Mycologia Iranica 7(1): 115 – 123, 2020 Original Article DOI: 10.22043/mi.2021.123686

New or less known ascomycete species associated with trunk and branches of trees in Guilan province, Iran

N. Mousavi
S. Mousanejad
S. A. Khodaparast ✉
Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

Abstract: In this study, we report some Ascomycete genera from Nectriaceae, Botryosphaeriaceae, Cryphonectriaceae and Hypocreaceae families from Guilan province, Iran. Cultural and morphological characteristics and sequence information of rDNA ITS and translation elongation factor 1-α encoding gene (*tef1*) were used for identification. Among isolates studied in this research, six species viz. *Microthia havanensis*, *Lasiodiplodia hormozganensis*, *Lasiodiplodia parva*, *Thyronectria austroamericana*, *Trichoderma cf. chromospermum* and *Trichoderma cf. longibrachiatum* are reported as new or less known species for the region. As a result, two genera *viz. Thyronectria* (Nectriaceae) and *Microthia* (Cryphonectriaceae) and two species (*Lasiodiplodia parva* and *Trichoderma* cf. *chromospermum*) are new reports for Iran mycobiota.

Keywords: Biodiversity, *Microthia*, Nectriaceae, *Thyronectria*, *Trichoderma*

INTRODUCTION

The phylum Ascomycota includes a large group of fungi that have adapted a variety of ways to complete their life cycle and take nutrition, such as sporophyte, parasitism, mutualistic symbiosis, etc. Tree trunk and branches harbor a large number of ascomycetes. Many of them are found on trees as lichen-forming ascomycetes; some of them are endophytes, some habit as parasites and some may live on wood as saprophytes and decomposers (Webster and Weber 2007). Interestingly, several species of the wood-inhabiting ascomycetes have been studied for their secondary metabolites (Quang et al. 2006; Kuhnert et al. 2015; Helaly et al. 2018; Stadler and Hellwig 2005). Quang et al. (2015) reported that Carneic acids A and B from *Hypoxylon carneum* have antibacterial and antifungal activities. In some cases, metabolites of wood inhabiting fungi showed antagonistic effects against plant pathogens (Pourmoghaddam et al. 2020).

Moreover, several species are well known woody plant pathogens causing canker, dieback or wilting. Some of these important phytopathogenic fungi have been previously reported from Iran, such as: *Cryphonectria parasitica* (chestnut blight), *Biscogniauxia mediterranea* (oak charcoal disease), *Ophiostoma novo-ulmi* (Dutch elm disease), *Phaeoacremonium* spp, *Botryosphaeria* spp, *Lasiodiplodia* spp, *Eutypella* spp (Ershad 1995, Kazempour et al. 2006; Roudsari 2007; Ghezi et al. 2010; Safae et al. 2017; Kazemzadeh Chakusary et al. 2017, 2019; Esparham et al. 2020; Sohrabi et al. 2020). In this study, we investigated fungal species associated with a forest tree trunk and branch cankers and introduced some new or less known species from Iran.

MATERIALS AND METHODS

Isolation and morphological characterization

Fungi were isolated directly by transferring ascospores or conidia of crushed fruiting bodies to appropriate media or culturing of small pieces of the margin between necrotic and apparently healthy wood tissues after surface-disinfection with sodium hypochlorite solution 1% for 2 minutes. For direct culture, spore suspensions were made in a drop of sterile distilled water and then streaked onto the surface of plates containing 2 % (w/v) water agar (WA), potato-dextrose agar [PDA: composed of potato extract (200 g), 20 g glucose, and 20 g agar/liter] or cornmeal agar + 2 % w/v dextrose (CMD); and incubated at 25 °C. Single germinating spores were transferred directly to PDA or CMD.

For the study of morphological characters, structures were mounted in lactic acid 50% and 30 measurements were taken for each structure. All photos were taken by using a digital camera attached to Leica DM 1000 microscope.

Submitted 4 Oct. 2019, accepted for publication 5 March 2020
✉ Corresponding Author E-mail: khodaparast@guilan.ac.ir

http://mij.areeo.ac.ir

The isolates were morphologically characterized on Potato Dextrose Agar, Synthetic Nutrient Agar [SNA: composed of KNO_3 (one g), KH_2PO_4 (one g), $MgSO_4.7H_2O$ (0.5 g), CMD, Cornmeal [CM: 10g cornmeal + 20 mL distilled water] (Hoegger et al. 2002, Hirooka et al. 2012). To induce sporulation of Botryosphaeriaceous species, fungi were cultured on 2 % water agar bearing double-autoclaved pine needles or poplar twig (Phillips et al. 2013).

For morphological identification, the following references were used: Roane et al. 1986; Rossman et al. 1999; Chaverri et al. 2003; Gryzenhout et al. 2006; Jaklitsch 2009; Jaklitsch 2011; Hirooka et al. 2012; Phillips et al. 2013; Jaklitsch et al. 2014).

