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SHORT COMMUNICATION

Erysiphe kenjiana: a newly arrived alien species causing powdery mildew on *Ulmus* trees in Hungary

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Abstract

Elm trees (*Ulmus* sp.) occurring naturally or planted as ornamentals in Europe are susceptible to powdery mildew (PM). In the past, the causal agent of the powdery mildew symptoms on the adaxial side of leaves of *Ulmus* trees was *Erysiphe ulmi* in Hungary. However, we identified *E. kenjiana* on *Ulmus pumila* leaves sampled from the urban area of Budapest. Identification was based on morphological characteristics and molecular phylogenetic analysis. Numerous spherical chasmothecia, which were 58–94 µm in diameter, were found on the samples. The apices of the appendages were characteristically spirally twisted and flattened. Anamorphs were found only on one sample, characterized by conidiophores developing ellipsoid–cylindrical conidia singly. The mycelium was epiphytic with lobed to multilobed hyphal appressoria, mostly in opposite pairs. The internal transcribed spacer region (ITS) of the nrDNA was amplified, and the BLAST search showed 100% similarity with *E. kenjiana* sequences in GenBank. In the maximum likelihood phylogenetic analysis, the sequences of the Hungarian samples grouped in one clade with the sequences of other *E. kenjiana* specimens collected in Europe and Asia. This is the first report of the non-native *E. kenjiana* causing powdery mildew on *Ulmus pumila* in Hungary.

Keywords *Erysiphe kenjiana* · *Ulmus pumila* · Internal transcribed spacer region · Phylogenetic analysis · First report

Introduction

Some elm species (such as *Ulmus laevis*, *U. minor* subsp. *minor* and *U. glabra*) are indigenous woodland trees in Hungary (Börcsök 2004). In addition to cultivars of these native species, horticultural centres also offer cultivars of species of Asian origin, such as *U. parvifolia* and *U. pumila,* the latter being especially popular as a hedge plant.

Causal agents of powdery mildew (PM) infections of elm trees were *Phyllactinia nivea* (infecting the abaxial side of the leaves) or *Erysiphe ulmi* (infecting the adaxial side of the leaves) in Europe in the past (Braun and Cook 2012). *Erysiphe ulmi* is also known to occur in Hungary (listed as "*Erysiphe clandestina*" (Nagy and Kiss 2006). In Asia, another species *E. kenjiana* infects elms, whereas a third species *E. parvifoliae* (Kirschner et al. 2020) is known to infect *U. parvifolia*.

Erysiphe kenjiana was originally described in China, and its distribution ranged from the Far East to Russia (Braun and Cook 2012). The species infects elms, i.e. *Ulmus davidiana, U. glaucescens, U. glabra, U. laevis, U. macrocarpa, U. minor, U. parvifolia* and *U. pumila* (Heluta et al. 2009; Bradshaw et al. 2023). In Europe, *E. kenjiana* has been identified in Ukraine (Heluta et al. 2009), the western part of Russia (Bulgakov 2008) and more recently in Romania (Chinan 2019).

In the present study, we identified *E. kenjiana* on *U. pumila* leaves sampled from the urban area of Budapest. To our knowledge, this is the first report of *E. kenjiana* in Hungary.

Materials and methods

Ulmus pumila leaves infected with powdery mildew on the adaxial side were collected in Budapest only at one restricted urban area infecting *U. pumila* hedges in 2018, 2019, 2023 and 2024. The forests surrounding Budapest were sampled

