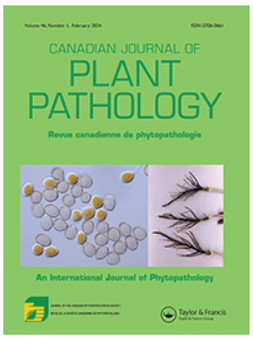

Taylor & Francis
Taylor & Francis Group

Canadian Journal of Plant Pathology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tcjp20

First report of *Melampsora ferrinii* (Pucciniales) on Babylon willow (*Salix babylonica*) in Iran

Mehrdad Abbasi, Gurcharn Singh Brar & M. Catherine Aime

To cite this article: Mehrdad Abbasi, Gurcharn Singh Brar & M. Catherine Aime (2024) First report of *Melampsora ferrinii* (Pucciniales) on Babylon willow (*Salix babylonica*) in Iran, Canadian Journal of Plant Pathology, 46:1, 11-18, DOI: 10.1080/07060661.2023.2266405

To link to this article: https://doi.org/10.1080/07060661.2023.2266405

Can. J. Plant Pathol., 2024
Vol. 46, No. 1, 11–18, https://doi.org/10.1080/07060661.2023.2266405

Disease Reports and Disease Diagnostics / Rapports des Maladies

First report of *Melampsora ferrinii* (Pucciniales) on Babylon willow (*Salix babylonica*) in Iran

MEHRDAD ABBASI[1], GURCHARN SINGH BRAR[1] AND M. CATHERINE AIME[2]

[1]*Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada*
[2]*Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA*

(Accepted 28 September 2023)

Abstract: As an agroforestry resource willow species (*Salix* spp.) becoming increasingly important in the protection of riverbanks, recovery of floodable lands, soil bioremediation, biomass production, and wicker furniture manufacture, Babylon willow (*Salix babylonica*) is widely planted all over the world and is found throughout Iran as part of the rural landscape and is mostly planted for ornamental or bioremediation purposes. Rust fungi in the genus *Melampsora* usually cause disease on *Salix* species. Identification of *Melampsora* species is often complicated because there are few differences in spore morphology and little is publicly available in comparative sequence data. Willow trees in Iran have been reported to be infected by 15 *Melampsora* species; however, most of these records are based on morphological characterization. During surveillance activities carried out in June 2007, a rust species was detected on isolated Babylon willow trees in an area in Northern Iran. Both the rust fungus and the host plant were identified on the basis of morphological features and DNA sequencing. Based on a combination of morphology, large subunit (LSU) rDNA sequencing and phylogenetic analyses, our rust specimen was identified as *Melampsora ferrinii*, which is newly reported from Iran on *Salix babylonica*. *Melampsora ferrinii* was originally described from the Americas and this is the first time that this rust has been observed on its telial host, Babylon willow, in Asia. The documentation of the rust fungus was performed using both scanning electron microscopy (SEM) and light microscopy. Additionally, an identification key for *Melampsora* species reported on *Salix* spp. in Iran is included.

Keywords: *Melampsora ferrinii*, rust fungi, Salicaceae, tree disease, Uredinales, weeping willow

Résumé: En tant que ressource agroforestière, les espèces de saules (*Salix* spp.) deviennent de plus en plus importantes dans la protection des berges, la récupération des terres inondables, la bioremédiation des sols, la production de biomasse et la fabrication de meubles en osier. Le saule de Babylone (*Salix babylonica*) est largement planté dans le monde entier et se trouve dans tout l'Iran dans le cadre du paysage rural principalement planté à des fins ornementales ou de bioremédiation. Les champignons de la rouille du genre *Melampsora* provoquent généralement des maladies sur les espèces de *Salix*. L'identification des espèces de *Melampsora* est souvent compliquée en raison du peu de différences dans la morphologie des spores et du peu de données de séquence comparative accessibles au public. Des saules en Iran ont été signalés comme étant infectés par 15 espèces de *Melampsora*, cependant, la plupart de ces signalements sont basés sur la caractérisation morphologique. Au cours des activités de surveillance menées en juin 2007, une espèce de rouille a été détectée sur des arbres isolés de saule de Babylone dans une zone située au nord de l'Iran. Le champignon de la rouille et la plante hôte ont été identifiés sur la base des caractéristiques morphologiques et du séquençage de l'ADN. Sur la base de la combinaison de la morphologie, du séquençage de l'ADNr des grandes sous-unités (LSU) et des analyses phylogénétiques, notre spécimen a été identifié comme *Melampsora ferrinii*, récemment signalé en Iran sur *Salix babylonica*. *Melampsora ferrinii* a été initialement décrite des Amériques et c'est la première fois que cette rouille est observée sur son hôte télien, le saule de Babylone, en Asie. La documentation du champignon de la rouille a été réalisée en utilisant à la fois la microscopie électronique à balayage (MEB) et la microscopie optique. De plus, une clé d'identification des espèces de *Melampsora* rapportée sur *Salix* spp. en Iran est inclus.

