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Taxonomy and phylogeny of *Pleurocordyceps* (Polycephalomycetaceae, Hypocreales) associated with ants and cicadas from Malaysia, including a new species and new records

MUHAMMAD SHAHBAZ[1,3], FIRDZA ZULKARNAIN MOHADDEN[1,4], JEREMIAH SIA YIAO RONG[2,5], YAP JING WEI[2,6] & JAYA SEELAN SATHIYA SEELAN[1,7,*]

[1] *Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan, UMS, 88400, Kota Kinabalu, Sabah, Malaysia*
[2] *Centre for Research on Sustainable Uses of Natural Resources (COR_SUNR), Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Hab Pendidikan Tinggi Pagoh, KM 1, Jalan Panchor, 84600 Panchor, Johor, Malaysia*
[3] *muhammad786943@gmail.com; https://orcid.org/0000-0002-6253-1769*
[4] *firdzazulkarnain@gmail.com; https://orcid.org/0009-0004-2001-5450*
[5] *Jeremiahsia@gmail.com; https://orcid.org/0009-0005-8925-0552*
[6] *jwyap@uthm.edu.my; https://orcid.org/0000-0002-3706-5954*
[7] *seelan80@ums.edu.my; https://orcid.org/0000-0002-0045-6206*
Corresponding author: (seelan80@ums.edu.my). (J. Seelan Sathiya Seelan)

Abstract

Pleurocordyceps is a group of hyperparasites that infects invertebrates, plants, and other fungi. It belongs to the family Polycephalomycetaceae, a newly established family separated from Ophiocordycipitaceae, order Hypocreales which includes several genera such as *Dingleyomyces, Paradingleyomyces, Perennicordyceps, Pleurocordyceps, and Polycephalomyces. Pleurocordyceps* currently comprises 26 species reported from South American and Asian regions In Asia, Malaysia has diverse forest ecosystems and rich fungal diversity; however, no previous studies have been reported on the occurrence of *Pleurocordyceps* species. The aim of the study is to explore the *Pleurocordyceps* diversity tropical rainforests of Malaysia using morpho-molecular analyses. During a fungal survey in Malaysia, fungicolous fungus associated with ants and cicadas nymphs were collected and subjected to morphological and molecular assessments. Two genetic loci—the internal transcribed spacer (ITS) region and the translation elongation factor 1-alpha (*TEF-1α*) gene—were used to construct both individual and concatenated phylogenetic trees. The study describes a new species, *Pleurocordyceps cornusynnemata sp. nov.* (BORH/F03651) from an ant, and two new records of *P. aurantiaca* (BORH/F03651), and *P. nipponica* (UTHM-CEF003.1) from Diptera and cicada nymph, respectively. These findings expand the knowledge of the geographic distribution of *Pleurocordyceps* and provide the first baseline data for the genus in Malaysia, thereby improving understanding of the diversity and evolutionary relationships within Polycephalomycetaceae.

Key words: arthropod hosts, entomopathogenic fungi, *Pleurocordyceps*, synnema, three taxa, typification

Introduction

Entomopathogenic fungi (EPF), is a fungal group consists of approximately more than 2,000 species across 90 genera (Saltamachia and Araujo, 2020). Molecular phylogenetic studies have clearly recognized the diversity of EPF within the order *Hypocreales*, which represents the largest group of insect and plant pathogens (Maharachchikumbura *et al.* 2016; Vinit *et al.* 2018). *Polycephalomyces* is an important entomopathogenic fungal genus in Polycephalomycetaceae, order Hypocreales established by Kobayasi (Kobayasi 1941) based on the asexual morph of *Po. formosus*. The species shows polycephalous synnemata with white to pale yellow conidial masses at the tip. *Polycephalomyces* species establish parasitic associations with a wide range of hosts, including insects, fungi and myxomycetes (Shrestha *et al.* 2017). The sexual morph of *Polycephalomyces* observed as fertile, capitulate, globose, tuberiform to pulvinate stromata and immersed, elongated pyriform perithecia (Matočec *et al.* 2014). Later, *Pleurocordyceps* was emerged as a new genus by (Wang *et al.* 2021) and *Polycephalomyces* was delineated as a distinct genus within Polycephalomycetaceae, which has recently been recognized as a distinct family of clavicipitoid fungi (Wang *et al.* 2020; Johnston and Park 2023; Wanhao and Yanfeng 2024). *Pleurocordyceps sinensis* was described as the type species of the *Pleurocordyceps* genus,

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which was isolated as hyper-parasites on *Ophiocordyceps sinensis* (Wang *et al.* 2021; Xiao *et al.* 2023). Exhibiting lateral fertile pulvinate stromata and distinct asexual morphs, which include two types of phialides and conidia are the key morphological characteristics of *Pleurocordyceps* associated species. Morphological attributes support to differentiate closely related genera within the family Polycephalomycetaceae (Xiao *et al.* 2024).

According to Index Fungorum (https://www.indexfungorum.org/Names/Names.asp accessed March 8, 2026), the genus *Pleurocordyceps* consists of 26 accepted species. The genus *Pleurocordyceps* has a worldwide distribution, mostly found in China, Thailand, and Ecuador. Different insect orders such as Coleoptera, Hymenoptera, and Lepidoptera have been reported as associated with *Pleurocordyceps* **(**Mongkolsamrit *et al.* 2024; Xiao *et al.* 2024). Members of Polycephalomycetaceae have a significant value and broad applications in the field of medicine, agriculture and other related areas (Liu *et al.* 2024; Xiao *et al.* 2023). *Pleurocordyceps* species exhibit significant potential for the production of diverse secondary metabolites (Xiao *et al.* 2024). Different biological activities such as antitumor and antibacterial activities, as well as bioactive compounds including cordytropolone and leptosphaerone A, have been reported from *Pleurocordyceps nipponica* (Thammawat *et al.* 2017; Sangdee *et al.* 2015, 2016; Surapong *et al.* 2019).

This study aimed to investigate the species diversity of *Pleurocordyceps* in Malaysia and expand the current understanding of its diversity in Southeast Asia. In this study, we introduced one new species, *Pleurocordyceps cornusynnemata sp. nov.* (BORH/F03651), and two new records of *Pleurocordyceps aurantiaca* (BORH/F03652) and *Pleurocordyceps nipponica* (UTHM-CEF003.1). We provide detailed morphological descriptions along with phylogenetic analyses using a combined two-locus gene region (ITS and *TEF-1* α), including appropriate taxonomic justifications to confirm the placements of the studied species. The discovery of two new records and one new species in this study highlights the expanding fungal diversity of *Pleurocordyceps* in Malaysia, a rich yet understudied region, and contribute to the species world-wide reported.

Materials and methods

Sample collection

A total of four samples were collected in which three were associated pathologically with ant-infecting *Ophiocordyceps* while one was associated with cicada's nymph that were collected from the Danum Valley Conservation Area, Lahad Datu, Sabah, Malaysia, Bukit Chabang Tiga, Johor (southern Peninsular Malaysia) and Air Terjun Termurun Forest Eco Park, Langkawi, Kedah, Malaysia, with lowland dipterocarp-dominated forests during a field survey in the wet season during 2024-2025 years (Fig. 1). A map showing the sample collecting sites was created using QGIS 3.44.3 software (https://qgis.org/download/). The samples were photographed, field codes were assigned, and collection details were noted including collection sites, elevation, longitude and latitude. The samples were transported to the laboratory using freezing tubes in an ice box. Upon arrival, the samples underwent initial observation such synnemata, conidiophores, phialides, and conidia through a Nikon ECLIPSE microscope.

Fungal isolation and culture preparation

A small piece of fresh conidia from the host was collected under a stereomicroscope using a sterile needle, and placed on solid PDA media containing 0.5g/L streptomycin and incubated at 25°C to obtain pure culture following the methods described by Wang *et al.* (2020). To obtain a well-grown fungal colony, fresh cultures were transferred to new PDA media and incubated at 25°C again. The fresh cultures were stored at 4°C in a refrigerator using beveled test tubes (Konopická *et al.* 2024). The herbarium specimens were deposited at BORNEENSIS, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia.

Morphological observations and descriptions

The fully grown pure cultures on PDA media were used to study morphological characters such as the color of front and reverse colony views, diameter, and growth rate after 14 days following the methods described by Xiao *et al.* (2023). Water mounted glass slides were prepared with structures taken from the colonies and microstructures such as phialides, conidia, and conidiophores were observed at different magnifications under a Nikon ECLIPSE microscope. For scanning electron microscopy (SEM), the fully grown culture from *Pleurocordyceps* sp. were fixed overnight at 48 ^{o}C in 4% glutaraldehyde in 0.05 M phosphate buffer (pH 7.3) and rinsed three times (10 min each) in phosphate buffer. Samples were then dehydrated in an ethanol series and critical point dried. Specimens were sputter-coated with gold, observed, and photographed in a HITACHI S-3000 N SEM at 16.5 6 4.6 kV (Wang *et al.* 2023).

