

FULL PAPER

Yoshiaki Shiroya · Chiharu Nakashima
Susumu Takamatsu

Erysiphe monascogera sp. nov., an unusual powdery mildew fungus found on fruits of *Styrax japonica*

Received: July 11, 2007 / Accepted: January 23, 2008

Abstract Morphological observations using light and scanning electron microscopes and molecular phylogenetic analysis revealed that the fungus growing on the surface of fruits or sepals of *Styrax japonica* collected at Nagano, Japan, is a new powdery mildew with an unusual morphology, described here as *Erysiphe monascogera*. This fungus has mainly a single ascus in a chasmothecium, but molecular phylogenetic analysis and the shape of the hyphal appressoria suggest that it is an *Erysiphe* species. *Erysiphe monascogera* is a sister-species to *E. nomurae* on *Symplocos chinensis* var. *leucocarpa* f. *pilosa*, although there are obvious morphological differences between the two species. This inconsistency between molecular phylogeny and morphology may be explained by the unique habitat of *E. monascogera*. *Erysiphe monascogera* and *E. nomurae* are included in a clade composed of the *E. alphitoides* complex, which suggests that these two species diverged by host jumping of the *E. alphitoides* complex, having oaks as major host plants.

Key words Erysiphaceae · Erysiphales · *Erysiphe* · Molecular phylogeny · New species

Introduction

The Styracaceae is a woody, dicotyledonous plant family containing 11 genera and about 160 species (Fritsch et al. 2001). This family is distributed widely in the world, from warm temperate to tropical areas of the Americas, the lands bordering the western Pacific Rim, and the Mediterranean region. *Styrax* is the largest genus in the Styracaceae, consisting of about 130 species of trees and shrubs distributed

in eastern and southeastern Asia, the New World (mainly tropical), and the Mediterranean region (Fritsch 1999). Japanese snowbell (*Styrax japonica* Siebold & Zucc.) is a deciduous tree endemic to eastern Asia, i.e., Japan, China, and Korea, and is frequently planted in gardens because it bears beautiful white flowers in the early summer season.

Four species (varieties) of powdery mildew fungi have been reported to occur on the Styracaceae: *Erysiphe miyabeana* (U. Braun) U. Braun & S. Takam. (≡ *Microsphaera miyabeana* U. Braun) and *E. togashiana* (U. Braun) U. Braun & S. Takam. var. *togashiana* (≡ *Uncinula togashiana* U. Braun var. *togashiana*) from the genus *Styrax*, and *E. togashiana* var. *rigida* (U. Braun & Tanda) U. Braun & S. Takam. (≡ *U. togashiana* var. *rigida* U. Braun & Tanda) and *Phyllactinia guttata* (Wallr.) Lév. from the genus *Pterostyrax* (Braun 1987; Braun and Takamatsu 2000). All these fungi colonize only leaves. In late September of 2005, we found a plentiful number of chasmothecia (fruiting bodies of powdery mildew fungi) on the surface of fruits and sepals of *Styrax japonica* at the campus of the Faculty of Agriculture, Shinshu University, Minami-Minowa Mura, Nagano Prefecture, Japan. Chasmothecia are found only on the surface of fruits and sepals, never on leaves. Morphological observation using light and scanning electron microscopes, and molecular phylogenetic analysis using rDNA internal transcribed spacer (ITS) and 28S ribosomal DNA (rDNA) sequences, revealed that the fungus is a new species of the powdery mildew fungi with an unusual morphology.

Materials and methods

Morphological studies

Specimens on fruits of *Styrax japonica* were examined by standard light microscopy (Axio Imager; Carl Zeiss, Göttingen, Germany) and differential-interference-contrast optical instruments and devices.

For scanning electron microscopic (SEM) observation, dried fruits with chasmothecia were cut into small pieces,

Y. Shiroya · C. Nakashima · S. Takamatsu (✉)
Graduate School of Bioresources, Mie University, 1577 Kurimamachiya, Tsu, Mie 514-8507, Japan
Tel. +81-59-231-9497; Fax +81-59-231-9450
e-mail: takamatu@bio.mie-u.ac.jp

fixed with OsO₄ gas at room temperature for 12 h, and then coated with gold using an ion-sputterer (model E-1010; Hitachi, Tokyo, Japan). Specimens were observed with a SEM (S-4000; Hitachi) at 15 kV accelerating voltage.

The specimens examined are deposited at MUMH (Herbarium, Faculty of Bioresources, Mie University, Tsu, Japan), TNS (Herbarium of the National Museum of Nature and Science, Tsukuba, Japan), and HAL [Martin-Luther-University, Institute of Biology, Geobotany and Botanical Garden, Herbarium, Halle (Saale), Germany].

