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ORIGINAL ARTICLE

Peronospora aquilegiicola sp. nov., the downy mildew affecting columbines in the UK is an invasive species from East Asia

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Abstract For diagnostics and control of emerging plant diseases, accurate species determination of the causal pathogens is a prerequisite. Downy mildew disease, caused by an unknown species of *Peronospora,* has speedily spread throughout numerous gardens and nurseries of the ornamental plant *Aquilegia* in the UK, but so far does not seem to have reached continental Europe. Apart from cultivated *Aquilegia*, downy mildew from wild columbines has only been reported from East Asia, where natural populations of *Semiaquilegia* are affected by downy mildew. To resolve the phylogenetic relationships of the causal pathogens on *Aquilegia* and *Semiaquilegia*, a phylogeny based on nine loci was performed. In addition, detailed morphological comparisons were carried out to determine if the downy mildew agents on *Aquilegia* and *Semiaquilegia* are conspecific and can be discriminated from related downy mildew species. Strong evidence was found that the downy mildew pathogens on *Aquilegia* and *Semiaquilegia* are conspecific, but distinct from other species of *Peronospora* affecting Ranunculaceae, Papaveraceae, and Saxifragaceae. Thus, a new species, *Peronospora aquilegiicola*, is introduced. The quick spread of the pathogen throughout the UK and the current absence from continental Europe highlights the importance to consider quarantine measures to restrict the further spread of this pathogen.

Keywords *Aquilegia* · Multi-locus phylogeny · Downy mildew · Quarantine · *Semiaquilegia*

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Introduction

Oomycetes cause commercially important downy mildew disease in numerous crop and ornamental plants worldwide. Recently, as the market size of ornamental plants grows larger and plants of new areas become

available for trade, the risk of the introduction of new downy mildew diseases is increasing. Thus the importance of accurate diagnosis and identification of these pathogens is crucial. As a result of efforts into this direction over the past decade, several new downy mildew species on economically relevant ornamental plants have been described, e.g. *Peronospora belbahrii* on basil (Thines et al. 2009), *P. apula* and *P. somniferi* on opium poppy (Voglmayr et al. 2014), *P. salviae-plebeiae* and *P. salviae-officinalis* on sages (Choi et al. 2009), and *Plasmopara destructor* and *Pl. velutina* on impatiens (Görg et al. 2017).

Members of the genus *Aquilegia* (Ranunculaceae) are widely grown in public and private gardens throughout the world (Nold and Nelson-Nold 2003; Stace 2010). Downy mildew disease has been first found on *Aquilegia* plants throughout England and Wales in 2013, but before on *Semiaquilegia* in Korea (Denton et al. 2015) and China (Yu et al. 1998). While it seems possible that *Aquilegia* and *Semiaquilegia* downy mildew (ASDM) is caused by a previously undescribed species of *Peronospora* (Denton et al. 2015), a decision regarding the conspecificity or distinctiveness of the species could not be reached due to uncertainty regarding the relationships of the downy mildew pathogens from *Aquilegia* and *Semiaquilegia*, and their relationships with other pathogens, in particular of other members of the Ranunculales. In the phylogenetic analyses based on ITS and LSU rDNA sequences presented by Denton et al. (2015), it was revealed that the ASDM collections formed a monophyletic cluster, but further clustered with several different species of *Peronospora* parasitic on Euphorbiaceae, Papaveraceae, Ranunculaceae, and Saxifragaceae, with high support values (Denton et al. 2015). As the ITS region often exhibits insufficient variability for phylogenetic distinction in closely related species, e.g. in *Peronospora* (Choi et al. 2007; Voglmayr et al. 2014), *Pseudoperonospora* (Choi et al. 2005), and *Phytophthora* (Goodwin et al. 1999; Cooke et al. 2000; Jung and Burgess 2009), it was uncertain, if the pathogens from *Aquilegia* and *Semiaquilegia* should be considered conspecific or not. Therefore, in the current study phylogenetic analyses were conducted using four nuclear (ITS, LSU, heat shock protein 90 [*hsp90*], β-tubulin [*β-tub*]) and five mitochondrial loci (cytochrome *c* oxidase subunit II and I [*cox2* and *cox1*], a spacer region between *cox2* and *cox1* genes [coxS], NADH dehydrogenase subunit I [*nad1*], ribosomal protein S10 and its flanking region [*rps10*]). In addition, detailed morphological comparisons were carried out in order to clarify the taxonomic identity of the downy mildew pathogens of *Aquilegia* and *Semiaquilegia*.

Materials and methods

Oomycete specimens

Five downy mildew specimens originating from *Aquilegia* and *Semiaquilegia* were analysed in this study. In addition, two *Peronospora* species parasitic on Euphorbiaceae, four on Papaveraceae, six on Ranunculaceae, and two on Saxifragaceae were included in the phylogenetic reconstructions. *Pseudoperonospora cubensis* was used as an outgroup. Information on the specimens sequenced in this study is shown in Table 1.

