

FULL PAPER

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Molecular phylogenetic analyses reveal a close relationship between powdery mildew fungi on some tropical trees and *Erysiphe alphitoides*, an oak powdery mildew

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Abstract To investigate the phylogenetic relationships among the powdery mildew fungi of some economically important tropical trees belonging to *Oidium* subgenus *Pseudoidium*, we conducted molecular phylogenetic analyses using 30 DNA sequences of the rDNA internal transcribed spacer (ITS) regions and 26 sequences of the domains D1 and D2 of the 28S rDNA obtained from the powdery mildews on *Hevea brasiliensis* (para rubber tree), *Anacardium occidentale* (cashew), *Bixa orellana*, *Citrus* spp., *Mangifera indica* (mango), and *Acacia* spp. The results indicate that the powdery mildew fungi isolated from these tropical trees are closely related to one another. These powdery mildews are also closely related to *E. alphitoides* (including *Erysiphe* sp. on *Quercus phillyraeoides*). Because of the obligate biotrophic nature of the powdery mildew fungi, the relationship between powdery mildews and their host plants is conservative. However, the present study suggests that a particular powdery mildew species has expanded its host ranges on a wide range of the tropical trees. This article also suggests that a powdery mildew fungus distributed in temperate regions of the Northern Hemisphere expanded its host ranges onto tropical plants and may be a good example of how geographical and host range expansion has occurred in the Erysiphales.

Key words *Acacia* · *Citrus* · Erysiphales · *Mangifera indica* · *Quercus phillyraeoides*

Introduction

The Erysiphales is an important group of plant pathogenic fungi that cause “powdery mildew” on about 10000 species of angiosperms. The host plant species are mainly distributed in temperate regions of the Northern Hemisphere. They also infect many plant species distributed in tropical or subtropical regions, including economically important cultivated plants such as *Hevea brasiliensis* (Willd. ex A. Juss.) Muell.-Arg. (para rubber tree) (Steinmann 1925; Beeley 1933; Mitra and Mehta 1938; Shaw 1967), *Anacardium occidentale* L. (cashew) (Viégas 1944; Sijaona et al. 2001), *Bixa orellana* L. (Bitancourt 1938; Viégas 1944; Capretti 1961; Peregrine and Siddigi 1972), *Citrus* spp. (oranges and lemons) (Petch 1915; Park 1933; Tamayo and Pordesino 1959; Yen 1967; Boesewinkel 1981), *Mangifera indica* L. (mango) (Palti et al. 1974; Boesewinkel 1980), and *Acacia* spp. (Tanaka 1986; Semangun 1992; Kawabe et al. 1998; Daidou and Ito 2001; Tamit 2003). The powdery mildews of these tropical trees cause early defoliation and discoloration or malformation of leaves, resulting in yield loss or reduction in product quality. However, ecology and classification of the powdery mildew fungi of these tropical trees are still uncertain because of the limited number of researchers working on this fungal group in tropical regions. Also, in the tropical region, powdery mildews usually lack teleomorphs, which are necessary for species identification. Although some of them have been described as *Oidium* species, such as *Oidium anacardii* Noack, *O. citri* (Yen) U. Braun, *O. heveae* Steinmann, and *O. mangiferae* Berthet, with descriptions of anamorphic characters, the identifications are mostly based on their host plants and the morphological descriptions are not adequate to distinctly delimit the species.

Recently, molecular analysis based on nucleotide sequences of the nuclear rDNA has been used to determine

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the phylogenetic relationship among powdery mildew fungi (Takamatsu et al. 1998, 1999, 2000, 2002; Saenz and Taylor 1999; Braun and Takamatsu 2000; Mori et al. 2000; Kiss et al. 2001; Okamoto et al. 2002; Matsuda and Takamatsu 2003; Takamatsu 2004). This technique can be applied to investigate the relationship between an anamorphic species and its suspected teleomorphic stage (Cunnington 2002; Cunnington et al. 2003). In this study, we conducted molecular phylogenetic analyses on the powdery mildew fungi belonging to *Oidium* subgenus *Pseudoidium* from some tropical trees to elucidate the phylogenetic relationships among the species and to link these anamorphic fungi with their allied teleomorphs.

