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Two *Erysiphe* species associated with recent outbreak of soybean powdery mildew: results of molecular phylogenetic analysis based on nuclear rDNA sequences

Abstract Serious outbreaks of powdery mildew by a fungus belonging to the mitosporic genus *Oidium* subgenus *Pseudoidium* have been reported on soybean (*Glycine max*) in a wide area of eastern Asia since 1998. The taxonomic and phylogenetic placement of the causal fungus has not yet been determined because of lack of the perfect stage. We found ascomata having mycelioid appendages on a single leaf of soybean infested by powdery mildew. Molecular phylogenetic analysis was conducted based on a total of 14 sequences of the rDNA internal transcribed spacer (ITS) region from 13 soybean and wild soybean (*Glycine soja*) materials collected in Japan, Korea, Vietnam, and the United States, combined with 47 sequence data obtained from the DNA databases. It was revealed that two *Erysiphe* species were associated with the outbreak of soybean powdery mildew. There was 16% difference between the two species in genetic divergence of the ITS sequence. One species with perfect stage has an ITS sequence identical to that of *Erysiphe glycines* on *Amphicarpaea* and is identified as *Erysiphe glycines* based on the ITS sequence and morphology of ascomata. The second species, without the perfect stage, is likely to be *Erysiphe diffusa* (= *Microsphaera diffusa*), known as the fungus causing soybean powdery mildew in the United States, because the ITS sequences are identical to those from materials collected in the United States. However, we need materials having ascomata of *E. diffusa* to confirm the species name.

Key Words Erysiphaceae · *Glycine max* · ITS · *Microsphaera* · *Oidium* subgenus *Pseudoidium*

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Introduction

Sawada et al. (1982) recorded the first occurrence of powdery mildew of soybean (*Glycine max* (L.) Merr.) in Japan in 1980, and identified the causal fungus as *Erysiphe pisi* DC. on the basis of its anamorphic and teleomorphic characters. After that occurrence, there was no report of the disease for 17 years in this country until a second outbreak occurred in Oita Prefecture, Kyushu Island, in 1998 (Hasama and Kato 2000). The disease has then expanded its distribution to a wide area of Kyushu and Honshu Islands within the past 4 years. Moreover, outbreak of the disease was first recorded in Korea in 1998 (Shin 2000) and in Vietnam in 2000 (N. Xuan Hong, personal communication). Morphological observation of anamorphic characters revealed that the causal fungus belongs to the mitosporic genus *Oidium* subgenus *Pseudoidium* (Cook et al. 1997). The detailed taxonomic and phylogenetic position of the fungus, however, is still unknown because the perfect stage is lacking.

Soybean powdery mildew caused by the fungus having the *Pseudoidium* anamorph, which is characterized by conidiophores producing conidia in solitary and lobed appressoria, was first reported in 1931 in the United States (Lehman 1931). The disease has since been reported in many countries of North and South America (Brasil, Canada, Peru, Puerto Rico, Venezuela, and the United States) (Amano 1986). Serious outbreaks of the disease were reported in southeastern and midwestern United States in 1970s (Arny et al. 1975; Dunleavy 1976; Leath and Carroll 1982). The causal fungus is known as *Erysiphe*

diffusa (Cooke & Peck) U. Braun & S. Takamatsu (syn. *Microsphaera diffusa* Cooke & Peck) (Paxton and Rogers 1974; McLaughlin et al. 1977). *Erysiphe diffusa* has characteristic dichotomously branched appendages on the ascomata, conspicuously differing from the ascomata of *E. pisi*, which has mycelioid appendages, although the two *Erysiphe* species share the *Pseudoidium* anamorph.

Our main questions in this study are (1) is a single causal fungus associated with the recent outbreak of this disease in a wide area of eastern Asia? (2) if so, is the fungus *E. diffusa* or *E. pisi*? and (3) how and why did the fungus cause outbreaks on soybean in recent years? To address these questions, we collected powdery mildew specimens on soybean and wild soybean (*G. soja* Sieb. & Zucc.) in Japan, Korea, Vietnam, and the United States, and conducted morphological and phylogenetic analyses of these specimens. During the study, we found formation of ascomata having mycelioid appendages on an infested soybean leaf. Analysis of the specimen revealed simultaneous occurrence of two *Erysiphe* species on a single individual of soybean.

Materials and methods

Sample sources

Ten powdery mildew isolates from soybean collected in Japan, Korea, Vietnam, and the United States, and two isolates from wild soybean collected in Japan and Korea were used in this study. Their designation, host plants, locations of collection, and accession numbers of the nucleotide sequence databases (DDBJ, EMBL, and GenBank) are given in Table 1.

Light microscopy

Hyphae, conidiophores, and conidia of fresh materials were stripped off the leaf surfaces with clear adhesive tape, mounted on a microscope slide with the fungal materials uppermost, and examined in water under a compound microscope. Herbarium materials were rehydrated before examination by boiling a small piece of infected leaf, with the fungal mycelium downward, in a drop of lactic acid on a slide as described by Shin and La (1993) and Shin (2000). After boiling, the rehydrated mycelium was scraped off the leaf and mounted in either lactic acid or cotton blue in lactic acid for light microscopy. The following information was noted during the examination: size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of the conidiophore, such as the size and shape of the foot cell and position of the basal septum; shape and position of hyphal appressoria; position of germ tubes of conidia, when found; and shape of appressoria on germ tubes of conidia.