DNA extraction, amplification and sequencing

Due to the limited budget for sequencing of all isolates, for each species, a representative was subjected to sequencing. DNA extraction was conducted by methods of Walsh et al. (1991) and Hirata and Takamatsu (1966). The rDNA internal transcribed spacers were amplified using the primer pairs ITS1 and ITS4 (White et al. 1990). PCR amplification was performed in 25 µL reaction volume containing 2.5 µL 10x PCR buffer (0.5 M KCl, 0.1 M Tris-HCl, pH 8.3, 0.015 M $MgCl_2$); 200 µM of each dNTPs, 0.2 µM of each primer; one unit of Taq DNA polymerase (Cinna Gen, Iran); 3–5 µL of extracted DNA solution. For the amplification, initial denaturation of 95°C for 1.5 min was followed by 30 PCR cycles consisting of 95°C for 30 s, 52°C for 30 s, 72°C for 30 s, followed by one extension period at 72°C for 6.5 min. The 5′ portions of translation elongation factor (*tef1*) (approximately 370–450 bp) were amplified and sequenced using EF1-728F (Carbone and Kohn 1999) and EF2R (O'Donnell et al. 1998) primers. For the amplification, an initial denaturation for 5 min at 95°C was followed by 30 PCR cycles consisting of 94°C for 45 s, 52°C for 30 s, 72°C for 90 s, followed by one extension period at 72°for 6 min (Khodaparast et al. 2020). The nucleotide sequences of polymerase chain reaction (PCR) products were obtained using direct sequencing in a Genetic Analyzer 3130XL (Applied Biosystems, USA) in Rouyan Zista Gene company (Iran, Tehran). Sequences were analyzed and edited using MEGA7.0 (Kumar et al. 2016). Similar sequences were searched with the available sequences in NCBI GenBank nucleotide database using a BLASTN search method. Several sequences (preferably type or reference sequences, if available) from GenBank were selected for comparison.

RESULTS AND DISCUSSION

During the present investigation, 20 fungal isolates belonging to Botryosphaeriaceae, Cryphonectriaceae, Hypocreaceae and Nectriaceae families (Ascomycota) were obtained from the trunk and branches of trees during 2017–2019. In this paper, we introduced some species that are new records to the mycobiota of Iran or less known from Guilan province. All specimens were deposited in Mycological Herbarium at the University of Guilan (GUM).

***Microthia havanensis* (Bruner)** Gryzenhout & M.J. Wingf., Studies in Mycology 55: 44 (2006)

Ascostroma on natural substrate semi-immersed to superficial, pulvinate, orange. Perithecia spherical to ovoid, 82–145×57–62 µm, in groups of 2 to 12, surrounded by host tissue. Asci fusiform to cylindrical, with 8 ascospores, 20–35×4–6 µm. Ascospores two-celled, hyaline, ellipsoid to fusoid, 6–7.5×1.5–3 µm. Anamorphic stromata 47–75×30–35 µm, semi-immersed to superficial, orange, uni- to multilocular, often occurring in the same stroma that contains perithecia. Conidiophores cylindrical with long paraphyses between them and 30–60×1 µm in length. Conidia hyaline, cylindrical to ellipsoid, aseptate, 2.5–3×1 µm.

On PDA, few pycnidia were produced after seven days of incubation at 25°C a. Pycnidia orange, slimy, subglobose. Conidia yellow, 4–6×1–2 µm. No pigmentation was observed on cornmeal after seven weeks at 25°C (Fig. 1).

Specimen examined. IRAN, Guilan Province, Fouman (Roudkhan castle), on the surface of branch and trunk of *Pterocarya fraxinifolia* (Poir.) Spach, 7 April 2017, N. Mousavi (GUM1561).

Note. This fungus was identified based on Roane et al. (1986) as *Cryphonectria havanensis* (Bruner) M.E. Barr. However, Gryzenhout et al. (2006) revised the genus *Cryphonectria* and transferred this species to *Microthia* based on its long paraphyses between conidiophores. Unfortunately, sequencing of ITS and *tef1* genes was failed for this species. This is the first report of this species from Iran.

Lasiodiplodia hormozganensis Abdollahzadeh, Zare & A.J.L. Phillips, Persoonia 25: 6 (2010)

Ascomata not observed. Conidiomata produced on pine needles on WA within 2–4 weeks, dark brown to black, covered with dense mycelium, globose, 0.8–1.2×0.5-0.7 mm. Paraphyses hyaline, cylindrical, 43–85×2–3 µm. Conidiogenous cells holoblastic, hyaline, cylindrical, 12–19×2–3 µm. Conidia initially hyaline, aseptate, ellipsoid to cylindrical, becoming pigmented, 1-septate with longitudinal striations, 18–23×8–15 µm.

On PDA, mycelium covering the 9 cm plate at 25°C after 72 hours, at first, the color was bright, after a week turned to light gray (Fig. 2).

Specimen examined. IRAN, Guilan Province, Sumaehsara (Sesar), on the barks of *Ailanthus altissima* (Mill.) Swingle, 5 Aug. 2016, N. Mousavi; *tef1* sequence GenBank: MW330392; (GUM1559).

Note. Phillips et al. (2013) revised Botryosphaeriaceae and have provided a key for species identification. Based on this key, our species was well distinguished by its dark brown conidia with longitudinal striations from closely related taxa.

BLAST queries of the sequence shared 99% identity with *Lasiodiplodia hormozganensis* (294/296 bp with two gaps, GU945343, IRAN1500C, holotype sequence, Abdollahzadeh et al. 2010). This species has already been reported from Hormozgan province (Abdollahzadeh et al. 2010) and this is a new report from Guilan province.

