Powdery Mildew of Prairie Gentian: Characteristics, Molecular Phylogeny and Pathogenicity

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ABSTRACT

In March 1999, we found prairie gentian (*Eustoma grandiflorum*) infected with powdery mildew in a greenhouse in Oita Prefecture, Japan. Morphological observation revealed that the causal fungus belongs to the mitosporic genus *Oidium* subgenus *Pseudoidium* [teleomorph: *Erysiphe sensu* Braun and Takamatsu (2000)]. Precise taxonomic position of the fungus, however, is uncertain due to lack of the perfect stage. We determined the nucleotide sequence of the rDNA ITS region of the fungus. Comparison of the sequence with those obtained from DNA databases of this fungal group revealed that the sequence is identical to those of powdery mildews from garden four-o'clock (*Mirabilis jalapa*) and broad bean (*Vicia faba*). Inoculation of an isolate from garden four-o'clock caused mildew on prairie gentian and broad bean, suggesting that the prairie gentian mildew originates from garden four-o'clock or broad bean. Molecular phylogenetic analysis indicated a close relationship of this fungus to *Erysiphe baeumleri* on *Vicia* spp. and *E. trifolii* on *Trifolium pratense*. From these results, we propose that prairie gentian mildew diverged from a Fabaceae-parasitic ancestor.

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Key words: broad bean, Erysiphaceae, *Eustoma grandiflorum*, garden four-o'clock, internal transcribed spacer, *Oidium* subgenus *Pseudoidium*.

Running title: Powdery mildew of prairie gentian

INTRODUCTION

Prairie gentian [*Eustoma grandiflorum* (Raf.) Shinners] is an annual or biennial plant belonging to the family Gentianaceae. Three species of *Eustoma*, *E. grandiflorum*, *E. exaltatum* Salisb., and *E. barkleyi* Standley & Shinners, have been recorded in South America. Of these species, *E. grandiflorum* has been used as a breeding source for a number of garden cultivars of prairie gentian, important cut flowers in Japan and elsewhere. In March 1999, we found prairie gentian infected with powdery mildew in a greenhouse in Oita Prefecture, Japan. Powdery mildew caused by *Leveillula taurica* (Lév.) Arnaud has been reported on this plant only in California, the United States[11]. Based on morphological observation, the current anamorph, however, is a species of *Oidium* subgenus *Pseudoidium*[3], which is clearly different from *L. taurica*, but the identity and origin of the anamorph remains uncertain because no fruiting bodies of the teleomorph were observed.

As a result of phylogenetic analyses of the Erysiphaceae, nucleotide sequence data for the ribosomal DNA (rDNA) region have been accumulating recently[7-9,12,15,19,20]. This nucleotide sequence is useful for identifying a fungus having no teleomorph, because precise phylogenetic relationships between meiosporic and mitosporic species can be studied. This kind of approach is effective for studies of the identity and origin of anamorphic powdery mildews newly discovered on a variety of horticultural plants such as tomato[10], soybean[22], baby's breath[21], *Rhododendron* sp.[23], and *Acacia* spp.[23] In this study, we determined the nucleotide sequence of the rDNA internal transcribed spacer (ITS) region of this new powdery mildew on prairie gentian. Comparison with a number of ITS sequences from powdery mildews having a *Pseudoidium* anamorph revealed that the sequence of this prairie gentian isolate is identical to those from powdery mildews on *Mirabilis jalapa* L. (garden four-o'clock, Nyctaginaceae) and *Vicia faba* L. (broad bean, Fabaceae). To confirm the result, we then inoculated prairie gentian and broad bean with an isolate from garden four-o'clock.

MATERIALS AND METHODS

Morphological observation Hyphae, conidiophores and conidia of fresh material were stripped off the leaf surface with clear adhesive tape, mounted on a slide glass , and examined in water using a light microscope. Conidial germ tubes were observed according to Hirata[6]. The epidermal layer from the inner surface of onion scales was stripped off a area ~~of~~ (1 cm^2) with forceps and dipped in 80% ethanol for more than 2 weeks. The cell layer rinsed with tap water for 30 min was put on a slide glass, removed excess water with filter paper, inoculated with conidia, and incubated at 20-25˚C for 24 hr until microscopic observation.

DNA extraction, PCR amplification, DNA sequencing and data analysis DNA was extracted from powdery mildew anamorphs on prairie gentian, garden four-o'clock, and broad bean (Table 1) using the 5% chelex method[25] as described in Hirata and Takamatsu[7]. The nuclear rDNA ITS region amplified by PCR was subjected to DNA sequencing as described in Takamatsu *et al*.[20]

The sequences were initially aligned using the Clustal V package[5]. The alignment was then visually refined with a word processing program, using color-coded nucleotides, and ambiguously aligned sites were removed from the data set in the following analyses. Phylogenetic trees were obtained from the data using parsimony and distance methods. For distance analysis, the most appropriate evolutionary model was determined for a given data set using PAUP* 4.0b8[17] and Modeltest 3.06[14]. A starting tree was obtained with the neighbor-joining method. With this tree, likelihood scores were calculated for 56 alternative models of evolution by PAUP*. The output file was then imported to Modeltest to compare the models with a likelihood ratio test. Once a model of evolution was chosen, it was used to construct phylogenetic trees with the minimum-evolution (ME) method using a heuristic search option in PAUP*.