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in 2023 (Table 1). Samples were placed in plastic bags and transferred to the laboratory for further investigation. Morphological characteristics were examined with bright-field or phase-contrast microscopy using a Nikon ECLIPSE Ni-U upright microscope (USA) equipped with a Nikon Digital Sight 10 (USA) camera. Chasmothecia were examined and covered in lactic acid, while the anamorphs were studied after boiling in lactic acid (Shin and La 1993). At least 20 measurements were taken for each fungal structure. For scanning electron microscopy (SEM), small pieces of dried leaves affected by powdery mildew were fixed on a metal disc and were coated with gold (2 nm thickness) (SafeMatic CCU-010 HV high-vacuum compact coating unit, Switzerland). Scanning electron micrographs were obtained using a Hitachi SV1000 FlexSEM scanning electron microscope (Hitachi High-Tech, Tokyo, Japan). The pathogen was identified based on morphology (Braun and Cook 2012) and based on sequences of the nrDNA internal transcribed spacer (ITS) (see below). The PM-infected leaves were deposited at the Herbarium of the Hungarian Natural History Museum (Table 1).

For species identification, DNA was extracted from a single chasmothecium following a described protocol (Pintye et al. 2020) or from anamorphic mycelium and conidia using a cellotape boiling method (Pintye et al. 2023). The ITS region was amplified according to Pintye et al. (2024). The resulting sequences were compared with the accessions in the National Center for Biotechnology Information database (NCBI, http://www.ncbi.nlm.nih.gov/Blast.cgi) using the BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1990). Sequences obtained in the present study were deposited in GenBank under accession numbers PQ084719-PQ084725 (Table 1).

Six newly obtained sequences were aligned with 30 other sequences (Table 2) retrieved from GenBank using the online version of MAFFT 7 (Katoh and Standley 2013) with the E-INS-i method. The alignments were examined and edited using MEGA 7 (Kumar et al. 2016). The dataset consisted of 36 sequences and 716 characters; *Erysiphe japonica* var. *japonica* and *Erysiphe japonica* var. *crispulae* were used as outgroup taxa. Maximum likelihood (ML) phylogenetic analysis was carried out with the raxmlGUI 1.5 implementation (Silvestro and Michalak 2012; Stamatakis 2014). A GTR + G nucleotide substitution model was used with ML estimation of base frequencies. Maximum likelihood bootstrap (BS) analysis with 1000 replicates was used to test the support of the branches. The phylogenetic tree was visualized and edited in TreeGraph 2.15 (Stöver and Müller 2010).

Results

Numerous chasmothecia but no conidiophores were found on the infected *Ulmus* sp. leaves sampled from September to November. Anamorphs were found only on the leaves collected in June 2024, and chasmothecia could also be observed on these leaves. Morphological features of the specimens identified as *E. kenjiana* based on the ITS sequences were as follows: Chasmothecia were spherical, measured 58–94 µm in diameter and had 9–17 equatorial straight or slightly curved appendages, and their length measured 62–103 µm. The apices of the appendages were characteristically spirally twisted and flattened (Fig. 1a, b). Asci were broadly ovoid, containing mostly two ascospores. Ascospores measured about 24–29 × 14–17.5 µm. Mycelium was epiphytic with lobed to multilobed hyphal appressoria, mostly in opposite pairs (Fig. 1d). The conidiophores arose singly, 80–111 µm long; foot cells were mainly curved at the base, followed by one or two shorter cells, and produced single, hyaline, ellipsoid–cylindrical conidia, measuring about 27–39 × 13–17 µm, without fibrosin bodies (Fig. 1e). Morphological features mostly agreed with those of *E. kenjiana* (Braun and Cook (2012), but slight differences were detected in the size of conidiophores and conidia.

The morphology of chasmothecia of the specimens identified as *E. ulmi* based on the ITS sequences was clearly distinguishable from that of the *E. kenjiana* specimens, as

