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FULL PAPER

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Phylogenetic relationships and generic affinity of *Uncinula septata* inferred from nuclear rDNA sequences

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Abstract Based on 18S, 5.8S, and 28S rDNA sequences, the phylogenetic position of *Uncinula septata* within the Erysiphales has been inferred. Although appendages of the ascomata are uncinula like, i.e., unbranched with curved-coiled apices, *U. septata* is situated at the very base of the large Erysiphales cluster, far away from the "pseudoidium clade" (*Erysiphe emend.*, including *Microsphaera* and *Uncinula*). Morphologically, *U. septata* differs from the species of *Erysiphe* sect. *Uncinula* (≡*Uncinula*) in having terminal, pluriseptate ascoma appendages, curved ascospores, and the absense of an anamorph. This species is a basal, tree-inhabiting powdery mildew with some additional ancestral characteristics, viz., uncinula-like appendages and 8-spored asci. The new genus *Parauncinula* with *U. septata* as the type species is proposed. *Uncinula curvispora* (≡*U. septata* var. *curvispora*) is tentatively maintained as a separate species, which is also assigned to *Parauncinula*.

Key words Erysiphales · Molecular phylogeny · New genus · *Parauncinula* · *Uncinula curvispora* · *Uncinula septata*

Introduction

Uncinula septata E.S. Salmon was described by Salmon (1900), based on ascomata with apically curved-coiled appendages collected in Japan on *Quercus serrata* Thunb. ex Murray. Hara (1915) added the new variety *curvispora* to this species, also described from Japan, but on *Fagus japonica*, which he later treated at species rank (Hara, in Tanaka 1919). Based on some morphological peculiarities of the ascomata, Hara (1936) placed the latter species in a separate genus, which he called *Uncinulella* Hara (nom. inval.). Most subsequent authors followed Salmon's (1900) and Hara's (in Tanaka, 1919) taxonomy and maintained two different species (e.g., Homma 1937; Braun 1987; Otani 1988; Nomura 1992, 1997). Chen et al. (1987) reduced *U. curvispora* (Hara) Hara to synonymy with *U. septata*, although this treatment was only based on the examination of Chinese collections on *Quercus* spp.

Molecular studies of numerous powdery mildew species of the *Erysiphe* DC. (sect. *Erysiphe*)–*Microsphaera* Lév.–*Uncinula* Lév. complex showed that they form a separate, monophyletic clade, which is characterized by having anamorphs belonging to *Oidium* subgen. *Pseudoidium* Jacz. and ascomata with numerous 3–8-spored asci (Takamatsu et al. 1999; Mori et al. 2000a). The shape of the ascoma appendages, which had been generally considered the basic feature for the generic taxonomy of the powdery mildew fungi since Léveillé (1851), proved to be less important at generic rank; consequently, Braun and Takamatsu (2000) introduced *Erysiphe* emend. (including *Microsphaera* and *Uncinula*) with pseudoidium anamorphs as a basic feature. However, *Uncinula septata* clustered far away from the "pseudoidium clade" at the very base of the large Erysiphales cluster (Takamatsu et al. 1999; Mori et al. 2000a), although this species seemed to be a typical member of *Uncinula* (≡*Erysiphe* sect. *Uncinula*) with unbranched, apically uncinate-circinate appendages. To solve this problem and to elucidate the phylogeny and generic affinity of *Uncinula septata*, additional molecular analyses have been conducted.

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Materials and methods

Light microscopic observation

Leaves of *Quercus serrata* with colonies of *U. septata* were collected once a month from June to November 1999 at the Magose Park, Owase-city, Mie Prefecture, Japan. Fresh

colonies were stripped off the leaf surfaces with clear adhesive tape, mounted on a microscope slide with the mycelium uppermost, and examined in water using phase-contrast light microscopy.

Scanning electron microscopic (SEM) observation

For cryo-SEM, fresh leaves with colonies were cut into small pieces, 0.5 cm square, with a razor blade. They were attached to a specimen stub and then frozen in liquid nitrogen. The specimens were then sputter-coated with gold and observed with a SEM (model S-4000; Hitachi, Tokyo, Japan) at 3 kV accelerating voltage.

