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Pyronema domesticum, a new species for the Funga of Iran

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Pyronema is a genus of fungi in the family Pyronemataceae (Pezizales, Ascomycota) (Yasin et al. 2016). It was described by German naturalist Carl Gustav Carus in 1835. Forty-six and forty-four of *Pyronema* species are recorded in the MycoBank (https://www.mycobank.org) and Index Fungorum (http://www.indexfungorum.org/names/names.asp) databases, respectively. About forty-six records of *Pyronema* species have been described by mycologists of various countries, out of them two are valid names under the genus. There are two currently accepted species of *Pyronema*: *P. domesticum* and *P. omphalodes* (= *P. confluens*). *Pyromena* species have been known to be rapid responders to fire and grow rapidly in situations where there are few competitors (Bruns et al. 2020). In April 2022, *Pyronema* ascocarps were observed on a sterile soil provided for tomato planting, in a greenhouse located at College of Agriculture, Razi University, Kermanshah, Iran. Ascocarps were measured, photographed and their macroscopic characters described. The voucher specimen was deposited in the Fungal Collection of the Iranian Research Institute of Plant Protection (IRAN18161F). Total genomic DNA was extracted using a fungi DNA isolation kit (CinnaGen, Iran) according to manufacturer's instructions. Universal primers of internal transcribed spacers (ITS) of nuclear ribosomal DNA (ITS1: 5'-TCCGTAGGT-GAACCTGCGG-3'; ITS4: 5'-TCCTCCGCTTATTGATA TGC-3') (White *et al*. 1990) were utilized for amplifying two specimens using T-Personal thermocycler (Biometra, Germany). The PCR was performed in a final volume of 25 µM reaction containing 20 ng genomic DNA, 1 µM of each primer, 100 µM of each dNTP, 0.5 U Taq DNA polymerase (CinnaGen, Iran), 1.5 mM of MgCl2, 2.5 µM of 10 × PCR buffer (CinnaGen, Iran), and 14.5 µM H2O. The PCR cycle included one cycle of initial denaturation at 94 for 3 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 60 s, and a final extension of 10 min at 72 °C after cycling. A portion (5 µM) of the amplified product was run on 1% TBE-agarose gel and the presence of a single band (ca. 600 bp) was considered as successful amplification.

Apothecia 0.5-1 mm across, cup-shaped, round to lenticular, sessile, pink to red, and forms a mass of few square mm to few square cm and resides on a white surface mycelium. Hymenium usually convex, pink-red with an almost smooth margin. Paraphyses unbranched, straight, cylindrical, hyaline, septate, enlarged at the apex up to 8.78-10.75 µm, and 170 (160-200) µm. Asci cylindrical, non-amyloid, thin-walled, uniseriate, eight-spored, hyaline, and 125 (120-160) × 12 (11-14) µm. Ascospores broadly elliptical, smooth, hyaline, not guttulate, 12.05-17 × 9.2-11.24 µm ($\bar{x}$ = 14.31 × 10.24 µm, n = 100), and the ratio of length/width (Q) 1.39 (Fig 1. a-i).

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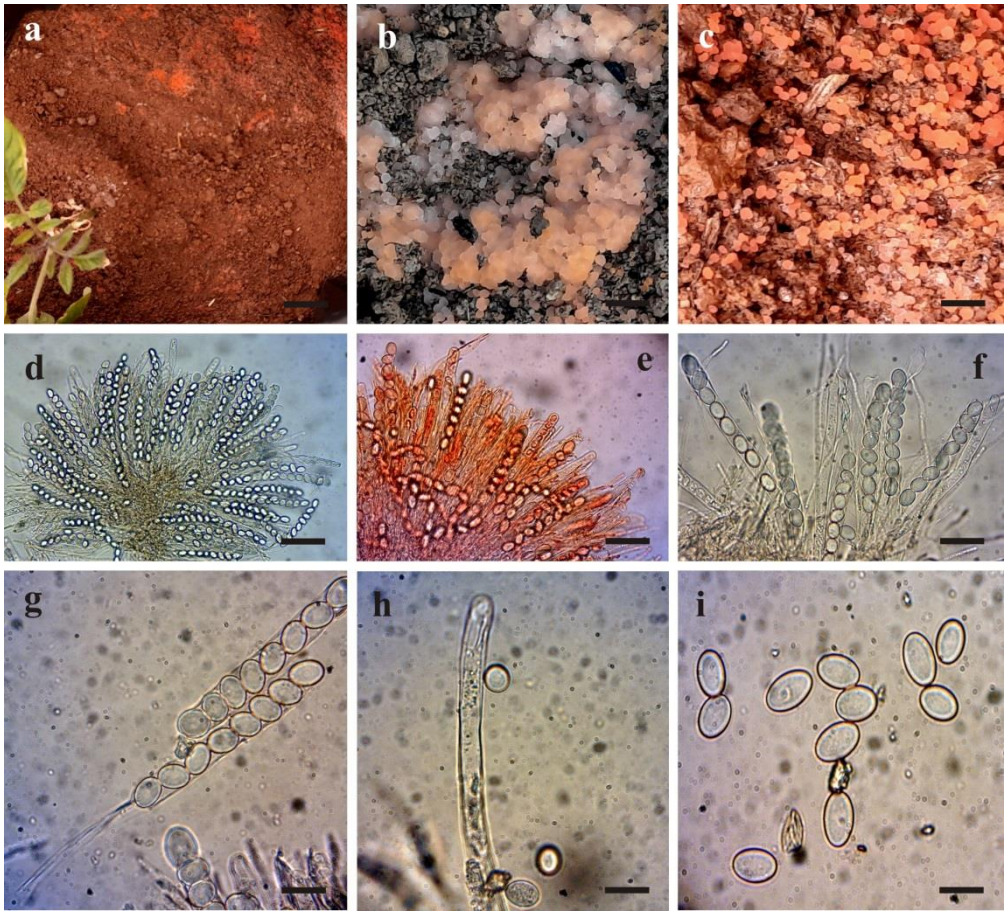

