Fungi causing powdery mildew on plants of a Botanical Garden in Southern Finland

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Abbreviations: PMCF, Powdery Mildew-Causing Fungi

Abstract

Fungi that cause powdery mildew on plants are plant pathogenic parasites (Erysiphales) and can significantly reduce the ornamental value of plants and cause significant yield losses among cultivated plants. In this study, 94 plant accessions infected with powdery mildew were observed in Kumpula Botanic Garden, Helsinki, Finland, in 2015. The taxonomic affiliation and species richness of powdery mildew fungi were investigated. Morphological studies by microscope distinguished only 14 fungal species, whereas further comparisons of internal transcribed spacer (ITS) sequences enabled the identification of 28 species. Hence, ITS sequencing improved the reliability of species determination, as compared with the use of morphological characteristics only. The vegetation in an area of six hectares supported a wide range of fungi that cause powdery mildew as well as hyperparasitic microbes, which may balance the impact of pathogens in host plants. The findings of this study emphasize the role of botanical gardens in protecting biological diversity in urban areas.
Introduction

The fungi in the order Erysiphales are obligate parasites that cause symptoms of powdery mildew and crop losses in a wide range of cultivated agricultural and horticultural crops, including ornamental plants (Glawe 2008). Globally, ca. 900 powdery mildew–causing fungi (PMCF) have been described (Braun & Cook 2012). However, diversity among PMCF in the Northern Hemisphere is more limited due to the short growing season and early death of host plants due to frost. In fact, diversity of PMCF in the Northern Hemisphere hasn’t been studied extensively. Botanical gardens in the Northern Hemisphere offer the possibility for studying the diversity of PMCF in this region, as they contain a wide range of plant species that are adapted to a northern climate. Such studies are relatively rare and have so far focused mainly on single taxa rather than on the analysis of overall diversity of PMCF within a certain area (Korytnanskaya 2010; Mieslerová et al. 2020a; Mieslerová et al. 2020b).

Over 100 PMCF have been identified and are listed in the Finnish Biodiversity Information Facility (Salo et al. 2019), and 256 natural host-fungus combinations associated with powdery mildew have been documented in Finland (Weltzien 1978). Kumpula Botanic Garden of University of Helsinki (subsequently referred to as KBG) hosts a collection of ca. 1500 plant taxa, consisting of plants that have been introduced from different areas of the Northern Hemisphere with bioclimatic habitats resembling Southern Finland (Schulman & Hällfors 2012). Powdery mildew is common and can significantly reduce the ornamental value of plants in the garden. The large and diverse collection of plant species in the garden may host a wide range of non-native PMCF from other countries.

PMCF can be cumbersome to study because hyphae may not be long-lasting, PMCF cannot grow on artificial media and need a living host to survive, and the characteristic structures used for identification by microscopy may be difficult to find and/or maintain. These obstacles can be alleviated by use of DNA-based methods, such as analysis of the internal transcribed spacer (ITS) sequences between the small and large subunit of ribosomal RNA genes. ITS sequences are used as universal barcodes for fungi (Schoch 2012) allowing identification of species based on their ITS sequences and comparisons with those deposited in gene banks (Benson et al. 2013).

The aim of this study was to use morphological characteristics and molecular techniques, such as barcodes based on ITS sequences, to determine the PMCF species infecting plants in KBG, to study species richness of PMCF, and to find out whether the population of PMCF differs from the species previously characterized in Finland.

Materials and methods

Collection of samples for analysis of morphology and ITS sequences

KBG is located at latitude 60.192059(N) and longitude 24.945831(E) in Southern Finland. In total, 94 plant accessions with symptoms of PMCF (Fig. 1) were observed during the growth season of 2015 (May 18th to September 30th), and 70 herbarium samples were collected. Plant disease symptoms were surveyed once a week. Samples were taken from symptomatic parts, dried between sheets of blotting paper, and preserved for further examination (Table 1). In addition, powdery mildew (mycelia) was scraped off of 42 plants, transferred into Eppendorf tubes, and frozen at -18 °C for DNA analysis.

Morphological examination of fungi

All dried samples from the herbarium were examined and measured with a Researcher Bino II light microscope (Bresser GmbH, Germany) that was equipped with a 3-megapixel camera and MicroCamLab software (v. 7.3.1.8). Fresh mycelia, were transferred onto a microscope slide covered with a piece of adhesive tape and placed on top of a drop of water (Heffer et al. 2006). Characteristics of the mycelium (color, width, growth habit, and shape of appressoria), anamorphs (size and shape of conidia and conidiophores; observation of conidiogenesis) and telemorphs stages (size and color of chas-
mothecia; shape of appendages; shape, size, and color of asci and ascospores) were recorded. Species identification of PMCF was based on the descriptions of Braun (1995) and Braun & Cook (2012).

DNA isolation and design of PMCF-specific primers

DNA was extracted from the fungal mycelia using DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). All 42 samples were screened first by polymerase chain reaction (PCR) using the universal primers ITS5/ITS4 (White et al. 1990) to ensure that samples contained fungal DNA. Subsequently, PMCF-specific primers were designed by downloading rDNA sequences of powdery mildew-causing fungi from NCBI GenBank (Benson et al. 2013): AB022346.1, AB022347.1, AB022349.1, AB022354.1, AB022364.1, AB022365.1, AB022366, AB022369.1, AB022373.1, AB022374.1, AB022398.1, AB022399.1, AB022402.1, AB022404.1, AB022405.1, AB022410.2, AB022411.1, AB022419.1, AB033477.1, AB033492.1, AB077619.1, AB077671.1, AB080411.1, AB080470.1, AB103069.1, AB103078.1, AB103370.1, AB193465.1, AB237812.1, and AF021796.1. These sequences were aligned with Mult-Alin software (Corpet 1988) and used to design primers for PCR (18S-2F, 28S-4R, and 28S-6R) and sequencing (5.8S-F, 5.8S-2F, 5.8S-R, and 5.8S-3R) (Fig. 2).

