

***Pustula lactucae* sp. nov. (Albuginales, Oomycota),
a new species causing white blister rust disease
on *Lactuca undulata***

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During 2016 and 2018, *Lactuca undulata* plants showing symptoms typical of white blister rust were collected in the province of South Khorasan, Iran. Phylogenetic analysis resolved the position of specimens in the genus *Pustula*. The specimens were characterised by oospore size and ornamentation, and size of secondary sporangia. In addition to morphology, phylogenetic analysis also suggests a new species for the genus distinct from other established *Pustula* species, which is described here as *Pustula lactucae* sp. nov. Scanning electron microscope illustrations are also provided.

Key words: Asteridae, obligate biotroph, *Peronosporomycetes*, SEM, semiarid region.

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V letech 2016 a 2018 byly v íránské provincii Jižní Chorásán sbírány rostliny *Lactuca undulata*, vykazující typické symptomy napadení bílou rzí. Z fylogenetické analýzy vyšlo zařazení sebraných položek do rodu *Pustula*. Jsou charakteristické velikostí a ornamentikou oospor, jakož i velikostí sekundárních sporangií. Vedle odlišnosti v morfologických znacích bylo i fylogenetickou analýzou potvrzeno, že jde o nový druh, odlišný od dosud známých druhů rodu *Pustula*, který je zde popsán jako *Pustula lactucae* sp. nov. Znaky nového taxonu jsou ilustrovány i snímky ze skenovacího elektronového mikroskopu.

INTRODUCTION

The family *Asteraceae* contains 10% of all flowering plant species with over 25,000 species and 1600 genera worldwide (Mandel et al. 2019, Rewicz et al. 2020). The desert plant *Lactuca undulata* Ledeb. (*Asteraceae*) is an annual herb belonging to the Irano-Turanian element with occurrence in Southwest and Central Asia to western China (Lebeda et al. 2004, Bano et Qaiser 2011).

Members of the genus *Pustula* (*Albuginaceae*) parasitise members of *Araliaceae*, *Asteraceae*, *Convolvulaceae*, *Gentianaceae* and *Goodeniaceae* (Ploch et al. 2011, Xu et al. 2018, Mirzaee et al. 2021).

Globally, several species of the genus *Pustula*, including *P. brasiliensis*, *P. cancriniae*, *P. centaurii*, *P. helianthicola*, *P. hydrocotyles*, *P. junggarensis*, *P. obtusata*, *P. spinulosa*, *P. swertiae*, *P. xinyuanensis* plus the recently described species *P. persica* from Iran, have been described thus far (Choi et al. 2012, Xu et al. 2016, Xu et al. 2018, Mirzaee et al. 2021).

There is one report of the occurrence of an *Albuginaceae* species (as *Albugo* sp.) on a member of the genus *Lactuca* from the USA in 1960 (Farr et Rossman on-line). Therefore, the phylogeny and taxonomic status of this pathogen group on *Lactuca* spp. is still unclear and needs to be resolved.

Among plant diseases, incidence of downy mildew and powdery mildew has been reported for *L. undulata* worldwide (Farr et Rossman on-line). There had not been reports of any pathogen infecting this host from Iran thus far (Ershad 2009).

The aim of the present study was to characterise the causal agent of white blister rust pathogen in the genus *Pustula* infecting *L. undulata* obtained from a semiarid region in eastern Iran.

MATERIAL AND METHODS

Material and morphological study. During 2016 and 2018, *Lactuca undulata* plants showing symptoms typical of white blister rust were collected in South Khorasan, eastern Iran. Cross-sections of infected leaves bearing sori were prepared, examined and photographed with an Olympus DP25 digital camera installed on an Olympus BX51 light microscope at 400× magnification. Measurements were performed as mentioned by Mirzaee et al. (2013).

In the preparation for scanning electron microscopy, a VEGA/TESCAN SEM (TESCAN, Brno, Czech Republic) operating at an accelerating voltage of 15 kV was used. Dried specimens were mounted on metallic stubs with double-sided tape and coated with gold in a sputtering chamber prior to investigation as mentioned by Salimi Moghadam et al. (2015).

