Unexpected ribosomal DNA internal transcribed spacer sequence variation within *Erysiphe aquilegiae sensu lato*

James H. Cunnington[1,2*], Ann C. Lawrie[2] and Ian G. Pascoe[1]

[1]Primary Industries Research Victoria (PIRVic), Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia
[2]Department of Biotechnology and Environmental Biology, RMIT University, PO Box 71, Bundoora, Victoria 3083, Australia

Cunnington, J.H., Lawrie, A.C. and Pascoe, I.G. (2004). Unexpected ribosomal DNA internal transcribed spacer sequence variation within *Erysiphe aquilegiae sensu lato*. Fungal Diversity 16: 1-10.

Erysiphe aquilegiae sensu lato is the most commonly occurring powdery mildew fungus (*Erysiphales*: Ascomycota) on the *Ranunculaceae*. Based on morphological characters, different authors have proposed the segregation of *Erysiphe aquilegiae* into two varieties or species. To determine if a genetic basis exists for these two taxa, the rDNA internal transcribed spacer regions of 26 specimens from five host genera, were compared by RFLP's and sequencing. The results failed to separate the two taxa, but revealed a third host-specialised taxon on *Delphinium*. Phylogenetic analysis revealed *E. aquilegiae* to be more closely related to *Oidium lycopersici* and *E. macleayae* than to the undescribed species on *Delphinium*.

Key words: *Delphinium*, *Erysiphales*, *Erysiphe ranunculi*, *Ranunculaceae*, rDNA, RFLP.

Introduction

Erysiphe aquilegiae DC. is a common powdery mildew infecting the *Ranunculaceae*. In his monograph of the *Erysiphales*, Braun (1987) accepted two varieties of this species, *Erysiphe aquilegiae* var. *aquilegiae* and *Erysiphe aquilegiae* var. *ranunculi* (Grev.) Zheng & Chen. These two taxa were differentiated by their cleistothecial appendages. Those of *E. aquilegiae var. ranunculi* are 0.5-4 times the diameter of the cleistothecia and mycelioid, while those of *E. aquilegiae* var. *aquilegiae* are (1)3-12 times the diam of the cleistothecia and straighter. According to Braun (1987) *Erysiphe aquilegiae* var. *aquilegiae* occurs on *Aquilegia*, *Anemonella*, *Actaea*, *Caltha* and some species of *Clematis*, *Thalictrum* and *Ranunculus,* while, *Erysiphe aquilegiae* var. *ranunculi* occurs on *Aconitum*, *Adonis*, *Anemone*, *Atragene*, *Cimicifuga*, *Clematis*, *Coptis*, *Delphinium*, *Isopyrum*, *Knowltonia*, *Nigella*, *Pulsatilla*,

* Corresponding author: J.H. Cunnington; e-mail: james.cunnington@dpi.vic.gov.au

Ranunculus, *Thalictrum*, *Trautvetteria* and *Trollis*. Zheng and Chen (1981) also recognised these two varieties.

Several authors have treated the two varieties as separate species. Junell (1967) first noted the difference in cleistothecial appendages and accepted two species: *Erysiphe aquilegiae* and *Erysiphe ranunculi* Grev. Examining the anamorphic states, Gorter and Eicker (1985) found that the conidiophore foot cells of *E. aquilegiae* var. *aquilegiae* were (25-)40(-50) µm long while those of *E. aquilegiae var. ranunculi* were (15-)25(-35) µm long. Based on morphological grounds, they argued that those two taxa merited species status. They also found that *E. aquilegiae* var. *aquilegiae* occasionally produced a conidium approximately twice the average size. Shin (2000) also recognised two species, but noted several other distinguishing characters. *Erysiphe ranunculi* had appressoria that were usually multilobed, shorter conidia, larger ascomata, more distinct peridial cells and shorter appendages with a more regular apex than *E. aquilegiae*. However, he did not find the foot-cell differences described by Gorter and Eicker (1985). By contrast, Boesewinkel (1979) noted no difference in the anamorphs of specimens on *Aquilegia* and *Ranunculus* and accepted only a single species.

