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FULL PAPER

Molecular phylogeny and evolution of subsection *Magnicellulatae* (Erysiphaceae: *Podosphaera*) with special reference to host plants

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Abstract The subsection *Magnicellulatae* of the genus *Podosphaera* section *Sphaerotheca* belongs to the tribe Cystotheceae of the Erysiphaceae, which has the characteristic of producing catenate conidia with distinct fibrosin bodies. In this study, we newly determined the nucleotide sequences of the D1/D2 domains of the 28S rDNA region and the sequences of the rDNA internal transcribed spacer (ITS) region to investigate the relationships between the phylogeny of this fungal group and their host plants. The results indicated that the 28S rDNA region is too conservative for phylogenetic analysis of this fungal group. The phylogenetic analysis using 95 ITS sequences demonstrated that two or more *Magnicellulatae* taxa often infect the same plant genus or species. Although there is a close relationship between *Magnicellulatae* and asteraceous hosts, this association seems to be not as strict as that between *Golovinomyces* and the Asteraceae. The difference between the two fungal groups may be explained by their different evolutionary timing.

Keywords Asteraceae · Erysiphales · Molecular clock · rDNA sequence · *Sphaerotheca*

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Introduction

The Erysiphaceae is a group of plant pathogenic fungi that cause powdery mildew disease on about 10,000 angiosperm species (Amano 1986); it consists of 16 genera and ca. 650 species (Braun and Takamatsu 2000; Braun et al. 2002; Takamatsu et al. 2005a, b; Liberato et al. 2006). The host range of this fungal group is strictly restricted to angiosperms, and the fungi have never been reported to infect ferns or gymnosperms (Amano 1986). The Erysiphaceae form a monophyletic group (Mori et al. 2000b; Lutzoni et al. 2004; Wang et al. 2007), all of which are obligate biotrophs of higher plants. This indicates that the Erysiphaceae diverged from an ancestor with an obligate biotrophic nature. Molecular phylogenetic analysis demonstrated that the Erysiphaceae belong to neither the Pyrenomycetes nor the Plectomycetes as was once thought (Saenz et al. 1994). They are currently classified in the Leotiomycetes, but the sister group to the Erysiphaceae is still controversial among reports (Mori et al. 2000b; Wang et al. 2006, 2007).

A molecular clock analysis demonstrated that the first radiation of the Erysiphaceae occurred within a short period near the Cretaceous/Tertiary boundary, resulting in the divergence of the five tribes, i.e., the Blumerieae, Cystotheceae, Erysipheae, Golovinomyceteae, and Phyllactinieae (Takamatsu and Matsuda 2004). Both tree-parasitic and herb-parasitic fungi are included in three of the five tribes, i.e., the Cystotheceae, Erysipheae, and Phyllactinieae. Tree-parasitic fungi usually take basal positions in the respective tribes, and herb-parasitic fungi have terminal positions. These results, as well as the fact that the most basal genera of the Erysiphaceae, i.e., *Parauncinula* and *Caespitotheca*, infect woody plants, suggest that the early host plants of the Erysiphaceae were woody plants

(Mori et al. 2000a). Multiple independent host shifts from trees to herbs may have then occurred during the Tertiary (Takamatsu 2004). Accompanying the host shifts, convergence of the morphology of chasmothecial appendages occurred, resulting in a simple, mycelioid type, probably due to the adaptation of their life cycles to herbs (Mori et al. 2000a; Takamatsu 2004). These multiple host shifts from trees to herbs may be important evolutionary events that occurred in the respective tribes of the Erysiphaceae.

The subsection *Magnicellulatae* of the genus *Podosphaera* section *Sphaerotheca* belongs to the tribe Cystotheceae, which is characterized by its production of catenate conidia with distinct fibrosin bodies. This subsection used to belong to the genus *Sphaerotheca* with the subsection *Sphaerotheca*. Molecular phylogenetic analyses demonstrated that the subsections *Magnicellulatae* and *Sphaerotheca* form each separate lineage evolved from different ancestors and that they form a monophyletic group with the genus *Podosphaera* together (Takamatsu et al. 2000). Thus, the genus *Sphaerotheca* was reduced to the genus *Podosphaera* (Braun and Takamatsu 2000). *Magnicellulatae* forms a monophyletic group within the genus *Podosphaera*, which is supported by the feature of unique conidial germ tubes called *Fuliginea*-type and large outer peridium cells of chasmothecia. *Magnicellulatae* infects more than 1,000 angiosperm species, spanning 40 families including Asteraceae, Scrophulariaceae, Cucurbitaceae, and Fabaceae, most of which are herbaceous plants (Amano 1986). Previous analyses (Takamatsu et al. 2000) demonstrated that the sister species to *Magnicellulatae* are *Podosphaera tridactyla* and *P. longiseta*, both of which are parasitic to woody plants of the genus *Prunus* (Rosaceae). This suggests that *Magnicellulatae* diverged by a host shift of these *Podosphaera* species onto herbaceous plants. Hirata et al. (2000) conducted molecular phylogenetic analyses of *Magnicellulatae* using 79 rDNA ITS sequences from 60 plant species covering 55 families. From the analyses, they suggested that *Podosphaera* on *Prunus* first switched their hosts onto herbaceous plants of Scrophulariaceae, and then acquired parasitism to the Asteraceae.