Scanning electron microscopy

Leaf specimens were prefixed in 2.5% glutaraldehyde and washed in 0.2 M Milonig's phosphate buffer. They were fixed in 1% osmium tetroxide (OsO_4) for 90 min and washed in deionized distilled water (DDW) three times. After dehydration through a graded ethyl alcohol series, they were treated with amylacetate. Subsequently, they were dried in a critical-point dryer, coated with gold, and finally observed by a scanning electron microscope (LEO 440, LEO Electron Microscopy, Cambridge, UK).

Table 1. Isolate, location, year of collection, and database accession number of the rDNA internal transcribed spacer (ITS) sequences of soybean and wild soybean powdery mildew used in this study

Isolate no.[a]	Location of collection	Year of collection	Database accession no.[b]
Glycine max (soybean)			
MUMH791	Oita, Japan	1999	AB078800
MUMH789	Kumamoto, Japan	1999	AB078801
MUMH793	Fukushima, Japan	1999	AB078802
MUMH878	Nara, Japan	1999	AB078803
MUMH1452	Shizuoka, Japan	2000	AB078804
MUMH1453	Okayama, Japan	2000	AB078805
MUMH1462 (conidia)	Mie, Japan	2001	AB078806
MUMH1462 (ascomata)	Mie, Japan	2001	AB078807
SMK17078	Chongju, Korea	1999	AB078808
MUMH1451	Hanoi, Vietnam	2000	AB078809
MUMH1463	Minnesota, USA	1999	AB078810
MUMH1464	Illinois, USA	1999	AB078811
Glycine soja (wild soybean)			
SMK15414	Chunchon, Korea	1998	AB078812
MUMH1162	Gifu, Japan	2000	AB078813

[a] Herbarium specimens at the following herbaria: MUMH, Mie University Mycological Herbarium, Japan; SMK, Mycological Herbarium, Department of Agricultural Biology, Korea University
[b] The nucleotide sequence data appear in the DDBJ, EMBL, and GenBank Database under the respective accession number

DNA extraction, polymerase chain reaction, and sequencing

Whole-cell DNA was isolated from mycelia by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). The nuclear rDNA region, including the internal transcribed spacer (ITS) regions (ITS 1 and ITS 2), and the 5.8S rRNA gene were amplified by polymerase chain reaction (PCR) using the primers ITS 5 (White et al. 1990) and P3 (Kusaba and Tsuge 1995). PCR reactions were performed in 50 μl volumes as previously described (Hirata and Takamatsu 1996). A negative control lacking template DNA was included for each set of reactions. One microliter of the first reaction mixture was used for the second amplification with the partial nested primer set ITS 1 (White et al. 1990) and P3. The PCR product was subjected to preparative electrophoresis in 1.5% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The DNA product of each amplification was then excised from the ethidium-stained gel and purified using the JETSORB kit (Genomed, Oeynhausen, Germany) following the manufacturer's protocol. Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in an Applied Biosystems 373A sequencer (Applied Biosystems, Foster City, CA, USA). The sequence reactions were conducted using the PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems) following the manufacturer's instructions.

Molecular phylogenetic analysis

The sequences were initially aligned using the Clustal V package (Higgins et al. 1992). The alignment was then visually refined with a word processing program, using color-coded nucleotides. The alignment was deposited in TreeBASE (http://www.herbaria.harvard.edu/treebase) as S746. Phylogenetic trees were obtained from the data using distance and parsimony methods. For distance analysis, the most appropriate evolution model was determined for a given data set using PAUP* 4.0b8 (Swofford 2001) and Modeltest 3.06 (Posada and Crandall 1998). 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 by likelihood ratio test. Once a model of evolution was chosen, it was used to construct phylogenetic trees with the minimum-evolution (ME) method by a heuristic search option of 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. All sites were treated as unordered and unweighted, 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 using 1000 replications (Felsenstein 1985) and by decay analysis (Bremer 1988; Donoghue et al. 1992).

Results

Morphology of anamorph

Irregular white patches are formed on the upper and lower sides of leaves, stems, and pods that finally cover the whole leaf surface and young stems. Hyphae are substraight to flexuous, branching at a right or narrow angle, with a septum near the branching point (Figs. 1, 2). Appressoria on hyphae are formed opposite in pairs or singly, and multi-lobed to moderately lobed (Fig. 2). Conidiophores are formed single or occasionally by twos on a hyphal cell, arising from the upper part of mother cells, 45–95 × 7–9 μm in size, straight or occasionally slightly flexuous at the base of foot cells, producing conidia singly followed by 1–3 cells, with a basal septum at the branching point of the mycelium (Fig. 1). Conidia are oval, ellipsoid, or cylindrical without conspicuous fibrosin bodies, 25–38(−44) × 13–18(−21) μm in size, producing *polygoni*-type germ tubes (Braun 1987) on the shoulder (Fig. 3). These characters, all in good agreement among all specimens observed, indicate that this fungus belongs to the mitosporic genus *Oidium* subgenus *Pseudoidium*.

