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Phlyctema yunnanensis (*Dermateaceae*, *Helotiales*), a novel species from herbaceous plants in grassland ecosystems of Yunnan, China

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Abstract

During a survey of grassland microfungi in Yunnan Province, China, a fungus with distinctive apothecia was collected. Initial BLAST analyses of its sequence data indicated a close affiliation with taxa in the *Dermateaceae*. Phylogenetic analyses based on a combined dataset of the internal transcribed spacer (ITS) and large subunit (LSU) of ribosomal DNA, RNA polymerase II second largest subunit (*rpb2*), and β-tubulin (*tub2*) genes confirmed its placement within the genus *Phlyctema* (*Dermateaceae*). The species is morphologically characterized by discoid, sessile apothecia, bitunicate, fissitunicate, 4-spored clavate asci, and subglobose to ellipsoid or oblong to obovoid, slightly inequilateral, hyaline, aseptate, and thin-walled ascospores. Based on both morphological features and multigene analyses, this fungus is introduced as a new species, *Phlyctema yunnanensis*.

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Introduction

Dermateaceae (*Helotiales*, *Leotiomycetes*) consists of 16 genera *Coleophoma* Höhn., *Corniculariella* P. Karst., *Cryptosporiopsis* Bubák & Kabát, *Davidhawksworthia* Crous, *Dermea* Fr., *Gelatinoamylaria* Prasher & R. Sharma, *Neodermea* W.J. Li, D.J. Bhat & K.D. Hyde, *Neofabraea* H.S. Jacks., *Neogloeosporidina* W.J. Li, Camporesi & K.D. Hyde, *Pezicula* Tul. & C. Tul., *Phlyctema* Desm., *Pseudofabraea* Chen Chen, Verkley & Crous, *Pseudoxenochalara* Iliushin & Kirtsideli, *Rhizodermea* Verkley & Zijlstra, *Verkleyomyces* Y. Marín & Crous, and *Xenochalara* M.J. Wingf. & Crous[1–6]. Species of *Dermateaceae* are predominantly reported from temperate regions of the world and occupy various ecological niches. They are reported as plant pathogens, endophytes, saprobes, wood destroyers, and soil inhabitants[2–4,6,7]. *Dermea* is the type genus of the family *Dermateaceae*. The sexual morph of *Dermea* is characterized by leathery, hard, dark brown to black apothecia. Its asci are 8-spored, cylindrical to clavate-cylindrical while the ascospores are aseptate to 3-septate, ellipsoid-fusiform to ellipsoidal and hyaline to yellowish-brown in colour[8–10]. The asexual morph exhibits diverse conidiomatal structures, which often accompany the apothecia. Two distinct types of conidia are typically observed: bacillary to filiform microconidia and elongate-fusiform to sickle-shaped macroconidia[8–10].

Phlyctema was introduced by Desmazières[11] with *P. vagabunda* Desm. as the type species. The sexual morph of *P. vagabunda* was previously classified under *Neofabraea alba* (E.J. Guthrie) Verkley, leading to the synonymization of *Phlyctema* with *Neofabraea*[12,13]. However, Chen et al.[2] later reinstated *Phlyctema* and *Neofabraea* as separate genera based on analyses of multi-genes and morphological traits. Subsequently, Li et al.[4] employed multi-gene phylogenetic analyses to further clarify the generic boundaries within *Dermateaceae*, providing support for the distinction between *Phlyctema* and *Neofabraea*. There are 61 accepted species in *Phlyctema*, although most have not been re-examined[14]. The sexual morph of *Phlyctema* is characterized by circular or irregular and merged, slightly convex, sessile, developing from acervuloid stromata apothecia, 8-spored, cylindrical-clavate asci, hyaline, elongated ellipsoid, straight or slightly curved, rounded or somewhat pointed at both ends, aseptate when young, septate at maturity ascospores, filiform, branched, slightly swollen at apical cell, with septate paraphyses[2,12,15]. However, the sexual morph is rarely been reported from the *Phlyctema* species. The asexual morph is characterized by having eustromatic, convulated, pulvinate to sporodochial conidiomata, branched, hyaline conidiophores, and phialidic conidiogenous cells that give rise to hyaline, aseptate, fusiform, straight to curved conidia[4,16]. *Phlyctema* species were reported as saprobic, parasitic, or endophytic on plant hosts in terrestrial habitats, such as *Aconitium napellus* L. (*Ranunculaceae*), *Coronilla* L. (*Fabaceae*), *Erigeron* L. (*Asteroideae*), *Libertia ixioides* (G. Forst.) Spreng. (*Iridaceae*), *Malus pumila* Mill., and *M. sylvestris* (L.) Mill. (*Rosaceae*)[2,4,16,17]. Although a considerable number of species have been reported in the genus *Phlyctema*, those associated with grassland vegetation are relatively scarce or nonexistent.

Grassland ecosystems across the world, including in China, offer numerous opportunities for fungal discovery[18–25]. For instance, bamboo ecosystems in China host more than 450 fungal species, accounting for approximately one-quarter of the global bambusicolous ascomycetes[26]. However, the investigation of fungi (such as in *Dermateaceae*) associated with short grass, bushes, and other grass vegetation remains underexplored. Extensive fungal sampling across a variety of grasslands in different geographical areas is urgently needed to mitigate species losses and understand their ecological significance. Therefore, we are continuously exploring the grassland-associated microfungi in Yunnan, China[25,27,28].