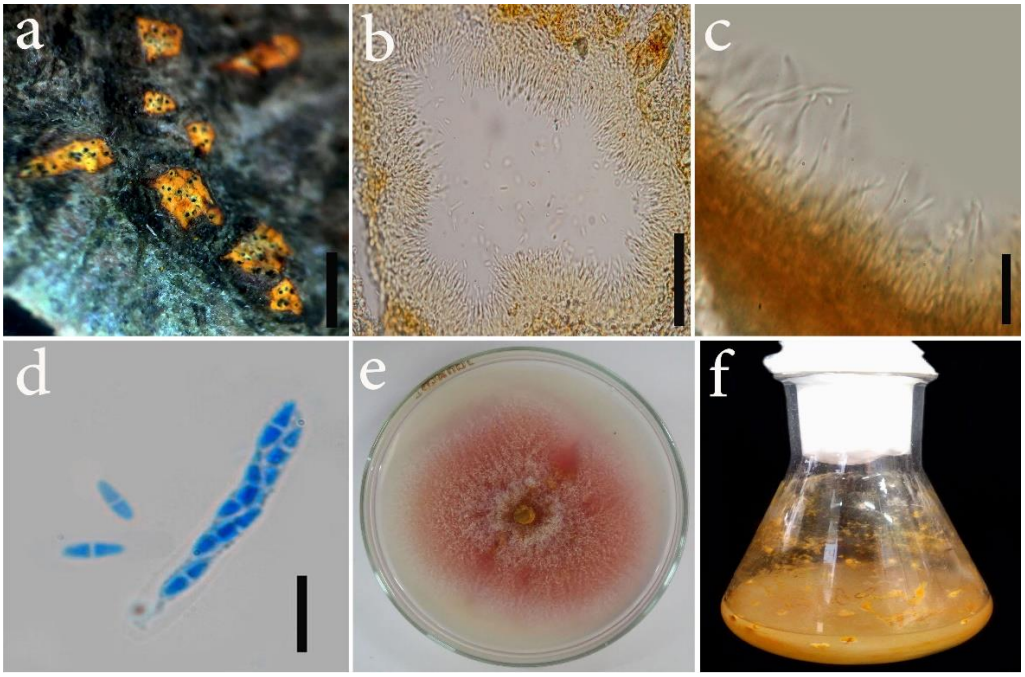
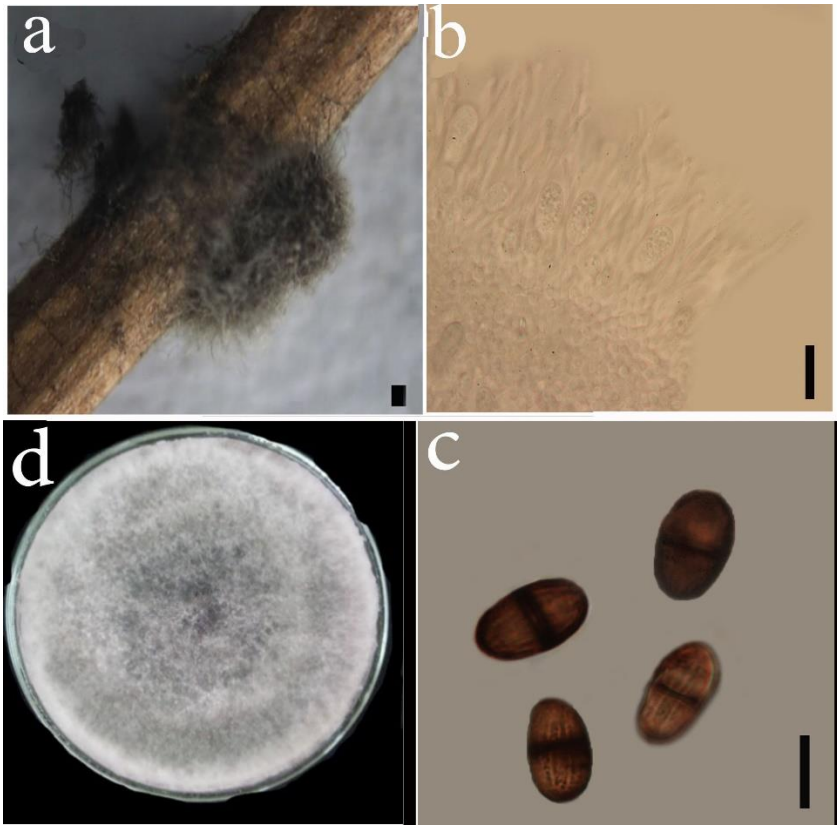


Fig. 1. *Microthia havanensis*. a. Ascostroma on bark; b. Longitudinal section of conidioma; c. Conidioma paraphyses; d. Asci and ascospores; e. Colony morphology in 90 mm dishes after two weeks at 25 °C in the dark regime on PDA; f. Colony on CM after seven weeks. — Scale bars: a = 100 µm; b = 40 µm; c = 20 µm; d = 20 µm.

Fig. 2. *Lasiodiplodia hormozganensis*. a. Conidiomata on pine needles in culture; b. conidiogenous cells and paraphyses; c. Conidia with longitudinal striations; d. Colony morphology on PDA after 3 days at 25 °C. — Scale bars: b = 40 µm; c = 20 µm).



