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A Species-Specific PCR Differentiates Two Causal Agents of Hazel Powdery Mildew and Reveals the Occurrence of *Erysiphe corylacearum*

Klementina Kalmár [1], Katalin Borostyán [2], Orsolya Molnár [2], János Ágoston [3], Éva Preininger [1] and Márk Z. Németh [2,*]

[1] Institute for Fruitgrowing, Hungarian University of Agriculture and Life Sciences, 1223 Budapest, Hungary; preininger.eva@uni-mate.hu (É.P.)
[2] Plant Protection Institute, HUN-REN Centre for Agricultural Research, 1022 Budapest, Hungary
[3] HUN-REN–SZE PhatoPlant-Lab, Széchenyi István University, 9200 Mosonmagyaróvár, Hungary
* Correspondence: nemeth.mark@atk.hun-ren.hu

Abstract: The demand for common hazel (*Corylus avellana*) fruit increases constantly. Powdery mildew (PM) on hazels in Hungary and throughout Europe was previously caused mainly by *Phyllactinia guttata*. However, less than a decade ago, another fungus of Asian origin, *Erysiphe corylacearum*, appeared on hazels in Europe, including Hungary. Our investigation aimed to develop a species-specific PCR (ssPCR) to aid the identification of *P. guttata* and *E. corylacearum*, and to assess the presence of the latter, non-native fungus in Hungary. For this study, 59 samples were collected from Hungary between 2021 and 2023. The chasmothecial morphology of the PM fungi was observed, and the internal transcribed spacer (ITS) of ribosomal DNA was sequenced in representative samples. Morphological analysis distinguished two types of chasmothecia. Parts of the chasmothecia, typical of *P. guttata*, were flattened and spherical with bristle-like appendages, while other chasmothecia, characteristic of *E. corylacearum*, were distinctly smaller, bearing appendages with branched apices. Sequence data also verified the presence of *P. guttata* and *E. corylacearum* in our samples. The developed ssPCR revealed that *E. corylacearum* was present in more than three-quarters of the samples, more than a quarter of the samples contained both fungi and about one-fifth carried solely *P. guttata*. The alien fungus *E. corylacearum* was found in all but one of the sampled regions and was found on *C. avellana* and also on *C. colurna*. *Erysiphe corylacearum* spreads rapidly and can be considered an invasive pathogen. Its practical importance lies in its ability to infect hazelnuts, potentially causing economic losses. Our ssPCR ensures accurate and quick identification of the fungus, which is essential for effective plant protection.

Keywords: morphology; powdery mildew; species-specific PCR; alien species; invasive fungi

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1. Introduction

The genus *Corylus* belongs to the family Betulaceae and subfamily Coryloideae [1]. One of the most well-known species, the European or common hazel (*Corylus avellana*), is a deciduous shrub, mostly multi-stemmed and up to five meters tall. Its native range includes Hungary. Its typical habitat is the edges of deciduous forests. *Corylus avellana* and its different cultivars are also widely planted as ornamentals.

Its fruit, the hazelnut, is one of the most important nuts globally, together with walnuts and almonds [2]. The current planted area of roughly 1.07 million hectares yields approximately 1.22 million tons [2], over 60% of which originate from Turkey [3]. Other countries with significant production quantities are Italy, the USA, Azerbaijan, Chile and Georgia [2].

In Hungary, hazel has long been a crop of cottage gardens and small farms, but now it has become, from an economic point of view, one of the most important nuts

in Hungary [2,4]. In addition to the cultivation of hazelnuts, a number of mycorrhizal plantations with the aim of growing truffles (mostly *Tuber aestivum*, and experimentally also *T. macrosporum* and *T. borchii*) are also being established [5].

A notable disease of hazel plantations in Hungary, and also in several other hazel-growing regions, is powdery mildew. At least nine species of powdery mildew fungi (*Erysiphaceae, Helotiales*) can infect *Corylus* species [6], but in Hungary, the disease is generally caused by the widespread pathogen *Phyllactinia guttata* [4,7]. The fungus is mostly found to infect plants belonging to the Betulaceae family, including the genus *Corylus* in Europe [8]. It has also been described from common lilac (*Syringa vulgaris*) [9] and is known to infect several other host plants as well [10]. The symptom of *P. guttata* infection is an effuse or evanescent, gray-white mycelial coating on the leaves of hazels [8], which is more likely to appear hypophyllously [8].

