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Two new hyphomycete species from petroleum–contaminated soils for mycobiota of Iran

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Abstract: During a study on fungal diversity in petroleum contaminated soils in Khuzestan province, two new hyphomycetes were isolated for the mycobiota of Iran. Based on the combination of morphological data and ITS–rDNA sequence, the isolates were identified as *Alternaria obovoidea* and *Emericellopsis pallida*. Morphological descriptions and illustrations are provided for the species. To our knowledge, this is the first report of these two species from petroleum contaminated soils in the world.

Key words: Petroleum pollution, fungal diversity, ITS–rDNA, new record

INTRODUCTION

Soil supports a bewildering diversity of microbes and fauna. Soil microorganisms play important roles in soil development, major biological elements (C, N, P) cycling, the flow of energy, recycling of wastes, and detoxification of environmental pollutants (Aislabie & Deslippe 2013). Fungi are one of the major microbial groups in the soil. Despite the important ecological role of soil fungi, the understanding of fungal community diversity and dynamics in soil is still in demand (De Boer et al. 2005).

Fungi can inhabit diverse ecological niches ranging from normal to extreme environments. The organisms able to grow in extreme environments are generally defined as extremophiles. Extremophiles have been adapted for a number of mechanisms which enable them to tolerate wide ranges of stresses (Palmer et al. 1990; Sterflinger 1998; Gorbushina et al. 2003; Ruibal 2004; Onofri et al. 2007; Gorbushina et al. 2008). There are a large number of physical, chemical and biological stress–inducing conditions in nature, including changes in temperature, osmotic pressure, pH and concentration of water, ions and solutes, as well as exposure to extremes of radiation, pressure and toxic chemicals, to oxidative conditions and to nutrient starvation (Moye–Rowley 2003).

Some fungal groups have adapted a number of features such as slow meristematic clumpy growth and thick–walled heavily melanised cells which enable them to grow in extreme conditions (Ruibal et al. 2009).

Heterogonous assemblages of fungi inhabit environments contaminated with aromatic compounds. Species in genera *Paecilomyces*, *Verticillium*, *Beauveria* and *Penicillium* species have been reported from petroleum–polluted marine water samples (Fedorak & Westlake 1986). Kim et al. (2010) have reported *Alternaria* species as the most frequently isolated fungi inhabiting creosote–treated wood. In another study, *Aspergillus flavus*, *A. niger*, *A. fumigatus* and *Alternaria* sp. have been isolated from petroleum–contaminated soils (Chaudhry et al. 2012). Zafra et al (2014) also observed *Acremonium*, *Fusarium*, and *Trichoderma* genera from petroleum–contaminated soils. According to Flayyih and AI–Jawhari (2014) *Aspergillus niger*, *A. fumigatus*, *Fusarium solani* and *Penicillium funiculosum* have been isolated from petroleum polluted soil.

In the present study, we describe two new fungal species namely *Alternaria obovoidea* and *Emericellopsis pallida* as new records for the mycobiota of Iran from petroleum contaminated soils in Khuzestan province based on a combination of morphological data and sequence data from ITS–rDNA region.

MATERIALS AND METHODS

Soil samples were collected from petroleum–contaminated soils in oil fields of Khuzestan province, Iran. Samples (approximately 25 g) were collected in depth of 15 cm from top soil. Fungal strains were isolated from soil samples following the protocol of Zhao et al. (2010).

Cultural and microscopic morphological features were examined on potato carrot agar (PCA) (Simmons 2007), malt extract agar (MEA) and oat meal agar (OMA) culture media. Fungal structures were mounted in distilled water and examined at 1000 X magni–fication using an Olympus–BX41 light microscope. Thirty measurements were taken of the relevant

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parameters of the conidiophores and conidia. Ninety five percentiles were derived for the measurements with the extremes given in parentheses. An Olympus digital camera system (DP 25) was used to capture high–resolution photographs of microscopic fungal structures.

