RESEARCH ARTICLE

First Report of the Entomopathogenic Fungus *Cordyceps koratensis* on Larvae of Lepidoptera in Korea

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ABSTRACT

The genus *Cordyceps*, a group of entomopathogenic fungi, comprises approximately 750 identified species, mainly distributed in Asia, Europe, and North America. During our investigation in 2024, fungal strains were isolated from larvae of Lepidoptera infected with entomopathogenic fungi on a Perilla farm in Korea. Fungal strains from the infected larvae were isolated and identified as *Cordyceps koratensis* based on their morphological traits and multi-locus phylogenetic analysis of the internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU), translation elongation factor 1-alpha (*TEF*), and the largest (*RPB1*) and second-largest (*RPB2*) subunits of RNA polymerase II loci. To our knowledge, this is the first report of *C. koratensis* infection in Korea. These findings may support the development of biocontrol agents within the framework of eco-friendly pest management for *C. koratensis*.

Keywords: *Cordyceps koratensis*, Entomopathogenic fungi, Identification

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INTRODUCTION

Entomopathogenic fungi (EPF) are microbiological insecticides with a broad host range [1,2]. Some EPF, such as *Beauveria bassiana* (Balsamo) Vuill (Hypocreales: Cordycipitaceae), *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha, Spatafora (Hypocreales: Cordycipitaceae), and *Cordyceps javanica* (Frieder. & Bally) Kepler, B. Shrestha & Spatafora, have been commercially produced as biocontrol agents [3,4]. Infection begins with the attachment of conidia or blastospores to the host cuticle, followed by the growth and penetration of the fungus through the host integument [5]. Fungal growth blocks the host's digestive and circulatory systems and produces toxins, leading to host death; eventually, aerial conidia are formed on the cadaver and disperse to infect new hosts [6,7]. However, the virulence of fungal strains may vary considerably both between and within species [8,9].

Cordyceps is a well-known genus of entomopathogenic fungi, with at least 700 known species. *Cordyceps* species reproduce through sexual (ascospores) or asexual (conidia) spores or both [10]. The

name *Cordyceps* derives from the Greek term "kordyle", which means "club", and the Latin etymon "ceps", which means "head" [11]. *Cordyceps* species invade insects and arthropods, and evade the host immune system by harmonizing the life cycle of their hosts with the intention of survival and multiplication. Many *Cordyceps* species play a significant role in the cycling of matter in ecological systems, have high environmental and economic value for biocontrol and bioactive compounds, and serve as a model system for research on fungal-insect pathology [12,13]. Known hosts of *Cordyceps* span seven orders of Arthropoda: Araneae, Coleoptera, Dermaptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera. Among them, Coleoptera and Lepidoptera are the two most significant host orders beyond the estimated 200 *Cordyceps* species [14–17].

A molecular phylogenetic investigation of Clavicipitaceae, with an emphasis on *Cordyceps*, was conducted by Sung et al. [18] and revealed that both Clavicipitaceae and *Cordyceps* were not monophyletic. Two additional families, Cordycipitaceae and Ophiocordycipitaceae, were recognized, and species previously classified as *Cordyceps* were supported as members of all three families. Clavicipitaceae and Ophiocordycipitaceae collectively formed a monophyletic group, whereas Cordycipitaceae, defined by the phylogenetic position of the type species of *Cordyceps*, *C. militaris*, shared a more recent common ancestor with Hypocreaceae [15]. The classification of *Cordyceps* species has been conventionally based on morphology [18]. However, significant changes in the taxonomy of *Cordyceps* have occurred since research on EPF entered the molecular era. Currently, multilocus phylogenetic analyses have gained importance for delimiting *Cordyceps* species [17,19–22].

In 2024, an EPF Lepidoptera larva was discovered in a Perilla field in Korea. The infected specimens were completely covered with mycelia, suggesting the presence of a potentially novel EPF species that was previously unreported in Korea. This study aimed to identify the fungal species using morphological characteristics and to provide the first report of this EPF species in Korea.

