PH.D. THESIS

BIODIVERSITY AND TAXONOMY OF POWDERY MILDEWS IN EAST ASIA

AND INDONESIA

GRADUATE SCHOOL OF BIORESOURCES

MIE UNIVERSITY

SISKA ARIE SANTY SIAHAAN

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CONTENTS

ABSTRACT

East Asia is one of the regions in the Northern Hemisphere which have abundant records of powdery mildew species in the world. Nevertheless, there are still plenty of powdery mildew genetic resources hidden in this area. *Erysiphe aucubae*, a new powdery mildew species found in this study, is an example of an endemic powdery mildew currently described from East Asian region.

Meanwhile, despite the rich biodiversity of host plants, there has been relatively little concern on powdery mildew research in Indonesia. Past researches have focused almost exclusively only on conventional taxonomic system and species identification have often been confounded due to the lack of sexual state, an important character for the identification in the conventional taxonomic system. This research was carried out to address the gaps between the conventional and the current taxonomic systems for the Indonesian powdery mildews by providing not only morphological information, but also host range data and inferred phylogenetic relationships based on molecular data.

In this study, surveys of the Indonesian powdery mildews were conducted several times during 2013-2017. Totally 109 specimens, consisted of 107 anamorphic and two teleomorphic specimens were collected mainly from North Sumatra, West Java and Bali provinces in Indonesia. Of these, four new powdery mildew species were proposed, i.e. *Erysiphe baliensis, E. sidae, Phyllactinia poinsettiae* and *Podosphaera perseae-americanae.* The latter species is currently proposed as a new genus, namely *Hommaea persea-americanae.* In addition, six new hosts were also recognized for *Erysiphe quercicola*, a northern hemisphere origin powdery mildew but has been hypothesized to expand its host ranges to tropical trees. Other powdery mildew isolates were described in detail based on morphological characteristics, molecular phylogenetic analyses and host

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range data.

CHAPTER I

General Introduction and outline of thesis

1.1. Background

Powdery mildews are some of the world's most frequently encountered plant pathogenic fungi, a fungal group belonging to the Erysiphaceae (Ascomycota; Erysiphales), all of which are exclusively obligate biothrophs of plants. They acquired the obligate biothropic nature only once in their ancestry and have retained it ever since (Takamatsu 2013). Powdery mildews are often conspicuous owing to the profuse production of conidia that give them their common name. They infect leaves, stems, flowers and fruits of ca. 10,000 species of angiosperms, but there are no records of any gymnosperms and ferns as hosts (Amano 1986). The host plants include many economically important plants, such as cereals and grasses, vegetables, ornamental, weeds, fruit-trees and broad-leaved shade and forest trees (Agrios 2005; Glawe 2008). Because of their obligate nature, researchers have not had the advantage of routinely cultivating these fungi on artificial media, although many powdery mildews have been grown on detached leaves of their hosts (Cook et al. 2015).

In general, the magnitude of fungal diversity is estimated to be 1.5 million species, but only around 5% of species have been described (Hawksworth 2001). Blackwell (2011) indicated that fungal diversity in the tropics is richer than that in temperate regions. However, this result is contradict to powdery mildews, as this fungal group is believed to be more diverse in the temperate region, since this area is believed to be the origin of this group. Among Asian countries, powdery mildews have been recorded abundantly in East Asia (subtropical to temperate regions) and fewer in Southeast Asia (tropical to subtropical regions). Amano (1986) noted that Eastern Asia region has been recognized to have especially many arboreal host plants, thus making it rich in several tree-parasitic powdery mildew genera, such as: *Microsphaera* and *Uncinula* (now *Erysiphe* sect. *Microsphaera* and sect. *Uncinula*) and *Phyllactinia*. In

addition, the basal genera in the Erysiphaceae are occupied by the genera *Parauncinula* and *Caespitotheca,* in which the former genus is known to be endemic in this region. (Takamatsu 2013; Meeboon et al. 2017).

