Discovery of a Cryptic Species, *Erysiphe Salicina* Sp. Nov., and Reconstruction of The Phylogeny of Powdery Mildews On *Populus* and *Salix* Spp.

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Research Article

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Abstract

Recently performed phylogenetic-taxonomic analyses of species belonging to *Erysiphe* sect. *Uncinula* on willows (*Salix* spp.) demonstrated a much higher diversity than previously assumed. Phylogenetic analyses and morphological examinations of Chinese *Erysiphe* collections on *Salix abscondita* (= *S. raddeana*), *S. sinica* and *S. taraikensis*, all belonging to *Salix* subgen. *Vetrix* sect. *Vetrix*, revealed an additional cryptic species in sister position to the *E. salicis* clade, which is described as *Erysiphe salicina*. The new species clearly distinguished from all allied species in morphological and genetical results. The phylogeny of the new species and closely related species on *Populus* and *Salix* spp. (Salicaceae) was reconstructed and discussed using a combined alignment of the internal transcribed spacer (ITS) regions and 28S rDNA sequences. The present phylogenetic analyses suggest that the recently described *E. salicicola* (on *Salix gracilistyla* in South Korea) has to be reduced to synonymy with *E. salicis* var. *salicis-gracilistylae* (º *Uncinula salicis-gracilistylae*).

Introduction

The genus *Salix* L. (Salicaceae), commonly known as willows, consists of 450–520 species, depending on the applied species concepts, which are mainly distributed throughout the Northern Hemisphere. It is one of the main groups of trees and shrubs in the North Temperate Zone (Fang et al. 1999; Skvortsov 1999; Argus 2010). *Salix* comprises valuable ecological species that are commonly used in conservation and environmental projects for habitat restoration and erosion control (Kuzovkina and Quigley 2005; Kuzovkina and Volk 2009). *Salix* is divided into three subgenera: *Salix*, *Chamaetia* and *Vetrix* (Skvortsov 1999; Chen et al. 2010). Species of *Salix* are diverse in China (275 spp., Fang et al. 1999). *Salix sinica* (K. S. Hao ex C. F. Fang & Skvortsov) G. Zhu is a species was originally described as *Salix caprea* var. *sinica* K. S. Hao (Zhu 1998). It is considered a species of its own, according to the current taxonomic concept recognized in Flora of China and belongs to *Salix* sect. *Vetrix* (http://www.iplant.cn/info/Salix%20sinica?t=z).

Salix spp. are commonly infected by powdery mildews almost worldwide. Four genera, six species, and two varieties of powdery mildews have been recorded parasitizing *Salix* spp., viz., *Podosphaera schlechtendalii* Lév., *Phyllactinia populi* (Jacz.) Y.N. Yu, *Pleochaeta salicicola* R.Y. Zheng and G.Q. Chen, *Erysiphe adunca* (Wallr.) Link var. *adunca* and var. *salicis-gracilistylae* (Homma) U. Braun and S. Takam., *E. capreae* DC. ex Duby, and *E. pseudoregularis* U. Braun (Braun and Cook 2012). The three species of *Erysiphe* sect. *Uncinula* on willows are mainly distributed in Asia (China, India, Japan, Korea, Russia [Siberia and Far East]), Europe and North America (Braun and Cook 2012). Recently, a comprehensive morphological and phylogenetic revision of the *E. adunca* complex is conducted by Darsaraei et al. (2021). They reinstated of *E. salicis* DC. (on *Salix* spp. belonging to various subgenera of *Salix* in Asia and Europe) as a recognized species of its own, introduced the new combination *Erysiphe salicis* var. *salicis-gracilistylae* (Homma) and reduced *Erysiphe pseudoregularis* to synonymy with *E. capreae*. The morphological characteristics of a powdery mildew found in China on *Salix sinica*, *S. taraikensis* and *S. abscondita* turned out to be different from all recognized *Erysiphe* spp. on *Populus* and *Salix* spp. in terms of morphology and phylogenetic position. Furthermore, the phylogenetic position and taxonomic status of *Erysiphe salicicola*, recently described from the Republic of Korea on *Salix gracilistyla* var. *melanostachys* (Boonmee et al. 2021), have been reassessed. Sequences retrieved from the new Chinese species and from *E. salicicola* have been added to the comprehensive phylogenetic analyses recently published by Darsaraei et al. (2021) in order to update the phylogeny of *Erysiphe* (sect. *Uncinula*) spp. on Salicaceae.