MATERIALS AND METHODS

Sample collection and fungal isolation

Lepidopteran larvae infected with an entomopathogenic fungus were collected from a perilla (*Perilla frutescens* L. Britton) farm in Gwangju, Korea (N 35.185835° E 126.783697°). Isolation was performed using a tissue transplantation technique. The samples were washed with sterile distilled water and sectioned into three pieces. The inner tissues of larvae were cut (5 × 5 mm^2), surface-sterilized by dipping in 1% sodium hypochlorite (NaOCl) for 1 min, and rinsed several times with sterile distilled water before being transferred onto the surface of potato dextrose agar (PDA). The mycelium growing out of the moth larvae tissue was sub-cultured on PDA and incubated at 25°C for 14 days. All single-spore cultures were transferred to PDA slants and preserved at 4°C. One of the isolated strains was deposited at the Korean Agricultural Culture Collection (KACC410975).

DNA extraction, polymerase chain reaction amplification, and sequencing

Fungal mycelia were scraped from 14-day-old cultures grown on PDA plates incubated at 25℃. Approximately 50 mg of fresh mycelia was used for DNA extraction using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

The primer pairs ITS5/ITS4, LROR/LR5, EF1-983F/EF1-2218R, RPB1AF/RPB1C, and RPB2-5f2/RPB2-7cr were used to amplify the internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU), translation elongation factor 1-alpha (*TEF*), and the largest (*RPB1*) and second-largest (*RPB2*) subunits of RNA polymerase II, respectively (Table 1). Each polymerase chain reaction (PCR) volume (25 μL) consisted of 12.5 μL MyTaq HS Mix, 1 μL (10 pmol) of each primer, 8.5 μL nuclease-free water, and 2 μL DNA template (100 ng/μL). PCR reactions were performed in an MJ Research PTC-200 Thermal Cycler (MJ Research, Ramsey, MN, USA) with an initial denaturation step at 94℃ for 5 min, followed by 30 cycles: denaturation at 94℃ for 30 s; annealing at 52℃ (ITS, LSU), 56℃ (*RPB1*, *RPB2*), and 54℃ (*TEF*) for 30 s; extension at 72℃ for 1 min; and a final extension at 72℃ for 10 min. PCR products were checked by gel electrophoresis before being sent to Macrogen (Seoul, Korea) for sequencing using the amplified primer pairs.

Table 1. Primer pairs used for PCR amplification and sequencing in this study

Gene	Product name	Primers	Direction	Sequences (5′–3′)	References
ITS	Internal transcribed spacer	ITS5	Forward	TCC GTA GGT GAA CCT GCG G	White et al. (1990)
		ITS4	Reverse	TCC TCC GCT TAT TGA TAT GC	White et al. (1990)
LSU	Nuclear ribosomal large subunit	LROR	Forward	ACC CGC TGA ACT TAA GC	Vilgalys & Hester (1990)
		LR5	Reverse	TCC TGA GGG AAA CTT CG	Vilgalys & Hester (1990)
TEF	Translation elongation factor 1α	EF1-983F	Forward	GCY CCY GGH CAY CGT GAY TTY AT	Rehner & Buckley (2005)
		EF1-2218R	Reverse	ATG ACA CCR ACR GCR ACR GTY TG	Rehner & Buckley (2005)
RPB1	RNA polymerase II	RPB1AF	Forward	GAR TGY CCD GGD CAY TTY GG	Matheny et al. (2002)
		RPB1C	Reverse	CCN GCD ATN TCR TTR TCC ATR TA	Matheny et al. (2002)
RPB2	RNA polymerase II	RPB2-5f2	Forward	GGG GWG AYC AGA AGA AGG C	Sung et al. (2007)
		RPB2-7cr	Reverse	CCC ATR GCT TGY TTR CCC AT	Liu, Whelen, & Hall (1999)

ITS, internal transcribed spacer; LSU, large subunit ribosomal RNA; *TEF*, translation elongation factor 1-alpha, *RPB1*, largest subunits of RNA polymerase II; *RPB2*, second-largest subunits of RNA polymerase II.

