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ORIGINAL ARTICLE

Characterisation and risk assessment of the emerging *Peronospora* disease on *Aquilegia*

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Abstract *Aquilegia* is a popular garden plant in the northern hemisphere as well as a native plant in the UK and continental Europe. In 2000, *Semiaquilegia adoxoides* was found infected by downy mildew in Korea, and since 2013 there have been several confirmed records of a *Peronospora* sp. affecting *Aquilegia* in the UK, with symptomatic plants being observed several years prior. Symptoms include yellow patches delineated by the veins on the leaves of affected plants, but systemically infected plants were also recorded, which are generally chlorotic and show curled leaf margins. A greyish down of conidiophores and conidia was observed on the lower side of infected leaves. Preliminary molecular phylogenetic analyses could not identify the causal agent at the species level, but revealed its affinities to other *Peronospora* species parasitic on the Ranunculales and Saxifragaceae. To our knowledge, this is the first occurrence of a downy mildew affecting a species of *Aquilegia*. Already, about 1 year after its confirmed first occurrence in the UK and 3 years after reported symptoms, a huge impact on infested gardens and nurseries has occurred. As oospore production has been observed and the pathogen can grow systemically, rendering seed transmission likely, this pathogen should be classified as a high risk pathogen for *Aquilegia*. Appropriate quarantine measures should be taken to restrict the pathogen from spreading.

Keywords *Aquilegia* · Downy mildew · *Peronospora* · Ranunculales · UK

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Introduction

Aquilegia is an herbaceous perennial in the buttercup family, Ranunculaceae, and is widely grown in United Kingdom (UK) gardens and elsewhere throughout the world (Nold 2003). The Ranunculaceae is a large family comprising at least 1800 species in over 50 genera (Glimn-Lacy and Kaufman 2006). The Royal Horticultural Society (RHS) Plant Finder (Cubey et al. 2015) lists 46 species and numerous cultivars of *Aquilegia* available to gardeners in the UK, but only *Aquilegia vulgaris* L. is considered as a UK native. However, it is difficult to determine wild populations as many garden escapes also occur (Stace 2010). In 2013, the RHS Gardening Advice service received its first record of downy mildew affecting *Aquilegia*. More records have followed in subsequent years. Cases have been recorded throughout England and Wales, including Berkshire, Buckinghamshire, Cardiff, Derbyshire, Devon, Dorset, Essex, East Sussex, Hampshire, Hertfordshire, Isle of Wight, Kent, Lancashire, London, Norfolk, North Yorkshire, Oxfordshire,

Somerset, South Yorkshire, Surrey, Swansea, West Midlands and Wiltshire.

Downy mildew pathogens can spread via rain splash and wind dispersal of spores. Air-borne spores (conidia or sporangia) are relatively short-lived and longer survival occurs via infection of perennial tissue or with the formation of oospores, which are produced in dying parts of the host. Oospores are readily produced in some species of *Peronospora* Corda but have not been found in others (Gustavsson 1959b). Many authors have confirmed seed transmission of downy mildews, including *Plasmopara halstedii* (Farl.) Berl. & De Toni on sunflower (*Helianthus annuus* L.) (Sackston 1981; Basavarju et al. 2004), *Peronospora effusa* (Grev.) Rabenh. on spinach (*Spinacia oleracea* L.) (Inaba et al. 1983), *Hyaloperonospora brassicae* (Gäum.) Göker, Voglmayr, Riethm, M. Weiss & Oberw. s.l. on radish (Jang and Safeeulla 1990), *Peronosclerospora sorghi* (W. Weston & Uppal) C.G. Shaw on maize (*Zea mays* L.) (Adenle and Cardwell 2000), *Peronospora variabilis* Gäum. on quinoa (Danielsen et al. 2004), *Peronospora belbahrii* Thines on sweet basil (Garibaldi et al. 2004; Belbahri et al. 2005), and *Peronospora meconopsidis* Mayor on opium poppy (Landa et al. 2007). In addition, Sackston (1981) demonstrated that secondary infections by sporangia of *Plasmopara halstedii* produced latent infections of sunflower plants showing no symptoms, but which may also produce seeds with latent infections.

To our knowledge, there are so far no records of downy mildew affecting *Aquilegia* worldwide,. However, there are 59 *Peronospora* species described from Ranunculales, i.e. 30 from Ranunculaceae, 27 from Papaveraceae, and 2 from Berberidaceae (Constantinescu 1991; Voglmayr et al. 2014; Voglmayr and Korytnianska 2015). *Peronospora* species are thought to be highly host-specific (Gäumann 1923; Constantinescu 1991), with most of them affecting only one or a few host species. However, previously, there have been conflicting species concepts for *Peronospora* species. De Bary (1863) recorded each *Peronospora* species depending on which host family it infected, whereas Gäumann (1923) mostly recognised one *Peronospora* species for each host species affected, dramatically increasing the number of *Peronospora* species recorded. Later, researchers have been divided over which approach to follow, with some, such as Yerkes and Shaw (1959), using a broader species concept, often because it was believed that the morphological features used by Gäumann (1923) for discrimination were insignificant. Others such as Gustavsson (1959a) and Constantinescu (1991) followed the concept of Gäumann in their monographic studies.

