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Blumeriella jaapii, a new fungal species on sour cherry in Guilan Province, Iran

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Abstract: Sour cherry trees in Guilan Province are infected by at least two fungi that cause leaf spots and shot holes namely *Wilsonomyces carpophilus* and *Pruniphilomyces circumscissus*. In the late summer and early autumn of 2023, a leaf spot in the form of small purple-red to brown lesions about 1-3 mm in size was seen on the sour cherry trees in Sumaehsara County, Guilan Province, Iran. Mature conidiomata, acervuli, were formed frequently on the abaxial leaf surface at the center of each lesion, producing asexual conidia in white masses. Morphological and molecular studies showed that *Blumeriella jaapii* is associated with the sour cherry leaf spot disease. This is the first report on this fungus and the first attempt to sequence the ITS-nrDNA as the primary barcode of *Blumeriella jaapii* in Iran.

Keywords: *Ascomycota*, Cherry foliar disease, *Cylindrosporium*, DNA-barcoding, *Drepanopezizaceae*.

INTRODUCTION

Cherries (*Prunus cerasus* L. and *Prunus avium* L.) are affected by several important pathogenic fungi, some of which cause important leaf spot diseases. When the cherry cultivar is susceptible and environmental conditions favor the disease, the leaves with numerous spots turn yellow and fall off the tree, causing poor fruit size and quality, reducing winter hardiness and tree decline and death (Holb 2009, Andersen et al. 2018). Some of these leaf spots are major diseases of cherries in North America, Europe, and India (Valiushkaite 2002, Khan et al. 2014, 2016). Although sour and sweet cherries are cultivated sporadically in most areas of Guilan Province (except for Talesh County) and are not considered an important garden crop, they are highly valued for their beautiful blossoms, which add charm to garden houses. Common shot hole diseases caused by *Wilsonomyces carpophilus* (Lév.) Adask., J. M. Ogawa & E.E. Butler and *Pruniphilomyces circumscissus* (Sacc.) Crous & Bulgakov are among the most important and prevalent cherry leaf diseases in Guilan Province (Ershad 2009, Bakhshi et al. 2021, personal observation). At the end of the summer and autumn of 2023, a leaf spot on sour cherry trees, which was slightly different from the previously reported leaf spots was observed in Sumaehsara County, Guilan Province. In this article, we report and describe *Blumeriella jaapii* (Rehm) Arx from sour cherry leaf spot symptoms for the first time in Iran.

MATERIALS AND METHODS

Leaf samples showing leaf spot symptoms were collected from sour cherry trees in Sumaehsara County, placed in new paper bags, and transferred into the laboratory. Fungal isolation was done by transferring conidial masses onto the PDA culture medium. Morphological examinations were done directly from naturally infected leaves. Small pieces containing mature acervuli, 4–5 mm wide and 2–3 cm long, were cut, and then fine cross sections were made by hand with a new razor blade. Cross sections were mounted in 50% lactic acid on a glass slide and examined with a light microscope (Olympus BH2, Japan) equipped with a Sony digital camera. The Fiji image processing package was used to measure the dimensions of fungal structures (Schindelin et al. 2012). For this purpose, several images of fungal structures were prepared and transferred to a computer equipped with Fiji software, and measurement was conducted according to software instructions. 15-30 fungal structures were used for measurements.

For molecular studies, DNA extraction was performed using the thermolysis protocol outlined by Zhang et al. (2010). Briefly, a small amount of fungal mycelium was harvested from the fungal culture on the PDA medium and transferred into 1.5 mL tubes containing 100 µL distilled water. The tubes, vortexed for 8-10 seconds and centrifuged at 10000 g for 1 min. After removing the supernatant, 100 µL of lysis buffer (containing sodium phosphate 50 mM at pH 7.4, EDTA 1 mM, and 5% glycerol) was added to the tube and incubated at 85 °C in the water bath for 30 min. The final crude extract

