Mycoscience (2005) 46:1–8
DOI 10.1007/s10267-004-0202-z



FULL PAPER

**Chaiwat To-anun · Sawwanee Kom-un**
**Aumpaiwan Sunawan · Wanwisa Fangfuk · Yukio Sato**
**Susumu Takamatsu**

# A new subgenus, *Microidium*, of *Oidium* (Erysiphaceae) on *Phyllanthus* spp.

Received: April 23, 2004 / Accepted: August 16, 2004

**Abstract** Powdery mildew fungi found on leaves, shoots, and stems of *Phyllanthus acidus*, *P. amarus*, and *P. reticulatus* proved to be a fungus having morphology unique in the Erysiphaceae. Light micrographs of a new germination pattern are added to discuss differences to other four germination patterns of the powdery mildews. The rDNA sequences (28S and 18S regions) of the fungi found on *Phyllanthus* spp. form a distinct monophyletic clade strongly supported by bootstrap (100%) in 18S + 28S trees, which indicates that the fungus is an isolated fungal group among the Erysiphaceae in tribal level. Because we cannot find the teleomorphic state of this fungus, a new subgenus *Microidium* of anamorphic genus *Oidium* is proposed to accommodate this organism.

**Key words** Euphorbiaceae · Molecular phylogeny · *Oidium phyllanthi* · Powdery mildew fungi · rDNA

## Introduction

Powdery mildew fungi occur in both anamorphic and teleomorphic forms. The characteristics of all structures of these fungi can be of taxonomic value. Especially among the earlier authors, the opinion was widely distributed that only a limited number of features should generally be used in erysiphacean taxonomy (Braun 1987). Salmon (1900) stressed that the conidial stages should not be used for systematic purposes, and he ignored the taxonomic value of the anamorphs entirely. Thus, he came to a very wide species concept and numerous authors followed him in neglecting the anamorphs.

The first systematic trial to identify the conidial states of powdery mildews at species level was made by Ferraris (1910), who grouped species of *Oidium* according to the size and shape of their conidia and created a key to its species. Foex (1913), Jaczewski (1927), and Brundza (1934) contributed to the classification of the conidiophore types in the genus *Oidium*. Jaczewski (1927) introduced the terms Euoidium and Pseudoidium for *Oidium* states with catenate and solitary conidia, respectively. Hirata (1942, 1955) provided comprehensive germination experiments with Japanese species of powdery mildews. Golovin (1956) reported extensive study on the anamorphs of the genus *Leveillula*. A survey on the Erysiphaceae, including the anamorphs, was given by Yarwood (1957). Boesewinkel (1977, 1980) provided the first real key based on a combination of more than 12 morphological characteristics observed on conidia, conidiophores, appressoria, haustoria, fibrosin bodies, and mycelium. Shin and La (1993) and Shin and Zheng (1998) introduced some new morphological features of taxonomic relevance.

A progressive report was provided by the work of Cook et al. (1997), who examined surface of conidia by scanning electron microscopy (SEM) and separated genus *Oidium* into eight subgenera. Braun (1999) discussed the classification of the Erysiphaceae proposed by Cook et al. (1997) and introduced some corrections and alterations. Comprehensive examinations of nucleotide sequences of the rDNA internal transcribed spacer (ITS) region of powdery mildew fungi have also been carried out (Takamatsu et al. 1998, 1999, 2000; Saenz and Taylor 1999; Mori et al. 2000). Because the results of these studies provided numerous molecular data as well as new SEM examinations (Cook et al. 1997), it was possible to rearrange the classification of the Erysiphaceae (Braun 1999; Braun and Takamatsu 2000). Recently, Braun et al. (2002) reported that the characteristics of the anamorphs are the base for the generic taxonomy of the Erysiphales and reflect the phylogeny in this fungal

C. To-anun (✉) · S. Kom-un · A. Sunawan · W. Fangfuk
Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiangmai 50200, Thailand
Tel. +66-53-944021; Fax +66-53-944666
e-mail: agppi006@chiangmai.ac.th

Y. Sato
College of Technology, Toyama Prefectural University, Toyama, Japan

S. Takamatsu
Faculty of Bioresources, Mie University, Tsu, Japan

group. The teleomorphs are less important for the generic taxomomy but provide useful features on the species level. Thus, it is possible to classify the powdery mildews in which the teleomorphic state generally is not found or rarely occurs, as in most of the powdery mildews in Thailand (Sontirat et al. 1994).

During 1999 to 2002, 73 powdery mildews in Thailand were collected and the morphology of conidial germ tubes was observed using the method described by Hirata (1942). rDNA sequence analysis of these powdery mildews was also carried out to classify the unidentified fungi. During the study, we found a unique fungus, *Oidium phyllanthi* J.M. Yen, isolated from three species of *Phyllanthus*, i.e., *P. acidus* Skeels, *P. amarus* Schum. & Thonn., and *P. reticulatus* Poir., that produces a new type of conidial germination. The germination type is designated as microidium-type (To-anun et al. 2002). The molecular characteristic supports the morphological result that *O. phyllanthi* on *Phyllanthus* spp. is a unique fungus. This is the first report of *O. phyllanthi* on *Phyllanthus* spp. in Thailand. Moreover, this is the first record of *O. phyllanthi* on *P. acidus* in the world. In this article, we present morphological and molecular characteristics of *O. phyllanthi* on *Phyllanthus* spp. collected in Thailand to propose a new subgenus *Microidium* for this fungus.