Mots clés: champignons de la rouille, maladie des arbres, *Melampsora ferrinii*, Salicaceae, saule pleureur, Uredinales

Correspondence to: Mehrdad Abbasi. E-mail: puccinia@gmail.com

Published online 18 Oct 2023

Introduction

Willows (*Salix* spp.) have been extensively planted all over the world as an agroforestry resource and have been utilized for amenity plantings, soil conservation, and horticultural shelter. Today, willows are found throughout the world as part of the rural landscape, becoming increasingly important in the protection of riverbanks, recovery of floodable lands, soil bioremediation, biomass production, and wicker furniture manufacture (Zapata 2016). The genus *Salix* L. in Iran comprises 38 taxa (including hybrids) of typically deciduous trees and shrubs, found primarily in cold and temperate regions of the country (Maassoumi et al. 2011). Most of the species are distributed in riverbanks and meadows created by rivers. However, some species are widely cultivated as ornamental trees across the country. *Salix babylonica* L. (Babylon willow) is a medium- to large-sized deciduous tree, growing up to 30 m tall, and is distinguishable from other *Salix* species by having long pendulous branches and yellowish green linear-lanceolate leaves that are up to 10 times as long as they are wide. The native range of this species is northeast China to Korea; however, it is grown across the world as an

Table 1. Overview of *Melampsora* species reported on *Salix* spp., in Iran based on Abbasi (2021a, 2021b) including *M. ferrinii* features from holotype (PUR N6740) and this study.

Species name	Host name	Features of uredinia	Features of urediniospres and uredinial paraphyses (uredinioparaphyses)
Melampsora abietis -caprearum	*Salix alba*	hypophyllous	13–20 × 12–16 µm, wall 1.5 µm thick, densely echinulate; paraphyses wall 1.5–3 µm thick
Melampsora allii-fragilis	*Salix alba; S.* sp.	mainly hypophyllous	22–33 × 13–15 µm, wall smooth at apex up to 3 µm thick; paraphyses wall 2–5 µm thick
Melampsora amygdalinae	*Salix excelsa*	hypophyllous	19–32 × 11–15 µm, wall smooth at apex 1.5 µm thick; paraphyses wall 3–5 µm thick
Melampsora caprearum	*Salix cinerea; S.* sp.	hypophyllous, 1–2 mm, up to 5 mm on young leaves	14–21 × 13–15 µm, wall evenly echinulate, 2–2.5 µm thick; paraphyses wall 5–6 µm thick at apex
Melampsora coleosporioides	*Salix babylonica*	mainly hypophyllous	18–27 × 13–19 µm, wall 1.5–2 µm thick, evenly echinulate or occasionally smooth at apex; paraphyses uniformly thickened (1.5–2 µm), some with apices thickened up to 5 µm
Melampsora cf. dimorphospora	*Salix alba*	mainly hypophyllous	19–30 × 15–25 µm, of two kinds with evenly echinulate or verrucose wall, wall thickness 2.5–3 µm; paraphyses wall 3–4 µm
Melampsora epitea	*Salix aegyptiaca; S. purpurea; S.* sp.	amphigenous, 0.5–1.5 mm	12–25 × 10–18 µm, wall 1.5–3 µm thick, evenly echinulate; paraphyses wall up to 3 µm
Melampsora euonymi-capraearum	*Salix carmanica*	hypophyllous	18–23 × 14–19 µm, wall evenly echinulate, up to 5 µm thick; paraphyses wall up to 8 µm thick at apex
Melampsora iranica	*Salix elbursensis; S.* sp.	mainly hypophyllous and on stems	17–25 × 15–20 µm, wall evenly echinulate, up to 3 µm thick; paraphyses wall up to 5 µm thick
Melampsora laricis -epitea	*Salix* sp.	amphigenous, up to 1.5 mm	12–25 × 9–19 µm, wall evenly echinulate up to 3 µm thick; paraphyses thick walled (8–10 µm)
Melampsora repentis	*Salix alba*	mainly hypophyllous	13–17 × 12–14 µm, wall evenly echinulate, 1.5 µm thick; paraphyses wall 3–5 µm thick
Melampsora ribesii -epitea	*Salix aegyptiaca*	hypophyllous, 0.5–1 mm	16–20 × 14–18 µm, wall evenly echinulate, 3–3.5 µm thick; paraphyses wall 2.5–4 µm thick
Melampsora ribesii -viminalis	*Salix* sp.	mainly hypophyllous, c. 0.2–0.3 mm	15–19 × 14–16 µm, wall evenly echinulate, 2 µm thick; paraphyses wall thickness mainly even, 1–2 µm
Melampsora salicis -acmophyllae	*Salix acmophylla*	hypophyllous	18–24 × 16–20 µm, wall evenly echinulate, 3–5 µm thick; paraphyses wall 7–9 µm thick at apex
Melampsora salicis -albae	*Salix alba; S. babylonica; S. excelsa; S. purpurea; S. triandra; S.* sp.	mainly hypophyllous and on young twigs, up to 5 mm long on young shoots	20–36 × 11–17 µm, wall smooth at apex, 2 µm thick; paraphyses wall thickness even, 2–3 µm
Melampsora ferrinii ***(this study)***	*Salix babylonica*	hypophyllous, 0.1 to 0.3 mm	15–32 × 10–21 µm, wall 1.5–3 µm thick, evenly echinulate; paraphyses wall up to 3.5 µm uniformly thick
Melampsora ferrinii ***(Holotype PUR N6740)***	*Salix babylonica*	hypophyllous, 0.1 to 0.3 mm	15–32 × 10–21 µm, wall 1.5–3 µm thick, evenly echinulate; paraphyses wall up to 3.5 µm uniformly thick

Table 2. Taxa and sequences used in phylogenetic analysis.