Phylogenetic analyses

The sequence datasets from each locus were individually aligned using the multiple sequence alignment program MAFFT version 11 (https://mafft.cbrc.jp/alignment/server/) with the G-INS-1 option. Poor terminal alignments were trimmed and further refined by visual inspection using MEGA 11 [23], followed by concatenation. Subsequently, a maximum likelihood (ML) phylogenetic tree was constructed using concatenated ITS, LSU, *TEF*, *RPB1*, and *RPB2* sequences. IQ-TREE was used for phylogenetic analysis, and the model employed was the best-fit model "TIM2 + F + I + G4" with 1,000 ultrafast bootstrap replicates. The sequence datasets included sequences from KACC 410975, 34 reference species in the

genus *Cordyceps*, and the outgroup (*Simplicillium lanosoniveum* CBS 704.86). Information on the reference sequences is provided in Table 2. All sequences obtained in this study were deposited in RDA-GenBank (http://genebank.rda.go.kr).

Table 2. List of species and GenBank accession numbers of sequences used in this study

Species	Strain no.	GenBank Accession no.				
		ITS	LSU	TEF	RPB1	RPB2
Cordyceps albocitrina	spat 07.174	–	–	MF416467	MF416629	–
Cordyceps amoenerosea	CBS 107.73^{T}	AY624168	MG665224	MF416494	MF416651	MG665234
Cordyceps bifusispora	EFCC 5690	–	EF468806	EF468746	EF468854	EF468909
Cordyceps blackwelliae	TBRC 7256^{T}	MF140736	MF140702	MF140822	MF140771	MF140795
Cordyceps cateniannulata	CBS 152.83^{T}	AY624172	MG665226	JQ425687	–	–
Cordyceps cateniobliqua	CBS 153.83^{T}	AY624173	–	JQ425688	–	MG665236
Cordyceps cf. *ochraceostromata*	ARSEF 5691	JN049849	EF468819	EF468759	EF468867	EF468921
Cordyceps cf. *takaomontana*	NHJ 12623	–	EF468838	EF468778	EF468884	EF468932
Cordyceps coleopterorum	CBS 110.73^{T}	AY624177	JF415988	JF416028	JN049903	JF416006
Cordyceps exasperata	MCA 2155	–	MF416542	MF416486	MF416643	–
Cordyceps farinosa	CBS 111113^{T}	AY624181	FJ765253	GQ250022	MF416656	GU979973
Cordyceps fumosorosea	CBS 107.10^{T}	AY624184	MG665227	HM161735	–	MG665237
Cordyceps ghanensis	CBS 105.73^{T}	AY624185	MH872340	–	–	–
Cordyceps javanica	CBS 134.22^{T}	NR111172	MF416558	MF416504	MF416661	MF416455
	NTUPPMCC 18-115	–	MT974284	MW025858	MW025904	MW025940
	NTUPPMCC 18-116	–	MT974309	MW025881	–	–
	TBRC 7259	MF140745	MF140711	MF140831	MF140780	MF140804
	TBRC 7260	MF140744	MF140710	MF140830	MF140779	MF140803
	TBRC 7261	MF140743	MF140709	MF140829	MF140778	MF140802
	TBRC 7262	MF140746	MF140712	MF140832	–	MF140733
Cordyceps kintrischica	ARSEF 7218^{T}	EU553278	–	GU734751	–	–
Cordyceps koratensis	NHJ 666.01^{T}	GQ250010	GQ249981	GQ250031	–	–
	KACC 410975	**PV158245**	**PV158246**	**PV165885**	**PV165886**	**PV165887**
Cordyceps lepidopterorum	TBRC 7263^{T}	MF140765	MF140699	MF140819	MF140768	MF140792
Cordyceps locastrae	NTUPPMCC 17-042^{T}	MT966044	MT974256	MW025837	MW025883	MW025917
Cordyceps polyarthra	MCA 996	–	MF416543	MF416487	MF416644	–
Cordyceps pseudotenuipes	YFCC 8404^{T}	–	OL468579	OL473527	OL739573	OL473538
Cordyceps qingchengensis	MFLU 17-1022^{T}	–	MK761211	MK770630	–	–
Cordyceps rosea	spat 09.053	–	MF416536	MF416480	MF416637	MF416442
Cordyceps siangyangensis	NTUPPMCC 18-149^{T}	MT966072	MT974299	MW025871	MW025910	–
Cordyceps simaoensis	YFCC 8406^{T}	–	OL468581	OL473529	OL739575	OL473540
Cordyceps spegazzinii	ARSEF 7850	DQ196435	DQ196435	GU734752	–	–
Cordyceps subtenuipes	YFCC 6051^{T}	–	MN576775	MN576945	MN576835	MN576891
Cordyceps takaomontana	BCC 12688	EU807996	MF416545	MF416489	MF416646	–
Cordyceps tenuipes	ARSEF 5135^{T}	AY624196	JF415980	JF416020	JN049896	JF416000
Cordyceps tiankengensis	KY11141^{T}	ON502831	ON502824	ON525440	–	–
Cordyceps yaoluopingensis	CGMCC 23076^{T}	ON311002	ON311006	ON314456	ON314458	ON314454
Simplicillium lanosoniveum	CBS 704.86	AJ292396	AF339553	DQ522358	DQ522406	DQ522464