## Materials and methods

### Light microscopy of fresh material

Hyphae, conidiophores, and conidia of fresh materials were stripped off the leaf surfaces with clear adhesive tape, mounted on a microscope slide with the fungal mycelium uppermost, and examined in water using light microscopy with phase contrast using 20×, 40×, and 100× oil immersion objectives. The following information was noted during the examination of the fresh specimens: size and shape of conidia, presence or absence of fibrosin bodies, nature of conidiogenesis, characteristics of the conidiophore, e.g., size and shape of foot cell, position of the basal septum, shape and position of hyphal appressoria, position of germ tubes, and shape of appressoria on germ tubes of conidia. Thirty conidia were measured for each specimen examined.

Observation of conidial germ tubes was carried out using the method of Hirata (1942). The inner surface cell layer of onion scales was cut with a razor in a size of 1 $cm^2$ and stripped off by a clean forceps. The cell layer was kept in 80% ethanol for more than 2 weeks and rinsed with tap water for 30 min before use. The cell layer was put on a microscope slide, followed by removing excess water with filter paper, and inoculated with the conidia. The inoculated cell layer was floated on distilled water in a Petri dish and incubated at 20°–25°C for 24 h until microscopic observation.

### DNA extraction and PCR amplification

Whole-cell DNA was isolated from fresh fungal specimens or herbarium specimens by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). The nuclear rDNA region was amplified twice or three times by the polymerase cham reaction (PCR) using nested primer sets. The following thermal cycling conditions were performed in a PCR thermal cycler SP (Takara, Kyoto, Japan): an initial denaturing step at 95°C for 2 min; thermocycling for 30 cycles, where each cycle consisted 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 cycle of 7 min at 72°C. A negative control lacking template DNA was included for each set of reactions. The PCR product was subjected to preparative electrophoresis in 1.5% agarose gel in TAE buffer. The DNA product of each amplification was then excised from the ethidium-stained gel and purified using the Jetsorb kit (Genomed, Löhne, Germany) following the manufacturer's instructions.

The oligonucleotide primers used in this study were as follows. The nucleotide sequences of NS1, NS2, NS3, NS6, NS7, and NS8 were obtained from White et al. (1990) and that of p3 from Kusaba and Tsuge (1995). The nucleotide sequence of TW14 was kindly provided by Dr. G.S. Saenz. The primers P1, P2, P3, P4, P6, P7, P8, NL1, NL2, and NL3 were designed by Mori et al. (2000) for the nucleotide sequences of the 18S rDNA region of the powdery mildew fungi. For amplification of the 18S rDNA, primer set T3/NS1 was used for the first amplification. Partial nested primer sets ITS2/NS1 and NS8/NS1 were then used for the second and third amplifications. The set of PM5/TW14, T4/TW14, and T2/TW14 was similarly used for amplification of the 5′-end of the 28S rDNA including D1 and D2 regions.

### DNA sequencing

Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in an Applied Biosystems 373A sequencer (Applied Biosystems, Foster City, CA, USA). The sequence reactions were conducted using the PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems) following the manufacturer's instructions. The primers P1, P2, P3, P4, P6, P7, P8, NS1, NS2, NS3, NS6, NS7, and NS8 were used for the sequencing of the 18S rDNA in both directions. Similarly, NL1, NL2, NL3, and TW14 were used for the sequencing of the 28S rDNA, respectively. The thermocycling was conducted for 30 cycles, where each cycle consisted of 20 s at 96°C, followed by 20 s at 50°C for annealing, and 4 min at 60°C for extension.

### Phylogenetic analysis

The obtained sequences were initially aligned using the Clustal V package (Higgins et al. 1992). The alignment was then defined visually with a word processing program with color-coded nucleotides. Phylogenetic trees were obtained

