MYCOBIOLOGY
2021, VOL. 49, NO. 2, 133–141
https://doi.org/10.1080/12298093.2021.1903130

RESEARCH ARTICLE

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Multigene Phylogeny and Morphology of *Ophiocordyceps alboperitheciata* sp. nov., A New Entomopathogenic Fungus Attacking Lepidopteran Larva from Yunnan, China

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ABSTRACT

A new fungus, *Ophiocordyceps alboperitheciata*, parasitic on the larva of Noctuidae (Lepidoptera) was identified from a survey of entomopathogenic fungi in Kunming Wild Duck Forest Park, Yunnan Province, China. It can be primarily distinguished from relatives by its longer fertile parts, sterile tips, superficial perithecia, narrower asci, and smaller septa of ascospores. As revealed from phylogenetic analyses inferred from nrSSU, nrLSU, tef-1α, rpb1, and rpb2 sequence data, *O. alboperitheciata* belongs to the *Hirsutella citriformis* clade in the genus *Ophiocordyceps* of Ophiocordycipitaceae, and forms a separated clade from other related species. The uniqueness of the taxon is significantly evidenced by both molecular phylogeny and morphology. Furthermore, the interspecific relationships in the *H. citriformis* clade are discussed.

ARTICLE HISTORY
Received 6 November 2020
Revised 3 February 2021
Accepted 10 March 2021

KEYWORDS
New species; *Hirsutella*; *Ophiocordyceps*; phylogenetic analyses

1. Introduction

The entomopathogenic *Ophiocordyceps* Petch is the largest genus with the maximum number of species in the family Ophiocordycipitaceae (Hypocreales) [1–3]. The genus was originally established by Petch based on four species, i.e., the type *O. blattae* Petch occurring on a cockroach, *O. unilateralis* (Tul. & C. Tul.) Petch on ants, *O. peltata* (Wakef.) Petch on a coleopteran larva, as well as *O. rhizoidea* (Höhn.) Petch on a coleopteran larva [4]. *Ophiocordyceps* was separated from *Cordyceps sensu lato* by the existing phylogenetic classification [5], accommodating over 270 names of accepted species to date [3,6,7]. Main hosts of *Ophiocordyceps* pertain to Lepidoptera, Coleoptera, Hymenoptera, Hemiptera, Diptera, Orthoptera, and Odonata, most of which are larvae of Lepidoptera or Coleoptera inhabiting wood or soil [5,8]. Species of *Ophiocordyceps* are distributed worldwide, primarily located in temperate, subtropical to tropical areas [9]. It is noteworthy that the species diversity of *Ophiocordyceps* appears to be the maximal in East and Southeast Asia [5].

Numerous associated-asexual morphs of *Ophiocordyceps* were reported, (e.g., *Hirsutella* Pat., *Hymenostilbe* Petch, *Paraisaria* Samson & B.L. Brady, and *Synglioclad*ium Petch) [2,3,5,10,11]. On the whole, asexual types of *Ophiocordyceps* are discovered from *Hymenostilbe* and *Hirsutella*, and the latter is recognized as the most common isolation source. The genus *Hirsutella* was erected by Patouillard based on the type species *H. entomophila* Pat., as suggested to attack an adult beetle collected from Ecuador [12]. First, the genus *Hirsutella* was defined as a clavariaceous hymenomycete with simple sterigmate basidia. Subsequently, this genus was critically investigated and then identified as anamorphic insect pathogens [13]. The generic name *Hirsutella* was affected by the ending of dual nomenclature for various morphs of pleomorphic fungi in 2011 [14]. Next, Simmons et al. initially adopted the *Ophiocordyceps* name for a novel species, *O. myrmicarum* D.R. Simmons & Groden, described only from the asexual *Hirsutella* morph [15]. The suppression of the generic name could positively impact the study on those groups in phylogeny and facilitate taxonomic revisions of the family Ophiocordycipitaceae. Thus far, over 100 asexual morphs of the genus *Ophiocordyceps* were identified (Index Fungorum: http://www.indexfungorum.org), as associated with more than 30 sexual species [16]. The *Hirsutella* consists of six groups, i.e., *H. citriformis* Speare, *H. thompsonii* F.E. Fisher, *H. nodulosa* Petch, *H. guyana* Minter & B. L. Brady, *H. sinensis*

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Liu, Guo, Yu & Zang, and *Hirsutella* ant pathogen clades.

A series of surveys were conducted to reveal the species diversity of entomopathogenic fungi in Kunming, Yunnan Province, China [16–20]. To be specific, the richness of cordycipitoid fungi was found to be relatively higher in Kunming Wild Duck Lake Forest Park. On the whole, 41 species were found here (with 20 species proposed as new species), belonging to eight genera of three families (i.e., Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae), which are *Flavocillium*, *Cordyceps*, *Beauveria*, *Samsoniella*, *Simplicillium*, *Ophiocordyceps*, *Polycephalomyces*, and *Metarhizium*. Among these species, a fungus attacking caterpillar was determined as a novel taxon of *Ophiocordyceps* by conducting the analyses of both morphology and molecular phylogeny. This study attempts to introduce the new species and investigate its biological and phylogenetic status.