In this study, a taxonomic survey of foliar fungal epiphytes was conducted on herbaceous plants from grasslands in Yunnan, China. A new species, *Phlyctema yunnanensis* is described and illustrated based on its morphological characteristics and DNA molecular evidence. Additionally, an updated multigene phylogenetic tree for *Phlyctema* is presented, incorporating combined ITS, LSU, *rpb*2, and *tub*2 sequence data, including the newly identified taxon.

Materials and methods

Sample collection, isolation, and morphological observations

Specimens were collected from grasslands in Zhaotong City, Yunnan Province, China in 2021. The local environment in Zhaotong is characterized by *Poaceae* as the predominant plant species and features typical plateau vegetation[24,25]. This area is influenced by a three-dimensional monsoon climate and reaches a maximum elevation of approximately 4000 m[29]. Specimens were stored in plastic Ziplock bags and returned to the mycology laboratory at the Kunming Institute of Botany, Chinese Academy of Sciences (Kunming, China). Samples were examined using an Olympus SZ-61 dissecting microscope. Fungal fruiting structures were manually sectioned with a sterilized razor blade and mounted in water on a slide for microscopic examination. Micro-morphological characteristics were examined using a Nikon ECLIPSE Ni-U complex microscope with differential interference contrast (DIC) and phase contrast (PC) illumination. Photos of microscopic structures were captured using a Nikon DS-Ri2 camera. Photo plates and measurements were processed using Adobe Photoshop CS6 Extended version 13.0.1 (Adobe Systems, CA, USA). Specimens were deposited in the herbarium of the Kunming Institute of Botany Academia Sinica (HKAS) (Kunming, China). The Index Fungorum and Faces of Fungi (FoF) numbers were obtained for the new fungus[14,30].

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fresh fruiting bodies using the E.Z.N.A.® Forensic DNA Kit-D3591 (Omega Bio-Tek, Inc, 400 Pinnacle Way, Suite 450 Norcross, Georgia 30071, USA) following the manufacturer's protocol. The DNA for polymerase chain reaction (PCR) was stored at 4 °C for regular use and at −20 °C for long-term usage. PCR amplifications were carried out for the partial 28S large subunit nuclear ribosomal DNA (LSU) and internal transcribed spacer region with intervening 5.8S nrRNA gene (ITS) were amplified with primers LR0R/LR5[31], ITS5/ ITS4[32], separately. The total volume of PCR mixtures for amplification was 25 μL containing 8.5 μL ddH_2O, 12.5 μL 2 × F8FastLong PCR MasterMix (Beijing Aidlab Biotechnologies Co. Ltd, Beijing, China), 2 μL of DNA template, and 1 μL of each forward and reverse primers (stock of 10 pM). The PCR thermal cycle profiles for LSU and ITS: the thermal conditions included initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 10 s, annealing temperatures at 55 °C for 15 s, elongation at 72 °C for 20 s, and final extension at 72 °C for 10 min. The amplified PCR fragments were sent to the Qingke Company, Kunming City, Yunnan Province, China, and Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., China, for sequencing. Sequences were deposited in the GenBank Database.

Sequence alignment, and phylogenetic analyses

Two phylogenetic analyses were performed based on two concatenated datasets of ITS-LSU-*rpb*2-*tub*2 and ITS-LSU. Sequences generated from this study were analyzed with other similar sequences obtained from GenBank (www.ncbi.nlm.nih.gov) (Table 1), and those derived from recent publications[2,4,6,17,33]. The newly generated sequences in this study were assembled by BioEdit 7.0.9.0[34]. Multiple sequence alignments with individual gene datasets were generated with the MAFFT v.7. online platform[35], and trimmed with TrimAl v.1.3[36] via the web server Phylemon2 (http://phylemon.bioinfo.cipf.es/utilities.html; accessed on August 10, 2024), and multi-gene alignments were manually aligned wherever necessary in SequenceMatrix program (1.7.8)[37]. Phylogenetic reconstructions of individual and combined datasets were performed using maximum likelihood (ML) and Bayesian inference (BI).

Maximum likelihood trees were inferred using RAxML-HPC v.8 on ACCESS (8.2.12) in CIPRES Science Gateway v. 3.3 online platform[38] under the GTRGAMMA nucleotide substitution model with 1,000 bootstrap replicates. The BI analysis was performed using MrModeltest v2.3[39] *via* PAUP v. 4.0b10[40]. Six simultaneous Markov chains were run for 10,000,000 generations, with trees sampled every 1000th generation. The run was configured to stop automatically when the critical value for the topological convergence diagnostic reached 0.01, and the first 25% of the trees were discarded as burn-in.

Tree topologies were visualized and exported using FigTree v. 1.4.0[41]. The phylogram was edited and annotated using Microsoft Office PowerPoint 2016 (Microsoft Inc., USA) and Adobe Photoshop CS6 Extended version 13.0.1 (Adobe Systems, CA, USA). Finally, the newly generated sequences were deposited in the GenBank database (Table 1).

Table 1. GenBank accession numbers of the strains used for phylogenetic analyses in this study.