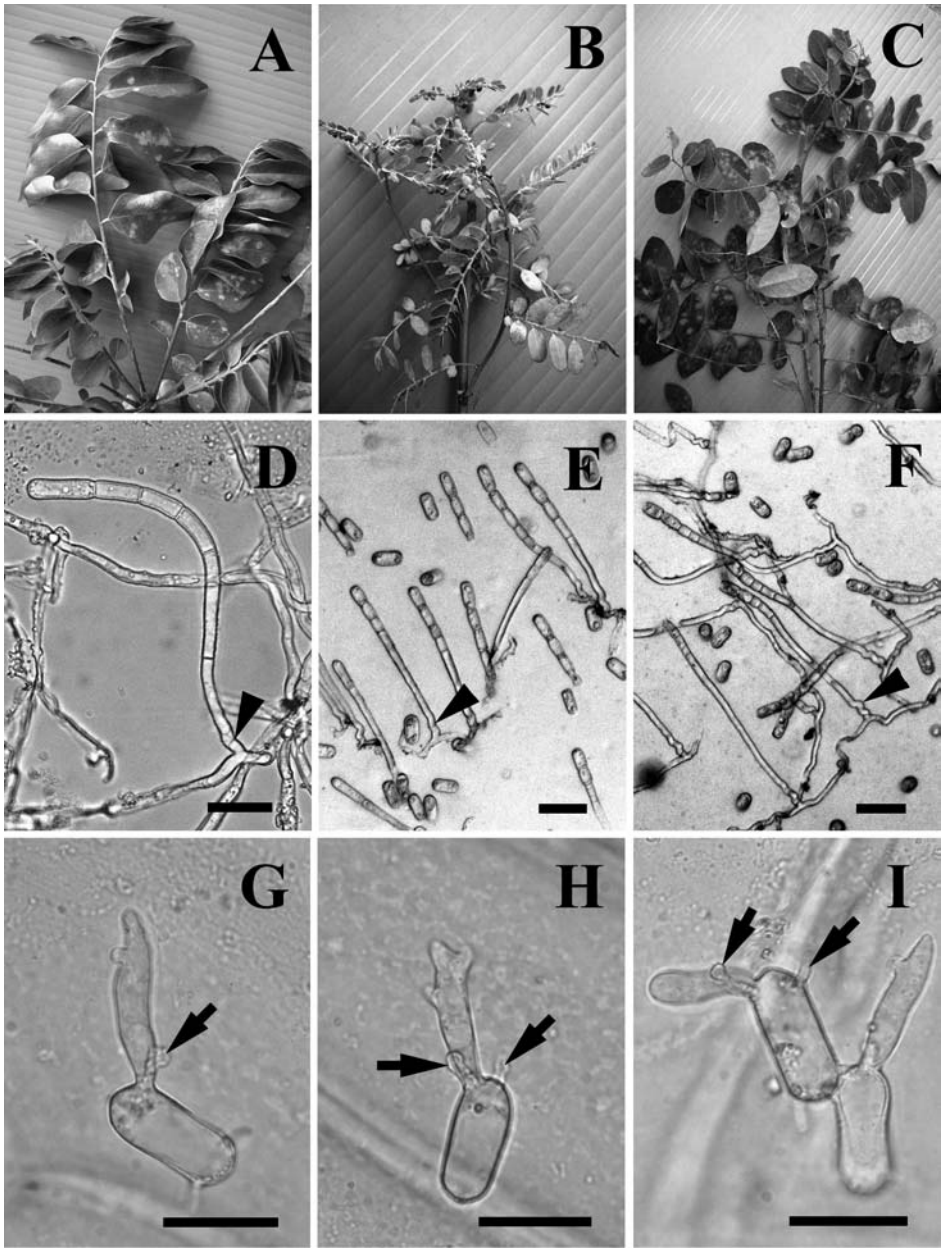


**Fig 1.** Symptom, conidiophore and conidia, and germ tubes of the powdery mildew *Oidium* (subgen. *Microidium*) *phyllanthi*, found on *Phyllanthus acidus* (**A**, **D**, **G**), *P. amarus* (**B**, **E**, **H**) and *P. reticulatus* (**C**, **F**, **I**), respectively. The fungus produces conidia in chains (**D**, **E**, **F**); foot-cell flexous or spiral twisted at the base (*arrows*). Conidia produced germ tubes on the shoulder (**G**, **H**, **I**), germ tubes broad club-shaped, terminating in nipple or lobed appressoria with hyaline germ tubes (*arrows*). *Bars* **D**, **G–I** 20 μm; **E**, **F** 30 μm

from the data using maximum-likelihood (ML), distance, and parsimony methods. For ML and distance 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 neighbor-joining method. With this tree, likelihood scores were calculated for 56 alternative models of evolution by PAUP*. The output file was then imported to 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 neighbor-joining (NJ) methods using PAUP*.

For the parsimony analysis, we used the maximum-parsimony (MP) method with 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. The partition homogeneity test (Farris et al. 1995) was conducted by PAUP* to determine whether the 18S and 28S data sets were in conflict, with 1000 replicates.

## Results

### Morphological observation

The colonies of powdery mildews found on *Phyllanthus acidus*, *P. amarus*, and *P. reticulatus* are whitish, covering the entire surface of the leaves, young shoots, and young stems (Fig. 1A–C). The fungus is characterized by forming catenate conidia (Fig. 1D–F). Conidia produce broad club-shaped germ tubes (Fig. 1G–I), without fibrosin bodies but containing oil drop granules (Fig. 2A–C), and foot-cells of the conidiophores are flexous or spirally twisted (Figs. 1D–F, 2C). Details of the main characteristic of the fungus on *Phyllanthus acidus* (MUMH1778) follow.

Mycelium amphigenous, white, effuse, confluent, forming irregular white patches or covering the whole leaf surface and on young stems, hyphae hyaline, branched, septate, 3.5–5 μm wide; appressoria well-developed, multilobed, opposite in pairs or single; conidiophores single on a hyphal cell, arising from the upper part of mother cells, position mostly central, 85–195 × 5–6 μm (average, 134 × 5.6 μm), with a long foot-cell, cylindrical, straight, twisted at the base, 20–90 × 4–6 μm (average, 61 × 4.9 μm), producing 3–9 conidia in chains, with a basal septum near the branching point of the mycelium or up to 15 μm away from it; conidia small, cylindrical to barrel-cylindrical, (20–)23–26 (–28) × (8–)9–10.5(–11) μm (average, 22.8 × 9.5 μm), with oil drop-like inclusion bodies but without conspicuous fibrosin bodies, producing germ tubes on the shoulder, germ tubes broad club-shaped, terminating in nipple or lobed appressoria with or without two to three small hyaline germ tubes at both ends (Fig. 1G).