Table 1 Data of the samples collected in the present study

Designation	PM species	Host plant	Date of collection	GPS coordinates	Herbarium inventory no.	Herbarium bar code	GenBank ITS accession no.
PM328	*Erysiphe kenjiana*	*U. pumila*	October 2018	47.51211, 19.017398	BP112650	HNHM-MYC 032043	PQ084719
PM273	*E. kenjiana*	*U. pumila*	November 2019	47.511860, 19.017232	BP112649	HNHM-MYC 032042	PQ084721
PM356	*E. kenjiana*	*U. pumila*	October 2023	47.511759, 19.017459	BP112761	HNHM-MYC 037081	PQ084720
PM336	*E. ulmi*	*Ulmus* sp.	September 2023	47.480894, 18.943381	BP112654	HNHM-MYC 032047	PQ084723
PM340	*E. ulmi*	*Ulmus* sp.	September 2023	47.52614, 18.95617	BP112657	HNHM-MYC 032050	PQ084724
PM343	*E. ulmi*	*Ulmus* sp.	September 2023	47.515535, 19.0257	BP112658	HNHM-MYC 032051	PQ084725
PM359	*E. kenjiana*	*U. pumila*	June 2024	47.511947, 19.017169	BP112762	HNHM-MYC 037082	PQ084722

Table 2 List of powdery mildew sequences obtained from GenBank and used for phylogenetic analysis

Fungal species	GenBank ITS accession number	Sample designation	Year of collection	Place of collection	Host species	References	Notes
Erysiphe ulmi	OQ821290	HAL2440A F	2004	Germany	*Ulmus* sp.	Bradshaw et al. (2023)	isoneotype
E. ulmi	MT218387	FR4674	2018	Germany	*Ulmus* sp.	Kirschner et al. (2020)	
E. ulmi	MT218388	FR4693	2018	Germany	*Ulmus* cf. *minor*	Kirschner et al. (2020)	
E. ulmi	MT218391	MP5423	2018	Germany	*Ulmus* cf. *laevis*	Kirschner et al. (2020)	
E. ulmi var. *ulmi*	OM574854	GUM1775	2020	Iran	*U. glabra*	Darsaraei et al. (2021)	
E. ulmi var. *ulmi*	OM574852	IRAN10827	1998	Iran	*Ulmus* sp.	Darsaraei et al. (2021)	
E. kenjiana	MK452611	HMJAU-PM91841	2017	China	*U. pumila*	Bradshaw et al. (2023)	epitype
E. kenjiana	AB475117	KW34758F	2007	Ukraine	*U. glabra*	Heluta et al. (2009)	
E. kenjiana	AB475118	KW34760F	2007	Ukraine	*U. minor*	Heluta et al. (2009)	
E. kenjiana	AB475119	KW34763F	2007	Ukraine	*U. pumila*	Heluta et al. (2009)	
E. kenjiana	MK518442	I184517	2017	Romania	*U. pumila*	Chinan (2019)	
E. parvifoliae	MG954111	FR4572	2017	Taiwan	*U. parvifolia*	Kirschner et al. (2020)	holotype
E. parvifoliae	MH427792	FR4612	2018	Taiwan	*U. parvifolia*	Kirschner et al. (2020)	
E. parvifoliae	AB475116	MUMH21	1994	Japan	*U. parvifolia*	Heluta et al. (2009)	
E. kusanoi	AB475120	MUMH20	1994	Japan	*Celtis sinensis*	Heluta et al. (2009)	epitype
E. kusanoi	KR048067	HMNWAFU-CF2013124	2013	China	*Celtis bungeana*	Bradshaw et al. (2023)	
E. celtidis	OM574855	GUM1770	2001	Iran	*Celtis caucasica*	Darsaraei et al. (2021)	
E. michikoae	AB693963	MUMH5055	2010	Japan	*Celtis jessoensis*	Meeboon et al. (2013)	holotype
E. zelkovae	AB475121	MUMH403	1997	Japan	*Zelkova serrata*	Heluta et al. (2009)	epitype
E. aphananthes	LC028971	MUMH 4648	2007	Japan	*Aphananthe aspera*	Takamatsu et al. (2015)	epitype
E. parvula	OQ821282	NCSLG15899	2002	USA	*Celtis laevigata*	Bradshaw et al. 2023	
E. parvula	OQ821283	FH01122118	1923	USA	*Celtis occidentalis*	Bradshaw et al. 2023	epitype
E. geniculata	OQ821274	BPI553955	1914	USA	*Morus rubra*	Bradshaw et al. 2023	epitype
E. geniculata	OQ821273	GAM00008958	1939	USA	*Morus rubra*	Bradshaw et al. (2023)	
E. couchii	OQ821264	BPI559095	1968	USA	*Quercus arizonica*	Bradshaw et al. (2023)	topotype
E. couchii	OQ821263	ARIZ-M-AN02491	1967	USA	*Quercus arizonica*	Bradshaw et al. (2023)	topotype
Erysiphe sp.	OQ821287	BPI563919	1968	USA	*Quercus hypoleucoides*	Bradshaw et al. (2023)	