It has now been accepted that Gäumann's (1923) narrow species hypothesis is more appropriate than the broader species concept of Yerkes and Shaw (1959), as phylogenetic studies have generally revealed phylogenetically distinct lineages specifically present only on a particular host species (Riethmüller et al. 2002; Voglmayr 2003; Göker et al. 2004, 2009; Voglmayr et al. 2004, 2009; Choi et al. 2007, 2008a, b, 2009, 2011, 2015; Thines et al. 2009, 2011; Constantinescu and Thines 2010).

The previously widespread application of the broad species concept had a profoundly negative impact on agriculture and horticulture, as has been highlighted by the misidentification of *Peronospora belbahrii* on basil as *Peronospora lamii* A. Braun (Gumedzoe et al. 1998; Heller and Baroffio 2003; Lefort et al. 2003; Martini et al. 2003). *Peronospora lamii* was thought to affect a variety of members of the Lamiaceae, but recent phylogenetic and morphological work (Belbahri et al. 2005; Thines et al. 2009; Choi et al. 2009; Gabler et al. 2012) revealed that the mint family was parasitized by several different species of *Peronospora*. However, there seem to be a few downy mildews with a broader host range, encompassing more than one host genus (Göker et al. 2004, 2009; Thines et al. 2011) or, in the exceptional case of *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev, even various genera of a host family (Waterhouse and Brothers 1981; Lebeda and Widrlechner 2003; Runge and Thines 2009, 2011, 2012; Kitner et al. 2015). Thus, it is necessary that new occurrences of *Peronospora* are investigated not only by the recording of new hosts but also by morphological and phylogenetic characteristics.

There are many downy mildews affecting plants in UK gardens. Francis and Waterhouse (1988) compiled a list of *Peronospora* spp. in the British Isles, listed by host family and then by host species, based on herbarium specimens and previous literature. The impact of these species differs depending on the aggressiveness of the downy mildew species and the host plant affected. For example, Impatiens downy mildew (*Plasmopara obducens* (J. Schröt.) J. Schröt. s.l.) has had a serious impact on UK gardens. The disease was first recorded affecting *Impatiens walleriana* Hook.f. (busy lizzies) in 2003 in the UK (Lane et al. 2005). It then remained at low levels or absent but reappeared in 2007 and 2008 (Beal 2012). The disease was controlled successfully in nurseries with a fungicide program using the active ingredient metalaxyl-M. But in 2011, a strain of *Plasmopara obducens* with resistance to metalaxyl-M was reported in the UK (Jennings 2014). As a consequence, few nurseries now sell *I. walleriana* and, therefore, the presence of this plant in UK gardens has decreased dramatically.

Given the aggressiveness of the newly occurring Aquilegia downy mildew, the aim of this study was to

report on the occurrence, biology, host preference, morphology and phylogenetic position of this emerging disease, with the goal of providing a basis for restricting the further spread of the pathogen.

Materials and methods

Plant material

Isolates used in this study were from symptomatic plants as listed in Table 1. Diseased samples were received by RHS Gardening Advice during 2013, 2014 and 2015 from at least 13 UK locations: Devon, East Sussex, Hampshire, Hertfordshire, North Yorkshire, Oxfordshire, South Yorkshire, Surrey, Swansea, West Midlands, and Wiltshire. The sample from Korea was collected from the Halla Arboretum in 2000.

Morphology

The morphology of conidiophores, conidia, and oospores from symptomatic plant tissue was investigated. Infected fresh leaf material was mounted in distilled water for observing conidiophores and conidia, while leaf material from herbarium specimens was first hydrated in 70 % ethanol and then mounted in distilled water. Oospores were directly observed following heating of rhizome tissue in 6 % KOH (w/v) and from infected leaves mounted in distilled water. Dimensions of 100 conidia, 30 conidiophores, 100 terminal branchlets (from 7 infected plants) and 30 oogonia (from 3 infected plants) were measured.

DNA isolation

Leaves, rhizomes and seeds of *Aquilegia* plants with downy mildew symptoms were tested. Plant tissue was ground to a fine powder using liquid nitrogen. Subsequently, genomic DNA was extracted using the Qiagen DNeasy plant mini-extraction kit (Qiagen, West Sussex, UK) according to the manufacturer's instructions, but with eluting in a final volume of 100 μl. Further purification was done using Micro Bio-Spin Chromatography columns (Bio-Rad Laboratories, Hertfordshire, UK) filled with Poly(vinylpolypyrrolidone), 110 μm particle size (Fluka Analytical; Sigma-Aldrich, Dorset, UK), pre-whetted with 600 μl sterile distilled water for