Characteristics of the other two fungi on *P. amarus* and *P. reticulatus* are almost the same as the fungus on *P. acidus*, excepting for the size of the conidia, conidiophore, foot cells and the shape of appressorium.

### Phylogenetic analysis

To clarify the phylogenetic placement of *O. phyllanthi*, complete nucleotide sequences of the 18S rDNA (~1.8-kb length) and the D1 and D2 regions of 28S rDNA (~800-bp) from 19 taxa covering all five tribes of the Erysiphaceae were used for the phylogenetic analysis. The data of the fungi and their accession numbers are listed in Table 1. Of these, nine complete sequences of the 18S rDNA and three sequences of the D1 and D2 regions of the 28S rDNA were newly determined in this study, in which three isolates of *O. phyllanthi* from different host species are included. Because the result of the partition homogeneity test showed no direct conflict between the 18S and 28S rDNA data, we combined these data into a single data set. *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx (18S: AB015776; 28S: U17912) was used as the outgroup taxon based on the result of Mori et al. (2000). The resulting large data set composed of 2526 sites was used to obtain phylogenetic trees. The alignment was deposited in TreeBASE (http://www.treebase.org/) under the study accession number of S1129 and matrix number of M1936.

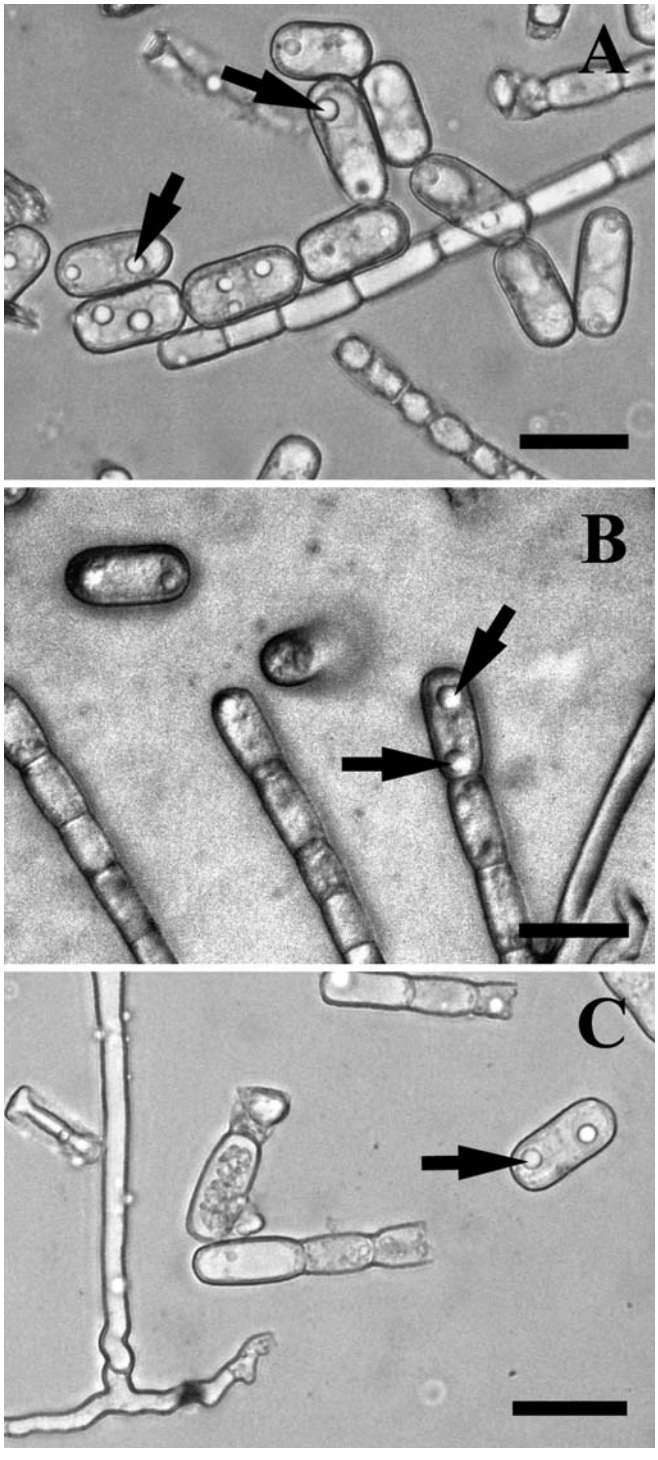


**Fig 2.** Conidia of *Oidium* (subgen. *Microidium*) *phyllanthi* found on *Phyllanthus acidus* (**A**), *P. amarus* (**B**), and *P. reticulatus* (**C**). The conidia of this fungus are small, cylindrical to barrel-cylindrical, with oil drop-like inclusion bodies (*arrows*) but without conspicuous fibrosin bodies. *Bars* 20 μm