However, another species of powdery mildew fungi, *Erysiphe corylacearum,* is also known to infect hazels [11,12]. Its symptoms develop in early spring on young shoots, leaves and young fruits [12]. The symptoms of infection mostly appear on the upper surface of the hazel leaf, but the fungus can also colonize the back of the leaf [8,11]. The leaves turn brown during infection, and symptoms also include necrosis of the leaves or leaf drop [12]. More importantly, hazelnut fruits can also be colonized by the powdery mildew fungus [12,13], rendering them shriveled and underdeveloped. The prevalence of symptoms can be as high as 100%, potentially leading to significant yield losses.

Erysiphe corylacearum is indigenous to Asia, where it appears on *C. heterophylla* (Asian hazel) and *C. sieboldiana* (Japanese hazel) [6]. This species was known to occur only in the Americas and Asia [8] until the beginning of the 2010s. The first reported outbreak outside of these regions was observed in Turkey in 2013 [12]. Then, the pathogen was observed on *C. avellana* in Azerbaijan [14]. The emergence of powdery mildew has also been reported in Iran [15], followed by its identification in Ukraine [16,17] and Georgia [18]. Thereafter, the fungus seemed to spread rapidly through Europe, appearing in different locations in Switzerland [19], Austria [20], Germany [21], Italy [13,22], Spain [23], Romania [24] and Hungary [4,11], where its reported hosts include *C. avellana* and *C. colurna*. Recent outbreaks of powdery mildew have also been reported in Slovenia, Bulgaria, Poland and the Czech Republic [25–28].

The two powdery mildew-causing species *P. guttata* and *E. corylacearum* can be differentiated by observing chasmothecia (fruiting bodies), as those of *P. guttata* are about 155–225 µm in diameter, with bristle-like appendages [8]. The chasmothecia of *E. corylacearum* are significantly smaller in size (80–115 µm) and have branched appendages with recurved tips [4,8]. However, when fruiting bodies are not available, differentiating the two species needs tedious preparation of microscopic slides and observation of anamorphic features.

In this study, we aimed to develop a species-specific PCR (ssPCR) for the molecular-based differentiation of *E. corylacearum* and *P. guttata*. Additionally, we aimed to investigate the occurrence of the fungus in Hungary using both the newly developed ssPCR method and morphological analysis.

2. Materials and Methods

2.1. Sampling and Morphological Analysis

A total of 59 hazel leaves showing PM symptoms were collected from Hungary from 12 counties and Budapest from 2021 to 2023. Details of these samples are shown in Table A1. The presence or absence of chasmothecia was noted, and the chasmothecial morphology of PM fungi was observed under a Zeiss Stemi 2000C stereomicroscope (Jena, Germany). The powdery mildew species present was identified based on descriptions of chasmothecial morphology [8,11,29].

2.2. DNA Extraction

Twelve control DNA extracts (three for both species, and the experiment was conducted twice, hence twelve) were created from the chasmothecia of *E. corylacaearum* and *P. guttata.* To create these control DNA extracts, five individual chasmothecia were carefully selected and collected individually from each species. The chasmothecia were then crushed and subjected to DNA extraction by incubating them in TE buffer at 97 °C [30]. The control DNA extracts were used to test species-specific primers.

From field samples not carrying chasmothecia, DNA was extracted with a Qiagen Plant Mini Kit (Qiagen GmbH; Hilden, Germany). For this, an approximately 1 cm^2 piece was cut from the leaf with powdery mildew symptoms and was used for DNA extraction. DNA extraction was conducted according to the manufacturer's instructions. From the samples with chasmothecia, DNA extracts were created from the fruiting bodies. For this, randomly chosen chasmothecia were collected and subjected to DNA extraction by incubating them in TE buffer as described above for the control DNA extracts.

