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Fungal species associated with leaf spot on mango trees in Iran

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Abstract: During a survey of leaf spots on tropical trees, symptomatic leaf tissue of mango trees was collected from several sites in the Hormozgan, and Sistan and Baluchestan provinces. Isolations were performed on potato dextrose agar (PDA) and wet paper media at 25 °C. As a result, several fungal species were obtained, that three isolates belonging to *Bartalinia* and four of *Beltrania*. Phylogeny based on DNA sequences of the large subunit of the ribosomal RNA (LSU) and internal transcribed spacer (ITS-rDNA) regions combined with morphological criteria revealed the species of *Bartalinia pini and Beltrania rhombica*. To our knowledge, both species, *B. pini* and *Be. rhombica* are new species for the Funga of Iran.

Keywords

Bartalinia pini, *Beltrania rhombica*, *Mangifera indica*, leaf spot, multilocus sequence analysis.

INTRODUCTION

Tropical fruit crops are valuable agricultural commodities (Ploetz 2003). Mango (*Mangifera indica* L.) is a famous fruit in tropical and subtropical regions (Usman et al. 2001; Berardini et al. 2005). Due to its tasty, high nutritional value, and economic importance in global markets, mango has increasingly been developed in traditional and non-traditional production countries such as the United Arab Emirates (UAE) (Nelson 2008; Saeed et al. 2017a). Iran has diverse climates and a significant temperature difference in the northern and southern regions. Therefore, various agricultural products are produced (Saboki et al. 2012). The south and southern areas of Iran, including large parts of Hormozgan and Sistan and Baluchistan provinces, are considered to be the most suitable regions of the country in the field of producing tropical products owing to their proximity to the equator, the Oman Sea, and the Indian Ocean. Although the history of cultivation of some of these products in the southern provinces of Iran reaches more than 300 years, it seems that the total product still has a significant difference from the ideal potential of the region (Saboki et al. 2012, 2014). Hence, these products' cultivation level has been considered in development programs of Iran (Saboki et al. 2012; FAO 2021).

Mango trees are affected by many pathogens causing various diseases that impact all parts of the tree and, therefore, reduce fruit quality and quantity (Prakash 2003). Furthermore, leaf spot diseases are among the important foliar diseases of tropical fruit trees, causing economic crop loss in these hosts (Okigbo and Osuinde 2003). Therefore, the precise identification of plant pathogenic fungi is a key step in studying epidemiology and, consequently, of the greatest importance for developing effective control strategies for plant diseases (Rajeshkumar et al. 2016; Zheng et al. 2020).

Beltrania is described by *B. rhombica*, from *Citrus limonum* (Penzig 1882), that has setae with radially lobed basal cells, conidiophores with dividing cells, and conidia with a hyaline transverse band and apical tubular appendage (Wang et al. 2017). Twenty-five epithets are confirmed for *Beltrania* (http://www.indexfungorum.org). The genus *Bartalinia* is established for *Bartalinia robillardoides* (Tassi 1900). The genus has colored, and erected setae, conidiophores rise to conidiogenous cells sympodially using short protruding denticles. Conidia are brown and bionic with an equatorial band of paler pigment and a single apical appendage (Seifert et al. 2011). Many hosts have recorded the genus effect on plant-causing leaf spots (Farr and Rossman 2017; Wijayawardene et al. 2017). Seventeen validated

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species for the genus are accepted, but the sexual morph of the genus is not recorded yet (Maharachchikumbura et al. 2016; Wijayawardene et al. 2017).

There are some sporadic reports of fungal species from tropical fruit crops in Iran (Ershad, 2022). The present study is a part of a more comprehensive study about fungi associated with leaf spot symptoms on tropical crops in the south of Iran that we described two new species of the genera *Bartalinia* and *Beltrania* for Iranian Funga.

MATERIALS AND METHODS

Sampling and fungal isolation

During a conducted survey on tropical and subtropical fruit trees in the summer season of 2021, seventy-five samples were collected from mango (*M. indica*) plants showing leaf spot symptoms. Infected leaf samples were collected from Hormozgan (Siaho district), Sistan and Baluchestan (Nikshahr, Ghasreghand, Rask, and Konarak districts) provinces located in the South and Southeast of Iran. Infected samples were transferred to the laboratory and kept in a refrigerator at 4°C in dry condition. For isolation, infected tissues were cut into 7-8 mm pieces, surface-disinfested using 2% sodium hypochlorite solution for 3 min, washed twice with sterile distilled water, dehydrated, and then transferred on 2% water-agar (2% WA) and wet filter paper at 25 °C. After 48 hours, conidia were grown on leaf pieces and were transferred to 2% WA. Hyphal tips produced from single conidia were moved to a potato dextrose agar (PDA) medium to obtain pure cultures (Pordel et al. 2015).