For ordinary SEM observation, fresh leaves with ascomata were cut into small pieces with a razor blade and then treated by the modified tannic acid fixation method (Kunoh et al. 1977). The specimens were fixed with 2% unbuffered glutaraldehyde containing 0.2% tannic acid at room temperature (RT) for 12 h, followed by further fixation in 2% unbuffered glutaraldehyde containing 2% tannic acid at RT for 12 h. After being washed in deionized water for 2 h, the specimens were treated in 1% unbuffered aqueous OsO_4 at RT for 12 h and washed in deionized water for 1 h. They were dehydrated in a graded ethanol series, then in a graded ethanol-*iso*-amyl acetate series and finally placed into 100% *iso*-amyl acetate. They were then dried in a critical-point dryer and coated with gold using an ion-sputter (model E-1010; Hitachi). Specimens were observed with a SEM at 20 kV accelerating voltage.

DNA extraction and polymerase chain reaction (PCR) amplification

Whole-cell DNA was isolated from ascomata by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). The respective nuclear rDNA regions, i.e., the 18S rDNA, internal transcribed spacer (ITS) region including the 5.8S rDNA, and D1 and D2 domains of the 28S rDNA, were separately amplified twice by the PCR using nested primer sets in a TaKaRa PCR Thermal Cycler SP (TaKaRa, Tokyo, Japan). PCR reactions were conducted in 50-μl volumes as previously described (Hirata and Takamatsu 1996). A negative control lacking template DNA was included for each set of reactions. The PCR product was subjected to preparative electrophoresis in a 1.5% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The DNA product of each amplification was then excised from the ethidium-stained gel and purified using the JETSORB kit (Genomed, Oeynhausen, Germany) following the manufacturer's instructions.

Annealing sites, nucleotide sequences, and references to the oligonucleotide primers used in this analysis are shown in Fig. 1. The primers US1 and US2 were newly designed in this study as specific primers for *U. septata*. For amplification of the 18S rDNA, primer set NS1/PM6 was used for the first amplification. The partial nested primer set NS1/PM4 was then used for the second amplification. For amplification of the ITS region, the primer sets ITS5/p3 and ITS5/ITS4 were used for the first and second amplifications, respectively. US1/TW14 and US2/TW14 were similarly used for amplification of the 5′-end of the 28S rDNA, including the D1 and D2 domains.

DNA sequencing

Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in a DNA sequencer CEQ2000XL (Beckman Coulter, Fullerton, CA, USA). The sequence reactions were conducted using the CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter, Fullerton, CA, USA) following the manufacturer's instructions. The primers, NS1, NS2, NS3, NS6, NS7, NS8, P1, P2, P3, P4, P6, P7, and P8 were used for sequencing the 18S rDNA in both directions. Similarly, ITS4, ITS5, T3, T4 and NL1, NL2, NL3, NLP2 were used for sequencing the ITS region and the 28S rDNA, respectively.

Phylogenetic analysis

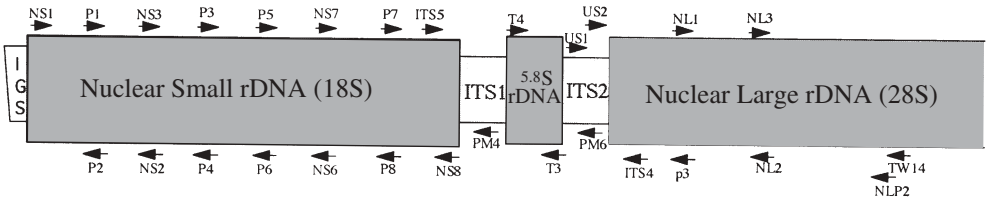
The sequences obtained were initially aligned using the Clustal X package (Thompson et al. 1997). The alignment was then improved visually with a word processing program with color-coded nucleotides. Phylogenetic trees were obtained from the data using maximum-likelihood (ML), neighbor-joining (NJ), and maximum-parsimony (MP) methods. For ML and NJ analyses, the most appropriate evolutionary model was determined for a given data set using PAUP* 4.0b8 (Swofford 2001) and Modeltest 3.06 (Posada and Crandall 1998). A starting tree was obtained with the NJ method. With this tree, likelihood scores were calculated for 56 alternative models of evolution by PAUP*. The output file was then imported into Modeltest to compare the models by using Akaike's (1974) information criterion (AIC). Once a model of evolution was chosen, it was used to construct phylogenetic trees with the ML and NJ methods using PAUP*.

