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Phylogenetic relationships of *Microsphaera* and *Erysiphe* section *Erysiphe* (powdery mildews) inferred from the rDNA ITS sequences*

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The genus *Microsphaera* has been considered to be derived from section *Erysiphe* of the genus *Erysiphe* by a single event. Cleistothecial appendages are the most distinct difference between the two genera and have an important role for overwintering. To understand the phylogenetic relationship between *Erysiphe* section *Erysiphe* and *Microsphaera* more precisely, phylogenetic trees were constructed using the nucleotide sequences of the rDNA ITS region from 11 *Erysiphe* (section *Erysiphe*) and 16 *Microsphaera* taxa. The phylogenetic trees indicated the close relationship between the two genera. However, the genera *Erysiphe* (section *Erysiphe*) and *Microsphaera* did not group into separate monophyletic lineages; instead, they formed several small clusters that were mixed together. This result suggests that the differentiations of the genera occurred two or more times independently. This also supports the idea that appendage morphology does not always accurately reflect the phylogeny of the powdery mildews because the morphology of appendages may evolve convergently under the selection pressure of their particular biotopes (host plants).

Key Words——*Erysiphe*; internal transcribed spacer; *Microsphaera*; phylogeny; powdery mildew.

The powdery mildew fungi are important plant pathogens which are obligately parasitic on the leaves, stems, fruits, and flowers of a wide range of angiosperm plants. Phylogenetic relationships between the genera of powdery mildews have been discussed based on the morphological characters of the teleomorph and anamorph (Blumer, 1933; Katumoto, 1973; Braun, 1987) as well as their host ranges (Amano, 1986). Blumer (1933) introduced a phylogenetic hypothesis of the powdery mildews, in which the genus *Erysiphe* (type species: *E. polygoni* DC.), which has hypha-like simple appendages on cleistothecia, was regarded as the most primitive genus amongst powdery mildews. Braun (1981) split *Erysiphe* into three sections based on the anamorphic features, i.e., section *Erysiphe* (conidia produced singly and lobed appressoria, Pseudoidium-type), section *Golovinomyces* (conidia produced in chains and nipple-shaped appressoria, Euoidium-type), and section *Galeopsidis* (conidia produced in chains and lobed appressoria, Euoidium-type). Some authors proposed dividing the genus *Erysiphe* into two separate genera because of the distinct anamorphic features (reviewed by Braun, 1987). However, Braun (1987) did not agree with them, because the section *Galeopsidis* was regarded as an intermediate between section *Erysiphe* and section *Golovinomyces*. Recently, Takamatsu et al. (1998) reported that the sections *Erysiphe* and *Golovinomyces* did not form monophyletic clusters in their phylogenetic trees, and that the section *Erysiphe* formed a monophyletic cluster with the genus *Microsphaera* (type species: *M. divaricata* (Wallr.) Lév.) The genus *Microsphaera* has conidia produced singly and lobed appressoria and thus has been considered to be closely related to *Erysiphe* section *Erysiphe* (Blumer, 1933; Braun, 1981). On the other hand, *Microsphaera* has been deemed to be clearly distinguished from the genus *Erysiphe* by the cleistothecial appendages with dichotomously branched tips (Braun, 1981). However, there are several species with appendages having intermediate characters between *Erysiphe* and *Microsphaera*, in which the appendages are sometimes irregularly branched at the apex and have longer, more open tips. These species were placed in section *Trichocladia* of the genus *Microsphaera* by Braun (1981) and have been regarded as intermediate between *Erysiphe* (section *Erysiphe*) and *Microsphaera*. Therefore, the two genera are considered to be a group having continuous morphological changes. Based on these morphological characters, Braun (1987) and Blumer (1933) considered that the genus *Microsphaera* was derived from *Erysiphe* section *Erysiphe*. However, a more objective and precise analysis is still necessary to understand the phylogenetic relationship between *Erysiphe* and *Microsphaera*.

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In this study, the phylogenetic relationship between *Microsphaera* and *Erysiphe* section *Erysiphe* is discussed based on the nucleotide sequences of the internal transcribed spacer (ITS) region including the 5.8S rRNA gene. We excluded *Erysiphe* section *Golovinomyces* from the current analysis because the section was revealed to be distantly related to both *Microsphaera* and *Erysiphe* section *Erysiphe* in our previous study (Takamatsu et al., 1998).

Materials and Methods

Sample sources Powdery mildew species used in this study, their original hosts, and accession numbers of the nucleotide sequence databases (DDBJ, EMBL, and GenBank) are given in Table 1. The data set includes 30 taxa, of which 11 taxa (6 species) belong to *Erysiphe* section *Erysiphe* and 16 taxa (13 species) belong to *Microsphaera*. Three other taxa, *Uncinula adunca* (Wallr.:Fr.) Lév. var. *adunca*, *U. mori* I. Miyake and *Uncinuliella simulans* (Salm.) Zheng et Chen var. *simulans*, were included as outgroups to the *Erysiphe* and

Table 1. Sources of fungal materials and sequence database accession numbers.