The ribosomal DNA internal transcribed spacer (ITS) region has been useful for discriminating fungi at the species level (Bruns *et al*., 1991) and has been used extensively for the identification and phylogenetic analysis of powdery mildews (Kiss *et al*., 2001; Cunnington *et al*., 2003; Hirata *et al*., 2000; Matsuda and Takamatsu, 2003; Mori *et al*., 2000). Given the difficulties for the morphological differentiation of the two varieties of *E. aquilegiae*, the aim of this study was to determine if they could be distinguished at the molecular level using RFLP's and sequence data of the ITS regions.

Materials and methods

Twenty-six specimens from the *Erysiphe aquilegiae* complex were obtained from Australia, Germany, Switzerland and Argentina (Table 1). Most of these were identified by their teleomorphic state according to Braun (1987), but some specimens were purely anamorphic and have therefore been referred to as *Oidium* sp. A small amount of the powdery mildew (approx 1 mm^3) was scraped from the infected leaf using a chisel shaped probe. This was put in a 1.5 mL Eppendorf tube containing 50 µL of 5% Chelex-100 (Biorad) containing 0.01% Triton X-100, and placed in a 94°C water bath for 1-2 hours. The tube was vortexed vigorously for 2-3 seconds and centrifuged for 10 minutes at 14000 rpm. The supernatant was used directly for the PCR.

An initial PCR was performed in 25 µL containing 1 µL DNA extract, 200 µM of each dNTP (Pharmacia Biotech), 1.5 mM $MgCl_2$, 2.5 µL 10×

Table 1. Specimens used to examine genetic diversity within *Erysiphe aquilegiae sensu lato*.

Specimen	Species	Host	Location	GenBank accession
VPRI 17280	*E. aquilegiae* var. *aquilegiae*	*Aquilegia vulgaris*	Victoria, Australia	-
VPRI 20807	*E. aquilegiae* var. *aquilegiae*	*Aquilegia* sp [B]	Victoria, Australia	-
VPRI 20820	*E. aquilegiae* var. *aquilegiae*	*Aquilegia* sp. [B]	Victoria, Australia	AY452800
VPRI 20859	*E. aquilegiae* var. *aquilegiae*	*Aquilegia* sp. [B]	Victoria, Australia	-
VPRI 22123	*E. aquilegiae* var. *aquilegiae*	*Aquilegia vulgaris*	Argentina	-
VPRI 21045	*E. aquilegiae* var. *aquilegiae*	*Clematis recta*	Geneva, Switzerland	AY452801
VPRI 21046	*E. aquilegiae* var. *ranunculi*	*Clematis integrifolia*	Geneva, Switzerland	AF154322
VPRI 19124	*E. aquilegiae* var. *ranunculi*	*Clematis pubescens*	Western Australia, Australia	-
VPRI 17736	*E. aquilegiae* var. *ranunculi*	*Delphinium* sp.[A]	Victoria, Australia	-
VPRI 18642	*Oidium* sp.	*Delphinium* sp. [A]	Victoria, Australia	-
VPRI 19613	*E. aquilegiae* var. *ranunculi*	*Delphinium* sp. [A]	Victoria, Australia	AY452802
VPRI 20819	*Oidium* sp.	*Delphinium* sp. [A]	Victoria, Australia	-
VPRI 22122	*E. aquilegiae* var. *ranunculi*	*Delphinium* sp.	Aubael, Germany	AY452803
VPRI 18533	*Oidium* sp.	*Nigella damascena*	Victoria, Australia	AY452805
VPRI 18808	*Oidium* sp.	*Nigella damascena*	Tasmania, Australia	-
VPRI 18627	*Oidium* sp.	*Ranunculus asiaticus*	Victoria, Australia	-
VPRI 19199	*Oidium* sp.	*Ranunculus repens*	Victoria, Australia	-
VPRI 19844	*Oidium* sp.	*Ranunculus repens*	Victoria, Australia	-
VPRI 19567	*Oidium* sp.	*Ranunculus acris*	South Australia, Australia	-
VPRI 19682	*Oidium* sp.	*Ranunculus asiaticus*	Victoria, Australia	-
VPRI 18740	*E. aquilegiae* var. *ranunculi*	*Ranunculus* sp.	Victoria, Australia	AY452805
VPRI 20338	*Oidium* sp.	*Ranunculus asiaticus*	Victoria, Australia	-
VPRI 20680	*Oidium* sp.	*Ranunculus* sp.	Victoria, Australia	-
VPRI 22125	*E. aquilegiae* var. *ranunculi*	*Ranunculus acris*	Naundorf, Germany	-
VPRI 22126	*E. aquilegiae* var. *ranunculi*	*Ranunculus acris*	Anhalt, Germany	-
VPRI 22127	*E. aquilegiae* var. *ranunculi*	*Ranunculus repens*	Germany	-
-	*E. aquilegiae* var. *ranunculi*	*Cimicifuga simplex*	Japan	AB000944
-	*E. aquilegiae* var. *ranunculi*	*Clematis terniflora*	Japan	AB015929

