한 국 균 학 회 지 The Korean Journal of Mycology Research Note

First Report of *Metacordyceps chlamydosporia* (*Cordyceps chlamydosporia*) Isolated from Soil in Korea

Hyun Seung Kim[1], Mahesh Adhikari[1], Dil Raj Yadav[1], Sang Woo Kim[1], Yong Hyun Um[1], Hyang Burm Lee[2] and Youn Su Lee[1]*

[1]*Division of Biological Resource Sciences, Department of Applied Plant Sciences, Kangwon National University, Chuncheon 24341, Korea*
[2]*Division of Applied Bioscience and Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, Korea*

ABSTRACT : A previously unrecorded species, *Metacordyceps chlamydosporia* KNU14-22, was isolated from soil in Korea. Identification of the fungal species was based on morphological and molecular characteristics. This species has not been previously reported in Korea and herein we present data with detailed descriptions and figures.

KEYWORDS : ***Metacordyceps chlamydosporia*, Molecular identification, Morphology**

Metacordyceps chlamydosporia is an entomophagous fungus belonging to the Clavicipitaceae family of the phylum Ascomycota and the order Hypocreales. *Metacordyceps chlamydosporia* was previously named *Cordyceps chlamydosporia* [1]. The genus *Metacordyceps*, which was erected from the genus *Cordyceps* along with other genera, *Ophiocordyceps* and *Elaphocordyceps*, contains more than 400 species attacking a range of insects and fungal hosts [2, 3]. *Metacordyceps* includes species with solitary or several simple and branched stromata, fleshy or tough stipe, partially or completely immersed perithecia in stromata, and cylindrical ascospores [3].

Metacordyceps species are particularly abundant and diverse in humid temperate and tropical forests of Asia. However, a wide range of entomopathogenic fungi are still hidden in the island and mountain regions of Korea. Ulleung-do, one of Korea's many islands, is located 150 km east of the Korean Peninsula. The island is volcanic in origin and has high variation in geographical and ecological indices, including temperature, altitude, biodiversity, soil physicochemical properties, etc. [4]. These factors contribute to a rich fungal diversity. Thus, examination of the fungal diversity of this solitary area so far away from the main land of Korea is important. The aim of this study was to isolate soil fungi from Ulleung-do, Korea, and confirm the first record of fungal species in Korea. The current study compares a previously unknown fungal species, *M. chlamydosporia* KNU14-22, with previously described *Metacordyceps* species with respect to morphological and phylogenetic characteristics.

Kor. J. Mycol. 2016 March, **44**(1): 48-50
http://dx.doi.org/10.4489/KJM.2016.44.1.48
pISSN 0253-651X • eISSN 2383-5249

***Corresponding author**
E-mail: younslee@kangwon.ac.kr

Received December 3, 2015
Revised December 16, 2015
Accepted December 20, 2015

Soil sample collection and isolation of fungi. Soil samples were collected in 2014 from the forest of Nari Basin, Ulleungdo Island, Korea (37° 31'06.67" N, 120° 51' 54.27" E). The samples were taken from a depth of 0~15 cm, air dried, and stored in plastic bags at 4°C until used. The fungus was isolated using a conventional dilution method [5] and grown on potato dextrose agar (PDA) for 5~7 days at 25°C. The isolate was preserved at 20°C on PDA slants for further studies.

Morphological characterization. The isolate KNU14-22 was cultured on PDA at 25°C for 14 days and colony characteristics including color, shape, and size were recorded. Photomicrographs were taken with an HK 3.1 CMOS digital camera (KOPTIC, Seoul, Korea) attached to an Olym-

Table 1. Morphological characteristics of *Metacordyceps chlamydosporia* isolated in this study

Characteristics		The Korean isolate *Mertacordyceps chlamydosporia*	*Metacordyceps chlamydosporia*[a]
Colony	Color	White and woolly growth appearances in surrounding	White to pale yellow
	Size	80~90 mm in 14 days and fast growth	15~40 mm in 10 days
	Texture	Pale white	Cream colored
Stromata	Size	11~14 × 0.4~1.14 mm	12~16 × 0.5~1.25 mm
	Shape and position	Becoming erumpent	Becoming erumpent and superficial
Perithecia	Color	White to pale white	Pale-yellow, occasionally brownish orange
	Shape	Broadly flask shaped	Broadly flask shaped

