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New or Unusual Disease Reports

## *Leveillula lactucae-serriolae* on *Lactuca serriola* in Jordan

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**Summary.** Jordan contributes significantly to the Near East plant biodiversity with numerous primitive forms and species of crops and their wild relatives. Prickly lettuce (*Lactuca serriola*) is a common species in Jordan, where it grows in various habitats. During a survey of wild *Lactuca* distribution in Jordan in August 2007, plants of *L. serriola* with natural infections of powdery mildew were observed at a site near Shobak (Ma'an Governorate). *Lactuca serriola* leaf samples with powdery mildew infections were collected from two plants and the pathogen was analyzed morphologically. Characteristics of the asexual and sexual forms were obtained. Sequence analyses of the rDNA ITS region and D1/D2 domains of the 28S rDNA were used to obtain phylogenetic data, and to reach taxonomic conclusions about these specimens. Molecular determination, performed by sequencing of the ITS region, proved its identity with the type material of *Leveillula lactucae-serriolae*. Sequencing of the 28S rDNA region provided the first verified GenBank record of *Leveillula lactucae-serriolae* deposited in this public nucleotide repository. This is the first taxonomically verified record of *L. lactucae-serriolae* on *L. serriola* growing wild in Jordan, and one of the first records of the fungus in the Near East.

**Keywords.** Lettuce powdery mildew, morphology, Near East, prickly lettuce, ITS and 28S rDNA region.

### INTRODUCTION

Powdery mildews are biotrophic pathogenic fungi from the order Erysiphales, which mostly exhibit exoparasitic life strategies (Braun and Cook, 2012). On the genus *Lactuca*, at least three biologically and ecologically different genera of powdery mildews (*Golovinomyces*, *Podosphaera*, *Leveillula*) are known (Lebeda and Mieslerova, 2011), and a *Pseudoidium* type of powdery mildew (without exact determination) was detected on *L. viminea* in the south France (Lebeda *et al.*, 2002). However, from this survey, it is also evident that the genus *Leveillula* is rare on *Lactuca* species. *Leveillula* has 40 species, and is an endoparasitic genus (Braun and Cook, 2012). It is considered to be adapted to xerophytic conditions, and is mainly distributed in arid and

warm areas of Africa, Asia, southern Europe, and southern North America to South America (Braun and Cook, 2012). The most known and common pathogen is *Leveillula taurica*, which has a broad host range. *Leveillula* spp. have been recorded on host 221 species in 78 genera of the Asteraceae. As a result, there is high genetic diversity in *Leveillula* spp. affecting Asteraceae (Palti, 1988).

Two different species concepts of *Leveillula* have been previously applied (Braun and Cook, 2012). These pathogens were regarded as highly specialized strains, as well as races which were able to infect a wide range of hosts belonging to various plant families. This ability was recently confirmed by the phylogenetic examinations of Khodaparast *et al.* (2001). In the past, Salmon (1900) recognized a single species, *Erysiphe taurica sensu latissimo*. He reduced all related taxa to synonymy with this species. Jaczewski (1927) divided *L. taurica* into numerous “formae” – i.e., one forma for each host genus. Golovin (1956) represented the other extreme in the treatment of *Leveillula*. He tried to split *Leveillula* into numerous species based on conidium shape and size as well as host range. He proposed one species for each host family, and his classification was, therefore, schematic and did not solve the phylo-taxonomic problems.

The detailed studies (including morphological and molecular analyses) of *Leveillula* were conducted mainly by Khodaparast and colleagues (Khodaparast *et al.*, 2001; Khodaparast *et al.*, 2007; Khodaparast *et al.*, 2010; Khodaparast *et al.*, 2012). In the most recent work, Khodaparast *et al.* (2012) performed phylogenetic analyses of ITS rDNA of 76 *Leveillula* specimens from different host families, and these suggested the maintenance of high phylogenetic variability in *Leveillula* on Asteraceae. The phylogenetic tree showed that powdery mildew on: a) *Carthamus*, *Crepis*, *Gundelia* and *Helianthus* established clade No. 1; b) *Cirsium*, *Lactuca serriola*, *Echinops* formed clade No. 2; c) *Centaurea*, *Launaea*, *Picris*, *Thevenotia* formed clade No. 5, and d) *Chondrilla*, *Acroptilon*, *Artemisia* and *Lactuca orientalis* were each in a separate clade. The phylogenetic variability confirmed that *Leveillula* spp. have probably colonized the Asteraceae several times during evolution, because isolates from this host family include genetically divergent taxa comprising several independent lineages (Khodaparast *et al.*, 2001).