For the MP analysis, we used a heuristic search using PAUP*. This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Transversions and transitions were treated as equal weight. All sites were treated as unordered, with gaps treated as missing data. The branch-swapping algorithm was tree-bisection-reconnection (TBR), the MULPARS option was in effect, and zero-length branches were collapsed.

The strength of the internal branches from the resulting trees was tested by bootstrap analysis (Felsenstein 1985) using 1000 replications in the NJ and MP analyses and 100 replications in the ML analysis. The partition homogeneity test (Farris et al. 1995) was conducted with PAUP* to determine whether the 18S, 5.8S, and 28S data sets were in conflict with 100 replicates.

Fig. 1. Annealing sites of primers used in this study (**A**) and their sequences (**B**). *ITS*, internal transcribed spacer

A. Primer map



B. Primer sequences

For 18S rDNA

NS1: 5'-GTAGTCATATGCTTGTCTC-3' (White et al. 1990)
NS2: 5'-GGCTGCTGGCACCAGACTTGC-3' (White et al. 1990)
NS3: 5'-GCAAGTCTGGTGCCAGCAGCC-3' (White et al. 1990)
NS6: 5'-GCATCACAGACCTGTTATTGCCTC-3' (White et al. 1990)
NS7: 5'-GAGGCAATAACAGGTCTGTGATGC-3' (White et al. 1990)
NS8: 5'-TCCGCAGGTTCACCTACGGA-3' (White et al. 1990)
P1: 5'-GGTTCATTCAAATTTCTGCC-3' (Mori et al. 2000a)
P2: 5'-GGCAGAAATTTGAATGAACC-3' (Mori et al. 2000a)
P3: 5'-TTTTGTTGGTTTCTAGGACC-3' (Mori et al. 2000a)
P4: 5'-GAAACCAACAAAATAGAACC-3' (Mori et al. 2000a)
P5: 5'-AACTTAAAGAAATTGACGGAAG-3' (Mori et al. 2000a)
P6: 5'-CTTCCGTCAATTTCTTTAAG-3' (Mori et al. 2000a)
P7: 5'-TCCCTGCCCTTTGTACACAC-3' (Mori et al. 2000a)
P8: 5'-GTGTGTACAAAGGGCAGGGA-3' (Mori et al. 2000a)
PM4: 5'-CCGGCCCGCCAAAGCAAC-3' (Takamatsu and Kano 2001)
PM6: 5'-GYCRCYCTGTCGCGAG-3' (Takamatsu and Kano 2001)

For ITS region

ITS4 : 5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990)
ITS5: 5'-GGAAGTAAAAGTCGTAACAAGG-3' (White et al. 1990)
T3: 5'-ACGCTCGAACAGGCATGCCC-3' (Hirata and Takamatsu 1996)
T4: 5'-TCAACAACGGATCTCTTGGC-3' (Hirata and Takamatsu 1996)
p3: 5'-GCCGCTTCACTCGCCGTTAC-3' (Kusaba and Tsuge 1995)

For 28S rDNA

TW14: 5'-GCTATCCTGAGGGAAACTTC-3' (G. Saenz, pers. commun.)
NL1: 5'-AGTAACGGCGAGTGAAGCGG-3' (Mori et al. 2000b)
NL2: 5'-TACTTGTTCGCTATCGGTCT-3' (Mori et al. 2000b)
NL3: 5'-AGACCGATAGCGAACAAGTA-3' (Mori et al. 2000b)
NLP2: 5'-GGTCCCAACAGCTATGCTCT-3' (Mori et al. 2000b)
US1: 5'-CCCGTAGCCCTGAGCGC-3' (This study)
US2: 5'-TCCAGGGCWCGCGTAGG-3' (This study)

Results

Microscopic observation

To determine whether *U. septata* forms conidia, fresh leaves of *Quercus serrata* with colonies of *U. septata* were collected once a month from June to November 1999 and observed under a light microscope and with cryo-SEM. Mycelia of *U. septata* were very thin and were found only on the abaxial surface of leaves. Neither conidia nor conidiophores were found on the mycelia during the period examined. Clavate appressoria, ca. 4 × 10 μm in size, were formed on the hyphae (Fig. 2). The morphology of this appressorial type is unique except for some *Phyllactinia* species (Babu et al. 1999; Shin 2000), and does not belong to any appressorium type known in the Erysiphales (Braun 1987).