For the parsimony analysis, we used the maximum-parsimony (MP) method with a heuristic search using PAUP*. This search was repeated 100 times with different random

starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Transversions were weighted over transitions by a 1.85:1 ratio by means of the USERTYPE STEPMATRIX. This ratio was obtained from analysis of the ME tree. All sites were treated as unordered, with gaps treated as missing data. The branch-swapping algorithm was TBR, the MULPARS option was in effect, and zero-length branches were collapsed. The strength of the internal branches from the resulting trees was tested by bootstrap analysis[4] using 1000 replications.

Inoculation test 1) Experiment 1 Prairie gentian cv. Azuma-no-Nami, perennial statice (*Limonium latiforium* x *L. belidifolium*) cv. Blue Fantasia 100, *Physalis alkekengi* L. var. *franchetii* (Masters) Hort., and sweet pea (*Lathyrus odoratus* L.) were inoculated with conidia produced on the leaves of prairie gentian cv. Exrosa Blue Flash. After incubation at 20˚C for 2 weeks, colony formation of powdery mildew was checked by eye.

2) Experiment 2 One hundred and twenty cultivars of prairie gentian, five cultivars of broad bean (Ryosai-issun, Kawachi-issun, Nintoku-issun, Sanuki-nagasaya-natamame, and Sanren), and a garden four-o'clock grown in a greenhouse at the Oita Prefectural Floricultural Research Center were inoculated with conidia produced on the leaves of garden four-o'clock. Colony formation of powdery mildew was checked 3 months after inoculation. When colony formation was observed, conidial formation was further checked under a stereomicroscope. Another set of these plants was grown in an independent greenhouse for the uninoculated control and similarly observed during the period. Conidia produced on the respective plants were back inoculated to the original host, garden four-o'clock, to check pathogenicity, and the anamorph was morphologically observed on the respective plants. Nucleotide sequences of the rDNA ITS region were determined for colonies that appeared on prairie gentian, broad bean, and garden four-o'clock.

RESULTS

Symptoms, morphology and pathogenicity

The powdery mildew on prairie gentian was found in a greenhouse of Sanko-mura, Oita Prefecture, in March 1999. Of five cultivars of prairie gentian grown in the greenhouse, mildew was found on four cultivars, Exrosa Blue Flash, Exrosa Blue Picoty, Exrosa Pink, and King of Snow. Although symptoms were most serious on Exrosa Blue Flash, the outbreak was found primarily on those plants grown near the entrance. Powdery mildew was not found on cultivar Hama-no-Utage, which was grown farthest from the entrance. White, powdery mycelial colonies appeared on upper leaves and peduncles, but not on lower leaves, stems, and petals (Plate I-A, B). Host tissue beneath the colonies had browned and leaves had distorted in some infected plants. The mildew disappeared next season, and no mildew has been observed since then.

Conidiophores arise from the upper part of mother cells, 80-132 x 5-7 µm, straight from foot cells, and produce conidia singly followed by 1-3 cells, with a basal septum at the branching point of the mycelium (Plate I-C). Conidia were cylindric to doliform, 33-52 x 13-20 µm, without conspicuous fibrosin bodies, and produce germ tubes terminating in multilobed appressoria (*polygoni*-type)[2)] ~~on the shoulder~~ (Plate I-D). Multilobed to moderately lobed appressoria are formed opposite in pairs or singly on hyphae (Plate I-E). Ascomata were not found. These characters indicate that this fungus belongs to the mitosporic genus *Oidium* subgenus *Pseudoidium*.

The fungus infected prairie gentian cv. Azuma-no-Nami producing conidia, but not perennial statice, *Physalis alkekengi* var. *franchetii*, or sweet pea.

Molecular phylogenetic analysis

In a comparison of the ribosomal DNA ITS sequence determined in the powdery mildew anamorphs from prairie gentian, other ITS sequences of *Oidium* subgenus *Pseudoidium* in DNA databases, the sequence is identical to those from garden four-o'clock and broad bean isolates. These three sequences were deposited in the DDBJ under the accession numbers AB079853 to AB079855 (Table 1).