The study of *Leveillula* species solely on Asteraceae was done mainly by Khodaparast *et al.* (2010). They evaluated *Leveillula* collections on Asteraceae from Iran and recognized six species, namely *L. guilanensis*, *L. lactucaae-serriolae*, *L. lactucarum*, *L. picridis*, *L. thevenotiae* and *L. taurica* s. lat., which could be separated into three morphological groups. Braun and Cook (2012) described nine *Leveillula* spp. on the family Asteraceae,

namely *L. asterisci*, *L. guilanensis*, *L. helichrysi*, *L. lactucaae-serriolae*, *L. lactucarum*, *L. lappae*, *L. osteospermi*, *L. picridis* and *L. thevenotiae*. The occurrence of *L. taurica* on Asteraceae was left as questionable. Among others, it was established that *Lactuca* spp., not only in Iran, are infected by at least two different *Leveillula* spp. (*L. lactucaae-serriolae* and *L. lactucarum*) (Khodaparast *et al.*, 2010; Braun and Cook, 2012).

The *Leveillula* species known currently (Braun and Cook, 2012) are distinguished from each other mainly by features of the conidia, especially by the shape and length/width ratio of primary and secondary conidia and by the form of conidium surfaces determined using scanning electron microscopy (SEM). The importance of conidial features was also proposed by Braun (1995), and before him by Golovin (1956), Rostam (1983), Durrieu and Rostam (1984), Heluta and Simonyan (1987, 1988), Simonyan and Heluta (1987, 1989). Voytyuk *et al.* (2009) performed detailed SEM examinations of *Leveillula* conidia and confirmed that variation in their surface structures provided taxonomically relevant traits allowing differentiation between allied taxa. Later, Khodaparast *et al.* (2012) acknowledged that many collections of *Leveillula* strains on different hosts showed conidial morphology which was usually consistent for a strain on a single host species. Besides the analyses completed by Khodaparast *et al.* (2001, 2012) of the ITS region, Voytyuk *et al.* (2009) also used *tub2* gene. However, resolutions in both ITS and *tub2* gene trees were not fully sufficient because taxa that are morphologically distinguishable are not well resolved genetically.

There is little known of the geographical distribution of *L. lactucaae-serriolae*. Voytyuk *et al.* (2009) reported this pathogen on *L. serriola* from Armenia, Iran and Israel. However, during field investigations in 2004–2007, this species was not recorded in Israel (Voytyuk *et al.*, 2009). In recent publications (Braun and Cook, 2012), *L. lactucaae-serriolae* is reported on *Lactuca azerbaijanica*, *L. scarioloides* and *L. serriola* from Asia (Iran, Israel, Lebanon, Turkmenistan) and Caucasus (Armenia), but not from Jordan. Currently, only Qasem and Abu-Blan, (1986) have reported a survey and identification of powdery mildews on economic and wild hosts in Jordan. Only *L. taurica* was confirmed in this survey, but this pathogen was not found on *Lactuca* spp. In the 1980s the taxonomy of powdery mildews and the possibilities of accurate species identification was generally on a low scientific level. From this time the attention of researchers in Jordan has been focused mainly on powdery mildews on economic crops (e.g., barley, tomato, cucurbits, grapes) (e.g., Abu-Blan and Khalil, 2001; Abdel-Ghani *et al.*, 2008; Mansour *et al.*, 2014).

The aim of the present study was to provide an accurate description and taxonomic position of *Leveillula* spp. found on wild *L. serriola* in Jordan.

MATERIALS AND METHODS

*Lactuca* spp. distribution

The character of the populations of wild *Lactuca* species in Jordan and the presence of powdery mildew on plants in their natural habitats was monitored during a field trip from 25–27 August 2007. Plants were studied at nine sites along a North (32°39'16,47"N) to South (30°19'48,59"N) transect, oscillating around the latitude of 35°40'E (Table 1). All sites were in the Mediterranean bioclimatic region. However, Shobak is located on its marginal part, near the Trans-Turanian region as defined by Al-Jaloudy (2006). While sites 1 to 7 were in northern areas with annual average rainfall of 200 to 400 mm, the annual average rainfall at site 9 (Petra) is 100 to 200 mm, and at site 8 (Shobak) is 50 to 100 mm (Fanack Water Editorial Team, 2017).

