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Research Article

New fungal canker pathogens of apple trees in Iran

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Abstract: Apples are the main horticultural crops grown in West Azarbaijan province of Iran. In recent surveys of apple orchards in Urmia and Miyandoab, West Azarbaijan Province, symptoms including branch and twig bark cracks, cankers, dieback and tree decline were commonly seen. Isolation of the fungi from cracked and cankered branches yielded fungal isolates with typical characteristics of the genera *Paecilomyces* and *Paramyrothecium*. Based on combination of morphological characteristics and sequence data obtained from ITS-rDNA and *β-tubulin* gene sequences for *Paecilomyces* isolates and ITS-rDNA region for *Paramyrothecium* isolates, they were identified as *Paecilomyces formosus* and *Paramyrothecium foliicola*. Results of the pathogenicity tests on detached shoots of 'Golden Delicious' and 'Red Delicious' cultivars showed that isolates of *P. formosus* were pathogenic only on 'Red Delicious' cultivar, but *Pa. foliicola* isolates were pathogenic on both cultivars and showed symptoms of infection. Re-isolation of the fungi from inoculated shoots confirmed Koch's postulates. To the best of our knowledge, the involvement of *P. formosus* and *Pa. foliicola* in the development of canker disease of apple trees is reported for the first time. Also, *Pa. foliicola* is a new record to Iran mycobiota.

Keywords: Apple disease, *Paecilomyces formosus*, *Paramyrothecium foliicola*, Iran

Introduction

Apple (*Malus* × *domestica* Borkh.) is the most important fruit crop in all temperate zones of both the northern and southern hemisphere, where winter temperatures allow adequate winter chilling and summer temperatures are not extreme (Cooke *et al.*, 2009; Turechek, 2004). More than 10000 cultivars of apple are documented worldwide, varying in their yield and ultimate size of the tree, of which a few are grown in commercial production (Elzebroek and Wind, 2008; Mir and Patel, 2018). World production of apples in 2017 was 83.3 million tonnes, with China, European Union, United States, Turkey, Poland, India and Iran as the major apple producers (FAOSTAT, 2019). In Iran, West Azarbaijan province is the major apple growing area with 60470 ha and total production of 970714 tonnes, ranking first in the country (Ahmadi *et al.*, 2018).

Several fungal diseases have been reported on apples, which reduce productivity of the orchards and constitute severe threat to their commercial production worldwide (Brown-Rytlewski and McManus, 2000; Xiao and Boal, 2005; Cloete *et al.*, 2011; Sutton *et al.*, 2014). Among the fungal diseases, canker, twig dieback and plant decline occur frequently in apple orchards. In Iran, several fungal pathogens have been reported with economic impacts (Ershad, 2009; Hanifeh *et al.*, 2013). *Paecilomyces formosus*, a segregated species from *Paecilomyces variotii* species complex, has been reported as the main causal

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agent of pistachio dieback disease in Iran (Ershad 2009; Heidarian *et al.*, 2018). In addition, the species was isolated from *Caesalpinia gilliesii*, *Haloxylon* sp., *Nerium oleander*, *Pistacia mutica*, *Prunus amygdalus*, *Punica granatum*, *Tamarix aphylla* and *Tamarix hispida* plants and the pathogenicity of the isolates was confirmed (Heidarian *et al.*, 2018). It has also been reported in Iran from *Prunus amygdalus* and *Sesamum indicum* seeds as *P. variotii* (Gooya *et al.*, 2000; Ershad, 2009). *Paramyrothecium foliicola* was described for the first time based on two strains, one strain from rotten leaf of unknown host in Brazil and the second strain from the air in Cuba (Lombard *et al.*, 2016). Fifteen species in the genus *Paramyrothecium* have been reported thus far (Lombard *et al.*, 2016; Krisai-Greilhuber *et al.*, 2017; Liang *et al.*, 2019), among them *Paramyrothecium roridum* is an important polyphagous plant pathogen infecting a wide range of host plants (Chavhan *et al.*, 2018; Farr and Rossman, 2019). *Paramyrothecium roridum* is commonly associated with necrotic leaf spot disease (Zhao *et al.*, 2010; Garibaldi *et al.*, 2016; Haudenshield *et al.*, 2018), although it was also reported as the causal agent of stem canker on watermelon, bark canker of coffee and crown canker of greenhouse muskmelon (Schieber and Zentmyer, 1968; Bruton and Fish, 2012; Chen *et al.*, 2018). In a most recent paper, the involvement of *Paramyrothecium foliicola*, which was previously considered as a saprophytic fungus, in the development of plant disease was reported for the first time (Matić *et al.*, 2019).

During the surveys of apple orchards in Urmia and Miyandoab in West Azarbaijan Province, the widespread occurrence of canker and tree decline symptoms with an economic impact to the growers was revealed. This study is aimed to isolate and identify fungal pathogens involved in apple canker and decline disease in West Azarbaijan province of Iran.