Many hypha-like structures (perithecial hyphae in Takamatsu et al. 1979) arising from the lower side of young, immature ascomata (Figs. 3, 4) and radiating onto the leaf surface (Fig. 4) were found. At first the ascomata were globose (Fig. 3), later depressed spheroidal (Figs. 4, 5), and finally discoid when fully mature (Figs. 6, 7), representing a unique type of ascoma development in the Erysiphales.

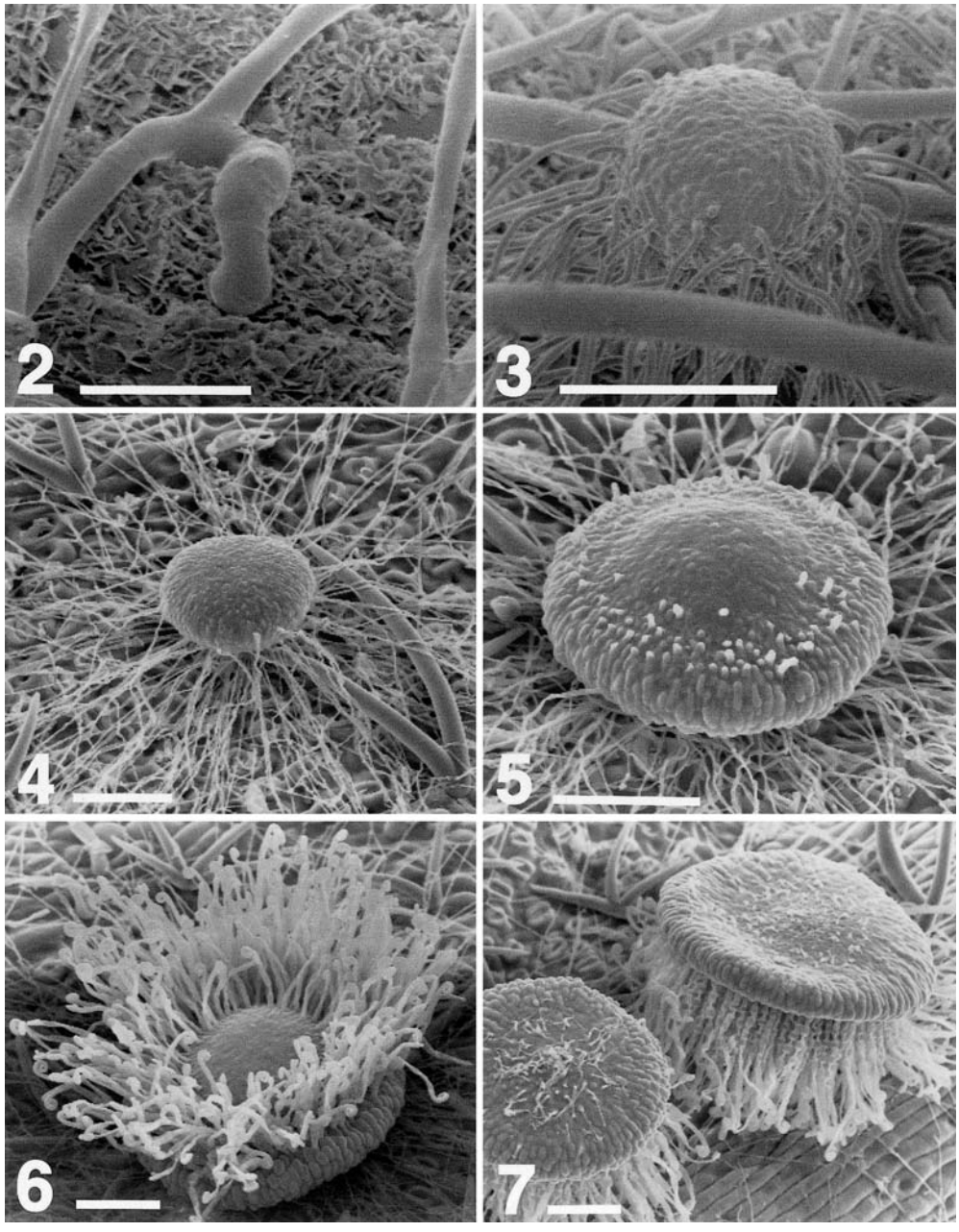


Fig. 2–7. Scanning electron micrographs of *Uncinula septata*. **2** Clavate appressoria formed on a hypha. **3** Young, immature ascoma with many hypha-like structures extending from the lower side. **4** Immature ascoma with many hypha-like structures growing radially onto the leaf surface. **5** Immature ascoma with appendage primordia emerging from the upper margin. **6** Mature ascoma and more than 100 unbranched appendages with curved-coiled apices. **7** Upside-down mature ascomata dislodged from the leaf surface and adhering again to a leaf with the appendages. Note that only traces of the hypha-like structures are found on the underside of mature ascomata. *Bars* **2** 10 μm; **3–7** 50 μm

