First Record of Two Egyptian Fungal Genera; The Myxomycete: Lepidoderma carestianum and the Ascomycete: Arthrinium bambusicola

Samah A. El-Debaiky (Samaheldebaiky@science.tanta.edu.eg)
Tanta University
Yehia A.G. Mahmoud
Tanta University

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Abstract

Background: Slime molds (Myxomycetes) considered a separate group of protozoal organisms, related to rhizopoda and primitive fungi. They are characterized by forming large multinuclear plasmodia devoid of cell walls and their complex life cycles. On the other hand, ascomycetous fungi are considered large group which includes diversity of fungal genera ranged between pathogenic and saprophytic habitat.

Results: The tested fungi*, Lepidoderma carestianum* which belongs to (Myxogastria, Amoebozoa) and the ascomycetous fungus; *Arthrinium bambusicola* were recorded for the first time in Egypt. Detailed morphological description and microscopic examination for these fungi were exhibited in this study.

Conclusions: Sporocarp of *L. carestianum* was appeared at cold climate at November 2021 on the surface of cultivated soil while, *A. bambusicola* was isolated from dead branch of *Melia azedarach* tree on PDA medium as a saprobe. The most obvious feature of the isolate colony was the presence of the black colour regions due to conidiomata embedded in the white mycelia. The fungal conidia are distinguished by dark brown colour, oval shaped with tapered ends. The definition of the tested *Lepidoderma* and *Arthrinium* species was confirmed by molecular characterization using COIF1/COIR1 primers for *Lepidoderma* and ITS1 and ITS4 primers for *Arthrinium*.

Background

Among soil-inhabiting protists, myxomycetes stand out by their macroscopic fructifcations True slime molds (Myxomycetes or Myxogastria), also called plasmodial slime molds considered a separate group of protozoal organisms, related to rhizopoda and primitive fungi. They are considered among soil-inhabiting protists having specialized fructifcations which have allowed studies on their ecology and distribution for more than two hundred years [1]. They are characterized by forming large multinuclear plasmodia devoid of cell walls and their complex life cycles. They found in a different habitats including deserts, forests, water pools to the surface of the snow in mountain ranges [2]. Also, these microorganisms characterized by the alternation of amoeboflagellate and plasmodial vegetative stages and by their ability to form spore bearing structures called sporocarps [3].

The genus *Lepidoderma* de Bary is an example of plasmodial slime molds which published for the first time by [4]. Also, it was described as sporangiate or plasmodiocarpous by [5]. The genus *Lepidoderma* belongs to the order Physarales, family Didymiaceae. It was recorded as a nivicolous myxomycete where it discovered by many researchers in snowy habitats. From few decades, the rare species *Lepidoderma crustaceum* was recorded from only a few localities in the world, especially the nivicolous situations: Casseron, Switzerland (leg. Meylan); Butte Co., California, USA; Mt. Rainier, Washington, USA [6], and the Italian Alps [7] and in the White Mountains (Lefka Ori) in western Crete [8]. Recently, the species *L. chailletii* with some other myxomycetes were recorded from the nivicolous environment of the Carpathian National Nature Park, East Carpathians, Ukraine [9].

The genus *Arthrinium* Gustav Kunze, which classified in family: Apiosporaceae, order: Xylariales, class Sordariomycetes in Ascomycota, was first represented and established more than 200years ago, with *Arthrinium caricicola* as a type species [10]. It is widespread and found in different habitats where it commonly occurs as a saprobic fungus on different parts of a range of many plant substrates [11]. Some species of *Arthrinium* have been reported as plant pathogens such as *A. arundinis* causing kernel blight of barley and *A. sacchari* causing damping-off of wheat [12, 13],. Some literatures reported *Arthrinium* as an endophyte in plant tissues, lichens, and marine algae [14–16]. Also, some species (*A. arundinis*, *A. phaeospermum*, *A. rasikravindrae*, *A. sacchari*, and *A. saccharicola*) have been recognized in both marine and terrestrial environments [17]. *Arthrinium* is a genus of 92 species that can be found in North and South America, Europe, Africa, Asia, and Oceania. A new species, *A. bambusicola* sp. nov., is described and illustrated in this paper. Oval to widely or irregularly round, medium brown, multi-guttulate to roughened, granular conidia with delicately pale slits in the outer borders characterize the new taxon [18].