Materials and Methods

Sampling and fungal isolation

Branch and twig samples with bark discoloration, cracks, canker and dieback symptoms were collected from different locations of Urmia and Miyandoab, West Azarbaijan Province, during the late spring to mid-fall of 2017 and 2018. The samples were washed thoroughly under running tap water, surface sterilized in 3% sodium hypochlorite solution for 5 minutes, washed again three times in sterile distilled water and blotted dry on sterile tissue papers. Small pieces of inner bark and wood, at the interfaces of healthy and discolored tissues were cut and transferred into Potato Dextrose Agar (PDA, Merck, 39 g/l^{-1}) plates amended with streptomycin sulfate and penicillin G antibiotics (150 ppm each) and incubated at 25 °C under daylight conditions. The growing fungi from the edge of plant samples were transferred into new PDA plates and purified based on hyphal tip culture or single spore methods. The purified fungi were grown on sterile filter papers and incubated at -20 °C for long-term preservation.

Morphological studies

Morphological characteristics of *Paramyrothecium* isolates were studied on PDA medium (Lombard *et al.*, 2016). Axenic cultures were inoculated on PDA and incubated at 23 ± 2 ºC, under day light diffusion for 14 days. Fungal structures were mounted in clear lactic acid and distilled water. Micromorphological measurements were made at × 400 and × 1000 magnifications using an Olympus X61 microscope with differential interference contrast (DIC) illumination. For morphological characterization of *Paecilomyces* isolates, the method adopted by Samson *et al.* (2009) was followed. The isolates were three-point inoculated on Czapek Yeast autolysate Agar (CYA), Malt Extract autolysate Agar (MEA) and Oatmeal Agar (OA) and incubated at 25 ºC for 7 days. Micromorphological characteristics were determined from MEA culture medium as indicated for *Paramyrothecium*. Pure cultures of all identified isolates were deposited in Fungal Culture Collections of Urmia University (FCCUU).

DNA extraction and PCR amplification

Total genomic DNA was extracted according to Zhong and Steffenson (2001). Polymerase chain reaction amplifications (PCR) of internal

transcribed spacer of ribosomal DNA (ITS–rDNA) and parts of *β-tubulin* (*TUB 2*) gene were performed using primer pairs ITS1/ITS4 (White *et al*., 1990) for ITS–rDNA and Bt2a/Bt2b (Glass and Donaldson, 1995) for parts of *β-tubulin* gene, respectively. Thermal conditions for PCR amplification consisted of an initial denaturation step at 95 °C for 4 min followed by 35 cycles of 45 s at 95 °C, 45 s at 57 °C and 45 s at 72 °C, and a final extension step at 72 °C for 6 min. The reaction mixtures consisted of 12.5 µl ready 2X Red Master Mix (1.5 mM $MgCl_2$, Ampliqon, Denmark), 0.4 µM of each primer and about 10 ng/µl of template DNA in a final volume of 25 µl. Following PCR amplification, amplicons were visualized on a 1 % agarose gel stained with GelRed™ (Biotium, Hayward, CA, USA), viewed under ultra-violet light and the sizes of amplicons were determined against a HyperLadder™ I molecular marker (Bioline). PCR products were cleaned up and sequenced by Macrogen Corporation (Macrogen Inc., Korea).

Phylogenetic analyses

The assembled consensus sequences were initially aligned with MAFFT v. 7 (https://mafft.cbrc.jp/alignment/server/) using default settings and adjusted manually where necessary (Katoh *et al*., 2017). The data were analyzed phylogenetically using distance and discrete methods. The distance matrix was calculated using Kimura's two-parameter model (Kimura, 1980) and analyzed with the neighbor-joining (NJ) method (Saitou and Nei, 1987) using the program MEGA version 7.0 (Kumar *et al*., 2016). Bootstrap values were generated with 1000 replicate heuristic searches to estimate support for clade stability of the consensus tree using the same program. Moreover, Maximum Parsimony (MP) analyses were performed using the ITS and *β-tubulin* combined datasets in PAUP 4.0 (Swofford, 2002). Trees were inferred using the heuristic search option with 1000 random sequence additions and branch swapping with tree-bisection-reconnection (TBR) algorithm and gaps treated as missing data. The bootstrap values with 1000 replicates were performed to determine branch support. Descriptive tree statistics [Tree Length (TL), Consistency Index (CI), Retention Index (RI) and Rescaled Consistency Index (RC)] were calculated for trees generated in the parsimony analysis. The generated tree was observed in TreeView v. 1.6.6 (Page, 1996). The newly generated sequences were deposited in the National Center for Biotechnology Information (NCBI).

Pathogenicity tests

Five isolates from each species were chosen randomly and used in pathogenicity tests. Healthy 1–2 years-old twigs of 'Golden Delicious' and 'Red Delicious' apple cultivars were obtained from an apple orchard and transferred into the laboratory. The twigs were washed thoroughly under running tap water, cut into fragments 20 cm in length, surface sterilized using 75% ethanol for 2 minutes and were kept under aseptic conditions for 30 min. A 6 mm diameter piece of bark was cut with a cork borer and inoculated with a 5 mm diameter plug of fungal mycelium taken from the edge of actively growing colonies. In the controls, PDA medium without fungal mycelium was used. The inoculated area was covered with a sterile wet cotton ball and wrapped with Parafilm to retain moisture. The inoculated twigs were placed in clean plastic containers lined with moistened filter paper and kept at 25 °C for 21 days. All the pathogenicity tests were repeated twice. Twigs were examined for the presence or absence of necrotic lesions around the inoculation site and if present, the length of the necrotic lesion was calculated. The symptomatic twigs were surface sterilized with 75% ethanol for 3 min, washed thrice with sterile distilled water, blotted dry, cut into small pieces and placed on PDA medium. The inoculated plates were kept at 25 °C for 7 days and checked for fungal growth. The growing fungi were isolated and studied morphologically as described above and compared with inoculated ones.