ITS, internal transcribed spacer; LSU, large subunit ribosomal RNA; *TEF*, translation elongation factor 1-alpha, *RPB1*, largest subunits of RNA polymerase II; *RPB2*, second-largest subunits of RNA polymerase II.
The accession numbers marked with T refer to sequences from ex-type strain. The sequences generated in this study are in bold.

Morphological observations

For morphological observation, the strain was grown on PDA and incubated at 25°C in the dark for 14 days. At the end of the incubation period, initial investigations included recording the colony diameters and specific characteristics. Colonies grown on PDA were examined microscopically. Slides were prepared by mounting with lactic acid and were observed under a Zeiss AXIO Imager A1 microscope with differential interference contrast (DIC) illumination equipped with a digital AxioCam ICc3 camera (Carl Zeiss, Oberkochen, Germany). The recorded microscopic characteristics included size, shape, conidial pigmentation, and conidiogenic properties.

RESULTS

Phylogenetic and morphological analyses

The phylogenetic position of strain KACC 410975 was analyzed using the concatenated sequence data of the ITS, LSU, *TEF*, *RPB1*, and *RPB2* loci (Fig. 1). The concatenated dataset alignment contained 4034 characters. The concatenated alignment consisted of 555 characters from ITS, 860 from LSU, 1006 from *TEF*, 711 from *RPB1,* and 902 from *RPB2*. During the BLASTn analyses of the sequences, the KACC 410975 sequence of ITS, LSU, and *TEF* showed 100% similarity with *C. koratensis* NHJ 666.01^{T}.

Phylogenetic analysis indicated that the isolate KACC 410975 was well clustered with the ex-type strain NHJ 666.01 of *C. koratensis*, supported by an ML bootstrap value of 97%, and was a sister of *C. javanica*.

The phylogenetic tree indicated that strain KACC 410975, an ex-type strain of *C. koratensis* (NHJ 666.01), and some strains (NTUPPMCC 18-115, NTUPPMCC 18-116, TBRC 7259, TBRC 7260, TBRC 7261, and TBRC 7262) previously regarded as *C. javanica* [26] were clustered in the same clade, supported by an ML bootstrap value of 97%. They are genetically distinct from *C. javanica* (ex-type strain CBS 134.22).

The morphological characteristics of KACC 410975 resembled those of *C. koratensis* (syn. *Parahevansia koratensis*, *Hevansia koratensis*, *Akanthomyces koratensis*) described by Hywel-Jones [24], as follows: phialides in a monolayer, single on basal lateral cells of synnemata, crowded, obovoid to ellipsoid with distinct necks.

Based on molecular phylogenetic and morphological analyses, the causative agent in lepidopteran larvae was identified as *C. koratensis*. This represents the first confirmed report of this species in Korea.

