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ACHIEVEMENTS

Publications :

1. Braun, U., Shi, A., Mmbaga, M., Takamatsu, S., **Divarangkoon, R.,** Chen, P. (2007) *Erysiphe abbreviata* on cherry bark oak—morphology, phylogeny and taxonomy. Mycologia, 99(5). pp. 655–663.
2. Takamatsu, S., Masuya, H., **Divarangkoon, R.,** Nomura, Y. (2008) *Erysiphe fimbriata* sp. nov.: a powdery mildew fungus found on *Carpinus laxiflora*. Mycoscience 49: 185–191.
3. Takamatsu, S., Heluta, V., Havrylenko, M., **Divarangkoon, R.** (2009) Four powdery mildew species with catenate conidia infect *Galium*: molecular and morphological evidence. Mycological Research 113: 117-129.
4. **Divarangkoon, R.**, Meeboon, J., Monkhung, S., To-anun, C., Takamatsu, S. (2011) Two new species of *Erysiphe* (*Erysiphales*, *Ascomycota*) from Thailand. Mycosphere, Vol.2 Issue.3 No.6: 231-238.

POSTER AND ORAL PRESENTATION

1. Takamatsu, S., Yamamoto, H., **Divarangkoon, R.,** Ito, M., Baba, H. (2007) Teleomorph and Taxonomic Revision of a powdery Mildew Fungus, *Erysiphe* (*Uncinula*) *nankinensis*, on *Acer buergerianum* (Abstracts of the Papers Presented at the Annual Meeting of the Society) [in Japanese] Annals of the Phytopathological Society of Japan, 73(3). 183. 20070825.
2. Araki, I., Motohashi, K., Meeboon, J., **Divarangkoon, R.,** Nachaiwiang, S., To Anun, C., Nakashima, C. (2007) Flora of Plant Parasitic Fungi in Northern Thailand. Abstracts of Papers Presented at the Meeting of the Mycological Society of Japan, Vol. 51 (2007) No. SPACE pp.49.
3. Monkhung, S., **Divarangkoon, R.**, To-anun, C., (2008) First report of Phyllactinia dalbergiae on Dalbergia lanceolaria (Fabaceae) in Thailand. The Annual Meeting of Thai Mycological Association (TMA) and Mycology conference in Thailand. October 10-11, 2008. Faculty of Agriculture. Khonkhan University, Khonkhan, Thailand.

4. Monkhung, S., **Divarangkoon, R.**, To-anun, C., (2009) Two Powdery Mildew Fungi on *Morus alba* in Thailand. The Annual Meeting of Thai Mycological Association (TMA) and Mycology conference in Thailand. October 24, 2009. Maejo University, Chiang Mai, Thailand.
5. **Divarangkoon, R.,** Monkhung, S., To-anun, C., (2009) First report of two *Brasiliomyces (Erysiphaceae)* on *Lithocarpus* spp. (Fagaceae) from Thailand. The Annual Meeting of Thai Mycological Association (TMA) and Mycology conference in Thailand. October 24, 2009. Maejo University, Chiang Mai, Thailand.
6. Monkhung, S., **Divarangkoon, R.**, To-anun, C. (2010) Morphology and Phylogeny of *Oidiopsis* (Erysiphaceae) found on *Capsicum* spp. in Thailand. The Thai Phytopathological Society. May 15, 2010. KU, Bangkok. Thailand.

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Erysiphe abbreviata on cherry bark oak—morphology, phylogeny and taxonomy

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Abstract: Powdery mildew on cherry bark oak (*Quercus falcate* var. *pagodifolia*) collected in Tennessee, USA, was determined to be *Erysiphe abbreviata*, a species confined to North America. The diagnostically important anamorph of this species is described for the first time. Sequence analyses of the rDNA ITS region and D1/D2 domains of the 28S rDNA were used to obtain phylogenetic data for and taxonomic conclusions about this species. The structure of the anamorph (*Oidium* subgen. *Pseudoidium*) and the molecular data support the placement of this species in *Erysiphe* emend. (including *Microsphaera*) as a species separate from the Eurasian *Erysiphe alphitoides*.

Key words: anamorph, cherry bark oak, *Erysiphe abbreviata*, *Erysiphe* sect. *Microsphaera*, internal transcribed spacer (ITS), powdery mildew, *Quercus falcata* var. *pagodifolia*, 28S rDNA

INTRODUCTION

Species in the genus *Quercus* L. (oak) in the Fagaceae are hosts to a number of powdery mildews, including numerous species of *Erysiphe* sect. *Microsphaera* (Lév.) U. Braun & Shishkoff. The causal agents of oak powdery mildews in North America (Braun 1984, 1987, Braun and Takamatsu 2000, Farr et al 1989) are *Erysiphe abbreviata* (Peck) U. Braun & S. Takam. (≡ *Microsphaera abbreviata* Peck), *E. calocladophora* (G.F. Atk.) U. Braun & S. Takam. (≡ *M. calocladophora* G.F. Atk.), *E. extensa* (Cooke & Peck) U. Braun & S. Takam. var. *extensa* (≡ *M. extensa* Cooke & Peck var. *extensa*) and *E. extensa* var. *curta* (U. Braun) U. Braun & S. Takam. (≡ *M. extensa* var. *curta* U. Braun). *E. calocladophora* also was recorded on oak from Japan (Nomura 1997).

The name *E. quercina* Schwein. (≡ *M. quercina* [Schwein.] Burrill), variously applied to North American oak powdery mildews, is doubtful and excluded. The type material is sterile (i.e. without ascomata, Braun 1987).

Erysiphe alphitoides (Griff. & Maubl.) U. Braun & S. Takam. (≡ *M. alphitoides* Griff. & Maubl.) is common and widespread in Asia and Europe and has been introduced into North America (Braun 1987, Chen et al 1987, Nomura 1997). *Erysiphe hypophylla* (Nevod.) U. Braun & J.H. Cunnington (≡ *M. hypophylla* Nevod.) was the second described Eurasian species of *Erysiphe* sect. *Microsphaera* on oaks. It was described from Russia and later spread rapidly westward and was recorded, discussed, described and illustrated in detail from various countries (Blumer 1967). Its taxonomic status was controversial (i.e. it was recognized either as separate species or reduced to synonymy with *E. alphitoides*, Braun 1995). However molecular comparisons support the recognition of *E. alphitoides* and *E. hypophylla* as separate species (Cunnington 2002, Cunnington et al 2003, Braun et al 2002). Asian collections of *E. alphitoides s. lat.* are morphologically and genetically heterogeneous (unpublished data) so the number of distinct species on oaks in Asia is unclear.

The taxonomy of North American oak powdery mildews has been discussed and treated with disagreement among authors. Most of the North American authors followed Salmon's (1900) wide species concept of the Erysiphales and assigned most taxa of Microsphaera Lév. (now *Erysiphe* sect. *Microsphaera*) to a single compound species, *M. alni* (Wallr.) G. Winter, which included *M. penicillata* (Wallr. : Fr.) Lév. (now *E. penicillata* [Wallr.] Link) as a synonym (Parmelee 1977, Farr et al 1989). *M. extensa* was treated as a variety of the latter species. *M. abbreviata* as well as *M. alphitoides* were considered to be conspecific and synonyms of *M. alni* (= *M. penicillata*). Chen et al (1987) cited *M. abbreviata* as synonym of *M. alphitoides*. On the other hand Braun

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(1987) considered *M. abbreviata* a separate North American species, and based on morphological examinations he cited numerous host species of *Quercus*.

This study was carried out to elucidate the taxonomy of the North American *E. abbreviata* and its relation to *E. alphitoides* with fresh material on *Quercus falcata* var. *pagodifolia* from Tennessee, USA. An anamorph was observed for the first time and provides diagnostically and taxonomically important details as noted for other species of this fungal group (Boesewinkel 1977, Braun 1995, Braun et al 2002, Shin 2000). The teleomorph and the hitherto unknown anamorph were examined carefully, and molecular sequence analyses of the rDNA region have been carried out and compared with sequences of *E. alphitoides* and other allied taxa.

MATERIALS AND METHODS

Materials.—Powdery mildew samples were collected from cherry bark oak leaves naturally infected with powdery mildew in McMinnville, Tennessee, in 2005 and 2006. Part of the fresh materials were used for microscopic observation and DNA extraction, and the remaining samples were stored as herbarium specimens in U. Braun, Fungi selecti exsiccati 31 (BPI, GZU, HAL, HMAS, IMI, KR, LE, M, PDD, SMK, VPIR; abbreviations according to Holmgren et al 1990) and in the Mie University Mycological Herbarium under specimen No. MUMH 3790.

DNA amplification and sequencing.—Powdery mildew mycelia and conidia for DNA analysis carefully were isolated from infected oak leaves with a scalpel; most samples contained some host tissue. Approximately 1–3 mg (fresh weight) of mycelia and conidia was used for DNA extraction with the DNeasy Plant Mini Kit (QIAGEN Inc, Valencia, California) following manufacturer protocols. Approximately 1–5 μg DNA was isolated from each sample. Each DNA sample was diluted to approximately 20 ng/μL for polymerase chain reaction (PCR).

The primers PMITS1 (tcggactggcccagggaga) and PMITS2 (tcactcgccgttactgaggt) (Cunnington et al 2003) were used to amplify a target region of the ITS operon. PCR amplification was performed in a Touchgene Thermal Cycler (Barloworld Scientific Ltd., United Kingdom). The thermocyler protocol was: Each 50 μL PCR reaction mixture consisted of 36 μL sterile ddH_2O, 5 μL 10× PCR buffer (Promega), 3 μL $MgCl_2$ (25 mM), 1.5 μL dNTP (10 mM total, 2.5 mM each), 1.5 μL each primer (20 ng/μL), 0.2 μL Taq polymerase (Promega) (5 U/μL) and 1.3 μL template DNA (20 ng/μL). PCR cycles consisted of an initial denaturation step at 94 C for 5 min followed by 40 cycles of 1 min at 93 C (denaturation), 1 min at 53 C (annealing), 2 min at 72 C (extension). An extension cycle at 72 C for 5 min was followed by a 4 C soak. PCR products were viewed with 1.5% agarose gel in 1× TBE stained with ethidium bromide. The resulting PCR products were sequenced directly with amplification primers (Davis Sequencing Inc., Davis, California).

Another sequence analysis was carried out independently at Mie University, Japan, with a specimen collected in 2005 (deposited in the Mie University Mycological Herbarium under specimen No. MUMH 3790) to confirm the robustness of the sequence obtained in USA. DNA extraction, PCR amplification and sequencing were carried out according to the procedures described in Takamatsu et al (2006).

Phylogenetic analysis.—Sequences were aligned initially with the Clustal X package (Thompson et al 1997). Alignment then was refined visually with a word processing program, using color-coded nucleotides, and ambiguously aligned sites were removed from the dataset in the analyses. The alignments were deposited in TreeBASE (http://www.treebase.org/) under accession No. SN3029. Phylogenetic trees were produced with maximum parsimony (MP) in PAUP* 4.0 (Swofford 2001) and Bayesian analysis in MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001). MP analyses were done 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 strength of the internal branches of the trees was tested with bootstrap analyses using 1000 replications (Felsenstein 1985). *Byssoascus striatisporus* (Barron & Booth) von Arx (U17912) was used as outgroup taxon based on Mori et al (2000).

For Bayesian phylogenetic analyses the best-fit evolutionary model was determined for each dataset by comparing different evolutionary models via the Akaike information criterion (AIC) with PAUP* and MrModeltest 2.2 (Nylander 2004). MrBayes was launched with random starting trees for 1.0×10^6 generations and Markov chains were sampled every 100 generations, which resulted in 1.0×10^4 sampled trees. To ensure that the Markov chain did not become trapped in local optima we used the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm, performing the estimation with four incrementally heated Markov chains. To establish whether the Markov chains had reached stationarity we plotted the likelihood scores of sampled trees against generation time. Stationarity was deemed to have been reached when the likelihood of the sample points reached a stable equilibrium (Huelsenbeck and Ronquist 2001).

Morphology.—The fungus was placed in a drop of distilled water with lactic acid on a microscope slide and examined under oil immersion by standard light microscopy. Colorless structures of the anamorph were stained with cotton blue for morphological examination.

RESULTS

Molecular phylogeny.—The primer pair PMITS1/PMITS2 produced a 779 bp DNA fragment and the resulting sequence data have been deposited in GenBank (DQ866999). The analysis in Japan resulted

in a sequence of 1298 bp and was included in the ITS region, 5.8S rDNA and D1/D2 domains of the 28S rDNA and deposited in DDBJ (AB271785).

These two independent extraction and sequence analyses carried out in USA and Japan resulted in identical ITS sequences. BLAST search analysis indicated that the ITS sequence from cherry bark oak powdery mildew had 97% (590/608) similarity to *Microsphaera alphitoides* (GenBank: AJ417497); 97% (666/684) to *Erysiphe euonymi-japonici* (GenBank: AB250228); 95% (708/744) to *Erysiphe cruciferarum* (GenBank: AF031283); 96% (658/684) to *Erysiphe syringae* (GenBank: DQ184478); 96% (596/620) to *Erysiphe elevata* (GenBank: AY587013); 95% (642/670) to *Oidium heveae* (GenBank: AB193607); 96% (580/598) to *Microsphaera pseudolonicerae* (GenBank: AB015915); and 95% (616/647) to *Microsphaera trifolii* var. *trifolii* (GenBank: AB163926).

We performed a total of four independent phylogenetic analyses with each 28S rDNA and ITS sequences by MP and Bayesian methods. In the 28S rDNA analyses a sequence from *E. abbreviata* was aligned with 53 sequences of the Erysiphales retrieved from the GenBank DNA database and outgroup sequence. The alignment data matrix consists of 55 taxa and 825 characters, in which 259 sites were variable and 176 sites were phylogenetically informative for parsimony analysis.

Forty-eight equally most parsimonious trees with 874 steps (CI = 0.4291, RI = 0.7149, RC = 0.3067) were constructed by the MP analysis. A tree having the highest likelihood score among the 48 trees is provided (FIG. 1). Although the branching order of the clades differed slightly among the 48 trees, the major clades commonly were supported in all MP trees. In the Bayesian analysis GTR + I + G was selected as the best-fit model for the given data matrix by AIC in MrModeltest. Bayesian analysis with MrBayes and the evolution model also resulted in a similar tree topology (not shown). The five tribes (viz. the *Erysipheae*, *Golovinomyceteae*, *Phyllactinieae*, *Cystotheceae* and *Blumerieae*, and *Oidium* subgenus *Microidium* To-anun & S. Takam.) recognized in the *Erysiphaceae* (Cook et al 1997, Braun 1999, Braun and Takamatsu 2000, Mori et al 2000, To-anun et al 2005) are supported as respective groups, although tribe Golovinomyceteae is paraphyletic and forms a large clade with *Oidium* subgenus *Microidium*. The basal position in the *Erysiphaceae* of the genera *Parauncinula* and *Caespitotheca* (Takamatsu et al 2005a, b) also is supported. Of the 54 28S rDNA sequences from the Erysiphales 15 sequences shown in bold letters are those from specimens on *Quercus*. These include five genera and nine species of the Erysiphales. These sequences are scattered in the tree and do not group together. This might indicate that the Erysiphales independently acquired parasitism to *Quercus* species many times. *Erysiphe abbreviata* is placed in the clade of tribe Erysipheae, which is coincident with the morphological charateristics. This fungus is closely related to *E. alphitoides* from *Quercus* spp. and *Erysiphe* sp. from *Q. phillyraeoides* but differs 9-base (98.7% similarity) from *E. alphitoides* AB237811 and 13-base (98.1% similarity) from *Erysiphe* sp. AB197135.

We used ITS sequence to investigate in detail the phylogenetic placement of *E. abbreviata* within tribe Erysipheae. In the analyses a sequence from *E. abbreviata* was aligned with 61 sequences of tribe Erysipheae retrieved from the GenBank DNA database. The alignment data matrix consists of 62 taxa and 607 characters, of which 47 at the 3′ end of the ITS2 region were excluded from the analyses due to ambiguity of the alignment. Of the remaining 560 characters, 186 sites were variable and 130 sites were phylogenetically informative for parsimony analysis. *Erysiphe glycines* Tai was used as outgroup taxon based on Takamatsu et al (1999). A total of 275 382 equally most parsimonious trees with 452 steps (CI = 0.5509, RI = 0.7456, RC = 0.4107) and 34 islands were constructed by MP analysis. One of the trees is shown (FIG. 2). Although the branching order of clades differs among the trees, major clades commonly were supported. In the Bayesian analysis SYM + I + G was selected as the best-fit model for the given data matrix by AIC in MrModeltest. Bayesian analysis with MrBayes and the SYM + I + G model also resulted in a similar tree topology (not shown).

