
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 \times , 40 \times , and 100 \times 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* (\equiv *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 monopერიდიата 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.

Erysiphe 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 ex-type: 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) \times (2.5–)3–6(–7.5) μm (\bar{x} = 33.1 \times 4.6 μm), colourless, aseptate, thin-walled, smooth. *Asci* sessile or short-stalked, (34–)36–58(–61) \times (24–)28–49(–52) μm (\bar{x} = 45.5 \times 37.8 μm), 4–6-spored. *Ascospores* ellipsoid-ovoid, hyaline, (11–)12.5–25(–26) \times (6–)7.5–13(–14.5) μm (\bar{x} = 20.3 \times 10.3 μm).

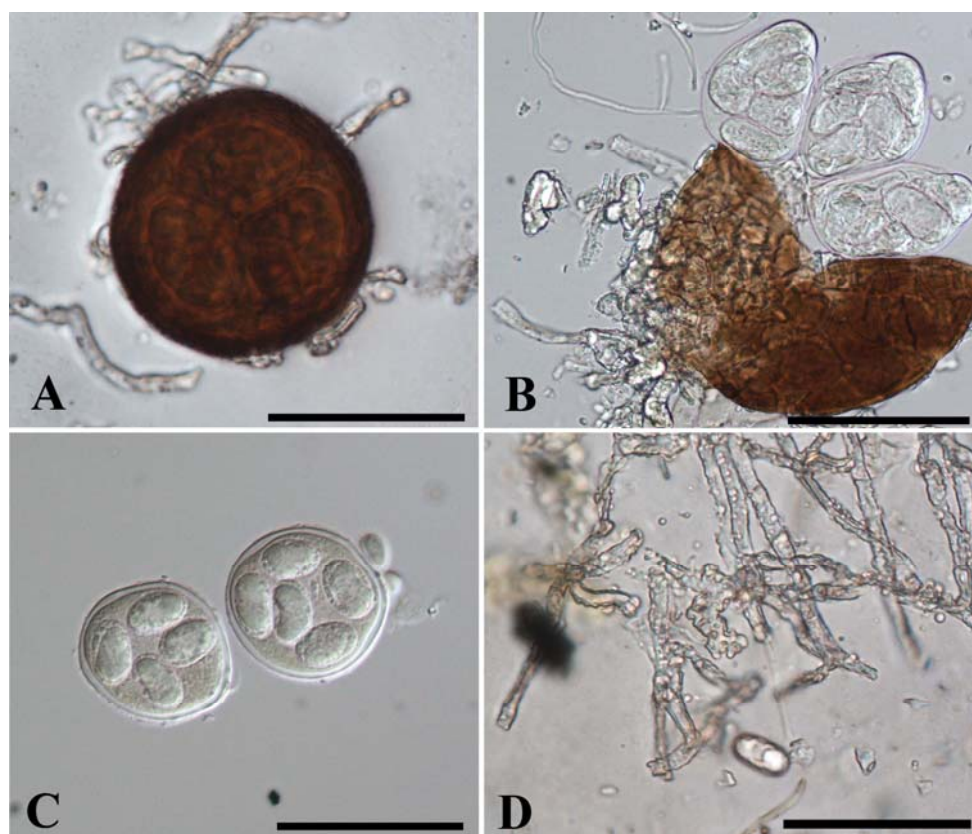


Fig. 1 – *Erysiphe monoperidiata*. **A** Chasmothecium. **B** Chasmothecia with asci and ascospores. **C** Asci and ascospores. **D** Appressoria. – Bars 50 μ m.

