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FULL PAPER

**Susumu Takamatsu · Hayato Masuya**
**Rangsi Divarangkoon · Yukihiko Nomura**

# *Erysiphe fimbriata* sp. nov.: a powdery mildew fungus found on *Carpinus laxiflora*

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**Abstract** Ascomata of a powdery mildew-like fungus have been found on *Carpinus laxiflora* in Tochigi Prefecture of Japan since 2003. The morphological and molecular characteristics of this fungus are reported, and a new species, *Erysiphe fimbriata*, is proposed. It has large chasmothecia (200–250 μm in diameter) with long (up to 4–5 mm in length), fimbriate appendages arising from the upper half of the chasmothecia and turning upward, and numerous asci (22–38 per chasmothecium). *Erysiphe fimbriata* is a unique fungus both genetically and morphologically.

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

## Introduction

The family Betulaceae (Fagales) is composed of six genera and up to 130 species of anemophilous shrubs and trees. Most species of the family are distributed in temperate regions of the Northern Hemisphere, i.e., Asia, Europe, and North America (Chen et al. 1999). More than 50% of the total species of the Betulaceae have been reported as hosts of powdery mildew fungi (Amano 1986). Because the ratio of hosts of powdery mildew fungi among the total species of angiosperms is about 4.5% (Amano 1986), this high ratio of hosts in the Betulaceae may indicate a close evolutionary affinity of this plant family with the powdery mildew fungi. *Carpinus* is one of the six genera of the Betulaceae (including Corylaceae), which is distributed in Asia, Europe, and North America, with a divergence center in China. Six species, i.e., *Erysiphe carpinicola* (Hara) U. Braun & S. Takam. [= *Uncinula carpinicola* (Hara) Hara], *E. ellisii* (U. Braun) U. Braun & S. Takam. (= *Microsphaera ellisii* U. Braun), *E. pseudocarpinicola* (Y. Nomura & Tanda) U. Braun & S. Takam. (= *U. pseudocarpinicola* Y. Nomura & Tanda), *E. wuyiensis* (Z.X. Chen & R.X. Gao) U. Braun & S. Takam. [= *U. wuyiensis* (Zhi X. Chen & R.X. Gao) U. Braun], *Oidium carpini* Foitzik, and *Phyllactinia guttata* (Wallr.) Lév., have been reported to occur on *Carpinus* (Braun 1987, 1995; Braun and Takamatsu 2000). Recently, *E. carpinicola* was divided into three species, *E. arcuata* U. Braun, Heluta & S. Takam. (host: *C. betulus* L. and *C. tschonoskii* Maxim.; anamorph: *O. carpini*), *E. carpinicola* (host: *C. japonica* Blume), and *E. carpini-laxiflorae* U. Braun, Heluta & S. Takam. [host: *C. laxiflora* (Siebold & Zucc.) Blume] based on morphological and molecular characteristics (Braun et al. 2006, 2007). Thus, a total of seven species of the powdery mildew fungi occur on *Carpinus* now.

In March 2003, we found powdery mildew-like ascomata attached to fallen twigs of *C. laxiflora* in litter of the Mikamoyama Park, Sano-shi, Tochigi Prefecture, Japan (see Fig. 3), which appeared to be strange because powdery mildew fungi are obligate biotrophs of plants. They usually infect living host tissues, and do not have saprophytic life stages. However, both morphological observations and a molecular phylogenetic analysis indicated that this fungus is a member of the powdery mildews. In this study, we report the morphological and molecular characteristics of this fungus that is described as a new species of the powdery mildew fungi with a unique morphology.

S. Takamatsu (✉)
Graduate School of Bioresources, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan
Tel. +81-59-231-9497; Fax +81-59-231-9540
e-mail: takamatu@bio.mie-u.ac.jp

H. Masuya
Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Ibaraki, Japan

R. Divarangkoon
Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand

Y. Nomura
Yotsukaido, Chiba, Japan

## Materials and methods

### Morphological studies

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

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

MUMH 3694, a paratype specimen of *Erysiphe fimbriata*, was used for molecular analysis. 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 the internal transcribed spacer (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 of AB333839.

