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First report of *Biscogniauxia mediterranea* causing canker on wild almond (*Amygdalus scoparia*)

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Abstract *Biscogniauxia mediterranea* causes charcoal disease on *Quercus castanifolia*, *Q. brantii* and *Zelcova carpinifolia* in Iran. This report introduces wild almond (*Amygdalus scoparia*) as a new host species for *B. mediterranea* in Iran. In 2015, symptoms of dieback of *A. scoparia* trees were observed for the first time in the Lorestan Forests of Iran. The causal fungus *B. mediterranea* was recovered from cankered tissues and identified based on morphology, and sequence analysis. Pathogenicity tests on two-year-old *A. scoparia* plants showed that *B. mediterranea* could cause lesions.

Keywords *Biscogniauxia mediterranea* · *Amygdalus scoparia* · Canker · Iran

The genus *Amygdalus*, includes approximately 45 species around the world (Cronquist 1981; Takhtajan 1997). Iran is the main diversity centre for the distribution of *Amygdalus* species but they can also be found in other countries, including Turkey, Russia, and Middle Eastern countries (Shishkin and Yuzepchuk 1941; Browwicz, 1972; Zohary 1972). *Amygdalus* spp. are distributed in the Irano-Turanian region of south west Asia and middle Asia and few species are seen in E Asia (Browicz and Zohary 1996). *Amygdalus scoparia* is a wild species of almond that occupies large areas in many parts of Iran and its neighbouring countries. In Iran, *Amygdalus* spp. are found in different ecological zones including the Irano-Turanian, Zagros and Khalij-*O-Omani regions* (Ghahreman and Attar 1999). *Biscogniauxia mediterranea* is one of the most frequent fungal pathogens in the Mediterranean Basin, causing charcoal canker. The pathogen can easily spread through large cavity vessels, colonise bark and woody tissues, and is able to kill the host in a single, growing season. *Biscogniauxia mediterranea* causes necrosis on stems and branches of *Quercus castaneifolia*, *Q. brantii* and *Zelkova carpinifolia* in Iran (Mirabolphathi 2013). During 2015, we observed canker and debarking in *Amegdalus scoparia* in the Kakasharaf region of the Lorestan Forests. Cankered branches and stems were noticed. When the bark was removed, the wood beneath the bark was discolored reddish-brown to brown-black. Brown-black cankers appeared surrounded and contained by callus wound wood, particularly on larger branches and trunks. The aim of this research is to identify the causal agent of the canker on wild almond in Iran.

Canker samples were collected from trees in the region under study, i.e., the Kakasharaf region (lat. 33° 20′ 54″ N, long. 48° 29′ 33″ E), Lorestan province, Iran. To isolate the causal agent, surface-sterilised bark pieces of the infected tissue were plated on potato dextrose agar (PDA). After purification, in order to obtain mycelium, pycnidia were transferred to potato dextrose broth culture medium (PDB) and placed on a shaker at 120 rpm at 25 °C for 21 days. After obtaining the

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Fig. 1 *Biscogniauxia mediterranea*. **a**-**b**: damage of charcoal disease on *Amygdalus scoparia*. **c** *B. mediterranea* canker. **d** Stromata exposed by dehiscence of overlying bark. **e** Stroma with perithecia. **f** Section through stroma; outer dehiscing layer and perithecia are visible. **g**-**h**: Ascospores of *Biscogniauxia mediterranea*. **i** Colony of *B. mediterranea* after 3–7 days. Bars: e, 5 mm. f, 1 mm. g, 40 μm. h, 10 μm

mycelium, the DNA was extracted from mycelium using the CTAB method (Murray and Thompson 1980) with some minor modifications (to adapt the procedure to the study of fungal material): Lyophilised mycelium (40–50 mg) was placed into a microcentrifuge tube and ground to powder. This mycelial powder was suspended in 700 μL of extraction buffer (NaCl 0.7 M; Tris-HCl 50 mM pH 8.0; EDTA 2 mM pH 8.0, 1 % CTAB) preheated to 65 °C. The suspension was thoroughly mixed and incubated for 1 h at 65 °C. After the suspension had cooled, 500 μL of chloroform/isoamyl alcohol (24:1 *v*/v) was added. The supernatant was gently mixed until an emulsion was obtained and centrifuged at 10,000 rpm for 20 min. The aqueous phase was transferred to a new sterile tube. A 10 % CTAB solution was added at one tenth of the volume of the aqueous phase and mixed. The supernatant was transferred to a new tube after a spin-down of 20 min. 700 μL of precipitation buffer (CTAB 1 %; Tris-HCl 50 mM pH 8.0; EDTA 10 mM pH 8.0) was then added to the supernatant, left at room temperature for 5–10 min and centrifuged. The aqueous phase was discarded and 300 μL of TEHS buffer (NaCl 1 M; Tris-HCl 10 mM pH 8.0; EDTA 1 mM pH 8.0) was added to the pellet to remove the CTAB from the DNA. The pellet was treated with ribonuclease A, incubated at 37 °C for 30 min, followed by addition of 750 μL of cold absolute ethanol and centrifuged at 10,000 rpm for 20 min. The supernatant was discarded and the pellet was washed in 500 μL 70 % (*v*/v) ethanol and air-dried at room temperature. The DNA pellet was then dissolved in 50 μL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0). The identity of the *B. mediterranea* isolate was confirmed by DNA sequence analysis.

Stromata on affected trees were slightly convex, ellipsoid, and elongate, 6.8 to 20.2 × 3.7 to 4.3 cm. The perithecia were ovoid to tubular, 0.73 to 0.79 × 0.11 to 0.14 mm; the asci were short and stipitate, 8.1 to 10.2 × 119.1 to 167.3 μm. The ascospores were ovoid, brownish-black, with narrowed and roundish ends, 6.5 to 8.9 × 13.4 to 19.3 μm. Symptomatic tissues plated onto potato dextrose agar (PDA) and incubated at 25 °C for 7 days produced colonies that were grey when viewed from the top and black when viewed from the dish underside. On the basis of these morphological characters, the fungal species was identified as *B. mediterranea* (Ragazzi et al. 2012).

The culture voucher has been deposited in the herbarium in the Department of Plant Protection, Faculty of Agricultural Sciences and Natural Resources, University of Lorestan, Khorramabad, Iran, (Accession number LU9402).

Traditional identification was further confirmed by sequence information from the rDNA ITS region. Blast searches of the NCBI GenBank nucleotide database were done using

ITS sequences derived from three cultures (CBS 129072 to 129,074). GenBank Accession Nos. JF295127 to JF295129 of the isolated fungus differed by one nucleotide from *B. mediterranea* (GenBank Accession No. AF280624).

Pathogenicity tests were performed by inoculation of two-year-old *Amygdalus scoparia* seedlings under greenhouse conditions; a single isolate of *B. mediterranea* was used and 18 seedlings in three replications were wounded and mycelium plugs from actively-growing cultures on PDA were placed on the wounds and sealed with Para-film, the controls were inoculated with sterile PDA plugs. After a further 28 days, brown-black stromata appeared on the bark and after 90 days, the bark became detached. The pathogen was reisolated from the lesions of 18 seedlings (on average lesion size was 0.9 mm in length), confirming Koch's postulates. No symptoms were observed on control seedlings, which presented healed wounds.

On the basis of our current knowledge, *A. scoparia* represents a new host of *B. mediterranea* from Iran and around the world, and this is the first report of this new host *A. scoparia* for *B. mediterranea* in the Lorestan Forests of Iran (Fig. 1).

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