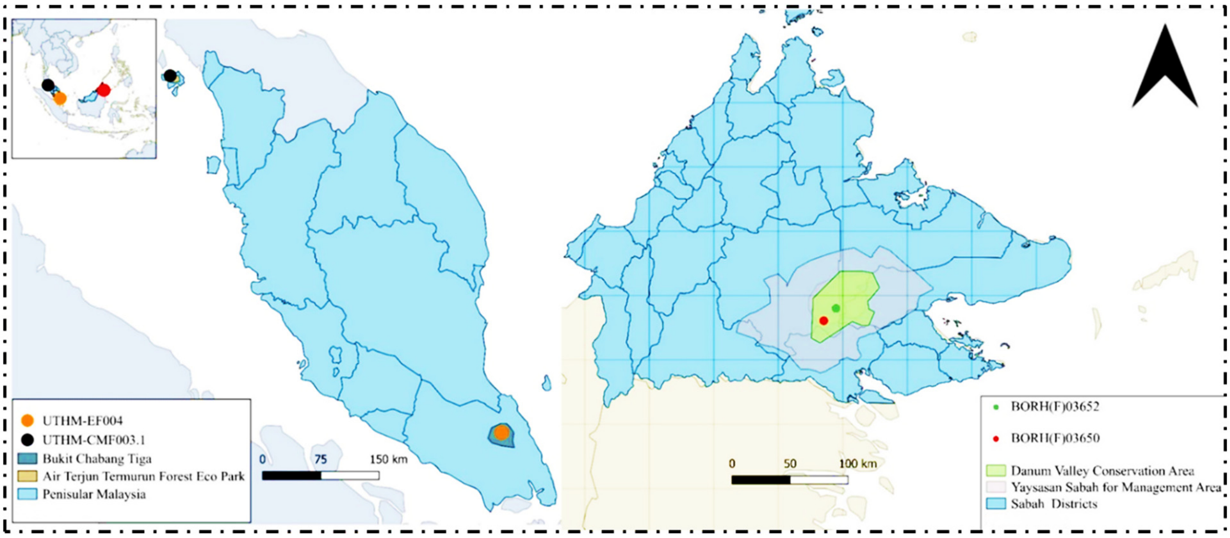

FIGURE 1. Distribution map of the collection sites of *Pleurocordyceps cornusynnemata sp. nov.* (BORH/F03650, BORH/F03651), *Pleurocordyceps aurantiaca* (BORH/F03652) and *Pleurocordyceps nipponica* (UTHM-CEF003.1) in Malaysia.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from cultures using the Fungal DNA extraction kit (E.Z.N. A® Plant & Fungal DNA Kit) following the manufacturer's instructions. The internal transcribed spacer gene region (ITS) and translation elongation factor 1-alpha gene region (*TEF-1*α) amplifications were performed using the ITS1/ITS4 and 983F/2218R primers, respectively (White *et al.* 1990; Hopple and Vilgalys, 1999; Vilgalys and Hester, 1990; Castlebury *et al.* 2004). The nuclear gene amplification reactions followed the protocol outlined by Yang *et al.* (2021). PCR products were purified and sequenced at Apical Scientific Sdn. Bhd, Selangor, Malaysia.

Phylogenetic Tree Construction

DNA sequences of this study were retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) and they were used with our newly generated sequences to analyze the major lineages within the family *Polycephalomycetaceae* and construct the phylogenetic tree. Two samples of *Perennicordyceps cuboidea* (NBRC 106330 and NBRC 100998) were selected as the outgroup taxa due to its close phylogenetic affinity and distinct to *Pleurocordyceps* (Xiao *et al.* 2023). The nucleotide sequences used in this study were listed in Table 1. Individual gene datasets (ITS and *TEF-1α*) (Supplementary Figure S1and S2) as well as the combined dataset included both newly generated and reference sequences. The concatenated tree (ITS-*TEF-1*α) is chosen as the main phylogenetic tree as it provides more stronger and reliable results (Fig. 2). Sequence alignments were performed using AliView (https://ormbunkar.se/aliview) (Larsson, 2014), and manual adjustments were made to ensure accuracy. The obtained sequences were edited and assembled using BioEdit v.7.1 (https://bioedit.software.informer.com/7.1) and ambiguous nucleotides were corrected. Multiple sequence alignment was conducted using MAFFT v7 (https://mafft.cbrc.jp/alignment/server/index.html), followed by manual refinement. The resulting alignments were trimmed to remove poorly aligned positions and excessive gaps to ensure high-quality datasets for subsequent phylogenetic construction. (Larget & Simon, 1999). In total, 103 sequences (54 ITS and 49 *TEF1-α*) were used in this study (Table 1), including 4 sequences of new species (2 ITS and 2 *TEF1-α*), and 10 sequences from the newly recorded species (5 ITS and 5 *TEF1-α*). The final ITS+ *TEF1-α* dataset contained 50 ITS and 50 *TEF1-α* sequences. ModelFinder (Kalyaanamoorthy *et al.* 2017) identified the best-fit likelihood model for both maximum likelihood (ML) and Bayesian inference (BI) analyses. Maximum Likelihood (ML) analysis considered bootstrap values ≥70% as strong phylogenetic support. The best-fit nucleotide substitution model was selected using jModelTest2 on the CIPRES Science Gateway (https://www.phylo.org/). The TrNef+G model was chosen with the following parameters: lset nst = 6; rates = gamma; prset revmatpr = fixed (1.0000, 1.7639, 1.0000, 1.0000, 3.1308, 1.0000); and shapepr = fixed (0.725) (Song *et al.* 2023). Bayesian Inference (BI) analyses were also performed on the CIPRES Science Gateway using MrBayes v3.2.7a (Kadam *et al*. 2024; Cheak *et al*. 2024; Masoudi *et al*. 2020). Five million generations of four Markov chains were executed, with a tree sample taken every 1,000 generations. The ITS alignment comprised 54 sequences with 511 characters, including 183 distinct patterns, 5 singleton sites, and 366 constant sites. The *TEF1-α* alignment included 49 sequences with 528 characters, 127 distinct patterns, 139 parsimony-informative sites, 2 singleton sites, and 387 constant sites. For the combined dataset (ITS+*TEF1-α*), the concatenated alignment contained 54 sequences with a total length of 1371 characters, showing 279 distinct patterns, 297 parsimony-informative sites, 73 singleton sites, and 1,001 constant sites. The concatenated dataset was used for both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses to infer the phylogenetic relationships among the taxa. Bootstrap values (BS) of ≥70% and posterior probabilities of (PP) ≥1.00 were considered to indicate significant support. Phylogenetic trees were visualized using FigTree v1.4.4 (https://evomics.org/resources/software/molecular-evolution-software/figtree/) and edited with Adobe Illustrator v27.6.1. The sequence alignments used for phylogenetic analyses have been submitted to TreeBASE (https://www.treebase.org) (submission ID: 32392).

TABLE 1. Sequences that used in this study including their species names, hosts, vouchers, countries, GenBank accession numbers and references.

Species	Host	Voucher No	GenBank accession number		Country	References
			ITS	*Tef-1α*		
Pleurocordyceps sp.	*O. nutans*	GZU 19-3019	-	OQ459740	China	Xiao *et al.* (2023)
P. agarica	*O. barnesii*	YHH PA1305^{T}	KP276651	KP276659	China	Wang *et al.* (2015a)
P. agarica	Lepidoptera	YHC PA1307	KP276654	KP276662	China	Wang *et al.* (2015a)
P. agarica	Lepidoptera	YHPA1307	-			
P. agarica	Limacodidae sp.	NTUCC 17-015	-	MK839228	Taiwan	Wang *et al.* (2015a)
P. agarica	Limacodidae sp.	NTUCC 17-018	-	MK839226	Taiwan	Wang *et al.* (2015a)
P. clavisynnema	*Ophiocordyceps* sp.	GZLG 23-102^{T}	OQ968788	OQ982009	China	Xiao *et al.* (2024)
P. clavisynnema	*Ophiocordyceps* sp.	GZCC 22-2042	OQ968789	OQ982008	China	Xiao *et al.* (2024)
P. lanceolata	Lepidoptera	GACP 17-2004^{T}	OQ172076	OQ459726	China	Xiao *et al.* (2023)
P. lianzhouensis	Cossidae	MFLU 17-1582^{T}	MG136920	MG136878	Thailand	Xiao *et al.* (2018)
P. lianzhouensis	*Gryllotalpa* sp.	HIMGD20918	-	OQ459735	China	
P. litangensis	*O. sinensis*	YFCC 06109293^{T}	PP410597	PP550103	China	Liu *et al.* (2024b)
P. litangensis	*O. sinensis*	YFCC 06109296	-	PP550105	China	Liu *et al.* (2024)
P. neoagarica	*Ophiocordyceps* sp.	GZCC 22-2043	OQ968791	OQ982007	China	Xiao *et al.* (2024)
P. nipponica	*Ophiocordyceps* sp.	BCC 18108	KF049657	MF416517	USA	Kepler *et al.* (2013)
Pl. nipponica	***Cicada nymph***	**UTHM-CEF003.1**	**PX530874**	**PX519663**	**Malaysia**	**This study**
Pl. nipponica	***Colobopsis* sp**	**UTHM-EF004**	**PX530881**	**PX519661**	**Malaysia**	**This study**
P. nipponica	Cicada	Cod NN1305	KX8277531	MG196129	Thailand	Liu *et al.* (2024)
P. nipponica	Cicada	Cod NN1306	KX827754	MG196130	Thailand	Liu *et al.* (2024)
P. nipponica	Cicada	Cod SN1401	KX827758	MG196134	Thailand	Liu *et al.* (2024)
P. nipponica	Cicada	AMHNO 10281	PQ357185	-	India	Liu *et al.* (2024)
P. nipponica	Cicada	Cod-NN1307	KX827755	MG196131	Thailand	Jaihan *et al.* (2021)
P. nutansis	*O. nutans*	GZU 191906		OQ459740	China	
P. nutansis	*O. nutans*	MFLU 21-0275^{T}	OQ172073	OQ459739	China	Xiao *et al.* (2023)
P. sinensis	*O. barnesii*	GZU 20-2304	-	OQ459724	China	Xiao *et al.* (2023)
P. sinensis	*O. neoacicularis*	MFLU 21-0268	-	OQ459743	China	Xiao *et al.* (2023)
P. sinensis	Ant	GZU 192301	KF049661	KF049689	China	Kepler *et al.* (2013)
P. sinensis	Ant	CN 80-2^{T}	-	HQ832890	China	
P. sinensis	Lepidoptera	MFLU 21-0269	-	OQ459742	China	Xiao *et al.* (2023)
P. phaothaiensis	Coleoptera	BCC 84553^{T}	MF959733	MF959742	Thailand	Crous *et al.* (2017a)
P. phaothaiensis	Coleoptera	BCC 84557	MF959732	MF959740	Thailand	Crous *et al.* (2017a)
P. longiphialis	Lepidoptera	CXCC0017^{T}	PX517735	PX622199	China	Dong *et al.* (2026)
P. longiphialis	Lepidoptera	CXCC0018	PX517736	PX622200	China	Dong *et al.* (2026)
P. puerensis	Coleoptera	GACPY117	PP627512	PP654214	China	Unpublished
P. ramosopulvinatus	*Ophiocordyceps* sp.	EFCC 5566	KF049658	KF049682	USA	Kepler *et al.* (2013)
P. ramosopulvinatus	*Ophiocordyceps* sp.	DQ118753	–	DQ118753	USA	Unpublished