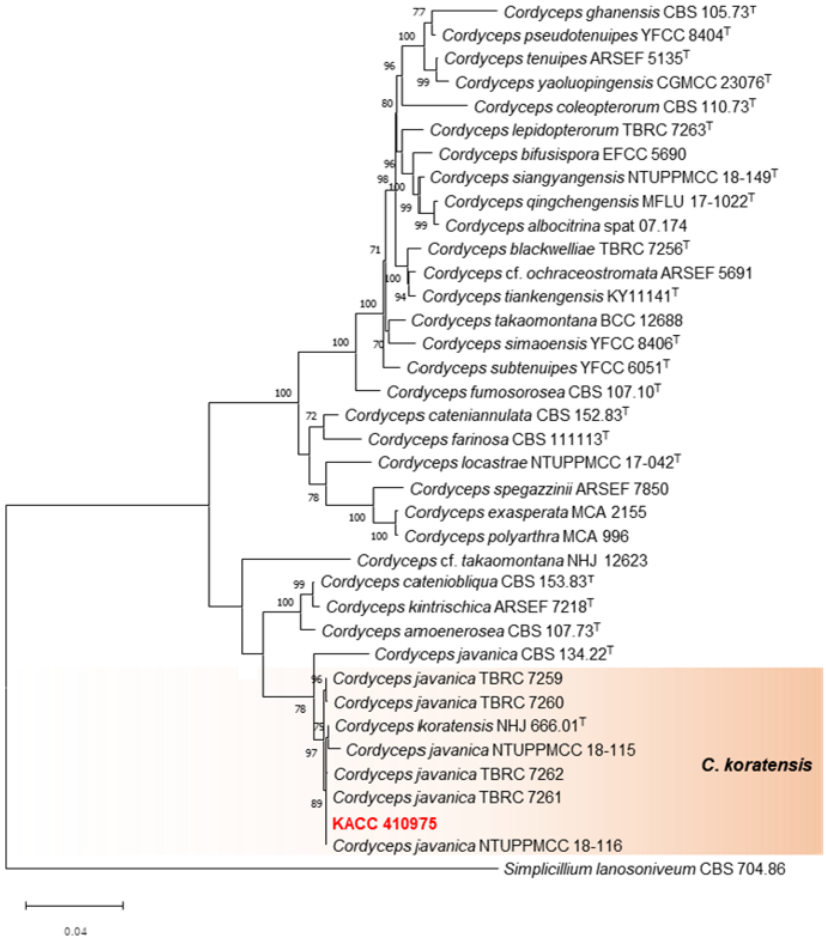

Fig. 1. Maximum likelihood tree of *Cordyceps* species based on a combined data set of the internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU), translation elongation factor 1-alpha (*TEF*), and the largest (*RPB1*) and second-largest (*RPB2*) subunits of RNA polymerase II gene regions. Bootstrap values ≥70 are indicated at each node. Scale bar indicates number of substitutions per nucleotide. The strain obtained in the present study are marked in red and bold. Ex-type strains are designated by ^{T}. The species *Simplicillium lanosoniveum* (CBS 704.86) was used as the outgroup.

Taxonomy

Cordyceps koratensis (Hywel-Jones) H.A. Ariyaw, M. Stadler, and Luangsa-ard (Figure 2) [MycoBank no.: 851851]

Colony morphology: Colonies on PDA reached 35–42 mm in diameter in 14 days at 25°C. Exhibited an entire margin and produced white mycelium with grey sporulation, becoming grey white, with a white to pale yellow reverse.

Micromorphology: Conidiophores arose from prostrate or aerial hyphae, consisting of solitary phialides along the hyphae or verticillate branches with two–four phialides in the whorls. Phialides were flask-shaped, measuring (6–)8–11(–13) × (2–)2.5–3(–3.5) μm, with an ovoid basal portion slightly tapering into a neck, 1 μm in width. Conidia were cylindrical to ellipsoidal, unicellular, and hyaline, measuring (3–)3.5–6(–7) ×

(1–)1.5–2(–2.5) µm.

Strain examined: KACC 410975

Diagnosis: *C. koratensis* is morphologically characterized by obovoid-to-ellipsoid phialides, whereas KACC 410975 produces flask-shaped phialides, similar to *C. javanica*.