Site 1 was in the Jordan Valley, sites 2 to 7 were in the North of the Irbid Plateau, and site 9 (Petra) was in the South. Sites 8 (Shobak) and 9 were both on the marginal part of Steppe, and were strongly influenced by the Eastern Desert (Badiyah).

Plants with morphological traits typical of *L. serriola* were observed at all of the sites except site 8. Plants with traits of *L. aculeata*, i.e., with dense and sharp spines on stems and cauline leaves, were observed at sites 1, 2, 3, 6 and 7. Plants with traits of *L. saligna*, i.e., with acute leaf apices and narrow lobes on cauline leaves, were observed at sites 1, 4, 7 and 8 (Table 1).

Plants naturally infected by powdery mildew were observed at site 8 (Shobak). Leaf samples with powdery mildew infections were collected from two different plants at this site.

*Morphological examination of powdery mildew*

Two powdery mildew samples collected on individual plants of *L. serriola* were used. Pieces of severely infected leaves were used for evaluation by light microscopy. As only dry leaf samples were analyzed, the modified method of Shin (2000) was used, i.e., heating of 'herbariumized' plant tissues in fuchsin in lactic acid. For statistical analyses (means, standard deviations and ranges), 30 measurements of each characteristic were used where possible (MS Excel, 2010).

*Molecular examination*

Genomic DNA was extracted from fungal mycelium scraped from two herbarium specimens of *L. serriola* (OL35561, OL35562) using the SDS extraction method (Edwards *et al.*, 1991). The ITS region (ITS1-5.8S rDNA-ITS2) and the 5' end of the 28S rDNA region (including D1 and D2 domains) were amplified separately by two polymerase chain reactions (PCRs) with nested primer sets. For amplification of the ITS region, the powdery mildew specific PMITS1/PMITS2 primers (Cunnington *et al.*, 2003) and ITS1-F/ITS4 primers (White *et al.*, 1990; Gardes and Bruns, 1993) were used. Amplification of D1/D2 domains of the 28S rDNA was performed according to Takamatsu *et al.* (2013), using primer sets PM3/TW14 and NL1/TW14 for the two nested PCR

**Table 1.** List of monitoring sites with wild *Lactuca* species in Jordan in 2007.

Site number	Name of location	Latitude	Longitude	Altitude (m a.s.l.)	Character of habitat	<i>Lactuca</i> <sup>a</sup> forma	Number of samples
1	Umm Qais, Cadara	32°39'16,47''N	35°40'45,72''E	353	stony slope	<i>serriola</i>	1
2	West from Jerash, Olive Branch hotel	32°17'46,30''N	35°51'17,01''E	932	stony slope, south exposition	segregating <i>serriola/integrifolia</i>	2
3	Old Jerash, entrance to Roman City	32°16'45,01''N	35°53'13,32''E	590	sandy soil	<i>Lser, Lint</i> [as above]	3
4	Mount Nebo, monastery, monument	31°46'02,08''N	35°43'33,21''E	680	near road	<i>Lser</i>	4
5	Mount Nebo	31°46'00,30''N	35°43'42,60''E	671	near road	<i>Lser</i>	1
6	Madaba	31°43'12,22''N	35°47'40,25''E	787	ruderal place	<i>Lser</i>	5
7	Madaba, archeological park	31°42'57,47''N	35°47'44,71''E	780	stony background	<i>Lser</i>	1
8	Shobak	30°31'30,45''N	35°35'21,33''E	1304	along the road	<i>Lser</i>	2
9	Petra, Canyon	30°19'48,59''N	35°26'26,77''E	877	stony background	<i>Lser</i>	1