These sequences were aligned with database DNA sequences of fungi with the *Pseudoidium* anamorph (Table 1). The alignment data matrix consists of 47 taxa and 605 characters, of which 42 sites were removed because of ambiguous alignment. Of the 563 remaining characters, 180 sites were variable and 121 sites were phylogenetically informative for parsimony analysis. Two isolates of *Erysiphe glycines* Tai were used as outgroup taxa based on Takamatsu *et al*.[19]. Using Modeltest[14] under likelihood-ratio test criterion, we concluded that the Tamura-Nei model[24], with equal base frequencies, a gamma-distributed rate heterogeneity model[26] (four rate categories, G = 0.9707) and an estimated proportion of invariant sites (0.4823) was the most appropriate model of evolution for this data set. A heuristic search with this model produced an ME tree with an ME score of 0.86. MP analysis found 24 equally parsimonious trees of 543.35 steps (CI = 0.591, RI = 0.754, RC = 0.446). Because no fundamental difference was found between the ME and MP trees in the phylogenetic position of the prairie gentian isolate, we show only the MP tree in Fig. 1.

The three *Oidium* on prairie gentian, garden four-o'clock and broad bean were situated in the most derived position of the phylogenetic tree and grouped into a clade with *E. baeumleri* (Magnus) U. Braun & S. Takamatsu on *Vicia* spp. and *E. trifolii* Grev. on *Trifolium pratense* L. with strong bootstrap supports (97% in MP tree and 98% in ME tree). The genetic distance of the prairie gentian isolate was 0.58% from *E. baeumleri* and 0.79% from *E. trifolii*. This clade further grouped with a clade consisting of *E. pisi* DC., *E. howeana* U. Braun and *Oidium* sp. on *Glycine max* (L.) Merr. to form a large clade. This large clade, however, was not supported by the bootstrap analysis. As indicated with solid circles in Fig. 1, seven of the ten members of the large clade are isolates from hosts of the Fabaceae, suggesting that the clade is a fungal group parasitic to fabaceous hosts.

Pathogenicity of garden four-o'clock isolate to prairie gentian and broad bean

Conidia produced on the leaves of garden four-o'clock were used to inoculate 120 cultivars of prairie gentian and five cultivars of broad bean. Mycelial formation of powdery mildew was observed on 81 of the 120 prairie gentian cultivars, and conidia were produced on

77 cultivars (Plate I-F). Of the four prairie gentian cultivars on which powdery mildew was observed in the greenhouse of Sanko-mura, three cultivars were included in the present inoculation test. Of these, mycelia and conidia formed on Exrosa Blue Flash and Exrosa Pink, but not on King of Snow. The garden four-o'clock isolate also formed colonies with conidia on all five cultivars of broad bean tested (Plate I-G). Host tissue browned beneath the colonies on all broad bean cultivars and on some cultivars of prairie gentian. Mycelia on broad bean were thin and scarce compared with those in the field, suggesting that broad bean is not completely susceptible to this fungus. Back inoculation tests revealed that these conidia retained pathogenicity to the original host (garden four-o'clock). No powdery mildew colony was ever found on uninoculated plants. The morphology of the anamorphs that formed on prairie gentian, broad bean and garden four-o'clock was the same as that of the field isolate from prairie gentian. The total DNAs that were extracted from these anamorphs to determine the nucleotide sequences of the rDNA ITS region were identical to each other and to the sequences of AB079853 to AB079855.

DISCUSSION

New occurrences of powdery mildews have been reported on a variety of cultivated plants in recent years[18,21-23]. In most cases, precise identification and origin of the causal agents have remained uncertain because of the lack of a perfect stage. Molecular phylogenetic analysis is a useful tool to study these powdery mildew anamorphs because phylogenetic relationships are able to be directly compared between meiosporic and mitosporic fungi. For instance, Kiss *et al.*[10] studied a new powdery mildew disease on tomato with scanning electron microscopy and molecular analysis and identified the causal fungus as a new mitosporic species *Oidium neolycopersici* L. Kiss, which is distantly related to *O. lycopersici* Cooke & Massee, a known pathogen of tomato. Takamatsu *et al.*[21], studying a powdery mildew anamorph newly found on baby's-breath (*Gypsophila paniculata* L.), showed that the

ITS sequence of the anamorph is identical to the sequences of *Erysiphe buhrii* U. Braun, a pathogen of *Gypsophila* in Europe. Takamatsu *et al*.[22] also determined the ITS sequences of 12 isolates of powdery mildew anamorph, which was recently found on soybean over a wide area of eastern Asia and suggested that the causal fungus was introduced to Asia from the New World.

In the case of prairie gentian mildew, introduction from a foreign country is not likely because powdery mildew caused by *Oidium* subgenus *Pseudoidium* has never been reported on this plant anywhere else in the world. Comparison of the ITS sequence with sequences obtained from DNA databases revealed that the sequence is identical to those of powdery mildew isolates from garden four-o'clock and broad bean. This result was unexpected for us because these plants are distantly related to each other. It has long been believed that most species of the Erysiphaceae have strict host specificity, in which a given species or race can infect and utilize a narrow range of host plants, or sometimes only a particular species of host[16]. The present molecular analysis suggests the presence of powdery mildews having a wide host range beyond the family of the host plant.