2.3. Species-Specific PCR

An ssPCR was developed as follows: Sequences of the internal transcribed spacer (ITS) region of *E. corylacearum* (isolate EC; MW031866 [20]) and *P. guttata* (voucher WTUF72463; MT162617 [31]) were downloaded from GenBank. The sequences were aligned with MEGA7 [32] and dissimilar regions of the ITS of the two species were identified. Primers E_coryl-f (CAGAGTGTGAGGCTCACTC) and E_coryl-r (TCCATGTGACTGGAGCAAAAG) were designed based on the *E. corylacearum* sequence to bind to the 5′ end and 3′ end of the ITS region, respectively, with the aid of SnapGene Viewer software (version 6.0.2; GSL Biotech, San Diego, CA, USA). A *Phyllactinia guttata*-specific primer PG-r3 (AAACGTGACTACGCGGAGAG [29]) was used along with a modified version of PG-f2 [29]. The latter primer was modified to avoid potential mismatches revealed after alignment of the original PG-f2 primer sequence to the ITS of *P. guttata* with MEGA7, resulting in PG-f2-alt (ACCCGTGTCGATTGTATCTTCTGT).

The control DNA extracts (see above), presumably containing DNA from one of the two species only, were assayed with both primer pairs. The ssPCR was started in a thermocycler (Tianlong; Xi'an, China) preheated to 95 °C to ensure specificity. PCRs were run with the following protocol: 95 °C for 3 min followed by 35 cycles of 94 °C for 20 s, 56 °C for 20 s and 72 °C for 1 min, and at the end, 72 °C for 5 min. Initially, a thermal gradient PCR (56–64 °C) was used to identify the optimal annealing temperature for providing sufficient yields and specificity at the same time. The reaction mix composition included 10 µL of DreamTaq Green PCR MasterMix (Thermo Fisher Scientific, Waltham, MA, USA), 0.6 µL of 10 µM forward and reverse primers, and 1 µL of DNA template, resulting in a final volume of 20 µL. To assure consistent results and avoid byproducts, a frozen PCR rack was used when setting up reaction mixes. A negative control (molecular biology grade water) was always included in each reaction. The resulting PCR products were run on 1% sodium borate [33] agarose gel and visualized under UV light.

To test the effectiveness of the method on DNA extracts containing both species, eleven artificial mixtures were created. These contained DNA extracts from each of the species: single chasmothecial DNA from *E. corylacearum* and *P. guttata* from the samples HPM19 and HPM28 were mixed in a 1:1 (v/v) ratio; and DNA from *E. corylacearum* chasmothecia from the samples HPM10, HPM24 and HPM25 and DNA from *P. guttata* chasmothecia from the samples HPM33, HPM40 and HPM41 were mixed in a 1:1 ratio in all nine possible combinations. These were then used as targets in the ssPCR. The experiment was conducted twice.

After the development of the ssPCR, the assay was used on all DNA samples originating from PM-infected *Corylus* leaf samples to determine which species of the two is present on each sample.

2.4. DNA Sequencing

To verify whether the ssPCR amplified the ITS of the targeted species only, and to verify the morphology-based identifications and the specificity of the ssPCR, six amplified ITS fragments from both species were sent for sequencing to LGC Genomics GmbH (Berlin, Germany). Sequencing was performed with the same primers used for the amplifications. Electropherograms were processed and individually checked using the CodonCode Aligner 8.0.2 (CodonCode Corporation; Centerville, MA, USA). The resulting sequences were analyzed using BLASTn to identify the most similar sequences in GenBank. If a sequence was identical or almost identical to reference sequences of *E. corylacearum* or *P. guttata*, it was considered to verify species identity. Representative ITS sequences determined in this study were deposited in GenBank (accession numbers PP825796-PP825800).

3. Results

In the morphological analysis, two types of chasmothecia could be distinguished (Figure 1). Parts of the chasmothecia, typical of *P. guttata*, were flattened and spherical, approximately 200 µm in diameter with bristle-like (acicular) appendages of about 400 µm. Other chasmothecia were distinctly smaller (less than 100 µm), bearing appendages with branched apices. These were characteristic of *E. corylacearum*. After observing 59 samples, chasmothecia were detected in 36 samples, and 23 samples did not have chasmothecia (Table A1). We identified only *E. corylacearum* fruiting bodies in 17 samples, those of *P. guttata* only were found in 6 samples and we had 13 samples with chasmothecia of both species (Table A1; Figure 1). Thus, a total of 30 samples had chasmothecia of *E. corylacearum* and 19 had those of *P. guttata*. Mostly, but not exclusively, *P. guttata* was found on the back (abaxial), while *E. corylacearum* was found on the upper (adaxial) side of the leaves.