Fig. 1. *Pyronema domesticum*: (a) apothecia on sterilized soil of tomato plant, (b, c) closeup of apothecia, (d-f) asci and paraphyses, (g) asci and ascospores, (h) paraphysid, (i) ascospores. Scale bars: (a) = 10 cm, (b) = 1 cm, (c) = 500 µm, (d, e) = 100 µm, (f) = 50 µm, (g) = 30 µm, (h,i) = 15 µm.

Based on morphological criteria such as the shape and color of the apothecia, spore size and shape, and size of asci and ascospores, the studied specimens found in sterilized soils of greenhouse were identified as *Pyronema* sp. (Sowerby) Sacc (Moore & Korf 1963). BLAST analysis of ITS region (at least 600 bp in length) revealed 100% identity of our specimens to *Pyronema domesticum* from Austria (GenBank HQ115722) (Gorfer et al. 2011) and Germany (GenBank MG098284) (Bußkamp et al. 2020). This confirmed the identity of the specimens. Phylogenetic analyses based on ITS sequence of our isolates and 10 selected isolates of *Pyronema* showed that our isolates are closely related to *P. domesticum* (Fig. 2). The DNA sequences of examining two specimens RU-PyDo1 and RU-PyDo2 were deposited in GenBank under accession numbers ON368078 and ON368079, respectively. *Pyronema domesticum* has been associated with charred soil, damp whitewash, wet walls and damp paper, and reported from Germany, India, Indonesia, USA, China, Finland, Ukraine and other countries (Moore & Korf 1963; Dougoud 2011; Dzhagan et al. 2020). *Pyronema domesticum* and *P. omphalodes* are indistinguishable microscopically, but in nutrient-rich culture only *P. domesticum* forms sclerotia (Moore & Korf 1963). In this study, sclerotia formed on potato dextrose agar (PDA) after seven days in slant tube (Fig. 3 a-d). To our knowledge, this is the first report of *P. domesticum* from Iran.