Molecular phylogenetic study

Isolation of whole-cell DNA was performed using the chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The 5'-end of the 28S rDNA, including the domains D1 and D2, and ITS region, including the 5.8S rDNA, were amplified by polymerase chain reaction (PCR) and then sequenced using direct sequencing as described in Takamatsu et al. (2006). DNA sequences determined in this study were deposited in DDBJ (DNA databank of Japan) under the accession numbers AB331645–AB331648.

The sequences were initially aligned using the Clustal X package (Thompson et al. 1997). The 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 the accession number S1882. 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 (Huelsenbeck and Ronquist 2001). 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 maximum tree number was set as 10⁴. The strength of the internal branches of the resulting trees was tested with bootstrap (BS) analyses using 1000 replications with the stepwise addition option set as simple (Felsenstein 1985). BS values higher than 70% are provided.

For Bayesian phylogenetic analyses, the best-fit evolutionary model was determined for each data set by comparing different evolutionary models via the Akaike information criterion (AIC) using PAUP* and MrModeltest 2.2 (Nylander 2004). MRBAYES was launched with random starting trees for 10⁶ generations and the Markov chains were sampled every 100 generations, which resulted in 10⁴ sampled trees. To ensure that the Markov chain did not

become trapped in local optima, we used the MCMCMC algorithm, performing the estimation with four incrementally heated Markov chains. Of the resulting 10⁴ trees, the first 2000 (burn-in) were discarded. The remaining 8000 trees were summarized in a majority-rule consensus tree, yielding the probabilities of each clade being monophyletic. Bayesian posterior probability (PP) values higher than 0.95 are provided.

Results

Phylogenetic placement of *Styrax* powdery mildew in the Erysiphaceae: 28S rDNA analysis

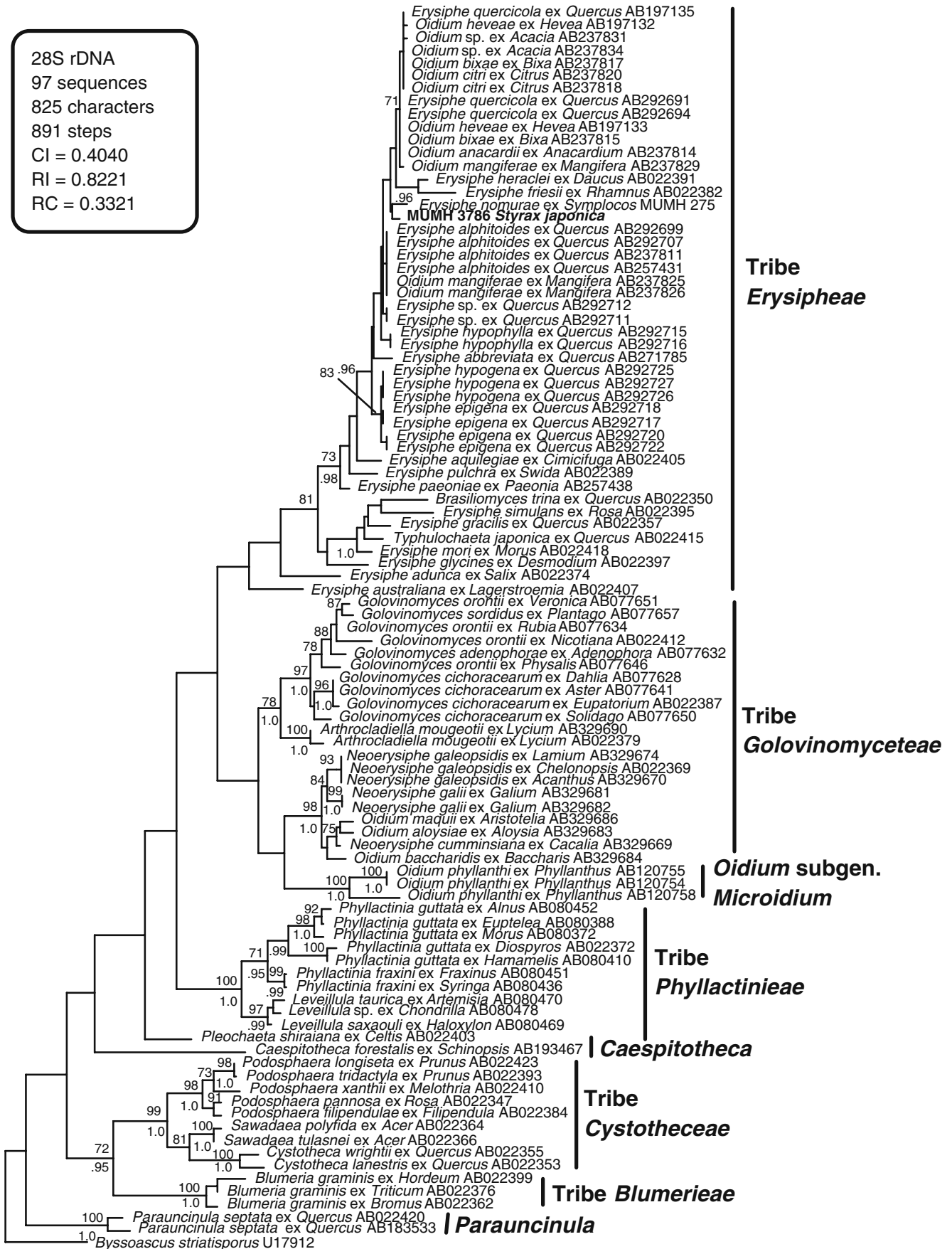
A total of 97 sequences of 28S rDNA, including a sequence from the *Styrax* powdery mildew, were used to construct the phylogenetic tree of the Erysiphaceae. *Bysoascus stratisporus* (G.L. Barron & C. Booth) Arx (Myxotrichaceae) was used as an outgroup taxon, based on Mori et al. (2000). The data set consisted of 825 characters, of which 239 characters were variable and 184 characters were phylogenetically informative for parsimony analysis. A total of 936 equally MP trees with 891 steps (CI = 0.4040, RI = 0.8221, RC = 0.3321) were constructed by the MP analysis. A tree with the highest likelihood score among the 936 MP trees is shown in Fig. 1. Most internal branches are supported in the strict consensus of the 936 trees. Bayesian analysis generated similar tree topology.

