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Short Article

New records of Hyphomycetes fungi from Iran

A. Pordel
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

A. Ahmadpour
Higher Education Center Shahid Bakeri Miyandoab, Urmia University, Miyandoab, Iran

M. Behnia
M. Javan-Nikkhah ✉
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract: In present study, two species of Hyphomycetes fungi including, *Cephaliophora tropica* (in soil), and *Monascus pilosus* (on corn silage) are reported for the first time to Iranian mycobiota. Descriptions of these species have been provided with morphological characteristics and ITS rDNA sequence analyses.

Key words: morphology, taxonomy, *Cephaliophora*, *Monascus*

INTRODUCTION

Hyphomycetes are the majority of fungi which are commonly called moulds, and some of these fungi regarded as the weeds of the fungal kingdom. The Hyphomycetes draw nourishment from living or dead organic matter, having been adapted to grow, reproduce, and survive in a wide range of ecological situations (Seifert et al. 2011). Many also cause economically important diseases in all type of vascular plants, especially agriculture and forestry crops. These fungi are primary pathogens of plants and weeds, causing root, stem and leaf necrosis, dieback, cankers, wilts and blights (Ellis 1971, 1976; Seifert et al. 2011). The first taxonomic research on hyphomycetes occurred in central and in northern Europe, and was followed by identification in North America. Many genera and species were described, regularly found from Caribbean, in the 1980s (Seifert et al. 2011). International interest in biodiversity stimulated studies of the hyphomyecets of Asia, South Africa, in the 1990, and in recent years in china. Most early mycologists, included hyphomycetes in their classification systems (Seifert et al. 2011).

Fungi have not been extensively investigated in Iran, and most reports of new taxa are limited to check lists without detailed descriptions (Ershad 2009, Abbasi & Aliabadi 2009). However, fungi of Iran have received more attention in the past few decades. Some of Iranian researchers studied on the identification of wood inhabiting hyphomycetes in Guilan province (Gharizadeh et al. 2004 a, b), and also biodiversity of hyphomycetes in soils of Urmia lake basin (Samadi et al. 2013). However, many hyphomycetes fungi have been reported in Iran (Ershad 2009, Abbasi & Aliabadi 2009).

In this study, two species of Hyphomycetes fungi including, *Cephaliophora tropica* (in soil), and *Monascus pilosus* (on corn silage) are reported for the first time to Iranian mycobiota based on morphological features and ITS rDNA sequence analyses.

MATERIALS AND METHODS

Infected samples were collected from different farms of Golestan and Mazandaran provinces during the summer of 2012. Soil samples were also collected from Cucurbitaceae farms simultaneously. Some infected corn samples, which were delivered to mycological laboratory of University of Tehran, were also used. Segments of infected plant tissues were washed under tap water for 10 minutes. The washed segments were sliced into smaller pieces and surface-sterilized by dipping in 10% Sodium Hypochlorite for one minute. The surface-sterilized pieces placed on water agar 2% and potato dextrose agar 2% and incubated at 25 ºC for 10 days. Fungi were also isolated using Blotter method. In this method segments of infected plant tissues were placed on wet sterile filter papers and incubated in darkness or nUV (near Ultra Violet) conditions at 25 ºC. Isolation from soil samples was done using Barron (1968, 1998) method. Single-conidial isolates were made on water agar 2%. In order to study morphological and microscopic characteristics of isolates, each isolate has been grown on potato dextrose agar (PDA) medium, Malt extract agar (MEA), Czapek yeast extract agar (CYA) and incubated at 25 ºC for 12 days. Morphological characteristics were studied and

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✉ Corresponding author: Email: jnikkhah@ut.ac.ir

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photographs were taken under Olympus microscope model BH2.

Genomic DNA was extracted using the method of Zhong and Stephenson (2001). Two isolates (Ct1, MP1) were subjected to PCR amplification of the internal transcribed spacer (ITS) 1 and ITS2 regions flanking the 5.8S rRNA gene. This was carried out with universal primers ITS1 and ITS4 according to the published protocol (White et al. 1990).The reaction mixture and PCR conditions were the same as described by Ahmadpour et al. (2012). The amplified products were cleaned and sequenced by Bioneer Inc. (Daejeon, South Korea).

RESULTS AND DISCUSSION

In this study two species including; *Cephaliophora tropica* and *Monascus pilosus* reported for the first time for mycobiota of Iran.