Key words: Pyronemataceae, Ascocarp Morphology,
Molecular identification

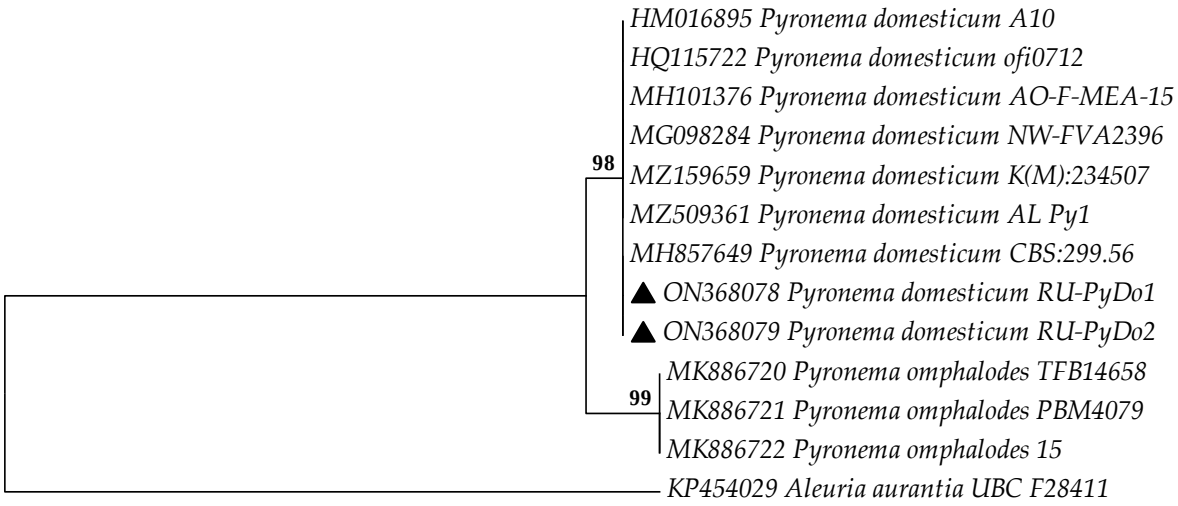

Fig. 2. Phylogeny of *Pyronema* species produced from maximum likelihood (ML) analysis of the ITS rDNA dataset. The triangle shapes refer to *Pyronema* in this study. Sequence of *Aleuria aurantia* was used as outgroup. .

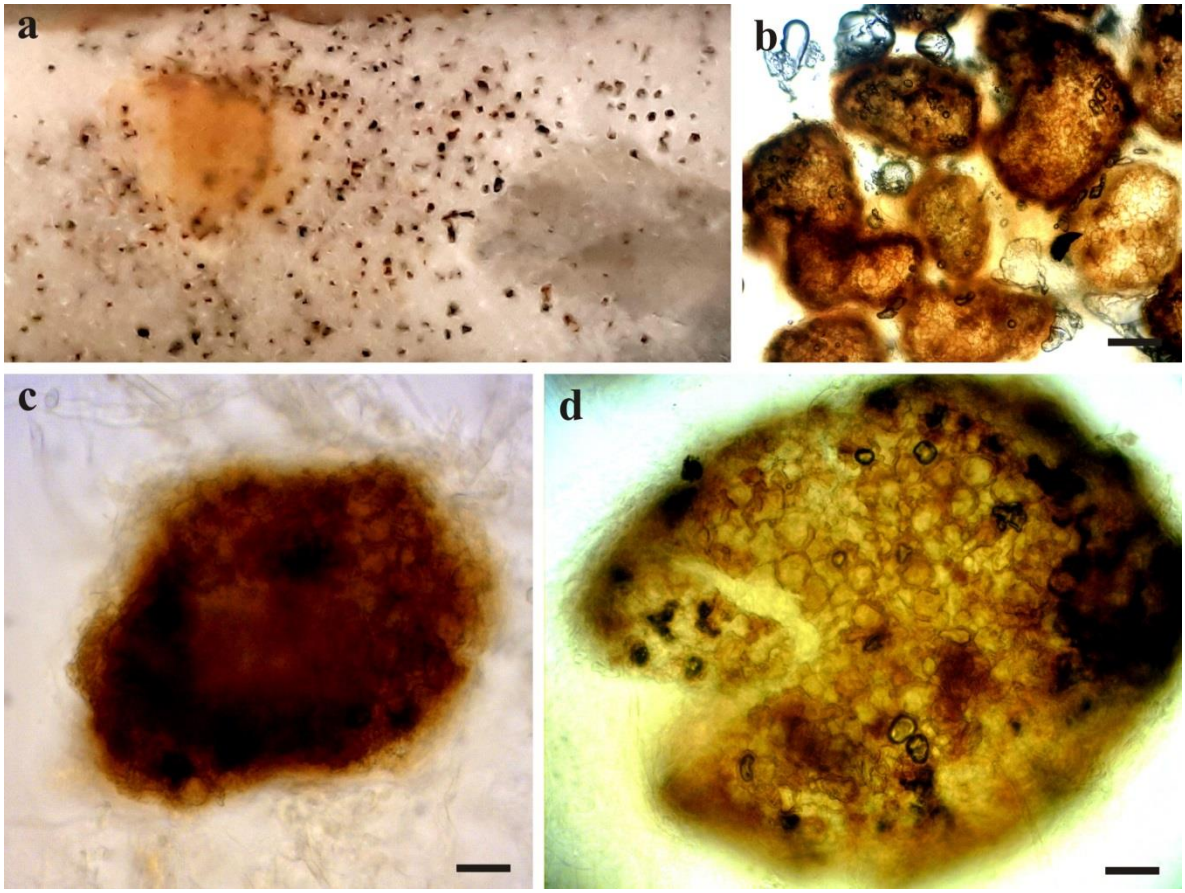

Fig. 3. Sclerotia on potato dextrose agar (PDA) after seven days. (a), sclerotia (b), close-up of sclerote (c, d). Scale bars: (b) = 50 µm, (c, d) = 20 µm.

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