PCR reactions

The whole ITS region (ITS1 + 5.8S + ITS2), which included partial 18S and 28S regions (800–900 bp), was amplified by PCR using the primer set 18S-2F/28S-4R or 18S-2F/28S-6R (Fig. 2). PCR reactions consisted of a final volume of 25 μl containing 1 μl DNA template, 2.5 μl of Optimized 10x DyNAzyme buffer, 0.5 μl of 10 mM dNTPs (Thermo Fisher Scientific Baltics, UAB, Vilnius, Lithuania), 1 μl of 10 μM forward primer (18S-2F), 1 μl of 10 μM reverse primer (28S-4R or 28S-6R), and 0.25 μl of 2 U/μl DyNAzyme II DNA polymerase (Thermo Fisher Scientific Baltics, UAB, Vilnius, Lithuania). Thermal cycling included an initial denaturation at 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 40 s and a final extension of 8 min at 72 °C. All PCR products were electrophoresed in an agarose gel with a 1% Tris-acetate-EDTA buffer (Sigma-Aldrich Co., St. Louis, MO, USA). Ethidium bromide was added (2.5 mg/ml) to the gel to visualize the PCR products.
Table 1. Ninety-four host plants from KBG (Helsinki, Finland) on which symptoms of powdery mildew were observed and the methods used for sampling.