The present study introduces the first record of the plasmodial slime mold *Lepidoderma* in Egypt where there is no any Egyptian records for this genus. In this study, the discovered species is identified as, *L. carestianum*. Its sporocarp was discovered on the surface of cultivated soil in the garden of faculty of science, Tanta University, Tanta, Egypt at November, 2021 where the temperature was between 8 and 20°C ± 2. Accordingly, the present study aimed to describe it in detail morphologically and microscopically. The identification of the tested myxomycete *L. carestianum* was confirmed by molecular characterization and DNA sequencing.

In the meantime, this study introduces the genus *Arthrinium* which isolated before from Egyptian habitat, but it identified as *A. sacchari* which isolated from the phylloplane of Guava (*Psidium guajava* L.) cultivated in El-Wady El-Assiuty, Assiut, Egypt [19]. While the present study introduces a first record of *A. bambusicola* from Egypt. It was isolated from dead branch of Melia azedarach tree, purified, and described morphologically in detail. The identification of the first recorded *A. bambusicola* was confirmed according to the molecular characterization.

Results And Discussion

Lepidoderma carestianum – Figs. 1&2.

GenBank accession number: OM630523.

1. Identification of the tested myxomycete:

The discovered myxomycete was identified as *L. carestianum* based on its morphological characters which then confirmed and supported by the molecular identification. This study is considered the first record of this myxomycete in the Egyptian environment. Recently, the climate in Egypt has become extremely cold in the winter season where minimum temperature reaches 6°C, so I predict that this cold environment encourages the appearance of this myxomycete in Egypt. This discussion is based on the previous records of *Lepidoderma* in extreme cold localities in the world [9].

1.1 Morphological characterization of *L. carestianum*:

Macromorphological features:

L. carestianum is characterized by flattened, branched, sessile, and irregular shaped sporocarp, 30-45 □ 60-65 mm diameter on the soil surface **(Fig. 1A)**. The entire surface is covered with thin layer, membranous, or sub-cartilaginous peridium which characterized by the presence of lime scales usually white, creamy, or grey color **(Fig. 1: B&C)**. The hypothallus appeared beneath the sporocarp contacted with the plant debris after removing it carefully from the soil surface, it looks thin, continuous, creamy white color **(Fig. 1D)**. This description of the sporocarp is agreed with [20].

Micromorphological features:

Dense masses of black powder are observed by broken the surface of the sporocarp lime scales **(Fig. 2)**. By microscopic examination, this powder is composed of globose to subglobose (rare), 10-10.2 □ 10-10.5 µ, dark brown, and spinose spores. and numerous motile, with different sizes and shapes cells **(Fig. 2F)**. Capillitium appeared as disrupted, reticulate, smooth, and thread like often with some globular swellings tissues among the spores and cells **(Ph. 2C).** My description of the microscopic features of the tested myxomycete is agreed with [5, 21] except in, they did not explore any findings of the motile cells appeared here in my investigation.

1.2 Molecular characterization:

The resulted data from COI sequencing process of the tested isolate were deposited in the GenBank with accession numbers listed in **Table 1**. The sequences producing significant alignments with the sequence of the tested myxomycete (1c1|Query_6927) indicated that it was 93.95 % identity to two isolates of *L. carestianum* (HE614609.1 and AM231296.1) with query cover 94% for each **(Table 1)**. Even the percent identity with these two strains smaller than the other strains mentioned in **Table 1**but, the tested strain is identified as *L. carestianum*. This result is based on all the investigations of morphological and microscopic examination with the query cover percent which exhibited the highest recorded value. The present strain along with another recorded strains of *L. carestianum* and other genera of myxomycetes were subjected to a phylogenetic tree analysis using available sequences data downloaded from GenBank **(Fig. 3)**. The Phylogenetic tree showed that my isolate clustered with *L. carestianum* isolates reported elsewhere. The studied strain was recorded in the GenBank with accession number: OM630523.