<sup>a</sup> Taxonomic status confirmed: *Lser* – *L. serriola* f. *serriola*; *Lint* – *L. serriola* f. *integrifolia*

runs. All PCR reactions were conducted in 15  $\mu\text{L}$  reaction volume, containing 1.2  $\mu\text{L}$  of DNA (50  $\text{ng } \mu\text{L}^{-1}$ ), 0.3  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ), 3  $\mu\text{L}$  of 10 $\times$  Reaction Buffer, 0.24  $\mu\text{L}$  of 10 mM dNTP's, 0.08  $\mu\text{L}$  of GoTaq G2 DNA Polymerase (Promega) and 9.88  $\mu\text{L}$  of PCR grade water, and were carried out in an Eppendorf Mastercycler ProS (Eppendorf). The following conditions were used for the PCRs: 5 min at 95°C; 35 cycles of 45 sec at 95°C, 45 sec at 60°C for the first PCR or 55°C for the second PCR, 1 min at 72°C, and a final extension (7 min at 72°C). PCR products were purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich) and sequenced (Macrogen Europe) using the following primers: ITS1-F/ITS4 for the ITS region and NL1/NL2/NLP2/TW14 for 28S rDNA (Takamatsu *et al.*, 2013). Geneious 7.1.8 (Biomatters Ltd) was used for contig assembly from partial reads, the editing of base calls and concatenation of partial genomic regions. The resulting nucleotide sequences were deposited in the NCBI database (accession numbers MG881818, MG881819, MG878434, MG878435) and used to search against the NCBI database using BLAST. All sequences having the similarity values equal or greater than 99% for ITS and 97% for 28S rDNA were compared using MEGA 7 software (Kumar *et al.*, 2016). Subsequently, Maximum Likelihood (ML) and Maximum Parsimony (MP) phylogenetic trees were constructed to trace the relationships among selected GenBank records and sequences obtained in this study. Moreover, for the ITS tree we used additional sequences representing the main *Leveillula* groups outlined by Khodaparast *et al.* (2012). The best-fit evolution model (*Tamura-3 parameter with gamma distribution*) was selected with Find Best DNA/Protein Models option implemented in MEGA 7. Alignment gaps were treated as missing information.

## RESULTS

### *Symptoms of infection*

Symptoms of powdery mildew infection consisted of extensive growth of white, superficial coatings on upper and lower leaf surfaces. Newly infected leaves had sparse coverings of powdery mildew. As the disease progressed, white mycelia completely covered both leaf surfaces (Figure 1).

### *Morphology of the fungus*

The morphological features of both herbarium samples of powdery mildew on *Lactuca* spp. are summarized as follows. Mycelium was external and (probably)



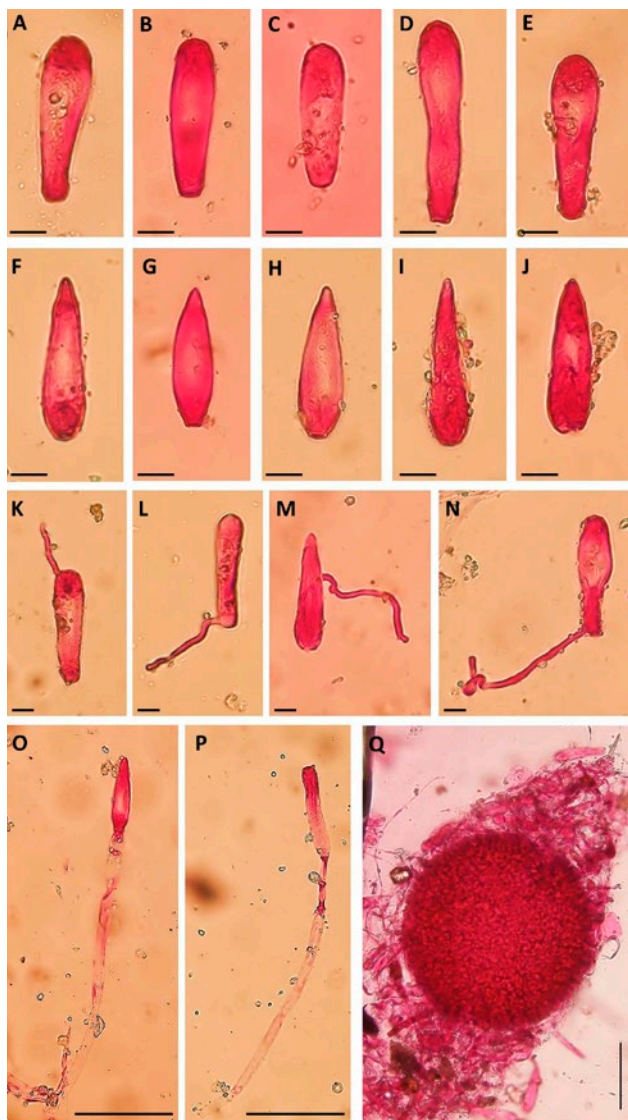
**Figure 1.** Symptoms of powdery mildew infections on lower sides of leaves of A, *Lactuca serriola* f. *integrifolia*, and B, *L. serriola* f. *serriola*.