2. Materials and methods

2.1. Specimen collection and strain isolation

In the present study, a specimen of the novel species was collected from the Kunming Wild Duck Lake Forest Park, Yunnan Province, China, in August 2018. The isolate was obtained with the methods presented by Wang et al. [18]. The specimen was deposited in Yunnan Herbal Herbarium (YHH), Yunnan University. The cultures were deposited at Yunnan Fungal Culture Collection (YFCC), Yunnan University. To describe the new species, the macro- and micromorphological characteristics were observed by complying with Wang et al. [20].

2.2. Morphological observations

The sample was photographed with a digital camera and Olympus SZ61 (Tokyo, Japan) stereomicroscope. Subsequently, the macromorphological characteristics were recorded (e.g., texture, shape, color, length, diameter of the stroma and color, shape, length, diameter of the fertile head, and host type). Furthermore, Olympus CX40 (Tokyo, Japan) and BX53 (Tokyo, Japan) microscopes were employed to observe the micromorphological characteristics of perithecia, asci, asci-caps and ascospores Next, the morphology of cultures was characterized by using the method presented by Wang et al. [16].

2.3. DNA extraction, PCR, and sequencing

The total genomic DNAs were extracted by employing the CTAB method of Liu et al. [21]. Five nuclear gene regions were amplified and sequenced, i.e., the small subunit of ribosomal DNA (nrSSU), the large subunit of ribosomal DNA (nrLSU), translation extension factor 1-gene (tef-1α), the largest subunit of RNA polymerase II (rpb1), as well as the second largest subunit of RNA polymerase II (rpb2) [5,22,23]. Polymerase chain reaction (PCR) was performed by adopting the method presented by Wang et al. [20]. Moreover, amplifications were conducted in 25 μL, and PCR conditions were referenced from Sung et al. [5]. Furthermore, PCR products were sequenced by the Beijing Genomics Institute (Shenzhen, China).

2.4. Phylogenetic analyses

Five-gene sequences (i.e., nrSSU, nrLSU, tef-1α, rpb1, and rpb2) of taxa pertaining to *Hirsutella*, *Ophiocordyceps,* and *Polycephalomyces* were downloaded from GenBank, and combined with those generated in here. Table 1 lists the specimen accession information and GenBank numbers of the five loci. Sequences were aligned by employing the programs Clustal X2.0 and MEGA5 [24]. Phylogenetic analyses were conducted with Bayesian inference (BI) and maximum-likelihood (ML) methods with the use of the programs MrBayes v.3.1.2 and RaxML7.0.3 [25,26], respectively. In addition, the BI analysis was conducted on MrBayes v.3.1.2 for five million generations with the GTR + G + I model, as determined by jModelTest version 2.1.4 [27]. Specific to the ML analysis based on RaxML7.0.3, GTR + I acted as the optimal model, and 500 fast bootstrap replications were conducted on the five-locus dataset. Trees were sampled every 100 generations. The first 25% trees were discarded as burn-in and the remaining trees were used to create a consensus tree using the sumt demand.

3. Results

3.1. Phylogenetic analyses

In ML and BI phylogenetic analyses, five-gene sequences of eighty taxa from *Hirsutella*, *Ophiocordyceps,* and the outgroup taxa *Cordyceps tenuipes* (Peck) Kepler, B. Shrestha & Spatafora and *C. militaris* (L.) Fr. were retrieved from GenBank, which were combined with those generated in the present study. The combined dataset consisted of 4082 bp (i.e., 794 bp for tef-1α, 859 bp for nrLSU, 999 bp for nrSSU, 543 bp for rpb1, as well as 887 bp for rpb2). Phylogenetic trees analyzed by ML and BI exhibited the nearly identical overall topologies (Figure 1). The mentioned results shared similar phylogenetic structures with existing analyses [14,15,18,19]. The phylogenetic trees recognized four statistically well-supported clades in

Table 1. Specimen information and GenBank accession number for sequences used in this study.