Table 2 (continued)

Fungal species	GenBank ITS accession number	Sample designation	Year of collection	Place of collection	Host species	References	Notes
E. trinae	OQ821289	FH00965658		USA	*Quercus agrifolia*	Bradshaw et al. (2023)	isotype
E. japonica var. *crispulae*	AB701301	MUMH4163	2005	Japan	*Quercus mangolica* var. *crispula*	Meeboon and Takamatsu (2013	
E. japonica var. *japonica*	AB701302	MUMH4582	2006	Japan	*Quercus serrata*	Meeboon and Takamatsu (2013)	epitype

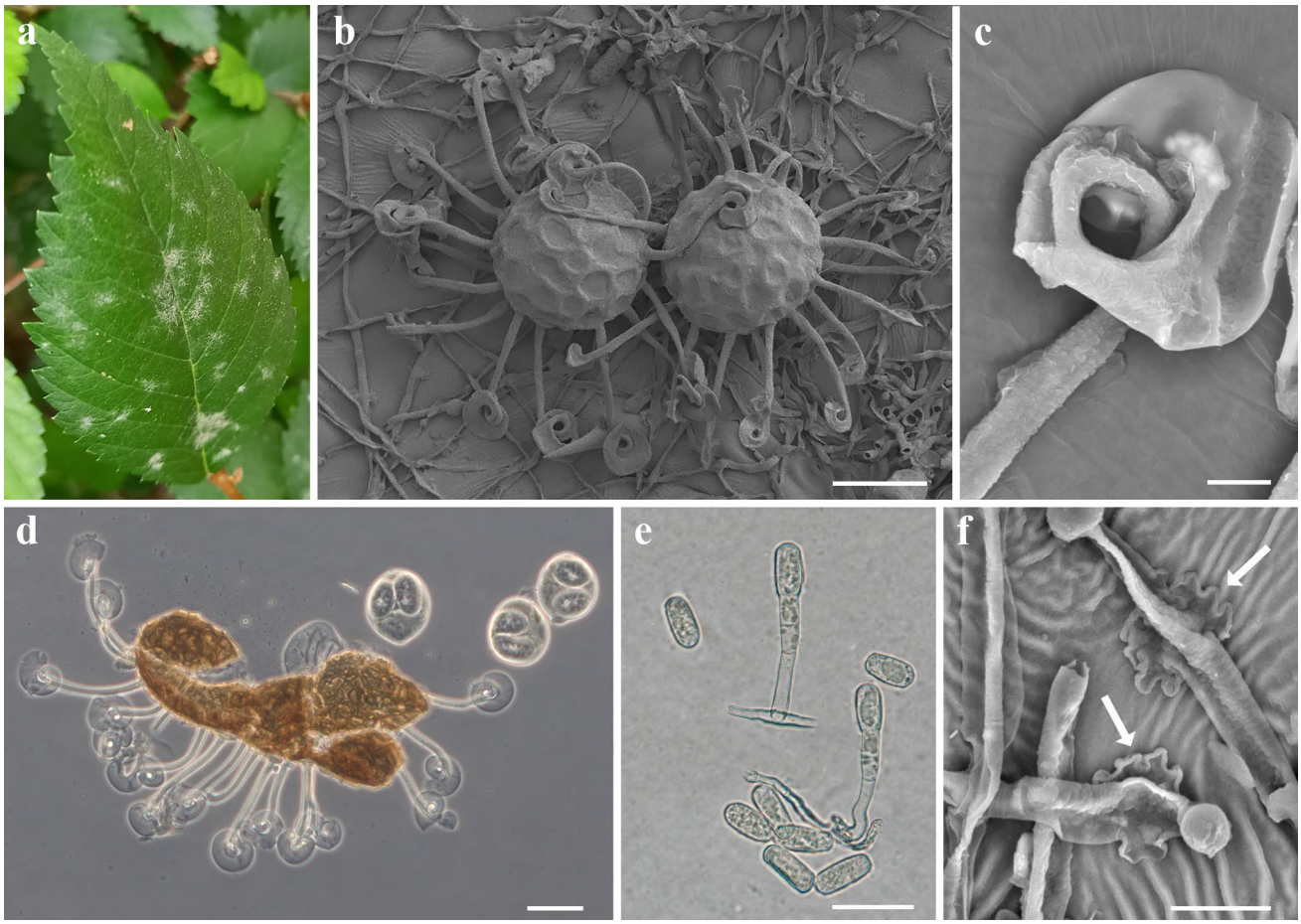


Fig. 1 *Erysiphe kenjiana* on *Ulmus pumila*. **a** Powdery mildew symptoms on the adaxial surface of the leaves, early infection in June. **b** Gregarious chasmothecia and mycelium on the surface of a leaf, SEM, bar = 50 µm. **c** Spirally twisted, flattened apex of an appendage, SEM, bar = 5 µm. **d** Squashed chasmothecium, asci with ascospores, phase-contrast image, bar = 50 µm. **e** Conidiophores and conidia, bright-field image, bar = 50 µm. **f** Multilobed hyphal appressoria in opposite pairs, SEM, bar = 10 µm

the appendages of the chasmothecia had uncinate–circinate apices, characteristic of *E. ulmi* as described by Braun and Cook (2012) (Fig. 2).

The ITS sequences of the Hungarian specimens from *U. pumila* were identical and showed 100% identity to ITS sequences of *E. kenjiana* in the BLAST search. The phylogenetic analysis revealed (Fig. 3) that the Hungarian samples grouped with the epitype of *E. kenjiana* (HMJAU-PM91841, accession no. MK452611, host *U. pumila*, China), and other specimens from Ukraine (AB475117, host *U. glabra*; AB475118, host *U. minor*; AB475119, host *U. pumila*) and from Romania (MK518442, host *U.*