Genus	Species	Voucher No. (Collection No.)	GenBank No.
Ceropsora	*weirii*	BPI910315	KY798397
Melampsora	*abietis-canadensis*	PURN22674 (MCA2842)	OQ947799
Melampsora	*abietis-canadensis*	PUR56615	JN934918
Melampsora	*abietis-canadensis*	PUR61512	JN934919
Melampsora	*aecidioides*	PURN15020 (URB1704)	OQ947800
Melampsora	*aecidioides*	PURF17322	JN934974
Melampsora	*aecidioides*	PURN4108	JN934975
Melampsora	*coleosporioides*	CJ01/1/01	AY652950
Melampsora	*coleosporioides*	JT98/01	AY652951
Melampsora	*euphorbiae-gerardianae*	BRIP39560	EF192199
Melampsora	*euphorbiae-gerardianae*	BPI877950 (U485)	OQ947801
Melampsora	*euphorbiae-gerardianae*	PURN22794 (U1642)	OQ947802
Melampsora	*ferrinii – Paratype*	PURN6739	KJ136566
Melampsora	*ferrinii – Holotype*	PURN6740 (MCA3522)	NG060305
Melampsora	*ferrinii – Paratype*	PURN6741 (MCA3581)	KJ136565
Melampsora	*ferrinii*	PURN6742 (MT225)	KJ136563
Melampsora	*ferrinii*	PURN6743 (MT275)	KJ136562
Melampsora	*ferrinii – Paratype*	PURN3995	KJ136567
Melampsora	*ferrinii – Paratype*	PURN3999	KJ136568
Melampsora	*ferrinii*	BPI863543 (U284)	OQ947803
Melampsora	*ferrinii*	PURN12884 (U1572)	OQ947804
Melampsora	***ferrinii***	**PURN23020 (UBC76)**	**OQ343720**
Melampsora	*hypericorum*	BPI871536 (U394)	OQ947806
Melampsora	*hypericorum*	PURN22470 (URB78)	OQ947807
Melampsora	*hypericorum*	PURN3971 (URB79)	OQ947808
Melampsora	*laricis*	PURN15091 (DJN184)	OQ947809
Melampsora	*laricis*	PURN15128 (DJN191)	OQ947810
Melampsora	*laricis*	PURN15118 (DJN201)	OQ947811
Melampsora	*laricis-epitea*	SAL46	OQ947812
Melampsora	*laricis-epitea*	PURN22968 (SAL52 cloneA)	OQ947813
Melampsora	*laricis-epitea*	PURN22968 (SAL52 cloneB)	OQ947814
Melampsora	*laricis-populina*	PURN15117 (DJN200)	OQ947815
Melampsora	*laricis-populina*	BPI841041 (U99)	JQ042251
Melampsora	*lini*	PURN11703 (MCA5267)	OQ947816
Melampsora	*lini*	PURN8817 (URB90)	OQ947817
Melampsora	*medusae*	BPI877955 (U840)	JQ042243
Melampsora	*medusae*	BPI877956 (U841)	JQ042244
Melampsora	*medusae*	BPI877952 (U842)	JQ042242
Melampsora	*occidentalis*	SAL 43	EF192207
Melampsora	*occidentalis*	PURN16026 (U1109)	JQ042239
Melampsora	*occidentalis*	U1218	JQ042236
Melampsora	*pakistanica*	U1645	OQ947818
Melampsora	*pakistanica*	PURN22996 (UBC7)	OQ947819
Melampsora	*pakistanica*	PURN22997 (UBC8)	OQ947820
Melampsora	*paradoxa*	SAL9 clone	OQ947821
Melampsora	*paradoxa*	PURN22982 (SAL44, cloneA)	OQ947822
Melampsora	*paradoxa*	PURN22982 (SAL44, cloneB)	OQ947823
Melampsora	*salicis-albae*	HMAAC4066	MK372197
Melampsora	*salicis-albae*	HMAAC4070	MK372201
Melampsora	*salicis-albae*	HMAAC4081	MK372203
Melampsora	*yoshinagai*	PURN16276 (URB101)	OQ947824
Melampsora	*yoshinagai*	PURN2 (URB102)	OQ947825
Melampsora	*yoshinagai*	PURN16478 (YUN58)	OQ947826