However, these results were obtained based on only the rDNA ITS sequences. Other DNA sequences were required to confirm these results. We thus determined the nucleotide sequences of the D1/D2 domains of the 28S rDNA region to investigate the relationships between the fungal phylogeny and their host plants. This study was conducted: (1) to evaluate the usefulness of the 28S rDNA region in phylogenetic analysis of the subsection *Magnicellulatae*, (2) to reconstruct the phylogenetic tree of *Magnicellulatae* by adding newly determined ITS sequences in this study, and (3) to discuss the phylogeny and evolution of this fungal group more precisely.

Materials and methods

Sample sources

The host plants, location of collection, and accession numbers for the nucleotide sequence databases (DDBJ, EMBL, and GenBank) are provided in Table 1. For most cases, the 28S rDNA sequences were determined using the same specimens studied in Hirata et al. (2000) for the ITS sequences. However, for some specimens where sequencing failed, other specimens of the same host species were used for sequencing of the 28S rDNA and were combined with the ITS sequences. Only the host plant names, not fungal names, are shown in this study, because the species delimitations of the subsection *Magnicellulatae* are still controversial (Hirata et al. 2000; Braun et al. 2001).

DNA extraction and amplification

Whole-cell DNA was isolated from chasmothecia or mycelia using the Chelex method (Walsh et al. 1991, Hirata and Takamatsu 1996). The ITS region including the 5.8S rDNA, and the 5′ end of the 28S rDNA including the variable domains D1 and D2 were amplified separately by two sequential polymerase chain reactions (PCRs) using partially nested primer sets. The PCR reactions were conducted using TaKaRa Taq DNA polymerase (TaKaRa, Tokyo, Japan) in a TP-400 thermal cycler (TaKaRa) under the following thermal cycling conditions: an initial denaturation step of 2 min at 95°C, 30 cycles of 30 s at 95°C, followed by 30 s at 52°C for annealing, and 30 s at 72°C for extension, and a final extension step of 7 min at 72°C. A negative control that lacked template DNA was included in each set of reactions. The PCR products were subjected to electrophoresis in a 1.5% agarose gel in TAE buffer, excised from the ethidium-bromide-stained gel, and purified using the JETSORB kit (Genomed, Oeynhausen, Germany) according to the manufacturer's protocol. The nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in a CEQ2000XL DNA sequencer (Beckman Coulter, Fullerton, CA, USA). The sequence reactions were conducted using the CEQ Dye Terminator Cycle sequencing kit (Beckman Coulter) according to the manufacturer's instructions.

For amplification of the ITS region, primers ITS5 (White et al. 1990) and P3 (Kusaba and Tsuge 1995) were used for the first amplification. One microliter of the first reaction mixture was used for the second amplification along with the partially nested primer sets ITS5 and ITS4 (White et al. 1990). The ITS5/ITS4 fragment was subjected to cycle sequencing using the primers ITS1, ITS4, T3, and T4 (Hirata and Takamatsu 1996). For amplification of the 28S rDNA, primers PM3 (Takamatsu and Kano

Table 1 Sources of *Magnicellulatae* material sequenced in this study and DNA database accession numbers