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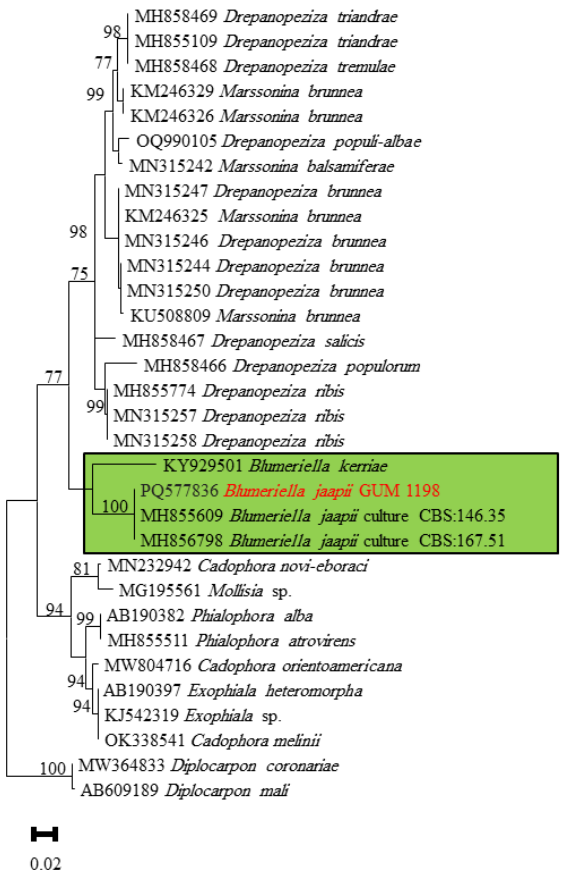

Fig. 1. A maximum likelihood tree shows the position of *Blumeriella jaapii* along with 31 taxa from *Drepanopeziza*ceae (*Helotiales, Ascomycota*). The tree with the highest log likelihood of -1631.69 is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers near the branches show the bootstrap support values (values below 70% were not shown).

containing DNA was stored at -20 °C for further use. Internal transcribed spacer regions of nuclear ribosomal DNA (ITS- nrDNA) were amplified using the ITS1 (5'–CTTGGT ATTTAGAGGAAGTAA–3') and ITS4 (5'–TCCTCCGCTTATTGATATGC–3') primers (White et al. 1990). The amplification was performed in a total volume of 30 µL. PCR mixture contained 15 µL of master mix (Pishgam Biotech, Iran) (including 10×PCR buffer, $MgCl_2$, dNTPs, Taq DNA Polymerase), 10.6 µL of double-distilled water, 1µL of each primer (10 µM), and 2.4 µL of DNA solution. PCR was run on an Eppendorf Thermal Cycler (Eppendorf Personal, Darmstadt, Germany) under the following conditions: an initial denaturation cycle at 94 ºC for 2 min, followed by 30 cycles of 94 ºC for 30 s, 52 ºC for 30 s, and 72 ºC for 30 s and a final extension cycle at 72 ºC for 5 min. PCR products were visualized on a 1% agarose gel under UV light using UVITEC Gel Documentation Systems (UVITEC, UK). Finally, the sequencing was carried out by Codon Genetic Group (Tehran, Iran). MEGA 11 software (Tamura et al. 2021) was used to make alignment. To do this we aligned our sequence along with 31 GenBank's hits to create a data matrix including 32 taxa of which *Diplocarpon coronariae* (MW364833) and *D. mali* (AB609189) were used as out-group taxa. The phylogenetic tree was inferred by the Maximum Likelihood method using MEGA11 (Tamura et al. 2021). The Kimura 2-parameter model was used as a substitution model. The percentage of trees in which the associated taxa clustered together was obtained by running a bootstrap analysis of 1000 replicates. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites. All positions with less than 95% site coverage were eliminated (partial deletion option). A total of 402 positions were remained in the final dataset. The new sequence generated in this study was deposited in GenBank under accession number: PQ577836.

RESULTS AND DISCUSSION

Culture of the fungi from symptomatic leaves yielded a slow-growing whitish colony that produced a few filiform, two-celled conidia. Amplification of the ITS-nrDNA region in the selected isolate resulted in a fragment about 560 bp long. BLASTN search in the GenBank database resulted in two hits of *B. jaapii* (CBS 146.35 and CBS 167.51) (Vu et al. 2019) that showed 100% identity with our sequence. In the phylogenetic tree (Fig. 1), our sequence was clustered well in the *Blumeriella* clade, and formed a distinct lineage with the representative strains of *B. jaapii*, with 100% bootstrap support. Based on the morphological characteristics and phylogenetic results, the studied isolate was identified as *B. jaapii*.