Morphology

Morphological characteristics of conidiophores, conidia, and oospores were investigated using dried herbarium specimens. A piece of infested leaf tissues was mounted on a drop of 70% lactic acid on a slide, gently warmed, covered with coverslips, and examined using an Olympus BX53 microscope (Olympus, Tokyo, Japan). Measurements were performed at 100–200× for conidiophores and at 400× for conidia, ultimate branchlets, and oospores, using the TCapture software (Tucsen Photonics, Fuzhou, China). The measurements were reported as follows; (minimum) – standard deviation towards the minimum – mean – standard deviation towards the maximum – (maximum) ($n = 50$). DIC micrographs were captured using an Olympus BX53 microscope equipped with a DigiRetina 16 M camera (Tucsen Photonics, Fuzhou, China) or using a Zeiss Imager M2 AX10 microscope with an AxioCam MRc5 camera (Carl Zeiss, Göttingen, Germany).

DNA extraction, PCR amplification, and sequencing

From herbarium specimens, 5–10 mg of infected leaf tissue was taken and disrupted in a mixer mill (MM2, Retsch, Germany), using two iron beads of 3 mm and five beads of 1 mm diameter per sample and shaking

Table 1 Information on downy mildew specimens used in this study

DNA No.	Herb. [a] No.	Species	Host	Family[b]	Year	Country	GenBank Acc. No.		
							18S + ITS	LSU D1–3	LSU D6–8
308	GLM75877	*Peronospora cyparissiae*	*Euphorbia cyparissias*	EUP	2005	Germany	MH730799	MH730860	MH730819
322	GLM75975	*Peronospora esulae*	*Euphorbia esula*	EUP	2005	Germany	MH730800	MH730840	MH730820
776	GLM64105	*Peronospora arborescens*	*Papaver rhoeas*	PAP	2004	Germany	MH730802	MH730842	MH730822
749	GLM63060	*Peronospora argemones*	*Papaver argemone*	PAP	2004	Germany	MH730801	MH730841	MH730821
240	GLM64102	*Peronospora bulbocapni*	*Corydalis cava*	PAP	2004	Germany	MH730818	MH730859	MH730839
2556	VK0210[a]	*Peronospora meconopsidis*	*Meconopsis cambrica*	PAP	2012	Greece	MH730809	MH730850	MH730830
2706	JK-F0514[b]	*Peronospora alpicola*	*Ranunculus aconitifolius*	RAN	2013	Italy	MH730811	MH730852	MH730832
974	GLM63033	*Peronospora ficariae*	*Ficaria verna*	RAN	2004	Germany	MH730804	MH730844	MH730824
948	GLM75690	*Peronospora hiemalis*	*Ranunculus acris*	RAN	2005	Germany	MH730803	MH730843	MH730823
2076	WU32790	*Peronospora illyrica*	*Ranunculus illyricus*	RAN	2004	Austria	MH730808	MH730849	MH730829
2681	JK-F0378[b]	*Peronospora pulveracea*	*Helleborus foetidus*	RAN	2012	Germany	MH730810	MH730851	MH730831
995	GLM73330	*Peronospora ranunculi*	*Ranunculus repens*	RAN	2002	Germany	MH730805	MH730845	MH730825
1015	GLM73678	*Peronospora ranunculi-sardoi*	*Ranunculus sardous*	RAN	2001	Germany	MH730806	MH730846	MH730826
3274	GLM116093	*Peronospora* sp.	*Aquilegia vulgaris*	RAN	2015	UK	MH730812	MH730853	MH730833
3277	KUS-F18143	*Peronospora* sp.	*Semiaquilegia adoxoides*	RAN	2000	Korea	MH730813	MH730854	MH730834
3278	KUS-F21042	*Peronospora* sp.	*Semiaquilegia adoxoides*	RAN	2004	Korea	MH730814	MH730855	MH730835
3279	KUS-F21756	*Peronospora* sp.	*Semiaquilegia adoxoides*	RAN	2006	Korea	MH730815	MH730856	MH730836
3280	KUS-F23265	*Peronospora* sp.	*Semiaquilegia adoxoides*	RAN	2008	Korea	MH730816	MH730857	MH730837
217	GLM73449	*Peronospora chrysosplenii*	*Chrysosplenium alternifolium*	SAX	2003	Germany	MH730817	MH730858	MH730838
1070	GLM75698	*Peronospora saxifragae*	*Saxifraga granulata*	SAX	2005	Germany	KT248937	MH730847	MH730827
1956	GLM49006	*Pseudoperonospora cubensis*	*Cucumis* sp.	CUC	1997	Germany	MH730807	MH730848	MH730828

