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Uncovering cryptic species diversity of *Ophiocordyceps* (*Ophiocordycipitaceae*) associated with *Coleoptera* from Thailand

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Abstract: This study advances our understanding of *Ophiocordyceps*, an extensively studied entomopathogenic fungus within the *Ophiocordycipitaceae*, particularly in Thailand. We introduce seven novel species associated with *Coleoptera* – *O. albostroma*, *O. brunnea*, *O. capilliformis*, *O. kohchangensis*, *O. phitsanulokensis*, *O. pseudovariabilis*, and *O. ratchaburiensis*. Remarkably, *O. brunnea*, *O. kohchangensis*, and *O. ratchaburiensis* exhibit ascomata on the subterminal region of the stromata, with the asexual form appearing at the apex of the stipe, reminiscent of *O. brunneipunctata*. In contrast, *O. phitsanulokensis* produces its ascomata in the upper region of the stipe. Shared traits include immersed perithecia and part-spores production. *Ophiocordyceps albostroma* and *O. pseudovariabilis* produce pseudo-immersed perithecia, with the former producing ascospores breaking into four part-spores, and the latter displaying 32 part-spores. *Ophiocordyceps capilliformis* is also introduced due to morphological distinctions from closely related species. Phylogenetic analyses based on multigene loci (LSU, *TEF1*, *RPB1*, *RPB2*) robustly confirm the placement of these new species within *Ophiocordyceps*. Additionally, we report a new record of *O. clavata* in Thailand.

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INTRODUCTION

Ophiocordyceps (*Ophiocordycipitaceae*, *Hypocreales*) was established as a genus of entomopathogenic fungi by Petch (1931) with *O. blattae* as the type species occurring on forest cockroaches, collected in Sri Lanka. This genus is one of the most populous genera of entomopathogenic fungi with a worldwide distribution. *Ophiocordyceps* spp. infect a wide range of insect orders, affecting insects at various life stages, including larvae and adults, which display a diverse range of morphological characteristics, spanning from soft, tough, and dark stromata to vividly coloured ones (ranging from yellow to red). The perithecia associated with these species can either be completely embedded or superficial. Their ascospores are filiform, whole with multiple septa, or they may form part-spores. The predominant asexual morphs associated with *Ophiocordyceps* are hirsutella-like, followed by hymenostilbe-like and synglioclad ium-like. Species in *Ophiocordyceps* are widely recognised as famous traditional Chinese medicine, and a rich herbal source of bioactive compounds *e.g.*, *O. formosana* on *Coleoptera* larvae and *O. sinensis* on the larvae of hepialid moths (*Lepidoptera*) (Wang *et al*. 2015, 2023, Wei *et al*. 2021). In recent years, other species in *Ophiocordyceps* have attracted increasing attention as a source of biologically active compounds, for example, *O. irangiensis* on ants has shown promising activity against *Mycobacterium tuberculosis*, and polysaccharides derived from *O. sobolifera* on cicada nymphs showing promise for potential biomedical applications (Zhang *et al* . 2020, Saepua *et al*. 2021, Tran *et al*. 2021). In addition, *Hirsutella citriformis* is being used to control hemipteran *Diaphorina citri* for agricultural insect pests (Pérez-

González *et al*. 2022, Cantú-Bernal *et al*. 2023).

Previous studies have revealed that many entomopathogenic fungi within *Ophiocordyceps* exist as diverse species complexes which makes it difficult to identify individual species using only morphological characteristics. The use of DNA sequences of conserved loci combined with morphological data has improved our knowledge of fungal phylogeny (Mongkolsamrit *et al*. 2020a, b, Kuephadungphan *et al*. 2022, Thanakitpipattana *et al*. 2022). Numerous studies have shown that phylogenetic analyses can help clarify the taxonomy and identification of *Ophiocordyceps* spp. New species of *Ophiocordyceps* are increasingly described based on molecular results in association with their insect hosts and ecology (Sanjuan *et al*. 2014, Araújo *et al*. 2020, Saltamachia & Araújo 2020, Tasanathai *et al*. 2020, Khao-ngam *et al.* 2021). Species in *Ophiocordyceps* discovered in the last decade occur on several insect orders such as *Blattodea*, *Coleoptera*, *Diptera*, *Hemiptera*, *Hymenoptera*, *Isoptera* and *Lepidoptera* (Luangsa-ard *et al*. 2018, Khonsanit *et al*. 2019, Tasanathai *et al*. 2019, 2022, Araújo *et al*. 2021, Mongkolsamrit *et al*. 2021, 2023).

Coleoptera, or beetles, are one of the most diverse and abundant groups of insects on Earth and are classified into 176 families, with an estimate of 350 000 to 400 000 described species of beetles worldwide (Bouchard *et al*. 2017). They are found in a wide range of terrestrial and freshwater ecosystems, with some species inhabiting marine environments (Perissinotto *et al*. 2016, Hodge *et al*. 2019). Many species of entomopathogenic fungi in *Cordyceps sensu lato* (*Hypocreales*, *Sordariomycetes*) have been found in forests occurring on *Coleoptera* (adults or larvae) including *Blackwellomyces calendulinus*, *B. minutus*, *Cordyceps chiangdaoensis*, *C. nirtolii*, *Metarhizium clavatum*, *Nigelia martiale*, *Paraisaria phuwiangensis*, *Perennicordyceps ryogamiensis*, among others (Negi *et al.* 2012, Matočec *et al*. 2014, Tasanathai *et al.* 2016, Luangsa-ard *et al*. 2018, Mongkolsamrit *et al*. 2019, 2020a, b). In two studies, Shrestha *et al*. (2016) and Zha *et al*. (2021) conducted a comprehensive review and taxonomic summary on the distribution of numerous *Ophiocordyceps* species associated with *Coleoptera*. Most of these species were originally reported from the Old World, such as *O. formosana*, *O. kuchinaraiensis* and *O. sporangifera* from Thailand (Xiao *et al*. 2019, Crous *et al*. 2023), *O. bidoupensis* from Vietnam (Zou *et al*. 2022) and *O. rubripunctata*, known from Congo and Ghana (Samson *et al*. 1982). However, they have also been found in the New World, including *O. melolonthae* and *O. michiganensis* from the USA. Currently, there are approximately 60 known species of *Ophiocordyceps* associated with *Coleoptera*, including both larvae and adults (www. indexfungorum.org, October 15, 2023).

In our study of the diversity of pathogenic fungi on adults and larvae of *Coleoptera* in Thailand, we examined fungal specimens provisionally identified as *Ophiocordyceps* spp. These specimens were sourced from the BIOTEC Bangkok Herbarium (BBH) and the BIOTEC Culture Collection (BCC). Applying an integrative approach, we conducted taxonomic investigations, leading to the discovery of seven new species and one new record, *Ophiocordyceps clavata*. All species introduced here are presented with both morphological and phylogenetic illustrations, along with the identification of their respective *Coleoptera* hosts.

MATERIALS AND METHODS

Collections and isolation

The fungal specimens were collected from different forests in Thailand, located in Chiang Mai, Nan, Nakhon Ratchasima, Phitsanulok, Ratchaburi and Trad Provinces following searches on the ground and in decayed wood. Specimens were placed singly in plastic boxes, returned to the laboratory, and examined under an Olympus SZ61 dissecting microscope. The protocol for isolating ascospores and conidia from fertile parts followed previous studies (Luangsa-ard *et al*. 2018, Mongkolsamrit *et al*. 2018) using potato dextrose agar (PDA) plates (PDA: freshly diced potato 200 g/L, dextrose 20 g/L, agar 15 g/L). For isolation of the sexual morph, the fertile head containing mature perithecia was placed over a fresh PDA plate to ensure it remained elevated and did not contact the agar surface. These setups were then placed in a plastic box with moist tissue paper and examined daily for discharged ascospores. In the isolation of the asexual morph, a flame-sterilised inoculation needle was used to collect conidia from sporulating structures. The conidia were transferred to PDA plates, which were then incubated in a plastic box at room temperature. Daily examinations using a dissecting microscope were conducted to observe germinated conidia. Any discharged ascospores and germinated conidia were carefully removed with a sterile needle from the agar and transferred to a fresh PDA plate without antibiotics. The cultures were deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology, Thailand. The *Coleoptera* hosts were identified, based on comparison of morphological characters of the dried specimens preserved at the Insect Museum Thailand of the Department of Agriculture, Thailand. All fungal specimens were dried in an electric food dryer (50–55 °C) overnight and accessioned in the BIOTEC Bangkok Herbarium (BBH), National Biobank of Thailand.

Morphological study

Macroscopic characters were observed based on natural specimens. Important microscopic characters such as perithecia, asci, ascospores, phialides and conidia from the specimens were mounted on a microscope slide containing a drop of lactophenol cotton blue solution. The shapes, sizes, and colours of individual characters were determined and measured according to Mongkolsamrit *et al.* (2020b). Fungal strains were grown on malt extract yeast agar (MYA, Gibco: malt extract 20 g/L, Gibco: yeast 2 g/L, agar 15 g/L), oatmeal agar (OA, Difco: oatmeal 60 g/L, agar 12.5 g/L) and PDA agar plates at 25 °C under light/dark conditions (L:D 14:10) for 30–35 d. Cultures were observed to compare morphological characters including synnematal characteristics, phialides, and colony pigmentation. The colours of fresh specimens and cultures incubated on MYA, OA and PDA were described and codified following the Royal Horticultural Society (RHS) Colour Chart (RHS Media 2015).

DNA extraction, amplification and sequencing

Genomic DNA was extracted either from actively growing mycelial mass of PDA or from small pieces of tissues of fungal specimens using the modified cetyltrimethylammonium bromide (CTAB) method (Doyle 1987) as previously described by Mongkolsamrit

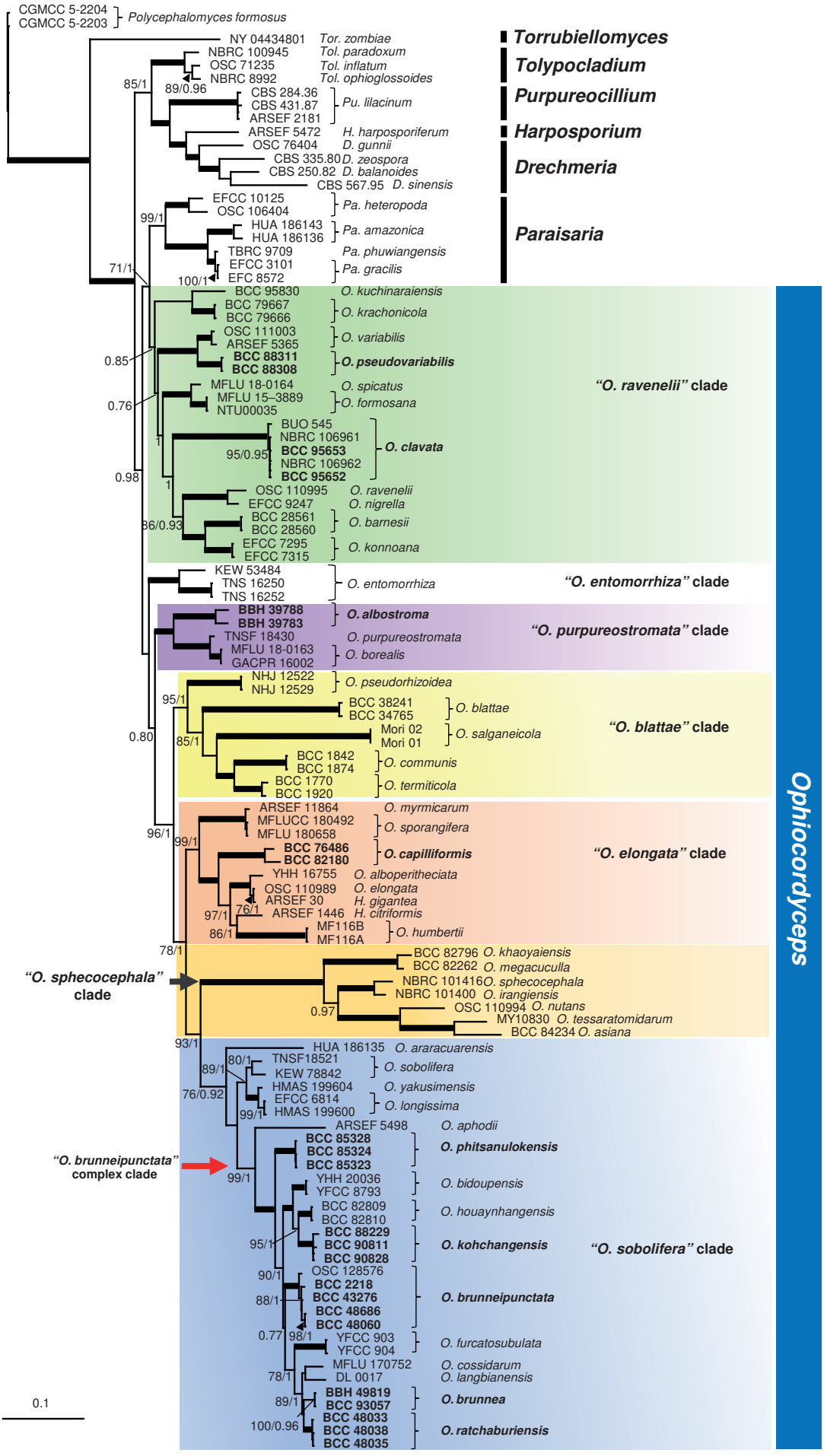

Fig. 1. RAxML tree of *Ophiocordyceps* and related genera in the *Ophiocordycipitaceae* from a combined LSU, *TEF1*, *RPB1* and *RPB2* dataset. Numbers at the major nodes represent Maximum Likelihood Bootstrap (MLB) support values and Bayesian Posterior Probabilities (BPP).

et al. (2020b). Nuclear loci, including the nuclear 28S rDNA (LSU), the translation elongation factor 1-α gene (*TEF1)* and the genes for RNA polymerase II largest (*RPB1*) and second largest (*RPB2*) subunits were amplified and sequenced. The primer pairs used were: LR0R/LR7 (Vilgalys & Hester 1990) for LSU, EF1-983f/EF1-2218r for *TEF1* (Rehner & Buckley 2005), CRPB1/RPB1Cr for *RPB1* (Castlebury *et al*. 2004) and RPB2-5F2/RPB2-7Cr for *RPB2* (Liu *et al*. 1999, O'Donnell *et al*. 2007). Amplification reactions were performed in 25 µL reaction volumes containing 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.4 M betaine, 200 µM of each of the four dNTPs, 0.5 µM of each forward and reverse primer, 1 U *Taq* DNA polymerase (Thermo Scientific) and 50–100 ng DNA templates. Amplifications were conducted under the following conditions: 2 min at 95 °C, 34 cycles of 1 min at 95 °C, 1 min at 55 °C, 2 min at 72 °C, with 2 min 72 °C for the *TEF1* region; 3 min at 94 °C, 34 cycles of 1 min at 94 °C, 1 min at 50 °C, 1.3 min at 72 °C, with 8 min 72 °C for the *RPB1* and *RPB2* regions. The purified PCR products were sequenced with PCR amplification primers for Sanger dideoxy sequencing (Macrogen Inc., Seoul, South Korea).

Phylogenetic analyses

The DNA sequences generated in this study were examined for ambiguous base calls using BioEdit v. 7.2.5; unambiguous sequences were then submitted to GenBank. Sequences of LSU, *TEF1*, *RPB1* and *RPB2* for alignment were collated to include sequences generated from previous studies, as shown in Table 1. Sequences were aligned using the ClustalW (Thompson *et al*. 1994) and manually edited employing BioEdit. The phylogenetic analyses for combined and single-locus alignments were performed using RAxML-HPC2 on XSEDE v. 8.2.12 (Stamatakis 2014) in the CIPRES Science Gateway portal, using the GTRGAMMA+I model with 1 000 bootstrap iterations. Bayesian inference (BI) of phylogenetic relationships was performed in MrBayes v. 3.2.7a (Ronquist *et al*. 2012), with best-fit models selected using MrModeltest v. 2.2 (Nylander 2004). The best model was GTR + G + I. Markov chain Monte Carlo (MCMC) simulations were run for 5 000 000 generations, sampling every 1 000 and discarding the first 10 % as burn-in after which the Bayesian posterior probabilities (PP) were calculated on the remaining trees. RAxML and BI output were imported into TreeView v. 1.6.6 to visualise the phylogenetic trees (Page 1996).

RESULTS

Molecular phylogeny

A total of 70 new sequences were generated (9 LSU, 19 *TEF1*, 23 *RPB1* and 19 *RPB2*) in this study (Table 1). The combined dataset comprised 109 taxa, with multi-loci sequences totalling an alignment length of 3 589 characters (bps), including gaps (LSU: 972 bps, *TEF1*: 979 bps, *RPB1*: 753 bps and *RPB2*: 885 bps). *Polycephalomyces formosus* (CGMCC5.2204

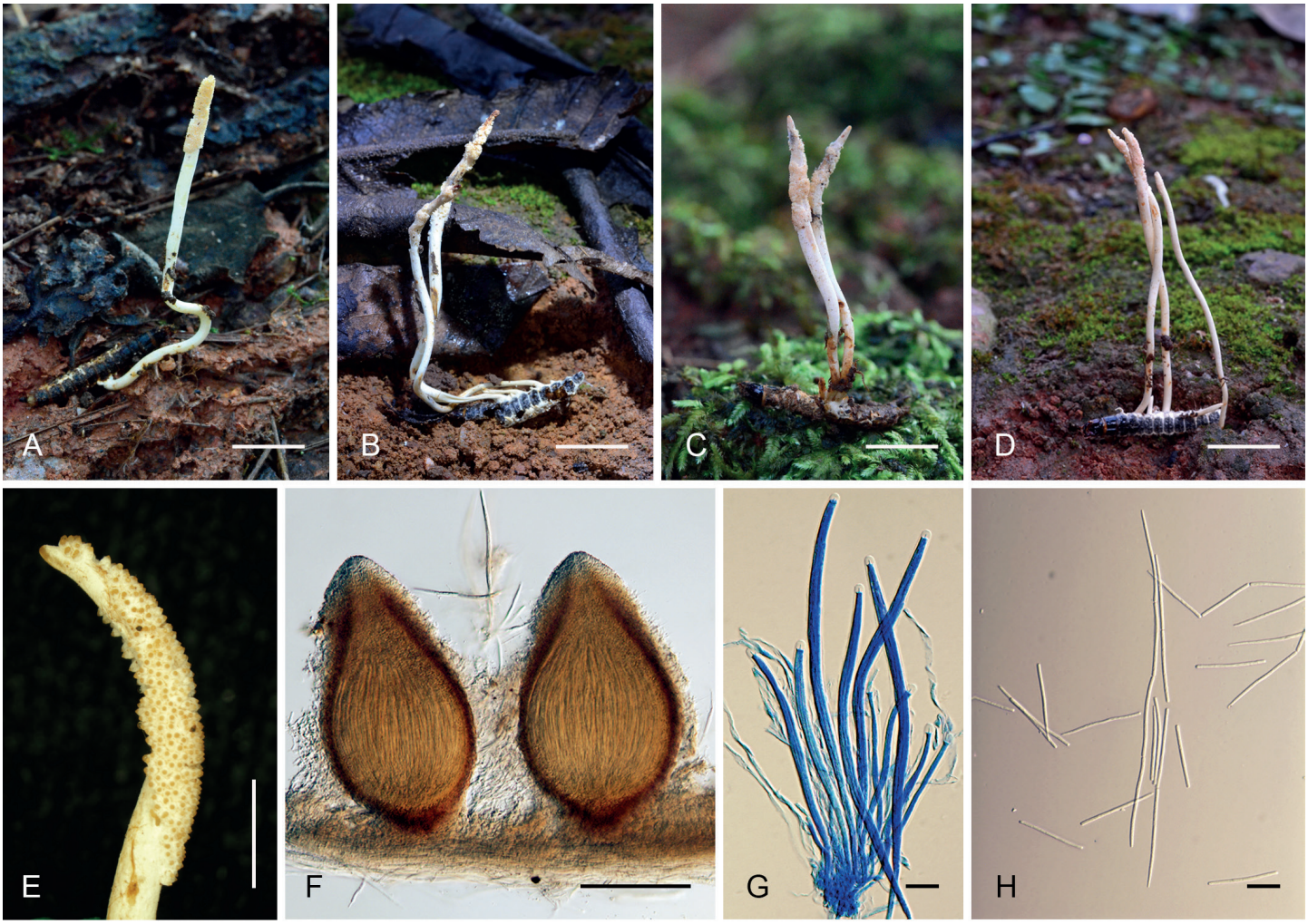

Fig. 2. *Ophiocordyceps albostroma.* **A–D.** Fungus on coleopteran larvae. **E.** Fertile part. **F.** Perithecia. **G.** Asci. **H.** Part-spores. Scale bars: A–D = 10 mm; E = 4 mm; F = 200 µm; G = 20 µm; H = 30 µm.

