

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

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**ABSTRACT:** In this study, we re-examined a collection from IRAN herbarium that was identified as *Erysiphe deutziae* on *Deutzia gracilis*. Precise morphological studies supplemented with rDNA ITS sequencing disclosed that the powdery mildew on this collection is a member of the genus *Arthrocladiella*. Moreover, re-examination of host plant confirmed that host plant has been misidentified and it belongs to the genus *Lycium*. *Arthrocladiella* is a monotypic member of family *Erysiphaceae* (Ascomycota, *Helotiales*), which has only been reported from *Lycium* spp. (*Solanaceae*). According to our findings, *Arthrocladiella mougeotii* infects *Lycium* sp. in Iran and is reported as a new genus for Iranian Mycobiota.

**KEYWORDS:** Biodiversity, Taxonomic Novelties, rDNA ITS, *Helotiales*, *Golovinomycetaceae*

### INTRODUCTION

*Arthrocladiella* is a monotypic genus within the family *Erysiphaceae* (Braun and Cook, 2012). Taxonomy of this genus date backs to Vassilkov who published *Arthrocladiella* for *Arthrocladia* Golovin (refer to Braun and Cook 2012). *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 encountered one collection from IRAN herbarium that was 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 is a member of the genus *Arthrocladiella*. Hence, we re-examined 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 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. Total 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'-GGCCGAAAGTTGTCCAAAC-3') (Bradshaw and Tobin 2020) and PM11 powdery mildew specific primers. PCR components and conditions were 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).

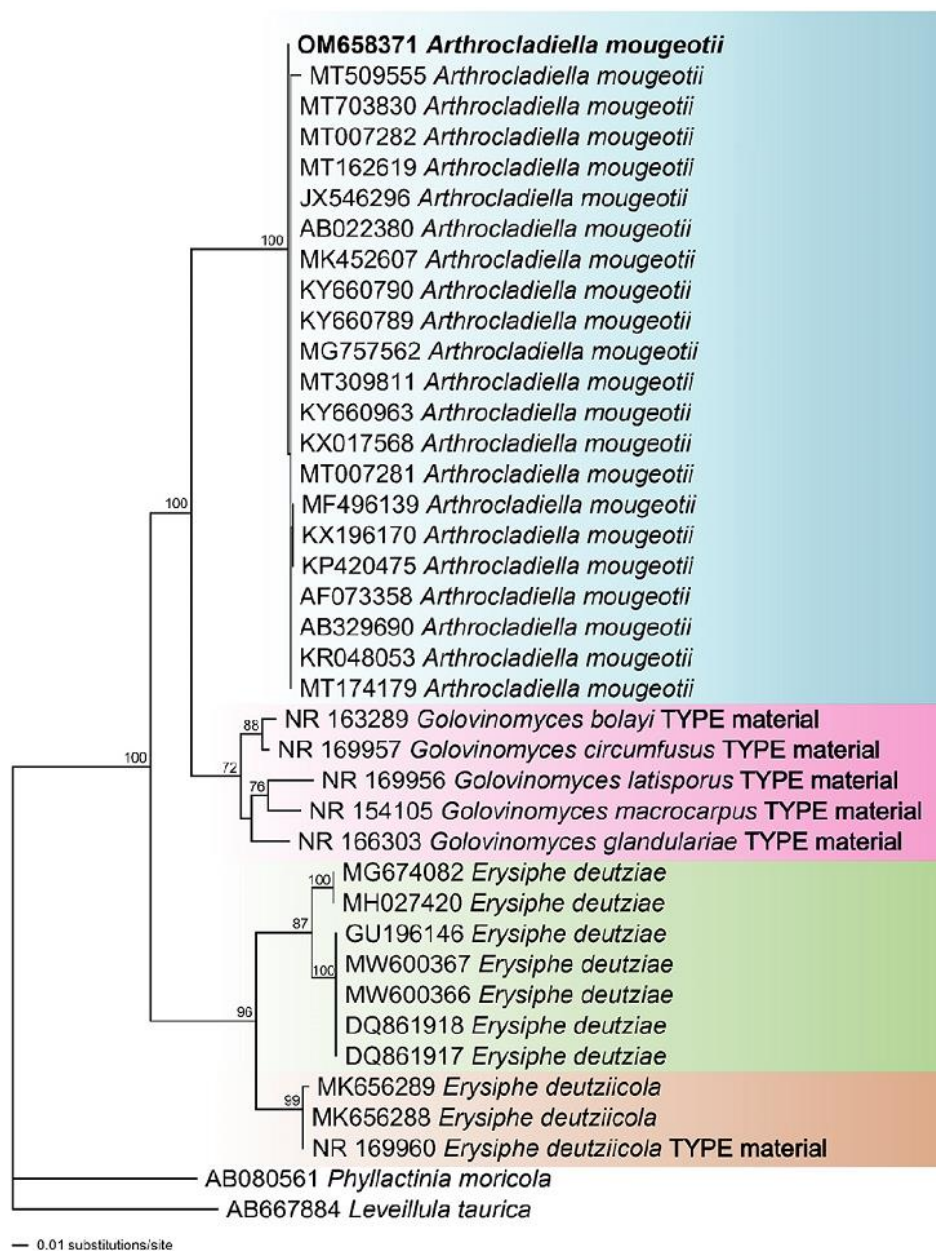
Obtained sequence was compared with the sequences available in the NCBI GenBank nucleotide database using a BLASTN search method. Several sequences from GenBank were selected for phylogenetic analyses. All sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA7 (Kumar et al. 2016). The 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.