internal, which was hard to confirm because of the age of the herbarium specimens. White, dense and persistent, mostly superficial mycelium occurred on leaves. Two types of conidia were produced separately on conidiophores (Figure 2, A-J). Primary conidia were lanceolate, long with pointed apices and rounded bases, and measured 39–56  $\times$  11–13  $\mu\text{m}$ , with length to width ratios of 3.2–4.9. Secondary conidia were mostly clavate, 39–66  $\times$  11–16  $\mu\text{m}$ , with length/width ratios of 2.9–4.9. Germ tubes were recorded on primary and secondary conidia; arising mostly from an end of each germinating conidium, rarely from the side. Germinating conidia usually had singly long germ tubes, the apices of which were mostly simple, but sometimes curved (Figure 2, K-N). Conidiophores with primary and secondary conidia were observed (Figure 2, O and P), and these were 117–244  $\mu\text{m}$  long, with foot cells measuring 46–175  $\mu\text{m}$  long, 6–8.5  $\mu\text{m}$  wide and mostly with 2–4 distal cells. Chasmothecia were also observed, but these were probably not mature. They were 92–219  $\mu\text{m}$  in diameter, with very short and few appendages, and without asci (Figure 2, Q).

The shape of primary and secondary conidia of the *Leveillula* found on the *Lactuca* spp. accessions in Jordan were very similar to micrographs of primary and secondary conidia of *L. lactucae-serriolae* from Iran, published by Khodaparast *et al.* (2012).

### *Molecular identification of powdery mildew*

The nucleotide sequences of the 28S rRNA gene and ITS regions were determined for the two analysed speci-



**Figure 2.** Micrographs of *Leveillula lactuca-serriolae*. A-E, shape variability of primary conidia; F-J, secondary conidia; K-N, germ development from primary and secondary conidia; O, conidiophore with primary conidia; P, conidiophore with secondary conidia; Q, chasmothecium. Bars = 10  $\mu$ m in A-N) and 50  $\mu$ m in O-Q).

mens. Identical nucleotide sequences for both specimens were obtained, with the total lengths of 673 bp for ITS and 875 bp long contig for 28S rRNA.

Comparison of the ITS from this study with sequences available in the GenBank database revealed 100% similarity with the “type” record of *L. lactuca-serriolae* (Accession no. AB044375) infecting *Lactuca serriola* from Iran (Khodaparast *et al.*, 2001). There is another ITS record in the GenBank database (Accession no. HQ821500) described as *L. lactuca-serriolae*, which was extracted from *Hexinia polydichotoma* by Xu