[A]Cultivated hybrids probably from *D. elatum*. [B]Cultivated hybrids probably from *A. vulgaris*.

buffer, 4 ng each of primers PMITS1 and PMITS2 (Cunnington *et al*., 2003), 6% Tween 20 (Labchem) and 0.5 units of Amplitaq Gold (Perkin-Elmer). Amplification was 10 min at 94°C, 35 cycles of: 1 min at 94°C, 1 min at 65°C, 2 min at 72°C, and a final extension of 10 min at 72°C. Amplified products were detected by loading 4 µL on a 1.4% agarose gel stained in EtBr. A nested PCR was performed in 50 µL as outlined above, but without Tween 20, using 8 ng of primers PMITS1 and ITS4 (White *et al*., 1990). The reaction mixture contained 1 µL of a 1:100 dilution of the first round product, or if no product was visible then an undiluted 1 µL was used. Cycling times were the same, but with an annealing temperature of 60°C.

Five microlitres of nested PCR product were digested in 10 µL reactions with six restriction endonucleases (Bresatec) *Msp*I, *Hinf*I, *Hin*6I, *Alu*I, *Csp*I and *Bsu*RI. Each 10 µL reaction contained 5 µL of nested PCR product, 10 U

of enzyme and 1 µL of 10x buffer. Digestions were performed at 37°C overnight, after which 1 µL of loading dye was added. Products were visualised on a 3% agarose gel.

Nested PCR products from several specimens from different host genera were selected for sequencing. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen). The products were sequenced directly using primers ITS5 (White *et al*., 1990) and ITS4, with an ABI PRISM® BIGDYE™ Terminator Cycle Sequencing Kit (Perkin-Elmer). Sequences were deposited on GenBank (Table 1).

The rDNA ITS sequences were compared with sequences on GenBank using Blast2 (Altschul *et al*., 1997). The most similar sequences were aligned with the sequences obtained here using ClustalX (Thompson *et al*., 1997). MEGA2 (Kumar *et al*., 2001) was used to construct a neighbour-joining tree using the Kimura-2-parameter method. Gapped sites were excluded from the analysis.

Results

All specimens produced a nested PCR product of approximately 740 bp and yielded an identical RFLP profile for all six restriction enzymes, except for the specimens on *Delphinium* (Fig. 1). For all specimens, except those from *Delphinium*, the resulting fragment sizes were 480 bp and 175 bp for *Msp*I; 500 bp and 250 bp for *Hinf*I; 280(x2) bp and 180 bp for *Hin*6I; 200 bp, 190 (x2) bp and 160 bp for *Alu*I; 320 bp, 200bp and 130 bp for *Bsu*RI; and 500 bp and 240 bp for *Csp*I. The specimens on *Delphinium* differed in the profiles for *Bsu*RI (520 bp and 130 bp), *Alu*I (340 bp, 200 bp and 190 bp) and *Csp*I (uncut).

The Blast2 search on GenBank revealed a high similarity between all the sequences obtained here and two other *E. aquilegiae* sequences, along with ITS sequences for *Oidium neolycopersici* (from *Lycopersicon esculentum*) and *E. maclaeyae* (from *Macleaya cordata*). The next most similar sequence was *E. symphoricarpi*, however this sequence was sufficiently different to serve as an outgroup for the phylogenetic analysis. The alignment of all these sequences revealed that the ITS region from all specimens in the *E. aquilegiae* complex that did not come from *Delphinium* had identical sequences, with the exception of *E. aquilegiae* var. *ranunculi* on *Cimicifuga simplex* (GenBank AB000944), which differed by 2 bases. Two sequences from *Delphinium* were identical, but differed from the other *E. aquilegiae* specimens by 11 bases and from *C. simplex* by 13 bases. The ITS sequences for *Oidium neolycopersici* and *E. macleayae* differed from *E. aquilegiae* sequences by two and one bases