Materials And Methods

Specimens examined

Twenty-eight specimens of *Erysiphe* species on *Salix* and *Populus* were collected in China and Russia. They are now deposited in the Herbarium of Mycology of Jilin Agricultural University (HMJAU). Details of these specimens are provided in Table 1.

Table 1
Information about specimens of *Erysiphe* species on *Salix* and *Populus* spp. used in this study.

Species	Herbarium No.	Host plant	Location	Coordinates	Altitude(m)	Collection date	ITS+28S rDNA Accession No.
Erysiphe salicina sp. nov.	HMJAU-PM91918	*Salix sinica*	Yichun, Heilongjiang Province, China	130°24'38"E; 48°53'42"N	177	2014/08/30	MZ474877
	HMJAU-PM91919	*S. sinica*	Tahe, Heilongjiang Province, China	124°41'28"E; 52°20'20"N	384	2018/10/06	MZ474891
	HMJAU-PM91920	*S. taraikensis*	Heihe, Heilongjiang Province, China	127°29'55"E; 49°57'47"N	114	2014/09/17	MZ474875
	HMJAU-PM91921	*S. sinica*	Heihe Heilongjiang Province, China	127°29'55"E; 49°57'47"N	114	2014/09/17	MZ474880
	HMJAU-PM91922	*S. sinica*	Jiagedaqi, Heilongjiang Province, China	124°7'29"E; 50°25'57"N	398	2015/09/01	MZ474876
	HMJAU-PM91923	*S. sinica*	Tahe, Heilongjiang Province, China	123°31'41"E; 52°47'5"N	776	2016/09/23	MZ474883
	HMJAU-PM91924	*S. sinica*	Tahe, Heilongjiang Province, China	125°16'41"E; 52°41'53"N	376	2016/09/25	MZ474881
	HMJAU-PM91925	*S. sinica*	Harbin, Heilongjiang Province, China	127°23'1"E; 45°29'35"N	374	2019/08/24	MZ474882
	HMJAU-PM91979	*S. taraikensis*	Heihe, Heilongjiang Province, China	127°29'55"E; 49°57'47"N	114	2014/09/17	MZ948832
	HMJAU-PM91989	*S. abscondita*	Beijing, China	115°34'19"E; 39°50'15"N	1133	2018/10/19	MZ948845
E. salicis var. *salicis-gracilistylae*	HMJAU-PM91964	*Salix* sp.	Harbin, Heilongjiang Province, China	126°35'19"E; 45°47'29"N	120	2019/09/14	OL739284
	HMJAU-PM91965	*S. wilhelmsiana*	Changchun, Jilin Province, China	116°29'16"E; 40°30'13"N	216	2018/09/17	OL763835