Table 1 *Aquilegia* samples affected by downy mildew disease (confirmed by morphology); Genbank accession numbers provided for selected samples used in this study

RHS accession number	Year	Origin of source material	Host material	Sequencing primers	Genbank accession number
RHS241064	2013	Oxfordshire, UK	Leaf	–	–
RHS242036	2013	Oxfordshire, UK	Leaf	ITS4 and ITS6	KP995426
RHS242036	2013	Oxfordshire, UK	Leaf	ITS4 and ITS6	KP995427
RHS273884	2014	Oxfordshire, UK	Leaf	ITS4 and ITS6	KP995428
RHS273884	2014	Oxfordshire, UK	Leaf	NL1 and NL4	KT072772
RHS273865	2014	Oxfordshire, UK	Leaf	NL1 and NL4	KT072773
RHS272097	2014	Hertfordshire, UK	Foliage	–	–
RHS273024	2014	Surrey, UK	Foliage	–	–
RHS275683	2014	Hampshire, UK	Foliage	–	–
RHS278753	2014	Cardiff, UK	Foliage	–	–
RHS278588	2014	Wiltshire, UK	Crown	ITS4 and ITS6	KP995429
RHS304539	2015	Swansea, UK	Seed	ITS4 and ITS6	KP995430
RHS304539	2015	Swansea, UK	Seed	ITS4 and ITS6	KP995431
RHS304539	2015	Swansea, UK	Rhizome	ITS4 and ITS6	KP995432
RHS304539	2015	Swansea, UK	Rhizome	ITS4 and ITS6	KP995433
RHS304539	2015	Swansea, UK	Rhizome	ITS4 and ITS6	KP995434
RHS307139	2015	North Yorkshire, UK	Whole plant	–	–
RHS309099	2015	Surrey, UK	Whole plant	–	–
RHS309109	2015	East Sussex, UK	Whole plant	–	–
RHS309880	2015	South Yorkshire, UK	Foliage	–	–
RHS310489	2015	Devon, UK	Whole plant	–	–
RHS310627	2015	West Midlands, UK	Whole plant	–	–
RHS311732	2015	Devon, UK	Whole plant	–	–
RHS312378	2015	Surrey, UK	Whole plant	–	–

5 min, and centrifuged at 6000 rpm for 6 min. The eluent was loaded onto the column, incubated at room temperature for 2 min, then centrifuged for 4 min at 6000 rpm. This eluate was used for subsequent Polymerase Chain Reactions (PCR). DNA for the Korean sample was extracted as reported in Choi et al. (2005).

PCR and DNA sequencing

The ITS nrDNA for the British samples was amplified using the semi-nested PCR, as outlined by Cooke et al. (2000), consisting of DC6 and ITS4 for the first round, followed by ITS6 and ITS4 (Table 2). Single amplicons of approximately 800 bp were excised and cleaned using a Qiaquick gel extraction kit (Qiagen) and then sequenced using primers ITS6 and ITS4 by Beckman Coulter Genomics using Sanger sequencing. The D1-D3 region of the LSU nrDNA (28S) was amplified using primers NL1 and NL4 following the method of Maier et al. (2003), and then sequenced as described in Riethmüller et al. (2002). The ITS region of the Korea sample was amplified as described earlier (Choi et al. 2005). Sequences were assembled and edited using Lasergene 10, SeqMan Pro (DNASTAR, Madison, WI, USA) and deposited in GenBank (Table 1).

Phylogenetic analyses

ITS and LSU sequences were searched at http://ncbi.nlm.nih.gov/blast/Blast.cgi, using the megablast algorithm and default search parameters (Altschul et al. 1990). All sequences of *Peronospora* species with high similarities for Aquilegia downy mildew were retrieved from GenBank, in addition, a sequence of *Hyaloperonospora parasitica* (Pers.) Constant. was downloaded to use as an outgroup. Each alignment for ITS and LSU regions was performed using MAFFT 7 (Katoh and Standley 2013) employing the Q-INS-i algorithm (Katoh and Toh 2008). Minimum Evolution (ME) Inference was done using MEGA6.0 (Tamura et al. 2013) using the Tamura-Nei (Tamura and Nei 1993) substitution model and performing 1000 bootstrap replicates. All other parameters were set to default values. Maximum Likelihood (ML) inference was computed using RAxML 7.0.3 (Stamatakis 2006), with default settings on the RAxML BlackBox webserver (Stamatakis et al. 2008) at http://embnet.vital-it.ch/raxml-bb/, performing 1000 bootstrap replicates.

Results

Macroscopic symptoms

An initial symptom of leaf infection is vein-delimited yellowing or chlorosis (Fig. 1a). These angular patches may enlarge over smaller veins, but more often multiple lesions are present on the leaves resulting in a mosaic appearance. The chlorotic lesions then darken to become purplish in colour (Fig. 1b). As the pathogen develops, a fine purplish/beige down of conidiophores and conidia becomes apparent on the lower leaf surface especially following a period of high humidity (Fig. 1d, f). Lesions eventually become necrotic and overcome with secondary bacterial and fungal infections, resulting in early leaf death.