The previous phylogenetic analyses of the Erysiphaceae demonstrate that five tribes and two basal genera are included in the family (Mori et al. 2000; Braun and Takamatsu 2000; Takamatsu et al. 2005a,b). The present analysis supports the monophyly of three tribes, i.e., tribes Erysipheae, Cystothecae, and Blumerieae. The tribe Phyllactinieae is paraphyletic to the tribes Erysipheae and Golovinomyceteae. The tribe Golovinomyceteae groups with *Oidium* subgenus *Microidium* (To-anun et al. 2005) to form a clade together. *Caespitotheca* S. Takam. & U. Braun is a sister to the large clade composed of the tribes Erysipheae, Golovinomyceteae, and Phyllactinieae. *Parauncinula* S. Takam. & U. Braun occupies the most basal position of the Erysiphaceae. The fungus MUMH 3786 on *Styrax japonica* is placed in the genus *Erysiphe* and is sister to *E. nomurae* (U. Braun) U. Braun (\equiv *Microsphaera nomurae* U. Braun) from *Symplocos chinensis* (Lour.) Druce var. *leucocarpa* (Nakai) Ohwi f. *pilosa* (Nakai) Ohwi, but this is not supported by BS and PP values. The fungus MUMH 3786 on *Styrax japonica* is also closely related to the *E. alphitoides* complex (Takamatsu et al. 2007).

Fig. 1. Phylogenetic analysis of the divergent domains D1 and D2 sequences of the 28S rDNA for 97 sequences from the Erysiphaceae covering all known tribes and one outgroup taxon. The tree is a phylogram of the maximum-likelihood tree among the 936 most parsimonious trees with 891 steps, which was obtained by a heuristic search employing the random stepwise addition option of PAUP*. Gaps were

treated as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. Percentage bootstrap support (1000 replications; >70%) and posterior probability (>0.95) are shown on and under branches, respectively