Fungal species	Abbreviation	Host plant	Specimen no.[a]	Database accession no.[b]
Uncinula adunca (Wallr.:Fr.) Lév. var. *adunca*	UADU	*Salix vulpina* Anders. (W)[c]	MUMH39	D84383
U. mori I.Miyake	UMOR	*Morus bombycis* Koidz. (W)	TPU-1832	AB000946
Uncinuliella simulans (Salm.) Zheng et Chen var. *simulans*	USIM	*Rosa multiflora* Thunb. (W)	TPU-3087	AB015926
Erysiphe aquilegiae DC. var. *rununculi* (Grev.) Zheng et Chen	EAQ1	*Cimicifuga simplex* Wormsk. (H)	TPU-495	AB000944
E. aquilegiae var. *rununculi*	EAQ2	*Clematis terniflora* DC. (H)	MUMH98	AB015929
E. glycines Tai em. Zheng var. *glycines*	EGG1	*Desmodium oxyphyllum* DC. (H)	MUMH52	AB015927
E. glycines var. *glycines*	EGG2	*Amphicarpaea edgeworthii* Benth. var. *japonica* Oliver (H)	MUMH56	AB015934
E. glycines var. *lespedezae* (Zheng et U. Braun) U. Braun et Zheng	EGL1	*Lespedeza cuneata* (Du Mont. d. Cours.) G. Don (H)	TPU-1762	AB015921
E. glycines var. *lespedezae*	EGL2	*L. thunbergii* (DC.) Nakai (H)	TPU-1761	AB015923
E. heraclei DC. sens. str.	ERHE	*Panax schin-seng* Nees (H)	MUMH73	AB000942
E. huayinensis Zheng et Chen	EHUA	*Plectranthus logotubus* Mig. (H)	MUMH30	AB015914
E. macleayae Zheng et Chen	EMAC	*Macleaya cordata* (Willd.) R.Br. (H)	TPU-1873	AB016048
E. weigelae Z. X. Chen et Luo	EWE1	*Weigela hortensis* (Sieb. et Zucc.) K. Koch (W)	TPU-1669	AB015931
E. weigelae	EWE2	*W. hortensis* (W)	MUMH28	AB015932
Microsphaera bäumleri Magnus	MBA1	*Vicia amoena* Fisch. (H)	YNMH12360-12	AB015933
M. bäumleri	MBA2	*V. cracca* L. (H)	YNMH12852-5	AB015920
M. blasti Tai	MBLA	*Lindera umbellata* Thunb. (W)	MUMH2	AB015918
M. friestii Lev. var. *dahurica* U. Braun	MFRI	*Rhamnus japonica* Maxim. (W)	MUMH6	AB000939
M. helwingiae Sawada	MHEL	*Helwingia japonica* (Thunb.) F. G. Dietr. (W)	MUMH110	AB015916
M. juglandis Golovin	MJUG	*Pterocarpa rhoifolia* Sieb. et Zucc. (W)	TPU-1745	AB015928
M. katumotoi U. Braun	MKAT	*Ligustrum obtusifolium* Sieb. et Zucc. (W)	MUMH14	AB015917
M. pseudolonicerae (Salm.) Blumer	MPSE	*Cocculus trilobus* (Thunb.) DC. (W)	MUMH86	AB015915
M. pulchra Cook et Peck var. *japonica* (P. Henn.) U. Braun	MPJ1	*Cornus controversa* Hemsley (W)	MUMH90	AB000941
M. pulchra var. *japonica*	MPJ2	*C. controversa* (W)	YNMH12992-4	AB015924
M. pulchra var. *pulchra*	MPUP	*C. kousa* Buerger ex Hance (W)	TPU-1731	AB015935
M. staphyleae Sawada	MSTA	*Staphylea bumalda* (Thunb.) DC. (W)	MUMH16	AB015922
M. syringae-japonicae U. Braun	MSYR	*Syringa vulgaris* L. (W)	TPU-1549	AB015920
M. trifolii (Grev.) U. Braun var. *trifolii*	MTRI	*Trifolium pratense* L. (H)	TPU-1546	AB015913
M. vanbruntiana Ger. var. *sambuci-racemosae* U. Braun	MVAN	*Sambucus sieboldiana* Bl. (W)	MUMH17	AB015925
M. wallrothii U. Braun et Tanda	MWAL	*Vaccinium hirtum* Thunb. (W)	TPU-1729	AB015930

a) MUMH=Mie University Mycological Herbarium; TPU=Herbarium of Toyama Prefectural University; YNMH=Yukihiko Nomura Mycological Herbarium

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

c) (W) and (H) indicate woody plant and herbaceous plant, respectively.

Microsphaera ingroup. Species were identified by morphological characters of the teleomorph according to the monographs of Nomura (1997) and Braun (1987). The specimens were preserved as herbarium specimens in Mie University Mycological Herbarium (MUMH), Herbarium of Toyama Prefectural University (TPU), and Yukihiko Nomura Mycological Herbarium (YNMH).

DNA extraction and amplification of rDNA ITS sequences Whole-cell DNA was isolated from cleistothecia or mycelia by the Chelex method (Walsh et al., 1991; Hirata and Takamatsu, 1996). The nuclear rDNA region including the ITS regions (ITS1 and ITS2) and the 5.8S rRNA gene were amplified by the polymerase chain reaction (PCR) using the primers ITS5 (White et al., 1990) and P3 (Kusaba and Tsuge, 1995). PCR reactions were conducted 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 ITS1 (White et al., 1990) and P3. The PCR product was subjected to preparative electrophoresis in 1.5% agarose gel in TAE buffer. The DNA product of each amplification was then excised from the ethidium-stained gel and purified using the JETSORB kit (GENOMED) following the manufacturer's protocol.

DNA sequencing Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in an Applied Biosystems 373A sequencer. The sequence reactions were conducted using the PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems) following the manufacturer's protocol. Six primers, ITS1, ITS2 (White et al., 1990), P3, T2, T3, and T4 (Hirata and Takamatsu, 1996), were used for the sequencing in both directions.