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 colour-coded nucleotides. The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number of S1942. 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 $10^4$. The strength of the internal branches of the resulting trees was tested with BS analyses using 1000 replications with the stepwise addition option set as simple (Felsenstein 1985). Bootstrap (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 $10^6$ generations and the Markov chains were sampled every 100 generations, which resulted in $10^4$ 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 $10^4$ 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

### Field observation

The occurrence of powdery mildew on *C. laxiflora* was observed from June to December 2006 in the Mikamoyama Park. No powdery mildew occurrence was found in early June. White powdery mildew colonies were found on the leaves of *C. laxiflora* in late September. The powdery mildew mainly colonized veins and their surrounding areas of the lower surface of leaves, and caused necrotic discolorations and distortions of the attacked host tissues. Colonies were not found on the upper leaf surface. Young, immature chasmothecia were produced on the colonies. In late October, mature chasmothecia were observed on the colonies together with immature ones. Long (up to 4–5 mm in length), fimbriate appendages rose from the upper half of the chasmothecia, which were easily observable by naked eye (see Fig. 4). By early December, almost all leaves had fallen on the ground. Obvious white colonies with mature chasmothecia were observed on the fallen leaves, but infections of twigs were not found during this period. Conidial formation was also not observed.

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

A total of 99 sequences of 28S rDNA, including a sequence from the new *Carpinus* powdery mildew, were used to construct the phylogenetic tree of the Erysiphaceae. *Byssoascus striatisporus* (G.L. Barron & C. Booth) Arx (Myxotrichaceae) was used as an outgroup taxon, based on Mori et al. (2000). The data set consisted of 831 characters, of which 245 characters were variable and 189 characters were phylogenetically informative for parsimony analysis. A total of $10^4$ equally MP trees with 995 steps (CI = 0.3789, RI = 0.8014, RC = 0.3037) were constructed by the MP analysis. To avoid the possibility that the heuristic search became trapped in local optima, we repeated similar analysis by the parsimony ratchet method (Nixon 1999) using PAUPRat (Sikes and Lewis 2001). The analysis also generated trees with 995 steps having topologies similar to the MP trees. Thus, one of the $10^4$ MP trees is shown in Fig. 1. Most internal branches are supported in the strict consensus of the $10^4$ trees. Bayesian analysis generated similar tree topology.

The previous phylogenetic analyses of the Erysiphaceae demonstrated that five tribes and two basal genera are included in the family (Mori et al. 2000; Braun and

