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New Record of the Genus *Calyptella* from Korea

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***Calyptella capula* is reported for the first time in Korea. It was found in spring in Chungcheongbuk-do, central Korea, growing in a dead stalk of *Sesamum indicum*. A description and molecular phylogenetic analyses are reported.**

KEYWORDS : *Calyptella capula*, Cyphelloid, Distribution, New record

Homobasidiomycetes are the most conspicuous group of fungi and includes approximately 16,000 described species of mushroom-forming fungi and related forms (Kirk *et al*., 2001). In Korea, 1424 species of Basidiomycotina from 330 genera, 82 families and 15 orders have been recorded (Lee, 2005). Members of the Homobasidiomycetes include gilled mushrooms, boletes, polypores, and puffballs. The Homobasidomycetes also contain a relatively obscure group called the cyphelloid fungi. Cyphelloid fungi include approximately 120 species in about 40 genera, but there is an estimated actual diversity of ca. 500 species (Agerer, 1978; Cooke, 1962; Donk, 1959; Reid, 1964; Singer, 1986). *Calyptella* species have been included in the cyphelloid fungi. Generally, cyphelloid fungi have minute, cup- to tube-shaped, often pendant fruiting bodies that are less than 2 mm in size. Cyphelloid fruiting bodies usually have a smooth, even hymenophore that lines their concave inner surface (Agerer, 1978a, 1983a: Donk, 1951, 1959, 1966).

In spring of 2006, a *Calyptella* species was collected by an author from a gingili plant at Chungcheongbuk-do in Korea, and identified as *Calyptella capula* (Holmsk.) Quélet following the descriptions of Redhead and Traquair (1981) and Breitenbach and Kränzlin (1986). To the best of our knowledge, this genus has not been reported in Korea. The morphology and genotypic characteristics of the isolate were studied.

Materials and Methods

Fungal isolates and morphology. Macroscopic and microscopic descriptions are based on fresh specimens. Color nomenclature refers to Manual of Color Names (Japan Color Research Institute 1973) and color codes in parentheses are from The Munsell Book of Color (Munsell Color, 1963). Microscopic characters were examined from material mounted in 5% KOH or 3% KOH + Congo red. A total of 50 basidiospores from mounted lamellae were examined for size. A minimum of 20 basidiospores, basidia and other structures were measured for each collection we examined. The strain is maintained in Herbarium Conservation Center Niast (HCCN). Cultures were grown in a biochemical oxygen-demand incubator at 25°C on potato dextrose agar.

Genomic DNA isolation, PCR and DNA sequencing. DNA was isolated from pure cultures of fungus using a SDS-CTAB (sodium dodecyl sulfate-cetyltrimethyl-ammonium bromide) method (Kim et al, 1990). ITS1 and ITS4 primer sets were used for amplification of internal transcribed spacer (ITS) regions (White *et al*., 1990). PCR amplifications were carried out in a Techne thermocycler (Techne LTD, Duxford, Cambridge, U.K.). PCR consisted of 35 cycles of 1 min at 94°C, 1 min at 58°C, and 2 min at 72°C. Contaminating primers and dNTPs were removed from PCR products using the High Pure PCR Product Purification Kit (Bioneer Co., Chungbuk, Korea). Big Dye Terminator Kit and ABI Prism 310 Genetic Analyzer (Perkin Elmer, New Jersey, USA) was used for sequencing.

Molecular phylogenetic analysis. The partial ITS region sequence (649nt) of strain CH3 was compared to the ITS region sequence available from GenBank using the BLAST program to determine an approximate phylogenetic affiliation. The gene sequence was then multiply aligned with those of closely related species using the program MEGALIGN (DNASTAR). Phylogenetic trees were constructed using the neighbour-joining method (Saitou and Nei, 1987) with the MEGA 3.0 program (Kumar *et al*., 2004); bootstrap percentages were based on 1000 replications (Felsenstein, 1985). The partial ITS