Data analysis The obtained sequences were initially aligned using the Clustal V package (Higgins et al., 1992). The alignment was then refined visually with a word processing program with color coded nucleotides, and unalignable regions were excluded from the analysis. The alignment is available from the authors upon request. Phylogenetic trees were obtained from the data by both distance and parsimony methods. For distance analysis, DNADIST in PHYLIP version 3.5 (Felsenstein, 1989) was used to obtain a matrix of Kimura's two-parameter distances (Kimura, 1980). The distance matrix was then analyzed by NEIGHBOR, which has algorithms based on Saitou and Nei's neighbor joining method (Saitou and Nei, 1987). The strength of the internal branches from the resulting trees was statistically tested by bootstrap analysis (Felsenstein, 1985) from 1000 bootstrap replications. For parsimony analysis, we used the maximum parsimony method with heuristic search of the computer package PAUP version 3.1.1 (Swofford, 1993). This search was repeated ten times from different random starting points using the stepwise addition option to make certain the most parsimonious tree was found. All nucleotide substitutions were equally weighted and unordered. Alignment gaps were treated as missing information.

Resulting trees were tested against a hypothetical phylogeny based on morphological characters by the log-likelihood ratio test (Kishino and Hasegawa, 1989). A user-defined constraint tree was first drawn with the MacClade program (Maddison and Maddison, 1992), and then the most parsimonious tree consistent with the constraint tree was found using the heuristic search described above. We then used the maximum likelihood program DNAML of PHYLIP to see whether our data were more likely to fit with the most parsimonious tree or the constraint trees. If log-likelihood of the most parsimonious tree was 2.0 standard deviations greater than the log-likelihood of any given constraint tree, we rejected the constraint tree and its underlying morphological hypothesis (Kishino and Hasegawa, 1989).

Results

Nucleotide length and GC content of the ITS region Nucleotide length of the *Erysiphe* (section *Erysiphe*) and *Microsphaera* species sequenced in this study ranged from 217 to 227 nucleotides in ITS1 and from 180 to 195 nucleotides in ITS2. No significant difference in ITS length was found between the two genera. GC contents ranged from 54.7 to 60.5% in ITS1 and from 55.4 to 62.0% in ITS2, indicating that both regions are comparatively GC-rich. Nucleotide length of the 5.8S rDNA was identical (154 nucleotides) in all taxa tested. Total length of the ITS regions and 5.8S rDNA ranged from 552 nucleotides in *E. aquilegiae* DC. var. *ranunculi* (Grev.) Zheng et Chen, *E. macleayae* Zheng et Chen, and *M. staphyleae* Sawada to 569 nucleotides in *E. glycines* Tai em. Zheng var. *glycines* on *Amphicarpaea edgeworthii* Benth. var. *japonica* and *E. huayinensis* Zheng et Chen, indicating 17 nucleotides difference between the shortest and longest taxa. We treated the nucleotide sequence data from two samples of *M. pulchra* Cook et Peck var. *japonica* (P. Henn.) U. Braun as one datum in the following analyses because the sequences were completely identical in the two samples.

Alignment of the sequences Alignment of ITS1-5.8S-ITS2 sequence positions resulted in a matrix of 608 characters. Of these, it was deemed necessary to delete 27 positions from ITS1 and 46 positions from ITS2 prior to phylogenetic analysis because of alignment ambiguities. Of the remaining unambiguously aligned 535 positions, 202 (37.8%) were variable, i.e., they possessed at least one nucleotide difference in at least one stretch of DNA sequence. Over half of this variation (55.4%) was in ITS1, as opposed to 42.6% in ITS2 and only 2.0% in the 5.8S rDNA. Of these variable characters, 134 (66.3%) were phylogenetically informative, possessing at least two nucleotide states each shared by at least two sequences. The ITS1 accounted for over half (59.7%) of this variation, compared with 38.8% in ITS2 and 1.5% in 5.8S.

Divergence between ITS1-5.8S-ITS2 sequences Pairwise percentages of sequence divergence of ITS1-5.8S-ITS2 region were calculated using PAUP for 11 *Erysiphe* (section *Erysiphe*) and 16 *Microsphaera* sequences.

Within the *Erysiphe* species, the sequence divergence between pairs of species ranged from 0% to 13.0% of nucleotides (Table 2A). *Erysiphe glycines* var. *glycines* was more divergent (10.4–13.0%) from all other *Erysiphe* species compared with the divergence within the other *Erysiphe* species (0.0–8.0%). The divergence between var. *glycines* and var. *lespedezae* of *E. glycines* was also high (10.8–12.6%), and the latter variety was more

Table 2. Matrix of percentage sequence divergences among ITS1-5.8S-ITS2 from 11 *Erysiphe* and 15 *Microsphaera* DNAs.

A. *Erysiphe*

	EAQ1	EAQ2	EGG1	EGG2	EGL1	EGL2	EHER	EHUA	EMAC	EWE1
EAQ2	0.0									
EGG1	11.0	11.0								
EGG2	12.0	12.0	3.6							
EGL1	6.0	6.0	10.8	12.6						
EGL2	5.0	5.0	11.2	12.4	1.0					
EHER	5.2	5.2	10.4	12.4	3.6	3.2				
EHUA	7.0	7.0	11.6	13.0	8.0	7.4	6.2			
EMAC	0.2	0.2	11.2	12.2	6.0	5.2	5.4	7.2		
EWE1	6.2	6.2	11.4	12.6	7.2	6.6	6.2	7.6	6.4	
EWE2	5.8	5.8	11.0	12.0	7.2	6.6	6.6	7.2	6.0	0.4