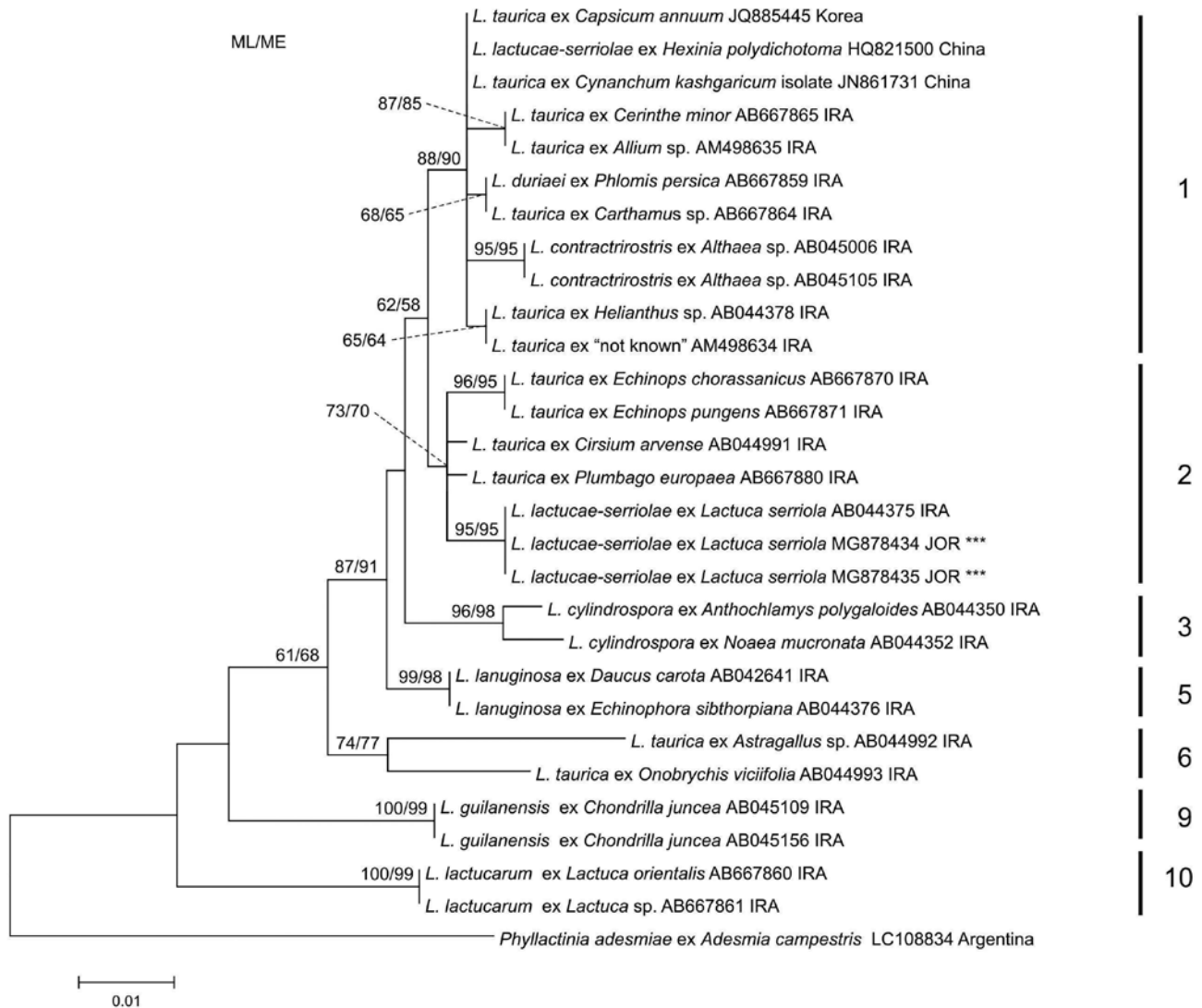
*et al.* (2011). To inspect these records in more detail, we aligned the above ITS sequences with selected sequences representing the main *Leveillula* groups outlined by Khodaparast *et al.* (2012), and performed phylogenetic analyses. The resulting ML tree proved the identity of all *Leveillula* samples originating from *L. serriola*, which formed a separate sub-group within clade No. 2, consisting of “type” specimens of *L. lactuca-serriolae* AB044375 and the two specimens sequenced in this study (Figure 3). On the other hand, the sequence HQ821500 extracted from *Hexinia polydichotoma* by Xu *et al.* (2011) fell within clade No. 1, together with *Leveillula taurica* samples originating from Korea and China.

The BLASTn search of part of the sequenced 28S rDNA region revealed 99% similarity to 28S rDNA sequences of different *Leveillula* species: *L. lactuca-serriolae* (HQ821501 ex *Hexinia polydichotoma*) reported by Xu *et al.* (2011); *L. duriaei* (AB080475 ex *Salvia nemorosa*) and *Leveillula* sp. (AB080478 ex *Chondrilla juncea*), reported by Takamatsu *et al.* (2008) (Figure 3).