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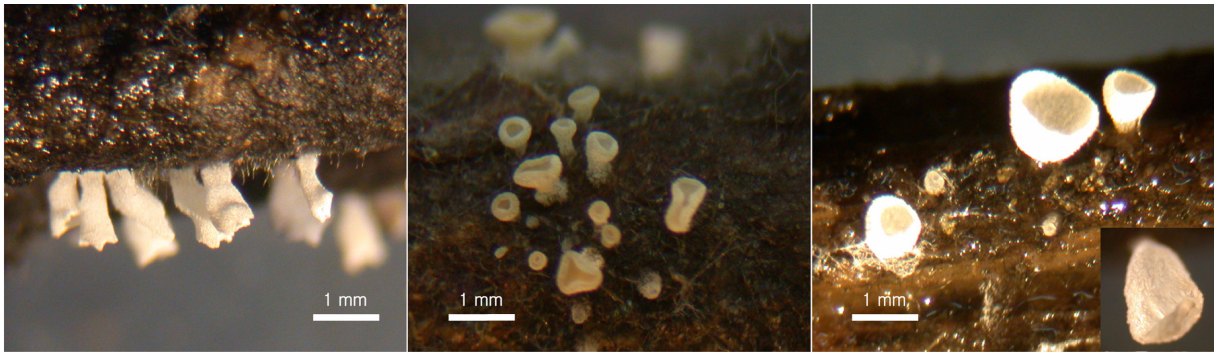

Fig. 1. Basidiomata of *Calyptella capula* (HCCN00010) in a habitat.

region sequence determined in this study has been deposited in the GenBank Database under accession numbers of EF374132 for strain CH3.

Results and Discussion

***Genus* Calyptella** Quélet, Enchir. F., p 216. 1886.
(Korean name: Eun-Bang-Ul-Beo-Seot-sok)
Type species: *Calyptella capula* (Holmsk.) Quélet
Calyptella capula (Holmsk.) Quélet, *Fl. mycol. France* (Paris): 25 (1886)
(Korean name: Eun-Bang-Ul-Beo-Seot)
Synonymy:
Calyptella laeta (Fr.) W.B. Cooke, Beih. Sydowia 4: 40 (1961)
Chaetocypha capula (Holmsk.) Kuntze, *Revis. gen. pl.* (Leipzig) 2: 847 (1891)
Chaetocypha laeta (Fr.) Kuntze, *Revis. gen. pl.* (Leipzig) 2: 847 (1891)
Chaetocypha pimii (W. Phillips) Kuntze, *Revis. gen. pl.* (Leipzig) 2: 847 (1891)
Cyphella capula (Holmsk.) Fr., *Epicr. syst. mycol.* (Upsaliae): 568 (1838)
Cyphella capula var. *flavescens* Pat., *Tabl. analyt. Fung. France* (Paris): 56 (1883)
Cyphella laeta Fr., *Epicr. syst. mycol.* (Upsaliae): 568 (1838)
Cyphella pimii W. Phillips, *Grevillea* 13(no. 66): 49 (1884)
Cyphella velenovskyi Pilát, *Annls mycol.* 22: 206 (1924)
Peziza capula Holmsk., 1: 286 (1799)

Basidiomes. Basically cupulate, dorsally stipitate, extremely fragile, solitary to gregarious, outer surface smooth, white to cream-colored, inner surface with the hymenium likewise smooth, white to cream-colored, discoid when young, becoming conical to campanulate, 1~4 mm wide, 1~3 mm deep, externally whitish to pale mouse grey, with age becoming blackish, glabrous or appearing frosted when magnified slightly, with incurved margins which flair with age and become slightly eroded.

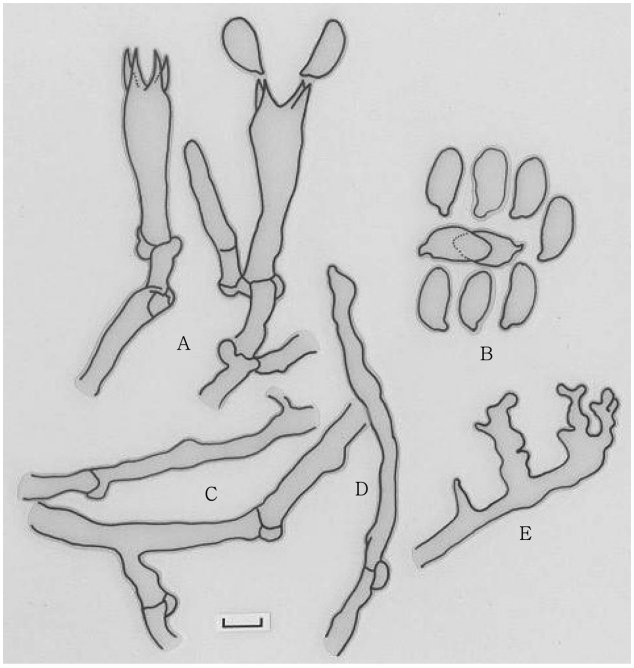

Fig. 2. Microscopic structures of *Calyptella capula* (HCCN00010). A, basidia; B, basidiospores; C, D, generative hyphae; E, hyphal ends of generative hyphae (Scale bar: 5 μm).