Figure 1. Infection of *Corylus avellana* by two powdery mildew fungi on same leaf. Sample HPM18 with chasmothecia of *E. corylacearum* (upper left) and *P. guttata* (lower right) is shown. Bar: 1 cm.

The developed ssPCR successfully differentiated *P. guttata* and *E. corylacearum*; by using DNA extracts obtained from *E. corylacearum*, the first primer set resulted in PCR amplicons, but DNA extracts from *P. guttata* did not, and vice versa for the second primer pair (Figure 2). All artificial targets resulting from mixing the DNA of both species gave positive results for both. Thus, the identity of the fungi present in the leaf samples could be determined based on the amplified DNA fragments' presence and size. The *E. corylacearum*-specific primer pair resulted in a product of ~550 bp, and the product of the *P. guttata*-specific primer pair was ~420 bp long. BLASTn analysis of the sequenced fragments

showed 100% identity to other ITS sequences of *E. corylacearum* and 99–100% similarity to other *P. guttata* samples, respectively.

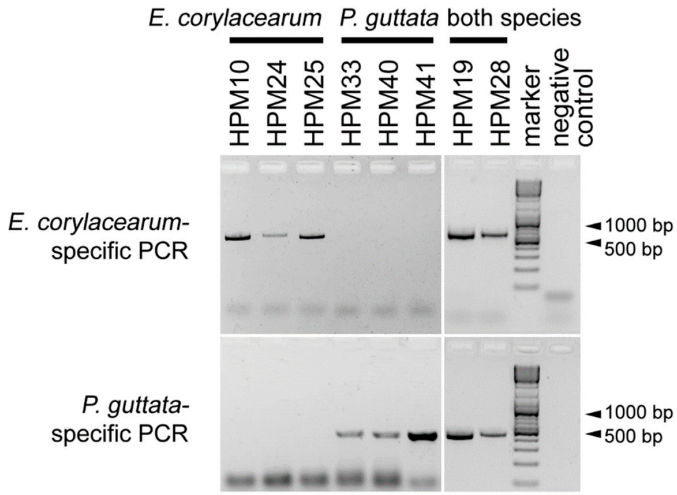

Figure 2. The representative results of the species-specific PCR developed to distinguish *Erysiphe corylacearum* and *Phyllactinia guttata*. Six DNA extracts and two DNA mixtures were assayed with *E. corylacearum*-specific (upper panel) and *P. guttata*-specific primers (lower panel). The first three samples were infected by *E. corylacearum*, and the following three samples were infected by *P. guttata*. In the next two wells, PCR products resulting from the amplifications of mixtures of DNA extracts from both species (mixtures of extracts originating from HPM19 and HPM28) are shown. The last sample was the negative control (molecular biology grade water).

The molecular-based identification of the 59 samples was carried out using the developed ssPCR. According to this identification, 12 samples carried solely *P. guttatta*, 22 were infected solely by *E. corylacearum* and 25 samples harbored both species. These results confirmed the results of the identification based on morphological analysis. All 30 samples carrying *E. corylacearum* chasmothecia as observed by microscopy were also positive for *E. corylacearum* in the ssPCR. Similarly, all samples observed to have *P. guttata* chasmothecia were positive in the ssPCR (Table A1).

The alien fungus *E. corylacearum* was found in all but one of the sampled regions (Budapest and eleven counties) based on observations of the chasmothecia and the results of the ssPCR and ITS sequence data (Table A1). Altogether, it was present on more than three-quarters (47 of 59) of the leaves. *Erysiphe corylacearum* was found on *C. avellana* and also on *C. colurna* (e.g., samples HPM12 and HPM37 in Table A1) but was not present on any of the *C. maxima* samples. In about half (25) of all of the samples (47) infected by *E. corylacearum*, the fungus was found together with *P. guttata* (Table A1).