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

Species	Strain	Host/Substratum	GenBank Accession no.*			
			LSU	*TEF1*	*RPB1*	*RPB2*
Drechmeria balanoides	CBS 250.82	*Nematoda*	AF339539[1]	DQ522342[2]	DQ522388[2]	DQ522442[2]
Drechmeria gunnii	OSC 76404	*Lepidoptera*	AF339522[1]	AY489616[3]	AY489650[3]	DQ522426[2]
Drechmeria sinensis	CBS 567.95	*Nematoda*	AF339545[1]	DQ522343[2]	DQ522389[2]	DQ522443[2]
Drechmeria zeospora	CBS 335.80	*Nematoda*	AF339540[1]	EF469062[4]	EF469091[4]	EF469109[4]
Hirsutella citriformis	ARSEF 1446	—	KM652106[5]	KM651990[5]	KM652031[5]	—
Hirsutella gigantea	ARSEF 30	—	JX566977[6]	JX566980[6]	KM652034[5]	—
Harposporium harposporiferum	ARSEF 5472	—	AF339519[1]	DQ118747[7]	DQ127238[7]	—
Ophiocordyceps albostroma	**BBH 39783**	***Coleoptera***	—	**OR855785**	**OR855805**	**OR855829**
	BBH 39788	***Coleoptera***	**OR805251**	**OR855786**	**OR855806**	**OR855830**
Ophiocordyceps alboperitheciata	YHH 16755	*Lepidoptera*	MT222278[8]	MT222279[8]	MT222280[8]	MT222281[8]
Ophiocordyceps aphodii	ARSEF 5498	*Coleoptera*	DQ518755[2]	DQ522323[2]	—	DQ522419[2]
Ophiocordyceps araracuarensis	HUA 186135	*Hemiptera*	KC610769[9]	KC610738[9]	KF658665[9]	KC610716[9]
Ophiocordyceps asiana	BCC 84234	*Coreidae*	MW280201[10]	MW292438[10]	—	—
Ophiocordyceps barnesii	BCC 28560	*Coleoptera*	—	—	EU408773[11]	EU418599[11]
	BCC 28561	*Coleoptera*	—	—	EU408774[11]	EU418572[11]
Ophiocordyceps bidoupensis	YHH 20036	*Coleoptera*	OK556893[12]	OK556897[12]	OK556899[12]	—
	YFCC 8793	*Coleoptera*	OK556894[12]	OK556898[12]	OK556900[12]	—
Ophiocordyceps blattae	BCC 34765	*Blattodea* (*Blattoidea*)	—	MT533484[13]	MT533478[13]	—
	BCC 38241	*Blattodea* (*Blattoidea*)	MT512657[13]	MT533485[13]	MT533479[13]	—
Ophiocordyceps borealis	MFLU 18 0163	*Coleoptera*	MK863051[14]	MK860189[14]	—	—
	GACPR 16002	*Coleoptera*	MK863052[14]	MK860190[14]	—	—
Ophiocordyceps brunnea	**BCC 93057**	***Coleoptera***	—	**OR855787**	**OR855807**	**OR855831**
	BBH 49819	***Coleoptera***	—	**OR855788**	**OR855808**	**OR855832**
Ophiocordyceps brunneipunctata	**BCC 48060**	***Coleoptera***	—	**OR855791**	**OR855811**	—
	BCC 48686	***Coleoptera***	—	**OR855792**	**OR855812**	**OR855835**
	OSC 128576	*Coleoptera*	DQ518756[2]	DQ522420[2]	DQ522369[2]	DQ522542[2]
	BCC 2218	***Coleoptera***	—	**OR855789**	**OR855809**	**OR855833**
	BCC 43276	***Coleoptera***	—	**OR855790**	**OR855810**	**OR855834**
Ophiocordyceps capilliformis	**BCC 82180**	***Coleoptera***	—	**OR855794**	**OR855814**	**OR855837**
	BCC 76486	***Coleoptera***	**OR805252**	**OR855793**	**OR855813**	**OR855836**
Ophiocordyceps clavata	**BCC 95652**	***Coleoptera***	—	**OR855795**	**OR855815**	—
	BCC 95653	***Coleoptera***	—	**OR855796**	**OR855816**	**OR855838**
	BUO 545	*Coleoptera*	MH879601[15]	MH879672[15]	MH885450[15]	—
	NBRC 106961	*Coleoptera*	JN941414[16]	—	JN992461[16]	—
	NBRC 106962	*Coleoptera*	JN941415[16]	—	JN992460[16]	—
Ophiocordyceps communis	BCC 1874	*Termitidae*	MH753679[17]	MK284267[17]	MK214109[17]	MK214095[17]
	BCC 1842	*Termitidae*	MH753680[17]	MK284266[17]	MK214110[17]	MK214096[17]
Ophiocordyceps cossidarum	MFLU 17-0752	*Lepidoptera*	MF398187[33]	MF928403[33]	MF928404[33]	—
Ophiocordyceps elongata	OSC 110989	*Lepidoptera*	EF468808[4]	EF468748[4]	EF468856[4]	—
Ophiocordyceps entomorrhiza	KEW 53484	*Coleoptera*	EF468809[4]	EF468749[4]	EF468857[4]	EF468911[4]
	TNS 16250	—	—	KJ878987[28]	KJ879021[28]	—
	TNS 16252	—	KJ878906[28]	KJ878986[28]	—	—
Ophiocordyceps formosana	NTU00035	*Coleoptera*	—	KT275192[19]	KT275190[19]	KT275191[19]
	MFLU 15-3889	*Coleoptera*	—	KU854950[20]	KU854948[20]	—
Ophiocordyceps furcatosubulata	YFCC 903	*Coleoptera*	MT774222[21]	MT774243[21]	MT774244[21]	MT774236[21]

Table 1. (continued)

Species	Strain	Host/Substratum	GenBank Accession no.*			
			LSU	*TEF1*	*RPB1*	*RPB2*
	YFCC 904	*Coleoptera*	MT774223[21]	MT774244[21]	MT774230[21]	MT774237[21]
Ophiocordyceps houaynhangensis	MY11460	*Coleoptera*	MH092908[22]	MH092899[22]	—	—
	MY11461	*Coleoptera*	MH092909[22]	MH092900[22]	—	—
Ophiocordyceps humbertii	MF116A	*Hymenoptera*	MK875537[23]	—	MK863828[23]	—
	MF116B	*Hymenoptera*	MK875536[23]	—	MK863829[23]	—
Ophiocordyceps irangiensis	NBRC 101400	*Hymenoptera*	JN941426[17]	—	JN992449[17]	—
Ophiocordyceps khaoyaiensis	BCC 82796	*Hymenoptera*	MH028153[24]	MH028187[24]	MH028165[24]	MH028175[24]
Ophiocordyceps kohchangensis	**BCC 88229**	***Coleoptera***	**OR805253**	—	**OR855817**	—
	BCC 90828	***Coleoptera***	**OR805255**	—	**OR855819**	**OR855840**
	BCC 90811	***Coleoptera***	**OR805254**	**OR855797**	**OR855818**	**OR855839**
Ophiocordyceps konnoana	EFCC 7315	*Coleoptera*	—	EF468753[4]	EF468861[4]	EF468916[4]
	EFCC 7295	*Coleoptera*	—	—	EF468862[4]	EF468915[4]
Ophiocordyceps krachonicola	BCC 79667	*Coleoptera*	MK632081[25]	MK632055[25]	MK632162[25]	MK632133[25]
	BCC 79666	*Coleoptera*	MK632080[25]	MK632054[25]	MK632161[25]	MK632132[25]
Ophiocordyceps kuchinaraiensis	BCC 95830	*Coleoptera*	OQ627397[26]	OQ625474[26]	—	OQ625475[26]
Ophiocordyceps langbianensis	DL 0017	*Coleoptera*	MT928306[27]	—	—	—
Ophiocordyceps longissima	HMAS 199600	—	—	KJ878972[28]	KJ879006[28]	KJ878949[28]
	EFCC 6814	*Hemiptera*	EF468817[4]	EF468757[4]	EF468865[4]	–
Ophiocordyceps megacuculla	BCC 82262	*Hymenoptera*	MH028161[24]	MH028191[24]	MH028172[24]	MH028180[24]
Ophiocordyceps myrmicarum	ARSEF 11864	*Hemiptera*	JX566965[6]	JX566973[6]	KJ680151[6]	–
Ophiocordyceps nigrella	EFCC 9247	*Lepidoptera*	EF468818[4]	EF468758[4]	EF468866[4]	EF468920[4]
	OSC 110994	*Hemiptera*	—	DQ522333[2]	DQ522378[2]	—
Ophiocordyceps phitsanulokensis	**BCC 85323**	***Coleoptera***	**OR805256**	—	**OR855820**	**OR855841**
	BCC 85324	***Coleoptera***	—	—	**OR855821**	**OR855842**
	BCC 85328	***Coleoptera***	**OR805257**	**OR855798**	**OR855822**	**OR855843**
Ophiocordyceps pseudorhizoidea	NHJ12522	Termite	EF468825[4]	EF468764[4]	EF468873[4]	EF468923[4]
	NHJ12529	Termite	EF468824[4]	EF468765[4]	EF468872[4]	EF468922[4]
Ophiocordyceps pseudovariabilis	**BCC 88308**	***Coleoptera***	—	**OR855799**	**OR855823**	—
	BCC 88311	***Coleoptera***	—	**OR855800**	**OR855824**	**OR855844**
Ophiocordyceps purpureostromata	TNSF18430	*Coleoptera*	KJ878897[28]	KJ878977[28]	KJ879011[28]	—
Ophiocordyceps ravenelii	OSC 110995	*Coleoptera*	DQ518764[2]	DQ522334[2]	DQ522379[2]	DQ522430[2]
Ophiocordyceps salganeicola	Mori01	*Blattodea*	MT741719[23]	MT759575[23]	MT759578[23]	MT759580[23]
	Mori02	*Blattodea*	MT741718[23]	MT759572[23]	MT759579[23]	MT759581[23]
Ophiocordyceps sobolifera	TNSF 18521	*Hemiptera*	KJ878898[28]	KJ878979[28]	KJ879013[28]	—
	KEW 78842	*Hemiptera*	EF468828[4]	—	EF468875[4]	EF468925[4]
Ophiocordyceps sphecocephala	NBRC 101416	—	JN941443[16]	—	JN992432[16]	—
Ophiocordyceps spicatus	MFLU 18-0164	*Coleoptera*	MK863054[14]	MK860192[14]	—	—
Ophiocordyceps sporangifera	MFLUCC 18-0492	*Lepidoptera*	MH725832[18]	MH727390[18]	MH727392[18]	—
	MFLU 18-0658	*Lepidoptera*	MH725831[18]	MH727389[18]	MH727391[18]	—
Ophiocordyceps termiticola	BCC 1770	*Termitidae*	MH753677[17]	MK284264[17]	MK214107[17]	MK214093[17]
	BCC 1920	*Termitidae*	MH753678[17]	MK284265[17]	MK214108[17]	MK214094[17]
Ophiocordyceps tessaratomidarum	MY 10830	*Tessaratomidae*	MW280218[10]	MW292434[10]	—	—
Ophiocordyceps ratchaburiensis	**BCC 48035**	***Coleoptera***	**OR805258**	**OR855803**	**OR855827**	**OR855847**
	BCC 48033	***Coleoptera***	**OR805259**	**OR855802**	**OR855826**	**OR855846**
	BCC 48038	***Coleoptera***	—	**OR855804**	**OR855828**	**OR855848**
Ophiocordyceps variabilis	OSC 111003	Dipteran	EF468779[4]	EF468885[4]	—	EF468933[4]

Table 1. (continued)

Species	Strain	Host/Substratum	GenBank Accession no.*			
			LSU	*TEF1*	*RPB1*	*RPB2*
	ARSEF 5365	Dipteran	DQ522340[2]	DQ522386[2]	—	DQ522437[2]
Ophiocordyceps yakusimensis	HMAS 199604	*Hemiptera*	KJ878902[28]	—	KJ879018[28]	KJ878953[28]
Paraisaria amazonica	HUA 186143	*Orthoptera*	KJ917571[9]	KM411989[9]	KP212902[9]	KM411982[9]
	HUA 186136	*Orthoptera*	—	KM411991[9]	KP212905[9]	—
Paraisaria gracilis	EFCC 3101	*Lepidoptera*	EF468810[4]	EF468750[4]	EF468858[4]	EF468913[4]
	EFCC 8572	*Lepidoptera*	EF468811[4]	EF468751[4]	EF468859[4]	**EF468912**[4]
Paraisaria heteropoda	EFCC 10125	*Hemiptera*	EF468812[4]	EF468752[4]	EF468860[4]	EF468914[4]
	OSC 106404	*Hemiptera*	AY489722[3]	AY489617[3]	AY489651[3]	—
Paraisaria phuwiangensis	TBRC 9709	*Coleoptera*	MK192057[29]	MK214082[29]	MK214086[29]	—
Polycephalomyces formosus	CGMCC 5-2203	*Coleoptera*	MN586844[30]	MN598059[30]	MN598050[30]	MN598066[30]
	CGMCC 5-2204	*Coleoptera*	MN586839[30]	MN598054[30]	MN598045[30]	MN598061[30]
Purpureocillium lilacinum	CBS 431.87	*Nematoda*	EF468844[4]	EF468791[4]	EF468897[4]	EF468940[4]
	CBS 284.36	Soil	AY624227[31]	EF468792[4]	EF468897[4]	EF468940[4]
	ARSEF 2181	—	AF339534[1]	EF468790[4]	EF468896[4]	—
Tolypocladium inflatum	OSC 71235	*Coleoptera*	EF469077[4]	EF469061[4]	EF469090[4]	EF469108[4]
Tolypocladium ophioglossoides	NBRC 8992	*Elaphomyces* sp.	JN941405[16]	—	JN992470[16]	—
Tolypocladium paradoxum	NBRC 100945	*Hemiptera*	JN941410[16]	—	JN992465[16]	—
Torrubiellomyces zombiae	NY 04434801	*O. camponoti-floridani*	ON493602[32]	ON513396[32]	ON513398[32]	ON513402[32]

*: [1]Sung *et al.* (2001), [2]Spatafora *et al.* (2007), [3]Castlebury *et al.* (2004), [4]Sung *et al.* (2007), [5]Simmons *et al.* (2015a), [6]Simmons *et al.* (2015b), [7]Chaverri *et al.* (2005), [8]Fan *et al.* (2021), [9]Sanjuan *et al.* (2015), [10]Khao-ngam *et al.* (2021), [11]Luangsa-Ard *et al.* (2010a), [12]Zou *et al.* (2022), [13]Mongkolsamrit *et al.* (2021), [14]Zha *et al.* (2021), [15]Chen *et al.* (2019), [16]Schoch *et al.* (2012), [17]Tasanathai *et al.* (2019), [18]Xiao *et al.* (2019), [19]Wang *et al.* (2015), [20]Li *et al.* (2016), [21]Wang *et al.* (2021a), [22]Crous *et al.* (2018), [23]Araujo *et al.* (2020), [24]Khonsanit *et al.* (2019), [25]Thanakitpipattana *et al*. (2020), [26]Crous *et al.* (2023), [27]Lao *et al*. (2021), [28]Quandt *et al.* (2014), [29]Mongkolsamrit *et al.* (2019), [30]Wang *et al.* (2021b), [31]Luangsa-ard *et al*. (2005), [32]Araújo *et al.* (2022), [33]Hyde *et al.* (2017).

and CGMCC5.2203) in *Polycephalomycetaceae* (Xiao *et al.* 2023) were used as outgroups. The phylogenetic tree from maximum likelihood phylogenetic analysis with maximum likelihood bootstrap (MLB) values is shown in Fig. 1. The nodes were also evaluated with Bayesian posterior probabilities (BPP). In the generated phylogenetic tree (Fig. 1), the family *Ophiocordycipitaceae* is represented by seven genera and seven subclades of *Ophiocordyceps* including 1) *"O. ravenelii"* (< 50 % MLB/0.85 BPP), 2) *"O. entomorrhiza"* (100 % MLB/1 BPP), 3) *"O. purpureostromata"* (100 % MLB/1 BPP), 4) *"O. blattae"* (95 % MLB/1 BPP), 5) *"O. elongata"* (99 % MLB/1 BPP), 6) *"O. sphecocephala*" (100 % MLB/1 BPP) and 7) *"O. sobolifera"* (76 % MLB/0.92 BPP) clades. Individual gene trees were generated using the same analytical tools as above and are shown in Supplementary Figs S1–S4. The sequence alignments for all datasets employed in this study were uploaded to Figshare at https://10.6084/m9.figshare.24495169.

The results from multi-locus phylogenetic analyses strongly support the placement of *O. clavata* and *O. pseudovariabilis* within the *"O. ravenelii"* clade, while *O. albostroma* and *O. capilliformis* are nested in the *"O. purpureostromata"* and *"O. elongata"* clades, respectively. The four newly identified taxa, namely *O. brunnea*, *O. kohchangensis*, *O. phitsanulokensis*, and *O. ratchaburiensis*, along with known species *O. aphodii*, *O. bidoupensis*, *O. houaynhangensis*, *O. furcatosubulata*, *O. cossidarum*, *O. langbianensis*, form distinct lineages within the *"O. brunneipunctata"* species complex subclade, which is part of the *"O. sobolifera"* clade. Based on these results, species descriptions are provided for seven novel species, including *O. clavata*, which is which is a newly recorded species.

Taxonomy

Ophiocordyceps albostroma Khonsanit, Mongkolsamrit, Noisripoom & Luangsa-ard, ***sp. nov.*** MycoBank MB 851828. Fig 2.

Etymology: Latin "*albus*" means white, referring to the colour of the stipes of fresh specimens.

Typus: **Thailand**, Chiang Mai Province, Chiang Dao Wildlife Sanctuary, Doi Chiang Dao Wildlife Research Station, on *Coleoptera* larva, buried in soil, 31 Oct. 2014, *A. Khonsanit*, *D. Thanakitpipattana*, *K. Tasanathai*, *S. Mongkolsamrit* & *W. Noisripoom* (**holotype** BBH 39783).

