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RESEARCH ARTICLE

A New Species of *Leptobacillium* and Additional Records of *Simplicillium* and *Leptobacillium* from Sabah, Malaysia

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ABSTRACT

Simplicillium and *Leptobacillium* are entomopathogenic fungal genera frequently associated with arthropods in tropical ecosystems and considered promising candidates for biological pest control. However, the diversity of these genera in tropical regions, particularly in Malaysia, has not been systematically investigated in the context of their taxonomy and phylogenetic relationships. This study aimed to investigate the taxonomic diversity and phylogenetic relationship of *Simplicillium* and *Leptobacillium* using integrated morphological and molecular analyses. During a survey of entomopathogenic fungi conducted in Malaysia from 2022–2023, parasitized insects were collected, including spiders and wasps infected by entomopathogenic fungi. Phylogenetic analyses were performed using concatenated datasets of the internal transcribed spacer (ITS) and translation elongation factor 1-alpha (*TEF1-α*) gene to construct phylogenetic trees. The study identified one novel species, *Leptobacillium geminatum* sp. nov., isolated from a spider. In addition, five species are reported here as new records for Malaysia: *S. formicae*, *L. leptobactrum. symbioticum,* and *L. coffeanum* isolated from spiders and *S. puwenense*, isolated from a wasp. Morphological characteristics together with molecular phylogenetic evidence supported the taxonomic placement of these taxa. These findings represent the first comprehensive documentation of *Simplicillium* and *Leptobacillium* species from Malaysia, significantly expanding the known diversity and geographical distribution of these entomopathogenic fungi in Southeast Asia.

1 | Introduction

The order *Hypocreales* (*Ascomycota*) includes a wide diversity of animal parasitic fungi; most of these lineages exploit insects, but others are known to parasitize spiders, nematodes, rotifers, and even immunocompromised humans (Northrup et al. 2024; Kepler et al. 2012; Luangsa-Ard, et al. 2018; Araújo and Hughes 2016). The arthropod parasitic lineages are mostly present in the families *Clavicipitaceae*, *Cordycipitaceae,* and *Ophiocordycipitaceae* (Kepler et al. 2017; Luangsa-Ard et al. 2018; Wei et al. 2021; Xiao et al. 2023; Mongkolsamrit et al. 2018), which infect various developmental stages of insects and arachnids (Peng et al. 2024). Among arachnids, spiders are by far the most diverse group (Agnarsson 2023). These cosmopolitan carnivorous arthropods play a pivotal role in maintaining the balance of ecological systems by killing 400–800 million tons of insects every year (Nyffeler et al. 2023; Veeramani et al. 2023). Nevertheless, they are predated by other animals, including spiders themselves (Pekár et al. 2023). Fungi are important but neglected natural enemies of spiders (Nyffeler and Hywel-Jones 2023). These fungi establish dense hyphal networks that destroy many taxonomically informative features of the host, making identification challenging. Thus, more than 80 hypocrealean fungal species from 13 genera were reported on infected spiders (Shrestha et al. 2019).

Most parasitic fungi associated with spiders belong to the family *Cordycipitaceae*, though some belong to *Ophiocordycipitaceae* (Shrestha et al. 2019; Kuephadungphan et al. 2022). *Cordycipitaceae* includes several spider-parasitic genera such as *Cordyceps* Fr., *Engyodontium* G.S. de Hoog., *Hevansia*, *Jenniferia* Luangsa-ard, Hywel-Jones & Spatafora., *Lecanicillium* Zare & W. Gams. and *Gibellula* Cavara (Mongkolsamrit et al. 2022). These lineages infect diverse spider families and occupy different ecological niches within the forest ecosystem (Mendes-Pereira et al. 2023).

The cordycipitaceous fungus genus *Simplicillium* Zare & W. Gams includes species that are primarily mycoparasitic but can also be entomopathogenic, symbiotic, or endophytic. Additional reports indicate that *S. sinense* X.Y. Yuan, Jing Z. Sun & H.W. Liu. has been isolated from human skin infection (James et al. 2016; Yan et al. 2023). They are commonly isolated from soil, decayed wood, air, and rocks (Nonaka et al. 2013; Wang et al. 2020; Okane et al. 2020; Custódio and Pereira 2025). Initially, the genus included a total of four species, i.e. *S. lanosoniveum* (Van Beyma), Zare & W. Gams, *S. obclavatum* (W. Gams, Zare & W. Gams., *S. lamellicola* Zare & Gams, *and S. wallacei* H.C. Evans (Khonsanit et al. 2024). The genus is characterized by solitary phialides, distinguishing it from *Lecanicillium* W. Gams and Zare. Over time, several species were added to the genus (Nonaka et al. 2013; Zhang et al. 2017; Gomes et al. 2018; Wei et al. 2019; Kondo et al. 2020; Wang et al. 2020). Though some (*S. coffeanum* A.A.M. Gomes & O.L. Pereira, *S. chinensis* F. Liu & L. Cai, and *S. filiforme* R.M.F. Silva, R.J.V. Oliveira, Souza-Motta, J.L. Bezerra & G.A. Silva) were reclassified into *Leptobacillium* Zare & Gams, while *S. wallacei* was transferred to *Gamszarea*, based on rDNA analyses (Zare and Gams 2008; Okane et al. 2020). According to Index Fungorum (https://www.indexfungorum.org/Names/Names.asp (accessed 20 April 2026)), the genus *Simplicillium* consists of 43 legitimate species.

Several species produce bioactive metabolites and show potential for their biological control (Li et al. 2022; Marin et al. 2025). For example, *S. lanosoniveum* (J.F.H. Beyma) Zare & W. Gams functions as a phytopathogen and mycoparasite and exhibits activity (Mukherjee et al. 2022), *L. chinense* has been reported to control plant-parasitic nematodes (Hussain et al. 2017; Luyen 2017), and *S. lamellicola* suppresses bacterial and gray mold diseases (Shin et al. 2017).

The genus *Leptobacillium* is phylogenetically distinct from *Simplicillium* within the family *Cordycipitaceae* and can be differentiated by both morphological and molecular characters (Lu et al. 2025). Species of *Leptobacillium* produce slimy, imbricate chains of short-ellipsoidal to subglobose or obclavate conidia (Zare and Gams 2001). Members of this genus are consistently associated with arthropods and are considered important entomopathogenic fungi within *Cordycipitaceae* (Bamisile et al. 2021). According to Index Fungorum (accessed 20 April 2026), the genus *Leptobacillium* currently consists of 13 legitimate species**.** Despite the increasing number of studies on cordycipitaceous fungi worldwide, the diversity and distribution of *Simplicillium* and *Leptobacillium* in tropical regions such as Malaysia remain limited due to lack of systematic surveys.

The objectives of this study were to: (1) collect parasitized spiders and wasps from different forests in Malaysia; (2) describe new taxa to accommodate species diversity within *Leptobacillium*; and (3) document new records of *Leptobacillium* and *Simplicillium* species in Malaysia.

2 | Materials and Methods

2.1 | Sample Collection

Surveys were conducted from February 2022 until October 2023 at different locations in Malaysia, that is, Danum Valley Conservation Area, INFAPRO, Sayap Substation, Kinabalu Park, Serinsim Substation, Kinabalu Park, and Kenaboi State Park, Negeri Sembilan. During the survey, six fungal-infected samples were collected, primarily from spiders and wasps (BORH/F03638, BORH/F03645, BORH/F03648, BORH/F03644, BORH/F03643, and UTHM-CMF001). A distribution map was generated using QGIS version 3.44.3 (https://qgis.org/download/) (Figure 1). The coordinates and information about sampling sites were noted. The survey locations included tropical lowland rainforests, secondary forests, and mixed forest–plantation areas. Environmental conditions at the time of collection, including temperature (25°C–32°C), and humidity (70%–90%), were recorded. Surveys covered in both dry and wet seasons. The samples were transported to the laboratory using freezing tubes in an icebox. Upon arrival, the samples underwent initial observations and isolation.