| Host plant a, b | Family         | Collected c | Accession   |
|-----------------|----------------|-------------|-------------|
| *Abelmoschus esculentus* | Malvaceae      | 2010-0348   |             |
| *Acer negundo (H) (Amp) (9)* | Sapindaceae   | 31.8.       | 1992-0060   |
| *Acer platanoides (H) (Amp)* | Sapindaceae   | 30.9.       | 00XX-0004   |
| *Acer platanoides* | Sapindaceae   | 30.9.       | 00XX-0167   |
| *Acer platanoides (H) (Amp)* | Sapindaceae   | 00XX-0166   |             |
| *Acer tataricum subsp. ginnala* | Sapindaceae   | 1994-0891   |             |
| *Acer tataricum subsp. ginnala (H) (Amp) (40)* | Sapindaceae | 30.9.       | 2008-0104   |
| *Acer tataricum subsp. tataricum (H)* | Sapindaceae | 3.8.        | 2005-0017   |
| *Acer tataricum subsp. tataricum (H) (Amp)* | Sapindaceae | 10.9.       | 2008-0161   |
| *Aconitum napellus (H) (13)* | Ranunculaceae | 20.8.       | 1995-0451   |
| *Alchemilla indet.* | Rosaceae       |             | 2008-1029   |
| *Arctium lappa var. edule (H) (Amp) (25)* | Asteraceae     | 20.8.       | 2010-0296   |
| *Avena sativa (H) (37)* | Poaceae        | 20.8.       | 2009-0567   |
| *Avena strigosa* | Poaceae        |             | 2014-0350   |
| *Brassica oleracea var. sabellica (H)* | Brassicaceae   | 29.9.       | 2013-0024   |
| *Campanula bononiensis (H)* | Campanulaceae | 30.9.       | 2012-0735   |
| *Centaurea phrygia (H) (Amp) (10)* | Asteraceae     | 30.9.       | 1992-0147   |
| *Clematis recta (H) (31)* | Ranunculaceae | 23.9.       | 2004-0502   |
| *Cucumis sativus (H) (35)* | Cucurbitaceae  | 20.8.       | 2013-0042   |
| *Cucurbita maxima (H)* | Cucurbitaceae  | 23.9.       | 2015-0123   |
| *Cucurbita moschata (H)* | Cucurbitaceae  | 23.9.       | 2015-0125   |
| *Cucurbita pepo (H) (Amp)* | Cucurbitaceae  | 23.9.       | 2015-0176   |
| *Cucurbita pepo (H)* | Cucurbitaceae  | 23.9.       | 2015-0175   |
| *Cucurbita pepo (H) (Amp) (36)* | Cucurbitaceae | 8.9.        | 2015-0124   |
| *Cucurbita pepo (H)* | Cucurbitaceae  | 23.9.       | 2015-0300   |
| *Delphinium elatum (H) (38)* | Ranunculaceae | 3.8.        | 2006-0690   |
| *Delphinium elatum (H)* | Ranunculaceae  | 31.8.       | 1995-0091   |
| *Delphinium indet. (H) (1)* | Ranunculaceae  | 3.8.        | 2008-0149   |
| *Echium maculatum (H) (28)* | Boraginaceae   | 3.8.        | 2012-0627   |
| *Echium vulgare* | Boraginaceae   |             | 2011-0721   |
| *Euonymus europaeus (H)* | Celastraceae   | 3.8.        | 1992-0099   |
| *Euonymus europaeus* | Celastraceae   |             | 1996-0337   |
| *Geranium pretense (H) (39)* | Geraniaceae    | 10.9.       | 2001-0068   |
| *Geranium sanguineum (H) (7)* | Geraniaceae    | 3.8.        | 1977-0512   |
| Host planta, b                        | Family            | Collected c | Accession |
|--------------------------------------|-------------------|-------------|-----------|
| Geum urbanum                         | Rosaceae          |             | 1996-0284 |
| Hieracium umbellatum (H)             | Asteraceae        | 30.9.       | 2010-1360 |
| Hordeum vulgare (H)                  | Poaceae           | 20.8.       | 2009-0568 |
| Hypericum ascyron (H) (12)           | Hypericaceae      | 10.9.       | 1993-0728 |
| Hypericum maculatum                  | Hypericaceae      |             | 2007-0699 |
| Hypericum perforatum (H) (18)        | Hypericaceae      | 23.9.       | 2003-0634 |
| Incarvillea delavayi (H) (26)        | Bignoniaceae      | 31.8.       | 2011-1215 |
| Lonicera tatarica                    | Caprifoliaceae    |             | 1997-0172 |
| Monarda didyma (H) (5)               | Lamiaceae         | 30.9.       | 1993-0230 |
| Mycelis muralis (H)                  | Asteraceae        | 30.9.       | 2010-1327 |
| Pismum sativum (H)                   | Fabaceae          | 23.9.       | 2005-0571 |
| Pismum sativum (H) (27)              | Fabaceae          | 8.9.        | 2012-1008 |
| Pismum sativum (H)                   | Fabaceae          | 23.9.       | 2013-0762 |
| Pismum sativum (H)                   | Fabaceae          | 23.9.       | 2010-0650 |
| Plantago lanceolata (H) (Amp) (20)   | Plantaginaceae    | 20.8.       | 2006-0888 |
| Plantago major subsp. major (H) (Amp) (33) | Plantaginaceae | 10.9.       | 2006-0889 |
| Plantago major subsp. major (H) (2)  | Plantaginaceae    | 20.8.       | 2008-1028 |
| Pulmonaria montana                   | Boraginaceae      |             | 2006-0654 |
| Quercus macrocarpa (H) (15)          | Fagaceae          | 30.9.       | 1997-0448 |
| Quercus macrocarpa (H)               | Fagaceae          | 23.9.       | 1996-0015 |
| Quercus robur (H) (29)               | Fagaceae          | 31.8.       | 00XX-0256 |
| Quercus robur (H)                    | Fagaceae          | 30.9.       | 00XX-0035 |
| Rhamnus frangula (H) (Amp) (16)      | Rhamnaceae        | 30.9.       | 1998-0201 |
| Rosa acicularis subsp. sayi (H) (6)  | Rosaceae          | 3.8.        | 1969-0232 |
| Rosa amblyotis                       | Rosaceae          |             | 1969-0282 |
| Rosa amblyotis                       | Rosaceae          |             | 1993-0734 |
| Rosa amblyotis (H) (11)              | Rosaceae          | 30.9.       | 1993-0721 |
| Rosa blanda                          | Rosaceae          |             | 1990-0064 |
| Rosa maximowiciana (H) (Amp) (17)    | Rosaceae          | 23.9.       | 1998-0600 |
| Rosa mollis                          | Rosaceae          |             | 1994-0443 |
| Rosa nutkana                         | Rosaceae          |             | 1992-0531 |
| Rosa villosa                         | Rosaceae          |             | 2012-0542 |
| Rosa virginiana (H)                  | Rosaceae          | 30.9.       | 1983-0565 |
| Salix caprea (H) (Amp) (30)          | Salicaceae        | 3.8.        | 1987-0943 |
| Salix caprea (H)                     | Salicaceae        | 23.9.       | 2010-0939 |
| Salix caprea (H) (Amp)               | Salicaceae        | 30.9.       | 00XX-0095 |
| Host plant a, b                   | Family            | Collected c | Accession     |
|----------------------------------|-------------------|-------------|---------------|
| Salix hastata (H) (42)           | Salicaceae        | 20.8.       | 1977-0871     |
| Salix repens subsp. rosmarinifolia (H) | Salicaceae     | 31.8.       | 1980-1929     |
| Salvia tesquicola                | Lamiaceae         |             | 2012-0751     |
| Sambucus racemosa (H)             | Adoxaceae         | 23.9.       | 2010-0946     |
| Sambucus racemosa var. melanocarpa (H) (Amp) (41) | Adoxaceae | 3.8.        | 1987-1194     |
| Solidago canadensis (H) (Amp) (14) | Asteraceae      | 30.9.       | 1995-0741     |
| Stachys palustris (H)             | Lamiaceae         | 30.9.       | 2010-1389     |
| Stachys sylvatica (H) (Amp) (21)  | Lamiaceae         | 8.9.        | 2007-0700     |
| Succisa pratensis (H) (Amp) (24)  | Dipsacaceae       | 30.9.       | 2010-1341     |
| Succisa pratensis (H) (19)        | Dipsacaceae       | 10.9.       | 2006-0844     |
| Symphytum officinale var. bohemicum (H) (Amp) (8) | Boraginaceae | 20.8.       | 1991-0452     |
| Thalictrum aquilegifolium         | Ranunculaceae     | 2013-0911   |               |
| Thalictrum aquilegifolium (H) (Amp) (22) | Ranunculaceae | 23.9.       | 2008-1011     |
| Thalictrum lucidum (H) (32)       | Ranunculaceae     | 8.9.        | 2006-0610     |
| Tragopogon capitatus              | Asteraceae        |             | 2009-0893     |
| Triticum aestivum                 | Poaceae           |             | 2009-0564     |
| Triticum boeticum                 | Poaceae           |             | 2015-0361     |
| Triticum compactum                | Poaceae           |             | 2015-0363     |
| Veronica jacquinii (H) (34)       | Plantaginaceae    | 23.9.       | 2012-0760     |
| Veronica longifolia var. longifolia (H) | Plantaginaceae | 30.9.       | 2010-1391     |
| Veronica spicata (H) (23)         | Plantaginaceae    | 8.9.        | 2010-0870     |
| Viburnum lantana (H) (Amp) (4)    | Adoxaceae         | 30.9.       | 00XX-0153     |
| Vicia sylvatica (H) (3)           | Fabaceae          | 8.9.        | 1991-0137     |