Materials and methods

Sample sources

Powdery mildew specimens that included one specimen on *A. occidentale* (cashew), four specimens on *B. orellana*, three specimens on *Citrus* spp., eight specimens on *Acacia* spp., ten specimens on *M. indica* (mango), two *Erysiphe*

alphitoides (Griffon & Maubl.) U. Braun & S. Takam. specimens on *Quercus* spp., and two *E. euonymi-japonici* (Vienn.-Bourg.) U. Braun & S. Takam. specimens on *Euonymus japonicus* Thunb. were used in this study. Five specimens on the para rubber tree (*Hevea barsiliensis*) and two specimens of *Erysiphe* sp. on *Q. phillyraeoides* Asa Gray reported by Limkaisang et al. (2005) were also used in this study. The powdery mildew on *Q. phillyraeoides* has been reported as *E. alphitoides* (Homma 1937; Nomura 1997). However, we found that the powdery mildew on *Q. phillyraeoides* differs from *E. alphitoides* on other *Quercus* species both in morphology and in molecular characteristics in this study. Therefore, this fungus is tentatively referred to *Erysiphe* sp. in this paper. Their herbarium accession numbers, host plants, locations, and accession numbers of the DNA databases (DDBJ, EMBL, and GenBank) are given in Table 1.

Morphological study

Herbarium materials were rehydrated before examination by boiling a small piece of infected leaf, with the fungal material downward, in a drop of lactic acid on a slide as

Table 1. Fungal name, host plant, designation, country of origin, and database accession number of internal transcribed spacer (ITS) and 28S rDNA sequence of powdery mildew specimens used in this study

Fungal name	Host plant	Herbarium accession no. ^a	Country of origin	Database accession no. ^b		
				ITS	28S	
<i>Erysiphe alphitoides</i>	<i>Quercus crispula</i>	MUMH242	Japan	AB237783	AB237811	
	<i>Quercus robur</i>	MUMH960	UK	AB237784	AB237812	
<i>E. euonymi-japonici</i>	<i>Euonymus japonicus</i>	MUMH133	Japan	AB250228	AB250230	
		MUMH2470	Argentina	AB250229	–	
<i>Erysiphe</i> sp.	<i>Quercus phillyraeoides</i>	MUMH885	Japan	AB193591	AB237813	
	<i>Anacardium occidentale</i>	MUMH781	Tanzania	AB237786	AB237814	
<i>Oidium anacardii</i>		MUMH3165	Argentina	AB237787	AB237815	
<i>O. bixae</i>	<i>Bixa orellana</i>	MUMH2606	Thailand	AB237788	–	
		MUMH3230	Thailand	AB237789	AB237816	
		MUMH3231	Thailand	AB237790	AB237817	
		VPRI30172	East Timor	AB237791	AB237818	
		VPRI30173	East Timor	AB237792	AB237819	
<i>O. citri</i>	<i>Citrus limon</i>	MUMH3210	Malaysia	AB237793	AB237820	
	<i>Citrus reticulata</i>	MUMH3188	Argentina	AB237794	AB237821	
<i>O. mangiferae</i>	<i>Mangifera indica</i>	VPRI18420	Australia	AB237795	AB237822	
		VPRI19251	Australia	AB237796	–	
		VPRI20332	Australia	AB237797	AB237823	
		VPRI20346	Australia	–	AB237824	
		VPRI20364	Australia	AB237798	AB237825	
		VPRI20379	Australia	AB237799	AB237826	
		MUMH3267	Thailand	AB237800	AB237827	
		MUMH3268	Thailand	AB237801	AB237828	
		MUMH3705	Thailand	AB237802	AB237829	
		MUMH2546	Malaysia	AB237803	AB237830	
<i>Oidium</i> sp.	<i>Acacia auriculiformis</i>	MUMH1805	Thailand	AB237804	AB237831	
		MUMH3241	Thailand	AB237805	AB237832	
		VPRI20468	Australia	AB237806	–	
		VPRI20374	Australia	AB237807	–	
	<i>Acacia holosericea</i>	VPRI20907	Australia	AB237808	AB237833	
		MUMH1183	Japan	AB237809	AB237834	
	<i>Acacia mangium</i>		MUMH3227	Indonesia	AB237810	AB237835
		<i>Acacia</i> sp.				

