FULL PAPER

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Identity of a powdery mildew fungus occurring on *Paeonia* and its relationship with *Erysiphe hypophylla* on oak

Abstract A powdery mildew fungus found on *Paeonia lutea* at the Botanical Garden of Geneva (Switzerland) was identified as *Erysiphe hypophylla* based on morphological observations. The occurrence of *E. hypophylla* on *Paeonia* seemed curious, because host plants of this species have been restricted to a few *Quercus* species of the family Fagaceae. In this study, we determined the rDNA sequences of the powdery mildew specimens on *Paeonia* and *E. hypophylla* on *Quercus* to confirm the identity of the *Paeonia* fungus. The three sequences from the specimens on *P. lutea* were identical to one another in both ITS and 28S rDNA regions and also to the sequences of *E. hypophylla* on *Q. robur*, which supports the identification that the fungus on *P. lutea* is *E. hypophylla*. However, these sequences were also identical to the sequences of *E. alphitoides* on *Quercus* spp. and *Oidium mangiferae* on mango. This result suggests a possibility that *E. hypophylla* is conspecific to *E. alphitoides*. Further study is required to clarify whether *E. hypophylla* is a synonym of *E. alphitoides* or a distinct species.

Key words Erysiphales · *Erysiphe alphitoides* · *Erysiphe paeoniae* · Molecular phylogeny · *Quercus*

Introduction

Paeonia (Paeoniaceae) comprises up to 35 species of shrubs and perennial herbs distributed widely in five disjunct areas in the Northern Hemisphere: eastern Asia, central Asia, the western Himalayas, the Mediterranean region, and Pacific North America (Sang et al. 1997). *Paeonia* has been further divided into three sections: *Moutan*, *Onaepia*, and *Paeonia*. Because of their great ornamental and medicinal value, peonies have been known as "king of flowers" in China and "queen of herbs" in Greece for more than 1000 years. Two kinds of powdery mildew fungi have been reported to occur on *Paeonia* (Braun 1987), viz. *Podosphaera paeoniae* (Z.Y. Zhao) U. Braun & S. Takam., distributed only in China, and *Erysiphe paeoniae* R.Y. Zheng & G.O. Chen, the most common species on *Paeonia* widespread in Europe, Asia, North America, and Australia. Since 1998, Bolay (2001) has observed and collected powdery mildews on a few species of *Paeonia* at the Botanical Garden of Geneva (Switzerland). The fungus on *Paeonia* species of the herbaceous section *Paeonia* has ascomata with mycelioid appendages and was identified as *E. paeoniae*, whereas the fungus on a woody *Paeonia* species (section *Moutan*), viz. *P. lutea* Franch., has appendages with 4–6 times dichotomously branched apices, and belongs to the section *Microsphaera* of the genus *Erysiphe* (formerly the genus *Microsphaera*). Bolay (2001) identified the fungus as *Erysiphe hypophylla* (Nevod.) U. Braun & J.H. Cunnington based on morphological observations. The occurrence of *E. hypophylla* on *Paeonia* seemed curious, because host plants of this species have been restricted to a few *Quercus* species of the family Fagaceae (Braun 1987). Further objective verifications, such as molecular analyses, are required to clarify the identity of the fungus. In this study, we determined the nucleotide sequences of the rDNA internal transcribed spacer (ITS) region and D1/D2 domains of the 28S rDNA for the powdery mildew specimens on *Paeonia* collected in Switzerland. We also determined the sequences of *E. hypophylla* on *Quercus* to compare them with those of the *Paeonia* fungus.

Another powdery mildew, *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam., has been reported to occur on *Quercus*. There are arguments for considering *E. hypophylla* as a synonym of *E. alphitoides* (Speer 1973; Záhorovská 1986) or as a distinct species (Blumer 1967; Boesewinkel 1979; Chen et al. 1987). Thus, we also

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determined the sequences of *E. alphitoides* collected at the Botanical Garden of Geneva to discuss the phylogenetic relationship between *E. alphitoides* and *E. hypophylla*.