DNA No.	GenBank Acc. No.						
	*cox*1	*cox*2 + 1 spacer[c]	*nad1*	*ypt1*	*hsp90*	*β-tub*	*rps10*
308	KJ654061	KJ654210	MH730881	MH730918	MH730798	MH730751	MH730884
322	KJ654062	KJ654211	MH730861	MH730919	MH730779	MH730752	MH730885
776	KJ654083	KJ654232	MH730863	MH730921	MH730781	–	MH730887
749	KJ654082	KJ654231	MH730862	MH730920	MH730780	MH730753	MH730886
240	KJ654056	KJ654205	MH730880	MH730917	MH730797	MH730750	MH730883
2556	KJ654120	KJ654269	MH730871	MH730909	MH730789	MH730759	MH730895
2706	KJ654137	KJ654286	MH730873	MH730911	MH730791	MH730761	MH730897
974	KJ654090	KJ654239	MH730865	MH730904	MH730783	MH730755	MH730889
948	KJ654089	KJ654238	MH730864	MH730903	MH730782	MH730754	MH730888
2076	KJ654155	KJ654304	MH730870	MH730908	MH730788	MH730758	MH730894
2681	KJ654134	KJ654283	MH730872	MH730910	MH730790	MH730760	MH730896
995	KJ654091	KJ654240	MH730866	MH730905	MH730784	MH730756	MH730890
1015	KJ654092	KJ654241	MH730867	MH730906	MH730785	MH730757	MH730891

Table 1 (continued)

DNA No.	GenBank Acc. No.							
	*cox*1	*cox*2 + 1 spacer[c]	*nad1*	*ypt1*	*hsp90*	*β-tub*	*rps10*
3274	MH730768	MH730774	MH730874	MH730912	MH730792	MH730762	MH730898
3277	MH730769	MH730775	MH730875	MH730913	MH730793	MH730763	MH730899
3278	MH730770	MH730776	MH730876	MH730914	MH730794	MH730764	MH730900
3279	MH730771	MH730777	MH730877	MH730915	MH730795	MH730765	MH730901
3280	MH730772	MH730778	MH730878	MH730916	MH730796	MH730766	MH730902
217	KJ654054	KJ654203	MH730879	–	–	–	MH730882
1070	KJ654095	KJ654244	MH730868	MH730907	MH730786	–	MH730892
1956	MH730767	MH730773	MH730869	MH730922	MH730787	JF304700	MH730893

[a] Acronyms of herbarium collections: GLM, Senckenberg Gesellschaft für Naturforschung; Senckenberg Museum für Naturkunde Görlitz, Görlitz, Germany; JK-F, Julia Kruse in Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main, Germany; KUS-F, Korea University, Seoul, Republic of Korea; VK, Volker Kummer in University Potsdam, Germany; WU, Universität Wien, Wien, Austria

[b] Host families: EUP, Euphorbiaceae; PAP, Papaveraceae; RAN, Ranunculaceae; SAX, Saxifragaceae; CUC, Cucurbitaceae

[c] the spacer region between *cox*2 and *cox*1 genes

twice at maximum speed for 15 min. Genomic DNA was extracted using the BioSprint 96 DNA Plant Kit (Qiagen) on a KingFisher Flex (Thermo Scientific) robot. Two nuclear (ITS, *hsp90*) and five mitochondrial markers (*cox2*, *cox1*, coxS, *nad1*, *rps10* and its flanking region) were amplified using oomycete-specific primers, as outlined previously by Choi et al. (2015b). In addition, two regions of LSU rDNA, D1–5 and D6–8, were amplified using two primer sets, LR0R (Vilgalys and Hester 1990) /LR6-O (Riethmüller et al. 2002) and LR6-OR2C (designed here; TAAGCTCGTCTGGC GATG)/LR9 (Hopple and Vilgalys 1999), respectively. In addition, as a forth nuclear gene *β-tub* was amplified using TUBUF2 (Kroon et al. 2004) and bTub1024R-O (Göker et al. 2007). In some cases, the *cox2* gene and the spacer region had to be separately amplified with two primer sets, cox2-F (Hudspeth et al. 2000) & cox2-RC4 (Choi et al. 2015a) for the gene, and FM79 & FMPh-10b (Martin et al. 2004) for the spacer region. Amplicons were sequenced at the Biodiversity and Climate Research Centre (BiK-F) laboratory using primers identical to those used for amplifications.

Phylogenetic analysis

Sequences were edited using the DNAStar software package (DNAStar, Inc., Madison, Wis., USA), version 5.05. An alignment of each locus was performed using MAFFT 7 (Katoh and Standley 2013) employing the Q-INS-i algorithm (Katoh and Toh 2008). SequenceMatrix 1.7.8 (Vaidya et al. 2011) was used for concatenating individual gene sequences and for checking unusually similar or divergent sequences. After making sure that no conflicting support was present in individual loci, three concatenation alignments for nuclear (ITS, LSU, *hsp9*, *β-tub*), mitochondrial (*cox2*, *cox1*, coxS, *nad1*, *rps10* and its flanking region), and all nine loci were constructed. Phylogenetic inference was done using two different methods, Maximum Likelihood (ML) and Minimum Evolution (ME). For ML analyses, 1000 rounds of random addition of sequences as well as 1000 fast bootstrap replicates were performed using RAxML 7.0.3 (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012) using the GTRCAT variant. ME analysis was done using MEGA 7.0 (Kumar et al. 2016), with the default settings of the program, except for using the Tamura-Nei model instead of the maximum composite likelihood model.