B. *Microsphaera*

	MBA1	MBA2	MBLA	MFRI	MHEL	MJUG	MKAT	MPSE	MPJ1	MPUP	MSTA	MSYR	MTRI	MVAN
MBA2	0.0													
MBLA	5.8	5.8												
MFRI	2.8	2.8	6.0											
MHEL	5.0	5.0	6.2	6.0										
MJUG	11.6	11.6	9.2	11.8	9.0									
MKAT	4.8	4.8	4.8	5.4	4.8	8.8								
MPSE	3.0	3.0	4.8	2.2	4.4	9.8	3.6							
MPJ1	6.6	6.6	6.4	7.2	6.2	10.2	5.0	5.8						
MPUP	5.4	5.4	5.4	6.0	4.6	9.0	3.2	4.2	4.6					
MSTA	4.8	4.8	5.6	5.6	4.6	8.6	3.2	4.4	4.6	3.8				
MSYR	4.2	4.2	6.0	3.6	6.8	11.8	5.4	2.8	7.2	6.0	6.0			
MTRI	1.0	1.0	6.0	2.6	5.6	11.6	5.0	2.8	6.8	5.6	5.0	4.0		
MVAN	6.6	6.6	6.0	7.2	4.0	9.8	4.8	5.6	6.2	5.0	4.8	6.4	6.8	
MWAL	2.8	2.8	4.6	2.0	4.2	10.0	3.4	0.2	5.6	4.0	4.2	2.6	2.6	5.4

C. *Erysiphe* versus *Microsphaera*

	EAQ1	EAQ2	EGG1	EGG2	EGL1	EGL2	EHER	EHUA	EMAC	EWE1	EWE2
MBA1	5.0	5.0	9.6	10.6	4.2	3.4	3.4	7.4	5.2	6.4	6.4
MBA2	5.0	5.0	9.6	10.6	4.2	3.4	3.4	7.4	5.2	6.4	6.4
MBLA	4.2	4.2	10.2	10.8	6.6	6.0	6.4	7.0	4.4	6.6	6.2
MFRI	5.0	5.0	10.2	11.4	3.0	2.2	2.2	7.2	5.2	6.8	6.8
MHEL	6.4	6.4	10.8	11.4	6.8	6.2	5.6	8.1	6.6	6.6	6.2
MJUG	9.6	9.6	11.0	11.8	12.0	11.4	11.2	11.6	9.8	11.0	10.6
MKAT	5.2	5.2	10.4	11.2	6.2	5.6	5.2	6.2	5.4	5.4	5.4
MPSE	4.2	4.2	10.0	11.0	3.2	2.6	2.8	6.2	4.4	5.2	5.2
MPJ1	7.2	7.2	10.2	11.8	7.0	6.8	7.0	7.6	7.4	7.2	6.8
MPUP	5.8	5.8	10.4	11.0	6.4	5.8	5.8	6.8	6.0	5.8	5.4
MSTA	5.2	5.2	9.2	11.0	6.2	6.0	5.2	6.4	5.4	5.6	5.2
MSYR	5.0	5.0	11.2	12.8	4.4	3.8	3.6	6.6	5.2	6.0	6.4
MTRI	5.0	5.0	9.8	10.8	4.0	3.2	3.6	7.6	5.2	6.6	6.6
MVAN	7.2	7.2	10.6	11.6	8.2	7.6	7.0	8.0	7.4	6.6	6.2
MWAL	4.0	4.0	9.8	10.8	3.0	2.4	2.6	6.0	4.2	5.0	5.0

Note. Sequences were compared only at unambiguously aligned positions that lacked ambiguous, missing, or polymorphic states. Names of taxa correspond to the abbreviations showed in Table 1.

closely related to the other *Erysiphe* species than to var. *glycines*. The divergence between two samples of *E. glycines* var. *glycines* on *Desmodium oxyphyllum* DC. and *Amphicarpaea edgeworthii* var. *japonica* was significantly higher (3.6%) than that between two samples of *E. glycines* var. *lespedezae* (Zheng et U. Braun) U. Braun et Zheng on *Lespedeza cuneata* (Du Mont. d. Cours.) G. Don and *L. thunbergii* (DC.) Nakai (0%). On the other hand, *E. aquilegiae* var. *ranunculi* and *E. macleayae* were closely related (0.2% in divergence) to each other, although these two species are parasitic to different plant families.

Within *Microsphaera* species except *M. juglandis* Golovin, the sequence divergence between pairs of species ranged from 0% to 7.2% of nucleotides (Table 2B). *M. juglandis* was distantly related (8.8–11.8% in divergence) to all other *Microsphaera* species. *Microsphaera pseudolonicerae* (Salm.) Blumer and *M. wallrothii* U. Braun et Tanda were closely related (0.2% in divergence) to each other, although these species infect different plant families.

The sequence divergence between *Erysiphe* (section *Erysiphe*) and *Microsphaera* species was similar to those within the respective genera (Table 2C). *Erysiphe glycines* var. *glycines* was also highly divergent compared with all of the *Microsphaera* species (9.6–12.8%) as well as with other *Erysiphe* species. Similarly, *M. juglandis* was distantly related to all of the *Erysiphe* species (9.6–12.0% in divergence) as well as to other *Microsphaera* species.