Sexual morph: *Stromata* solitary or several, cylindrical, gradually tapering toward the apex, yellowish white (155D), arising from head, thorax and abdomen of *Coleoptera* larvae, 30–60 mm long. *Stipes* cylindrical, smooth, white to white cream, 0.5–2 mm wide. *Fertile parts* developing at middle to upper region of

stipes, occasionally produced on one side of surface of stipes, light yellow (17D). *Perithecia* pseudo-immersed, tightly packed, ovoid, (390–)406–456(–490) × (220–)235.5–267.5(–295) µm. *Asci* cylindrical, (123–)155–213(–250) × (4–)5–6.5(–8) µm with asci caps, 2–3 × 3.5–4 µm. *Ascospores* filiform, (118–)134–160(–178) × 1–2 µm, breaking into 4 part-spores, (25–)31–44(–55) × 1–2 µm. *Asexual morph*: Unknown.

Distribution: Found in the northern region of Thailand.

Additional materials examined: **Thailand**, Chiang Mai Province, Chiang Dao Wildlife Sanctuary, Doi Chiang Dao Wildlife Research Station, on *Coleoptera* larvae, buried in soil, 31 Oct. 2014, *A. Khonsanit*, *D. Thanakitpipattana*, *K. Tasanathai*, *S. Mongkolsamrit* & *W. Noisripoom* (specimens BBH 39781; BBH 39782; BBH 39784; BBH 39785; BBH 39786; BBH 39787; BBH 39788; BBH 39789; BBH 39790).

Notes: This is a fastidious species, and no cultures could be obtained from any of the collected specimens, hence DNA extraction was performed on the stroma. *Ophiocordyceps albostroma* has exclusively been found in the Chiang Dao Wildlife Sanctuary in northern Thailand. The phylogenetic analyses presented in Fig. 1 clearly show a close genetic affinity between *O. albostroma* and its counterparts, *O. purpureostromata* and *O. borealis*. The latter two were originally found on *Elateridae* larvae in Japan and Russia, respectively (Kobayasi & Shimizu 1980, Zha *et al*. 2021). A distinctive characteristic among these species is the densely packed structures of perithecia on the stipes, occasionally found on one side of the stipes. However, they notably differ in stromata coloration: *O. albostroma* exhibits white to cream stromata, *O. purpureostromata* displays purple stromata, and *O. borealis* produces grey stromata. These three species share the presence of part-spores. *Ophiocordyceps albostroma* produces ascospores that break into four part-spores, similar to *O. purpureostromata*, while the number of part-spores for *O. borealis* is not indicated. However, the part-spores in *O. albostroma* are longer than in the other two species (Table 2).

Ophiocordyceps brunnea Mongkolsamrit, Himaman, Noisripoom & Luangsa-ard, ***sp. nov*.** MycoBank MB 851829. Fig. 3.

Etymology: Referring to the brown colour of the ascomata.

Typus: **Thailand**, Nan Province, Ban Sali deciduous dipterocarp forest trail, on *Elateridae* larva (*Coleoptera*), buried in soil, 6 Aug. 2020, *B. Sakolrak* & *W. Himaman* (**holotype** BBH 49817, culture ex-type BCC 93057).

Sexual morph: *Stromata* solitary or in pairs, cylindrical, gradually tapering toward the apex, brownish orange (166B–C), 20–35 mm long. *Stipes* cylindrical, smooth, pale yellow (161D), 0.7–1 mm wide. *Rhizoids* flexuous, 2–3 cm long buried under the ground, arising from head and thorax of *Coleoptera* larvae. *Fertile parts* distinctly subterminal with asexual morph at the apex. *Ascomata* subterminal, cylindrical, moderate reddish brown to brownish orange (166B–C), crowded perithecia, 2–4 mm long, 1.2–2 mm wide. *Perithecia* completely immersed, obpyriform, (450–)455–485(–500) × (150–)160–220(–240) µm. *Asci* cylindrical, (125–)135–185(–200) × 5–8 µm with asci caps 1.5–2 × 5 µm. *Ascospores* cylindrical, 125–195 µm long, breaking into 32 small truncate part-spores, 4–5(–6) × 1–1.5(–2) µm. *Asexual morph*: *Conidiogenous cells* hirsutella-like, mono- or polyphialidic, cylindrical basal portion, 5–22 × 3–5 µm with a distinct, filiform, long neck up to 21 µm long. *Conidia* hyaline, smooth-walled, globose, 3–5 µm diam.

Culture characteristics: Colonies on MYA attaining a diam of 6–8 mm in 30 d, light yellowish pink (36A), colonies reverse dark greyish yellow brown (N199B). *Conidiogenous cells* hirsutella-like, phialidic, arising from either the lateral or terminal regions of the hyphae, cylindrical or ellipsoidal basal portion, 10–22(–25) × 3–4.5(–5) µm with a distinct, filiform, long neck up to 15 µm long. *Conidia* hyaline, smooth-walled, globose, 2–2.5(–3) µm diam. Poor sporulation was observed. Colonies on OA attaining a diam of 10–16 mm in 30 d, yellowish white (156D) to greyish red (182B), colonies reverse dark greyish yellow brown (N199B), sparse synnemata producing on edge of colonies, white, 2–3 mm long, 200–300 µm wide. *Conidiogenous cells* hirsutella-like, phialidic, emerging along the synnemata, subglobose, cylindrical or ellipsoidal basal portion, (8–)10.5–21.5(–25) × 3–4.5(–5) µm with a distinct, filiform, long neck up to 20 µm long. *Conidia* hyaline, smooth-walled, globose, 2–3 µm diam. Colonies on PDA attaining a diam of 10–15 mm in 30 d, white, greyish red (182B) in the centre of colonies, colonies reverse white and dark greyish yellow brown (N199B) in the centre of colonies. *Conidiogenous cells* hirsutella-like, phialidic, arising from either the lateral or terminal regions of the hyphae, cylindrical or ellipsoidal basal portion, (9.5–)10–17.5(–20) × 2–4 µm with a distinct, filiform, long neck up to 18 µm long. *Conidia* hyaline, smooth-walled, globose, 2–3 µm diam. Poor sporulation was observed.

Distribution: Found in the northern region of Thailand.

Additional materials examined: **Thailand**, Nan Province, Ban Sali deciduous dipterocarp forest trail, on *Coleoptera* larva, buried in soil, 6 Aug. 2020, *B. Sakolrak* & *W. Himaman* (BBH 48915, **paratype**, culture ex-paratype BCC 93059); *idem*., (BBH 49818, culture BCC 93058); *idem*., (BBH 49819).

Notes: Based on macro-morphology in the field, *O. brunnea* appears similar to *O. brunneipunctata* and *O. ratchaburiensis* as it produces ascomata on the subterminal region of the stroma, with the asexual form appearing at the apex of the stipe. All three species have immersed perithecia, and their ascospores break into 32 part-spores. However, molecular phylogenetic analysis (Fig. 1) strongly supports the close relationship between *O. brunnea* and *O. ratchaburiensis*, but not forming a monophyletic clade with *O. brunneipunctata*. When comparing the colour of the stromata, those of *O. ratchaburiensis* are pale yellow, while those of *O. brunnea* range from brown to light brown. Furthermore, when examining the characteristics of colonies on three different media (MYA, OA, and PDA), it is observed that *O. brunnea* and *O. ratchaburiensis* generally produce reproductive structures on those media. However, an exception is noted for *O. ratchaburiensis* on MYA, where it exhibits flattened white mycelium without any accompanying reproductive structures (Supplementary Fig. S5). *Ophiocordyceps brunnea* produces sparse synnemata on OA, a characteristic not observed in *O. ratchaburiensis*. Conversely, synnemata were observed in PDA for *O. ratchaburiensis,* a feature not observed in *O. brunnea*.

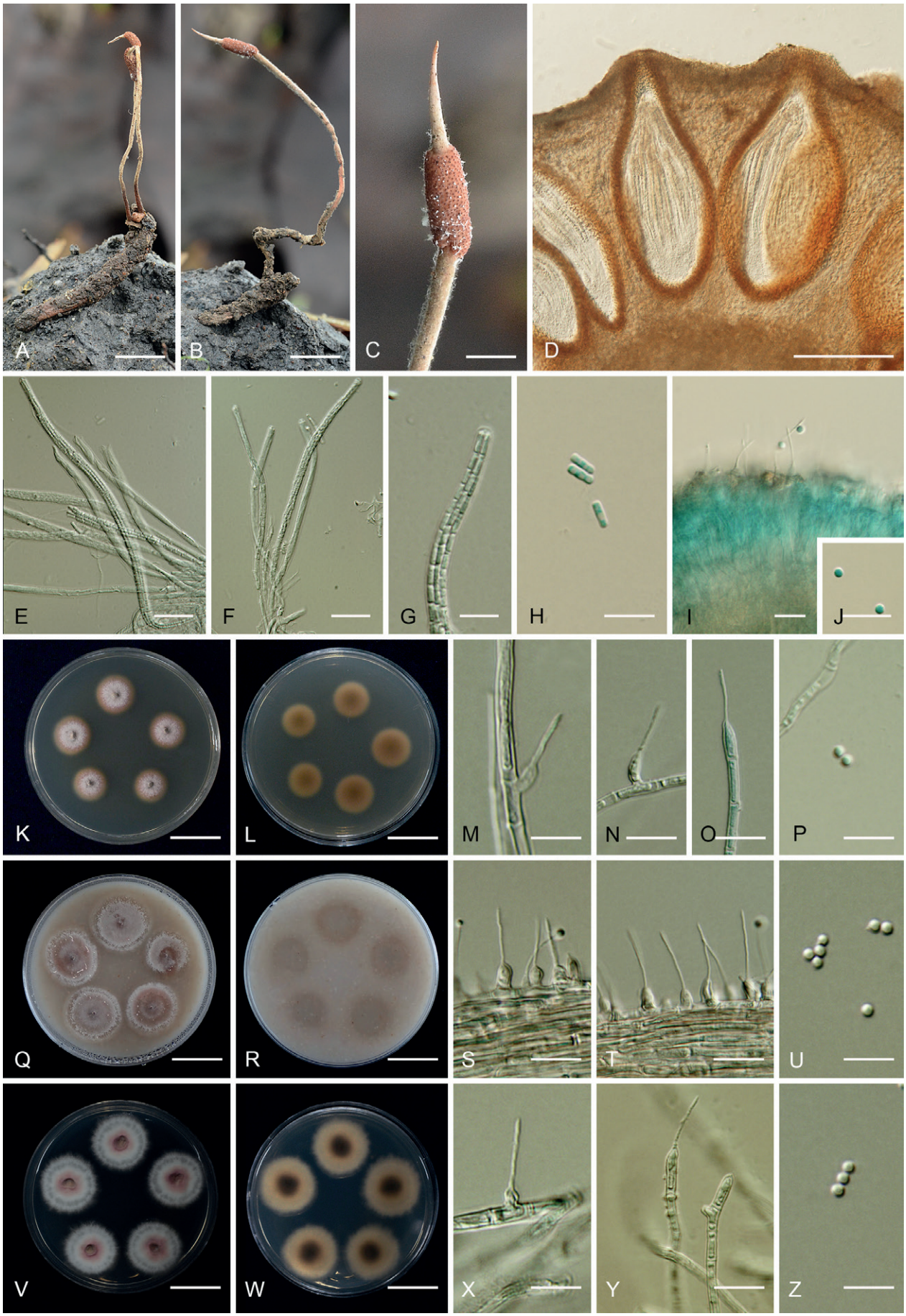

Fig. 3. *Ophiocordyceps brunnea.* **A, B.** Fungus on coleopteran larvae. **C.** Close-up of fertile head. **D.** Perithecia. **E, F.** Asci. **G.** Ascus. **H.** Part-spores. **I, J.** Phialides with conidia. **K, L.** Colonies on MYA at 30-d-old (**K** obverse, **L** reverse). **M–O.** Phialides with conidia on MYA. **P.** Conidia on MYA. **Q, R.** Colonies on OA at 30-d-old (**Q** obverse, **R** reverse). **S, T.** Phialides on OA. **U.** Conidia on OA. **V, W.** Colonies on PDA at 30-d-old (**V** obverse, **W** reverse). **X, Y.** Phialides on PDA. **Z.** Conidia on PDA. Scale bars: A, B = 5 mm; C = 1 mm; D = 200 µm; E, F, I, J, M–O, S, T, X, Y = 20 µm; G, H, P, U, Z = 10 µm; K, L, Q, R, V, W = 15 mm.

Table 2. Morphological comparisons between *Ophiocordyceps* species occurring on *Coleoptera* and other hosts.

Species	Host	Stromata/Synnemata	Perithecia	Asci	Ascospores	Conidiogenous cells	Conidia	Reference
Ophiocordyceps albostroma	Larva of *Coleoptera*	Solitary, several, cylindrical, yellowish white, 30–60 mm long, 0.5–2 mm wide	Pseudo-immersed, 390–490 × 220–295 µm	Cylindrical, 123–250 × 4–8 µm	Filiform, breaking into 4 part-spores 25–55 × 1–2 µm	—	—	This study
O. alboperitheciata	Larva of *Noctuidae* (*Lepidoptera*)	Two, brown to dark brown, unbranched, 69–71 × 0.6–1.2 mm	Superficial, 408–549 × 233–321 µm	Cylindrical, 144–246 × 3.5–4.7 µm	Cylindrical, multiseptate, 1.1–1.3 × 0.5–0.6 µm. Part-spores were not seen.	—	—	Fan *et al*. (2021)
O. bidoupensis	Larva of *Elateridae* (*Coleoptera*)	Solitary, solid, cylindrical, yellow, 11.8–22.5 cm long	Immersed, pyriform to lanceolate, brown-yellow, 213.4–405.9 × 74.8–192.4 µm	Slender, 116.1–192.7 × 4.8–7.5 µm	Filiform, multiseptate	Cone, hyaline, spetate, smooth-walled, forming on hyphae, with a hypertrophic base, tapering abruptly into a thin neck, smooth-walled, 13.8–46.4 × 0.42–5.13 µm	Oval or briolette, 2.24–3.61 × 1.49–2.70 µm	Zou *et al.* (2022)
O. borealis	Larva of *Elateroidea* (*Coleoptera*)	Single or paired, grey, 10–13 mm long, 0.25–0.6 mm wide	Immersed, obliquely or at right angles to the surface of stipe, pyriform, 220–290 × 120–150 µm	Cylindrical, 6–8 µm wide	Cylindrical, part-spore, 10–15 × 2 µm	—	—	Zha *et al*. (2021)
O. brunnea	Larva of *Elateridae* (*Coleoptera*)	Solitary, or pairs, cylindrical, gradually tapering toward the apex, brownish orange, 20–35 mm long, 0.7–1 mm wide	Completely immersed, obpyriform, 450–500 × 150–240 µm	Cylindrical, 125–200 × 5–8 µm	Cylindrical, 125–195 µm long, breaking into 32 part-spores, 4–6 × 1–2 µm	Monophialidic, polyphialidic, cylindrical basal portion, 5–22 × 3–5 µm with a distinct, filiform, long neck up to 21 µm long	Globose, 3–5 µm diam.	This study
O. brunneipunctata	Larva of *Elateridae* (*Coleoptera*)	Solitary, rarely up to 3, simple, 25–90 mm high	Immersed, ovate to pyriform, brown-walled, 270–335 × 110–160 µm	Cylindric, 280–295 × 6–7 µm	Filiform, multiseptate, 4–6 × 1–1.5 µm	Monophialidic, rarely polyphialidic, hyaline, 5.5–7.5 × 2.5–3 µm at the base, cylindrical base, up to 15 × 0.5 µm above	Spherical, 1.5–2.5 µm diam., enveloped by a mucous sheath	Hywel-Jones (1995)

Table 2. (continued)

Species	Host	Stromata/Synnemata	Perithecia	Asci	Ascospores	Conidiogenous cells	Conidia	Reference
O. capilliformis	Larva of *Meracantha* sp. (*Tenebrionidae*, *Coleoptera*), adult of *Platydracus* sp. (*Staphylinidae, Coleoptera*)	Solitary, greyish reddish brown to moderate brown 80–100 mm long, 0.8–1.2 mm wide. Synnemata indeterminate, multiple, produced along the stipe below the ascomata, cylindrical, up to 8 mm long	Completely immersed, pyriform, 395–420 × 190–250 µm	Cylindrical, 125–200 × 5–8 µm	Whole, filiform, 130–210 × 2–3 µm with 5- or 6-septa	Hirsutella-like, solitary, cylindrical to ovoid basal portion, 29–33 × 4–5 µm, tapering to a narrow neck up to 25–28 µm long	Fusiform, occasionally slightly curved, 9–15 × 3–8 µm with a mucous sheath	This study
O. clavata	Larva of *Tenebrionidae* (*Coleoptera*)	Multiple, clavate, pale yellow, 2–10 mm long, 0.5–1.5 mm wide	Immersed, broad ovoid, 400–500 × 250–350 µm	Cylindrical, 200–300 × 6–8 µm	Cylindrical, 200–250 µm long, breaking into 32 part-spores, 5–10 × 1.5–3 µm	—	—	This study
O. cossidarum	Larva of *Cossidae* (*Lepidoptera*)	Solitary, simple, 40–70 mm high	Immersed, red, ovate to phialide, red-walled, 355–454 × 136–171 µm	Cylindrical, 8-spores with a thickened apex, 174–221 × 5.7–7 µm	Filiform, multiseptated, 131–153 × 1.8–2.2 µm, breaking into 32 part-spores	—	—	Hyde *et al.* (2017)
O. elongata	Pupae and Larva of *Apatela americana* (*Lepidoptera*)	The stalk is flexuose, longitudinally sulcate and twisted, 110 mm long	Immersed, ovatoconoid, 0.5 × 0.3 mm	220 µm long, 8 µm wide	Cylindrical, 2 um wide, with septa, 4–12 µm apart. Part-spores were not seen.	—	—	Petch (1937)
O. formosana	Larva of *Tenebrionoidea* (*Coleoptera*)	Several, yellow to orange, 14 mm, 2-5 mm wide, simple or branched	Completely immersed, 453–546 × 265–298 µm	366–498 × 8–11 µm	Cylindrical, part-spores, 2–6 × 1–3 µm	—	—	Li *et al.* (2016)
O. furcatosubulata	Larva of *Elateridae* (*Coleoptera*)	Single, solid, yellow to brown, 40–80 mm long, 1.5–2.2 mm wide	Immersed, long ovoid or pyriform, 289.6–405.8 × 87–159.2 µm	Cylindrical, 138.8–202.5 × 4.3–6 µm	Filiform, multiseptated, finally breaking into secondary ascospores, 3.7–5.3 × 1.3–2 µm	Polyphialidic, forming on conidiophores or side branches, with a slender or subulate base, tapering gradually, smooth-walled or verruculose, 3.5 –15.8 × 0.9–1.7 µm	Solitary, aseptate, smooth-walled, broadly ellipsoid or ellipsoid, 1.5–2.5 × 1.2–1.9 µm	Wang *et al.* (2021a)

Table 2. (continued)