Materials and methods

Sample sources

Powdery mildew specimens consisting of five specimens on *Paeonia* spp. and two *E. hypophylla* and one *E. alphitoides* specimens each from *Quercus* spp. were used in this study. All the specimens were collected in Switzerland, and identified by A. Bolay based on morphological observations and deposited in his personal herbarium as well as Mie University Mycological Herbarium (MUMH). An ITS sequence (AF298538) from an *E. alphitoides* specimen collected at the Botanical Garden of Geneva was retrieved from the DNA database and included in the analyses. An *E. paeoniae* specimen collected in Japan was also included in the analyses. 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

Anamorph. Fresh hyphae, conidiophores, and conidia were stripped off from the leaf surfaces with clear adhesive tape, mounted on a microscope slide with the fungal materials uppermost, and examined in water using a light microscope. The following information was noted during the examination: size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of the conidiophore, e.g., size and shape of foot cell, position of the basal septum; shape and position of hyphal appressoria; and position of germ tubes of conidia and shape of appressoria on germ tubes of conidia.

Teleomorph. Ascomata were transferred onto a microscope slide with a needle under a dissecting microscope and observed in 3% NaOH using a light microscope. The following information was noted during the examination: size and shape of ascomata, asci, and ascospores; characteristics of appendages, e.g., number, length, color, shape of the apex; and number of asci and ascospores.

Molecular phylogenetic study

Whole-cell DNA was extracted from ascomata by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). Polymerase chain reaction (PCR) amplification and sequencing were conducted according to Limkaisang et al. (2006).

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 S1595. 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 ten 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 tree bisection/reconnection (TBR), the MulTrees option was in effect, zero-length branches were collapsed, and MaxTrees setting was 200. In distance analysis, the most appropriate evolution model was determined for a given data set using

Table 1. Fungal name and host plant of the powdery mildews on *Quercus* and *Paeonia* used in this study with voucher and sequence accession numbers

Fungal name	Host plant	Location of collection and voucher[a]	Date	Database accession no.[b]
Erysiphe alphitoides	*Quercus crispula*	Shiga, Japan; MUMH 242	31 Oct 1996	AB237783 (AB237811)[c]
	Quercus petraea	Geneva, Switzerland; MUMH 1448	21 Oct 1998	AB257435[d]
	Quercus robur	Cauterbug, UK; MUMH 960	Oct 1999	AB237784 (AB237812)[c]
	Quercus robur	Geneva, Switzerland; VPRI 22226	6 Oct 1995	AF298538
Erysiphe hypophylla	*Quercus robur*	Tochigi, Japan; VPRI 22120	Jan 1981	AF298544
	Quercus robur	Geneva, Switzerland; MUMH 1442	21 Oct 1997	AB257430[d]
	Quercus robur	Nyon, Switzerland; MUMH 1443	30 Oct 2000	AB257431[d]
	Paeonia lutea var. *ludlowii*	Geneva, Switzerland; MUMH 1444	27 Oct 1999	AB257432[d]
	Paeonia lutea var. *ludlowii*	Geneva, Switzerland; MUMH 1445	7 Nov 2000	AB257433[d]
	Paeonia lutea	Montreux, Switzerland; MUMH 1446	13 Sept 2000	AB257434[d]
Erysiphe paeoniae	*Paeonia coriacea* var. *atlantica*	Geneva, Switzerland; MUMH 1449	27 Oct 1998	AB257436[d]
	Paeonia lactiflora	Nara, Japan; MUMH 146	16 Sept 1996	AB257438[d]
	Paeonia wittmanniana	Geneva, Switzerland; MUMH 1450	27 Oct 1998	AB257437[d]

[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

[c] Sequences of the internal transcribed spacer (ITS) and 28S rDNA were separately deposited in DNA database under the accession numbers; parentheses indicate the accession number of 28S rDNA sequence

[d] Sequence newly determined in this study

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 best fit model for the data matrix was used to construct trees with the neighbor-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