Fourteen sequences from *Erysiphe* species parasitic to *Quercus* hosts shown in bold letters are closely related. However they do not group into a clade. *Erysiphe abbreviata* has a unique ITS sequence that differs 17-base (96.8% similarity) from *E. alphitoides* AJ309200, 26-base (95.5% similarity) from *E. hypophylla* AF298544 and 28-base (95.0% similarity) from *Erysiphe* sp. AB193590.

Morphology.—Symptoms and characters of the teleomorph of *E. abbreviata* were typical of those described by Braun (1987), who did not observe an anamorph. The following first description of the conidial state and a redescription of chasmothecia of *E. abbreviata* are based on the examination of the fresh collections from Tennessee.

Erysiphe abbreviata (Peck) U. Braun & S. Takam., Schlechtendalia 4:4, 2000. FIGS. 3–4

≡ *Microsphaera abbreviata* Peck, Rep. (Annual) New York State Mus. Nat. Hist. 28:64, 1876.

= *M. alni* auct. Amer. bor. p.p.

= *M. penicillata* auct. Amer. bor. p.p.

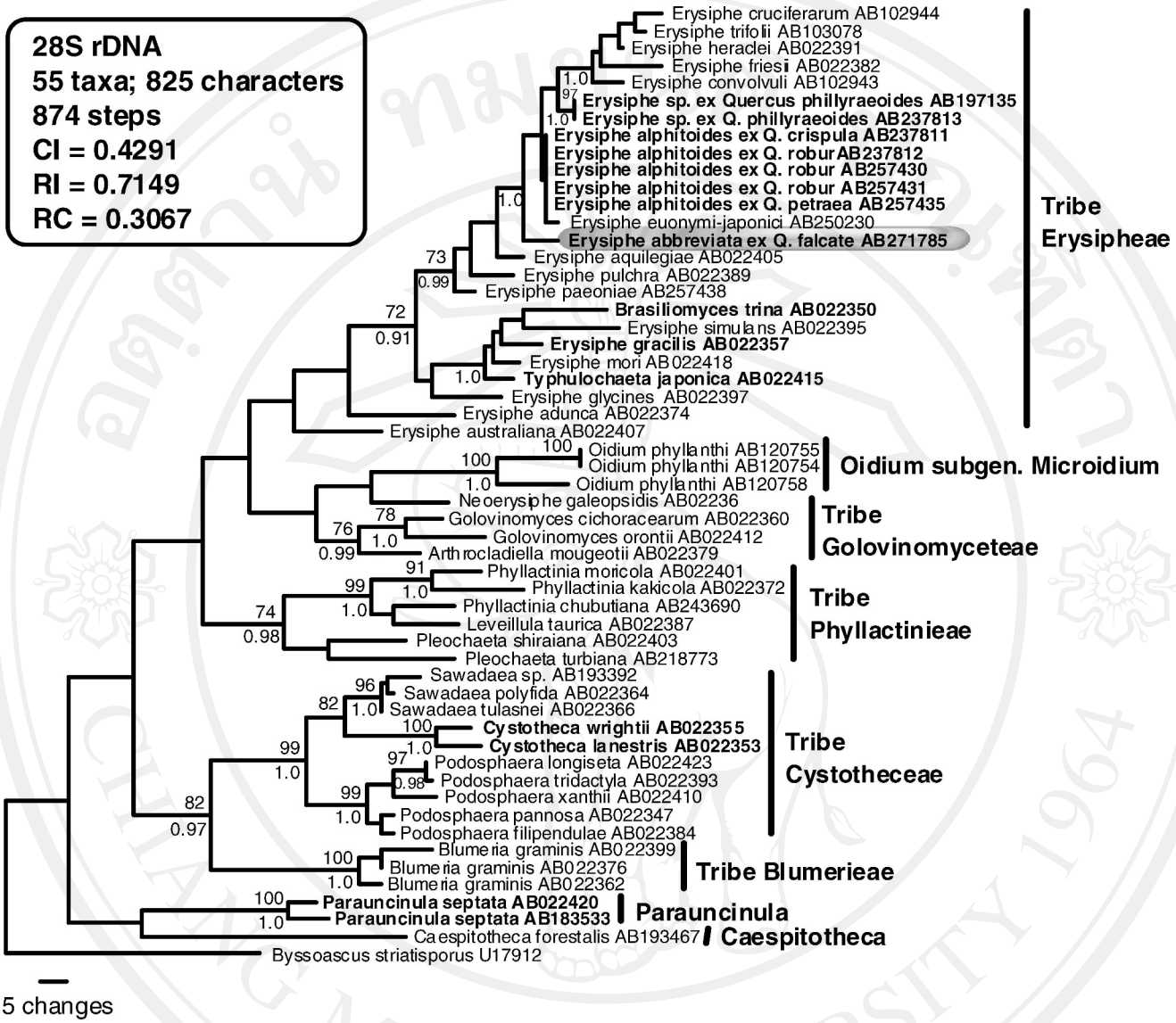

FIG. 1. Phylogenetic analysis based on the 28S rDNA dataset for *Erysiphe abbreviata* on cherry bark oak and 53 taxa of the Erysiphales covering all known tribes, and outgroup taxon. The tree is a phylogram of one of the 48 most parsimonious trees with 874 steps, which was obtained with a heuristic search employing 100 times random stepwise addition option of PAUP* treating gaps as missing data. This tree is also the maximum likelihood tree among the 48 most parsimonious trees. Horizontal branch lengths are proportional to the number of nucleotide substitutions inferred to have occurred along a particular branch of the tree. Greater than 70% maximum-parsimony bootstrap and >0.95 Bayesian posterior probability values are shown on and under the respective branch. The respective groups of the Erysiphales are shown on the right of the tree. AB257430 and AB257431 originally were deposited in DNA database as *E. hypophylla* and re-identified as *E. alphitoides* by A. Bolay.

Mycelium external, superficial, forming white patches or effuse. Hyphae branched, straight to strongly sinuous, 2–6 µm wide, smooth, thin-walled. Appresoria solitary, nipple-shaped to lobed, 3–8 µm diam. Conidiophores solitary, arising from external hyphal cells, position between two septa more or less central, erect, straight, usually 60–120 µm long, occasionally somewhat longer, foot-cells subcylindrical, straight, somewhat curved to frequently sinuous-helicoid at the base 40–70 × 5–10 µm, followed by 1–3 mostly shorter cells, occasionally following cells about as long as the foot cells. Conidia formed singly, occasionally 2–3 conidia adhering in short chains, ellipsoid-ovoid, doliiform to cylindrical, 25–45 × 10–20 µm, length/width ratio 2.2–2.9, apex rounded to subtruncate, base subtruncate, germ tubes terminal or subterminal, short, straight to sinuous, terminating in a somewhat lobed appressorium or without any appressorium.

Chasmothecia hypophyllous, scattered to subgregar-

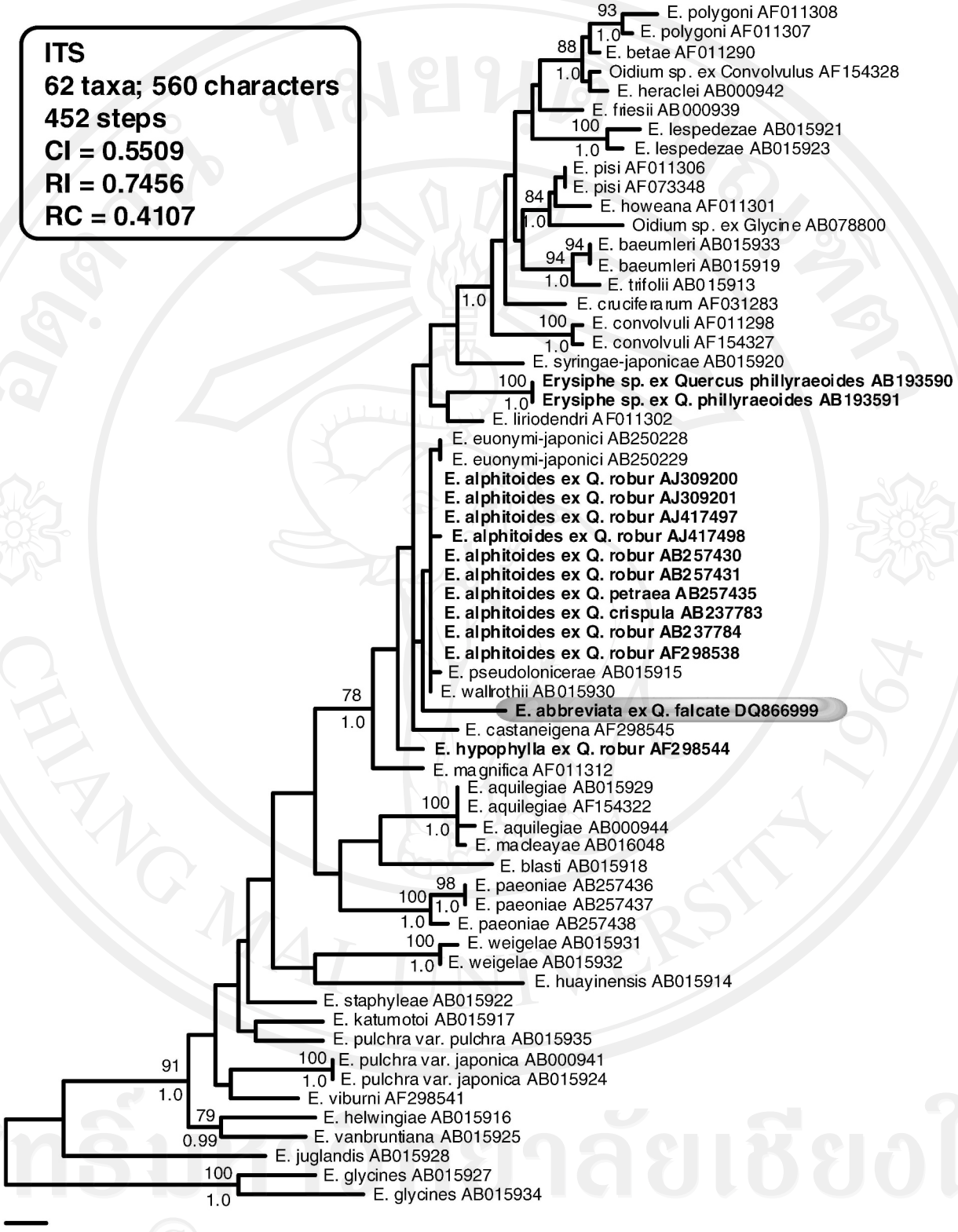

FIG. 2. Phylogenetic analysis based on the rDNA ITS dataset for *Erysiphe abbreviata* on cherry bark oak and 61 taxa of the tribe Erysipheae. The tree is a phylogram of one of the 275 382 most parsimonious trees with 452 steps, which was found with a heuristic search employing 100 times random stepwise addition option of PAUP* treating gaps as missing data. Horizontal branch lengths are proportional to the number of nucleotide substitutions inferred to have occurred along a particular branch of the tree. Greater than 70% maximum-parsimony bootstrap and >0.95 Bayesian posterior probability values are shown on and under the respective branch. AB257430 and AB257431 originally were deposited in DNA database as *E. hypophylla* and re-identified as *E. alphitoides* by A. Bolay.

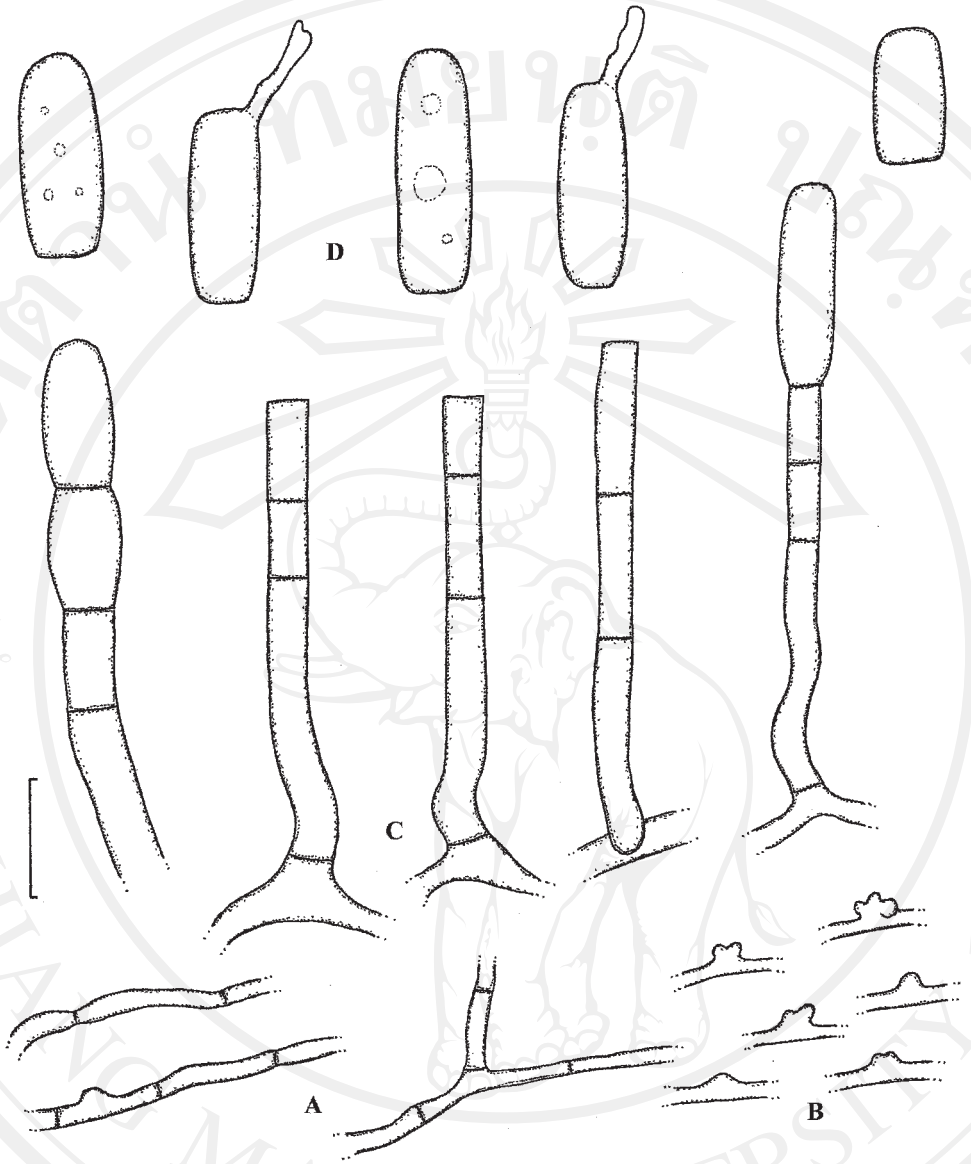

FIG. 3. Anamorph of *Erysiphe abbreviata*. A. Hyphae. B. Appresoria. C. Conidiophores. D. Conidia with and without germ tubes. Bar = 20 µm.

ious, 70–125(–130) µm diam. Peridial cells irregularly polygonal in outline or walls somewhat sinuous, 8–30 µm diam. Appendages more or less equatorial, 3–15, rarely more, stiff, straight to somewhat curved, short, 0.5–1(–1.25) times as long as the chasmothecial diam, moderately thick-walled throughout or thick-walled at the base and thin-walled toward the apex, hyaline or only pigmented at the very base, continuous or with a single basal septum, smooth to rough-walled, above all in the lower half, 7–10 µm wide near the base, apex 4–6 times closely and regularly dichotomously branched, primary branches occasionally slightly elongated, ultimate tips in mature appendages recurved. Asci 3–6, sessile to short-stalked, saccate, broadly ellipsoid-ovoid or often subglobose, 50–70 × 35–55 µm, wall of the apex (oculus) thinner, ca. 20–25 µm diam, not distinct, 3–6-spored, ascospores ellipsoid-ovoid(-subglobose), 20–32 × 12–21 µm.

Material examined: USA, Tennessee, McMinnville, Tennessee State University, Nursery Research Center, 12 Oct 2005, A. Shi, in U. Braun, Fungi selecti exsiccati 31 (BPI, GZU, HAL, HMAS, IMI, KR, LE, M, PDD, SMK, VPIR; abbreviations according to Holmgren et al 1990); and 6 Jun 2006 (HAL).