Results

Molecular studies

The PCR amplicon size of ITS and *GAPDH* sequences ranged from 545–556 and 456 base

pairs, respectively. Results of neighbor-joining (NJ) and the maximum parsimony (MP) analyses yielded the same tree topology. The combined ITS and *β-tubulin* sequence alignment containing 27 isolates of *Paecilomyces* species (26 ingroup and *Thermoascus crustaceus* CBS 181.67 as outgroup) includes 981 positions, of which 683 were constant, 81 were variable and parsimony uninformative and 217 were parsimony informative. MP and NJ trees were similar in terms of major clades and topology. Nine equally most parsimonious trees were generated and one of them was used to represent the molecular phylogeny as Fig. 1 (RI = 0.897; CI = 0.758; HI = 0.242; Tree length = 545). Topologies of the individual gene trees were determined to be congruent and no conflicts were observed in species delimitation (data not shown). In the phylogenetic tree (Fig. 1), MP analyses of the combined dataset revealed that the studied isolates (FCCUU111 and FCCUU115) clustered well with *P. formosus* type isolates with 100% bootstrap value.

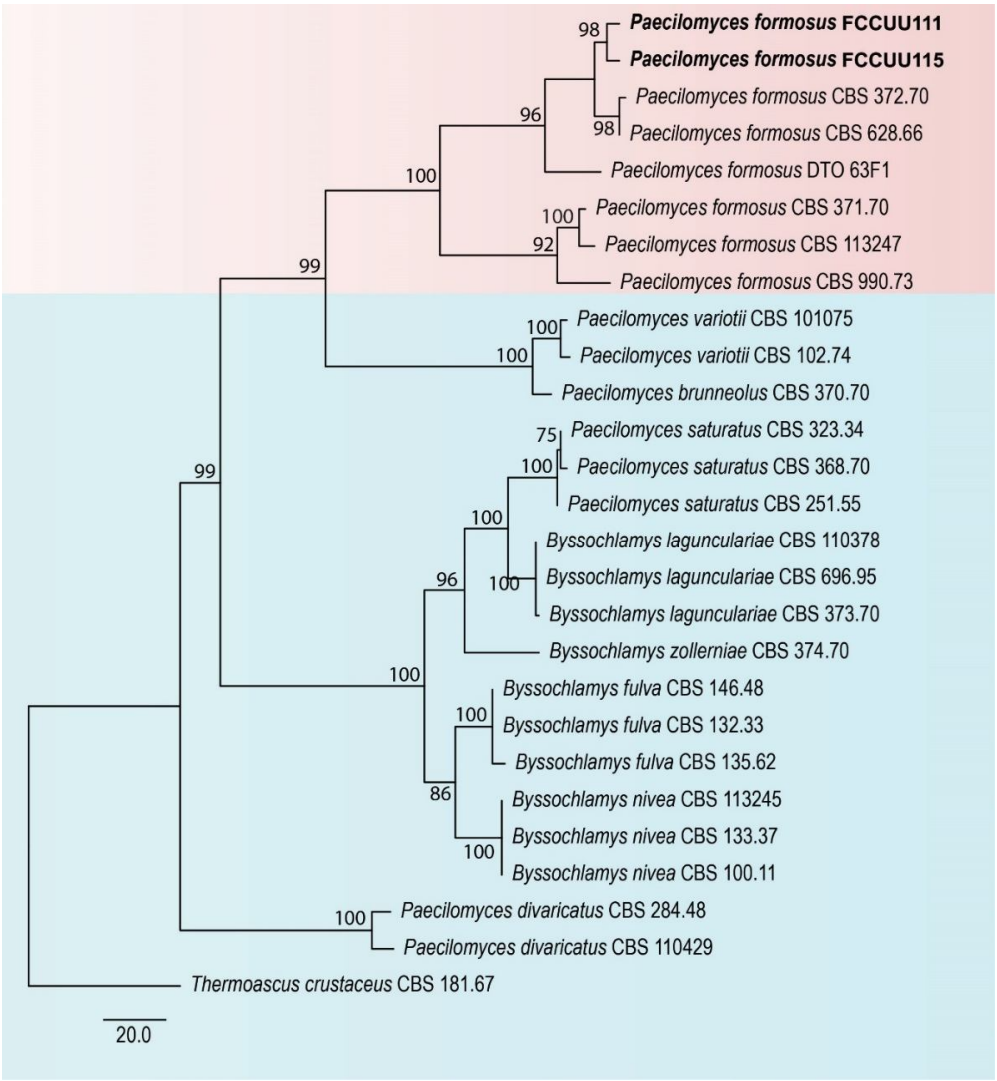

Figure 1 Maximum Parsimony (MP) Phylogenetic tree based on combination of ITS–rDNA and partial sequences of *β-tubulin* gene data for *Paecilomyces* species. Bootstrap values ≥ 50% are shown above the branches. The tree is rooted with *Thermoascus crustaceus* (CBS 181.67). Scale bar indicates the number of nucleotide substitutions per site.