Systemic infection leads to a more uniform yellowing and chlorotic appearance of the leaves, which are often lighter in colour than with primary infections. Predominantly, the whole leaves are chlorotic but a few may show partial discolouration which appears to originate from the pathogen working from the petiolule towards to outer leaflet edge (Fig. 1c). Systemic infections result in stunted growth, producing shorter plants with smaller leaves that are often curled (Fig. 1d, e, g). Aquilegia downy mildew has proven to be an aggressive disease causing death of plants over only one, sometimes two, seasons. Conidiophores and conidia develop over the whole lower surface of chlorotic leaves, particularly under humid conditions.

Flowers of systemically infected plants take on a water-soaked appearance, may become distorted and decolourized, eventually turning brown. Overall flower development is inhibited. The flower stalks often develop purple or brown

Table 2 Primers used for analysis

Primer name	Primer sequence	Region	Reference
DC6	GAGGGACTTTTGGGTAATCA	ITS	Cooke et al. 2000
ITS4	TCCTCCGCTTATTGATATGC	ITS	White et al. 1990
ITS6	GAAGGTGAAGTCGTAACAAGG	ITS	White et al. 1990
NL1	GCATATCAATAAGCGGAGGAAAAG	LSU	Maier et al. 2003
NL4	GGTCCGTGTTTCAAGACGG	LSU	Maier et al. 2003

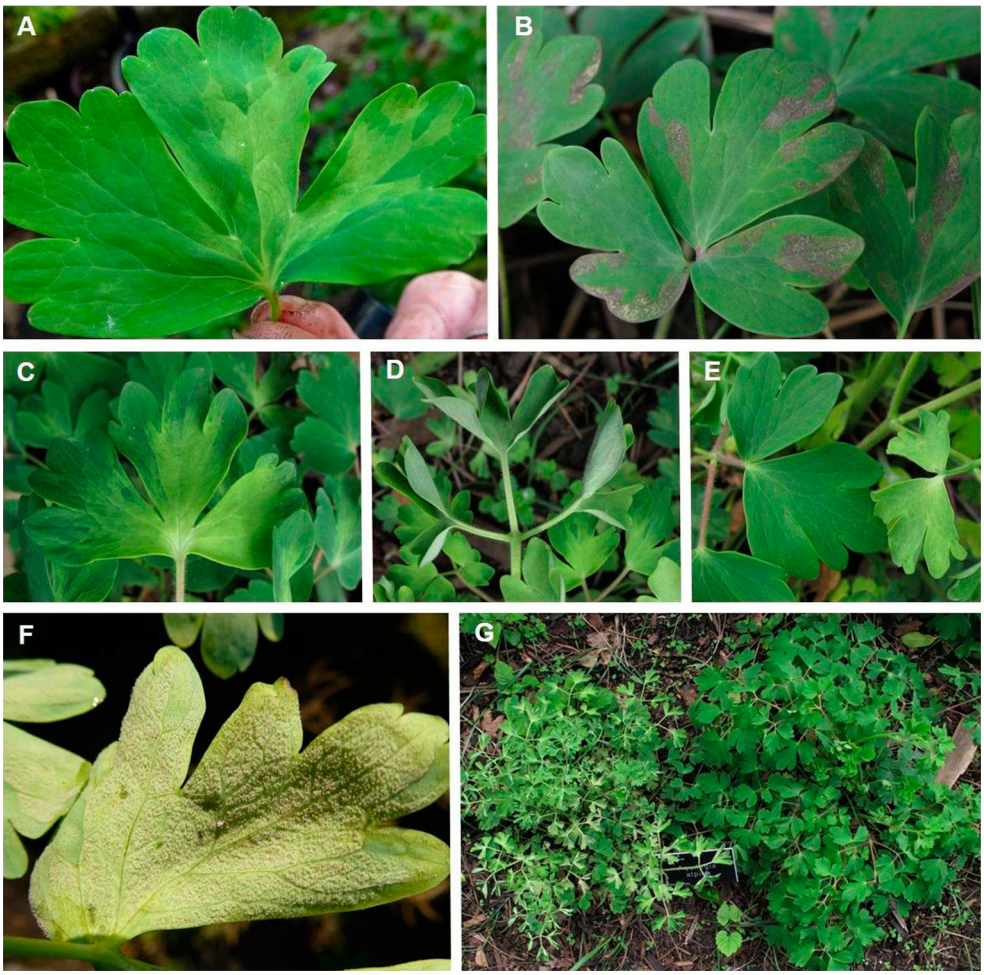

Fig. 1 Macroscopic features of primary and systemic infections on *Aquilegia* spp. **a** Primary infection showing vein delimited chlorosis on the adaxial leaf surface. **b** Chlorotic lesions later become purplish coloured. **c** Systemic leaf infection showing chlorosis originating from the petiole working towards the outer leaflet edge. **d** Infected leaflets curl. **e** Infected foliage of a systemically infected plant (*right*), stunted and chlorotic. **f** Purplish/ beige bloom of conidiophores on abaxial leaflet surface. **g** Systemically infected *Aquilegia alpina* plant (*left*) showing stunted growth, chlorotic foliage and die-back

blotches, and occasionally Aquilegia downy mildew causes kinks in the stems. If the infection occurs post-flowering, seed pods develop brown patches and may fail to set seed.