^aMUMH, Mie University Mycological Herbarium, Japan; VPRI, Plant Disease Herbarium, Institute for Horticultural Development, Victoria, Australia

^bDDBJ, EMBL, and GenBank database accession number of the nucleotide sequence data

described by Shin and La (1993) and Shin (2000). After the boiling process, the rehydrated material was scraped off the leaf surface into a drop of lactic acid on a slide and covered by a coverslip for light microscopy. The following information was recorded: size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of the conidiophore, e.g., size and shape of the foot cell, 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. *Oidium* species were tentatively identified based on the host.

PCR amplification and sequencing

Whole-cell DNA was extracted from conidia and mycelia and from cleistothecia for samples that have a teleomorphic stage by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). The internal transcribed spacer (ITS) regions of the nuclear rDNA including the 3'-end of the 18S (small subunit) rRNA gene, the 5.8S rRNA gene, and 5'-end of the 28S (large subunit) rRNA gene were amplified by polymerase chain reaction (PCR) with the primer pair ITS5 (White et al. 1990) and P3 (Kusaba and Tsuge 1995) for the first amplification. The first PCR products were used for the templates of the second PCR using the nested primer set ITS5/ITS4 or ITS1/ITS4 (White et al. 1990). For PCR amplification of the D1 and D2 domains of the 28S rRNA gene, the primer pair PM3 (Takamatsu and Kano 2001)/TW14 (Mori et al. 2000), PM3/NLP2 (Mori et al. 2000), and PM3/NLP1 (Mori et al. 2000) were used for the first, second, and third amplifications, respectively. PCR reactions were conducted in 50- μ l volumes as previously described (Hirata and Takamatsu 1996; Mori et al. 2000). The PCR amplicons were separated by electrophoresis on 1.5% agarose gels in TAE buffer. The desired band was visualized under long-wavelength ultraviolet light and cut out of the gel. Purification of the DNA fragment was performed utilizing the JETSORB Kit (GENOMED, Oeynhausen, Germany), as described by the manufacturer's protocol.

For ITS rDNA sequencing, both strands of the amplicons were sequenced using the primers ITS5, ITS4, ITS2 (White et al. 1990), and T4 (Hirata and Takamatsu 1996). The primers PM3 (Takamatsu and Kano 2001), NL1, NL2, NL3, and NLP2 (Mori et al. 2000), and NLP1 (5'-CACCTRCGTTCACTTTTCATTC-3') were used for 28S rDNA sequencing. The sequence reactions were conducted using the PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions and run on an Applied Biosystems 373A sequencer (Applied Biosystems).

Phylogenetic analyses

The sequences determined in this study were aligned with the sequences of the genus *Erysiphe* obtained from the DDBJ database using the Clustal V package (Higgins et al.

1992). The alignment was manually edited in MacClade 4.0 (Maddison and Maddison 2002). The alignment files of the ITS and the 28S rDNA were deposited in TreeBASE (<http://www.treebase.org/treebase/>) as S1565. Phylogenetic trees were obtained from the data using parsimony and distance methods. For parsimony analysis, we used the maximum-parsimony (MP) method with the heuristic search using PAUP* 4.0 (Swofford 2001). 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. Gaps were treated as missing data. The branch-swapping algorithm was TBR, the MulTrees option was in effect, and zero-length branches were collapsed. In distance analysis, the most appropriate evolution model was determined for a given data set using PAUP* and Modeltest 3.06 (Posada and Crandall 1998). A starting tree was obtained with the neighbor-joining (NJ) method (Saitou and Nei 1987). 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 using Akaike's (1974) information criterion (AIC). The Symmetrical Model (SYM; Zharkikh 1994) was chosen to construct trees with the neighbour-joining method. The strength of the internal branches from the resulting trees was tested by bootstrap analysis using 1000 replications (Felsenstein 1985) in both parsimony and distance analyses.

