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Pseudoidium javanicum, a new species of powdery mildew on *Acalypha* spp. from Indonesia

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ABSTRACT

Pseudoidium javanicum is proposed as a new species based on analyses of 28S, ITS and IGS rDNA sequences, and morphological data. This new species was found on *Acalypha wilkesiana* var. *marginata*, *A. argentea*, and *A. cristata* collected from Cibodas Botanical Garden, Bogor (West Java Province, Indonesia). Our analyses showed that all these specimens have identical rDNA sequences and similar morphological characteristics. They form a distinct clade separated from other species of Erysiphaceae. *Pseudoidium javanicum* differs from *Erysiphe acalyphae* by having shorter conidiophores and foot cells 1–3 times as long as the 0–2 following cells. The conidial size of *Ps. javanicum* is also smaller than that of *E. jatrophae*.

1. Introduction

Acalypha L. is one of the largest genera in the plant family Euphorbiaceae comprising about 462 species (Qin et al. 2006). The genus consists of herbs, shrubs, and trees and it is mainly distributed in tropical and subtropical regions, and a few species are found in temperate areas (Atha 2008). Five powdery mildew species have been recorded on hosts belonging to the genus *Acalypha*, namely, *Erysiphe acalyphae* (F.L. Tai) R.Y. Zheng & G.Q. Chen, *E. jatrophae* Doidge, *Podosphaera euphorbiae-hirtae* (U. Braun & Somani) U. Braun & S. Takam., *Golovinomyces sparsus* (U. Braun) V.P. Heluta, and *Fibroidium acalyphae* (Chidd.) U. Braun & R.T.A. Cook (Amano 1986; Braun 1987; Braun and Cook 2012).

During visits in the Cibodas Botanical Garden (West Java, Indonesia) in March 2011 and 2012, three species of *Acalypha* – *A. wilkesiana* var. *marginata* E. Morren, *A. argentea* Hort., and *A. cristata* Radcl.-Sm. – were found to be infected by powdery mildews. Morphological examination confirmed that the causal agents belong to the genus *Pseudoidium* [anamorph of *Erysiphe* R. Hedw. ex DC. emend. U. Braun & S. Takam.], but all specimens are distinct from *E. acalyphae* (Tai 1946) by having shorter conidiophores and foot cells 1–3 times as long as the 0–2 following cells. All nucleotide sequences from internal transcribed spacer (ITS), intergenic spacer (IGS) and 28S regions of the ribosomal DNA showed that all specimens on *Acalypha* spp. collected in Indonesia form an independent lineage separated from other *Erysiphe*

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species. Therefore, the fungus concerned has to be considered a new species.

2. Materials and methods

2.1. *Morphological examination*

Specimens were collected at Cibodas Botanical Garden, Bogor (West Java Province, Indonesia) in March 2011 and 2012. Details of host names, collection dates, localities, and collectors were recorded. Herbarium samples were rehydrated before examination by boiling a small piece of infected leaf with the fungal mycelium downwards in a drop of lactic acid on a slide as described by Shin and La (1993). After boiling, the rehydrated mycelium was scraped off and mounted in lactic acid using a light microscope with phase contrast. Thirty conidia, conidiophores, foot cells and mother cells were measured per sample. Specimens were deposited at the National Museum of Nature and Science (TNS) and Mie University Mycological Herbarium (MUMH) [Japan] and Herbarium Bogoriense (BO) [Indonesia].