Species	Isolate no./ specimen no.	Host	GenBank accession no.				
			nrSSU	nrLSU	tef1-α	rpb1	rpb2
Ophiocordyceps alboperitheciata	YHH 16755	Lepidoptera		MT222278	MT222279	MT222280	MT222281
Hirsutella citriformis	CHE-CNRCB 335		KY587216		KY587203	KY587213	
Hirsutella citriformis	CHE-CNRCB 339		KY587217		KY587204	KY587214	
Hirsutella citriformis	ARSEF 490	Hemiptera		KM652103	KM651987		
Hirsutella citriformis	ARSEF 591	Hemiptera		KM652104	KM651988		
Hirsutella citriformis	ARSEF 1035	Hemiptera	KM652064	KM652105	KM651989	KM652030	
Hirsutella citriformis	ARSEF 1446	Hemiptera	KM652065	KM652106	KM651990	KM652031	
Hirsutella citriformis	ARSEF 2598	Hemiptera		KM652107	KM651991		
Hirsutella citriformis	CHE-CNRCB 375		KY587218		KY587205	KY587215	
Hirsutella cryptosclerotium	ARSEF 4517	Hemiptera	KM652066	KM652109	KM651992	KM652032	
Hirsutella eleutheratorum	ARSEF 13375		MH057734		MH057732	MH057733	
Hirsutella fusiformis	ARSEF 5474	Coleoptera	KM652067	KM652110	KM651993	KM652033	
Hirsutella gigantea	ARSEF 30	Hymenoptera		JX566977	JX566980	KM652034	
Hirsutella guyana	ARSEF 878	Hemiptera	KM652068	KM652111	KM651994	KM652035	
Hirsutella haptospora	ARSEF 2226	Ixodida			KM651995	KM652036	
Hirsutella illustris	ARSEF 5539	Hemiptera	KM652069	KM652112	KM651996	KM652037	
Hirsutella kirchneri	ARSEF 5551	Ixodida	KM652070	KM652113	KM651997		
Hirsutella lecaniicola	ARSEF 8888	Hemiptera	KM652071	KM652114	KM651998	KM652038	
Hirsutella liboensis	ARSEF 9603	Lepidoptera	KM652072	KM652115			
Hirsutella necatrix	ARSEF 5549	Ixodida	KM652073	KM652116	KM651999	KM652039	
Hirsutella nodulosa	ARSEF 5473	Lepidoptera	KM652074	KM652117	KM652000	KM652040	
Hirsutella radiate	ARSEF 1369	Diptera	KM652076	KM652119	KM652002	KM652042	
Hirsutella rhossiliensis	ARSEF 3747	Tylenchida	KM652080	KM652123	KM652006	KM652045	
Hirsutella satumaensis	ARSEF 996	Lepidoptera	KM652082	KM652125	KM652008	KM652047	
Hirsutella sp.	ARSEF 8378	Hemiptera	KM652084	KM652127	KM652010	KM652049	
Hirsutella stilbelliformis var. *myrmicarum*	IMI 396397	Hymenoptera		GQ866966	GQ866964		
Hirsutella strigosa	ARSEF 2197	Hemiptera	KM652085	KM652129	KM652012	KM652050	
Hirsutella subulata	ARSEF 2227	Lepidoptera	KM652086	KM652130	KM652013	KM652051	
Hirsutella thompsonii	ARSEF 2800	Ixodida	KM652095	KM652142	KM652023	KM652058	
Hirsutella thompsonii	ARSEF 3323	Ixodida	KM652096	KM652143	KM652024	KM652059	
Hirsutella thompsonii	ARSEF 1947	Ixodida		KM652146	KM652026		
Hirsutella versicolor	ARSEF 1037	Hemiptera	KM652102	KM652150	KM652029	KM652063	
Hirsutella shennongjiaensis	GZUIFR-Snj121022			KY945357		KY945364	
Ophiocordyceps aciculari	OSC 128580	Coleoptera	DQ522543	DQ518757	DQ522326	DQ522371	DQ522423
Ophiocordyceps agriotidis	ARSEF 5692	Arthropoda	DQ522540	DQ518754	DQ522322	DQ522368	DQ522418
Ophiocordyceps aphodii	ARSEF 5498	Coleoptera	DQ522541	DQ518755	DQ522323		DQ522419
Ophiocordyceps appendiculata	NBRC 106959		JN941729	JN941412	AB968578	JN992463	AB968540
Ophiocordyceps brunneipunctata	OSC 128576	Coleoptera	DQ522542	DQ518756	DQ522324	DQ522369	DQ522420
Ophiocordyceps coenomyia	NBRC 106964		AB968385	AB968413	AB968571		AB968533
Ophiocordyceps elongata	OSC 110989	Lepidoptera		EF468808	EF468748	EF468856	
Ophiocordyceps entomorrhiza	KEW 53484	Coleoptera	EF468954	EF468809	EF468749	EF468857	EF468911
Ophiocordyceps formicarum	TNS F18565		KJ878921	KJ878888	KJ878968	KJ879002	KJ878946
Ophiocordyceps formosana	TNM F13893		KJ878908		KJ878956	KJ878988	KJ878943
Ophiocordyceps forquignonii	OSC 151908		KJ878922	KJ878889		KJ879003	KJ878947
Ophiocordyceps gracilis	EFCC 8572	Lepidoptera	EF468956	EF468811	EF468751	EF468859	EF468912
Ophiocordyceps gracilis	OSC 151906	Lepidoptera	KJ878923	KJ878890	KJ878969		
Ophiocordyceps heteropoda	EFCC 10125	Hemiptera	EF468957	EF468812	EF468752	EF468860	EF468914
Ophiocordyceps humbertii	MF116A		MK874747	MK875537		MK863828	
Ophiocordyceps humbertii	MF116B		MK874748	MK875536		MK863829	
Ophiocordyceps irangiensis	OSC 128577		DQ522546	DQ518760	DQ522329	DQ522374	DQ522427
Ophiocordyceps irangiensis	OSC 128578		DQ522556	DQ518770	DQ522345	DQ522391	DQ522445
Ophiocordyceps kniphofioides	MF90	Hymenoptera	MK874746	MK875538		MK863827	
Ophiocordyceps konnoana	EFCC 7295	Coleoptera	EF468958			EF468862	EF468915
Ophiocordyceps konnoana	EFCC 7315	Coleoptera	EF468959		EF468753	EF468861	EF468916
Ophiocordyceps lanpingensis	YHOS 0705	Lepidoptera	KC417458	KC417460	KC417462	KC417464	KC456333
Ophiocordyceps lloydii	OSC 151913		KJ878924	KJ878891	KJ878970	KJ879004	KJ878948
Ophiocordyceps longissima	EFCC 6814	Hemiptera		EF468817	EF468757	EF468865	
Ophiocordyceps myrmicarum	CG1357	Hymenoptera	MG922559	MG922561	MG922554	MG922556	
Ophiocordyceps nigrella	EFCC 9247		EF468963	EF468818	EF468758	EF468866	EF468920
Ophiocordyceps nutans	OSC 110994		DQ522549	DQ518763	DQ522333	DQ522378	
Ophiocordyceps pseudocommunis	NHJ 12581	Isoptera	EF468973	EF468831	EF468775		EF468930
Ophiocordyceps ravenelii	OSC 151914		KJ878932		KJ878978	KJ879012	KJ878950
Ophiocordyceps rhizoidea	NHJ 12522		EF468970	EF468825	EF468764	EF468873	EF468923
Ophiocordyceps rubiginosiperitheciata	NBRC 106966		JN941704	JN941437	AB968582	JN992438	AB968544
Ophiocordyceps sinensis	EFCC 7287	Lepidoptera	EF468971	EF468827	EF468767	EF468874	EF468924
Ophiocordyceps sinensis	ARSEF 6282	Lepidoptera	KM652083	KM652126	KM652009	KM652048	
Ophiocordyceps sobolifera	KEW 78842	Hemiptera	EF468972	EF468828		EF468875	EF468925
Ophiocordyceps sp.	OSC 151904		KJ878935	KJ878899	KJ878980	KJ879014	
Ophiocordyceps sp.	OSC 151909		KJ878937	KJ878900	KJ878981	KJ879016	KJ878952
Ophiocordyceps sphecocephala	OSC 110998		DQ522551	DQ518765	DQ522336	DQ522381	DQ522432
Ophiocordyceps unilateralis	OSC 128574	Hymenoptera	DQ522554	DQ518768	DQ522339	DQ522385	DQ522436
Ophiocordyceps unituberculata	YHH HU1301		KY923213		KY923215	KY923217	KY923219
Ophiocordyceps unituberculata	YFCC HU1301		KY923214		KY923216	KY923218	KY923220