Species	Strain number	ITS	LSU	*rpb* 2	*tub*2
Coleophoma caliginosa	CBS 124806^T	KR859090	KR858881	KR859330	KR859293
Coleophoma camelliae	CBS 101376^T	KU728481	KU728521	NA	KU728597
Coleophoma coptospermatis	CPC 19864^T	KU728483	KU728523	NA	NA

(to be continued)

Table 1. (continued)

Species	Strain number	ITS	LSU	*rpb* 2	*tub*2
Coleophoma cylindrospora	CBS 591.70^T	KU728486	KU728526	NA	NA
Coleophoma ericicola	CBS 301.72^T	KU728488	KU728528	NA	NA
Coleophoma eucalypticola	CBS 124810^T	GQ303279	GQ303310	KR859331	KR859294
Coleophoma eucalyptorum	CPC 19294	KF251240	KF251743	NA	NA
Coleophoma paracylindrospora	CBS 109074^T	KU728491	KU728531	NA	NA
Coleophoma parafusiformis	CBS 132692^T	KU728494	KU728534	NA	NA
Coleophoma proteae	CBS 132532^T	JX069866	JX069850	NA	NA
Corniculariella rhamni	MFLUCC 16-1446^T	MT185495	MT183457	MT432216	NA
Davidhawksworthia ilicicola	CBS 734.94^T	NR_154008	NG_067307	NA	NA
Davidhawksworthia ilicicola	CBS 261.95	KU728516	KU728555	NA	NA
Dermea cerasi	MFLU 16-0929	MT185502	MT183464	MT432221	NA
Dermea cerasi	MFLUCC 16-1147^T	MT185501	MT183463	MT432220	NA
Infundichalara microchona	CBS 175.74^T	KR859078	HQ609479	KR859318	KR859284
Neodermea rossica	MFLUCC 17-2506^T	MT185530	MT183493	MT432236	NA
Neofabraea actinidiae	CBS 121403^T	KR859079	KR858870	KR859319	KR859285
Neofabraea brasiliensis	CNPUV499^T	KR107002	KR107002	NA	KR107012
Neofabraea brunneipila	MFLU 15-0231^T	MK584984	MK592004	NA	NA
Neofabraea inaequalis	CBS 326.75^T	KR859081	KR858872	KR859321	KR859287
Neofabraea kienholzii	CBS 126461^T	KR859082	KR858873	KR859322	KR859288
Neofabraea krawtzewii	CBS 102867	KR859084	KR858875	KR859324	AF281459
Neofabraea malicorticis	CBS 122030^T	KR859086	KR858877	KR859326	KR859291
Neofabraea perennans	CBS 275.29	KR859088	KR858879	KR859328	KR859292
Neofabraea perennans	CBS 453.64	KR859089	KR858880	KR859329	AF281474
Neofabraea salicina	CBS 148445^T	OK664708	OK663747	OK651161	NA
Neogloeosporidina pruni	MFLU 16-2153^T	NR_169721	MT183501	MT432239	NA
Pezicula acericola	CBS 239.97	KR859093	KR858884	KF376214	KF376283
Pezicula aurantiaca	CBS 201.46	KR859102	KR858893	KF376210	KF376335
Pezicula californiae	CBS 124805^T	KR859104	KR858895	KR859332	KR859295
Pezicula carpinea	CBS 923.96^T	KR859108	KR858899	KF376158	KF376279
Pezicula cinnamomea	CBS 239.96	KR859124	KR858915	KF376165	KF376323
Pezicula cinnamomea	CBS 240.96	KR859125	KR858916	KF376163	KF376325
Pezicula cornina	CBS 285.39	KR859163	KR858955	KR859333	KR859296
Pezicula eucrita	CBS 259.97	KR859179	KR858971	KF376205	KF376333
Pezicula fagacearum	CBS 112400^T	KR859201	KR858993	KR859335	KR859298
Pezicula frangulae	CBS 100244	KR859204	KR858996	KF376211	KF376285
Pezicula italica	MFLU 16-1284^T	NR_170044	NG_073839	NA	NA
Pezicula microspora	CBS 124641^T	KR859212	KR859004	KR859337	KR859300
Pezicula neocinnamomea	CBS 100248^T	KR859213	KR859005	KF376209	KF376328
Pezicula neoheterochroma	CBS 127388^T	KR859221	KR859013	KR859338	KR859301
Pezicula neosporulosa	CBS 101.96^T	KR859223	KR859015	KF376193	KF376305
Pezicula ocellata	CBS 268.39	KR859232	KR859024	KR859339	KR859302
Pezicula pseudocinnamomea	CBS 101000^T	KR859235	KR859027	KR859340	KR859303
Pezicula rubi	CBS 253.97	KR859250	KR859042	KF376204	KF376329
Pezicula sporulosa	CBS 224.96^T	NR_137161	NG_069840	KF376201	KF376326
Phlyctema phoenicis	MFLU 15-1243	MT449717	MT449705	MT432248	NA
Phlyctema phoenicis	CPC 29372^T	KY173432	KY173522	NA	NA
Phlyctema phoenicis	CBS 147066	ON811524	ON811581	NA	NA
Phlyctema vagabunda	CBS 109875^T	KR859275	KR859069	KR859346	AY064702
Phlyctema vagabunda	CBS 304.62	KR859276	KR859070	KR859347	KR859310
Phlyctema vagabunda	ATCC 38338	AF281366	NA	NA	AF281456
Phlyctema vincetoxici	CBS 102469	KR859277	KR859071	KR859348	KR859311
Phlyctema vincetoxici	CBS 123727^T	KR859278	KR859072	KR859349	KR859312
Phlyctema yunnanensis	**HKAS 128745^T**	**PQ456177**	**PQ456179**	NA	NA
Phlyctema yunnanensis	**HKAS 128746**	**PQ456178**	**PQ456180**	NA	NA
Pseudofabraea citricarpa	CBS 130297^T	KR859279	KR859073	KR859350	KR859313
Pseudofabraea citricarpa	CBS 130532	KR859280	KR859074	KR859351	KR859314
Pseudoxenochalara grumantiana	CBS 148028	OM774424	OM776920	OM782293	NA
Rhizodermea veluwensis	CBS 110605^T	KR859282	KR859076	KR859353	KR859316
Rhizodermea veluwensis	CBS 110615	KR859283	KR859077	KR859354	KR859317
Xenochalara juniperi	MEA-B5-SW	JX869564	NA	NA	NA
Xenochalara juniperi	CBS 670.75	AF184887	NA	NA	NA