Results

Morphological analysis

Based on morphology of observable structures the downy mildew pathogens on *Aquilegia* and *Semiaquilegia* could not be distinguished. However, they could be discriminated from all other related pathogens (Table 2). The dimensions of conidia and oospores were observed to be the most useful characters distinguishing between ASDM and other *Peronospora* species. The mean conidial length in ASDM was of 17.6 μm and, thus, smaller than in other *Peronospora* species (more than av. 20 μm) previously recorded on members of the Ranunculaceae (Gäumann 1923; Gustavsson 1959). Similarly, the diameter of oospores (av. 24.0 μm) was also smaller than any other report for a *Peronospora* species on this family (Gäumann 1923). In the morphological comparison to four phylogenetically closely related species, *P. bulbocapni* and *P. meconopsidis* from the Papaveraceae, as well as *P. chrysosplenii* and *P. saxifragae* from the Saxifragaceae, ASDM was distinguishable from the former two species again by smaller conidia (av. 17.6 μm in ASDM but av. 22 μm in *P. bulbocapni* (Beck 1886) and av. 24.8 μm in *P. meconopsidis* (Voglmayr et al. 2014). In addition, the diameter of its oospores (av. 24.0 μm) was also smaller (Beck 1886; Voglmayr et al. 2014). Similarly, the dimensions of conidia were smaller in ASDM than in *P. chrysosplenii* (17–28 × 15–20 μm in (Kochman and Majewski 1970); 22–30 × 16–23 μm in (Stanyavichene 1984)) and *P. saxifragae* (av. 24.5 × 20.8 μm in (Gäumann 1923); (20–)22–30 × 17–25 μm in (Kochman and Majewski 1970)). Moreover, ASDM exhibited smaller oospores than those of *P. chrysosplenii* (Kochman and Majewski 1970; Stanyavichene 1984). Two *Peronospora* species parasitic to the Euphorbiaceae differed from ASDM by larger conidia of (17–)21–23(−27) μm in *P. cyparissiae* and 19–24 μm in *P. esulae* (Stanyavichene 1984). In addition, *P. cyparissiae* exhibited larger oogonia (37–46 μm) and oospores (30–35 μm) (Stanyavichene 1984).

Multi-locus phylogeny

Trees based on the concatenated alignment of four nuclear loci compared to that of five mitochondrial loci each showed no strong conflicting support with a phylogeny based on the concatenated alignment of all nine loci. Thus, only the phylogeny based on concatenation of all loci is shown in Fig. 1. The final concatenated alignment had 8122 total characters, including 1464 variable characters, 887 of which were parsimony-informative. Since the dataset revealed no significant conflicts in the topologies derived from ML and ME analyses, only the tree from the ML inference is shown in Fig. 1, with bootstrap support from both analyses.

In the multi-gene tree, all ASDM sequences were identical in sequence and formed a monophyletic group with maximum support, demonstrating the genetic homogeneity of the pathogen, despite originating from two different host genera, *Aquilegia* and *Semiaquilegia*, and having been collected from two distant countries, U.K. and Korea. The grouping of ASDM with two *Peronospora* species from the Saxifragaceae (*P. chrysosplenii* and *P. saxifragae*) and two species from the Papaveraceae (*P. bulbocapni* and *P. meconopsidis*), was well resolved in the present study. The further grouping with two *Peronospora* species from the Euphorbiaceae (*P. cyparissiae* and *P. esulae*) was weakly supported in ML and not supported in ME analyses. Interestingly, except for ASDM, all other species of *Peronospora* parasitic on Ranunculaceae formed another large clade with maximum support that was further split into several different lineages, corresponding to a particular host genus or species.

Taxonomy

Peronospora aquilegiicola Thines, G. Denton & Y.J. Choi, sp. nov. [MB 827342] (Fig. 2).

Etym.: '*aquilegiicola*' refers to the host plant, aquilegia.

Infected *Aquilegia* spp. leaf tissue first yellowish or chlorotic, later often darkening to become purplish, vein-delimited if not fully systemic, resulting in a polyangular, mosaic appearance on leaves. Down present on the lower leaf surface of leaves, dense, felt-like, purplish or beige due to the colour of conidia. At systematic infection lesions more uniform, rims of infected leaves often curling outwards. Flowers are water-soaked, distorted, decolourised to brown. Overall, flower development is negatively affected. Mycelium in leaves, shoots and rhizomes.