Lasiodiplodia parva A.J.L. Phillips, A. Alves & Crous, Fungal Diversity 28: 9 (2008)

Ascomata not seen. Conidiomata produced on pine needles and poplar twig on WA within 2–4 weeks, dark brown to black, covered with dense mycelium, globose to subglobuse, 0.7–1 × 0.5–0.7 mm. Paraphyses hyaline, cylindrical, usually 45–75 × 2–4 µm on a pine needle, but reach to 130 µm. Conidiogenous cells holoblastic, hyaline, cylindrical, 10-15 × 3–5 µm. Conidia initially hyaline, aseptate, ellipsoid to cylindrical, becoming pigmented, 1-septate with longitudinal striations, 19–24 × 12–14 µm.

On PDA, mycelium covering the 9 cm plate at 25°C after 72 hours of incubation, at first, the color was bright after a week turned into light gray (Fig 3).

Specimen examined. IRAN, Guilan Province, Fouman (Roudkhan castle), on the barks of *Pterocarya fraxinifolia* (Poir.) Spach, 7 April 2017, N. Mousavi; *tef1* sequence GenBank: MW330393; (GUM1560)

Note. The sequence of *tef1* showed 99.4 % similarity (302/304 identity) with the sequence of the type material of *L. parva* (EF622063, CBS456.78, Alves et al. 2008). This is a new record for mycobiota of Iran.

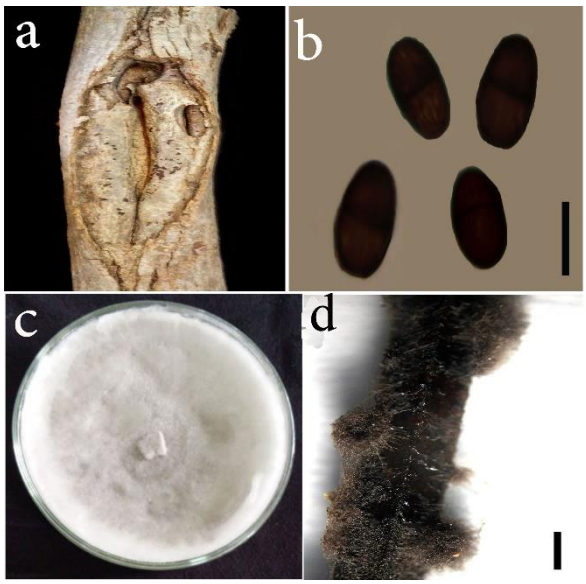


Fig. 3. *Lasiodiplodia parva*. a. Disease symptoms on stem; b. Conidia with longitudinal striations; c. Colony morphology on PDA after three days at 25°C; d. Conidiomata on pine needles in culture. — Scale bar = 40 µm.

Thyronectria austroamericana (Speg.) Seeler, Journal of the Arnold Arboretum 21: 405 (1940)

On the natural substrate, ascomata and pycnidia were formed separately or on the same stroma. Stromata variable in size, partly immersed, reaction to KOH and lactic acid (LA) negative. Perithecia were subglobose to globose 20–48×20–33 µm, aggregated in groups of 4–160, dark brown to black. Asci narrowly clavate, 57.6–68.4 × 7.2–12 µm, 8-spored, ascospores subglobose to ellipsoidal, muriform, light yellow, (11)12–19 × 7–10 (12) µm. Pycnidial stromata erumpent through epidermis or developing in the stroma with ascomata, orange to peach. Pycnidia dimorphic, superficial and immersed in the stroma, KOH-, LA-. Superficial pycnidia multilocular, brown, immersed pycnidia multilocular, irregular multiple chambers with shared walls, solitary or aggregated in groups of 2–4. Conidiophores 2–5 times branched, 8–13.5 × 1–2 µm. Sterile hyphae mixed with phialides and acicular, straight or slightly curved, unbranched, sometimes 1–2 branched, aseptate, 20–45 × 1–1.5 µm. Conidia light yellow, ellipsoidal to obovate, aseptate, 2–3×1–1.5 µm.

On PDA, after 7 d at 25°C, colonies 35–45 mm. Colony surface with radial growth and sometimes wavy, peach odor on PDA slightly fruity. Sporulation on SNA rare. Conidiophores abundant, unbranched, 12.5–31 × 1–2 µm. Conidia hyaline, KOH+, LA-, oblong or ellipsoidal, straight or slightly curved, rounded at both ends, not germinating and budding on media, 4.5–13.5(–17) × 2–3(–4) µm (Fig 4).

Specimen examined. IRAN, Guilan Province, Fouman (Alian), on the branches and barks of *Gleditsia caspia* Desf., 30 Mar. 2016, N. Mousavi; ITS sequence GenBank: MW325661; (GUM1544)

Note. Recently Jaklitsch and Voglmay (2014) revised *Thyronectria s*pecies and provided a dichotomous key for species identification. Based on this key, the above species is well distinguished by its muriform, subglobose to ellipsoid ascospore from closely related species. Moreover, the rDNA ITS sequence of our isolate showed 100% homology (548/548 identity) with a reliable accession number KJ570691 that has been submitted by Jaklitsch and Voglmay (2014). This is the first report of the species from Iran.