Voucher specimens were deposited in the herbarium of the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection (IRIPP), Tehran. Their accession numbers are listed in Tab. 1.

Tab. 1. Taxa, specimen information and GenBank accession numbers determined in this study.

Taxon	Host	Origin	Herbarium accession no.	GenBank accession no.	
				<i>cox2</i>	ITS
<i>Pustula obtusata</i> s.l.	<i>Tragopogon graminifolius</i>	Iran, Khusf, Fedeshk, leg. M.R. Mirzaee, May 2019	IRAN17923F	MZ182087	–
<i>Pustula obtusata</i> s.l.	<i>Tragopogon graminifolius</i>	Iran, Khusf, Siyujan, leg. M.R. Mirzaee, Mar 2018	IRAN18017F	MZ182088	–
<i>Pustula lactucae</i>	<i>Lactuca undulata</i>	Iran, Birjand, Mohammadih, leg. M.R. Mirzaee, Apr 2018	IRAN17919F [†]	MZ182085	MZ494190
<i>Pustula lactucae</i>	<i>Lactuca undulata</i>	Iran, Birjand, Esfahroud, leg. M.R. Mirzaee, Apr 2016	IRAN17944F	MZ182086	MZ494191

Molecular analyses. Genomic DNA was isolated from individual sori removed from infected plant tissues using the Chelex method by Walsh et al. (1991) modified by Hirata et Takamatsu (1996).

Amplification of the rDNA and *cox2* mtDNA gene regions was obtained using primer pairs DC6 and LR0 (Moncalvo et al. 1995, Cooke et al. 2000) and *cox2f/cox2r* (Hudspeth et al. 2000, Ploch et al. 2010), respectively. The PCR conditions were carried out according to Choi et al. (2007).

The amplified ITS and *cox2* mtDNA gene regions were purified and sequenced, without prior cloning of the PCR products, at the Microsynth Company (Balgach, Switzerland) with primers LR0 and *cox2r*, respectively. Sequences were visually inspected with BioEdit ver. 7.2.5 (Hall 1999) and compared with sequences of related sequences available in GenBank. The sequences for *cox2* of the four *Pustula* specimens infecting *L. undulata* and *Tragopogon graminifolius* were deposited in NCBI GenBank (Tab. 1). The ITS sequences of two specimens of *Pustula* on *L. undulata* were also deposited in GenBank but were not included in the phylogenetic analysis for reason of low availability of *Pustula* ITS sequences in GenBank.

The nucleotide sequences of this study along with retrieved sequences from datasets provided by Ploch et al. (2011), Xu et al. (2018) and Mirzaee et al. (2021) were aligned using MAFFT version 7 (mafft.cbrc.jp/alignment/server/), with G-INS-i strategy (1PAM/k = 2 scoring matrix) (Katoh et Standley 2013). All alignments were manually checked with the MEGA version 5.0 software and edited if needed. The phylogenetic tree based on *cox2* gene sequences was constructed using the minimum evolution (ME) algorithm in MEGA v.5 on the basis of the

Tamura-Nei substitution model and the partial deletion method (85% cutoff) with 1000 bootstrap replicates (Tamura et al. 2011).

Further, the TrEase webserver (Mishra et al. on-line) was applied to build a Bayesian inference through MrBayes v3.2 (Ronquist et al. 2012) with the 6 GTR substitution model, running for 1 million generations and generating posterior probability values after discarding the first 30% of the sampled trees.

To confirm the identity of plant host species, genomic DNA was extracted from leaf tissue of a *P. lactucae*-infected plant sample, as mentioned above. PCR was carried out using primer pair ITS4/ITS5 (White et al. 1990) with cycling conditions as mentioned by Vahedi-Darmiyan et al. (2017).

