The genus Arthrocladiella: a new report of powdery mildew fungi from Iran

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Abstract

Arthrocladiella is a monotypic member of family Erysiphaceae (*Ascomycota, Helotiales*), which has only been reported from *Lycium* spp. (Solanaceae). Morphological data supplemented with rDNA ITS sequence confirmed that the *Arthrocladiella mougeotii* infects *Lycium* sp. in Iran. Moreover, *Arthrocladiella* is reported as a new genus for Iranian Mycobiota.

Introduction

Arthrocladiella is a monotypic genus within the family Erysiphaceae (Braun and Cook, 2012). Taxonomy of this genus date backs to Vassilkov (1960) who published *Arthrocladiella* for *Arthrocladia* Golovin 1956. *Arthrocladiella mougeotii* is the only species that infects various species of *Lycium* in Solanaceae (Glawe et al. 2004, Braun and Cook. 2012, Wang et al. 2015, Özer et al. 2016, Kiss et al. 2018, Schmidt and Braun. 2020, Zhu et al. 2020). Occurrence of this species has been confirmed on *Lycium* species worldwide such as Russia, Europe, China, Japan, New Zealand, USA, Turkey, Israel (Braun and Cook 2012). As we are aware, there is no report of the occurrence of this fungus outside of the Solanaceae family.

In a comprehensive work for re-examination of powdery mildew fungi belonging to the Genus *Erysiphe*, we encounter one collection from IRAN herbarium that already identified as *E. deutziae* on *Deutzia gracilis*. *Deutzia gracilis (*Hydrangeaceae) is a flowering plant which is native to Japan. This deciduous shrub is planted for ornamental purposes in Iran. Six *Erysiphe* species (Braun and Cook. 2012, Qiu et al. 2019), as well as a single *Golovinomyces*, a single *Pseudoidium*, and two *Phyllactinia* species have been reported on Hydrangeaceae so far (Braun and Cook. 2012). Precise morphological studies supplemented with rDNA ITS sequencing disclosed that the powdery mildew on this collection in Iran is a member of the genus *Arthrocladiella*. Hence, we re-examine plant material and compared it with type material of *Deutzia gracilis* and two authentic herbarium specimens of *Lycium europaeum*, in the JSTOR Global Plants database, to re-examine the previous host plant diagnosis. As a result, we made sure that host plant has already been misidentified and it belongs to the genus *Lycium*. In this paper *Arthrocladiella mougeotii* is re-described and illustrated for the first time from Iran.

Materials And Methods

For microscopic preparation, a small piece of mycelia (including conidia and conidiophores) was transferred to a microscopic slide equipped with a drop of 1:1 glycerin: lactic acid using a clear adhesive tape, slightly heated, and then examined using Sairan BM22 biological microscope. At least 30 fungal structures were examined for measurements. The DNA was extracted using Thermolysis buffer (Zhang et al. 2010). The polymerase chain reaction (PCR) was done using semi-nested method. The first reaction was done using powdery mildew specific primers PMITS1 (5'-TCGGACTGGCCYAGGGAGA-3') (Cunnington et al. 2003) and PM11 (5'-TACCGCTTCACTCGCCGTTA-3') (Bradshaw and Tobin 2020). The second reaction was done using PM10 (5'-GGCCGGAAAGTTGTCCAAAC-3') (Bradshaw and Tobin 2020) and

PM11 powdery mildew specific primers. PCR components and conditions was designed according to Darsaraei et al (2022). The amplicons were then sent to Codon Genetic Group (Tehran, Iran) for sequencing using PM10 primer. The sequence was submitted to NCBI GenBank (accession number OM 658371).

Obtained sequence was compared with the sequences available in the NCBI GenBank nucleotide database using a BLASTN search method. Several sequences from GenBank were selected for phylogenetic analyses. All sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA7 (Kumar et al. 2016). The phylogenetic reconstruction was held using Maximum Likelihood approach. The ML tree was conducted in RAxML (Silvestro and Michalak 2012) under a GTRGAMMA model. The bootstrap analysis (Felsenstein 1985) consists of 1000 pseudoreplicates followed by a search for the tree with the highest likelihood. *Phyllactinia moricola* (AB080561) and *Leveillula taurica* (AB667884) were used as the outgroup sequences.