Morphology of powdery mildews on *Paeonia* and *Quercus*

Morphological characters of the powdery mildew fungus found on *Paeonia lutea* Switzerland are shown in Table 2 with those of the related species. Two different powdery mildews were found on *Paeonia* spp. One species was identified as *Erysiphe paeoniae* based on the mycelioid appendages and the characteristics of teleomorph and anamorph. The second species on *P. lutea* differed distinctly from *E. paeoniae* by the appendages with 4–6 times dichotomously branched apices and belongs to the section *Microsphaera* of genus *Erysiphe* (formerly the genus *Microsphaera*). Characteristics of the powdery mildew on *P. lutea* were similar to *E. hypophylla* on *Quercus robur* collected at the botanical garden and description of the species by Braun (1987)

(Table 2). Therefore, the fungus found on *P. lutea* was identified as *E. hypophylla*.

Molecular phylogenetic study (ITS analyses)

The ITS sequences of the nine powdery mildew specimens on *Paeonia* spp. and *Quercus* spp. were aligned with 64 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 73 taxa and 607 characters, in which 220 (36.2%) characters were variable and 155 (25.5%) characters were informative for parsimony analysis. This parsimony analysis using PAUP* generated 200 equally parsimonious trees of 562 steps [consistency index (CI) = 0.5463, retention index (RI) = 0.7794, rescaled consistency index (RC) = 0.4258]. Tree topologies were almost consistent among the 200 trees, except for small branching orders of the terminal branches and branch length. One of the 200 trees with the highest log-likelihood value is shown in Fig. 1. The tree topology of the NJ tree was similar to the MP tree (tree not shown). The two *E. paeoniae* sequences collected in Switzerland formed a clade with a sequence of *E. paeoniae* collected in Japan with 100% bootstrap support. There were seven nucleotide substitutions (1.3% in diversity) between the sequences from Switzerland and Japan in the ITS region. The three ITS sequences from the specimens on *P. lutea* were identical to one another and differed from *E. paeoniae* in 35 nucleotides (6.3% in diversity). These se-

Table 2. Morphological characters of powdery mildews on *Paeonia* and *Quercus*

Morphological characters	*Erysiphe hypophylla* ex *Paeonia lutea* (MUMH1444–1446)	*E. paeoniae* ex *Paeonia* spp. (MUMH1449–1450)	*E. hypophylla* ex *Quercus robur* (MUMH1442–1443)	*E. hypophylla* (Braun 1987)	*E. alphitoides* (Braun 1987)
Mycelium	amphigenous, mostly hypophyllus	amphigenous	amphigenous, mostly hypophyllus	amphigenous, mostly hypophyllus	amphigenous
Appressorium	lobed, multilobed	simple lobed	lobed, multilobed	multilobed	lobed
Foot-cell of conidiophore	15–30 × 6–7 μm flexuous	10–40 × 5–8 μm	20–28 × 7.5 μm flexuous	20–28 × 7.5 μm flexuous	15–30 × 6–9(–10) μm straight
Conidium	solitary, cylindrical-ellipsoid	solitary ellipsoid, cylindrical or doliform	solitary, cylindrical, ellipsoid	solitary, cylindrical (-ellipsoid)	solitary ellipsoid-ovoid to doliform
	25–39 × 10–14.5 μm	25–40 × 10.5–16 μm	25–45 × 12–21 μm	30–45 × 12–17.5 μm l/w ratio 2.3–3.3	25–40 × 13–23 μm l/w ratio 1.4–2.3
Ascoma	scattered to gregarious 90–110(–125) μm	(70–)90–120 μm	scattered to gregarious 80–110(–140) μm	scattered, subgregarious (70–)80–140(–155) μm	scattered, subgregarious 70–180 μm
Appendage	2–20 per ascoma equatorial 0.7–1.3 times long of ascomatal diameter 4–6(–8) times branched	0.25–1.0 times long of ascomatal diameter mycelioid	10–30 per ascoma equatorial 1–1.5 times long of ascomatal diameter 4–8 times branched	5–25 per ascoma equatorial 1–1.5 times long of ascomatal diameter 5–6 times branched	4–28 per ascoma equatorial 0.75–1.5 times long of ascomal diameter 4–6(–7) times branched
Ascus	not observed	(3–)4–6(–8) per ascoma 50–70 × 30–45 μm	(3–)4–6 per ascoma	4–10 per ascoma 50–70 × 30–50 μm	5–16 per ascoma 45–80 × 30–55 μm
Ascospore	ellipsoid-ovoid 17.5–25 × 7–12.5 μm	(2–)3–5(–7) per ascus ellipsoid, rarely ovoid 18–25 × 10–13.5 μm	6–8 per ascus ellipsoid-ovoid 14–26 × 10–14 μm	(4–)6–8 per ascus ellipsoid-ovoid 16–25 × 9–14.5 μm	(4–)6–8 per ascus mostly 8 ellipsoid-ovoid to subglobose (14–)16–26 × 9–15 μm