When the diameter of the ascomata reached approximately 100 μm, appendages began to develop from the upper margin of the ascomata (Fig. 5). More than 100 unbranched appendages with curved-coiled apices were formed on mature ascomata (Figs. 6, 7). The mature ascomata were easily dislodged from the leaf surface and adhered to twigs or leaves upside down with the appendages (Fig. 7). In this stage, the hypha-like structures disappeared, so that only traces on the underside of mature ascomata could be observed.

Phylogenetic analysis

To clarify the phylogenetic placement of *U. septata*, complete nucleotide sequences of the 18S rDNA (~1.8 kb length), the D1 and D2 domains of 28S rDNA (~800 bp), and 5.8S rDNA (154 bp) from 21 taxa covering all five tribes of the Erysiphales and *Oidium* subgenus *Microidium* (To-anun et al. 2005) were used for the phylogenetic analysis. Sequences of the ITS spacer region were excluded from the analysis because they were too variable to align unambiguously. Fungal collection data and DNA sequence accession numbers are listed in Table 1. Of these, two complete sequences of the 18S rDNA and one sequence of the ITS region, including the 5.8S rDNA and domains of D1 and D2 of the 28S rDNA of *U. septata*, were newly determined in this study. *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx (18S: AB015776; 5.8S: AF062817; 28S: U17912) was used as outgroup taxon based on the results of Mori et al. (2000a). A partition homogeneity test with the 18S, 5.8S, and 28S rDNA data was run in PAUP* with 100 replicates;

Table 1. List of the species of Erysiphales used for molecular phylogenetic analysis

Fungus	Host plant	Isolate and location[a]	Database accession no.[c] 18S rDNA/5.8S rDNA/28S rDNA
Arthrocladiella mougeotii	*Lycium chinense*	MUMH135, Ibaraki, Japan	AB033477/AB022380/AB022379
Blumeria graminis f.sp. *bromi*	*Bromus catharticus*	MUMH117, Mie, Japan	AB033475/AB000935/AB022362
B. graminis f.sp. *hordei*	*Hordeum vulgare*	L.I.[b]	AB033480/D84379/AB022399
Cystotheca wrightii	*Quercus glauca*	MUMH137, Mie, Japan	AB120747/AB000932/AB022355
Erysiphe friesii var. *dahurica*	*Rhamnus japonica* var. *decipiens*	MUMH6, Mie, Japan	AB033478/AB000939/AB022382
E. glycines var. *glycines*	*Desmodium podocarpum* subsp. *oxyphyllum*	MUMH52, Nara, Japan	AB120748/AB015927/AB022397
E. mori	*Morus australis*	MUMHS77, Toyama, Japan	AB033484/AB000946/AB022418
Golovinomyces orontii	*Nicotiana tabacum*	L.I.[b]	AB033483/AB022413/AB022412
Leveillula taurica	*Capsicum annuum* var. *grossum*	MUMH124, Kochi, Japan	AB033479/AB000940/AB022387
Neoerysiphe galeopsidis	*Chelonopsis moschata*	MUMHS132, Toyama, Japan	AB120749/AB022370/AB022369
Oidium phyllanthi	*Phyllanthus acidus*	MUMH1778, Nan, Thailand	AB120753/–[d]/AB120754
O. phyllanthi	*P. amarus*	MUMH1782, Chiang Mai, Thailand	AB120756/–[d]/AB120755
O. phyllanthi	*P. reticulatus*	MUMH1761, Nan, Thailand	AB120757/–[d]/AB120758
Phyllactinia moricola	*Morus australis*	MUMH35, Mie, Japan	AB033481/D84385/AB022401
Pleochaeta shiraiana	*Celtis sinensis* var. *japonica*	MUMH36, Mie, Japan	AB120750/D84381/AB022403
Podosphaera longiseta	*Prunus grayana*	MUMH70, Kanagawa, Japan	AB120751/AB000945/AB022423
P. xanthii	*Melothria japonica*	MUMH68, Mie, Japan	AB033482/D84387/AB022410
Sawadaea polyfida var. *japonica*	*Acer palmatum*	MUMH47, Mie, Japan	AB033476/AB000936/AB022364
Typhulochaeta japonica	*Quercus cuspidata*	MUMHS76, Toyama, Japan	AB120752/AB022416/AB022415
Uncinula septata	*Q. cuspidata*	MUMH197, Niigata, Japan	AB183531[e]/AB022421/AB022420
U. septata	*Q. serrata*	MUMH585, Mie, Japan	AB183530[e]/AB183532[e]/AB183533[e]