[a]Source of description [1].

pus BX50F-3 microscope (Olympus, Tokyo, Japan) and a scanning electron microscope (LEO Model 1450VP; Carl Zeiss, Oberkochen, Germany). The morphological characteristics of the identified species are summarized in Table 1. The colony attained a diameter of 80~90 mm. The colony was white to pale white in color, floccose mycelium, margin entire, and lacking exudate (Fig. 1A, 1B). Phialides were usually scanty and produced on prostrate aerial hyphae, solitary, sometimes in whorls of 2~3 numbers, tapered towards the tip and 15~25 μm in diameter. Conidia were ellipsoidal, smooth walled, one-celled, and 2.5~3.5 μm in size. Dichlomydospores were abundant, produced in the aerial mycelium, and stalked (Fig. 1C~1F).

Genomic DNA extraction, sequencing, and data analysis. The genomic DNA of the isolate was extracted using the DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. The internal transcribed spacer (ITS) region of rDNA was amplified using the ITS1 and ITS4 primers [6]. The purified DNA was sequenced directly using the Macrogen Sequencing Service (Macrogen, Seoul, Korea). The obtained 579-

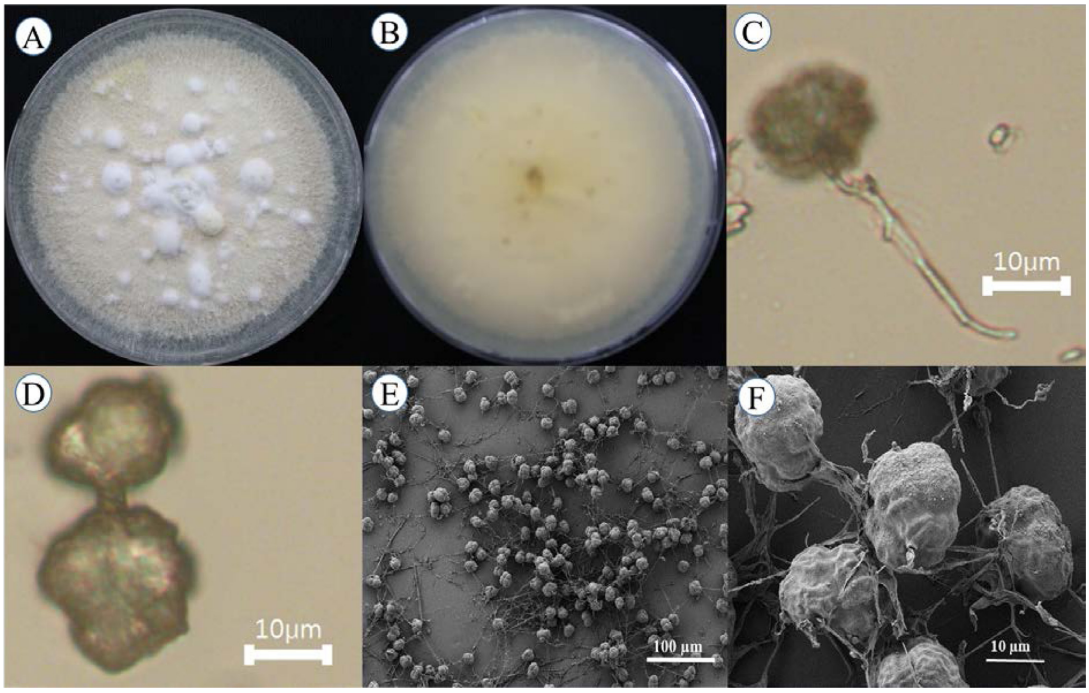

Fig. 1. Morphological characterization of *Metacordyceps chlamydosporia* KNU14-22 using a compound microscope and scanning electron microscope (SEM). A, Colony in front; B, Colony in reverse; C, D, Microscopic pictures of dichlamydospores; E, F, SEM of dichlamydospores.