Roots often look healthy well after all top growth has died and regrowth stops. Surface depressions are seen in the crown and upper rhizome sections following infection, with dark brown lesions beneath the surface. Oogonia are present in the rhizome lesions and also in necrotic leaf tissue.

Pathogen morphology

Samples received early in the growing season had only conidia and conidiophores (Fig. 2a) present, which were observed protruding from stomatal pores on the underside of leaves. Direct germination of the conidia with a germ tube was observed. Plants received later in the growing season were additionally found to have abundant oogonia (Fig. 2d, e) present within both foliage and rhizomes. Based on available data, the conidia sizes of Aquilegia downy mildew were generally smaller than other *Peronospora* species previously recorded on the Ranunculaceae and instead fit closer to *Peronospora* species on the Papaveraceae (Voglmayr et al. 2014). Oogonia and oospores dimensions of the present samples were smaller than other *Peronospora* species recorded on Ranunculaceae by Gäumann (1923), although the comparison is limited due to limited data available for oospore dimensions.

Mycelia are found in leaves, shoots and rhizomes. Conidiophores are erect, hyaline, 132.0–382.0 μm long (mean= 240.0 μm) with straight trunk, 5.0–10.5 μm wide, almost uniform in width throughout, sometimes swollen at the base, up to 12.5 μm. Branches of conidiophores arise from the main axis in up to seven orders, and are straight to somewhat curved. Ultimate branchlets are straight to curved and have pointed tips. The length of longer ultimate branchlets ranges from 5.0 to 37.5 μm (mean=10.9 μm); the length of shorter ultimate branchlets ranges from 3.0 to 25.5 μm (mean= 8.0 μm). The ratio of longer to the shorter ultimate branchlets ranges from 1.0 to 3.0 (mean=1.4). Conidia are ovoid to ellipsoidal, 14.0–33.0 μm (mean=17.6 μm) long and 11.0–29.0 μm (mean=14.4 μm) wide, with a length to width ratio ranges from 1.0 to 1.4 (mean=1.2). Oogonia are light brown, with diameters ranging from 24.0 to 37.0 μm (mean= 32.0 μm) and oospore diameters range from 19.5 to

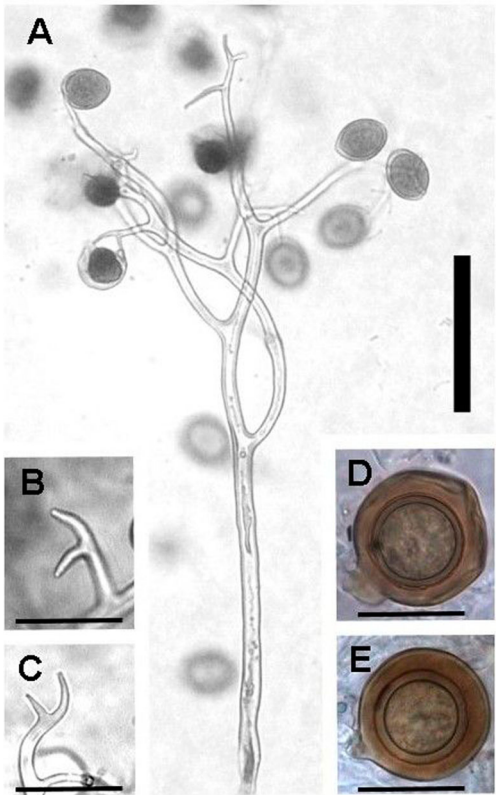

Fig. 2 Morphological features of *Peronospora* sp. on *Aquilegia* spp. **a** conidiophore and conidia, **b**, **c** ultimate branchlets, **d**, **e** oogonia and oospores. *Scale bars* (**a**) 100 μm, (**b**–**e**) 25 μm

32.0 μm (mean=23.8 um). The oospore wall was 1.5–4.5 μm (mean=2.6 μm) thick, surface smooth.

Molecular detection

From all symptomatic leaves and rhizomes, as well as seeds collected from infected plants, amplicons of approximately 800 bp were observed after semi-nested PCR for ITS rDNA. Sequencing of the excised amplicons confirmed their identity as a *Peronospora* species.