The new sequences are indicated in bold. The ex-type strains are indicated with 'T'. 'NA' indicates that the sequence is unavailable.

Results

Phylogenetic analysis

Since we failed to obtain *rpb*2 and *tub*2 under various PCR conditions, the analysis was initially conducted using only ITS and LSU data. A total of 64 strains of taxa are included in the combined analyses. The aligned dataset contained 1,253 characters (ITS: 1–476 bp, LSU: 477–1,253 bp). *Infundichalara microchona* (CBS 175.74) was used as the outgroup taxon. The best-scoring RAxML tree with a final likelihood value of -6894.552551 is presented. The matrix had 337 distinct alignment patterns, with 3.47% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.233464, C = 0.235945, G = 0.285797, T = 0.244794; substitution rates AC = 1.549458, AG = 2.488198, AT = 0.862983, CG = 0.557730, CT = 6.974513, GT = 1.000000, gamma distribution shape parameter α = 0.531475, 0.543158, Tree-Length = 1.065237 (Fig. 1).

To follow up with the recent phylogenetic analyses of this family by Iliushin & Kirtsideli[6] a second analysis of *Dermateaceae* was constructed using sequence data from ITS, LSU, *rpb*2, and *tub*2 sequence data. The total sequence dataset comprised 64 strains, *Infundichalara microchona* (CBS 175.74) was selected as the outgroup taxon. The aligned dataset contained 2918 characters (ITS: 1–476 bp, LSU: 477–1,253 bp, *rpb*2: 1,254–2,126 bp, *tub*2: 2,127–2,918 bp). Phylogenetic relationships were inferred by conducting analyses using both ML and BI methods. The best-scoring RAxML tree was selected to represent the relationships amongst taxa, with a final likelihood value of -20239.20371. The matrix contained 1,007 distinct alignment patterns, with a 24.36% proportion of gaps and completely undetermined characters. The estimated base frequencies of A = 0.240797, C = 0.243480, G = 0.260336, T = 0.255387; substitution rates AC = 2.098811, AG = 4.359330, AT = 1.121395, CG = 0.807926, CT = 11.035437, GT = 1.000000, gamma distribution shape parameter α = 0.756016, I = 0.553584, Tree-Length = 1.966413 (Fig. 2).

Based on the phylogenetic analyses of the combined LSU and ITS sequence data, the novel species *Phlyctema yunnanensis* (HKAS 128745 and HKAS 128746) is closely related to *P. vagabunda* (CBS 109875, CBS 304.62, and ATCC 38338) with 75% ML and 0.99 PP statistical support (Fig. 1). The multigene phylogenetic analyses of the concatenated ITS, LSU, *rpb*2, and *tub*2 sequence data showed that all representative species formed well-resolved clades in the present study. *Phlyctema yunnanensis* (HKAS 128745 and HKAS 128746) is a sister to *P. vagabunda* (CBS 109875, CBS 304.62, and ATCC 38338) with 88% ML and 1.00 PP support values (Fig. 2).

Taxonomy

Phlyctema yunnanensis Y. Gao, H. Gui & K.D. Hyde, sp. nov.

Index Fungorum number: IF 902420; **Facesoffungi numbers**: FoF 16782; Fig. 3.

Etymology: The specific epithet 'yunnanensis' refers to Yunnan Province (China), where the holotype was collected.

Holotype: HKAS 128745.

Saprobic on a decaying stem of herbaceous plants. **Sexual morph**: *Apothecia* 360–440 μm diam × 130–180 μm high ($\bar{x}$ = 410 × 160 μm, n = 10), superficial, solitary, discoid, sessile, disc circular, elliptical to irregular, slightly convex, concave when dry. *Hymenium* 70–130 μm thick ($\bar{x}$ = 105 μm, n = 25) bright yellow to yellowish-brown, receptacle near and at the margin concolorous with the hymenium, brownish at flanks and towards the base, margin entire or slightly torn. *Excipular* 30–60 μm wide ($\bar{x}$ = 44 μm, n = 25), hyaline cells of *textura angularis*. *Paraphyses* slightly swollen to 2.5–5 μm wide ($\bar{x}$ = 4 μm, n = 25) at the apex, branched, 1.5–3 μm wide ($\bar{x}$ = 2.5 μm, n = 30), wide below, septate, hyaline. *Asci* 65–90 × 8.5–13 μm ($\bar{x}$ = 77 × 10 μm, n = 25), 4-spored, bitunicate, fissitunicate, clavate, with apex rounded or blunt, hyaline. *Ascospores* 13–19 × 6–9.5 μm ($\bar{x}$ = 16 × 8 μm, n = 25), uniseriate, varied in shape, subglobose to ellipsoid or oblong to obovoid, slightly inequilateral, hyaline, aseptate, rough-walled, covered with a thin mucilagenous sheath. **Asexual morph:** Not observed.