In contrary, tropical fungi have traditionally been underresearched and their taxonomic placement has been confounded, often misidentified with temperate fungi. Limkaisang et al. (2006) stated that the ecology and classification of the powdery mildew fungi on tropical trees are still uncertain, not only because of the limited number of researchers working on this fungal group in tropical regions but also the lack of teleomorphic state, which are necessary for species identification. Furter, they stated that the identification of tropical powdery mildew fungi are mostly in their host plants and anamorphic state, which are not adequeate to distinctly delimit the species. Furthermore, Arnold (2011) stated that the exploration of tropical fungi is thus limited by (i) the extensive training needed for sampling of complex tropical habitats, (ii) the paucity of newly trained systematics specializing in tropical mycology, and (iii) traditional difficulties in delineating species boundaries. Similarly, all these points suggest that the degree of exploration of powdery mildews in Southeast Asian countries is comparably low, undoubtedly due to a relatively small number of mycologists dealing with powdery mildews compared with Europe, North America and eastern Asia and also because sexual morph necessary for reliable identifications of species is mostly lacking in subtropical and tropical areas.

There are very few reports regarding on powdery mildews in Indonesia. Those reports were mainly written based on the conventional taxonomical system, based on anamorph and not supported by molecular data. Moreover, the information of powdery mildews in Indonesia is scattered and quite limited despite the diversity of its plants. However, recently the exploration in this area, especially Indonesia is rapidly increasing. By

combining morphological, molecular and host range data, eight new species have been reported from this country since 2011 (Meeboon et al. 2012a, c-d; Siahaan et al. 2015; 2016a-c). Those reports indicated that Indonesia has potentially many unique, undescribed and probably endemic powdery mildew species, playing an important role in providing additional information of the tropical powdery mildews, especially from Indonesia, for a better understanding of the geographical distribution and evolution of powdery mildews in the world. Therefore, the survey on the diversity of this group of fungi and its distribution in Indonesia should be carried out to provide a comprehensive database of the fungi based on the current generic and/or species concept.

1.2. Taxonomical review of powdery mildews

Powdery mildews were recognized and named at least as early as 1753 by Linnaeus (Braun 1987, 2011; Glawe 2006), when he published *Mucor erysiphe* for a powdery mildew, now known as *Phyllactinia guttata* (Braun 2011) without any definite description. From then on, several other scientists kept on debating which morphological characters were important for species identification and those information has been described in detail by Braun and Cook (2012). Apart from the morphological characteristics, Amano (1986), the first to consider that host range is another substantial factor in determining species identification of powdery mildews, listed and described the occurrence, host range and geographical distribution of worldwide powdery mildews in his book based on nearly 4000 references. To this date, his book remains as the most comprehensive list of host range of powdery mildews in the world.

The current classification of the Erysiphales is described by Braun and Cook (2012) is presented in Table 2.1. The five major clades within the order are designated as the the tribes Erysipheae, Golovinomyceteae, Cystotheceae, Phyllactinieae and Blumerieae.

Several species, however, remain unassigned to tribes. For example, To-anun et al. (2005) described a new subgenus of *Oidium* that appeared most closely related to tribe Golovinomyceteae but clearly distinct based on molecular analysis thus this genus was not regarded as a member of tribe Golovinomyceteae. Another similar case is the recently described genera, i.e. *Parauncinula* and *Caespitotheca*. *Parauncinula*, a genus with uncinated chasmothecial appendages, occupied a basal position within the Erysiphales (Takamatsu et al. 2005a) and *Caespitotheca* , a genus with apically grouped uncinated chasmothecial appendages with *Euoidium* anamorph with coralloid appressoria (Takamatsu et al. 2005b), are not assigned to any tribes in the Erysiphales.

In the current system, morphological structures of both teleomorph and anamorph play important role for species identification, supported by host range information and molecular data. Some critical characteristics for morphological observations are: (1) number of asci in an ascoma, e.g. one or several; (2) number of ascospores in an ascus; (3) morphology of appendages; (4) conidiogenesis, conidia maturing in chains, catenescent (*Euoidium*-type) or maturing one at a time (*Pseudoidium*-type); (5) mycelium, ectophytic or endophytic; (6) the presence of fibrosin body in fresh conidia (Braun 1987; Braun and Cook 2012).