2.2 | Fungi Isolation

Fresh fungal-infected spider specimens were collected during field surveys. When the specimens were in a fresh state, spores present on the surface of the fungal structures were gently shaken onto Potato Dextrose Agar (PDA; Merck, Germany) plates supplemented with antibiotics to reduce bacterial contamination. The Petri dishes were incubated at room temperature under dark conditions for 2 weeks until fungal growth appeared (Barra-Bucarei et al. 2019). All specimens were deposited at BORNEENSIS, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Kota Kinabalu.

2.3 | Morphological Identification of Isolated Fungi

For morphological identification, initially isolated cultures were re-cultured on PDA at room temperature under dark conditions. After 14 days, cultures were inspected visually, and the colony aspects, e.g., color, diameter, growth rate, and margins, were recorded. The compound microscope (Leica DM2500 LED,

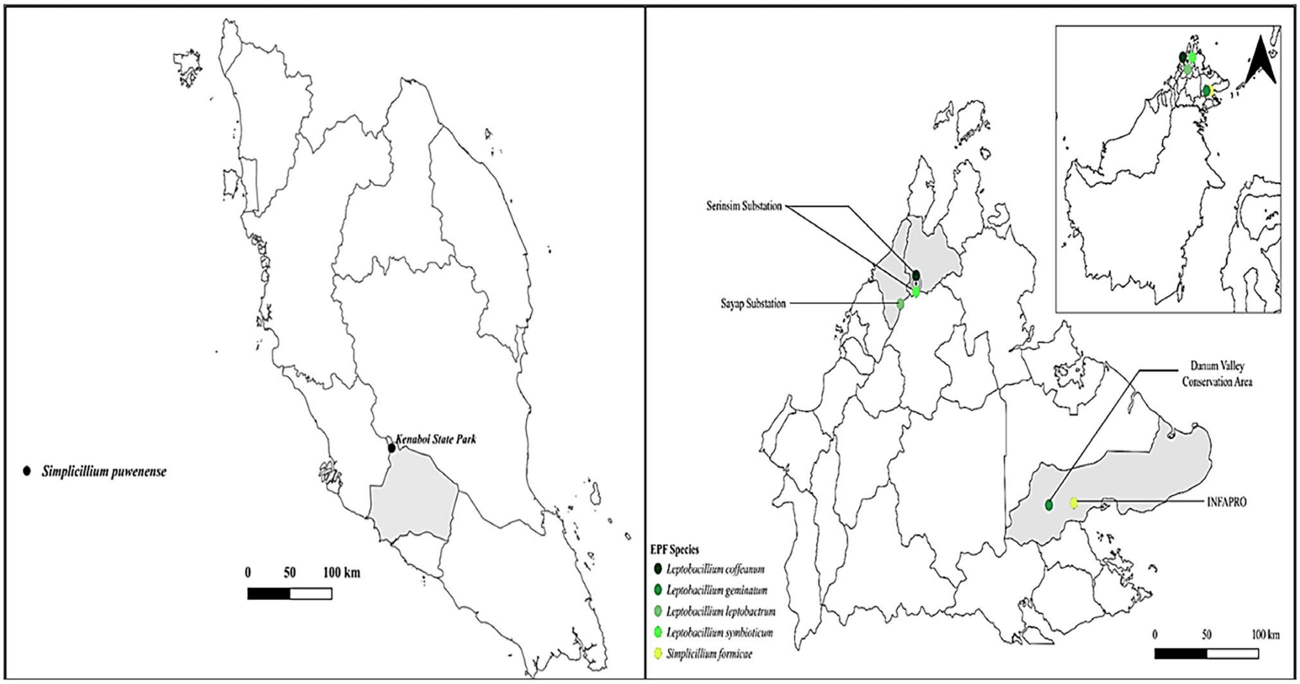

FIGURE 1 | Sampling sites and collection of samples. A distribution map was generated using QGIS version 3.44.3 (https://qgis.org/download/).

Germany) was used at magnifications up to ×100 for the investigation of their microscopic features. A small piece of fungal tissue was mounted on a glass slide with 5% KOH and 1% Congo Red dye to observe and measure taxonomically informative structures (Chang et al. 2026).

2.4 | Fungal DNA Extraction, Amplification and Sequencing

DNA extraction was carried out with 7-day-old cultures using the E.Z.N.A. Plant & Fungal DNA Kit (Omega Bio-tek, USA) in accordance with Liang et al. (2022). The extracted DNA was stored at −20°C. The internal transcribed spacer (ITS) region was amplified using primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as described by White et al. (1990). The translation elongation factor 1-alpha (*TEF1-α*) region was amplified using primers EF1-983F (5′-GGTGACGGTGACCTTGATGGCACG-3′) and EF1-2218R (5′-AAACAGGGTGGTGACCGTGATGGGTTG-3′) (Rehner and Buckley 2005). The PCR amplification conditions for the ITS and *TEF1* regions were as follows: an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for ITS and 58°C for *TEF1* for 30 s, and extension at 72°C for 45 s. A final extension step was carried out at 72°C for 2 min (Chang et al. 2026). PCR products were purified and sequenced at Apical Scientific Sdn. Bhd., Selangor, Malaysia.

2.5 | Sequence Alignment and Phylogenetic Tree Construction

The newly generated ITS and *TEF1-α* sequences were manually inspected and were subjected to BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis for quality control. Consensus sequences were obtained by sequencing each locus in both directions and de novo assembled using BioEdit (https://bioedit.software.informer.com/7.1). A total of 18 sequences (9 ITS and 9 *TEF-1α*) were generated in this study, including six (3 ITS and 3 *TEF-1α*) from a newly described species and 12 (6 ITS and 6 *TEF-1α*) from the newly recorded species (Table 1), and two strains of the genus *Beauveria*, *B. bassiana* (ARSEF 1564) and *B. brongniartii* (ARSEF 617), were selected as the outgroup, as they have phylogenetically close but distinct lineages outside *Simplicillium and Leptobacillium,* which is helpful for evolutionary direction within the ingroup (Lu et al. 2025), and alignments were edited with Aliview software (https://ormbunkar.se/aliview) (Katoh and Standley 2013). For phylogenetic analyses, we used reference sequences from (https://www.ncbi.nlm.nih.gov/genbank/) based on studies by Nonaka et al. (2013), Zhang et al. (2017), Gomes et al. (2018), Wei et al. (2019), and Kondo et al. (2020). The sequences were manually edited and aligned using AliView (Larsson 2014). RAxML-HPC2 on XSEDE v. 8.2.12, 1000 nonparametric bootstrap iterations were used to conduct maximum likelihood (ML) analysis (Kalyaanamoorthy et al. 2017). Bootstrap values ≥70% were considered for significant branch support. Posterior probabilities (PP) were estimated using MrBayes via XSEDE v3.2.7a with five million generations and sampling every 1000 generations (Ronquist and Huelsenbeck 2003). Phylogenetic trees were visualized using FigTree v1.4.4 and edited in Adobe Illustrator v27.6.1. Sequence alignments were deposited in TreeBASE (Submission ID: 32 428).

3 | Results

3.1 | Phylogenetic Analysis

According to the concatenated phylogenetic tree, four spider-associating species from genus *Leptobacillium* were recorded from Malaysia (Figure 2). Three isolates, BORH/F03645,

TABLE 1 | Strains, voucher specimens, and selected GenBank sequences used in this study.