Material examined: China, Yunnan Province, Zhaotong City, grassland (26°55'3''N, 103°8'36''E), on a decaying stem of herbaceous plants, 21 August 2021, Ying Gao, ZG16A (HKAS 128745, holotype); *ibid.*, ZG16B (HKAS 128746).

Notes: *Phlyctema yunnanensis* is introduced as a new species based on its distinct morphology and phylogenetic analysis of combined ITS, LSU, *rpb*2, and *tub*2 sequence data. The new isolate clustered with *P. vagabunda* (strains CBS 304.62, CBS 304.62, and ATCC 38338) with support in ML and BI analyses (88% ML, 100 PP; Fig. 2). A pairwise nucleotide comparison showed that *P. yunnanensis* differs from *P. vagabunda* (CBS 109875, holotype) in 3/836 bp of LSU (0.4%, without gaps) and 8/510 bp of ITS (1.6%, without gaps). The sexual morph of *P. vagabunda* (a synonym of *Pezicula alba* E.J. Guthrie) appears to have been collected only once[12,13,15]. *Phlyctema yunnanensis* differs from *P. vagabunda* in apothecia size (360–440 μm diam × 130–180 μm high vs. 1 mm diam), asci (65–90 × 8.5–13 μm, 4-spored, clavate, with a rounded apex vs. 125–150 × 13–24, 8-spored, cylindrical-clavate), and ascospores (13–19 × 6–9.5 μm, subglobose to ellipsoid or oblong to obovoide, aseptate vs. 20–30 × 7–10 μm, elongated ellipsoid, 3–5-septate)[4,12]. Therefore, based on the guideline for a polyphasic approach recommended for species boundary delimitation[42,43], *P. yunnanensis* is introduced as a novel taxon.

Discussion

The phylogenetic analysis presented in this study confirms the placement of *Phlyctema* within the *Dermateaceae*, providing robust molecular evidence for the classification of *P. yunnanensis* (Figs 1, 2). The data indicate that *P. yunnanensis* can be differentiated from other *Phlyctema* species based on both phylogenetic and morphological analyses. Similar to the concatenated ITS-LSU-*rpb*2-*tub*2 dataset phylogeny, the phylogenetic analysis of the ITS-LSU data also placed the sequences of *P. yunnanensis* into a supported subclade in both methods. To date, the only known sexual morph within *Phlyctema* is that of the type species, *P. vagabunda*[2,12,15]. Notably, *P. yunnanensis* exhibits morphological differences from *P. vagabunda*, particularly in its asci and ascospores. However, it is important to highlight that the type material of *P. vagabunda* is in relatively poor condition, which may limit the accuracy of morphological comparisons[12]. The asexual form of *P. yunnanensis* remains unidentified and warrants further investigation. In contrast, the asexual morph of *P. vagabunda* is well-documented as a pathogen responsible for bull's eye rot in apples and pears, with a prevalence reported in Australia, the USA, and Chile[44,45]. It is also implicated in causing coin canker on ash trees[46,47], and fruit spots on olives[48]. Additionally, *P.*