ornamental tree. Because of their simple propagation, rapid growth, and distinctive drooping branches, Babylon willows are highly prized as ornamental trees in landscaping. Moreover, these trees exhibit significant promise for bioremediation purposes, as they thrive in wet and flooded environments and possess the ability to amass heavy metals

and other undesirable substances from polluted ecosystems (Toome and Aime 2015). In Iran this species is widely planted in parks and gardens, but has never been collected in the wild habitat by botanists (Maassoumi 2009). Leaf rust, caused by various *Melampsora* species, stands as the most prevalent, significant, and widely spread leaf disease among willow trees in Iran. To date, 15 *Melampsora* species have been documented on *Salix* species within Iran (Table 1). Among these, only two species, viz. *M. coleosporioides* Dietel and *M. salicis-albae* Kleb., have been observed on *Salix babylonica* (Abbasi 2021b). During a study on the biodiversity of rust fungi (Pucciniales) in Iran, we studied a herbarium specimen of *S. babylonica* infected by *Melampsora* sp. using morphological features and molecular analysis. This study revealed the presence of *M. ferrinii* Toome & Aime on Babylon willow in Iran, which has not been previously reported. *Melampsora ferrinii* is a heteromacrocyclic rust and its aecial state has been previously reported on *Corydalis acuminata* Franch., C. *edulis* Maxim. and *C. racemosa* (Thunb.) Pers., of family Papaveraceae in Shaanxi province China (Peng et al. 2022). However, a natural infection of rust on a telial host has never been reported from Asia.

Materials and methods

Sample collection and host identification

Rust-infected material in this study was observed and collected on cultivated *Salix babylonica* from Iran, Mazandaran province, Babolsar towards Babol, 6 km south of Babolsar (N 36.624689790130624; E 52.641549110412605; elevation −13 m), on 1 June 2007. We collected rusted plant specimens in a paper bag and pressed them in the field using a field press. Voucher material was deposited in the Arthur Fungarium (PUR), Purdue University, Indiana, USA under the accession no. PURN23020.

Morphological characterization

The host species was identified using Maassoumi et al. (2011). The rust sori shape, colour, and size were studied and measured under a Nikon stereomicroscope model SMZ1500 (Nikon, Japan). Lactic acid in glycerol mounting medium was used for slide preparation. Microscopic slides were studied using Differential Interference Contrast (DIC) of Nikon compound microscope model Eclipse 80i (Nikon, Japan). The length and width of 50 spores were randomly

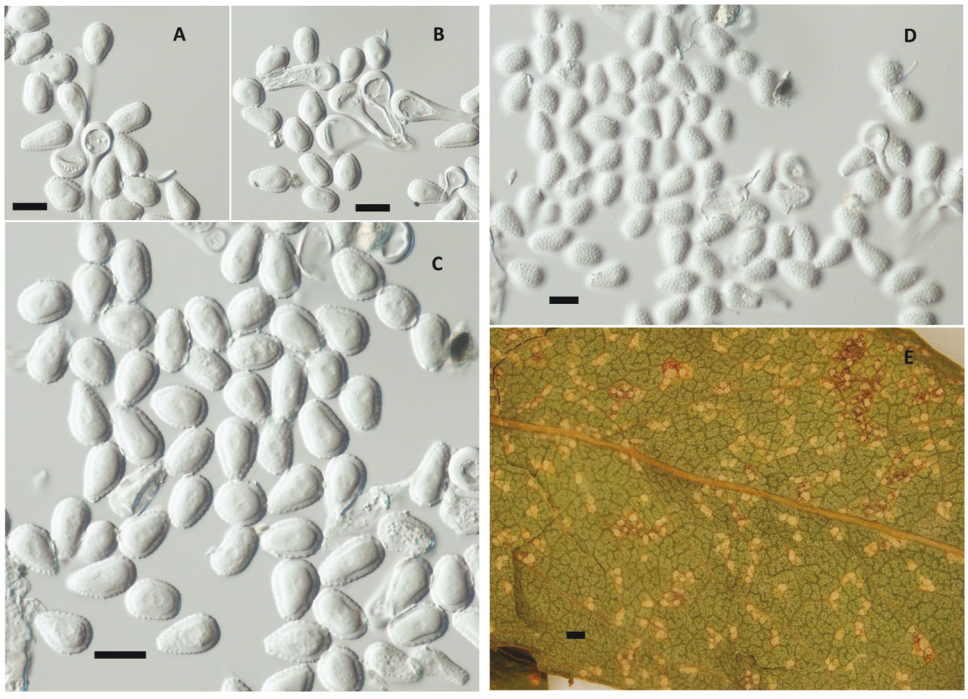

Fig. 1 (Colour online) Uredinial paraphyses (**a** & **b**) and urediniosores (**c**) of *Melampsora ferrinii*, scale bar = 20 µm. Surface ornamentation (echinulate) of urediniospores (**d**) and hypophyllous minute uredinia (**e**) of *Melampsora ferrinii* on *Salix babylonica* from Iran, (**d**) scale bar = 20 µm; (**e**) scale bar = 0.5 mm.