In many cases, although both teleomorphic and anamorphic states are important in determining the classification of powdery mildews, they may occasionally experience lack of life cycle, either the teleomorph or anamorph and this may vary within the same species. In temperate regions, the life cycles may involve both teleomorphic and anamorphic states. However, this phenomenon is rather difficult to be found in subtropical and tropical areas, where teleomorphic state occurs infrequently. The lack of teleomorphic or anamorphic state makes it difficult to discuss more closely about the relationships between anamorphic and teleomorphic state. In addition, some species might share only

very slight morphological differences within cryptic species. These species were hardly separated by means of classical morphological methods. Indeed, current taxonomic research on powdery mildews is characterized by new morphological and molecular approaches to solve the problems (Braun et al. 2002).

1.3. Molecular phylogeny and evolution of powdery mildews

Morphological characters may not reflect phylogenetic relationships among powdery mildews species (Shenoy *et al.* 2007). Molecular techniques such as RFLP, RAPD-PCR and DNA sequencing might support the classical morphological criteria and host range data used in identification of powdery mildews (Bruns *et al.* 1991). However, these techniques were not applicable to powdery mildews since these obligately parasitic fungi cannot grow on artificial media and the amount of DNA obtainable from the target fungi is often insufficient for analysis using these techniques (Hirata and Takamatsu 1996; Takamatsu and Kano 2001).

The taxonomy of powdery mildews based on molecular data began appearing in the 1990s. Saenz et al. (1994) determined the sequences of the 18S rDNA of *Blumeria graminis* (DC.) Speer and at once resolved the ambiguities of the taxonomic placement of the Erysiphales, that this fungal group is closely related to Leotiales than to Plectomycetes or Pyrenomycetes as previously assumed. Hirata and Takamatsu (1996) successfully provided sequences of rDNA internal transcribed spacer (ITS) of four powdery mildew genera and the first to emphasize that this region, along with other noncoding region such as intergenic region (IGS), are more variable and suited for phylogenetic studies among closely related taxa.

Table 1. Key to the genera of the Erysiphales

Holomorph taxon	Anamorph genus	Chasmothecium	Appendages	Conidia	Conidial germ. type	Appressoria
Tribe *Erysipheae*						
Genus *Erysiphe* emend.						
a. Section *Erysiphe*	*Pseudoidium*	Epicortex chasmothecia present, thick walled	Mycelioid, flexuous, simple to irregularly branched	Single without fibrosin bodies	*Pseudoidium* type, e*xtensitubus* pattern	Lobate
b. Section *Californiomyces*	Unknown	Thin peridium cell, composed of only one conspicuous layer, yellowish to light brown walls, only on Fagaceae	Lacking or few, mycelioid	Unknown	Unknown	Unknown
c. Section *Microsphaera*	*Pseudoidium*	Epicortex chasmothecia present,thick walled	Dichotomously branched, short, setiform, stiff to long and flexuous, apically branched	Single without fibrosin bodies	*Pseudoidium* type, alobate to *extensitubus* pattern	Lobate
d. Section *Typhulochaeta*	Unknown	Peridium multilayered, pigmented, pigmented, dark, or thin, yellowish to light brown, semitransparent, base concave when dry, polyascal	True appendages lacking, special apical cells arising from the upper half, irregularly spread or concentric circles	Unknown	Unknown	Unknown
e. Section *Uncinula*	*Pseudoidium*	Chasmothecia small < 150µm	Equatorially inserted; tips uncinate-circinate	Single without fibrosin bodies	*Pseudoidium* type, *extensitubus* pattern	Lobate