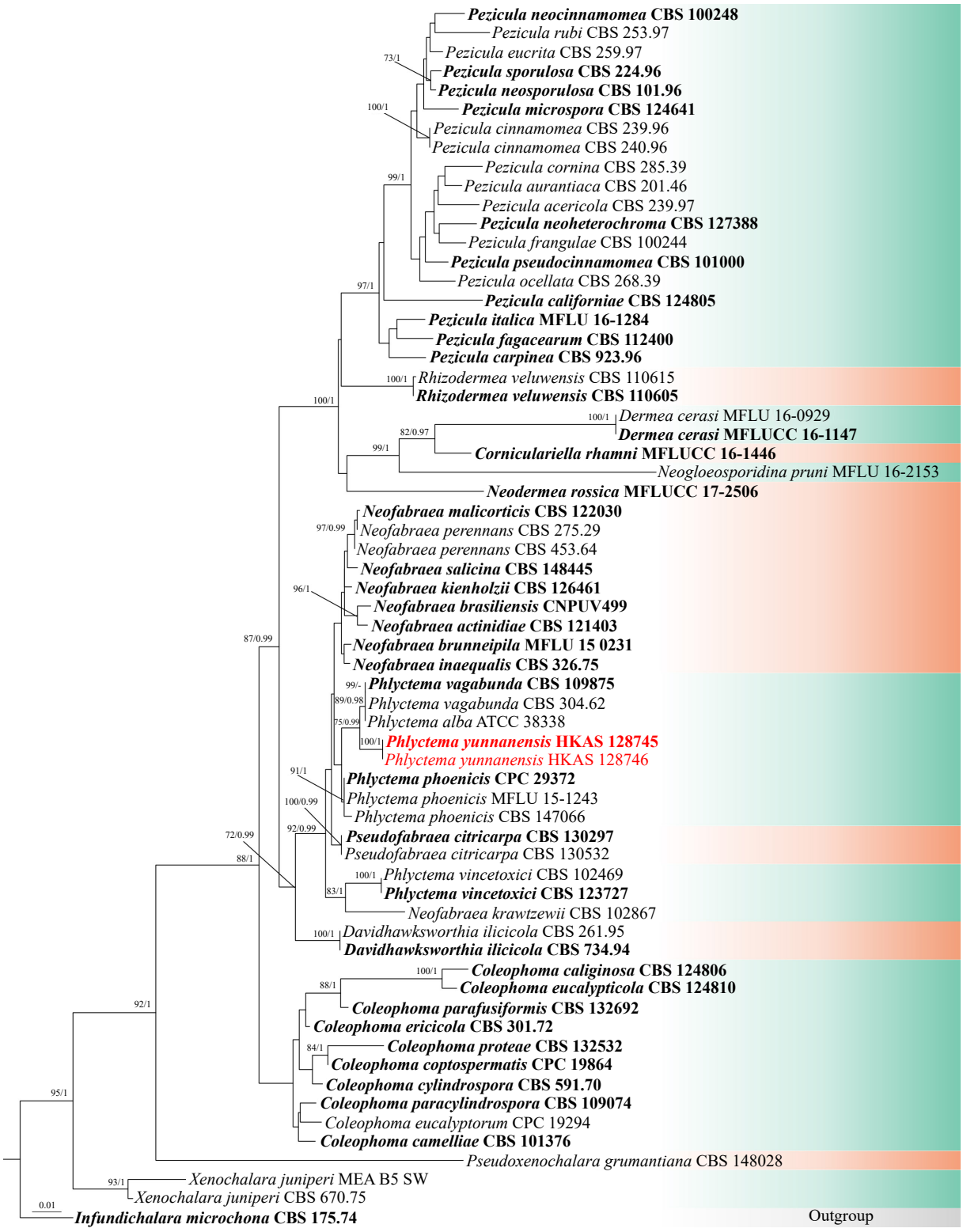

Fig. 1 Phylogram generated from ML analysis based on ITS and LSU sequence data representing *Dermateaceae*. The tree topology of the ML analysis is similar to the Bayesian analysis. Bootstrap values for ML equal to or greater than 70% and BYPP values greater than 0.90 (the rounding of values to two decimal proportions) are labeled at the nodes. Strains of the newly described species are in red, while type strains are in bold.

vagabunda has been identified as a saprobe on various woody and herbaceous plants[12].

The phylogenetic analyses reveals that *Phlyctema coronillae* clusters within *Phlyctema phoenicis* (Figs 1, 2). *Phlyctema phoenicis* was introduced by Crous et al.[49] from *Phoenix canariensis* (*Arecaceae*) in New Zealand whereas *P. coronillae* was described by Li et al.[4] from dead aerial branches of *Coronilla emerus* (*Fabaceae*) in Italy. However, Li et al.[4] did not