To confirm the results of the molecular analysis, we tested pathogenicity of a garden four-o'clock isolate on prairie gentian and broad bean. The isolate successfully infected both plants and formed conidia on the colonies, suggesting that the powdery mildew of prairie gentian originates from the fungus on garden four-o'clock or broad bean. Powdery mildew of broad bean was a new disease in 1999 in Kagoshima Prefecture[18], and has never been found in other areas of Japan. A garden four-o'clock isolate produced only scarce mycelia and conidia on broad bean with browning of host tissues, while the powdery mildew of broad bean produces a dense mycelial mat without browning the host tissues in the field. In addition, a preliminary inoculation test showed that a broad bean isolate failed to infect prairie gentian (data not shown). These observations suggest that powdery mildew of broad bean differs in pathogenicity from those of prairie gentian and garden four-o'clock, although they have identical ITS sequences. It is thus unlikely that the prairie gentian mildew originates from

broad bean.

Powdery mildew of garden four-o'clock, first reported by Nomura[13] in 1964, is now found widely in Japan including Oita Prefecture. We found that the conidia of powdery mildew of garden four-o'clock infect prairie gentian. Powdery mildew of prairie gentian was observed at a single greenhouse in 1999, and then disappeared the next season. This observation and the browning of host tissues both in the field and after artificial inoculation suggest that prairie gentian is not the original host of this fungus. Thus, we propose that powdery mildew of garden four-o'clock was able to infect this plant under special conditions, such as in a greenhouse. To confirm this hypothesis, we should conduct cross-inoculations among isolates from the three host plants. However, we could not use the isolate of prairie gentian from the present investigation because fresh material of the fungus is currently not available.

Molecular phylogenetic analysis revealed that the closest relative of the prairie gentian mildew is *E. baeumleri* on *Vicia* spp. Sequence identity for the rDNA ITS region is more than 99% between these fungi. The family Fabaceae is an important host family of powdery mildews having the *Pseudoidium* anamorph. More than 800 species of Fabaceae have been recorded as hosts of this group of fungi in the world[1]. Most of the fungi parasitic to Fabaceae are closely related to each other, and situated at a derived position in the phylogenetic tree of *Pseudoidium*. This suggests that most of the Fabaceae mildews have a relatively recent origin. The prairie gentian mildew is placed in this Fabaceae-mildew group. It is thus likely that the fungus has recently diverged from a Fabaceae-parasitic ancestor.

With the exception of the dormant stage, the life cycle of the powdery mildew fungi completely depends on living hosts, from which they obtain nutrients without killing the host cells. The association between the powdery mildew fungi and their hosts is therefore expected to have been conserved during the course of their evolution. If fungal lineages remain associated with their hosts over a long time, events that isolate the host populations may also isolate the populations of their associated fungi, which may eventually result in co-speciation

of the parasites and their hosts. In this context, the phylogeny of the powdery mildew fungi has long been suggested to be concordant with that of its host plants[2]. The present study, however, suggests that host switching beyond the level of the plant family also occurs in this fungal group. The outbreak of powdery mildew on prairie gentian may be a temporary event occurring only a special environment. However, repeating this event might trigger the establishment of a new host-parasite relationship eventually resulting in the expansion of the host range of the powdery mildew fungi.

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LITERATURE CITED

1. Amano (Hirata), K. (1986). Host Range and Geographical Distribution of the Powdery Mildew Fungi. Japan Scientific Societies Press, Tokyo, Japan.
2. Braun, U. (1987). A monograph of the Erysiphales (powdery mildews). Beih. Nova Hedwigia 89 : 1-700.
3. Cook, R.T.A., Inman, A.J. and Billings, C. (1997). Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range. Mycol. Res. 101 : 975-1002.
4. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39 : 783-791.
5. Higgins, D.G., Bleaby, A.J. and Fuchs, R. (1992). CLUSTAL V: Improved software for multiple sequence alignment. Comput. Appl. Biosci. 8 : 189-191.

6. Hirata, K. (1942). On the shape of the germ tubes of Erysipgaceae. Bull. Chiba Coll. Hort. 5 : 34-49 (in Japanese).
7. Hirata, T. and Takamatsu, S. (1996). Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37 : 265-270.
8. Hirata, T., Cunnington, J.H., Paksiri, U., Limkaisang, S., Shishkoff, N., Grigaliunaite, B., Sato, Y. and Takamatsu, S. (2000). Evolutionary analysis of subsection *Magnicellulatae* of *Podosphaera* section *Sphaerotheca* (Erysiphales) based on the rDNA ITS sequences with special reference to host plants. Can. J. Bot. 78 : 1521-1530.
9. Khodaparast, S.A., Takamatsu, S. and Hedjaroude, G.-A. (2001). Phylogenetic structure of the genus *Leveillula* (Erysiphales: Erysiphaceae) inferred from the nucleotide sequences of the rDNA ITS region with special references to the *Leveillula taurica* species complex. Mycol. Res. 105 : 909-918.
10. 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 an *Oidium* subgenus *Reticuloidium*, infecting tomato in different parts of the world. Mycol. Res. 105 : 684-697.
11. Koike, S.T., Tjosvold, S.A. and Greene, I.D. (1995). Powdery mildew of Eustoma caused by *Leveillula taurica* in California. Plant Dis. 79 : 426.
12. Mori, Y., Sato, Y. and Takamatsu, S. (2000). Evolutionary analysis of the powdery mildew fungi (Erysiphales) using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92 : 74-93.
13. Nomura, Y. (1964). Erysiphaceae found in the south-western parts of the Kanto district. Trans. Mycol. Soc. Japan 7 : 346-349 (in Japanese).
14. Posada, D. and Ceandall, K.A. (1998). Modeltest: Testing the model of DNA substitution. Bioinformatics 14 : 817-818.
15. Saenz, G.S. and Taylor, J.W. (1999). Phylogeny of the Erysiphales (powdery mildews)