Identification of the fungal isolates

Morphological examination

The pure isolates were cultures on PDA and malt extract agar (MEA) and incubated at 25 °C for seven days. Colony morphology and microscopic characteristics were examined, measured, and photographed using seven days old cultures. Colony color was rated using a mycological color chart (Rayner 1970). Then, morphological identification was done by Zheng et al. 2020 and Liu et al. 2019 descriptions. Finally, the pure cultures were kept in the Herbarium of Mycological Laboratory of the University of Jiroft, Kerman (UJFM).

Phylogenetics analyses

Seven isolates were selected for DNA extraction using the protocol of Cenis, 1972. The following PCR program was used to amplify the large subunit of the ribosomal RNA gene (LSU) and internal transcribed spacer (ITS-rDNA) regions: initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 50 s, 72 °C for 1 min, and finally 10 min at 72 °C (Lin et al. 2017). The obtained sequences from amplifying a part of the genomic region were compared with the reference sequences in the GenBank using the BLASTn search tool.

The obtained sequences were edited by CLC Genomic Workbench 10.1 software and separately aligned with MUSCLE in Mega 6.10 software along with references genes (Edgar 2004; Tamura et al. 2011). Phylogenetic analyses for *B. pini* and *Be. rhombica* determined the most suitable substitution models in PhyML 3.0 (Guindon et al. 2010). Maximum likelihood (ML) trees were computed in MEGA v. 6.10 (Tamura et al. 2011).

RESULTS

Sampling and isolation

Seventy-five samples were collected from different sites in Hormozgan, and Sistan and Baluchestan provinces. Seven isolates that represent the morphological characteristics of *artalinia* and *Beltrania* were selected for further actions and phylogenetic studies. These isolates were obtained from mango trees that showed leaf spot symptoms.

Identification of fungal isolates obtained from symptomatic mango trees

Phylogenetics analyses

The *Bartalinia* sequence contains LSU (800 bp) and ITS (510 bp) sequence data for 17 taxa and one outgroup taxon which 290 are parsimony informative, 112 are parsimony-uninformative, and 1110 characters were aligned. The maximum likelihood (ML) analysis, based on combined LSU and ITS sequence data, resulted in the isolates (UJF59M1, UJF59M2, and UJF59M3) grouped with *B. pini* (strains CBS 143819) (Fig. 1; Table 1). The BLAST analysis of the LSU and ITS gene regions showed 100% (LSU: CPC 24328) and 99/97 (ITS: CBS143891) similarity with ex-type sequences.

The phylogenetic placement of *Beltrania rhombica* isolates, within other Beltraniaceae, was assessed by combining the LSU and ITS sequences. In these analyses (character set LSU: 1–801, *ITS1*: 802–1290) 32 sequences including four new ones generated in this study (Table 1) and 38 taxa of the type or ex-type strain, from GenBank (Wang et al. 2017), were used. The phylogenetic tree showed our isolates (UJFM26AA1, UJFM26AB2, UJFM26C, and UJFM26AB) lay in a clade with the *Beltrania rhombica* CBS: 12358 (Fig. 2). Furthermore, the BLAST analysis of the LSU and ITS gene regions showed 100 % similarity with ex-type sequence (LSU: CBS12358, ITS: CBS12250).

Morphological criteria

Identifying *Bartalinia* and *Beltrania* is traditionally based on morphological characters mentioned in

Zheng et al. 2020 and Liu et al. 2019. Here, we described and illustrated the *Bartalinia pini* and *Beltrania rhombica* as new species for the Funga of Iran.