(*continued*)

Table 1. Continued.

Species	Isolate no./ specimen no.	Host	GenBank accession no.				
			nrSSU	nrLSU	tef1-α	rpb1	rpb2
Ophiocordyceps variabilis	ARSEF 5365	Diptera	DQ522555	DQ518769	DQ522340	DQ522386	DQ522437
Ophiocordyceps variabilis	OSC 111003	Diptera	EF468985	EF468839	EF468779	EF468885	EF468933
Ophiocordyceps yakusimensis	HMAS 199604		KJ878938	KJ878902		KJ879018	KJ878953
Ophiocordyceps pulvinata	TNS-F-30044			GU904208	GU904209	GU904210	
Ophiocordyceps crinalis	GDGM 17327		KF226253	KF226254	KF226256	KF226255	
Cordyceps militaris	OSC 93623		AY184977	AY184966	DQ522332	DQ522377	
Cordyceps tenuipes	TBRC 7265			MF140707	MF140827	MF140776	MF140800

Ophiocordyceps, designated here as *Ophiocordyceps* clades A (the *Hirsutella* clade), B (the *O. sobolifera* clade), C (the *O. ravenelii* clade), and D (the *O. sphecocephala* clade) (Figure 1). The *Ophiocordyceps* clade A (*Hirsutella* clade) consisted of six major subclades, i.e., *H. nodulosa*, *H. citriformis*, *H. thompsonii*, *H. guyana*, *H. sinensis*, as well as *Hirsutella* ant pathogen clades. As revealed from phylogenetic analyses, the new species *O. alboperitheciata* clustered into the *H. citriformis* subclade and isolated a distinct clade from other related species with 100% statistical support.

3.2. Taxonomy

Ophiocordyceps alboperitheciata H. Yu, Q. Fan & Y.B. Wang, sp. nov. (Figure 2).

MycoBank: MB 834082

Etymology: *alboperitheciata*, indicating to the color of perithecia from the type specimen, "*albo*" means white.

