

New record of *Lecanicillium aphanocladii* family: Cordycipitaceae from Egypt

Abstract

New record of the entomopathogenic fungus, *Lecanicillium aphanocladii* (Zare & W. Gams) for the first time in Egypt. The detailed description of the cultural and morphological features of *L. aphanocladii* using PDA plates was exhibited in this study. In the meantime, a measurement of its microscopic structures (hypha, phialides and conidia) was carried out using ocular micrometer. The definition of *L. aphanocladii* was confirmed by molecular characterization by 18S rRNA gene sequences using [ITS1] and [ITS4] primers.

Keywords: *lecanicillium aphanocladii*, entomopathogen, molecular characterization, ITS, conidia and aphanophialides

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Introduction

The fungus *Lecanicillium* is belonging to the order Hypocreales and is described as anamorphic *Cordycipitaceae*. This genus is known by its ability to infect other species of fungi such as *Agaricus spp.*, *Sphaerotheca spp.*,¹ *Puccinia spp.*² On the other hand, it was recorded as entomopathogenic fungus as it can parasitize some species of insects like *Mosquito larvae*³⁻⁶ *Bombyx mori*, *Trialeurodes vaporariorum*. In the meantime, it is reported from leaf litter of *Abelmoschus esculentus* and *Acacia karroo*. Currently, there are 21 described species of *Lecanicillium*.⁷ In the past, this genus was described as *Verticillium lecanii* (Zimmerman) Viegas.

The species *L. aphanocladii* was described for the first time by Zare and Gams, 2001⁸ as fungicolous fungus causing cobweb and spotting in the cultivated *Agaricus* mushroom. There are two synonyms (Ex-epitypes) of *L. aphanocladii* where it was earlier identified as:

- I. *Acremonium aranearum* (Petch): preserved in MycoBank under the strain number: MB#144303.
- II. *Aphanocladium aranearum* (Petch) W.Gams: preserved in MycoBank under the strain number: MB#308804. This species was also known as *Aphanocladium album* (sensu stricto).

The present study introduces a new record of *L. aphanocladii* in Egypt. It was discovered as a contaminant of some prepared PDA plates which stored in the laboratory at room temperature (about 26°C±2). This contamination was predicted to be due to the passage of some small insects like mites within the PDA plates. Accordingly, the present study aimed to purify the fungus and describing its cultural and morphological features in detail. The identification of the new recorded *L. aphanocladii* was confirmed according to molecular characterization and DNA sequencing.

Materials and methods

Preparation of potato dextrose agar medium (PDA):

The used medium in this study was PDA⁹ which composed of (g/L), potato tubers, 200, D-Glucose, 20, agar, 20. The potato tubers were peeled, divided to small pieces and boiled in 1L distilled water for 1h.

After boiling, the potatoes were removed by sieve and the filtrate was added to glucose and agar then the mixture volume was completed to one liter with distilled water. Chloramphenicol antibiotic (0.5g/L) was added to prevent bacterial contamination before sterilization in autoclave at 121°C, 1.5 atm. for 15min.

Identification of the tested fungus

Morphological characterization: The tested fungus was investigated as a laboratory contaminant on pre-prepared PDA plates. Then it was sub-cultured on new PDA plates in aseptic conditions and incubated for 5days at 26±°C. After the incubation period, the macroscopic and microscopic features of the tested fungus were recorded. The macroscopic features included the colony color and appearance, pigmentation at the bottom of the plates while the microscopic features included size and shape of conidia, conidiogenous cells (phialides) and their arrangement on the hyphae.