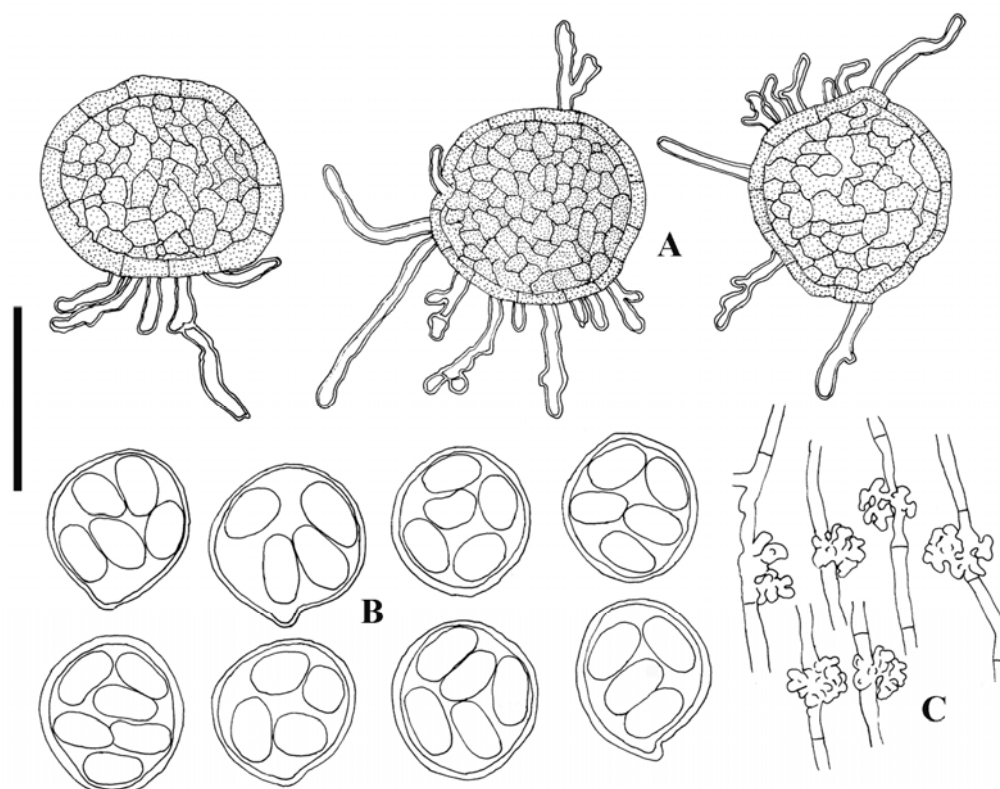


Fig. 2 – Drawing of *Erysiphe monoperidiata*. **A** Chasmothecia. **B** Asci and ascospores. **C** Appressoria. Bar – 50 μ m.

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.

Erysiphe 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 monopendiata* 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. monopendiata* 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. monopendiata*