***Blumeriella jaapii* (Rehm) Arx, Phytopath. Z. 42(2): 164 (1961)**

Synonyms: *Pseudopeziza jaapii* Rehm, Annls mycol. 5(6): 465 (1907); *Higginsia jaapii* (Rehm) Nannf., Nova Acta R. Soc. Scient. upsal., Ser. 4 8(no. 2): 175 (1932); *Ascochyta padi* Lib., Pl. crypt. Arduenna, Fasc. (Liège) 2 (nos 101-200): no. 153 (1832); *Septoria padi* (Lib.) Thüm., Fungi austr. exsicc. 11-13: no. 1186 (1874); *Phloeosporella padi* (Lib.) Arx, Phytopath. Z. 42(2): 163 (1961); *Cylindrosporium padi* P. Karst., Meddn Soc. Fauna Flora fenn. 11: 159 (1884); *Phlyctema padi* (P. Karst.) Petr., Annls

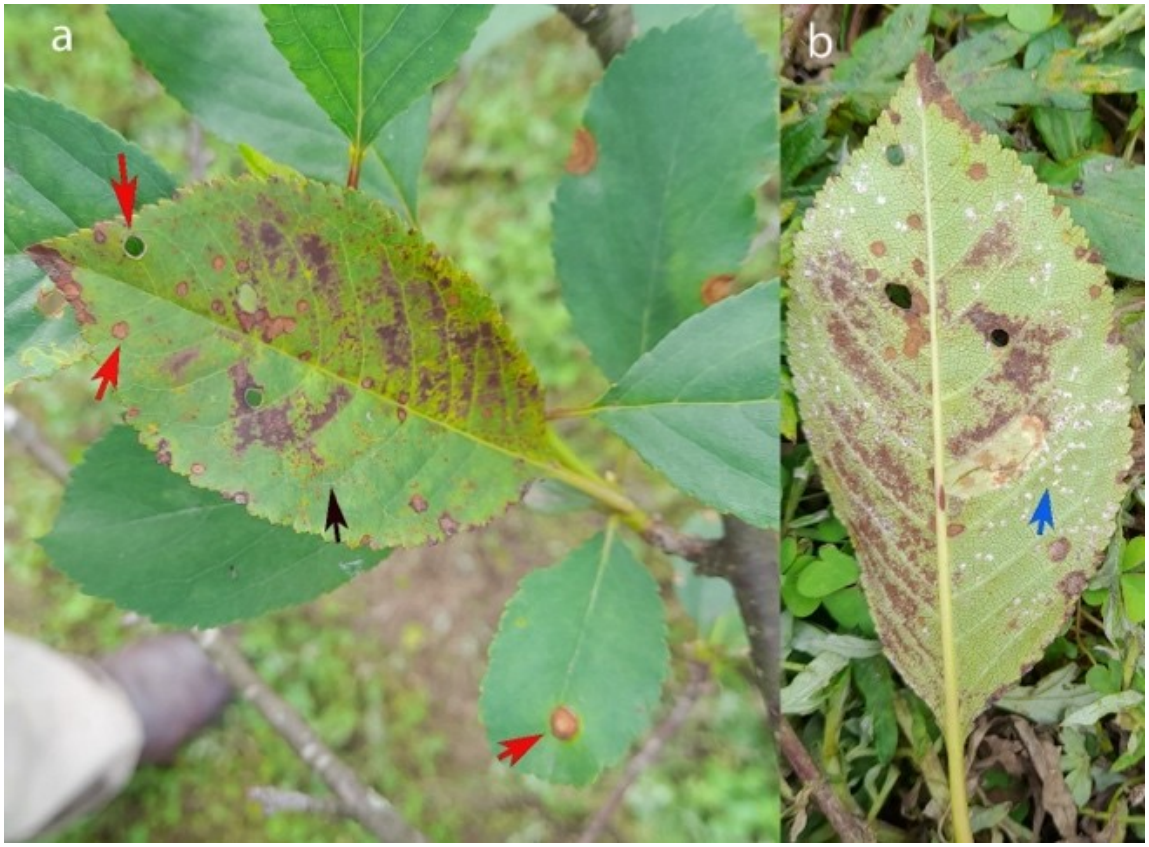

Fig. 2. *Blumeriella jaapii*: (a) symptoms on leaves (upper side, black arrow) (b), acervuli on the abaxial leaf surface, seen as whitish masses of conidia (blue arrow). Symptoms caused by *Pruniphilomyces circumscissus* (as mixed infections) can be seen on these leaves as larger necrotic and shot hole spots (red arrow).