......*continued on the next page*

TABLE 1. (Continued)

Species	**Host**	**Voucher No**	**GenBank accession number**		**Country**	**References**
			ITS	***Tef-1α***		
P. sanduensis	*Ophiocordyceps* sp.	GZLG 23-104^{T}	OQ968786	OQ982005	China	Xiao *et al.* (2024)
P. sanduensis	*Ophiocordyceps* sp.	GZCC 22-2044	OQ968787	OQ982006	China	Xiao *et al.* (2024)
P. yunnanensis	*O. nutans*	GZCC 24-0148	-	PQ666777	China	Wang *et al.* (2020)
P. yunnanensis	*O. nutans*	YHHPY1006^{T}	KF977849	KF977851	China	Wang *et al.* (2015b)
Pl. cornusynnemata	**Ant**	**BORH (F) 03650**	**PX482945**	**PX233248**	**Malaysia**	**This study**
Pl. cornusynnemata	**Ant**	**BORH (F) 03651**	**PX482947**	**PX233249**	**Malaysia**	**This study**
Pl. aurantiaca	**Ant**	**UTHM-CMF003.1**	**PX482749**	**PX519664**	**Malaysia**	**This study**
Pl. aurantiaca	**Ant**	**UTHM-CMF003.2**	**PX482752**	**PX519665**	**Malaysia**	**This study**
Pl. aurantiaca	**Ant**	**BORH (F) 03652**	**PX482944**	**PX233250**	**Malaysia**	**This study**
Pl. aurantiaca	*O. barnesii*	MFLU 17-1394	OQ172069	MG136876	Thailand	Xiao *et al.* (2023)
Pl. aurantiaca	*O. buquetii*	MFLU 21-0276	OQ17207	OQ459714	Thailand	Xiao *et al.* (2023)
P. ramosus	*Ophiocordyceps* sp.	NBRC 109985	MN58682	MN598053	China	Wang *et al.* (2021)
P. ramosus	*Ophiocordyceps* sp.	NBRC 109984	MN58682	MN598052	China	Wang *et al.* (2021)
P. ramosus	*Ophiocordyceps* sp.	NBRC 101760	MN58682	MN598051	China	Wang *et al.* (2021)
P. vitellina	*O. nigrella*	KUMCC 3006	OQ172089	OQ459730	China	Xiao *et al.* (2023)
P. vitellina	*O. nigrella*	KUMCC 3007	OQ172088	OQ459729	China	Xiao *et al.* (2023)
P. formosus	Coleoptera	CGMCC 5.22048	PQ666784	PQ666776	China	Xiao *et al.* (2023)
P. formosus	Coleoptera	CGMCC 5.2207	MN58683	MN598058	China	Wang *et al.* (2021)
P. formosus	Coleoptera	CGMCC 5.2204	MN586826	PQ446421	China	Wang *et al.* (2021)
P. formosus	Coleoptera	CGMCC 5.2206	MN59804	MN598057	China	Wang *et al.* (2021)
Pl. shibingensis	Coleoptera	HKAS149965	PX692977	PX694731	China	Zhang *et al.* (2026)
Pl. shibingensis	Coleoptera	HKAS149966	PX692978	PX694732	China	Zhang *et al.* (2026)
Pl. tengchongensis	*Lepidoptera*	HKAS149971	PX692975	PX694741	China	Zhang *et al.* (2026)
Pl. tengchongensis	*Lepidoptera*	HKAS149972	PX692976	PX694742	China	Zhang *et al.* (2026)
P. albiramus	*Gryllotalpa* sp.	GZUCC 21XS08	OQ172093	OQ459736	China	Xiao *et al.* (2023)
P. albiramus	*Gryllotalpa* sp.	GZU 21 XS08	OQ172092	OQ459735	China	Xiao *et al.* (2023)
Perennicordyceps cuboidea	beetles	NBRC 106330	JN94333	KF049684	Japan	Ban *et al.* (2015
Perennicordyceps cuboidea	beetles	NBRC 100998	JN943331	AB972951	Japan	Ban *et al.* (2015)

Results

Phylogeny

Maximum likelihood phylogenetic tree based on ITS data set, two isolates BORH/F03650 and BORH/F03651 formed distinct clade with 97% bootstrap support value and a Bayesian posterior probability (PP) of 1.00. The formation of distinct clade revealed as *Pleurocordyceps cornusynnemata* as a new species. Similarly, *P. aurantiaca* (BORH/F03652, UTHM-CMF003.1, and UTHM-CMF003.2) are grouped with already reported *Pl. aurantiaca* (MFLU 171394, MFLU 210276) from Thailand producing 98% ML bootstrap support value and a Bayesian posterior probability (PP) of 1.00. *P. nipponica* (UTHM-CEF003.1, UTHM-EF004) aligned with *P. nipponica* (CodRE1201, CodRE1202) forming 100% bootstrap value and a Bayesian posterior probability (PP) of 1.00 (Supplementary Figure S1). In *TEF-1*α-based phylogenetic tree, two isolates of *Pl. cornusynnemata sp. nov.* BORH/F03650 and BORH/F03651 also form a separate clade with strong ML=100% and (PP=1.00. Similarly, *P. aurantiaca* (BORH/F03652, UTHM-CMF003.1, UTHM-CMF003.2) and *P. nipponica* (UTHM-CEF003.1, UTHM-EF004) are aligned together in their respective clades with supportive bootstrap value and Bayesian posterior probability (Supplementary Figure S2).

The phylogenetic tree of two combine genes data based revealed that isolates BORH/F03650 and BORH/F03651 exhibited distinct clade with ML= 100% and PP = 1), forming a sister clade with *Pl. agaricus* (YHPA1407, YHHPA1307, and YHHPA1305) and *Pl. neoagarica* (GZCC 222043) from China. BORH/F03652, UTHM-CMF003.1, and UTHM-CMF003.2 grouped with *Pl. aurantica* with (ML = 100%, PP = 1) and forming a sister clade to *Pl. phaothaiensis* (BCC 84557, BCC 84553). The isolates (UTHM-CEF003.1) and (UTHM-CEF003.2) formed a clade with *Pl. nipponica* (NN1307) reported from Thailand with (ML = 98%, PP = 1.00) (Fig. 2).