Trichoderma* cf. *chromospermum P. Chaverri & Samuels, Studies in Mycology 48: 51(2003)

On the natural substrate, stromata scattered or gregarious, pulvinate to discoidal with a narrowly large margin, pale yellow. When dry 0.1–1.7×0.8–1.3 mm. Perithecia only rarely projecting, ostiolar dots 30–70 µm, conspicuous, numerous, brown to dark green or black when mature, orange-yellow after addition of KOH. Perithecia 60–125×20–100 µm, subglobose or flask-shaped. A cortical layer consisting of a textura angularis of light orange, thin-walled cells, (6–)7–9(–10)×4–6 µm, often only of 1–2 layers of coarse cells; uppermost layer partly collapsing. Subcortical tissue subhyaline, thin-walled cells, 7–13×4–7(–10) µm. Subperithecial tissue sub-hyaline, thin-walled cells, 10–25×7–13 µm. Asci 60-91×5–5.5 µm. Ascospores yellowish green to green, coarsely tuberculate, cells dimorphic with little difference in shape and size, distal cells subglobose, (3.6–)4.16(–5.2)×3.6–4 µm, proximal cells subglobose to oblong, 4.16–5.2×(3–) 3–4, including warts.

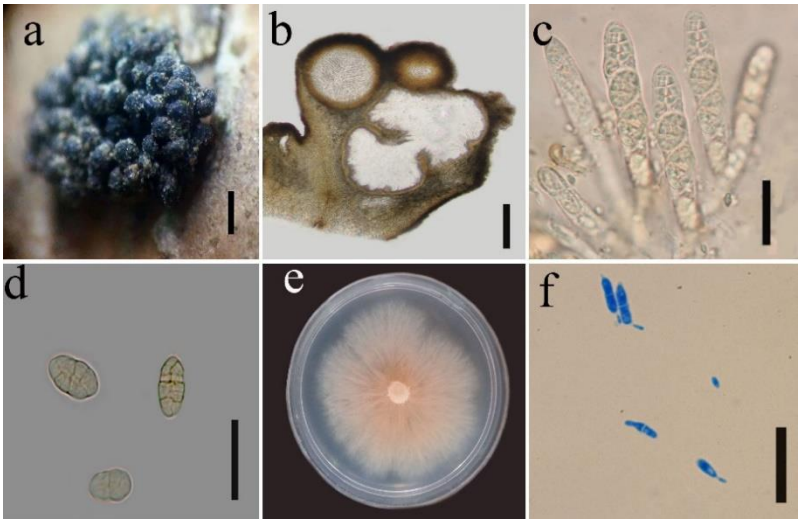


Fig. 4. *Thyronectria austroamericana*. a. Teleomorph on natural substrata; b. Cross section of superficial pycnidia; c. Asci; d. Ascospores; e. Colony morphology on PDA after 2 weeks at 22 °C; f. Conidia on SNA media. — Scale bars: a = 100 µm; b-f = 20 µm.

On PDA, after 72 h of incubation 40–42 mm at 25°C, 20–22 mm at 30°C after two weeks and did not grow at 35°C after a month; mycelium covering the plate after ten days. Colony dense, thin, silky, not zonate. The surface is covered by a white cottony with pale green conidia. Agar not pigmented; no distinctive odor. Autolytic excretion absent. On SNA after 72 h 34–40 mm at 25°C, 30–35 mm at 30°C after two weeks and did not grow at 35°C after a month; mycelium covering the plate after two weeks. Colony hyaline, thin, dense, with a wavy margin, hyphae with the radial arrangement, thin, with low variation in width. Autolytic activity absent. On CMD after 72 h 48–56 mm at 25°C, 25–28 mm at 30°C after two weeks and did not grow at 35°C after a month. Colony hyaline, thin, radial, shiny, little mycelium on the agar surface, yellow-green to citrine green. Autolytic activity absent. Agar not pigmented

Conidiophores short, with verrucose base, 30–60×3–4 µm, hyaline. Phialides arising in more or less narrow angles from barrel-shaped metulae on the secondary or tertiary branches, or directly from the main branch, flask-shaped or lageniform, tapering uniformly from base to tip, slender, sometimes twisted and short, formed singly or in pairs, 10–18 µm long, 3–4 µm wide at the widest point, 2–3 µm at the base. Conidia unicellular, pale green, smooth, ellipsoidal to oblong, 3–6× (2.5–)3(–3.5) µm (Fig 5).

Specimen examined. IRAN, Guilan Province, Fouman (Roudkhan castle), on the branches and barks of *Pterocarya fraxinifolia* (Poir.) Spach, 6 Sept. 2017, A. Mousavi.; *tef1* sequence GenBank: MW330394; (GUM1563).

Note. According to Chaverri and Samuels's description (Chaverri and Samuels 2003), morphologically, this fungus was identified as *T. chromospermum*. After Blast search, the sequence of *tef1* showed 98-100 % similarity to eight sequences of *T. chromospermum* available in GenBank (such as KF923291, KF923292, KF923287, Zhu and Zhuang 2015.). However, it showed 96 % similarity (523/544 identity, with eight gaps) with one sequence of this species (AY737728, strain BPI 749362, Samuels 2005). Other sequences in GenBank showed less than 80 % similarity with this fungus. According to our knowledge, this is a new record for Iran mycobiota.

Trichoderma* cf. *longibrachiatum Rifai, Mycological papers 116; 42 (1969)

On the natural substrate, stromata scattered or gregarious, pulvinate to discoidal with a narrowly large margin, isabelline-hazel, when dry 0.1–1.7×0.8–1.3 mm. Perithecia only rarely projecting, ostiolar dots 30–50 µm, conspicuous, numerous, brown to dark green or black when mature, no change seen in 3% KOH. Perithecia 100–170 × 70–130 µm, subglobose or flask-shaped. Cortical layer 4–5(–6) µm thick, consisting of a textura angularis of light yellow to hyaline, thin-walled cells, subcortical tissue subhyaline, thin-walled cells 5–7 × (3–)4–5 µm. Subperithecial tissue subhyaline, thin-walled cells, 11–30 × 5–11 µm. Asci 50–73×5–6 µm. Ascospores hyaline, coarsely tuberculate, cells 3–4 × 3–4 µm including warts.