Discovery and morphology of perfect stage

In November 2001, we found ascomata on a part of soybean leaf covered with whitish powdery mildew colonies at a soybean field of Mie University. Several mature dark brown and many immature yellowish ascomata were embedded in colonies forming aerial hyphae without conidial formation (Fig. 4). Matured ascomata are depressed globose, dark brown, 125–130 μm in diameter (Fig. 5). Appendages are mycelioid, subhyaline, and thin-walled throughout, interwoven with each other or with mycelia. Asci are 5–6 in an ascoma, 47–68 × 25–39 μm in size with short stalks (Fig. 6). Ascospores are 5–7 in an ascus, oval, 16–23 × 9–14 μm in size. These characters indicate that this fungus belongs to *Erysiphe* section *Erysiphe*.

To obtain more specimens with ascomata, we kept some individual soybean plants in the field and observed these for ascomata formation twice a month until late January 2002. Unfortunately, we could not find further formation of ascomata. Most colonies continued to produce conidia vigorously until mid-December 2001. This observation suggested to us that the colonies with ascomata are derived from a species different from that producing only conidia. To evaluate this possibility, we then carried out molecular analysis of the material with other powdery mildew materials of soybean collected in eastern Asia and the United States.

Figs. 1–3. Scanning electron micrographs of the anamorph of soybean powdery mildew fungus. **1** Conidiophores arising from the upper part of mother cells, straight or occasionally flexuous at the base of foot cells, producing conidia singly followed by 1–3 cells. **2** Appressoria on hyphae, formed opposite in pairs, multilobed to moderately lobed. **3** Conidia of oval, ellipsoid, and cylindrical shape, producing *polygoni*-type germ tubes. *Bars* **1–3** 20 μm
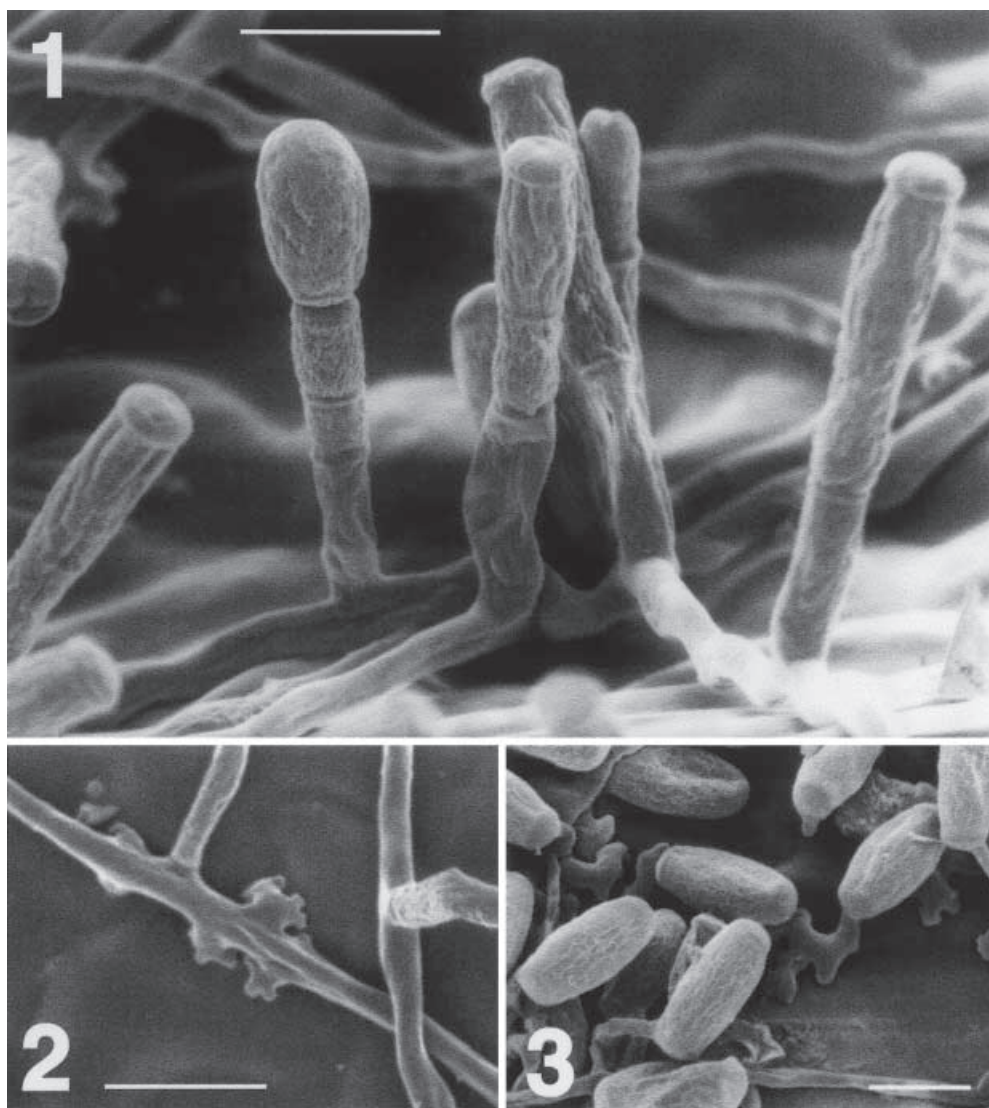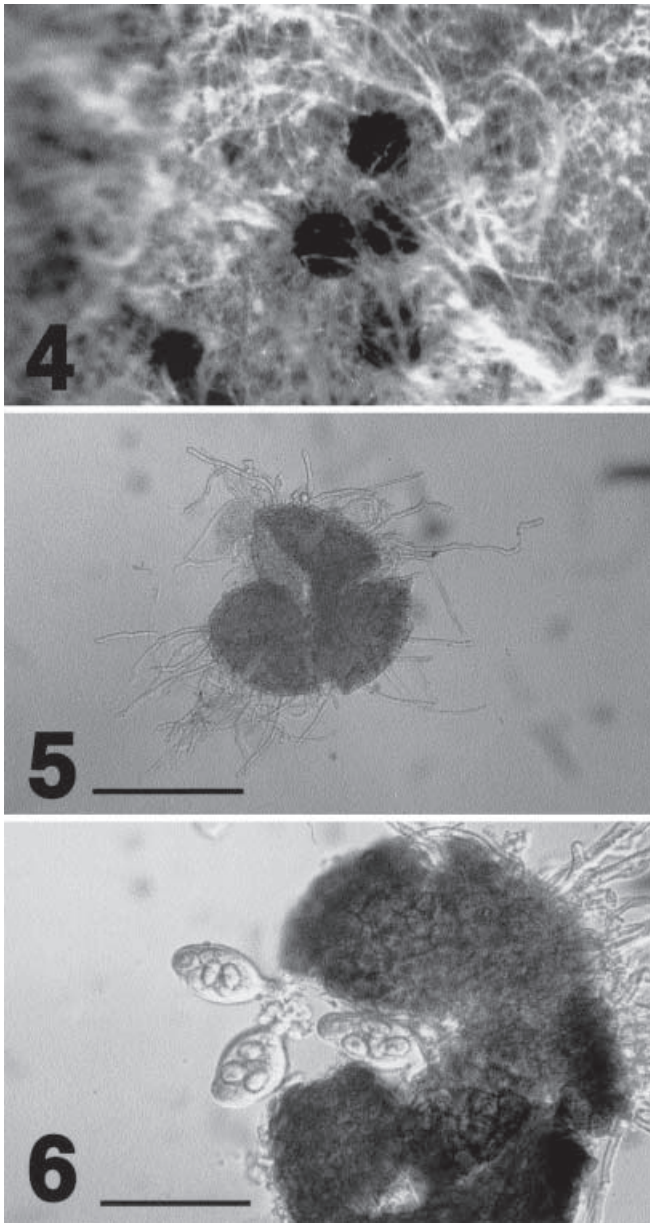