Bartalinia pini F. Liu, L. Cai & Crous, in Liu, Bonthond, Groenewald, Cai & Crous, Stud. Mycol. 92: 309 (2018)

Colonies on PDA cream with yellowish merge, covered with dense aerial mycelium and attaining a diam of 7 cm after a week in the dark at 25° C (Fig. 3a). Mycelium immersed and superficial. Conidiomata acervular, stromatic, single or aggregated, glabrous (Fig. 3b). Conidiogenous cells phialidic, ampulliform, hyaline, septate, 4 –10 (–11) × 1–2.5 µm. Conidia (14–) 15 – 19 × (1–) 1.5 – 3 µm, cylindrical to allantoid, hyaline, becoming pale brown at maturity, guttulate, mostly 4-septate, occasionally 3-septa, constricted at septa; with basal cell obconic, slightly truncate at the base and appendaged; 2 median cells subcylindrical, apical and basal cells each with a single, simple, unbranched, filamentous appendage at the ends (Fig. 3,c).

Specimen examined: Iran, Hormozgan province, Siaho city, on leaves of mango trees, 6 Aug. 2021, Kowsar Dehghani.

Note— *Bartalinia pini* is morphologically similar to *B. lateripes, B. pondoensis,* and *B. rosicola* in having 4-septate conidia, but its conidia are thinner and shorter than *B. lateripes, B. pondoensis,* and *B. rosicola* (Nag Raj 1993, Arzanlou et al. 2012, Wanasinghe et al. 2018). *B. bella* are pretty diverse in conidia shape with 3 septa and size (Marincowitz et al. 2010). This species is reported for the first time from Iran.

Beltrania rhombica Penz., Michelia 2(8): 474. 1882. Fig. 1

Colony on PDA in center greyish and margin white and reaching up to 7.5 cm diam. after a week in dark at 25° C. Conidiophores arising in groups from the terminal cells of the immersed mycelium, erect, ascending, straight or flexuous, unbranched, thick-walled, 4-septate, mid-brown to dark brown, geniculate, conidial scars visible after the secession of the conidia, 1–1.5 (–2) µm (Fig. 4, c and d). Setae are visible. Conidiogenous cells polyblastic, integrated, terminal, determinate, and cylindrical, 8–22 (–23) × (4–) 4.5–6 µm. Conidia solitary, dry, acrogenous or acropleurogenus, simple, biconic, smooth, slightly truncate at the base, with a rostrum, subhyaline to light brown, with a hyaline median pale transverse band, 21 – 26 (–27) × 8 – 10 (–11) µm, rostrum 4.5–7 µm long (Fig. 4,e).

Specimen examined: Iran, Sistan and Baluchestan Province, Nikshahr city, on leaves of mango trees, 2 Nov. 2021, Adel Pordel.

Note—*Beltrania rhombica* is easily recognizable by its rostrum conidia. The specimen fits well with the original description of *Be. rhombica* by Penzig (1882). *Beltrania* species, especially the type species (*Be. rhombica*), have mainly been isolated from decaying plant materials, indicating a possible saprobic lifestyle (Ellis 1971, 1976; Seifert 2011; Zheng et al. 2020). This species differs from *B. pseudorhombica* in shorter setae and more elongated conidia (Crous et al. 2014). The present data show the genus *Belterania* and *Be. rhombica* are both new report taxa for the Iranian Funga.

Table 1. Species name, strain code, source, host, and GenBank accession numbers of the strains studied

Species	Culture collection	Source	Hosts	LSU	ITS
Bartalinia pini	UJF59M1	Hormozgan, Iran	*Mangifera indica*	OP642483	OP630882
Bartalinia pini	UJF59M2	Hormozgan, Iran	*Mangifera indica*	OP642484	OP630883
Bartalinia pini	UJF59M3	Hormozgan, Iran	*Mangifera indica*	OP642485	OP630884
Beltrania rhombica	UJFM26AA1	Sistan and Baluchestan, Iran	*Mangifera indica.*	OP630848	OP630833
Beltrania rhombica	UJFM26AB2	Sistan and Baluchestan, Iran	*Mangifera indica.*	OP630849	OP630834
Beltrania rhombica	UJFM26C	Sistan and Baluchestan, Iran	*Mangifera indica*	OP630850	OP630835
Beltrania rhombica	UJFM26AB	Sistan and Baluchestan, Iran	*Mangifera indica*	OP630851	OP630836