Typus: China. Yunnan Province: Kunming City, the Wild Duck Forest Park, at 25°13′N, 102°87′E, alt. 2100 m, on a larva of Noctuidae (Lepidoptera) buried in fallen leaves, 12 August 2018, Hong Yu (holotype, YHH 16755; ex-holotype living culture, YFCC 7203).

Sexual morph: Stromata arising in pairs from the larva of Noctuidae buried in fallen leaves, cylindrical, flexible, light brown to dark brown, unbranched, gradually tapering toward the apex, 69–71 × 0.6–1.2 mm, with a sterile tip, remaining unchanged in 3% KOH. Stipes cylindrical, smooth, dark brown, 0.6–1.2 mm wide. Fertile parts clavate, pale brown, covered by a spinous surface, reaching up to 4.1–4.5 × 0.8–1.4 mm. Perithecia superficial, subtranslucent, scattered or crowded, nearly ovoid, white to pale brown, exhibiting an unequal distribution on the middle of the stromata, covering densely the lower part and aggregating loosely at the upper of stromata, arranged in a disordered manner, 408–549 × 233–321 µm. Asci hyaline, cylindrical, eight-spores, 144–246 × 3.5–4.7 µm, with a hemispheric apical cap of 3.2–4.2 × 2.3–2.5 µm. Ascospores hyaline, cylindrical, multiseptate, 0.5–0.6 µm diameter, with septa of 1.1–1.3 µm long. Part-spores were not examined.

Asexual morph: Colonies on PDA growing very slowly, exhibiting 3.0–3.6 cm diameter in 21 days at 25 °C, fan split, dark brown at the centrum, and white at the edge. Reverse dark brown. Hyphae hyaline, branched, septate, smooth-walled, 1.37–2.05 µm wide. Conidiogenous cells and conidia were not detected.

Host: Larva of Noctuidae (Lepidoptera).

Habitat: Buried in fallen leaves.

Type locality: The Wild Duck Lake Forest Park, Kunming City, Yunnan Province, China.

4. Discussion

The particularity of *O. alboperitheciata* is revealed by morphological and ecological comparisons with eight other closely related species that possess *Hirsutella* morphs (Table 2). *Ophiocordyceps alboperitheciata* is noticeably inconsistent with eight other related species of *H. citriformis* clade in five aspects: (1) its fertile parts are long, rod-shaped, 4.1–4.5 × 0.8–1.4 mm; (2) its perithecia are superficial and scattered or crowded, which is nearly white; (3) its asci are slender; (4) its septa of ascospores are smaller; (5) it is associated with the larva of Noctuidae buried in fallen leaves. In the *H. citriformis* clade, sexual morphs of species have been rarely reported, except for *O. elongata* and *O. humbertii* Petch [13,28,29]. It is noteworthy, *O. alboperitheciata* and *O. elongata* are closely clustered together, whereas the latter exhibits greater sizes of stromata (110 mm long), asci (220 × 8 µm), ascospore septa (4–12 µm long), and immersed perithecia. *Ophiocordyceps alboperitheciata* synthesizes relatively shorter stromata (54–65 mm long) with sterile tips and fertile parts, stromata in pairs, superficial perithecia, shorter asci (144–246 × 3.5–4.7 µm), as well as ascospore septa (1.1–1.3 µm). As revealed from the mentioned distinct features above indicated that *O. alboperitheciata* was considerably different from other related species. The hosts comprised five orders of insects in the *H. citriformis* clade, in which *O. alboperitheciata*, *O. elongate,* and *H. gigantean*