Molecular phylogenetic analysis

A total of 14 rDNA ITS sequences of powdery mildews were determined in this study, of which 12 are from soybean and 2 from wild soybean. In the material collected in Mie Prefecture, DNAs were separately extracted from colonies producing conidia or ascomata. The data of the fungi sequenced in this study and their accession numbers are listed in Table 1. Of the 14 ITS sequences, 13 sequences, extracted from conidia-producing colonies, are identical to each other, having 559 nucleotides in length in total of ITS 1, ITS 2, and 5.8S rRNA regions. The DNA extracted from the ascomata-forming colony has an ITS sequence different from other sequences, 566 nucleotides in length. Identity between the former and latter sequences is 84%.

These sequences were aligned with the sequences of *Erysiphe* sections *Erysiphe* and *Microsphaera* that were obtained from DNA databases (Table 2). The alignment data matrix consisted of 61 taxa. *Erysiphe nishidana* (Homma) U. Braun & S. Takamatsu (syn. *Uncinula*

nishidana Homma) from *Firmiana simplex* (L.) W.F. Wight was used as the outgroup taxon based on our unpublished data. Of the 605 aligned characters, 220 sites are variable and 159 sites are phylogenetically informative for parsimony analysis. Using Modeltest (Posada and Crandall 1998) under the likelihood ratio test criterion, we concluded that the Tamura–Nei model (Tamura and Nei 1993), with equal base frequencies, a gamma-distributed rate heterogeneity model (four rate categories, $G = 0.9458$; Yang 1994), and an estimated proportion of invariant sites (0.4620) was the most appropriate model of evolution for this data set. A heuristic search with this model produced a ME tree with a ME score of 1.11 (Fig. 7). MP analysis found 63 equally parsimonious trees of 561 steps (CI = 0.553, RI = 0.796, RC = 0.440). Strict consensus of the MP trees is shown in Fig. 8.