measured, as well as the diameter of the heads of paraphyses. Measuring shrinking spores or those that were not on the side view was avoided. Close-up photos from sori on infected leaves were taken by Nikon Digital Sight DS-Ri1 camera attached to the Nikon stereomicroscope. All photomicrographs were taken at 400× magnification using a Nikon Digital Sight DS-Fi1 camera attached to the Nikon compound microscope. For scanning electron microscopy (SEM), sori and urediniospores from dried leaves were mounted on SEM stubs with double-sided tape and then coated with gold using a Leica EM ACE600 High Vacuum sputter coater (Leica Microsystems, Wetzlar, Germany). Surface features of urediniospores were then examined with a Zeiss Crossbeam350 Cryo-FIB scanning electron microscope (Carl Zeiss, Germany) operated at 5 kV, and photographed with a digital camera at 3,800× to 7,500× magnification.

Molecular characterization and phylogenetic analysis

To confirm morphological host identification, the internal transcribed spacer rDNA was sequenced. Identity of the rust was also confirmed with DNA sequencing of the 28S rDNA, which is the preferred rust barcode locus (McTaggart and Aime 2018). For DNA extraction, sori were aseptically removed from a single infected leaf and placed in 2 mL Bead Solution tubes of the UltraClean Plant DNA Isolation Kit and extracted as per the manufacturer's instructions (MoBio Laboratories, Inc.). The 5' end of the nuclear 28S rDNA (LSU) was amplified with rust-specific primer Rust2inv (5′- GATGAAGAACACAGTGAAA, based on Aime 2006) and LR6 (5′- CGCCAGTTCTGCTTACC based on Vilgalys and Hester 1990), and sequenced following previously published protocols (Aime 2006; Aime et al. 2018). The newly generated 880 bp sequence was deposited in GenBank (OQ343720) and was compared with other sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/) using the BLASTn algorithm. To build a phylogenetic tree, the Iranian LSU sequence was analyzed within a dataset of 16 species of *Melampsora*, including all other taxa known to occur on weeping willows, and which also included sequences from the holotypes and paratypes of *M. ferrinii*. *Ceropsora weirii* was included as outgroup based on Aime and McTaggart (2020). Newly generated sequences were obtained in the same manner as described above. Table 2 provides a list of all specimens and sequences selected for phylogenetic analyses. Sequences were aligned with MAFFT, and analyzed by maximum likelihood within Geneious Prime 2023 (https://www.geneious.com).

Results

Morphological characteristics of the rust on Salix babylonica

Host identification was initially carried out by observing the overall appearance of the tree's weeping pattern, analyzing leaf characteristics, and considering relevant background information regarding the trees. Concerning rust fungus identification, only the uredinial state was present on infected leaves. Uredinia were minute, hypophyllous, scattered over leaf surfaces, pale yellow on dried material, erumpent, pulverulent, and measured 0.1 to 0.3 mm in diameter. Urediniospores were 15–32 × 10–21 µm, mostly obovoid, sometimes globose or ellipsoid, and the wall was 1.5–3 µm thick, uniformly and evenly echinulate with finely pointed spines, 1.5–2 µm apart (Figs 1 and 2). Uredinial paraphyses were mostly capitate, occasionally clavate, 15–18 µm wide at upper part (paraphysis head), the wall up to 3.5 µm uniformly thick,

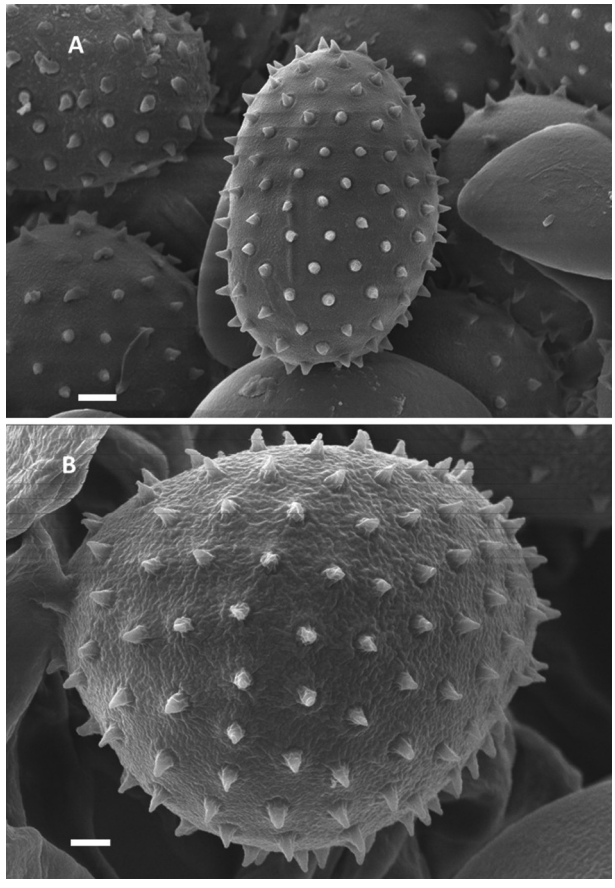

Fig. 2 SEM view of urediniospores of *Melampsora ferrinii* (PURN23020) showing spines evenly distributed over the surface; (**a**) scale bar = 2 µm; (**b**) scale bar = 1 µm.

