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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*). Due to the obligate biotrophic nature of the powdery mildew fungi, the relationship between powdery mildews and their host plants is conservative. But, the present study suggests that a particular powdery mildew species expanded its host ranges on a wide range of the tropical trees. This paper 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 geographic 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 ten thousands 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, 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 due to a limited number of researchers working on this fungal group in tropical regions. Also, in tropical region, powdery mildews are usually lack teleomorphs, which are necessary for species identification. Although some of them have been described as *Oidium* species, like *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 enough to distinctly delimit the species.

Recently, molecular analysis based on nucleotide sequences of the nuclear rDNA has been used to determine 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 in order to elucidate the phylogenetic relationships among the species and to link these anamorphic fungi with their allied teleomorphs.

Materials and Methods

Sample sources

The powdery mildew specimens that include 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 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 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 leave, with the fungal material downwards, in a drop of lactic acid on a slide as described by Shin and La (1993) and Shin (2000). After boiling process, the rehydrated material was scrapped off the leave surface into a drop of lactic acid on a slide, and covered by cover–slip 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 which have 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 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 the 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 amplifications of

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 µl 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 a long wavelength ultraviolet light and cut out of the gel. Purification of the DNA fragment was performed utilizing the JETSORB Kit (GENOMED, 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'-CACCTRCGTTCACTTTCATTC-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 SN2526. 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 neighbour-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 (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., *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. 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 are 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*.

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 obtained in this study were split into two different groups. Group I comprised *O. anacardii*, *O. bixae*, *O. citri*, *O. heveae*, *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 analysed in this study are shown in Table 2. The *Oidium* species from six tropical cultivated trees were 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 twenty-five ITS and twenty-two 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, thirty ITS and twenty-six 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 observation and inoculation test to show that *O. citri*, a powdery mildew on *Cirus* 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, Philippine, 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 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 of these fungi occur on herbaceous plants. This may be explained by considering the evolutionary history of the powdery mildew fungi. The powdery mildews were tree-parasitic in the early stage of 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 may be the first report 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 late 19th 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 with the global cooling of the earth 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. This 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). Since 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 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 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 this 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. This 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. This suggests 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 variation. *Erysiphe hypophylla* is another powder mildew species that infects *Quercus* that mainly infects the lower surface of *Quercus* leaves, whereas *E. alphitoides* infects both the lower and upper surfaces. They differ in 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; Záhorovská 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 few collections of *E. alphitoides* were sequenced in his study, the whole variability of

this species is still unclear. In this study, we reported 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*); 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 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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FIGURE LEGENDS

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. 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.

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. Sequences newly determined in this study are shown in boldface. CI = 0.6609, RI = 0.7668, RC = 0.5068.

Table 1 Fungal name, host plant, designation, country of origin and database accession number of 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
Erysiphe alphitoides	*Quercus crispula*	MUMH242	Japan	AB237783	AB237811
	Quercus robur	MUMH960	UK	AB237784	AB237812
E. euonymi-japonici	*Euonymus japonicus*	MUMH133	Japan	AB250228	AB250230
	Euonymus japonicus	MUMH2470	Argentina	AB250229	–
Erysiphe sp.	*Quercus phillyraeoides*	MUMH885	Japan	AB193591	AB237813
Oidium anacardii	*Anacardium occidentale*	MUMH781	Tanzania	AB237786	AB237814
O. bixae	*Bixa orellana*	MUMH3165	Argentina	AB237787	AB237815
		MUMH2606	Thailand	AB237788	–
		MUMH3230	Thailand	AB237789	AB237816
		MUMH3231	Thailand	AB237790	AB237817
O. citri	*Citrus limon*	VPRI30172	East Timor	AB237791	AB237818
	Citrus reticulata	VPRI30173	East Timor	AB237792	AB237819
	Citrus sinensis	MUMH3210	Malaysia	AB237793	AB237820
O. mangiferae	*Mangifera indica*	MUMH3188	Argentina	AB237794	AB237821
		VPRI18420	Australia	AB237795	AB237822
		VPRI19251	Australia	AB237796	–
		VPRI20332	Australia	AB237797	AB237823

Table 1 (Continue)