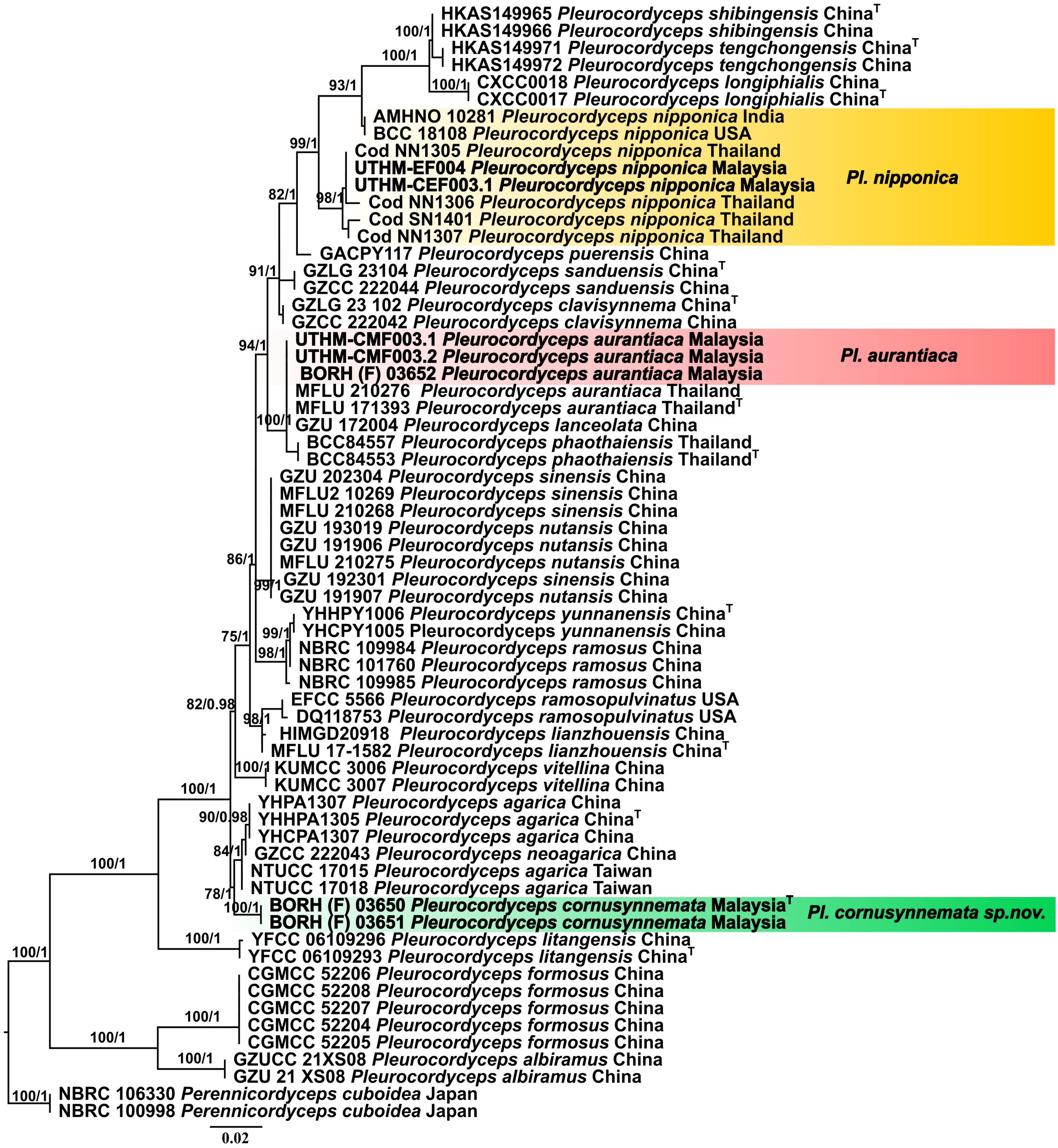

FIGURE 2. Maximum likelihood phylogenetic tree generated with RAxML based on concatenated ITS and *TEF-1α* sequences. Maximum likelihood bootstrap support values ≥ 70% and Bayesian inference posterior probabilities (PP) 0.90 mentioned only. The tree was rooted with two isolates of *Perennicordyceps cuboidea* (NBRC 106330 and NBRC 100998). The line segment with the value '0.02' indicates the length of the branch, which signifies a 0.02 genetic alteration.

Taxonomy

Pleurocordyceps aurantiaca (Y.P. Xiao *et al.*) Y.H. Wang, S. Ban, W.J. Wang, Yi Li, Ke Wang, P.M. Kirk & Y.J. Yao, 2020**.** (Fig. 3)

Basionym*:* *Polycephalomyces aurantiacus* Y.P. Xiao, T.C. Wen & K.D. Hyde, 2018

Holotype: *Pleurocordyceps aurantiaca* MFLU:17-1393

Description: *Pleurocordyceps aurantiaca* (BORH/F03652) parasitizing Diptera host exhibiting a dark brown to black exoskeleton with a rigid exoskeleton. Multiple synnemata measuring 1.5–3.5 mm long ($\bar{x}$ = 2.5 mm, n = 10) emerge from the thorax, abdomen, and legs. These synnemata are slender, cylindrical to slightly tapering, and display a distinct orange to orange-yellow (4C8–6B8) pigmentation, sharply contrasting with the dark host surface (Fig. 3A). The upper regions of synnemata are vividly pigmented and smooth, while the basal regions are slightly darker.

Sexual Morph: Undetermined.

Asexual Morph: A section of conidium dome-shaped to flask-like, measuring 50–90 µm in diameter ($\bar{x}$ = 70 µm, n = 20) (Fig. 3B). The conidiophores, measuring 23–28.4 × 1.1–1.7 µm ($\bar{x}$ = 25.7 × 1.4 µm, n = 15) are slender and slightly branched, and the phialides measuring 3–8.1 × 0.3–1.5 µm ($\bar{x}$ = 5.5 × 0.9 µm, n = 20), are flask-shaped or cylindrical, typically borne at the conidiophore tips (Fig. 3C). Conidia were not observed.

Cultural characteristics: The culture front colony is circular and white (N9) that produced upright synnemata measuring 4–12 mm long, white to creamy (1A1–2A3) at the base, becoming pale orange to brownish (4C8–7F8) towards the tip and distributed evenly at each colony that formed in the margin of the colony after 10 days and observation of long synnemata on 30 days. Fertile head usually arises from the middle of the synnemata. The stipe of synnemata is white to off-white (4C8–7F8) while tip is light orange to ochre (4C8–5D8). The colony attained a diameter of 40–45 mm after 14 days at 25°C. The culture reverse colony is also circular with regular margins forming a distinct orange to dark amber (4C8–7D8) coloration, especially concentrated in the central region of each colony (Fig. 3D-E). Conidial masses covered the surface of the colony as pale-yellow color (3A4–4A4) (Fig. 3F). Under scanning electron microscope, synnemata are cylindrical and elongated, with densely packed phialides forming a compact apex (Fig. 3G and H). α-phialides measuring 7.5–12.2 × 1.4–1.8 µm ($\bar{x}$ = 9.9 × 1.6 µm, n = 20) are slender, cylindrical to slightly tapering at the apex. Similarly, *α*-conidia measuring 3.2–5.8 × 3.1–3.2 µm ($\bar{x}$ = 4.5 × 3.15 µm, n = 30) are ellipsoidal to cylindrical, single celled measuring and attached to the tips of *α*-phialides (Fig. 3I and J). *β*-phialides measuring 10.1–18.3 × 1.1–2.2 µm ($\bar{x}$ = 14.2 × 1.7 µm, n = 20), are shorter, curved or hook-like structures, narrower at the base and slightly widened at the apex approximately7–12 × 1.5–2.5 µm ($\bar{x}$ = 9.5 × 2.0 µm, n = 20). *β*-conidia measuring 4.2–6.8 × 1.3–2.1 µm ($\bar{x}$ = 5.5 × 1.7 µm, n = 30), are small, curved allantoid or sickle-shaped, appear smooth-walled, single-celled, and hyaline. Usually formed singly or in slimy heads approximately 4–6 × 1.5–2 µm ($\bar{x}$ = 5.0 × 1.75 µm, n = 20) (Fig. 3K).

Known distribution: Thailand (Xiao *et al.* 2023), Malaysia (This study).

Species examined: Malaysia, Sabah (Northern Borneo), Danum Valley Conservation Area, Lahad Datu (N° 4.906' E° 117.455'). 12 September, 2024, Jaya Seelan Sathiya Seelan, BORH/F03652, GenBank accession: ITS (PX482944) = *TEF1-α* **= (**PX233250)

Ecology: *Pleurocordyceps* found on *Ophiocordyceps neouguetii* (Ophiocordycipitaceae) as hyperparasites on queen of green tree ant (Formicidae, *Oecophylla smaragdina)*, in the hollow of tree.

Remarks: The position of *Pl. aurantica* (BORH/F03652) in our study is on green leaves while *Pl. aurantica* (MFLU 21-0276) as the type material attached to dry, woody surface. Current study host appears dark brown to nearly black than *Pl. aurantica* (MFLU 21-0276) shows a lighter, reddish-brown. The appearance of synnemata is different in both samples of *Pleurocordyceps aurantica*. In *Pl. aurantica* (BORH/F03652), synnemata are longer (4–12 mm), slender and extrude from head and thorax regions of the host whereases *Pl. aurantica* (MFLU 21-0276) possess relatively shorter, slightly thicker (1.3–2.2 µm), and more densely clustered around the thorax. According to phylogenetic analyses, *P. aurantiaca* (BORH/F03652, UTHM-CMF003.1, and UTHM-CMF003.2) shared same clade with already reported *Pl. aurantiaca* (MFLU 171394, MFLU 210276) from Thailand producing 98% ML bootstrap support value and a Bayesian posterior probability (PP) of 1.00.