Holomorph taxon	Anamorph genus	Chasmothecium	Appendages	Conidia	Conidial germ. type	Appressoria
Tribe *Golovinomyceteae*						
Subtribe *Neoerysiphinae*						
f. Genus *Neoerysiphe*	*Striatoidium*	Epicortex chasmothecia present,thick walled; mature after overwintering	Mycelioid, flexuous, simple to irregularly branched	Chain, without fibrosin bodies	*Striatoidium* type	Lobate
Subtribe *Golovinomycetinae*						
g. Genus *Golovinomyces*	*Reticuloidium*	Epicortex chasmothecia present,thick walled	Mycelioid, flexuous, simple to irregularly branched	Chain, without fibrosin bodies	*Euoidium* type	Nipple-shaped
- Section *Golovinomyces*	*Reticuloidium*	Epicortex chasmothecia present,thick walled	Mycelioid, flexuous, simple to irregularly branched	Chain, without fibrosin bodies	*Euoidium* type	Nipple-shaped
- Section *Depressi*	*Reticuloidium*	Epicortex chasmothecia present,thick walled	Mycelioid, flexuous, simple to irregularly branched	Chain, without fibrosin bodies	*Euoidium* type, *longitubus* pattern	Nipple-shaped
h. Genus *Arthrocladiella*	*Graciloidium*	Epicortex chasmothecia present,thick walled	Setiform, stiff, dichotomously branched	Chain, without fibrosin bodies	*Graciloidium* similar to *Euoidium*	Nipple-shaped
Tribe *Cystotheceae*						
Subtribe *Cystothecinae*						
i. Genus *Cystotheca*	*Setoidium*	Single ascus; wall of chasmothecium composed of two layers easily separated	Mycelioid / absent	Chain, fibrosin bodies present	*Fibroidium* type, *orthotubus* subtype	Indistinct to nipple-shaped

	Holomorph taxon	Anamorph genus	Chasmothecium	Appendages	Conidia	Conidial germ. type	Appressoria
j.	Genus *Podosphaera*						
-	Section *Podosphaera*	*Fibroidium*	Single ascus, wall of chasmothecium simple	Stiff, setiform and terminally dichotomously branched	Chain, fibrosin bodies present	*Fibroidium* type, *orthotubus* subtype	Indistinct to nipple-shaped
-	Section *Sphaerotheca*	*Fibroidium*	Single ascus, wall of chasmothecium simple	Mycelioid, simple to irregularly branched	Chain, fibrosin bodies present	Fibroidium type	Indistinct to nipple-shaped
	- Subsect. *Sphaerotheca*	*Fibroidium*	Single ascus, wall of chasmothecium simple, outer peridial cell small	Mycelioid, simple to irregularly branched	Chain, fibrosin bodies present	*Fibroidium* type, *orthotubus* subtype	Indistinct to nipple-shaped
	- Subsect. *Sphaerotheca*	*Fibroidium*	Single ascus, wall of chasmothecium simple, outer peridial cell small	Mycelioid, simple to irregularly branched	Chain, fibrosin bodies present	*Fibroidium* type, *orthotubus* subtype	Indistinct to nipple-shaped
	- Subsect. *Magnicellulatae*	*Fibroidium*	Single ascus, wall of chasmothecium simple, outer peridial cell large	Mycelioid, simple to irregularly branched	Chain, fibrosin bodies present	*Fibroidium* type, *brevitubus* subtype	Indistinct to nipple-shaped
Subtribe *Sawadaeinae*							
k.	Genus *Sawadaea*	*Octagoidium*	Polyascal chasmothecia; end walls whorled; outer wall with vein-like embossed strips	Dichotomously or trichotomously branched; tips uncinate-circinate	Conidia dimorphic, chain, fibrosin bodies present	*Fibroidium* type, *orthotubus* subtype	Indistinct to nipple-shaped