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 frequen-

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

| Fungal species[a] | Host plants | Voucher collection and locations[b] | Database accession no.[c] 18S rDNA / 28S rDNA |
|---|---|---|---|
| *Arthrocladiella mougeotii* (Lév.) Vassilkov | *Lycium chinense* Mill. | MUMH135, Ibaraki, Japan | AB033477, AB022379 |
| *Blumeria graminis* (DC.) Speer f. sp. *bromi* | *Bromus catharticus* Vahl | MUMH117, Mie, Japan | AB033475, AB022362 |
| *B. graminis* (DC.) Speer f. sp. *hordei* | *Hordeum vulgare* L. (Barley) | L.I.[d] | AB033480, AB022399 |
| *Cystotheca wrightii* Berk. & M.A. Curtis | *Quercus glauca* Thunb. ex Murray | MUMH137, Mie, Japan | AB120747[e], AB022355 |
| *Erysiphe friesii* Lév. var. *dahurica* (U. Braun) U. Braun & S. Takam. | *Rhamnus japonica* Maxim. var. *decipiens* Maxim. | MUMH6, Mie, Japan | AB033478, AB022382 |
| *E. glycines* F.L. Tai em. Zheng var. *glycines* | *Desmodium podocarpum* DC. subsp. *oxyphyllum* (DC.) H. Ohashi | MUMH52, Nara, Japan | AB120748,[e] AB022397 |
| *E. mori* (I. Miyake) U. Braun & S. Takam. | *Morus australis* Poir. (Mulberry) | MUMHS77, Toyama, Japan | AB033484, AB022418 |
| *Golovinomyces orontii* (Castagne) V.P. Gelyuta | *Nicotiana tabacum* L. (Tobacco) | L.I.[d] | AB033483, AB022412 |
| *Leveillula taurica* (Lév.) G. Arnaud | *Capsicum annuum* L. var. *grossum* Sendtn. | MUMH124, Kochi, Japan | AB033479, AB022387 |
| *Neoerysiphe galeopsidis* (DC.) U. Braun | *Chelonopsis moschata* Miq. | MUMHS132, Toyama, Japan | AB120749,[e] AB022369 |
| *Oidium phyllanthi* J.M. Yen | *Phyllanthus acidus* Skeels | MUMH1778, Nan, Thailand | AB120753,[e] AB120754[e] |
| *O. phyllanthi* | *P. amarus* Schum. & Thonn. | MUMH1782, Chiang Mai, Thailand | AB120756,[e] AB120755[e] |
| *O. phyllanthi* | *P. reticulatus* Poir. | MUMH1761, Nan, Thailand | AB120757,[e] AB120758[e] |
| *Phyllactinia moricola* (Henn.) Homma | *Morus australis* Poir. (Mulberry) | MUMH35, Mie, Japan | AB033481, AB022401 |
| *Pleochaeta shiraiana* (Henn.) Kimbr. & Korf | *Celtis sinensis* Pers. var. *japonica* (Planch.) Nakai | MUMH36, Mie, Japan | AB120750,[e] AB022403 |
| *Podosphaera longiseta* Sawada | *Prunus grayana* Maxim. | MUMH70, Kanagawa, Japan | AB120751,[e] AB022423 |
| *P. xanthii* (Castagne) U. Braun & Shishkoff | *Melothria japonica* (Thunb.) Maxim. ex Cogn. | MUMH68, Mie, Japan | AB033482, AB022410 |
| *Sawadaea polyfida* (C.T. Wei) R.Y. Zheng & G.Q. Chen var. *japonica* U. Braun & Tanda | *Acer palmatum* Thunb. | MUMH47, Mie, Japan | AB033476, AB022364 |
| *Typhulochaeta japonica* S. Ito & Hara | *Quercus mongolica* Fish. ex Turcz. var. *crispula* (Blume) H. Ohashi | MUMHS76, Toyama, Japan | AB120752,[e] AB022415 |

[a] Fungi were identified using Braun (1987) and Nomura (1997)
[b] MUMH, Mie University Mycological Herbarium
[c] The nucleotide sequence data will appear in the DDBJ, EMBL, and GenBank Database under the respective accession number
[d] Isolate maintained as a living fungus in the Laboratory of Plant Pathology, Mie University
[e] Sequence newly determined in this study

cies, a gamma-distributed rate heterogeneity model (four rate categories, G = 0.6037; Yang 1994), and an estimated proportion of invariant sites (0.7632) was the most appropriate model of evolution for this data set. A NJ tree generated by using the data set and the evolution model is shown in Fig. 3. The ML analysis found a ML tree with a log-likelihood score of −7038.8210 (tree not shown). MP analysis found a single MP tree of 626 steps and six trees of 627 steps belonging to two different islands. A total of the seven trees were subjected to the Kishino–Hasegawa test (Kishino and Hasegawa 1989) using the above evolution model to select the tree with the highest likelihood. As a result, a tree having 627 step with a log-likelihood score of −7032.1216 was selected as the best MP tree [consistency index (CI) = 0.6204; retention index (RI) = 0.6722; rescaled consistency index (RC) = 0.4170; tree not shown].

All three tree-constructing methods with different algorithms resulted in similar tree topologies. We thus show only the NJ tree in Fig. 3, and indicate only bootstrap values of the three analyses on the tree. All five tribes recognized in the Erysiphaceae (Cook et al. 1997; Braun 1999; Braun and Takamatsu 2000) are again supported as the respective monophyletic groups in the NJ analysis (Fig. 3). In the ML and MP analyses, although the tribes Erysipheae, Phyllactinieae, Cystotheceae, and Blumerieae are also supported as monophyletic groups, the tribe Golovinomyceteae becomes paraphyletic. Three isolates of *Oidium phyllanthi* from *Phyllanthus* spp., i.e., *P. acidus*, *P. amarus*, and *P. reticulatus*, formed a distinctive clade with a bootstrap support of 100% in all three tree-constructing methods. The *O. phyllanthi* clade is not included in any of the five tribes. The present molecular characteristic supports the morphological result that *O. phyllanthi* on *Phyllanthus* spp. is a unique fungal group. Moreover, the present phylogenetic analysis indicates that *O. phyllanthi* is an isolated group among the Erysiphaceae in tribal level.