sometimes slightly thickened at the top (Fig. 1). The aforementioned morphological characteristics clearly showed that the rust fungus attacking *S. babylonica* belongs to the genus *Melampsora*. Table 1 lists the different *Melampsora* species reported on *Salix* spp. in Iran including host name, features of uredinia, urediniospores, and uredinial paraphyses. Comparison of morphological features of the rust on *S. babylonica* from Iran with the protologue (Toome and Aime 2015) and holotype of *M. ferrinii* (PUR N6740; see Table 1), confirmed the identity of the Iranian rust on Babylon willow as *M. ferrinii*.

Identification of the host and rust fungus by molecular analysis

Molecular characterization of host plant was performed by sequencing the internal transcribed spacer rDNA. The obtained sequence (GenBank OQ343723) shared 100% identity (654/654 bp) with the available sequence of *S. babylonica* in GenBank (KC415496). Concerning the studied rust fungus, BLASTn analysis using the LSU sequence amplified in this study (OQ343720) shares 100% identity (880/880 and 870/870 bp) with six publicly available sequences of *M. ferrinii* including paratypes (KJ136563, KJ136562, KJ136566) and the holotype (KJ136564), and

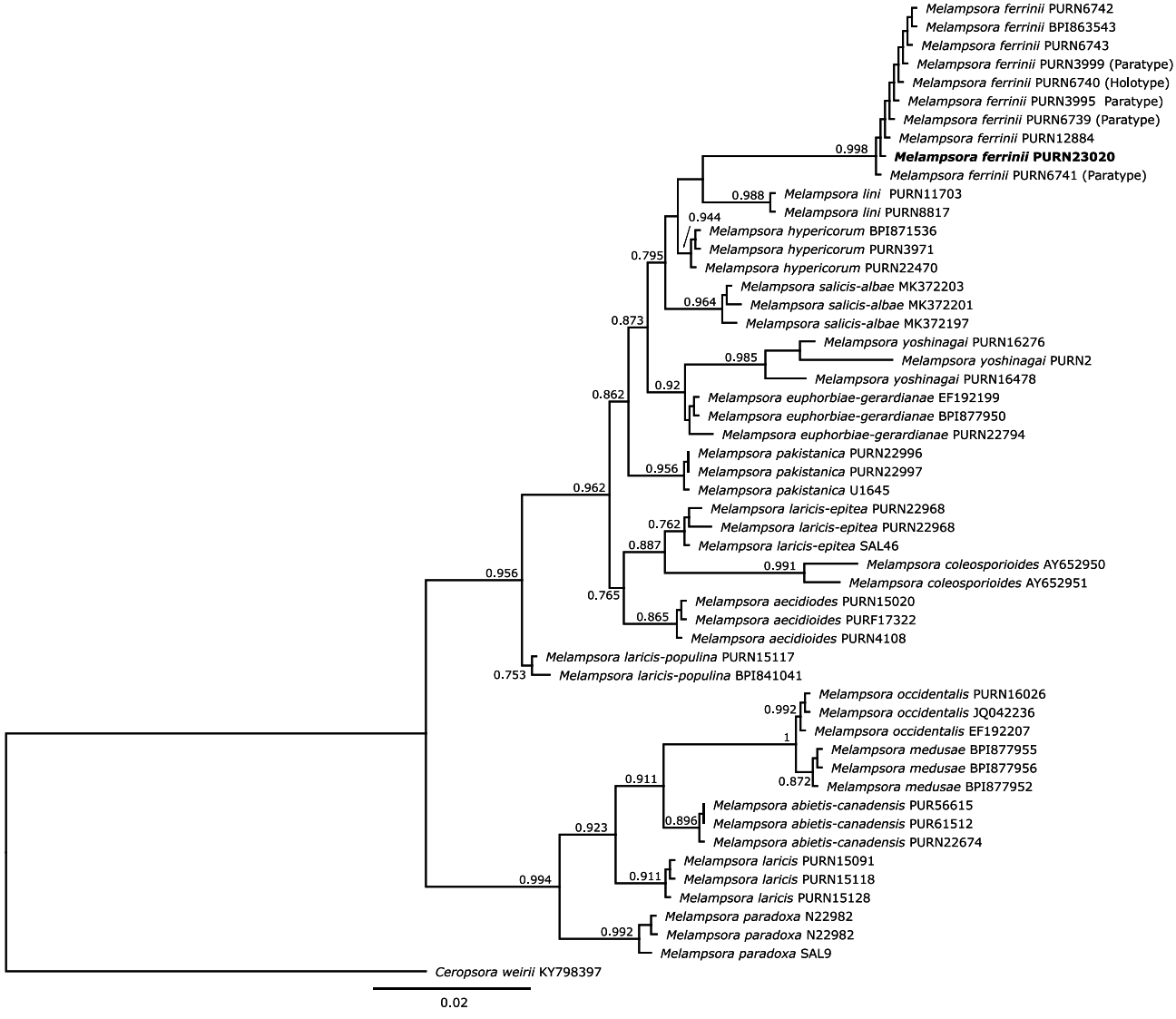

Fig. 3 Phylogenetic tree of LSU region obtained through maximum likelihood (ML). ML bootstrap values (values >70% are shown) are given at each node. Our own sequence is in bold. LSU region of *Ceropsora weirii* was used as the outgroup.