DISCUSSION

In previous taxonomic treatments the North American *Erysiphe abbreviata* and the Eurasian *E. alphitoides* were assigned respectively to the compound species

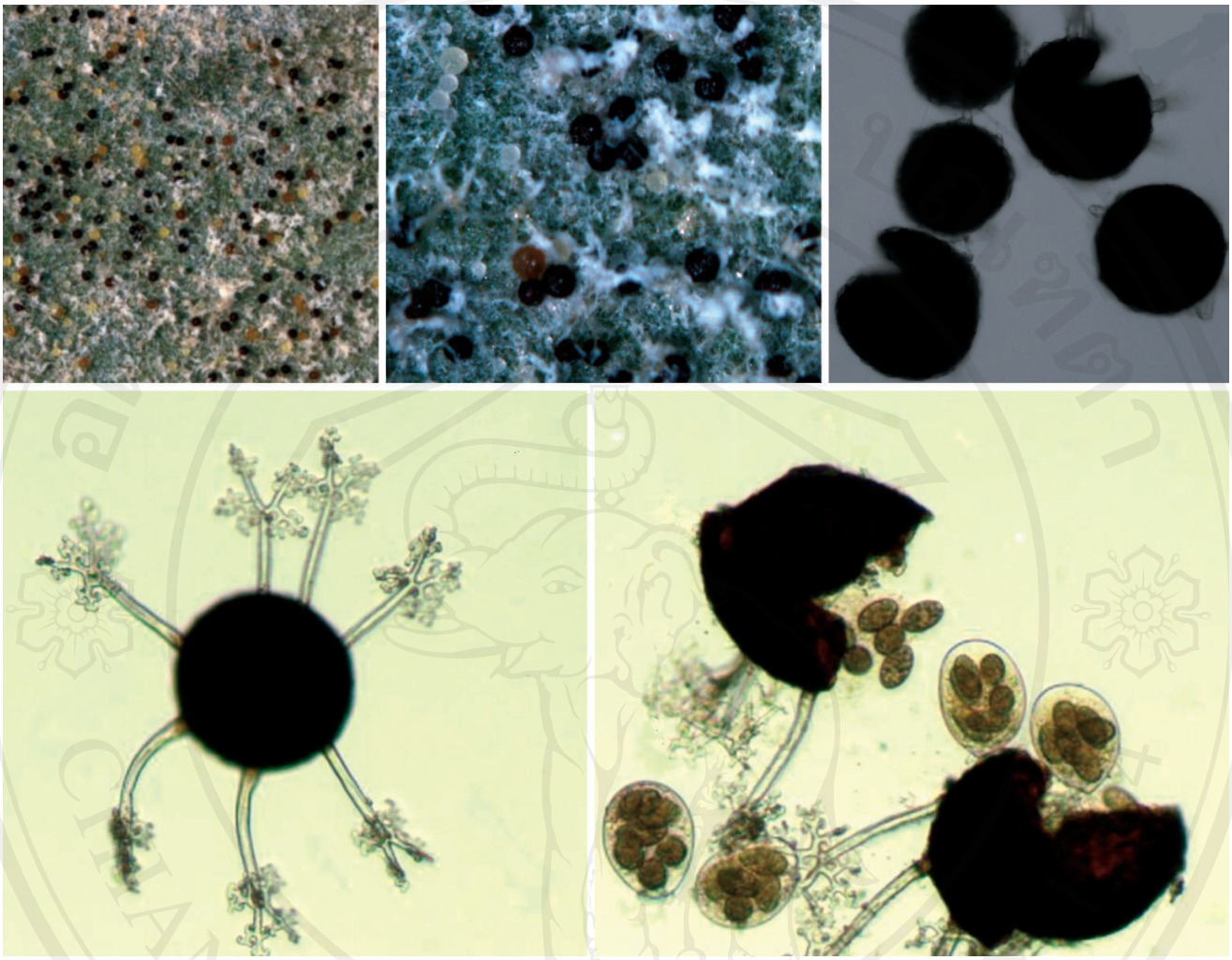

FIG. 4. Chasmothecia, asci and ascospores of *Erysiphe abbreviata*.

Microsphaera alni and *M. penicillata* (Farr et al 1989, Parmelee 1977, Salmon 1900). Chen et al (1987) cited *M. abbreviata* as a synonym of *E. alphitoides*. The present molecular analysis of rDNA ITS sequence data of *E. abbreviata* clearly places this species in the *Erysiphe* emend. clade, characterized by having anamorphs belonging in *Oidium* subgen. *Pseudoidium* (Y.S. Paul & J.N. Kapoor) R.T.A. Cook et al (appresoria lobed, conidia formed singly) and ascomata with numerous 3–8-spored asci (Braun et al 2002, Cook et al 1997, Mori et al 2000, Takamatsu et al 1999). Comparisons with GenBank data for *E. alphitoides* and other *Erysiphe species* shows that species from oak all are within the same tribe/subcluster of *Erysiphe* but they are not necessarily sibling species. Sequence analyses by BLAST indicated that the sequence of *E. abbreviata* from this study was closest to *E. alphitoides* (GenBank: AJ417497) and had 97% (590/608) similarity. In the context of powdery mildew fungi ITS differences of 3–5% are indicative of clear differentiation between closely allied species. Similarities in ITS data of 98–99% are not unusual in mophologically distinct species of fungi, and the discrimination of closely allied taxa often is not based solely on ITS data (Takamatsu et al 1999).

The molecular results are strongly supported by morphological data. In monographic studies on powdery mildew fungi Braun (1987) treated *E. abbreviata* and *E. alphitoides* as two distinct species, described and illustrated the teleomorphs in detail, pointed out differences and provided a key. The chasmothecia of *E. abbreviata* are easily distinguishable from those of *E. alphitoides* by having few, short appendages (3–15, 0.5–1[–1.25] times as long as the chasmothecial diameter vs. 4–28, 0.5–2 times as long as the chasmothecial diameter in *E. alphitoides*) and 3–6-spored asci (vs. [4–]8-spored in *E. alphitoides*).

The chasmothecia of *E. abbreviata* are hypophyllous whereas those of *E. alphitoides* are usually epiphyllous. In addition this first observation of the anamorph of *E. abbreviata* allowed a direct comparison with the conidial form of *E. alphitoides*, demonstrating that conidiophores and conidia of the two species are also distinct. The foot cells of the conidiophores of *E. abbreviata* are 40–70 µm long and mostly curved to sinuous-helicoid, and mature conidia are ellipsoid-ovoid, doliiform to cylindrical, 25–45 × 10–20 µm, a length/width ratio of 2.2–2.9 (vs. foot cells 15–30 µm long and usually cylindrical, straight, mature conidia mostly doliiform, limoniform, 25–40 × 13–25 µm, length/width ratio of 1.4–2.3, usually <2, in *E. alphitoides*).

In conclusion *E. abbreviata*, the North America oak powdery mildew, and *E. alphitoides*, the Eurasian taxon, are two clearly distinct species, genetically well discriminated, with easily distinguishable anamorphs and teleomorphs. These results clearly support the narrow species concept for powdery mildew fungi as introduced and adopted by authors in Asia and Europe (Blumer 1967, Braun 1987, Bunkina 1991, Chen et al 1987, Junell 1967, Nomura 1997, Shin 2000) and reject Salmon's (1900) broad species concept, which is popular in the North American mycological and phytopathological powdery mildew literature. Sequence analyses and examinations of anamorphs of *E. calocladophora* and *E. extensa* ought to be the next step to elucidate the phylogeny and taxonomy of North American oak powdery mildews.

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FULL PAPER

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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.

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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

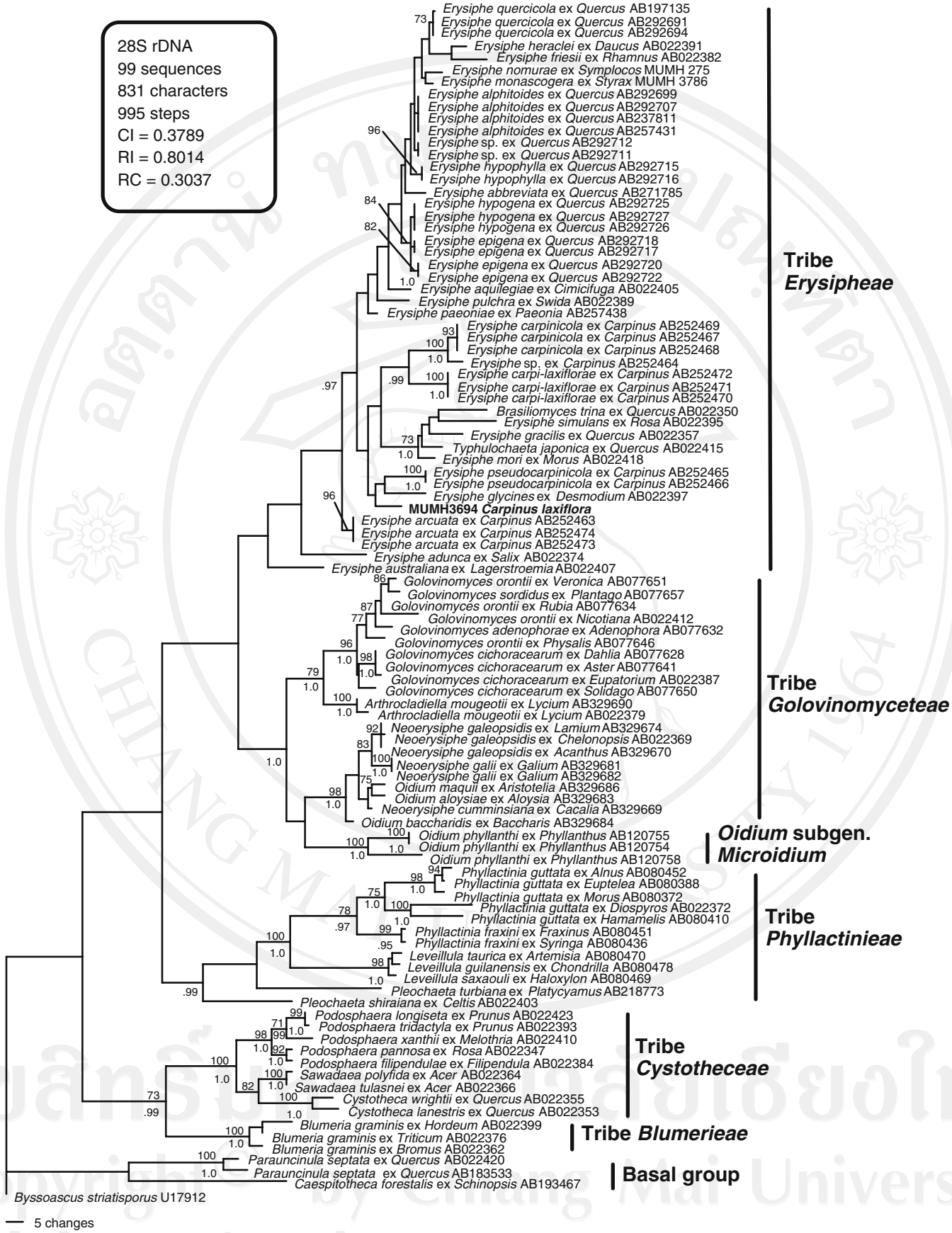


Fig. 1. Phylogenetic analysis of the divergent domains D1 and D2 sequences of the 28S rDNA for 99 sequences from the Erysiphaceae covering all known tribes and one outgroup taxon. The tree is a phylogram of the maximum-likelihood tree among the 10^4 most parsimonious trees with 995 steps, which was obtained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

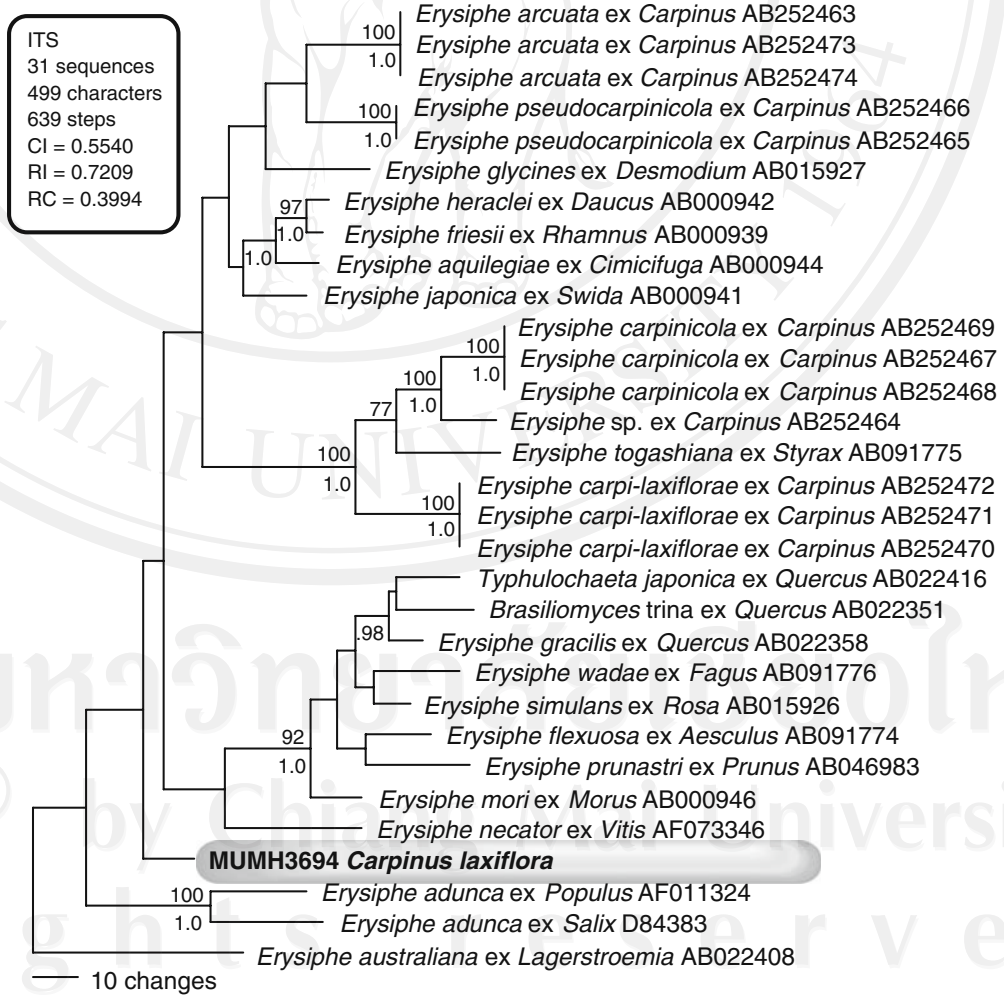
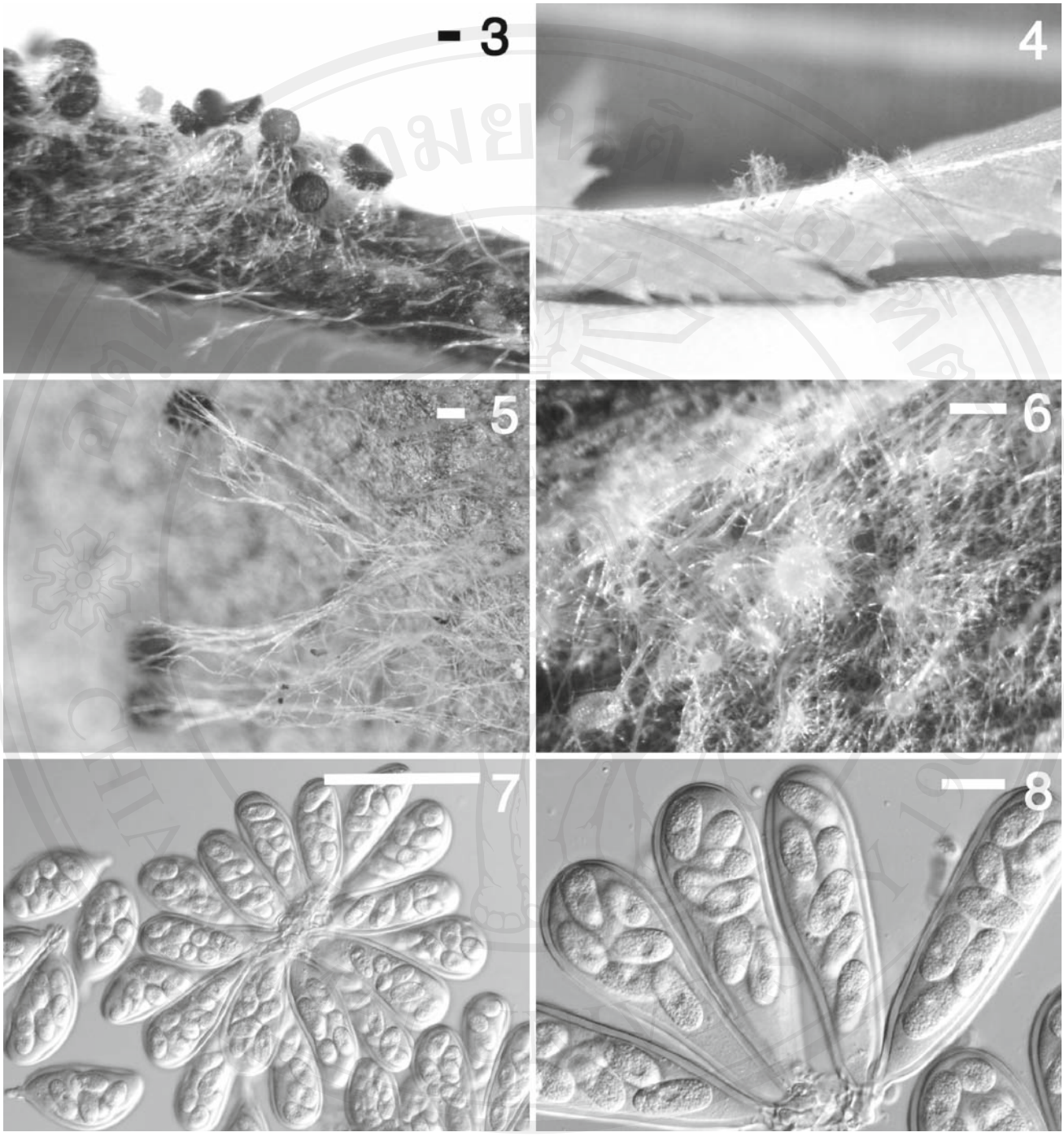


Fig. 2. Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for 31 sequences from *Erysiphe*. The tree is a phylogram of the maximum-likelihood tree among the 106 most parsimonious trees with 639 steps, which was obtained by a heuristic 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.