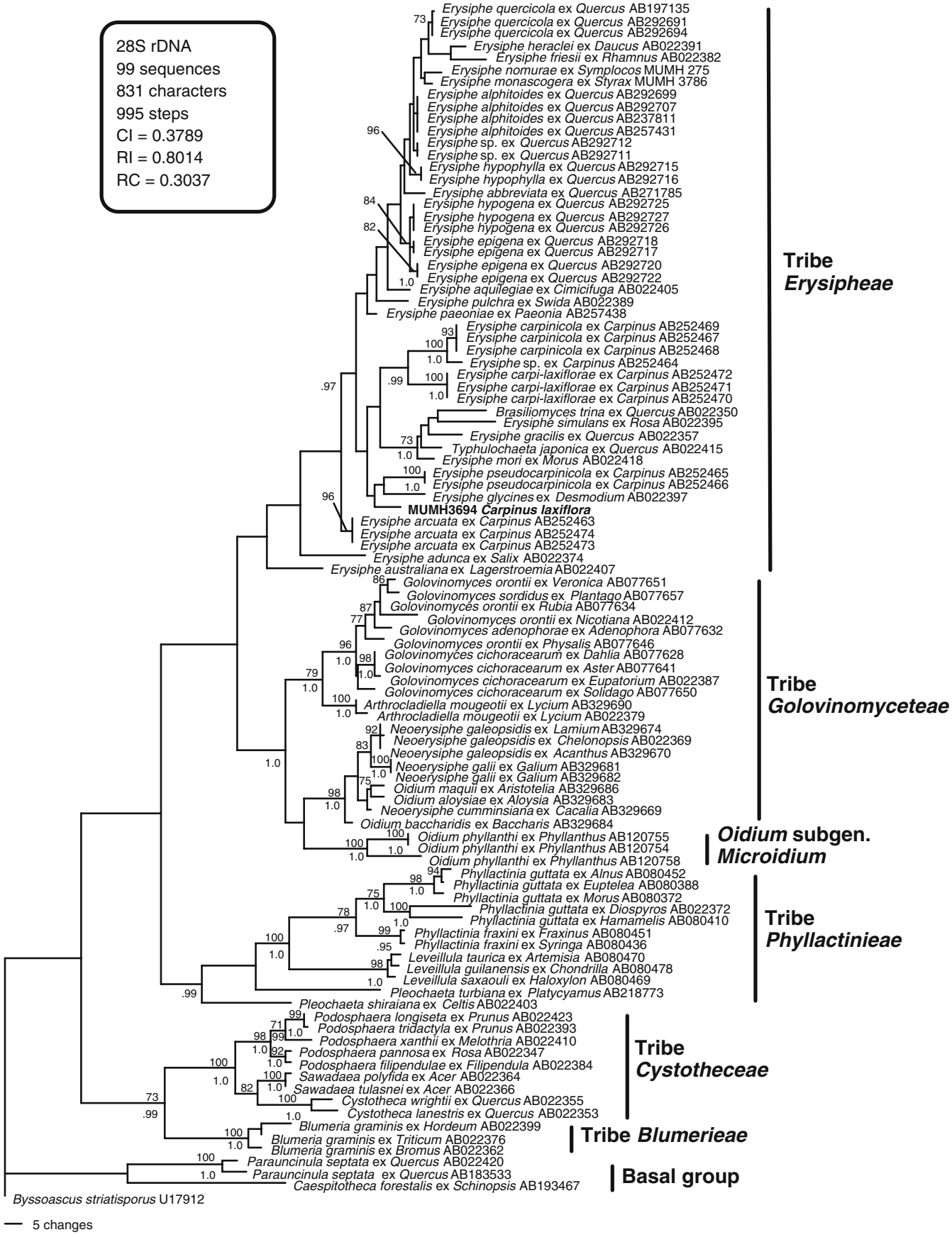



**Fig. 1.** Phylogenetic analysis of the divergent domains D1 and D2 sequences of the 28S rDNA for 99 sequences from the Erysiphaceae covering all known tribes and one outgroup taxon. The tree is a phylogram of the maximum-likelihood tree among the $10^4$ most parsimonious trees with 995 steps, which 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

Takamatsu 2000; Takamatsu et al. 2005a,b). The present analysis supports the monophyly of four tribes, i.e., the tribes Blumerieae, Erysipheae, Cystotheceae, and Phyllactinieae. The tribe Golovinomyceteae groups with *Oidium* subgenus *Microidium* To-anun & S. Takam. (To-anun et al. 2005) to form a clade together. *Caespitotheca* S. Takam. & U. Braun and *Parauncinula* S. Takam. & U. Braun take basal positions within the Erysiphaceae. The fungus MUMH 3694 on *C. laxiflora* is placed in the genus *Erysiphe* DC. and groups with *E. pseudocarpinicola* (= *Uncinula pseudocarpinicola*) from *C. cordata* Blume and *E. glycines* F.L. Tai var. *glycines* from *Desmodium podocarpum* DC. subsp. *oxyphyllum* (DC.) Ohashi, but this is supported by neither BS nor PP values.

### Phylogeny within *Erysiphe*: ITS analysis

A total of 31 ITS sequences from *Erysiphe*, including a sequence from the new *Carpinus* powdery mildew, were used to construct the phylogenetic *Erysiphe* tree. The data set consisted of 711 characters, of which 212 characters were removed from the analysis because of ambiguous alignment. Of the remaining 499 characters, 225 characters were variable, and 161 characters were phylogenetically informative for parsimony analysis. A total of 106 equally MP trees with 639 steps (CI = 0.5540, RI = 0.7209, RC = 0.3994) were constructed by the MP analysis. A tree with the highest likelihood score among the 106 MP trees is shown in Fig. 2. Most internal branches are supported in the strict consensus of the 106 trees. Bayesian analysis generated similar tree topology.

The ITS sequence from MUMH3694 on *C. laxiflora* is sister to all *Erysiphe* species excluding *E. australiana* (McAlpine) U. Braun & S. Takam. (= *U. australiana* McAlpine) and *E. adunca* (Wallr.) Fr. var. *adunca* [= *U. adunca* (Wallr.) Lév. var. *adunca*], but this is supported by neither BS nor PP values.