inferred from internal transcribed spacer ribosomal DNA sequences. Can. J. Bot. 77 : 150-169.

16. Schmitt, J.A. (1955). The host specialization of *Erysiphe cichoracearum* from zinnia, phlox and cucurbits. Mycologia 47 : 688-701.
17. Swofford, D.L. (2001). PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0b8. Sinauer, Sunderland, MA.
18. Taishi, S., Nojima, H., Hosokawa, Y., Sakaki, R. and Sato, Y. (2000). New record of the powdery mildew on *Vicia faba* L. in Japan. Ann. Phytopathol. Soc. Jpn. 66 : 255 (Abstr. in Japanese).
19. Takamatsu, S., Hirata, T., Sato, Y. and Nomura, Y. (1999) Phylogenetic relationships of *Microsphaera* and *Erysiphe* section *Erysiphe* (powdery mildews) inferred from the rDNA ITS sequences. Mycoscience 40 : 259-268.
20. Takamatsu, S., Hirata, T., and Sato, Y. (2000). A parasitic transition from trees to herbs occurred at least twice in tribe Cystotheceae (Erysiphaceae): Evidence from nuclear ribosomal DNA. Mycol. Res. 104 : 1304-1311.
21. Takamatsu, S., Paksiri, U., Matsuura, S., Okamoto, J. and Braun, U. (2000). Classification and origin of the powdery mildew of baby's-breath (*Gypsophila paniculata* L.) inferred from the DNA sequence of the rDNA ITS region. Ann. Phytopathol. Soc. Jpn. 66 : 101-102 (Abstr. in Japanese).
22. Takamatsu, S., Shin, H.-D., Paksiri, U., Limkaisang, S., Taguchi, Y., Thi Binh, N. and Sato, Y. (2002). Two *Erysiphe* species associated with recent outbreak of soybean powdery mildew: consequence from molecular phylogenetic analysis based on nuclear rDNA sequences. Mycoscience 43 (in press).
23. Takamatsu, S., Limkaisang, S., Kom-un, S., Nishimura, T., Daido, T. and Ito, S. (2002). Powdery mildews of *Rhododendron* sp. and *Acacia* spp., New record in Japan. Ann. Phytopathol. Soc. Jpn. 68 : 78 (Abstr. in Japanese).

24. Tamura, K. and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10 : 512-526.
25. Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10 : 506-513.
26. Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. J. Mol. Evol. 39 : 306-314.

FIGURE LEGENDS

Fig. 1. A maximum parsimonious tree based on ITS data for 47 taxa of *Oidium* subgenus *Pseudoidium*. This tree is also the maximum likelihood tree among 24 trees obtained by the maximum-parsimony analysis. Percentage bootstrap support (1000 replications) is shown above nodes. The consistency index (CI) is 0.591; retention index (RI) is 0.754; and rescaled consistency index (RC) is 0.446. Solid circle indicates isolate from legume plant.

EXPLANATION OF PLATE

Plate I

A. Powdery mildew mycelia on the peduncle of prairie gentian.

B. Mycelia, conidophores and conidia produced on prairie gentian.

C. Conidia produced singly on conidiophores (*Pseudoidium* type) (bar = 20 µm).

D. A germ tube from conidium terminating in multilobed appressoria (*polygoni*-type) (bar = 20 µm).

E. A lobed appressorium formed on hypha (bar = 10 µm).

F. Mycelia, conidophores and conidia produced on prairie gentian inoculated with a powdery mildew isolate from garden four-o'clock.

G. Mycelia, conidophores and conidia produced on broad bean inoculated with a powdery mildew isolate from garden four-o'clock.

Table 1. Fungal materials and sequence database accession numbers used for phylogenetic analysis.