For molecular identification, in a rapid DNA extraction protocol, a small piece of mycelium scraped from the surface of the plate, then was added to a PCR tubes with 50 µL TE Buffer, then mashed with a sterile pipette tip or toothpick. The samples were placed in thermocycler at 95°C for 5 min. DNA extracts were incubated at –20 °C prior to use. The primers ITS1–F, 5′–CTTGGTCATTTAGAGGAAGTAA–3′ and ITS4–R 5′–TCCTCCGCTTATTGATATGC–3′ (White et al. 1990; Gardes & Bruns 1993) were used to amplify ITS region of rDNA. ITS amplicons were generated for all strains by using the primer pairs ITS1–Fand ITS4–R. PCR reactions were performed in 25 µL volumes containing 1 µL of template DNA, 2.5 µL of 10 × reaction PCR buffer, 1 µL of 10 µM dNTP, 1 µL of each primer and 0.3 µL of 5.0 U Taq DNA polymerase. Ultrapure water was added to increase the volume to 25 µL. Amplification of ITS was performed under the following PCR conditions: 95 °C for 5 min, followed by 36 cycles consisting of 94 °C for 45 sec, 57 °C for 30 sec and 72 °C for 90 sec, with a final 10 min extension step at 72 °C. Sequencing was done on a 3730 DNA Analyzer sequencer at Plant–Microbe Genomics Facility (PMGF) at the Ohio State University. The obtained sequences were compared with those included in the GenBank database by using Basic Local Alignment Search Tool (BLAST) in NCBI.

RESULTS and DISSCUSION

Alternaria obovoidea and *Emericellopsis pallida* were identified based on a combination of morphological and sequence data from ITS–rDNA region. These species are reported as new records for mycobiota of Iran. Description of these two species is illustrated below.

***Alternaria obovoidea* (E.G. Simmons) Woudenberg et al., Studies in Mycology 75: 171–212 (2013)**
Basionym: *Ulocladium obovoideum* E.G. Simmons, Mycotaxon 37: 104. 1990. Section Ulocladioides

Colony on PCA attaining a diameter of 35–45 mm after 7 days at 25 °C. Texture woolly, grayish brown, dark blackish brown. Hyphae brown to golden, 4.42 µm in width as they grew old. Conidiophores brown, golden and curved due to production of conidiogenous cells, 45.26 µm in length and 4.25 µm in width. Conidia, solitary, golden or dark brown and punctuate roughened, forming beak 8 µm in length with 1 to 3 longitudinal and 2–5 transverse septa, subglobose in immature conidia, obovoid to broadly obovoid in those produced at 4–7 days (mature conidia). Conidial showed an average 32.85 µm in length and 13 µm in width (Fig.1).

Alternaria obovoidea has been originally described as *U. obovoideum* however, based on the recent multi–gene phylogenetic analysis a new combination has been proposed as *A. obovoidea* (Lawrence et al. 2013; Woudenberg et al. 2013). *Alternaria obovoidea* can be distinguished from other *Alternaria* species based on morphological characteristics summarized in Simmons (1990, 2007). In this species, conidia are obovoid to broadly obovoid, single or in chains. However, *A. obovoidea* resembles *A. alternata* and *A. arborescens* by producing geniculate conidiophores with secondary conidiophores and fundamentally septate conidia. Conidia of *A. alternata* and *A. arborescens* are smaller than those of *A. obovoidea* (Simmons 1990, 2007).

The identity of this species was confirmed as *A. obovoidea* based on 100% sequence identity of ITS sequence to the type strain of *A. obovoidea* CT4D (GenBank accession KP794083.1); while, substan–tially deviated from the other species of the genus namely *A. alternata*, *A. arborescens*, and *A. infectoria*. As regards, ITS–rDNA is not useful for robust identification within the *Alternaria* species (Pryor et al. 2002; Andrew et al. 2009; Lawrence et al. 2013); hence, in this study the morphological characteristics were preferred and assigned for identification of *A. obovoidea* species. Pure culture was established using a hyphal tip technique and was deposited in culture collection of Tabriz University (CCTU 1156), and in Iranian Fungal Culture Collection with accession numbers (IRAN 2744C) at the Iranian Research Institute of Plant Protection, Tehran, Iran.

***Emericellopsis pallida* Beliakova, Mikologiya i Fitopatologiya 8: 386 (1974)**

Colonies on OMA reached to 65 mm diameter after 10 days. MEA with slower growing, showed 45 mm diameter after 10 days. Colonies on OMA were white–pink to salmon–colored. Hyphae hyaline, branched, septate had 1–3 µm wide. Conidiophores macronematous, simple, sometimes lateral branched, septate, smooth–walled, 25–45 × 2–3 µm, and borne a terminal phialide. Conidiation abundant, conidia aseptate, hyaline, ellipsoidal, oval, smooth–walled and 3.5–6.0 × 1.8–2.2 µm gathering in colorless slime at the tips of the phialides, chlamydospores absent (Fig. 2).