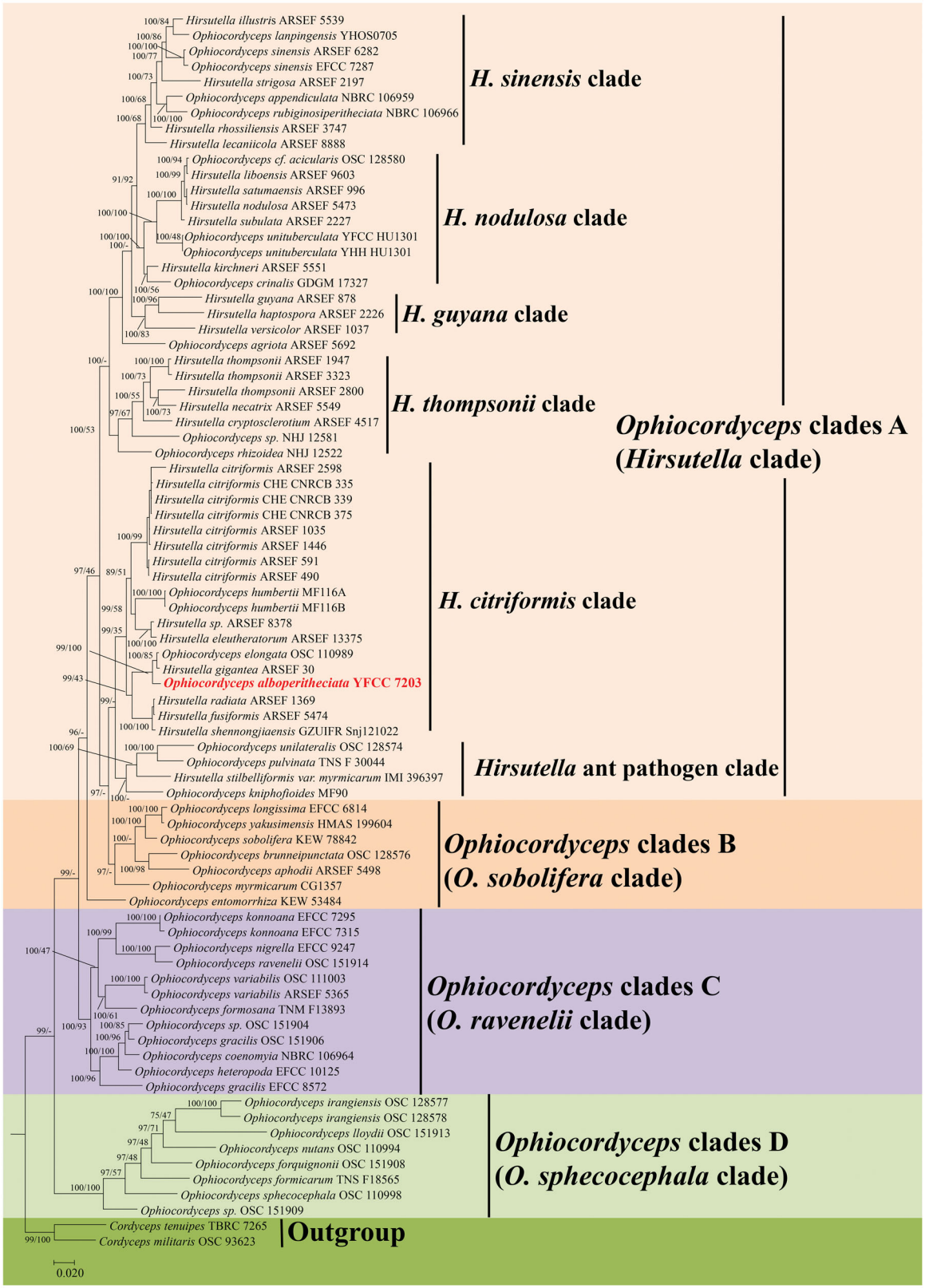

Figure 1. Phylogenetic placement of *Ophiocordyceps alboperitheciata* infered from BI and ML analyses based on five-gene (nrSSU, nrLSU, tef-1α, rpb1, and rpb2) sequence dataset. Values at the nodes before and after the backslash are BI posterior probabilities and ML bootstrap proportions, respectively. Support values of ML bootstrap proportions greater than 40% are indicated at the nodes.

clustered together and linked to Lepidoptera, other six species displayed the respective association with Hymenoptera, Hemiptera, Diptera, Orthoptera, Dermaptera, and Anoplura.

The family Ophiocordycipitaceae was proposed according to the type genus *Ophiocordyceps* with the sexual morph characterized by the production of whole septate ascospores, which usually did not