For host plant identification the JSTOR Global Plants database (2022) was used.

Results And Discussion

Molecular phylogeny

A total of 39 sequences consisting of 679 characters were included in the phylogenetic analysis. The alignment includes 37 sequences of *A. mougeotii*, *E. deutziae*, *E. deutziicola*, and some Type sequences of *Golovinomyces* spp., as well as two outgroup sequences. As shown in Fig. 1, the desired sequence placed in *Arthrocladiella* clade, and forms a sister clade with *Golovinomyces* spp. This sequence showed one base substitution when compared with other sequences of *A. mougeotti* retrieved from GenBank.

Morphology

Mycelium amphigenous, *hyphal appressoria* nipple-shaped, *conidiophores* arising from upper surface of the mother cell, erect, 60–130 × 9–11 µm, sometimes there is a concavity below the mother cell, *foot-cells* straight to slightly sinuous, 28–44 × 8–13 µm, followed by 1–4 (5) shorter cells (mostly 3), forming conidia in chain, *conidia* ellipsoid, obovoid, cylindrical, 28–37 × 10–16 µm, conidial germination ± terminal, from one or both ends, sometimes two sides of one end (Fig. 3).

We found some immature chasmothecia on examined specimens. However, there is a brief description of the sexual state of this specimen in Iran (Abbasi et al 2013): It seems these authors found more chasmothcia and used them for their examination. They have described the sexual state for this specimen as follows: chasmothecia were 100–160 µm diam., asci often obovoid or rather clavate, 50–56 × 22–26 µm, the appendages mostly tend to point to one direction, dichotomously or trichotomously branched, primary branches deeply divided and the following branchlets are shorter. Apex of the appendages are straight. According to above mentioned features it seems they observed some

morphological characteristics fits well with *A. mougeotii* especially the features related to the appendages.

After fungal identification and having molecular support for *A. mougeotii*, we re-examined the host plant specimen (Fig. 2) using JSTOR Global Plants database, and compared Iranian specimen with type material of *Deutzia gracilis* (L0176009) and two authentic specimens of *Lycium europaeum* (MA108036 & B -W 04474 -01 0). The above comparison also confirmed the fungus ID and showed that host plants is definitely belong to the genus *Lycium*.

Specimen examined: on*Lycium* sp., J. Boujari, 3/Dec/2012, Tehran, Iran (IRAN 16117 F)

Based on host plant and primary investigations, this specimen was regarded as a member of *Erysiphe deutziae* species complex, but current molecular studies shed light on the mysterious state of this specimen. The genus *Arthrocladiella* is reported as a new genus for the Mycobiota of Iran.

Declarations

Acknowledgement

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Authors' contributions Seyed Akbar Khodaparast, planned and supervised the work; Hamideh Darsaraei and Seyed Akbar Khodaparast performed DNA sequencing and Phylogenetic analysis; Hamideh Darsaraei provided the drawings and color photos; Mehrdad Abbasi, provided powdery mildew specimens. All authors reviewed the manuscript and commented on the manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on on reasonable request. All other relevant data are within the paper.

Conflict of interest The authors declare no conflicts of interest.