Host	Location and year	Designation[a]	Accession no.[b]	
			ITS	28S rDNA
Acanthaceae				
Peristrophe japonica	Fukui, Japan; 1996	MUMH 313	AB026135	
	Gifu, Japan; 2005	MUMH 4056		AB462795[c]
Asteraceae				
Arctium lappa	Mie, Japan; 1996	MUMH 311	AB040310	AB462767[c]
Aster iinumae	Ishikawa, Japan; 1997	MUMH s143	AB040353	AB462760[c]
Aster microcephalus var. *ovatus*	Aichi, Japan; 1996	MUMH 333	AB040335	AB462779[c]
Aster tataricus	Mie, Japan; 1996	MUMH 316	AB040341	
	Shiga, Japan; 2005	MUMH 4019		AB462793[c]
Bidens frondosa	Mie, Japan; 1996	MUMH 314	AB040295	
	Shiga, Japan; 2004	MUMH 3464		AB462790[c]
Calendula officinalis	Mie, Japan; 1996	MUMH 304	AB040317	AB462762[c]
Conyza canadensis	Nara, Japan; 1996	MUMH 322	AB040313	AB462772[c]
Cosmos bipinnatus	Mie, Japan; 1996	MUMH 315	AB040299	
	Aichi, Japan; 2005	MUMH 4177		AB462797[c]
Gerbera hybrida	Mie, Japan; 1996	MUMH 305	AB040309	AB462763[c]
Helianthus annuus	Toyama, Japan; 1996	MUMH 324	AB040311	AB462774[c]
	Toyama, Japan; 1997	MUMH 337	AB040312	AB462781[c]
Lactuca indica	Nara, Japan; 1996	MUMH 323	AB040294	AB462773[c]
Lactuca raddeana var. *elata*	Niigata, Japan; 1996	MUMH 328	AB040352	AB462776[c]
Rudbeckia sp.	Niigata, Japan; 1996	MUMH s142	AB040337	AB462798[c]
Taraxacum albidum	Mie, Japan; 1996	MUMH 306	AB040342	AB462764[c]
Taraxacum officinale	Toyama, Japan; 1994	–	AB026148	
	Aichi, Japan; 2005	MUMH 4112		AB462796[c]
Zinnia elegans	Toyama, Japan; 1997	MUMH 338	AB040355	AB462782[c]
Balsaminaceae				
Impatiens balsamina	Aomori, Japan; 2001	MUMH 1608	AB462803[c]	AB462788[c]
	Kagoshima, Japan; 2000	MUMH 1125	AB462799[c]	
	Mie, Japan; 2000	MUMH 1225	AB462800[c]	
Impatiens noli-tangere	Hokkaido, Japan; 2003	MUMH 2658	AB462805[c]	AB462789[c]
	Hokkaido, Japan; 2003	MUMH 2890	AB462807[c]	
	Iwate, Japan; 2003	MUMH 2841	AB462806[c]	
	Niigata, Japan; 1996	MUMH 227	AB040318	
Impatiens textori	Nagano, Japan; 2000	MUMH 1231	AB462801[c]	AB462787[c]
	Akita, Japan; 2001	MUMH 1496	AB462802[c]	
	Niigata, Japan; 1996	MUMH 245	AB040344	
Cucurbitaceae				
Cucumis sativus	Mie, Japan; 1995	MUMH 65	AB026146	
	Mie, Japan; 2005	MUMH 3702		AB462791[c]
Cucurbita maxima	Kyoto, Japan; 1996	MUMH 309	AB040315	AB462766[c]
Trichosanthes kirilowii var. *japonica*	Niigata, Japan; 1996	MUMH 246	AB040316	
	Niigata, Japan; 1997	MUMH 434		AB462786[c]
Zehneria japonica	Mie, Japan; 1994	MUMH 68	D84387	
	Nagano, Japan; 2005	MUMH 3864		AB462792[c]
Euphorbiaceae				
Acalypha australis	Mie, Japan; 1996	MUMH 319	AB040306	AB462770[c]

Table 1 continued

Host	Location and year	Designation[a]	Accession no.[b]	
			ITS	28S rDNA
Fabaceae				
Crotalaria juncea	Toyama, Japan; 1997	MUMH 342	AB040304	AB462785[c]
Dunbaria villosa	Toyama, Japan; 1996	MUMH 326	AB040334	AB462775[c]
Glycine max subsp. *max*	Toyama, Japan; 1997	MUMH 339	AB040305	AB462783[c]
Vigna angularis var. *angularis*	Nara, Japan; 1996	MUMH 320	AB040297	AB462771[c]
Vigna unguiculata	Toyama, Japan; 1997	MUMH 340	AB040340	AB462784[c]
Lamiaceae				
Ajuga reptans	Tokyo, Japan; 1996	MUMH s131	AB026142	
	Shiga, Japan; 2005	MUMH 4021		AB462794[c]
Lycopus lucidus	Aichi, Japan; 1996	MUMH 332	AB040343	AB462778[c]
Scrophulariaceae				
Veronicastrum japonicum	Niigata, Japan; 1996	MUMH 303	AB026144	AB462761[c]
Solanaceae				
Solanum melongena	Mie, Japan; 1996	MUMH 318	AB040333	AB462769 [c]
Urticaceae				
Boehmeria nivea var. *nipononivea*	Fukui, Japan; 1996	MUMH 312	AB026139	AB462768[c]
Verbenaceae				
Clerodendrum trichotomum	Aichi, Japan; 1996	MUMH 331	AB026145	AB462777[c]
Verbena bonariensis	Mie, Japan; 2002	MUMH 1899	AB462804[c]	
Verbena × *hybrida*	Aichi, Japan; 1996	MUMH 334	AB040347	AB462780[c]
Vitaceae				
Cayratia japonica	Chiba, Japan; 1996	MUMH 307	AB026151	AB462765[c]

[a] MUMH, Mie University, Mycological Herbarium, Japan

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

[c] Sequence determined in this study

2001) and TW14 (Mori et al. 2000a), and NL1 (Mori et al. 2000a) and TW14 were used for the first and second amplifications, respectively. Primers NL1, NL2, NL3, and NLP2 (Mori et al. 2000a) were used for cycle sequencing.