ITS sequence comparison of the studied isolates showed 99–100% identity to *Pa. foliicola* type isolate in NCBI BLAST searches. According to the neighbor-joining tree derived from ITS-rDNA sequences made in this study for *Paramyrothecium* species, our isolates (FCCUU120 and FCCUU126) formed a monophyletic lineage (73% bootstrap value) with the *Pa. foliicola* isolates (KU846293 and KU846294) (Fig. 2).

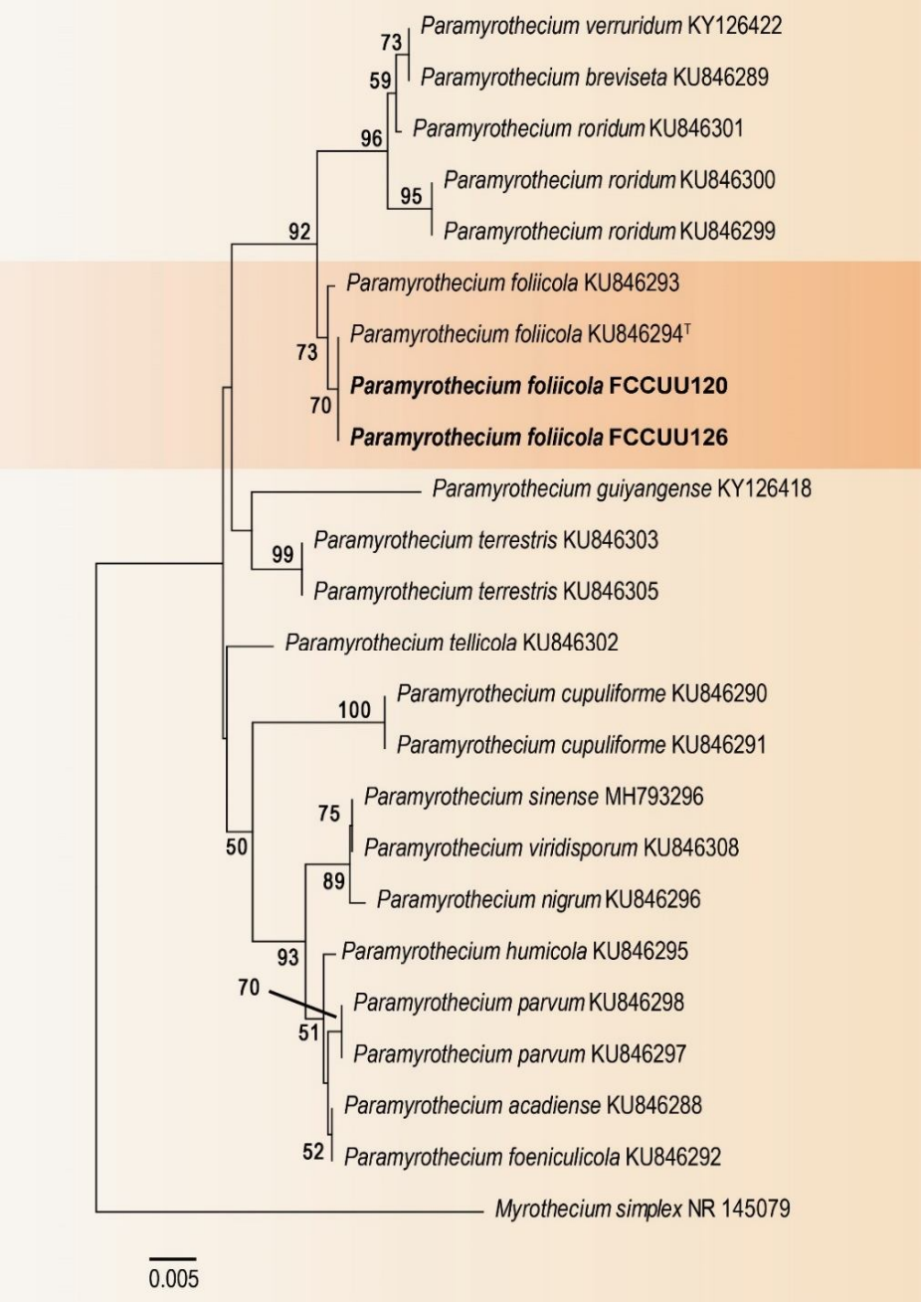

Figure 2 A neighbor-joining (NJ) tree inferred from the ITS–rDNA sequences for *Paramyrothecium* species. Bootstrap values ≥ 50% are shown above the branches. The tree is rooted with *Myrothecium simplex* (NR 145079). Scale bar indicates the number of nucleotide substitutions per site.

Morphological studies

Twenty-seven isolates with typical characteristics of *Paramyrothecium* (12 isolates) and *Paecilomyces* (15 isolates) were obtained from cultured samples. Based on cultural and morphological characteristics, isolates representing the genus *Paecilomyces,* were identified as *P. formosus* and those representing the genus *Paramyrothecium*, were identified as *Pa. foliicola.* The morphological identifications were confirmed well by molecular data. Description of the species are indicated below.

- *Paecilomyces formosus* (Sakag., May. Inoue & Tada) Houbraken & Samson, Persoonia 22: 21 (2009)

Colonies on CYA cottony, pale luteous at center, white at margins, 25 mm diam. after 7 days, reverse primrose (Fig. 3a); on OA cottony, ochreous at center, white at margins, 21 mm diam. after 7 days, reverse salmon (Fig. 3b) and on MEA at 25 ºC fast-growing, cottony, luteous with frequent white aerial mycelium, 75 mm diam. after 7 days, reverse pale luteous (Fig. 3c).