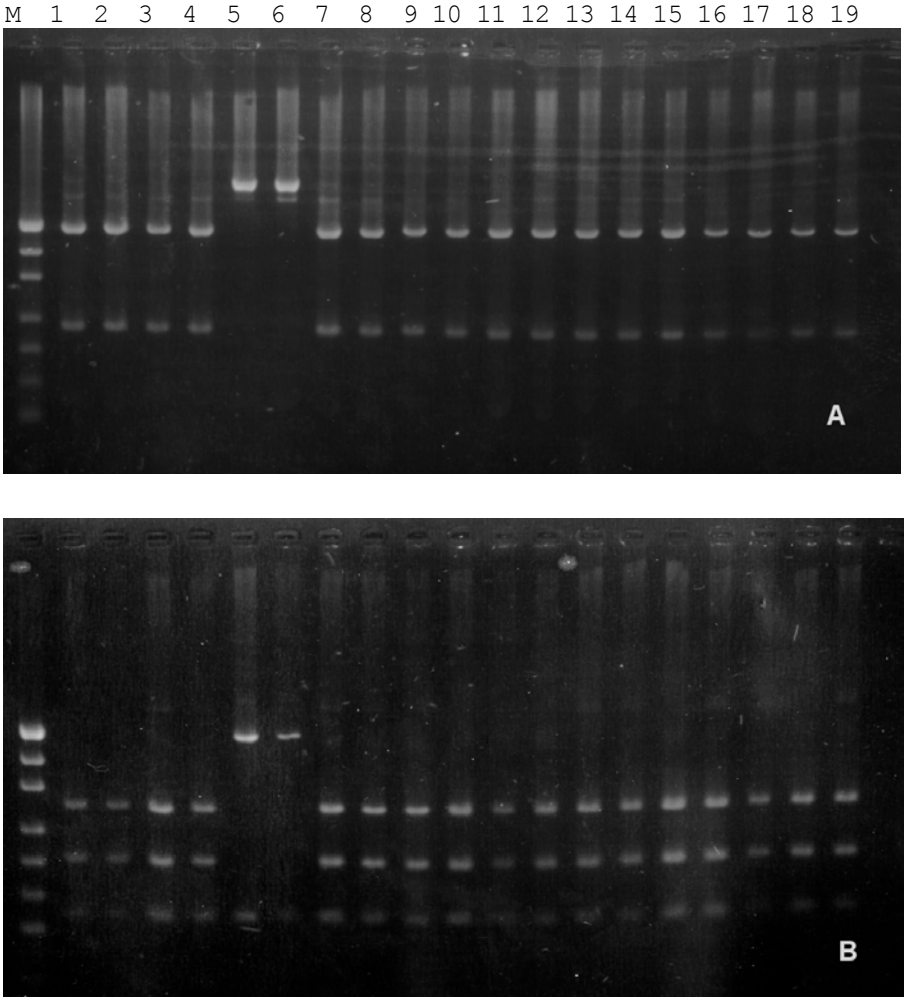

Fig. 1. Restriction digests of the rDNA ITS region from specimens of *E. aquilegiae*, using the endonucleases *Csp*I (A) and *Bsu*RI (B). Lanes 1-19: VPRI 17280, 20807, 22123, 21045, 17736, 19613, 18533, 18808, 18627, 19199, 19844, 19567, 19682, 18740, 20338, 20680, 22125, 22126, 22127. M = molecular weight marker.