Phylogenetic analysis

The sequences were initially aligned using the Clustal X package (Thompson et al. 1997). Alignment was then visually refined with a word processing program using color-coded nucleotides. The alignments were deposited in TreeBASE (http://www.treebase.org/) under accession number S2410. Phylogenetic trees were obtained from the data using the maximum-parsimony (MP) method in PAUP* 4.0 (Swofford 2001) and Bayesian analysis in MRBAYES 3.1.1 (Ronquist and Huelsenbeck 2003). MP analyses were performed with the heuristic search option using the "tree-bisection-reconstruction" (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analyses using 1,000 replications with the stepwise addition option set as simple and maximum tree number as 100 (ITS + 28S data set) or 10 (ITS data set) to save analysis time (Felsenstein 1985).

For Bayesian phylogenetic analyses, the best-fit evolutionary model was determined for each dataset by comparing different evolutionary models via a likelihood ratio test (LRT) using PAUP* and MrModeltest 2.2 (Nylander 2004). MRBAYES was launched with random starting trees, and Markov chains were sampled every 100 generations. To ensure that the Markov chain did not become trapped in local optima, we used the MCMCMC algorithm and performed the estimation with four incrementally heated Markov chains. The average standard deviation of split frequencies (ASDSF) was observed to verify that the values dropped below 0.01. Support for individual nodes was tested by posterior probabilities (PP) obtained from a 50% majority-rule consensus.

The partition homogeneity test (Farris et al. 1995) was conducted by PAUP* with 1,000 replicates to determine

whether the ITS and 28S rDNA data sets were in conflict. An LRT was performed to assess whether the molecular clock hypothesis was applicable to the ITS data set.

Results

Phylogeny inferred from the 28S rDNA region

About 700 nucleotides of the D1/D2 domains of the 28S rDNA region were determined for 38 specimens in this study. By adding two outgroup sequences and a sequence of *Magnicellulatae* retrieved from the DNA database, a total of 41 sequences were used to construct the ITS, 28S rDNA, and their combined data sets. The ITS data set consisted of 484 characters, of which 68 characters were variable and 41 characters were phylogenetically informative for parsimony analysis. Of these 41 informative characters, 20 characters were still informative within *Magnicellulatae*. The 28S rDNA data set consisted of 678 characters, of which 32 characters were variable and 16 characters were informative. Of these, only five characters were informative within *Magnicellulatae*. MP analysis using the ITS data set generated three equally parsimonious trees with 87 steps. The BS supports were 86%, 94%, and 84% for the major nodes A, B, and C, respectively (Table 2; Fig. 1). The 28S rDNA data set generated 12 equally parsimonious trees with 37 steps. The BS supports were lower than 50% for all three of the major nodes.

Table 2 Results of the molecular phylogenetic analyses of ITS, 28S rDNA, and their combined data

	ITS	28S rDNA	ITS + 28S
Total characters	484	678	1162
Variable site	68 (14.0%)	32 (4.7%)	100 (8.6%)
Informative site			
Total 41 taxa	41 (8.5%)	16 (2.4%)	57 (4.9%)
Within *Magnicellulatae*	20 (4.1%)	5 (0.7%)	25 (2.2%)
Number of trees	3	12	3
Tree length	88	37	126
Consistency index	0.9318	0.8919	0.9127
Retention index	0.9580	0.9216	0.9433
Rescaled consistency index	0.8927	0.8219	0.8609
Bootstrap support (%)			
Node A	86	<50	84
Node B	94	<50	93
Node C	84	<50	91
Posterior probability			
Node A	1.0	<0.5	1.0
Node B	1.0	<0.5	1.0
Node C	1.0	0.54	1.0

Because the result of the partition homogeneity test showed no conflict between the ITS and 28S rDNA data ($P = 0.25$), the ITS and 28S rDNA data sets were combined to make a combined data set. The MP tree constructed by using the combined data set is shown in Fig. 1. The tree topology based on the combined data set was almost the same as the tree based on the ITS data set. MrModeltest selected HKY + G model and GTR + I model as the best for the ITS and 28S rDNA data partitions, respectively. Using these evolution models separately for the respective partitions, MRBAYES were run for 2,000,000 generations, resulting in 20,001 sampling trees. The first 4,700 trees were discarded (burn-in) because ASDSF dropped below 0.01. The remaining 15,301 trees were summarized in a majority-rule consensus tree, yielding the probability of each clade being monophyletic. The tree topology by Bayesian analysis was identical to the MP tree, and thus the tree is not shown.