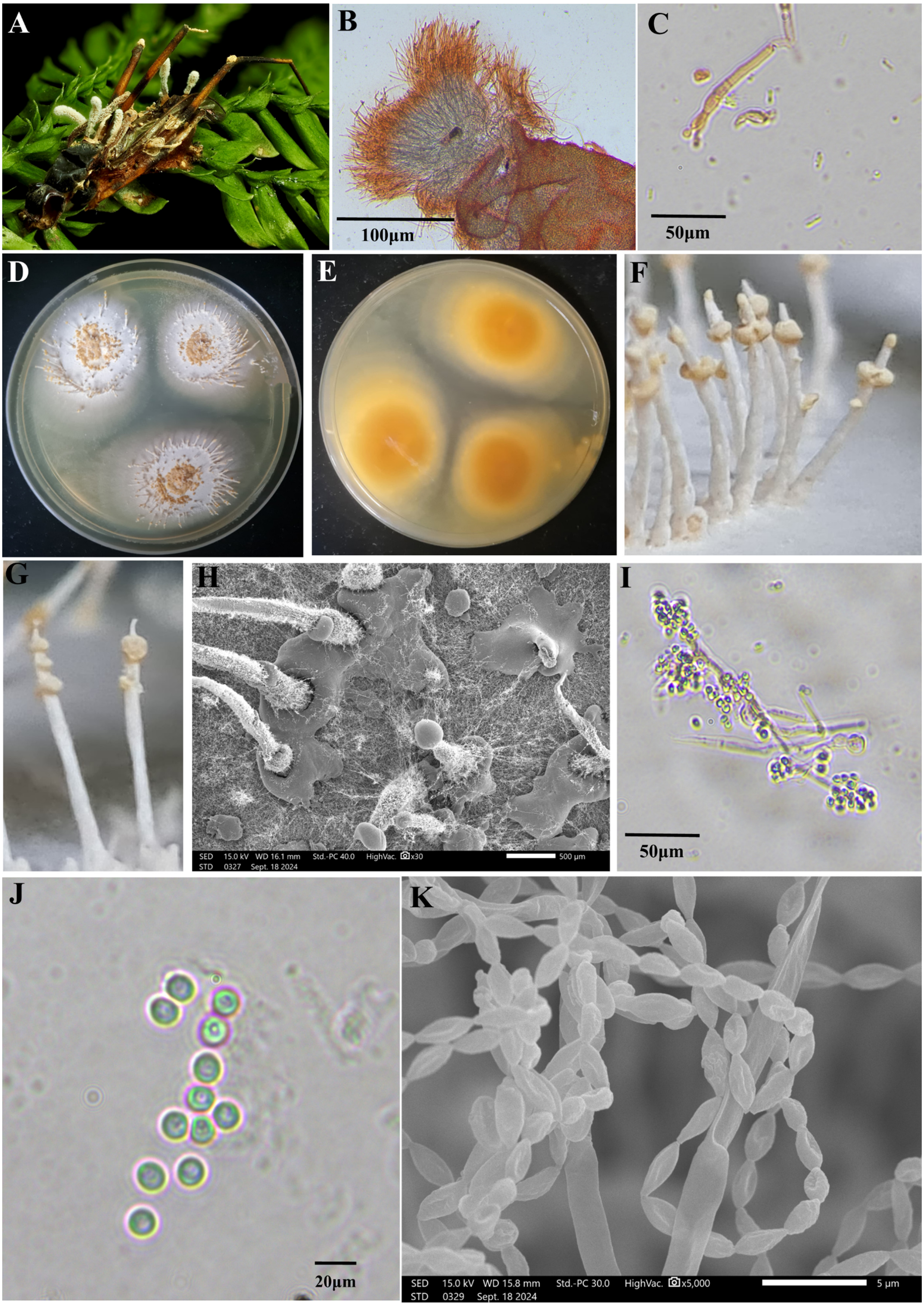

FIGURE 3. *Pleurocordyceps aurantiaca* (BORH/F03652). (A) *Pl. aurantiaca* (BORH/F03652) as a parasitic fungus on Diptera. (B) Section conidioma, (C) Phialides and conidia, (D) Culture on PDA, (E) Culture reverse colony on PDA media (F) Conidial mass on the culture (G) Synnemata emerging from culture, (H) Scanning electron microscopy showing synnemata, (I) α-Phialides bearing conidia (J) α- conidia (K), β-phialides and β-conidia.

Pleurocordyceps cornusynnemata M. Shahbaz, F.Z. Mohadden, and J.S Sathiya Seelan *sp. nov.* (Fig. 4)
MycoBank: 861608

Etymology: *cornu* meaning "horn" synnema "Synnema like structures" from ant "*Dinomyrmex gigas*"

Holotype: BORH/F03650

Paratype: BORH/F03651

Description: *Pleurocordyceps cornusynnemata sp. nov.* growing on an ant approximately 16–2 mm long. The insect host exhibits a metallic dark brown to black (6F8–8F8) coloration with a slight greenish-blue (6B7–7B7) iridescent sheen, especially on the thorax and wings. Its legs and antennae are dark brown (7F8–8F8), providing a strong contrast with the reddish-brown (5F8–6F8) stromata emerging from the body, particularly from the thoracic and abdominal regions, forming an antler-like appearance each measuring about 5–15 mm in length and 1.3–1.5 mm in width. The lower parts of the stromata are distinctly reddish to dark brown, gradually becoming lighter toward the apex, which exhibits a yellow to ochre coloration where the fertile structures are concentrated. The host body appears rigid and completely colonized by the fungus, and the stromata surface is velvety to finely hairy, particularly near the apices forming globose to spiny clusters with a radiating, star-like pattern, representing the fertile apex of the structure. The apices are ochre to light yellow (4D8–5A4), contrasting with the dark brown (7F8–8F8) basal region. The stromata are cylindrical to slightly tapered, occasionally branched, with expanded apices (Fig. 4A–C).

Sexual morph**:** Mature perithecia are globose to subglobose, immersed in the centre showing dark-brown to reddish colour (7F8–5F8/6F8), produced lighter reddish (4F8–5F8) radiating hyphae, measuring 350–500 µm in diameter ($\bar{x}$ = 425 µm, n = 20) (Fig. 4D). The asci are cylindrical to clavate measuring 14.5–95.3 µm × 1.2–2.6 µm ($\bar{x}$ = 54.9 × 1.9 µm, n = 30), and ascospores are hyaline, filiform, measuring 22.5–42.5 µm × 1.2–2.5 µm ($\bar{x}$ = 32.5 × 1.85 µm, n = 30) (Fig. 4E&F).

Asexual morph**:** Undetermined.

Culture characteristics**:** Colonies on PDA forms form circular shapes with a dense central mycelial mat that appears white to cream (1A1–2A2) colour in the central zone while, becoming slightly yellowish to pale brown towards margins. The texture is cottony to floccose, and the margins are regular. The colony size measures approximately 25-35 mm after 14 days at 25°C. The reverse colony appears smooth, light yellow to pale brown that is more concentrated towards centre and lighter colour towards margins (Fig. 4G&H). According to (Fig. 4I), synnemata emerging from culture are erect, cylindrical to clavate, arising upright forming colony surface. These synnemata are appears yellowish to reddish-brown (3A4–6F8), with slightly brighter apices with rough texture. *α*-conidia which are hyaline, smooth-walled, single celled and oval to ellipsoid in shape and measuring 1–1.5 × 1–1.5 µm ($\bar{x}$ = 1.25 × 1.25 µm, n = 20) (Fig. 4J). α-phialides responsible e for producing α-conidia that are hyaline, smooth-walled, and typically cylindrical to flask-shaped (lageniform), tapering towards a narrow apex where conidia are extruded and measuring 1.2–3.3 × 1–1.2 µm ($\bar{x}$ = 2.25 × 1.1 µm, n = 20) (Fig. 4K). β-phialides are associated with their β-conidia measuring 2.2–4.3 × 2.1–3.3 µm (mean 3.2 × 2.7 µm, n = 20) are hyaline, slender, and filiform to needle-like. β-conidia are hyaline, very small, and curved (Fig. 4L).

Material examined: Malaysia, Sabah (Northern Borneo), Danum Valley Conservation Area, Lahad Datu (N°4.81' E° 117.359), 13 October 2024, Jaya Seelan Sathiya Seelan, BORH/F03650 (Holotype), BORH/F03651(Paratype).

Distribution: *Pleurocordyceps cornusynnemata sp. nov.* (BORH/F03650) were collected from Danum Valley Conservation Area, Lahad Datu.

Remarks: On the basis of phylogenetic analysis using concatenated ITS and *TEF-1α* genes, our two strains *Pl. cornusynnemata* (BORH/F03650, and BORH/F03651) form a distinct clade with ML bootstrap values of 100% and Bayesian posterior probabilities of 1.00 and these two samples appeared as a sister clade of *Pl. agarica* (NTUCC 17015) and (NTUCC 17018) from Taiwan region, China as well as *Pl. agaricus* (YHPA 1407, YHHPA 1307, and YHHPA 1305) and *Pl. neoagarica* (GZCC 222043) from Guizhou Province, China. *Pluerocordyceps agaricus* (YHPA1407, YHHPA1307, and YHHPA1305) is featured with agaric-shaped synnemata, pileus-like slimy conidial masses, fairly short stipes, two types of conidia (α- and β-conidia), and phialides (α- and β-phialides) while our study specimen *Pl. cornusynnemata sp. nov.* (BORH/F03650, and BORH/F03651) possesses Antler-shaped synnemata-like structures, with dense emergence of synnemata from the culture whereas in *Po. agaricus* (YHPA 1407, YHHPA 1307, and YHHPA 1305) synnemata are scattered and form a wider colony on PDA as compared to our study specimen. The upgrowth of synnemata either from host in the form antler-like structures or from the culture on PDA as densely clustered are distinct features.