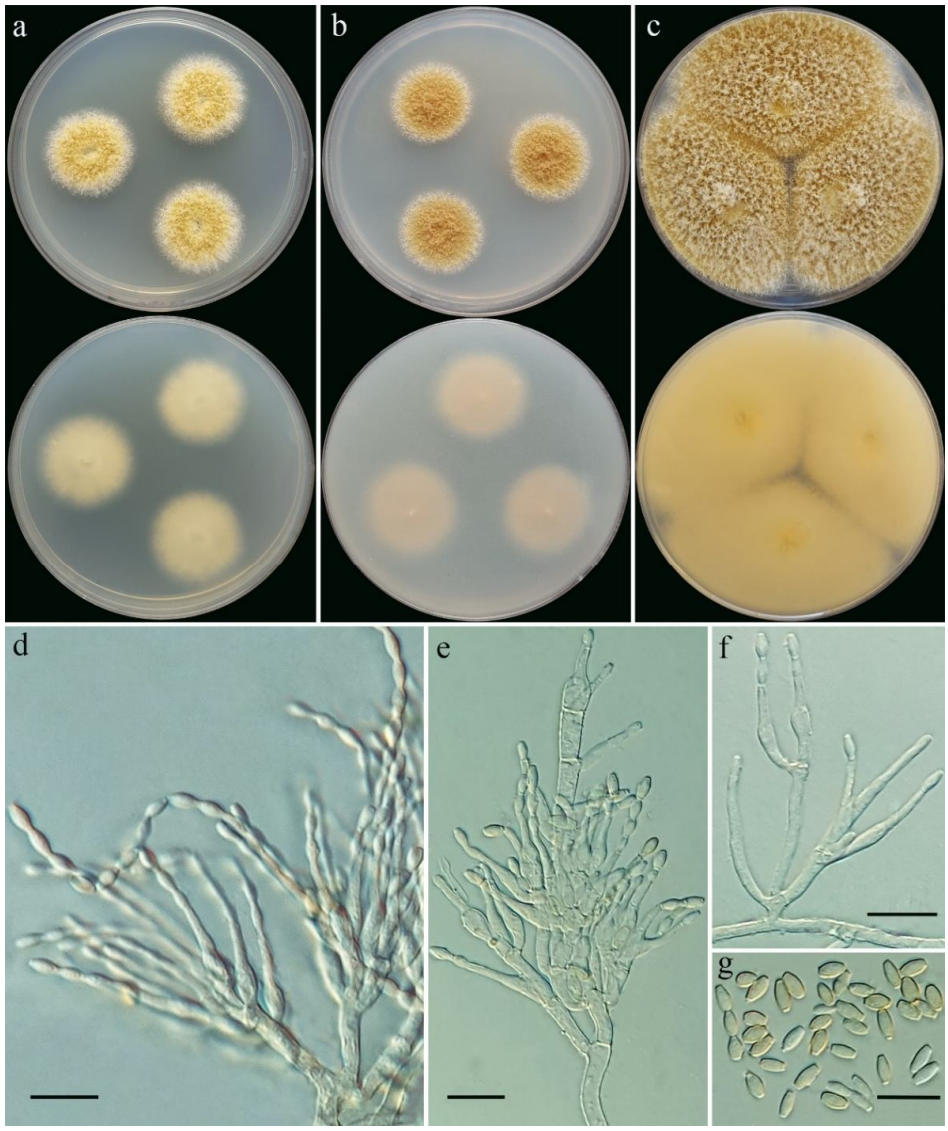

Figure 3 *Paecilomyces formosus*. a–c, Colonies after 7 days on CYA (a), OA (b) and MEA media (c). d–g, conidiophores, phialides and conidia. Scale bar 10 µm.

Conidiophores irregularly branched, hyaline, smooth, with penicillate conidiogenous cells. Conidiogenous cells phialidic, with narrow necks (1 µm diam.), hyaline, 12–17 × 2–3 µm. Conidia ellipsoid to cylindrical, with truncate ends, sub-hyaline to olive-brown, formed in basipetal chains, 4–5 × 1.5–2 µm (Fig. 3d–g).

Specimen examined–IRAN. West Azarbaijan, Urmia city, Torkaman, on 'Red Delicious' apple cultivar, 29 July 2017, Razmig Azizi, isolate FCCUU111. GenBank accession numbers: MN809137, ITS; and MN809631, *TUB 2*.

Additional specimen examined–IRAN. West Azarbaijan, Urmia city, Goytapeh, on 'Red Delicious' apple cultivar, 10 July 2017, Razmig Azizi, isolate FCCUU115.

- *Paramyrothecium foliicola* L. Lombard & Crous, in Lombard, Houbraken, Decock, Samson, Meijer, Réblová, Groenewald & Crous, Persoonia 36: 209 (2016)

Colonies on PDA at 25 ºC were initially white, then becoming yellowish at center, floccose, 23 mm diam. after 7 days and 38 mm diam. after 14 days, reverse luteous (Fig. 4a).