Holomorph taxon	Anamorph genus	Chasmothecium	Appendages	Conidia	Conidial germ. type	Appressoria
l. Genus *Takamatsuella*	Unknown	Polyascal, peridium multilayered, pigmented, cells irregularly shaped to polygonal	Setiform, aseptate with uncinated-circinate tips, arising from the lower half	Unknown	Unknown	Unknown
Tribe *Phyllactinieae*						
m. Genus *Leveillula*	*Oidiopsis*	Chasmothecia large, multilayered, non-transparent peridium, asci 2 spored	Mycelioid; simple or irregularly branched	Conidia dimorphic, single	*Ovulariopsis*-type	Alobate to multilobate to coralloid shaped
n. Genus *Phyllactinia*	*Ovulariopsis*	Large, >150μm; apical part of chasmothecium has numerous penicillate cells	Equatorially inserted; straight, rigid, acicular with bulbous swelling at the base	Single with various shapes; sometimes dimorphic	*Ovulariopsis*-type	Alobate to multilobate to coralloid shaped
o. Genus *Pleochaeta*	*Ovulariopsis*	Chasmothecium large, turbinate, subglobose to somewhat flattened at the base; asci 2-5 spored	Numerous appendages, attached around the equatorial zone or somewhat in the upper half; tips uncinate-circinate	Conidia dimorphic; single	*Ovulariopsis*-type	Lobate
p. Genus *Queirozia*	Unnamed (*Queirozia* anamoroph)	Chasmothecium large, turbinate, subglobose to somewhat flattened at the base; asci 2-spored	A few appendages; tips uncinate-circinate	Conidia dimorphic; single	Not-distinguished	Indistinct to nipple shaped
Tribe *Blumeriae*						
q. Genus *Blumeria*	*Oidium s. str.*	Chasmothecium large, peridium multilayered	Short, mycelioid, simple	Chain	Two distinct types on the same conidium (*Blumeria* type)	Nipple-shaped

The determination of powdery mildew species based on molecular study is now being done using nucleotide sequences of the ribosomal DNA (rDNA) internal transcribed spacer (ITS), 28S, 18S and Intergenic spacer (IGS) regions and several other multi-locus analyses, such as b-tubulin gene (*tub2*), the cytochrome P450 14α-demethylase gene (*CYP51*), and the chitin synthase gene (*Chs1*), as well as by light and scanning electron microscopes (Cook et al. 1997; Heluta et al. 2005, 2010; Lebeda et al. 2008; Takamatsu et al. 2010; Seko et al. 2011; Braun 2011). The application of molecular technique in the identification of powdery mildews is useful for identifying the major lineages of powdery mildews and to better identify several morphologically indistinct species such as *Erysiphe syringae* and *E. syringae-japonicae*, a lilac powdery mildew (Seko et al. 2011). In addition, Braun (1987) reported approximately 435 species of powdery mildews based on morphological characters, but this number has increased to 769 species in accordance with the progress of molecular phylogeny application (Kirk et al. 2008).

Nowadays many scientists are collaborating in the project of higher classification of fungi called AFTOL (Assembling the Fungal Tree of Life). Scientists are trying to root the fungal kingdom by constructing phylogenetic tree using several gene sequences (Spatafora et al. 2006; Hibbet et al. 2007). In this case, the study of powdery mildews (Erysiphales) is also included. Based on this project, the Erysiphales is included in the Leotiomycetes, although only several taxa of the Erysiphales were included in the analyses (Lutzoni et al. 2004; Blackwell et al. 2006; Spatafora et al. 2006; Wang et al. 2006; Zhang & Wang 2015).

The study on the phylogenetic of powdery mildews continued with more taxa and/or genera included, and the evolution of powdery mildews began to be revealed. Using 45 taxa of ten genera of powdery mildews, Saenz & Taylor (1999) began the phylogenetic analysis of powdery mildews and compare the morphological features with the evolutionary hypothesis inferred from phylogenetic analysis of the ITS nucleotide sequences. In their analysis, the simple, mycelioid appendages distributed throughout the