Ethics approval This article does not contain any studies with human

participants or animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

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Figures

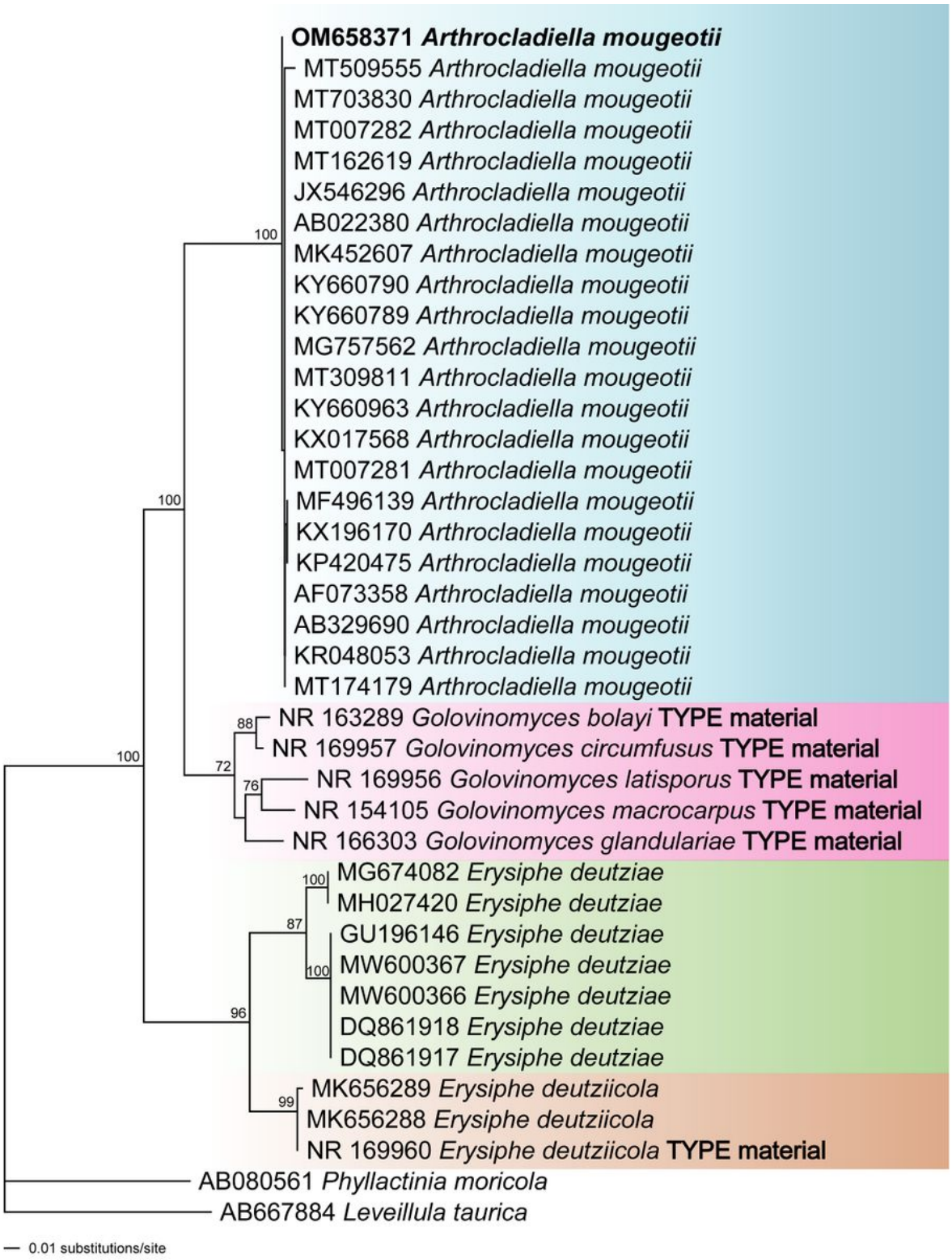

Figure 1

Phylogenetic analysis of ITS region for 39 sequences in RAxML software. Bootstrap values (>70%) are shown above the branches. Branch length represents the rate of substitution per site.