## Discussion and taxonomy

According to the aforementioned characters, the fungus found on three species of *Phyllanthus* is in accord with those of *Oidium phyllanthi* reported by Yen (1967). He first established *O. phyllanthi* on *Phyllanthus urinaria* L. (Euphorbiaceae) collected in Taiwan in 1966. In his description, he provided a drawing and described two types of conidia and conidiophores: a primary conidiophore producing primary conidia as well as a secondary conidiophore producing secondary conidia. The primary conidium is oval, cylindrical to oval, whereas the secondary conidium is cylindrical. Narayanswamy and Ramakrishnan (1969) reported *O. phyllanthi* on *P. niruri* collected in India. Unfortunately, they did not provide a drawing or measurements; only the size of the conidia (14.5–20 × 2.2–9.1 μm) was provided in their description. Braun (1987) commented in his monograph that these slender conidia are very unusual for powdery mildews. In the same paper, Braun (1987) described this fungus as follows: mycelium amphigenous, white, effuse, confluent, hyphae hyaline, branched, septate, 3.6–4.2 μm wide, appressoria lobed, conidiophores erect, 3–8-septate, cylindrical, flexuous below, 93.4–156 × 7.2–8.4 μm,

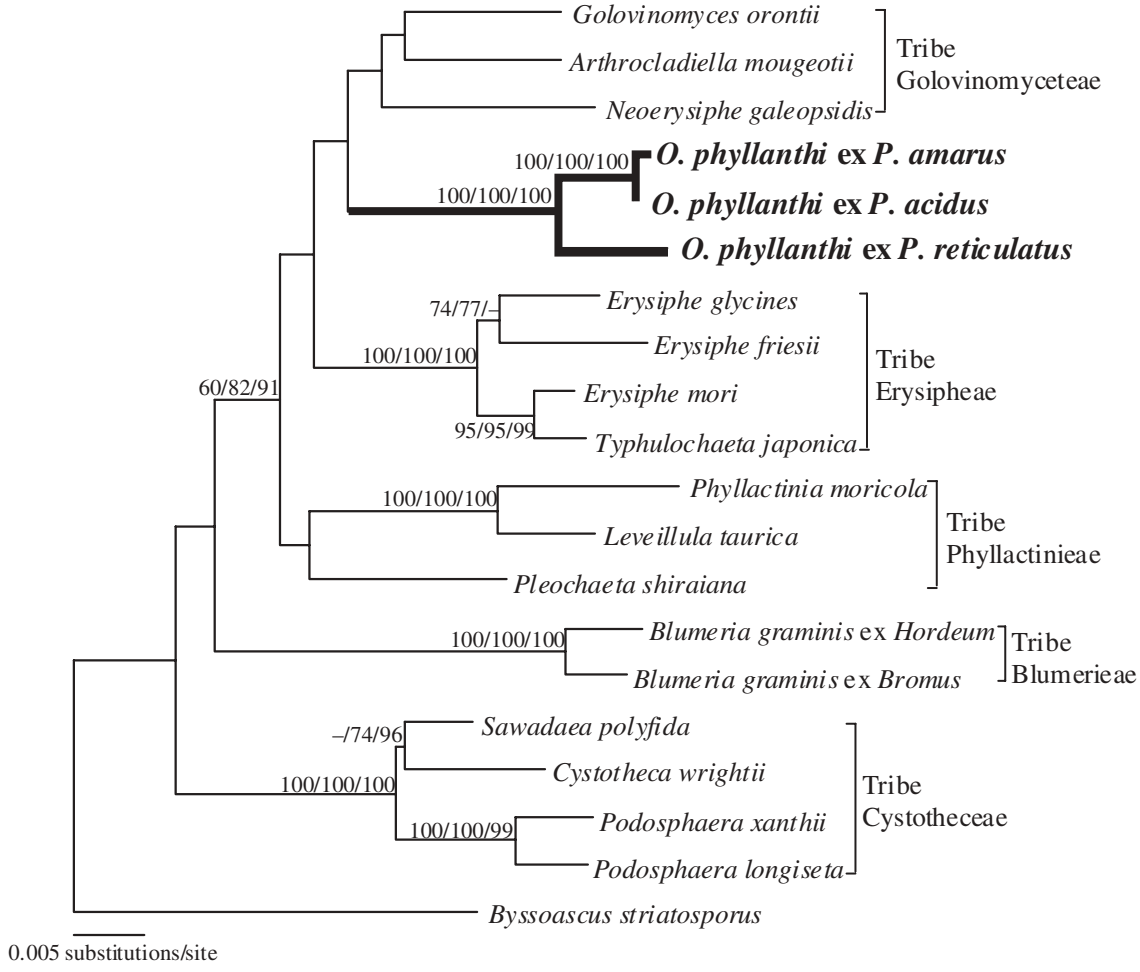


**Fig 3.** A neighbor-joining (NJ) tree based on the combined data of the 18S and 28S rDNA (D1 and D2 regions) sequences for three isolates of *Oidium* (subgen. *Microidium*) *phyllanthi*, 15 taxa of the Erysiphaceae covering all known tribes, and an outgroup taxon. Model parameters: unequal base frequencies with rate heterogeneity; gamma shape parameter = 0.5574; proportion of invariable sites = 0.7553; six rate categories; general time-reversible (GTR) model (Rodriguez et al. 1990) with transformation parameters [A–C] = 1.0000, [A–G] = 2.2991, [A–T] = 0.5279, [C–G] = 0.5279, [C–T] = 5.1365, [G–T] = 1.0000. Bootstrap values (>50%) for NJ/maximum likelihood (ML)/ maximum-parsimony (MP) analyses are given *above nodes*