Table 2 Morphological comparison between *Peronospora* sp. and other phylogenetically or morphologically close species

Species	Host (Family)[a]	Conidiophores	Conidia	
		Length (μm)	Length (μm)	Width (μm)
Peronospora sp.	*Aquilegia*, *Semiaquilegia* (RAN)	(163–)189–235–278(−389)[b]	(13.7–)16.3–17.6–18.9(−20.6)	(11.3–)13.6–14.9–16.1(−17.3)
P. odessana	*Gymnospermium odessanum* (BER)	(180–)215–275(−310)	(20–)23–26.5(−29.5)	(15.5–)18–21(−22.5)
P. cyparissiae	*Euphorbia cyparissia* (EUP)	150–400	(17–)21–23(−27)	(12–)14–18(−19)
P. esulae	*Euphorbia esula* (EUP)	300–350	19–24	(8–)12–18(−23)
P. apula	*Papaver apulum* (PAP)	(170–)270–430(−500)	(14–)16.5–18.3–20(−22)	(12–)14.5–15.8–17(−19)
P. arborescens	*Papaver* spp. (PAP)	(290–)360–600(−720)	(14–)16.5–18.3–20(−24)	(12.5–)15–16.1–17.5(−20)
P. argemones	*Papaver argemone* (PAP)	(220–)290–490(−590)	(16.5–)19–21.1–23.5(−26)	(14.5–)16.5–18.1–20(−23)
P. bulbocapni	*Corydalis cava* (PAP)	–	(17–)20–22–24(−28)[c]	(14–)17–19–21(−23)[c]
P. cordalyis–intermediae	*Coryalis intermedia* (PAP)	400–550	(9–)16–16.77–19(−24)	(9–)13–15.65–17(−22)
P. dicentrae	*Dicentra canadensis* (PAP)	300–500	(12–)20–22.24–24(−34)	(10–)19–20.64–23(−29)
P. meconopsidis	*Meconopsis cambrica* (PAP)	(230–)240–510(−730)	(17–)22.5–24.8–27.5(−35.5)	(15–)19–20.6–22.5(−26)
P. somniferi	*Papaver somniferum* (PAP)	(280–)320–510(−660)	(15.5–)19–21.1–23(−28)	(14.5–)16.5–17.7–19(−22.5)
P. alpicola	*Ranunculus aconitifolius* (RAN)	200–600	(22–)30–32.28–33(−42)	(16–)22–24.22–26(−34)
P. ficariae	*Ficaria verna* (RAN)	–	av. 25.86	av. 21.6
P. hiemalis	*Ranunculus acer* (RAN)	250–650	(12–)21–23.07–25(−30)	(9–)16–17.86–20(−26)
P. illyrica	*Ranunculus illyricus* (RAN)	300–500	(16–)22–23.78–24(−32)	(14–)21–22.98–24(−30)
P. pulveracea	*Helleborus foetidus* (RAN)	–	av. 30.18	av. 24.67
P. ranunculi	*Ranunculus repens* (RAN)	200–800	(12–)24–26.09–28(−32)	(9–)19–21.49–24(−29)
P. chrysosplenii	*Chrysosplenium alternifolium* (SAX)	130–500	17–28	15–20
P. chrysosplenii	*Chrysosplenium alternifolium* (SAX)	226.8–361	22.3–24.6–26.9	20.6–21.4–22.2
P. saxifragae	*Saxifraga granulata* (SAX)	200–400	(20–)22–30	17–25
P. saxifragae	*Saxifraga granulata* (SAX)	–	av. 24.45	av. 20.83

Species	Conidia	Resting organs		Reference
	length / width	Oogonia (μm)	Oospores (μm)	
Peronospora sp.	(1.11–)1.15–1.22–1.29(−1.38)	(24.0–)28.0–32.0–36.0(−37.05)	(19.0–)21.0–24.0–27.0(−32.5)	The present study
P. odessana	(1.11–)1.21–1.37(−1.6)	(38–)45–55(−59)	(24–)28–34(−43)	Voglmayr & Korytnianska 2015
P. cyparissiae	–	37–46	30–35	Ul'yanishchev, 1985
P. esulae	–	–	–	Ul'yanishchev, 1985
P. apula	(1.01–)1.07–1.25(−1.43)	(29–)40–48(54)	(21–)25–30(−34)	Voglmayr et al. 2014
P. arborescens	(1.01–)1.07–1.21(−1.45)	(37–)43–51(−59)	(21–)26–30(−33)	Voglmayr et al. 2014
P. argemones	(1.02–)1.1–1.23(−1.37)	(42–)48–59(−66)	(25–)28–34(−38)	Voglmayr et al. 2014

Table 2 (continued)