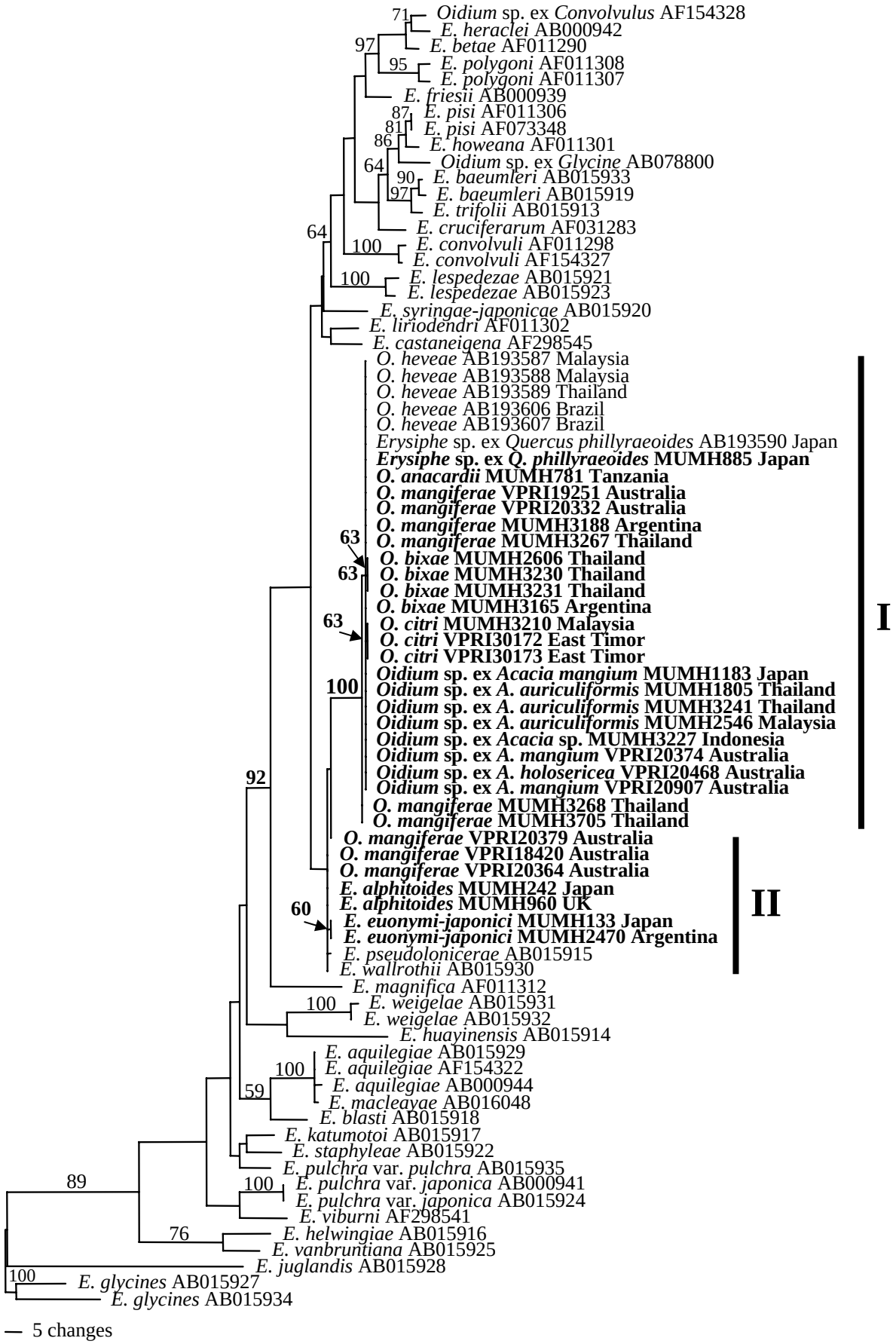
Fungal name	Host plant	Herbarium accession no.[a]	Country of origin	Database accession no.[b] ITS	28S
		VPRI20346	Australia	–	AB237824
		VPRI20364	Australia	AB237798	AB237825
		VPRI20379	Australia	AB237799	AB237826
		MUMH3267	Thailand	AB237800	AB237827
		MUMH3268	Thailand	AB237801	AB237828
		MUMH3705	Thailand	AB237802	AB237829
Oidium sp.	*Acacia auriculiformis*	MUMH2546	Malaysia	AB237803	AB237830
		MUMH1805	Thailand	AB237804	AB237831
		MUMH3241	Thailand	AB237805	AB237832
	Acacia holosericea	VPRI20468	Australia	AB237806	–
	Acacia mangium	VPRI20374	Australia	AB237807	–
		VPRI20907	Australia	AB237808	AB237833
		MUMH1183	Japan	AB237809	AB237834
	Acacia sp.	MUMH3227	Indonesia	AB237810	AB237835

[a] MUMH: Mie University Mycological Herbarium, Japan; VPRI: Plant Disease Herbarium, Institute for Horticultural Development, Victoria, Australia.