Species	Herbarium No.	Host plant	Location	Coordinates	Altitude(m)	Collection date	ITS+28S rDNA Accession No.
	HMJAU-PM91972	*S. babylonica*	Harbin, Heilongjiang Province, China	126°35'19"E; 45°47'29"N	120	2019/09/14	OL763833
	HMJAU-PM91914	*S. ernestii*	Harbin, Heilongjiang Province, China	127°21'51"E; 45°30'14"N	374	2019/08/24	OL763836
E. salicis var. *salicis*	HMJAU-PM91968	*S. abscondita*	Erdao Baihe, Jilin Province, China	128°5'46"E; 42°40'44"N	678	2020/09/05	OL770127
Erysiphe capreae	HMJAU-PM91963	*Salix acutifolia*	Russia	——	——	2018/10/06	OL739394
	HMJAU-PM91973	*S. wallichiana*	Heihe, Heilongjiang Province, China	127°29'36"E; 49°57'28"N	112	2014/09/17	OL763832
	HMJAU-PM91974	*S. wallichiana*	Heihe, Heilongjiang Province, China	127°28'43"E; 49°57'11"N	123	2017/09/01	OL739522
	HMJAU-PM91975	*S. wallichiana*	Heihe Heilongjiang Province, China	127°28'43"E; 49°57'11"N	123	2017/09/01	OL763834
	HMJAU-PM91976	*S. eriocarpa*	Erdao Baihe, Jilin Province, China	127°52'30"E; 42°27'29"N	681	2020/09/02	OL739580
	HMJAU-PM91977	*Salix* sp.	Erdao Baihe, Jilin Province, China	127°52'30"E; 42°27'29"N	681	2020/09/02	OL739581
	HMJAU-PM91986	*S. wallichiana*	Heihe, Heilongjiang Province, China	127°29'36"E; 49°57'28"N	112	2014/09/17	OL739609
	HMJAU-PM91987	*Salix* sp.	Mohe, Heilongjiang Province, China	122°21'58"E; 53°29'15"N	291	2016/09/26	OL739612
E. adunca	HMJAU-PM91910	*Populus ×canadensis*	Russia	——	——	2018/11/03	OL744369
	HMJAU-PM91980	*P. davidiana*	Erdao Baihe, Jilin Province, China	128°4'33"E; 42°24'48"N	668	2020/09/04	OL744370

Species	Herbarium No.	Host plant	Location	Coordinates	Altitude(m)	Collection date	ITS+28S rDNA Accession No.
	HMJAU-PM91981	*P. koreana*	Erdao Baihe, Jilin Province, China	128°6'52"E; 42°26'12"N	682	2020/09/06	OL744371
E. mandshurica	HMJAU-PM91982	*P. koreana*	Mohe, Heilongjiang Province, China	122°21'58"E; 53°29'15"N	291	2016/09/26	OL744376
	HMJAU-PM91983	*P. adenopoda*	Shennongjia, Hubei Province, China	110°0'12"E; 31°29'12"N	1727	2020/09/27	OL744377

Morphological examinations

Anamorphs and teleomorphs of dried herbarium specimens were examined in lactic acid using a light microscope (Axio Scope A1, ZEISS, Germany). The sizes of diverse structures of the anamorphs and teleomorphs were measured with at least 30 repetitions. If possible, measurements of microscopic structures were made at a magnification of ×1000, and 95% confidence intervals were determined.

DNA extraction, PCR, and sequencing

Whole-cell DNA was extracted by the Chelex-100 method (Walsh et al., 1991; Hirata and Takamatsu, 1996) from mycelia and chasmothecia. The complete internal transcribed spacer (ITS) region (including 5.8S rDNA) and 5'-end of the 28S rDNA (including D1 and D2 regions) of the fungi were amplified by polymerase chain reaction (PCR) using the primer sets ITS5/ITS4, PM10/PM2, PM5/PM6 for ITS region and LSU1/LSU2, PM3/TW14 and PM28F/PM28R for partial of 28S rDNA region. The details of primers are presented in Table 2. The reaction components were 2 μL of the total genomic DNA, 12.5 μL Premixed PCR mixtures (TaKaRa, Tokyo, Japan), 1 μL of each primer (10 μM) and sterile ddH_2O up to a final volume of 25 μL. The PCR reactions were conducted under the following thermal cycling conditions: an initial predenaturation step of 5 min at 95 ℃, 35 cycles of 30 s at 95 ℃, followed by 1 min at 56 ℃ for annealing, and 30 s at 72 ℃ for extension, and a final extension step of 8 min at 72 ℃. The PCR products were subjected to electrophoresis in a 1.2% agarose gel in 0.5 × TBE buffer. The amplicons were sent to Sangon Biotech (Shanghai, China) for direct sequencing in the forward and reverse direction using the same primers as for the PCR. The accession numbers are shown in Table 1. Details of the sequences retrieved from GenBank are shown in Supplementary Table 1.