Cephaliophora tropica Thaxt., Bot. Gaz. 35: 157 (1903) Fig. 1a–f

Colonies were white to cottony with aerial mycelia on PDA. Conidiophores single, erect, broad, short. Fertile vesicles are globose and multi-position for producing conidia (polyblastic). Conidia are born all over the vesicle and are solitary, cumulative over conidiophores, pale brown, darker in septa, septa near the point attachment usually appearing, clavate to cylindrical, 3–4 septa, 27–37 × 7–15 µm. Based on these data, the fungus was identified as *Cephaliophora tropica* Thaxter (Ellis 1971, Seifert et al. 2011).

Specimen examined: IRAN, Golestan province, Gorgan city, on soil, 22 July. 2012, A. Ahmadpour.

The fungus includes six species; *C. tropica*, *C. irregular*, *C. uniformis*, *C. navicularis*, *C. musicola* and *C. longiospora* (Ellis 1971, Seifert et al. 2011). The species are found on cacao, dung, beans, wood, soil, etc. (Ellis 1971, Seifert et al. 2011). Some *Cephaliophora* species can capture rotifers with specialized adhesive peg (Tanabe et al. 1999, Tzean & Barron 1983). The sequence (MP1 isolate) was deposited into GenBank (accession number KR809561). The ITS sequence comparison showed 99% identity to *C. tropica* type strain xsd08001 (GenBank accession FJ792583). According to morphological and molecular analysis, the isolates were identified as *C. tropica*. In our knowledge, this is the first report of *Cephaliophora* genus from Iran.

Note: this species is distinguished from other *Cephaliophora* species by the difference in conidial shape, sizes, septation, and habitat (Ellis 1971, Seifert et al. 2011).

Monascus pilosus K. Satô ex D. Hawksw & Pitt, Aust. J. Bot. 31(1): 54 (1983) Fig. 2a–j

Colonies are 20–23 mm diam. on PDA at 25 °C in 7 days. Velvety radiate, dark orange as colonies mature, pale at the margins, but deep orange at the center, exudates and soluble pigment absent. Colonies are 32–34 mm diam. on MEA at 25 °C in 7 days. Light orange as colonies mature, yellow at the margins, but deep orange at the center, exudates and soluble pigments absent. Colonies are 19–22 mm in diam. on CYA at 25 °C in 7 days. Irregular shape due to variable growth rates, orange to light orange, become reddish as colonies mature, exudates and soluble pigments absent. The fungus did not grow on CYA, PDA, and MEA at 5 °C in 7 days. Mycelia developed rather sparsely, hyphae hyaline. Cleistothecia globose, arising singly from distinct stalk-like hyphae, 20–33 µm diam. Asci evanescent at an early stage, ascomata filled by spores. Ascospores ellipsoid, hyaline, 5–6 × 4–5 µm. Aleurioconidia born terminally on hyphae and laterally on pedicels, in short chains or singly, obpyriform to globose, light brown and clearly truncated at the base, 7–11 × 7–9 µm. Intercalary chlamydoconidia present (7–12 × 6–9 µm). Based on these data, the fungus was identified as *Monascus pilosus* (Hawksworth & Pitt 1983, Stchigel et al. 2004).The sequence (Ct1 isolate) was deposited into GenBank (accession number KR809560). The ITS sequence comparison showed 98% identity to *M. pilosus* type strain FRR 2194 (GenBank accession GU733334). According to morphological and molecular analysis, the isolates were identified as *M. pilosus*.

Specimen examined: IRAN, Alborz province, Karaj city, on corn silage, 22 Dec. 2012, A. Pordel.

Note: this species is distinguished from *Monascus ruber* by the difference in colony pigmentation and conidia and ascospores sizes. It also differs from *M. purpures* in colony characteristics. *Monascus pilosus* produces smaller cleistothecia and narrower ascospores than *M. purpures* (Hawksworth & Pitt 1983, Stchigel et al. 2004).