Molecular phylogenetic analysis

All *Peronospora* isolates found affecting *Aquilegia* and *Semiaquilegia* revealed no sequence differences in ITS region, but significantly differed from other *Peronospora* sequences retrieved from GenBank. In the ITS-based tree (Fig. 3), all isolates from *Aquilegia* and *Semiaquilegia* causing downy mildew grouped with two *Peronospora* species from the Saxifragaceae (*P. chrysosplenii* Fuckel and *P. saxifragae* Bubák), two species from the Papaveraceae (*P. bulbocapni* Reichardt and *P. dicentrae* Syd.), and a species from Berberidaceae (*P. odessana* Voglmayr & Korytnianska), with high supporting values of 90 (ML) and 86 (ME). This clade further grouped with five species from the Ranunculaceae (*P. alpicola* Gäum., *P. hiemalis* Gäum., *P. illyrica* Gäum., *P. pulveracea* Fuckel, and *P.* aff. *ranunculi*), with low to moderate support. Similarly, in the LSU tree (Fig. 4), isolates from *Aquilegia* causing downy mildew grouped with *Peronospora* species, originated from the Papaveraceae (*P. bulbocapni*), Ranunculaceae (*P. alpicola, P. ficariae* Tul., *P. hiemalis*, and *P. pulveracea*), and Berberidaceae (*P. odessana*), without significant support. However, there are no LSU sequences available for *Peronospora* species from the Saxifragaceae. Both ITS and LSU phylogenies demonstrate that the present isolates are distinct from all available sequence data for *Peronospora* species from other Ranunculales.

Known host range

Current records of downy mildew on *Aquilegia* in Britain are predominantly from *Aquilegia vulgaris* and its hybrids, *A. alpina* L., *A. flabellata* Siebold & Zucc., and *A. viridiflora* Pall. Where specific cultivars were identifiable, these included 'Alaska', 'Colorado', 'Florida', Louisiana', 'Virginia' and *A. flabellata* 'Georgia', although this is unlikely to be an exhaustive list. In Korea, *Semiaquilegia adoxoides* (DC.) Malino was affected by downy mildew.

Discussion

Aquilegia downy mildew has become rapidly established in several parts of the UK. It is currently unclear whether the conidia, which are produced abundantly in moist conditions, are primarily serving as an inoculum which infects other plants locally, or if the conidia are also important for dispersal over longer distances. Rhizome infection, demonstrated by the observation of oogonia in below-ground rhizome parts and the occurrence of systemically infected plants when the first leaves emerge, seems to be of major importance for the overwintering of the pathogen. The role of oospores from decayed plant tissue for the epidemiology of the disease is currently unclear and requires further investigation. Initial PCR-testing of seeds indicated the presence of *Peronospora* DNA warranting further studies to establish, to which degree seed transmission is an important method of dispersal over large distances by human activity. Given the fact that several downy mildews are known to be distributed with infected seeds (Sackston 1981; Inaba et al. 1983; Garibaldi et al. 2004; Belbahri et al. 2005), this mode of transmission seems likely, and thus, seeds should not be exported from areas in which the downy mildew has been observed. In addition to seed trade, the trade with rhizomes could also lead to a further spread of the pathogen, as latent infection of plants in a previous season might result in symptomatic infections in the following season.