Species	Conidia	Resting organs		Reference
	length / width	Oogonia (μm)	Oospores (μm)	
P. bulbocapni	av. 1.17[c]	–	26-30[d]	Beck 1886; Gustavsson 1959
P. cordalyis–intermediae	–	–	25–35	Gäumann 1923
P. dicentrae	–	–	–	Gäumann 1923
P. meconopsidis	(1.03–)1.13–1.28(−1.47)	(29–)40–48(−54)	(21–)25–30(−34)	Voglmayr et al. 2014
P. somniferi	(1.01–)1.11–1.28(−1.48)	(31–)40–48(−57)	(19–)24–28(−34)	Voglmayr et al. 2014
P. alpicola	–	–	–	Gäumann 1923
P. ficariae	av. 1.2	–	–	Gäumann 1923
P. hiemalis	av. 1.29	–	30–39	Gäumann 1923
P. illyrica	av. 1.03	–	–	Gäumann 1923
P. pulveracea	av. 1.22	–	–	Gäumann 1923
P. ranunculi	av. 1.21	–	25–40	Gäumann 1923
P. chrysosplenii		42–58	32–43	Kochman and Majewski 1970
P. chrysosplenii	–	ca. 54	36–40	Stanyavichene 1984
P. saxifragae	–	–	–	Kochman and Majewski 1970
P. saxifragae	–	–	–	Gäumann 1923

[a] Host family: *BER* Berberidaceae, *EUP* Euphorbiaceae, *PAP* Papaveraceae, *RAN* Ranunculaceae, *SAX* Saxifragaceae

[b] The measurements available are reported as follows; (minimum–) standard deviation towards the minimum – mean – standard deviation towards the maximum (−maximum)

[c] measurements of Gustavsson (1959)

[d] measurement of Beck (1886)

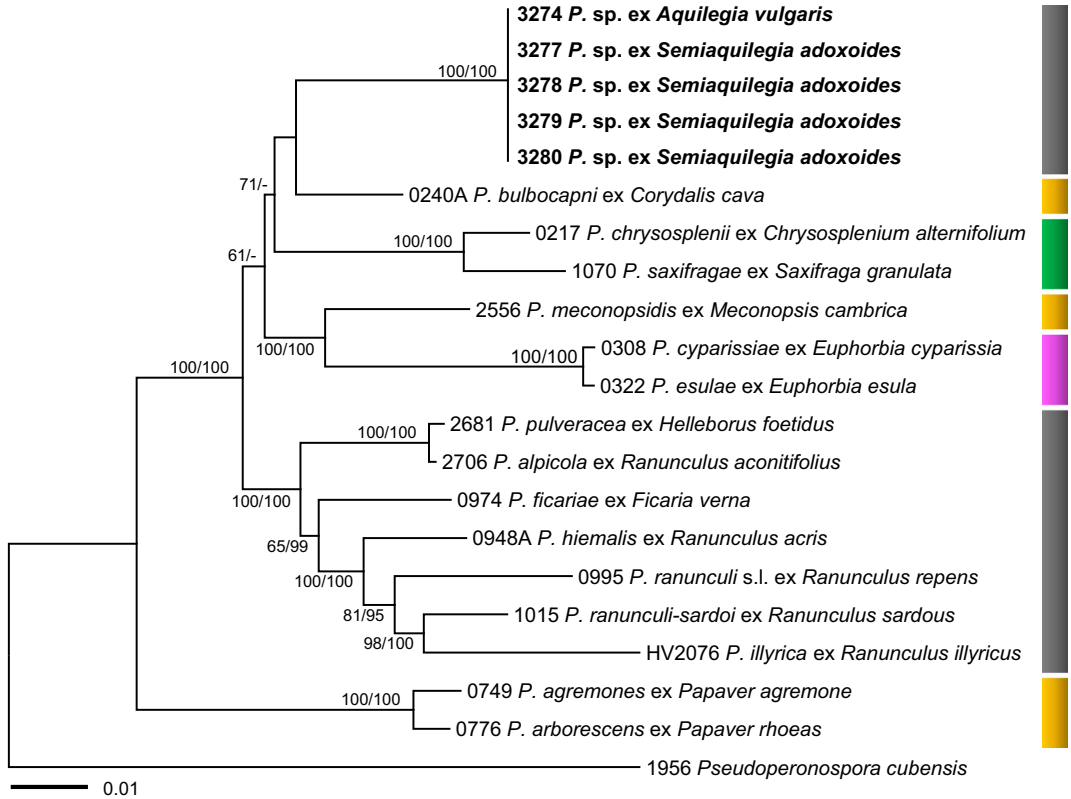

Fig. 1 Maximum Likelihood tree of a concatenated alignment of four nuclear (ITS, LSU, heat shock protein 90 [*hsp90*], β-tubulin [*β-tub*]) and five mitochondrial loci (cytochrome *c* oxidase subunit II and I [*cox2* and *cox1*], NADH dehydrogenase subunit I [*nad1*], ribosomal protein S10, and its flanking region [*rps10*]). Bootstrapping support values of Maximum Likelihood and Minimum Evolution methods higher than 60% are given above or below the branches. The scale bar equals the number of nucleotide substitutions per site. Specimens originating from different host families are marked with grey (Ranunculaceae), pink (Euphorbiaceae), yellow (Papaveraceae), and green (Saxifragaceae) bars