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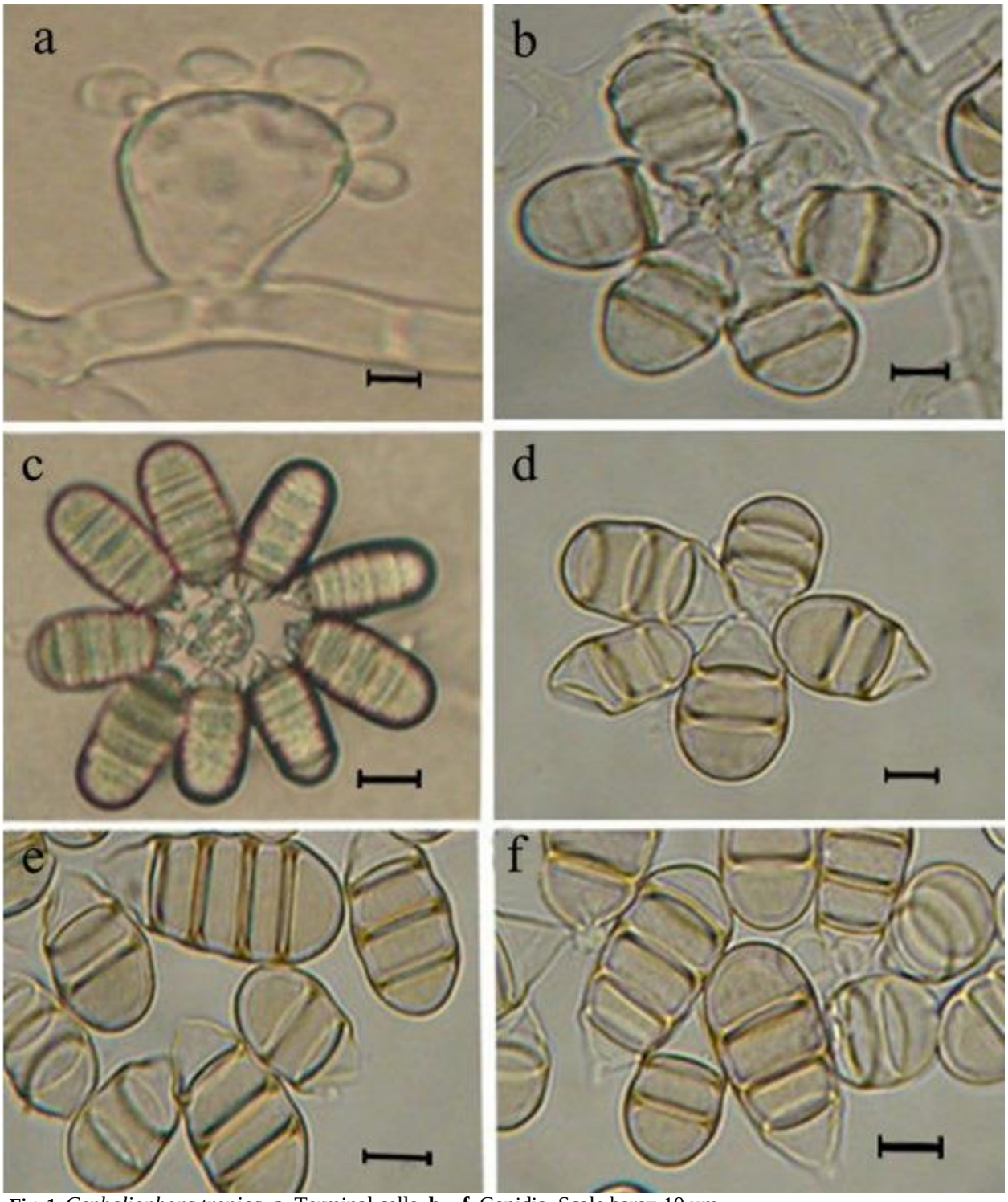

Fig. 1. *Cephaliophora tropica*. **a.** Terminal cells, **b – f.** Conidia. Scale bars= 10 µm.