Molecular characterization: Molecular characterization of the fungal strain was carried out by sequencing of rRNA gene with the help of Solgent Company, Daejeon South Korea. The fungal mycelium was grown on PDA plates at 28°C for 7days. A small portion of the mycelium was scraped and suspended in 100µl autoclaved distilled water in 2ml sterile vials and boiled at 100°C for 15minutes. DNA was extracted and purified from the non-living fungal mycelia using SolGent purification bead. Prior to the sequencing, the ribosomal rRNA gene (rDNA) was amplified using the polymerase chain reaction (PCR) technique. Two universal fungal primers ITS1 (forward) and ITS4 (reverse) were incorporated in the reaction mixture in PCR technique.¹⁰ The sequence of each primer is: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5' - TCC TCC GCT TAT TGA TAT GC -3'). The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. Then these bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Each sample was sequenced in the sense and antisense directions using ITS1 and ITS4 primers.¹⁰ Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

Results and discussion

Identification of the tested fungus

The tested fungus was identified as *L. aphanocladii* based on its morphological characters which then confirmed and supported by the molecular characterization. The culture of the new recorded *L. aphanocladii* was deposited at the mycological center, Assiute University, Assiute, Egypt with strain number (AUMC11913).

Morphological characterization of *L. aphanocladii*

Macroscopic features: Colonies of *L. aphanocladii* were fast growing reached 3-5cm in 4days on PDA medium. They were characterized by their white color and high on the surface of the medium (Figure 1A). The bottom of the plates appeared with reddish reverse (Figure 1B).

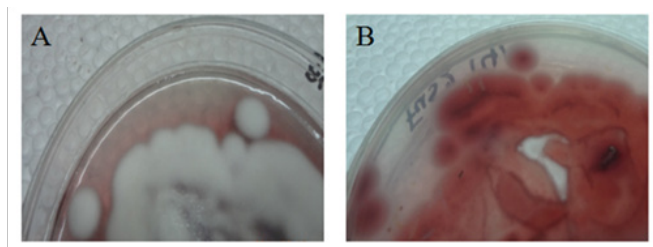


Figure 1 Culture appearance of *L. aphanocladii* on PDA plates (A) and its reverse (B).

Microscopic features: The phialides of *L. aphanocladii* were characteristic as aphanophialides which protrude solitary, paired or in cluster on the prostrate hyphae (Figure 2A, 2C & 2D). The aphanophialides were cylindrical shaped (11.5*2.6µ), oval shaped with small neck (3.6-4.7*1.5-1.9µ) or flask shaped with long tapered neck (7*1.7µ). Solitary conidium was observed attached to the apex of each phialide. Conidia were characteristic by different shapes; sub-globose (3.5*2.9µ), oval (4.9*3.2µ) or ellipsoidal shaped (5.7*2.4µ). They were generated either as phialo-conidia (Figure 2A, 2C & 2D) or directly on the hyphae (Figure 2B). These morphological

characteristics of *L. aphanocladii* agreed the description recorded by Zare.⁸

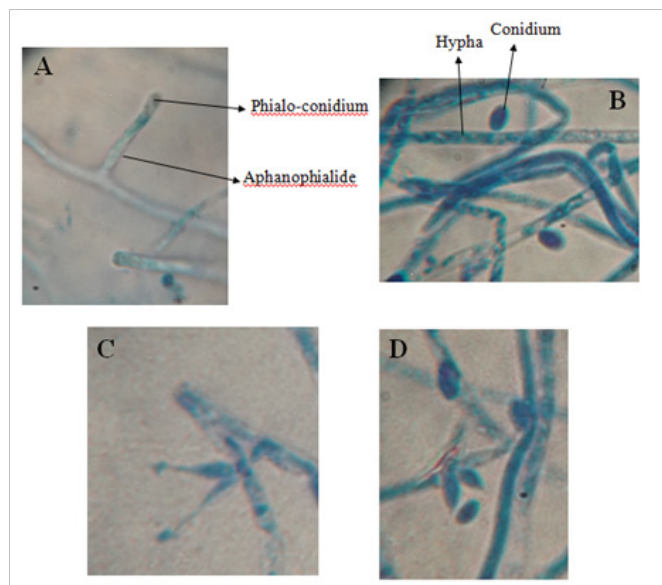


Figure 2 Microscopic structures of *L. aphanocladii*. Cylindrical shaped aphanophialide carrying phialo-conidium (A), conidia generated directly on the hypha (B), paired aphanophialides (C) and aphanophialides in cluster (D).