A samples 1–42 for ITS sequencing. Accession numbers refer to the KBG database (Kotka) and are also used to identify isolates subjected to DNA isolation.

b Hyperparasitic fungus (Ampelomyces quisqualis) was observed from the sample with a microscope (Amp).

c Dates of collection (in the year 2015) for inclusion in herbarium (H).
Sequencing

PCR products were purified using the E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek, Georgia, USA). The purified DNA products were sequenced by Macrogen Inc. (Amsterdam, The Netherlands). Five samples (isolates 4, 8, 9, 28, and 33) were cloned into pGEM-T Easy vector (Promega Corporation, Madison) using *E. coli* 5α cells and sequenced with universal M13F-pUC primer. This approach was used, because direct sequencing did not produce good-quality sequences (data not shown). All ITS1 and ITS2 sequences were deposited in European Nucleotide Archive (ENA) GenBank (accessions LT794916–LT795001).

**Analysis of sequences**

All ITS sequences obtained in this study were examined with BioEdit (v. 7.2.5, http://www.mbio.ncsu.edu/BioEdit/bioedit.html) software (Ibis Biosciences, Carlsbad, CA, USA), and all ambiguous regions within the sequences were manually removed. The sequences were processed with the program ITSx (Bengtsson-Palme et al. 2013) to remove conservative rDNA sequences (18S, 5.8S, and 28S) and separate the ITS1 and ITS2 sequences of each isolate. ITS1 and ITS2 of the same sample were joined together using a text editor, followed by comparison with the sequences deposited in GenBank by applying the BLASTn tool (Altschul et al. 1990). Sequences (ITS1 + ITS2) were aligned with AliView software (v. 1.18) (Larsson 2014), and a phylogenetic tree was built using MEGA software (v. 7) (Kumar et al. 2016). The maximum likelihood method with 1000 bootstrap replicates (Felsenstein 1985) was used to estimate branch support. The evolutionary distances were counted with Kimura’s two-parameter model (Kimura 1980). The ITS sequences of a closely related fungus, accession no. KT970793 (Baturo-Ciesniewska et al. 2017) from order Helotiales, were used as an outgroup.

**Fig. 2.** Primers used for PCR and sequencing. The approximate binding sites of primers used for amplification of ITS1 and ITS2 regions are shown.
Fig. 3. Phylogenetic tree built from PMCF sequences determined in this study using maximum likelihood method in MEGA 7 software. The ITS sequence of *Sclerotinia sclerotiorum* (accession no. KT970793.1) was chosen as an outgroup. KBG accession numbers are provided for the host plants corresponding to the sequenced specimens. The bar indicates 50 nucleotide changes within 1000 nucleotides.
Results and discussion

Morphological characteristics and phylogenetic analysis

Altogether, 94 observations of powdery mildew were made on different host plants grown in KBG during the growth season of 2015 (Table 1), which represented 70 plant species from 24 families. Among the 70 herbarium samples collected, 14 species of PMCF were detected and identified. Their identification was based on morphological characteristics only (Supplementary Tables 1 and 2). A hyperparasitic fungus (Ampelomyces quisqualis sensu lato) was detected in 23 herbarium samples with a microscope (Table 1). Using the PMCF-specific primers, we were able to amplify rDNA from 37 of the 42 isolates (Table 2). The ITS regions were PCR-amplified from five PMCF samples in which multiple fungi were present and were cloned into pGEM-T Easy vector (Promega Corporation, Madison).

A phylogenetic tree of PMCF including six fungal genera was generated based on the identified sequences (Fig. 3) using BLASTn (Altschul et al. 1990). A few isolates lacked polymorphisms within their ITS sequences. They were assumed to be different species based on their minor morphological variations and previous knowledge of their known hosts as mentioned in the literature (Braun 1995; Braun & Cook 2012).

Taxonomy

In this study, 28 species of PMCF from six genera were identified based on their morphological characteristics, ITS sequences, or both (Table 3). All PMCF determined in this study were previously known fungi in Finland. One of the identified fungi (Erysiphe hedwigii) lacks native hosts in Finland, yet it may thrive on ornamental host plants that are not native to Finland. The results of phylogenetic analyses and species determination based on morphological characteristics were largely consistent. The somewhat ambiguous species are discussed below.

Erysiphe spp

Eleven species of PMCF in the genus Erysiphe were identified based on knowledge of their morphological characteristics and analysis of ITS sequences (Table 3). A common feature in genus Erysiphe is production of a single conidium (Fig. 4a), whereas in the majority of other powdery mildew fungi the conidial-producing structures are characterized as catenescence (Fig. 4b). The appressoria in Erysiphe are typically lobed (Fig. 4c), and this feature helps to identify some samples. The fungal species E. hedwigii (isolate 00XX-0153) lacks a native host in Finland but may thrive on its ornamental hosts.

Erysiphe aquilegiae sensu lato infects plants within the family Ranunculaceae and is found throughout most of the world (Braun & Cook 2012). There are two known variants of the fungus: var. aquliegiae, which infects Thalictrum and Clematis, and var. ranunculi, which infects Aconitum, Clematis, Delphinium, and Thalictrum (Braun & Cook 2012). In this study, the morphological characteristics were not sufficient to distinguish between these variants on their hosts, but the two clusters of E. aquilegiae branches in the phylogenetic tree (Fig. 3) support the variants mentioned by Braun & Cook (2012). In contrast, Cunnington et al. (2004) showed that the ITS sequences are not the best candidates for distinguishing between these variants. On the same phylogenetic tree, isolates Erysiphe knautiae 2006-0844 and 2010-1341 showed 100% identity with isolates 2006-0610 and 2008-1011 of E. aquilegiae and 95% identity with E. aquilegiae isolate 2004-0502. Takamatsu et al. (2015) showed that E. knautiae belongs to the homogenous clade E. aquilegia, which includes at least 15 fungal species that are identical or differ very little among their rDNA sequences. It is assumed that E. knautiae does not infect Ranunculaceae hosts but does infect hosts belonging to family Dipsacaceae (Braun & Cook 2012). In Finland, E. knautiae is known to infect Knautia arvensis and Succisa pratensis (Braun 1995). The unexpected morphology of the appressoria of E. knautiae isolated in this study showed that appressoria in the observed samples were opposite (Fig. 4c) rather than single lobed, although they were described as being single lobed by Braun & Cook (2012).