Phylogenetic analysis Phylogenetic trees obtained by the neighbor joining and maximum parsimony methods are shown in Figs. 1 and 2, respectively. Because 84 equally parsimonious trees which required 438 steps were obtained by the maximum parsimony method, strict consensus of the 84 trees is shown in Fig. 2. The

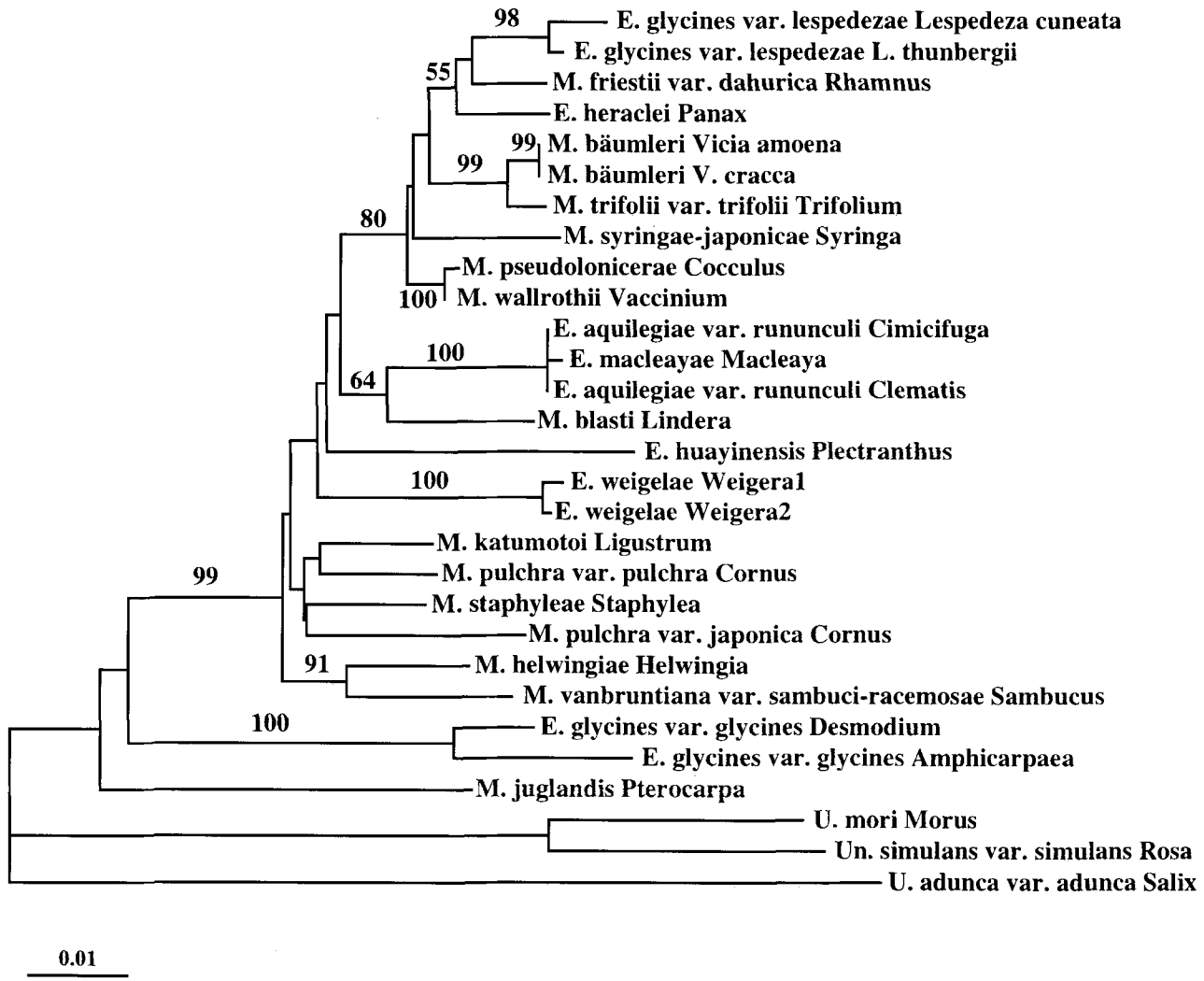

Fig. 1. A neighbor-joining tree based on distances derived from sequences of ITS1, ITS2, and the 5.8S rRNA gene from 11 *Erysiphe* section *Erysiphe* and 16 *Microsphaera* DNAs plus three outgroup taxa. The bar indicates a distance of 0.01 (one base change per 100 nucleotide positions). The numbers above the branches represent the proportion (percent) of 1000 bootstrap replications in which the groups to the right were placed together.