On PDA, forming concentric rings of whitish mycelium, fluffy and dense, with velvety to slightly granular zones, greenish yellow to yellowish green, yellow diffusing pigment formed within 24 h at 25, 30, and 35°C, odor indistinct. Autolytic excretion absent. On CMD, forming concentric rings of whitish, often submerged mycelia, with granular deep green zones, pustules at first white then became green to pale green, diffusible pigment not formed.

On SNA, autolytic excretion common in marginal hyphae, light yellow. Conidiophores usually consisting of a distinctive main axis, 60–80 × 3–4 µm, hyaline and smooth walled, sterile hairs not formed, phialides borne singly and laterally on the main axis or from side branches, divergent in small whorls of 2 to 3 phialides arising from supporting cells, less commonly solitary, cylindrical to lageniform 6–10 µm long, 2–3 µm the widest point and 1–2 µm at the base. Conidia unicellular, brown, smooth, round to elliptical, (3–)4–5(–6)×2.5–3.5 µm (Fig 6).

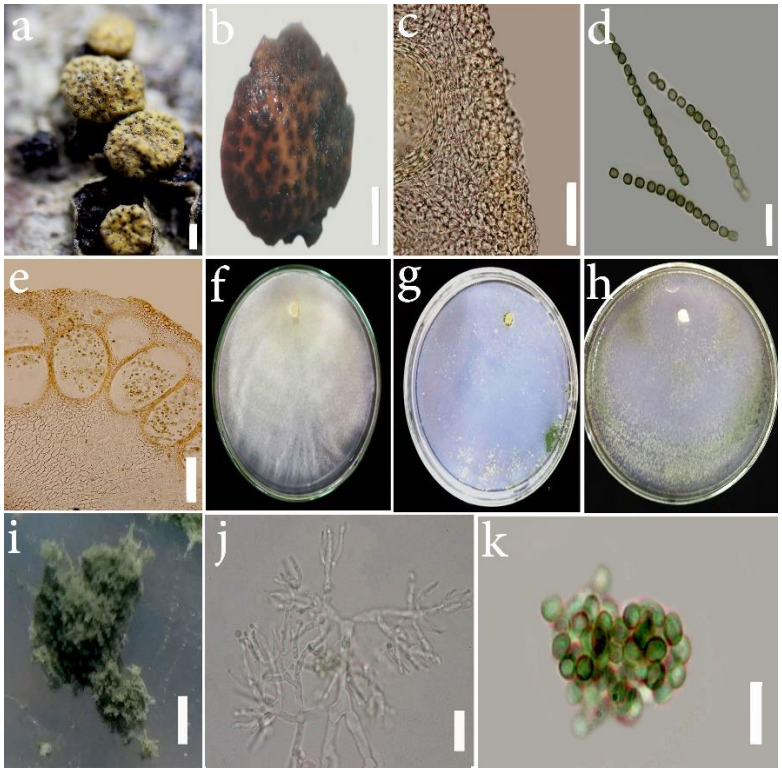


Fig. 5. *Trichoderma* cf. *chromospermum*. a. Fresh stromata; b. Mature stroma in 3 % KOH after reconstitution in water; c. Cortical tissue in section; d. Asci with ascospores; e. Cross section of perithecium; f-h. Cultures at 25°C after 14 days (f. on PDA, g. on SNA, h. on CMD); i. Hyphae on agar surface (SNA, 25°C, 7 days); j. Conidiophores; k. Conidia (14 days). — Scale bars: a = 1000 µm; b = 500 µm; c = 1000 µm; d, e = 20 µm; i, j = 200 µm; k = 10 µm.

