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Myxotrichum, a new genus for the funga of Iran

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Abstract

An isolate of an ascomycetous fungus collected during a field trip in the Zagros Forest, Kermanshah Province (West of Iran) was identified as *Myxotrichum*. Based on the morphological and molecular characters the isolate was identified as *M. deflexum*. Genomic DNA was extracted and a nuclear rDNA region, containing the internal transcribed spacers 1, 2 and 5.8S gene of rDNA (ITS) were amplified and PCR product was sequenced. Amplicon was purified, sequenced and submitted to the GenBank (Acc. No. OR047187). The BLAST search results showed highest similarity of this isolate to other isolates of *M. deflexum* from GenBank. By reviewing the literature on ascomycetous fungi, *Myxotrichum* is found as a new genus for the funga of Iran.

Keywords: *Ascomycota*, *Helotiales*, *Leotiomycete*, molecular identification, *Myxotrichum deflexum*

Myxotrichum، جنس جدیدی برای قارچ‌های ایران

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خلاصه

جنس *Myxotrichum* از جمله قارچ‌های آسکومیست در راسته *Helotiales* و رده *Leotiomycetes* است که زیستگاه معمول گونه‌های آن، لایه‌های هوموس در جنگل‌ها و تعدادی نیز از خاک، چوب، مواد سلولزی و سایر مواد پوسیده گزارش شده‌اند. بیشتر گونه‌های این جنس در دمای پایین رشد کرده و به صورت پوده‌رست در تجزیه مواد گیاهی نقش مؤثری ایفا می‌کنند. تاکنون ۲۰ گونه معتبر از این جنس در جهان گزارش و تایید شده است ولی در ایران تا کنون مطالعه‌ای در مورد جنس مذکور صورت نگرفته و نیز گزارشی از این جنس و گونه‌های آن وجود ندارد. طی بازدیدهای دوره‌ای از جنگل‌های بلوط در استان کرمانشاه، برگ‌های پوسیده درختان جمع‌آوری و با کشت آن‌ها یک آرایه قارچی با مشخصات زیر به دست آمد. پرگنه روی محیط کشت عصاره سیب زمینی-دکستروز-آگار رشد بسیار کمی داشت و پس از چهار هفته در دمای ۲۵ درجه سلسیوس به ۲۰ میلی‌متر رسید. پرگنه در ابتدا سفید رنگ و با گذشت زمان به قهوه‌ای متمایل به قرمز تغییر یافت. رنگ پشت پرگنه قرمز شرابی بود. رنگدانه‌های صورتی تا قرمز نیز در محیط کشت تولید شد. آسکوکارپ‌ها کروی و با زواید قهوه‌ای تیره و منشعب پوشیده شده بودند. انشعابات زواید راست و تعدادی نیز برگشته و وارونه و انتهای آن‌ها تا حدودی شفاف بودند. آسک‌ها شفاف، دارای هشت آسکوسپور، کروی تا نیمه‌کروی و به ابعاد ۱۵/۷-۱۲/۲ × ۱۰/۱۲-۷/۸ میکرومتر بودند. آسکوسپورها شفاف، بیضوی و به ابعاد ۴/۴-۳/۳ × ۳-۲ میکرومتر بودند. مرحله میتوسپوری قارچ در محیط کشت مشاهده نشد. توصیف این جنس با توصیف سایر محققان در مورد جنس *Myxotrichum* و گونه *M. deflexum* Berk. منطبق بود. به علاوه، هویت گونه مذکور، با استفاده از توالی‌یابی ناحیه نسخه‌برداری شده داخلی دی.ان.ای. ریبوزومی و واکاوی فیلوژنتیکی انجام شد.

واژه‌های کلیدی: شناسایی مولکولی، *Ascomycota*، *Helotiales*، *Leotiomycete*، *Myxotrichum deflexum*

Introduction

The *Helotiales* is an order of the class *Leotiomycetes* within the phylum *Ascomycota*. The order encompasses 33 families, among which the *Myxotrichaceae* with its three distinct genera, namely, *Byssoascus*, *Myxotrichum*, and *Oidiodendron* placed in the said order (Wijayawardene *et al.* 2020). Members belonging to the *Myxotrichaceae* possess fusiform or ellipsoidal ascospores that are striate or smooth. They possess the ability to decompose cellulosic substrates, and some have *Geomyces* or *Malbranchea* anamorphs (Eriksson *et al*. 2001). Twenty species of *Myxotrichum* are recorded in the Index Fungorum database (Wijayawardene *et al*. 2020). In forest soils, *Myxotrichum* species are common inhabitants of the humus layers, and since they can grow and sporulate at low temperatures, they might be used to colonize colder environments as saprophytes helping in the digestion of the plant material (Uchiyama *et al*. 1995).