Figure 2

Lycium sp. (IRAN 16117 F) infected with *Arthrocladiella mougeotii*

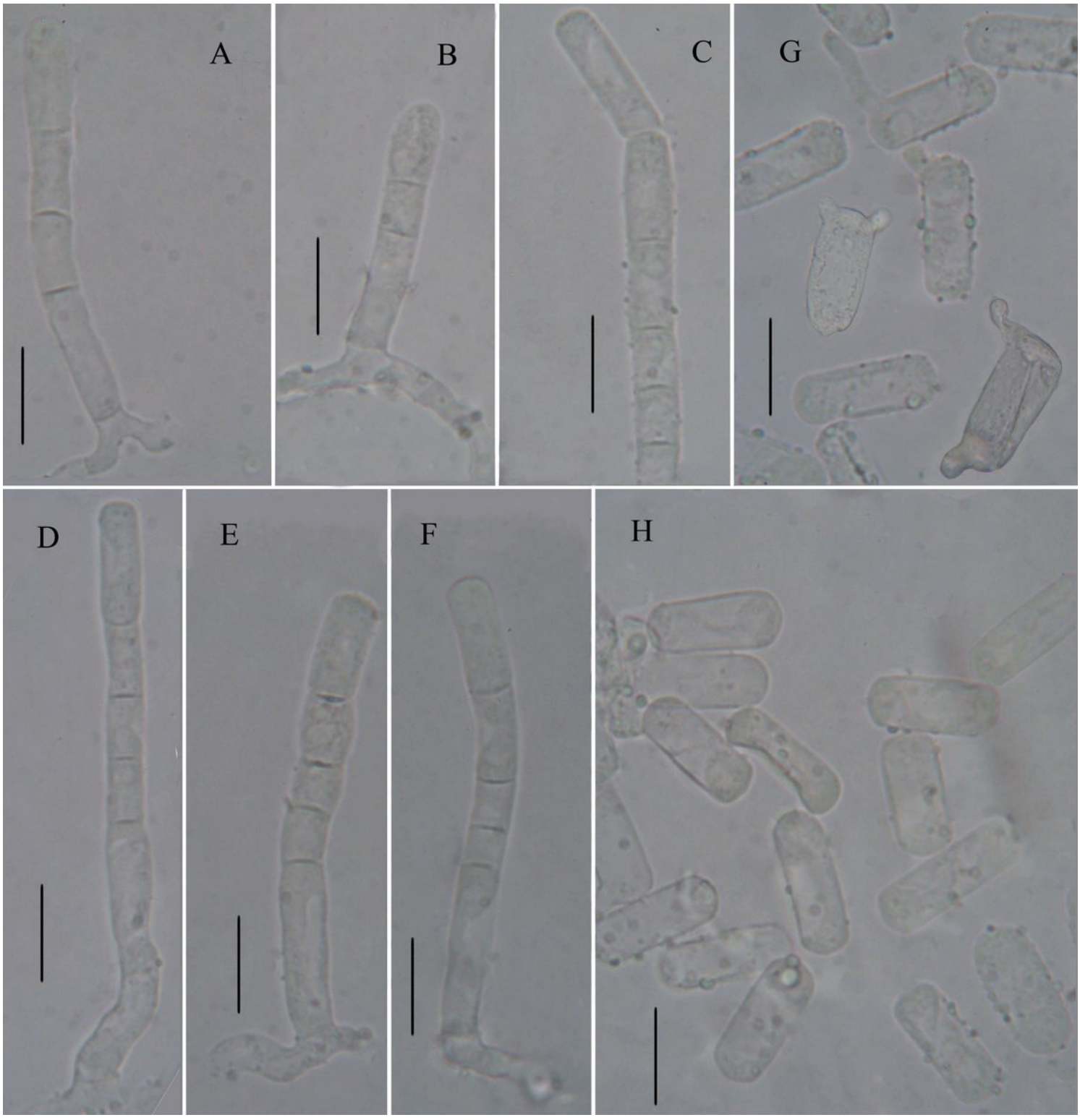

Figure 3

Arthrocladiella mougeotii *on* Lycium *sp., A-F Conidiophores, G conidial germination, H conidia. Scale bars = 20 µm.*