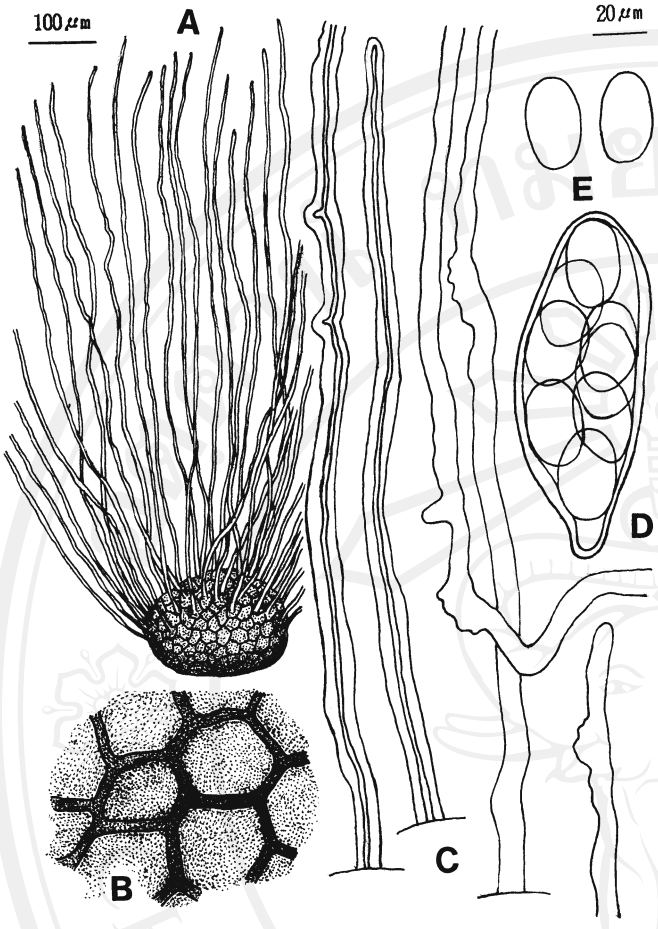


Fig. 9. *Erysiphe fimbriata.* **A** Chasmothecium. **B** Peridial cells. **C** Appendages. **D** Asci and ascospores. **E** Ascospores. *Bars* **A** 100 μm; **E** 20 μm (for **B–E)**

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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Four powdery mildew species with catenate conidia infect *Galium*: molecular and morphological evidence

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ABSTRACT

The *Erysiphaceae* are a group of obligately biotrophic fungi that cause powdery mildew disease of angiosperms. Due to their inability to be cultured on artificial media, the taxonomy of the *Erysiphaceae* has generally been based on the morphological characteristics of fresh and herbarium specimens. Thus, several morphological species with wide host ranges have long been maintained in this family, even though they clearly consist of several biological species. *Erysiphe galii* has been known as a powdery mildew of *Galium* spp. Recently, the former *E. galii* var. *galii* has been reassessed as *Neoerysiphe galii* and *E. galii* var. *riedliana* as *Golovinomyces riedlianus*, along with a taxonomic revision of the generic concept of the *Erysiphaceae*. The present study was conducted to evaluate the validity of the taxonomic revision of the two varieties of *E. galii*. During the course of this study, we found that the *Galium* powdery mildews consist of at least four different species, viz. *Neoerysiphe galii*, *Golovinomyces orontii*, *G. riedlianus*, and an unknown species collected in Argentina. The latter species is described as a new species, *Golovinomyces calceolariae*. The three species belonging to *Golovinomyces* are morphologically very similar to each other, i.e. the discrimination between them is rather difficult. The morphological differences of the three *Golovinomyces* species of *Galium* are discussed.

Introduction

The *Erysiphaceae* are a group of obligately biotrophic fungi that cause powdery mildew disease on about 10K angiosperm species (Amano 1986); the group consists of 16 genera and about 650 species (Braun 1999; Braun & Takamatsu 2000; Braun *et al.* 2002; Takamatsu *et al.* 2005a, 2005b; Liberato *et al.* 2006). Because they cannot be cultured on artificial media, the taxonomy of the *Erysiphaceae* has generally been based on the morphological characteristics of fresh and herbarium specimens. Thus, several morphological species with wide host ranges, such as *Golovinomyces cichoracearum*, *Leveillula taurica*, *Phyllactinia guttata*, and *Podosphaera fusca*, have long been maintained in the *Erysiphaceae*, even though they clearly represent several distinct biological species (Braun 1987). Taxonomic revisions on generic or higher levels have recently been conducted based on molecular data and anamorphic features (Cook *et al.* 1997; Braun 1999; Braun & Takamatsu 2000;

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Takamatsu et al. 2005a, 2005b). However, various revisions on species level remain to be performed for this family. Molecular data revealed that more than one species with similar morphology often infect the same host species. For instance, *Erysiphe alphitoides* (section *Microsphaera*) occurs on *Quercus serrata*, an oak tree common in Japan. Molecular and morphological analyses revealed that *E. alphitoides* is a compound species, consisting of three different *Erysiphe* species, *viz. E. alphitoides* s. str., *E. hypophylla* and *E. quercicola*; these species often occur simultaneously on the same leaf of *Q. serrata* (Takamatsu et al. 2007). Two *Erysiphe* species, *viz. E. syringae* and *E. syringae-japonicae*, occur on *Syringa* spp. (lilacs). These species are clearly discriminated by their rDNA sequences, despite their similar morphology. Biogeographical studies using molecular markers revealed that *E. syringae-japonicae* was recently introduced into Europe from East Asia (Seko et al. 2008). Therefore, molecular techniques have made it possible to study the distribution and migration of fungal species that are barely indistinguishable from each other by morphological characteristics (Matsuda & Takamatsu 2003; Takamatsu et al. 2008a, 2008b; Jankovics et al. 2008).

The cosmopolitan genus *Galium*, belonging to *Rubiaceae*, consists of about 300 herbaceous plant species. Two powdery mildew species with catenate conidia without fibrosin bodies (tribe *Golovinomyceteae*), *viz. E. galii* (Blumer 1933) and *E. riedliana* (Speer 1969), were known to occur on 36 *Galium* species (Amano 1986). These two *Erysiphe* species are characterized by chasmothecia with mycelioid appendages and catenate conidia. Braun (1983) regarded the morphological variation between the two species to be below the species level, and thus *E. riedliana* was reduced to a variety of *E. galii*. Variety *galii* has ascospores that develop after overwintering and lobed hyphal appressoria, whereas var. *riedliana* has ascospores that develop within the year and nipple-shaped hyphal appressoria. Later, the genus *Erysiphe* was divided into three genera, *viz. Erysiphe* s. str., *Golovinomyces* and *Neoerysiphe*, based on characteristics of the anamorph, as well as on molecular phylogenetic analyses (Heluta 1988; Braun 1999). *Erysiphe galii* var. *galii* was reassessed as *N. galii* (Braun 1999) and *E. galii* var. *riedliana* was reapproved as *G. riedlianus* (Heluta 1988). However, the validity of this taxonomic treatment has not yet been proven using DNA sequence analyses.

The present study was conducted to fill this gap. To this end, we collected *Galium* powdery mildew specimens from Europe, Asia, and South America, and determined the nucleotide sequences of the rDNA ITS regions, as well as the divergent domains, D1 and D2, of the 28S rDNA of the specimens. During the course of this study, we discovered that the *Galium* powdery mildews consist of at least four different groups. The results of molecular analyses as well as morphological observations are described in this paper.

Materials and methods

DNA extraction and amplification

Sources of the powdery mildew specimens used for the molecular analyses and the database accession numbers of their DNA sequences are listed in Table 1. Voucher specimens were deposited in BCRU, Institutional Herbarium of Centro Regional Universitario Bariloche, San Carlos de Bariloche, Argentina, in KW, National Herbarium of Ukraine, M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Kiev, Ukraine, or in the Mie University Mycological Herbarium (MUMH), Japan.

Whole-cell DNA was isolated from chasmothecia or mycelia using the chelex method (Walsh et al. 1991; Hirata & Takamatsu 1996). The ITS region, including the 5.8S rDNA and the 5′ end of 28S rDNA, which includes the variable domains D1 and D2, were amplified separately by two sequential PCR reactions using partially nested primer sets. PCR reactions were conducted using TaKaRa Taq DNA polymerase (TaKaRa, Tokyo) in a TP-400 thermal cycler (TaKaRa) under the following thermal cycling conditions: an initial denaturation step of 2 min at 95 °C, 30 cycles of 30 s at 95 °C, followed by 30 s at 52 °C for annealing, and 30 s at 72 °C for extension, and a final extension for 7 min at 72 °C. A negative control that lacked template DNA was included in each set of reactions. PCR products were subjected to electrophoresis in a 1.5 % agarose gel in TAE buffer, excised from the ethidium bromide-stained gel, and purified using the JETSORB Kit (Genomed, Oeynhausen) according to the manufacturer's protocol. Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in a DNA sequencer CEQ2000XL (Beckman Coulter, Fullerton, CA). The sequencing reactions were conducted using the CEQ Dye Terminator Cycle Sequencing Kit (Beckman Coulter) according to the manufacturer's instructions.

To amplify the ITS region, the primers ITS5 (White et al. 1990) and P3 (Kusaba & Tsuge 1995) were used for the first round of amplification. One microlitre of the first reaction mixture was then used for the second amplification, along with the partially nested primer sets ITS5 and ITS4 (White et al. 1990). The ITS5/ITS4 fragment was subjected to cycle-sequencing using primers ITS1, ITS4, T3, and T4 (Hirata & Takamatsu 1996). To amplify the 28S rDNA, primers PM3 (Takamatsu & Kano 2001) and TW14 (Mori et al. 2000), and NL1 (Mori et al. 2000) and TW14 were used for the first and second amplifications, respectively. Primers NL1, NL2, NL3 (Mori et al. 2000), and NLP2 were used for cycle-sequencing.

Phylogenetic analysis

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 S2130. Phylogenetic trees were obtained from the data using the MP method in PAUP 4.0 (Swofford 2001) and Bayesian analysis in MRBAYES 3.1.1 (Ronquist & Huelsenbeck 2003). MP analyses were performed with the heuristic search option using the tree bisection–reconnection (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The maximum tree number was set as 10K. The strength of the internal branches of the resulting trees was tested with BS analyses using 1K replicates with the step-wise addition option set as simple (Felsenstein 1985). BS values higher than 70 % were shown. To evaluate the

Table 1 – Sources of *Galium* powdery mildew and related materials used for phylogenetic analyses and DNA database accession numbers

Host	Location	Date	Voucher no.[a]	Accession no.[b]
Calceolaria polyrrhiza	Argentina: Río Negro, Parque Nacional Nahuel Huapi, Cerro Challhuaco	18 Apr 2001	BCRU 4527 MUMH 1934	AB430810
Galium album	Switzerland: CH, VD, Prangins, Aérodrome	3 Sep 1997	MUMH1301	AB430811
G. aparine	Argentina: Río Negro, Parque Nacional Nahuel Huapi, Cerro Challhuaco	18 Apr 2001	MUMH1879	AB430812
G. aparine	Lithuania	17 Jul 2000	MUMH 946	AB329681[c]
G. aparine	UK: Surrey	2004	K(M)129497	DQ359696[c]
G. aparine	Ukraine: Odessa region	29 Jun 1978	KW 11877 MUMH 3225	AB430813
G. aparine	Ukraine: Poltava region, Kobelyaky distr.	13 Jun 2002	MUMH 3222	AB329682[c]
G. odoratum	Ukraine: Crimea, Ai-Petri Mt	20 Jul 1982	KW 28570 MUMH 3219	AB329689[c]
G. odoratum	Ukraine: Zakarpattia region, Karpatsky reserve	18 Aug 1984	KW 28586 MUMH 3216	AB329688[c]
G. ruthenicum	Ukraine: Crimea, Opuk Mt	28 Sep 2003	KW 33932 MUMH 3223	AB430814
G. spurium var. *echinospermon*	Japan: Aichi, Nagoya	19 May 2000	MUMH 826	AB430815
G. spurium var. *echinospermon*	Japan: Okayama-shi	10 May 2004	MUMH 2622	AB430816
G. spurium var. *echinospermon*	Japan: Okayama-shi	6 Jun 2004	MUMH 2623	AB430817
G. spurium var. *echinospermon*	Japan: Okayama-shi	6 Jun 2004	MUMH 2624	AB430818
G. verum var. *asiaticum*	Japan: Gifu, Sekigahara	12 Nov 2000	MUMH 1148	AB430819
G. verum	Ukraine: Kyiv region, Borodianka distr.	6 Sep 1979	KW 11879 MUMH 3217	AB430820
Phuopsis stylosa	Iran: Guilan		No voucher	AB104525[c]

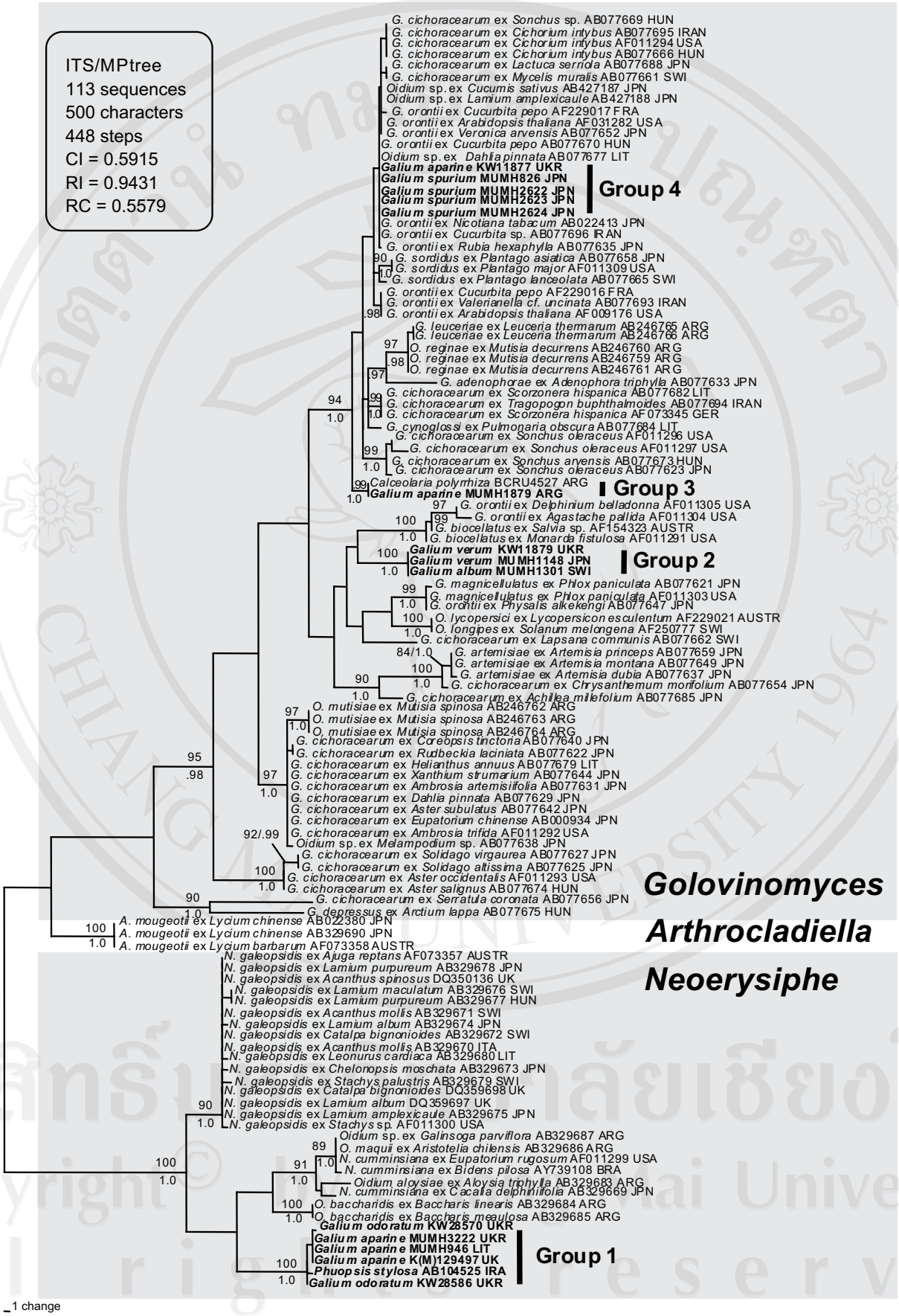
a Sources: BCRU, Institutional Herbarium of Centro Regional Universitario Bariloche, San Carlos de Bariloche, Argentina; K(M), Mycological collections in K(M) at Kew, UK; KW, National Herbarium of Ukraine, M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Kiev, Ukraine; MUMH, Mie University, Mycological Herbarium, Japan.
b DDBJ, EMBL, and GenBank database accession numbers of the nucleotide sequence data.
c Sequence retrieved from DNA database.

robustness of the trees obtained by the MP methods, we also constructed MP trees with the parsimony ratchet method (Nixon 1999) in PAUP and PAUPRat ver 1 (Sikes & Lewis 2001).