RESULTS AND DISCUSSION

The causal agent of white blister rust on *Lactuca undulata* exhibits characteristics considered typical of the genus *Pustula*, including prominent equatorial thickening of secondary sporangia, reticulate oospores and host preference for a member of the *Asteridae* (Thines et Spring 2005, Choi et al. 2012). The oospore ornamentation in *Pustula* on *L. undulata* is characterised by a verrucose-reticulate pattern with wart-like tubercles pattern (Fig. 2).

MOLECULAR ANALYSES

A BLAST search using the *cox2* sequences (GenBank accession numbers MZ182085 and MZ182086) belonging to the *Pustula* specimens infecting *L. undulata* showed highest similarity to described species *P. junggarensis* (GenBank acc. no. KX589299) and *P. obtusata* (GenBank acc. no. GU292167) with 96.36% and 96.18%, respectively.

Based on phylogenetic analysis using *cox2* sequences, the two specimens of *Pustula* sp. parasitic to *Lactuca undulata* are placed into a distinct clade outside any of the other lineages. This clade was composed of two *Pustula* specimens infecting *L. undulata* with maximum support in minimum evolution (ME) and Bayesian inference (BI) analyses.

Two specimens of *P. obtusata* from *Tragopogon graminifolius* included in this study constituted a clade along with *P. obtusata* on *T. pratensis* and *T. porri-folius* with strong bootstrap support in ME and maximum support in BI analysis (Fig. 1).