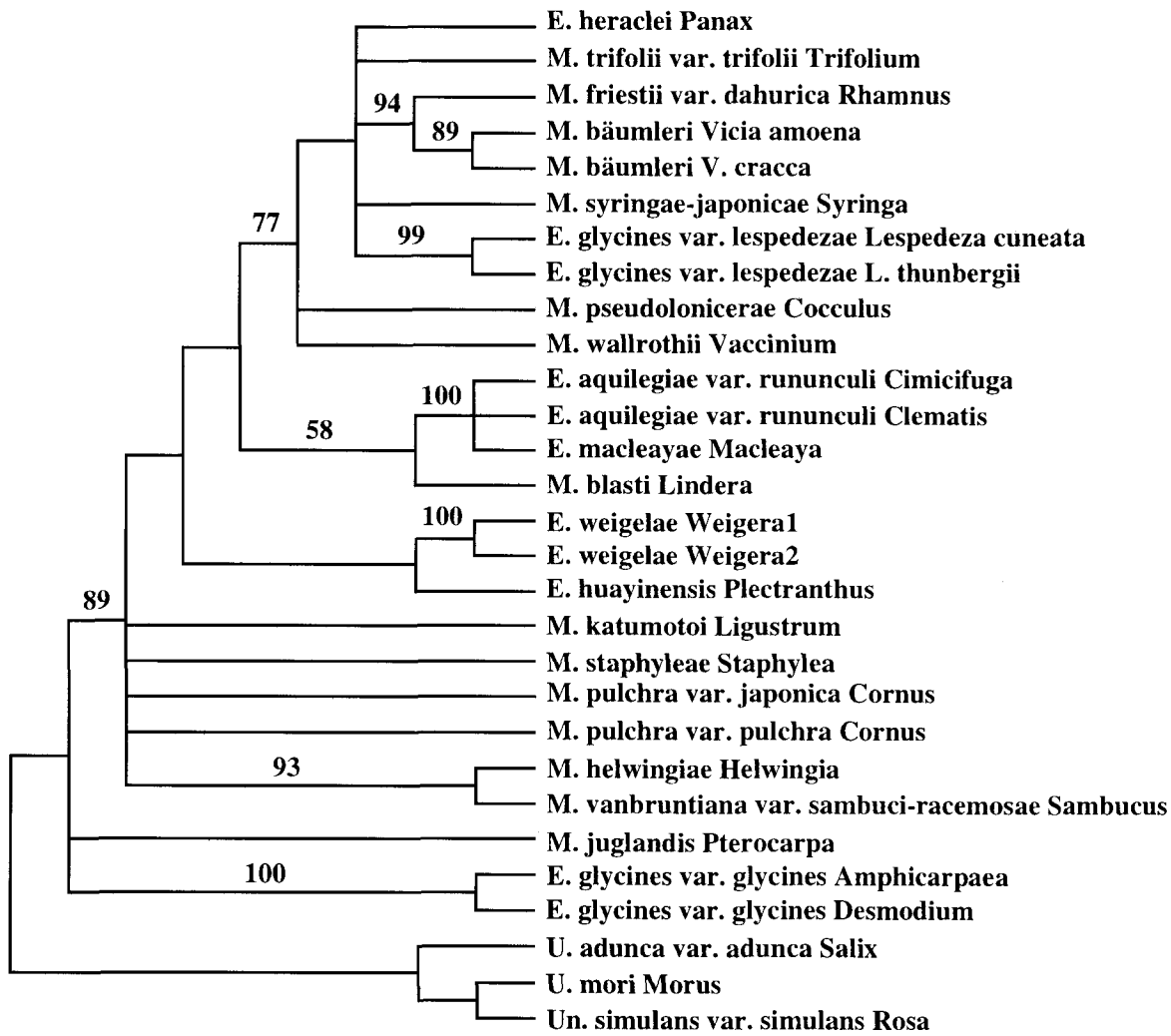

Fig. 2. A strict consensus of 84 equally parsimonious trees inferred from sequences of ITS1, ITS2, and the 5.8S rRNA gene from 11 *Erysiphe* section *Erysiphe* and 16 *Microsphaera* DNAs plus three outgroup taxa. The numbers above the branches represent the proportion (percent) of 1000 bootstrap replications in which the groups to the right were placed together. The consistency index is 0.621; the retention index is 0.667; and the rescaled consistency index is 0.414.

neighbor joining tree and the most parsimonious tree displayed similar topology.

The *Erysiphe* (section *Erysiphe*) and *Microsphaera* species analysed in this study did not form separate monophyletic clusters in the phylogenetic trees, instead, the majority of the *Erysiphe* (section *Erysiphe*) and *Microsphaera* species formed a distinct cluster together. *Erysiphe glycines* var. *glycines* and *M. juglandis* were not included in this cluster and were placed at the base of the phylogenetic tree. The cluster was strongly supported by the bootstrap analysis in both the neighbor joining (99%) and maximum parsimony (89%) methods. In the cluster, *M. friestii* Lév. var. *dahurica* U. Braun, *M. trifolii* (Grev.) U. Braun var. *trifolii*, *M. bäumleri* Magnus, *M. pseudolonicerae*, and *M. wallrothii* formed a sub-cluster with *E. heraclei* DC. sens. str. and *E. glycines* var. *lespedezae*. The bootstrap value of the sub-cluster was 80% by the neighbor joining method and 77% by the maximum parsimony method. Similarly, *M. blasti* clustered with *E. aquilegiae* var. *rununculi* and *E. macleayae*, which was supported 64% and 58% by the neighbor joining and the maximum parsimony methods, respectively. On the other hand, some *Microsphaera* species, i.e., *M. katumotoi* U. Braun, *M. staphyleae*, *M. pulchra* var. *pulchra*, *M. pulchra* var. *japonica*, *M. helwingiae* Sawada, and *M. vanbruntiana* Ger. var. *sambuci-racemosae* U. Braun did not cluster with *Erysiphe* species nor with themselves, except for the cluster of *M. helwingiae* and *M. vanbruntiana* var. *sambuci-racemosae*.

Constraint trees were made based on the following hypotheses: 1) *Erysiphe* and *Microsphaera* could be divided into separate groups (Fig. 3A); 2) *Microsphaera* could be derived from *Erysiphe*, placing section *Trichocladia* in intermediate position between the genera (Fig. 3B). These hypothetical trees were compared with the most parsimonious tree shown in Fig. 2 by the log-likelihood ratio test. As a result, the differences of log-likelihood of the phylogenetic trees increased to 3.4–4.1 times that of