Table 1

Molecular identification of the tested myxomycete strain (1c1|Query_6927) and percent identity with related strains accessed from the GenBank.

Arthrinium bambusicola – Fig. 4.

GenBank accession number: ON076927.

Tested strain	Name	Accession No.	Query cover (%)	Percent Identity (%)
1c1|Query_6927	*Lepidoderma carestianum*	HE614609.1	94	93.95
	Lepidoderma carestianum	AM231296.1	94	93.95
	Physarum vernum	KC759102.1	49	95.33
	Physarum vernum	KC759101.1	49	95.33
	Physarum nivale	DQ903680.2	49	95.33
	Diderma crustaceum	JQ277927.1	49	95.02
	Mucilago crustacea	MH348907.1	50	94.12

1. Morphological and microscopic description:

Pure colonies on PDA plates are recognized with white colour, fast spreading growth, with abundant aerial mycelia and colourless reverse except from black spots due to formation of conidiomata. Conidiomata are irregular shaped surrounded by lot of brown septate hyphae. Conidia hyaline at first then turn brown at maturity, smooth walled and oval shaped occasionally with tapered ends **(Fig. 5)**. These characteristic features are in accordance with those reported by [17].

2. Molecular characterization:

The molecular weight of the PCR product of the tested strain (Arth) was detected 250 bp by comparison to the used marker as shown in **Fig. 5**. Phylogenetic analysis of the tested strain nucleotide sequences data of the ITS region was obtained to confirm its identification as *A. bambusicola*. The present isolate along with another recorded isolates of *Arthrinium* were subjected to a phylogenetic analysis using available ITS sequence data downloaded from GenBank. The BLAST search results of ITS regions of the obtained sequences with accession No. ON076927 reflected 95% similarity between the tested isolate (Arth) with the recorded strain *A. bambusicola* (MFLU 20-0528 ITS). The Phylogenetic tree of ITS sequences showed that the tested isolate clustered with *A. bambusicola* isolates reported elsewhere **(Fig. 6)**. The resulted data of molecular and phylogenetic analyses supported and confirmed the morphological identification. Recently, DNA sequences of different genes like ITS, TEF, and TUB were employed to delimit and recognize closely related *Arthrinium* species where species identification based on morphological features is problematic [22].

Methods

1. Diagnosis and morphological identification of the tested fungi

Mature sporocarp of *L. carestianum* was found associated with small plant debris on the surface of cultivated soil, with *faba* bean plants, during field experiment in the garden of faculty of science, Tanta University, Egypt. This soil was composed of clay, manure, 10% sand. Detailed description and measurements of its macromorphological characters were recorded included dimensions, color, surface texture and appearance. While the microscopic examination of micromorphological structures was investigated using binocular biological light microscope (Model: XSZ-107BN) at different magnification powers (40, 100, 400 and 1000 ×).

A.bambusicola was grown sapropically on a dead cut branch of *Melia azedarach* tree. The fungal growth was appeared as olivaceous green velutinous spores' masses on the dead branch **(Fig. 8)**. The tested branch was transferred to the laboratory where the isolation process of *A. bambusicola* was performed. Under sterilization condition, small part of spores' masses was picked from the infected branch by sterilized needle and transferred to previously prepared PDA plates supplied by antibiotic chloramphenicol as antibacterial agent. The inoculated plates were incubated at 26 °C ± 2 for 7 d. Sub-culture process was repeated several times till getting pure cultures of the tested fungus. The morphological characterization of the pure culture including colour, reverse and texture was recorded concurrently with the microscopic investigation of different fungal structures (hyphae, conidiomta and conidia) according to [18, 23, 24].