Phylogeny inferred from ITS sequences

To investigate the detailed phylogenetic relationships within *Magnicellulatae*, a total of 14 ITS sequences, consisting of 9 sequences newly determined in this study and 5 sequences retrieved from the DNA database, were combined with the data set used in Hirata et al. (2000). *Podosphaera tridactyla* and *P. longiseta* were used as outgroup taxa based on Takamatsu et al. (2000). The data set consisted of 95 sequences and 484 characters, of which 80 characters were variable and 45 characters were informative for parsimony analysis. An MP analysis generated 34 equally parsimonious trees with 115 steps. Of these 34 trees, a tree with the highest likelihood value is shown in Fig. 2. MrModeltest selected GTR + G model as the best for this data set. Using this evolution model, MRBAYES were run for 2,000,000 generations, resulting in 20,001 sampling trees. The first 11,230 trees were discarded (burn-in) because ASDSF dropped below 0.01. The remaining 8,771 trees were summarized in a majority-rule consensus tree, yielding the probability of each clade being monophyletic. The tree topology by the Bayesian analysis was identical to the MP tree and thus the tree is not shown.

Of the 93 ITS sequences of *Magnicellulatae*, the deepest branches were shared by two Scrophulariaceae isolates. The remaining 91 sequences, including an isolate from *Melampyrum nemorosum* (Scrophulariaceae), formed a large clade with strong supports (BS = 92%; PP = 1.0). This large clade was further divided into two well-supported clades and a basal paraphyletic assemblage. Group 1 was situated at the basal position of the large clade and consisted of ten isolates from the Asteraceae,

