Mycoscience (2009) 50:409–414
DOI 10.1007/s10267-009-0499-8

FULL PAPER

Yoshiaki Shiroya · Susumu Takamatsu

Erysiphe corylopsidis sp. nov., a new powdery mildew fungus found on *Corylopsis spicata* and *C. pauciflora*

Received: February 2, 2009 / Accepted: May 13, 2009

Abstract A powdery mildew fungus occurring on leaves of *Corylopsis pauciflora* and *C. spicata* in Japan is described as a new species, *Erysiphe corylopsidis*. This species is characterized by fewer than 15 appendages on a chasmothecium, primary branches of the appendages occasionally elongated, and a relatively small number (2–5) of ascospores per ascus. Molecular phylogenetic analyses based on rDNA ITS and 28S rDNA sequences indicate that this fungus forms an independent lineage in the genus *Erysiphe*.

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

Introduction

The Hamamelidaceae is a woody, dicotyledonous plant family containing 30 genera and 144 species (Zhang and Lu 1995). This family is distributed in tropical, subtropical, and temperate areas in both the Old World and New World. *Corylopsis* is a genus in the Hamamelidaceae that consists of 29 species of deciduous trees or shrubs distributed in Asia, especially in East Asia (Endress 1993; Zhang and Lu 1995). *Corylopsis pauciflora* Siebold & Zucc. is distributed in Japan and Formosa, and *Corylopsis spicata* Siebold & Zucc. is an endemic species in Japan, especially in Kochi Prefecture. Both species have yellow flowers in early spring and are cultivated in Japan as garden trees.

Five powdery mildew species have been reported to occur on hosts of the Hamamelidaceae, viz. *Podosphaera biuncinata* Cooke & Peck and *Phyllactinia guttata* (Wallr.: Fr.) Lév. on *Hamamelis* spp., *Erysiphe variabilis* (R.Y. Zheng & G.Q. Chen) U. Braun & S. Takam. (≡*Uncinula variabilis* R.Y. Zheng & G.Q. Chen) and *E. liquidambaris* (R.Y. Zheng & G.Q. Chen) U. Braun (≡*U. liquidambaris* R.Y. Zheng & G.Q. Chen) on *Liquidambar* spp., and *Phyllactinia corylopsidis* Y.N. Yu & S.J. Han on *Corylopsis* and *Fortunaria* spp. (Braun 1987; Nomura 1997; Braun and Takamatsu 2000). Only *Ph. corylopsidis* is reported on *Corylopsis*, and there was no report of *Erysiphe* sect. *Microsphaera* (formerly the genus *Microsphaera*) on *Corylopsis* or on its plant family. In autumn 1997, Sato and Horie (1998) found a powdery mildew fungus belonging to sect. *Microsphaera* on *C. pauciflora* in Tokyo. Recently, a species of *Erysiphe* sect. *Microsphaera* has been collected on *C. pauciflora* in Kanagawa, Tochigi, and Aichi prefectures and on *C. spicata* in Ibaraki and Aichi prefectures. Morphological and molecular phylogenetic analyses were done to determine whether this fungus was a new species of powdery mildew.

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

Materials and methods

Morphological studies

Specimens on *C. spicata* and *C. pauciflora* were examined by standard light microscopy (Axio Imager; Carl Zeiss, Göttingen, Germany) and differential interference contrast optical instruments and devices. We examined 50 samples of chasmothecia from *C. spicata* and *C. pauciflora* and 50 samples of conidia from *C. pauciflora*.

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 the internal tran-

scribed 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 AB478984-AB478991.

The sequences were initially aligned using the Clustal X package (Thompson et al. 1997). The alignment was then visually refined with MacClade ver. 4.08 (Maddison and Maddison 2005). The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number S2382. 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 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 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

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

A total of 82 sequences of 28S rDNA, including four sequences from the *Corylopsis* powdery mildews, were used to construct a 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 825 characters, of which 238 characters were variable and 185 characters were phylogenetically informative for parsimony analysis. A total of 288 equally parsimonious trees with 914 steps (CI = 0.399, RI = 0.800, RC = 0.320) were constructed by the MP analysis. A tree with highest likelihood score among the 288 trees is shown in Fig. 1. Most internal branches are supported in the strict consensus of the 288 trees. Bayesian analysis generated similar tree topology.