Fungus	Host plant	Isolate[a)]	Country of origin	Database accession no.[b)]
Erysiphe aquilegiae var. *ranunculi*	*Cimicifuga simplex*	TPU-495	Japan	AB000944
E. aquilegiae var. *ranunculi*	*Clematis terniflora*	MUMH98	Japan	AB015929
E. aquilegiae var. *ranunculi*	*C. integrifolia*	VPRI 21046	Switzerland	AF154322
E. baeumleri	*Vicia amoena*	YNMH12360-12	Japan	AB015933
E. baeumleri	*V. cracca*	YNMH12852-5	Japan	AB015920
E. betae	*Beta vulgaris*	UC1512312	USA	AF011290
E. blasti	*Lindera umbellata*	MUMH2	Japan	AB015918
E. convolvuli	*Convolvulus arvensis*	UC1512307	USA	AF011298
E. convolvuli	*C. arvensis*	VPRI20227	Switzerland	AF154327
E. cruciferarum	*Arabidopsis thaliana*	UEA1	USA	AF031283
E. friesii var. *dahurica*	*Rhamnus japonica* var. *decipiens*	MUMH6	Japan	AB000939
E. glycines	*Desmodium podocarpum* subsp. *oxyphyllum*	MUMH52	Japan	AB015927
E. glycines	*Amphicarpaea bractaeta* subsp. *edgeworthii* var. *japonica*	MUMH56	Japan	AB015934
E. lespedezae	*Lespedeza juncea* var. *subsessilis*	TPU-1762	Japan	AB015921
E. lespedezae	*L. thunbergii*	TPU-1761	Japan	AB015923
E. helwingiae	*Helwingia japonica*	MUMH110	Japan	AB015916
E. heraclei	*Daucus carota*	MUMH73	Japan	AB000942
E. howeana	*Oenothera biennis*	UC15123012	USA	AF011301
E. huayinensis	*Rabdosia longituba*	MUMH30	Japan	AB015914
E. hypophylla	*Quercus robur*	VPRI22120	Japan	AF298544
E. juglandis	*Pterocarya rhoifolia*	TPU-1745	Japan	AB015928
E. katumotoi	*Ligustrum obtusifolium*	MUMH14	Japan	AB015917
E. liriodendri	*Liriodendron tulipifera*	UC1512306	USA	AF011302
E. macleayae	*Macleaya cordata*	TPU-1873	Japan	AB016048
E. magnifica	*Magnolia liliflora*	UC1512303	USA	AF011312
E. pisi	*Lathyrus latifolius*	UC1512315	USA	AF011306

E. pisi	*Pisum sativum*	VPRI19688	Australia	AF073348
E. polygoni	*Rumex crispus*	UC1512308	USA	AF011308
E. polygoni	*Polygonum arenastrum*	UC1512295	USA	AF011307
E. pseudolonicerae	*Cocculus trilobus*	MUMH86	Japan	AB015915
E. pulchra var. *japonica*	*Swida controversa*	MUMH90	Japan	AB000941
E. pulchra var. *japonica*	*S. controversa*	YNMH12992-4	Japan	AB015924
E. pulchra var. *pulchra*	*Benthamidia japonica*	TPU-1731	Japan	AB015935
E. sinensis	*Castanea crenata*	VPRI20272	Korea	AF298545
E. sparsa	*Viburnum opulus*	VPRI22168	Switzerland	AF298541
E. staphyleae	*Staphylea bumalda*	MUMH16	Japan	AB015922
E. syringae-japonicae	*Syringa vulgaris*	TPU-1549	Japan	AB015920
E. trifolii var. *trifolii*	*Trifolium pratense*	TPU-1546	Japan	AB015913
E. vanbruntiana var. *sambuci-racemosae*	*Sambucus racemosa* subsp. *sieboldiana*	MUMH17	Japan	AB015925
E. wallrothii	*Vaccinium hirtum* var. *pubescens*	MUMH56	Japan	AB015934
E. weigelae	*Weigela hortensis*	TPU-1669	Japan	AB015931
E. weigelae	*W. hortensis*	MUMH28	Japan	AB015932
Oidium sp.	*Convolvulus erubescens*	VPRI20708	Australia	AF154328
Oidium sp.	*Glycine max*	MUMH791	Japan	AB078800
Oidium sp.	*Eustoma grandiflorum*	DNA565 [c)]	Japan	AB079855[d)]
Oidium sp.	*Mirabilis jalapa*	MUMH85	Japan	AB079853[d)]
Oidium sp.	*Vicia faba*	MUMH837	Japan	AB079854[d)]

[a)] MUMH = Mie University Mycological Herbarium, Japan; TPU = Herbarium of Toyama Prefectural University, Japan; YNMH = Yukihiko Nomura Mycological Herbarium, Japan; VPRI = Plant Disease Herbarium, Institute for Horticultural Development, Victoria, Australia; UC = University of California Herbarium, USA; UEA = University of East Anglia, UK.

[b)] DDBJ, EMBL, and GenBank database accession number of the nucleotide sequence data.

[c)] Isolate preserved as extracted DNA.

[d)] Sequence determined in this study.