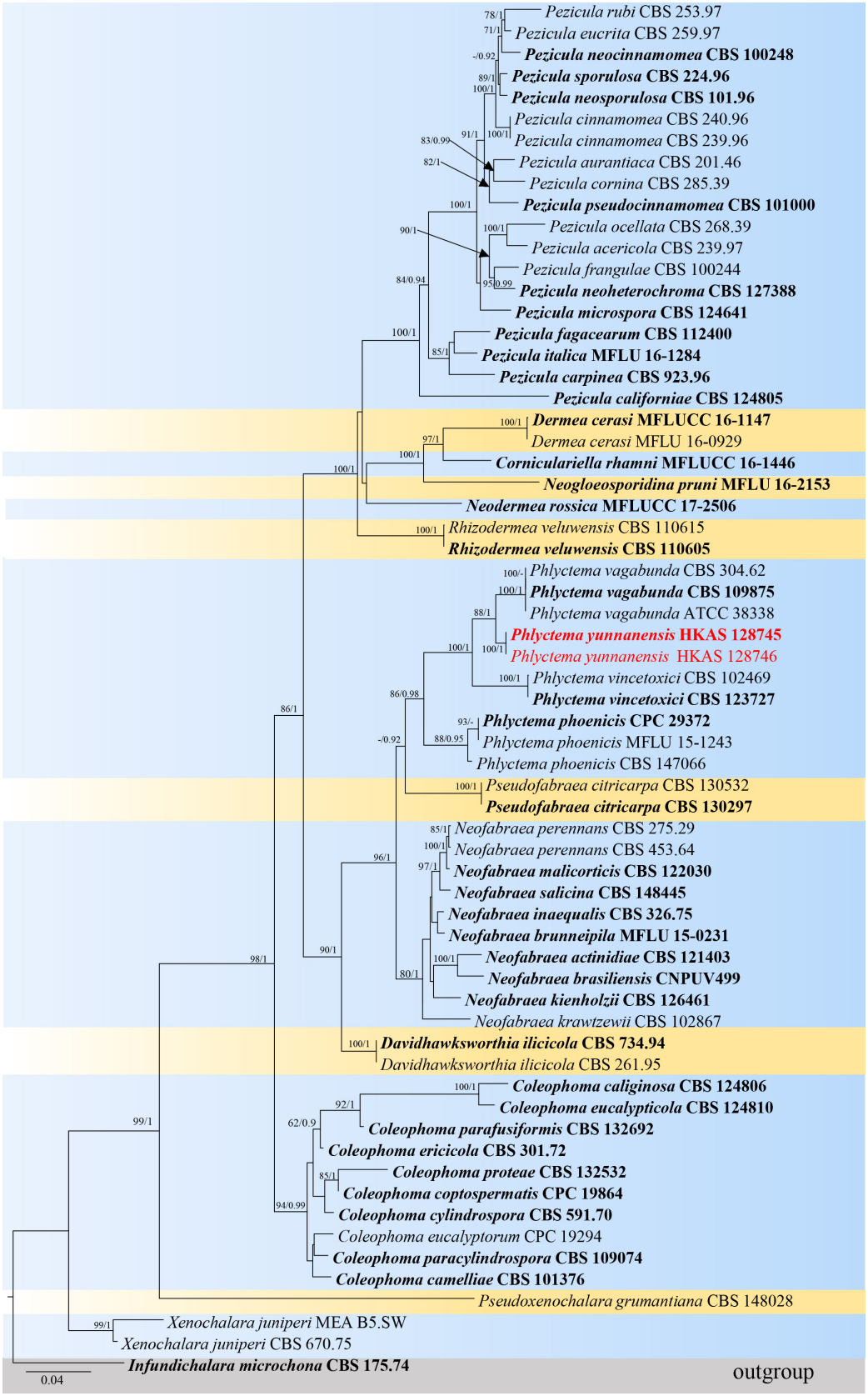

Fig. 2 Phylogenetic tree based on analysis of a combined ITS, LSU, *rpb*2, and *tub*2 sequence dataset. Bootstrap support values for ML equal to or greater than 70% and BYPP equal to or greater than 0.90 are shown as ML/BI above the nodes. The ex-type strains are in bold and the new isolate in this study is in red. The tree is rooted with *Infundichalara microchona* (CBS 175.74). The scale bar represents the expected number of nucleotide substitutions per site.