Species	Host	Location	Voucher number	ITS	*TEF1-α*	Reference
Beauveria bassiana	*Hyphantria cunea*	Italy	ARSEF 1564	NR_111594	HQ880974	Lu et al. 2025
B. brongniartii	*Melolontha melolontha*	Italy	ARSEF 617	NR_111595	HQ880991	Lu et al. 2025
Leptobacillium chinense	*Juniperus seravschanica*	China	ZJ06C02	PP385591	NA	Unpublished
L. chinense	*J. seravschanica*	China	JX18E06	PP385169	NA	Unpublished
L. coffeanum	Coffee plants	Brazil	CDA734	MF066034	MF066034	Gomes et al. 2018
L. coffeanum	Coffee plants	Brazil	CDA735	MF066035	NA	Gomes et al. 2018
L. coffeanum	**Spider**	**Malaysia**	**BORH/F03643**	**PP976482**	**PX233247**	**This study**
L. filiforme	NA	South Korea	DUCC19020	OL871297	NA	Unpublished
L. filiforme	*Acronicta rumicis*	Estonia	TUF:133292	OK649257	NA	Unpublished
L. filiforme	*Citrullus lanatus*	Brazil	URM 7918	NR171744	NA	Unpublished
***L. geminatum* sp. nov.**	**Spider**	**Malaysia**	**BORH/F03645**^{T}	**PP976484**	**PX233242**	**This study**
***L. geminatum* sp. nov.**	**Spider**	**Malaysia**	**BORH/F03646**	**PP976485**	**PX233243**	**This study**
***L. geminatum* sp. nov.**	**Spider**	**Malaysia**	**BORH/F03647**	**PP976481**	**PX233244**	**This study**
L. latisporum	Cave environment	Thailand	BRC 16288^{T}	NR_190959	NA	Shen et al. 2026
L. leptobactrum	Spider	Malaysia	BORH/F03648	PP976487	PX233245	This study
L. leptobactrum var. *calidius*	NA	China	KUNCC 8679	NA	OQ943222	Zare and Gams 2016
L. leptobactrum var. *calidius*	NA	China	KUMCC 8679	OQ926715	NA	Zare and Gams 2016
L. leptobactrum var. *leptobactrum*	*Beta vulgaris* soil	Netherlands	CBS 771.69	EF641868	NA	Zare and Gams 2016
L. longiphialidum	Spider	China	YFCC 24079491	PQ509281	PQ560996	Lu et al. 2025
L. longiphialidum	Spider	China	YFCC 23039272	PQ509282	PQ560997	Lu et al. 2025
L. symbioticum	*Phakopsora pachyrhizi* sori	Japan	NBRC 104297	LC485674	NA	Okane et al. 2020
L. symbioticum	*P. pachyrhizi* sori	Japan	NBRC 113865^{T}	NA	LC485673	Okane et al. 2020
L. symbioticum	Prostigmata (mite)	Japan	OPTF00168	LC485675	NA	Okane et al. 2020
L. symbioticum	**Spider**	**Malaysia**	**BORH/F03644**	**PP976483**	**PX233246**	**This study**
L. xianyushanense	*Araneae* sp.	China	RCEF7511	NA	PV166472	Chang et al. 2024
L. xianyushanense	*Araneae* sp.	China	RCEF6795	NA	PV097798	Chang et al. 2024
Simplicillium aogashimaense	*Blumeria graminis* f. sp. *tritici*	Netherlands	CBS 41370A	PV407385	PV414573	Nonaka et al. 2013
S. aogashimaense	*B. graminis* f. sp. *tritici*	Netherlands	CBS 317.70A	PV40738	PV414572	Nonaka et al. 2013

(Continues)

TABLE 1 | (Continued)

Species	Host	Location	Voucher number	ITS	*TEF1-α*	Reference
S. araneicola	Spider	China	DY11251	PV082783	PV171301	Unpublished
S. calcicola	Rock	China	LC5586	KU746706	KX855252	Zhang et al. 2017
S. calcicola	Rock	China	LC5371	KU746705	KX855251	Zhang et al. 2017
S. cicadellidae	Carpenter worm	China	GY11011	MN006243	MN022263	Chen et al. 2019
S. cicadellidae	Carpenter worm	China	GY11012	MN006244	MN022264	Chen et al. 2019
S. coccinellidae	NA	China	DY101791	MT453861	MT471342	Chen et al. 2019
S. cylindrosporum	Soil	Japan	JCM 18169^T	AB603989	NA	Kondo et al. 2020
S. cylindrosporum	Soil	Japan	JCM 18170	AB603994	LC496906	Kondo et al. 2020
S. formicae	Formicidae	Thailand	MFLUCC 18–1379	NR168789	NA	Wei et al. 2019
S. formicae	Formicidae	Thailand	MFLUCC 18–1379^T	MK766511	MK926451	Wei et al. 2019
S. formicae	**Spider**	**Malaysia**	**BORH/F03638**	**PZ196262**	**PX233240**	**This study**
S. formicae	**Spider**	**Malaysia**	**BORH/F03639**	**PZ196263**	**PX233241**	**This study**
S. hymenopterorum	Spider	China	DY101692	NA	MT471338	Unpublished
S. hymenopterorum	Spider	China	DY101691	NA	MT471337	Unpublished
S. lamellicola	*Brassica napus*	China	JC-1	MT807908	MT826785	Unpublished
S. lamellicola	Wood	Venezuela	CBS101912	NA	OQ471194	Unpublished
S. lamellicola	Wood	Venezuela	CBS101911	NA	OQ471193	Unpublished
S. lanosoniveum	NA	USA	CBS 704.86	AF339602	DQ522358	Sung et al. 2007
S. lepidopterorum	*Salvinia rotundifolia*	China	GY29132	NA	MN022266	Chen et al. 2019
S. lepidopterorum	*S. rotundifolia*	China	GY29131	NA	MN022265	Chen et al. 2019
S. minatense	Soil	Japan	JCM 18176	NR111025	NG068380	Nonaka et al. 2013
S. neoaraneae	NA	China	ZY06261	PV082789	PV171307	Unpublished
S. obclavatum	Air	China	CBS 31174^T	MH860859	EF468798	Sung et al. 2007
S. pseudocercosporicola	Spider	Brazil	COAD 3687	PP836713	NA	Unpublished
S. pseudocercosporicola	Spider	Brazil	COAD 3688	PP836712	NA	Unpublished
S. puwenense	NA	China	YFCC 23069492	PQ508798	PQ537124	Lu et al. 2025
S. puwenense	NA	China	YFCC 23129490	PQ508796	PQ537122	Lu et al. 2025
S. puwenense	**Wasp**	**Malaysia**	**UTHM-CMF001**	**PX488893**	**PX551695**	**This study**
S. salviniae	*Salvinia auriculata*	Taiwan	NTUCC 20–074	NR200377	MW200240	Unpublished
S. scarabaeoidea	*Araneae* sp.	China	RCEF7549	NA	PV166487	Unpublished
S. subtropicum	NA	Japan	IBCB 79	MT822178	NA	Unpublished

(Continues)

TABLE 1 | (Continued)

Species	Host	Location	Voucher number	ITS	*TEF1-α*	Reference
S. subtropicum	Soil	Japan	JCM 18180^{T}	LC496895	LC496910	Kondo et al. 2020
S. subtropicum	Soil	Japan	JCM 18181	LC496896	LC496911	Kondo et al. 2020
S. sympodiophorum	Soil	Japan	JCM 18184^{T}	NA	LC496912	Unpublished
S. wudangense	Spider	China	WD04131	PV082791	PV171309	Unpublished
S. zunyiense	Spider	China	ZY06581	PV082793	PV171311	Unpublished

BORH/F03646, and BORH/F03647 from Malaysia formed a clear sister clade to *L. longiphialidum* from China with strong nodal values (ML = 100% and PP = 1), a clear indication for a novel species. Therefore, *L. geminatum* sp. nov. is proposed to address the new species. Along with *L. geminatum* sp. nov, the other three species of *Leptobacillium* discovered were *L. leptobactrum* (BORH/F03648), *L. symbioticum* (BORH/F03544), and *L. coffeanum* (BORH/F03643). On the other hand, three isolates of two *Simplicillium* species were also recorded in Malaysia, namely, BORH/F03638, BORH/F03639, and UTHM-CMF001. Based on the concatenated tree, BORH/F03638 and BORH/F03639 formed a clade with *S. formicae*, while UTHM-CMF001 claded with *S. puwenense*.