phylogenetic tree and occurred in every major clade. Based on this result, they hypothesized that the simple mycelioid appendages was the ancestral to all other appendages. However, when Mori et al. (2000b) constructed phylogenetic analysis comprises of 18 powdery mildew genera described in Braun (1987), the result showed that *Uncinula septata* (now *Parauncinula septata*) positioned at the basal placement of Erysiphales, thus emphasizing that the morphology of appendages, which is uncinated-circinate, as the most primitive in the Erysiphales. This appendage character is widespread within the Erysiphales and all fungal taxa that occupied basal positions of several lineages have similar appendages. This result is supported by the fact that some members of Myxotrichaceae of the Onygenales, a sister group of the Erysiphales, also have appendages with circinate tips (Alexopoulos et al. 1996), indicating that both Erysiphales and Myxotrichaceae have a common ancestor (Braun and Takamatsu 2000; Mori et al. 2000a,b; Takamatsu 2004; Takamatsu et al. 2005a). Using molecular clock analysis of the 18S rDNA sequences, Takamatsu (2004) reported that splitting time between the Erysiphales from its sister, Myxotrichaceae, was calculated about 100 myr ago and the first divergence within the Erysiphales occurred about 76 myr ago. This splitting period is consistent with the timing of the first large-scale radiation of the angiosperms (Crane et al. 1995).

Speaking of evolution of powdery mildews, it can never be separated from the evolution of host and earth's environmental changes. Host plants are an absolute prerequisite for the survival of an obligate biotroph like powdery mildew. In addition, earth's environmental changes affect the evolution of powdery mildews, directly or indirectly through their effects on host plants. When Hirata (1971) divided the world into four regions, i.e. Europe, North America, East Asia and West & Central Asia, he found out that powdery mildews distributed in Europe was closely related to those in North America, while the powdery mildews in North America was closely related to East Asia. Takamatsu (2013) explained that the North Atlantic Land Bridge (NALB) and the Bering Land Bridge (BLB) formed in the Paleogene period may have allowed the exchange of

powdery mildews between Europe and America and between the North America and East Asia, respectively. In addition, the tree parasitic *Uncinula* lineage, considered as the old origin of the powdery mildews, was recorded to be more abundantly in East Asia than in Europe or in North America (Amano 1986, Braun & Cook 2012, Takamatsu et al. 2015). This strengthen the hypothesis that the distribution of powdery mildews in East Asia is unique and that of North America is intermediate between Europe and East Asia.

Based on taxonomical view, Blumer (1933) hypothesized that powdery mildews with mycelioid appendages as the most primitive genus amongst powdery mildews. But then, Mori et al. (2000a) proposed that the uncinated-circinate appendages as the ancestor of the powdery mildews after they compared their phylogenetic analysis with morphological aspects and found out that the uncinated-circinate appendage appears not only at the basal part of the Erysiphales, but also occurs in the basal part of each tribe, except the Golovinomyceteae (Takamatsu 2013). For example, in the Erysipheae, the basal part was occupied by *E. australiana* and *E. adunca* that have uncinate-circinate appendages. In the Phyllactinieae and Cystotheceae, *Pleochaeta shiraiana* and the genus *Sawadaea* occupied that position respectively. The fact that all these species infect trees also support the hypothesis that powdery mildews were originally tree parasitic. Mori et al. (2000a) also pointed out the possibility that the morphology of the appendages was a result of the fungal adaptation to their hosts, since appendages have an important role in overwintering by chasmothecia. Considering that the circinate-uncinate tips as the ancestral taxa of powdery mildews, it is more likely that mycelioid appendage has convergently evolved at multiple times accompanied by host expansion to herbaceous plants, and this event occurred independently in each tribe of the Erysiphales (Takamatsu et al. 1999, 2000, 2005, 2010; Takamatsu 2004, 2013).