The genus *Emericellopsis* belongs to the order Hypocreales (Sordariomycetes, Hypocreomycetidae), includes 22 species and varieties derived from various ecological niches (Lumbsch & Huhndorf 2007). Currently, *Emericellopsis* comprises saprobic cleistothecial species with acremonium–like conidiation (Crous et al. 2004). *Emericellopsis* species can be distinguished based on a combination of morphological and molecular data (Zuccaro et al. 2004; Duc et al. 2009; Grum–Grzhimaylo et al. 2013).

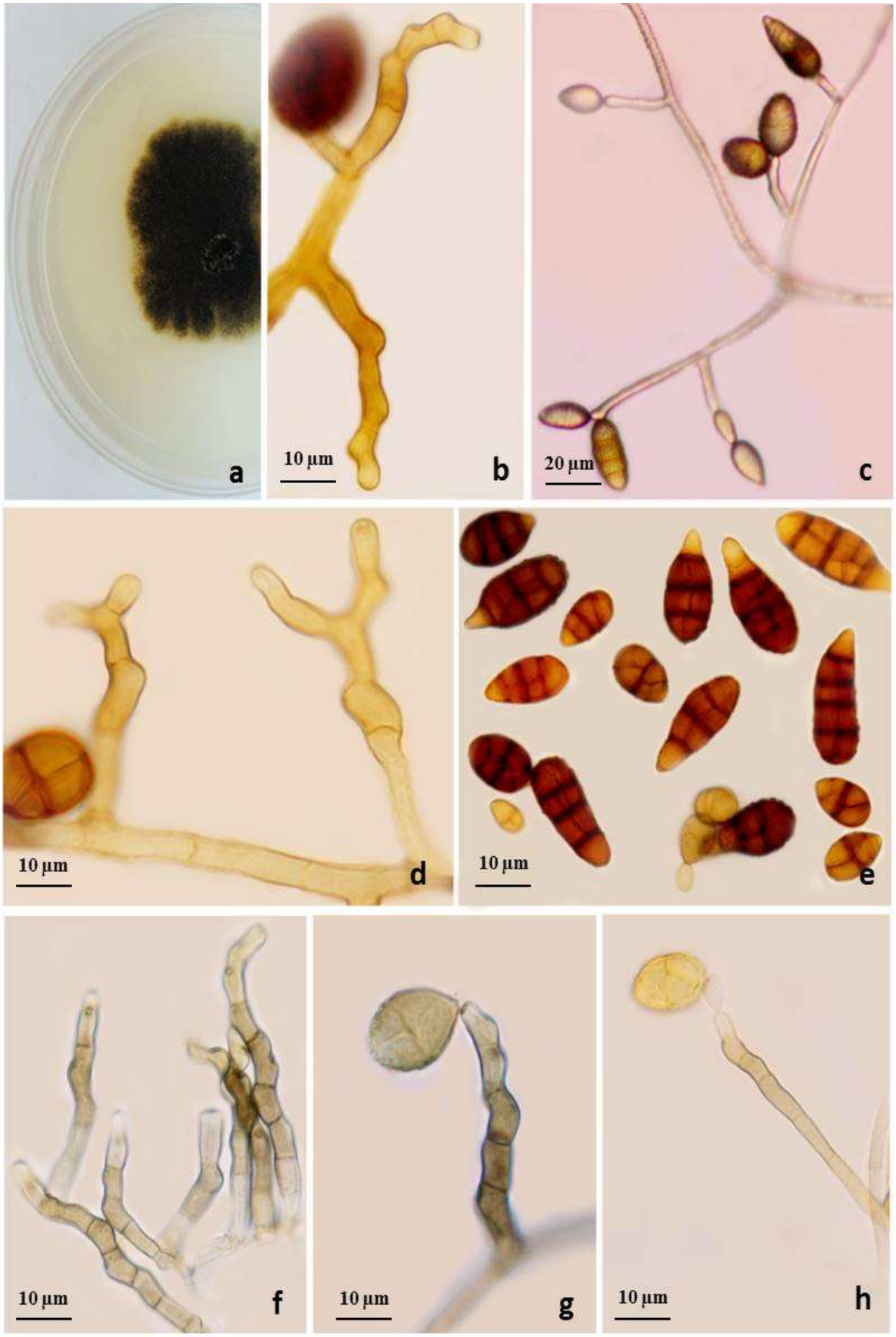

Fig. 1. *Alternaria obovoidea*. a. 7–d–old colony on PCA; b–f. Conidiophores and conidia; g–h. Sporulation.