#### 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. deuziicola*, and some type sequences of *Golovinomyces* spp., as well as two outgroup sequences. As shown in Figure 1, the desired sequence placed in *Arthrocladiella* clade, and forms a sister clade with *Golovinomyces* spp.



**Fig.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.

This sequence showed one base substitution when compared with other sequences of *A. mougeotii* retrieved from GenBank.

## RESULTS AND DISCUSSION

### Morphology

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

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 chasmothecia and used them for their examination.

They have described the sexual state for this specimen as follows: chasmothecia were  $100\text{-}160 \mu\text{m}$  diam., asci often obovoid or rather clavate,  $50\text{-}56 \times 22\text{-}26 \mu\text{m}$ , 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 the host plant definitely belongs to the genus *Lycium*.

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

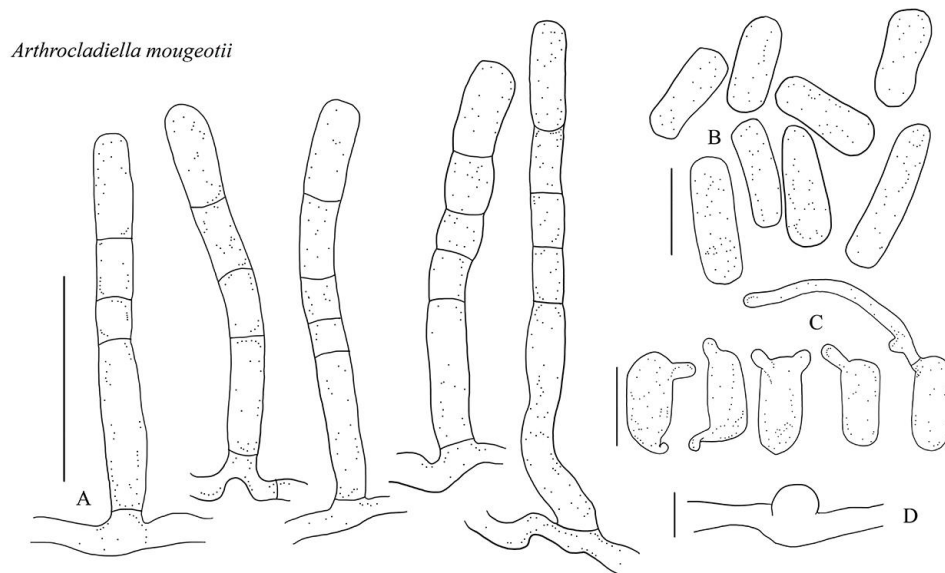
Based on host plant and primary investigations, this specimen was regarded as a member of *Erysiphe deutziae* species complex, but current molecular studies shed light on the mysterious state of this specimen. The genus *Arthrocladiella* is reported as a new genus for the Mycobiota of Iran.



**Fig. 2** *Lycium* sp. (IRAN 16117 F) infected with *Arthrocladiella mougeotii*



**Fig. 3** *Arthrocladiella mougeotii* on *Lycium* sp., A-F Conidiophores, G conidial germination, H conidia. Scale bars = 20  $\mu$ m.



**Fig. 4** An illustration of *Arthrocladiella mougeotii* on *Lycium* sp. A conidiophores, B conidia, C conidial germination, D hyphal appressoria. Scale bars for A = 50  $\mu$ m, B, C = 20  $\mu$ m, D = 10  $\mu$ m.

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## جنس *Arthrocladiella*: یک گزارش جدید برای قارچ‌های عامل سفیدک‌های پودری در ایران

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**چکیده:** در این مطالعه یک نمونه از هرباریوم ایران که با نام *Erysiphe deutziae* از روی گیاهی با نام *Deutzia gracilis* ثبت شده بود مجدداً بررسی شد. مطالعات دقیق بر اساس ریخت‌شناسی و توالی‌یابی rDNA ITS آشکار ساخت که این گونه از قارچ‌های مولد سفیدک‌های پودری به جنس *Arthrocladiella* تعلق دارد. علاوه بر این بررسی مجدد نام میزبان نیز ثابت کرد که میزبان قبلاً اشتباه تشخیص داده شده بود و به جنس *Lycium* تعلق دارد. *Arthrocladiella* یک جنس تک گونه داخل تیره‌ی *Erysiphaceae* (*Ascomycota, Helotiales*) است که فقط از روی گونه‌های *Lycium* (*Solanaceae*) گزارش شده است. بر اساس یافته‌های ما، *Arthrocladiella* گونه‌ی *Lycium* sp. را در ایران آلوده می‌کند و یک جنس جدید برای فهرست قارچ‌های ایران است.

**کلمات کلیدی:** تنوع زیستی، آرایه‌ی جدید، rDNA ITS، *Helotiales, Golovinomycetaceae*