respectively. The majority of these differences were in the ITS1 region (Fig. 2). The neighbour-joining tree (Fig. 3) clearly shows *E. aquilegiae* to be more closely related to both *Oidium neolycopersici* and *E. macleayae* than to the taxon on *Delphinium*.

```
                    |ITS1→
AB000944            ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
AB015929            ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
VPRI 18533          ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
VPRI 21045          ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
VPRI 18740          ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
VPRI 20820          ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
AF154322            ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
E. macleayae        ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
O. neolycopersici   ACAGAGCGTGAGGCTCAGTCGTGGCGTCAGCTGCGTGCTGGGCCGACCCT
VPRI 19613          ACAGAGCGTGAGGCTCAGTCGTGGCATCTGCTGCGTGCTGGGCCGACCCT
VPRI 22122          ACAGAGCGTGAGGCTCAGTCGTGGCATCTGCTGCGTGCTGGGCCGACCCT
E. symphoricarpi    ACAGAGCGTGAGGCTCAGTCGTGGCGTCTGCTGCGTGCTGGGCCGACCCT
                                            *  *

AB000944            CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
AB015929            CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
VPRI 18533          CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
VPRI 21045          CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
VPRI 18740          CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
VPRI 20820          CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
AF154322            CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
E. macleayae        CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
O. neolycopersici   CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
VPRI 19613          CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
VPRI 22122          CCCACCCGTGTCGATTTCTATCTTGTTGCTTTGGCGGGCCGGGCTACGTC
E. symphoricarpi    CCCACCCGTGTCGATTTATATTTTGTTGCTTTGGCGGGCCGGGTTACGTC
                                  *   *                         *

AB000944            GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
AB015929            GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
VPRI 18533          GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
VPRI 21045          GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
VPRI 18740          GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
VPRI 20820          GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
AF154322            GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
E. macleayae        GTCGCTGCCCGTACGGACATGCGTCGGCCGCCCACCGGTTTCGACTGGAG
O. neolycopersici   GTCGCTGCCCGTACGGACATGTGTCGGCCGCCCACCGGTTTCGACTGGAG
VPRI 19613          GTCGCTGACCATTAGGACATGCGTCGGCCGCCCACCGGTTTCGGCTGGAG
VPRI 22122          GTCGCTGACCATTAGGACATGCGTCGGCCGCCCACCGGTTTCGGCTGGAG
E. symphoricarpi    GTCGCTGTCCGCAAGGACAGACGTCGGCCTCCCACCGGTTTCGACTGGAG
                           *  ****     ***       *               *

AB000944            CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
AB015929            CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
VPRI 18533          CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
VPRI 21045          CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
VPRI 18740          CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
VPRI 20820          CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
AF154322            CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
E. macleayae        CGCGTCCGCCAAAGACCTAACCAAAACGCATGTTGTCTTTGTCGTCTCAG
O. neolycopersici   CGCGTCCGCCAAAGACCTAACCAAAACTCATGTTGTCTTTGTCGTCTCAG
VPRI 19613          CGCGCCCGCCAAAGACCTATACAAAACTCATGTTGTCTTTGTCGTCTCAG
VPRI 22122          CGCGCCCGCCAAAGACCTATACAAAACTCATGTTGTCTTTGTCGTCTCAG
E. symphoricarpi    CGCGTCCGCCAAAGACCCAATCAAAACTCATGTTGTCTTTGCAGTCTCAG
                          *           * **     *           **
```

Fig. 2. Alignment of the rDNA ITS1 sequences for *E. aquilegiae* specimens, *E. macleayae*, *O. lycopersici* and *E. symphoricarpi*. * indicates a variable site.

```
                                        |5.8S→
AB000944            GTTTATTATAGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
AB015929            CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
VPRI 18533          CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
VPRI 21045          CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
VPRI 18740          CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
VPRI 20820          CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
AF154322            CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
E. macleayae        CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
O. neolycopersici   CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
VPRI 19613          CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
VPRI 22122          CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
```

```
E. symphoricarpi   CTTTATTATTGAATTGATAAAACTTTCAACAACGGATCTCTTGGCTCTGG
                   *       *
```

Fig. 2 continued. Alignment of the rDNA ITS1 sequences for *E. aquilegiae* specimens, *E. macleayae*, *O. lycopersici* and *E. symphoricarpi*. * indicates a variable site

Discussion

The lack of any ITS sequence difference between *E. aquilegiae* var. *aquilegiae* and *E. aquilegiae* var. *ranunculi* (with the exception of the material on *Delphinium*) was unexpected, but may indicate how very recently these two taxa diverged. In this respect, Boesewinkel's (1979) decision to accept only a single species and Braun's (1987) decision to retain the two taxa as varieties of one species seems to be justified by their genetic similarity (at least in the ITS region). Only the Japanese specimen on *Cimicifuga simplex* showed some variation; however, without further ITS sequences from specimens collected from *Cimicifuga* it cannot be determined whether this reflects the presence of a distinct taxon or random genetic variation. Other, more sensitive molecular based techniques, such as RAPD's (Bardin *et al*., 1997) or examination of ß-tubulin genes sequences (Wyand and Brown, 2003) should be examined in the future in an attempt to differentiate these taxa.