Materials and Methods

In 2022, the presence and frequency of ascomycetous fungi was studied in oak Zagros forest, Kermanshah Province (West of Iran). Oak litters were surface disinfected by immersing in 1% solution on NaOCl for 30 sec, rinsed in sterile distilled water and plated on potato-dextrose-agar (PDA) amended with chloramphenicole (25 µg/ml). The *Myxotrichum* fungus was isolated from oak litter samples. Pure cultures were obtained by monosporic isolation. A representative isolate, RU-MyDe1, was used for morphological and molecular characterization and deposited in the Fungal Reference Collection of the Iranian Research Institute of Plant Protection (Tehran, Iran). For confirmation of morphological identification, the internal transcribed spacer region of the nuclear ribosomal DNA (ITS-rDNA) was amplified using primer pairs ITS1 (CCGTAGGTGAACCTGCGC) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al*. 1990). The PCR product was submitted for sequencing to a capillary sequencing machine (Microsynth Co., Swiss). ITS aligned sequences dataset was used for phylogenetic analysis of *Myxotrichum* species. Phylogenetic analyses were performed using Maximum Parsimony (MP) method in the MEGA Ver. 5 software (Tamura *et al*. 2011).

Results and Discussion

In this study, one isolate of *Myxotrichum* on oak litter was found in Zagros forest, Kermanshah Province (West of Iran) in 2022 with the following characteristics: Colony on potato-dextrose-agar (PDA) was slow growing, attaining a diameter of 20 mm in four weeks on PDA at 25 °C, at first white, then turned to reddish brown, diffused pink pigment on PDA and frequently developed pink or red patches (Fig. 1a, b). The colony on the reverse side of the agar plate was hyaline to dark pink (Fig. 1c). Ascomata cleistothecial, globose, covered by septate, thick-walled, dark brown, and branched appendages (Fig. 2a–c). Appendage branches were straight or deflexed upward or downward, borne along almost the entire length of the appendage, with hyaline apices (Fig. 2d–f). Asci hyaline, globose or subglobose, 8-spored, 7.8–10.12 × 12.2–15.7 µm (Fig. 2g, h). Ascospores hyaline, ellipsoidal, 2–3 × 3.3–4.4 µm (Fig. 2i). Asexual morph was not formed on PDA medium. The characteristics of *Myxotrichum* sp. isolate agreed with the descriptions reported by Currah (1985), Uchiyama *et al.* (1995), and Fernando *et al*. (2005).

Specimen examined: IRAN: Kermanshah Province, on oak leaf litter (oak forest), 02.05.2022, S. Jamali (IRAN 4845C).