2.2. *Phylogenetic analysis*

DNA extraction of the powdery mildew specimens was conducted according to 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), ITS region including the 5.8S rDNA, and IGS region were amplified by polymerase chain reaction (PCR) using the respective primer pairs: PM3/TW14 (28S), ITS5/PM6 (ITS fragment 1) and PM7/ITS4 (ITS fragment 2), and IGS-12A/NS1R (IGS; Carbone and Kohn 1999). KOD FX Neo DNA polymerase (Toyobo, Japan) was used in the PCR reaction according to the manufacturer's protocol. The amplicons of 28S rDNA, ITS and IGS were sent to SolGent Co. Ltd. (Daejeon, South Korea) for sequencing using primers NL1 and NLP2 (28S), ITS1 and ITS4 (ITS), and IGS-12A and NS1R (IGS). Representative sequences determined in this study were deposited in the DNA DataBase of Japan (DDBJ) under the accession numbers of AB733586 – AB733597 (Table 1). Sequences generated from the 28S rDNA region were aligned with other sequences of Erysiphaceae retrieved from DNA databases (DDBJ, EMBL, NCBI) using MEGA 5 (Tamura et al. 2011). The alignment was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S12969. Phylogenetic trees were calculated from the dataset using neighbor joining (NJ) (Saitou and Nei 1987), maximum parsimony (MP), and maximum likelihood (ML) methods in PAUP* 4.0b10 (Swofford 2002). For ML and NJ analyses, the most appropriate evolutionary model was determined for a given dataset using PAUP* and Modeltest 3.06 (Posada and Crandall 1998). A starting tree was obtained with the NJ method. With this tree, likelihood scores were calculated with PAUP* for 56 alternative models of evolution. The output file was then imported into Modeltest to compare the models using Akaike's (1974) information criterion (AIC). Once a model of evolution was chosen, it was used to construct phylogenetic trees with the NJ and ML method using PAUP*. The GTR+G+I model (Tavaré 1986) was selected as the best evolution model to construct trees. For MP analyses, the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm, the MULPARS option was in effect, and zero-length branches were collapsed. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting tree was tested with bootstrap (BS) analysis using 1000 replications (Felsenstein 1985) for all analyses.

3. Results

3.1. *Taxonomy*

Pseudoidium javanicum Meeboon & S. Takam. sp. nov. Fig. 1.

MycoBank no.: MB 800883.

Differs from *E. acalyphae* by having shorter conidiophores [(46–)48–69(–83) × (5–)5.5–7(–8) μm] and foot cells 1–3 times as long as the 0–2 following cells. The conidial size [(19–)20–25(–27) × (8–)9–12(–13) μm] is smaller than in *E. jatrophae*.

Type: on *A. wilkesiana* var. *marginata* E. Morren (Euphorbiaceae), Indonesia, West Java province, Cibodas Botanical Garden, Bogor, 7 March 2012 (Holotypus, TNS-F-46915; Isotypus, MUMH 5559).

Ribosomal DNA sequence ex holotype: AB733593 (ITS), AB733597 (28S), AB733586 (IGS).

Etymology: the new species is named after the place (Java Island) where the fungus was collected.

Mycelium amphigenous, mostly epiphyllous, almost persistent, effuse; hyphal appressoria solitary, rarely in

Table 1 – Sources of *Pseudoidium javanicum* material used for molecular analyses and DNA database accession numbers.

Host	Specimen no.	Location and year	Accession no.		
			ITS	28S	IGS
Acalypha wilkesiana var. marginata	MUMH5559	Indonesia: West Java province; 2012	AB733593	AB733597	AB733586
A. cristata	MUMH5560	Indonesia: West Java province; 2012	–	–	AB733587
A. argentea	MUMH5561	Indonesia: West Java province; 2012	–	–	AB733588
A. wilkesiana var. marginata	MUMH5149	Indonesia: West Java province; 2011	AB733594	AB733589	–
A. wilkesiana var. marginata	MUMH5150	Indonesia: West Java province; 2011	AB733595	AB733590	–
A. cristata	MUMH5151	Indonesia: West Java province; 2011	AB733591	–	–
A. argentea	MUMH5152	Indonesia: West Java province; 2011	AB733596	AB733592	–