The differentiation of a separate form genotype on *Delphinium* was unexpected. Even though only five specimens on *Delphinium* were examined, the consistently identical ITS sequences (and RFLP patterns) among those isolates and difference in sequence composition from other samples analysed indicates that it represents a distinct taxon. Even though morphological studies were not conducted here, it is expected that it will be difficult to find consistent characters to delineate the species on *Delphinium* from *E. aquilegiae* var. *ranunculi*, given the large number of taxonomic studies already conducted on the *E. aquilegiae* complex (e.g. Braun, 1987; Junell, 1967; Shin, 2000; Gorter and Eicker, 1985). However, Junell (1967) noted that specimens from *Delphinium* produced 3-6 ascospores per ascus, while those on *Ranunculus* produced only (2-)3-5. Ascospore number is a useful taxonomic character for

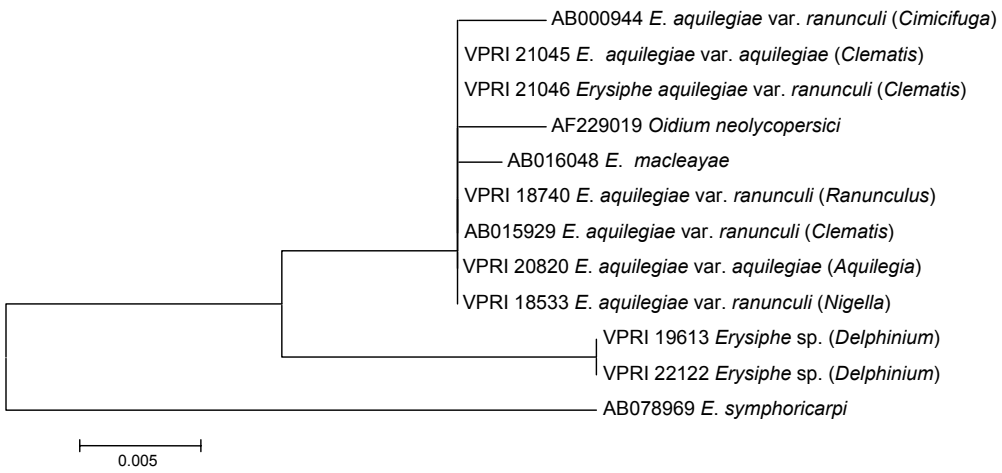

Fig. 3. Neighbour joining tree from ITS sequences showing the relationships between *E. aquilegiae*, *O. lycopersici*, *E. macleayae* and an undescribed taxon on *Delphinium*. Bar = 5 changes per 1000 bases.

differentiating species of powdery mildews (Braun, 1987). This character needs to be examined further when a revision of this group is undertaken.

Phylogenetic analysis emphasised the evolutionary distance between *E. aquilegiae* and the taxon on *Delphinium*. *Erysiphe macleayae* and *Oidium neolycopersici* are more closely related to other isolates of *E. aquilegiae* (var. *ranunculi* and var. *aquilegiae*) than to *E. aquilegiae* isolates from *Delphinium*. Thus, it is surprising that sufficient morphological differences have not developed to a state where the taxon on *Delphinium* has previously been recognised as different species. The phylogenetic similarity between *O. neolycopersici*, *E. macleayae* and *E. aquilegiae* was previously noted by Kiss *et al*. (2001). The ITS sequence variation between these three species is comparable to the intraspecific variation reported for several powdery mildews by Cunnington *et al*. (2003). Despite the lack of variation found here, it is likely that the ITS region will continue to be commonly used for molecular taxonomic studies for the *Erysiphales* as the absence of specific primers for other gene regions requires that relatively large amounts of fresh material be available, rather than dried specimens which are usually mixed with contaminating fungi.

Given that only five host genera (plus *Cimicifuga*) were examined in this study, it is possible that more cryptic species exist within this complex. For example, *E. aquilegiae* has never been collected in Australia on most of the host plants listed in Braun's monograph (herb. VPRI, DAR and BRIP records).