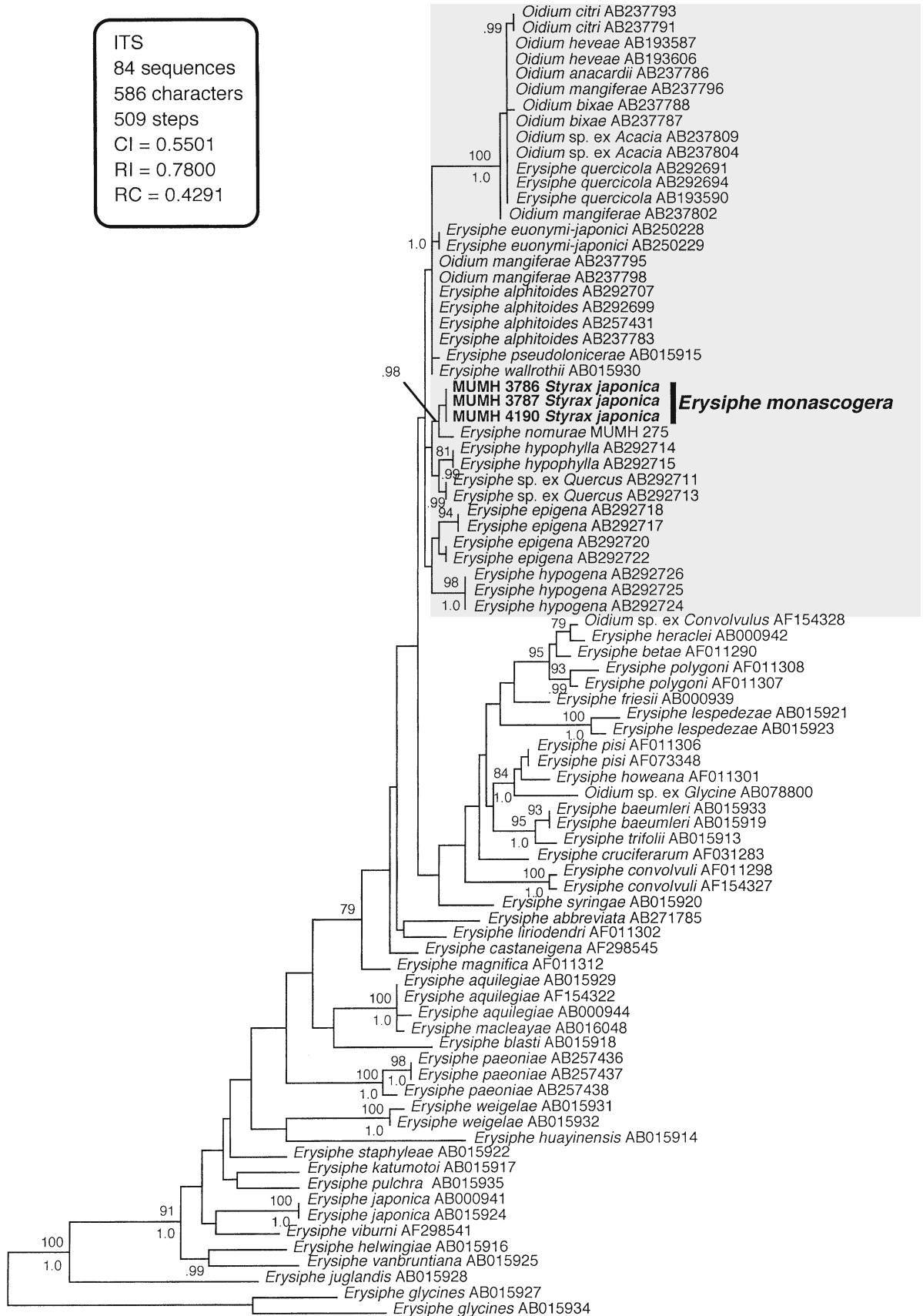
28S rDNA
 97 sequences
 825 characters
 891 steps
 CI = 0.4040
 RI = 0.8221
 RC = 0.3321



— 5 changes

Erysiphe alphitoides group

ITS
 84 sequences
 586 characters
 509 steps
 CI = 0.5501
 RI = 0.7800
 RC = 0.4291



— 5 changes

Phylogeny within *Erysiphe*: ITS analysis

A total of 81 ITS sequences from *Erysiphe* (sections *Erysiphe* and *Microsphaera*) were aligned with three sequences of the fungus on *Styrax japonica*. The data set consisted of 607 characters, of which 21 characters were removed from the analysis because of ambiguous alignment. Of the remaining 586 characters, 208 characters were variable and 146 characters were phylogenetically informative for parsimony analysis. A total of 1805 equally MP trees with 509 steps (CI = 0.5501, RI = 0.7800, RC = 0.4291) were constructed by the MP analysis. A tree with the highest likelihood score among the 1805 MP trees is shown in Fig. 2. Most internal branches are supported in the strict consensus of the 1805 trees. Bayesian analysis generated similar tree topology.

The three ITS sequences from the *Styrax* fungus are identical to each other and group with *E. nomurae* again. This finding is supported with a PP value of 0.98, but the support of BS is less than 50%. Both the *Styrax* fungus and *E. nomurae* are included in the clade of the *E. alphitoides* species complex group.

Taxonomy

Erysiphe monascogera Shiroya, C. Nakash. & S. Takam., sp. nov. Figs. 3–9
Mycobank no.: MB510967

Mycelio epigeno in fructibus, etiam in sepalis, nunquam in foliis, hyalino, appressoriis multilobatis, solitariis vel oppositis; chasmotheciis adpersis, fusco-brunneis, globosis vel subglobosis, 61–86(–92) μm diametro, ex cellulis polygonis irregularibus 7–11 \times 11–20 μm diametro compositis; appendicibus ex zona aequatoria vel prope basin chasmothecii 2–6 nascentibus, simplicibus, mycelioidibus, raro ramosis, sinuosis vel geniculatis, (4–)5–9 μm latis, diametro ascumatis 0.3–3plo longioribus, 2–3-septatis, fusco-brunneis, deorsum hyalinis; ascis saepe singularibus, raro duobus, subglobosis, sessilibus, 36–50.5 μm ; ascosporis 6–8, ovatis, hyalinis, 7.8–12.4(–14.4) \times (13.2–)14.2–19.6 μm .