3.2 | Taxonomy

Leptobacillium geminatum **M. Shahbaz & J.S. Sathiya Seelan, sp. nov.**

Figure 3

***MycoBank*:** 861 545

Holotype: Malaysia, Sabah (Northern Borneo), INFAPRO (N04°58.865′, E117°51.327), 23 February 2022 Jaya Seelan Sathiya Seelan, BORH/F03645.

Etymology: *geminatum* (Latin) = "paired" refers to the presence of distinct adjacent phialides.

***Description**:* A dense, whitish fungal growth appears on spider producing white mycelial mat, that is cottony to powdery in appearance (Figure 3A). No sexual morph observed. Conidiophore 17.4–35.4 × 1.3–3.6 µm ($\bar{x}$ = 26.7–1.9 µm, n = 10) is relatively short, cylindrical, and hyaline that arises from a septate hyphal filament (Figure 3B). Conidia long, hyaline, ellipsoidal to allantoid, smooth-walled, single-celled, and aseptate (Figure 3C). Conidia are oval shaped with rounded ends that are not perfectly symmetrical.

Culture morphology: Morphologically, *L. geminatum* sp. nov. forms the front colony circular with a dense, cottony to floccose texture that radiates outward, forming entire margins. It shows rapid growth of 20–35 mm after 2 weeks at 25°C. The surface of the colony appears largely white or slightly creamy that shows rapid growth, and the reverse colony appears pale yellow with orange pigmentation (Figure 3D,E). Two phialides that are narrow, cylindrical to slightly flask-shaped possess adjacent positions measured 23–44 µm × 1–1.7 µm ($\bar{x}$ = 30.0 × 1.23 µm, n = 30) (Figure 3F). Conidia ellipsoidal to slightly curved, smooth-walled, hyaline and aseptate, 4.3–8.5 × 0.4–2.9 µm ($\bar{x}$ = 6.2 × 1.38 µm, n = 30), rounded at both ends, single-celled, and cylindrical (Figure 3G,H).

Distribution: Found in INFAPRO, Danum Valley Conservation Area (DVCA), Sabah, Malaysia.

***Materials examined*:** Malaysia, Sabah (Northern Borneo), INFAPRO (N04°58.865’, E117°51.327), 23 February 2022, Jaya Seelan Sathiya Seelan. Holotype: Living culture BORH/F03645; GenBank accession: ITS = PP976484, *TEF1-α* = PX233242. Culture derived from the holotype strain: BORH/F03646; GenBank accession: ITS = PP976485, *TEF1-α* = PX233243. Additional material: BORH/F03647; GenBank accession: ITS = PP976481, *TEF1-α* = PX233244.

Notes: Phylogenetically, three samples of *Leptobacillium geminatum* sp. nov were grouped together on a single branch, forming a monophyletic clade. Our specimen is identified as a sister group to *L. longiphialidum* with Bayesian posterior probabilities and the maximum likelihood analysis (ML = 100% and PP = 1). Microscopic examination revealed two adjacent phialides that are slender, elongated, and have slightly tapered ends. The distinct adjacent position of phialides in our study specimens is not reported from other *Leptobacillium* species. The adjacent position of two phialides in *L. geminatum* sp. nov. is a distinct feature. Similar to *L. longiphialidum* is the conidia shape that is long with a cylindrical or columnar shape, but the conidia length of *L. geminatum* sp. nov. is almost twice the conidia length of *L. longiphialidum*. Besides that, *L. longiphialidum* has longer phialides than *L. geminatum* sp. nov.

Leptobacillium leptobactrum (W. Gams) Zare & W. Gams, Mycol. Progr. 15: 1003 (2016)

Figure 4

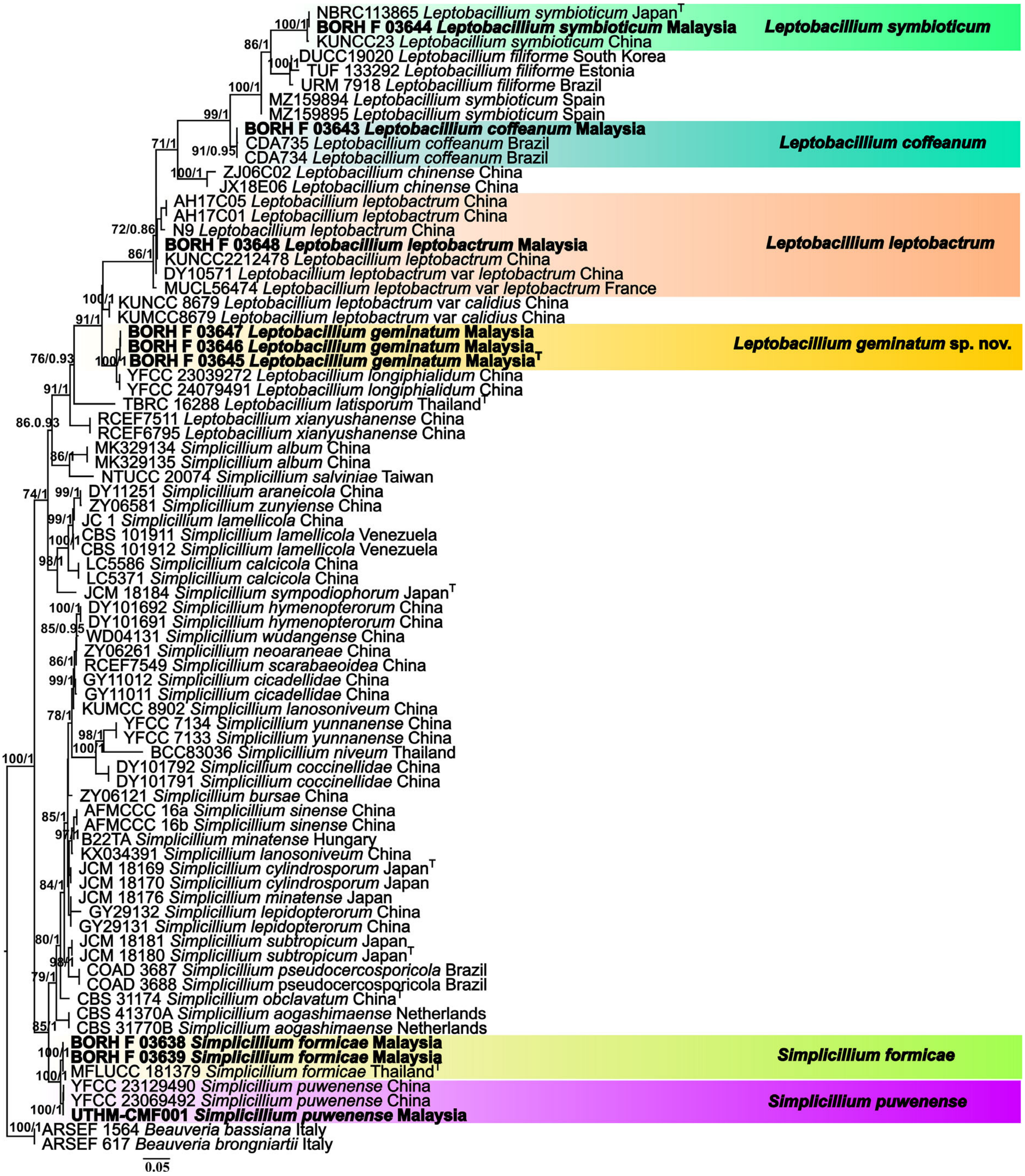

FIGURE 2 | Maximum likelihood phylogenetic tree generated with RAxML based on concatenated ITS and *TEF-1α* sequences of genera *Simplicillium* and *Leptobacillium*. The value on nodes is described as upper left, maximum likelihood bootstrap (ML); upper right, Bayesian posterior probabilities (PP).