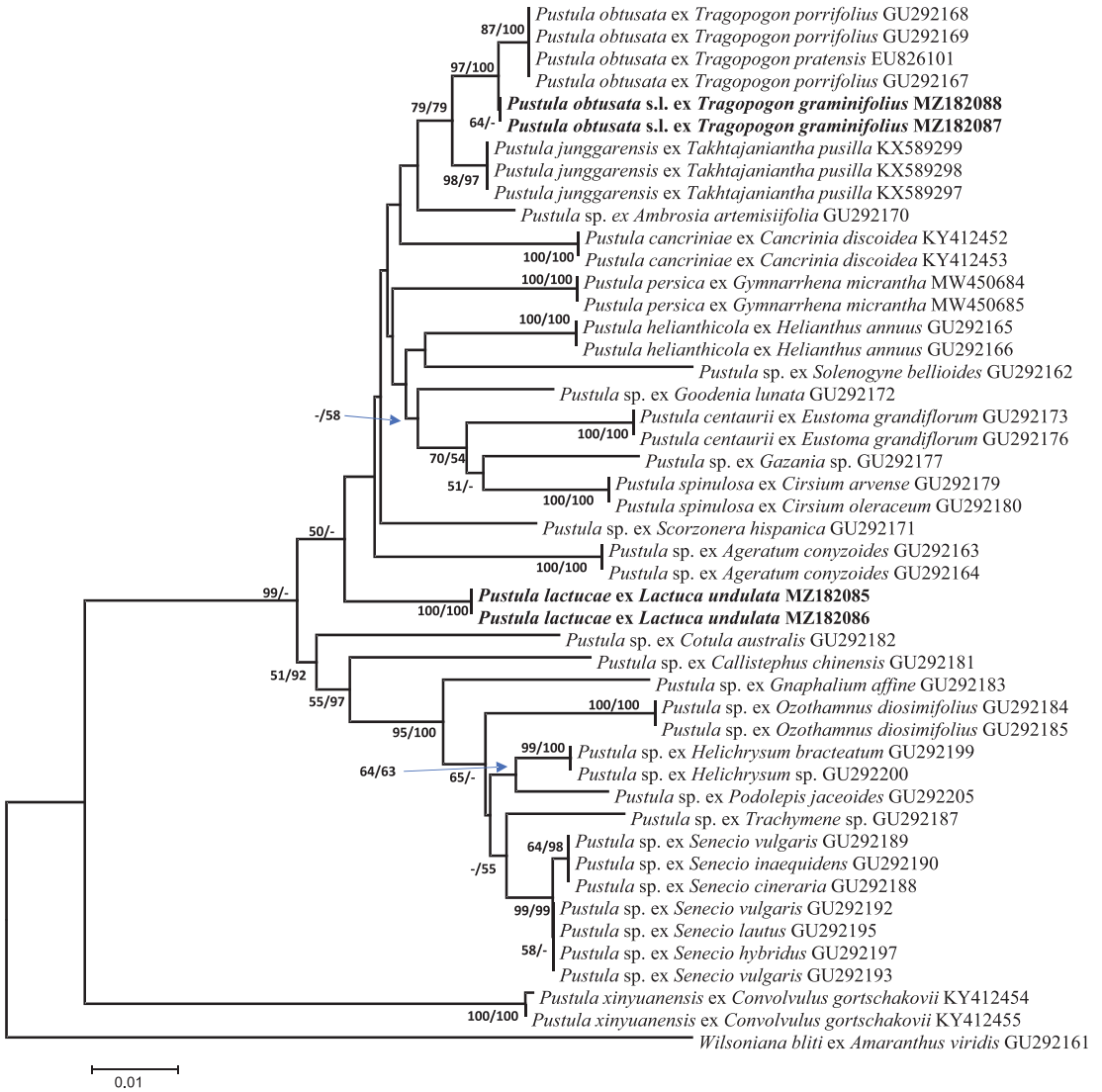


Fig. 1. Phylogenetic tree based on minimum evolution (ME) analysis of the *cox2* mtDNA gene sequences of *Albuginaceae* species. Bootstrap values of minimum evolution and Bayesian (BI) analyses are shown on the left on the branches. Bootstrap values of minimum evolution and Bayesian (BI) analyses $\geq 50\%$ are shown at the nodes. Sequences obtained in this study are presented in bold. *Wilsoniana bliti* (DAR 76991) was used as an outgroup.

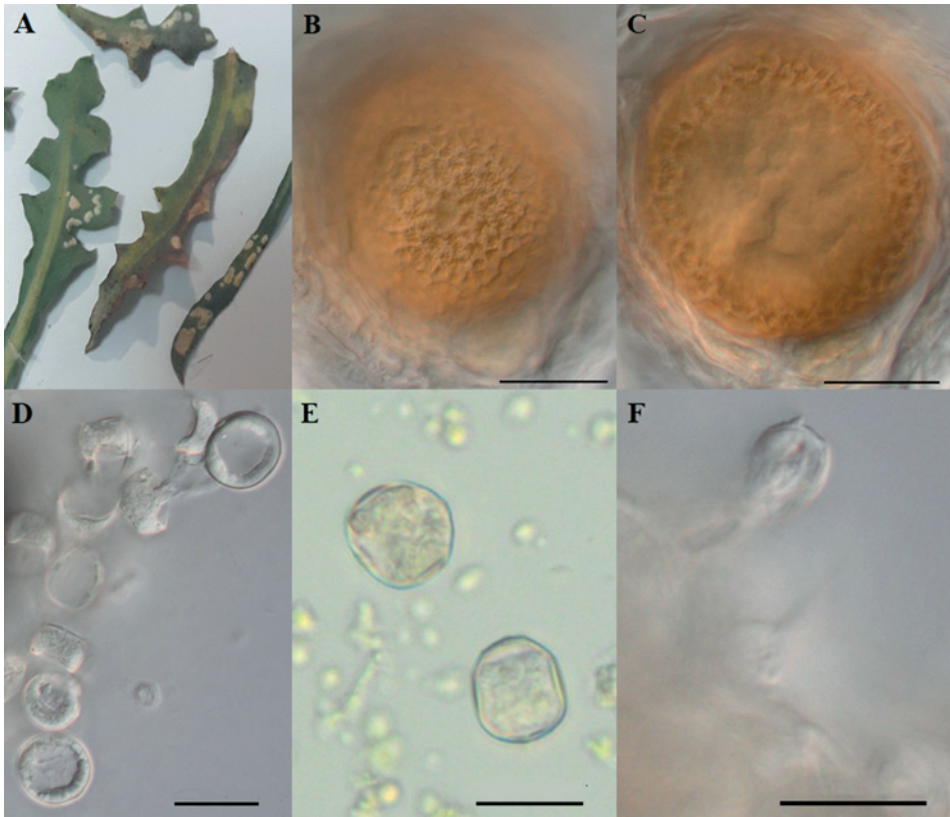


Fig. 2. *Pustula lactucae* (IRAN17919F). **A** – white blister rust symptoms on leaves of *Lactuca undulata*, **B–C** – surface ornamentation of oospores, **D** – primary sporangia, **E** – secondary sporangia, **F** – sporogenous hyphae. Scale bars = 20 µm. Photos M.R. Mirzaee.