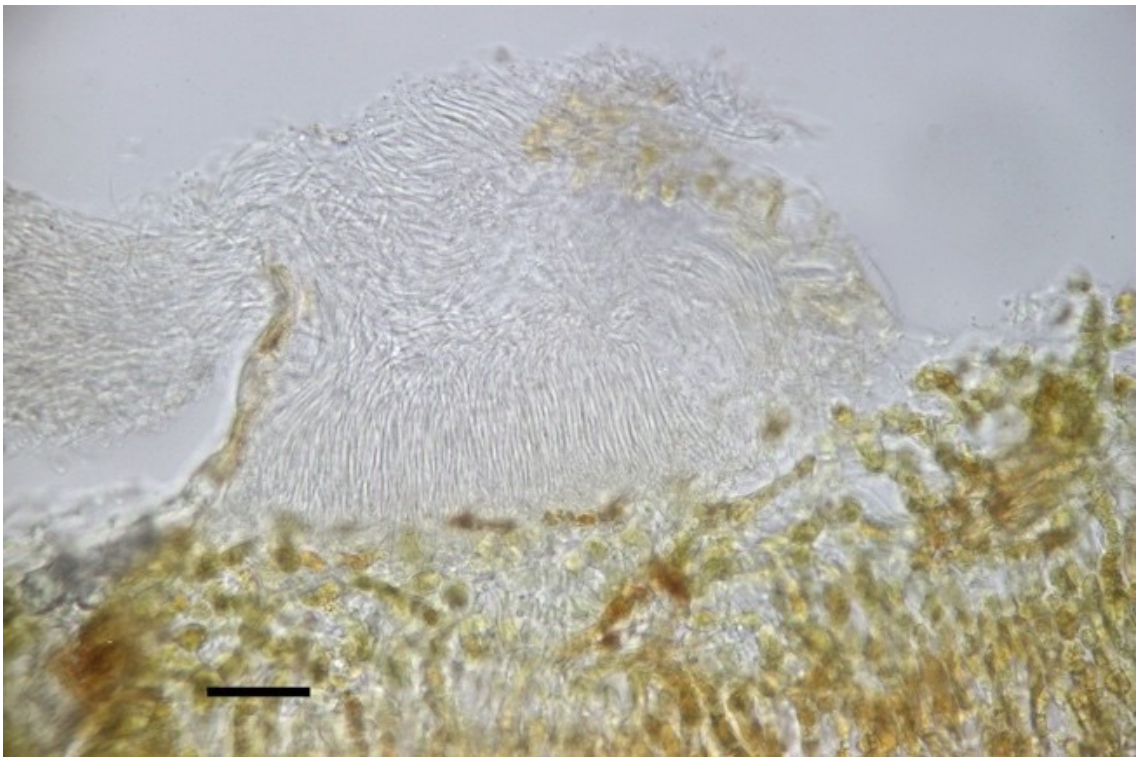

Fig. 3. *Blumeriella jaapii*: Cross-section from an acervulus, scale bar = 20 μm.