Species	Host	Stromata/Synnemata	Perithecia	Asci	Ascospores	Conidiogenous cells	Conidia	Reference
O. houaynhangensis	Larva of *Coleoptera*	Solitary, cylindrical, cream, up to 11 cm long, 1.5–2.5 mm wide	Completely immersed, obclavate, 300–450 × 80–170 µm	Cylindrical, 100–250 × 4–7.5 µm	Cylindrical, part-spores, 4–7 × 1–2 µm	Monophialidic, phialides flasked-shaped with long necks, up to 30 µm long and 2–4 µm in breadth; phialide necks up to 18 µm long and 0.5 µm in breadth	Spherical, 2–3 µm	Crous *et al.* (2018)
O. kohchangensis	Larva of *Elateridae* (*Coleoptera*)	Solitary, cylindrical, gradually tapering toward the apex, moderate yellow to light yellow, up to 75 mm long, 1–1.5 mm wide	Completely immersed, pyriform, 320–400 × 100–200 µm	Cylindrical, 90–200 × 4–6 µm	Cylindrical, breaking into 32 part-spores, 4–7 × 1.5–2 µm	Hirsutella-like, monophialidic or polyphialidic, 5–40 × 3–5 µm with a distinct, filiform, long neck up to 25 µm long	Globose, 2–4 µm	This study
O. krachonicola	Nymph of *Gryllotalpa orientalis* (*Gryllidae*)	Solitary, cylindrical, dark grey, 37–40 × 1–1.5 mm	Completely immersed, ovoid to obclavate, 460–580 × 180–300 µm	Cylindrical, 250–400 × 4–5 µm	Cylindrical, breaking into 64 part-spores, 4–10 × 1 µm	—	—	Thanakitpipattana *et al.* (2020)
O. kuchinaraiensis	Larva of *Coleoptera*	Single, or double, brown, cylindrical, dark brown to black, slightly curved, 5.5–10 cm long, 2–3 mm wide	Semi-immersed, obclavate, 630–820 × 210–300 µm	Cylindrical, 305–525 × 4.5–5.5 µm	filliform, multiseptate, 430–615 × 1–2 µm, breaking into 64 part-spores, 5–20 × 1–2 µm	—	—	Crous *et al.* (2023)
O. langbianensis	Larva of *Coleoptera*	Solitary, cylindrical, 40–100 mm long, pale yellow, 0.7–1 mm wide,	Immersed, ovate or pyriform, 260–400 × 100–190 µm	Cylindrical, 200–250 × 5–6 µm	Cylindrical, part-spores, 5–7.5 × 1.3–2 µm	—	—	Lao *et al*. (2021)
O. phitsanulokensis	Larva of *Elateridae* (*Coleoptera*)	Solitary, multiple, cylindrical, greyish red to reddish brown, up to 15 cm long, 1–2 mm wide	Completely immersed, narrowly ovoid, 480–550 × 110–170 µm	Cylindrical, up to 250 µm long, 5–7 µm wide	Cylindrical, slightly curved, breaking into 32 part-spores, 5–8 × 1–1.5 µm	Hirsutella-like, polyphilalidic, cylindrical to subcylindrical basal part, occasionally slightly curved, 5–15 × 2.5–5 µm with a short neck up to 2–5 µm long	Fusoid, 5–8 × 1–2 µm	This study

Table 2. (continued)

Species	Host	Stromata/Synnemata	Perithecia	Asci	Ascospores	Conidiogenous cells	Conidia	Reference
O. pseudovariabilis	*Lycidae* larva (*Coleoptera*)	Solitary, multiple, brownish orange to moderate orange, 4–18 mm long, 0.5–1 mm wide	Pseudo-immersed, pyriform, 380–480 × 220–320 µm	Cylindrical, up to 360 µm long, 5–8 µm wide	Cylindrical, breaking into 32 small truncate part-spores, 5–10 × 1–1.5 µm	Hirsutella-like, monophialidic, polyphialidic, cylindrical basal portion, 4–15 × 2–5 µm	Fusoid, 4–8 × 0.5–1 µm	This study
O. purpureostromata	Larva of *Elateridae* (*Coleoptera*)	Cylindrical, purple, 7–23 mm, 0.6–1 mm wide	Immersed, pyriform, 430–450 × 220–250 µm	150–160 × 10 µm	13–23 × 2.5–3 µm	—	—	Kobayasi & Shimizu (1980)
O. ratchaburiensis	Larva of *Coleoptera*	Solitary, or pairs, cylindrical, gradually tapering toward the apex, strong orange yellow to moderate yellow, up to 25 mm long, 0.5–1 mm wide	Completely immersed, pyriform, 220–530 × 110–280 µm	Cylindrical, 153–225 × 5–10 µm	Cylindrical, part-spores, 3–9 × 1.5–2 µm	Monophialidic, polyphialidic, cylindrical basal portion, 8–19 × 3–4 µm with a distinct, filiform, long neck up to 20 µm	Globose, subglobose, 2–3.5 × 2–3 µm, a mucous sheath observed in mature conidia	This study
O. spicatus	*Tenebrionoidea* larva (*Coleoptera*)	Single, yellow, 5 mm in length	Partially immersed, obliquely or at right angles to the surface of stipe, broadly pyriform, 200–250 × 170–200 µm	Cylindrical, 5–9 µm	Cylindrical, part-spores cylindrical, 3.5–6.5 × 1.7–2 µm	—	—	Zha *et al*. (2021)
O. sporangifera	Larva of *Elateridae* (*Coleoptera*)	Primary synnemata; 9–18 cm high, 1–2 mm wide, brown to deep brown	—	—	—	Hirsutella-like, solitary, 25–40 × 1.3–2.5 µm, with narrow	Subglobose, reniform, 6.7–9.8 × 2.5–3.8	Xiao *et al*. (2019)
O. variabilis	*Xylophagidae* (*Diptera*)	Solitary, several, chrome yellow, 2–24 mm long, 0.2–3 mm wide	Immersed, obpyriform, 350–590 × 200–370 µm	Cylindrical, 210–330 × 6 µm	Cylindrical, part-spores cylindrical, 5–10 × 1.5–3 µm	Strongly hooked or bent, 10–19.2 × 2.5 µm, with subcylindric base and abruptly narrowing to form a short, tapering neck	Subcylindrical, 8–12.4 × 1.9–3.1 µm	Hodge *et al*. (2016)

Ophiocordyceps capilliformis Tasanathai, Mongkolsamrit, Noisripoom & Luangsa-ard, ***sp. nov.*** MycoBank MB 851830. Fig 4.

Etymology: Refers to synnemata of the fungus which exhibit an outer appearance resembling hair.

Typus: **Thailand**, Phitsanulok Province, Ban Phaothai community forest, on larva of *Meracantha sp.* (*Tenebrionidae*, *Coleoptera*), buried in soil, 3 Aug. 2016, *K. Tasanathai*, *S. Mongkolsamrit*, *A. Khonsanit*, *W. Noisripoom*, *D. Thanakitpipattana* & *R. Somnuk* (**holotype** BBH 41761, culture ex-type BCC 82180).

Sexual morph: *Stromata* solitary, greyish reddish brown (200B) to moderate brown (200C), 80–100 mm long. *Stipe* slender, slightly curved, 0.8–1.2 mm wide. *Rhizoids* flexuous, 3–4 cm long buried under the ground, arising from head of larva of *Meracantha sp. Ascomata* on upper part of the stipe, cylindrical, strong yellowish brown (N199D), densely packed perithecia, 20–25 mm long, 1.5–2 mm wide. *Perithecia* completely immersed, pyriform, (395–)400–410(–420) × 190–240(–250) µm. *Asci* cylindrical, (125–)148–193(–200) × 5–8 µm with asci caps, 3–5 × 5–7 µm. *Ascospores* whole, filiform, (130–)143–190(–210) × 2–3 with 5- or 6-septa. *Asexual morph*: *Synnemata* indeterminate, multiple, slender projections produced along the stipe below the ascomata, light brownish grey (200C), or light yellow (162C), up to 8 mm long, 80–120 mm wide. *Conidiogenous cells* hirsutella-like, solitary, arising individually from synnemata, cylindrical to ovoid basal portion, 29–33 × 4–5 µm, tapering to a narrow neck up to 25–28 µm long. *Conidia* hyaline, smooth-walled, fusiform, occasionally slightly curved, (9–)10–13(–15) × 3–5(–8) µm with a mucous sheath. Some strains are found only producing their asexually reproductive form by producing abundant synnemata along the stipes.

Culture characteristics: Colonies on MYA attaining a diam of 18–20 mm in 30 d, pale yellow (161D), strong orange yellow (163B) in the centre of colonies, colonies reverse strong orange yellow (163B). *Conidiogenous cells* monophialidic, arising from either the lateral or terminal regions of the hyphae, up to 50 µm long, 2–3 µm wide, narrow cylindrical basal portion, gradually tapering from the base to the apex. *Conidia* hyaline, smooth-walled, fusiform, (7–)9–12(–14) × 2–3.5(–5) µm. Colonies on OA attaining a diam of 15–20 mm in 30 d, white, moderate brown (200C) in the centre of colonies, colonies reverse moderate brown (200C). *Synnemata* multiple, slender, moderate brown, arising from the centre of colonies, up to 20 mm long, 100–200 µm wide. *Conidiogenous cells* monophialidic, arising from terminal region of the hyphae. *Conidia* hyaline, smooth-walled, fusiform, 9–11(–12) × 2–3 µm, a mucous sheath observed in mature conidia. Colonies on PDA attaining a diam of 12–15 mm in 30 d, white, brownish orange (166C) in the centre of colonies, colonies reverse brownish orange (171B). *Conidiogenous cells* monophialidic, arising from terminal region of the hyphae. *Conidia* hyaline, smooth-walled, fusiform, (9–)10–12(–13) × 2–3(–4) µm, a mucous sheath observed in mature conidia.

Distribution: Found in the central and northern regions of Thailand Thailand.

Additional material examined: **Thailand**, Chiang Mai Province, Ban Hua Thung community forest, on adult beetle *of Platydracus* sp. (*Staphylinidae*, *Coleoptera*), in leaf litter, 30 Oct. 2014, *K. Tasanathai*, *A. Khonsanit*, *W. Noisripoom*, *D. Thanakitpipattana*, *P. Srikitikulchai* & *S. Wongkanoun* (**paratype** BBH 41871, culture ex-paratype BCC 76486).

Notes: In terms of asexual morphology, *O. capilliformis* closely resembles *O. sporangifera*, both characterised by the production of multiple slender synnemata along the stipe. *Ophiocordyceps capilliformis* distinguishes itself by the absence of sclerotia at the apex of the stipe. Additionally, while both species have conidia enveloped in a mucous sheath, *O. capilliformis* features fusiform conidia, while *O. sporangifera* exhibits subglobose to reniform conidia. *Ophiocordyceps sporangifera* is commonly found on *Elateridae* larvae, whereas *O. capilliformis* was typically associated with larva of *Meracantha* sp. (*Tenebrionidae*), which is buried in the soil, as well as on adult beetles of *Platydracus* sp. (*Staphylinidae*) in the leaf litter. It is noteworthy that sexual morph have been observed in *O. capilliformis*, whereas *O. sporangifera* has not exhibited any sexual morph. The sexual morph morphology of *O. capilliformis* resembles that of *O. obtusa*, characterised by the production of cylindrical ascomata, immersed perithecia, and filiform ascospores. *Ophiocordyceps obtusa* was discovered on *Coleoptera* larvae in Java, Indonesia. Upon comparison, it is noted that the perithecia and ascospores in *O. capilliformis* are larger and longer than those reported for *O. obtusa* (395–420 × 190–250 µm *vs* 360–370 × 110–150 µm; 130–210 × 2–3 µm *vs* 150 × 1.5 µm, respectively) (Penzig & Saccardo 1904).

Ophiocordyceps clavata (Kobayasi & Shimizu) G.H. Sung *et al.*, *Stud. Mycol*. **57**: 40. 2007. MycoBank MB 504237. Fig. 5.

The description and illustrations are based on *O. clavata* specimens collected in Thailand.

Sexual morph: *Stromata* multiple, clavate, pale yellow (161C–D), arising from head, thorax and abdomen of *Tenebrionidae* larvae (*Coleoptera*), 2–10 mm long. *Stipes* cylindrical, smooth, pale yellow (161D), 0.5–1.5 mm wide. *Ascomata* formed on upper part of the stipe, or subterminal, occasionally located on one side of the stipe, pale yellow (161C–D), densely packed perithecia, 2–4 mm long, 1.5–2 mm wide. *Perithecia* immersed, tightly packed, broad ovoid, 400–470(–500) × (250–)275–335(–350) µm. *Asci* cylindrical, (200–)207–300 µm long, 6–8 µm wide with asci caps 3.5–7 × 3.5–8 µm. *Ascospores* cylindrical, 200–250 µm long, breaking into 32 small truncate part-spores, (5–)6.5–8.5(–10) × 1.5–2(–3). *Asexual morph*: Unknown.

Culture characteristics: Colonies on MYA attaining a diam of 10–15 mm in 30 d, white, colonies reverse moderate orange yellow (164B). *Synnemata* multiple, white. *Conidiogenous cells* synglíocladium-like, monophialidic, cylindrical at the base or slightly flask-shaped, slightly curved, 5.5–15.5(–20) × 2–3 µm. *Conidia* hyaline, smooth-walled, cylindrical, 5–8.5(–10) × 1.5–2.5(–3) µm, mostly aggregated in slimy heads at the apex phialides. Colonies on OA attaining a diam of 5–8 mm in 30 d, mycelium sparse, white to moderate orange yellow (165C), colonies reverse moderate orange yellow (164B). *Conidiogenous cells* monophialidic, cylindrical basal portion, slightly curved, (5–)7–14(–18) × 2–3 µm. *Conidia* hyaline, smooth-walled, cylindrical, (6–)7–14(–18) × 1.5–2.5(–3) µm, not aggregated in

slimy heads at the apex phialides. Poor sporulation was observed. Colonies on PDA attaining a diam of 12–15 mm in 30 d, white, colonies reverse greyish brown (166A). *Conidiogenous cells* monophialidic, cylindrical basal portion with acute end, 5–9.5(–15) × (1–)2–3 µm. *Conidia* hyaline, smooth-walled, cylindrical, (5–)7–8.5(–10) × 1.5–2.5(–3) µm, not aggregated in slimy heads at the apex phialides. Poor sporulation was observed.

Distribution: Found in Japan, known from Daisen mountain in Tottori Prefecture, Ryōkami mountain in Saitama Prefecture (Kobayasi & Shimizu 1980); Thailand, known from Doi Inthanon National Park and Phu Suan Sai National Park; China (Chen *et al*. 2019).

Additional materials examined: **Thailand,** Chiang Mai Province, Doi Inthanon National Park, on larvae of *Tenebrionidae* (*Coleoptera*), buried in the bark of a living tree. 24 Aug. 2022, *J. Luangsa-ard*, *S. Mongkolsamrit*, *W. Noisripoom*, *U. Pinruan*, *S. Sommai*, *P. Khamsuntorn & C. Suriyachadkun* (BBH 49815, culture BCC 95652); *idem*., (BBH 49816, culture BCC 95653); Loei Province, Phu Suan Sai National Park, on *Coleoptera* larva, buried in soil, 16 Jul. 2008, *K. Tasanathai*, *S. Mongkolsamrit*, *B. Thongnuch*, *P. Srikitikulchai*, *A. Khonsanit*, *N.T. Toan* & *N.T. Vui* (BBH 24029).

Notes: According to the multi-gene phylogenetic analysis presented in Fig. 1, our study revealed that *O. clavata* strains (BCC 95652 and BCC 95653) cluster together with *O. clavata* previously reported from China (BUO545) and Japan (NBRC106961, NBRC 10962). *Ophiocordyceps clavata* was initially described and illustrated based on Japanese specimens infecting *Coleoptera* larvae by Kobayasi & Shimizu (1980). When comparing the of morphological features of the Thai and Japanese materials, it becomes evident that the size of both the perithecia and part-spores are in the same range (400–500 × 250–350 µm *vs* 420–550 × 230–330 µm; 5–10 × 1.5–3 µm *vs* 5–9 × 1.5 µm, respectively). We obtained specimens of this species from Chiang Mai and Loei Provinces. In this study, we present the morphological characters through a colour figure for this species for the first time.

Ophiocordyceps kohchangensis Mongkolsamrit, Noisripoom, Himaman & Luangsa-ard, ***sp. nov.*** MycoBank MB 851832. Fig. 6.

Etymology: Refers to the locality where the type specimen was found, Mu Koh Chang National Park.

Typus: **Thailand**, Trad Province, Mu Koh Chang National Park. on *Elateridae* larva (*Coleoptera*), buried in soil, 9 May 2018, *J. Luangsa-ard*, *S. Mongkolsamrit*, *W. Noisripoom*, *B. Sakolrak* & *W. Himaman* (**holotype** BBH 44483, culture ex-type BCC 88229).

Sexual morph: *Stromata* solitary, cylindrical, gradually tapering toward the apex, moderate yellow to light yellow (162B–C), up to 75 mm long. *Stipes* cylindrical, smooth, pale yellow (162C–D), 1–1.5 mm wide. *Rhizoids* flexuous, 5 cm long buried under the ground, arising from head of *Elateridae* larva (*Coleoptera*). *Fertile parts* distinctly subterminal with asexual morph at apex. *Ascomata* subterminal, cylindrical, moderate yellow to light yellow (162B–C), 10–20 mm long, 2–2.5 mm wide. *Perithecia* completely immersed, pyriform, (320–)340–380(–400) × (100–)105–160(–200) µm. *Asci* cylindrical, 90–150(–200) × 4–5.5(–6) µm with asci caps, 2–3 × 3–5 µm. *Ascospores* cylindrical, breaking into 32 small truncate part-spores, (3–)4–5.5(–7) × 1.5–2 µm. *Asexual morph*: *Synnemata* solitary, cylindrical, produced at upper region of the stipe, up to 60 mm long. *Conidiogenous cells* hirsutella-like, monophialidic or polyphialidic, cylindrical basal portion, (5–)6.5–23(–40) × 3–4.5(–5) µm with a distinct, filiform, long neck up to 25 µm long. *Conidia* hyaline, smooth, globose, (2–)2.5–4 µm.

Culture characteristics: Colonies on MYA attaining a diam of 10–14 mm in 30 d, white, colonies reverse pale yellow (161C). *Conidiogenous cells* polyphialidic, cylindrical to subcylindrical basal portion, (5–)7–23(–40) × 3–4.5(–5) µm with a distinct, filiform, long neck up to 25 µm. *Conidia* hyaline, smooth-walled, globose 3–4 µm. Colonies on OA attaining a diam of 14–17 mm in 30 d, white, colonies reverse pale yellow (164D). *Mycelium* hyaline, septate, smooth-walled. *Conidiogenous cells* polyphialidic, cylindrical to subcylindrical basal portion, (10–)13–37(–45) × 3–4(–5) µm with a distinct, filiform, long neck up to 38 µm long. *Conidia* hyaline, smooth-walled, globose, 2–3(–5) µm. Colonies on PDA attaining a diam of 15–18 mm in 30 d, white, colonies reverse brownish orange (165B). *Conidiogenous cells* polyphialidic, cylindrical to subcylindrical basal portion, 10–29(–45) × 3–4(–5) µm with a distinct, filiform, long neck up to 35 µm long. *Conidia* hyaline, smooth-walled, globose, (2–)3–4 µm.

Distribution: Found in the eastern region of Thailand.

Additional materials examined: **Thailand**, Trad Province, Mu Koh Chang National Park, on *Elateridae* larvae (*Coleoptera*), buried in soil, 9 July 2019, *K. Tasanathai*, *S. Mongkolsamrit*, *B. Sakolrak*, *W. Himaman* & *P. Jangsantear* (**paratype** BBH 48326, culture ex-paratype BCC 90828); *idem*., (BCC 90811); *idem*., 9 May 2018, *J. Luangsa-ard*, *S. Mongkolsamrit*, *W. Noisripoom*, *B. Sakolrak* & *W. Himaman* (BBH 88234); *idem*., (BBH 88226, culture BCC 44479); *idem*., (BBH 88227, culture BCC 44480).