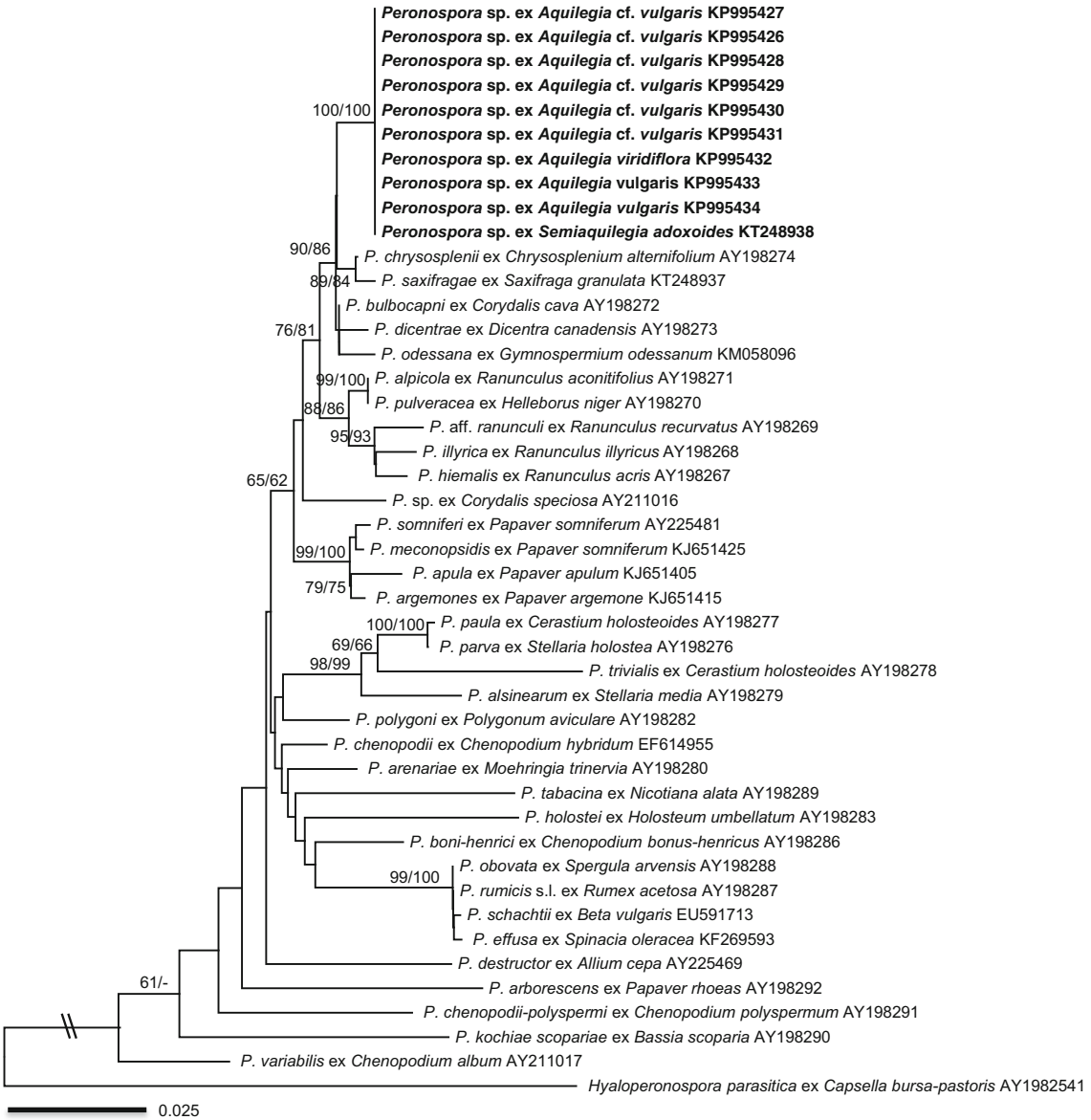

Fig. 3 Minimum Evolution tree of the complete ITS region (ITS1, 5.8S rDNA, and ITS2). Bootstrap support values of Minimum Evolution and Maximum Likelihood greater than 50 % are given above or below the branches. The *scale bar* equals the number of nucleotide substitutions per site

The foliar symptoms and disease progression of the *Peronospora* species on *Aquilegia* bears striking similarity to Gustavsson's (1959b) account of *Peronospora ficariae* on *Ranunculus ficaria* L. He said "The undersides of the infected leaves are always completely covered by a dense layer of conidiophores, the blades are somewhat smaller than usual, and they turn yellowish green". Although causing severe symptoms, Gustavsson (1959b) notes that this does not appear to kill the plants, which is unlike the *Peronospora* present on *Aquilegia*. However, other pathogens of Ranunculaceae (e.g. *Peronospora ranunculi* Gäum.) and Papaveraceae (e.g. *Peronospora corydalis* de Bary) also exhibit very similar symptoms and sometimes lead to the death of the infected plant.

Interestingly, based on initial DNA analysis, the present pathogen on *Aquilegia* was most closely related to *Peronospora* spp. found to infect members of the Berberidaceae, Papaveraceae and Saxifragaceae rather than *Peronospora* spp. found on other members of the Ranunculaceae. While it seems possible that Aquilegia downy mildew is caused by a previously undescribed species of *Peronospora*, further molecular and morphological studies will be needed to ascertain this. Interestingly, a specimen from *Semiaquilegia adoxoides* in Korea from gardens was identical in ITS rDNA sequences to specimens from *Aquilegia* from the