Erysiphe hyperici (Wallr.) S. Blumer and E. trifoliorum (Wallr.) U. Braun were identified based on knowledge of their hosts (Braun 1995; Braun & Cook 2012) and ITS sequences. The ITS sequences show
Table 2. The samples identified based on their ITS sequence identities, as compared with previously described fungi using the BLASTn tool.

| Fungus                  | Host plant            | Isolate   | Sample |
|-------------------------|-----------------------|-----------|--------|
| Blumeria graminis f. sp. avenae | Avena sativa          | 2009-0567 | 37a    |
| Erysiphe adunca          | Salix caprea          | 1987-0943 | 30a    |
| Erysiphe adunca          | Salix hastata         | 1977-0871 | 42a    |
| Erysiphe alphitoides     | Quercus macrocarpa    | 1997-0448 | 15a    |
| Erysiphe alphitoides     | Quercus robur         | 00XX-0256 | 29a    |
| Erysiphe aequilegiae     | Delphinium indet.     | 2008-0149 | 1a     |
| Erysiphe aequilegiae     | Aconitum napellus     | 1995-0451 | 13a    |
| Erysiphe aequilegiae     | Thalictrum aquilegfolium | 2008-1011 | 22a    |
| Erysiphe aequilegiae     | Clematis recta        | 2004-0502 | 31a    |
| Erysiphe aequilegiae     | Thalictrum lucidum    | 2006-0610 | 32a    |
| Erysiphe aequilegiae     | Delphinium elatum     | 2006-0690 | 38a    |
| Erysiphe dwaricata       | Rhamnus frangula      | 1998-0201 | 16b    |
| Erysiphe hedwigii        | Viburnum lantano      | 00XX-0153 | 4c     |
| Erysiphe hyperici        | Hypericum ascyron     | 1993-0728 | 12a    |
| Erysiphe hyperici        | Hypericum perforatum  | 2003-0634 | 18a    |
| Erysiphe knautiae        | Succisa pratensis     | 2006-0844 | 19a    |
| Erysiphe knautiae        | Succisa pratensis     | 2010-1341 | 24d    |
| Erysiphe pisi           | Pisi sativum          | 2012-1008 | 27a    |
| Erysiphe trifoliorum     | Vicia sylvatica       | 1991-0137 | 3a     |
| Erysiphe vanbruntiana    | Sambucus racemosa var. malanocarpa | 1987-1194 | 41a |
| Galovinomyces asterum var. solidaginis | Solidago canadensis   | 1995-0741 | 14a |
| Galovinomyces cynoglossi | Symphytum officinale var. bohemicum | 1991-0452 | 8c |
| Galovinomyces depressus  | Arctium lappa         | 2010-0296 | 25a    |
| Galovinomyces depressus  | Echium maculatum      | 2012-0627 | 28c    |
| Galovinomyces monordae   | Monarda didyma        | 1993-0230 | 5a     |
| Galovinomyces montagnae  | Centaurea phrygia     | 1992-0147 | 10a    |
| Galovinomyces orontii    | Incarvillea delavayi  | 2011-1215 | 26e    |
| Galovinomyces orontii    | Veronica jacquini     | 2012-0760 | 34a    |
| Galovinomyces orontii    | Cucumis sativus       | 2013-0042 | 35a    |
| Galovinomyces sardidus   | Plantago major subsp. major | 2008-1028 | 2a |
| Galovinomyces sardidus   | Plantago major subsp. major | 2006-0889 | 33c |
| Neorysiphe galeapsids    | Stachys sylvatica     | 2007-0700 | 21a    |
| Podosphaera fugax        | Geranium sanguineum   | 1977-0512 | 7a     |
| Podosphaera fugax        | Geranium pratense     | 2001-0068 | 39d    |
| Podosphaera fuliginea    | Veronica spicata      | 2010-0870 | 23a    |
| Podosphaera pannosa      | Rosa acicularis subsp. sayi | 1969-0232 | 6a |
| Podosphaera pannosa      | Rosa ambylotis       | 1993-0721 | 11a    |
| Podosphaera pannosa      | Rosa maximowicziana  | 1998-0600 | 17b    |
| Podosphaera plantaginis  | Plantago lanceolata   | 2006-0888 | 20a    |
| Podosphaera xanthii      | Cucurbita pepo        | 2015-0124 | 36a    |
| Sawadaea bicornis        | Acer negundo         | 1992-0060 | 9c     |
| Sawadaea tulasnei        | Acer tataricum subsp. ginnala | 2008-0104 | 40a |