The previous phylogenetic analyses of the Erysiphaceae demonstrated that five tribes and two basal genera, *Caespitotheca* and *Parauncinula*, are included in the family (Braun and Takamatsu 2000; Mori et al. 2000; Takamatsu et al. 2005a,b). The present analysis supports the monophyly of three tribes, i.e., tribes Erysipheae, Cystotheceae, and Blumerieae. The tribe Phyllactinieae is paraphyletic to the tribes Erysipheae and Golovinomyceteae. The tribe Golovinomyceteae grouped with *Oidium* subgenus *Microidium* (To-anun et al. 2005) to form a clade together. *Caespitotheca* is a sister to the large clade composed of the tribes Erysipheae, Golovinomyceteae, and Phyllactinieae. *Parauncinula* occupies the most basal position of the Erysiphaceae. The four 28S rDNA sequences from the isolates on *Corylopsis spicata* and *C. pauciflora* were identical and formed a distinct clade with strong statistical support (BS = 100%; PP = 1.0) in the genus *Erysiphe*. They were sister to *E. pisi* DC. on *Medicago sativa* L. and *E. aquilegiae* var. *ranunculi* (Grev.) R.Y. Zheng & G.Q. Chen on *Cimicifuga simplex* Wormsk. ex DC., but this grouping was not supported by BS and PP values.

Phylogeny within *Erysiphe*: ITS analysis

A total of 70 ITS sequences from *Erysiphe* sections *Erysiphe* and *Microsphaera* were aligned with four sequences of the fungi on *C. spicata* and *C. pauciflora*. The data set consisted of 586 characters, of which 214 characters were variable and 147 characters were phylogenetically informative for parsimony analysis. A total of 7566 equally parsimonious trees with 525 steps (CI = 0.554, RI = 0.777, RC = 0.431) were constructed by the MP analysis. A tree with the highest likelihood score among the 7566 trees is shown in Fig. 2. Bayesian analysis generated similar tree topology.

The four ITS sequences from the isolates on *C. spicata* and *C. pauciflora* are identical and formed a distinct clade with strong BS and PP supports (BS = 100%; PP = 1.0). *Erysiphe katumotoi* (U. Braun) U. Braun & S. Takam. (≡*Microsphaera katumotoi* U. Braun) from *Ligustrum obtusifolium* Siebold & Zucc. was sister to the clade, but this association is not supported by BS and PP values.

Taxonomy

Erysiphe corylopsidis Shiroya & S. Takam., sp. nov.

Figs. 3–10

Mycobank no.: MB 513004

Mycelio hypophyllo, raro amphigeno, in foliis vivis, hyalino, evanescenti; appressoriis simpliciter lobulatis; conidiophoris erectis, rectis, ex cellulis aequalibus 1–3-septatis compositis; cellulis pedibus cylindricis, (26–)32–61 (–64) × 5–11 μm; conidiis solitariis, ellipsoideis, 23–43 × 15–22 μm, sine corpusculis fibrosis, germinatio "typi