[a] MUMH, Mie University Mycological Herbarium
[b] Isolate maintained as a living fungus in the Laboratory of Plant Pathology, Mie University
[c] The nucleotide sequence data will appear in the DDBJ, EMBL, and GenBank Database under the respective accession numbers
[d] Unpublished sequence
[e] Sequence newly determined in this study

the result showed no direct conflict among these data. Thus, we combined these data into a single data set consisting of 2723 sites and used it to obtain phylogenetic trees. The aligned data set was deposited in TreeBASE (http://www.treebase.org/) under accession number S1181/M2045. Separate analyses of the 18S, 5.8S, and 28S rDNA were also conducted to confirm the result of the combined analysis.

Using Modeltest (Posada and Crandall 1998) under the AIC, we concluded that the general time-reversible (GTR) model (Rodriguez et al. 1990), with unequal base frequencies, a gamma-distributed rate heterogeneity model (four rate categories, G = 0.5814; Yang 1994), and an estimated proportion of invariant sites (0.756) was the most appropriate model of evolution for this data set. A NJ tree was generated by using the data set and the evolution model. The ML analysis found a ML tree with a log-likelihood score of −7796.26. MP analysis found a single most parsimonious tree of 712 steps (CI = 0.6124; RI = 0.6703; RC = 0.4104). Three trees were subjected to the Kishino–Hasegawa test (Kishino and Hasegawa 1989) using the above evolution model to select a tree with the highest likelihood. As a result, the ML tree was selected as the best tree (Fig. 8).

All three tree-constructing methods using different algorithms yielded similar tree topologies, in which four tribes, viz. the Erysipheae, Phyllactinieae, Cystotheceae, and Blumerieae, recognized in the Erysiphales (Cook et al. 1997; Braun 1999; Braun and Takamatsu 2000; Mori et al. 2000a, 2000b) were again supported as respective monophyletic groups, although the bootstrap supports of the Phyllactinieae were relatively low (53% or lower). Tribe Golovinomyceteae is paraphyletic. *Oidium* subgenus *Microidium*, proposed by To-anun et al. (2005) as a new subgenus of the mitosporic powdery mildew genus *Oidium*, formed an independent clade, clearly separated from the other five tribes. *Uncinula septata* is placed at the very base of the Erysiphales and is a sister to all other erysiphaceous species. All separate analyses of the 18S, 5.8S, and 28S rDNA regions also supported the basal and independent position of *U. septata*.

Discussion and taxonomic conclusion

In all molecular analyses (18S, 5.8S, and 28S rDNA), *Uncinula septata* proved to be an ancestral species clustering at the very base of a large clade composed of all powdery mildew fungi**,** far away from the "pseudoidium cluster" (*Erysiphe* emend.), so that this species is undoubtedly not congeneric with the latter genus (Takamatsu et al. 1999; Mori et al. 2000a; Braun et al. 2002). The occurrence of pseudoidium anamorphs is the basic characteristic of *Erysiphe* emend. However, all attempts to find the anamorph of *U. septata* were unsuccessful. Numerous authors examined and described collections of this species (Homma 1937; Braun 1987; Chen et al. 1987; Otani 1988; Nomura 1992, 1997) but failed to find any conidiophores and conidia. Wada (1989) described two-celled blastospores