Typus: Japan, Nagano Prefecture, Minami-Minowa Mura, campus of Faculty of Agriculture, Shinshu University, on *Styrax japonica* Siebold & Zucc. (Styracaceae), 25 Sep 2005, leg. Y. Shiroya and S. Takamatsu (Holotypus, TNS-F-15700; isotypus, MUMH 3787).

rDNA sequence ex isotype (MUMH 3787): AB331646.

Etyymology: “*monascogera*” refers to having a single ascus per chasmothecium.

Colonies: Mycelia on the surface of fruits and sepals, not on leaves, hyaline. Appressoria multilobed, single or opposite in pairs.

Teleomorph: Chasmothecia scattered, 61–86(–92) μm diameter, blackish-brown, globose or subglobose. Wall cells irregularly polygonal, 7–11 \times 11–20 μm diameter. Appendages 2–6, arising from the equatorial zone or lower half of the chasmothecia, simple, mycelioid, rarely irregularly branched, faintly sinuous to geniculate, (4–)5–9 μm wide, variable in length, usually 0.3–3 times as long as the chasmothecium diameter, 2–3 septate, dark brown from the base up to the 1st or 2nd septum and becoming hyaline upward, thin-walled. Asci single or very rarely two per chasmothecium, 36–50.5 μm , globose, sessile. Ascospores 6–8 per ascus, ovate, subhyaline, 7.8–12.4(–14.4) \times (13.2–)14.2–19.6 μm .

Anamorph: Unknown.

Host range and distribution: on the surface of fruits and sepals of *Styrax japonica*, Japan.

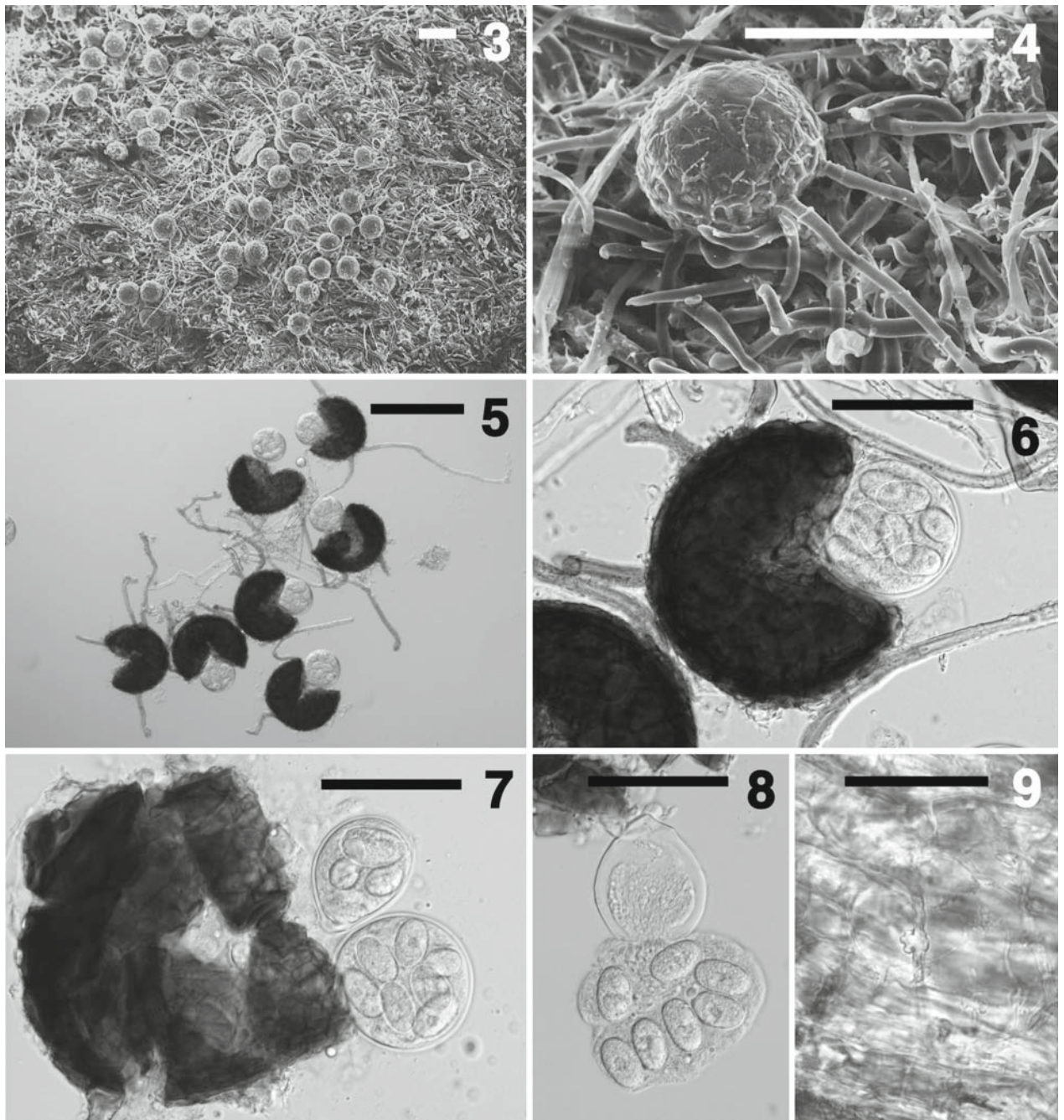
Additional materials examined: Japan, Nagano Prefecture, Minami-Minowa Mura, campus of Faculty of Agriculture, Shinshu University, 30 Sep 2005, leg. Y. Shiroya and S. Takamatsu, MUMH 3786, HAL 1943 F; 14 Aug 2006, leg. Y. Shiroya, MUMH4190, HAL 1944 F.