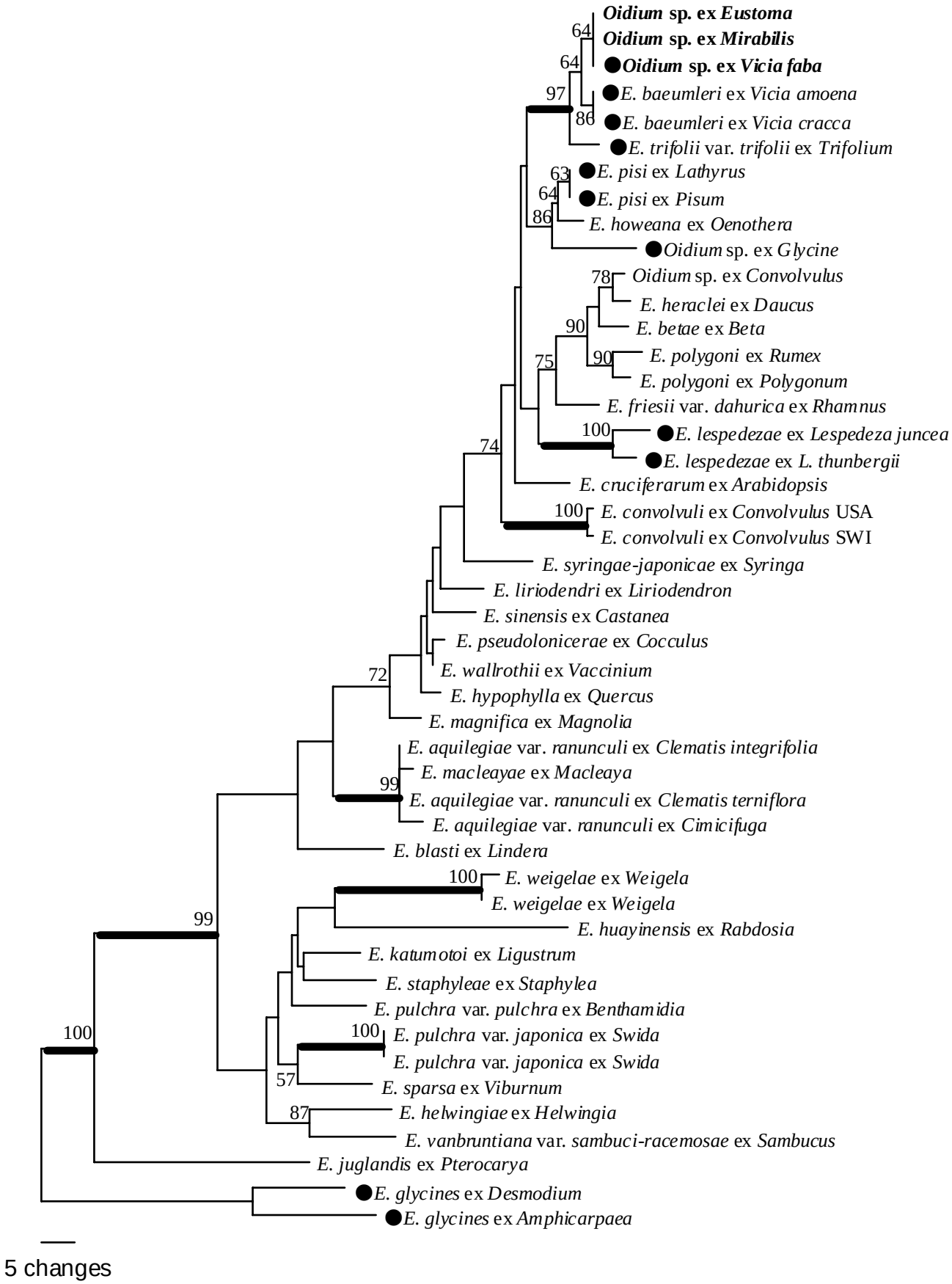
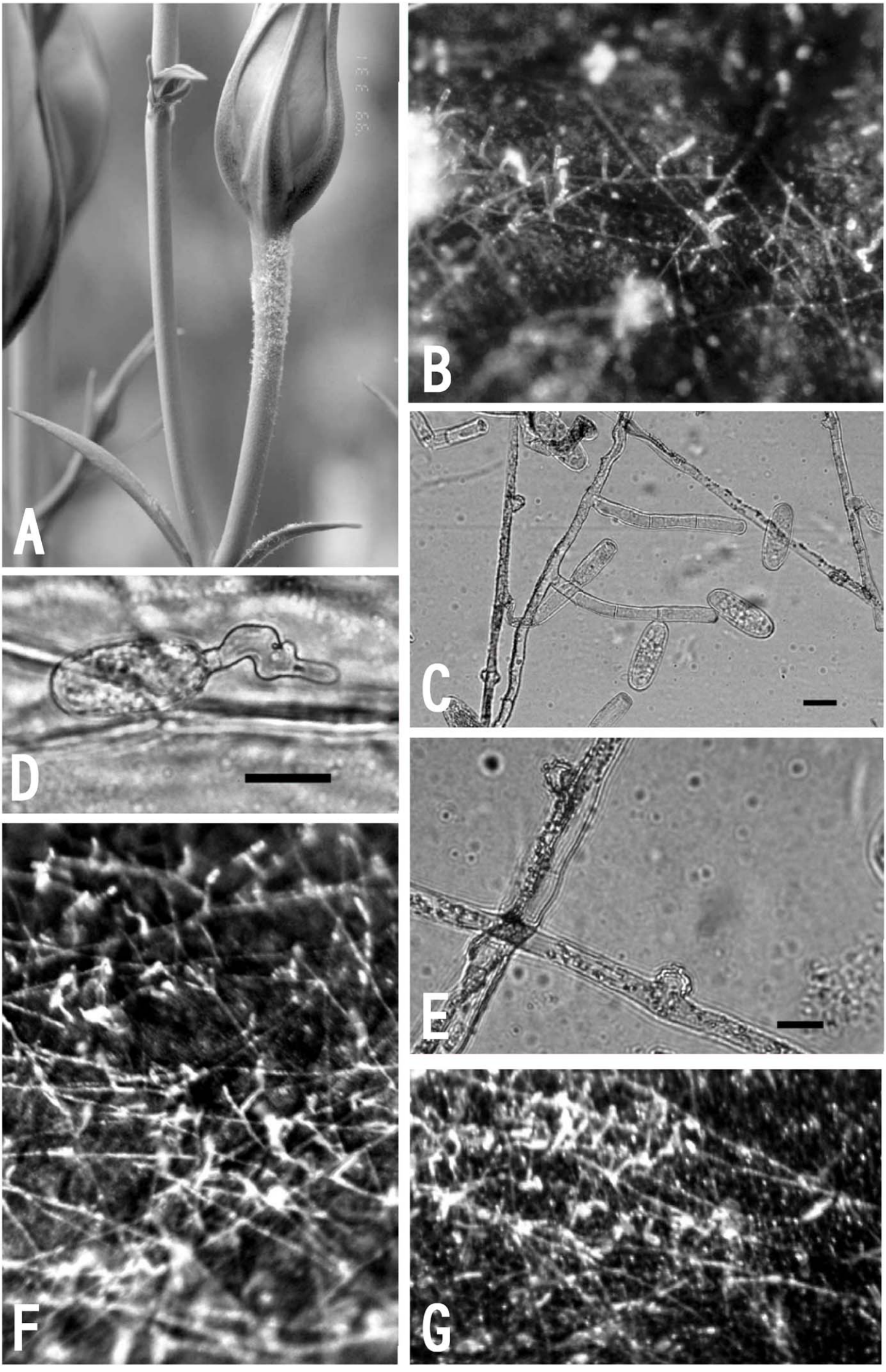