2. Molecular Identification

Where the found myxomycetous species does not record before in the Egyptian environments so, it was subjected to the molecular identification and recording in National Center for Biotechnology Information (NCBI). Genetic analysis of the tested myxomycete was carried out using total DNA genome of spores where the mycelia were not available. For this purpose, the fungal spores were collected from crushed sporocarp and grind. Then, DNA was extracted from the grind spores according to manufacturer protocol of E.Z.N.A.® Fungal DNA Mini Kit (D3390-01, Omega BIO-TEK,USA). Molecular characterization of the tested myxomycete was carried out by sequencing of COI gene with the help of Micron-Corp Company, Korea. This mitochondrial genetic marker was chosen because it is useful for barcoding and phylogenetic reconstructions in dark-spored myxomycetes [25].

The genomic DNA of *A. bambusicola* was extracted from the fungal cells which grown for one-week-old PDA culture using DNeasy Plant Mini Kit (Supplied by QIAGEN) according to the manufacturer's instructions. Prior to the sequencing of extracted DNA of *L. carestianum*, the COI gene was amplified using the polymerase chain reaction (PCR) technique [26].

The tested fungal isolate: *A. bambusicola* was identified by ITS sequencing analysis. The genomic DNA was used as a template for PCR amplification of a segment of its ITS gene. Primer ITS4: R-(5'-GCTGCGTTCTTCATCGATGC-3') and ITS1 F-(5'-CTTGGTCATTTAGAGGAAGTAA-3') were used to analyse the ITS sequence. The ITS sequences obtained were added to publicly available GenBank sequences and integrated into the database with the automatic alignment tool. Phylogenetic tree was generated by

performing distance matrix analysis using neighbor joining method neighbor-joining (NJ) trees were constructed in MEGA x [27].

Conclusions

This study introduces first record of two fungal genera from Egyptian habitat. The psychrophilic myxomycete, *L. carestianum* was recorded for the first time in the Egyptian environment through very cold weather at November 2021. The appeared sporocarps on cultivated soil surface were collected and identified based on its morphological characters then confirmed and supported by the molecular identification. The other fungus belongs to ascomycetes was found grown sapropically on a dead cut branch of *Melia azedarach* tree and identified as *A.bambusicola* based on its morphological features and molecular characterization.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material: All data were mentioned with transparency.

Availability of data and material: All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Figures

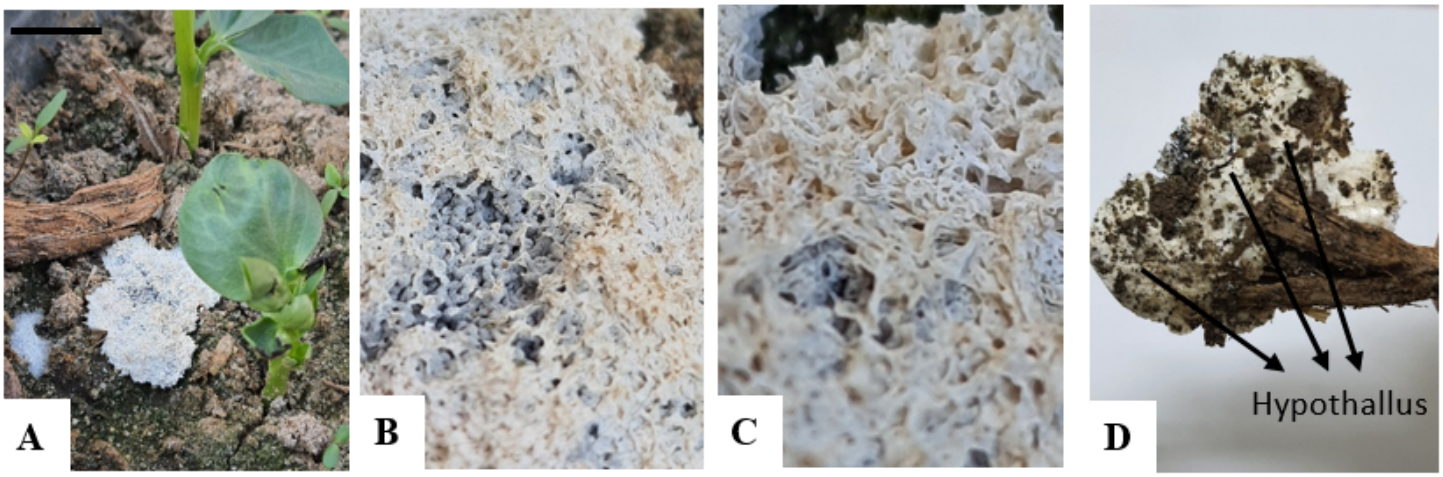

Figure 1

Sporocarp of *L. Carestianum*: **A.** On the soil surface. **B&C.** lime scales covered the peridium of sporocarp. **D.** Hypothallus appeared associated with plant debris after removing the sporocarp carefully from the soil surface.