Discussion

Of the 15(–16) genera in the Erysiphaceae, only 2 genera, *Cystotheca* and *Podosphaera*, have monoascus-type chasmothecia. In *Cystotheca*, the wall of the chasmothecia is composed of two layers that are easily separable from one another, and this character is not found in the present fungus. Thus, based on the morphological characteristic of the monoascus-type chasmothecia, the present fungus seems to be most similar to the genus *Podosphaera*. However, both ITS and 28S rDNA sequences indicate that the *Styrax* fungus does not belong to the genus *Podosphaera*, but to *Erysiphe*. The main difference between *Erysiphe* and *Podosphaera* with regard to their teleomorphs has been considered that *Erysiphe* has multiple asci and *Podosphaera* has a single ascus per chasmothecium (Braun 1987). Although the morphology of the appendages of chasmothecia has long been regarded as an important character to distinguish genera of the Erysiphaceae, it is now regarded as a character to distinguish sections or species (Braun et al. 2002). Thus, *Erysiphe* and *Podosphaera* can no longer be distinguished by the morphology of their appendages. In their anamorphic stages, the two genera are differentiated by

Fig. 2. Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for 84 sequences from *Erysiphe* sections *Erysiphe* and *Microsphaera*. The tree is a phylogram of the maximum-likelihood tree among the 1805 most parsimonious trees with 509 steps, which was obtained by a heuristic search employing the random stepwise addition option of PAUP*.

Gaps were treated as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions that were inferred to have occurred along a particular branch of the tree. Percentage bootstrap support (1000 replications; >70%) and posterior probability (>0.95) are shown on and under branches, respectively



Figs. 3–9. *Erysiphe monascogera*, from holotype. **3, 4** Chasmothecia (fruiting bodies). **5, 6** Chasmothecia with a single ascus and mycelioid type appendages. **7** Chasmothecium with two asci. **8** Ascospores. **9** Lobed-type hyphal appressorium. Bars **3–5** 100 μm ; **6–9** 50 μm

conidiogenesis and appressoria on hyphae. *Erysiphe* produces solitary conidia without distinct fibrosin bodies, whereas *Podosphaera* produces conidia in a chain with distinct fibrosin bodies (Braun et al. 2002). Hyphal appressoria of *Erysiphe* are usually lobed; those of *Podosphaera* are nipple-shaped or indistinct. Hyphal appressoria of the present fungus are distinctly lobed, which supports the result of molecular analysis that this fungus is an *Erysiphe* species. Observation of the conidiogenesis would provide further evidence concerning the identity of the *Styrax* powdery mildew. We thus visited Shinshu University several

times from spring to autumn of 2006 to examine the conidiogenesis of this fungus, but we could not find any conidial formation. Until mid-August, the fungus produced a plentiful number of chasmothecia on the surface of fruits of the hosts. In general, powdery mildew fungi begin to produce chasmothecia from mid-September in Japan, with a few exceptions. Thus, the present fungus seems to produce chasmothecia earlier than the other Japanese powdery mildew fungi. Further observations of chasmothecia reveal that the chasmothecia rarely contain two asci, which suggests that this fungus has an ability to produce more than one ascus

per chasmothecium. Molecular phylogenetic analyses suggest that ancestral taxa of the Erysiphaceae usually have multiple asci per chasmothecium and that taxa with monoascus chasmothecia have diverged only once at the lineage leading to *Podosphaera* and *Cystotheca* (Mori et al. 2000; Takamatsu 2004). However, as *E. symplocicola* U. Braun & S. Takam. (\equiv *M. symploci* Y.N. Yu & Y.Q. Lai), an *Erysiphe* species, occasionally produces monoascus chasmothecia, it may be possible that the monoascus-type species evolves not only at the lineage leading to *Podosphaera* and *Cystotheca*, but also within the genus *Erysiphe*. Therefore, we propose to place the new species on *Styrax* in the genus *Erysiphe* as "*E. monascogera*."