Notes: *Ophiocordyceps kohchangensis* produces ascomata on the subterminal region of the stroma, with the asexual form appearing at the apex. It closely resembles *O. houaynhangensis*, *O. bidoupensis* and *O. langbianensis*. *Ophiocordyceps kohchangensis*, *O. houaynhangensis*, *O. bidoupensis* and *O. langbianensis* share remarkable similarities, including their stromata being pale yellow-brown and the size of their perithecia, asci, and ascospores falling within the same range. Difficulties in using morphological discrimination has often been encountered in these fungi since there is an overlap in the measurements of important morphological characters such as phialides, conidia, and ascospores. Furthermore, these species are associated with the larval stages of *Coleoptera*. It is noteworthy that only *O. bidoupensis* and *O. kohchangensis* have been reported to occur on *Elateridae* larvae (*Coleoptera*). However, the results of a molecular phylogenetic analysis clearly indicate that *O. kohchangensis* forms a distinct clade separate from the previously mentioned species (Fig. 1).

Ophiocordyceps phitsanulokensis Mongkolsamrit, Noisripoom, Lamlertthon & Luangsa-ard, ***sp. nov.*** MycoBank MB 851833. Fig 7.

Etymology: Refers to the locality where the type specimen was found, Phitsanulok Provice.

Fig. 4. *Ophiocordyceps capilliformis.* **A.** Fungus on coleopteran larva. **B.** Close-up of ascoma region. **C.** Perithecia. **D.** Asci. **E.** Whole ascospore. **F–H.** Phialide and conidia. **I, J.** Colonies on MYA at 30-d-old (**I** obverse, **J** reverse). **K, L.** Phialides with conidia on MYA. **M, N.** Conidium on MYA. **O, P.** Colonies on OA at 30-d-old (**O** obverse, **P** reverse). **Q.** Phialide with conidium on OA. **R–T.** Conidia on OA. **U, V.** Colonies on PDA at 30-d-old (**U** obverse, **V** reverse). **W, X.** Phialides with conidia on PDA. **Y, Z.** Conidium on PDA. Scale bars: A, I, J, O, P, U, V = 15 mm; B = 5 mm; C = 200 µm; D = 15 µm; E, K = 20 µm, F, H, L = 10 µm; G, M, N, Q–T = 5 µm; W, X = 12 µm; Y, Z = 4 µm.

Typus: **Thailand**, Phitsanulok Province, Ban Phaothai community forest, on *Coleoptera* larva, buried in soil, 28 Jun. 2017, *S. Mongkolsamrit*, *K. Tasanathai*, *W. Noisripoom* & *U. Pinruan* (**holotype** BBH 44437, culture ex-type BCC 85328).

Sexual morph: *Stromata* solitary or multiple, cylindrical, greyish red (178A–B) to reddish brown (175A), arising from head region of *Elateridae* larvae (*Coleoptera*), up to 15 cm long. *Stipes* cylindrical smooth, moderated orange yellow (165C), 1–2 mm wide. *Rhizoids* flexuous, 3–8 cm long buried under the ground. *Ascomata* terminal, cylindrical, 20–40 mm long, 1–3.5 mm wide. *Perithecia* completely immersed, narrowly ovoid, (480–)490–540(–550) × (110–)120–155(–170) µm. *Asci* cylindrical, up to 250 µm long, 5–7 µm with asci caps, 2.5–3 × 5–6 µm. *Ascospores* cylindrical, slightly curved, breaking into 32 small truncate part-spores, 5–7(–8) × 1–1.5 µm. *Asexual morph*: *Synnemata* solitary

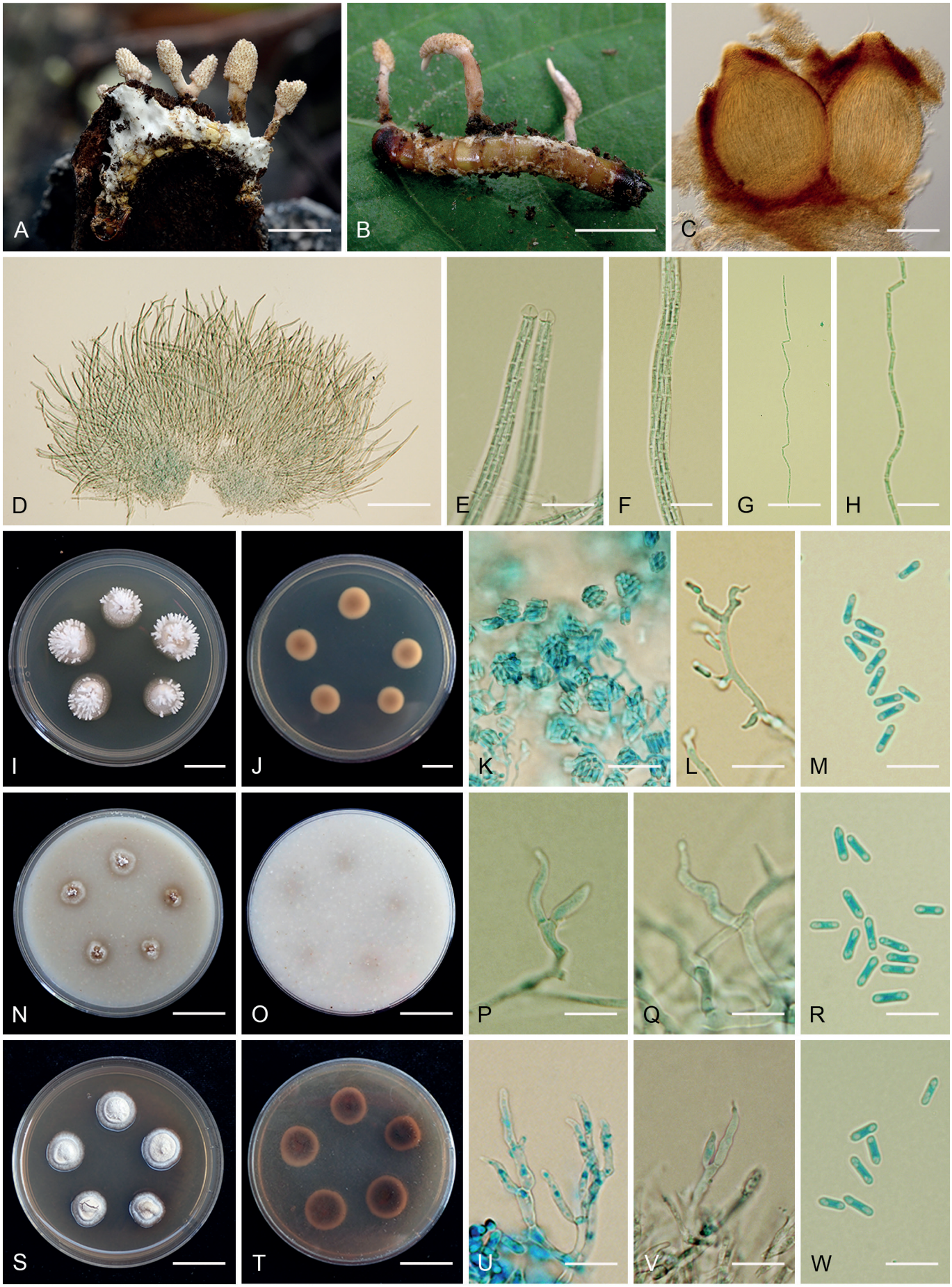

Fig. 5. *Ophiocordyceps clavata*. **A, B.** Fungus on coleopteran larvae. **C.** Perithecia. **D.** Asci. **E.** Ascus tips. **F–H.** Part-spores. **I, J.** Colonies on MYA at 30-d-old (**I** obverse, **J** reverse). **K, L.** Phialides with conidia on MYA. **M.** Conidia on MYA. **N, O.** Colonies on OA at 30-d-old (**N** obverse, **O** reverse). **P, Q.** Phialides on OA. **R.** Conidia on OA. **S, T.** Colonies on PDA at 30-d-old (**S** obverse, **T** reverse). **U, V.** Phialides on PDA. **W.** Conidia on PDA. Scale bars: A, B = 5 mm; C = 150 µm; D = 100 µm; F, M, P–R, U–W = 10 µm; G, K = 50 µm; E, H, L = 20 µm; I, J, N, O, S, T = 15 mm.

or multiple, cylindrical, produced at upper region of the stipe, up to 12 cm long. *Conidiogenous cells* hirsutella-like, polyphialidic, cylindrical to subcylindrical basal part, occasionally slightly curved, 5–15 × 2.5–5 µm with a short neck up to 2–5 µm. *Conidia* hyaline, smooth-walled, fusoid, 5–7(–8) × 1–1.5 µm. Some strains are occasionally found producing the asexual morph at the subterminal part of stipe connected with the ascomata.

Culture characteristics: Colonies on MYA attaining a diam of 8 mm in 30 d, brownish orange (165B), low mycelial density, colonies brownish orange (165B). *Conidia* and reproductive structures not observed (Supplementary Fig. S5). Colonies on OA attaining a diam of 5–8 mm in 30 d, greyish red (182C), with low mycelial density, colonies reverse light reddish brown (177B). *Conidiogenous cells* hirsutella-like, monophialidic or polyphialidic, cylindrical, swollen basal portion, (5–)6–16(–25) × (2–)3–5 µm, forming a short neck up to 2 µm long. *Conidia* hyaline, smooth-walled, fusoid, 5–7 × 1–2.5 µm. Colonies on PDA attaining a diam of 10–15 mm in 30 d, white to pale greenish yellow (9D), colonies reverse greyish brown (166A). *Conidiogenous cells* hirsutella-like monophialidic or polyphialidic, awl-shaped, or swollen basal portion, (5–)6.5–18.5(–30) × 2–4 µm, forming a short neck up to 2.5 µm long. *Conidia* hyaline, smooth-walled, fusoid, 5–6.5(–8) × 1.5–2.5 µm.

Distribution: Found in the central region of Thailand.

Additional materials examined: **Thailand**, Phitsanulok Province, Ban Phaothai community forest, on *Coleoptera* larva, buried in soil, 28 Jun. 2017, *S. Mongkolsamrit*, *K. Tasanathai*, *W. Noisripoom* & *U. Pinruan* (**paratype** BBH 43368, culture ex-paratype BCC 85323); *idem*., (BBH 43336, culture BCC 85324); *idem*., (BBH 43369, culture BCC 85325); *idem*., (BBH 43337, culture BCC 85326); *idem*., (BBH 43338, culture BCC 85327); *idem*., (BBH 44438, culture BCC 85329); *idem*., (BBH 44439, culture BCC 85333); *idem*., (BBH 44440, culture BCC 85334); *idem*., (BBH 44441, culture BCC 85335).

Notes: Based on the molecular phylogenetic analyses (Fig. 1), *O. phitsanulokensis* is closely related to *O. bidoupensis*, *O. houaynhangensis*, *O. kohchangensis*, *O. brunneipunctata*, *O. furcatosubulata*, *O. langbianensis*, *O. cossidarum*, *O. brunnea* and *O. ratchaburiensis*. When examining these natural specimens, it becomes evident that *O. phitsanulokensis* shares morphological similarities with these species, characterised by immersed perithecia and ascospores disarticulating into part-spores. However, the fertile components of the ascomata in *O. phitsanulokensis* are located at the terminal end of the stipe, while the above-mentioned species produce fertile components of the ascomata at the subterminal region of the stipe. Moreover, *O. phitsanulokensis* resembles *O. longissima*, particularly in the production of the long solitary stroma. The fertile component of its ascomata exhibits cylindrical structures located at the terminal end of the stipe, displaying a reddish brown colour. However, they differ significantly in their hosts, with *O. phitsanulokensis* found on *Coleoptera* larvae, while *O. longissima* was found on cicada nymphs (Luangsa-ard *et al*. 2010b).

Ophiocordyceps pseudovariabilis Mongkolsamrit, Noisripoom, Himaman & Luangsa-ard, ***sp. nov.*** MycoBank MB 851834. Fig. 8.

Etymology: Named after the similarity to *Ophiocordyceps variabilis.*

Typus: **Thailand**, Nakhon Ratchasima Province, Khao Yai National Park, on *Lycidae* larva (*Coleoptera*), buried in decaying wood, 31 May 2018, *J. Luangsa-ard*, *K. Tasanathai*, *S. Mongkolsamrit* & *W. Noisripoom* (**holotype** BBH 47453, culture ex-type BCC 88308).

Sexual morph: *Stromata* solitary or multiple, cylindrical, gradually tapering toward the apex, brownish orange to moderate orange (172C–D), arising from head, thorax and abdomen of *Lycidae* larvae (*Coleoptera*), 4–18 mm long. *Stipes* cylindrical, smooth, brownish orange, 0.5–1 mm wide. *Ascomata* distinctly subterminal, developed lateral cushion on stipes, slightly pulvinate, densely packed perithecia, 1.5–2 mm diam, 1.5 mm thick. *Perithecia* pseudo-immersed, pyriform, (380–)388–460(–480) × (220–)290–320 µm. *Asci* cylindrical, up to 360 µm long, 5–6(–8) µm wide with asci caps, 2–5 × 5–6 µm. *Ascospores* cylindrical, breaking into 32 small truncate part-spores, (5–)6–9(–10) × 1–1.5 µm. *Asexual morph*: *Synnemata* multiple, cylindrical, brownish orange to pale yellow, up to 8 mm long, 0.5–1 mm wide, occasionally located at the terminal of stipe connected with the ascomata. *Conidiogenous cells* hirsutella-like, monophialidic or polyphialidic, swollen basal portion, 4–15 × 2–5 µm, gradually tapering from the base to the apex up to 8 µm long. *Conidia* hyaline, smooth-walled, fusoid, (4–)5–7.5(–8) × 0.5–1 µm.

Culture characteristics: Colonies on MYA attaining a diam of 8–10 mm in 30 d, pale yellow (20C), colonies reverse moderate reddish brown (166B). *Synnemata* arising on edge of colonies, up to 8 mm long, 0.5–1 mm wide. *Conidiogenous cells* synglocladium-like, phialidic, cylindrical to subcylindrical basal portion, slightly curved, (5–)6.5–12.5(–15) × 1.5–2 µm. *Conidia* hyaline, smooth-walled, falcate, slightly *truncate at the ends*, (9–)9.5(–11) × 2–3 µm. Colonies on OA attaining a diam of 5–8 mm in 30 d, white, low mycelial density, colonies reverse uncoloured. *Conidia* and reproductive structures not observed (Supplementary Fig. S5). Colonies on PDA attaining a diam of 10 mm in 30 d, strong orange yellow (N163D), reverse deep orange yellow (163A). *Conidiogenous cells* syngliocladium-like, phialidic, cylindrical to subcylindrical basal portion, slightly curved, (5–)8–14.5(–15) × 1.5–2 µm, forming a short neck up to 3 µm long. *Conidia* hyaline, smooth-walled, falcate, slightly truncate at the ends, (8–)8.5(–10) × 2–3.5 µm.

Distribution: Found in the northeastern region of Thailand.

Additional materials examined: **Thailand**, Nakhon Ratchasima Province, Khao Yai National Park, on *Lycidae* larva (*Coleoptera*), buried in decaying wood, 31 May 2018, *J. Luangsa-ard*, *K. Tasanathai*, *S. Mongkolsamrit* & *W. Noisripoom* (**paratype** BBH 47458, culture ex- paratype BCC 88311); *idem*., (BBH 47454, culture BCC 88309); *idem*., (BBH 47455, culture BCC 88310); *idem*., (BBH 43791, culture BCC 88268).

Notes: In Thailand, *O. pseudovariabilis* was rarely documented in natural forests. Despite extensive surveys conducted in various forests throughout the country at different times of the year, our collections were limited to Khao Yai National Park in May. The small size of the natural specimens adds to the challenge of distinguishing *O. pseudovariabilis* from *Pleurocordyceps*

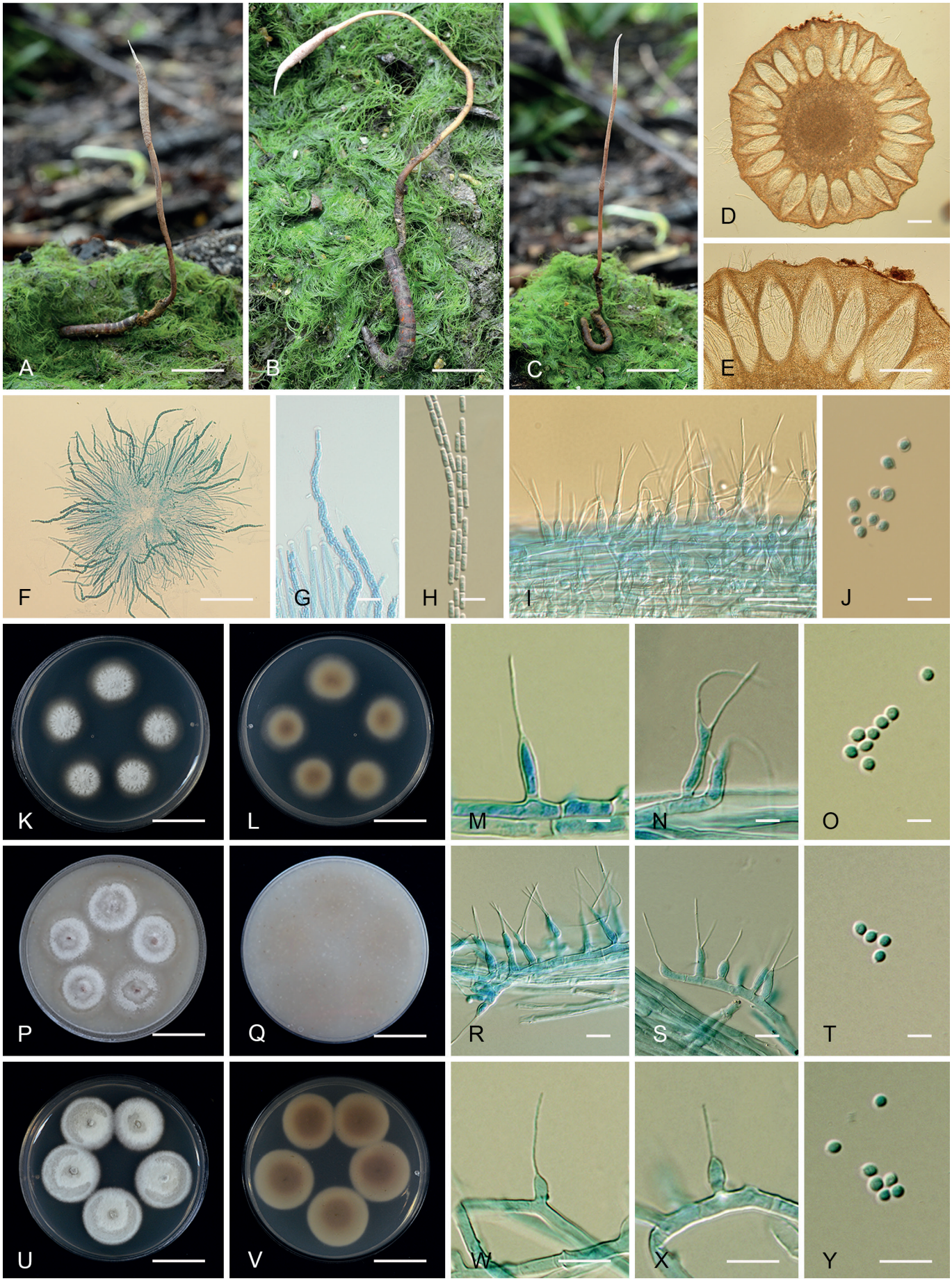

Fig. 6. *Ophiocordyceps kohchangensis.* **A–C.** Fungus on coleopteran larvae. **D, E.** Perithecia. **F, G**. Asci. **H.** Part-spores. **I.** Phialides. **J.** Conidia. **K, L.** Colonies on MYA at 30-d-old (**K** obverse, **L** reverse). **M, N.** Phialides on MYA. **O.** Conidia on MYA. **P, Q.** Colonies on OA at 30-d-old (**P** obverse, **Q** reverse). **R, S.** Phialides on OA. **T.** Conidia on OA. **U, V.** Colonies on PDA at 30-d-old (**U** obverse, **V** reverse). **W, X.** Phialides on PDA. **Y.** Conidia on PDA. Scale bars: A, C, K, L, P, Q, U, V = 15 mm; B = 10 mm; D, E = 200 µm; F = 100 µm; G, R, S, Y = 10 µm; H, J, M–O, T = 5 µm; I, W, X = 20 µm.

phaothaiensis, based on macromorphology, as both produce stroma with subterminal perithecial plates, and their ascospores form part-spores (Crous *et al.* 2017, Wang *et al*. 2021b). However, the asexual morphologies observed on PDA culture differ. *Ophiocordyceps pseudovariabilis* forms synglioclad ium-like colonies, with falcate conidia. In contrast, *P. phaothaiensis* exhibits verticillate branches or singly arranged phialides, and its conidia come in two types: fusoid to globose and fusiform in chains.