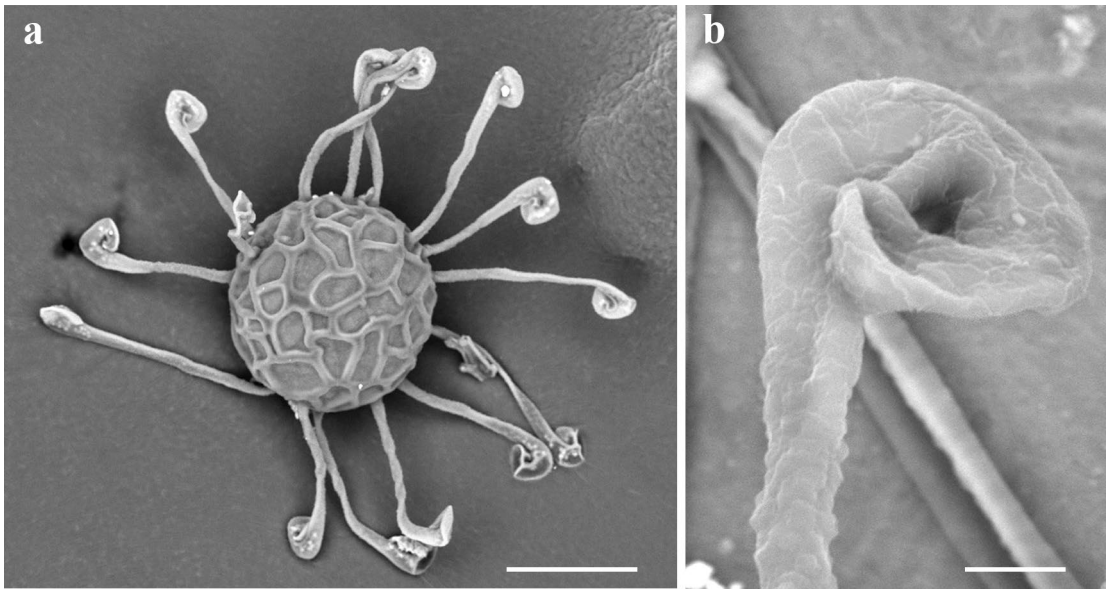


Fig. 2 *Erysiphe ulmi* on *Ulmus* sp. scanning electron micrograph of **a** a chasmothecium (bar = 50 µm) and **b** the apex of an appendage (bar = 5 µm)