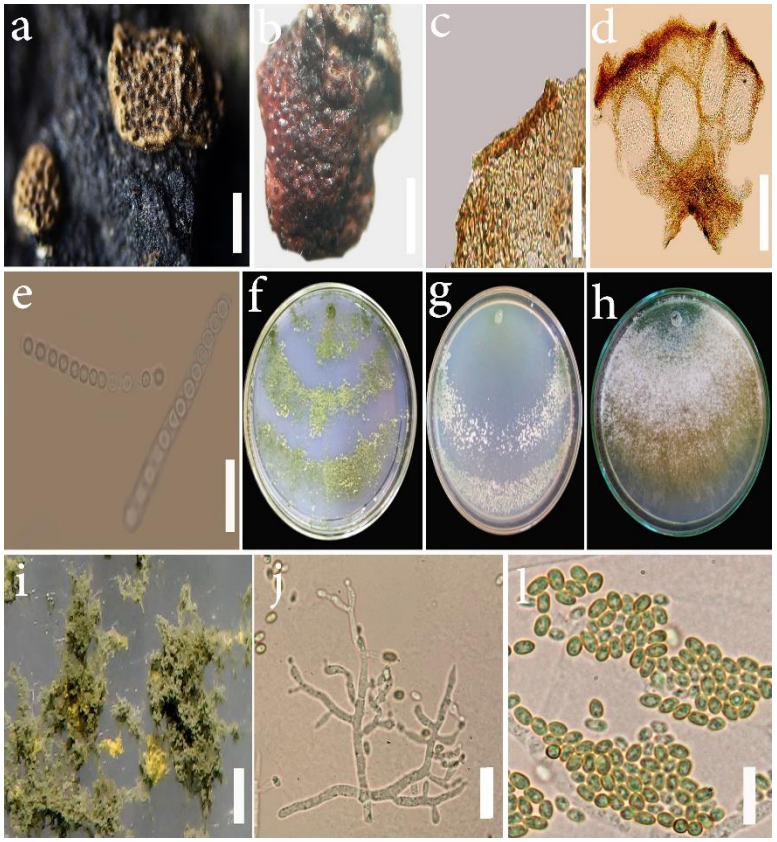


Fig. 6. *Trichoderma* cf. *longibrachiatum*. a. Fresh stromata; b. Mature stroma in 3 % KOH after reconstitution in water; c. Cortical tissue in section; d. Cross section of perithecium; e. Asci with ascospores; f-h. Cultures at 25°C (f. on PDA, 3 days; g. on SNA, 3 days; h. on CMD, 7 days); i. Hyphae on agar surface with autolytic excretion. (SNA, 25°C, 7 days); j. Conidiophores; k. Conidia (3 days). — Scale bars: a, b = 1000 µm; c, j= 50 µm; d, i = 300 µm; e = 100µm; k= 30 µm.

This species already reported from Iran (Ershad 1995), however, the sexual state of the species is recorded for the first time from Iran.

Specimen examined. IRAN, Guilan Province, Rasht, on the barks of *Populus* sp., 4 Oct. 2017, A. Mousavi., *tef1* sequence GenBank: MW330395; (GUM1564).

Note. After blast search, *tef1* sequence showed more than 99% similarity to several sequences such as EU401590 provided by Druzhinina et al. (2008).

However, this sequence showed many differences when compared with the ex-holotype of *Trichoderma longibrachiatum* provided by Druzhinina et al. (2008) in the same paper. Druzhinina et al. (2008) considered all such sequences in *Trichoderma longibrachiatum.*

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deputy of Research and Technology of the University of Guilan, Rasht Iran.

REFERENCES

Abdollahzadeh J, Javadi A, Goltapeh EM, Zare R, Phillips AJL. 2010. Phylogeny and morphology of four new species of Lasiodiplodia from Iran. Persoonia: Molecular Phylogeny and Evolution of Fungi 25: 1–10.

Alves A, Crous PW, Correia A, Phillips AJL. 2008. Morphological and molecular data reveal cryptic speciation in Lasiodiplodia theobromae. Fungal Diversity 28: 1–13.

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556.

Chaverri P, Samuels GJ. 2003. Hypocrea/Trichoderma (Ascomycota, Hypocreales, Hypocreaceae): Species with green ascospores. Studies in Mycology 48:1–116.

Druzhinina IS, Komoń-Zelazowska M, Kredics L, et al. 2008. Alternative reproductive strategies of Hypocrea orientalis and genetically close but clonal Trichoderma longibrachiatum, both capable of causing invasive mycoses of humans. Microbiology 154: 3447–3459.

Ershad D. 2009. Fungi of Iran. 3rd edn. Iranian Research Institute of Plant Protection, Tehran, Iran.

Esparham N, Mohammadi H, Gramaje D. 2020. A Survey of Trunk Disease Pathogens within Citrus Trees in Iran. Plants 9: 754.

Ghezi E, Khodaparast SA, Zare R. 2010. Distribution and severity of damage by Cryphonectria parasitica in the chestnut stands in Guilan province, Iran. Forest Pathology 40: 450–457.

Gryzenhout M, Myburg H, Hodges CS, Wingfield BD, Wingfield MJ. 2006. Microthia, Holocryphia and Ursicollum, three new genera on Eucalyptus and Coccoloba for fungi previously known as Cryphonectria. Studies in Mycology 55: 35–52.

Helaly SE, Thongbai B, Stadler M. 2018. Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. Natural Product Reports 35: 992–1014.

Hirata T, Takamatsu S. 1996. Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37: 283–288.

Hirooka Y, Rossman AY, Samuels GJ, Lechat C, Chaverri P. 2012. A monograph of Allantonectria, Nectria, and Pleonectria (Nectriaceae, Hypocreales, Ascomycota) and their pycnidial, sporodochial, and synnematous anamorphs. Studies in Mycology 71: 1–210.

Hoegger PJ, Rigling D, Holdenrieder O, Heiniger U. 2002. Cryphonectria radicalis: rediscovery of a lost fungus. Mycologia 94: 105–115.

Jaklitsch WM. 2009. European species of Hypocrea. Part I. The green-spored species. Studies in Mycology 63: 1–91

Jaklitsch WM. 2011. European species of Hypocrea part II: species with hyaline ascospores. Fungal Diversity 48: 1–250.

Jaklitsch WM, Voglmayr H. 2014. Persistent hamathecial threads in the Nectriaceae, Hypocreales: Thyronectria revisited and re-instated. Persoonia (Molecular Phylogeny and Evolution of Fungi) 33: 182–211.

Kazempour MN, Khodaparast SA, Arefipour M, Salehi M, Amanzadeh B, Ramazanie M, Shiraz BK. 2006. Occurrence of Cryphonectria parasitica the causal agent of chestnut blight in Iran. Plant Pathology 55: 815.