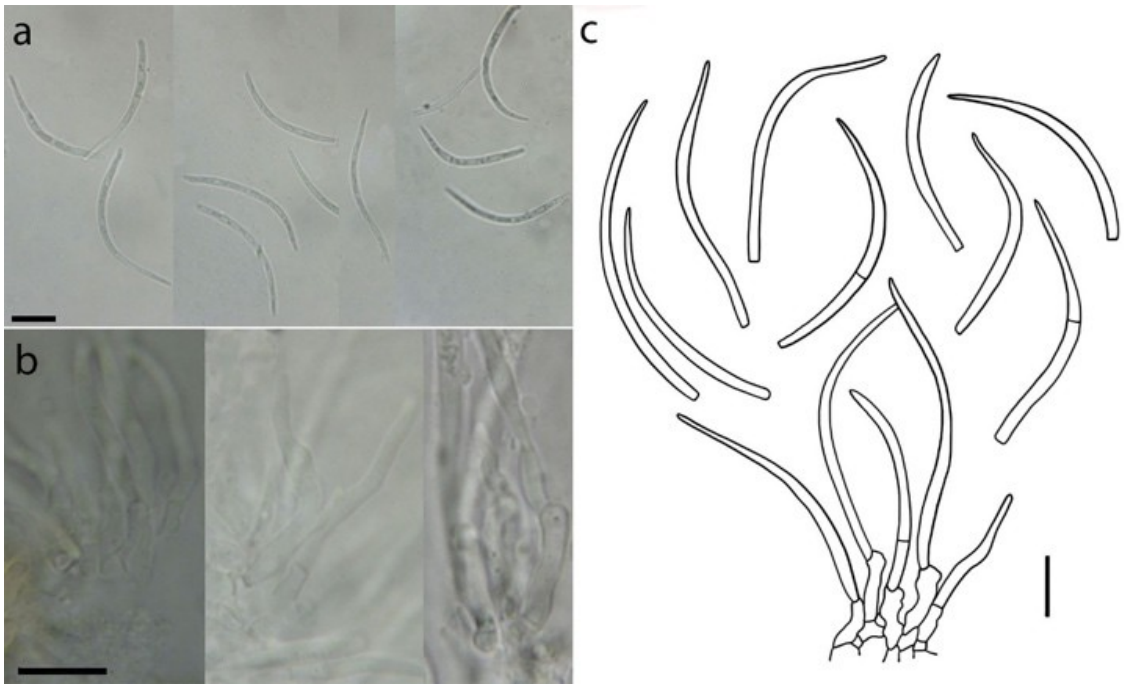

Fig. 4. *Blumeriella jaapii*: a. conidia, b. conidiogenous cells, c. A drawing from conidiogenous cells and conidia, scale bar a = 20 and b, c = 10 µm.

mycol. 17(2/6): 72 (1920); *Coccomyces hiemalis* B.B. Higgins, Science, N.Y. 37: 638 (1913); *Higginsia hiemalis* (B.B. Higgins) Nannf., Nova Acta R. Soc. Scient. upsal., Ser. 4 8(no. 2): 174 (1932); *Blumeriella hiemalis* (B.B. Higgins) K. Põldmaa, Fitopatolennye Mikromitsety Severnoi Estonil (Phytopathogem c Micromycetes of North Estonia) (Tallin): 227 (1967); *Phloeosporella hiemalis* (B.B. Higgins) K. Põldmaa, Fitopatolennye Mikromitsety Severnoi Estonil (Phytopathogem c Micromycetes of North Estonia) (Tallin): 227 (1967); *Coccomyces lutescens* B.B. Higgins, Am. J. Bot. 1(4): 166 (1914); *Higginsia lutescens* (B.B. Higgins) Nannf., Nova Acta R. Soc. Scient. upsal., Ser. 4 8(no. 2): 174 (1932); *Cylindrosporium hiemalis* B.B. Higgins, Am. J. Bot. 1(4): 164 (1914); *Coccomyces prunophorae* B.B. Higgins [as 'prunophora'], Am. J. Bot. 1(4): 165 (1914); *Higginsia prunophorae* (B.B. Higgins) Nannf., Nova Acta R. Soc. Scient. upsal., Ser. 48(no. 2): 174 (1932); *Blumeriella prunophorae* (B.B. Higgins) Becer., Simeria & Creţiou, Analele Institutului de Cercetări pentru Protecţia Plantelor 17: 82 (1983).

Disease symptoms appeared as irregular purple-red to brown spots on the upper leaf surface, 1–3 mm diameter, sometimes turning yellow around the spots (Fig. 2). The asexual fruiting bodies, acervuli, formed frequently on the abaxial leaf surface, containing large masses of whitish conidia at the center. Acervuli up to 350 µm diam. and 240 µm in height; conidiogenous cells sympodial, hyaline, cylindrical to irregular, 5–15 × 1.5–2.5 µm; conidia elongated, hyaline, two-celled, strongly to slightly curved, flexuous, rounded at the base and tapered toward the apex, 48–77 µm length, 2.5–3.5 µm at the widest part and 1–1.5 (–2) µm near the apex (Figs. 3, 4).