Results

Molecular phylogenetic study (ITS analyses)

The ITS sequences of the 30 powdery mildew specimens (see Table 1) were 556–557 bp in length. These sequences were aligned with 49 sequences representing sections *Microsphaera* and *Erysiphe* of the genus *Erysiphe* (anamorph, *Oidium* subgenus *Pseudoidium*) obtained from DNA databases. *Erysiphe glycines* Tai was used as an outgroup taxon based on Takamatsu et al. (1999). The alignment data matrix consisted of 77 taxa and 607 characters, in which 218 (35.9%) characters were variable and 151 (24.9%) characters were informative for parsimony analysis. This parsimony analysis using PAUP* generated 119 equally parsimonious trees of 531 steps (CI = 0.5650, RI = 0.8064, RC = 0.4556). Tree topologies were almost consistent among the 119 trees, except for small branching orders of the terminal branches and branch length. One of the 119 trees with the highest log likelihood value is shown in Fig. 1. The tree topology of the NJ tree was very similar to the MP tree (tree not shown). The 30 sequences obtained in this study were divided into two groups. Group I consisted of *O. anacardii*, *O. bixae*, *O. citri*, *Oidium* sp. on *Acacia* spp., and *O. heveae*, six *O. mangiferae* sequences, and two sequences of *Erysiphe* sp. on *Q. phillyraeoides* with strong bootstrap supports (100% in MP and NJ analyses). The sequences of *Erysiphe* sp. on *Q. phillyraeoides* were identical to *O.*

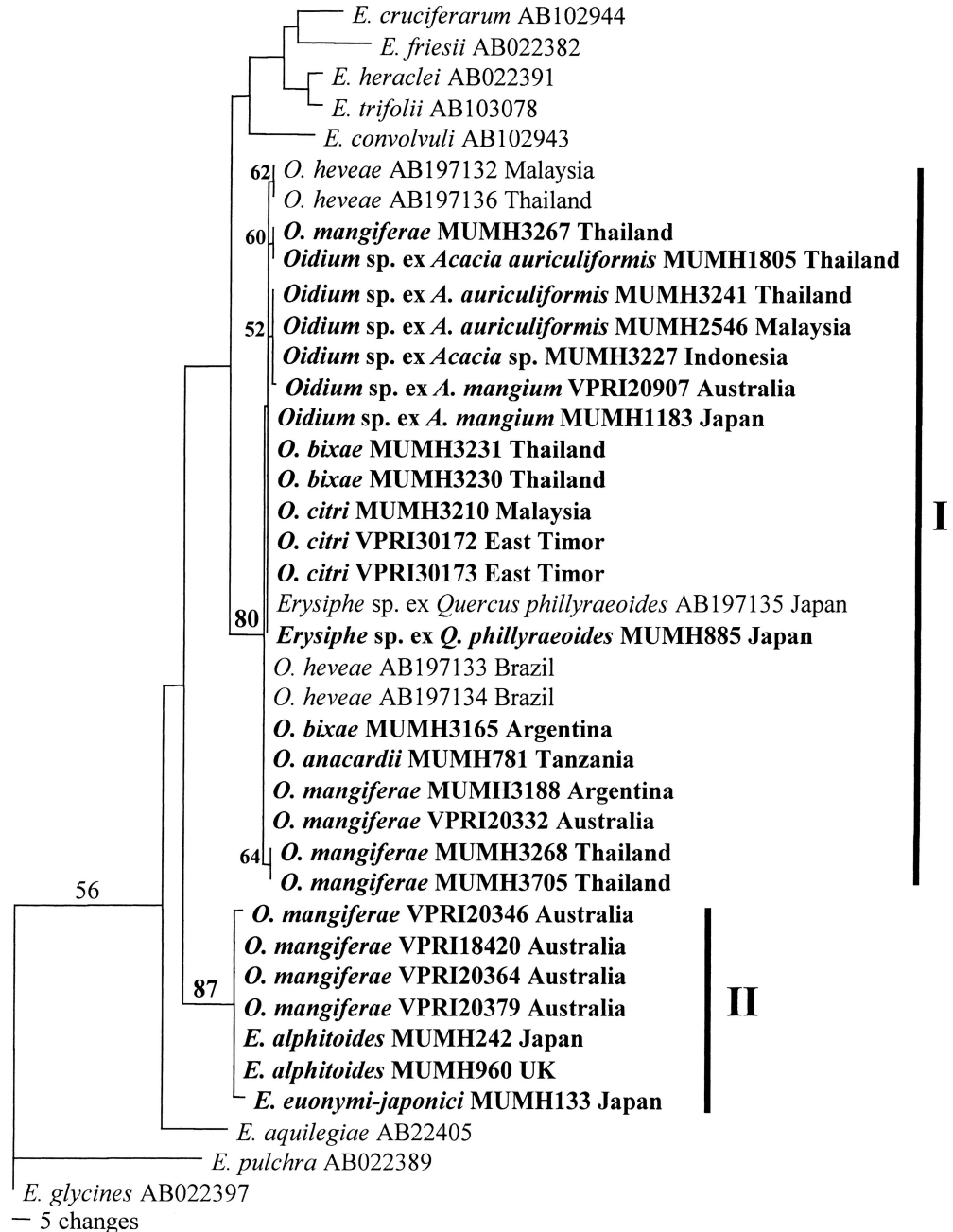
Fig. 1. One of the most parsimonious trees based on the internal transcribe spacer (ITS) sequences from 77 taxa of *Oidium* subgenus *Pseudoidium*. The tree is also the maximum-likelihood tree among the 119 equally parsimonious trees with 531 steps. The tree was obtained by a heuristic search employing the random stepwise addition option of PAUP*. Gaps were treated as missing data. Percentage bootstrap support (1000 replications) is shown above branches. Roman numerals at right of the tree show groups of powdery mildew fungi sequenced in this study. Two specimens of *Erysiphe glycines* were used as outgroup taxa. Sequences newly determined in this study are shown in *boldface*. CI = 0.5650, RI = 0.8064, RC = 0.4556