Kazemzadeh Chakusary M, Mohammadi H, Khodaparast SA. 2017. Decline-associated Phaeoacremonium species occurring on forest trees in the north of Iran. Forest Pathology 47(6) e12368.

Kazemzadeh Chakusary M, Mohammadi H, Khodaparast SA. 2019. Diversity and pathogenicity of Botryosphaeriaceae species on forest trees in the north of Iran. European Journal of Forest Research 138: 685–704.

Khodaparast SA, Pourmoghaddam MJ, Amirmijani A. Byrami F. 2020. Phylogenetic structure of the Iranian capnodiaceous sooty mould fungi inferred from the sequences of rDNA regions and TEF1-α. Mycological Progress 19: 155–169.

Kuhnert E, Surup F, Sir EB, Lambert C, Hyde KD, Hladki AI, Romero AI, Stadler M. 2015. Lenormandins A–G, new azaphilones from Hypoxylon lenormandii and Hypoxylon jaklitschii sp. nov., recognised by chemotaxonomic data. Fungal Diversity 71: 165–184.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95: 2044–2049.

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW. 2013. The Botryosphaeriaceae: genera and species known from culture. Studies in Mycology 76: 51–167.

Pourmoghaddam MJ, Lambert C, Surup F, Khodaparast SA, Krisai-Greilhuber I, Voglmayr H, Stadler M. 2020. Discovery of a new species of the Hypoxylon rubiginosum complex from Iran and antagonistic activities of Hypoxylon spp. against the Ash Dieback pathogen, Hymenoscyphus fraxineus, in dual culture. MycoKeys 66: 105-133.

Quang DN, Stadler M, Fournier J, Asakawa Y. 2006. Carneic acids A and B, chemotaxonomically significant antimicrobial agents from the Xylariaceous Ascomycete Hypoxylon carneum. Journal of Natural Products 69: 1198–1202.

Roane MK, Griffin GJ, Elkins JR. 1986. Chestnut blight, other Endothia diseases, and the genus Endothia. The American Phytopathological Society, USA.

Rossman AY, Samuels GJ, Rogerson CT, Lowen R. 1999. Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Studies in Mycology 42: 1–248.

Roudsari NF, Dustmorad Z, Khodaparast SA, Rouhani H. 2007. Introducing some new species of Trichoderma for Iran. Rostaniha 8: 67–82.

Safaee D, Khodaparast SA, Mirabolfathy M, Sheikholeslami M. 2017. Some aspects of biology and host range of Biscogniauxia mediterranea, one of the causal agent of oak charcoal disease. Mycologia Iranica 4: 121–129.

Samuels GJ. 2005. Changes in taxonomy, occurrence of the sexual stage and ecology of Trichoderma spp. Phytopathology 96: 195-206.

Sohrabi M, Mohammadi H, León M, Armengol J, Banihashemi Z. 2020. Fungal pathogens associated with branch and trunk cankers of nut crops in Iran. European Journal of Plant Pathology, doi.org/10.1007/s10658-020-01996-w

Stadler M, Hellwig V. 2005. Chemotaxonomy of the Xylariaceae and remarkable bioactive compounds from Xylariales and their associated asexual stages. Recent Research Developments in Phytochemistry 9: 41–93.

Walsh PS, Metzer DAR, Higuchi R. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506-513.

Webster J, Weber R. 2007. Introduction to fungi. Third edition, Cambridge University Press. UK.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. In: PCR protocols: a guide to methods and applications. (MA Innis, DH Gelfand, JJ Sninsky, TJ White, eds). 315–322. Academic Press, San Diego, California, USA

Zhu ZX and Zhuang WY. 2015. Trichoderma (Hypocrea) species with green ascospores from China. Persoonia: Molecular Phylogeny and Evolution of Fungi 34: 113-129.

آسکومیست‌های ناشناخته یا کمتر شناخته شده همراه تنه و شاخه درختان در استان گیلان

نسترن موسوی، صدیقه موسی نژاد، سید اکبر خداپرست✉
گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه گیلان، رشت

چکیده: در این مطالعه چند جنس آسکومیستی از تیره‌های Botryosphaeriaceae، Cryphonectriaceae، Hypocreaceae و Nectriaceae از استان گیلان گزارش می‌شوند. ویژگیهای کشت، شکل‌شناسی و توالی ناحیه ITS و *tef1* برای تشخیص استفاده شدند. در میان جدایه‌های به دست آمده در این مطالعه شش گونه شامل *Microthia havanensis*، *Lasiodiplodia hormozganensis*، *Lasiodiplodia parva*، *Thyronectria austroamericana*، *Trichoderma cf. chromospermum* و *Trichoderma cf. longibrachiatum* به عنوان گونه‌های ناشناخته یا کمتر شناخته شده در منطقه معرفی می شوند. در نتیجه دو جنس *Microthia* (Cryphonectriaceae) و *Thyronectria* (Nectriaceae) و نیز گونه‌های *Lasiodiplodia parva* و *Trichoderma cf. chromospermum* به عنوان آرایه‌های جدید از ایران گزارش می‌شوند.

کلمات کلیدی: تنوع زیستی، میکروتیا، نکتریاسه، تیرونکتریا، تریکودرما

مکاتبه کننده: سید اکبر خداپرست Email: khodaparast@guilan.ac.ir
تاریخ دریافت: ۱۳۹۸/۰۷/۱۳ تاریخ پذیرش: ۱۳۹۹/۱۲/۱۴