For Bayesian phylogenetic analyses, the best-fit evolutionary model was determined for each dataset 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 1M generations and Markov chains were sampled every 100 generations, resulting in 10K 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 10K trees, the first 2K (burn-in) were discarded. The remaining 8K trees were summarized in a majority-rule consensus tree, yielding the probability of each clade being monophyletic. PP values higher than 0.9 are shown.

Table 2 – Size of asci and ascospores of *Galium* and *Calceolaria* powdery mildew samples

Fungal species	Host, location, voucher no.	Size of ascus		Size of ascospore	
		Range (mean), μm	L:b ratio	Range (mean), μm	L:b ratio
Golovinomyces riedlianus (Group 2)	*Galium verum*, Ukraine, KW 11879	47–67.5 × 26.5–34 (57.5 × 30.5)	1.89	27.5–33 × 17–21 (30.5 × 19)	1.61
	G. ruthenicum, Ukraine, KW 33932	41.5–73.5 × 24–45.5 (57.5 × 34.5)	1.68	25.5–28.5 × 15.5–22.5 (27 × 19)	1.44
	G. ruthenicum, Ukraine, KW 32987	46.5–69.5 × 31.5–46 (58 × 38.5)	1.5	24.5–36.5 × 14.5–23 (29 × 18.5)	1.57
	G. verum, Austria (Speer 1969)	60–85 × 28–38		25–28 × 13–16	
G. calceolariae (Group 3)	*Calceolaria polyrrhiza*, Argentina, BCRU 4527	47.5–69 × 26–34.5 (58 × 30)	1.94	22.5–29.5 × 14.5–19.5 (26 × 17)	1.5
G. orontii (Group 4)	*G. aparine*, Ukraine, KW 11877	47.5–57 × 26–37.5 (52.5 × 32)	1.65	19.5–24.5 × 13–17.5 (22 × 15.5)	1.45
	G. spurium, Japan, MUMH 2622	37.5–57.5 × 23.5–37.5 (47.5 × 30.5)	1.58	15.5–22 × 14.5–17.5 (19 × 15)	1.22
	G. spurium, Japan, MUMH 2624	43.5–59.5 × 29–40 (51.5 × 35)	1.48	17.5–23.5 × 14.5–17.5 (20.5 × 16)	1.29



Morphological analysis

Powdery mildew specimens used in the morphological analysis are listed in Table 2. The morphological features of the specimens were examined and photographed in phase contrast using a light microscope MBI-6 (LOMO, Saint-Petersburg, Russia) and a digital camera, model EOS 350D (Canon, Tokyo). Each morphological feature was measured 30 times. The resulting data were then processed statistically. Limits of feature variation were determined as $M \pm 1.96s$, where M was a simple average and s is a standard deviation.

Results

ITS phylogeny

A total of 113 sequences of the ITS region, including 14 sequences from *Galium* powdery mildews, were used to construct the phylogenetic tree of the tribe *Golovinomyceteae*. The dataset consisted of 535 characters, of which 35 characters were removed from the analysis due to ambiguous alignment. Of the remaining 500 characters, 179 characters were variable and 154 characters were phylogenetically informative for parsimony analysis. A total of 10K equally MP trees with 448 steps (CI = 0.5915, RI = 0.9431, RC = 0.5579) were constructed by the MP analysis. One of the 10K MP trees is shown in Fig 1. The deepest root of the tree was determined based on a previous report (Mori *et al.* 2000). Most internal branches were supported in the strict consensus of the MP trees. Parsimony ratchet analysis generated trees with the same tree length and similar tree topologies. Therefore, we concluded that the tree shown in Fig 1 is not the result of a local optimum. Bayesian analysis generated a similar tree topology. The three genera of the tribe *Golovinomyceteae*, *viz. Golovinomyces*, *Arthrocladiella*, and *Neoerysiphe*, formed each a distinct monophyletic clade, although the statistic support of the *Golovinomyces* clade was low. The 14 sequences from the *Galium* mildews were placed into four different groups. Group 1, consisting of six sequences from mildews on *Galium* and *Phuopsis* collected in Europe and West Asia (Iran), belonged to the genus *Neoerysiphe* and was supported with high BS (100 %) and PP (1) values. The remaining three groups belonged to the genus *Golovinomyces*. Group 2 consists of three sequences from *Galium* mildews collected in Europe and East Asia (Japan) and formed an independent clade (BS = 100 %, PP = 1). Group 3 was composed of a sequence from *Galium* mildew collected in Argentina. This sequence was identical to the sequence from a *Calceolaria* mildew that was collected at the same location and time as the *Galium* mildew. These two sequences formed an independent clade (BS = 100 %, PP = 1). Group 4 consisted of five sequences of *Galium* mildews collected in Japan and Ukraine. These sequences were identical to those from *G. orontii* on *Cucurbita pepo*, *Cucurbita* sp., and *Nicotiana tabacum*, and *Oidium* sp. on *Dahlia pinnata*, and only one base differed from the sequences of *G. orontii*, *G. cichoracearum*, and *Oidium* sp. on a wide range of host plants.

28S phylogeny

A total of 84 sequences of the 28S rDNA, including 14 sequences from *Galium* powdery mildews, were used to construct the phylogenetic tree of the tribe *Golovinomyceteae*. The dataset consisted of 724 characters, of which 92 were variable and 73 characters were phylogenetically informative for parsimony analysis. A total of 10K equally MP trees with 190 steps (CI = 0.6053, RI = 0.9352, RC = 0.5660) were constructed by the MP analysis. One of the 10K MP trees is shown in Fig 2. The deepest root of the tree was determined based on a previous report (Mori *et al.* 2000). Most internal branches were supported in the strict consensus of the MP trees. Parsimony ratchet analysis generated trees with the same tree length and similar tree topologies. Therefore, we concluded that the tree shown in Fig 2 is not the result of a local optimum. Bayesian analysis generated a similar tree topology. The 28S rDNA tree was almost consistent with the result of the analysis using the ITS sequences. Group 1 belonged to the genus *Neoerysiphe* and was sister to *N. galeopsidis*, with strong BS (88 %) and PP (0.94) supports. Group 2 consisted of four sequences from *Galium* mildews that originated from Europe and Asia (Japan). Group 3 was composed of two sequences from *Galium* and *Calceolaria* mildews collected in Argentina, and formed an independent clade. There was only one base difference between the sequences. The sequences of group 4 were identical to the sequences from *G. orontii* on *Nicotiana tabacum* and *Rubia hexaphylla*.

Morphology

Group 1 belonging to the genus *Neoerysiphe* is easily distinguishable from other groups belonging to *Golovinomyces*. This group has a fine white primary mycelium with nipple-shaped to slightly lobed appressoria at the early stage of infection (Voytyuk *et al.* 2004) and quickly develops moderately dense white secondary mycelium, in which chasmothecia are formed. In contrast to the *Golovinomyces* species on *Galium*, chasmothecia of group 1 are hemispherical, distinctly depressed in the lower part, and have appendages arising from the basal position. The appendages are barely visible, hyaline or brownish at the base. They are neither interlaced with the secondary mycelium nor twined around the chasmothecia. In addition, asci of this fungus mature only after overwintering and contain up to four ascospores.

In contrast to *Neoerysiphe* (group 1), the three groups belonging to *Golovinomyces* have unlobed appressoria (Braun 1987) and are morphologically very similar to each other, so

Fig 1 – Phylogenetic analysis of the nucleotide sequences of the ITS region, including the 5.8S rDNA, for 113 sequences from the tribe *Golovinomyceteae*, including the *Galium* mildews. The tree is one of the 10K MP trees with 448 steps, and was obtained by a heuristic search 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 BS support (1K replicates; ≥70 %) and the PP value (≥0.9) are shown above and below the branches, respectively.

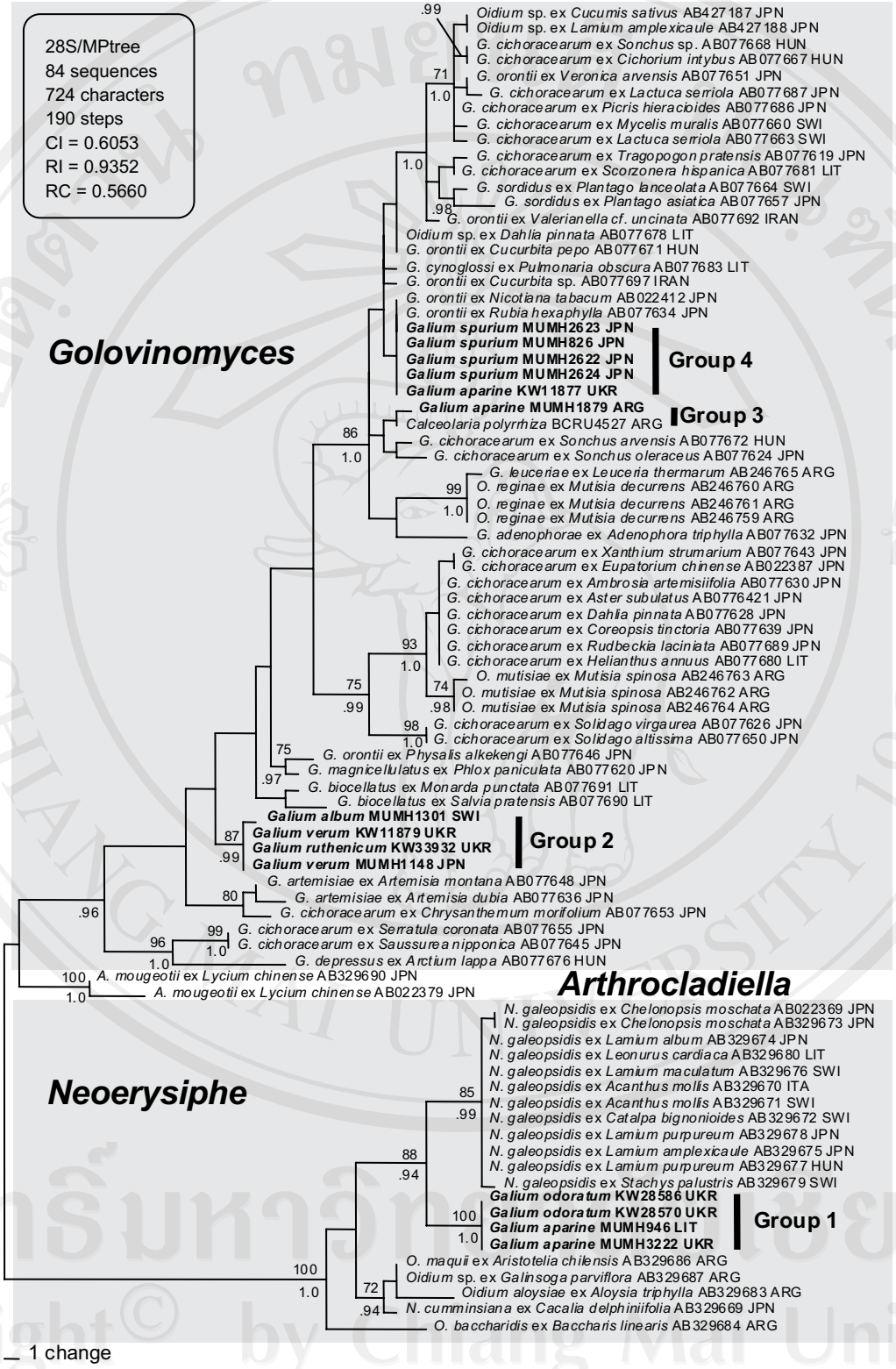


Fig 2 – Phylogenetic analysis of the divergent domains D1 and D2 sequences of the 28S rDNA for 84 sequences from the tribe *Golovinomyceteae*, including the *Galium* mildews. The tree is one of the 10K MP trees with 190 steps, and was obtained by a heuristic search of PAUP. Gaps were treated as missing 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 BS support (1K replicates; ≥70 %) and the PP value (≥0.9) are shown above and below the branches, respectively.

that the discrimination between them is rather difficult. However, it is possible to distinguish them using reflected light under a stereomicroscope. Group 4 collected on *Galium* has fine greyish primary mycelium. Simultaneously with chasmothecia, moderately compact white secondary mycelium develops. Sometime later, brownish appendages interlace with the mycelium and form a coloured, loose felt, especially on the lower side of leaves. The chasmothecia are spherical, and occasionally somewhat flattened, but not depressed at the lower part. Appendages arise from the entire surface of the chasmothecia, and mainly twine around chasmothecia; they are numerous and interlaced both with each other and with the secondary mycelium, forming a moderately dense felt, especially on the lower side of leaves. The Japanese specimens have less-developed appendages, but the tendency to form a felt was also observed. The asci are mainly ellipsoid, sometimes broadly clavate, stipitate, and always 2-spored. In contrast to group 2, ascospores are shorter, and often almost globose [length:-breadth (l:b) ratio 1.22–1.45 on average; Fig 3A–G, Table 2].

Group 2 has a fine, white, dense primary mycelium forming a high number of conidiophores. Sometime later, pure white secondary mycelium mixed with conidiophores develops, in which chasmothecia similar to those in *Leveillula* are formed. Chasmothecia are initially spherical, and then somewhat depressed at the lower part. The appendages mainly arising from the lower part of the chasmothecia are very numerous, brownish at the base, and interlaced with hyphae of the secondary mycelium. Sometimes, appendages arise from the upper part of the chasmothecia, especially if the secondary mycelium is poorly developed. In this case, the appendages twine around chasmothecia. Asci of this species are always 2-spored, stipitate, and very variable in shape, but mainly oblong ellipsoid. However, numerous ellipsoid-clavate or clavate asci are also present. Ascospores of this group are mainly ellipsoid, and more rarely nearly globose (l:b ratio 1.44–1.61 on average; Fig 3H–O, Table 2), but only one type of ascospores, either ellipsoid or almost globose, is contained in a single chasmothecium, i.e. the two types of ascospore are not mixed in a single chasmothecium in this species. In addition, this group is confined to fine-leaved *Galium* species.

Group 3 on *G. aparine* and *Calceolaria polyrrhiza* collected in Argentina has poorly developed, almost invisible mycelia. The chasmothecia are spherical, with relatively few, brownish, extremely fragile appendages, in comparison with the other *Golovinomyces* groups on *Galium*. They form a delicate interlacement, which is not merged into a continuous felt layer. Asci of this species are mainly ellipsoid-clavate or clavate, more elongated (l:b ratio 1.94 on average, in contrast to 1.48–1.89 in the other *Golovinomyces* groups; Table 2), and always 2-spored (Fig 4A–L). Spores are mainly ellipsoid, occasionally subglobose.