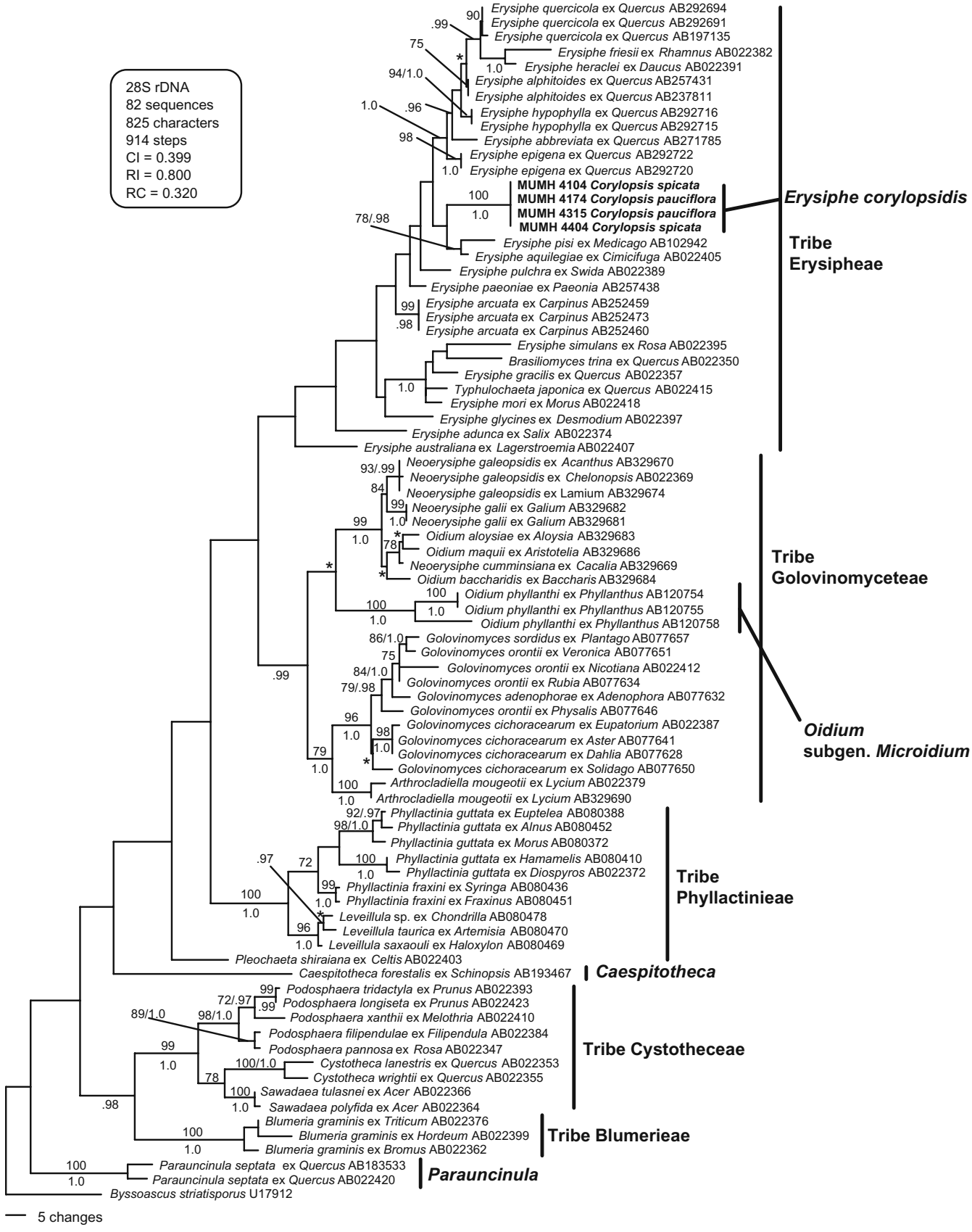


Fig. 1. Phylogenetic analysis of the divergent domains D1 and D2 sequences of the 28S rDNA for 82 sequences from the Erysiphaceae covering all known tribes and one outgroup taxon. The tree is a phylogram of the maximum-likelihood tree among the 288 most parsimonious trees with 914 steps, which was obtained 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. *Asterisks* on the branches mean that they were collapsed in a strict consensus tree. Percentage bootstrap support (1000 replications; ≥70%) and posterior probability (≥0.95) are shown *on* and *under branches*, respectively