anacardii, *Oidium* sp. on *Acacia* spp., *O. heveae*, one *O. bixae*, and four *O. mangiferae* sequences, and differed only in one base to the sequences of two *O. bixae* from Thailand, *O. citri* from Malaysia and Indonesia, and two *O. mangiferae* from Thailand. Group II comprised three *O. mangiferae* sequences collected in Australia, *E. alphitoides*

from *Q. crispula* Blume and *Q. robur* L., *E. euonymi-japonici*, *E. pseudolonicerae* (E.S. Salmon) U. Braun & S. Takam., and *E. wallrothii* (U. Braun & S. Tanda) U. Braun & S. Takam. The sequences of *O. mangiferae* were identical to or different in only one base from the sequences of *E. alphitoides* on *Q. crispula* and *Q. robur*.

Fig. 2. One of the most parsimonious trees based on the 28S rDNA sequences from 38 taxa of *Oidium* subgenus *Pseudoidium*. The tree is also the maximum-likelihood tree among the two equally parsimonious trees with 174 steps. The tree was obtained by a heuristic search employing the random stepwise addition option of PAUP*. Gaps were treated as missing data. Percentage bootstrap supports (1000 replications) are shown above branches. Roman numerals at right of the tree show groups of powdery mildew fungi sequenced in this study. Sequences newly determined in this study are shown in *boldface*. CI = 0.6609, RI = 0.7668, RC = 0.5068



Molecular phylogenetic study (28S analyses)

Twenty-six sequences obtained in this study were aligned with the sequences of nine *Erysiphe* spp. and four *O. heveae* retrieved from DNA databases. *Erysiphe glycines* was used as the outgroup. Of the 667 characters, 63 (9.4%) were variable and 36 (5.4%) were informative for parsimony analysis. The parsimony analysis using PAUP* generated two equally parsimonious trees of 174 steps (CI = 0.6609, RI = 0.7668, RC = 0.5068). The two trees have a very similar topology, differing only in the branching orders of the terminal branches and branch length. One of these two trees, the tree with the higher log-likelihood value, is shown in Fig. 2. The results were very similar to the results obtained by the analysis of the ITS sequences. The sequences ob-

tained in this study were split into two different groups. Group I comprised *O. anacardii*, *O. bixae*, *O. citri*, *O. heveae*, and *Oidium* sp. on *Acacia* spp., five *O. mangiferae* sequences, and *Erysiphe* sp. on *Q. phillyraeoides* (80% or more bootstrap support in both MP and NJ analyses). Group II comprised four *O. mangiferae* sequences collected in Australia, *E. alphitoides* on *Q. crispula* and *Q. robur*, and *E. euonymi-japonici*.