## DISCUSSION

As mentioned above, the only study of powdery mildews occurring in Jordan was that of Qasem and Abu-Blan (1986), where only *Leveillula taurica* was confirmed. However, occurrence of powdery mildew species is rarely limited by state borders. In surrounding Near East countries, powdery mildews have been surveyed, but not with the same intensity in various countries. In the reviews of El-Kazzaz *et al.* (1989), Voytyuk *et al.* (2009), Severoglu and Ozyigit (2012) and Kabaktepe *et al.* (2015), the most common powdery mildew on Asteraceae was *Leveillula taurica*. However, in Turkey *L. lactuca-serriolae*, *L. lactucarum* and *L. picridis* were also recorded (Kabaktepe *et al.*, 2015). Voytyuk *et al.* (2009) reported *Leveillula lactuca-serriolae*, *L. picridis* and *Leveillula* spp. on Asteraceae in Israel. *Leveillula* spp. was separated into three species, and *L. osteospermi* and *L. wasserii* were described as new species (Voytyuk *et al.*, 2009; Braun and Cook, 2012). Detailed studies of powdery mildews in Iran were completed by Khodaparast *et al.* (2001, 2007, 2010, 2012, 2016), and the pathogens were mainly in *Leveillula*. These authors introduced some new species of *Leveillula*, including *L. guilanensis* and *L. lactuca-serriolae*, on Asteraceae (Khodaparast *et al.*, 2002).

According to our observations, the shapes of primary and secondary conidia of *Leveillula* found on *Lactuca* spp. accessions in Jordan are very similar to those in micrographs of *L. lactuca-serriolae* published by



**Figure 3.** Maximum-Likelihood tree based on ITS nucleotide sequences of 28 taxa of *Leveillula* and single outgroup taxa. Numbers above branches indicate bootstrap values based on 1000 replications of ML and ME phylogeny (Bootstrap values less than 50% are not shown). Numbers to the right indicate the clades designated by Khodaparast *et al.* (2012). \*\*\* indicates specimens analysed in the present study.

Khodaparast *et al.* (2012). Differences were found in size, but not in shape, since in our observations (Table 2) the sizes of both types of conidia were smaller than those described by Khodaparast *et al.* (2012). Khodaparast *et al.* (2002), who first introduced *L. lactucae-serriolae*, described this species as morphologically very close to *L. taurica*, since the primary conidia of the two species are similar, but *L. lactucae-serriolae* differed in having more distinctly clavate (widest in the upper half) secondary conidia. However, this species is genetically clearly distinct, forming a separate clade (Khodaparast *et al.*, 2001), so it cannot be conspecific with *Leveillula taurica*. According to Braun and Cook (2012), *L. lactucae-serriolae* occurs on *Lactuca* (*L. azerbaijanica*, *L. scarioloides*,

*L. serriola*) in Asia (Iran, Israel, Lebanon, Turkmenistan, and Armenia).

Another newly described powdery mildew species on *Lactuca* spp. in Jordan is *Leveillula lactucarum*, known for 30 years, and described by Braun and Cook (2012) on *Chondrilla*, *Hexinia*, and *Lactuca* (*L. orientalis*, *L. tatarica*, *L. viminea*) in Asia (Afghanistan, China, Iran, Kazakhstan, Kyrgyzstan, Turkey, Turkmenistan, Armenia, and Azerbaijan), and Europe. These authors suspected that other collections belonged to this group. *Leveillula lactucarum* is well characterized as having subcylindrical primary conidia which are narrowed toward pointed apices. This contrasts with the long, lanceolate primary conidia and more or less subclavate secondary

**Table 2.** The main morphological characteristics of *Leveillula lactucae-serriolae* samples originated from Jordan (collected by A. Lebeda, in 2007) and Iran (Khodaparast *et al.*, 2002).