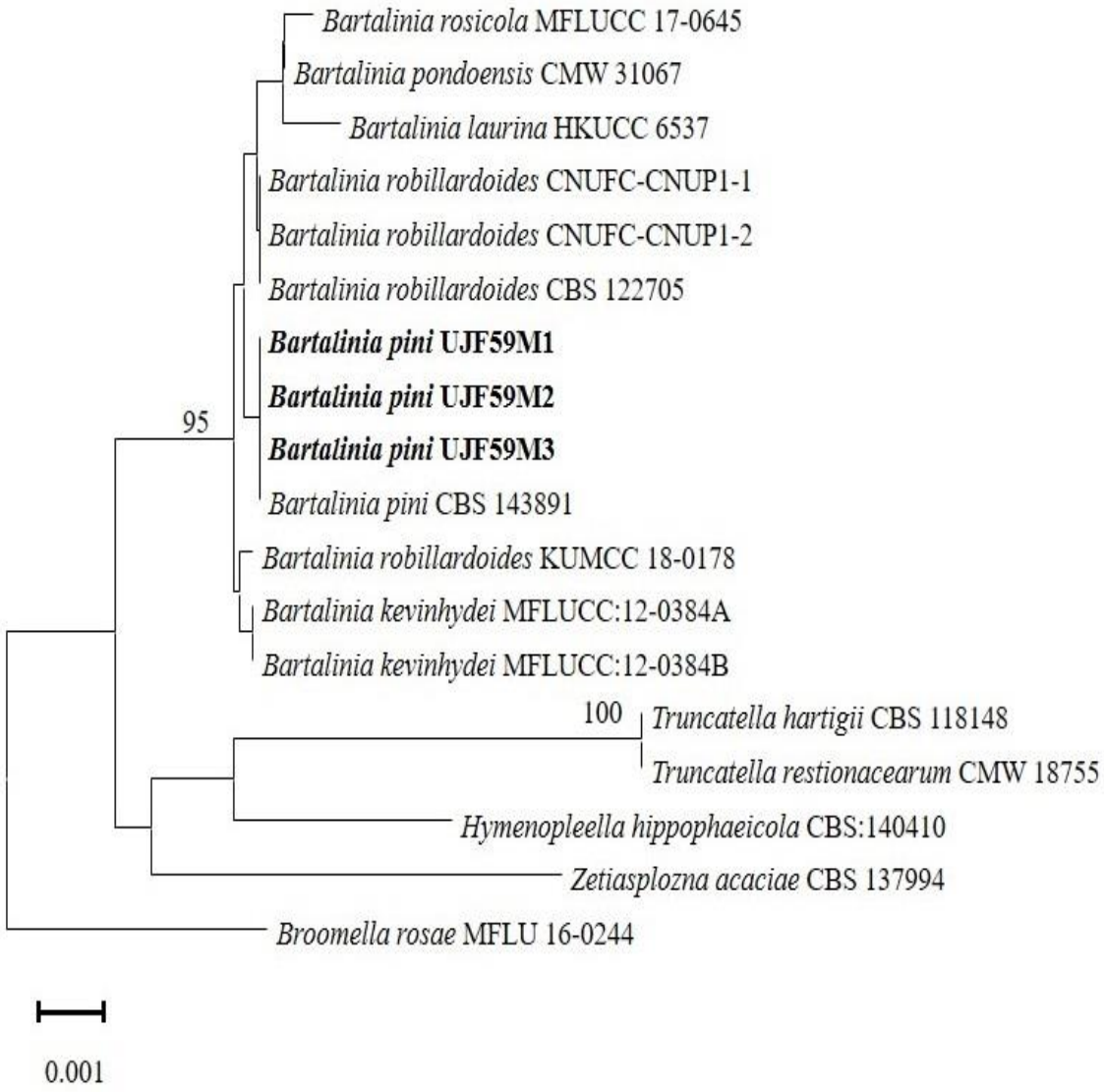

Fig. 1 Phylogenetic analysis of combined LSU and ITS gene regions of *Bartalinia pini* isolates by Maximum likelihood methods.

DISCUSSION

In a survey of the leaf spots on tropical trees in the south and southeast of Iran, we collected seventy-five samples (three isolates belonging to *Bartalinia pini* and four of *Beltrania rhombica*) with leaf spots. *Mangifera indica* is commercially one of the most important trees in the south and east south of Iran. Many fungi could cause leaf spots on mango trees as part of symptoms, including *Colletotrichum gloeosporioides* (Anthracnose), *Pseudoidium anacardii* (Powdery mildew), *Elsinoe mangiferae* (Mango Scab), *Verticillium dahlia* (Verticillium Wilt), *Fusarium oxysporum*, *Phoma mangiferae*, and *Aureobasidium melanogenum* (Okigbo and Ikediugwu 2001; Zainab and Shinkaf 2016; Shinde 2020).