Morphological study

The morphological characteristics of the powdery mildew fungi analyzed in this study are shown in Table 2. The *Oidium* species from six tropical cultivated trees were

Table 2. Morphological characteristics of *Oidium* spp. on six plants and *Erysiphe* sp. on *Quercus phillyraeoides*

	<i>O. anacardii</i> (MUMH781)	<i>O. bixae</i> (MUMH2606)	<i>O. citri</i> (MUMH3210)	<i>O. heveae</i> (Limkaisang et al. 2005)	<i>O. mangiferae</i> group 1 (MUMH3267, VPRI20332)	<i>O. mangiferae</i> group 2 (VPRI20364, VPRI20379)	<i>Oidium</i> sp. on <i>Acacia</i> spp. (MUMH118, MUMH1805)	<i>Erysiphe</i> sp. on <i>Quercus</i> <i>phillyraeoides</i> (Limkaisang et al. 2005)
Conidia:								
Shape	Ellipsoid-cylindrical	Ellipsoid-cylindrical	Ellipsoid-cylindrical	Ellipsoid-cylindrical	Ellipsoid-cylindrical	Ellipsoid-cylindrical	Ellipsoid-ovoid	Ellipsoid-cylindrical
Length (µm)	25.3–36.3	28.8–40	24.8–41.3	25.1–43.6	21.4–54	19.1–38.1	24–38	30.8–50
Width (µm)	14.1–19.1	15–20.5	13.1–17.4	13.4–23.3	14.2–25.7	9.2–16.1	13–19	15.5–21.8
l/w ratio	1.5–2.3	1.7–2.4	1.7–2.9	1.4–2.5	1.4–2.4	1.5–3.8	1.5–2.5	1.4–2.9
Germ tube	–	Polygoni-type	Polygoni-type	Polygoni-type	Polygoni-type	–	Polygoni-type	Polygoni-type
Conidiophore:								
Foot cell length (µm)	24–66.2	11.3–43.8	10.4–45.2	13.4–61.6	25.7–120.9	14.4–40.1	32–64	15.3–46.2
Foot cell width (µm)	5–7.7	6.3–8.8	6.5–8.7	7–9.7	5.8–7.8	4.4–7.8	7.5	7.3–9.7
Foot cell base	Straight	Straight	Straight	Straight	Straight	Flexuous	Straight	Straight
No. of additional cells	1–3	1–3	1–3	1–3	1–3	1–3	1–3	1–3
Appressoria	Lobed	Lobed	Lobed	Lobed	Lobed	Lobed	Lobed	Lobed

similar to each other and to *Erysiphe* sp. on *Q. phillyraeoides*. The bases of foot cells were straight on the fungi belonging to group I but flexuous on the *O. mangiferae* specimens in group II.

Discussion

We determined 25 ITS and 22 28S sequences from powdery mildew fungi isolated on tropical trees in this study. When the sequences of *O. heveae* determined in a previous study are added, 30 ITS and 26 28S sequences are included in total. They comprise six *Oidium* species belonging to subgenus *Pseudoidium* isolated from six host plant genera covering five families and four orders. The most interesting result in this study is that these sequences from different *Oidium* species and distantly related host plants are identical or closely related to each other. Another interesting result is that the DNA sequences from the powdery mildews on these tropical cultivated trees are identical or closely related to that of *E. alphitoides* and *Erysiphe* sp. on *Quercus* species.

There are some reports on the morphological similarities between powdery mildews on tropical trees and *E. alphitoides*. Boesewinkel (1980) reported that the morphology of *O. mangiferae* is consistent with *E. alphitoides* on *Q. robur* and that *O. mangiferae* can infect *Q. robur*. Braun (1987) reported that *O. anacardii* on *A. occidentale* (cashew) and *O. bixae* are similar to *O. alphitoides*, the anamorph of *E. alphitoides*. Thankamma (1968) reported that *O. heveae* on *H. brasiliensis* (rubber tree) can infect *B. orellana*, a host of *O. bixae*. These results suggest that the powdery mildews on mango, rubber tree, cashew, and *Bixa* are closely related to one another and also to *E. alphitoides*, an oak powdery mildew. These reports support the results of the present phylogenetic analyses. Boesewinkel (1981) conducted morphological observations and an inoculation test to show that *O. citri*, a powdery mildew on *Citrus* spp., is conspecific with *E. euonymi-japonici*. However, there is no report to suggest a close relationship between *O. citri* and *E. alphitoides* or between these species and powdery mildews on other tropical fruits and trees. The present molecular analyses revealed a close relationship between *O. citri*, the powdery mildew fungi on tropical trees, and *Erysiphe* sp. on *Q. phillyraeoides*, but not with *E. euonymi-japonici*.