Of the samples without any chasmothecia, eleven were positive for *E. corylacearum* and six for *P. guttata*. There were six samples (HPM5, HPM15, HPM43-HPM45 and HPM51; Table A1) that did not contain any fruiting bodies but were diagnosed as positive for both species in the ssPCR.

In some cases, fruiting bodies of only *E. corylacearum* were detected; however, both species were present according to the ssPCR results (samples HPM22, HPM46-HPM49 and HPM54; Table A1).

4. Discussion

Our morphological analysis and the developed ssPCR distinguished *P. guttata* and *E. corylacearum* on hazel leaves. Notably, *E. corylacearum* was prevalent across sampled regions, not only on *C. avellana* but also on *C. colurna*. To our knowledge, the fungus was not detected on the latter host in Hungary earlier.

Erysiphe corylacearum has been spreading from its region of origin into Europe in the last decade. The spread is remarkably fast, as judged by the first reports of the fungus in

different countries. The process was called an "epidemic spread" [21]. In Hungary, it has been present since 2020 at least, and by 2023, it was found in all but one of the sampled regions in the present work. Most probably, by now, its area of occurrence includes the whole country. As shown by our data and by other studies, the symptoms of *E. corylacearum* usually occur earlier in the vegetation period ([12], Table A1). As we also found in several samples, the fungus also readily co-occurs with *P. guttata* ([4], Table A1). These traits are characteristic of an invasive pathogen [16,23], possibly contributing to its spreading potential. It was also assumed, however, that the spread of the fungus is facilitated by human activities, such as the import of infected propagating material [13]. *Erysiphe corylacearum* also infects *Corylus* species which are predominantly native to Asia. As some of the species, such as *C. colurna*, are also widely planted in Hungary as ornamentals, and rootstocks of *Corylus* species for hazelnut growing are imported, it is reasonable to hypothesize that trade plays a significant role in the spread of the fungus.

In general, powdery mildew fungi are among the most commonly reported alien species in European countries [34,35]. Indeed, the occurrence of *E. corylacearum*, a species from Asia, on native hazels shows remarkable similarities to that of other newly reported alien species, such as *E. salmonii*, which infects *Fraxinus* trees [36], *E. syringae-japonicae* on lilacs [37] and *E. kenjiana* occurring on *Ulmus* sp. [38]. In all of these cases, there were established or native powdery mildew fungi regularly occurring on the respective host plants, and new pathogens originating from Asia infecting the same hosts were introduced.

Unlike *P. guttata, E. corylacearum* is also able to infect hazelnuts [7,12], which makes it potentially significantly more devastating than *P. guttata* [13]. Therefore, it may be essential to identify the pathogen quickly, preferably using a cost-effective method. Our ssPCR technique allows for the rapid and accurate identification of *E. corylacearum*, even in cases when fruiting bodies are not present or when *P. guttata* is also infecting the plant. This latter is a clear advantage of our method over another assay [39], which was not tested with samples containing both species. Thus, it is unknown how it performs in detecting *P. guttata* and, consequently, whether it detects both species in cases of coinfections. Our ssPCR method can aid the development of effective management strategies and allow for the monitoring of *E. corylacearum* in the affected regions.

The emergence and spread of *E. corylacearum* highlight the importance of continued research to find a suitable management strategy and assaying the susceptibility of different varieties to mitigate the impact of powdery mildew on hazelnut cultivation.

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Data Availability Statement: All relevant data except for the determined sequences are available in the manuscript. The determined sequences are deposited in GenBank under accession numbers PP825796-PP825800.

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Appendix A

Table A1. List of samples collected during this study. "+" denotes chasmothecia present, "−" denotes chasmothecia not detected.