Conidiophores protruding from stomata on the underside of infected leaf tissue, erect, hyaline, monopodially branched up to 7 orders, (163–)189–235–278(−389) μm long, (4.1–)4.6–7.6–10.6(−20.1) μm wide, sometimes swollen at the base, up to 12.5 μm; trunk (46–)59–90–121(−188) μm. Ultimate branchlets slightly curved to substraight, mostly in pairs (90%), but rarely in single (10%), branched at an angle of 80–110°, with different lengths, (5.4–)6.59–9.08–11.57(−16.8) μm for the longer ones, (2.5–)4.91–7.23–9.55(−12.32) μm for the shorter ones, a ratio of longer to the shorter ultimate branchlets of (0.82–)1.08–1.30–1.52(−2.01) μm, (0.80–)1.37–1.75–2.13(−2.6) μm wide at the base, tip obtuse or pointed. Conidia broadly ellipsoidal, (13.7–)16.3–17.6–18.9(−20.6) μm long, (11.3–)13.6–14.9–16.1(−17.3) wide, with a length to width ratio of (1.11–)1.15–1.22–1.29(−1.38), directly germinating with a germ tube. Resting organs present in the necrotic leaf tissue but also in rhizome lesions with brownish dots. Oogonia light brown, (24.0–)28.0–32.0–36.0(−37.05) μm in diameter. Oospores (19.0–)21.0–24.0–27.0(−32.5) μm in diameter, wall 2.0–4.5 μm (mean = 3.0 μm) thick, surface smooth.

Typus: UK; Cymru, Swansea, Killay, Cyne Vallay Cottages, Touchwood, in a public garden, on living leaves of *Aquilegia vulgaris* affected by downy mildew disease, April 2015, Carrie Thomas (GLM-F116093–holotypus).

Habitat: On living leaves of *Aquilegia alpina*, *A. buergeriana*, *A. flabellata*, *A. viridiflora*, *A. vulgaris* and *Semiaquilegia adoxoides* (Ranunculaceae).

Distribution: South Korea, the UK, and China (?)

Additional specimens examined: see Table 1.

Note: This pathogen seems to have been first found on *Semiaquilegia adoxoides* in China (Yu