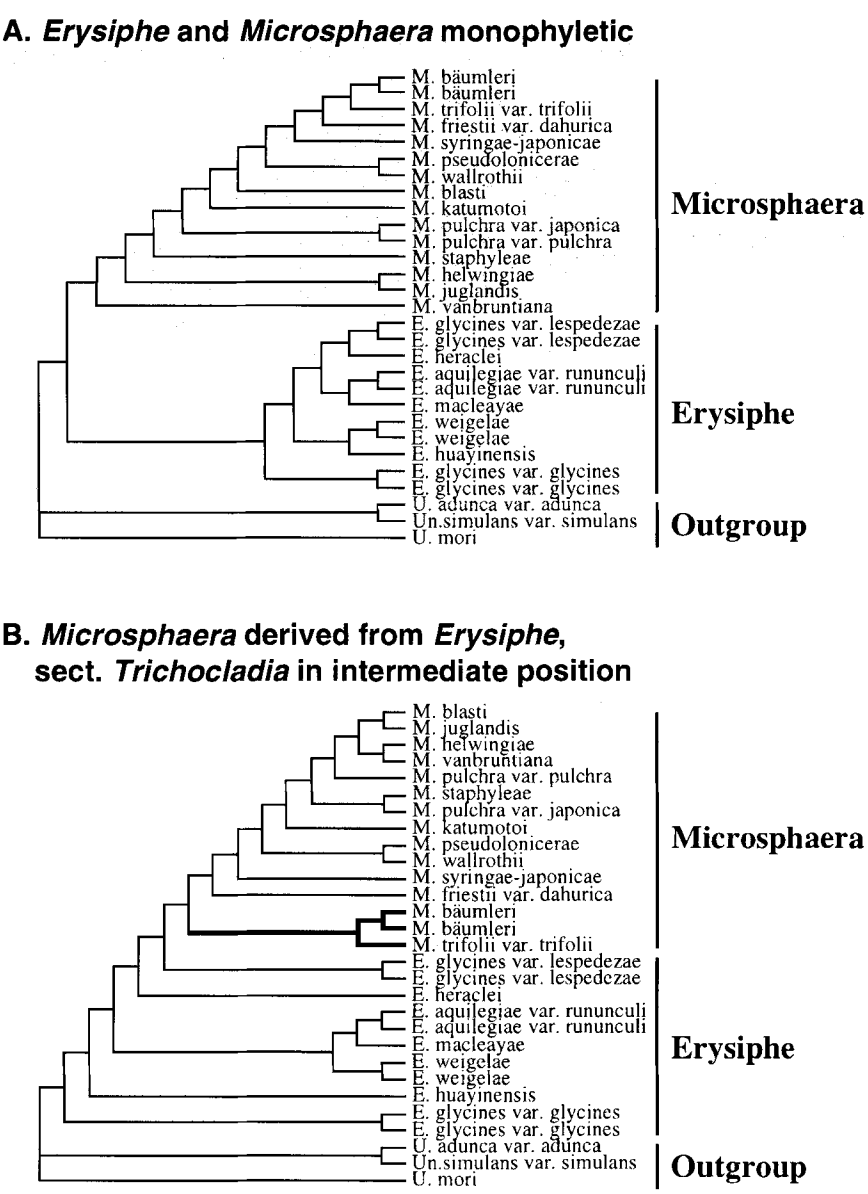

Fig. 3. Constraint trees representing the following hypotheses: A) *Erysiphe* section *Erysiphe* and *Microsphaera* species group into separate monophyletic clusters; B) *Microsphaera* is derived from *Erysiphe*, and section *Trichocladia* (bold line) occupies an intermediate position between the genera.

Table 3. Log-likelihood ratio test.

Constraint hypothesis tree	Parsimony tree length	Ln L[a]	Difference Ln L[b]	Standard deviation[c]	T-value[d]	Significantly worse?[e]
Most parsimonious tree	438	−3080.14				
Erysiphe and *Microsphaera* monophyletic	465	−3191.59	111.45	27.20	4.10	Yes
Microsphaera derived from *Erysiphe*, sect. *Trichocladia* in intermediate position	462	−3173.27	93.13	27.44	3.39	Yes

a) All numbers are rounded to the second decimal place.
b) Difference in log-likelihood compared to that of the best tree.
c) The standard deviation in log-likelihood.
d) The T-value is determined by dividing the difference in log-likelihood by the standard deviation.
e) The constraint hypothesis tree is considered to be significantly worse if the difference in log-likelihood is more than twice the standard deviation.

the standard deviations, and thus both hypothetical trees were significantly rejected (Table 3).

Discussion

Zheng (1983) placed the powdery mildews affecting *Amphicarpaea*, *Desmodium*, *Glycine*, *Phaseolus*, and *Vicia* in *E. glycines*, and raised *E. lespedezae* for the powdery mildews affecting *Lespedezae*. Later, Braun (1985) reduced them to a single species, *E. glycines*, and placed *E. glycines* of Zheng (1983) in *E. glycines* var. *glycines* and *E. lespedezae* in *E. glycines* var. *lespedezae*, because he considered that the morphological difference between the two taxa was too small for them to be recognized as different species. The present analysis indicated that there was high diversity (10.8–12.6%) between var. *glycines* and var. *lespedezae* of *E. glycines* and also that var. *lespedezae* was more closely related to the other *Erysiphe* species than to var. *glycines*. This result suggests that the difference between the two taxa is more than that of species level, because interspecies diversity of *Erysiphe* species usually ranged between 3–8% in this study. Sato (1990) reported that the size of conidia and the shape of conidial germ tubes of powdery mildews on *Lespedeza* spp. are different from those of the other *Erysiphe* species affecting legume plants. The present result as well as the Sato's report suggests that *E. glycines* var. *glycines* and var. *lespedezae* should be revised into separate species. Further, we analyzed two samples of *E. glycines* var. *glycines* isolated from *D. oxyphyllum* and *A. edgeworthii* var. *japonica* in this study, and found comparatively high sequence diversity (3.2%) between them. This high intraspecies diversity of *E. glycines* var. *glycines* as well as its basal placement in the phylogenetic tree might indicate that this species appeared early in the evolution of *Microsphaera* and *Erysiphe* (section *Erysiphe*).