99.89% identity (879/880 bp) with two additional *M. ferrinii* accessions (KJ13656, KY798349). The phylogenetic tree clearly showed that the LSU sequence of the *Melampsora* species on *Salix babylonica* in Iran is conspecific with *M. ferrinii* (Fig. 3).

Discussion

Of the 15 *Melampsora* species previously reported on the genus *Salix* in Iran, only two species, *M. coleosporioides* and *M. salicis-albae*, have been reported on *S. babylonica*. Here, we add *M. ferrinii* on *S. babylonica* to the known mycota of Iran. We also provide a comprehensive list of distinguishing characters (Table 1) and a revised identification key to these species.

In this study, the combination of morphological features and molecular analysis revealed the presence of *M. ferrinii* on *S. babylonica* in Iran. Comparison of morphological features of the rust on *S. babylonica* from Iran with the protologue (Toome and Aime 2015) and holotype of *M. ferrinii* (PUR N6740) (see Table 1), confirmed the identity of the Iranian rust on Babylon willow as *M. ferrinii*. Phylogenetic analysis (Fig. 3) showed that the Iranian *Melampsora* species on Babylon willow was placed in a clade of *Melampsora ferrinii* including holotype, paratypes, and also isolates from USA and South America. Based on the provided identification key and Toome and Aime (2015), *Melampsora salicis-albae* can be easily distinguished from *M. ferrinii* by having larger uredinia (0.5–2 mm vs 0.1–0.3 mm) and urediniospores which are completely smooth at the apex. *Melampsora coleosporioides* is a species with small hypophyllous uredinia and overlapping published urediniospore morphology and measurements (Toome and Aime 2015). However, urediniospore walls of *M. coleosporioides* are occasionally smooth at the apex (Spiers and Hopcroft 1996; Pei 2005). *Melampsora ferrinii* can be easily distinguished from *M. coleosporioides* based on sequence data, as they share only 96.18% identity (453/471 bp; AY65295), and by lacking partly bald apices on urediniospores (Figs 2 and 3).

To our knowledge, this is the first report of *M. ferrinii* on *S. babylonica* from the continent of Asia. Peng et al. (2022) reported the aecial state of *M. ferrinii* on *Corydalis* spp. (Papaveraceae) from China. However, they did not observe the uredinial and telial states on *Salix* in nature. *Melampsora ferrinii* was described for the first time from the USA (Toome and Aime 2015). However, the authors (Toome and Aime 2015) believed that this rust has been infecting weeping willows in the Americas for at least 20 years. This rust has also been reported from Argentina, Brazil, Chile, and China in addition to the USA (Farr and Rossman 2022). *Salix babylonica* is native to eastern Asia, but has been introduced across the world mainly as an ornamental plant. It is very probable that *M. ferrinii* was accidentally introduced with the host where it is found outside of Asia. Herbarium specimens from plant pathology and mycological herbaria have been widely used to identify fungal plant pathogens and track their distribution and natural host range (Ristaino 2020). This study shows how herbarium specimens are providing new

1- urediniospores wall with two types of ornamentations, echinulate or verrucose	*M. dimorphospora*
1- urediniospore walls only echinulate	2
2- uredinia on leaves and on young shoots and twigs	3
2- uredinia only on leaves	4
3- urediniospores 17–25 × 15–20 µm, wall evenly echinulate	*M. iranica*
3- urediniospores 20–36 × 11–17 µm, wall smooth at apex	*M. salicis-albae*
4- urediniospores wall smooth at apex	5
4- urediniospores wall evenly echinulate	7
5- urediniospore walls thick (> 3 µm) distinctly elongated often thickened at apex, pear shaped, 22–33 × 13–15 µm	*M. allii-fragilis*
5- urediniospore walls thin (≤ 2 µm)	6
6- urediniospores elongated, ovoid to clavate, 19–32 × 11–15 µm, wall thickness 1.5 µm	*M. amygdalinae*
6- urediniospores obovate to pyriform, 18–27 × 13–19 µm, wall 1.5–2 µm thick	*M. coleosporioides*
7- uredinia on both sides of the leaf (amphigenous)	8
7- uredinia restricted to lower side of the leaf (hypophyllous)	9
8- urediniopараphyses thick walled (8–10 µm)	*M. laricis-epitea*
8- urediniopараphyses with thinner walls (up to 3 µm)	*M. epitea*
9- uredinia 1–2 mm, up to 5 mm on young leaves	*M. caprearum*
9- uredinia ≤ 1 mm	10
10- wall of urediniopараphyses up to 9 µm thick	11
10- wall of urediniopараphyses ≤ 5 µm thick	12
11- urediniospores 18–24 × 16–20 µm, wall 3–5 µm thick, on *Salix acmophylla*	*M. salicis-acmophyllae*
11- urediniospores 18–23 × 14–19 µm, wall up to 5 µm thick, on *S. carmanica*	*M. euonymi-caprearum*
12- urediniospores length frequently > 20 µm	***M. ferrinii***
12- urediniospores length frequently ≤ 20 µm	13
13- urediniospore walls 3–3.5 µm thick	*M. ribesii-epitea*
13- urediniospore walls ≤ 2 µm thick	14
14- urediniospore ornamentation dense (> 0.6 spines/µm^2)	*M. abietis-caprearum*
14- urediniospore ornamentation less dense (0.3–0.5 spines/µm^2)	15
15- urediniopараphyses thin walled (1–2 µm)	*M. ribesii-viminalis*
15- walls of urediniopараphyses thicker (3–5 µm)	*M. repentis*