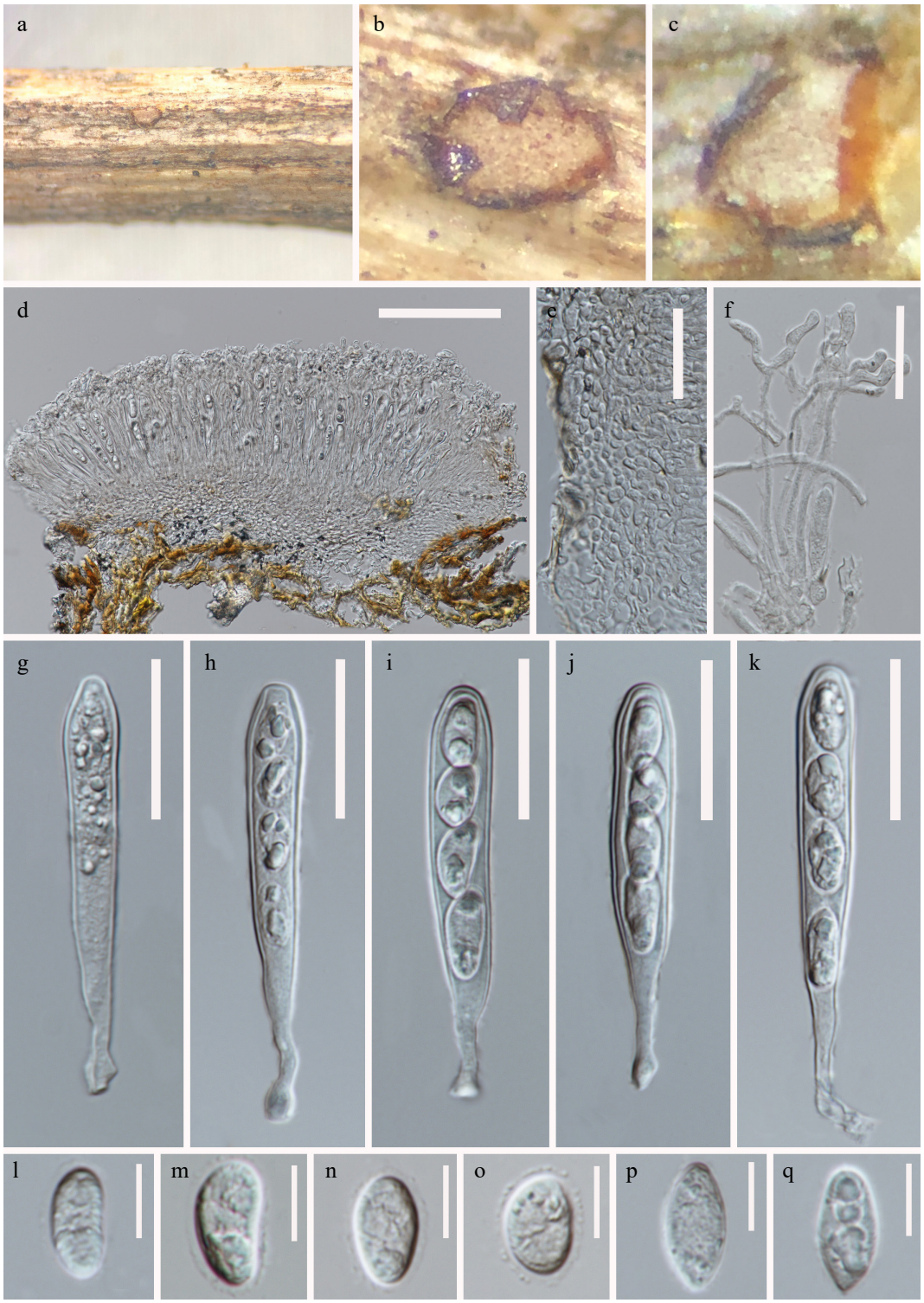

Fig. 3 *Phlyctema yunnanensis* (HKAS 128745, holotype). (a), (b) Apothecia on natural substrate. (c) Sections through apothecium. (d) Longitudinal section of apothecium. (e) Excipular structure of margin. (f) Paraphyses. (g)–(k) Asci. (l)–(q) Ascospores. Scale bars: (d) = 100 μm, (e)–(k) = 30 μm, (l)–(q) = 10 μm.

include the holotype of *Phlyctema phoenicis* in their phylogenetic analyses. Later, Crous et al.[17] re-analyzed the phylogeny of *Phlyctema* and included both *Phlyctema phoenicis* and *P. coronillae*. Despite their co-occurrence in the same clade, no explanation was provided to distinguish between these two species. To clarify their relationship, a comparative study was conducted based on molecular and morphological data. Pairwise nucleotide comparison shows that *Phlyctema phoenicis* (CPC 29372) and *P. coronillae* (MFLU 15-1243) differ by only 2/548 bp (0.36%, with 1 gap) in ITS and show no variation 0/827

bp in LSU. These minimal differences indicate no significant molecular distinction between the two taxa. Morphologically, *Phlyctema phoenicis* and *P. coronillae* are also similar[4,49]. Based on the combined molecular and morphological evidence, we propose that *Phlyctema coronillae* is a synonym of *P. phoenicis*. The present analyses also confirm that *Neofabraea*, *Phlyctema,* and *Pseudofabraea* form distinct, well-supported clades in the phylogenetic trees (Figs 1, 2). Previously, species within these genera were grouped under *Neofabraea*. However, Chen et al.[2] demonstrated significant morphological variation among species, leading to the recognition of these three as separate genera. The present phylogenetic results support this distinction, with *Neofabraea*, *Phlyctema,* and *Pseudofabraea* forming independent lineages.

In this study, the phylogenetic tree of *Dermateaceae* was updated based on the most recent literature and the available sequence data, including Iliushin & Kirtsideli[6], who introduced a new genus, *Pseudoxenochalara*. Specifically, the phylogenetic analysis of *Phlyctema* was expanded by incorporating findings from Li et al.[4] and Crous et al.[17]. The updated tree includes *Neofabraea*, *Phlyctema,* and *Pseudofabraea*, which formed three distinct clades (Figs 1, 2). This separation reflects the morphological and genetic diversity among these genera, previously grouped under *Neofabraea*[2]. The present study highlights some differences in the results derived from analyses of the ITS and LSU dataset compared to the multigene sequence dataset (ITS-LSU-*rpb2*-*tub2*). The multigene phylogenetic analysis provided better resolution, successfully delineating *Neofabraea*, *Phlyctema,* and *Pseudofabraea* as separate genera. In contrast, the ITS and LSU datasets alone resulted in unresolved clades for some species, including *Phlyctema vincetoxici*, which formed a distinct clade with *Neofabraea krawtzewii* and *Pseudofabraea citricarpa*, which clustered within the *Phlyctema* clade (Fig. 1). These results emphasize that ITS and LSU markers alone are insufficient for accurately distinguishing these genera due to limited phylogenetic resolution, necessitating the use of multigene datasets for robust analysis. The updated phylogenetic tree also provides new insights into the relationships among genera within the *Dermateaceae*. Many genera included in this study show unresolved clades in ITS and LSU-based analyses. This pattern reflects inherent limitations in single-locus analyses and reinforces the importance of multigene phylogenies. By presenting ITS and LSU phylogenetic trees alongside multigene trees, we validate that our species identification results are consistent across methods while demonstrating the added resolution provided by the latter approach.

The present study marks the first report of the sexual morph of *Phlyctema yunnanensis* on herbaceous plants from grasslands in China. The present findings enhance the taxonomic diversity within the *Phlyctema* genus and contribute to the broader understanding of fungal diversity in this group. Furthermore, several recent studies[24,25,50] have documented new species from Yunnan grasslands, suggesting a wealth of undiscovered species remains in this region, highlighting the need for continued exploration of fungal diversity.

Author contributions

The authors confirm their contribution to the paper as follows: study conception and design, data collection, draft manuscript preparation: Gao Y; analysis and interpretation of results: Gao Y, Zhong T, Wanasinghe DN; review and editing: Wanasinghe DN, Eungwanichayapant PD, Jayawardena RS, Hyde KD, Gui H. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data generated for this study are available in NCBI, Index Fungorum, and Facesoffungi databases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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