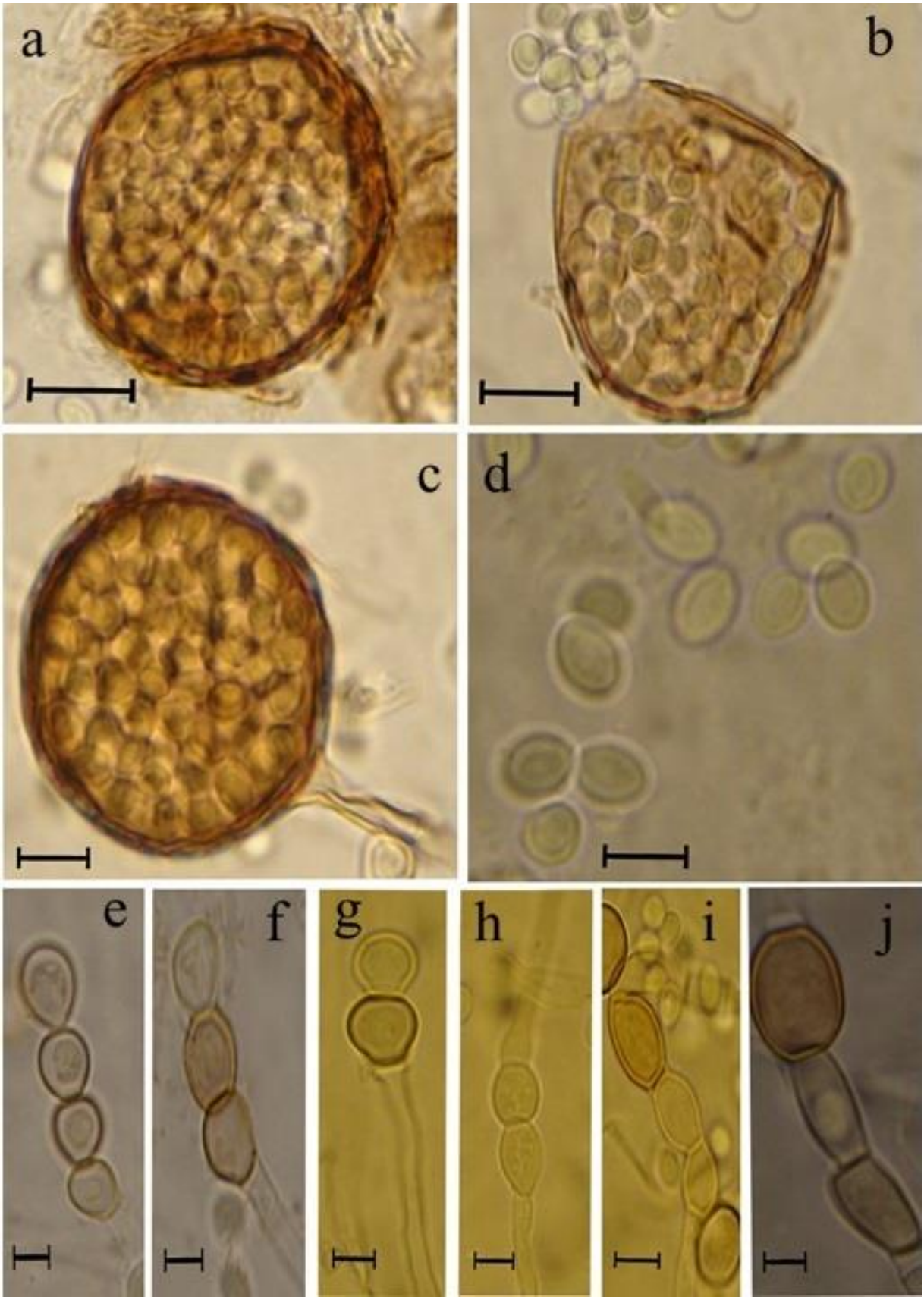

Fig. 2. *Monascus pilosus*. **a – c.** Cleistothecia, **d.** Ascospores, **e – g.** Aleurioconidia, **h – j.** Chlamydoconidia. Scale bars = 10 µm.

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گزارش جدیدی از قارچ‌های هیفومیست در ایران

عادل پردل[1]، عبداله احمدپور[2]، مهگل بهنیا[1] و محمد جوان نیکخواه[1] ✉

۱- گروه گیاهپزشکی، پردیس کشاورزی و منابع طبیعی دانشگاه تهران، کرج

۲- مرکز آموزش عالی شهید باکری میاندوآب، دانشگاه ارومیه، میاندوآب

چکیده: در این مطالعه دو گونه از قارچ‌های هیفومیست شامل *Cephaliophora tropica* (جدا شده از خاک) و *Moascus pilosus* (جدا شده از برگ ذرت انبار شده برای علوفه) برای اولین بار برای میکوبیوتای ایران گزارش می‌گردد. توصیف گونه‌ها بر اساس خصوصیات مورفولوژیکی و توالی ناحیه ITS – rDNA ارائه شده است.

واژه‌های کلیدی: مورفولوژی، تاکسونومی، *Cephaliophora*، *Monascus*

مکاتبه کننده: محمد جوان نیکخواه Email: jnikkhah@ut.ac.ir

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