Table 2
Primers used in this study.

Fragment	Name	Direction	Primer sequences (5' → 3')	Reference
ITS region	ITS5	Forward	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	White et al. (1990)
	PM10	Forward	GGCCGGAAAGTTGTCCAAAC	Bradshaw and Tobin (2020)
	PM2	Reverse	TCACTCGCCGTTACTGAGGT	Cunnington et al. (2003)
	PM5	Forward	TTGCTTTGGCGGGCCGGG-	Takamatsu and Kano (2001)
	PM6	Reverse	GYCRCYCTGTCGCGAG	Takamatsu and Kano (2001)
partial 28S rDNA	LSU1	Forward	ACCCGCTGAACTTAAGCATA	Scholin et al. (1994)
	LSU2	Reverse	CCTTGGTCCGTGTTTCAAGA	Scholin et al. (1994)
	PM3	Forward	GKGCTYTMCGCGTAGT	Takamatsu and Kano (2001)
	TW14	Reverse	GCTATCCTGAGGGAAACTTC	Mori et al. (2000)
	PM28F	Forward	TAACGGCGAGTGAAGCGGTA	Bradshaw and Tobin (2020)
	PM28R	Reverse	ACGTTCACTTTCATTCCGCG	Bradshaw and Tobin (2020)

Molecular phylogenetic analysis

The obtained sequences, including the complete ITS and partial 28S rDNA from eight herbarium specimens, were aligned with other sequences retrieved from GenBank. Multiple sequence alignments were conducted by MUSCLE implemented in MEGA-X (Kumar et al. 2018). Alignments were further manually refined and deposited in TreeBASE (http://www.treebase.org/) under the accession number S28710. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods. MP analysis was performed in PAUP 4.0 (Swofford 2002) with the heuristic search option using the tree bisection reconnection (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analysis using 1000 replications with the step-wise addition option set as simple (Felsenstein 1985). Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. The ML analysis was performed using raxmlGUI 2.0 (Edler et al. 2020), under a GTRGAMMA model. The BS supports and trees were obtained by running rapid bootstrap analysis of 1000 pseudo-replicates followed by a search for the tree with the highest likelihood.

Results

Taxonomy

Erysiphe salicina J. Feng, S.-Y. Liu & Y. Li, **sp. nov.**

Mycelium epiphyllous, effuse or in patches, sometimes confluent and covering the entire leaf surface, white, moderately thick, evanescent to persistent (Fig. 1a–c). Hyphal cells about 3–6 µm wide; hyphal appressoria solitary or in opposite pairs, lobed to multilobed, coral-like (Fig. 1d–g); conidiophores arising from the upper surface of the mother cell, erect, 48–87 µm long, foot-cells cylindrical, straight, occasionally slightly sinuous at the base, 17–59 × 4–7 µm, followed by 0–2 usually shorter cells, forming conidia singly (Fig. 1h); conidia ellipsoid-cylindrical, usually 20–29 × 8–14 µm, with a length/width ratio varying from 1.8 to 2.9 (Fig. 1i–l), germ tubes almost terminal, short, conidial appressoria multilobate, some showing longitubus pattern, produced laterally, near the middle or in perihilar position (Fig. 1m–n). Chasmothecia gregarious or

scattered, 112–149 µm diam. (Fig. 1o); peridium cells irregularly polygonal to rounded, 8–16 µm diam. (Fig. 1p); appendages numerous, 22–53 per chasmothecium, rarely more, ± equatorial, straight and stiff, simple, 104–169 µm long (0.8–1.3 times as long as the chasmothecial diam.), width almost equal throughout or somewhat wider above, i.e., coiled part somewhat enlarged, width usually about 5–8 µm near the base, 13–19 µm towards the coiled tip (Fig. 1q–s), aseptate, hyaline, wall thin throughout, smooth, occasionally somewhat rough at the base, apices tightly or loosely uncinate-circinate or subhelicoid; asci 5–7, ellipsoid-obovoid, saccate, 57–79 × 29–50 µm, short-stalked, sometimes almost sessile, 4–7-spored (Fig. 1t–w); ascospores oblong ellipsoid-ovoid, 24–34 × 11–17 µm, colourless (Fig. 1x).