Known distribution: Japan (Kondo et al. 2020), Poland (Vu et al. 2019), France (Vu et al. 2019), Netherlands (Zare and Gomez 2016), Malaysia (This study).

Ecology: The original specimen was associated with decaying wood from Poland.

Materials examined: Malaysia, Sabah (Northern Borneo), Sayap Substation, Kinabalu Park (N06°9.8733' E116°33. 93744'), 20 December 2022, Jaya Seelan Sathiya Seelan. Herbarium specimen: BORH/F03648; living culture: BORH/F03648 (ex-type); GenBank accession: ITS = PP976487, *TEF1-α* = PX233245.

Notes: BORH/F03648 formed a clade within the *L. leptobactrum* clade in the concatenated ITS-*TEF1-α* phylogenetic tree and possesses a 98% identical sequence within this clade. Therefore, we referred to BORH/F03648 as *L. leptobactrum* (Figure 2). Culture on PDA of BORH/F03648 has similar characteristics with the type specimen (CBS 774.69) from Poland. Likewise, both CBS 774.69 and BORH/F03648 showed similar growth rates on PDA (Figure 4). However, BORH/F03648's phialides are shorter

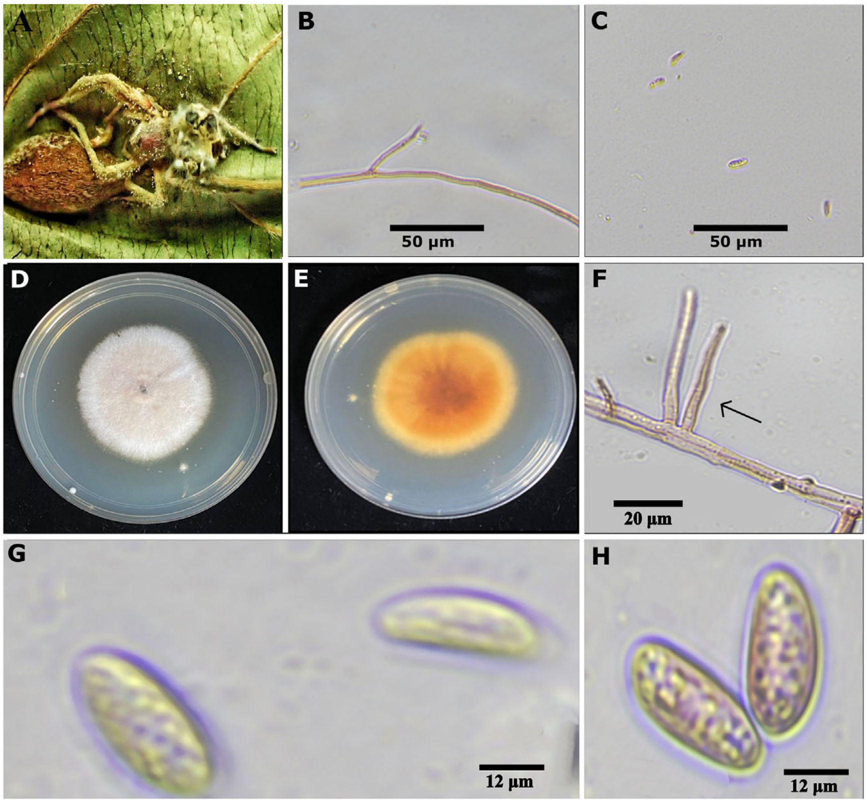

FIGURE 3 | *Leptobacillium geminatum* sp. nov. (A) *Leptobacillium geminatum* sp. nov. on spider. (B) Conidiophore associated with host; (C) Conidia; (D) Front colony of culture; (E) Reverse colony on PDA; (F) Conidiophore and phialides; and (G,H) Conidia.

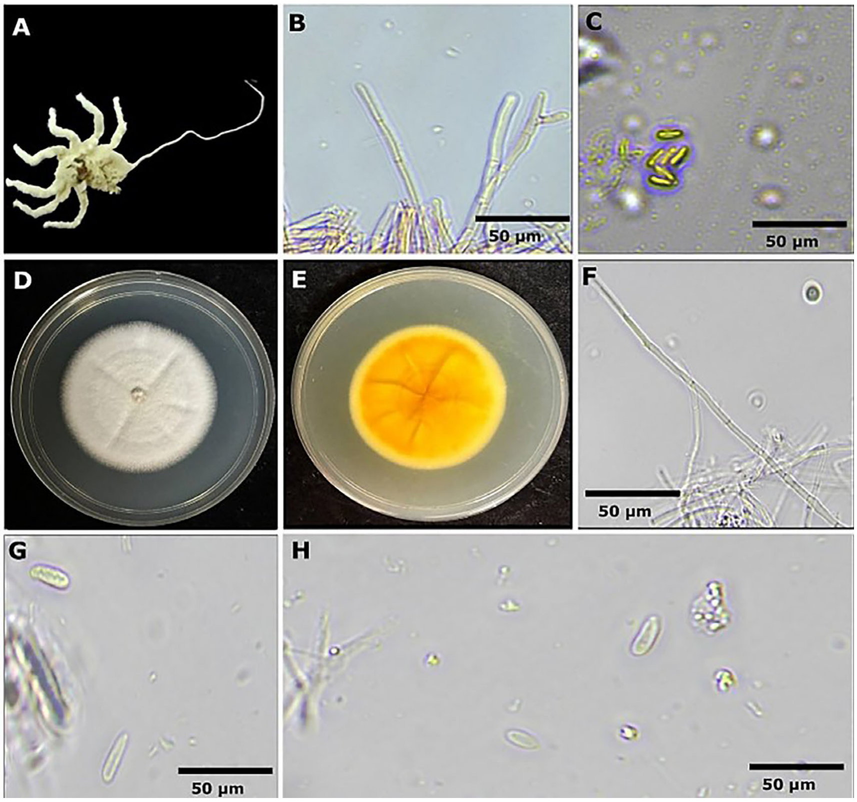

FIGURE 4 | *Leptobacillium leptobactrum* (A) *Leptobacillium leptobactrum* on spider. (B) Conidiophore emerging from host. (C) Conidia. (D) Front colony. (E) Reverse colony. (F) Phialides. (G,H) Conidia.

TABLE 2 | Comparison of *Leptobacillium* species.