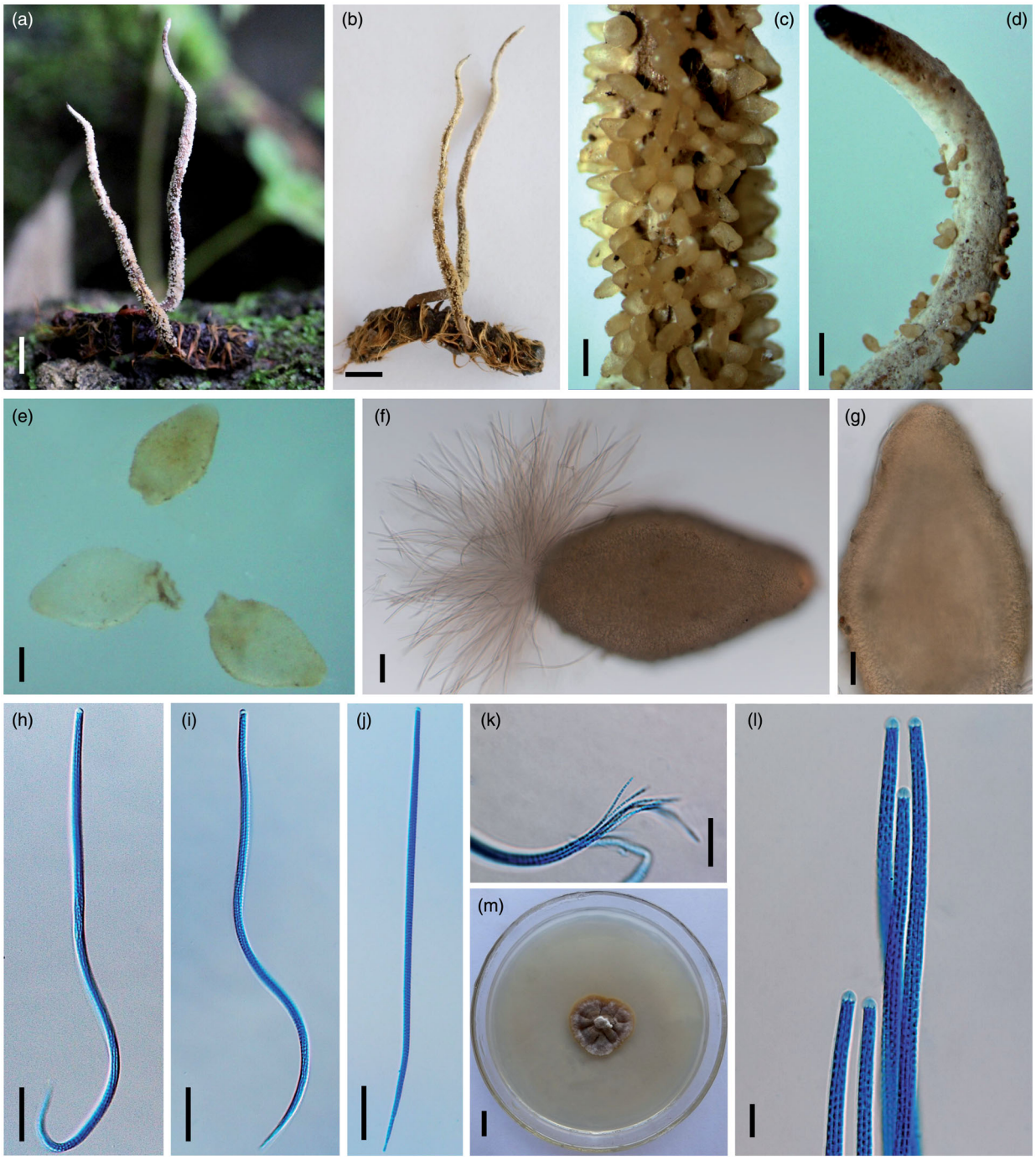

Figure 2. Morphological characteristics of *Ophiocordyceps alboperitheciata*. (a, b) Stromata on a larva of Noctuidae; (c) Fertile part; (d) Sterile tip; (e–g) Perithecia; (h–j, l) Asci; (k) Ascospores; (m) Clony on PDA. Scale bars: a, b = 1 cm; c = 600 µm; d = 1 mm; e–f = 100 µm; g–j = 50 µm; k, l = 20 µm; m = 1 cm.

disarticulate into part-spores at maturity, and asci had an apical hemispheric cap. The *Hirsutella*, as the old asexual generic name associated with the *Ophiocordyceps*, is synonymized under *Ophiocordyceps*, most species occurring from adult insects are formerly employed in the *Ophiocordyceps* clade A [1,3,5]. Phylogenetic studies of *Hirsutella* species from the USA were conducted by three loci providing evidence for taxonomic revisions under novel rules [14,15]. The available molecular data have facilitated the use of the mentioned fungi and associated data to conduct in-depth phylogenetic classification studies on *Hirsutella* and *Ophiocordyceps*. The phylogenetic tree of *Hirsutella* and *Ophiocordyceps* of this study complies with the existing studies of Ophiocordycipitaceae [5,10,14,15]. The genus *Ophiocordyceps* with *Hirsutella* morph comprises six distinct groups, i.e., *H. citriformis*, *H. thompsoni*, *H. nodulosa*, *H. guyana*, *H. sinensis,* and *Hirsutella* ant pathogen clades. The insect pathogen *O. alboperitheciata* pertains to the *H. citriformis* clade, which is obviously separated from other allied species.

The present phylogenetic tree covers nine species cluster in the *H. citriformis* clade. Our result is

Table 2. A morphological comparison of *Ophiocordyceps alboperitheciata* and its related species.