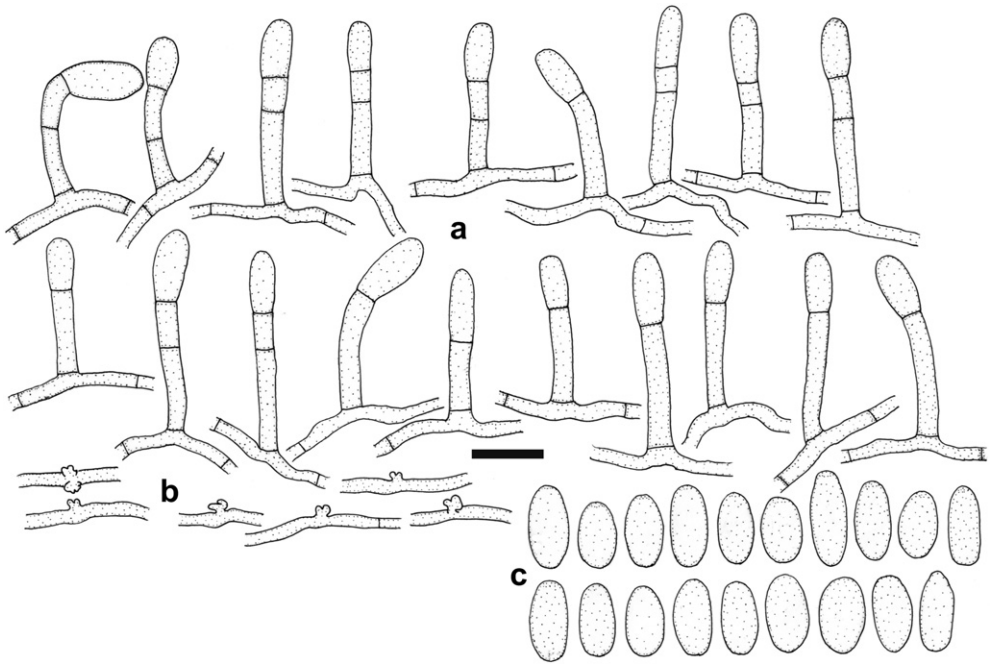

Fig. 1 – Line drawing of *Pseudoidium javanicum* (TNS-F-46915). a Conidiophores. b Appressoria. c Conidia. *Bar* 30 μm.

opposite pairs, lobed; mother cells (18–)20–32(–65) × (3.5–)4–5 μm; conidiophores (46–)48–69(–83) × (5–)5.5–7(–8) μm, erect, hyaline, arising from the upper part of the mother cell, position central or non-central; foot cells (12–)15–30(–42) × (4–)5–7.5(–8) μm, 1–3 times as long as the 0–2 following cells, straight, with a basal septum at the branching point of the mycelium or very slightly elevated (up to 3 μm), forming conidia singly; conidia ellipsoid-ovoid or doliiform, hyaline, (19–)20–25(–27) × (8–)9–12(–13) μm.

Additional collections examined (paratypes): on *A. wilkesiana* var. *marginata*, (BO-22665, 22666; MUMH5149, 5150), *A. argentea* Hort. (MUMH5152), *A. cristata* Radcl.-Sm. (BO-22668, MUMH 5151), from the same locality, 14 March 2011; *A. argentea* Hort. (BO-22667, MUMH5561), *A. cristata* Radcl.-Sm. (MUMH 5560), 7 March 2012.

3.2. *Phylogenetic analysis*

The alignment of the 28S rDNA consisted of 31 sequences and 698 total characters, of which 572 characters were constant, 43 characters were variable and parsimony-uninformative, and 83 characters were parsimony-informative. All NJ, MP and ML analyses revealed that the sequences of *Ps. javanicum* belong to the genus *Erysiphe* (anamorph *Pseudoidium*) and forms an independent lineage separated from other sequences of the tribe Erysipheae with 100% BS support. However, the phylogenetic placement of *Ps. javanicum* within the genus *Erysiphe* was not consistent depending on the tree constructing methods. In NJ analysis, *Ps. javanicum* was sister to other *Erysiphe* species except for *E. australiana* (McAlpine) U. Braun & S. Takam. and *E. adunca* (Wallr.) Fr. (Fig. 2a). In ML analysis, *Ps. javanicum* grouped with *E. pisi* Boerema & Verh., but this was not supported by BS (Fig. 2c). Furthermore, the branch length leading to *Ps. javanicum* was unusually long, which suggests long-branch attraction. In MP analysis, 64 equally parsimonious trees with 281 steps were generated. The strict consensus tree (Fig. 2b) supported the NJ and ML analyses that phylogenetic placement of *Ps. javanicum* differed among the equally parsimonious trees.