information on plant pathogens and their host range and distribution.

A key to *Melampsora* species on willows (*Salix* spp.) in Iran when only uredinia are present:

Acknowledgments

We would like to thank Dr. Karen Golinski, collections curator of Bryophytes, Lichens & Fungi, and Spencer Goyette, curatorial assistant at the UBC Herbarium, for making it possible for the first author to use microscope and photomicrography system.

Funding

Funding in part was provided by the U.S. National Science Foundation [DEB-2127290 & DEB-1458290], the U.S. Department of Agriculture [AP20PPQS & T00C077], and USDA Hatch #1010662. This study was also supported by the funding by the University of British Columbia (UBC), BC Peace River Grain Industry Development Council, and by Saskatchewan Wheat Development Commission.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

Abbasi M. 2021a. Biodiversity of rust fungi in Iran, part 2: identification keys for Iranian rust fungi. Tehran: Iranian Research Institute of Plant Protection.

Abbasi M. 2021a. A checklist of rust fungi (Pucciniales) in Iran. J Crop Prot. 10(2):175–259.

Aime MC. 2006. Toward resolving family-level relationships in rust fungi (Uredinales). Mycoscience. 47(3):112–122. doi: 10.1007/S10267-006-0281-0.

Aime MC, Bell C, Wilson AW. 2018. Deconstructing the evolutionary complexity between rust fungi (Pucciniales) and their plant hosts. Stud Mycol. 89:143–152. doi: 10.1016/j.simyco.2018.02.002.

Aime MC, McTaggart AR. 2020. A higher-rank classification for rust fungi, with notes on genera. Fungal Syst Evol. 7:21–47. doi: 10.3114/fuse.2021.07.02.

Farr DF, Rossman AY 2022. Fungal databases. U.S. National Fungus Collections, ARS, USDA; [accessed 2022 Sep 29]. https://nt.ars-grin.gov/fungaldatabases.

Maassoumi AA. 2009. Experimental taxonomy of the genus *Salix* L. (Salicaceae) in Iran. Iran J Bot. 15(1):3–20.

Maassoumi AA, Assadi M, Hemmati A. 2011. Salicaceae. In: Assadi M, Maassoumi MM, Mozaffarian V, editors. Flora of Iran. Vol. 74. Tehran: Research Institute of Forest and Rangelands Publication.

McTaggart AR, Aime MC. 2018. The species of coleosporium (Pucciniales) on Solidago in North America. Fungal Biol. 122 (8):800–809. doi: 10.1016/j.funbio.2018.04.007.

Pei MH. 2005. A brief review of *Melampsora* rusts on *Salix*. In: Pei MH, McCraken A, editors Rust diseases of Willow and Poplar. Wallingford: CABI Publishing; pp. 11–28.

Peng Z, Xiong C, Luo Z, Hu X, Yu Z, Chen T, Xu Y, Wang B. 2022. First report of alternate hosts of willow rust disease caused by Melampsora ferrinii in China. Plant Dis. 106:324. doi: 10.1094/PDIS-05-21-0958-PDN.

Ristaino JB. 2020. The importance of mycological and plant herbaria in tracking plant killers. Front Ecol Evol. 7:1–11. doi: 10.3389/fevo.2019.00521.

Spiers AG, Hopcroft DH. 1996. Morphological and host range studies of *Melampsora* rusts attacking Salix species in New Zealand. Mycol Res. 100(10):1163–1175. doi: 10.1016/S0953-7562(96)80176-6.

Toome M, Aime MC. 2015. Reassessment of rust fungi on weeping willows in the Americas and description of Melampsora ferrinii sp. nov. Plant Pathol. 64(1):216–224. doi: 10.1111/ppa.12237.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol. 172(8):4238–4246. doi: 10.1128/jb.172.8.4238-4246.1990.

Zapata M. 2016. First report of *Melampsora ferrinii* causing willow leaf rust in Chile. New Dis Rep. 34:25. doi: 10.5197/j.2044-0588.2016.034.025.