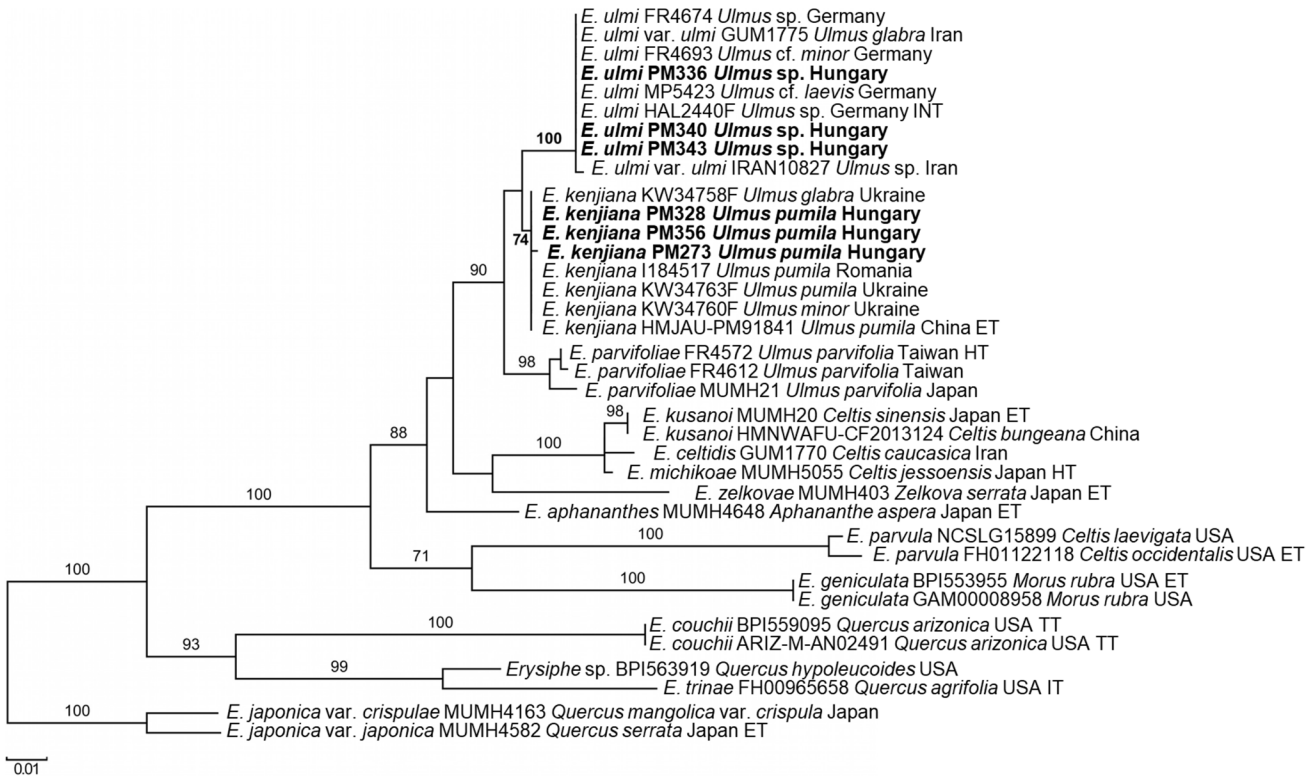


Fig. 3 Phylogenetic tree with the highest likelihood value resulting from the maximum likelihood analysis of ITS sequences of *Erysiphe* sp. infecting *Ulmus* sp. and other host plant species. Sequences obtained in the current study are shown in bold. Culture collection numbers, host species and locations are provided. ITS sequences of *Erysiphe japonica* var. *japonica* and *Erysiphe japonica* var. *crispulae* served as outgroup taxa. (INT = ex isoneotype; ET = ex epitype; HT = ex holotype; TT = ex topotype.) The bar indicates 0.01 expected changes per site per branch

pumila). The second subset of ITS sequences of Hungarian specimens from *Ulmus* sp. were also identical and showed 100% identity to ITS sequences of *E. ulmi*. The ITS of those Hungarian samples grouped with the isoneotype of *E. ulmi* (HAL 2440A F, accession no. OQ821290, host *Ulmus* sp., Germany), other specimens from Germany (MT218387, host *Ulmus* sp.; MT218388, host *U.* cf. *minor*; MT218391, host *U.* cf. *laevis*) and *E. ulmi* var. *ulmi* specimens from Iran (OM574854, host *U. glabra;* OM574852, host *Ulmus* sp.). Both morphological examination and phylogenetic analysis have verified the presence of *E. ulmi* and have shown that there is a newly occurring species of powdery mildew fungi, *E. kenjiana,* that infects elm in Hungary.

Discussion

Only *E. ulmi* was reported to infect the adaxial sides of elm leaves in Europe until the first appearance of *E. kenjiana*, a PM fungus of Asian origin in 2007 (Heluta et al. 2009). Since then, *E. kenjiana* has been found in the western part of Russia (Bulgakov 2008) and in Romania (Chinan 2019). Several *Erysiphe* species have been reported to spread in a similar way. *Erysiphe corylacearum*, causing PM on hazel (Kalmár et al. 2023), and *E. salmonii*, causing PM on ash trees (Pintye et al. 2024), are both of Asian origin and have recently spread rapidly westwards.