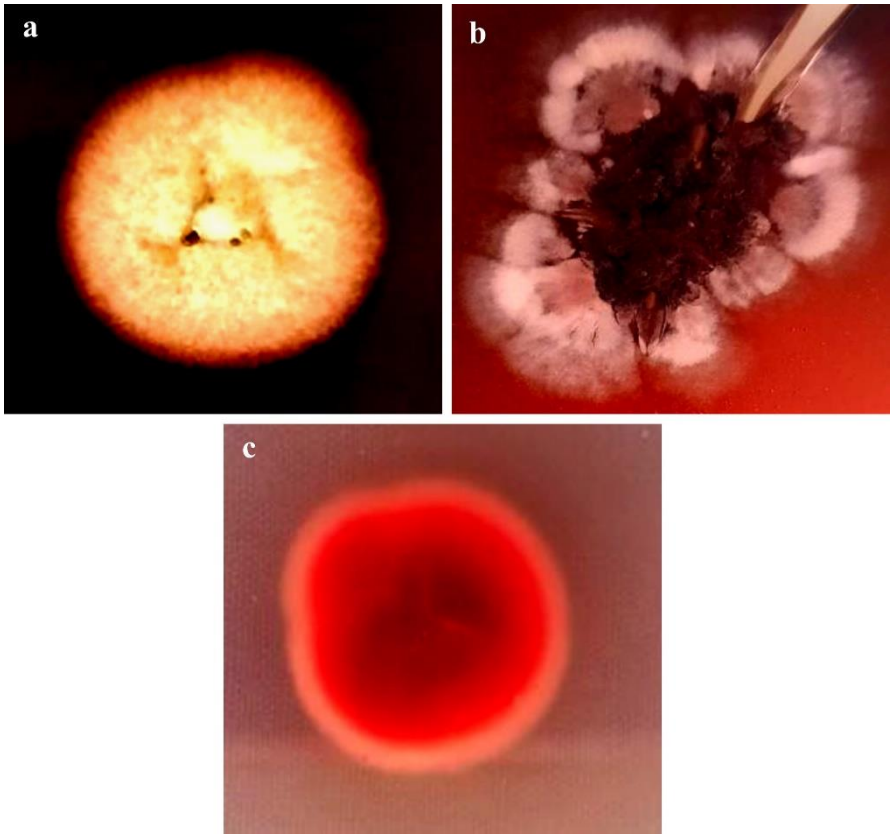

Fig. 1. *Myxotrichum deflexum* colony on PDA: a. One week, b. Four weeks, c. Colony reverse.

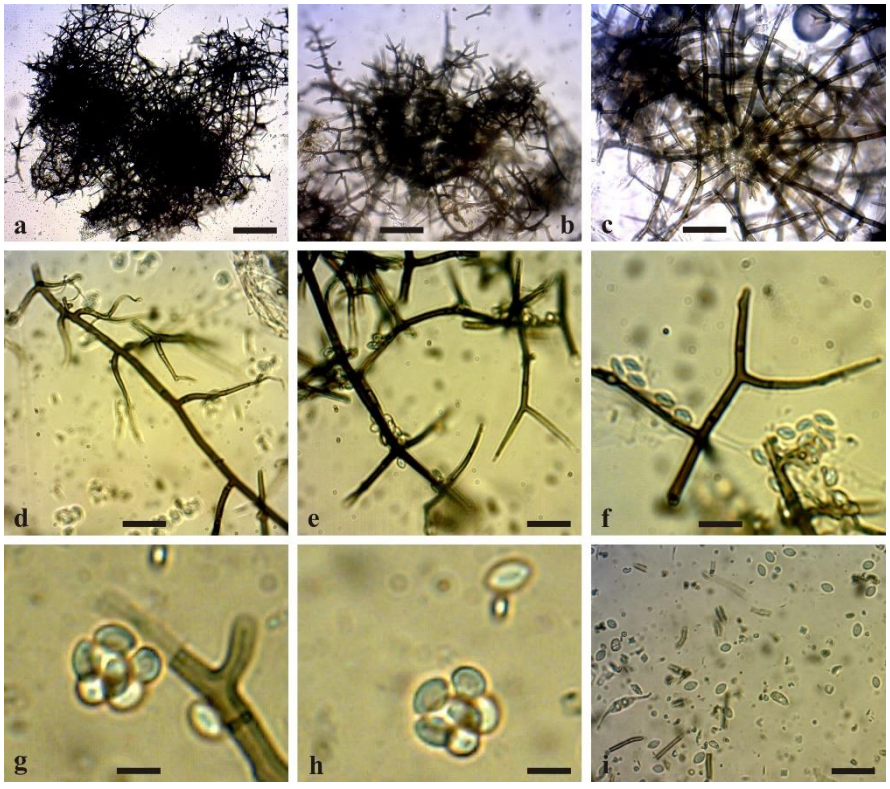

Fig. 2. *Myxotrichum deflexum* Ascocarp: a–c. Appendages, d–f. Asci, g, h. Ascospores (Bars: a–c = 20 µm, d–h = 5 µm, i = 10 µm).

For confirmation of morphological identification, the internal transcribed spacer region of the nuclear ribosomal DNA (ITS-rDNA) was amplified using primer pairs ITS1 (CCGTAGGTGAACCTGCGC) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al*. 1990). The PCR product was submitted for sequencing to a capillary sequencing machine (Microsynth Co., Swiss). The sequence generated in this study was deposited in GenBank under accession number OR047187. BLAST analysis revealed a 99.6% nucleotide identity with the ITS region of *Myxotrichum deflexum* Berk. (OW987173, OW987189, JX160056, OU989414, and OW987171) that were reported from Belgium, France, and the UK (Becker *et al*. 2014). ITS aligned sequences dataset was used for phylogenetic analysis of *Myxotrichum* species. Phylogenetic analyses were performed using Maximum Parsimony (MP) method in the MEGA Ver. 5 software (Tamura *et al*. 2011). Phylogenetic analyses based on ITS sequences of the present study isolate together with 18 selected isolates of *Myxotrichum* showed that, this isolate is closely related to *M. deflexum* (Fig. 3). The isolate formed a well-supported clade with the authentic reference isolates of *M. deflexum* (Fig. 3) and placed separately from the other species of *Myxotrichum*. The result of the phylogenetic analysis was in accordance with the molecular identification based on DNA sequences in BLAST search, thus resolving the morphological identification.