foot-cell cylindrical, 72–103 × 5–6 μm, conidia in chains, 3–8 conidia, ovoid to cylindric-ovoid or cylindrical, 20–28 × 7–10 μm. The other records of this fungus were also found on *P. amarus* from Ghana, on *P. niruri* from Ceylon, Ghana, Java, and Mauritius, on *P. reticulatus* from Ceylon, and on *P. rheedii* Wight from India. Five powdery mildews, *Oidium* sp., *O. phyllanthi*, *G. cichoracearum* (DC.) V.P. Gelyuta, *Erysiphe* sp., and *E. phyllanthi* (Tanda & U. Braun) U. Braun & S. Takam., have been recorded on 12 species of *Phyllanthus* in the world (Amano 1986). The morphological characteristics of powdery mildew on *Phyllanthus* spp. in Thailand are in good agreement with *O. phyllanthi*. Therefore, the fungus on *Phyllanthus* spp. should be identified as *O. phyllathi*. The corresponding teleomorphic state has not been recorded.

Cook et al. (1997) examined the surface of conidia by SEM and separated genus *Oidium* into eight subgenera. The characteristics of *O. phyllanthi* differ from those of the eight subgenera. Interestingly, the fungus on *Phyllanthus* spp. has some unique characters, i.e., cylindrical to barrel-cylindrical conidia, conidium initials gradually developing into conidia, conidium initials and conidia hardly separable, and mature conidia containing two oil drop-like inclusion bodies but without conspicuous fibrosin bodies. Moreover, further studies of the conidial germ tube revealed that the powdery mildew on *Phyllanthus* spp. produces a unique germination type, the microidium type (To-anun et al. 2002), in which the conidia produce germ tubes on the shoulder or at the end of conidia, germ tube short, ~0.8–1.2 times as long as conidial length, terminating in a broad club-shaped, with nipple-shaped swelling, or a slightly lobed appressoria, with or without two or three smaller germ tubes at the end of conidia. This germination type is not identical to those of any other powdery mildews reported by Hirata (1955) and Braun (1987). Therefore, this fungus should be introduced into a new subgenus of *Oidium* on the basis of these unique characteristics.

Molecular phylogenetic analysis based on the 18S and 28S rDNA sequences supported the morphological uniqueness of *O. phyllanthi*. The fungus formed a distinct clade

and did not belong to any of the five tribes recognized in the Erysiphaceae (Braun et al. 2002). The result strongly suggests that this fungus should be placed in a new genus and new tribe of the Erysiphaceae. However, because of lacking teleomorph, it is impossible to propose new meiosporic genus for this fungus. We thus propose a new subgenus *Microidium* of mitosporic genus *Oidium* to accommodate this organism.

## Taxonomy

***Oidium*** subgenus ***Microidium*** C. To-anun & S. Takamatsu, subgen. nov.

Mycelio albo, effuso, confluenti; hyphis repentibus, tortuosis, ramosis, septatis, hyalinis; appressoriis lobatis; conidiophoris ex hyphis oriundis, erectis, rigidioribus, cylindraceis, hyalinis, cellula pedis cylindracea, inferne tortili praeditis; conidiis aliquot catenulatis, hyalinis, membrana levi, unicellularibus, cylindraceis vel doliiformibus, apice utrinque rotundatis vel leviter truncatis, cum corporibus guttae oreis similibus, sine corporibus fibrosaceis; tubis gemninalibus e partibus humeli utrinque vel e apicibus conidii nascentibus, brevibus, cum tumore late clavati mammifori vel appressoriis leviter lobatis ferentibus.

Type species: *Oidium phyllanthi* J.M. Yen.

Description: Mycelium amphigenous, appressoria multilobed, conidiophores erect, simple, foot-cells long, straight, slightly twisted at the base. Conidia small, unicellular, hyaline, cylindrical to barrel-cylindrical, with oil drop-like inclusion bodies, without fibrosin bodies, producing germ tubes on the shoulder, germ tubes broad club-shaped, terminating in nipple-shaped or lobed appressoria (microidium type).