Ophiocordyceps ratchaburiensis Mongkolsamrit, Khonsanit, Noisripoom & Luangsa-ard, ***sp. nov*.** MycoBank MB 851835. Fig. 9.

Etymology: Refers to the locality where the type specimen was found, Ratchaburi Province.

Typus: **Thailand**, Ratchaburi Province, Chaloem Phrakiat Thai Prachan National Park, on *Coleoptera* larva, buried in soil, 26 May 2011, *K. Tasanathai*, *P. Srikitikulchai*, *A. Khonsanit* & *W. Noisripoom* (**holotype** BBH 30544, culture ex-type BCC 48033).

Sexual morph: *Stromata* solitary or in pairs, cylindrical, gradually tapering toward the apex, strong orange yellow to moderate yellow (163B–C), up to 25 mm long. *Stipes* cylindrical, smooth, light orange yellow (22B), 0.5–1 mm wide. *Rhizoids* flexuous, 1–2 cm long buried under the ground, arising from head of *Elateridae* larvae (*Coleoptera*). *Fertile parts* distinctly subterminal with asexual morph at apex. *Ascomata* subterminal, strong orange yellow (163B), crowded perithecia, 2–6 mm long, 1.5–2 mm wide. *Perithecia* completely immersed, pyriform, (220–)316–446(–530) × (110–)154–222(–280) µm. *Asci* cylindrical, (153–)162–198(–225) × (5–)5.5–8.5(–10) µm with asci caps, 1.5–2.5 × 3–4 µm. *Ascospores* cylindrical, breaking into truncate part-spores, (3–)4–6(–9) × 1.5–2 µm. *Asexual morph*: *Conidiogenous cells* monophialidic or polyphialidic, cylindrical basal portion, (8–)9.5–15(–19) × 3–4 µm with a distinct, filiform, long neck up to 20 µm. *Conidia* hyaline, smooth-walled, globose to subglobose, (2–)2.5–3(–3.5) × 2–3 µm diam, a mucous sheath observed in mature conidia.

Culture characteristics: Colonies on MYA attaining a diam of 8 mm in 30 d, white, flattened, colonies reverse moderate orange yellow (164B). *Conidia* and reproductive structures not observed (Supplementary Fig. S5). Colonies on OA attaining a diam of 10–15 mm in 30 d, white, greyish red (182B) in the middle of colonies, colonies reverse greyish reddish orange (177C). *Conidiogenous cells* monophialidic or polyphialidic, arising from either the lateral or terminal regions of the hyphae, cylindrical basal portion, (5–)8–29.5(–42) × (2–)3–4 µm with a distinct, filiform, long neck up to 25 µm. *Conidia* hyaline, smooth-walled, globose to subglobose, 2–3(–4) × (2–)2.5–3.5(–4) µm with a mucous sheath. Colonies on PDA attaining a diam of 15–20 mm in 30 d, white, colonies reverse moderate reddish brown (177A). *Synnemata* white to dark pink (182C), in the middle of colonies. *Conidiogenous cells* monophialidic or polyphialidic, arising from either the lateral or terminal regions of the hyphae, cylindrical basal portion, 10–28.5(–44) × 2–4 µm with a distinct, filiform, long neck up to 28 µm. *Conidia* hyaline, smooth-walled, globose to subglobose, (1.5–)2–3.5(–4) × 1.5–2(–3.5) µm with a mucous sheath.

Distribution: Found in the central region of Thailand.

Additional materials examined: **Thailand**, Ratchaburi Province, Chaloem Phrakiat Thai Prachan National Park, on *Coleoptera* larvae, buried in soil, 26 May 2011, *K. Tasanathai*, *P. Srikitikulchai*, *A. Khonsanit* & *W. Noisripoom* (**paratype** BBH 30553, culture ex-paratype BCC 48037); *idem*., (BBH 30545, culture BCC 48034); *idem*., (BBH 30546, culture BCC 48035); *idem*., (culture BCC 48873); *idem*., (BBH 30551, culture BCC 48036); *idem*., (BBH 30552, culture BCC 48682); *idem*., (BBH 30554, culture BCC 48039).

Notes: *Ophiocordyceps ratchaburiensis* exhibits the formation of ascomata on the subterminal portion of its stipe, with the asexual morph located at the apex, similar to *O. brunnea*, *O. brunneipunctata* and *O. houaynhangensis*. However, *O. ratchaburiensis* distinguishes itself significantly from *O. brunnea* and *O. brunneipunctata*, by featuring pale orange stromata, whereas *O. brunnea* and *O. brunneipunctata* exhibit stromata in shades of dark brown to cinnamon. Moreover, *O. ratchaburiensis* shares a stroma colour similar to *O. houaynhangensis*, characterised by shades of yellow to orange. Nevertheless, molecular phylogenetic analysis supports the clear distinction of *O. ratchaburiensis* from *O. brunnea*, *O. brunneipunctata* and *O. houaynhangensis*.

DISCUSSION

In the present study, we introduce seven new species and document one new record of *Ophiocordyceps clavata* associated with *Coleoptera* in Thailand. These findings are the result of comprehensive phylogenetic analyses that incorporate sequences from LSU, *TEF1*, *RPB1*, and *RPB2*, complemented by compelling morphological evidence. Notably, the type of *Ophiocordyceps* is *O. blattae*, known for parasitising a cockroach, identified as *Blatta germanica* (Araújo *et al*. 2021). Initially, only two specimens were collected in Sri Lanka (Petch 1924) with more recent records from Thailand (Luangsa-ard *et al*. 2018, Mongkolsamrit *et al.* 2021), placing them in the *"O. blattae"* clade. *Ophiocordyceps blattae* forms cylindrical ascoma producing elongated-fusoid ascospores that do not disarticulate into part-spores. The seven proposed taxa, distributed into four subclades in *Ophiocordyceps*, are discussed below.

Species discovered in the *"O. ravenelii"* clade

This clade was recognised based on *Ophiocordyceps ravenelii* (Sung *et al*. 2007). Our comprehensive multi-locus analyses clearly show species like *O. clavata*, *O. formosana*, *O. spicatus*, *O. pseudovariabilis*, *O. variabilis*, *O. krachonicola*, and *O. kuchinaraiensis* are nested within this distinct clade. Most species in this clade infect larvae of *Coleoptera*, except for *O. krachonicola* on *Gryllotalpa orientalis* nymphs and *O. variabilis* on *Xylophagidae* larvae, *Diptera*. The seven aforementioned species share common features, such as solitary or multiple yellowish, orange to brown stromata and have ascospores that break into cylindrical part-spores, with overlapping sizes within a similar range. The phylogenetic analyses (Fig. 1) reveal a close relationship between *O. pseudovariabilis* and *O. variabilis* forming a sister group, closely related to the Thai species *O. kuchinaraiensis* and *O. krachonicola* (Thanakitpipattana *et al.*

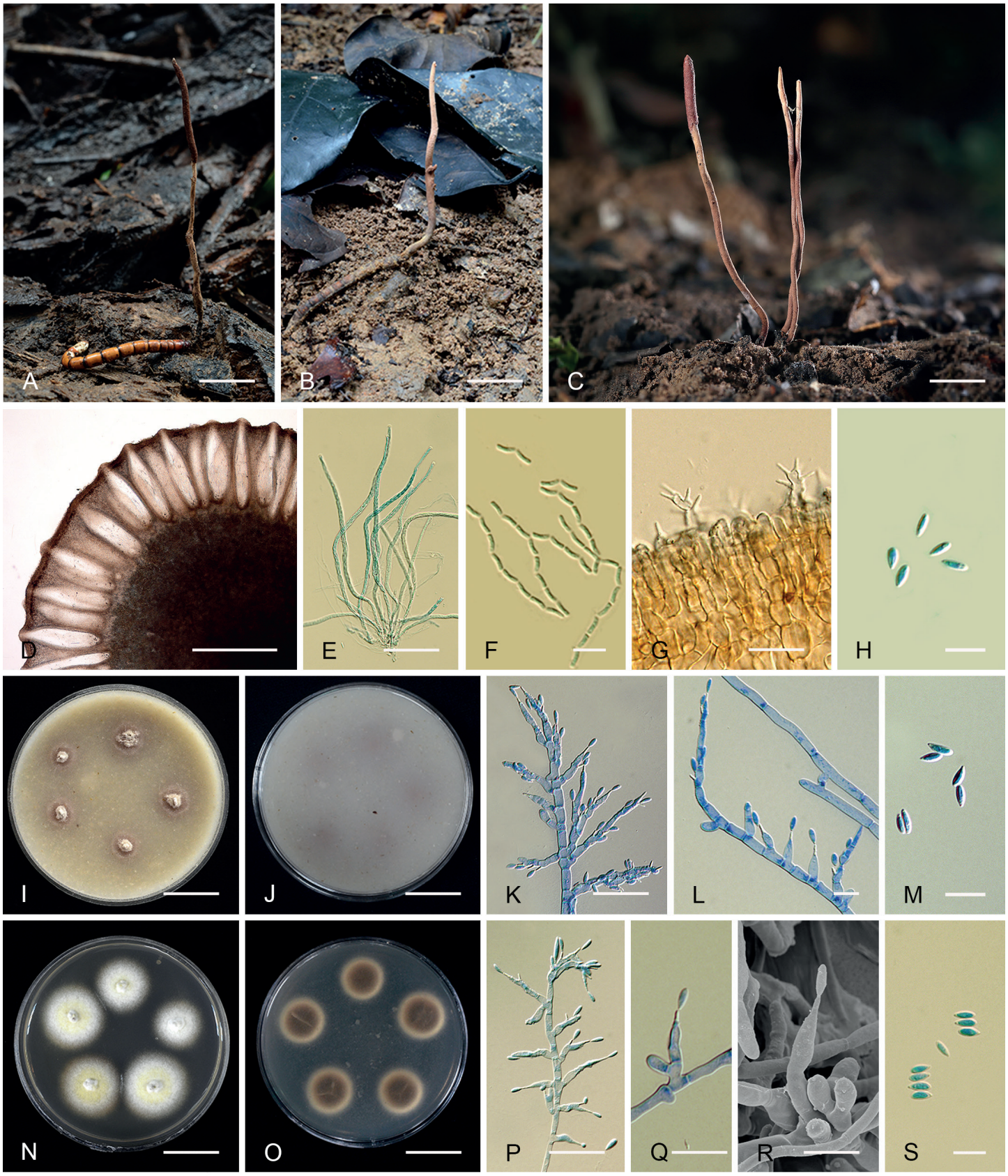

Fig. 7. *Ophiocordyceps phitsanulokensis.* **A–C.** Fungus on coleopteran larvae. **D.** Perithecia. **E.** Asci. **F.** Part-spores. **G.** Phialides. **H.** Conidia. **I, J.** Colonies on OA at 30-d-old (**I** obverse, **J** reverse). **K, L.** Phialides and conidia on OA. **M.** Conidia on OA. **N, O.** Colonies on PDA at 30-d-old (**N** obverse, **O** reverse). **P–R.** Phialides and conidia on PDA. **S.** Conidia on PDA. Scale bars: A–C = 10 mm; D = 500 µm; E = 50 µm; F, G, H, L, M, Q–S, K = 10 µm; P = 20 µm; I, J, N, O = 15 mm.

2020, Crous *et al.* 2023), as well as the Chinese *O. spicatus* and the Taiwanese *O. formosana*. Both *O. pseudovariabilis* and *O. variabilis* produce perithecial cushions on the upper end of the stipe, but *O. pseudovariabilis* has smaller perithecia compared to *O. variabilis*. *Ophiocordyceps pseudovariabilis* displays a hirsutella-like asexual morph on the synnemata in natural samples, while a synglіocladium-like morph is observed in cultures on MYA and PDA. On the other hand, *O. variabilis* displays a synglіocladium-like asexual form either on immature stromata or on the mycelium covering the host. Cultures on OA

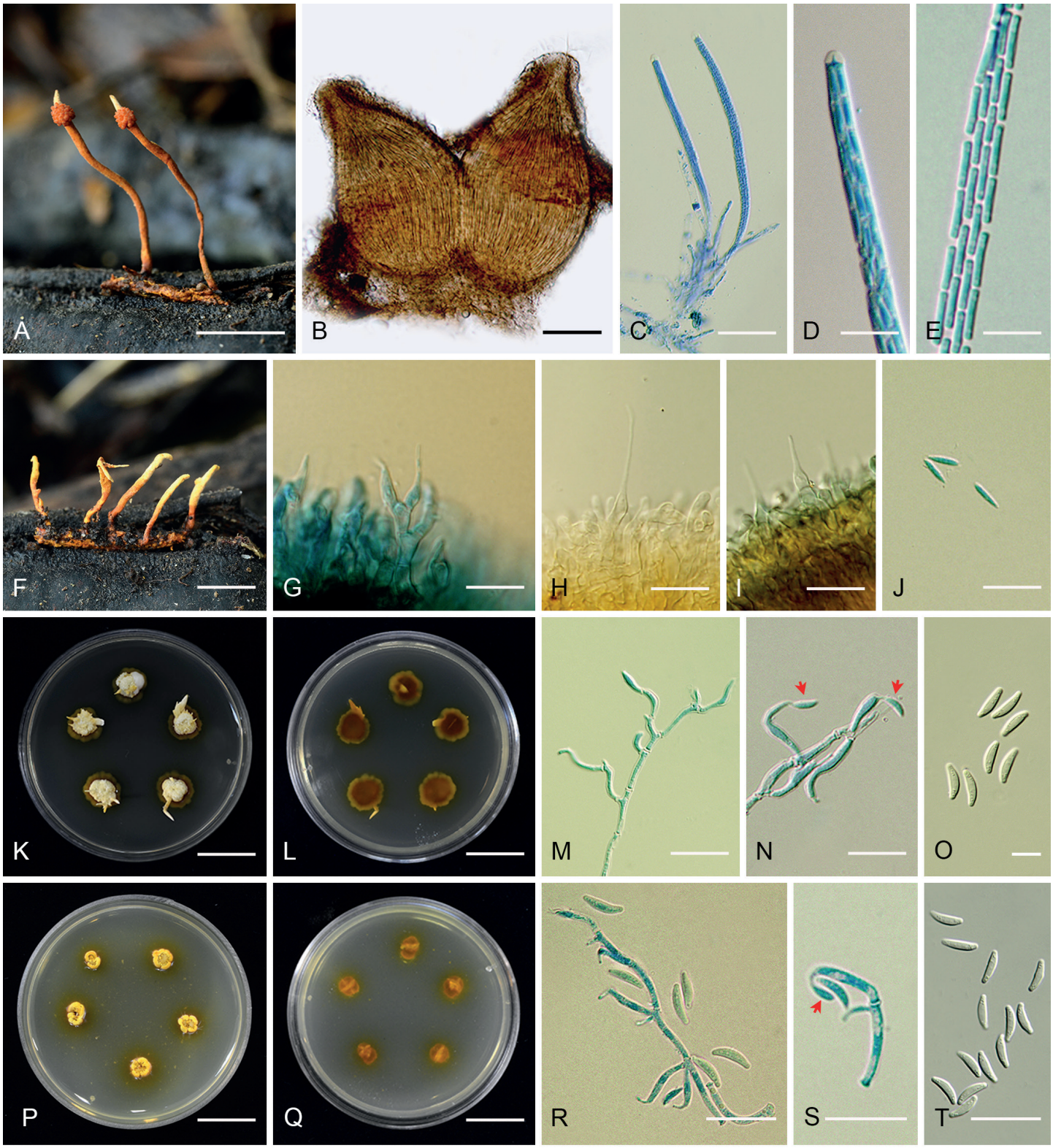

Fig. 8. *Ophiocordyceps pseudovariabilis.* **A, F.** Fungus on coleopteran larvae. **B.** Perithecia. **C.** Asci. **D.** Ascus tip. **E.** Part-spores. **G–I.** Phialides. **J.** Conidia. **K, L.** Colonies on MYA at 30-d-old (**K** obverse, **L** reverse). **M, N.** Phialides with conidia and immature conidia (arrows) on MYA. **O.** Conidia on MYA. **P, Q.** Colonies on PDA at 30-d-old (**P** obverse, **Q** reverse). **R, S.** Phialides with conidia and immature conidium (arrow) on PDA. **T.** Conidia on PDA. Scale bars: A, F = 5 mm; B = 100 µm; C = 50 µm; D, E, G–J, N, O = 10 µm; K, L, P, Q = 15 mm; M, R–T = 20 µm.

and mOA have revealed blunt orange synnemata with abundant conidiogenesis and slime production, as observed by Hodge *et al*. (1998). These two species, commonly found in decaying wood, differ in their preferred insect hosts, with *O. variabilis* associated with *Xylophagidae* larvae (*Diptera*) and *O. pseudovariabilis* found on *Lycidae* larvae (*Coleoptera*).