Using a BLAST search, the sequence of the host plant showed 99.68% identity in the ITS region (accession no. MZ264208) to *Lactuca undulata* from China (accession no. KF485647).

TAXONOMY

The new species *Pustula lactucae* on *Lactuca undulata* is included in the *Albuginaceae* based on morphological criteria, phylogenetic data and host specificity.

***Pustula lactucae* Mirzaee, sp. nov.**

Figs. 2, 4

Mycobank MB 839887

Ety m o l o g y. The name reflects the host genus *Lactuca*.

H o l o t y p e. Iran, South Khorasan Province, village of Mohammadih near the town of Birjand, on *Lactuca undulata*, 19 April 2018, leg. M.R. Mirzaee (IRAN17919F).

Sequences ex holotype: GenBank no. MZ182085 (*cox2*), MZ494190 (ITS nrDNA).

D e s c r i p t i o n. Hyphae intercellular. Sori mostly on abaxial leaf surfaces of lower leaves, 0.5–2.5(5) mm diam., distinct or confluent, rounded or irregular, white to cream in colour. Sporogenous hyphae hyaline, clavate to cylindrical, 25–57.5 × 12.5–15 µm. Sporangia in basipetal chains, hyaline, of two kinds. Primary sporangia hyaline, globose to subglobose, wall surface with reticulate pattern, (12.5)16.2–20.8(25) µm (av. 18.5 µm). Secondary sporangia hyaline, subglobose to cylindrical, with equatorial wall thickening, wall surface showing striate ornamentation, sometimes with fine verrucose pattern, (12.5)15.4–18.6(21) µm (av. 17 µm). Oogonia globose, sometimes irregular in shape, (70)75.7–85.3(87.5) µm (av. 80.5 µm) in diameter. Oospores globose, pale brown to dark brown, (60)62–68(72) µm (av. 65 µm) in diameter, pattern reticulate with wart-like protuberances.

H a b i t a t. On lower living leaves of *Lactuca undulata*.

D i s t r i b u t i o n. Asia: Iran.

D i s t i n g u i s h i n g c h a r a c t e r s. *Pustula obtusata* (IRAN18017F) differs from *Pustula lactucae* in having smaller oospores [(45)46–52(57) µm (av. 49 µm)]. *Pustula junggarensis* differs from *Pustula lactucae* in having smaller oospores [(45.4)54.6–65.4(68.5) µm (av. 62.5 µm)] and larger secondary sporangia [(15.3)18.2–21.8(23.4) µm (av. 20.9 µm)].

The species differentiation between *P. obtusata* and *P. lactucae* is also indicated based on oospore patterns. *Pustula obtusata* oospores on *Tragopogon graminifolius* exhibit a more regular protuberance arrangement. Also, the oospore surface of *P. lactucae* shows some confluent protuberances, in contrast to *P. obtusata* oospores on *Tragopogon graminifolius*, which exhibit mainly isolated protuberances (Fig. 3).

Under SEM, the primary sporangial wall of *P. lactucae* shows a reticulate pattern, whereas that of *P. obtusata* has denticulate structures (Fig. 4).

Additional specimens examined

Pustula lactucae* ex *Lactuca undulata

I r a n. South Khorasan Province, village of Esfahroud near the town of Birjand, 1 April 2016, leg. M.R. Mirzaee (IRAN17944F).

Pustula obtusata* ex *Tragopogon graminifolius

I r a n. South Khorasan Province, village of Siyujan near the town of Khusf, 24 March 2018, leg. M.R. Mirzaee (IRAN18017F). – Village of Fedeshk near the town of Khusf, 7 May 2019, leg. M.R. Mirzaee (IRAN17923F).