## Taxonomy

***Erysiphe fimbriata*** S. Takam., Masuya & Y. Nomura, sp. nov. Figs. 3–9

MycoBank no.: MB511033

Mycelio hypophyllo, in venis et prope venas habitanti, hyalino, persistensi; discolorationem cum necrose et torsionem folii efficienti; chasmotheciis hypophyllis, dispersis vel subgregariis, fusco-brunneis, 200–250 µm diametro; peridiis

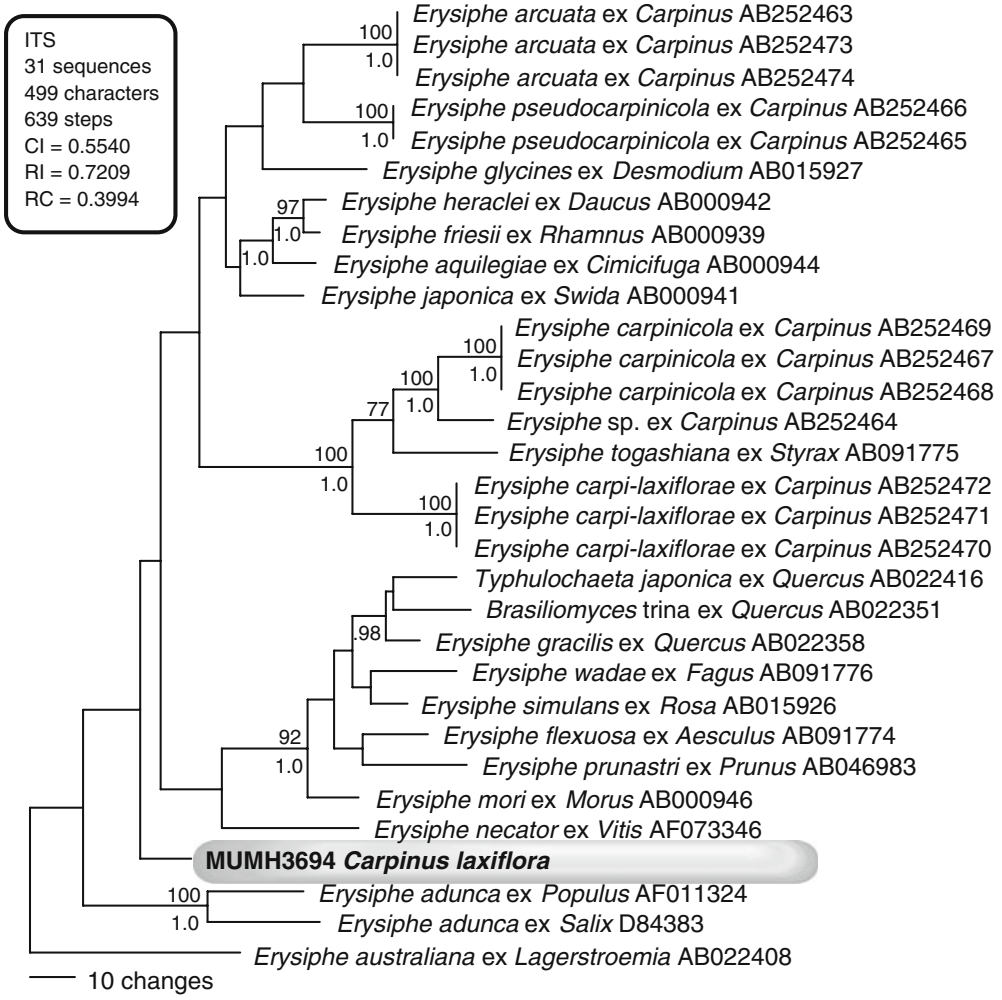
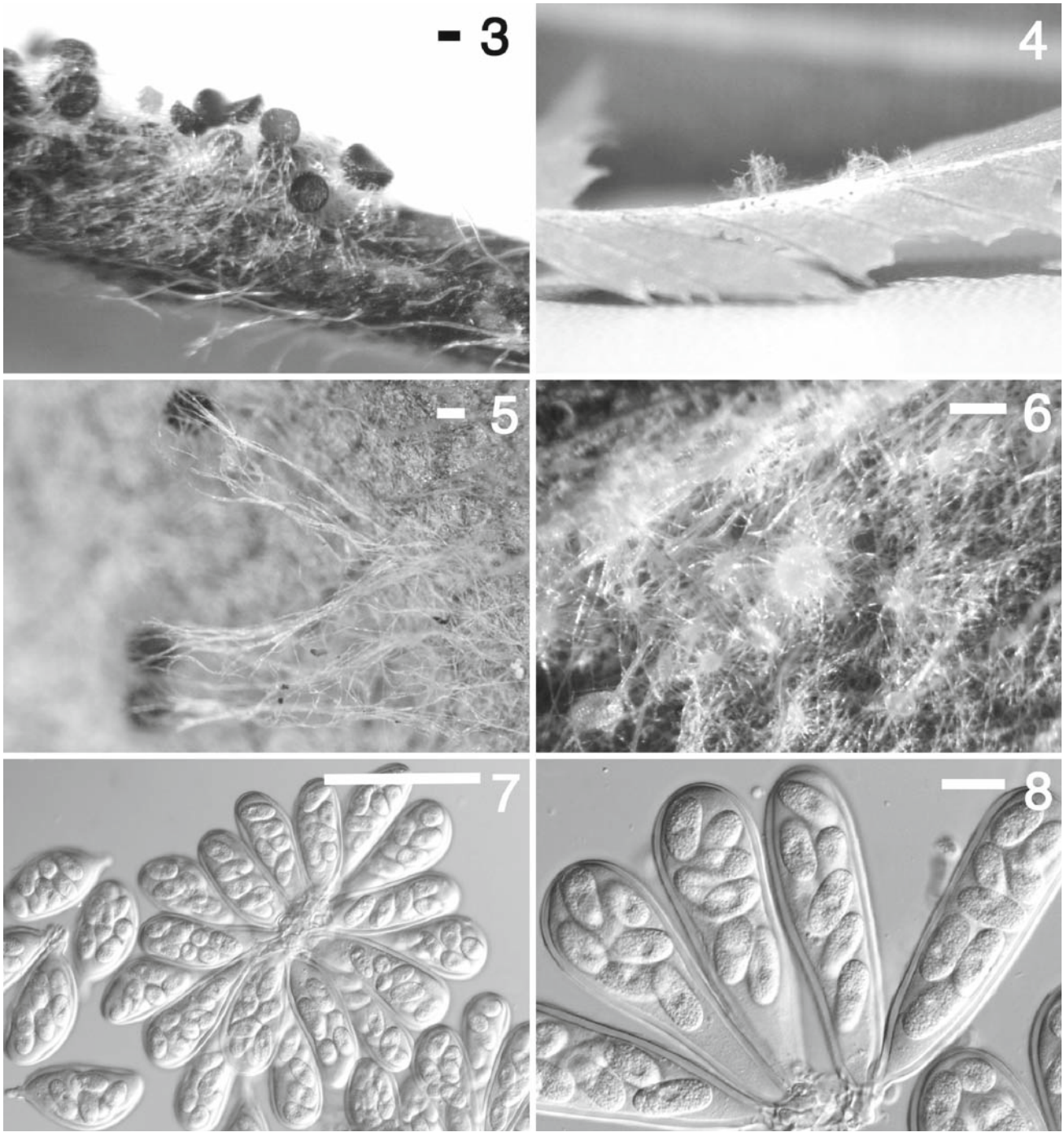