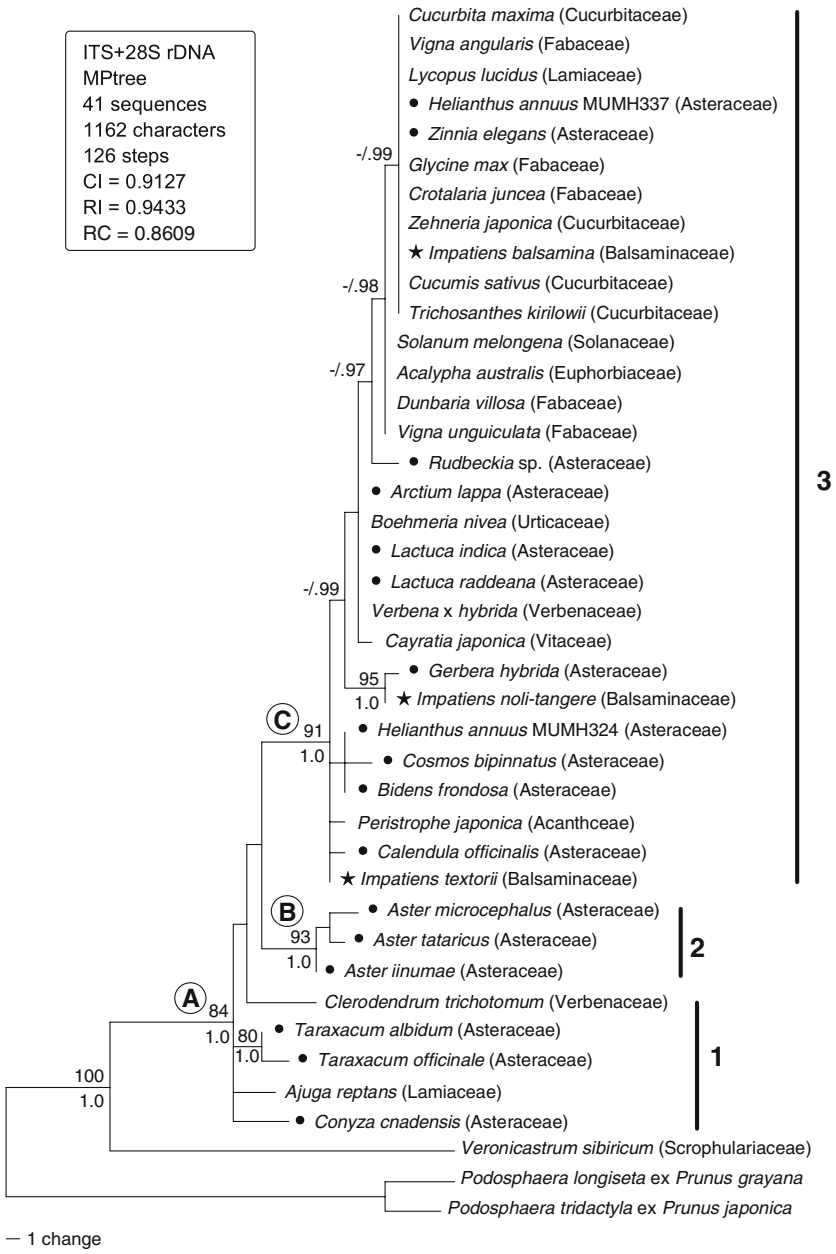

Fig. 1 Phylogeny of *Magnicellulatae* inferred from the combined data set of the rDNA ITS sequences and the D1 and D2 domain sequences of the 28S rDNA. *Solid circles* and *solid stars* at the *left* of the taxon labels indicate isolates from the Asteraceae and *Impatiens*, respectively. The percentage bootstrap support (1,000 replications; ≥70%) and the Bayesian posterior probability value (≥0.95) are shown on and under the branches, respectively

Lamiaceae, Scrophulariaceae, and Verbenaceae. Group 2 comprised four isolates from the tribes Astereae and Inuleae of the Asteraceae and was supported by 85% BS and 1.0 PP values. Group 3 was a large clade composed of 77 isolates from 13 families including the Asteraceae, Cucurbitaceae, and Fabaceae. This group was supported moderately (62%) by BS and strongly (1.0) by PP values.

Timing of evolutionary events

Because an LRT significantly rejected the molecular clock of the ITS data set of 95 sequences, 8 sequences with extremely long or short terminal branches were removed from the data set. The molecular clock hypothesis of the reduced data set consisting of 87 ITS sequences was not rejected by the LRT and thus was used to construct unweighted pair-group method with arithmetic mean (UPGMA) tree. The Kimura two-parameter model (Kimura 1980) was used to calculate genetic distances. The evolutionary timing estimated by the molecular clock of the ITS region of the Erysiphales (2.52×10^{-9} substitutions/site/year; Takamatsu and Matsuda 2004) suggested that the major radiation of *Magnicellulatae* occurred less than 2 million years ago in the Quaternary Era (Fig. 3).