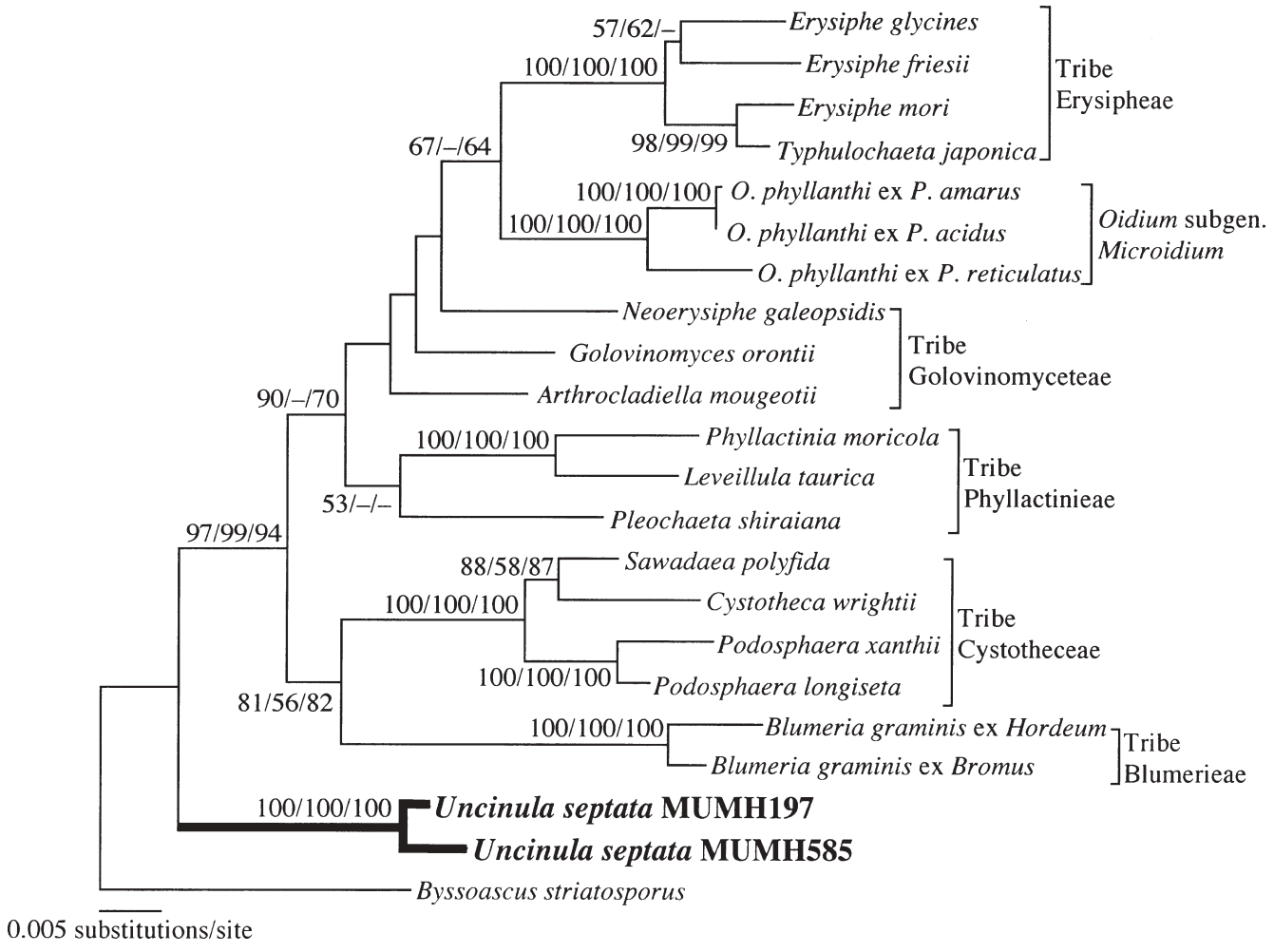


Fig. 8. A maximum-likelihood (ML) tree based on the combined data (2723 sites) of the 18S, 5.8S, and 28S rDNA (D1 and D2 domains) sequences for two isolates of *Uncinula septata*, 19 taxa of the Erysiphales covering all known tribes, and an outgroup taxon. Model parameters: unequal base frequencies with rate heterogeneity; gamma shape parameter = 0.5814; proportion of invariable sites = 0.756; six rate categories; GTR model (Rodriguez et al. 1990) with transformation parameters [A–C] = 1.0000, [A–G] = 2.2748, [A–T] = 0.5744, [C–G] = 0.5744, [C–T] = 5.1183, [G–T] = 1.0000. Bootstrap values (>50%) for ML/NJ/MP analyses are given above or below nodes