Morphological features of our isolate are in full agreement with original description of *E. pallida*. The identity of our isolates was further confirmed as *E. pallida* based on 99% identity to the type strains of *E. pallida* XJURML–3 (GenBank accession EU045572.1); while deviated from the sequence data for other *Emericellopsis* and *Acremonium* species available in GenBank namely *E. terricola*, *A. zonatum* and *A. sclerotigenum*. This species is distinguished from other *Emericellopsis* and *Acremonium* species by the differences in conidial shape, sizes, conidiogenous structures and colony characteristics (Crous et al. 2004; Zuccaro et al. 2004; Lumbsch & Huhndorf 2007; Grum–Grzhimaylo et al. 2013). Pure culture was established using a hyphal tip technique and was deposited in culture collection of the University of Tabriz (CCTU 1132), and in Iranian Fungal Culture Collection with accession numbers (IRAN 2782C) at the Iranian Research Institute of Plant Protection, Tehran, Iran.

The sequences are available in Genbank with accession numbers KY053135 and KY039291 for *A. obovoidea* and *E. pallida* respectively.

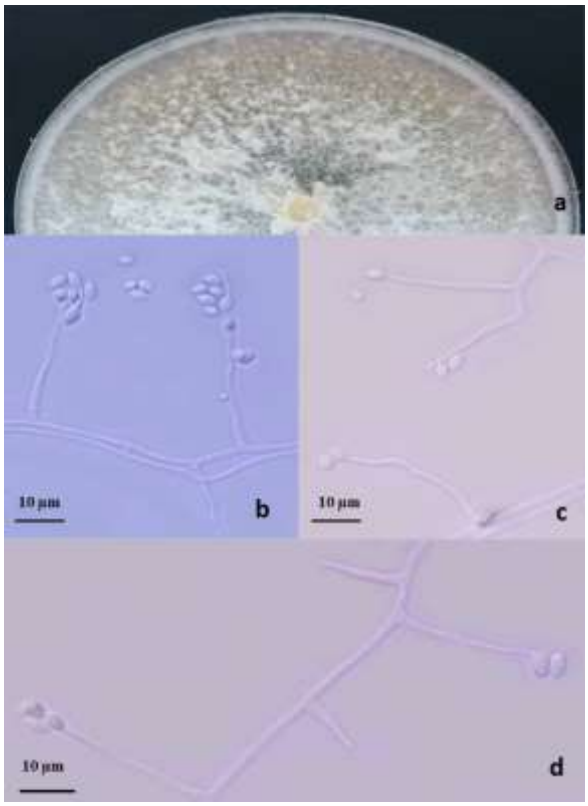

Fig. 2. *Emericellopsis pallida*. a. 10–d–old colony on oatmeal agar (OA); b–d. Branched conidiophores and conidia.

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REFERENCES

Aislabie J, Deslippe JR. 2013. Soil microbes and their contribution to soil services. In: Ecosystem Services in New Zealand – Conditions and Trends (Dymond JR, eds). Manaaki Whenua Press, Lincoln, New Zealand: 143–161.

Andrew M, Peever TL, Pryor BM. 2009. An expanded multilocus phylogeny does not resolve species among the small–spored *Alternaria* species complex. Mycologia 101:95–109.

Beljakova LA. 1974. Genus *Emericellopsis* van Beyma (Eurotiaceae). Mikologiya i Fitopatologiya 8: 385–396.

Chaudhry S, Luhach J, Sharma V, Sharma C. 2012. Assessment of diesel degrading potential of fungal isolates from sludge contaminated soil of petroleum refinery, Haryana. Research Journal of Microbiology 7: 182–190.

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology 50: 19–22.

De Boer W, Folman LB, Summerbell RC, Boddy L. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29: 795–811.

Duc PM, Hatai K, Kurata O, Tensha K, Yoshitaka U, Yaguchi T, Udagawa S. 2009. Fungal infection of mantis shrimp (Oratosquilla oratoria) caused by two anamorphic fungi found in Japan. Mycopathologia 167: 229–247.

Fedorak PM, Westlake DW. 1986. Fungal metabolism of n–alkylbenzenes. Applied and Environmental Microbiology 51: 435–437.

Flayyih I, AI–Jawhari H. 2014. Ability of some soil fungi in biodegradation of petroleum hydrocarbon. Journal of Applied and Environmental Microbiology 2: 46–52.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for Basidiomycetes: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Gorbushina AA, Whitehead K, Dornieden T, Niesse A, Schulte A, Hedges JI. 2003. Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock–dwelling microcolonial fungi. Canadian Journal of Botany 81: 131–138.

Gorbushina AA, Kotlova ER, Sherstneva OA. 2008. Cellular responses of microcolonial rock fungi to long–term desiccation and subsequent rehydration. Studies in Mycology 61: 91–97.