Species	Phialides (μm)	Conidia (μm)	Colony diameter (mm, 2 weeks)	Country	Reference
Leptobacillium coffeanum	**12.4–43.5 × 1–2.0**	**1.2–3.2 × 0.4–1.7**	**35**	**Malaysia**	**This study**
L. coffeanum	11–44 × 1–2.4	2.2–3.8 × 0.8–1.5	30–33	Brazil	Gomes et al. 2018
***L. geminatum* sp. nov.**	**23–44 × 1–1.7**	**4.3–8.5 × 0.4–2.9**	**20–35**	**Malaysia**	**This study**
L. var. calidius	18.4–60 × 0.7–2.0	3.0–5.7 × 0.7–1.7	22–32	Ghana	Zare and Gams (2016)
L. leptobactrum	**3.4–5.3 × 0.6–1.4**	**12–36 × 0.4–1.5**	**20–25**	**Malaysia**	**This study**
L. leptobactrum	20–45 × 1–2	4.5–8 × 0.8–1.5	10–25	Poland	Zare and Gams 2016
L. symbioticum	**14.3–29.4 × 3.4–5.4**	**4.9–12.2 × 2.1–5.2**	**25–38**	**Malaysia**	**This study**
L. symbioticum	7.1–30.6 × 1.6–3.5	4–6.9 × 0.7–1.6	33–53	Japan	Okane et al. 2020

than the Polish's. Conidia shapes and sizes of BORH/F03648 also differ from CBS 774.69 (refer to Table 2).

Leptobacillium symbioticum Okane, Nonaka, Kurihara, J.P. Abe & Yamaoka, Mycoscience 61(4): 167 (2020)

Figure 5

Known distribution: Japan, China, Taiwan, Spain, Malaysia (This study).

Ecology: *Leptobacillium symbioticum* was associated with the soybean rust fungus from Japan.

Materials examined: Malaysia, Sabah (Northern Borneo), Serinsim Substation, Kinabalu Park (N6°17.2275948' E116°42.3593616'), 13 October 2022, Jaya Seelan Sathiya Seelan. Herbarium specimen: BORH/F03644; living culture: BORH/F03644 (ex-type); GenBank accession: ITS = PP976483, *TEF1-α* = PX233240, PX233241.

Notes: BORH/F03644 formed a clade with the type specimen of *L. symbioticum* (NBRC 113 865) from Japan and other *L. symbioticum* specimens from other countries (Figure 2). The sequences of BORH/F03644 and NBRC113865 are 99% identical. Therefore, our sample BORH/F03644 is identified as *L. symbioticum.* Culture characteristics of BORH/F03644 and NBRC 113 865 are similar. Phialides' width of BORH/F03644 are slightly thicker than NRBC 113 865's. Conidia of BORH/F03644 are quite different from NRBC 113 865 in shapes and sizes (refer to Table 2). The host of BORH/F03644 is also

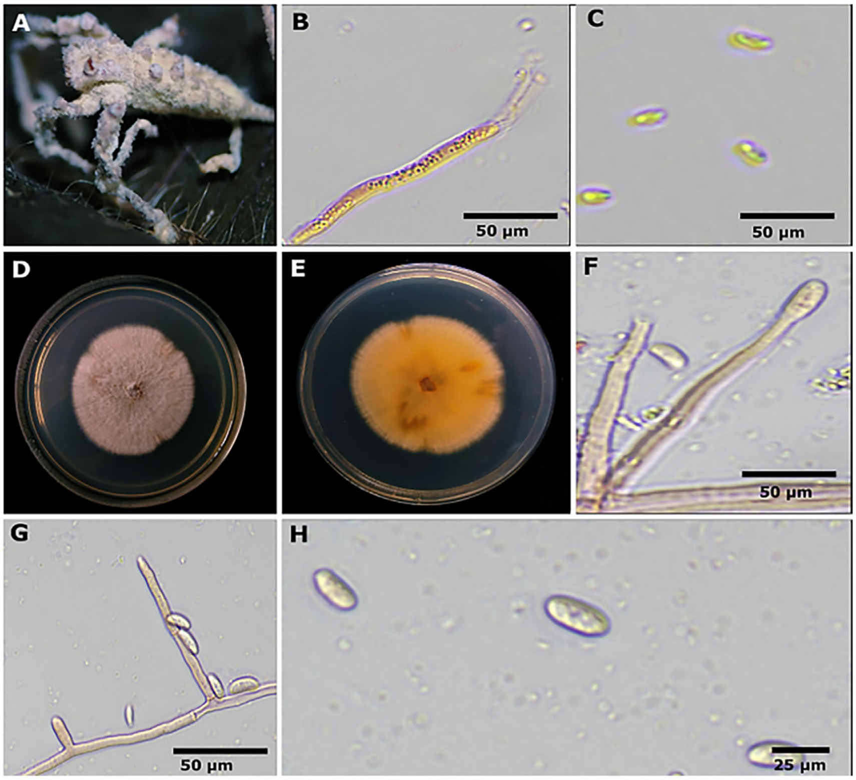

FIGURE 5 | *Leptobacillium symbioticum* (A) *Leptobacillium symbioticum* parasitizing spider. (B) Conidiophore. (C) Conidia. (D) Fungus front colony. (E) Reverse side on PDA media. (F,G) Phialides. (H) Conidia.

different from NBRC 113 865 which was isolated from soybean rust fungus (Figure 5).

Leptobacillium coffeanum (A.A.M. Gomes & O.L. Pereira) Okane, Nonaka, Kurihara, J.P. Abe & Yamaoka, Mycoscience 61(4): 169 (2020)

Figure 6

Known distribution: Japan, Brazil, Malaysia (This study).

Materials examined: Malaysia, Sabah (Northern Borneo), Serinsim Substation, Kinabalu Park (N6°16.8817554' E116°41.6922396'), 13 October 2022, Jaya Seelan Sathiya Seelan. Herbarium specimen: BORH/F03643; living culture: BORH/F03643 (ex-type); GenBank accession: ITS = PP976482, *TEF1-α* = PX233247.

Ecology: *Leptobacillium coffeanum* found on branches of *Coffea arabica* from Brazil.

Notes: Our study sample BORH/F03643 formed a sister clade to *L. coffeanum* (CDA734 and CDA735) from Brazil, and the sequence is completely identical to *L. coffeanum* (CDA734 and CDA735). Therefore, our sample BORH/F03643 is designated as *L. coffeanum* (BORH/F03643). The type specimen of *L. coffeanum* was discovered to associate endophytically with a healthy *Coffea arabica* while BORH/F03643 was found to associate pathologically with a spider host (Figure 6). The phialides and conidia on PDA of both BORH/F03643 and the type specimen have similar shapes and sizes (Table 2). However, the macroconidia were not observed.

Simplicillium formicae D.P. Wei & K.D. Hyde, in Wei, Wanasinghe, Hyde, Mortimer, Xu, Xiao, Bhunjun & To-anun, MycoKeys 60: 78 (2019).

Figure 7

Known distribution: Thailand (Wei et al. 2019), Malaysia (This study).

Materials examined: Malaysia, Sabah (Northern Borneo), Danum Valley Conservation Area (N04°59.587' E118°04.380'), 20 February 2022, Jaya Seelan Sathiya Seelan. Herbarium specimens: BORH/F03638, BORH/F03639; living cultures: BORH/F03638, BORH/F03639 (ex-type); GenBank accession: ITS = PZ196262, PZ196263, *TEF1-α* = PX233240, PX233241.

Notes: The ITS-*TEF1*-**α** concatenated tree showed BORH/F03639 claded with MFLUCC 181 379 (*S. formicae*) from Thailand. Besides the slightly thicker phialides observed in the Thai specimen, the morphologies of both specimens are similar (refer to Table 3). Therefore, BORH/F03639 is regarded as *S. formicae* (Figure 7). However, the host of BORH/H03641 in our study is a spider that is distinct from previous reports. Previous reports described this fungus from ants and plant leaves (Wei et al. 2019; Nada et al. 2025).