The 13 powdery mildew sequences from conidia-producing colonies are situated at the most derived position in both ME and MP analyses. They form a clade with *E. pisi* and *E. howeana* U. Braun with high bootstrap supports (89% in ME and 86% in MP) and decay index (3). The

MP trees. Although they form a clade with *E. glycines* from *Desmodium podocarpum* DC. subsp. *oxyphyllum* (DC.) Ohashi with high bootstrap support (100%), the genetic distance between the isolates from soybean and *Desmodium* is relatively high (7.8%).

Discussion

Of the 14 ITS sequences of soybean and wild soybean powdery mildews determined in this study, 13 sequences extracted from conidia-producing colonies are identical to each other. The remaining 1 sequence extracted from the ascomata-forming colony is distantly related to these 13 sequences (16% in genetic distance) and is identical to the sequences of *E. glycines* from *Amphicarpaea*. Based on the morphological characters of the ascomata and the sequence results, we identify the latter isolate as *E. glycines*. Because *E. glycines* belongs to *Oidium* subgenus *Pseudoidium* in its anamorph, this result clearly indicates that two different powdery mildew species sharing the *Pseudoidium* anamorph are associated with this widespread outbreak of soybean powdery mildew.

Because ascomata formation seems to be rare on soybean, it is possible that *E. glycines* is not the original pathogen of soybean and incidentally infects the plant. However, formation of ascomata having mycelioid appendages was also observed in Okayama Prefecture, Japan, in 2000 (Y. Nomura and K. Fujioka, personal communication). Sawada et al. (1982) reported a serious outbreak of soybean powdery mildew in Tokyo and identified the causal fungus as *E. pisi sensu* Homma (1937). We cannot directly compare the *E. pisi* of Sawada et al. (1982) with our isolate because their specimen is not available. Sawada described that his soybean isolate was very similar to the isolate from *Amphicarpaea* in both teleomorphic and anamorphic characters (Sawada 1984). Because *E. glycines* is included in *E. pisi sensu* Homma (1937), the causal fungus of the outbreak in Tokyo is likely to be *E. glycines*. These data suggest that *E. glycines* is an original pathogen of soybean powdery mildew.

The identical ITS sequence between isolates from soybean and *Amphicarpaea* suggests that the pathogen came from *Amphicarpaea*, although we must conduct an inoculation test to confirm this idea. *Erysiphe glycines* of *Amphicarpaea* is commonly found in the understory of forests in Japan. Conidia of *E. glycines* may easily arrive at a soybean field and infect the soybean if the soybean cultivars are susceptible to the fungus. The question as to why powdery mildew has not been common on soybean until this recent outbreak, however, still remains to be answered. Because many reports (Demski and Phillips 1974; Grau and Laurence 1975; Dunleavy 1976; Mignucci and Chamberlain 1978; Mignucci and Lim 1980; Lohnes and Nickell 1994; Hasama et al. 2000) indicate that the soybean powdery mildew is cultivar specific, the question might be addressed by difference of susceptibility of soybean cultivars to the fungus.

Figs. 4–6. Teleomorph (ascomata) of soybean powdery mildew fungus found in Mie Prefecture, Japan. **4** Mature dark brown ascomata embedded in colonies forming aerial hyphae. **5** An ascoma having mycelioid appendages. **6** Asci and ascospores. *Bars* **5** 100 μm; **6** 50 μm

genetic distance is 2.4% between soybean fungi and *E. pisi*, and 2.9% between soybean fungi and *E. howeana*. This clade weakly clusters with other legume-parasitic species, *E. lespedezae* Zheng & U. Braun, *E. baeumleri* Magn., and *E. trifolii* Grev. var. *trifolii* in the ME tree (less than 50% in bootstap value), whereas *E. lespedezae* is situated out of the group in the MP tree.

The sequence of DNA extracted from the ascomata-forming colony is identical to that of *E. glycines* Tai from *Amphicarpaea bractaeta* (L.) Felnald subsp. *edgeworthii* (Benth.) Ohashi var. *japonica* (Oliver) Ohashi, and the fungus is situated at the basal position in both ME and

Table 2. 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. bäeumleri	*Vicia amoena*	YNMH12360-12	Japan	AB015933
E. bäeumleri	*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. cruciferarum	*Brassica rapa*	VPRI20803	Australia	–[c]
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
O. mangiferae	*Mangifera* sp.	VPRI20364	Australia	–[c]

[a] MUMH, Mie University Mycological Herbarium; TPU, Herbarium of Toyama Prefectural University; YNMH, Yukihiko Nomura Mycological Herbarium; VPRI, Plant Disease Herbarium, Institute for Horticultural Development, Victoria, Australia; UC, University of California Herbarium
[b] DDBJ, EMBL, and GenBank database accession number of the nucleotide sequence data
[c] Unpublished sequence provided by J. Cunnington

The 13 anamorphic specimens have ITS sequences identical to each other and similar anamorphic characters. Because this fungus is commonly isolated from every place of outbreak of soybean powdery mildew, this may be the major causal fungus of the recent outbreak of the disease. Molecular phylogenetic analysis indicates that pea powdery mildew, *E. pisi*, is the closest relative of this fungus. However, there is 2.4% genetic distance between the soybean fungus and *E. pisi*. We further compared the sequence of the soybean powdery mildew with 143 ITS sequences of fungi belonging to *Erysiphe* sections *Erysiphe* and *Microsphaera*, including 30 sequences from legume-parasitic species (unpublished data). There is no sequence identical to the sequence of soybean fungus, which suggests that the fungus is unique to soybean and wild soybean, and was introduced from outside Japan.