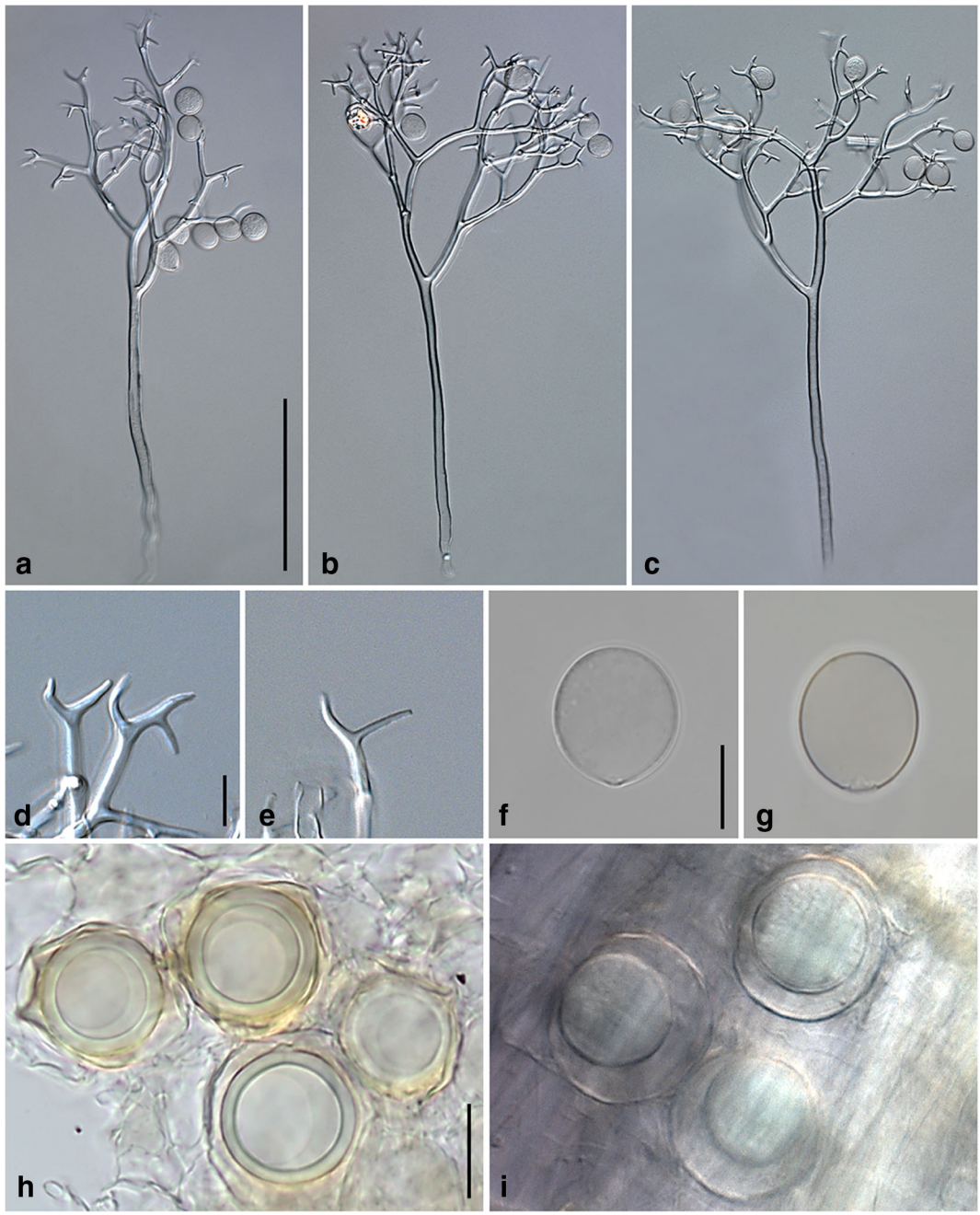

Fig. 2 *Peronospora aquilegiicola* sp. nov. parasitic on *Aquilegia vulgaris*. **a**–**c** Conidiophores (bar = 100 μm); **d** & **e** Ultimate branchlets (bar = 10 μm); **f** & **g** Conidia (bar = 10 μm); **h** & **i** Resting organs inside a leaf (**h**) and a rhizome (**i**) (bar = 20 μm). Source: GLM-F116093

et al. 1998) as "*Peronospora ficariae*". In a simple description of the pathogen on *S. adoxoides*, the measurements of conidiophores (196–511 μm in length, 5.7–8.5 μm in width) and oospores (22.3–25 μm in diameter) are in line with measurements for *P. aquilegiicola* performed in this study. However, larger conidia of 11.4–42.6 × 12.8–34 μm have been reported than the present study, requiring further investigation, before a conspecificity can be ascertained.

Discussion

Downy mildew of *Semiaquilegia* was found in natural host populations in South Korea and presumably in China, which precedes the year 2013, when *Aquilegia* downy mildew emerged in the UK. However, because *Semiaquilegia* plants are found only for a short time in spring, and the infested plants are often almost indistinguishable from the healthy ones, the disease may be easily overlooked. Thus, it is most likely that *P. aquilegiicola* is an indigenous species in northeast Asia. The genetic similarity of *P. aquilegiicola* collections from Korea and the UK suggests that this pathogen has been quite recently introduced to the UK, presumably by trade with infected plants or seeds from East Asia, the origin of *Semiaquilegia*. There it might have jumped onto non-indigenous *Aquilegia* plants or the indigenous species, *A. buergeriana*, which are grown for ornamental purpose. The two genera, *Aquilegia* and *Semiaquilegia*, are morphologically similar (Tucker and Hodges 2005; Damerval and Nadot 2007) and phylogenetically close (Wang and Chen 2007), and the host plant *S. adoxoides* was often classified under *Aquilegia*.

The market size for *Aquilegia* is increasing due to growing demand for this ornamental plant in gardens and parks. *Peronospora aquilegiicola* should be seen as a high-risk pathogen due to its aggressiveness, its production of oospores that outlast unfavourable conditions, and the production of air-borne conidia which are produced abundantly. In the past, some of the most devastating oomycete diseases have emerged by a transfer of infected plants and seeds. For example, basil downy mildew caused by *Peronospora belbahrii*, resulted in significant losses in global sweet basil production (Belbahri et al. 2005; Thines et al. 2009; Wyenandt et al. 2015). When the basil downy mildew was first discovered, the causal agent was attributed to *Peronospora lamii*, which is widely distributed throughout the world together with its host, *Lamium purpureum*. Inevitably, few countries have taken quarantine measures against infested basils or seeds, resulting in its spread over most of the world (Thines and Choi 2016). At the moment, as *Aquilegia* downy mildew seems to be still restricted to the UK, *P. aquilegiicola* should be considered to be a quarantine organism, to block further spread of this pathogen. If measures are taken quickly, it might be possible to avoid the advent of another global epidemic and render *P. aquilegiicola* the first downy mildew to be successfully halted from spreading.

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Compliance with ethical standards

Conflict of interest All authors herewith declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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