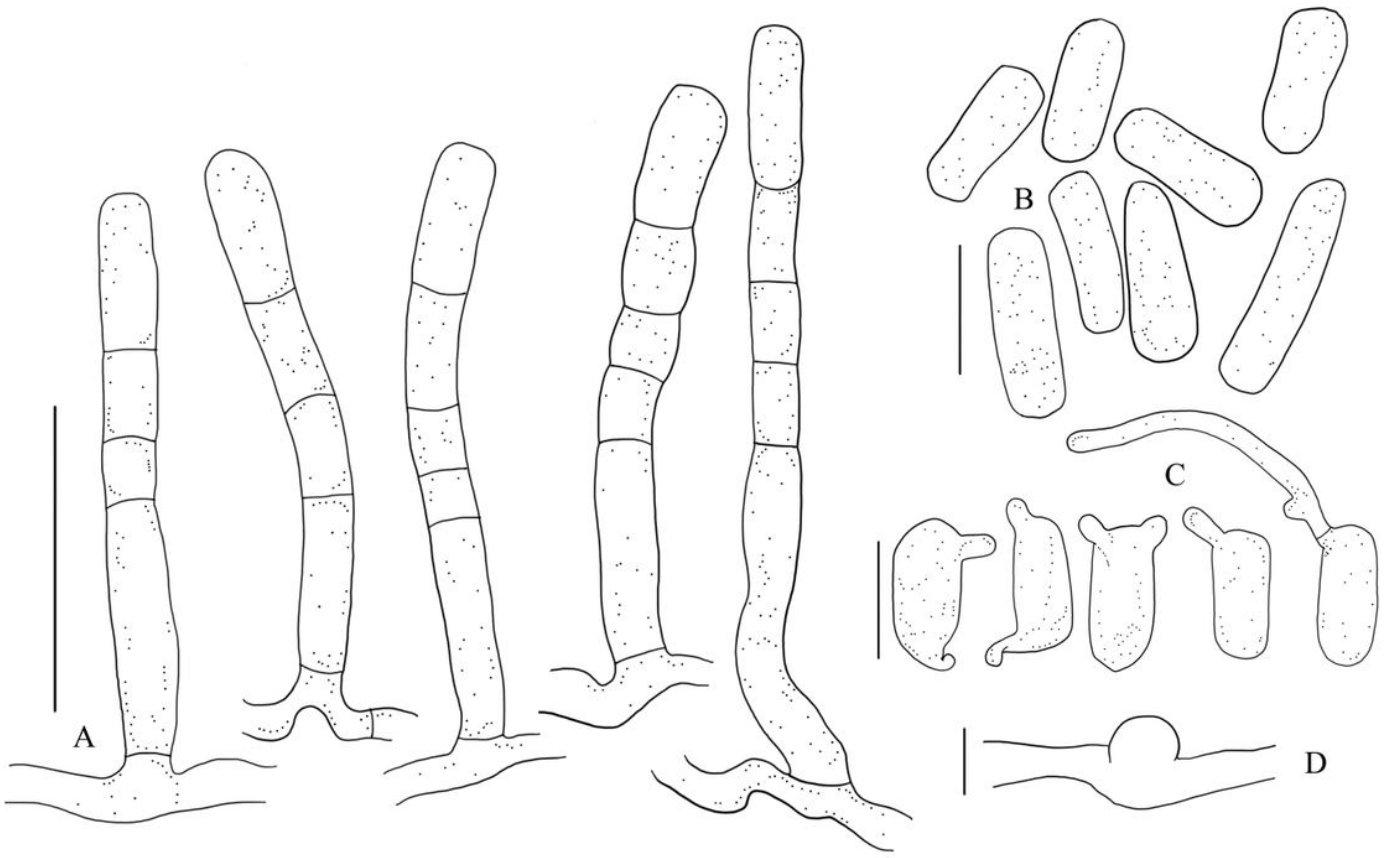

Figure 4

An illustration of Arthrocladiella mougeotii on Lycium sp. A conidiophores, B conidia, C conidial germination, D hyphal appressoria. Scale bars for A =50 µm, B, C = 20 µm, D = 10 µm.