Powdery mildew commonly occurs on *Acacia* spp. in Asian countries such as Bangladesh, Indonesia, Malaysia, Papua, Philippines, Thailand, and Vietnam (Tanaka 1986; Semangun 1992; Kawabe et al. 1998; Daidou and Ito 2001; Tamit 2003). *Erysiphe acaciae* S. Blumer occurs on *Acacia* spp. and belongs to the subgenus *Pseudoidium* of the genus *Oidium* in its anamorph (Braun 1987). But, because the powdery mildew of *Acacia* rarely produces a teleomorph, the fungus is usually identified as *Oidium* sp., and the relationships of this fungus with other powdery mildews on tropical trees are unknown. This study is the first report to indicate a close relationship between the powdery mildew

on *Acacia* spp. and the powdery mildews on other tropical trees.

Because the powdery mildews are obligately biotrophic fungi, their life cycle completely depends on living hosts, from which they obtain nutrients without killing the host cells and without which they are unable to survive. To maintain the biotrophic life cycle, powdery mildew fungi have developed highly specific and sophisticated mechanisms to avoid the resistance system of the host, to obtain nutrient resources from the host without injuring the host cells, and to synchronize their life-cycle parameters to those of the host, etc. (Aist and Bushnell 1991; Bushnell and Gay 1978; Giese et al. 1997). As a natural outcome, most species of the powdery mildew fungi show strict host specificity, in which a given species or race can infect and utilize a narrow range of host plants, or sometimes only a particular species of host (Yarwood 1957, 1978; Zheng and Chen 1981). However, recent molecular phylogenetic studies (Hirata et al. 2000; Matsuda and Takamatsu 2003) suggest that powdery mildew fungi can sometimes jump host to distantly related plants and that there are some powdery mildews with wide host ranges; these include *Golovinomyces orontii* (Castagne) V. P. Heluta (Braun 1987), *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff on Cucurbitaceae and Fabaceae (Hirata et al. 2000), and *Leveillula taurica* (Lév.) Arnaud. (Palti 1988). All these fungi occur on herbaceous plants. This expansion may be explained by considering the evolutionary history of the powdery mildew fungi. The powdery mildews were tree-parasitic in the early stage of their evolution. Then, host expansion from trees to herbs occurred numerous times during the Tertiary along with the radiation of herbaceous plants (Takamatsu 2004). In general, herb-parasitic powdery mildews have a recent origin compared with tree-parasitic species. It is possible that plant pathogens with a recent origin have wide host ranges and then specialize onto a narrow range of hosts for a long time. In this study, we suggested that *E. alphitoides* (including *Erysiphe* sp. on *Q. phillyraeoides*) is a powdery mildew with a wide host range, which is able to infect numerous plant species including tropical trees. This report may be the first to suggest the presence of a tree-parasitic powdery mildew with a wide host range.

Biogeography is an attractive subject in evolutionary biology. Boesewinkel (1980) suggested that *E. alphitoides* might have originated in the tropics and was introduced to Europe with infected mango plants, because powdery mildew of mango already occurred in the late 19th century in India. This explanation apparently looks reasonable. However, another explanation would be also possible. Host species of the powdery mildews are abundant in temperate regions of the Northern Hemisphere, but rather few are found in tropical regions and the Southern Hemisphere (Amano 1986; Braun 1987). Molecular phylogenetic analyses of the Erysiphales suggested that the hosts of the early evolutionary stage of the Erysiphales were deciduous trees such as Betulaceae, Aceraceae, Ulmaceae, Fagaceae, and Salicaceae (Mori et al. 2000; Takamatsu 2004). These trees were distributed in high-latitude areas of the Northern Hemisphere in the early Tertiary when the first radiation of

the Erysiphales occurred. The Erysiphales may have migrated southward, accompanying the global cooling of the earth that occurred during the mid and late Tertiary (Tiffney and Manchester 2001). Thus, the general geographical direction of spreading may be from north to south in the Erysiphales, which suggests that the powdery mildews originated in the Northern Hemisphere and then migrated into tropical regions and the Southern Hemisphere (Boesewinkel 1979a; Takamatsu et al. 2006). Because the genus *Quercus*, the main host genus of *E. alphitoides*, occurs in the temperate regions of the Northern Hemisphere, it may be more likely that *E. alphitoides* on *Quercus* (including *Erysiphe* sp. on *Q. phillyraeoides*) expanded its host range to tropical trees than the opposite direction. This idea suggests that, when powdery mildews expand their host range onto tropical plants, a particular powdery mildew species can jump to a wide range of host plants independent of the phylogenetic relationships of the original hosts. Further studies are required to prove whether this is a common phenomenon in the strategy of host expansion for powdery mildews or if it occurred only in this case.