a PCR amplified with primer set 18S-2F/28S-4R and sequenced with primers 5.8SF and 5.8SR
b PCR amplified with primer set 18S-2F/28S-4R and 28S-6R, and sequenced with primers 5.8SF and 5.8S-3R
c PCR amplified with primer set 18S-2F/28S-4R and sequenced with M13F-pUC primer
d PCR amplified with primer set 18S-2F/28S-4R and sequenced with primers 5.8SF and 5.8S-3R
e PCR amplified with primer set 18S-2F/28S-4R and sequenced with primers 5.8S-2F and 5.8S-3R
| Accession (ENA) | ITS1 | ITS2 | Length (bp) | BLASTn (ITS1 + ITS2) Identity (%) | Accession (ENA) |
|----------------|------|------|-------------|-----------------------------------|----------------|
| LT794935       | LT794977 | 324 | 100         |                                  | AJ313140.1     |
| LT794916       | LT794958 | 396 | 100         |                                  | LC028970.1     |
| LT794917       | LT794959 | 396 | 100         |                                  | LC028970.1     |
| LT794918       | LT794960 | 396 | 100         |                                  | AB292705.1     |
| LT794919       | LT794961 | 396 | 100         |                                  | AB292705.1     |
| LT794922       | LT794964 | 392 | 99          |                                  | AY452802.1     |
| LT794920       | LT794962 | 392 | 99          |                                  | AY452802.1     |
| LT794923       | LT794965 | 392 | 100         |                                  | LC010016.1     |
| LT794921       | LT794963 | 392 | 99          |                                  | LC010016.1     |
| LT794924       | LT794966 | 392 | 100         |                                  | LC010016.1     |
| LT794925       | LT794967 | 392 | 100         |                                  | AY452802.1     |
| LT794926       | LT794968 | 400 | 99          |                                  | LC009956.1     |
| LT794934       | LT794976 | 395 | 100         |                                  | AF298539.1     |
| LT794928       | LT794970 | 398 | 100         |                                  | LC010027.1     |
| LT794927       | LT794969 | 398 | 100         |                                  | LC010027.1     |
| LT794929       | LT794971 | 392 | 100         |                                  | LC010042.1     |
| LT794930       | LT794972 | 392 | 100         |                                  | LC010042.1     |
| LT794931       | LT794973 | 398 | 100         |                                  | LC009890.1     |
| LT794932       | LT794974 | 398 | 100         |                                  | FJ378884.1     |
| LT794933       | LT794975 | 395 | 99          |                                  | LC009909.1     |
| LT794936       | LT794978 | 349 | 100         |                                  | KC513763.1     |
| LT794937       | LT794979 | 345 | 100         |                                  | AB077684.1     |
| LT794938       | LT794980 | 345 | 100         |                                  | AB077675.1     |
| LT794939       | LT794981 | 345 | 100         |                                  | AB077675.1     |
| LT794940       | LT794982 | 353 | 100         |                                  | LC076842.1     |
| LT794941       | LT794983 | 350 | 99          |                                  | AB077656.1     |
| LT794943       | LT794985 | 346 | 100         |                                  | AB769464.1     |
| LT794944       | LT794986 | 346 | 100         |                                  | AB769464.1     |
| LT794942       | LT794984 | 346 | 100         |                                  | AB769464.1     |
| LT794945       | LT794987 | 346 | 100         |                                  | AB077658.1     |
| LT794946       | LT794988 | 346 | 100         |                                  | AB077658.1     |
| LT794949       | LT794991 | 344 | 99          |                                  | KX231842.1     |
| LT794951       | LT794993 | 318 | 98          |                                  | AB525922.1     |
| LT794950       | LT794992 | 316 | 98          |                                  | AB525922.1     |
| LT794952       | LT794994 | 316 | 98          |                                  | AB046986.1     |
| LT794953       | LT794995 | 317 | 98          |                                  | AB525937.1     |
| LT794954       | LT794996 | 317 | 98          |                                  | AB525937.1     |
| LT794955       | LT794997 | 317 | 100         |                                  | KX842352.1     |
| LT794956       | LT794998 | 316 | 100         |                                  | JX442063.1     |
| LT794957       | LT794999 | 316 | 100         |                                  | KX369541.1     |
| LT794947       | LT794989 | 313 | 99          |                                  | AB193380.1     |
| LT794948       | LT794990 | 303 | 99          |                                  | AB193385.1     |
Table 3. Summary of 28 PMCF species identified in the host plant samples collected from KBG and their identification methods. The accession numbers refer to the origin of the samples in the database of the KBG (https://kotka.luomus.fi).