Fig. 1. One of the most parsimonious trees based on the internal transcribed spacer (ITS) sequences from 73 taxa of *Oidium* subgenus *Pseudoidium*. The tree is also the maximum-likelihood tree among the 200 equally parsimonious trees with 562 steps. The tree was obtained by a heuristic search employing the random stepwise addition option of PAUP* (Swofford 2001). 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*. Consistency index (CI) = 0.5463, retention index (RI) = 0.7794, rescaled consistency index (RC) = 0.4258
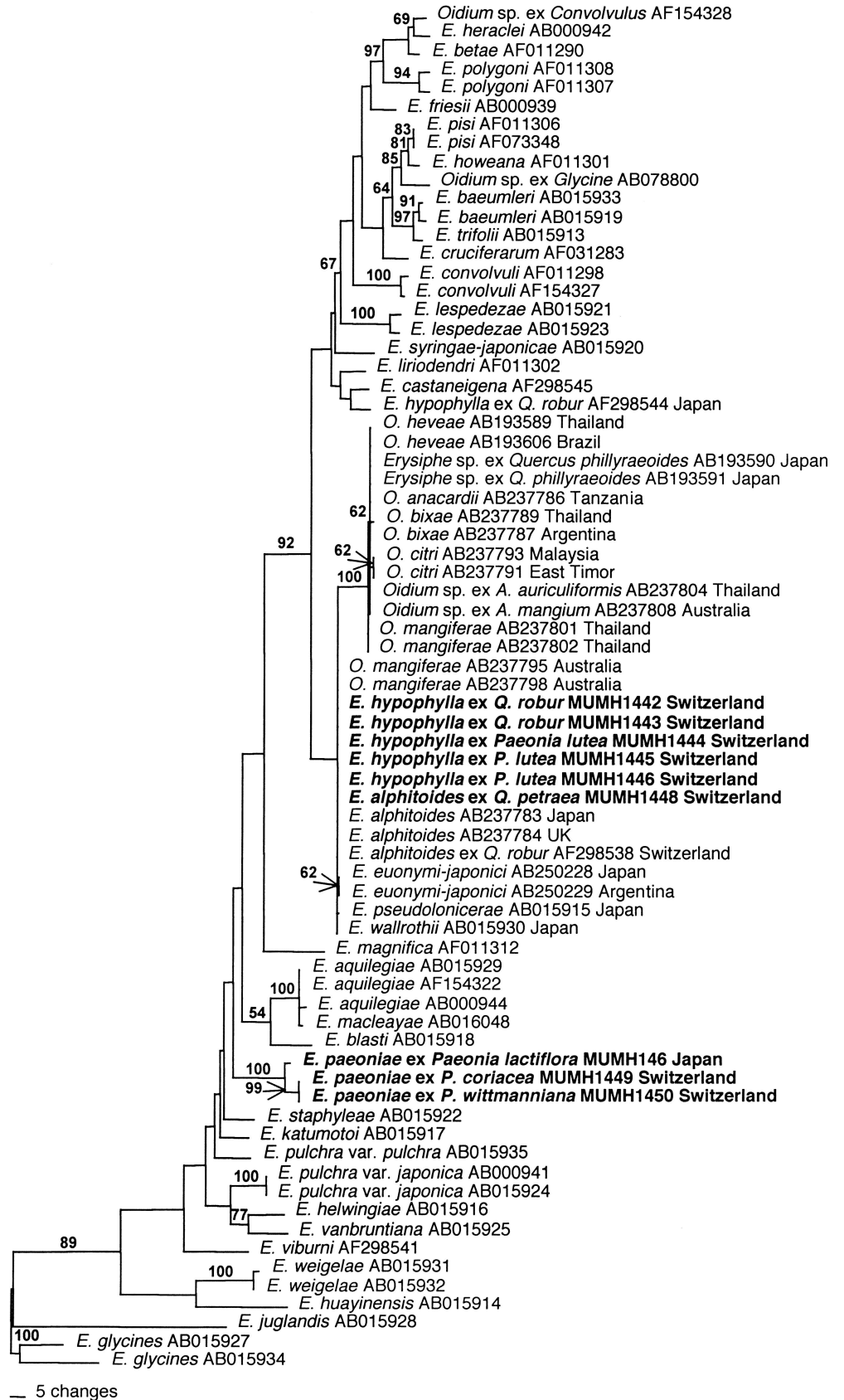
quences were identical to the sequences of *E. hypophylla* on *Q. robur* and also to the sequences of *E. alphitoides* collected in Europe and Japan and *O. mangiferae* collected in Australia. However, they differed from the sequence (AF298544) of *E. hypophylla* collected in Japan (VPRI 22120) in ten nucleotides.