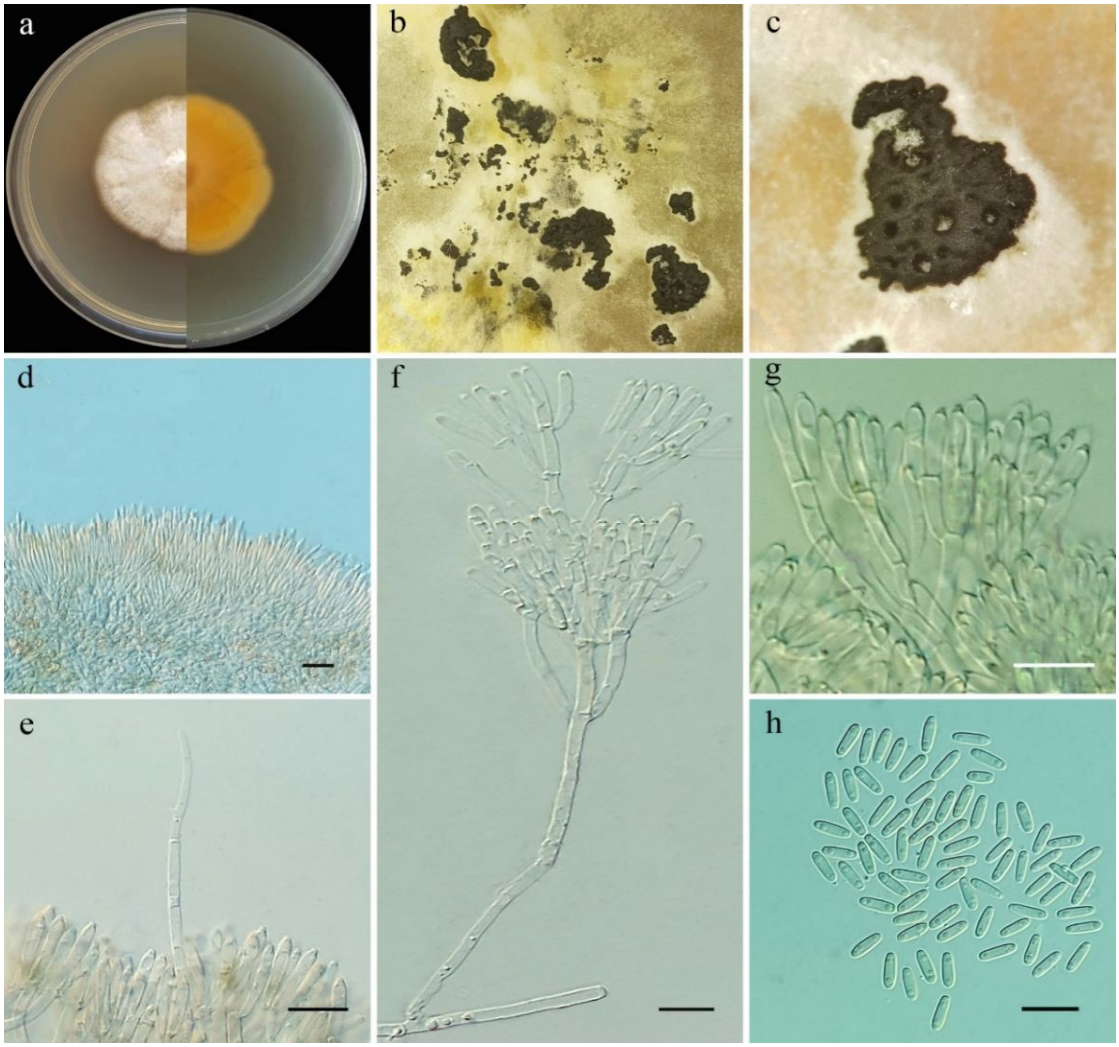

Figure 4 *Paramyrothecium foliicola*. a) Colony on PDA medium after 7 days. b–c, Sporodochia formed on agar surface. d, Cross section of sporodochia. e, Setae. f–g, Conidiophores and conidiogenous cells. h, Conidia, scale bars 10 µm.

Sporodochia were formed after 15 days in concentric rings, superficial, scattered or gregarious, covered with olivaceous green to dark green slimy conidial mass (Fig. 4b–d). Setae hyaline, 1–4 septate, straight or flexuous, sinuous above apical septum, 30–40 × 2 µm (Fig. 4e). Conidiophores macronematous, consisting of a hyaline stipe and a penicillately branched conidiogenous apparatus. Primary branches aseptate, unbranched, smooth, 12–23 × 2–3 µm. Secondary branches aseptate, unbranched, smooth, 10–21 × 1.5–2 µm. Conidiogenous cells phialidic, cylindrical to subcylindrical, hyaline, smooth, straight to slightly curved, 10–20 × 1.5–2 µm with conspicuous collarettes and periclinal thickening (Fig. 4f–g). Conidia cylindrical to ellipsoidal, one-celled, hyaline to pale green, smooth, rounded at both ends, 6–8 × 1.5–2 µm (Fig. 4h).

Specimen examined–IRAN. West Azarbaijan, Barrogh city, Ghareh Sagghal, on 'Red Delicious' apple cultivar, 1 Sept 2017, Razmig Azizi, isolate FCCUU120. GenBank accession number: MN809138, ITS.

Additional specimen examined–IRAN. West Azarbaijan, Miyandoab city, Ebrahim Naieb, on 'Red Delicious' apple cultivar, 18 Sept 2017, Razmig Azizi, isolate FCCUU126.