Fig. 7. A minimum-evolution (ME) tree based on internal transcribed spacer (ITS) data for 14 isolates from soybean and wild soybean, 39 taxa of *Erysiphe* sections *Erysiphe* and *Microsphaera*, and 1 outgroup taxon. Model parameters: equal base frequencies with rate heterogeneity; gamma shape parameter = 0.9458; proportion of invariable sites = 0.4620; six rate categories; Tamura–Nei model (Tamura and Nei 1993) with transformation parameters [A–C] = 1.0000, [A–G] = 2.8140, [A–T] = 1.0000, [C–G] = 1.0000, [C–T] = 3.9310, and [G–T] = 1.0000. Percent bootstrap support (1000 replications) is indicated above or below nodes. *Solid circles* indicate isolate from legume plant
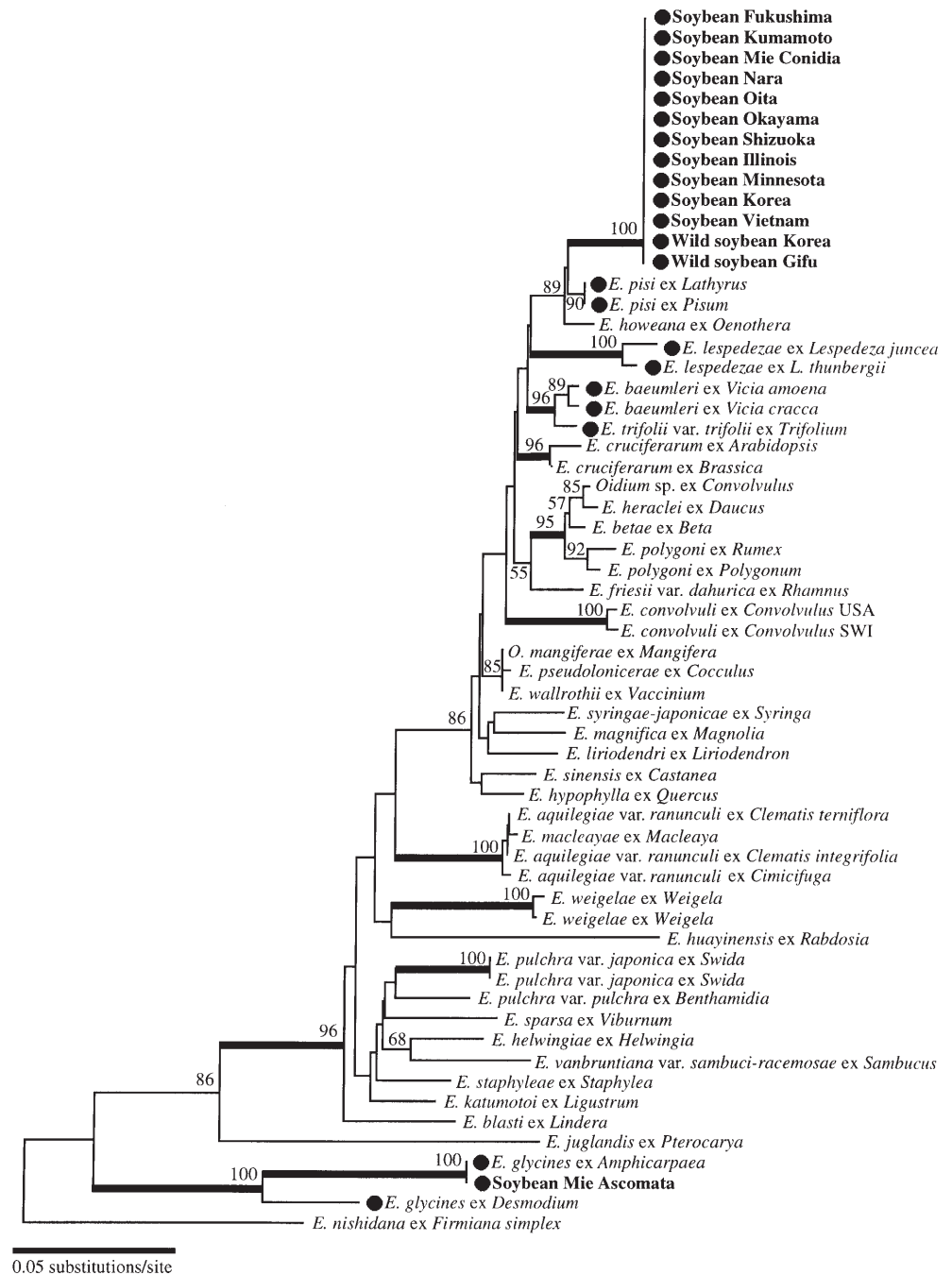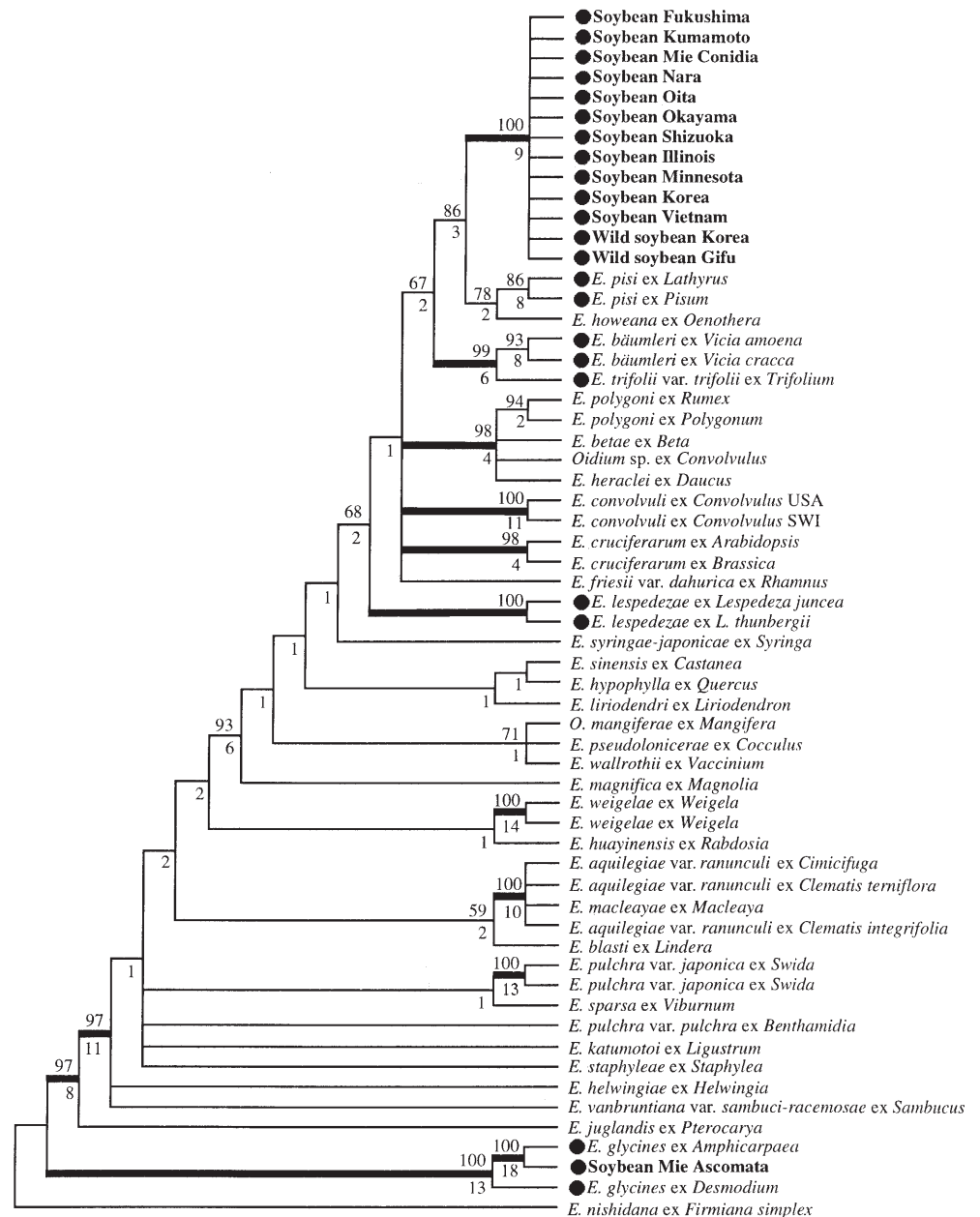
The causal fungus of soybean powdery mildew is known as *Erysiphe* (*Microsphaera*) *diffusa* in the United States (McLaughlin et al. 1977). *Erysiphe diffusa* has dichotomously branched appendages on its ascomata, which is conspicuously different from *E. glycines*, which has mycelioid appendages (Braun 1987). Powdery mildews having dichotomously branched appendages have long been classified as the genus *Microsphaera* and those having mycelioid appendages as the genus *Erysiphe*. However, *Microsphaera* and *Erysiphe* (section *Erysiphe*) share the *Pseudoidium* anamorph and cannot be differentiated from each other by anamorphic characters. Molecular phylogenetic analyses (Takamatsu et al. 1999) have indicated that *Microsphaera*