Ulmus pumila is a species of Asian origin (Solla et al. 2005). This tree species was introduced in several regions outside its native range as it tolerates drought, alkaline or saline soils and frosts (Heybroek 1979). Nevertheless, *U. pumila* was found to be resistant to Dutch elm disease (Solla et al. 2005) as well. In most regions where it has been introduced, *U. pumila* is now listed as an alien invasive plant (Hirsch et al. 2015). This is also the case in Hungary, although it is still cultivated and marketed (Bartha 2021). *Erysiphe kenjiana* was reported on *U. pumila* only in urban regions in Ukraine (Heluta et al. 2009), Romania (Chinan 2019) and Hungary (this study). Infestations by *E. kenjiana* of elm trees native to Europe, such as *U. laevis*, *U. minor* and *U. glabra*, have so far only been reported by Heluta et al. (2009). In the case of these *Ulmus* species, *E. kenjiana* caused less severe symptoms. We could find *E. kenjiana* infecting *U. pumila* hedges only in one restricted urban area, however, it was found in several years (2018–2024, Table 1). The other leaves, presumably not of *U. pumila* based on morphological characteristics, and collected in the hilly regions around Budapest, were infected only by *E. ulmi* (Table 1).

Considering all these facts, in the future, *E. kenjiana* may spread from *U. pumila* to the native elm species in Hungary as well, as it has already happened in Ukraine. Subsequently, the new species should also be expected to occur in forest ecosystems in Hungary.

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