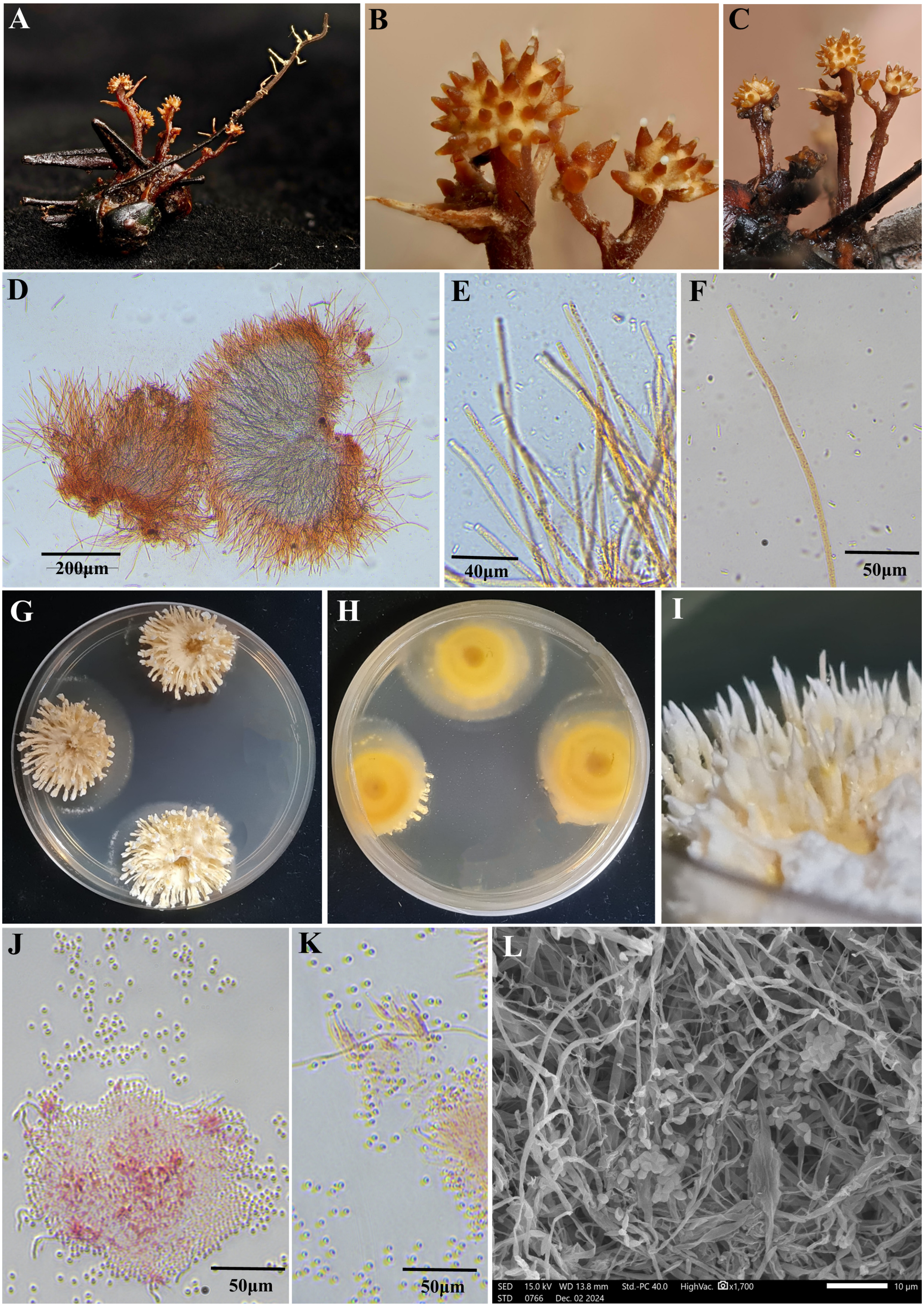

FIGURE 4. Morphology of *Pleurocordyceps cornusynnemata sp. nov.* (Holotype, BORH/F03650) (A) *Pleurocordyceps cornusynnemata sp. nov.* on host ant (B&C) Stromata emerging from host. (D) Perithecia. (E&F) Asci and Part of ascospores. (G&H) Culture front and reverse colony on PDA media. (I) Synnemata emerging from culture. (J&K) *α*-conidia and *α*-phialides. (L) β-Conidia and β-phialides under scanning electron microscope.

Pleurocordyceps nipponica (Kobayasi) Y.H. Wang, S. Ban, W.J. Wang, Yi Li, Ke Wang, P.M. Kirk & Y.J. Yao (Fig. 5)

Synonym: *Cordyceps nipponica* Kobayasi, 1941

Homotypic synonym: *Polycephalomyces nipponica* (Kobayasi) Kepler & J. W. Spatafora, 2013

Basionym: *Cordyceps nipponica* Kobayasi, 1941

Holotype: Japan, Prov. Owari, Nisi-kasngaigun, Sinkawa-mati, on *Graptopsaltria nigrofuscata* (Hemiptera), July 1936, A. Hayakawa, Type in Kobayasi Herbarium (Fungal Names FN570680).

Description

Pleurocordyceps nipponica specimens were found infected two host species of different orders; one infected a cicada nymph and the other an ant host (Fig. 5A and 5K).

The cicada nymph was buried 20–30 mm underground, with stromata emerging from the head.

Sexual Morph: Stromata erect, dark brown, 5.8–5.9 cm long ($\overline{x}$ = 5.85 cm, n = 5) and 75–171 µm wide ($\overline{x}$ = 123 µm, n = 5), cylindrical to slightly tapering, infrequently branched, more regularly branched towards the apex, terminating in rust-brown ascomata. Perithecia solitary along the stromata near the apex, asymmetrically arranged, mostly immersed, thick-walled, elongated pyriform, 560–830 µm long ($\overline{x}$= 714 µm, n=30) and 172-300 µm wide ($\overline{x}$= 235 µm, n = 30) (Fig. 5B–C). Asci 100–339 µm long ($\overline{x}$ = 219.5 µm, n = 30) × 2.7–6.2 µm wide ($\overline{x}$ = 4.45 µm, n = 30) (Fig. 5D–E), not forcibly discharged in water. Ascospores fragment into hyaline, smooth-walled, cylindrical part spores, 2.6–5.3 µm long ($\overline{x}$ = 4.0 µm, n = 30) × 0.9–1.3 µm wide ($\overline{x}$ = 1.1 µm, n = 30) (Fig. 5F).

Asexual Morph: Not observed

Fungal structures emerged from joints, abdominal fissures, and dorsal neck, forming concave, cup-like structures lacking a distinguishable stipe (Fig. 5L). Fungus light brown with α-phialides crowded along the edge forming a white ring that turns brown with age (Fig. 5M).

Sexual Morph: Not observed.

Asexual Morph: α-Phialides hyaline, smooth-walled, lageniform, 5–12 × 1–2 µm ($\overline{x}$ = 8.5 × 1.5 µm, n = 20); α-conidia hyaline, smooth-walled, globose to subglobose, 2–3.5 µm diameter ($\overline{x}$ = 2.7 µm, n = 30).

Culture Characteristics: Colonies on agar appear light orange, with the intensity of the orange fading towards the colony margins (Fig. 5O). No synnemata were formed during 21 days of incubation. Colony diameter 28–30 mm ($\overline{x}$ = 29 mm, n = 5), circular on both front and reverse, raised, floccose, clumpy in texture, with entire margins (Fig. 5P). The primary asexual morph predominantly formed at the colony centre, where the orange coloration is most intense, occasionally developing among the proliferating mycelia. α-Phialides hyaline, smooth-walled, lageniform, 4.1–15 µm long ($\overline{x}$ = 9.55 µm, n = 20) × 2.1–2.3 µm wide ($\overline{x}$ = 2.2 µm, n = 20) (Fig. 5H, 5R). α-Conidia hyaline, smooth-walled, globose to subglobose, produced singly at the apex of phialides, 2.3–3.23 µm long ($\overline{x}$ = 2.76 µm, n = 30) × 2–2.5 µm wide ($\overline{x}$ = 2.25 µm, n = 30) (Fig. 5G, 5Q). α-type is the dominant anamorph, forming in larger quantities than β-type.

The secondary asexual morph formed only among proliferating mycelia. β-Phialides acicular, slightly tapering at the apex, occasionally almost cylindrical, 8–33 µm long ($\overline{x}$ = 20.5 µm, n = 20) × 1.1–2.5 µm wide ($\overline{x}$ = 1.8 µm, n = 20) (Fig. 5J, 5T). β-Conidia hyaline, smooth-walled, catenulate at phialide apex, with diverse shapes: fusiform (dominant), narrow fusiform, elongated elliptical, sub-elliptical, long-oval, and long pyriform; 3.4–5.9 µm long ($\overline{x}$ = 4.65 µm, n = 30) × 1.5–2.4 µm wide ($\overline{x}$ = 1.95 µm, n = 30) (Fig. 5I, 5S).

Known distribution: Japan (Jaihan *et al.* 2021), Thailand (Xiao *et al*. 2018), Malaysia (This study).

Material examined: Malaysia, Johor (southern Peninsular Malaysia), Bukit Chabang Tiga (N 2.2164°, E 103.6514°), 6 Aug 2025, Yap Jing Wei, UTHM-CEF003.1, Malaysia, Kedah, Langkawi (northern Peninsular Malaysia), Air Terjun Termurun Forest Eco Park (N 6°26′7″, E 99°43′15″), 9 Sep 2025, Yap Jing Wei, UTHM-EF004.

Ecology: *Pleurocordyceps nipponica* were found on two different host species from different orders. One on an ant*, Colobopsis* sp. (Hymenoptera, Formicinae), found bitten unto the underside of a leaf and the other on a cicada nymph (Hemiptera, Cicadidae) buried underground with the fungal fertile part exposed.