Grum–Grzhimaylo AA, Georgieva ML, Debets AJM, Bilanenko EN. 2013. Are alkalitolerant fungi of the *Emericellopsis* lineage (*Bionectriaceae*) of marine origin? IMA Fungus 4: 213–228.

Kim MJ, Lee H, Choi YS, Kim GH, Huh NY, Lee S, Lim YW, Lee SS, Kim JJ. 2010. Diversity of fungi in creosote–treated crosstie wastes and their resistance to polycyclic aromatic hydrocarbons. Antonie Van Leeuwenhoek 97: 377–387.

Lawrence DP, Gannibal PB, Peever TL, Pryor BM. 2013. The sections of *Alternaria*: Formalizing species–groups concepts. Mycologia 105: 530–546.

Lumbsch HT, Huhndorf, SM. 2007. Notes on ascomycete systematics. Nos. 4408– 4750. Myconet 13: 59 – 99.

Moye–Rowley W S. 2003. Regulation of the transcriptional response to oxidative stress in fungi: similarities and differences. Eukaryot Cell 2: 381–9.

Onofri S, Zucconi L, Selbmann L, Hoog GS de, de los Ríos A, Ruisi S, Grube M. 2007. Fungal Association at the cold edge of life. In: Algae and Cyanobacteria in Extreme Environments. Series: Cellular Origin, Life in Extreme Habitats and Astrobiology (Seckbach J, eds). Springer, Berlin, Germany: 735–757.

Palmer FE, Staley JT, Ryan B. 1990. Ecophysiology of microcolonial fungi and lichens on rocks in Northeastern Oregon. New Phytologist 116: 613–620.

Pryor BM, Michailides TJ. 2002. Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with Alternaria late blight of pistachio. Phytopathology. 92 (4): 406–416.

Ruibal C. 2004. Isolation and characterization of melanized, slow–growing fungi from semiarid rock surfaces of central Spain and Mallorca. Ph.D. dissertation. Universidad Autonoma de Madrid/Merck, Sharp and Dohme de Espana, Madrid, Spain.

Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, Groenewald JZ, et al. 2009. Phylogeny of rock–inhabiting fungi related to Dothideomycetes. Studies in Mycology 64: 123–133.

Simmons EG. 1990. *Alternaria* themes and variations. Mycotaxon 37: 79–119.

Simmons EG. 2007. *Alternaria*: an identification manual. CBS Biodiversity Series 6. CBS Fungal Biodiversity Centre, Utrecht, Netherlands.

Sterflinger K. 1998. Temperature and NaCl–tolerance of rock–inhabiting meristematic fungi. Antonie van Leeuwenhoek 74: 271–281.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a Guide to methods and applications (Innis MA, Gelfandm DH, Sninsky JJ, White TJ. eds). Academic Press, USA: 315–322.

Woudenberg J, Groenewald J, Binder M, Crous P. 2013. *Alternaria* redefined. Studies in Mycology 75: 171–212.

Zafra G, Absalon AE, Cuevas MC, Cortes Espinosa DV. 2014. Isolation and selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil contaminated soils. Water, Air, and Soil Pollution 225: 1826.

Zhao J, Zeng J, De Hoog GS, Attili Angelis D, Prenafeta Boldu FX. 2010. Isolation and identification of black yeasts by enrichment on atmospheres of monoaromatic hydrocarbons. Microbial Ecology 60: 149–156.

Zuccaro A, Summerbell RC, Gams W, Schroers H–J, Mitchell JI. 2004. A new Acremonium species associated with Fucus spp., and its affinity with a phylogenetically distinct marine Emericellopsis clade. Studies in Mycology 50: 283–297.

گزارش دو گونه جدید از قارچ‌های هیفومیستی جداسازی شده از خاک‌های آلوده به نفت برای مایکوبیوتای ایران

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چکیده: طی مطالعه تنوع قارچی خاک‌های آلوده به نفت استان خوزستان دو گونه جدید از هیفومیست‌ها برای مایکوبیوتای ایران جداسازی شدند و بر اساس داده‌های ریخت‌شناختی و توالی ITS–rDNA به عنوان گونه‌های *Alternaria obovoidea* و *Emericellopsis pallida* شناسایی و توصیف شدند. این مطالعه اولین گزارش از جداسازی و شناسایی این دو گونه از خاک‌های آلوده به نفت در دنیا می‌باشند.

واژه‌های کلیدی: آلودگی نفتی، تنوع قارچی، ITS، گزارش جدید

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