This may indicate additional host-specialised taxa that have not been introduced into Australia. Jaczewski (1927) introduced many formal *formae* according to host genus within *E. aquilegiae*, but as they were not the result of inoculation experiments, there seems little chance that they could form the basis of a revision of this group. Sequence analysis from ITS regions has made some progress towards better understanding the taxonomy of *E. aquilegiae*, but further molecular analysis using more sensitive techniques to assess genetic variation will be required to determine the extent to which different taxa are present within this complex.

Acknowledgements

We thank A. Bolay (Geneva, Switzerland) for providing the Swiss material and U. Braun (Martin-Luther Universität, Germany) for the specimens from Germany and Argentina.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, Z., Zhang, Z., Miller, W. and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389-3402.

Bardin, M., Nicot, P.C., Normand, P. and Lemaire, J.M. (1997). Virulence variation and DNA polymorphism in *Sphaerotheca fuliginea*, causal agent of powdery mildew of cucurbits. European Journal of Plant Pathology 103: 545-554.

Boesewinkel, H.J. (1979). Erysiphaceae of New Zealand. Sydowia 32: 13-56.

Braun, U. (1987). A monograph of the Erysiphales (powdery mildews). Nova Hedwigia 89: 1-700.

Bruns T.D., White, T.J. and Taylor, J.W. (1991). Fungal molecular systematics. Annual Revue of Ecology and Systematics 22: 525-564.

Cunnington J.H., Takamatsu S., Lawrie A.C., and Pascoe I.G. (2003). Molecular identification of anamorphic powdery mildew fungi. Australasian Plant Pathology 32: 421-428.

Gorter, G.J.M.A. and Eicker, A. (1985). New South African records of Erysiphaceae from the Transvaal II. South African Journal of Botany 51: 339-343.

Hirata, T., Cunnington, J.H., Paksiri, U., Limkaisang, Shishkoff, N., Grigaliunaite, B., Sato, Y and Takamatsu, S. (2000). Evolutionary analysis of subsection *Magnicellulatae* of *Podosphaera* section *Sphaerotheca* (Erysiphales) based on the rDNA internal transcribed spacer sequences with special reference to host. Canadian Journal of Botany 78: 1521-1530.

Jaczewski, A. (1927). Karmannyj opredelitel' gribov, II. Mučnisto-rosjanye griby, Leningrad.

Junell, L. (1967). *Erysiphaceae* of Sweden. Symbolae Botanicae Upsaliensis 19: 1-117.

Kiss, L., Cook, R.T.A., Saenz, G.S., Cunnington, J.H., Takamatsu, S., Pascoe, I., Bardin, M., Nicot, P.C., Sato, Y., and Rossman, A.Y. (2001). Identification of two powdery mildew fungi, *Oidium neolycopersici* sp. nov. and *O. lycopersici*, infecting tomato in different parts of the world. Mycological Research 105: 684-697.

Kumar, S., Tamura, K., Jakobsen, I.B. and Nei, M. (2001). MEGA2: Molecular Evolutionary Genetics Analysis software. Bioinformatics 17: 1244-1245.

Matsuda, S. and Takamatsu, S. (2003). Evolution of host-parasite relationships of *Golovinomyces* (Ascomycetes: Erysiphaceae) inferred from nuclear rDNA sequences. Molecular Phylogenetics and Evolution 27: 314-327.

Mori, Y., Takamatsu, S. and Sato, Y. (2000). Evolutionary analysis of the powdery mildew fungi (Erysiphales) using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92: 74-93.

Shin, H.D. (2000). *Erysiphaceae of Korea*. National Institute of Agricultural Science and Technology, Korea.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876-4882.

White, T.J., Bruns, T.D., Lee, S. and Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: a guide to methods and applications*. (eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White). Academic Press, USA.

Wyand, R.A. and Brown, J.K.M. (2003). Genetic and *forma specialis* diversity in *Blumeria graminis* of cereals and its implications for host-pathogen co-evolution. Molecular Plant Pathology 4: 187-198.

Zheng, R.-y. and Chen, G.Q. (1981). The genus *Erysiphe* in China. Sydowia 34: 214-327.

(Received 5 November 2003; accepted 9 February 2004)