We also determined five ITS and three IGS sequences of *Ps. javanicum*. All these sequences were identical among the isolates, which indicates that all of them belong to a single species. We tried to use these sequences for phylogenetic analyses. However, the sequences of *Ps. javanicum* have too many substitutions to construct unambiguous alignments with other *Erysiphe* species. For example, FASTA search using EMBL DNA database showed that the highest similarity of ITS sequences was only 78.8% with 459 nucleotide overlap per 637 total length in *E. mori* (I. Miyake) U. Braun & S. Takam., followed by *E. magellanica* (Thaxt.) U. Braun & S. Takam. (76.3% with 532 nt overlap), *E. nothofagi* (Thaxt.) U. Braun & S. Takam. (75.9% with 543 nt overlap), and *E. patagoniaca* Havryl. & S. Takam. (79.5% with 452 nt overlap). Similarly, the highest similarity in IGS sequences was only 72.6% with 368 nt overlap per 391 total length in *E. necator* Schwein. We thus did not make phylogenetic trees of ITS and IGS sequences.

4. Discussion

Erysiphe acalyphae, *E. jatrophae*, *P. euphorbiae-hirtae*, *G. sparsus*, and *F. acalyphae* have been recorded as powdery mildews found on various species of *Acalypha* (Amano 1986; Braun 1987; Braun and Cook 2012). Among them, only *E. acalyphae* and *E. jatrophae* are linked to *Pseudoidium* anamorphs (Tai 1946;