| Fungus | Host plant (accession) | Identification | Morphology | ITS |
|--------|------------------------|----------------|------------|-----|
| Blumeria graminis (DC.) Speer | Avena sativa (2009-0567) | × | × | |
| | Hordeum vulgare (2009-0568) | × | | |
| Erysiphe adunca (Wallr.) Fr. | Salix caprea (1987-0943) | × | × | |
| | Salix hastata (1977-0871) | × | | |
| | Salix caprea (00XX-0095) | × | | |
| | S. repens subsp. rosmarinifolia (1980-1929) | × | | |
| | Salix caprea (2010-0939) | × | | |
| Erysiphe alphitoides (Griffon & Maubl.) U. Braun & S. Takam. | Quercus macrocarpa (1997-0448) | × | × | |
| | Quercus robur (00XX-0256) | × | | |
| | Quercus robur (00XX-0035) | × | | |
| Erysiphe aquilegiae DC. | Aconitum napellus (1995-0451) | × | × | |
| | Clematis recta (2004-0502) | × | | |
| | Delphinium elatum (2006-0690) | × | | |
| | Delphinium indet. (2008-0149) | × | | |
| | Thalictrum aquilegifolium (2008-1011) | × | | |
| | Thalictrum lucidum (2006-0610) | × | | |
| | Delphinium elatum (1995-0091) | × | | |
| Erysiphe divaricata (Wallr.) Schltdl. | Rhamnus frangula (1998-0201) | × | | |
| Erysiphe euonymi DC. | Euonymus europaeus (1992-0099) | × | | |
| Erysiphe hedwigii (Lév.) U. Braun & S. Takam. | Viburnum lantano (00XX-0153) | × | | |
| Erysiphe hyperici (Wallr.) S. Blumer | Hypericum ascyron (1993-0728) | × | | |
| | Hypericum perforatum (2003-0634) | × | | |
| Erysiphe knautiae Duby | Succisa pratensis (2006-0844) | × | | |
| | Succisa pratensis (2010-1341) | × | | |
| Erysiphe pisi DC. | Pisum sativum (2012-1008) | × | | |
| Erysiphe trifoliorum (Wallr.) U. Braun | Vicia sylvatica (1991-0137) | × | | |
| Erysiphe vanbruntiana (W.R. Gerard) U. Braun & S. Takam. | Sambucus racemosa (2010-0946) | × | | |
| | S. racemosa var. melanocarpa (1987-1194) | × | | |
| Golovinomyces asterum var. solidaginis U. Braun | Solidago canadensis (1995-0741) | × | | |
| Golovinomyces cichoracearum (DC.) V.P. Heluta | Hieracium umbellatum (2010-1360) | × | | |
| Golovinomyces cynoglossi (Wallr.) V.P. Heluta | S. officinale var. bohemicum (1991-0452) | × | | |
| Fungus                                      | Host plant (accession)       | Identification Morphology | ITS |
|--------------------------------------------|------------------------------|---------------------------|-----|
| Golovinomyces depressus (Wallr.) V.P. Heluta | Arctium lappa (2010-0296)    |                           |     |
|                                            | Echium maculatum (2012-0627) |                           |     |
| Golovinomyces monardae (G.S. Nagy) M. Scholler | Monarda didyma (1993-0230)   |                           |     |
|                                            |                             |                           |     |
| Golovinomyces montagnei U. Braun           | Centaurea phrygia (1992-0147)|                           |     |
| Golovinomyces orontii (Castagne) V.P. Heluta | Cucumis sativus (2013-0042)  |                           |     |
|                                            | Incarvillea delavayi (2011-1215) |                 |     |
|                                            | Veronica jacquinii (2012-0760) |                           |     |
| Golovinomyces sordidus (L. Junell) V.P. Heluta | Plantago major subsp. major (2006-0889) |    |     |
|                                            | Plantago major subsp. major (2008-1028) |   |     |
| Neoerysiphe galeopsidis (DC.) U. Braun     | Stachys sylvatica (2007-0700) |                           |     |
| Podosphaera fugax (Penz. & Sacc.) U. Braun & S. Takam. | Geranium pratense (2001-0068) |                           |     |
|                                            | Geranium sanguineum (1977-0512) |                       |     |
| Podosphaera fuliginea (Schltld.) U. Braun & S. Takam. | Veronica spicata (2010-0870) |                           |     |
| Podosphaera pannosa (Wallr.) de Bary       | Rosa acicularis subsp. sayi (1969-0232) |                      |     |
|                                            | Rosa amblyotis (1993-0721)    |                           |     |
|                                            | Rosa maximowicziana (1998-0600) |                        |     |
|                                            | Rosa virginiana (1983-0565)   |                           |     |
| Podosphaera plantaginis (Castagne) U. Braun & S. Takam. | Plantago lanceolata (2006-0888) |                      |     |
| Podosphaera xanthii (Castagne) U. Braun & Shishkoff | Cucurbita pepo (2015-0124)  |                           |     |
| Sawadaea bicornis (Wallr.) Homma           | Acer negundo (1992-0060)     |                           |     |
| Sawadaea tulasnei (Fuckel) Homma           | Acer platanoides (00XX-0004)  |                           |     |
|                                            | Acer platanoides (00XX-0166)  |                           |     |
|                                            | Acer tataricum subsp. ginnala (2008-0104) |                    |     |
|                                            | Acer tataricum subsp. tataricum (2005-0017) |                 |     |
|                                            | Acer tataricum subsp. tataricum (2008-0161) |                    |     |
100% similarity among all three isolates (1991-0137 on Vicia sylvatica, 1993-0728 on Hypericum ascyron, and 2003-0634 on H. perforatum) although they were collected from hosts belonging to different plant families. The morphological characteristics were not sufficient to distinguish between species. E. hyperici is a common fungus of Hypericum species in Asia, the Caucasus, Europe, and North America (Braun & Cook 2012). There are also a few native hosts (Hypericum spp.) in Finland (Braun 1995; Mäkinen 1965; Rauhala 1957). In contrast, E. trifoliorum is known to infect only Fabacean hosts in Africa, North America, the Caucasus, and Europe. It has spread to South America, Australia, and New Zealand as well (Braun & Cook 2012). The native hosts in Finland for E. trifoliorum are Succisa pratensis and several Lathyrus and Trifolium species (Ahti 1967; Braun 1995; Mäkinen 1965; Rauhala 1957). Also, genus Caragana is among the host plants in Finland (Braun 1995; Rauhala 1957), but Erysiphe palczewskii (Jacz.) U. Braun & S. Takam. is known to have displaced E. trifoliorum on Caragana host plants in Finland (Huhtinen et al. 2001). Takamatsu et al. (2015) presented the clade E. trifoliorum sensu lato, which includes both E. hyperici and E. trifoliorum.

**Golovinomyces SPP.** Eight species of PMCF were identified as Golovinomyces with the methods previously mentioned (Table 3). A common morphological feature for the fungi in this genus is caten escent conidial formation (Fig. 4b). Similar to the Erysiphe species, all of the identified Golovinomyces species had been previously identified in Finland (Salo et al. 2019). The species Golovinomyces monardae (G.S. Nagy) M. Scholler (= G. biocellatus Ehrenb.) does not have any native Monarda plants as its host in Finland, but it is found on non-native ornamental hosts and on several native or non-native Lamiaceae hosts. In earlier publications, it is usually referred to as G. biocellatus, but Scholler et al. (2016) showed in their study that G. monardae can be excluded from the wider G. biocellatus complex and is classified as its own species.