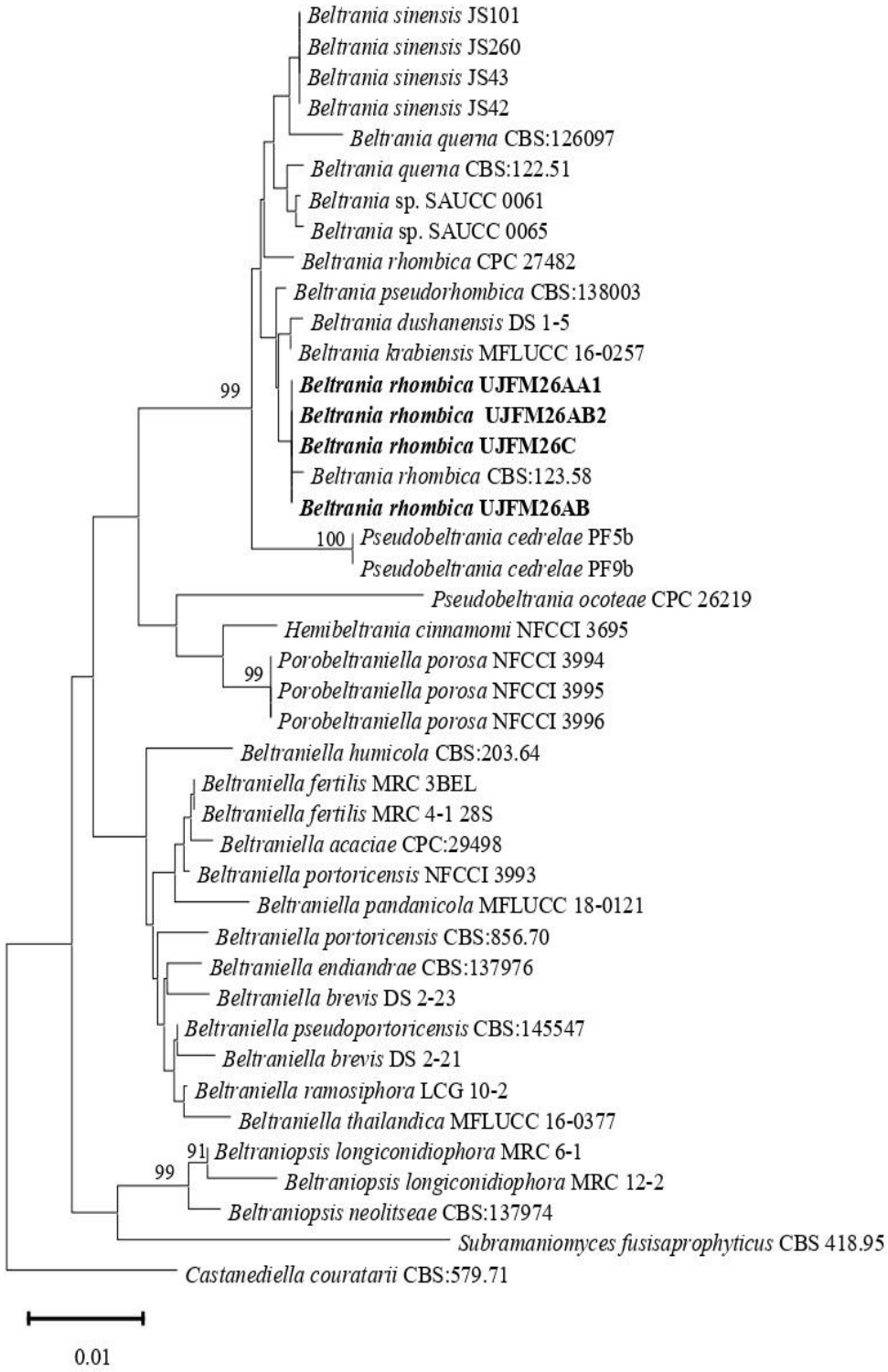

Fig. 2 Phylogenetic tree constructed with combined LSU and ITS gene regions of *Beltrania rhombica* isolates by Maximum likelihood methods.