Taxonomy

Golovinomyces calceolariae Havryl., S. Takam. & Heluta, **sp. nov.** (Figs 4 and 5)

MycoBank no.: MB 511672

Etym.: *calceolariae*, from host plant genus.

Anamorph: *Oidium* subgenus *Reticuloidium*.

Species nostra *Golovinomyces brunneopunctato* similis est tamen mycelio secundario admodum delicato, ascis solum bisporis et sporis ellipsoideis interdum subglobosis, non subcylindricis, bene differt.

Typus: **Argentina**: *Provincia de Río Negro*: Parque Nacional Nahuel Huapi, Cerro Challhuaco, on *Calceolaria polyrrhiza*, 18 Apr 2001, *M. Havrylenko & S. Takamatsu* (BCRU 4527—holotypus; BCRU 4528, MUMH 1934, KW 34470—isotypi). rDNA sequence ex-type: AB430810.

Primary mycelium on leaves, amphigenous, hyaline, effuse or forming patches. *Secondary mycelium* formed by brown, thin-walled hyphae, 4–8 μm diam, growing around mature ascomata as a very delicate web. *Appressoria* indistinct. *Conidiophores* erect. *Foot cells* straight, cylindrical, 73–105 × 11–15 μm, followed by two shorter cells. *Conidia* catenate, doliiform to subcylindrical, 28–35(−39) × 13–15 μm, l:b ratio mostly above 2, without conspicuous fibrosin bodies. *Germ tubes* long, simple, arising subapically. *Chasmothecia* spherical, loosely grouped, 90–110(−130) μm diam. Cells of peridium 10–32 μm diam, irregularly shaped. *Appendages*15–30, arising equatorially and in the upper half, brown throughout when mature, with 4–7 septa, mycelioid, simple, geniculate, length variable, 1–3 times as long as chasmothecial diameter, 8–10 μm wide at the base, very fragile. *Asci* 6–25, mostly 12 per chasmothecium, ellipsoid, ellipsoid-clavate with irregular outline, 47.5–69(−73) × 26–34.5 μm, with well developed stalk, 2-spored. *Ascospores* ellipsoid, occasionally almost globose, hyaline, (19–)22.5–29.5 × (13–)14.5–19.5 μm (in pressed asci).

Additional materials studied: **Argentina**: *Provincia de Río Negro*: Parque Nacional Nahuel Huapi, Cerro Challhuaco, on *Galium aparine*, 18 Apr 2001, *M. Havrylenko & S. Takamatsu* (BCRU 4341, KW 34471, MUMH 1879).

Host range and distribution: on *Calceolaria polyrrhiza* (*Calceolariaceae*) and *Galium aparine* (*Rubiaceae*), South America, Argentina.

Comments: There is only one record of powdery mildew on *Calceolaria* registered as *Erysiphe galeopsidis* on *C. plantaginea* in Mendoza Province (Argentina) (Spegazzini 1909). In addition, the same fungus was listed on *Calceolaria* spp. in Argentina by Amano (1986). However, *E. galeopsidis* is not comparable with the present fungus in morphology because, according to the current taxonomic concept of the *Erysiphales*, the two fungi belong to different genera, *viz. Neoerysiphe* and *Golovinomyces*.

G. calceolariae is close to *G. brunneopunctatus* on *Mimulus guttatus* described by Braun (1984), but differs by always having two smaller ascospores, longer appendages, and hyaline and doliifom to subcylindric conidia and conidiophores. Secondary mycelium is very scarce, and only developed as few thin hyphae around chasmothecia, which are hardly distinguishable from the appendages. *Golovinomyces* on *Galium aparine* collected in the same area was previously referred to as *Golovinomyces riedlianus* (Havrylenko & Takamatsu 2005). The present study clarified that this specimen belongs to *G. calceolariae*.

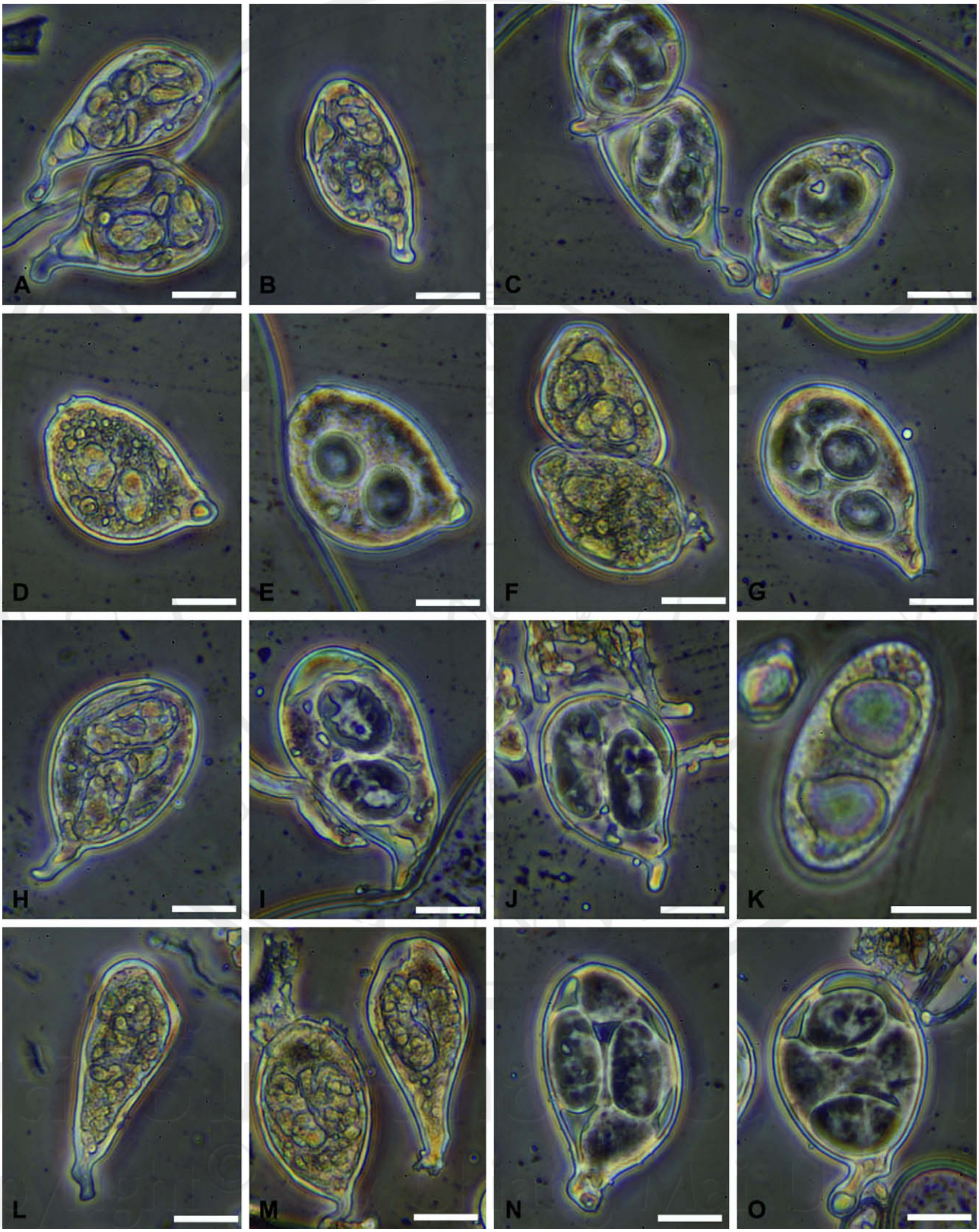


Fig 3 – Asci and ascospores of *Galium* powdery mildews. (A–C) *Golovinomyces orontii* on *Galium aparine* (Ukraine). (D–G) *Golovinomyces orontii* on *Galium spurium* (Japan). (H–K) *Golovinomyces riedlianus* on *Galium ruthenicum* (Ukraine). (L–O) *Golovinomyces riedlianus* on *Galium verum* (Ukraine). (C, E, G, I, J, N, O) Asci pressed by the cover glass. Bars = (A–J, L–O) 20 μm; (K) 10 μm.

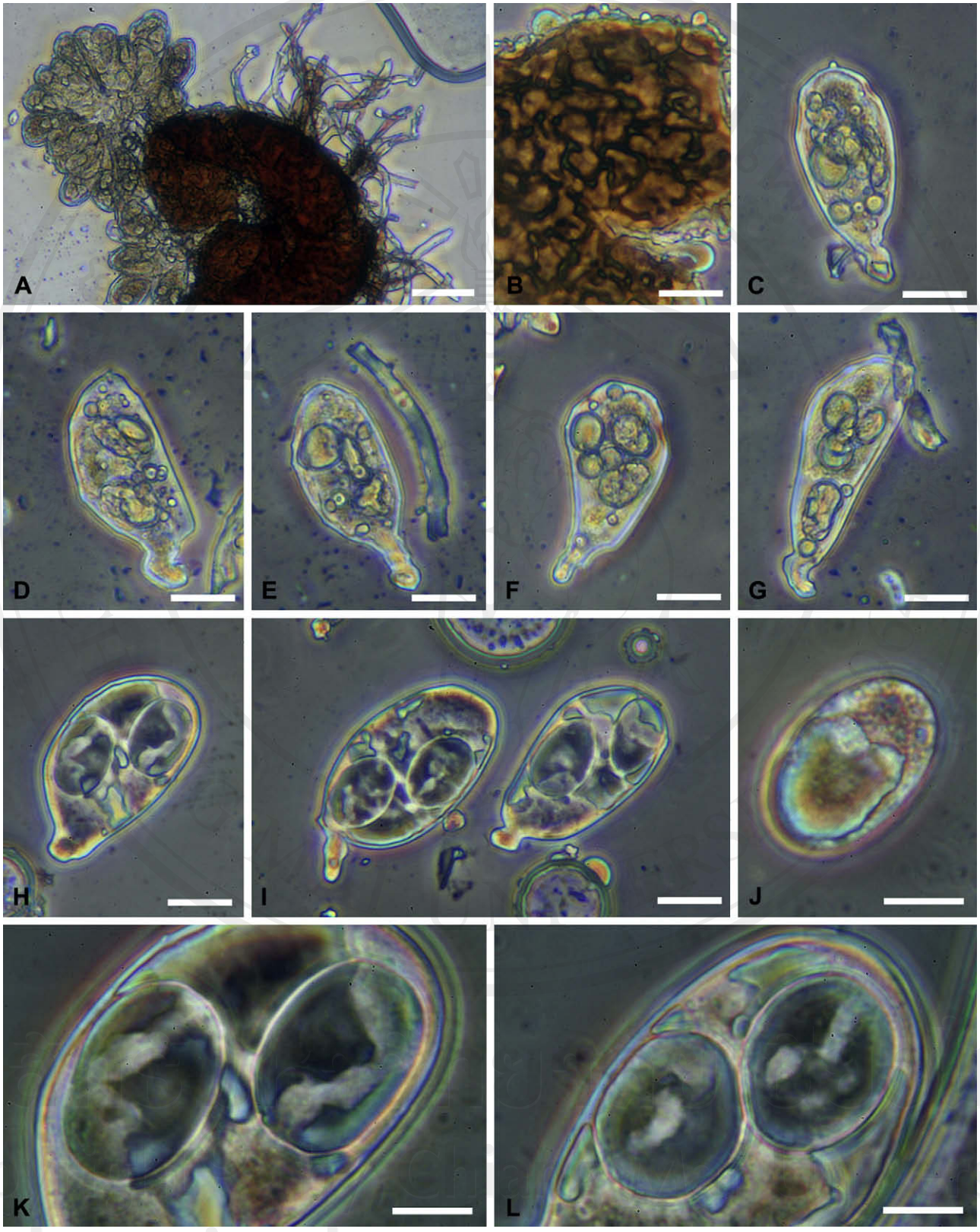


Fig 4 – *Golovinomyces calceolariae* on *Calceolaria polyrrhiza* (KW 34470). (A) Crushed chasmothecium with fascicle of asci and the rest of the appendages. (B) Peridium cells. (C–G) Asci in normal state. (H–I) Asci pressed by the cover glass. (J) Ascospore. (K–L) Ellipsoid and subglobose ascospores in asci pressed by the cover glass. Bars = (A) 50 μm; (B–I) 20 μm; (J–L) 10 μm.

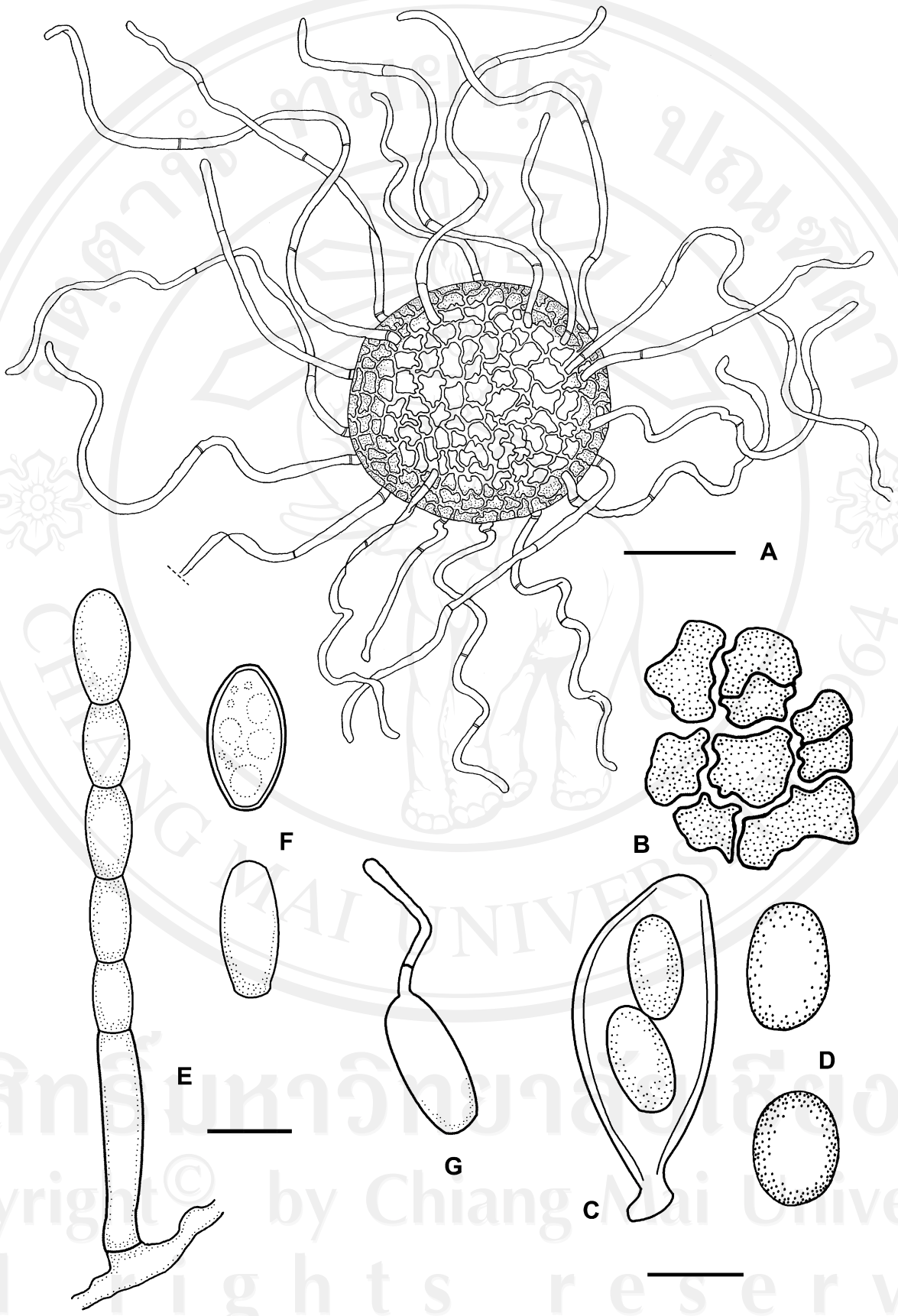


Fig 5 – Line drawings of *Golovinomyces calceolariae* (BCRU 4527). (A) Chasmothecium with appendages. (B) Peridium cells. (C) Ascus. (D) Ascospores. (E) Conidiophore. (F) Conidia. (G) Germinating conidium. Bars = (A) 50 μm; (B–G) 20 μm.