Pathogenicity studies

The results of pathogenicity tests showed that the isolates of *P. formosus* were pathogenic only on twigs of 'Red Delicious' apple cultivar, which exhibited distinct necrotic lesions around the inoculated site and wood discoloration, while in twigs of 'Golden Delicious' cultivar and those of the control treatments, there were not any symptoms of necrotic lesions or wood discoloration (Fig. 5a–b). In the case of *Pa. foliicola* isolates, inoculated twigs of both apple cultivars exhibited distinct necrotic lesions and wood discoloration around the inoculated site, but without any symptoms on control treatments (Fig. 5c–f). Re-isolation of the inoculated fungi from the newly infected twigs and their identification as the inoculated fungi fulfilled Koch's postulates and confirmed their pathogenicity.

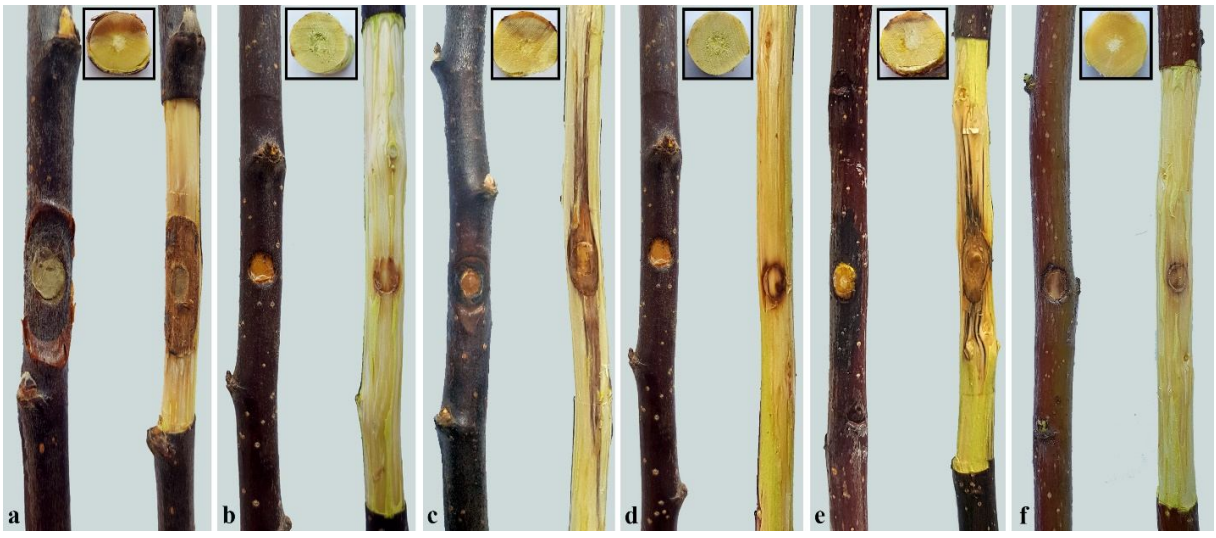

Figure 5 Pathogenicity tests on detached apple shoots. a, apple 'Red Delicious' cultivar inoculated with an isolate of *Paecilomyces formosus*, and b, control treatment. c, apple 'Red Delicious' cultivar inoculated with an isolate of *Paramyrothecium foliicola* and d, control treatment. e, apple 'Golden Delicious' cultivar inoculated with the same isolate of *Pa. foliicola* and f, control treatment.

Discussion

Apple is commercially the most important horticultural crop grown widely in West Azarbaijan province, Iran. This crop is affected by many diseases causing economic losses to the growers, so that more than 70 diseases caused by different organisms have been reported thus far (Sutton *et al.*, 2014; Sikdar *et al.*, 2019). In Iran, about 60 fungal species have been reported from apple trees (Ershad, 2009; Hanifeh *et al.*, 2013). In recent years, apple trees in the studied area have encountered new problems in the form of cankers, twig diebacks, tree decline and ultimately tree death. Because of economic importance of apple trees and in order to effectively manage the diseases, special attention should be paid to the correct identification, characterization and pathogenicity determination of causal pathogens. In this study, we isolated several fungal isolates from symptomatic plants, which based on morphological and molecular characteristics, were identified as *Paecilomyces formosus* and *Paramyrothecium foliicola* and their pathogenicity were confirmed on apple trees based on Koch's postulates. *Paecilomyces formosus* was segregated from *P. variotii* complex based on polyphasic approach (Samson *et al.*, 2009). The species has been isolated from different substrates including tropical and subtropical soils, wood, sponge, food products, air, clinical samples and potting soil (Samson *et al.*, 2009; Barker *et al.*, 2014; Moreira *et al.*, 2018). It is also reported as a cutaneous infection in an extremely premature infant (Kuobi *et al.*, 2016). As plant pathogen, *P. formosus* is a serious causal agent of dieback and canker disease of pistachio trees in Iran (Heidarian *et al.*, 2018; Torabi *et al.*, 2019). It is also reported as a pathogenic fungus from wild almonds and oak trees showing dieback and decline symptoms (Mirabdollahi Shamsi *et al.*, 2019; Sabernasab *et al.*, 2019). Also, in the host range studies under laboratory conditions and using excised branch inoculation procedure, *P. formosus* isolates obtained from oak, wild pistachio and hawthorn produced cankers on cornelian cherry, fig, hawthorn, Montpellier maple, wild almond and wild pistachio (Sabernasab *et al.*, 2019). In this study, the species is reported as pathogenic fungus from apple trees with canker, dieback and decline symptoms, which expands the host range of *P. formosus*.