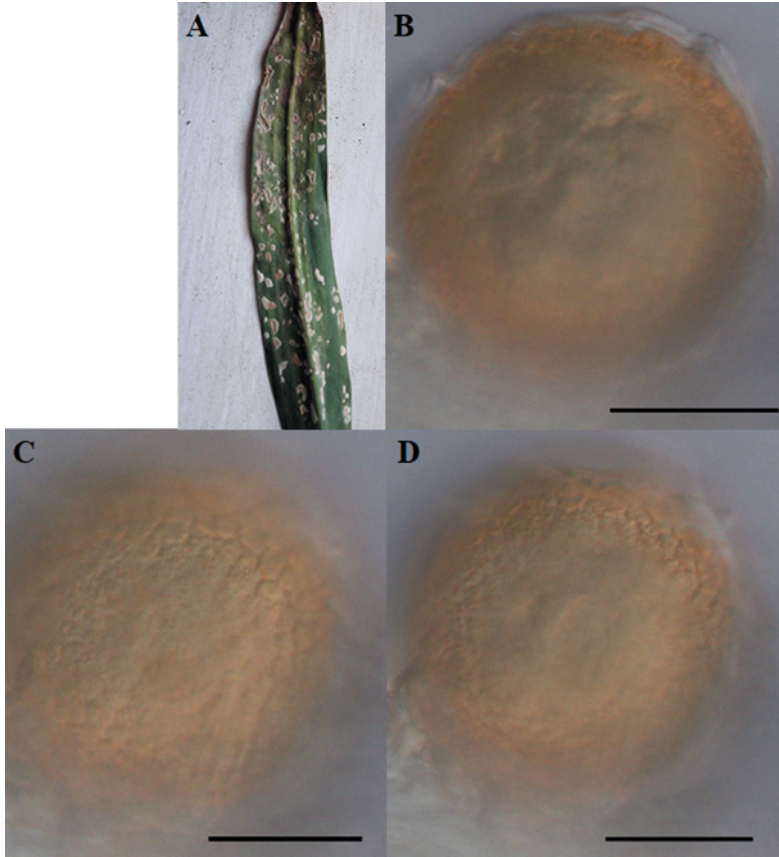


Fig. 3. *Pustula obtusata* (IRAN17923F). **A** – white blister rust symptoms on leaves of *Tragopogon graminifolius*, **B–D** – surface ornamentation of oospores. Scale bars = 25 µm. Photos M.R. Mirzaee.

Notes. The white blister rust disease incidence on *Lactuca undulata* and *Tragopogon graminifolius* was assessed again during the growing season of 2021 on sites where the specimens of *Pustula lactucae* and *P. obtusata* had previously been collected. However, no disease incidence was observed. Significantly low precipitation during the growing season might have impacted the lack of disease incidence, indicating that environment variables play a determinative role in discovering these specimens in semiarid regions.

Considering the fact that species within *Pustula* are mostly host genus specific (Ploch et al. 2011), and also due to the richness of *Asteraceae* in Iran (Noroozi et al. 2019), an increasing number of species within this genus is expected to be described on this host family in diverse habitats.