Key to* Galium *powdery mildew species

1 Chasmothecia hemispherical, distinctly depressed in the lower part, appendages arising only from the basal position, neither interlaced with the secondary mycelium nor twined around chasmothecia; asci mature only after wintering, containing up to 4 ascospores… ..... **Neoerysiphe galii**

Chasmothecia spherical or somewhat depressed at the lower part, appendages mainly arising from the lower part of the chasmothecia, sometimes also from the upper part, but then appendages twined around the chasmothecia; asci mature in the current season, always 2-spored…..... 2

2(1) Mycelium poorly developed, almost invisible, appendages brownish, extremely fragile, forming a delicate interlacement, not merging in a continuous felt layer; asci elongated (l:b ratio 1.94 on average)..... …**Golovinomyces calceolariae**

Mycelium well-developed, appendages moderately fragile, not forming a delicate interlacement, but interlaced with the secondary mycelium, forming a pigmented loose felt; asci less elongated (l:b ratio 1.48–1.89)…..... 3

3(2) Chasmothecia at first spherical, then somewhat depressed at the lower part, formed mainly within white secondary mycelium, similar to *Leveillula*; ascospores mainly ellipsoid, l:b ratio 1.44–1.61 on average; confined to fine-leaved *Galium* species… ..... **Golovinomyces riedlianus**

Chasmothecia spherical, occasionally somewhat flattened but not depressed at the lower part, brownish appendages interlaced with secondary mycelium, forming a pigmented loose felt that can surround chasmothecia; ascospores shorter, often almost globose (l:b ratio 1.22–1.45); confined to broad-leaved *Galium* species…... **Golovinomyces orontii**

Discussion

The present study revealed that the powdery mildew fungi with catenate conidia occurring on *Galium* spp. can be divided into four distinct groups. One of these groups belongs to the genus *Neoerysiphe* and was identified as *N. galii*. The other three groups belong to the genus *Golovinomyces*. Therefore, this study supports the taxonomic revision of Heluta (1988, 1989) and Braun (1999) in which the powdery mildews of *Galium* include both *Golovinomyces* and *Neoerysiphe* species. Moreover, the present study indicates that the fungi belonging to *Golovinomyces* can be divided into three groups.

Group 2 forms a distinct clade and combines the powdery mildew fungi that parasitize *Galium album*, *G. ruthenicum*, and *G. verum*. These hosts with fine leaves belong to the two close sections *Leiogalium* (*G. album*) and *Galium* (two latter species) (Bobrov *et al.* 1978). One of the species, *viz. G. verum*, is the type host of *Golovinomyces riedlianus* described as *Erysiphe riedliana* from Austria (Speer 1969). Our specimens agree relatively well with the description of *G. riedlianus*; in particular, the mycelium appearance, shape of asci, shape and size of ascospores coincided with *G. riedlianus*. However, it should be noted that the asci of the specimens studied were smaller than those given in the original description (Table 2). In order to observe ascospores, it is necessary to press them by the cover glass. As a result, the asci and ascospores may change in size; specifically, the width of the ascus increases by a factor of 1.09 and the length by a factor of 1.2 times, on average. It is likely that Speer (1969) measured pressed asci. The sizes of asci agreed very well with those of Speer's *G. riedlianus* if these coefficients were applied to our measurements. The size of ascospores coincided well with the original description of this species, probably because we had to measure ascospores in pressed asci, as they were almost invisible in intact asci. Taking into consideration the affinity of the hosts and the geographical distribution, the fungi included in group 2 were identified as *Golovinomyces riedlianus*. However, the geographical distribution of this fungus remains unclear. Until now, this species was recorded only from Austria, Ukraine, and Japan. A powdery mildew fungus on *Galium* sp. from Pakistan (IBA 5302) has been examined, which also belongs to *G. riedlianus*. The majority of records of this fungus is from Ukraine (Heluta 1989), where this species was found in different plant communities, but mainly confined to forest vegetation. In the northern Ukraine, this fungus was collected in forest glades, but in the extreme south, it was only found in mountainous regions. Although *Galium* species are common in Ukraine, *G. riedlianus* is relatively rare.

Group 4 has a DNA sequence identical to sequences from the fungi infecting *Rubia hexaphylla* (*Rubiaceae*), *Nicotiana tabacum* (*Solanaceae*), *Cucurbita pepo,* and *Cucurbita* sp. (*Cucurbitaceae*), and *Dahlia pinnata* (*Asteraceae*). These fungi are considered to be *Golovinomyces orontii* characterized by having a wide host range (Braun 1987; Vági *et al.* 2007). Voytyuk *et al.* (2004) suggested that a fungus similar to *G. cichoracearum* and *G. orontii* may infect *Galium,* in addition to *N. galii* and *G. riedlianus*. The present result is consistent with their report. *Golovinomyces orontii* is found on *Galium aparine* in Ukraine and on *G. spurium*, which is closely related to *G. aparine*, in Japan. Both species have comparatively wide leaves and belong to the section *Aparine*. The fungus frequently infects *Galium* in Japan, but was found only once in Ukraine during the course of the present study. In Ukraine, *G. aparine* and other related *Galium* species are affected mainly by *Neoerysiphe galii*.

The DNA sequences of the fourth lineage (group 3), a fungus on *G. aparine* collected in Argentina, are identical or just one base different from the sequences of a fungus on *Calceolaria polyrrhiza* collected at the same location. *Galium aparine* is a herbaceous plant originating from the Northern

Hemisphere, which is naturalized in America, growing from Alaska to Tierra del Fuego (Bacigalupo 1999). *Calceolaria* is a genus of native perennial herbaceous plants distributed along the Andean range, from Neuquén to Santa Cruz Provinces in Argentina (Rosow 1999). *Calceolaria* has previously been classified as a genus of the *Scrophulariaceae*. However, recent molecular analyses have revealed that the *Scrophulariaceae* are polyphyletic, and *Calceolaria* is now assigned to the family *Calceolariaceae* (Olmstead *et al*. 2001, APG II 2003). It is unknown whether this fungus was introduced into Argentina with *G. aparine* and infected *Calceolaria* or if a fungus on *Calceolaria* expanded its host range onto *G. aparine*. Analyses of additional specimens are needed to address this question because we used only one specimen each from the respective hosts in this study. The fungi collected on *Galium* and *Calceolaria* in Argentina were also morphologically identical. *Golovinomyces calceolariae* differs from *G. orontii* in many features, but is similar to *G. riedlianus* both in its shape and in the sizes of its asci and ascospores (Table 2).

In conclusion, molecular and morphological evidence reveal that at least four powdery mildew taxa with catenate conidia, *viz*. *Neoerysiphe galii*, *Golovinomyces orontii*, *G. riedlianus* and the new species *G. calceolariae*, are able to infect *Galium* species.

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Two new species of *Erysiphe* (*Erysiphales*, *Ascomycota*) from Thailand

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During a survey of powdery mildews in northern Thailand, two morphologically unique powdery mildews were collected on *Castanopsis* and *Lithocarpus*. Both powdery mildews have a thin, single layer of peridium cells of chasmothecia, which is a morphological character of the genus *Brasiliomyces*. However, recent molecular phylogenetic analyses indicates that *Brasiliomyces* is polyphyletic and shows that the two powdery mildews from Thailand belong to the *Erysiphe* lineage with *Oidium* subgenus *Pseudoidium* anamorphs. Therefore, they are described as *Erysiphe monoperidiata* sp. nov. and *E. asiatica* sp. nov.

Key words – *Brasiliomyces* – fungi – powdery mildew – taxonomy

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Introduction

The *Erysiphales* is a fungal group causing important plant diseases (powdery mildew) on about ten thousand angiosperm plants including many economically important cultivated plants (Amano 1986, Braun 2011). The biodiversity of the *Erysiphales* is less explored in tropical and subtropical regions compared with temperate regions of the Northern Hemisphere (Hirata 1976), probably because of fewer scientists working on this fungal group in these regions. In order to estimate the biodiversity of the powdery mildews in tropical regions, we have been working on the biodiversity of the *Erysiphales* in northern Thailand since 1999. This investigation revealed that there are still many undescribed and unique powdery mildew species in this region (To-anun et al. 2003, 2005). Therefore, exploring the *Erysiphales* in subtropical and tropical regions is important for further understanding of biodiversity, phylogeny and evolution of these organisms.

In this paper, we describe two new *Erysiphe* species recently found in northern Thailand. Both species have distinct morphological characteristics of the genus *Brasiliomyces*. However, recent molecular phylogenetic analyses revealed that *Brasiliomyces* is polyphyletic (Takamatsu *in litt*.) and the delimitation of this genus needs to be revised. Due to the phylogenetic position of the two new taxa within the *Erysiphe* clade, we prefer to assign them to *Erysiphe*.

Methods

Morphological examination

Specimens were collected in northern Thailand between November 2004 and March 2010. Details of host name, collection date, place, and collector were noted. Morphological

examinations were carried out as outlined in To-anun et al. (2003). Hyphae, chasmothecia, appendages, asci, and ascospores were stripped off from the leaf surfaces with a clean needle, mounted on a microscope slide, and examined in 3% NaOH using a light microscope with phase contrast 20×, 40×, and 100× objectives. The following data were recorded during the examination of the specimens: size and shape of chasmothecia, presence or absence of appendages, structure and size of peridial cells, number of asci per ascus, number of ascospores per asci, size and shape of asci and ascospores, and shape and position of hyphal appressoria. Thirty chasmothecia were measured per sample. Specimens were deposited at the National Museum of Nature and Science (TNS) and Mie University Mycological Herbarium (MUMH), Japan.

Phylogenetic analysis

Whole-cell DNA was extracted from chasmothecia by the chelex method (Walsh et al. 1991, Hirata & Takamatsu 1996). The rDNA internal transcribed spacer (ITS) region including 5.8S rDNA was amplified using primers ITS5 (White et al. 1990) and p3 (Kusaba & Tsuge 1995) for the first amplification. The ITS5/p3 fragment was subjected to the second amplification using powdery mildew specific primer sets ITS5/PM6 and PM5/p3 according to the procedure of Takamatsu & Kano (2001). The ITS5/PM6 and PM5/p3 fragments were sent to SolGent Co. (Daejeon, South Korea) for sequencing using ITS1 and ITS4 (White et al. 1990) as sequence primers, respectively. Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of AB622211–AB622218.

Eight sequences of the rDNA ITS region determined in this study were aligned manually using MS Word ver.5.1 and colour-coded nucleotides with 23 sequences from the genus *Erysiphe*, including *Typhulochaeta japonica* and *Erysiphe trinae* (≡ *Brasiliomyces trini*) used in Heluta et al. (2009). This data set consisted of 31 sequences and 674 sites, of which 180 ambiguously aligned sites were removed from the following phylogenetic analysis. The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number of S11366. Maximum parsimony analysis was done with the parsimony ratchet (Nixon 1999) in PAUP* 4.0 (Swofford 2002) and PAUPRat ver. 1 (Sikes & Lewis 2001) with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting trees was tested with bootstrap analyses using 1000 replications (Felsenstein 1985). Tree scores, including tree length, CI, RI, and RC, were also calculated.

Results

Taxonomy

Erysiphe monoperidiata Meeboon, R. Divarangkoon & S. Takamatsu, **sp. nov.** Figs 1, 2
MycoBank 561124

Etymology – *monoperidiata*, refers to the chasmothecia of this species with a single peridium cell layer.

Erysiphes trinae similis, sed ascis 4–6-sporis distinguitur.

Typus – on *Castanopsis tribuloides* A.DC. (Fagaceae), THAILAND, Mae Hong Son Province, Huai Nam Dang National Park, 1 March 2010 (TNS-F-39216, holotype; MUMH 4988, isotype). rDNA sequence extype: AB622214 (ITS).

Colonies amphigenous, mainly epiphyllous, persistent, forming irregular white patches on the host surfaces. *Hyphae* hyaline, superficial, 4–6 µm wide, branching. *Appressoria* well developed, coral-like, single or occasionally opposite in pairs. *Conidiophores* and *conidia* unknown.

Chasmothecia scattered to gregarious, (55.5–)58–82.5(–85) µm diameter ($\bar{x}$ = 68.9 µm), containing 2–4 asci. *Peridium* thin, one conspicuous layer, yellowish to light brown, semitransparent, appendages present, poorly developed, often branched, rarely absent, mycelioid, (15.5–)18–66(–75) × (2.5–)3–6(–7.5) µm ($\bar{x}$ = 33.1 × 4.6 µm), colourless, aseptate, thin-walled, smooth. *Asci* sessile or short-stalked, (34–)36–58(–61) × (24–)28–49(–52) µm ($\bar{x}$ = 45.5 × 37.8 µm), 4–6-spored. *Ascospores* ellipsoid-ovoid, hyaline, (11–)12.5–25(–26) × (6–)7.5–13(–14.5) µm ($\bar{x}$ = 20.3 × 10.3 µm).

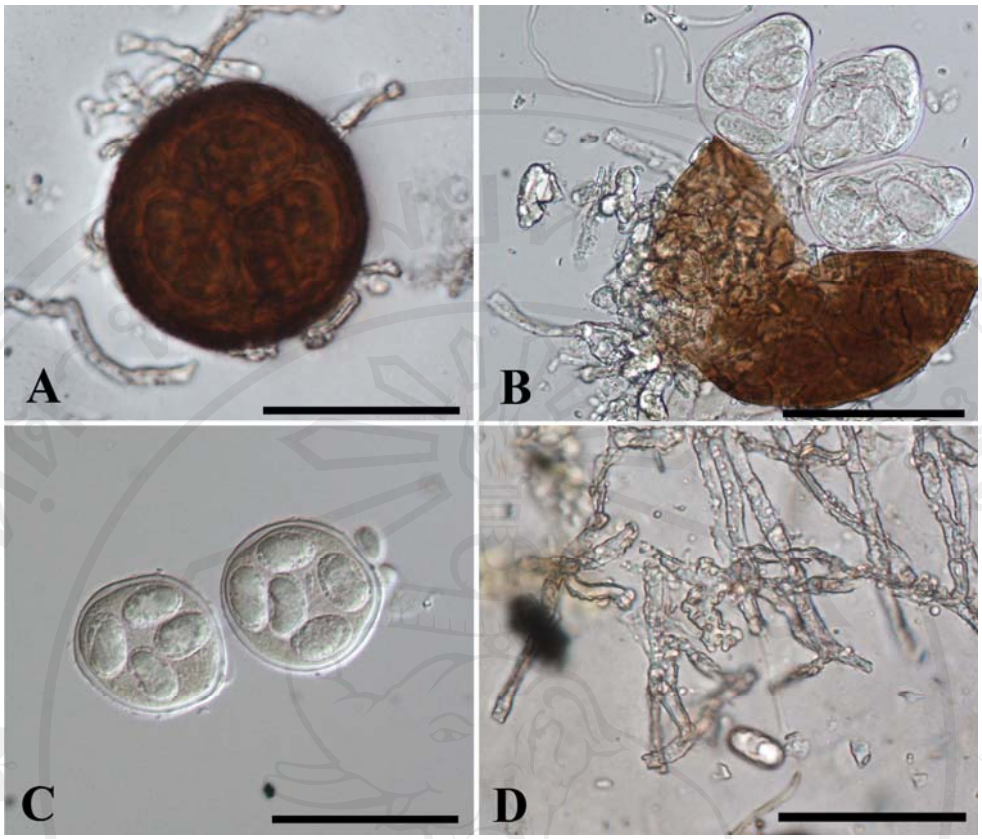

Fig. 1 – *Erysiphe monoperidiata*. **A** Chasmothecium. **B** Chasmothecia with asci and ascospores. **C** Asci and ascospores. **D** Appressoria. – Bars 50 µm.