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

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

	O. anacardii (MUMH781)	*O. bixae* (MUMH2606)	*O. citri* (MUMH3210)	*O. heveae* (Limkaisang et al. 2005)	*O. mangiferae* group 1 (MUMH3267, VPRI20332)	*O. mangiferae* group 2 (VPRI20364, VPRI20379)	*Oidium* sp. on *Acacia* spp. (MUMH118, MUMH1805)	*Erysiphe* sp. on *Quercus phillyraeoides* (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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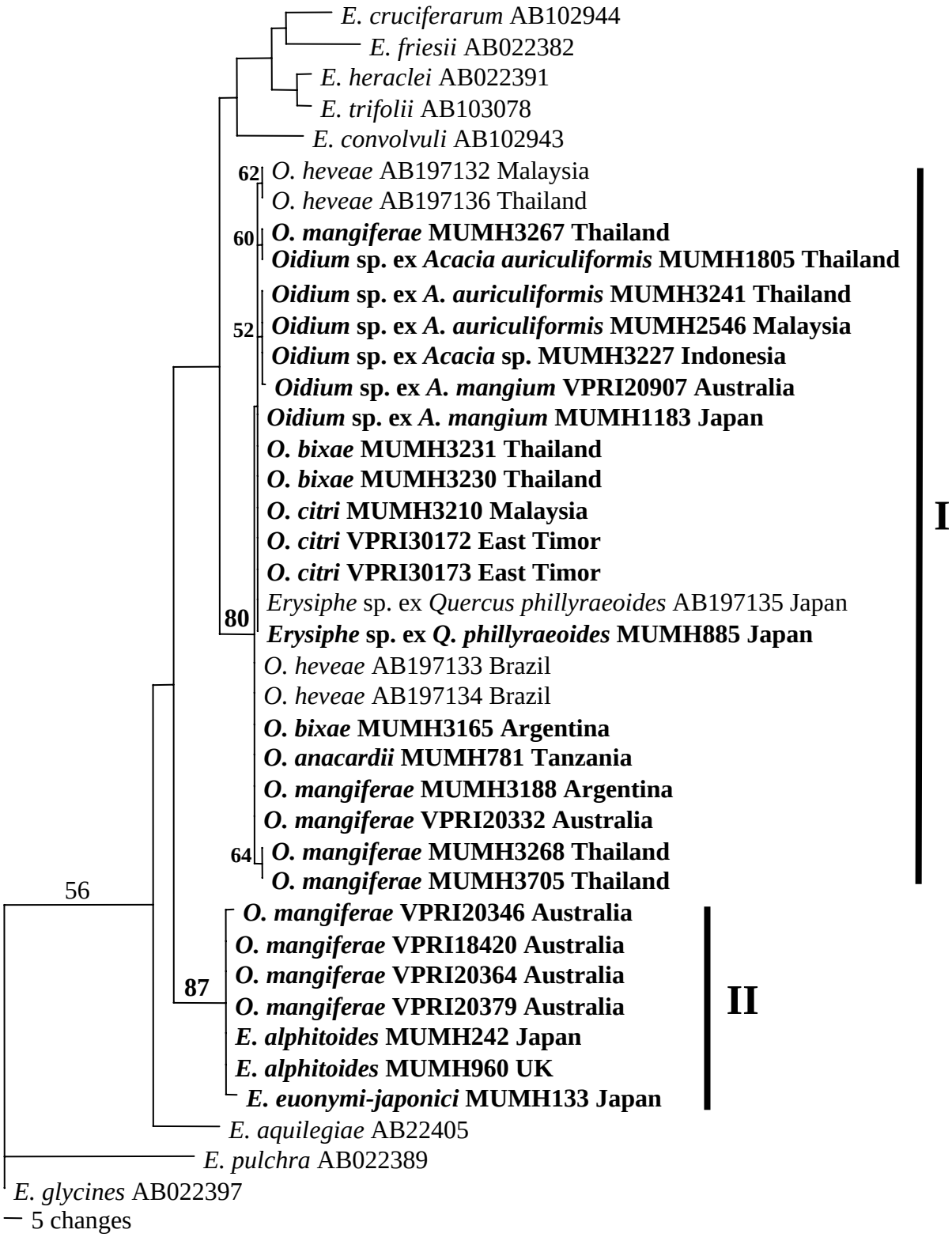
E. betae AF011290
E. polygoni AF011308
E. polygoni AF011307
E. friesii AB000939
E. pisi AF011306
E. pisi AF073348
E. howeana AF011301
Oidium sp. ex Glycine AB078800
E. baeumleri AB015933
E. baeumleri AB015919
E. trifolii AB015913
E. cruciferarum AF031283
E. convolvuli AF011298
E. convolvuli AF154327
E. lespedezae AB015921
E. lespedezae AB015923
E. syringae-japonicae AB015920
E. liriodendri AF011302
E. castaneigena AF298545
O. heveae AB193587 Malaysia
O. heveae AB193588 Malaysia
O. heveae AB193589 Thailand
O. heveae AB193606 Brazil
O. heveae AB193607 Brazil
Erysiphe sp. ex Quercus phillyraeoides AB193590 Japan
Erysiphe sp. ex Q. phillyraeoides MUMH885 Japan
O. anacardii MUMH781 Tanzania
O. mangiferae VPRI19251 Australia
O. mangiferae VPRI20332 Australia
O. mangiferae MUMH3188 Argentina
O. mangiferae MUMH3267 Thailand
O. bixae MUMH2606 Thailand
O. bixae MUMH3230 Thailand
O. bixae MUMH3231 Thailand
O. bixae MUMH3165 Argentina
O. citri MUMH3210 Malaysia
O. citri VPRI30172 East Timor
O. citri VPRI30173 East Timor
Oidium sp. ex Acacia mangium MUMH1183 Japan
Oidium sp. ex A. auriculiformis MUMH1805 Thailand
Oidium sp. ex A. auriculiformis MUMH3241 Thailand
Oidium sp. ex A. auriculiformis MUMH2546 Malaysia
Oidium sp. ex Acacia sp. MUMH3227 Indonesia
Oidium sp. ex A. mangium VPRI20374 Australia
Oidium sp. ex A. holosericea VPRI20468 Australia
Oidium sp. ex A. mangium VPRI20907 Australia
O. mangiferae MUMH3268 Thailand
O. mangiferae MUMH3705 Thailand
O. mangiferae VPRI20379 Australia
O. mangiferae VPRI18420 Australia
O. mangiferae VPRI20364 Australia