**Acknowledgments** This work was financially supported by the Hitachi Scholarship Foundation, the Thailand Research Fund (PDF/47/2542), JICA (ATRACT), and a Grant-in-Aid for Scientific Research (Nos. 13660047, 14255004, and 15405021) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References

Akaike H (1974) A new look at the statistical model identifications. IEEE Trans Automat Control AC-19:716–723

Amano (Hirata) K (1986) Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo

Boesewinkel HJ (1977) Identification of Erysiphaceae by conidial characteristics. Rev Mycol 41:493–507

Boesewinkel HJ (1980) The morphology of the imperfect states of powdery mildews (Erysiphaceae). Bot Rev (Lancaster) 46:167–224

Braun U (1987) A monograph of the Erysiphales (powdery mildews). Beih Nova Hedwigia 89:1–700

Braun U (1999) Some critical notes on the classification and generic concept of the Erysiphaceae. Schlechtendalia 3:49–55

Braun U, Takamatsu S (2000) Phylogeny of *Erysiphe, Microsphaera, Uncinula* (Erysipheae) and *Cystotheca, Podosphaera, Sphaerotheca* (Cystotheceae) inferred from rDNA ITS sequences: some taxonomic consequences. Schlechtendalia 4:1–33

Braun U, Cook RTA, Inman AJ, Shin HD (2002) The taxonomy of the powdery mildew fungi. In: Bélanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) The powdery mildews: a comprehensive treatise. APS Press, St. Paul, pp 13–55

Brundza K (1934) Beiträge zur Kenntnis der Erysiphaceen. Litauens Z U Akad Metras 2:107–197

Cook RTA, Inman AJ, Billings C (1997) Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycol Res 101:975–1002

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of incongruence. Cladistics 10:315–319

Felsenstein J (1985) Confidence limits on phylogenies; an approach using the bootstrap. Evolution 39:783–791

Ferraris T (1910) Flora Italica Cryptogama, part I Fungi. Rocca San Casciano, Florence, Italy, pp 591–916

Foex E (1913) Recherches sur *Oidiopsis taurica* (Lév.). Arn Bull Soc Mycol Fr 29:577–588

Golovin PN (1956) Morfologiceskij obsor roda *Leveillula* Arnaud (mucnisto-rosjanye gribysem Erysiphaceae). Tr Bot Inst VL Komarova An SSSR Ser II 10:193–308

Higgins DG, Bleaby AJ, Fuchs R (1992) CLUSTAL V: improved software for multiple sequence alignment. Comput Appl Biosci 8:189–191

Hirata K (1942) On the shape of the germ tubes of Erysipheae. Bull Chiba Coll Hortic 5:34–49

Hirata, K (1955) On the shape of the germ tubes of Erysipheae (II). Bull Fac Agric Niigata Univ 7:24–36

Hirata T, Takamatsu S (1996) Nucleotide sequence diversity of rDNA internal transcribed spacer extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37:265–270

Jaczewski A (1927) Karmannyj opredelitel' gribov 2. Mucnisto-rosjanye Griby, Leningrad

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J Mol Evol 29:170–179

Kusaba M, Tsuge T (1995) Phylogeny of *Alternaria* fungi know to produce host-specific toxins on the basis of variation in internal transcribed spacers of ribosomal DNA. Curr Genet 28:491–498

Mori Y, Sato Y, Takamatsu S (2000) Evolutionary analysis of the powdery mildew fungi (Erysiphales) using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92:74–93

Narayanaswame P, Ramakrishnan K (1969) Powdery mildews of Coimbatore, Madras State. Madras Univ J 38:84–99

Nomura Y (1997) Taxonomical study of Erisiphaceae of Japan. Yokendo, Tokyo

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Rodriguez F, Oliver JL, Martin A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theor Biol 142:485–501

Saenz GS, Taylor JW (1999) Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer (ITS) ribosomal DNA sequences. Can J Bot 77:150–169

Salmon E (1900) A monograph of the Erysiphaceae. Mem Torrey Bot Club 9:1–292

Shin HD, La YJ (1993) Morphology of edge lines of chained immature conidia on conidiophores in powdery mildew fungi and their taxonomic significance. Mycotaxon 26:445–451

Shin HD, Zheng RY (1998) Anamorphic morphology of *Uncinula* and allied genera (I). Mycotaxon 66:243–266

Sontirat P, Pitakpaiwan P, Khamhangridthirong T, Choobamroong W, Kueprakone U (1994) Host index of plant diseases in Thailand, 3rd edn. Plant Pathology and Microbiology Division, Department of Agriculture, Bangkok, Thailand

Swofford DL (2001) PAUP: Phylogenetic analysis using parsimony (and other methods) 4.0b8. Sinauer, Sunderland, MA

Takamatsu S, Hirata T, Sato Y (1998) Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). Mycoscience 39:441–453

Takamatsu S, Hirata T, Sato Y, Nomura Y (1999) Phylogenetic relationships of *Microsphaera* and *Erysiphe* sect. Erysiphe (powdery mildews) inferred from the rDNA ITS sequences. Mycoscience 40:259–268

Takamatsu S, Hirata T, Sato Y (2000) A parasitic transition from trees to herbs occured at least two times in tribus Cystotheceae (Erysiphaceae): evidence from nuclear ribosomal DNA. Mycol Res 104:1304–1311

To-anun C, Sunawan A, Limkaisang S, Khom-un S, Sato Y, Takamatsu S (2002) New germination type of conidia of powdery mildews found on *Phyllanthus* spp. In: Summary of the first international conference on tropical and subtropical plant diseases (TPS 2002), November 5–8, 2002. Chiang Mai, Thailand

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506–513

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322

Yang Z (1994) Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J Mol Evol 39:306–314

Yarwood CE (1957) Powdery mildews. Bot Rev 13:235–301

Yen JM (1967) Etude sur les champignons parasites du Sud-Est asiatique. VIII. Quelques especes d'*Oidium* de Formose. Cah Pac 11:75–116