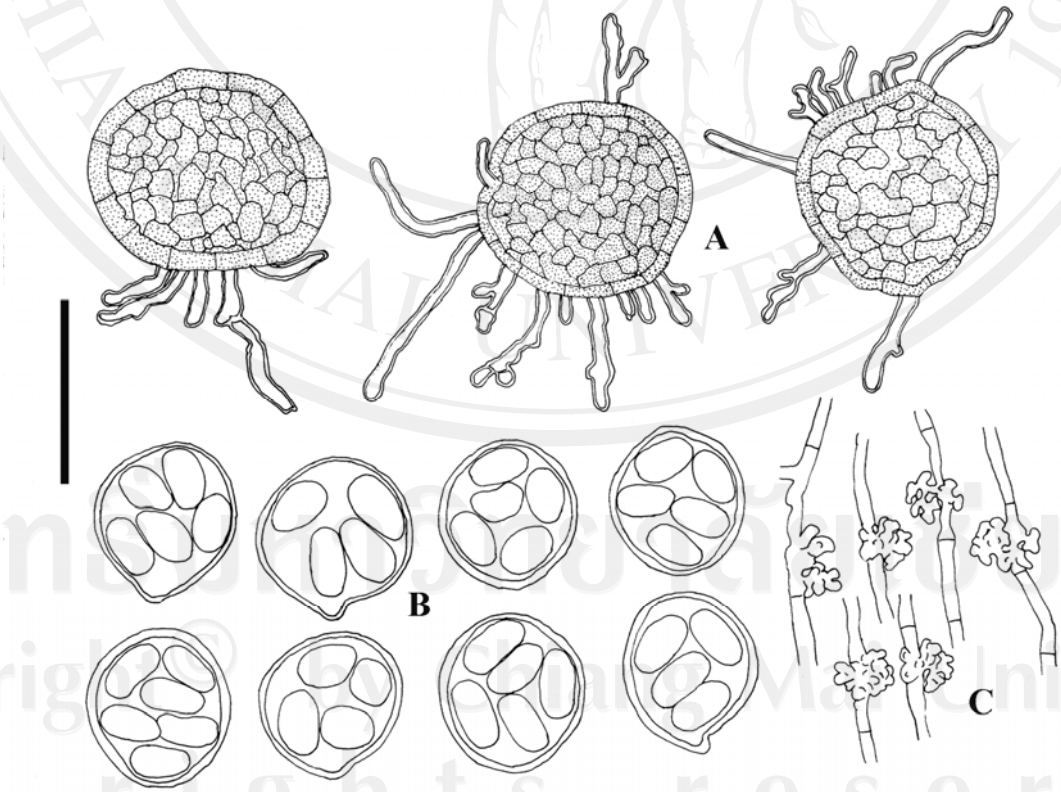

Fig. 2 – Drawing of *Erysiphe monoperidiata*. **A** Chasmothecia. **B** Asci and ascospores. **C** Appressoria. Bar – 50 µm.

Additional collections examined – on *Lithocarpus polystachyus* Rehder (Fagaceae), Thailand, Mae Hong Son Province, 1 March 2010 (MUMH 4986); on *Lithocarpus elegans* (Blume) Hatus. ex Soepadmo, Thailand, Mae Hong Son Province, 1 March 2010 (MUMH 4985); on *Castanopsis argyrophylla* King ex Hook.f. (Fagaceae), Thailand, Chiang Mai Province, Doi Khuntan, 21 March 2010 (MUMH 4987); on *Castanopsis indica* A.DC., Thailand, Chiang Mai Province, Botanical Garden, 10 March 2010 (MUMH 4990); on *Castanopsis calathiformis* Rehder & E.H.Wilson, Thailand, Chiang Rai Province, Khun Chae National Park, 5 March 2010 (MUMH 4991).

Host range and distribution – on *Castanopsis argyrophylla*, *C. calathiformis*, *C. indica*, *C. tribuloides*, *Lithocarpus elegans*, and *L. polystachyus* (Fagaceae), Asia, Thailand.

Erysiphe asiatica Meeboon, R. Divarangkoon & S. Takamatsu, **sp. nov.** Figs 3, 4
MycoBank 561125

Etymology – *asiatica*, a fungus found in Asia.

Erysiphes trinae similis, sed ascis 6–8-sporis distinguitur.

Typus – on *Castanopsis diversifolia* King ex Hook.f., THAILAND, Chiang Mai Province, Doi Pui National Park, 1 March 2010 (TNS-F-39215, holotype; MUMH 4992, isotype). rDNA sequence ex-type: AB622218 (ITS).

Colonies hypophyllous, persistent, forming irregular white patches on host surfaces. *Hyphae* hyaline, superficial, 4–6 µm wide. *Appressoria* well-developed, coral-like, single or occasionally opposite in pairs. *Conidiophores* and *conidia* unknown. *Chasmothecia* scattered, (51–)57–74(–78) µm diameter ($\bar{x}$ = 65.9 µm), containing only 2 asci. *Peridium* thin, one conspicuous layer, yellowish to light brown, semitransparent, chasmothecial appendages often absent or rudimentary, if present poorly developed, mycelioid, (31–)45–51(–66) × (4–)4.5–5(–5.5) µm ($\bar{x}$ = 48.6 × 4.8 µm), branched, hyaline, aseptate, thin-walled, smooth. *Asci* sessile or short-stalked, (45–)46–59(–62) × (38–)40–53(–57.5) µm ($\bar{x}$ = 51.5 × 45.6 µm), 6–8-spored. *Ascospores* ellipsoid-ovoid, olivaceous brown, (16–)18–25(–28) × (8.5–)9–15 (–16.5) µm ($\bar{x}$ = 21.5 × 12.2 µm).

Additional collections examined – *Castanopsis echinocarpa* Miq., Thailand, Chiang Mai Province, Phu Ping Palace, 19 March 2010 (MUMH 4989).

Host range and distribution – *Castanopsis diversifolia*, *C. echinocarpa* (Fagaceae), Asia, Thailand.

Phylogenetic analysis

Of the 494 total characters used in this analysis, 331 characters were constant, 61 characters were variable and parsimony-uninformative and 102 characters were parsimony-informative. A total of 174 equally parsimonious trees with 311 steps (CI = 0.675, RI = 0.799, RC = 0.539) were generated by the parsimony ratchet analysis. One of the best trees is shown in Fig. 5. *Erysiphe monoperidiata* and *E. asiatica* each formed separate clades with 97% and 100% bootstrap support, respectively. These two clades further formed a larger clade with 99% bootstrap support. This large clade grouped with *Typhulochaeta japonica*, *Erysiphe trinae* and *E. gracilis*, but with weak (54%) bootstrap support.

Discussion

Both *E. monoperidiata* and *E. asiatica* have a single layer of chasmothecial peridium cells, which is a morphological characteristic of the genus *Brasiliomyces* (Zheng 1984, Braun 1987). However, unpublished results of our recent phylogenetic study clearly indicate that the genus *Brasiliomyces* is polyphyletic, consisting of at least two independent lineages. This result urgently requires revision of the generic concept of *Brasiliomyces*. Because the current phylogenetic analysis indicates that both species belong to the *Erysiphe* clade with *Oidium* subgenus *Pseudoidium* anamorphs, together with *E. trinae* and *Typhulochaeta japonica*, we propose to assign these two new species to *Erysiphe*.

A total of eight *Brasiliomyces* species have been reported in the world, especially from subtropical and tropical regions (Harkness 1886, Viégas 1944, Marasas 1966, Boesewinkel 1980, Hanlin & Tortolero 1984, Hodges 1985, Kuo et al. 1992, Ahmad et al. 1998, To-anun et al. 2003). Three of the eight species occur on Fagaceae. Of these, *B. cyclobalanopsidis* is distinct from *E. monoperidiata*

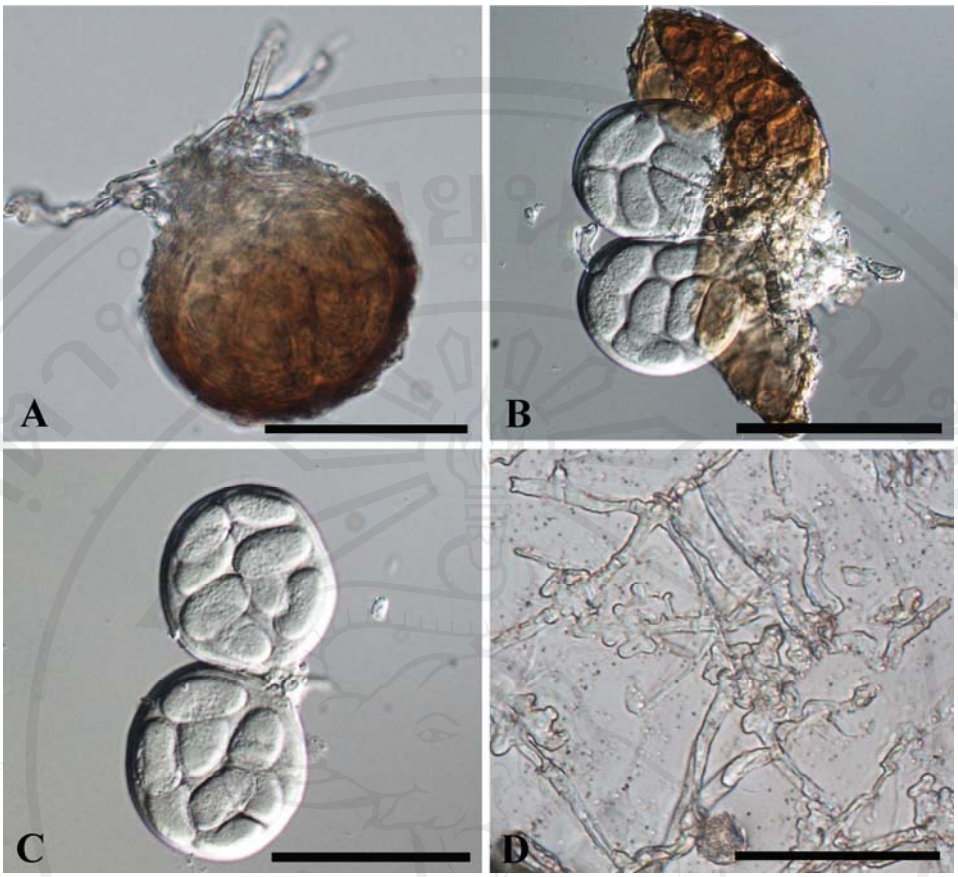

Fig. 3 – *Erysiphe asiatica*. **A** Chasmothecium. **B** Chasmothecia with asci and ascospores. **C** Asci and ascospores. **D** Appressoria. Bars = 50 µm.

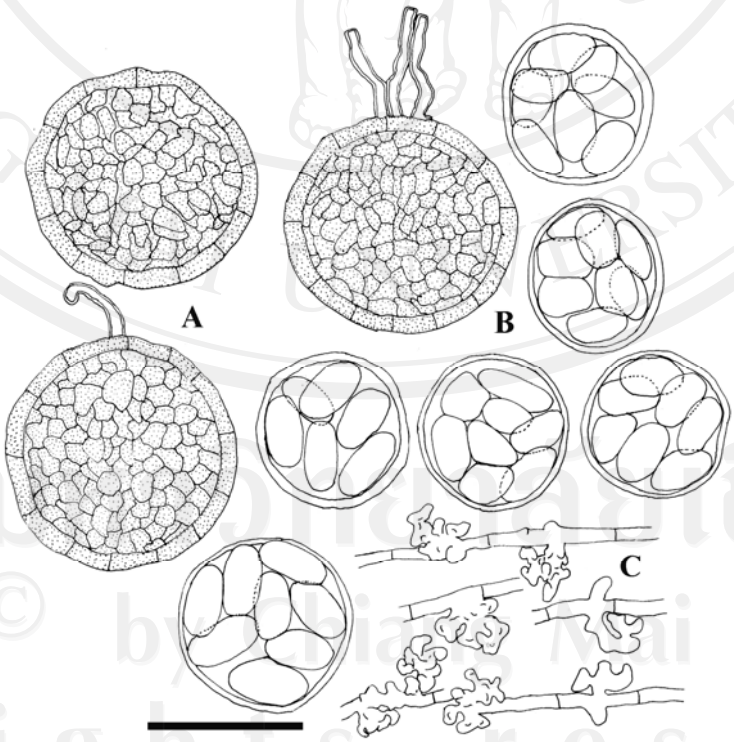

Fig. 4 – Drawing of *Erysiphe asiatica*. **A** Chasmothecia. **B** Asci and ascospores. **C** Appressoria. Bar = 50 µm.

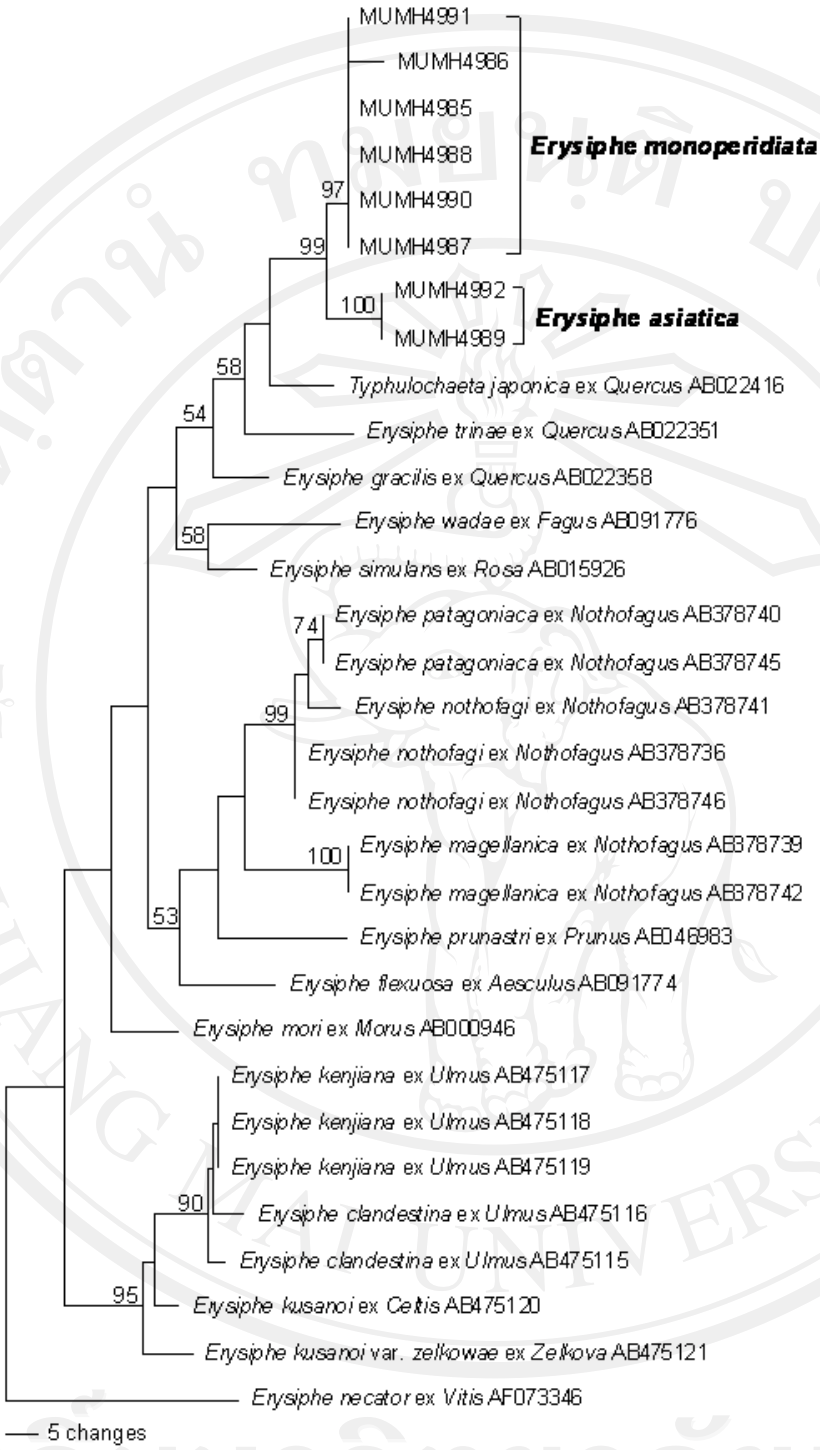

Fig. 5 – Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for eight newly determined sequences and 23 sequences from *Erysiphe* species and *Typhulochaeta japonicae*. The tree is one of the 174 equally parsimonious trees with 311 steps, which was obtained by the parsimony ratchet method. 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; ≥50%) is shown on branches.

and *E. asiatica* by its much smaller ascospores. Epiphyllous mycelia of *B. kumanoensis* are shared by *E. monosperidiata*, but the former species differs from the latter one by its larger chasmothecia (80–90 μm). The present phylogenetic analysis indicates that *E. monoperidiata* and *E. asiatica* are closely related to *E. trinae* occurring on *Quercus agrifolia* in North America. However, they did not form a clade together in the phylogenetic tree (Fig. 5). In addition, *E. trinae* usually has 2-spored asci, which differs from *E. monoperidiata* and *E. asiatica* having 4–6-spored and 6–8-spored asci, respectively.

The present phylogenetic analysis indicates that *E. monoperidiata* and *E. asiatica* form a clade together with *T. japonica*, *E. trinae* and *E. gracilis* infecting *Fagaceae*. This clade belongs to a lineage consisting of fungi with uncinuloid appendages that formerly belonged to the genus *Uncinula*. Interestingly, *E. monoperidiata*, *E. asiatica*, *E. gracilis* and *E. trinae* have mycelioid appendages, and *T. japonica* has unique club-shaped appendages, which indicates that none of the species belonging to this clade has uncinuloid appendages. This result suggests that these different appendage shapes and a single layered peridium cells evolved on fagaceous hosts. Molecular phylogenetic analysis using more sequences from *B. cyclobalanopsidis* and *B. kumanoensis* is required for further and deeper discussions.

Acknowledgements

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