Diagnosis: Differs from all known *Erysiphe* species on hosts belonging to *Salix* in having hyphal appressoria in opposite pairs, shorter conidiophores, up to 87 µm, smaller conidia, 20–29 × 8–14 µm, chasmothecia with fewer and shorter appendages and larger ascospores, and by forming a monophyletic clade separate to all other *Erysiphe* species in phylogenetic analyses.

Type: China, Heilongjiang Province, Yichun, Jiayin Maolanggou National Forest Park, on *Salix sinica*, 16 Aug. 2014, Shu-Yan Liu and Feng-Yun Zhao (HMJAU-PM91918 – holotype; HMAS249775 – isotype, MycoBank, MB 840551); ex-holotype sequence – MZ474877 (ITS+28S).

Etymology: Composed of the name of the host genus, *Salix*, + Latin adjectival suffix -inus = belonging to.

Host range and distribution: On *Salix abscondita* (= *S. raddeana*), *S. sinica* and *S. taraikensis* (*Salix* subgen. *Vetrix* sect. *Vetrix*, Salicaceae), Asia (China).

Specimens examined: China, Heilongjiang Province, Tahe County, Qixia Mountain Botanical Garden, on *S. sinica*, 6 Oct. 2018, Feng-Yun Zhao, Zhen-Zhen Wang, Wei-Wen Yan (HMJAU-PM91919); Heilongjiang Province, Heihe City, Aihui Town, Aihui National Forest Park, 17 Sep. 2014, Feng-Yun Zhao, Jian Liu, Shu-Rong Tang, Peng-Lei Qiu (HMJAU-PM91920, HMJAU-PM91921, HMJAU-PM91963); Heilongjiang Province, Jiagedaqi, Beishan Park, 1 Sep. 2015, Wen-Tao Jiang, Jia-Ni Li, Lei Zhao, Feng-Yun Zhao (HMJAU-PM91922); Heilongjiang Province, Tahe County, Zhangling Forest Farm, 23 Sep. 2016, Feng-Yun Zhao, Guan-Xiu Guan, Jia-Ni Li, Peng-Lei Qiu (HMJAU-PM91923); Heilongjiang Province, Tahe County, Lvhai Holiday Inn, 25 Sep. 2016, Feng-Yun Zhao, Guan-Xiu Guan, Jia-Ni Li, Peng-Lei Qiu (HMJAU-PM91924); Heilongjiang Province, Harbin, Jinlongshan National Forest Park, 24 Aug. 2019, Jing Feng, Han-Xing Gao (HMJAU-PM91925). Heilongjiang Province, Heihe City, Aihui Town, Aihui National Forest Park, on *S. taraikensis*, 17 Sep. 2014, Feng-Yun Zhao, Jian Liu, Shu-Rong Tang, Peng-Lei Qiu (HMJAU-PM91920, HMJAU-PM91963); Beijing, Baihua Mountain, on *S. abscondita* (= *S. raddeana*), 19 Oct. 2018, Shu-Rong Tang, Li Liu, Peng-Lei Qiu (HMJAU-PM91989).