Molecular characterization

The resulted data from rDNA sequencing process of the tested isolate were deposited in the GenBank with accession numbers listed in Table 1. A BLAST search for the 18S sequences of the tested isolate, *L. aphanocladii* (AUMC11913) indicated that it was 100% similar to three Chinese isolates of *L. aphanocladii* (JX241644, JF911775 and KX530079) Table 1. The present isolate along with another recorded seven isolates of *L. aphanocladii* were subjected to a phylogenetic analysis using available ITS rDNA sequence data downloaded from GenBank (Table 1).

Table 1 Molecular identification of the tested fungus (AUMC11913) and percentage similarities with related strains accessed from the GenBank

Strain No.	Identification	Closely related strains			
		Strain no.	Source/Country	Accession no.	Similarity (%)
AUMC 11913	<i>Lecanicillium aphanocladii</i>	GL0836	Apple / China	JX241644	100
		HQ69	Lake / China	JF911775	100
		-----	Red spot of Tremella / China	KX530079	100
		FS13	Leaves of Hupezia / China	KP689216	99.09
		FJTL	Tobacco / China	KJ162356	99.88
		DPI 1LA	Leaves mining insects / Lithuania	KC574075	99.66
		Outgroup strain= <i>Lecanicillium lecanii</i>	PA196	Entomopathogenic	AF172335

The Phylogenetic tree of 18S sequences showed that my isolate clustered with *L. aphanocladii* isolates reported elsewhere (Figure 3). The isolate *L. lecanii* AF172335 was included as an out-group strain where it does not cluster with the *L. aphanocladii* isolate reported in this study. The resulted data of molecular and phylogenetic analyses supported the morphological identification of my isolate as *L. aphanocladii*.

Later and for a long time, the fungus *L. aphanocladii* has been confused with the species, *Aphanocladium album* (Preuss) W. Gams, but the genetics studies on the compatibility among fungal species improved that the two taxa are phylogenetically quite unrelated.^{11,12} On the other time, a recent study revealed that there is a close relationship between *L. aphanocladii* and *L. psalliotae* based on the ITS phylogeny analysis.¹³

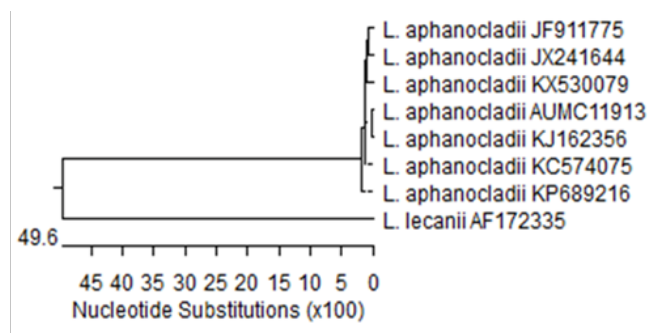


Figure 3 Phylogenetic tree of 18S sequences of the fungal strain isolated in the present study (*L. aphanocladii* AUMC 11913; arrowed) aligned with closely related sequences of strains accessed from the GenBank. *L.* = *Lecanicillium*, *L. lecanii* AF172335 is included as an out-group strain.

Conclusion

In the present study, *L. aphanocladii* was recorded for the first time in Egypt. The fungus was observed as a laboratory contaminant in stored PDA plates. It was identified morphologically according to its culture appearance and microscopic structures. This identification was confirmed and supported by the molecular characterization of the fungus using the 18S sequences where it shows 100% similarities with other 6 isolates of the fungus recorded in the GenBank.

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Conflict of interest

The author declares no conflict of interest.

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