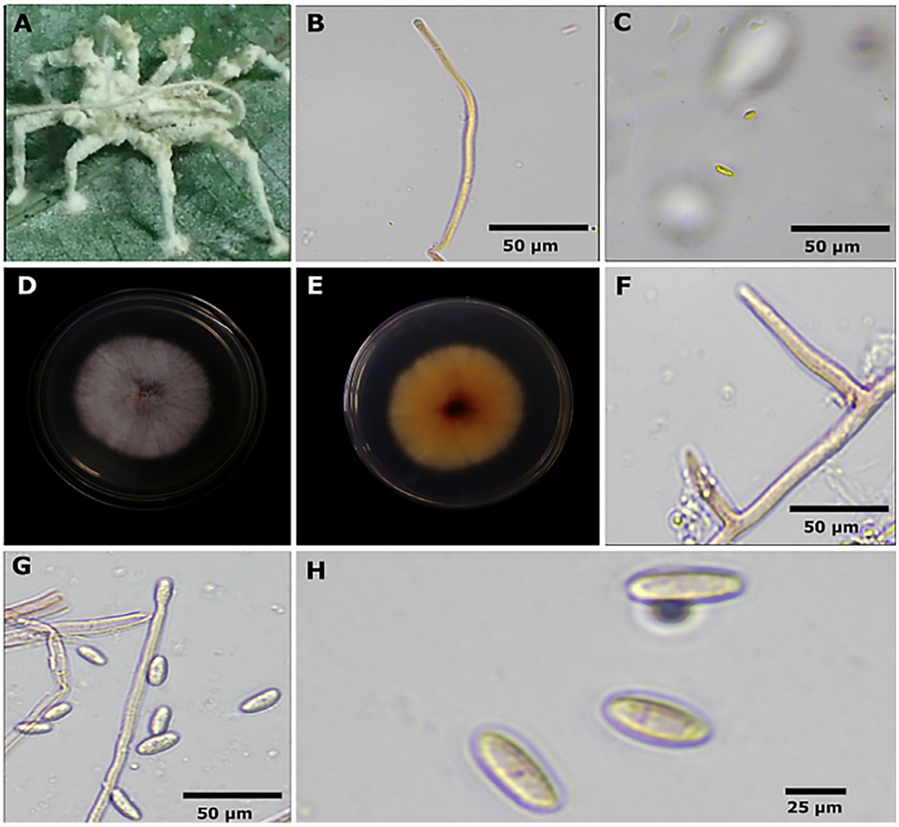

FIGURE 6 | *Leptobacillium coffeanum* (A) *Leptobacillium coffeanum* on spider. (B) Ascus. (C) Conidia; (D) Fungus culture front view; and (E) Reverse side on PDA plates; (F,G) Conidiophore and Phialides; and (H) Conidia.

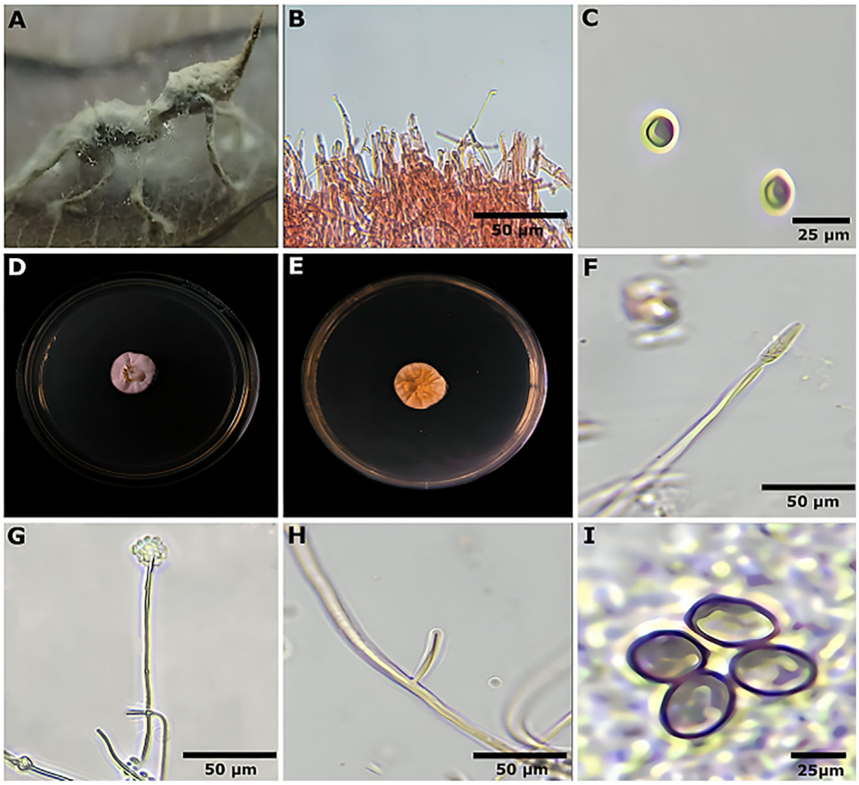

FIGURE 7 | *Simplicillium formicae* (A) *S. formicae* on spider; (B) Conidiophore; (C) Conidia; (D) Front side; and (E) Reverse side of the colony on PDA media; (F–H) Phialides with conidia; and (I) Conidia.

TABLE 3 | Comparison of *Simplicillium* species.

Species	Phialides (µm)	Conidia (µm)	Colony diameter (mm, 2 weeks)	Country	Reference
Simplicillium formicae	**20.5–47.4 × 0.2–0.5**	**1.2–3.5 × 1.5–3.5**	**30–40**	**Malaysia**	**This study**
S. formicae	25–100 × 0.5–1.5	2–3.5 × 1.5–2.5	25–30	Thailand	Wei et al. 2019
S. puwenense	**8.6–109.8 × 0.7–1.3**	**1.25–3.1 × 1.25–1.83**	**20–25**	**Malaysia**	**This study**
S. puwenense	3.37–52.57 × 0.5–1.6	1.19–2.41 × 0.88–1.6	32–35	China	Lu et al. 2025

Simplicillium puwenense Hong Yu bis, Y.L. Lu & Jing Zhao bis, in Lu, Li, Liu, Zhao, Yu, Liang, Li, Huang, Li & Yu, MycoKeys 115: 195 (2025) Figure 8

Known distribution: China, Yunnan Province (Lu et al. 2025)

Species examined: Malaysia, Negeri Sembilan (Southwestern Peninsular Malaysia), Kenaboi State Park (N3°10′54.498″ E101°58’53.909”), 17 August 2023, Shahrul Nizam bin Ariffin. Herbarium specimen: UTHM-CMF001; living culture: UTHM-CMF001 (ex-type); GenBank accession: ITS = PX488893, *TEF1-α* = PX551695. ***Ecology***: UTHM-CMF001 isolated from *Ophiocordyceps* sp. that infected a wasp biting on a leaf.

Notes: The isolate UTHM-CMF001 forms a clade with *S. puwenense* (YFCC 23 129 490) from China (Figure 2). Besides, the ITS and *TEF1-α* sequences of UTHM-CMF001 and YFCC 23 129 490 are completely identical. Therefore, we refer to UTHM-CMF001 as *S. puwenense*. The culture characteristics on PDA of UTHM-CMF001 differ slightly from YFCC 23 129 490 with the reverse of the Chinese specimen appearing light brown while the Malaysian sample appeared light yellow (Figure 8). The shapes of the phialides for both specimens are similar but the sizes differ slightly (refer to Table 3). Lastly, the Chinese specimen was found to be enteropathogenic, but the Malaysian specimen was associated with an *Ophiocordyceps* sp. that infected a wasp. However, the relationship with the entomopathogen is unclear.