Genetic confirmation of *O. clavata* strains from China, Japan, and Thailand indicates a shared genetic identity (Fig. 1). The morphological characteristics of perithecia and part-spores observed in Japanese and Thai specimens fall within the same range, suggesting that these populations represent the same species (Kobayasi & Shimizu 1980). This finding suggests a broader distribution for *O. clavata*, transcending geographical boundaries. Interestingly, this shared genetic identity is

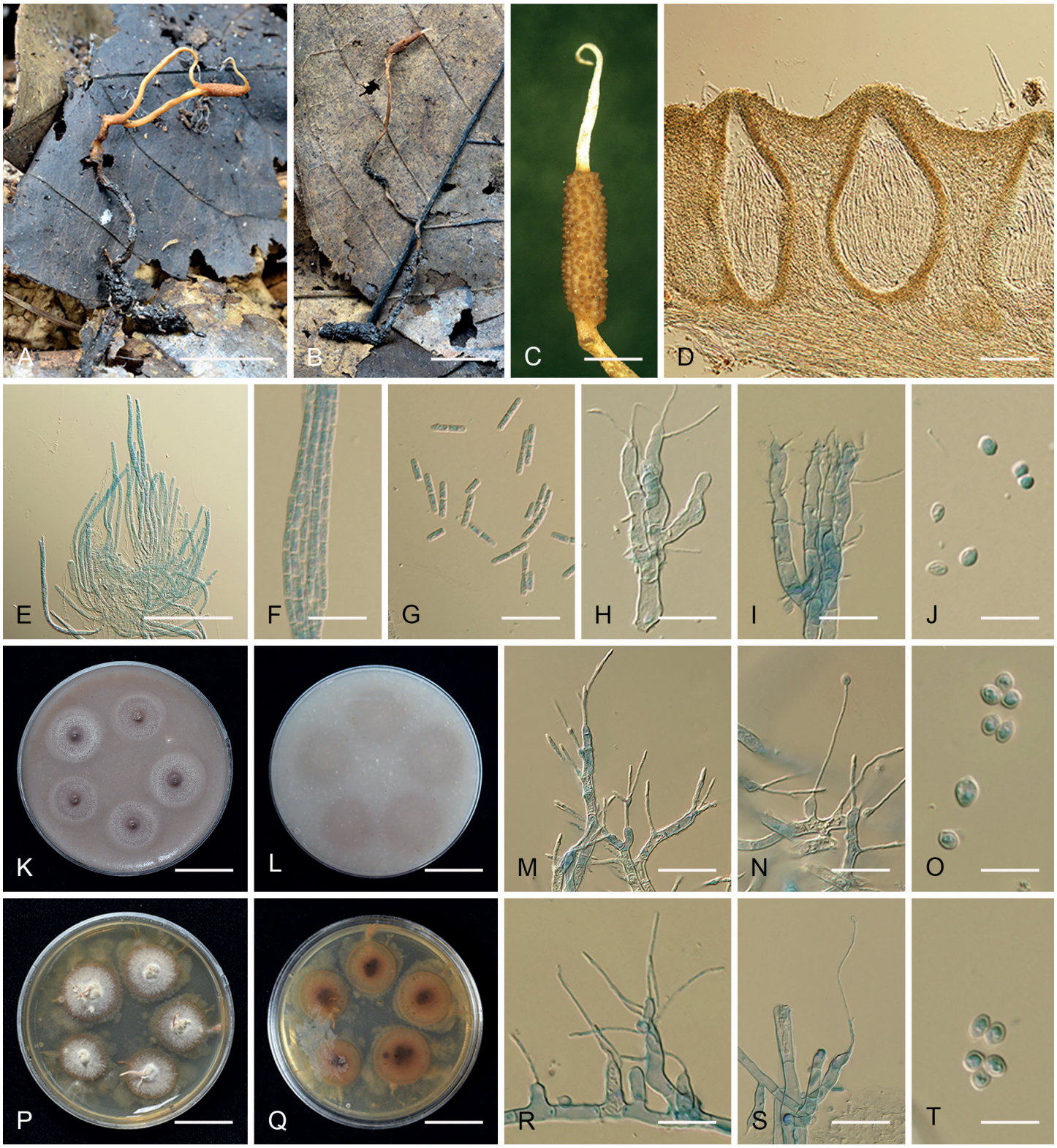

Fig. 9. *Ophiocordyceps ratchaburiensis.* **A, B.** Fungus on coleopteran larvae. **C.** Fertile part, **D.** Perithecia. **E.** Asci. **F, G.** Part-spores. **H, I.** Phialides. **J.** Conidia. **K, L.** Colonies on OA at 30-d-old (**K** obverse, **L** reverse). **M, N.** Phialides on OA. **O.** Conidia on OA. **P, Q.** Colonies on PDA at 30-d-old (**P** obverse, **Q** reverse). **R, S.** Phialides on PDA. **T.** Conidia on PDA. Scale bars: A, B = 10 mm; C = 2 mm; D, E = 100 µm; F–J, O, T = 10 µm; K, L, P, Q = 15 mm; M, N, R, S = 20 µm.

not exclusive to *O. clavata*; it is a phenomenon observed in various *Ophiocordyceps* species. Examples include *O. buquetii*, present in Brazil, China, Ghana, Taiwan and Thailand, while *O. spataforae* occurs in Thailand and the USA (Luangsa-ard *et al*. 2018, Araújo *et al*. 2020, Mongkolsamrit *et al*. 2023). These instances underscore similar genetic signatures, emphasizing the potential for widespread distribution and gene flow among these entomopathogenic fungi. The genetic uniformity observed across diverse geographic regions offers valuable insights into the dispersal mechanisms and ecological adaptability of *Ophiocordyceps* species (Castillo *et al*. 2018, Dai *et al.* 2020).

In this study, careful examination revealed that the majority of collected specimens showcase both sexual and asexual

morphs, except *O. clavata*, which exclusively displayed the sexual morph. For *O. pseudovariabilis*, the natural specimens exhibited a hirsutella-like asexual morph. We used three different media to study the asexual morphological traits in these collections. The results revealed that both *O. clavata* and *O. pseudovariabilis* produce synglioclad ium-like asexual morphs, with both species displaying the synglioclad ium-like morphology when cultured on MYA and PDA. The variations observed in morphologies between specimens in their natural habitat and those cultured may arise from distinctions in nutritional conditions, a phenomenon that has been observed in many invertebrate pathogenic fungi. For instance, cultures of *Polystromomyces araneae* and *Metarhizium rileyi* exhibit a yeast-like form when cultivated (Boucias *et al*. 2016, Mongkolsamrit *et al.* 2022, Iwanicki *et al*. 2023). Among the synglioclad ium-like asexual morphs, reports have been rare, and *O. variabilis* was found on immature stromata or in tufts or patches on the surface of the host (Hodge *et al*. 1998). The presence of this asexual morph represents a distinctive feature of *O. pseudovariabilis* and *O. clavata*, as revealed through comprehensive morphological and ecological comparisons with closely related species. Understanding this distinctiveness is important for generic or species circumscription.

New species in the *"O. purpureostromata"* clade

The "*O. purpureostromata*" clade encompasses three distinct species: *O. albostroma*, *O. borealis*, and *O. purpureostromata*, all occurring on *Coleoptera* larvae. The relationship of these three species that morphologically look the same but are genetically distinct represents a significant contribution to the taxonomy and understanding of this clade that it comprises a species complex. A noteworthy shared unique characteristic within this clade is the production of ascospores that divide into four part-spores, featuring long part-spores, as observed in *O. purpureostromata* (13–23 µm) (Kobayasi & Shimizu 1980) and *O. albostroma* (25–55 µm). Although the specific number of part-spores was not specified for *O. borealis*, it was mentioned that these part-spores are notably long (10–15 µm) (Zha *et al*. 2021). In Thailand, the production of elongated part-spores in *Ophiocordyceps* species is a rare occurrence. The first formally recorded instance of this characteristic is attributed to *O. barnesii*, as reported by Luangsa-ard *et al*. (2010a). To enhance our understanding of the *"O. purpureostromata"* clade, it is required to collect more samples, including additional isolates and a broader spectrum of insect hosts, for a comprehensive analysis. This approach is essential for refining taxonomy, elucidating evolutionary history, and uncovering the ecological roles of these fungi with various insect hosts.

New species in the *"O. elongata"* clade

The distinctiveness of *O. capilliformis* is evident through morphological comparisons with seven closely related species exhibiting the hirsutella-like asexual morph. *Ophiocordyceps capilliformis* stands out conspicuously from the seven other species within the *"O. elongata"* clade (Fig. 1). Sexual morphs in the "*O. elongata"* clade species are rarely reported, with exceptions including *O. elongata* and *O. humbertii*. A recent study by Fan *et al*. (2021) further described *O. alboperitheciata* from China. It is notable, that *O. capilliformis* shares comparable perithecia and ascospore morphology with *O. elongata* by having immersed perithecia and whole, filiform, multiseptate ascospores (Petch 1937). Nevertheless, the perithecia in *O. capilliformis* are smaller than those reported in *O. elongata*. Conversely, the distance between each septum in *O. capilliformis* is longer than that in *O. elongata* (20–35 µm *vs* 4–12 µm). Examining the morphology of perithecia and ascospores of the sexual morph of this clade reveals diverse characteristics. In the case of *O. alboperitheciata*, which is closely related to *O. elongata*, the perithecia are superficial, and the distances between septa in the ascospores are short (1.1–1.3 µm). On the other hand, in *O. humbertii*, perithecia are pseudo-immersed, with whole ascospores that are filiform and multiseptate (Petch 1935, Luangsa-ard *et al*. 2012). *Ophiocordyceps capilliformis* and *O. sporangifera* share similar features, producing multiple synnemata along stipes. However, *O. capilliformis* distinguishes itself by the absence of sclerotia at the apex of its stipe. The observed similarities and distinctions in asexual morphology between these two species raise interesting questions about their ecological roles and host specificity. Sclerotia form from dense aggregations of fungal hyphae (Smith *et al*. 2015). Sclerotia production was not reported among the species within the "*O. elongata*" clade. However, instances of sclerotia production were noted in *Hirsutella cryptosclerotium* and *H. subramaniiani* var. *myrmicarum*, which are part of the "*H. thompsonii*" clade and "*Hirsutella ant-pathogen*" clade presented by Simmons *et al*. (2015). The *"O. elongata"* clade reveals a wide range of insect hosts spanning various orders. *Ophiocordyceps alboperitheciata*, *O. elongata* and *H. gigantea* cluster together, predominantly associated with *Lepidoptera*, while *O. myrmicarum* and *O. humbertii* are linked with *Hymenoptera*. *Ophiocordyceps sporangifera* and *O. capilliformis* are affiliated with *Coleoptera*. Additionally, *H. citriformis* exhibits an association with *Hemiptera*. According to multigene phylogenies presented by Sanjuan *et al*. (2015) and Araújo *et al*. (2018), it is evident that *O. unilateralis sensu lato* and *O. sinensis* belong to the "*O. elongata*" clade. *Ophiocordyceps unilateralis sensu lato* is well known for its role as a common pathogenic fungus that infects ants (*Hymenoptera*) (Araújo *et al*. 2015, 2018, Kobmoo *et al*. 2015, Tang *et al*. 2023), while *O. sinensis* is an important component of the famous traditional Chinese medicine associated with larvae of *Hepialidae* (*Lepidoptera*) (Dai *et al*. 2020, 2024).

Diversity of new cryptic species in the "*O. brunneipunctata* complex" subclade in the *"O. sobolifera"* clade

The *"O. sobolifera"* clade encompasses fungi that are pathogens associated with cicada nymphs such as *O. sobolifera*, *O. yakusimensis*, *O. longissima*, which also include an endosymbiotic lineage (Sanjuan *et al*. 2015, Matsuura *et al*. 2018). Phylogenetic analyses revealed that *O. brunnea*, *O. kohchangensis*, *O. phitsanulokensis* and *O. ratchaburiensis* are nested well within the "*O. sobolifera*" clade (Fig. 1), forming a cluster with *O. aphodii*, *O. bidoupensis*, *O. houaynhangensis*, *O. brunneipunctata*, *O. langbianensis*, *O. cossidarum* and *O. furcatosubulata*. Within this clade, we identified a distinct subclade (supported by 99 % MLB/1 BPP) known as the *"O. brunneipunctata* complex" subclade. All species within this subclade primarily infect *Coleoptera* larvae, with the exception of *O. cossidarum*, which targets the larvae of *Cossidae* (*Lepidoptera*) (Hyde *et al.* 2017). The stromata typically exhibit a colour range from reddish brown to pale yellow, showcasing shared morphological traits including cylindrical structures, immersed perithecia, and

mostly an asexual morph situated at the end of the stroma. Additionally, all species feature ascospores with short part-spores (Table 2). Our fungal collections feature *O. brunnea* from Nan (Northern Thailand), *O. brunneipunctata* from Khao Yai National Park (Northeast Thailand), *O. ratchaburiensis* from Ratchaburi Province (West-central Thailand), *O. phitsanulokensis* from Phitsanulok Province (North-central Thailand), and *O. kohchangensis* from Mu Koh Chang National Park, an island in eastern Thailand. The observation of these five species within this subclade, each exhibiting apparent specificity to distinct locations and possibly hosts in Thailand, implies an ecological preference or adaptation to the specific conditions prevalent in each location. Based on natural specimens, we recognise *O. brunnea* as a newly discovered cryptic species that parasitises coleopterans. Despite their morphological similarities, such as red-brown stromata, these species were previously misclassified under *O. brunneipunctata*. Molecular phylogenetic analyses revealed that *O. brunnea* forms a sister lineage with *O. ratchaburiensis*. The asexual morph of both is closely associated, characterised by the production of mono- or polyphialidic, cylindrical basal portion, while the asexual morph in *O. brunneipunctata* produces a hirsutella-like asexual morph. However, all three species produce conidia in globose to subglobose forms. The sexual morph of *O. phitsanulokensis* exhibits morphological traits shared with several species within the *"O. brunneipunctata* complex" subclade. However, upon observing the asexual morph of *O. phitsanulokensis*, a distinct characteristic emerges. The phialides are produced at the subterminal part of the stipe, which is connected with the ascomata or formed independently of the sexual morph. Notably, the phialides in *O. phitsanulokensis* significantly differ from those observed in related species in Thailand, as they produce a hirsutella-like asexual morph with polyphialidic structure, and the conidia exhibit a fusoid form both in natural specimens and on culture media. The identification of cryptic species in this fungal study has become more attainable, owing to the widespread integration of molecular data and the consistent application of phylogenetic species concepts, aligning with previous research findings (Xiao *et al*. 2019, Lao *et al*. 2021, Zha *et al*. 2021, Zou *et al*. 2022).

Diversity of host life stages and habitats

Understanding the host range and habitat preferences of entomopathogenic fungi provides helpful information for their initial taxonomic classification, particularly at the familial or genus level (Araújo *et al*. 2020, Mongkolsamrit *et al.* 2020a, Kobmoo *et al*. 2023, Xiao *et al*. 2023). For example, species in *Ophiocordyceps* exhibit a diverse range of nutritional preferences, infecting diverse orders of insect hosts and host stages, as extensively studied (Luangsa-ard *et al.* 2018, Tasanatai *et al.* 2020, 2021, Khao-ngam *et al*. 2021, Fan *et al*. 2024). In this study, the majority of *Ophiocordyceps* species were found predominantly buried in the soil or lying on the leaf litter. However, exceptions were noted for *O. pseudovariabilis* and *O. clavata*, which were discovered on decaying wood and in the bark of living trees, respectively. Identifying insect hosts during their larval stages, especially those from the *Coleoptera* or *Lepidoptera*, has posed challenges due to the limited availability of taxonomic features for classification as well as the availability of expert entomologist in the area. However, certain species of entomopathogenic fungi have coleopteran larvae hosts that can be classified at the genus level, as seen with examples like *Metarhizium clavatum* and *M. purpureum* infecting larvae of *Oxynopterus* sp. (Mongkolsamrit *et al*. 2020a). Similarly, *O. sinensis* infects lepidopteran larvae belonging to the ghost moth *Thitarodes* sp. (Wang *et al*. 2020, Dai *et al.* 2024). Based on the review conducted by Zha *et al*. (2021) and this study, *Ophiocordyceps* species associated with *Coleoptera* were primarily identified as belonging to the subfamilies *Elateridae* and *Tenebrionidae*, we hypothesise that *Ophiocordyceps* species associated with *Coleoptera* might have a broader range of hosts and manifest their host-specificity at the genus or family level. This stands in contrast to the compelling evidence of high host-specificity observed in *Ophiocordyceps unilateralis* complex species on ant species in the tribe Camponotini (Evans *et al.* 2011, Araújo *et al*. 2015, 2018, Kobmoo *et al*. 2012, 2015). To enhance our understanding of the relationships between fungi associated with *Coleoptera*, it is crucial to collect molecular data directly from the host, particularly during its larval stages.

Regarding *O. capilliformis*, its occurrence across disparate beetle host families and different stages of host development is fascinating, including adult beetles of *Platydracus* sp. and larvae of *Meracantha* sp. A similar phenomenon, where the sexual morph is found on larval stages while the asexual morph occurs on the adult of *Coleoptera*, was reported in *Beauveria thailandica* (Kobmoo *et al*. 2021). Additionally, *M. chaiyaphumense* also exhibits a similar pattern, with the sexual morph found on cicada nymphs buried in the soil and the asexual morph found on adult cicadas attached to the underside of leaves (Luangsa-ard *et al*. 2017).

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Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

Araújo JPM, Evans HC, Fernandes IO, *et al*. (2020). Zombie-ant fungi cross continents: II. Myrmecophilous hymenostilboid species and a novel zombie lineage. *Mycologia* **112**: 1138–1170.

Araújo JPM, Evans HC, Geiser DM, *et al*. (2015). Unravelling the diversity behind the *Ophiocordyceps unilateralis* (*Ophiocordycipitaceae*) complex: three new species of zombie-ant fungi from the Brazilian Amazon. *Phytotaxa* **220**: 224–238.

Araújo JPM, Evans HC, Kepler R, *et al.* (2018). Zombie-ant fungi across continents: 15 new species and new combinations within *Ophiocordyceps*. I. Myrmecophilous hirsutelloid species. *Studies in Mycology* **90**: 119–160.

Araujo JPM, Lebert BM, Vermeulen S, *et al*. (2022). Masters of the manipulator: two new hypocrealean genera, *Niveomyces* (*Cordycipitaceae*) and *Torrubiellomyces* (*Ophiocordycipitaceae*), parasitic on the zombie ant fungus *Ophiocordyceps camponoti-floridani*. *Persoonia* **49**: 171–194.

Araújo JPM, Moriguchi MG, Uchiyama S, *et al.* (2021). *Ophiocordyceps salganeicola*, a parasite of social cockroaches in Japan and insights into the evolution of other closely related *Blattodea*-associated lineages. *IMA Fungus* **12**: 3.

Bouchard P, Smith ABT, Douglas H, *et al*. (2017). Biodiversity of *Coleoptera*. In: *Insect Biodiversity: Science and Society* (Foottit RG, Adler PH. eds). John Wiley & Sons, New York, USA: 337–417.

Boucias D, Liu S, Meagher R, *et al.* (2016). Fungal dimorphism in the entomopathogenic fungus *Metarhizium rileyi*: Detection of an *in vivo* quorum-sensing system. *Journal of Invertebrate Pathology* **136**: 100–108.

Cantú-Bernal SH, Gomez-Flores R, Flores-Villarreal RA, *et al.* (2023). Adult *Diaphorina citri* biocontrol using *Hirsutella citriformis* strains and gum formulations. *Plants* **12**: 3184.

Castillo LP, Osorio A, Vargas N, *et al*. (2018). Genetic diversity of the entomopathogenic fungus *Cordyceps tenuipes* in forests and butterfly gardens in Quindío, Colombia. *Fungal Biology* **122**: 891–899.

Castlebury LA, Rossman AY, Sung GH, *et al.* (2004). Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycological Research* **108**: 864–872.

Chaverri P, Bischoff J, Evans H, *et al*. (2005). *Regiocrella*, a new entomopathogenic genus with a pycnidial anamorph and its phylogenetic placement in the *Clavicipitaceae*. *Mycologia* **97**: 1225–1237.

Chen Z, Wang Y, Dai Y, *et al.* (2019). Species diversity and seasonal fluctuation of entomogenous fungi of *Ascomycota* in Taibaoshan forest park in western Yunnan. *Biodiversity Science* **27**: 993–1001.