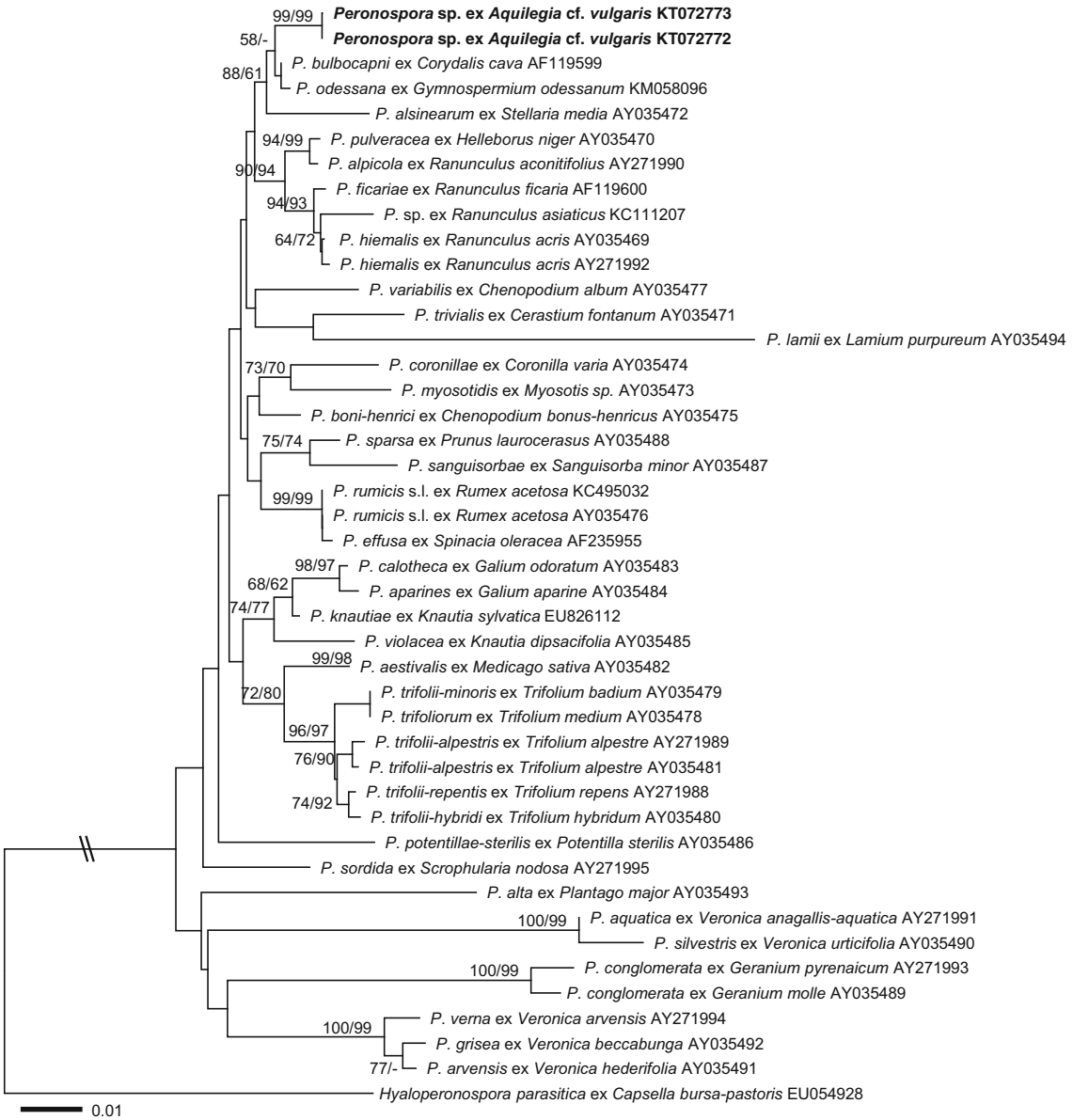

Fig. 4 Minimum Evolution tree of the partial LSU nrDNA. Bootstrap support values of Minimum Evolution and Maximum Likelihood greater than 50 % are given above or below the branches. The *scale bar* equals the number of nucleotide substitutions per site

UK. As the collection from *Semiaquilegia* in Korea predates the occurrence of *Aquilegia* in the UK, it seems possible that the pathogen originated from East Asia, but further investigation is necessary to explore this possibility.

There are currently no fungicidal controls available to home gardeners in the UK labelled for use against downy mildew. Initial infections might be controlled by prompt removal of infected plants. Cool damp conditions favour rapid onset of disease symptoms and light levels may also be of importance, as with other downy mildew species (Cohen et al. 2013). Deeper knowledge of the environmental conditions that favour Aquilegia downy mildew progression may facilitate an integrated management approach to this disease in a garden situation.

The impact of the disease to UK gardens and the wider environment is likely to be significant. *Aquilegia* is a widely grown herbaceous perennial and *A. vulgaris* is a UK native as well as being naturalised from gardens (Stace 2010). In continental Europe, *Aquilegia* is also widely grown as an ornamental and is an iconic flower in some undisturbed natural habitats. Therefore, it is important to reduce further spread of the disease by quarantine measures, including restrictions in seed movement and in the trade of plant material from regions where the downy mildew has been found. The Aquilegia downy mildew can be seen as a high risk pathogen due to its aggressiveness, production of oospores that can outlast unfavourable conditions and the likelihood that it can be distributed with infected seeds or rhizomes. In the past, some of

the most devastating oomycete diseases have emerged by transfer of infected plants and seeds, such as *Phytophthora infestans* (Mont.) de Bary (Yoshida et al. 2013, 2014), *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni (Viennot-Bourgin 1981), *Plasmopara halstedii* (Cohen and Sackston 1974; Ioos et al. 2007), and, in more recent years, e.g. *Peronospora belbahrii* (Garibaldi et al. 2004; Belbahri et al. 2005; Thines et al. 2009). Only if the trade in seeds and plants is restricted quickly and disease incidence is kept low by disposing of infected plant material carefully, might it be possible to restrict the disease and avoid it becoming another globally present plant disease. As Aquilegia downy mildew has been observed to be present in plants for sale in nurseries, it seems high time to consider programmes and measures to restrict the further spread of the pathogen.

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