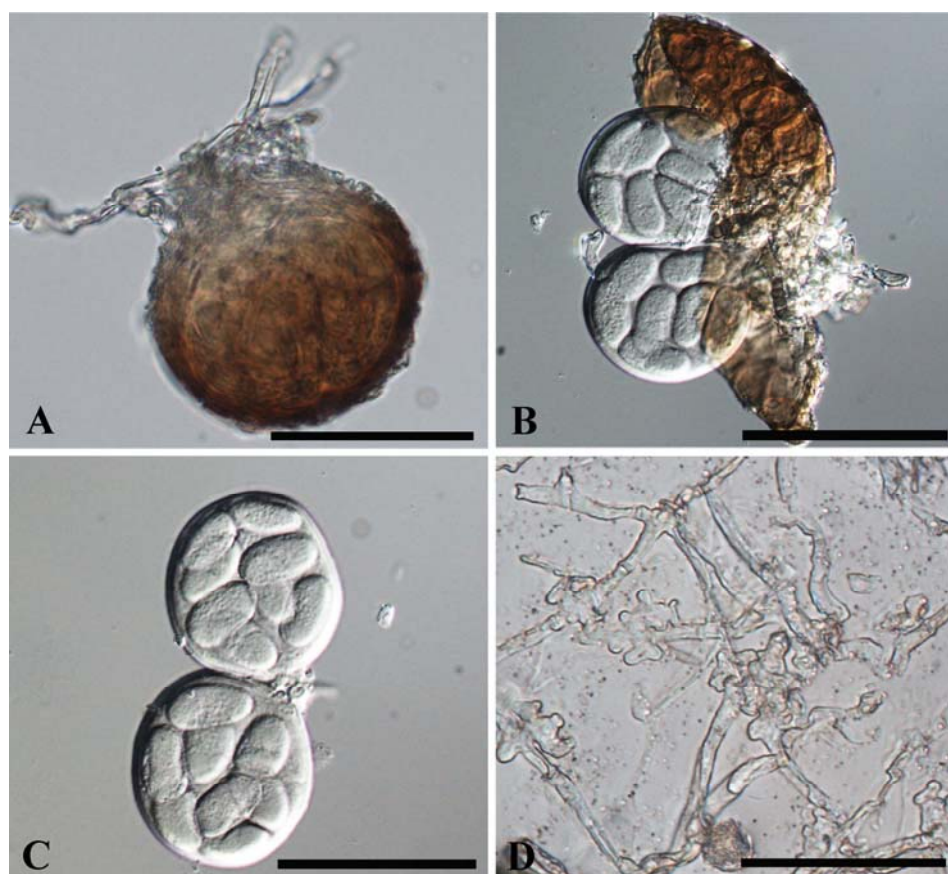


Fig. 3 – *Erysiphe asiatica*. **A** Chasmothecium. **B** Chasmothecia with asci and ascospores. **C** Asci and ascospores. **D** Appressoria. Bars = 50 μ m.

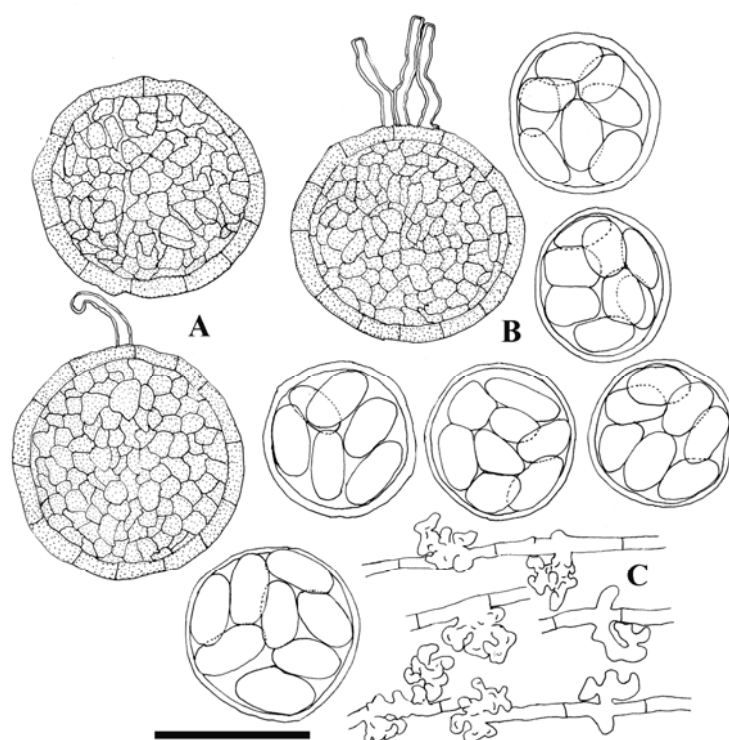


Fig. 4 – Drawing of *Erysiphe asiatica*. **A** Chasmothecia. **B** Asci and ascospores. **C** Appressoria. Bar = 50 μ m.