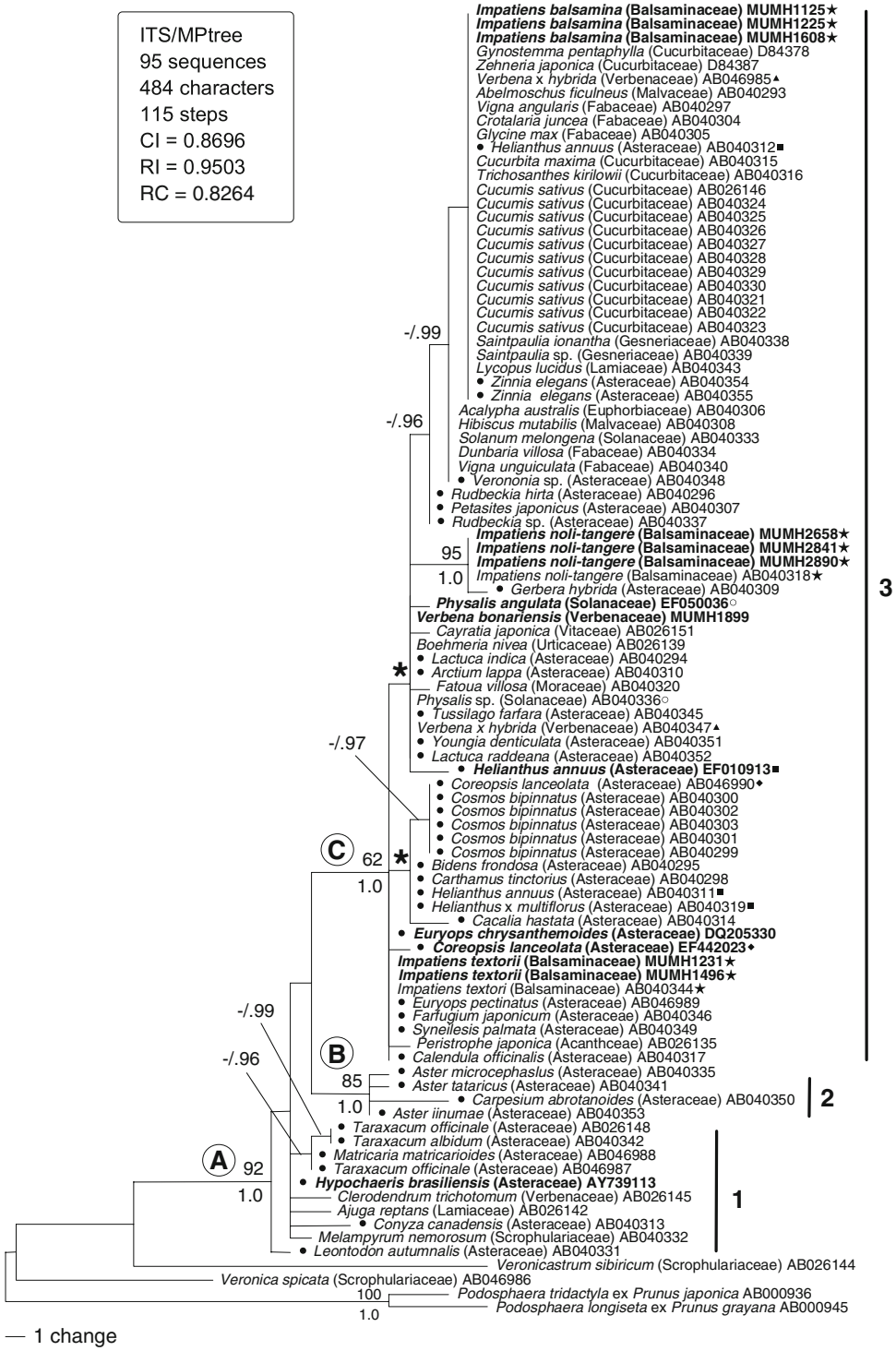

Fig. 2 Phylogeny of *Magnicellulatae* inferred from the rDNA ITS sequences. *Solid circles* at the *left* of the taxon labels indicate isolates from the Asteraceae. *Solid stars*, *solid triangles*, *solid squares*, *open circles*, and *solid diamonds* at the *right* of the taxon labels indicate sequences from powdery mildews on *Impatiens*, *Verbena*, *Helianthus*, *Physalis*, and *Coreopsis*, respectively. *Bold type* indicates sequences determined in this study. *Asterisks* on the branches indicate that the branches were collapsed in a strict consensus tree. The percentage bootstrap support (1,000 replications; ≥70%) and the Bayesian posterior probability value (≥0.95) are shown on and under the branches, respectively

Discussion

Phylogenetic value of the 28S rDNA region

This study was conducted to determine a more accurate phylogeny of the subsection *Magnicellulatae* by combining newly determined 28S rDNA sequences with the ITS data reported previously (Hirata et al. 2000). The combined data set generated a tree topology almost the same as the tree constructed by using the ITS data set. However, the 28S rDNA data set showed only 5 informative sites present in the 39 *Magnicellulatae* ingroup. This result indicates that the 28S rDNA region is too conservative for phylogenetic analysis of the subsection *Magnicellulatae*. More-

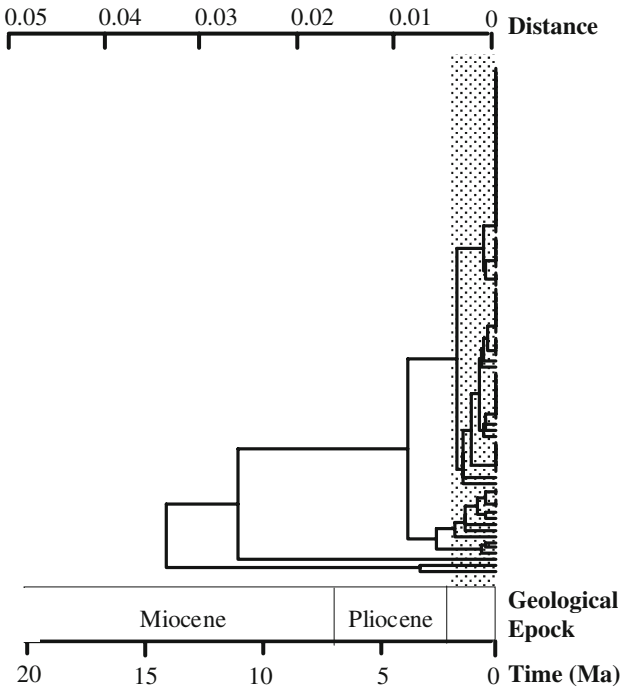

Fig. 3 Estimated time of divergence of the subsection *Magnicellulatae* of the genus *Podosphaera* section *Sphaerotheca* based on the nucleotide sequences of the rDNA ITS region and the nucleotide substitution rate of Erysiphaceae (2.52×10^{-9} substitutions/site year^{-1}) reported by Takamatsu and Matsuda (2004). *Ma* million years ago

variable DNA regions may be required for further phylogenetic analyses of this fungal group.