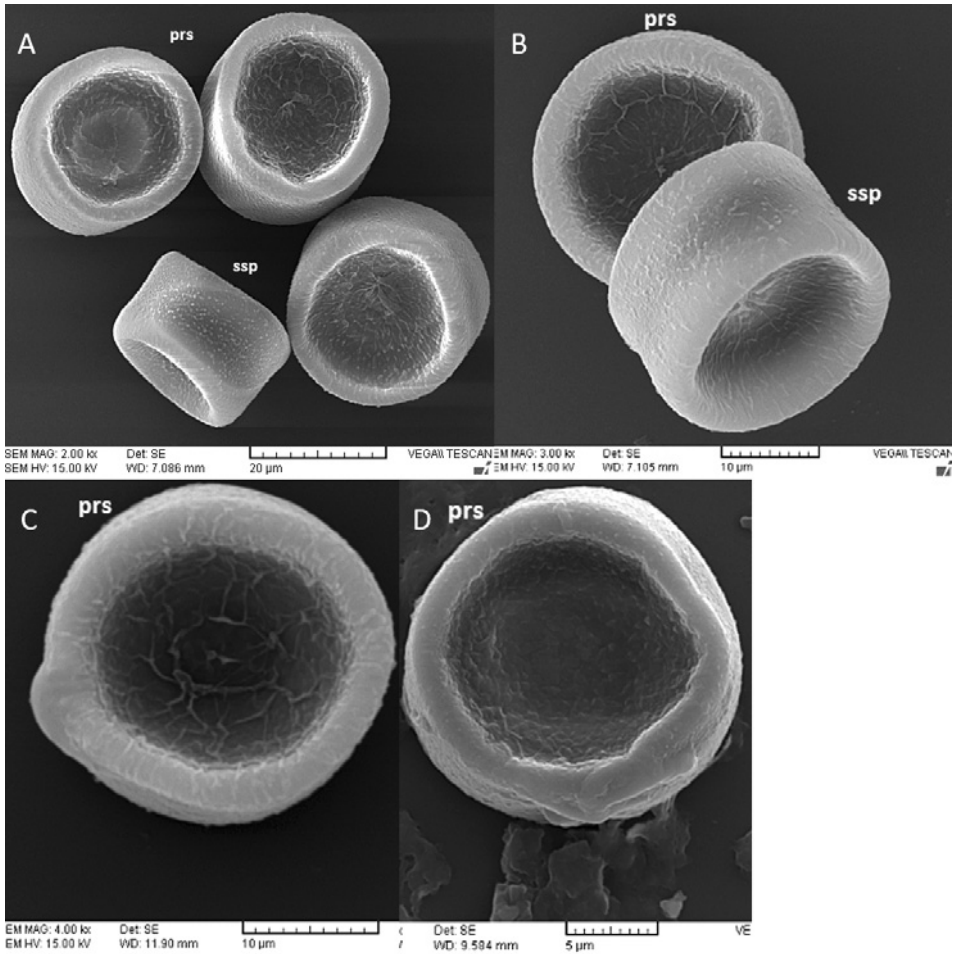


Fig. 4. Scanning electron micrographs of the sporangial wall in *Pustula lactucae* (IRAN17919F) and *P. obtusata* (IRAN17923F). **A–C** – *P. lactucae*, **D** – *P. obtusata*; prs = primary sporangia, ssp = secondary sporangia. Scale bars = 20 µm (A), 10 µm (B, C), 5 µm (D). Photos Razi Metallurgical Research Center.

The increasing number of *Pustula* species discovered in deserts (Xu et al. 2016, Xu et al. 2018, Mirzaei et al. 2021) predicts high species richness to be found in similar habitats. This finding contributes to the knowledge of biodiversity of plant pathogens and their effects on the structuring of plant communities in desert and similar habitats. Besides climate and habitat conditions, the pathogens can have direct and indirect effects on plant community structure (Dobson et Crawley 1994, Wang et al. 2020).

Within oomycetes, potential sources of resistance derived from wild *Lactuca* species have been developed for resistance to downy mildew (Lebeda et al. 2014). This suggests also a strategy for selecting sources of race-specific resistance from wild *Lactuca* species against the white blister rust pathogen on *Lactuca sativa* (lettuce) as a potential host for this pathogen (Farr et Rossman on-line) and *L. undulata*. The reason for highlighting the latter species is that the necessity of domestication of *L. undulata* has recently been considered because it has important medicinal properties and is threatened with extinction in some regions of Iran (Mofid bojnoordi et al. 2020).

Because *Lactuca undulata* is an ephemeral plant with a short life cycle, it is suggested to study the adaptation mechanism of *P. lactucae* to the short life of the host in the future. Such a mechanism has been elucidated for adaptation of smut fungus *Moesziomyces penicillariae* to the short cycle of its host, pearl millet (Diagne-Leye et al. 2013).

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