Molecular phylogenetic study (28S analyses)

Seven sequences obtained in this study were aligned with 36 sequences representing sections *Microsphaera* and *Erysiphe* of the genus *Erysiphe* (anamorph, *Oidium* subgenus *Pseudoidium*) obtained from DNA databases. *Erysiphe glycines* was used as the outgroup taxon. Of the 667 characters, 63 (9.4%) were variable and 35 (5.2%) were informative for parsimony analysis. The parsimony analysis using PAUP* generated nine equally parsimonious trees of 186 steps (CI = 0.6183, RI = 0.7739, RC = 0.4785). The nine trees have a very similar topology, differing only in the branching orders of the terminal branches and branch length. One of these nine trees, the tree with the highest log-likelihood value, is shown in Fig. 2. The tree topology of the NJ tree was similar to the MP tree (tree not shown). The three 28S sequences from the specimens on *P. lutea* were identical to one another and differed from *E. paeoniae* in 17 nucleotides (2.9% in diversity). These sequences were identical to the sequences of *E. hypophylla* on *Q. robur*, and also to the sequences of *E. alphitoides* collected in Europe and Japan and *O. mangiferae* collected in Australia.

Discussion

Two powdery mildew species, viz. *Podosphaera paeoniae* and *Erysiphe paeoniae*, have been reported on *Paeonia* spp. (Braun 1987). The fungus on *P. lutea* found in Switzerland distinctly differs from the two species in its appendages with 4–6 times dichotomously branched apices and belongs to the section *Microsphaera* of the genus *Erysiphe*. Based on the morphological comparisons with species of the section *Microsphaera*, Bolay (2001) identified the fungus on *P. lutea* as *E. hypophylla*, a powdery mildew species on *Quercus* (oak tree). Powdery mildew fungi are obligate biotrophs of plants, and their host ranges are usually restricted in a narrow range of host plants. It was curious that a single powdery mildew species occurs on distantly related plants such as *Quercus* (Fagaceae) and *Paeonia* (Paeoniaceae). We thus conducted molecular phylogenetic analyses of the fungus on *P. lutea* to confirm the identification of Bolay (2001). We determined the nucleotide sequences of the rDNA for each five and three samples of the powdery mildew fungi on *Paeonia* and *Quercus*, respectively, collected in Switzerland, and used them for phylogenetic analyses with other available sequences of the genus *Erysiphe* retrieved from DNA database. The three sequences from *E. hypophylla* on *P. lutea* differed from *E. paeoniae* in both ITS and 28S rDNA regions and were identical to the sequences of *E.*
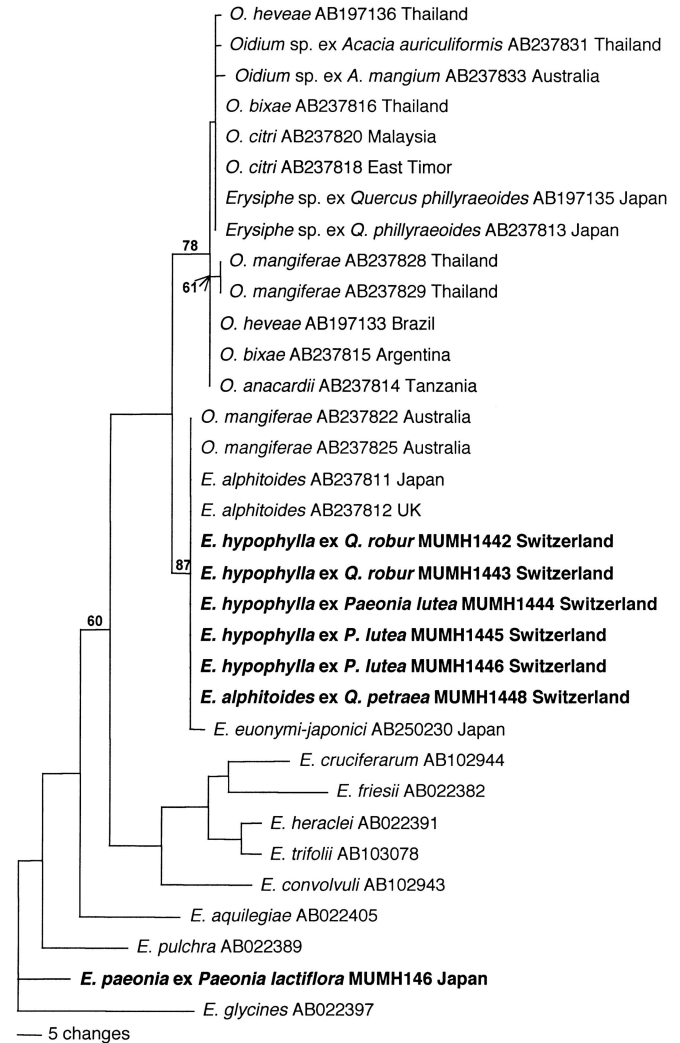

Fig. 2. One of the most parsimonious trees based on the 28S rDNA sequences from 33 taxa of *Oidium* subgenus *Pseudoidium*. The tree is also the maximum-likelihood tree among the nine equally parsimonious trees with 186 steps. The tree was obtained by a heuristic search employing the random stepwise addition option of PAUP* (Swofford 2001). 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 = 0.6183, RI = 0.7739, RC = 0.4785

hypophylla on *Q. robur*, supporting the identification of Bolay (2001), who referred the fungus on *P. lutea* to *E. hypophylla*.

The genus *Paeonia* is divided into three sections, viz. *Moutan*, *Onaepi*, and *Paeonia*, which are supported by molecular phylogenetic analyses (Sang et al. 1997; Tang and Sang 2001). Occurrence of powdery mildew has been reported only on species of the section *Paeonia*, whereas powdery mildews have not been recorded on species of the sections *Moutan* and *Onaepi* (Amano 1986; Braun 1987). *Paeonia lutea*, belonging to the woody section *Moutan*, is distributed in Yunnan and Sichuan of China and in southeastern Tibet. However, there is no record of powdery mildew on this plant in these regions. The record in Switzerland (Bolay 2001) is the first of a powdery mildew

on a host from the section *Moutan*, suggesting that *E. hypophylla* expanded its host range to *P. lutea* from *Quercus* in Switzerland.