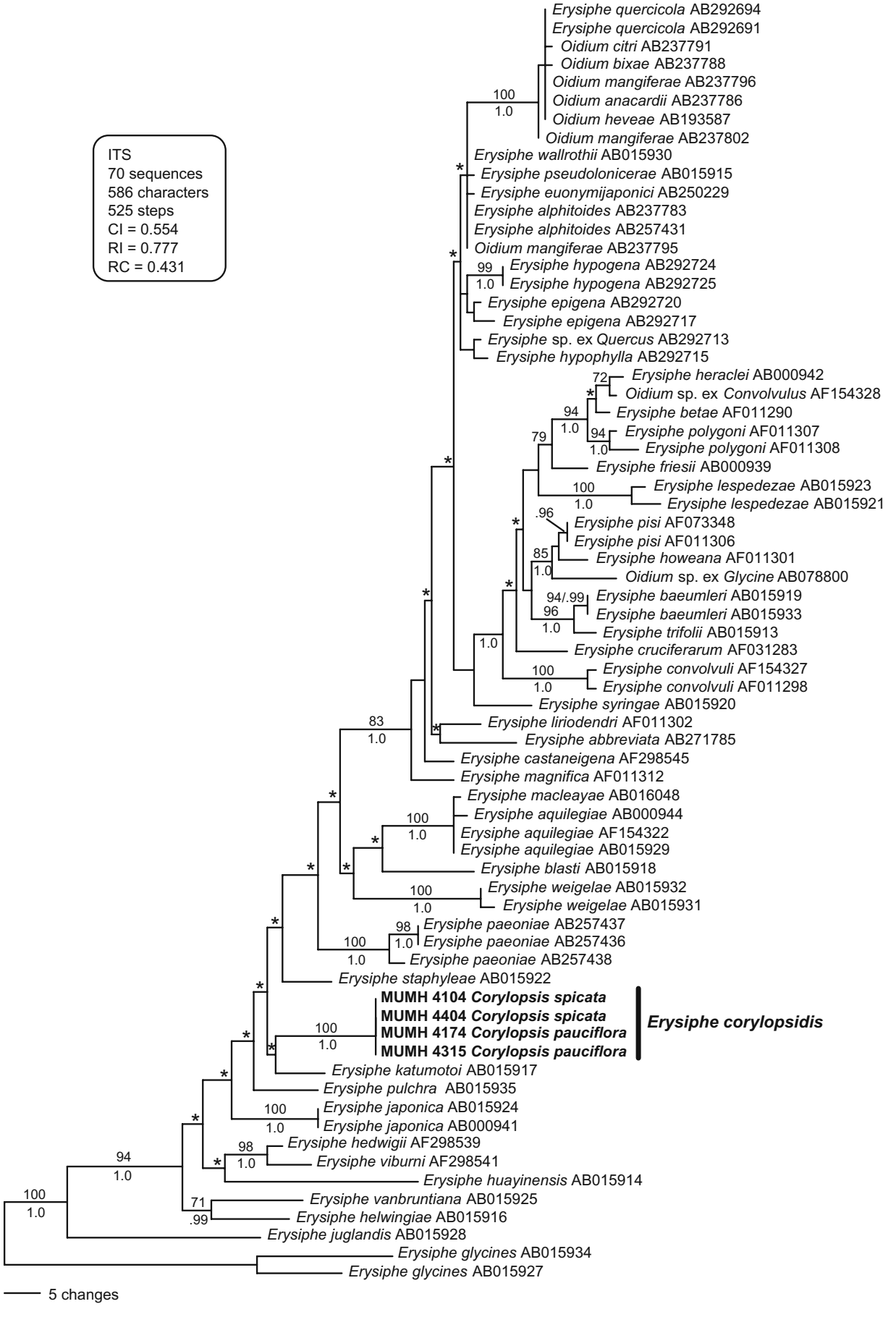
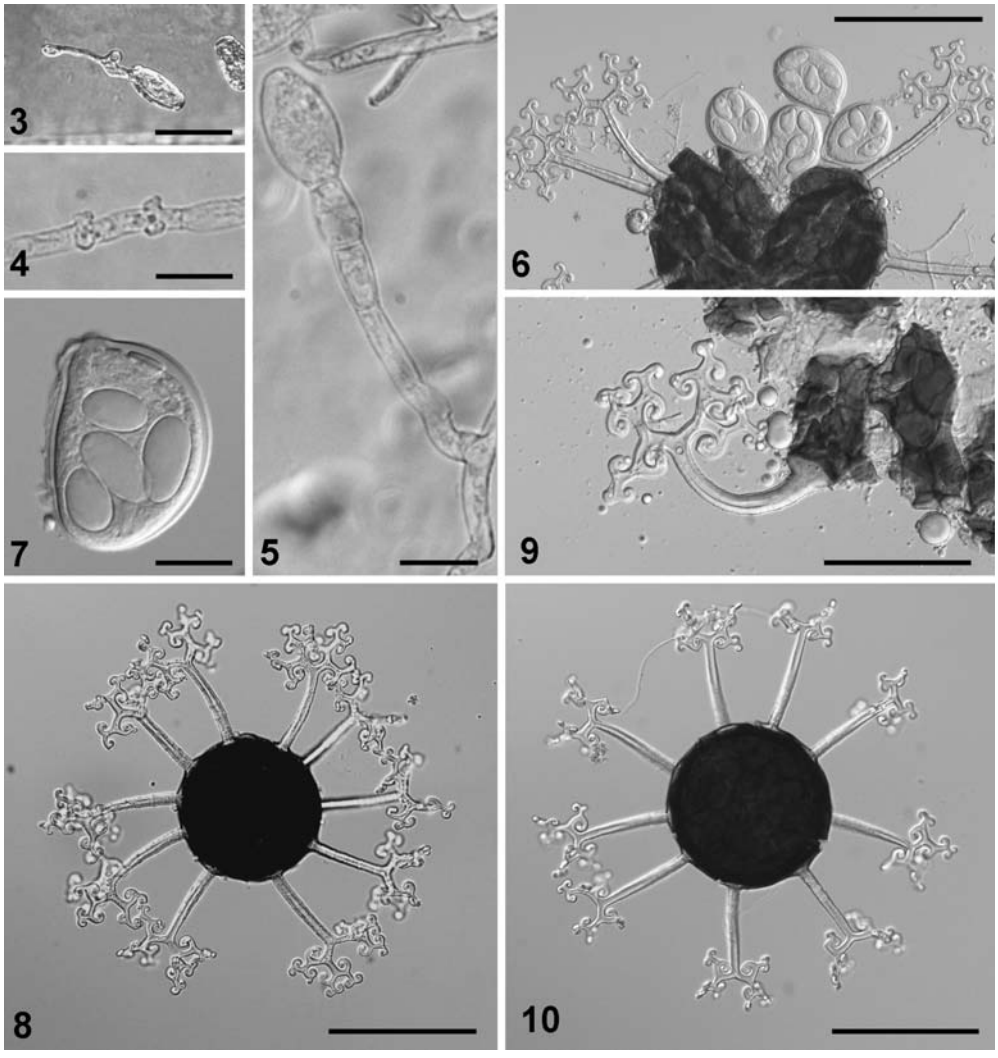