Myxotrichum deflexum is recognized as a chromogenous fungus (Nugari 2005). Many researchers have recorded this species from Argentina, Canada, England, France, Israel, Japan, and USA, on cow dung, feathers, fingernails, paper-based materials, plaster board, rotten rug, rotting board, rotten straw, sand, soil, wood, wood shavings, and ringworm lesions on a dog (Pasquariello *et al.* 2005, Sato *et al*. 2014). To the best of author's knowledge, *Myxotrichum* is a new genus record for funga of Iran. A subculture of this fungus is kept at the Iranian Fungal Culture Collection of the Iranian Research Institute of Plant Protection (Tehran, Iran) under accession number IRAN 4845C.

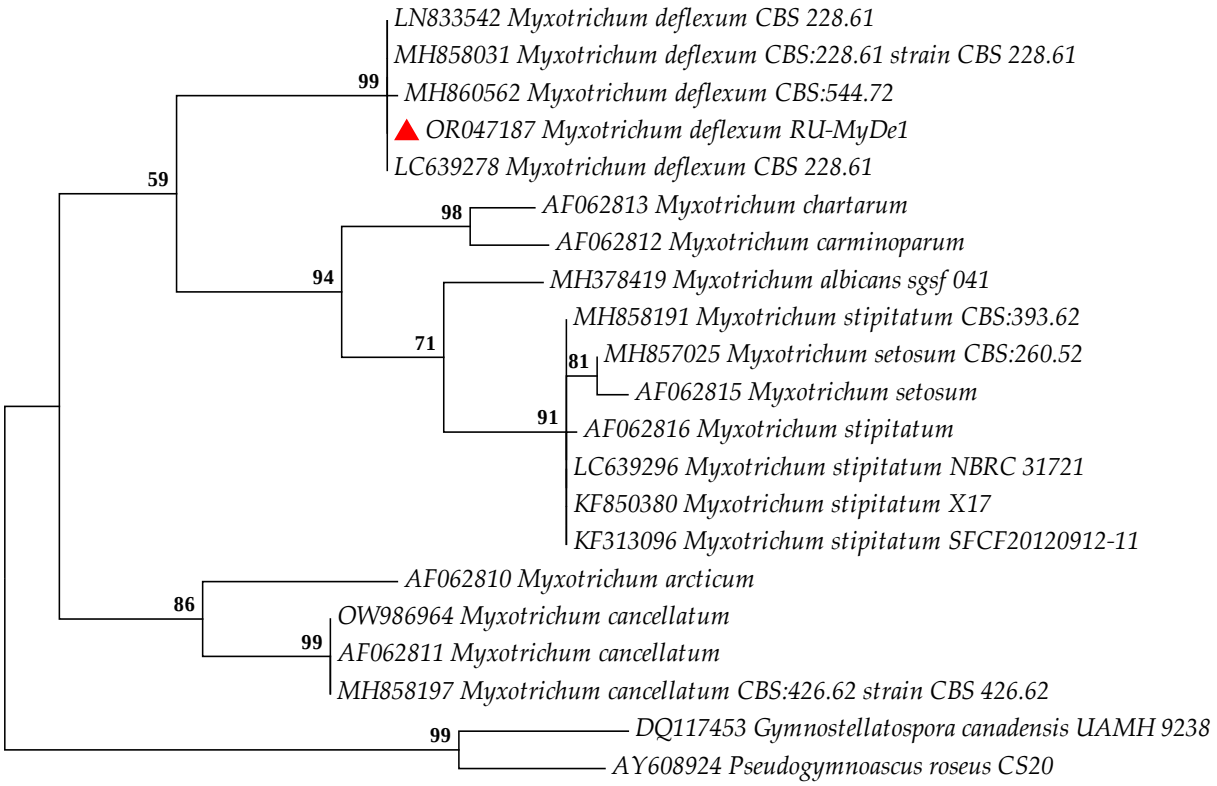

Fig. 3. Maximum Parsimony phylogram generated in Mega Ver. 5 from the alignment of 18 combined ITS1+5.8S+ITS2 regions of the genomic ribosomal DNA sequences of *Myxotrichum* isolates (CI = 0.76, RI = 0.88, RCI = 0.68, iRCI = 0.64, and Tree length = 243). The triangle (in red) refers to *Myxotricum* sp. isolate from Iran.

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