Five *Erysiphe* species belonging to the subgenus *Microsphaera* have been recorded on *Quercus* spp. (Braun 1987). Of these, three species, viz. *Erysiphe abbreviata* (Peck) U. Braun & S. Takam., *Erysiphe extensa* (Cooke & Peck) U. Braun & S. Takam., and *Erysiphe calocladophora* (G.F. Atk.) U. Braun & S. Takam., are distributed in North America, whereas *E. alphitoides* and *E. hypophylla* are widely distributed around the world. *Erysiphe hypophylla* mainly infects the lower surface of *Quercus* leaves, whereas *E. alphitoides* infects both the lower and upper surfaces. They distinctly differ in l/w ratio of conidia: 2.3–3.3 in *E. hypophylla* and 1.4–2.3 in *E. alphitoides* (Blumer 1967; 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* as a synonym of *E. alphitoides* (Speer 1973; Záhorovská 1986) or as a distinct species (Blumer 1967; Boesewinkel 1979; Chen et al. 1987). Biological investigations have been urgently required to determine the identity of the two taxa concerned (Braun 1987).

In this study, we determined the rDNA sequences of two *E. hypophylla* specimens on *Q. robur* and one *E. alphitoides* specimen on *Q. petraea*. Unexpectedly, the three rDNA sequences of *E. hypophylla* and *E. alphitoides* on *Quercus*, and three sequences of *E. hypophylla* on *P. lutea*, were identical to one another, and also to the sequences of *E. alphitoides* and *Oidium mangiferae* retrieved from DNA database. In contrast, an ITS sequence of *E. hypophylla* (AF298544) reported by Cunnington (2002) collected in Japan differed from our *E. hypophylla* sequences in ten nucleotides and belonged to a different clade (see Fig. 1). We identified *E. hypophylla* and *E. alphitoides* according to the monograph of Bolay (2005) as follows. The infections caused by *E. hypophylla* are only visible from middle August to October, when the ascomata are present on the undersurface of the leaves. The disease can be recognized only from a very short distance (less than 1 m); mycelium hypophyllous, rarely amphigenous, thin, effuse, evanescent; infected leaves neither deformed nor necrotic. Conidia rare, cylindrical-ellipsoid, 25–48(–60) × 10–21 μmm. On the other hand, the infections caused on the leaves by *E. alphitoides* are visible early in the year, in Switzerland in May–June. The disease can be recognized from a long distance (>100 m); mycelium on the leaves first epiphyllous, then amphigenous, persistent, mostly patchy, dense, often covering the entire leaf surface; leaves often deformed, distorted and with necroses; infection also on bark of the top of new shoots. Numerous conidia are produced during the entire growing period of the oak trees. They are ellipsoid, ovoid, or doliiform, 20–40 × 10–25 μm. However, the present result suggests that these characteristics are not appropriate to distiguish *E. hypophylla* from *E. alphitoides*. An alternative explanation could be that *E. hypophylla* and *E. alphitoides* are conspecific and the morphological differences are an intraspecific variation of *E. alphitoides*. The morphological characters of *E. hypophylla* VPRI 22120 (DNA sequence AF298544) are unkown because Cunnington (2002) did not describe the morphology of the specimen. We thus cannot evaluate the validity of the identification of Cunnington (2002).

Various questions are still unanswered. *Erysiphe hypophylla* first occurred in and was described from Russia in east Europe and spread gradually westward in the 1950s (Blumer 1967). The origin of this species is still unclear, but can be supposed to be in Asia, possibly China or Japan. It seems that *E. hypophylla* has later been gradually replaced by *E. alphitoides*. During the past 25 years, *E. hypophylla* has not been recollected in Germany (U. Braun, personal communication). All "suspect" specimens proved to be hypophyllous samples of *E. alphitoides*, always formed as so-called mixed infections. Further studies are required to determine whether *E. hypophylla* is a synonym of *E. alphitoides* or a distinct species. We are now conducting a comprehensive study of *E. alphitoides* and its relatives using molecular analyses. The results will be reported elsewhere.

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