**Fig. 2.** Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for 31 sequences from *Erysiphe*. The tree is a phylogram of the maximum-likelihood tree among the 106 most parsimonious trees with 639 steps, which was obtained by a heuristic search employing the random stepwise addition option of PAUP*. Gaps were treated as missing data. This tree is also the maximum-likelihood tree among the 106 most parsimonious trees. 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. 3–8.** *Erysiphe fimbriata*. **3** Chasmothecia attaching on a twig of *Carpinus laxiflora*. **4** Long, fimbriate appendages growing upward from leaf surface. **5** Chasmothecia with long appendages. **6** Anchoring hyphae arising from whole surface of young, immature chasmothecia. **7, 8** Asci and ascospores. *Bars* **3–7** 100 µm; **8** 20 µm

ex cellulis angulatis vel irregularibus 20–25 × 15–17.5 µm compositis; appendicibus ex parte superne chasmothecii oriundis, surgentibus, (17–)20–50, simplicibus, mycelioidibus, rectis, interdum sinuosis vel geniculatis, interdum torulosis, raro ramosis, (3–)4–8(–12.5) µm latis, 4–5 mm longis, septatis, crassitunicatis, hyalinis, raro ad basim pallide brunneis; hyphis anchoriformibus ex superficie omnino chasmothecii oriundis, intertextis; ascis numerosis, 22–38, pedunculatis, 75–105 × 35–37.5 µm, ellipsoideis vel ovoideis; ascosporis 6–8, late ellipsoideis vel ovoideis, hyalinis, 20–25 × 12.5–17.5 µm.