ITS
70 sequences
586 characters
525 steps
CI = 0.554
RI = 0.777
RC = 0.431
Erysiphe quercicola AB292694
Erysiphe quercicola AB292691
Oidium citri AB237791
Oidium bixae AB237788
Oidium mangiferae AB237796
Oidium anacardii AB237786
Oidium heveae AB193587
Oidium mangiferae AB237802
Erysiphe wallrothii AB015930
Erysiphe pseudolonicerae AB015915
Erysiphe euonymijaponici AB250229
Erysiphe alphitoides AB237783
Erysiphe alphitoides AB257431
Oidium mangiferae AB237795
Erysiphe hypogena AB292724
Erysiphe hypogena AB292725
Erysiphe epigena AB292720
Erysiphe epigena AB292717
Erysiphe sp. ex Quercus AB292713
Erysiphe hypophylla AB292715
Erysiphe heraclei AB000942
Oidium sp. ex Convolvulus AF154328
Erysiphe betae AF011290
Erysiphe polygoni AF011307
Erysiphe polygoni AF011308
Erysiphe friesii AB000939
Erysiphe lespedezae AB015923
Erysiphe lespedezae AB015921
Erysiphe pisi AF073348
Erysiphe pisi AF011306
Erysiphe howeana AF011301
Oidium sp. ex Glycine AB078800
Erysiphe baeumleri AB015919
Erysiphe baeumleri AB015933
Erysiphe trifolii AB015913
Erysiphe cruciferarum AF031283
Erysiphe convolvuli AF154327
Erysiphe convolvuli AF011298
Erysiphe syringae AB015920
Erysiphe liriodendri AF011302
Erysiphe abbreviata AB271785
Erysiphe castaneigena AF298545
Erysiphe magnifica AF011312
Erysiphe macleayae AB016048
Erysiphe aquilegiae AB000944
Erysiphe aquilegiae AF154322
Erysiphe aquilegiae AB015929
Erysiphe blasti AB015918
Erysiphe weigelae AB015932
Erysiphe weigelae AB015931
Erysiphe paeoniae AB257437
Erysiphe paeoniae AB257436
Erysiphe paeoniae AB257438
Erysiphe staphyleae AB015922
MUMH 4104 Corylopsis spicata
MUMH 4404 Corylopsis spicata
MUMH 4174 Corylopsis pauciflora
MUMH 4315 Corylopsis pauciflora
Erysiphe corylopsidis
Erysiphe katumotoi AB015917
Erysiphe pulchra AB015935
Erysiphe japonica AB015924
Erysiphe japonica AB000941
Erysiphe hedwigii AF298539
Erysiphe viburni AF298541
Erysiphe huayinensis AB015914
Erysiphe vanbruntiana AB015925
Erysiphe helwingiae AB015916
Erysiphe juglandis AB015928
Erysiphe glycines AB015934
Erysiphe glycines AB015927
5 changes