In contrast, a few cases were found of extremely low sequence diversity between different species. Sequence diversity between *M. pseudolonicerae* and *M. wallrothii* was only 0.2%, and that between *E. aquilegiae* var. *ranunculi* and *E. macleayae* was also 0.2%. The host plants of these four species belong to different plant families. Since powdery mildews have long been considered as specialized parasites, those which affect plants belonging to separate families have been usually regarded as separate species. In particular, in the taxonomic system of Chen et al. (1987), host plant species was an important criterion to separate the species of powdery mildews. However, the present finding that powdery mildews affecting different plant families are closely related suggests the possibility that powdery mildews sometimes expand their host ranges to other plant families.

The present analysis as well as our previous report (Takamatsu et al., 1998) clearly showed the close relationship between *Erysiphe* (section *Erysiphe*) and *Microsphaera*, but did not support the traditional evolutionary hypothesis (Blumer, 1933; Braun, 1987) that *Microsphaera* was derived from *Erysiphe* section *Erysiphe* by a single event. In our trees the genera *Erysiphe* (section *Erysiphe*) and *Microsphaera* did not group into separate monophyletic lineages, instead, they formed several small clusters that were mixed together. This result suggests that alternative differentiations in both *Erysiphe* (section *Erysiphe*) and *Microsphaera* occurred two or more times independently. Among the taxa tested in this study, the two species, i.e., *M. trifolii* var. *trifolii* and *M. bäumleri*, belong to the section *Trichocladia*. If these species are intermediate between *Erysiphe* and *Microsphaera*, they should be placed in an intermediate position between the genera in the phylogenetic trees. Although the two species made a distinct cluster, indicating the close relationship between them, they were not placed at an intermediate position between the genera. Thus, the section *Trichocladia* is not considered as an intermediate between *Erysiphe* and *Microsphaera* phylogenetically, although it is intermediate morphologically. To evaluate the robustness of the present results, we constructed constraint trees based on the hypotheses that the genera *Erysiphe* (section *Erysiphe*) and *Microsphaera* make separate monophyletic lineages and the section *Trichocladia* is intermediate between them. The hypothetical trees were compared with the most parsimonious tree shown in Fig. 2 by the log-likelihood ratio test (Kishino and Hasegawa, 1989). As a result the hypothetical trees were significantly rejected, indicating that *Erysiphe* (section *Erysiphe*) and *Microsphaera* do not make separate monophyletic lineages and the section *Trichocladia* is not intermediate between them.

Amano (1986) reported that powdery mildews split into two groups based on their parasitism: groups mainly affecting herb plants (*Erysiphe*, *Sphaerotheca*, *Leveillula*, etc.) and mainly affecting woody plants (*Microsphaera*, *Uncinula*, *Podosphaera*, *Phyllactinia*, etc.). The appendages of the herb-parasitic genera are simple and hypha-like, whereas those of the woody plant-parasitic genera usually have more specialized morphology such as the dichotomously branched of *Microsphaera* and *Podosphaera* or the uncinate of *Uncinula* and *Sawadaea*. The most specialized appendages are found in the genus *Phyllactinia*, in which there are two types of appendages, bristle-like ones and penicillate ones (penicillate cell). The bristle-like appendages of *Ph. moricola* bend downward and lift cleistothecia off the leaf surface when the cleistothecia are matured (Itoi et al., 1962). These cleistothecia are easily dislodged and blown off the leaf surface by wind or rain, adhere to the bark of twigs by the sticky penicillate cells, and function as primary infection sources for the next year. Similarly, mature cleistothecia of grape powdery mildew, *Uncinula necator*, readily disperse in rain from infected tissues, adhere to the bark of the vine by the appendages, and overwinter there (Gadoury and Pearson, 1988; Cortesi et al., 1995). For powdery mildews infecting deciduous trees, overwintering on the bark of twigs may be far more advantageous than falling to the ground with infected leaves (Itoi et al., 1962; Pearson and Gadoury, 1987). Morphology of the appendages of woody plant-parasitic genera seems to be differentiated for clinging or adhering

to the bark of twigs. By contrast, the appendages of the herb-parasitic genera are hypha-like and interweave with the hyphae of the surrounding mildew colony. The cleistothecia of the herb-parasitic genera remain on the leaf surface even after maturing. Thus, the behavior of the cleistothecia is quite different between herb- and woody plant-parasitic genera, and cleistothecial appendages have an important role in this behavior. This suggests the possibility that the morphology of the appendages receives selection pressure from the environments of the hosts. Therefore, the hypha-like appendages of *Erysiphe*, which mainly affects herbs, and the dichotomously-branched appendages of *Microsphaera*, which mainly affects woody plants, may not imply monophyly of the respective genus, but instead might be the result of convergence due to selection pressure of the biotopes.

In summary, our previous report (Takamatsu et al., 1998) pointed out that the highly variable sequences of the ITS regions were not suitable for phylogenetic comparison between distantly related genera of powdery mildews. However, the regions showed a moderate level of variation between closely related genera like *Erysiphe* and *Microsphaera*, and between species. The phylogenetic analysis of *Erysiphe* section *Erysiphe* and *Microsphaera* based on the ITS sequences did not support the traditional hypothesis that *Microsphaera* was derived from *Erysiphe* section *Erysiphe* by a single event, and suggested that the multiple differentiations of the genera occurred two or more times independently. We pointed out the possibility that the morphology of the appendages, which is the most distinct difference between the genera, was under a selection pressure depending on the habit of their host plants, woody plants or herbs, since appendages have an important role in overwintering of the fungi by cleistothecia. This means that, because of convergence, the morphology of appendages does not always reflect the phylogeny of powdery mildews. Molecular data from other species of *Erysiphe* section *Erysiphe* and *Microsphaera*, and from other DNA regions are necessary to confirm the present result. Morphological and ecological reinvestigations of the genera will be also necessary for future studies.

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