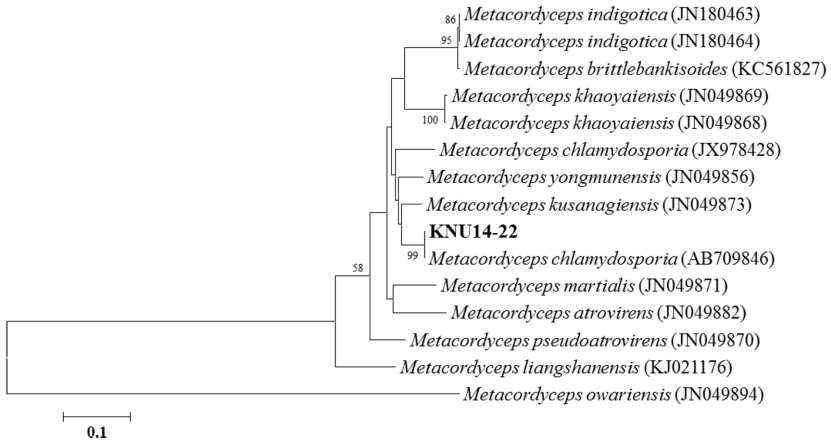

Fig. 2. Neighbor-joining tree of the partial 18S, ITS1-5.8S-ITS2 and partial 28S rDNA sequence of *Metacordyceps chlamydosporia* KNU14-22. The sequence was compared with the reference sequences in NCBI GenBank. The sequences obtained in the present study are shown in boldface. Numerical values (>50) above/below branches are the percentage of 1,000 bootstrap replicates that support the branch. *Metacordyceps owariensis* was used as an outgroup. The scale bar represents the number of substitutions per site.

bp sequence was deposited in GenBank at the National Center for Biotechnology Information under accession no. KP055595. In a GenBank BLAST search the ITS sequence showed 99% similarity with *M. chlamydosporia* (AB 709846). For phylogenetic analyses, all available ITS rDNA sequences of reference species belonging to the *Metacordyceps* were retrieved from GenBank. *M. owariensis* (JN 049894) was used as an outgroup taxon. All retrieved sequences were aligned using the Multalin program. The phylogenetic tree was constructed using neighbor-joining method with Kimura 2-parameter model in MEGA6 software [7]. Bootstrap analysis was performed with 1,000 replications to determine the support for each clade. The ITS sequence of KNU14-22 matched with *M. chlamydosporia* (AB709846) with 99% similarity (576/576 bp) and clustered together in a clade with a 99% bootstrap value (Fig. 2).

This fungal species is a new record for Korea and previous research results have proved that *M. chlamydosporia* is pathogenic to nematodes and insects. Thus, further investigations on the biocontrol aspect of the current isolate would be worthwhile.

Acknowledgements

This work was supported by a grant (NIBR2014-01205) from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea for projects on the survey and discovery of indigenous Korean fungal species.

REFERENCES

1. Zare R, Gams W, Evans HC. A revision of *Verticillium* section *Prostrata*. V. The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia 2001;73:51-86.
2. Kepler RM, Sung GH, Ban S, Nakagiri A, Chen MJ, Huang B, Li Z, Spatafora JW. New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. Mycologia 2012;104: 182-97.
3. Sung GH, Hywel-Jones NL, Sung JM, Luangsa-Ard JJ, Shrestha B, Spatafora JW. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Stud Mycol 2007;57:5-59.
4. Ryu SH, Jang KH, Choi EH, Kim SK, Song SJ, Cho HJ, Ryu JS, Kim YM, Sagong J, Lee JH, et al. Biodiversity of marine invertebrates on rocky shores of Dokdo, Korea. Zool Stud 2012;51:710-26.
5. Davet P, Rouxel F. Detection and isolation of soil fungi. Enfield: Science Publishers; 2000.
6. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
7. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30:2725-9.