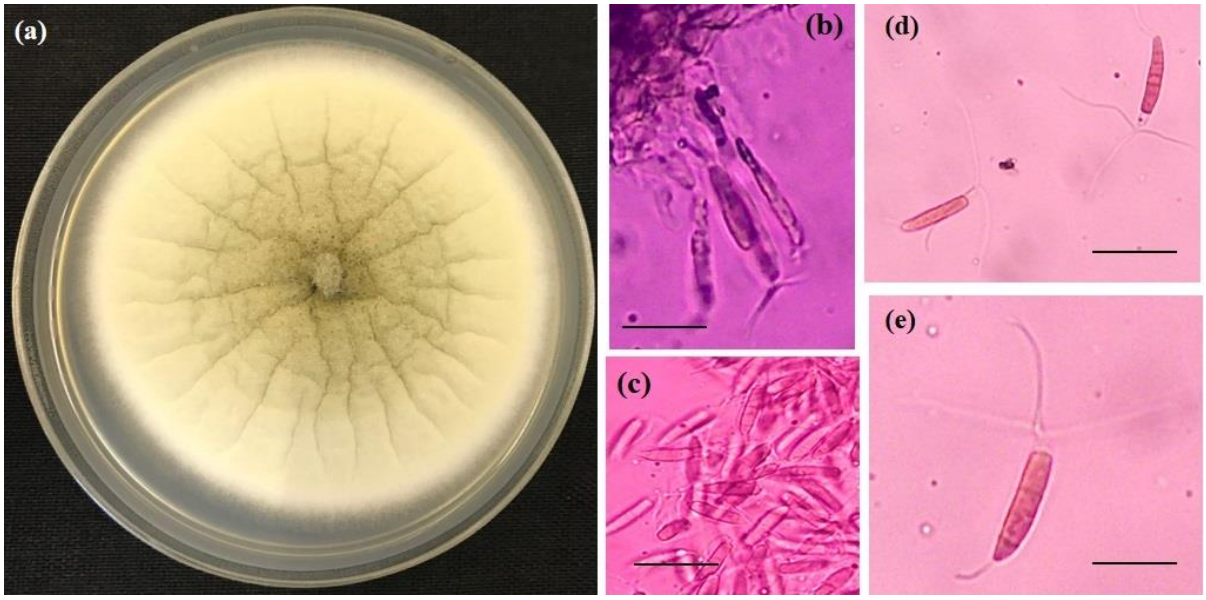

Fig. 3 *Bartalinia pini*. (a) Colony on PDA. (b) Conidiogenous cells. (c-e) Conidia. – Scale bars = 10 µm.