4 | Discussion

Morphological and molecular analyses in this study confirmed that the examined specimens belong to the genera *Leptobacillium* and *Simplicillium* within the family *Cordycipitaceae*. Our phylogenetic analyses based on combined ITS and *TEF 1-α* sequences supported the placement of these taxa within their respective genera and revealed the presence of one novel species of *Leptobacillium* along with additional records of *Simplicillium* from Malaysia. These findings expand the known

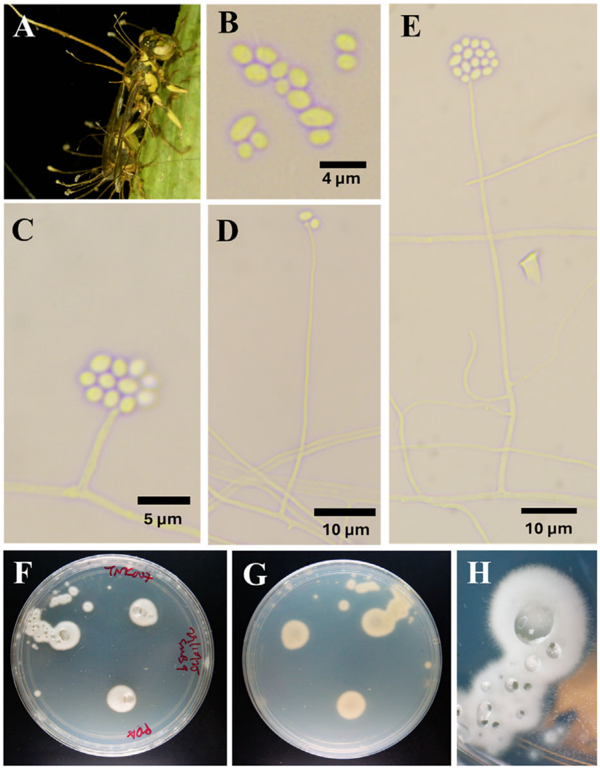

FIGURE 8 | *Simplicillium puwenense.* (A) A wasp infected by *Ophiocordyceps* sp. where *Simplicillium puwenense* was isolated; (B) Conidia; (C–E); Phialide-bearing conidia; (F) Colonies on PDA obverse; (G) Colonies on PDA reverse; (H) Cuttation observed on PDA.

diversity and geographical distribution of these genera and highlight the rich but still underexplored diversity of arthropod-associated fungi in Malaysian tropical forests.

Phylogenetic evidence supports the taxonomic placement of the isolates within *Leptobacillium* and clarifies their relationship with closely related species. Phylogenetic analysis based on the combined ITS and *TEF1-α* region sequences suggests that *Leptobacillium* is monophyletic and distinct from *Simplicillium*. In addition, the genus *Leptobacillium* can be morphologically distinguished from *Simplicillium* by its long, slender, and rod-shaped conidia (Zare and Gams 2016).

Leptobacillium coffeanum and *L. symbioticum* showed slightly smaller phialides than those previously reported from Brazil and Japan, respectively (Gomes et al. 2018; Okane et al. 2020). However, these differences likely represent intraspecific morphological variation and do not affect the taxonomic placement of the isolates, which is strongly supported by phylogenetic analyses.

In the case of *Leptobacillium coffeanum*, the Malaysian specimen presents an interesting ecological shift compared to previous reports. While the type specimen was described as an endophyte associated with *Coffea arabica*, the present study records it from a spider host, suggesting a broader ecological amplitude. This shift from plant-associated endophytes to arthropod association may indicate ecological potential opportunistic behavior. Similarly, *Leptobacillium symbioticum* demonstrates notable ecological and morphological variation. The Malaysian isolate was isolated from a spider, in contrast to earlier reports where the species was associated with soybean rust fungi. This indicates that *L. symbioticum* may exhibit variations in ecological strategies, potentially functioning as both a mycoparasite and an entomopathogen. For *Leptobacillium leptobactrum*, although the phylogenetic placement clearly supports its identification, the Malaysian specimen shows morphological differences, particularly in having shorter phialides and variation in conidial dimensions. The differences may reflect environmental adaptations to tropical conditions or host-specific influences.

The potentially *new L. geminatum* sp. nov. described in this study exhibited distinct morphological features compared with closely related taxa such as *L. calidius*. *Leptobacillium leptobactrum* had reduced phialides compared to a previous report from Japan (Okane et al. 2020). The phylogenetic placement of *Leptobacillium geminatum* sp. nov. as a lineage closely related to *Leptobacillium longiphialidum* supports its recognition as a distinct species within the genus. This relationship, together with morphological differences observed in phialide arrangement and conidial characteristics, further confirms the taxonomic distinctiveness of the new species. *Leptobacillium geminatum* sp. nov. is distinguished by closely positioned phialides and lin cylindrical conidia, nearly twice the length of those in *L. longiphialidum.* In contrast, *L. longiphialidum* possesses noticeably longer phialides. Importantly, the presence of paired adjacent phialides in *L. geminatum* sp. nov. represents a unique morphological trait not previously reported in the genus, further supporting its novelty. This feature may have functional implications in conidia production.

Species of *Simplicillium* are ecologically versatile, acting as mycoparasites and antagonists of plant pathogens, with some species also occurring as endophytes (Gauthier et al. 2014; Nonaka et al. 2013; Gomes et al. 2018). In the case of *Simplicillium formicae*, the Malaysian specimens were isolated from spiders, whereas previous reports primarily associated this species with ants and plant substrates. This represents a notable host expansion and suggests that *S. formicae* may have a broader host range than previously reported species. Such host plasticity may indicate an adaptive advantage in diverse tropical ecosystems.

The isolation of *S. puwenense* in the present study represents an interesting ecological observation. The type specimen was reported from China and associated with a spider host. In contrast, our specimen was isolated from an *Ophiocordyceps* species infecting a wasp, suggesting a more complex host–parasite interaction. The ecological relationship between *S. puwenense* and the entomopathogenic host fungus remains unclear and warrants further investigation. Morphologically, the Malaysian specimen differs slightly from the Chinese specimen. The Malaysian isolate possesses longer phialides while maintaining similar phialide width, and the conidia are also relatively larger. This pattern of association suggests the possibility of hyperparasites, where *S. puwenense* may parasitize another entomopathogenic fungus rather than directly infecting the insect's host. Such interactions are increasingly recognized within *Cordycipitaceae* and highlight the complexity of fungal ecological networks in tropical environments.

5 | Conclusion

In this study, one new species and five newly recorded spider- and wasp-associated entomopathogenic fungi were described from forests of Malaysia using morphological and phylogenetic analyses. These EPF were two species of genus *Simplicillium*, i.e. *S. formicae* and *S. puwenense*, and four species of genus *Leptobacillium*, i.e. *L. geminatum* sp. nov., *L. leptobactrum*, *L. symbioticum*, and *L. coffeanum*.

Author Contributions

All authors contributed to the conception and design of the study. **Muhammad Shahbaz, Jaya Seelan Sathiya Seelan, Fredrick C. De Went, Yee Shin Tan, Sumaiyah Abdullah**, and **Qian-Qun Koid** contributed to material preparation and data collection. Morphological characteristics were examined by **Muhammad Shahbaz, Jaya Seelan Sathiya Seelan**, and **Qian-Qun Koid**. 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 **Jaya Seelan Sathiya Seelan** which was then improved by changes, edits, suggestions, and comments from **Tom Fayle, Kishneth Palaniveloo, Jaya Seelan Sathiya Seelan, Jeremiah Sia Yiao Rong, Yap Jing Wei**. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The nucleotide sequences generated in this study have been deposited in the NCBI GenBank database (https://submit.ncbi.nlm.nih.gov/subs). The accession numbers will be made publicly available upon publication of this article. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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