Typus: Japan, Tochigi Prefecture, Sano-shi, Mikamo-yama Park, on fallen leaves of *Carpinus laxiflora* (Siebold & Zucc.) Blume (Betulaceae), 4 Dec 2006, leg. S. Takamatsu (Holotypus, TNS-F-16170; isotypus, MUMH 4592 and HAL 2051 F).

Etymology: "*fimbriata*" refers to the long, fimbriate appendages.

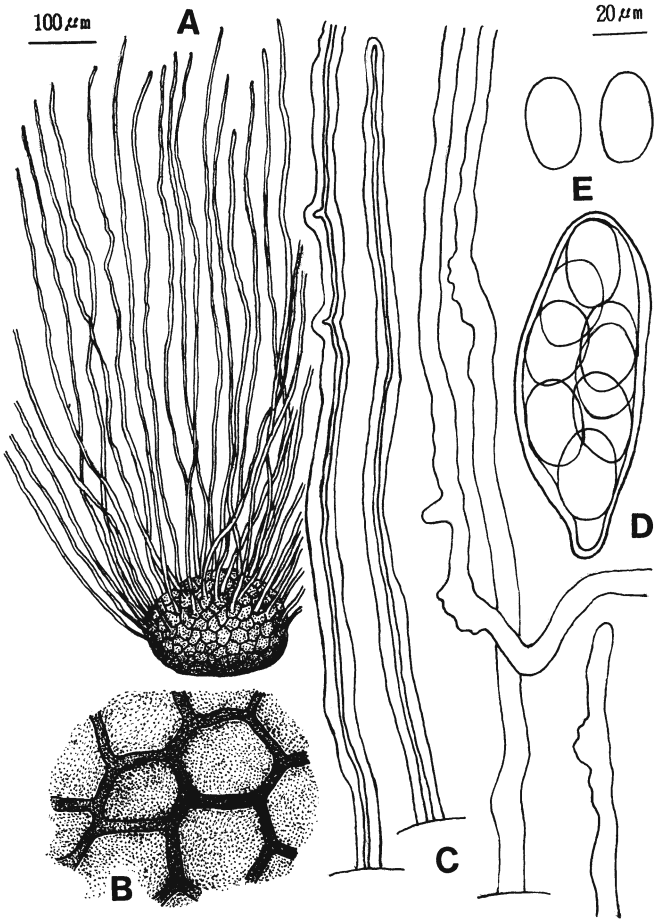



**Fig. 9.** *Erysiphe fimbriata.* **A** Chasmothecium. **B** Peridial cells. **C** Appendages. **D** Asci and ascospores. **E** Ascospores. *Bars* **A** 100 μm; **E** 20 μm (for **B–E)**

Mycelia hypophyllous, colonizing veins and the surrounding leaf area, hyaline, persistent, causing necrotic discoloration and distortion of the attacked host tissue. Chasmothecia hypophyllous, scattered to subgregarious, blackish brown, 200–250 μm diameter, peridial cells angular-irregular in outline, 20–25 × 15–17.5 μm. Appendages (17–) 20–50, arising from the upper part of chasmothecia, turning upward, simple, mycelioid, straight, sometimes slightly sinuous to geniculate, sometimes having small projections, rarely branched, (3–)4–8(–12.5) μm wide, long (up to 4–5 mm), aseptate, thick-walled, hyaline, rarely pale brown at the base. Anchoring hyphae arise from whole surface of chasmothecia, interwoven with vegetative hyphae. Asci numerous, 22–38 per chasmothecium, stalked, 75–105 × 35–37.5 μm, ellipsoid to ovoid, (6–)8-spored. Ascospores broadly ellipsoid to ovoid, colorless, 20–25 × 12.5–17.5 μm. Anamorph unkown.

Host range and distribution: On the leaves of *Carpinus laxiflora*, Asia, Japan.

Additional materials examined: Japan, Tochigi Prefecture, Sano-shi, Mikamoyama Park, on *Carpinus laxiflora*, 19 Mar 2003, leg. H. Masuya, MUMH 3694; 4 Mar 2006, leg. H. Masuya, MUMH 3813; 20 Sep 2006, leg. S. Takamatsu, MUMH 4303; 22 Oct 2006, leg. S. Takamatsu, Y. Shiroya, and M. Ito, MUMH 4416.