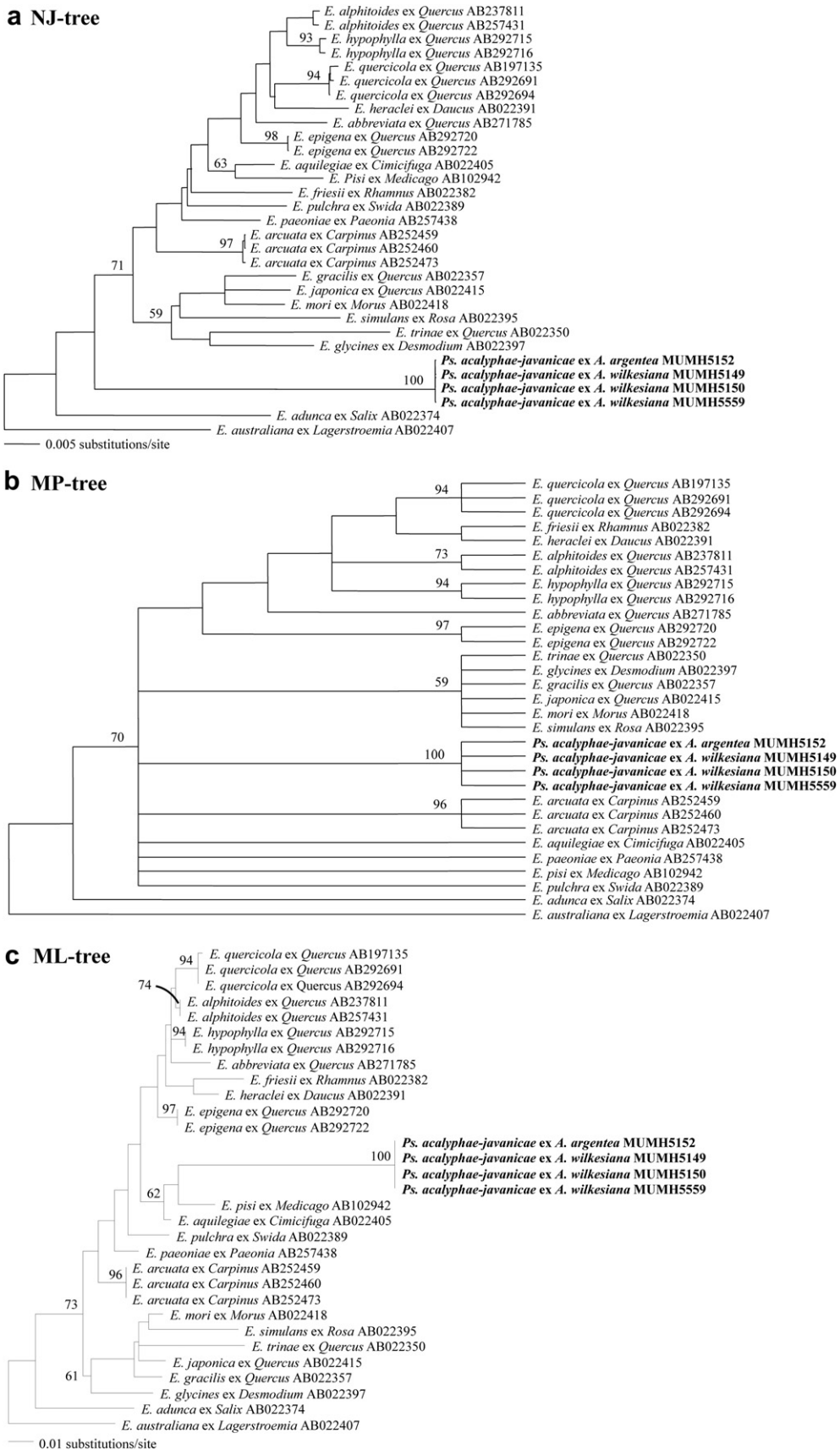

Fig. 2 – Phylogeny of *Pseudoidium javanicum* inferred from 28S rDNA sequences. a Neighbor joining tree, b strict consensus tree of the 64 equally parsimony trees with 281 steps and c maximum likelihood tree. Percentage bootstrap supports (1000 replications; ≥50%) are shown on the branches.

Doidge 1948; Braun and Cook 2012). *Erysiphe acalyphae* is distributed in Asia (China, Taiwan, India) and Africa (Ghana, Mauritius, South Africa, Sudan, Tanzania, Uganda, Zambia, Zimbabwe) while *E. jatrophae* is endemic to South Africa (Braun 1987; Braun and Cook 2012). Both species belong to *E.* sect. *Erysiphe* (Braun and Cook 2012). Because no teleomorph has been found for the present fungus, only the anamorph can be described in details. Tai (1946) described the anamorph of *E. acalyphae* (≡ *Uncinula acalyphae* F.L. Tai) found on *A. brachystachya* to have solitary conidia (16–30 × 10–16 μm), long and slender conidiophores (46–113 μm, up to 243 μm long) with the basal cell 4–6 times longer than the following cells. This species was also found on *A. indica*, *A. ciliata*, *A. lanceolata*, *A. sinensis*, and *A. superba* [= *A. brachystachya*] (Braun and Cook 2012). *Pseudoidium javanicum* is distinct from *E. acalyphae* by having shorter conidiophores [(46–)48–69(–83) × (5–)5.5–7(–8) μm], foot cells 1–3 times as long as the 0–2 following cells, and being found on *A. wilkesiana* var. *marginata*, *A. argentea*, and *A. cristata*. Furthermore, the conidial size of the current new species [(19–)20–25(–27) × (8–)9–12(–13) μm] is smaller than that of *E. jatrophae* on *A. angustata* (30–40 × 15–20 μm) (Doidge 1948; Braun and Cook 2012).

In the molecular analyses, all ITS, IGS and 28S rDNA sequences strongly supported that *Ps. javanicum* belongs to the genus *Erysiphe* (including *Pseudoidium* anamorphs). However, since the fungus is too distantly related to other *Erysiphe* species, the phylogenetic placement of this species in the genus *Erysiphe* is presently uncertain.

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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