Remarks: The specimen found in Langkawi is a typical representation *Pl. nipponica*, with the stromata emerging from underground and the host being a cicada. However, the *Pl. nipponica* from Johor has a completely different morphology. It is the first record of anamorphic exhibition in nature for *Pl.* nipponica. It is also the first report of an ant susceptible to infection of *Pl. nipponica*, further widening its host range. Interestingly, the infected ant host seemingly has also gone through the behavioural manipulation that has been commonly observed from ant host.

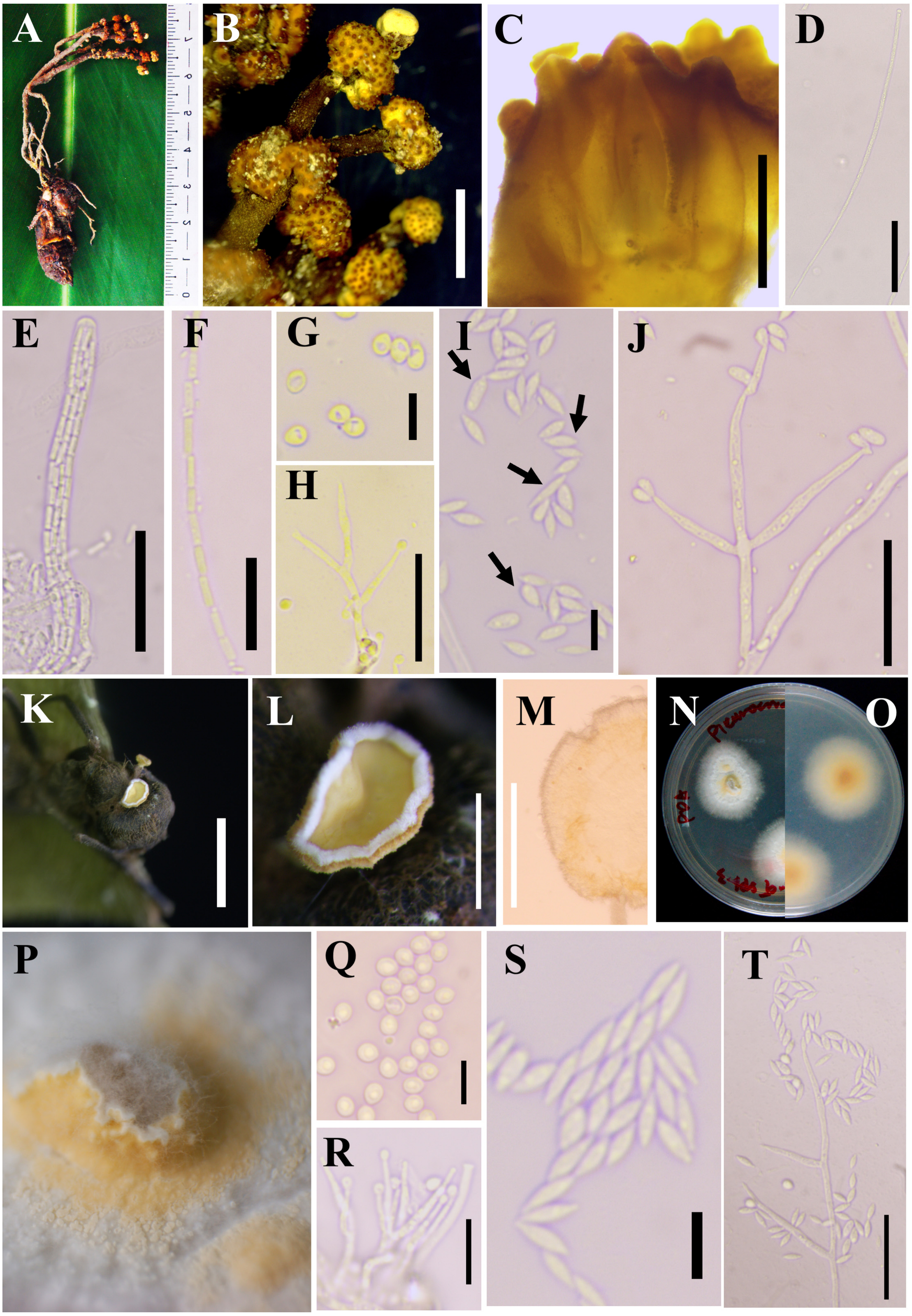

FIGURE 5. Morphology of *Pleurocordyceps nipponica*. Sexual morph observed on the cicada host: (A) *P. nipponica* UTHM-EF004 infecting a cicada nymph; (B) terminal ascomata; (C) arrangement of perithecia; (D) entire ascus; (E) ascus cap; (F) part spores. Asexual morph observed on the ant host and in culture: (G) α-conidia; (H) α-phialides; (I) diverse shapes of β-conidia (arrows indicate types); (J) β-phialides; (K) fertile structures emerging from the dorsal neck of an ant biting a leaf; (L) microscopic view of the fertile part (α-type); (M) fertile part under microscope; (N) obverse of culture on PDA; (O) reverse of culture on PDA; (P) orange coloration at the center of colonies; (Q) α-conidia from culture; (R) α-phialides from culture; (S) β-conidia from culture; (T) β-phialides from culture. **Scale bars:** B 1 mm; C 0.5 mm; D 50 µm; E 20 µm; F 5 µm; G 5 µm; H 20 µm; I 5 µm; J 20 µm; K 2 mm; L 0.5 mm; M 200 µm; Q 5 µm; R 10 µm; S 5 µm; T 20 µm.

TABLE 2. Morphological comparison sexual morphs of different species within genus *Pleurocordyceps*.

Species	**Host**	**Habitat**	**Stromata (mm)**	**Asci (µm)**	**Perithecia (µm)**	**Ascospores (µm)**	**References**
Pl. clavisynnema	*O. neogryllotalpae*	Soil	2–5	NA	NA	NA	Xiao *et al.* (2024)
Pl. multisynnema	*Paraisaria* sp.	Leaf litter	2–6	NA	NA	NA	Xiao *et al.* (2023)
Pl. neoagarica	*O. neogryllotalpae*	Soil	0.5–3	NA	NA	NA	Xiao *et al.* (2023)
Pl. sanduensis	*O. neogryllotalpae*	Soil	0.1–0.5	NA	NA	NA	Xiao *et al.* (2023)
Pl. elaphomyceticola	*Elaphomyces* sp.	Soil	51–62 × 5–7	164–173 × 3.1–5.5	259–519 × 152–291	55.1–105 × 0.8–1.2	Xiao *et al.* (2023)
Pl. parvicapitata	*Elaphomyces* sp.	Soil	50–150 × 1–5	322–399 × 4.8–6.8	577–751 × 207–281	318–396 × 1.2–1.9	Xiao *et al.* (2023)
Pl. heilongtanensis	*Ophiocordyceps* sp.	Leaf litter	3–10 × 0.5–2	NA	NA	NA	Xiao *et al.* (2023)
Pl. aurantiaca	**Ant**	**Leaf**	**Undetermined**	**Undetermined**	**Undetermined**	**Undetermined**	**This study**
***Pl. cornusynnemata* sp. nov.**	**Ant**	**On ant**	**5–15 × 1.3–1.5**	**14.5–95.3 × 1.2–2.6**	**350–500**	**22.5–42.5 × 1.2–2.5**	**This study**
Pl. nipponica	Cicadidae nymph	Soil	20–70	NA	800–950 × 300–370	NA	Kobayasi (1939)
Pl. nipponica	Homoptera/Neuroptera	Soil	40	NA	580–720 × 200–250	3–3.5	Luangsa-ard *et al.* (2008)
Pl. nipponica	Cicadidae nymph	Soil	15–80 × 1–3	66–866	100–284	150–331 × 0.5–6	Jaihan *et al.* (2021)
Pl. nipponica	**Cicada nymph**	**Soil**	**58–59 × 7.5–17.1**	**100–339 × 2.7–6.2**	**560–830 × 110–280**	**2.6–5.3 × 0.9–1.3**	**This study**

TABLE 3. Morphological comparison asexual morphs of different species within genus *Pleurocordyceps*.