Figure 2

A. Black powder inside sporocarp of *L. carestianum*. **B.** Dark spores. **C.** Capillitium thread with swellings **D.** Dark spores and masses of motile cells. **E.** Enlarged spores with spinose walls.**F.** Enlarged motile cells.

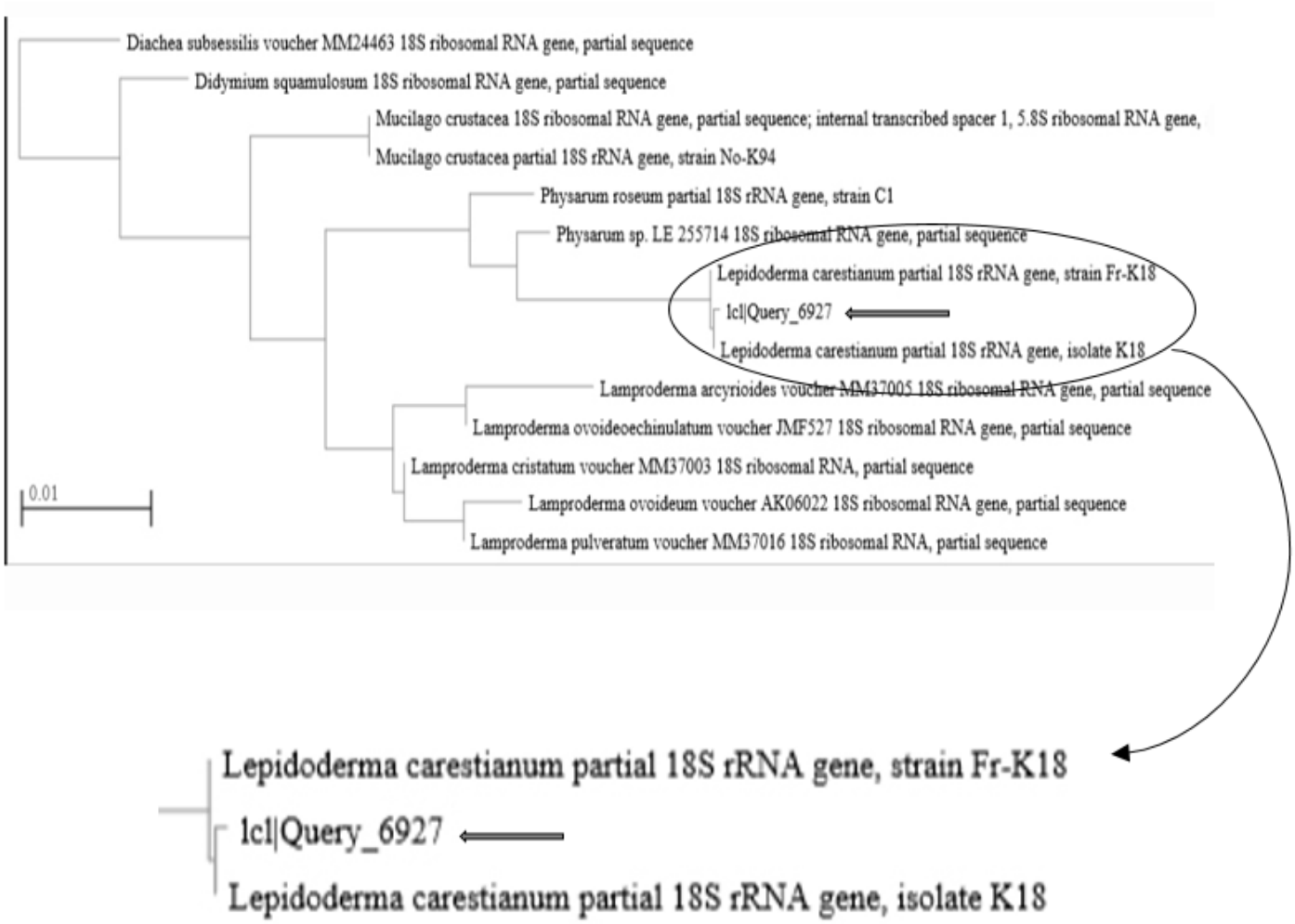

Figure 3

Phylogenetic tree of COI sequences of tested myxomycete strain (1c1|Query_6927) aligned with some related sequences of other myxomycetes.