Golovinomyces depressus (Wallr.) V.P. Heluta was identified in two species (Arctium lappa 2010-0296 and Echium maculatum 2012-0627) based on ITS sequencing. This may indicate that Echium maculatum is a new host for the fungus G. depressus, although the morphological characteristics were not enough to confirm this. In addition, pathogenicity was not tested to provide further proof. It is currently understood that G. depressus infects Arctium and Centaurea species in Asia, the Caucasus, Europe, and North America (Braun & Cook 2012), but not Boraginaceae plants such as Echium spp. In Finland, the known G. depressus observations are from Arctium, Centaurea, and Onopordum acting as host plants (Braun 1995; Rauhala 1957).

**Blumeria, Neoërysiphe, Podosphaera, and Sawadaea SPP.** One Blumeria sp., one Neoërysiphe sp., five Podosphaera spp., and two Sawadaea spp. were identified (Table 3). Among them, Podosphaera and Sawadaea are the only species that have fibrosin bodies (Fig. 4e) inside their conidia, which can be used as a supportive feature for identification based on morphological characteristics. If teleomorphs are present, the species in genus Podosphaera are the only ones that have a single ascus inside each chasmothecia (Fig. 4d). Among all PMCF, Blumeria is the only genus that is capable of infecting monocot plants. Blumeria spp. share a common morphological feature of having a bulbous swelling (Fig. 4f) that develops from the hyphae and forms the base of the conidium. The species identified in these four genera are PMCF that had been previously identified in Finland (Salo et al. 2019).

Podosphaera fugax (Penz. & Sacc.) U. Braun & S. Takam. was identified in two Geranium samples (G. sanguineum 1977-0512 and G. pratense 2001-0068) based on morphological characteristics as well as ITS sequence similarity. Knowledge of the known hosts of P. fugax (Braun & Cook 2012) was also used for species determination. Among ITS sequences from the fungus Podosphaera pannosa (Wallr.) de Bary isolated from three Rosa spp. (1969-0232, 1993-0721, and 1998-0600) there were differences at only a few nucleotides. All three P. pannosa sequences were 317 nucleotides in length but showed minor nucleotide polymorphisms among the analyzed isolates (1969-0232, 1993-0721, and 1998-0600). P. pannosa was also identified on a Rosa virginiana (1983-0565) herbarium sample based on morphological characteristics. P. fugax is known to infect Geranium species in Africa, Asia, the Caucasus, Europe, and North America, and it has been introduced into New Zealand (Braun & Cook 2012). P. fugax is also a common fungus on Geranium spp. in the wild in Finland (Ahti 1967; Braun...
Fig. 4. Morphologically important characteristics of powdery mildew fungi. (a-g) The following are examples of structures observed among the 14 morphologically identifiable species of PMCF (host plant accession numbers are included): (a) a single conidiospore (1991-0137), bar = 20 µm; (b) a catenescence conidial formation (1993-0230), bar = 10 µm; (c) a lobed appressorium (2006-0844), bar = 10 µm; (d) a chasmothecium and single ascus containing multiple ascospores (1983-0565), bar = 40 µm; (e) microconidia and fibrosin bodies inside them (2005-0017), bar = 10 µm; (f) a bulbous swelling (2009-0568), bar = 20 µm; and (g) a hyperparasite of powdery mildew (1995-0741), bar = 20 µm.

1995; Rauhala 1957). Takamatsu et al. (2010) placed both *P. fugax* and *P. pannosa* in the same Rosoideae group, as they seem to be evolutionarily closely related. *P. pannosa* has spread all over the world (Braun & Cook 2012) and has been found on several *Rosa* spp. in Finland (Braun 1995; Mäkinen 1965; Rauhala 1957).

*Podosphaera plantaginis* (Castagne) U. Braun & S. Takam. was identified from one plant of *Plantago lanceolata* (isolate 2006-0888) based on ITS sequence similarity. *P. plantaginis* is known to infect plants in Asia, the Caucasus, Europe, and North America (Braun & Cook 2012). The common host for the fungus in Finland is *Plantago lanceolata* (Braun 1995; Rauhala 1957; Jousimo et al. 2014). *Ampelomyces quisqualis* Ces. is a well-studied hyperparasitic fungus that infects *P. lanceolata* (Tollenaere et al. 2014) in Finland. It grows inside the mycelial structures of the powdery mildew fungus (*Fig. 4g*) (Kiss et al. 2004). The ITS sequence of *A. quisqualis* was obtained in this study while sequencing the isolate from *Plantago major* 2006-0889. The *A. quisqualis* ITS sequence was deposited in ENA GenBank (ITS1: LT795000; ITS2: LT795001).

Taken together, the results of this study show that an area of six hectares can support a wide range of fungal populations, such as PMCF, when compatible host plants are present. Furthermore, the hyperparasitic fungus *A. quisqualis* sensu lato was commonly present based on microscope analysis. The 28 PMCF determined in this study are likely to represent just a subset of PMCF in Finland. However, they were isolated by one of the few surveys carried out in a botanical garden using morphological characteristics.
and molecular methods for identification. The higher number of PMCF identified with ITS sequences relative to morphological characteristics is supported by a previous study carried out elsewhere (Cunnington et al. 2003). The results of this study also show that botanical gardens maintain not only the genetic diversity of plants but also their pathogens, such as hyperparasitic fungi, which may help to regulate the impact of plants and microbes in gardens.

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**SUPPLEMENTARY TABLES**

**Table S1.** Fungal isolates observed at the anamorph stage with a focus on their mycelia and conidia.

**Table S2.** Fungal isolates observed at the teleomorph stage with a focus on chasmothecia, asci, and ascospores.