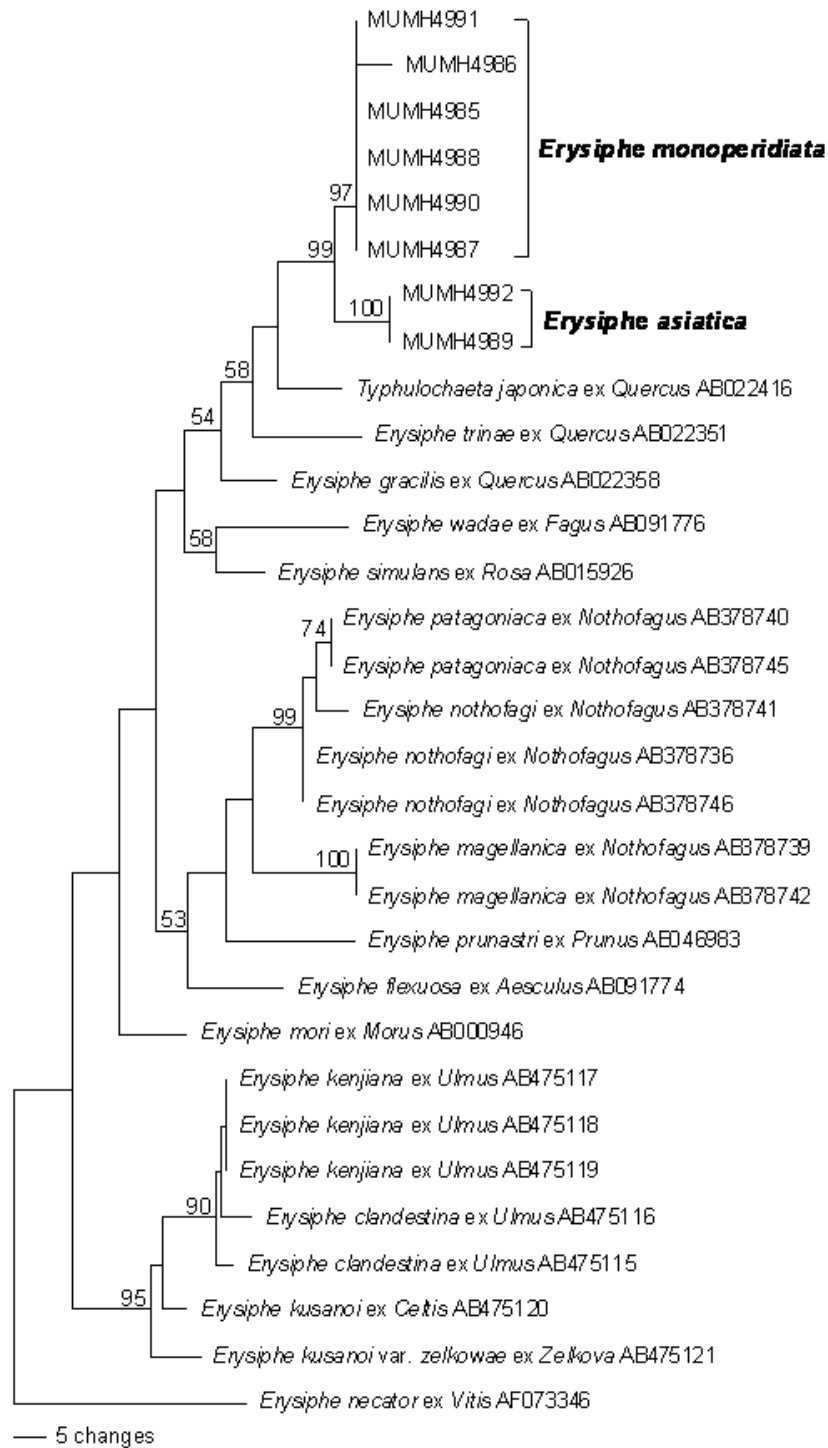


Fig. 5 – Phylogenetic analysis of the nucleotide sequences of the internal transcribed spacer (ITS) region including 5.8S rDNA for eight newly determined sequences and 23 sequences from *Erysiphe* species and *Typhulochaeta japonicae*. The tree is one of the 174 equally parsimonious trees with 311 steps,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

and *E. asiatica* by its much smaller ascospores. Epiphyllous mycelia of *B. kumanoensis* are shared by *E. monoperidiata*, 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.

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