Hymenium. 0.2~2.0 mm long, smooth to radially furrowed or folded, whitish to pale grey or yellowish grey. Pseudostipe; short to elongate, usually eccentric, solid, often frosted to pubescent, concolorous or darker than the receptacle.

Basidia. 20~25 × 5~8 μm, cylindrical when young, becoming clavate, 4-sterigmata, basal clamped.

Basidiospores. 6~9 × 3~4.5 μm, ellipsoid to obovoid, inequilateral, somewhat flattened on one side, thin-walled, hyaline, nonamyloid, smooth, with a prominent apiculus.

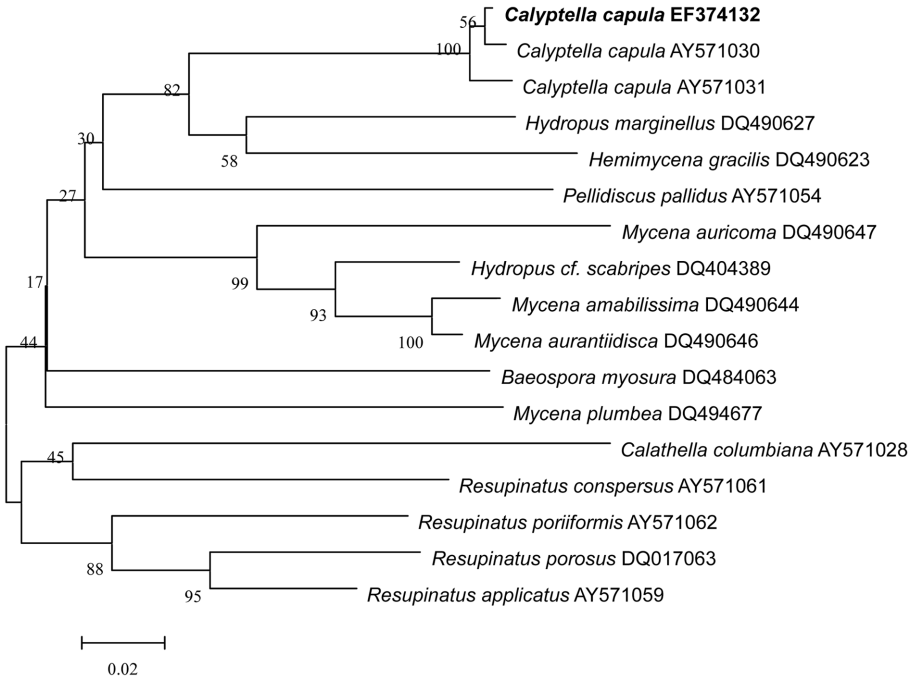

Fig. 3. Neighbor joining phylogenetic dendrogram of *Calyptella* species based on internal transcribed spacer (ITS) sequence. Numbers on the branches represent percentage of 1000 bootstrap frequencies.

Cystidia. Cystidia not seen.

Hyphal system. Monomitic, hyphae in the subhymenium 2~4 μm across, septa with clamps, hyphae in the stalk and in the trama up to 15 μm across, hyphal ends in the marginal part of the cup sinuous-gnarled with outgrowths, some exserted.

Habitat. Spring, dead stems on sesame (*Sesamum indicum*).

Materials examined. Eumseong-gun, Chungbuk, 22 May 2006. (Herbarium No. : HCCN00010).

Molecular chatacterization. The rDNA ITS region of the *Calyptella* isolate was amplified with conserved fungal primers ITS1 and ITS4. The size of the ITS fragment was approximately 649 bp, which includes ITS1, 5.8S and ITS4 regions. The ITS region was sequenced and the sequence data was submitted to GenBank nucleotide database (accession number EF374132). The sequence was compared with the published Cyphelloid fungi sequences available in the NCBI database. The strain is closely related to strains of the type species of *C. capula* (Fig. 3). This strain showed 96% ITS region sequence similarity to *C. capula,* according to blast search for homologous. Bodensteiner *et al*. (2001) reported Cyphelloid species *C. capula* (the type species of Calyptella) is the sister group of the resupinatus clade, but this placement received weak bootstrap support. *C. capula* produces cupulate fruiting bodies covered by surface hyphae with multiply branched, coralloid excrescences that lack a crystal covering (Cooke, 1962; Donk, 1951; Reid, 1961; Singer, 1962, 1986).

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