Fig. 2. Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for 70 sequences from *Erysiphe* sections *Erysiphe* and *Microsphaera*. The tree is a phylogram of the maximum-likelihood tree among the 7566 most parsimonious trees with 525 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. *Asterisks* on the branches mean that they were collapsed in a strict consensus tree. Percentage bootstrap support (1000 replications; ≥70%) and posterior probability (≥0.95) are shown *on* and *under* branches, respectively



Figs. 3–10. *Erysiphe corylopsidis*. **3** Conidial germ tube. **4** Hyphal appressoria. **5** Conidiophore and conidium. **6** Appendages and asci. **7** Ascus and ascospores. **8**, **10** Chasmothecium. **9** Appendage. **3–8** from *Corylopsis pauciflora*; **9–10** from *Corylopsis spicata*. *Bars* **3** 40 µm; **4**, **5**, **7** 20 µm; **6**, **8**, **10** 100 µm; **9** 50 µm

Pseudoidii"; chasmotheciis gregariis vel subsparsis, globosis, 75–121(–130) µm in diametro; pariete peridii ex cellulis irregularibus, 12–25(–29) × 8–22 µm composito; appendicibus 6–14, aequatorialibus, rectis vel curvatis, 71–118 µm longis, laevibus vel scruposis, leptotunicatis, ad basim crassitunicatis, 0–1-septatis, hyalinis, raro ad basim brunneis, ad apicem 3–6 dichotome ramosis, apicibus recurvatis, raro cum ramulis primariis elongatis; ascis 2–7, breviter pedicellatis vel sessilibus, 32–62 × 26–47 µm; ascosporis 2–5, ellipsoideis vel oblongo-ovoideis, 13–25.5 × 7.5–15 µm.

Type specimen: on leaves of *Corylopsis spicata* Siebold & Zucc. (Hamamelidaceae), Japan, Aichi Prefecture, Nagoya-shi, Higashiyama Zoo and Botanical Gardens, 21 Nov. 2005, collected by S. Takamatsu and R. Divarangkoon (TNS-F-11719, holotypus; MUMH 4174, HAL 2311 F, isotypus). rDNA sequence ex-type: AB478984 (28S rDNA), AB478988 (ITS).

Etymology: The specific epithet "*corylopsidis*" is derived from the host genus name.

Mycelia on leaves hypophyllous, rarely amphigenous, hyaline, evanescent. Appressoria on mycelium simply lobed, solitary or opposite in pairs (Fig. 4).

Anamorph: Conidiophores erect, straight, uniform, 1–3-septate; foot-cells cylindrical, (26–)32–61(–64) × 5–11 µm (Fig. 5). Conidia solitary, ellipsoid, 23–43 × 15–22 µm, without conspicuous fibrosin bodies. Germ tubes of the *Pseudoidium* type (Fig. 3; Cook and Braun 2009).