Specimen examined: Iran, Guilan Province, Sumaehsara, on *Prunus cerasus* L., 1 Nov. 2023; S.A. Khodaparast (GUM 1198).

Our investigations showed that an atypical sour cherry leaf spot in the Guilan Province was caused by *B. jaapii*, a fungus in the family *Drepanopezizaceae* (*Helotiales, Ascomycota*), which differs from the common cherry leaf spots reported previously in this region. *B. jaapii* was first introduced as *Cylindrosporium padi* P. Karst, on *Prunus padi* from Mustiala (Finland) based on anamorphic state (Karsten 1884). For many years, *Cylindrosporium padi* has been regarded as the asexual state for this fungus (Sutton 1980). According to Rossman & Johnston (2016), *Pseudopeziza jaapii* Rehm is the basionym for the fungal agent of this cherry disease. After one fungus and one name rule, *B. jaapii* was proposed as a conserved name against *Cylindrosporium padi* (Rossman & Johnston 2016). During the last century, *B. jaapii* has been reported from almost all over the world such as Australia, Bulgaria, Canada, Greece, India, Italy, Slovakia, China, Lithuania, Mexico, Scotland, South Africa, Turkey, and the USA (Farr et al. 2021, Khan et al. 2014, 2016, Tezean 2008, Valiushkaite 2002). Many researchers investigated different aspects of the fungus and the disease it causes including fungal pathogenicity, its life cycle, host resistance, and disease management (Holb 2009, Andersen et al. 2018, McManus 2007, Schuster 2004, Vu 2019). Recently, Peng et al. (2020) have provided

the complete genome sequence of *B. jaapii* (strain 11BO-GW45). They hope that the information obtained from the whole genome sequencing will help to characterize the resistance mechanisms of this fungus against fungicides and expand our knowledge of the phytopathogenic fungi in the family *Drepanopezizaceae*.

Blumeriella is a small genus in *Drepanopezizaceae* and contains five species (April 2024; https://www.speciesfungorum.org). There are three sequences named *Blumeriella* available in the GenBank database; two of these, published by Vu et al. (2019), belong to *B. jaapii*. Although there have been numerous reports of this fungus worldwide, this species has not yet been documented in Iran. This study marks the first record of the species in Iran and the first effort to obtain ITS-nrDNA sequences as the primary DNA barcode.

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Blumeriella jaapii یک گونه قارچی جدید روی درختان آلبالو در استان گیلان، ایران

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چکیده: در استان گیلان حداقل دو قارچ به نام‌های *Wilsonomyces carpophilus* و*Pruniphilomyces circumscissus* روی درختان آلبالو لکه برگی و غربالی ایجاد می کنند. در اواخر تابستان و اوایل پاییز سال ۱۴۰۲ لکه‌هایی روی برگ به شکل ضایعات کوچک ارغوانی مایل به قرمز تا قهوه‌ای به اندازه ۱ تا ۳ میلی‌متر بر روی درختان آلبالو در شهرستان صومعه سرا، استان گیلان مشاهده شد. اندامهای بارده بالغ به شکل آسروول، به فراوانی روی سطح پایینی برگ تشکیل شده و کنیدیوم های فراوان به صورت یک توده سفیدرنگ تولیده کرده بودند. مطالعات مورفولوژیکی و مولکولی نشان داد که *Blumeriella jaapii* با این بیماری روی برگ درختان آلبالو همراه است. این قارچ یک بیمارگر شناخته شده روی درختان گیلاس و آلبالو در دنیا است. این اولین گزارش در مورد این قارچ و اولین تلاش برای تعیین توالی ITS-nrDNA به عنوان بارکد اولیه *Blumeriella jaapii* در ایران است.

کلمات کلیدی: آسکومیکوتا، بیماری برگی آلبالو، خط شناسه *Drepanopezizaceae*، *Cylindrosporium*، DNA

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