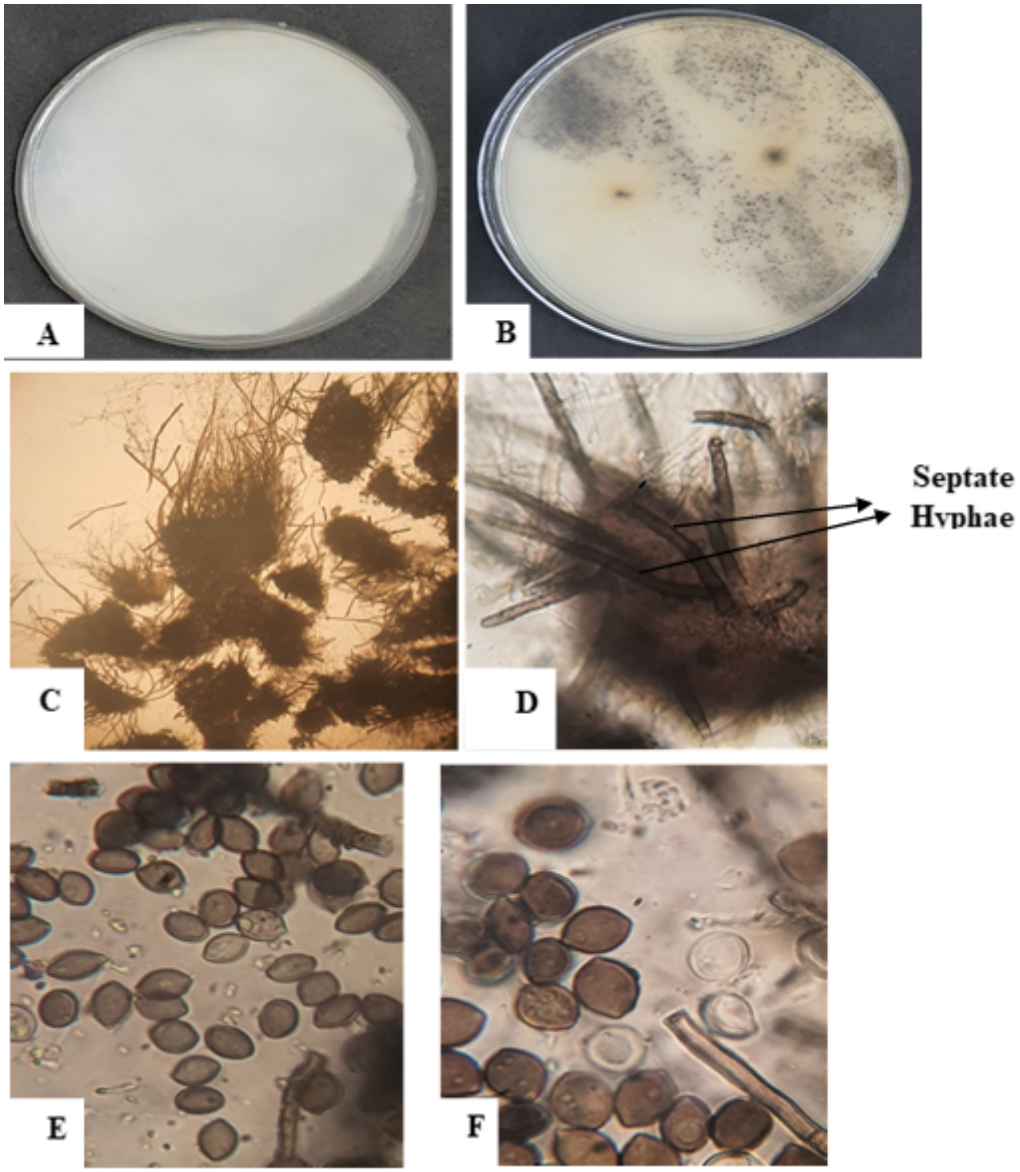

Figure 4

Morphology and microscopic examination of *A. bambusicola*: **A.** White colony. **B.** Reverse with black spots due to conidiomata. **C&D.** Conidiomata surrounded by septate hyphae. **E&F.** Conidia. Objectives 10 and 40 X.