and *Erysiphe* (section *Erysiphe*) are grouped together in a clade and cannot be separated from one another in phylogeny. Braun and Takamatsu (2000) proposed to combine the genera *Erysiphe* (section *Erysiphe*), *Microsphaera*, and *Uncinula* into the single genus *Erysiphe* based on molecular phylogenetic studies (Takamatsu et al. 1999; Saenz and Taylor 1999; Mori et al. 2000) and scanning electron microscopy of conidia (Cook et al. 1997).

It is possible that the second species of soybean powdery mildew is *E. diffusa*, based on the following reasons: (1) the ITS sequences of isolates in eastern Asia are identical to the sequences of the two isolates collected in the United States and (2) anamorphic characters of the present fungus

Fig. 8. Strict consensus of 63 equally parsimonious trees based on ITS data for 14 isolates from soybean and wild soybean, 39 taxa of *Erysiphe* sections *Erysiphe* and *Microsphaera*, and 1 outgroup taxon. Percent bootstrap support (1000 replications) and decay indices are shown above and below nodes, respectively. The consistency index (CI) is 0.553, the retention index (RI) is 0.796, and the rescaled consistency index (RC) is 0.440. *Solid circles* indicate isolate from legume plant

● Soybean Fukushima
● Soybean Kumamoto
● Soybean Mie Conidia
● Soybean Nara
● Soybean Oita
● Soybean Okayama
● Soybean Shizuoka
● Soybean Illinois
● Soybean Minnesota
● Soybean Korea
● Soybean Vietnam
● Wild soybean Korea
● Wild soybean Gifu
● *E. pisi* ex *Lathyrus*
● *E. pisi* ex *Pisum*
E. howeana ex *Oenothera*
● *E. bäumleri* ex *Vicia amoena*
● *E. bäumleri* ex *Vicia cracca*
● *E. trifolii* var. *trifolii* ex *Trifolium*
E. polygoni ex *Rumex*
E. polygoni ex *Polygonum*
E. betae ex *Beta*
Oidium sp. ex *Convolvulus*
E. heraclei ex *Daucus*
E. convolvuli ex *Convolvulus* USA
E. convolvuli ex *Convolvulus* SWI
E. cruciferarum ex *Arabidopsis*
E. cruciferarum ex *Brassica*
E. friesii var. *dahurica* ex *Rhamnus*
● *E. lespedezae* ex *Lespedeza juncea*
● *E. lespedezae* ex *L. thunbergii*
E. syringae-japonicae ex *Syringa*
E. sinensis ex *Castanea*
E. hypophylla ex *Quercus*
E. liriodendri ex *Liriodendron*
O. mangiferae ex *Mangifera*
E. pseudolonicerae ex *Cocculus*
E. wallrothii ex *Vaccinium*
E. magnifica ex *Magnolia*
E. weigelae ex *Weigela*
E. weigelae ex *Weigela*
E. huayinensis ex *Rabdosia*
E. aquilegiae var. *ranunculi* ex *Cimicifuga*
E. aquilegiae var. *ranunculi* ex *Clematis terniflora*
E. macleayae ex *Macleaya*
E. aquilegiae var. *ranunculi* ex *Clematis integrifolia*
E. blasti ex *Lindera*
E. pulchra var. *japonica* ex *Swida*
E. pulchra var. *japonica* ex *Swida*
E. sparsa ex *Viburnum*
E. pulchra var. *pulchra* ex *Benthamidia*
E. katumotoi ex *Ligustrum*
E. staphyleae ex *Staphylea*
E. helwingiae ex *Helwingia*
E. vanbruntiana var. *sambuci-racemosae* ex *Sambucus*
E. juglandis ex *Pterocarya*
● *E. glycines* ex *Amphicarpaea*
● Soybean Mie Ascomata
● *E. glycines* ex *Desmodium*
E. nishidana ex *Firmiana simplex*

(straight or occasionally flexuous conidiophore at the base, and conidial shape and size) are in good agreement with the description of *E. diffusa* (Braun 1987). However, it cannot be confirmed yet because the specimens collected in the United States do not have ascomata. We thus do not know whether these U.S. materials are *E. diffusa*. Materials having ascomata of *E. diffusa* are essential to confirm the species name.

In conclusion, we showed that two *Erysiphe* species are associated with the recent outbreak of soybean powdery mildew in eastern Asia, one species having only the conidial stage and another having a perfect stage. It has been reported that soybean powdery mildew has strict cultivar specificity (Demski and Phillips 1974; Grau and Laurence 1975; Dunleavy 1976; Mignucci and Chamberlain 1978; Mignucci and Lim 1980; Lohnes and Nickell 1994; Hasama

et al. 2000). Because specificity may differ between the fungal species, the causal fungus should be identified to investigate the reaction of soybean cultivars to powdery mildew. The two species can simultaneously infect a single individual or a single leaf of soybean. Separate or simultaneous infection of these two species may cause various different reactions of soybean cultivars. Hasama et al. (2000) investigated reactions of soybean cultivars to powdery mildew in fields in 1998 and 1999, and reported that the results differed significantly between the years. This difference might be attributed in species responding to an outbreak of the disease during the 2 years.

The easiest and most effective method to differentiate the two species of soybean powdery mildews may be molecular techniques using PCR. Because there is 16% sequence divergence between the two species, it will be easy

to design PCR primers to separate these species. Alternatively, differentiation based on anamorphic characters may also be possible, although not easy. Sawada (1984) reported that the branching angle of hyphae is different between *E. pisi* on pea and soybean fungus. Further detailed observation of the fungi might make it possible to differentiate the species based on anamorphic features.

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