Sample Identifier	Fruiting Bodies of *E. corylacearum*	Fruiting Bodies of *P. guttata*	Powdery Mildew Fungus	Host Plant Species	Place of Collection (City, County)	GPS Coordinates	Date of Collection
HPM1	−	−	*E. corylacearum*	*Corylus avellana*	Budapest	47.505302, 19.138174	4 June 2021
HPM2	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Érd, Pest	47.345625, 18.859505	8 August 2021
HPM3	+	−	*E. corylacearum*	*C. avellana*	Dobogókő, Pest	47.719439, 18.892749	30 October 2021
HPM4	−	−	*E. corylacearum*	*C. avellana*	Kerepes, Pest	47.576529, 19.264852	23 June 2022
HPM5	−	−	*E. corylacearum, P. guttata*	*C. colurna*	Budapest	47.500904, 19.031150	24 June 2022
HPM6	−	−	*E. corylacearum*	*C. avellana*	Kerepes, Pest	47.576572, 19.265041	27 June 2022
HPM7	−	−	*E. corylacearum*	*C. avellana*	Szekszárd, Tolna	46.330652, 18.666095	5 July 2022
HPM8	−	−	*E. corylacearum*	*C. avellana*	Szekszárd, Tolna	46.330609, 18.665910	5 July 2022
HPM9	−	−	*E. corylacearum*	*C. avellana*	Szekszárd, Tolna	46.330496, 18.665554	5 July 2022
HPM10	+	−	*E. corylacearum*	*C. avellana*	Budakeszi, Pest	47.511878, 18.933464	7 July 2022
HPM11	−	−	*E. corylacearum*	*C. avellana*	Budakeszi, Pest	47.508907, 18.931212	7 July 2022
HPM12	−	−	*E. corylacearum*	*C. colurna*	Budakeszi, Pest	47.511938, 18.930508	7 July 2022
HPM13	−	−	*E. corylacearum*	*C. avellana*	Budapest	47.505037, 19.138470	11 July 2022
HPM14	−	−	*E. corylacearum*	*C. avellana*	Mende, Pest	47.434124, 19.457578	15 July 2022
HPM15	−	−	*E. corylacearum, P. guttata*	*C. avellana*	Garáb, Nógrád	47.963556, 19.746401	23 July 2022
HPM16	+	−	*E. corylacearum*	*C. avellana*	Szada, Pest	47.635653, 19.333720	August 2022
HPM17	−	−	*E. corylacearum*	*C. avellana*	Jánossomorja, Győr-Moson-Sopron	47.787065, 17.150850	30 August 2022
HPM18	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Gyula, Békés	46.664682, 21.263174	19 September 2022
HPM19	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Püspökladány, Hajdú-Bihar	47.335130, 21.091024	24 September 2022
HPM20	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Püspökladány, Hajdú-Bihar	47.334187, 21.090440	24 September 2022
HPM21	+	−	*E. corylacearum*	*C. avellana* 'Heterophylla'	Püspökladány, Hajdú-Bihar	47.335080, 21.091447	24 September 2022
HPM22	+	−	*E. corylacearum, P. guttata*	*C. avellana*	Dobogókő, Pest	47.719446, 18.892284	2 October 2022
HPM23	+	−	*E. corylacearum*	*C. avellana*	Dobogókő, Pest	47.719278, 18.895112	2 October 2022
HPM24	+	−	*E. corylacearum*	*C. avellana*	Dobogókő, Pest	47.718488, 18.904377	2 October 2022
HPM25	+	−	*E. corylacearum*	*C. avellana*	Vál, Fejér	47.359523, 18.681418	3 October 2022
HPM26	+	−	*E. corylacearum*	*C. avellana*	Diósd, Pest	47.411191, 18.936779	3 October 2022
HPM27	+	+	*E. corylacearum, P. guttata*	*C. avellana* 'Pendula'	Balatonvilágos, Somogy	46.962783, 18.163501	6 October 2022
HPM28	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Balatonboglár, Somogy	46.754358, 17.665625	6 October 2022
HPM29	+	−	*E. corylacearum*	*C. avellana*	Szekszárd, Tolna	46.330563, 18.666124	12 October 2022
HPM30	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Szekszárd, Tolna	46.330320, 18.665476	12 October 2022