Crous PW, Luangsa-ard JJ, Wingfield MJ, *et al.* (2018). Fungal Planet description sheets: 785–867. *Persoonia* **41**: 238–417.

Crous PW, Osieck ER, Shivas RG, *et al.* (2023). Fungal Planet description sheets: 1478–1549. *Persoonia* **50**: 158–310.

Crous PW, Wingfield MJ, Burgess TI, *et al*. (2017). Fungal Planet description sheets: 625–715. *Persoonia* **39**: 270–467.

Dai Y, Chen S, Wang Y, *et al.* (2024). Molecular phylogenetics of the *Ophiocordyceps sinensis*-species complex lineage (*Ascomycota*, *Hypocreales*), with the discovery of new species and predictions of species distribution. *IMA Fungus* **15**: 2.

Dai Y, Wu C, Yuan F, *et al.* (2020). Evolutionary biogeography on *Ophiocordyceps sinensis*: An indicator of molecular phylogeny to geochronological and ecological exchanges. *Geoscience Frontiers* **11**: 807–820.

Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.

Evans HC, Elliot SL, Hughes DP (2011). Hidden diversity behind the zombie-ant fungus *Ophiocordyceps unilateralis*: four new species described from carpenter ants in Minas Gerais, Brazil. *PLoS ONE* **6**: e17024.

Fan Q, Wang YB, Zhang GD, *et al*. (2021). Multigene phylogeny and morphology of *Ophiocordyceps alboperitheciata sp. nov*., a new entomopathogenic fungus attacking Lepidopteran Larva from Yunnan, China. *Mycobiology* **49**: 133–141.

Fan Q, Yang T, Li H, *et al.* (2024). Molecular phylogeny and morphology reveal two new entomopathogenic species of *Ophiocordyceps* (*Ophiocordycipitaceae*, *Hypocreales*) parasitic on termites from China. *MycoKeys* **103**: 1–24.

Hall T (1999). BioEdit. A user-friendly biological sequence alignment editor and analysis program forWindows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Hodge KT, Humber RA, Wozniak CA (1998). *Cordyceps variabilis* and the genus *Synglioclad*ium. *Mycologia* **90**: 743–753.

Hodge S, Marris J, Brown SDJ, *et al*. (2019). *Coleoptera* found in marine strandlines on New Zealand beaches: species diversity, seasonal trends and the effect of beach substrate. *New Zealand Entomologist* **42**: 47–66.

Hyde KD, Norphanphoun C, Abreu VP, *et al.* (2017). Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Diversity* **87**: 1–235.

Hywel-Jones NL (1995). *Cordyceps brunneipunctata sp. nov*. infecting beetle larvae in Thailand. *Mycological Research* **99**: 1195–1198.

Iwanicki NSA, Júnior ID, Carvalho LLB de, *et al*. (2023). Comparative transcriptomics of growth metabolism and virulence reveal distinct morphogenic profiles of yeast-like cells and hyphae of the fungus *Metarhizium rileyi. Fungal Genetics and Biology* **164**: 103766.

Khao-ngam S, Mongkolsamrit S, Rungjindamai W, *et al*. (2021). *Ophiocordyceps asiana* and *Ophiocordyceps tessaratomidarum* (*Ophiocordycipitaceae*, *Hypocreales*), two new species on stink bugs from Thailand. *Mycological Progress* **20**: 341–353.

Khonsanit A, Luangsa-ard JJ, Thanakitpipattana D, *et al*. (2019). Cryptic species within *Ophiocordyceps myrmecophila* complex on formicine ants from Thailand. *Mycological Progress* **18**: 147–161.

Kobayasi Y, Shimizu D (1980). *Cordyceps* species from Japan 3. *Bulletin of the National Science Museum, Tokyo, Series B. Botany* **6**: 125–146.

Kobmoo N, Arnamnart N, Pootakham W, *et al*. (2021). The integrative taxonomy of *Beauveria asiatica* and *B. bassiana* species complexes with whole-genome sequencing, morphometric and chemical analyses. *Persoonia* **47**: 136–150.

Kobmoo N, Mongkolsamrit S, Tasanathai K, *et al*. (2012). Molecular phylogenies reveal host-specific divergence of *Ophiocordyceps unilateralis sensu lato* following its host ants. *Molecular Ecology* **21**: 3022–3031.

Kobmoo N, Mongkolsamrit S, Wutikhun T, *et al.* (2015). New species of *Ophiocordyceps unilateralis*, an ubiquitous pathogen of ants from Thailand. *Fungal Biology* **119**: 44–52.

Kobmoo N, Tasanathai K, Araújo JPM, *et al*. (2023). New mycoparasitic species in the genera *Niveomyces* and *Pseudoniveomyces gen. nov.* (*Hypocreales*: *Cordycipitaceae*), with sporothrix-like asexual morphs, from Thailand. *Fungal Systematics and Evolution* **12**: 91–110.

Kuephadungphan W, Petcharad B, Tasanathai K, *et al.* (2022). Multilocus phylogeny unmasks hidden species within the specialized spider-parasitic fungus, *Gibellula* (*Hypocreales*, *Cordycipitaceae*) in Thailand. *Studies in Mycology* **101**: 245–286.

Lao T D, Le T AH, Truong NB (2021). Morphological and genetic characteristics of the novel entomopathogenic fungus *Ophiocordyceps langbianensis* (*Ophiocordycipitaceae*, *Hypocreales*) from Lang Biang Biosphere Reserve, Vietnam. *Scientific Reports* **11**: 1412.

Li GJ, Hyde KD, Zhao RL, *et al*. (2016). Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* **78**: 1–237.

Liu YJ, Whelen S, Hall BD (1999). Phylogenetic relationships among Ascomycetes: Evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution* **16**: 1799–1808.

Luangsa-Ard JJ, Hywel-Jones NL, Manoch L, *et al*. (2005). On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycological Research* **109**: 581–589.

Luangsa-ard JJ, Mongkolsamrit S, Thanakitpipattana D, *et al*. (2017). Clavicipitaceous entomopathogens: new species in *Metarhizium* and a new genus *Nigelia*. *Mycological Progress* **16**: 369–391.

Luangsa-ard JJ, Ridkaew R, Mongkolsamrit S, *et al*. (2010a). *Ophiocordyceps barnesii* and its relationship to other melolonthid pathogens with dark stromata. *Fungal Biology* **114**: 739–745.

Luangsa-ard JJ, Tasanathai K, Mongkolsamrit S, *et al.* (2010b) *Atlas of invertebrate-pathogenic fungi of Thailand.* Volume 3. National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand.

Luangsa-ard JJ, Tasanathai K, Mongkolsamrit S, *et al*. (2012). *Atlas of invertebrate-pathogenic fungi of Thailand.* Volume 4. National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand.

Luangsa-ard JJ, Tasanathai K, Thanakitpipattana D, *et al.* (2018). Novel and interesting *Ophiocordyceps* spp. (*Ophiocordycipitaceae*, *Hypocreales*) with superficial perithecia from Thailand. *Studies in Mycology* **89**: 125–142.

Matočec N, Kušan I, Ozimec R (2014). The genus *Polycephalomyces* (*Hypocreales*) in the frame of monitoring Veternica cave (Croatia) with a new segregate genus *Perennicordyceps*. *Ascomycete.org* **6**: 125–133.

Matsuura Y, Moriyama M, Łukasik P, *et al.* (2018). Recurrent symbiont recruitment from fungal parasites in cicadas. *Proceedings of the National Academy of Sciences of the USA* **115**: E5970–E5979.

Mongkolsamrit S, Khonsanit A, Thanakitpipattana D, *et al.* (2020a) Revisiting *Metarhizium* and the description of new species from Thailand. *Studies in Mycology* **95**: 171–251.

Mongkolsamrit S, Noisripoom W, Arnamnart N, *et al.* (2019). Resurrection of *Paraisaria* in the *Ophiocordycipitaceae* with three new species from Thailand. *Mycological Progress* **18**: 1213–1230.

Mongkolsamrit S, Noisripoom W, Hasin S, *et al.* (2023). Multi-gene phylogeny and morphology of *Ophiocordyceps laotii sp. nov*. and a new record of *O. buquetii* (*Ophiocordycipitaceae*, *Hypocreales*) on ants from Thailand. *Mycological Progress* **22**: 5.

Mongkolsamrit S, Noisripoom W, Pumiputikul S, *et al.* (2021). *Ophiocordyceps flavida sp. nov*. (*Ophiocordycipitaceae*), a new species from Thailand associated with *Pseudogibellula formicarum* (*Cordycipitaceae*), and their bioactive secondary metabolites. *Mycological Progress* **20**: 477–492.

Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, *et al.* (2018). Disentangling cryptic species with *Isaria*-like morphs in *Cordycipitaceae*. *Mycologia* **110**: 230–257.

Mongkolsamrit S, Noisripoom W, Tasanathai K, *et al.* (2020b). Molecular phylogeny and morphology reveal cryptic species in *Blackwellomyces* and *Cordyceps* (*Cordycipitaceae*) from Thailand. *Mycological Progress* **19**: 957–983.

Mongkolsamrit S, Noisripoom W, Tasanathai K, *et al.* (2022). Comprehensive treatise of *Hevansia* and three new genera *Jenniferia*, *Parahevansia* and *Polystromomyces* on spiders in *Cordycipitaceae* from Thailand. *MycoKeys* **91**: 113–149.

Negi PS, Singh R, Koranga PS, *et al*. (2012). Two new for science species of genus *Cordyceps* Fr. (*Ascomycetes*) from Indian Himalaya. *International Journal of Medicinal Mushrooms* **14**: 501–506.

Nylander JAA (2004). MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

O'Donnell K, Sarver BA, Brandt M, *et al.* (2007). Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *Journal of Clinical Microbiology* **45**: 2235–2248.

Page RD (1996). TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.

Penzig O, Saccardo PA (1904). *Icones fungorum Javanicorum*. Buchhandlung and Druckerei E.J. Brill, Leiden, The Netherlands.

Pérez-González O, Gomez-Flores R, Tamez-Guerra P (2022). Insight into biological control potential of *Hirsutella citriformis* against Asian citrus psyllid as a vector of citrus huanglongbing disease in America. *Journal of Fungi* **8**: 573.

Perissinotto R, Bird MS, Bilton DT (2016). Predaceous water beetles (*Coleoptera*, *Hydradephaga*) of the Lake St Lucia system, South Africa: biodiversity, community ecology and conservation implications. *Zookeys* **595**: 85–135.

Petch T (1924). Studies in entomogenous fungus. IV. Some Ceylon *Cordyceps*. *Transactions of the British Mycological Society* **10**: 28–45.

Petch T (1931). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **16**: 55–75.

Petch T (1935). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **19**: 161–194.

Petch T (1937). Notes on entomogenous fungi. *Transactions of the British Mycological Society* **21**: 34–67.

Quandt CA, Kepler RM, Gams W, *et al.* (2014). Phylogenetic-based nomenclatural proposals for *Ophiocordycipitaceae* (*Hypocreales*) with new combinations in *Tolypocladium*. *IMA Fungus* **5**: 121–134.

RHS Media (2015). *Royal Horticultural Society (RHS) Colour Chart*, 6th ed. Royal Horticultural Society, UK.

Rehner SA, Buckley E (2005). A *Beauveria* phylogeny inferred from nuclear ITS and *EF1-α* sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **97**: 84–98.

Ronquist F, Teslenko M, Mark P, *et al.* (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Saepua S, Kornsakulkarn J, Auncharoen P, *et al.* (2021). Secondary metabolites from cultures of the ant pathogenic fungus *Ophiocordyceps irangiensis* BCC 2728. *Natural Product Research* **35**: 3556–3561.

Saltamachia SJ, Araújo JPM (2020). *Ophiocordyceps desmidiospora*, a basal lineage within the "Zombie-Ant Fungi" clade. *Mycologia* **112**: 1171–1183.

Samson RA, Evans HC, Hoekstra S (1982). Notes on entomogenous fungi from Ghana, VI. The genus *Cordyceps*. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C* **85**: 589–605.

Sanjuan TI, Franco-Molano AE, Kepler RM, *et al*. (2015). Five new species of entomopathogenic fungi from the Amazon and evolution of neotropical *Ophiocordyceps*. *Fungal Biology* **119**: 901–916.

Sanjuan T, Tabima J, Restrepo S, *et al.* (2014). Entomopathogens of Amazonian stick insects and locusts are members of the *Beauveria* species complex (*Cordyceps sensu stricto*). *Mycologia* **106**: 260–275.

Schoch CL, Seifert KA, Huhndorf S, *et al.* (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences of the USA* **109**: 6241–6246.

Shrestha B, Tanaka E, Hyun MW, *et al.* (2016). Coleopteran and lepidopteran hosts of the entomopathogenic genus *Cordyceps sensu lato*. *Journal of Mycology* **2016**: 1–14.
Simmons DR, Kepler RM, Rehner SA, *et al*. (2015a). Phylogeny of *Hirsutella* species (*Ophiocordycipitaceae*) from the USA: remedying the paucity of *Hirsutella* sequence data. *IMA Fungus* **6**: 345–356.
Simmons DR, Lund J, Levitsky T, *et al.* (2015b). *Ophiocordyceps myrmicarum*, a new species infecting invasive *Myrmica rubra* in Maine. *Journal of Invertebrate Pathology* **125**: 23–30.
Smith ME, Henkel TW, Rollins JA (2015). How many fungi make sclerotia? *Fungal Ecology* **13**: 211–220.
Spatafora JW, Sung GH, Sung JM, *et al*. (2007). Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Molecular Ecology* **16**: 1701–1711.
Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
Sung GH, Hywel-Jones NL, Sung JM, *et al.* (2007). Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* **57**: 5–59.
Sung GH, Spatafora JW, Zare R, *et al*. (2001). A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the *Clavicipitaceae*. *Nova Hedwigia* **72**: 311–328.
Tang D, Huang O, Zou W, *et al.* (2023). Six new species of zombie-ant fungi from Yunnan in China. *IMA Fungus* **14**: 9.
Tasanathai K, Khonsanit A, Noisripoom W, *et al*. (2022). Hidden species behind *Ophiocordyceps* (*Ophiocordycipitaceae*, *Hypocreales*) on termites: four new species from Thailand. *Mycological Progress* **21**: 86.
Tasanathai K, Noisripoom W, Chaitika T, *et al.* (2019). Phylogenetic and morphological classification of *Ophiocordyceps* species on termites from Thailand. *MycoKeys* **56**: 101–129.
Tasanathai K, Thanakitpipattana D, Himaman W, *et al.* (2020). Three new *Ophiocordyceps* species in the *Ophiocordyceps pseudoacicularis* species complex on *Lepidoptera* larvae in Southeast Asia. *Mycological Progress* **19**: 1043–1056.
Tasanathai K, Thanakitpipattana D, Noisripoom W, *et al*. (2016). Two new *Cordyceps* species from a community forest in Thailand. *Mycological Progress* **15**: e28.
Thanakitpipattana D, Mongkolsamrit S, Khonsanit A, *et al*. (2022). Is *Hyperdermium* congeneric with *Ascopolyporus*? phylogenetic relationships of *Ascopolyporus* spp. (*Cordycipitaceae*, *Hypocreales*) and a new genus *Neohyperdermium* on scale insects in Thailand. *Journal of Fungi* **8**: 516.
Thanakitpipattana D, Tasanathai K, Mongkolsamrit S, *et al*. (2020). Fungal pathogens occurring on *Orthopterida* in Thailand. *Persoonia* **44**: 140–160.
Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.
Tran TVT, Tran VK, Ho XAV, *et al*. (2021). Chemical structure of a novel heteroglycan polysaccharide isolated from the biomass of *Ophiocordyceps sobolifera*. *Journal of Molecular Structure* **1232**: 129986.
Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.
Wang Y, Wei S, Lian H, *et al.* (2023). Neutral polysaccharide from spores of *Ophiocordyceps gracilis* regulates oxidative stress via NRF2/FNIP1 pathway. *International Journal of Molecular Sciences* **24**: 14721.
Wang Y, Wu HJ, Tran NL, *et al*. (2021a). *Ophiocordyceps furcatosubulata*, a new entomopathogenic fungus parasitizing beetle larvae (*Coleoptera*: *Elateridae*). *Phytotaxa* **482**: 268–278.
Wang YH, Ban S, Wang WJ, *et al.* (2021b). *Pleurocordyceps gen. nov*. for a clade of fungi previously included in *Polycephalomyces* based on molecular phylogeny and morphology. *Journal of Systematics and Evolution* **59**: 1065–1080.
Wang YW, Hong TW, Tai YL, *et al.* (2015). Evaluation of an epitypified *Ophiocordyceps formosana* (*Cordyceps s.l*.) for its pharmacological potential. *Evidence-Based Complementary and Alternative Medicine* **2015**: 189891.
Wang Z, Li M, Ju W, *et al.* (2020). The entomophagous caterpillar fungus *Ophiocordyceps sinensis* is consumed by its lepidopteran host as a plant endophyte. *Fungal Ecology* **47**: 100989.
Wei Y, Zhang L, Wang J, *et al*. (2021). Chinese caterpillar fungus (*Ophiocordyceps sinensis*) in China: Current distribution, trading, and futures under climate change and overexploitation. *Science of the Total Environment* **755**: 142548.
Xiao YP, Hongsanan S, Hyde KD, *et al.* (2019). Two new entomopathogenic species of *Ophiocordyceps* in Thailand. *MycoKeys* **47**: 53–74.
Xiao YP, Wang YB, Hyde KD, *et al.* (2023). *Polycephalomycetaceae*, a new family of clavicipitoid fungi segregates from *Ophiocordycipitaceae*. *Fungal Diversity* **120**: 1–76.
Zha LS, Kryukov VY, Ding JH, *et al*. (2021). Novel taxa and species diversity of *Cordyceps sensu lato* (*Hypocreales*, *Ascomycota*) developing on wireworms (*Elateroidea* and *Tenebrionoidea*, *Coleoptera*). *MycoKeys* **78**: 79–11.
Zhang, L, Fasoyin OE, Molnár I, *et al*. (2020). Secondary metabolites from hypocrealean entomopathogenic fungi: Novel bioactive compounds. *Natural Product Reports* **37**: 1181–1206.
Zou W, Tang D, Xu Z, *et al*. (2022). Multigene phylogeny and morphology reveal *Ophiocordyceps hydrangea sp. nov.* and *Ophiocordyceps bidoupensis sp. nov.* (*Ophiocordycipitaceae*). *MycoKeys* **92**: 109–130.

Supplementary Material: http://fuse-journal.org/

Fig. S1. RAxML tree of *Ophiocordyceps* species based on LSU sequences. Numbers at the major nodes represent maximum likelihood bootstrap (MLB) support values. The new species presented here are indicated in blue font.
Fig. S2. RAxML tree of *Ophiocordyceps* species based on *TEF1* sequences. Numbers at the major nodes represent maximum likelihood bootstrap (MLB) support values. The new species presented here are indicated in blue font.
Fig. S3. RAxML tree of *Ophiocordyceps* species based on *RPB1* sequences. Numbers at the major nodes represent maximum likelihood bootstrap (MLB) support values. The new species presented here are indicated in blue font.
Fig. S4. RAxML tree of *Ophiocordyceps* species based on *RPB2* sequences. Numbers at the major nodes represent maximum likelihood bootstrap (MLB) support values. The new species presented here are indicated in blue font.
Fig. S5. Culture characteristics of *Ophiocordyceps phitsanulokensis* on MYA, *O. pseudovariabilis* on OA and *O. ratchaburiensis* on MYA at 30 d.