Origin (host plant species and country)	Conidia <sup>a</sup>				Comidiophores <sup>a</sup>				Chasmothecia <sup>a</sup>	
	Primary		Secondary		Mean length (µm) ± SD (min – max)		Mean basal cell (µm) ± SD (min – max)			Mean diameter (µm) ± SD (min – max)
	Mean length (µm) ± SD (min – max)	Length/ Width Ratio	Mean length (µm) ± SD (min – max)	Length/ width Ratio	Mean length (µm) ± SD (min – max)	SD (range)				
<i>Lactuca serriola</i> f. <i>integrifolia</i> (Jordan)	50.8 ± 3.3 (43.9 – 53.7)	4 ± 0.2 (3.6 – 4.2)	52.8 ± 5.5 (39 – 61)	13.9 ± 1.1 (10.9 – 15.9)	146 ± 38 (117.1 – 200)	70.7 ± 23.5 (46.3 – 102.5)	2.7 ± 0.4 (2 – 4)	154.1 ± 43.9 (92.7 – 219.6)		
<i>Lactuca serriola</i> f. <i>serriola</i> (Jordan)	50.4 ± 4.6 (39 – 56)	4.1 ± 0.4 (3.2 – 4.9)	52.4 ± 5.5 (39 – 65.9)	13.2 ± 1.1 (11 – 15.9)	190 ± 40.5 (126.9 – 244)	108.4 ± 32.6 (53.7 – 175.7)	2.8 ± 0.8 (2 – 4)	154.5 ± 13.8 (134.2 – 170.8)		
<i>Lactuca serriola</i> (Iran)	53 – 75	3.1 – 5	49 – 75	13 – 18	n.a.	n.a.	n.a.	187 – 225		

<sup>a</sup> Means ± SDs and ranges, where available.

<sup>b</sup> Information not available.

conidia of *L. lactucae-serriolae*. Furthermore, the micrographs of primary and secondary conidia of *L. lactucarum* on *Lactuca* (e.g. *L. orientalis*) presented by Khodaparast *et al.* (2012) show conidia that are very different from our samples.

*Leveillula lactucae-serriolae* and *L. lactucarum* are well supported molecularly and morphologically. The taxonomic positions of several species were generally well supported by morphology especially that of primary conidia (Khodaparast *et al.*, 2012). *Leveillula lactucae-serriolae* was placed in clade No. 2, while *L. lactucarum* was placed in clade No. 10, different from *Leveillula taurica* (clade No. 1).

Nucleotide sequences of two genomic regions were determined in the present study. ITS proved that all *Leveillula* samples originating from *L. serriola* (including “type” material from Iran), formed a well resolved *L. lactucae-serriolae* sub-clade on the ML phylogenetic tree (Figure 3). In contrast, GenBank record HQ821500 deposited as *L. lactucae-serriolae* extracted from *Hexinia polydichotoma* fell within the *L. taurica* samples. Although Xu *et al.* (2011) stated that the BLASTn search of HQ821500 returned 99% similarity with “type” *L. lactucae-serriolae* record AB044375, these authors ignored 100% identity of HQ821500 to 11 *Leveillula taurica* ITS records (JN861731, JQ885445). It is evident that the taxonomic denomination of HQ821500 is doubtful and should be corrected to *L. taurica* or *Leveillula* sp.

We cannot perform direct comparison of the 28S rDNA sequence to the “type” material of *L. lactucae-serriolae* since it is not deposited in GenBank. Nevertheless, the inspection of 28S rDNA nucleotide alignment of *Leveillula* sequences obtained in the present study with the most similar GenBank records (i.e. HQ821501, AB080475, AB080478 having 99% identity; alignment not shown) proved that none of the analysed sequences is identical with the two *L. lactucae-serriolae* specimens from Jordan. These differ from others in a single SNP with record HQ821501 (*L. lactucae-serriolae* ex *Hexinia polydichotoma*) and another SNP with *L. duri-aei* (AB080475 ex *Salvia nemorosa*). The two deposited sequences (HQ821501 and AB080475) are also not identical, and there is high probability that each of these sequences represents a different taxon. Moreover, the record of HQ821501 published by Xu *et al.* (2011) is linked to the problematic ITS record of HQ821500 discussed above, and most likely does not represent 28S rDNA of *L. lactucae-serriolae*. Therefore, it should be corrected to *Leveillula* sp.

In conclusion, the sequencing of the ITS region of powdery mildew obtained from two *Lactuca serriola* plants in Jordan proved their identity with “type” mate-

rial of *Leveillula lactucae-serriolae* described by Khodaparast *et al.* (2012). Sequencing of 28S rDNA region provided the first verified GenBank record of *Leveillula lactucae-serriolae* deposited in a public nucleotide repository. The sequencing of both genomic regions clearly throws doubt on the *L. lactucae-serriolae* record on *Hexinia polydichotoma*. This first record of *L. lactucae-serriolae* on wild *Lactuca* species in Jordan is, however, not surprising because the environmental conditions in this area favoured the occurrence of powdery mildew of this genus.

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