Occurrence of two or more *Magnicellulatae* fungi on the same host genus or species

The powdery mildew fungi are obligate biotrophs of plants. Thus, the relationships between the fungi and their host plants have long been considered to be conservative in that, in most cases, a single host species is expected to be infected by a single powdery mildew taxon. However, recent molecular phylogenetic analyses demonstrated that two or more fungal species with similar morphology often infect a single host genus or species (Havrylenko 1993; Takamatsu et al. 2006, 2009). The present study also demonstrated that two or more *Magnicellulatae* taxa often infect a single plant genus or species. For instance, the four isolates from *Helianthus* collected in Japan and Taiwan were divided into three groups (Fig. 2, solid square). There were three nucleotide substitutions between two isolates from *Verbena* × *hybrida* collected in Japan and the USA (Fig. 2, solid triangle). Shishkoff (1999) reported that the fungus collected in the USA (MUMH 808) was more similar in morphology to *Podosphaera xanthii* on cucumber than to *P. verbenae* (=*Sphaerotheca verbenae*), a species known to occur on *V.* × *hybrida*. This report is consistent with the present molecular analysis. There were three nucleotide substitutions between the two isolates from *Coreopsis lanceolata* sequenced in Japan and Taiwan (Fig. 2, solid diamond). There was also one nucleotide difference between the two isolates from *Physalis* sequenced in Taiwan and Australia (Fig. 2, open circle).

The fungi occurring on *Impatiens balsamina*, *I. noli-tangere* and *I. textorii* (Balsaminaceae) have been regarded as a single species, *Podosphaera balsaminae* (Braun 1987; Zheng and Yu 1987; Nomura 1997). Hirata et al. (2000) reported that there are four nucleotide substitutions in the ITS region between isolates from *I. noli-tangere* and *I. textorii*. We newly sequenced three specimens on *I. noli-tangere* and *I. textorii* and also sequenced three isolates from *I. balsamina* in this study. The sequences from the same *Impatiens* species are identical to each other, and the sequences from the different *Impatiens* species each formed different clades (Fig. 2, solid star). This suggests that the fungi on the three *Impatiens* species do not share a single ancestor and acquired parasitism to each *Impatiens* species independently. Nomura (1974) reported that an isolate from *I. noli-tangere* did not infect *I. textorii*. Similarly, Wolcan (2004) reported that *P. balsaminae* did not show cross-infection between *I. balsamina* and *I.* × *hawker*. These reports support the present molecular analysis that the isolates from different species of *Impatiens* form distinct clades. However, we could not find any morphological differences between these specimens (data not shown). Further morphological observations including anamorphic stages are required to conduct a taxonomic revision of the powdery mildews of *Impatiens*.

Phylogeny of *Magnicellulatae* and their host plants

The powdery mildew fungi were tree parasitic in their early stage of evolution, and host shifts occurred independently from woody plants to herbaceous plants multiple times along with the radiation of herbaceous plants on the Earth (Mori et al. 2000a; Takamatsu 2004). Therefore, in general, herb-parasitic powdery mildew groups have a recent origin compared with woody-plant-parasitic groups. Among the 16 genera of the Erysiphales, three genera, i.e., *Golovinomyces*, *Leveillula*, and *Magnicellulatae*, are herb parasitic, and the Asteraceae is the family with the largest number of host species for these genera. Especially, the genus *Golovinomyces* has intimate relationships with the Asteraceae, in which *Golovinomyces* from each tribe of the Asteraceae form a separate clade (Matsuda and Takamatsu 2003). In the case of *Magnicellulatae*, isolates from the Asteraceae were scattered throughout the phylogenetic trees (Figs. 1, 2). Therefore, the relationship between

Magnicellulatae and the Asteraceae seems to be not as strict as that between *Golovinomyces* and the Asteraceae.

Both *Golovinomyces* and *Magnicellulatae* commonly infect herbaceous plants and have Asteraceae as the most major host family, but the relationships between the fungi and their hosts are different between these two fungal groups. Calibration of the evolutionary timing of the *Magnicellulatae* using the molecular clock of the ITS region indicates that the major radiation of the *Magnicellulatae* occurred less than 2 million years ago, in the Quaternary Era. This result suggests that the divergence of the major asteraceous tribes was already finished when *Magnicellulatae* acquired parasitism to the Asteraceae. Therefore, factors such as geographic closeness, susceptibility, and similarity of chemical contents of host plants might have been involved more strongly in the host shifts for the *Magnicellulatae* than the phylogenetic relationships of the hosts. Further comprehensive phylogenetic analyses including *Leveillula*, another fungal genus having Asteraceae as the major host family, are required to verify the above speculation.

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