Species	Host	Habitat	Synnemata/stromata	Perithecia	Asci	Ascospores	Conidiogenous cells	Conidia	References
O. alboperitheciata	Larva of Noctuidae (Lepidoptera)	Buried in fallen leaves	Stromata in pairs, rigid, the stalk is smooth, unbranched, long 54–65 mm, light brown to dark brown, with a clavate fertile part, white to light brown, 4.1–4.5 × 0.8–1.4 mm, and a sterile tip.	Perithecia superficial, scattered or crowded, size 0.41–0.55 × 0.23–0.32 mm, nearly ovoid, white nearly light brown.	Asci hyaline, cylindrical, 8-spores, 144–246 × 3.5–4.7 μm, with a hemispheric apical cap, 3.2–4.2 × 2.3–2.5 μm.	Ascospores hyaline, cylindric, multiseptate, 0.5–0.6 μm diameter, with septa 1.1–1.3 μm apart. part-spores were not seen.	Undetermined	Undetermined	This study
O. elongata	Pupae and larvae of *Apalela americana* (Lepidoptera).	Unknown	The stalk is flexuose, longitudinally sulcate and twisted, 110 mm long, pale brown.	The perithecia are immersed, scattered or crowded, ovato-conoid, size 0.5 × 0.3 mm, apex subacute, wall yellow by transmitted light.	The asci are 220 μm long, 8 μm diameter.	Ascospores cylindric, 2 μm diameter, with septa 4–12 μm apart. Part-spores were not seen.	Unknown	Unknown	[28]
O. humbertii	Hymenoptera	Unknown	Several, 7 mm long, dark brown, with an oval swelling, 1 × 0.4 mm.	Perithecia, scattered, dark amber, subtranslucent, flask-shaped with a truncate apex, 275 × 120 μm.	The asci are 130 μm long, 10 μm diameter, capitate, fusoid or narrow-clavate.	Ascospores are 75 μm long, 25 μm diameter, narrow-fusoid, septate at intervals of 6–16 μm, not dividing into part-spores.	Unknown	Unknown	[29]
H. gigantea	Pupae and larvae of Apatela Americana (Lepidoptera)	On wood	Branched, longitudinally sulcate, glabrous, ashy and minutely setose above, size 40 × 0.6 mm, brown below.	None	None	None	Phialider up to 40 μm high, with a flask-shaped base, 16–20 × 8–9 μm, and a long, stout sterigma, 1 μm diameter.	The spore cluster is lemon-shaped, 10 × 6 μm, becoming globose, 10 μm diameter, and the separate conidia are broadly cymbiform with obtuse tips, 9–10 × 3–4 μm	[28]
H. citriformis	Adult of Fulgoridae (Hemiptera)	Unknown	Synnemata usually long, flexible, simple or branched, branches often short and stumpy, and easily detached, brown in color	None	None	None	Sporophores simple, sessile or subsessile, with rather short, delicate sterigmata 20–30 μm	Spores fusoid, hyaline, 5.5–8.5 × 1.5–18 μm	[13]
H. radiata	Fly (Diptera)	Unknown	Rrigid, branched, size 18–19 mm, dark brown or rufous brown, cinercous toward the tips, with a matt surface	None	None	None	The phialides have a conical base, 5–8 × 3–4 μm, merging into a stout sterigmata, 9–14 μm long, or a cylindrical base, 6–18 × 2 μm, with a sterigmata 6 μm long	The spore cluster is oval, 9–11 × 6–7 μm, and the individual conidia are cymbiform, 6–9 × 2–2.5 μm, or oval, 7–8 × 3–4 μm	[28]

(*continued*)

Table 2. Continued.

Species	Host	Habitat	Synnemata/stromata	Perithecia	Asci	Ascospores	Conidiogenous cells	Conidia	References
H. fusiformis	Cricket adult (Orthoptera)	Unknown	Synnemata erect, straight, unbranched, uniform in height, measuring 4–5 mm, nearly black in color	None	None	None	Sporophores simple, sessile, the inflated basal portion tapering gradually to rather short 25–35 µm sterigmata	Spores fusoid cylindrical, hyaline, size 9–10 × 2 µm	[28]
H. shennongjiaensis	Earwigs (Dermaptera)	Unknown	Synnemata cylindrical, size 60.0 × 1.0–2.0 mm, brown	None	None	None	Conidiogenous cells solitary, phialides cylindrical or awl-like, 14.4–26.1 or 6.3–14.4 µm	Conidia hyaline, aseptate, smooth, sausage-shaped, single or double from the apex of the neck, 6.3–10.8 × 3.6–6.3 µm	[30]
H. eleutheratorum	Colaoptera (Anoplura)	Unknown	Synnemata simple or branching, 3–5 mm, cinereous to violaceous gray to dull brown, often paler at the apex	None	None	None	Conidiogenous cells ellipsoid, base 8–10 × 5–6 µm, tapering rather abruptly into a long neck, 30–35 µm long	Conidia cymbiform to narrow ellipsoid, 4–7 × 1–2 µm, forming citriform clusters 8 × 6 µm	[31]

consistent with existing findings, i.e., *H. radiata, H. fusiformis,* and *O. shennongjiaensis,* and *H. gigantean* and *O. elongate* group cluster closely, respectively [14,15,32]. Three species, i.e., *O. alboperitheciata, O. elongate*, and *H. gigantea*, are closely clustered together, whereas they are noticeably inconsistent with each other in morphological and ecological characteristics. According to both molecular phylogeny and morphology, a consistent relationship between *O. alboperitheciata* and other relatives in the *H. citriformis* clade is evidenced. Thus, the novel species *O. alboperitheciata* is proposed in genus *Ophiocordyceps.*

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was funded by the National Natural Science Foundation of China [No. 31760011 and 31870017], and the Department of Science and Technology of Yunnan Province [No. 2018FY001(-006)].

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