Teleomorph: Chasmothecia gregarious to subscattered, 75–121(–130) µm in diameter; peridial wall cells irregularly polygonal, obscure, 12–25(–29) × 8–22 µm. Appendages 6–

14, equatorial, straight or curved, 71–118 µm long, smooth to rough, thin walled, thick walled toward the base, 0–1-septate, hyaline, sometimes brown at the base, ~4–9 µm wide, apex 3–6 times dichotomously branched, primary branches occasionally elongated, tips recurved (Figs. 8–10). Asci 2–7, sessile or short stalked, 32–62 × 26–47 µm, 2–5-spored (Figs. 6, 7). Ascospores ellipsoid to oblong-ovoid, 13–25.5 × 7.5–15 µm.

Host range and distribution: on the leaves of *Corylopsis pauciflora* and *C. spicata* (Hamamelidaceae), Japan.

Additional materials examined: Japan, Kanagawa Prefecture, Yokohama-shi, on *C. pauciflora*, Oct. 1994, coll. S. Takamatsu, MUMH 69; Japan, Aichi Prefecture, Nagoya-shi, Higashiyama Zoo and Botanical Gardens, on *C. pauciflora*, 6 June 2005, coll. T. Inuma, MUMH 3682; 26 May 2008, coll. Y. Shiroya and I. Araki, MUMH 4924; on *C. spicata*, 6 June 2005, coll. T. Inuma, MUMH 3687; 14 Nov. 2005, coll. S. Takamatsu and R. Divarangkoon, MUMH 4104, HAL 2312 F; Japan, Tochigi Prefecture, Nikko-shi, Botanical Gardens, Nikko Graduate School of Science, The University of Tokyo, on *C. pauciflora*, 21 Sept. 2006, coll. S. Takamatsu, MUMH 4315; Japan, Ibaraki Prefecture, Tsukuba-shi, University of Tsukuba, on *C. spicata*, 21 Oct. 2006, coll. S. Takamatsu, Y. Shiroya, and M. Ito, MUMH 4404.

Discussion

The DNA sequences from four *E. corylopsidis* isolates on *C. pauciflora* and *C. spicata* were identical in both ITS and 28S rDNA regions and formed a distinct clade (BS = 100%, PP = 1.0 in both ITS and 28S trees). This clade was supported by the identical morphological characteristics of these isolates. *Erysiphe katumotoi* was sister to *E. corylopsidis* in the ITS tree, but this was supported by neither BS nor PP values. Preliminary analyses including other unpublished DNA sequences of the genus *Erysiphe* also suggested that *E. corylopsidis* forms an independent lineage.

The morphological characteristics of *E. corylopsidis*, viz. appendages dichotomously branched several times at the apex and multiple asci in a chasmothecium, indicate that the fungus belongs to the section *Microsphaera* in the genus *Erysiphe*. Among five powdery mildew species known to occur on the Hamamelidaceae, there is no species belonging to the section *Microsphaera* (Braun 1987; Nomura 1997; Braun and Takamatsu 2000). Therefore, this is the first species of section *Microsphaera* occurring on a host of the Hamamelidaceae.

The characteristics of *E. corylopsidis* with fewer than 15 appendages per chasmothecium and the primary branches occasionally elongated are shared by *E. clethrae* (U. Braun) U. Braun & S. Takam., *E. erlangshanensis* (Y.N. Yu) U. Braun & S. Takam., *E. katumotoi*, *E. miyabeana* (U. Braun) U. Braun & S. Takam., and *E. tiliae* (Eliade) U. Braun & S. Takam. (Braun 1987). *Erysiphe corylopsidis*, having 2–5-spored asci, differs from these species, which possess 5 or more ascospores per ascus. In addition, the host ranges of these species are restricted and confined to unrelated hosts, viz. *Clethra barbinervis* Siebold & Zucc. (Clethraceae) for *E. clethrae*, *Lonicera* spp. (Caprifoliaceae) for *E. erlangshanensis*, *Ligustrum obtusifolium* (Oleaceae) for *E. katumotoi*, *Styrax japonica* Siebold & Zucc. (Styracaceae) for *E. miyabeana*, and *Tilia* spp. (Tiliaceae) for *E. tiliae*.

Acknowledgments We thank Dr. Uwe Braun for his critical reading of the manuscript, Dr. Chiharu Nakashima for the Latin description, and Drs. Yukio Sato and Hiromichi Horie for helpful comments during the work. The experiments comply with the current laws of Japan. The authors have no conflict of interest.

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