In a recent phylogenetic study on the family *Stachybotryaceae*, the previously polyphyletic genus *Myrothecium* was divided into 13 new genera, and *Paramyrothecium* was established with *Pa. roridum* as type species (Lombard *et al.*, 2016). Among the 15 species identified in the genus *Paramyrothecium*, *Pa. roridum* was the only species considered as plant pathogenic fungus with a wide host range causing leaf spot, stem and crown canker and fruit rot symptoms (Zhao *et al.*, 2010; Han *et al.*, 2014; Ben *et al.*, 2016; Pelayo-Sánchez *et al.*, 2017; Borges *et al.*, 2018; Chavhan *et al.*, 2018), while other species are considered mostly as soil-borne fungi with saprophytic lifestyle (Lombard *et al.*, 2016; Krisai-Greilhuber *et al.*, 2017; Liang *et al.*, 2019). In a study to determine occurrence and pathogenicity of foliar pathogens on cucurbits in South Carolina, the two species *Pa. foliicola* and *Pa. humicola* were isolated from cowpea, tomato and watermelon and their pathogenicity was confirmed on these three vegetable host plants for the first time (Rennberger, 2018). In another study involving leaf spot disease of leafy vegetable and ornamental crops in Northern and Southern Italy, three species including *Pa. foliicola*, *Pa. nigrum* and *Pa. roridum* were isolated and identified as causal agents of leaf spot disease on basil, lamb's lettuce, lettuce, spinach and cultivated and wild rocket plants (Matić *et al.*, 2019). In the present study, *Pa. foliicola* was isolated from apple trees with canker and decline symptoms and its pathogenicity was confirmed. This is the first report of the occurrence and pathogenicity of *Pa. foliicola* on tree plants. The results of these recent studies confirm the idea that there is a need to reconsider the concept of saprophytic life style in *Paramyrothecium* species (Rennberger, 2018). To date, only *Pa. roridum* was reported from Iran (as *Myrothecium roridum*) based on the revised species concept of *Myrothecium* s.l. (Ershad, 2009). *Myrothecium verrucaria* and *M. brachysporum* which were reported from sugar

beet cyst nematodes (Khezrinejad *et al.*, 2007) and nutsedge plants (Farzaneh *et al.*, 2009) respectively, were renamed as *Albifimbria verrucaria* and *Striaticonidium brachysporum* (Lombard *et al.*, 2016). This is the first report of *Pa. foliicola* to Iran mycobiota. Further studies are needed to determine the distribution of these diseases, the host range of the identified species in the studied area, reaction of different apple cultivars grown commonly in the region to these fungal pathogens, assess their economic impacts and present effective management strategies in order to control the disease.

Conflict of Interest

The authors declare that they have no conflict of interest

Author's Contribution

All the authors have contributed the same to this work

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بیمارگرهای جدید قارچی مولد شانکر درختان سیب در ایران

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چکیده: درختان سیب مهم‌ترین محصول باغبانی کشت شده در استان آذربایجان غربی محسوب می‌شوند. طی بررسی‌های اخیر باغات سیب شهرستان‌های ارومیه و میاندوآب، علائم متنوعی شامل ترک‌خوردگی و زخم‌های سطحی در شاخه‌ها و سرشاخه‌ها، شانکرها، خشکیدگی سرشاخه‌ها، زوال و مرگ درختان مشاهده گردید. جداسازی قارچ‌ها از شاخه‌ها و سرشاخه‌های دارای علائم ترک‌خوردگی و شانکر منجر به شناسایی جدایه‌های قارچی با ویژگی‌های متداول جنس‌های *Paecilomyces* و *Paramyrothecium* گردید. براساس ترکیبی از ویژگی‌های ریخت‌شناختی و داده‌های حاصل از توالی‌یابی ناحیه ITS-rDNA و بخشی از ژن بتاتوبولین برای جدایه‌های جنس *Paecilomyces* و ناحیه ITS-rDNA برای جدایه‌های جنس *Paramyrothecium*، گونه‌های *Paecilomyces formosus* و *Paramyrothecium foliicola* شناسایی شدند. نتایج آزمون‌های بیماری‌زایی روی شاخه‌های بریده سیب ارقام Red Delicious و Golden Delicious، نشان داد که جدایه‌های گونه *P. formosus* تنها روی رقم Red Delicious بیماری‌زا بودند، درحالی‌که جدایه‌های گونه *Pa. foliicola* روی هر دو رقم مورد بررسی بیماری‌زا بوده و علایم تغییر رنگ پوست و چوب را نشان دادند. جداسازی مجدد قارچ‌های مایه‌زنی شده و تأیید ویژگی‌های ریخت‌شناختی آن‌ها، اجرای اصول کخ را اثبات نمود. براساس اطلاعات نگارندگان، ایجاد بیماری شانکر در درختان سیب توسط گونه‌های *Pa. foliicola* و *P. formosus* برای اولین بار گزارش می‌شود. هم‌چنین گونه *Pa. foliicola* ثبت جدید برای بیوتای قارچی ایران است.

واژگان کلیدی: بیماری سیب، *Paecilomyces formosus*، *Paramyrothecium foliicola*، ایران