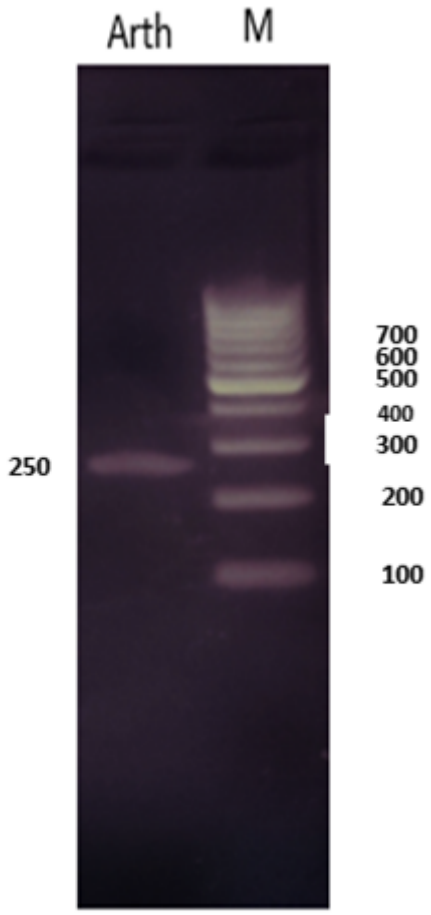

Figure 5

PCR products obtained from fungal isolate; Arth using ITS1 and ITS4 primers. Lane M: Molecular weight markers, PCR products of isolate Arth (250 Bp). Samples were run on 2% agarose gel.