Species	Host	Habitat	Synnemata (mm)	Conidiophore (µm)	Phialides (µm)	α-conidia (µm)	β-conidia (µm)	References
Pl. clavisynnema	*O. neogryllotalpae*	Soil	2–5	21–39	α: 8.3–14.5 × 0.9–1.7; β: 12.3–21.6 × 0.8–1.8	1.7–2.6	3.1–4.1 × 1.6–2.2	Xiao *et al.* (2023)
Pl. multisynnema	*Paraisaria* sp.	Leaf litter	2–6	NA	α: 9–15 × 1.1–2.2; β: 19.8–25.9 × 1.7–2.6	2.1–2.5	2.9–3.8 × 1.3–2.2	Xiao *et al.* (2023)
Pl. neoagarica	*O. neogryllotalpae*	Soil	0.5–3	42–63	α: 11.6–17.4 × 1.1–1.9	2.1–2.9	NA	Xiao *et al.* (2023)
Pl. sanduensis	*O. neogryllotalpae*	Soil	0.1–0.5	12–23	α: 9.5–18.7 × 0.8–2.1; β: 19–33.4 × 0.9–1.8	2.1–3.1	3.3–5.5 × 1.5–2.1	Xiao *et al.* (2023)
Pl. sinensis	Lepidoptera larvae	Leaf litter	2–8 × 0.2–0.5	NA	α: 10–25 × 1–1.2; β: 10–18 × 1.1–1.8	1.6–2.2	3–4.5 × 1.3–2	Xiao *et al.* (2024)
Pl. fusiformispora	*Ophiocordyceps* sp.	Soil	12–15 × 0.1–0.5	NA	α: 8.4–20.9 × 1.3–2.9; β: 8.7–14.8 × 2.5–3.1	2.9–4.8 × 1.3–3.1	2.8–4.4 × 1.7–2.8	Xiao *et al.* (2024)
Pl. heilongtanensis	*Ophiocordyceps* sp.	Leaf litter	2–10 × 0.1–1	12.1–21.4	α: 10.5–24.8 × 1.3–2.6; β: 12.3–28.5 × 1.6–3.2	2.4–3.3 × 1.4–2.1	2.7–4.6 × 1.5–2.5	Xiao *et al.* (2024)
Pl. parvicapitata	*Elaphomyces* sp.	Soil	10–12 × 1–2	21–30	α: 11.7–16.3 × 1.1–1.6	2.8–3.6 × 2.4–3	NA	Xiao *et al.* (2023)
Pl. aurantiaca	**Ophiocordyceps sp.**	**On ant**	**1.5–3.5**	**23–28.4 × 1.1–1.7**	**α: 7.5–12.2 × 1.4–1.8;** **β: 10.1–18.3 × 1.1–2.2**	**3.2–5.8 × 3.1–3.2**	**4.2–6.8 × 1.3–2.1**	**This study**
***Pl. cornusynnemata* sp. nov.**	***Dinomyrmex gigas***	**On ant**	**5–15 × 0.2–1.5**	NA	**α: 1.2–3.3 × 1–1.2;** **β: 2.2–4.3 × 2.1–3.3**	**1–1.5 × 1–1.5**	**NA**	**This study**
Pl. nipponica	Cicada nymph	Soil	N/A	N/A	α: N/A; β: N/A	1.8-2.6× 1.1-2.2	2.0-3.5× 0.9-1.8	Ban (2016)
Pl. nipponica	***Cicada***	**Soil**	NA	NA	**α: 4.1–15 × 2.1–2.3;** **β: 8–33 × 1.1–2.5**	**2.3–3.23 × 2–2.5**	**3.4–5.9 × 1.5–2.4**	**This study**

Discussion

To date, genus *Pleurocordyceps* comprises 26 species reported from China, Thailand, Japan, Ecuador. In this study, we describe one new species and two new records of extant species of *Pleurocordyceps* from Malaysia, providing new insights into the species diversity of the family *Polycephalomycetaceae* in this region. None of these species have previously been reported from Malaysia. The newly described species is *Pleurocordyceps cornusynnemata sp. nov.* BORH/F03650, while *Pl. aurantiaca* BORH/F03652 and *Pl. nipponica* UTHM-CEF003.1 represent new records previously known from Japan, China, and Thailand. The discovery of these species in Malaysia is significant, as it expands the global distribution of *Pleurocordyceps* and provides important baseline data for future mycological studies in the region. Malaysia likely remains less explored for *Pleurocordyceps* due to a combination of ecological and logistical factors. Its tropical rainforest ecosystems, high rainfall, and diverse insect fauna provide suitable habitats and host availability for *Pleurocordyceps* species, yet limited targeted surveys have been conducted compared to regions such as Thailand, China, and Japan. The high diversity of potential insect hosts (ants, cicadas, and other arthropods) suggests that Malaysia may harbor additional undescribed species which shows the importance of systematic sampling in these habitats.

Phylogenetic approaches based on ITS and *TEF-1α* (Supplementary Figures S1–S2), as well as combined sequence data set of ITS and *TEF-1α* (Fig. 2) not only validated the distinct clade of *Pl. cornusynnemata* with strong ML bootstrap support (100%) and Bayesian posterior probability (1.00), but also indicate that these loci are sufficient for initial species delimitation in *Pleurocordyceps*. However, we acknowledge that ITS may have limited resolution in closely related species within the genus, and additional loci or genomic data may further refine phylogenetic placement and species boundaries. Morphological characters of *Pl. cornusynnemata* sp. nov (BORH/F03650) differs from closely associated species i.e. *Pl. agarica* (NTUCC 17015) and (NTUCC 17018) from China (Taiwan region) as well as *Pl. agaricus* (YHPA1407, YHHPA1307, and YHHPA1305) and *Pl. neoagarica* (GZCC 222043) from Guizhou Province, China. Formation of antler-like stroma measuring 5–15 × 1.3–1.5 mm, with robust perithecia (350–500 µm) and long, filiform ascospores (22.5–42.5 × 1.2–2.5 µm) distinguished it with already reported species such as *Pl. clavisynnema*, and *Pl. sanduensis* which lack sexual morph structures (Wang *et al.* 2015a; Wang *et al.* 2021). Additionally, *Pl. cornusynnemata* produces two types of phialides (α and β) and conidia, comparable to *Pl. fusiformispora* and *Pl. multisynnema* (Xiao *et al.* 2024; Liu *et al.* 2024), but differs in host specificity and the arrangement of synnemata. Together, morphological and molecular evidence support the recognition of this novel species.

Phylogenetic analysis based on ITS, *TEF-1α* (Supplementary Figures S1–S2) and concatenated phylogenetic tree (ITS- *TEF-1α*) (Fig. 2) placed the two isolates i.e. BORH/F03652 and UTHM-CEF003.1 within well supportive clades to their reference species i.e. *Pl. aurantica* (MFLU 21-0276) and *Pl. nipponica* (NN1307) respectively. Therefore, *Pl. aurantiaca* BORH/F03652 and *Pl. nipponica* UTHM-CEF003.1 confirming their conspecific identity as two new records from Malaysia. The morphological characters of *Pl. aurantiaca* BORH/F03652 and *Pl. nipponica* UTHM-CEF003.1 corresponds with the features of *Pl. nipponica* (NN1307) and *Pl. aurantica* (MFLU 21-0276) from Thailand (Xiao *et al*. 2023; Wang *et al.* 2014). *Pleurocordyceps aurantiaca* has elongated α-phialides (up to 18.3 µm) and β-conidia ranging from 4.2 to 6.8 µm. Similarly, *Pl. nipponica* shared morphological attributes corresponds to formerly reported from Japan and Thailand. It includes asexual morphs, smooth hyaline phialides, and globose to fusiform conidia. Colonies were white to light orange and floccose, dominated by primary asexual morph. For *Pl. nipponica*, observed differences such as darker stromata, cup-shaped structures without a stipe, and hosts (cicada nymph and ant *Colobopsis* sp.) likely reflect phenotypic plasticity influenced by substrate, host, or culture conditions, rather than cryptic species. Nonetheless, since only ITS and *TEF*-1α loci were analyzed, we interpret these morphological deviations cautiously, and further multi-locus or genomic analyses could clarify potential intraspecific variation or hidden diversity. All isolates were obtained from host tissue and from cultures derived from the same host specimen. Only single isolates per species were examined; therefore, some observed morphological variability may reflect sampling limitations, which shows the need for further collections and repeated surveys

Future research should focus on expanding the survey of *Pleurocordyceps* diversity in Malaysia, particularly targeting varied insect hosts in tropical forests. Comparing Malaysian *Pleurocordyceps* communities with neighboring regions (Thailand, China, and Japan) may reveal biogeographic patterns and host-specificity trends, contributing to a broader understanding of fungal diversity and evolution in Southeast Asia.

Conclusion

The current study described one novel species, *Pleurocordyceps cornusynnemata sp. nov.*, and two new records, *Pl. aurantiaca* and *Pl. nipponica*, from Malaysia for the first time based on detailed morphological characterization and phylogenetic analyses. These findings expand the global understanding of *Pleurocordyceps* diversity and identify Malaysia as a previously unexplored region for these fungi.

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Author contribution: All authors contributed to the conceptualization and design of the study. **Muhammad Shahbaz, Jeremiah Sia Yiao Rong and Jaya Seelan Sathiya Seelan**: contributed to material preparation or data collection. Morphological characteristics were examined by **Muhammad Shahbaz, Firdza Zulkarnain Mohadden**. Molecular lab work and phylogenetic analyses were conducted by **Muhammad Shahbaz** and **Jaya Seelan Sathiya Seelan**. The first draft of the manuscript was written by **Muhammad Shahbaz** and **Jeremiah Sia Yiao Rong** which was then improved by changes, edits, suggestions, and comments from **Yap Jing Wei**, **Jaya Seelan Sathiya Seelan, Jeremiah Sia Yiao Rong, and Yap Jing Wei**. All authors read and approved the final version of the manuscript

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Declarations

Competing interests the authors state no competing interests.

Conflict of interest the authors do not have any conflict of interest.

Ethical approval current research work does not require any ethical approval.

Data availability The nucleotide sequences have been deposited in NCBI GenBank database (https://submit.ncbi.nlm.nih.gov/subs) Under the following accession numbers: UTHM-CEF003.1 (ITS: PX530874; *TEF-1α*: PX519663), UTHM-EF004 (ITS: PX530881; *TEF-1α*: PX519661), UTHM-CMF003.1 (ITS: PX482749; *TEF-1α*: PX519664), UTHM-CMF003.2 (ITS: PX482752; *TEF-1α*: PX519665), BORH (F) 03650 (ITS: PX482945; *TEF-1α*: PX233248), BORH (F) 03651 (ITS: PX482947; *TEF-1α*: PX233249), and BORH (F) 03652 (ITS: PX482944; *TEF-1α*: PX233250).

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Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper:

Supplementary Figures S1–S2 provide additional ITS and *TEF-1α* phylogenetic tree.