Sixteen species of powdery mildew fungi covering six genera have been reported on the genus *Quercus*, Fagaceae (Hirata 1968; Braun 1987). There is no other plant genus that is infected by so many powdery mildew species, which suggests a close relationship between powdery mildews and *Quercus*. Of these powdery mildew species on *Quercus*, *E. alphitoides* is the most common and widely distributed around the world. According to Braun (1987), the Hippocastanaceae and the Anacardiaceae are also listed as host families of *E. alphitoides*, along with the Fagaceae, suggesting the possibility that *E. alphitoides* has a wide host range. *Erysiphe alphitoides* is also morphologically variable (Speer 1973; Shin 2000; Ufnalski and Przybyl 2004). In this study, we showed that *O. mangiferae* is split into two different groups based on the rDNA sequences. These groups also differed in the morphology of foot-cells. Shin (2000) and Cunnington (2002) reported that the foot-cells of *E. alphitoides* are mostly straight, but occasionally curved to flexuous. The former author regarded this difference as an intraspecific variation. Further studies are required to prove whether this variation of the foot-cells is interspecific or intraspecific. *Erysiphe hypophylla* is another powder mildew species infecting *Quercus* that mainly infects the lower surface of *Quercus* leaves, whereas *E. alphitoides* infects both the lower and upper surfaces. They differ in length/width (l/w) ratio of conidia: 2.3–3.3 in *E. hypophylla* and 1.4–2.3 in *E. alphitoides* (Braun 1987). In contrast, it is difficult to distinguish the two species by the morphology of their teleomorphs. There are arguments to consider *E. hypophylla* a synonym of *E. alphitoides* (Speer 1973; Záhorská 1986) or two distinct species (Blumer 1967; Boesewinkel 1979b; Chen et al. 1987). Biological investigations are urgently required to determine their identity (Braun 1987).

Cunnington (2002) first reported the genetic variation of *E. alphitoides*. However, because only a few collections of *E. alphitoides* were sequenced in his study, the whole variability of this species is still unclear. In this study, we re-

ported the rDNA sequences of *E. alphitoides* on *Q. crispula* and *Q. robur*, and *Erysiphe* sp. on *Q. phillyraeoides*. The powdery mildew on *Q. phillyraeoides* has been reported as *E. alphitoides* (Homma 1937; Nomura 1997). However, the present study shows that there are 11–13 substitutions (97.7%–98.2% similarity) in the ITS region and 7–9 substitutions (98.8%–99.1% similarity) in the D1/D2 domains of 28S rDNA between *E. alphitoides* on *Q. crispula* and *Q. robur*, and *Erysiphe* sp. on *Q. phillyraeoides*. Homma (1937) reported that the appendages of the fungus on *Q. phillyraeoides* are shorter than those of *E. alphitoides* on other *Quercus* species. Therefore, we tentatively classify the fungus on *Q. phillyraeoides* as *Erysiphe* sp. in this study. The taxonomic identity of this fungus will be discussed elsewhere.

In conclusion, we can report the following results based on the molecular phylogenetic analyses: (1) the powdery mildew fungi isolated from a wide range of tropical trees are closely related to each other; (2) these powdery mildews are also closely related to *E. alphitoides* (including *Erysiphe* sp. on *Q. phillyraeoides*); and (3) it is likely that *E. alphitoides* expanded its host range onto tropical trees. This is the first report to suggest that a powdery mildew fungus distributed in temperate regions of the Northern Hemisphere has expanded its host range onto tropical plants, and this may be a good example of the way in which the Erysiphales expand their geographical distribution and host ranges. Further studies are required to understand why the fungus on *Quercus* (*E. alphitoides*) has expanded its host range onto tropical trees.

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