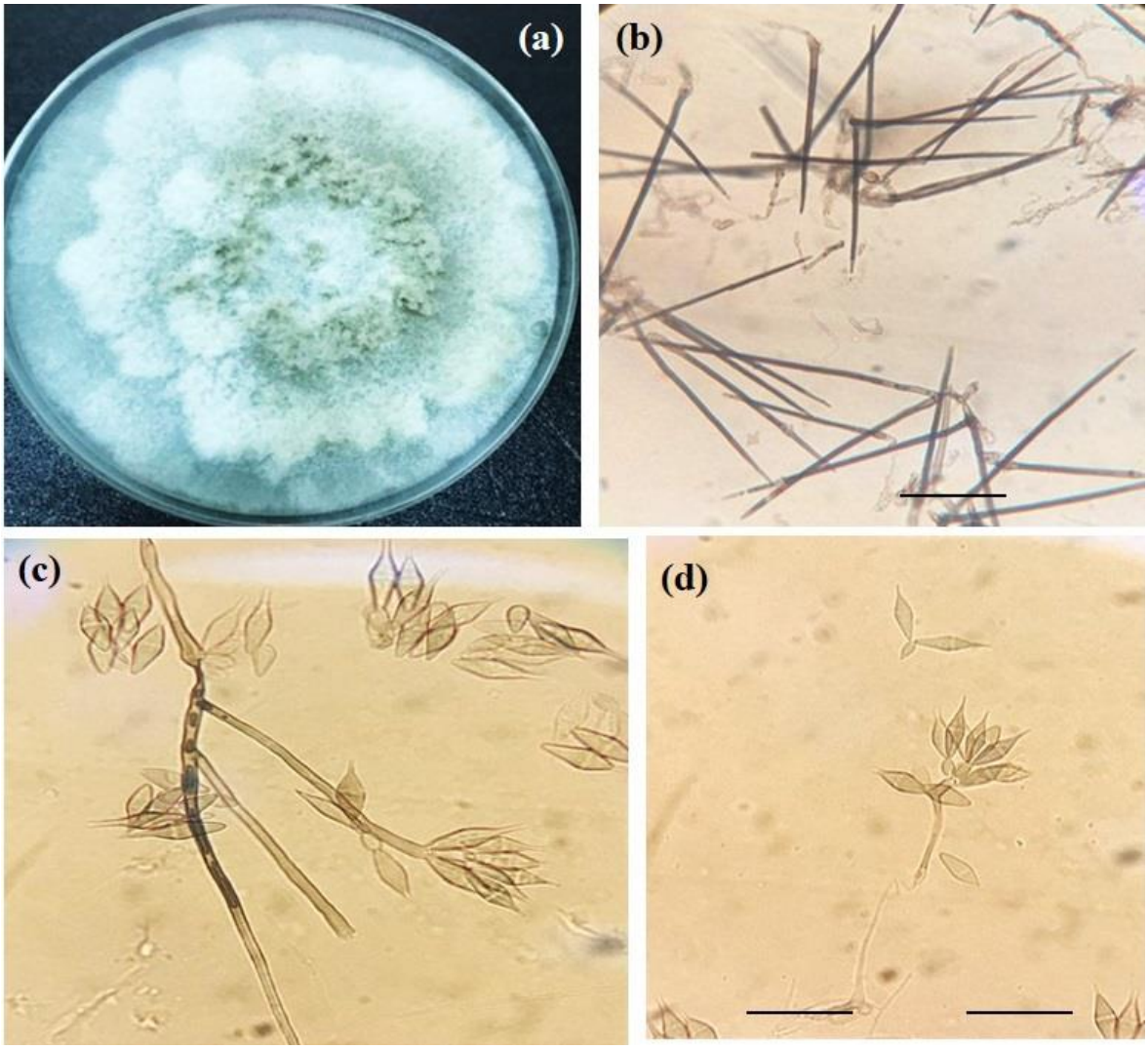

Fig. 4 *Beltrania rhombica*, (a) Colony on PDA. Setae (b) Conidiophores. (c) Conidia. Scale bars = 10 µm

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In this paper, we described and illustrated *Bartalinia pini* and *Beltrania rhombica* as new species for the Funga of Iran by morphological and molecular data. Our isolates were entirely similar to the description provided by Marincowitz et al. (2010) (*B. pini*) and Penzig (1882) (*Be. rhombica*). Furthermore, the nrLSU and ITS rDNA sequences showed these species separated from their closed species. Moreover, these species can produce leaf spot symptoms in *Mangifera indica* (data unpublished).

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قارچهای همراه با علایم لکه برگی درختان انبه در ایران

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۲-بخش تحقیقات گیاه‌پزشکی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی بلوچستان (ایرانشهر)، سازمان تحقیقات، آموزش و ترویج کشاورزی، ایرانشهر، ایران

چکیده: طی بازدید از درختان گرمسیری مناطق مختلف استانهای هرمزگان و سیستان و بلوچستان، از برگهای درختان انبه دارای علایم لکه‌برگی نمونه‌برداری صورت پذیرفت. جداسازی جدایه‌ها روی محیط کشت PDA و کاغذ مرطوب در دمای ۲۵ درجه سلسیوس انجام شد. در نتیجه سه جدایه متعلق به جنس *Bartalinia* و چهار جدایه از جنس *Beltrania* به دست آمد. تجزیه و تحلیل فیلوژنتیکی داده‌های به دست آمده از توالی‌یابی بخشی از نواحی ژنومی ITS-rDNA و LSU و صفات ریخت‌شناسی، نشان داد این جدایه ها متعلق به گونه‌های *Bartalinia pini* و *Beltrania rhombica* می‌باشند. بر اساس اطلاعات ما، هر دو گونه *B. pini* و *Be. rhombica* گزارش‌های جدیدی برای قارچ‌های ایران هستند.

کلمات کلیدی: *Bartalinia pini*، *Beltrania rhombica*، انبه (*Mangifera indica*)، تجزیه و تحلیل چند ژنی، لکه‌برگی

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