## Discussion

Of the seven powdery mildew species known to occur on *Carpinus*, five belong to *Erysiphe* section *Uncinula* (Lév.) U. Braun & Shishkoff, which has appendages with uncinate-circinate apex. *Erysiphe ellisii* belongs to the section *Microsphaera* (Lév.) U. Braun & Shishkoff, which has appendages with apex dichotomously branched several times. *Erysiphe fimbriata*, having simple, mycelioid appendages, belongs to the section *Erysiphe*. Thus, *Carpinus* is affected by *Erysiphe* species of all sections known. However, appendages of species of the section *Erysiphe* usually arise from the lower part of the chasmothecia and are interwoven with hyphae on the surface of the leaves. In contrast, appendages of *E. fimbriata* arise from the upper part of chasmothecia and turn upward, which is quite different from the appendages of most species of the section *Erysiphe*, except for some species that are intermediate between the sections *Erysiphe* and *Microsphaera*, as, for instance, *Erysiphe tortilis* (Wallr.) Link: Fr. and *E. trifolii* Grev., and allied species in which the appendages turn upward (toward one direction). The phylogenetic analysis also supports that *E. fimbriata* belongs to the lineage of section *Uncinula*, but not to section *Erysiphe*. In species of *Erysiphe* section *Uncinula*, the appendages mostly arise equatorially, but in *Uncinula forestalis* Mena, now *Caespitotheca forestalis* (Mena) S. Takam. & U. Braun, the terminal appendages turn toward one direction. Moreover, the large size of chasmothecia and numerous asci of *E. fimbriata* demonstrate that this species is a unique fungus among the genus *Erysiphe*. *Phyllactinia guttata* (Wallr.: Fr.) Lév., one of the powdery mildews that infect *Carpinus*, also has large chasmothecia (150–250 μm diameter). This fungus has needle-shaped appendages with a bulbous base, which is quite different from the mycelioid appendages of *E. fimbriata*. Therefore, *E. fimbriata* differs from any other powdery mildew species known to occur on *Carpinus*, and also differs from any other powdery mildew species. We thus propose *E. fimbriata* as a new species of the Erysiphaceae.

Molecular phylogenetic analyses support that *E. fimbriata* belongs to the genus *Erysiphe*, which is consistent with the morphological characteristics of this fungus having multi-asci chasmothecia and mycelioid appendages. Molecular analyses also demonstrate that the phylogenetic position of *E. fimbriata* is ambiguous within *Erysiphe*, i.e., there is no *Erysiphe* species closely related to *E. fimbriata*. *Erysiphe* species most closely allied to *E. fimbriata* are *E. paeoniae* R.Y. Zheng & G.Q. Chen and *E. arcuata* in 28S rDNA (97.5% similarity). ITS sequence similarities of *E. fimbriata* are less than 90% to all other *Erysiphe* species used in this study. Sequence similarity of *E. fimbriata* to *Erysiphe* species parasitic on *Carpinus* are 95.3%–97.5% in the 28S rDNA and 77.4%–87.3% in ITS regions, which indicates that *E. fimbriata* is distantly related to all other

*Erysiphe* species reported on *Carpinus*. These data suggest that *E. fimbriata* is a unique fungus genetically as well as morphologically.

*Erysiphe fimbriata* was first found as chasmothecia attached on twigs of *C. laxiflora* in litter. Our first assumption was that *E. fimbriata* colonized the twigs of *C. laxiflora* and later formed chasmothecia there. To confirm this assumption, we visited the Mikamoyama Park several times from May to December in 2006. However, we failed to observe the fungus colonizing on twigs during this period. Therefore, the chasmothecia might be transferred from leaves to twigs after maturation in some unknown way. The evidence that the chasmothecia are attached to the twigs upside down may support this assumption. The long appendages turning upward from the chasmothecia might have some function in the dispersal process.

*Erysiphe carpini-laxiflorae* also occurs on *C. laxiflora* in the Mikamoyama Park. *Erysiphe carpini-laxiflorae* usually occurs on young seedlings of *C. laxiflora*, but not on the adult tree, whereas *E. fimbriata* occurs on a large tree, about 15–20 m tall. Thus, the two powdery mildews that occur on *C. laxiflora* seem to have different ecological niches. However, additional detailed ecological studies of these fungi are required.

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