Notes: *Erysiphe salicina* morphologically differs from *E. capreae*, *E. salicis* var. *salicis* and *E. salicis* var. *salicis-gracilistylae* in having shorter conidiophore foot-cells [17–59 × 4–7 µm vs (25–)35–90(–110) × 5–9 µm in *E. capreae* and 15–45 × 5–9 µm in *E. salicis*] and smaller conidia (20–29 × 8–14 µm vs 25–45 × 10–24 µm in *E. capreae* and 23–40 × 10–18 µm in *E. salicis*). The ascospores are larger (24–34 × 11–17 µm) compared to *E. salicis* var. *salicis* (16–29 × 9–16 µm) and *E. salicis* var. *salicis-gracilistylae* (19–24 × 10–18 µm). In addition, *Erysiphe salicina* has chasmothecia with less appendages per chasmothecium [22–53 vs (33–)50–400 in *E. capreae*, and 25–95 in *E. salicis*], and appendages with enlarged uncinate-circinate apices (vs tightly uncinate-circinate, not enlarged apices in *E. salicis*). Furthermore, the appendages are regular, i.e., without constrictions and swellings, compared to *E. salicis* var. *salicis-gracilistylae*, and they arise equatorially, as in *E. salicis*, compared to *E. capreae* that are not equatorial or only somewhat in the upper half, and have a tendency to point upwards with maturity. *Erysiphe adunca* s. str. on *Populus* spp. (Salicaceae) is genetically as well as morphologically also distinguished from *E. salicina* by having much longer conidiophores, 70–150 µm, and foot-cells, 40–110 µm, larger conidia, 28–38 × 14–18 µm, and much longer chasmothecial appendages, 1–3 times as long as the chasmothecial diam. (Darsaraei et al. 2021).

Phylogenetic analysis

Twenty-eight nucleotide sequences spanning the ITS1-5.8S rDNA-ITS2 and 28S rDNA D1/D2 domains were generated in this study. These sequences were aligned with 42 sequences of *E. adunca* s. lat. taken from Darsaraei et al. (2021) and 2 sequences of *E. salicicola* (Boonmee et al. 2021). A sequence of *E. australiana* (AB022408) was used as an outgroup taxon in accordance with Takamatsu et al. (2015). The alignment consisted of 73 sequences and 1269 characters, of which 133 characters were variable and 141 were informative for parsimony analysis. The maximum parsimony tree (TL = 397, CI = 0.8615, RI = 0.9726, RC = 0.8378) with the highest likelihood value is shown in Figure 2. The ML tree topology was almost identical to the MP tree and only bootstrap supports are shown in the MP tree.The 72 ingroup sequences are divided into five groups. Three groups (Group , and) consist of sequences from powdery mildews on *Salix* spp. and the other two groups (Group and) are composed of sequences from powdery mildews on *Populus* spp. All five groups are supported by BS analyses with 70% or higher. Group contains all *Erysiphe salicina* sequences obtained from powdery mildews on *Salix* spp. and forms an independent clade supported by a bootstrap value of 100%. Group contains two clades, viz., *E. salicis* var. *salicis-gracilistylae*, including two sequences obtained from *E. salicicola*, and *E. salicis* var. *salicis* supported by a bootstrap value of 100%.

Discussion

Salix is a common host genus for powdery mildews of various genera. Four genera, seven species and two varieties of powdery mildews are currently recognized on *Salix* spp. (Zheng and Chen 1978; Yu and Lai 1979; Braun and Cook 2012; Darsaraei et al. 2021; Boonmee et al. 2021). Powdery mildews in the genus *Erysiphe* the predominant species infecting *Salix* hosts and are geographically widespread. Molecular phylogenetic analyses plays an important role in the fungal taxonomy of the powdery mildews. Genetic evaluations are the main method to discover new species, above all cryptic species within complexes (Kirschner et al. 2020; Qiu et al. 2020; Yamaguchi et al. 2021). The recently published phylogenetic-taxonomic revision of the *Erysiphe adunca* complex (Darsaraei et al. 2021) is a case in point, and provides the basis for the present examinations and analyses. The comparative morphology and phylogenetic analysis from the current study elucidate several issues with this group of fungi including the recent publication of the species *E. salicicola*. The morphology of *E. salicicola* is consistent with *E. salicis* var. *salicis-gracilistylae* (≡ *Uncinula salicis-gracilistylae*), and sequences retrieved from *E. salicicola* cluster within the *E. salicis* var. *salicis-gracilistylae* subclade of the *E. salicis* clade, suggesting that *E. salicicola* has to be reduced to synonymy with *E. salicis* var. *salicis-gracilistylae*.