for this species, but these could not be confirmed. A genetic connection between the anamorph seen by Wada (1989) and *U. septata* has not been proven experimentally or genetically and is considered doubtful. Conidiophores and conidia seem to be lacking in *U. septata*. Furthermore, the ascomata of this species possess some peculiar features, viz., pluriseptate appendages arising from the upper half of the fruit bodies and curved ascospores. However, based only on these morphological peculiarities, erecting a new genus for *U. septata* seems unwarranted. However, in combination with the clear molecular results, these features and differences support the exclusion of this species from *Erysiphe* sect. *Uncinula*. These results support the placement of *U. septata* in a separate new genus. Validation of the invalid name *Uncinulella* would be one option for a new genus for *U. septata*. However, this opinion is rejected because the name *Uncinulella* is likely to be confused with *Uncinuliella* R.Y. Zheng & G.Q. Chen. Hence, we prefer to introduce a new generic name.

Parauncinula S. Takamatsu & U. Braun, gen. nov.

Sicut *Erysiphe* sect. *Uncinula* sed anamorphus ignotus ascomata depresso-globosa vel leviter lenticularia appendices ad apicem ascomatis circulatim nascentes pluriseptatae ascosporae curvatae.

Species typica: ***Parauncinula septata*** (E.S. Salmon) S. Takamatsu & U. Braun, comb. nov.

Basionym: *Uncinula septata* E.S. Salmon, J. Bot. 37: 426 (1900).

Descriptions: Homma (1937: 361), Braun (1987: 464), Chen et al. (1987: 422), Otani (1988: 245–246), Nomura (1992: 186–187; 1997: 101).

Illustrations: Homma (1937: Pl. IX, fig. 10), Braun (1987: 465, fig. 215 A), Chen et al. (1987: 424, fig. 245), Otani (1988: 246, fig. 161), Nomura (1992: 187, fig. 123; 1997: 102, fig. 124).

Molecular data for *Uncinula curvispora* are not yet available. Based on some morphological differences, we follow the traditional taxonomy of this fungus and maintain it tentatively as a separate species.

Parauncinula curvispora (Hara) S. Takamatsu & U. Braun, comb. nov.

Basionym: *Uncinula septata* var. *curvispora* Hara, J. Forest. Assoc. Jpn 392: 62 (1915).

Synonyms: *Uncinula curvispora* (Hara) Hara, in Tanaka, Mycologia 11: 80 (1919).

≡*Erysiphe curvispora* (Hara) U. Braun & S. Takam., Schlechtendalia 4: 18 (2000).

Descriptions: Homma (1937: 360), Braun (1987: 464–465), Otani (1988: 245), Nomura (1992: 188; 1997: 102).

Illustrations: Hara (1936: 133, fig. 56), Homma (1937: Pl. IX, fig. 9), Braun (1987: 465, Pl. 215 B), Otani (1988; 245, fig. 160).

Parauncinula is undoubtedly one of the most ancestral genera that exhibits various basic features, viz., the occurrence on trees, uncinula-like appendages, and 8-spored asci. In all previous powdery mildew classifications, ascomata with mycelium-like appendages were considered to be ancestral (Blumer 1933, 1967; Braun 1987). Jaczewski (1927) was the first author to consider *Erysiphe* the basic genus in the Erysiphales. Saenz and Taylor (1999), based on an analysis of ITS nucleotide sequences, postulated that mycelioid ascoma appendages are ancestral to all other types because they are distributed throughout the parsimony tree and occur in all major clades. However, uncinula-like appendages are also widespread within the Erysiphales [*Erysiphe* sect. *Uncinula*, *Pleochaeta* Sacc. & Speg., *Sawadaea* Miyabe (in Sawada 1914)]. Mycelium-like appendages, which are now considered to be derived (Takamatsu et al. 1999; Mori et al. 2000a; Braun et al. 2002), may have developed in connection with the colonization of herbaceous plants. Ancestral powdery mildews occur on woody plants, and uncinula-like appendages appeared at the base of the "pseudoidium clade" and at the very base of the whole Erysiphales cluster (Mori et al. 2000a). It is noteworthy that Sugiyama et al. (1999) and Mori et al. (2000a) discussed phylogenetic relationships between the Erysiphales and the Myxotrichales. *Byssoascus striatisporus* was placed at the base of the whole Erysiphales cluster. Some species belonging to the Myxotrichales form ascomata with appendages that are uncinate-circinate at the apex, and *Oidiodendron* Robak, the anamorph genus of *Byssoascus* Arx, resembles *Oidium* Link by forming one-celled, doliform arthroconidia (meristem-arthroconidia in *Oidium*).

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