HPM31	+	−	*E. corylacearum*	*C. avellana*	Szekszárd, Tolna	46.329513, 18.665134	12 October 2022
HPM32	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Szekszárd, Tolna	46.329911, 18.665134	12 October 2022
HPM33	−	+	*P. guttata*	*C. avellana*	Szekszárd, Tolna	46.329359, 18.665081	12 October 2022
HPM34	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Budapest	47.513955, 19.010434	14 October 2022
HPM35	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Budakeszi, Pest	47.511878, 18.933464	15 October 2022
HPM36	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Budakeszi, Pest	47.508907, 18.931212	15 October 2022
HPM37	+	+	*E. corylacearum, P. guttata*	*C. colurna*	Budakeszi, Pest	47.511938, 18.930508	15 October 2022
HPM38	−	+	*P. guttata*	*C. colurna*	Budakeszi, Pest	47.519600, 18.929327	15 October 2022
HPM39	−	+	*P. guttata*	*C. avellana*	Budapest	47.512486, 19.014810	2 November 2022
HPM40	−	+	*P. guttata*	*C. avellana*	Budapest	47.514257, 19.010399	17 November 2022
HPM41	−	+	*P. guttata*	*C. avellana*	Budapest	47.501734, 19.034448	21 November 2022
HPM42	+	−	*E. corylacearum*	*C. avellana*	Galgahévíz, Pest	47.620339, 19.543198	21 November 2022
HPM43	−	−	*E. corylacearum, P. guttata*	*C. avellana*	Budapest	47.513344, 19.011459	20 June 2023
HPM44	−	−	*E. corylacearum, P. guttata*	*C. avellana*	Nagyrákos, Vas	46.822309, 16.460806	14 July 2023
HPM45	−	−	*E. corylacearum, P. guttata*	*C. avellana*	Dunaújváros, Fejér	46.954716, 18.949894	3 September 2023
HPM46	+	−	*E. corylacearum, P. guttata*	*C. avellana*	Szalkszentmárton, Bács-Kiskun	46.924567, 19.118414	4 September 2023
HPM47	+	−	*E. corylacearum, P. guttata*	*C. avellana*	Táborfalva, Pest	47.135971, 19.477080	4 September 2023
HPM48	+	−	*E. corylacearum, P. guttata*	*C. avellana*	Szigetújfalu, Pest	47.234927, 18.937678	4 September 2023
HPM49	+	−	*E. corylacearum, P. guttata*	*C. avellana*	Zalaháshágy, Zala	46.882798, 16.631994	8 September 2023
HPM50	−	−	*P. guttata*	*C. avellana*	Nagyrákos, Vas	46.822472, 16.460779	8 September 2023
HPM51	−	−	*E. corylacearum, P. guttata*	*C. avellana*	Kerekegyháza, Bács-Kiskun	46.930966, 19.473140	8 September 2023
HPM52	−	−	*P. guttata*	*C. avellana*	Nagykarácsony, Fejér	46.872177, 18.767247	8 September 2023
HPM53	−	−	*P. guttata*	*C. avellana*	Berettyóújfalu, Hajdú-Bihar	47.213776, 21.541546	10 September 2023

Table A1. *Cont.*

Sample Identifier	Fruiting Bodies of *E. corylacearum*	Fruiting Bodies of *P. guttata*	Powdery Mildew Fungus	Host Plant Species	Place of Collection (City, County)	GPS Coordinates	Date of Collection
HPM54	+	–	*E. corylacearum, P. guttata*	*C. avellana*	Érd, Pest	47.394408, 18.931385	11 September 2023
HPM55	–	–	*P. guttata*	*C. maxima* 'Purpurea'	Csemő, Pest	47.147483, 19.712353	11 September 2023
HPM56	–	+	*P. guttata*	*C. maxima* 'Purpurea'	Szentes, Csongrád-Csanád	46.695962, 20.211840	12 September 2023
HPM57	–	–	*P. guttata*	*C. avellana*	Szentes, Csongrád-Csanád	46.727203, 20.227526	12 September 2023
HPM58	–	–	*P. guttata*	*C. avellana*	Kiskunfélegyháza, Bács-Kiskun	46.669697, 19.815522	12 September 2023
HPM59	+	+	*E. corylacearum, P. guttata*	*C. avellana*	Decs, Tolna	46.282690, 18.760560	25 September 2023

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