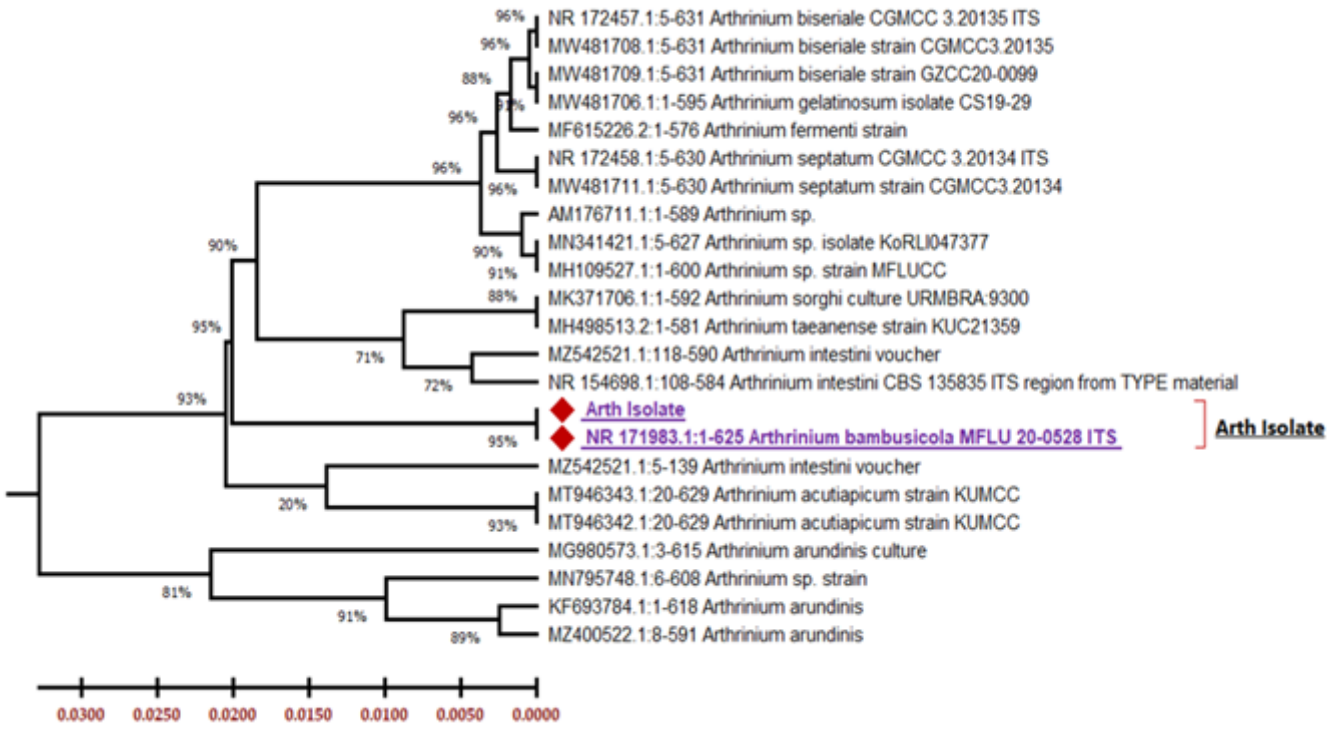

Figure 6

Phylogenetic tree based on ITS sequences, showing the relationship between the tested isolate (Arth) and other species belong to the genus *Arthrinium*. The tree was constructed using the MEGAX and neighbor-joining method.

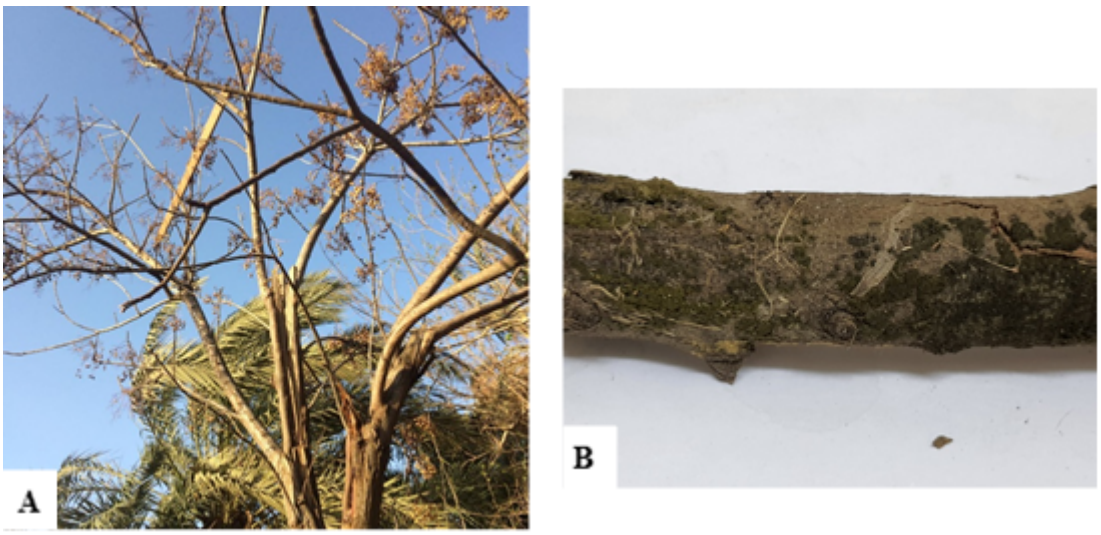

Figure 7

Melia azedarach tree: **A.** Healthy tree. **B.** olivaceous green growth of *A. bambusicola* on the dead branch.