Both ITS and 28S rDNA sequences demonstrate that *E. nomurae* is the sister of *E. monascogera*. They are highly similar in DNA sequences: 99.1% in ITS region and 99.0% in the D1/D2 domains of the 28S rDNA. *Symplocos chinensis* var. *leucocarpa* f. *pilosa*, the host plant of *E. nomurae*, belongs to the family Symplocaceae, and *Styrax japonica* belongs to the Styracaceae. These two families form a monophyletic group with the Diapensiaceae in the Ericales (Schönenberger et al. 2005). Powdery mildew fungi parasitic to closely related host plants are often closely related to each other (Matsuda and Takamatsu 2003; Inuma et al. 2007). Thus, the close relationship of the host families may support the sister-relationship between *E. nomurae* and *E. monascogera*. In contrast, morphological characteristics are distinctly quite different in the two species. For instance, the size of the chasmothecia is larger in *E. nomurae* (70–115 μ m; Braun 1987) than in *E. monascogera* (61–86 μ m). The number of asci is 3–6 in *E. nomurae* and only 1 in *E. monascogera*. Furthermore, the appendages of *E. nomurae* are dichotomously branched several times at the apex; those of *E. monascogera* are simple, viz. mycelioid. These morphological differences could be explained by the unique habitat of *E. monascogera*. This fungus colonizes only on the surface of fruits or sepals, but not on leaves. *Erysiphe vernalis* P. Karst. on *Alnus* spp. and *E. weigela* Z.X. Chen & S.B. Luo on *Weigela* spp. often colonize on the surface of fruits of host plants (Braun 1987; Mori et al. 2000), and share simple, mycelioid appendages with *E. monascogera*. Mori et al. (2000) reported that the mycelioid appendage of the powdery mildew fungi is a derived character that evolved as a result of convergence to adapt their life cycle on herbs, evergreen trees, or twigs. This unique habitat on a fruit surface might be a causal factor of mycelioid appendages for these fungi. An ecological study of *E. monascogera* is necessary to prove this possibility.

The molecular data demonstrate that *E. nomurae* and *E. monascogera* are included in the lineage of the *E. alphitoides* complex having oaks as major host plants. *Symplocos chinensis* var. *leucocarpa* f. *pilosa*, the host of *E. nomurae*, and *Styrax japonica*, the host of *E. monascogera*, have sympatric distribution with oaks in Japan. Thus, it is likely that host jumping has occurred between these plants. Fungi belonging to the *E. alphitoides* complex are extremely variable in morphology as well as in genetics and were recently divided into five species (Takamatsu et al. 2007). One of these species, *E. quercicola* S. Takam. & U. Braun, has a

(nearly) identical rDNA sequence with the powdery mildew fungi parasitic on a variety of tropical trees, i.e., *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg. (para rubber tree), *Anacardium occidentale* L. (cashew), *Bixa orellana* L., *Citrus* spp., *Mangifera indica* L. (mango), and *Acacia* spp. (Limkaisang et al. 2005, 2006; Takamatsu et al. 2007). Similarly, *E. alphitoides* sensu stricto has a rDNA sequence similar to several powdery mildew species parasitic to trees distributed in temperate areas, i.e., *E. wallrothii* (U. Braun & Tanda) U. Braun & S. Takam. (\equiv *M. wallrothii* U. Braun & Tanada) on *Vaccinium* spp., *E. pseudolonicerae* (E.S. Salmon) U. Braun & S. Takam. [\equiv *M. pseudolonicerae* (E.S. Salmon) Homma] on *Cocculus trilobus* (Thunb.) DC., and *E. euonymi-japonici* (Vienn.-Bourg.) U. Braun & S. Takam. (\equiv *M. euonymi-japonici* Vienn.-Bourg.) on *Euonymus japonicus* Thunb. Thus, the *E. alphitoides* complex seems to be extremely variable not only in morphology but also in its parasitic ability. *Erysiphe nomurae* and *E. monascogera* might be powdery mildew species diverged by host jumping of the *E. alphitoides* complex.

Erysiphe miyabeana and *E. togashiana* var. *togashiana* are known to occur on *Styrax japonica*, and *E. symplocicola* has been reported on *Symplocos paniculata* (Thunb.) Miq. (Symplocaceae) in China. Of these, *E. togashiana* var. *togashiana*, of which an ITS sequence is available in DNA database (AB091775; Takamatsu et al. 2003), is distantly related to *E. monascogera* (79.5% similarity). DNA sequences of *E. miyabeana* and *E. symplocicola* are not yet available. Both species, having appendages dichotomously branched several times at the apex, distinctly differ in morphology from *E. monascogera*, which has mycelioid appendages. However, *E. symplocicola* occasionally has small (62–94 μ m diameter), monoascus-type chasmothecia (Braun 1987). These characteristics are similar to *E. monascogera*, which also usually has small (61–86 μ m diameter), monoascus-type chasmothecia. This finding suggests the possibility of a close relationship between *E. symplocicola* and *E. monascogera*, but molecular analyses of *E. symplocicola* are required to prove the affinity of the two species.