Powdery mildews found on multiple *Salix* spp. in China have been morphologically and phylogenetically examined. The species is genetically and morphologically distinct from all allied *Erysiphe* species on *Populus* and *Salix* spp. (see details above under notes) and is thus described as *E. salicina* sp. nov. At first glance, the result was unexpected, because the most common host plant of the new species, *S. sinica*, was originally described as *S. caprea* var. *sinica*, i.e., this taxon is closely related to *S. caprea* and both species pertain to *Salix* subgen. *Vetrix* sect. *Vetrix*. *Salix caprea* is the principal host of *E. capreae*, which is common in the northern hemisphere. Before our analysis we expected that *E. capreae* also be on *S. sinica*. The occurrence of a separate species on two hosts belonging to *Salix* subgen. *Vetrix* sect. *Vetrix*, which is morphologically and phylogenetically close to *E. salicis*, suggests a higher and not yet fully elucidated diversity of *Erysiphe* on *Salix* spp. in Asia, and in particular China. We hypothesize that future research will reveal additional cryptic species, hidden under previous reports as *Erysiphe* (*Uncinula*) *adunca*.

There are additional open questions that will require analyses. For example, Zhu (1998) supposed that *S. sinica* collections from northern and northeastern China have often been misidentified as *S. caprea*. *Erysiphe* spp. on these hosts require further examinations in northeastern China. *Erysiphe salicis* var. *salicis* has not yet been verified for China and future collection efforts are needed to elucidate whether *E. salicis* var. *salicis* is distributed in China. Additionally, *Erysiphe adunca* on *Populus* spp. in China needs to be evaluated. It is currently known that *E. adunca* is a common and widespread species on a wide range of *Populus* spp. in Asia, Europe and North America (Darsaraei et al. 2021). Several *Uncinula* spp., based on Chinese specimens on poplars, have been described (Zhen and Chen 1977), but all names were reduced to synonymy with *E. adunca* (Braun and Cook 2012, Darsaraei et al. 2021). It is still unclear whether all Chinese *Erysiphe* collections on *Populus*

spp. pertain to *E. adunca*. It cannot be excluded that, comparable to *Erysiphe* on willows, the diversity of *Erysiphe* on poplars is much higher than previously assumed, most notably in China.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Jing Feng, Shu-Yan Liu and Uwe Braun were involved in planning and supervised the work; Jing Feng performed DNA sequencing and color photos; Susumu Takamatsu, Michael Bradshaw and Seyed Akbar Khodaparast performed phylogenetic analysis; Timur Bulgakov, Guan-Xiu Guan, Feng-Yun Zhao, Shu-Rong Tang provided part of powdery mildew specimens. All authors reviewed the manuscript and commented on the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. Moreover, alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number S28710.

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Figures

Figure 1

Symptoms and morphology of *Erysiphe salicina* on *Salix sinica* a. Infected plant; b. Dried infected leaves (HMJAU-PM91918); c. Close-up view of mycelia and chasmothecia; d–g. Hyphal appressoria; h. Conidiophore; i–l. Conidia; m–n. Perihilar germ tubes; o. Chasmothecia; p. Peridium cells; q–s. Appendages; t–w. Asci; x. Ascospores. Bars: o = 50 μm; h, p–x = 20 μm; d–g, i–n = 10 μm.

Figure 2

Phylogenetic analysis of combined data of the ITS and 28S rDNA region for 73 sequences from *Erysiphe salicina, E. adunca* s. lat. and an outgroup sequence from *E. australiana*. The tree was constructed using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS (70%) values by the maximum parsimony (MP) and maximum likelihood (ML) are shown on the branches. Sequences in red and bold were from *E. salicicola*.

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