Oidium sp. ex Eustoma
Oidium sp. ex Mirabilis
Oidium sp. ex Vicia faba
E. baeumleri ex Vicia amoena
E. baeumleri ex Vicia cracca
E. trifolii var. trifolii ex Trifolium
E. pisi ex Lathyrus
E. pisi ex Pisum
E. howeana ex Oenothera
Oidium sp. ex Glycine
Oidium sp. ex Convolvulus
E. heraclei ex Daucus
E. betae ex Beta
E. polygoni ex Rumex
E. polygoni ex Polygonum
E. friesii var. dahurica ex Rhamnus
E. lespedezae ex Lespedeza juncea
E. lespedezae ex L. thunbergii
E. cruciferarum ex Arabidopsis
E. convolvuli ex Convolvulus USA
E. convolvuli ex Convolvulus SWI
E. syringae-japonicae ex Syringa
E. liriodendri ex Liriodendron
E. sinensis ex Castanea
E. pseudolonicerae ex Cocculus
E. wallrothii ex Vaccinium
E. hypophylla ex Quercus
E. magnifica ex Magnolia
E. aquilegiae var. ranunculi ex Clematis integrifolia
E. macleayae ex Macleaya
E. aquilegiae var. ranunculi ex Clematis terniflora
E. aquilegiae var. ranunculi ex Cimicifuga
E. blasti ex Lindera
E. weigelae ex Weigela
E. weigelae ex Weigela
E. huayinensis ex Rabdosia
E. katumotoi ex Ligustrum
E. staphyleae ex Staphylea
E. pulchra var. pulchra ex Benthamidia
E. pulchra var. japonica ex Swida
E. pulchra var. japonica ex Swida
E. sparsa ex Viburnum
E. helwingiae ex Helwingia
E. vanbruntiana var. sambuci-racemosae ex Sambucus
E. juglandis ex Pterocarya
E. glycines ex Desmodium
E. glycines ex Amphicarpaea
64
64
97
86
63
64
86
78
90
90
75
100
74
100
72
99
100
99
100
57
87
5 changes

Fig. 1


A
B
C
D
E
F
G