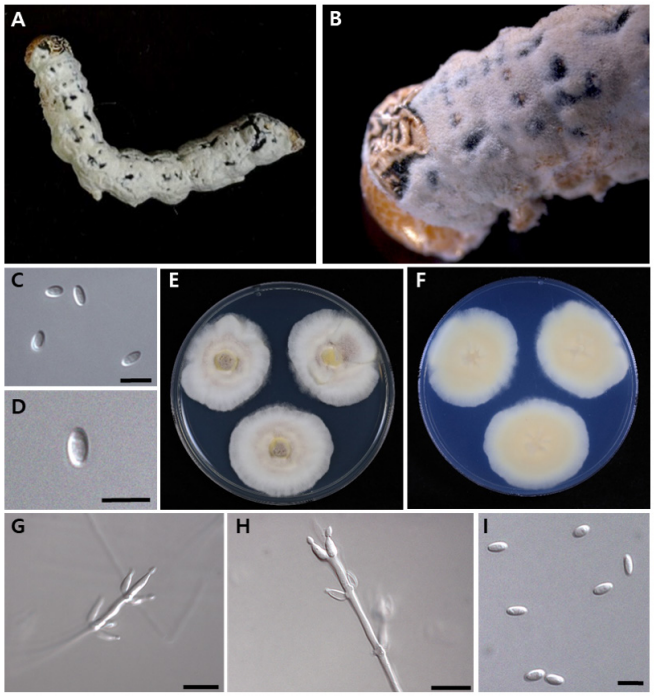

Fig. 2. Morphology of *Cordyceps koratensis* (KACC 410975). **A, B:** Mycelium arising from larva of Lepidoptera. **C, D:** Conidia on the host. **E:** Obverse of colonies on potato dextrose agar (PDA) at 14 d. **F:** Reverse of colonies on PDA at 14 d. **G, H:** Phialides on PDA. **I:** Conidia on PDA culture. Scale bars: C, D = 5 µm, G, H = 10 µm, I = 5 µm.

DISCUSSION

We aimed to identify an entomopathogenic fungus infecting the larvae of Lepidoptera based on a combination of morphological characteristics and multi-locus sequence analysis. *Cordyceps koratensis* was originally described as *Akanthomyces koratensis* [24], later transferred to *Hevansia koratensis* [15] and then to *Parahevansia koratensis* [25]. Mongkolsamrit et al. [25] concluded that two strains of *P. koratensis* (NHJ 2662 and NHJ 666.01) formed an independent clade with strong support from the *Hevansia* clade and in proximity to *Cordyceps* species. The morphology of *C. koratensis* described by Hywel-Jones [24] exhibited significant variation compared to strains NTUPPMCC 18-115, NTUPPMCC 18-116, TBRC 7259, TBRC 7260, TBRC 7261, and TBRC 7262, previously identified as *C. javanica* by Chuang et al. [26]. However, the phylogenetic classification of cordycepitoid fungi showed that most diagnostic characteristics used in the current classifications of *Cordyceps* species (e.g., host, arrangement of perithecia, ascospore fragmentation, conidiogenous structures, conidial shape, and size) are not phylogenetically informative [15,17,18,27]. Therefore, strain KACC 410975 and six previously studied strains (NTUPPMCC 18-115,

NTUPPMCC 18-116, TBRC 7259, TBRC 7260, TBRC 7261, and TBRC 7262) were identified as *C. koratensis* by multilocus sequence analysis (Fig. 1).

In summary, the current study identified an entomopathogenic fungus infecting the larvae of lepidopterans as *Cordyceps koratensis*. To date, 59 Cordyceps species have been identified worldwide. Among these, *C. ampullacea*, *C. bifusispora*, *C. brongniartii*, C. *coccidiocapitata*, *C. isarioides*, *C. kyusyuensis*, *C. militaris*, *C. ninchukispora*, *C. ochraceostromata*, *C. ootakiensis*, *C. pruinosa*, *C. rosea*, and *C. tenuipes* have been recorded in Korea [28]. To our knowledge, this study represents the first record of *C. koratensis* in Korea. These results help expand our current knowledge of *Cordyceps* diversity in Korea and supplement the available bioresources in Korea from the *Cordyceps*. Moreover, this finding will help develop biocontrol agents within the framework of ecofriendly pest management for *C. koratensis*.

CONFLICT OF INTERESTS

The authors pronounce that they have no potential conflict of interest.

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