E. alphitoides MUMH242 Japan
E. alphitoides MUMH960 UK
E. euonymi-japonici MUMH133 Japan
E. euonymi-japonici MUMH2470 Argentina
E. pseudolonicerae AB015915
E. wallrothii AB015930
E. magnifica AF011312
E. weigelae AB015931
E. weigelae AB015932
E. huayinensis AB015914
E. aquilegiae AB015929
E. aquilegiae AF154322
E. aquilegiae AB000944
E. macleayae AB016048
E. blasti AB015918
E. katumotoi AB015917
E. staphyleae AB015922
E. pulchra var. pulchra AB015935
E. pulchra var. japonica AB000941
E. pulchra var. japonica AB015924
E. viburni AF298541
E. helwingiae AB015916
E. vanbruntiana AB015925
E. juglandis AB015928
E. glycines AB015927
E. glycines AB015934
I
II
5 changes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E. heraclei AB022391
E. trifolii AB103078
E. convolvuli AB102943
62
O. heveae AB197132 Malaysia
O. heveae AB197136 Thailand
60
***O. mangiferae* MUMH3267 Thailand**
***Oidium* sp. ex *Acacia auriculiformis* MUMH1805 Thailand**
***Oidium* sp. ex *A. auriculiformis* MUMH3241 Thailand**
52
***Oidium* sp. ex *A. auriculiformis* MUMH2546 Malaysia**
***Oidium* sp. ex *Acacia* sp. MUMH3227 Indonesia**
***Oidium* sp. ex *A. mangium* VPRI20907 Australia**
***Oidium* sp. ex *A. mangium* MUMH1183 Japan**
***O. bixae* MUMH3231 Thailand**
***O. bixae* MUMH3230 Thailand**
***O. citri* MUMH3210 Malaysia**
***O. citri* VPRI30172 East Timor**
***O. citri* VPRI30173 East Timor**
Erysiphe sp. ex *Quercus phillyraeoides* AB197135 Japan
80
***Erysiphe* sp. ex *Q. phillyraeoides* MUMH885 Japan**
O. heveae AB197133 Brazil
O. heveae AB197134 Brazil
***O. bixae* MUMH3165 Argentina**
***O. anacardii* MUMH781 Tanzania**
***O. mangiferae* MUMH3188 Argentina**
***O. mangiferae* VPRI20332 Australia**
64
***O. mangiferae* MUMH3268 Thailand**
***O. mangiferae* MUMH3705 Thailand**
I
56
***O. mangiferae* VPRI20346 Australia**
***O. mangiferae* VPRI18420 Australia**
***O. mangiferae* VPRI20364 Australia**
87
***O. mangiferae* VPRI20379 Australia**
***E. alphitoides* MUMH242 Japan**
***E. alphitoides* MUMH960 UK**
***E. euonymi-japonici* MUMH133 Japan**
II
E. aquilegiae AB22405
E. pulchra AB022389
E. glycines AB022397
5 changes