Acknowledgments We thank Dr. Uwe Braun for critical reading of the manuscript.

References

- Braun U (1987) A monograph of the Erysiphales (powdery mildews). Beih Nova Hedwigia 89:1–700
- Braun U, Takamatsu S (2000) Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysiphaceae) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystothecaceae) inferred from rDNA ITS sequences: some taxonomic consequences. *Schlechtendalia* 4:1–33
- Braun U, Cook RTA, Inman AJ, Shin HD (2002) The taxonomy of the powdery mildew fungi. In: Bélanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) *The powdery mildews: a comprehensive treatise*. APS Press, St. Paul, MN, pp 13–55
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Fritsch PW (1999) Phylogeny of *Styrax* based on morphological characters, with implications for biogeography and infrageneric classification. *Syst Bot* 24:356–378

- Fritsch PW, Morton CM, Chen T, Meldrum C (2001) Phylogeny and biogeography of the Styracaceae. *Int J Plant Sci* 162:S95–S116
- Hirata T, Takamatsu S (1996) Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. *Mycoscience* 37:283–288
- Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755
- Inuma T, Khodaparast SA, Takamatsu S (2007) Multilocus phylogenetic analyses within *Blumeria graminis*, a powdery mildew fungus of cereals. *Mol Phylogenet Evol* 44:741–751
- Limkaisang S, Kom-un S, Furtado EL, Liew KW, Salleh B, Sato Y, Takamatsu S (2005) Molecular phylogenetic and morphological analyses of *Oidium heveae*, a powdery mildew of rubber tree. *Mycoscience* 46:220–226
- Limkaisang S, Cunnington JH, Liew KW, Salleh B, Sato Y, Divarangkoon R, Fangfuk W, To-anun C, Takamatsu S (2006) Molecular phylogenetic analyses reveal a close relationship between powdery mildew fungi on some tropical trees and *Erysiphe alphitoides*, an oak powdery mildew. *Mycoscience* 47:327–335
- Matsuda S, Takamatsu S (2003) Evolution of host–parasite relationships of *Golovinomyces* (Ascomycete: Erysiphaceae) inferred from nuclear rDNA sequences. *Mol Phylogenet Evol* 27:314–327
- Mori Y, Sato Y, Takamatsu S (2000) Evolutionary analysis of the powdery mildew fungi using nucleotide sequences of the nuclear ribosomal DNA. *Mycologia* 92:74–93
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala
- Schönenberger J, Anderberg AA, Sytsma KJ (2005) Molecular phylogenetics and patterns of floral evolution in the Ericales. *Int J Plant Sci* 166:265–288
- Swofford DL (2001) PAUP: phylogenetic analysis using parsimony (and other methods) 4.0b8. Sinauer, Sunderland, MA
- Takamatsu S (2004) Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences. *Mycoscience* 45:147–157
- Takamatsu S, Sato Y, Mimuro G, Kom-un S (2003) *Erysiphe wadae*: a new species of *Erysiphe* sect. *Uncinula* on Japanese beech. *Mycoscience* 44:165–171
- Takamatsu S, Braun U, Limkaisang S (2005a) Phylogenetic relationships and generic affinity of *Uncinula septata* inferred from nuclear rDNA sequences. *Mycoscience* 46:9–16
- Takamatsu S, Niinomi S, Cabrera de Álvarez MG, Álvarez RE, Havrylenko M, Braun U (2005b) *Caespitotheca* gen. nov., an ancestral genus in the Erysiphales. *Mycol Res* 109:903–911
- Takamatsu S, Matsuda S, Niinomi S, Havrylenko M (2006) Molecular phylogeny supports a northern hemisphere origin of *Golovinomyces* (Ascomycota: Erysiphales). *Mycol Res* 110:1093–1101
- Takamatsu S, Braun U, Limkaisang S, Kom-un S, Sato Y, Cunnington JH (2007) Phylogeny and taxonomy of the oak powdery mildew *Erysiphe alphitoides* sensu lato. *Mycol Res* 111:809–826
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882
- To-anun C, Kom-un S, Limkaisang S, Fangfuk W, Sato Y, Takamatsu S (2005) A new subgenus, *Microoidium*, of *Oidium* (Erysiphaceae) on *Phyllanthus* spp. *Mycoscience* 46:1–8
- Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10:506–513