1.4. Review of powdery mildews in Indonesia

Reports on diversity of powdery mildews in Indonesia are quite limited despite of the rich biodiversity of its plants. Raciborski (1900) was the first author reported the

occurrence of powdery mildew in Java island. He reported two species of powdery mildews on six host plants. Palm (1921) observed that *Oidium* sp. attacked native grown tobacco in Java and Sumatra islands, which he inferred as *Erysiphe cichoracearum*. He could find only the anamorphic state of the fungus, so it was not possible to do exact identification. Schwarz (1926) reported powdery mildew occurred on stem and both leaf surface of *Physalis minima*. He provided average dimension of its mycelium and conidia. However, no further information was available about this fungus. One year later, he reported powdery mildew on an ornamental plant, *Verbena laciniata* (Schwarz 1927). This time, not only was the average dimension provided, he also pointed out this fungus as a new powdery mildew species, *Oidium verbenae*. In the following year, Schweizer (1928) enumerated 35 host plants of powdery mildews in Java. He was the first who observed the occurrence of powdery mildew, caused by *Leveilulla taurica* (*Oidiopsis*), on *Capsicum annuum*. Based on his observations, he concluded that the fungus was not as stated by Salmon (1906), a xerophytic nature of organism, since he found it in the middle of rainy season. On the other hand, he indicated that Javanese powdery mildew host plants were mostly attacked during dry seasons. Powdery mildew may also cause leaf fall on cultivated plant (*Hevea brasiliensis* (A.Juss)), caused by *Oidium heveae* (Hubert 1957). Since the damage caused by this fungus seemed to be the worst foliage disease in Indonesia, he implied that it was responsible for the low yields in rubber production at that time. Some additional reports of the powdery mildews in Indonesia were also published by Spaulding (1961), Reddy (1970), Hirata (1986), Aryuti & Rifai (1987) and Semangun (1992).

Unfortunately, those general information and database of this fungal group were written based on the conventional taxonomical system of powdery mildews, with no support of teleomorphic features, important for identification of powdery mildews, host range information or molecular information. This condition caused difficulties to precisely identify the species of powdery mildews. However, in the recent years, the biodiversity of powdery mildews in Indonesia started to be explored using combination

of morphological and molecular data, as well as geographical distribution and host ranges information (Meeboon et al. 2012a-d; Siahaan et al. 2015, 2016 a-c). All these reports proved that there must be many more undescribed and unique powdery mildews exist in Indonesia, waiting to be discovered. This dissertation will provide the information of Indonesian powdery mildews collected since 2013 until 2018.

1.5. Objectives of the study

The aims of this study are:

1) to study the biodiversity of powdery mildews in Indonesia, including the description of new species based on morphology and molecular phylogeny data

2) to provide a comprehensive review of the species and biogeographic distribution of powdery mildews found in Indonesia

The information contain in this study could provide useful information regarding biodiversity of powdery mildew in Eastern Asia and Indonesia. Particularly for Indonesia, the information will be of interest and utility to other scientists interested in the phylogeny and biogeography of Indonesian powdery mildews, especially in identification, surveillance and prevention of emergence and invasion from other regions. Moreover, precise identification of powdery mildews will allow us to be able to determine the best step to biologically control the occurence of the fungus which has not been widely applied in Indonesia.

1.6. Outline of thesis

The powdery mildews collected from Indonesia are mostly in their anamorphic state. As the morphological features of anamorphic states are almost similar, especially to those of closely related species, thus, the identification to species level of the Indonesian powdery mildew fungus required both morphological and molecular sequences.

Chapter 1 gives an introduction to powdery mildews (Erysiphales), including the taxonomy of the Erysiphales and the development of molecular tools to help identifying

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species level of this fungal group. The current situation of powdery mildew research in Indonesia is also provided.

Chapter 2 provides information of a new powdery mildew species, *Phyllactinia poinsettiae* Siahaan & S. Takam., on ornamental plant, *Euphorbia pulcherrima* Willd. Ex Klotzsch, Euphorbiaceae, in Indonesia. It describes and discusses on how the new fungus is morphologically and phylogenetically differs from its closely related species.

Chapter 3 provides detail information on two new powdery mildew fungi described from Indonesia. The first one is *Erysiphe baliensis* Siahaan & S. Takam. on *Gliricidia sepium* (Jacq.) Kunth ex Walp., Fabaceae, in Indonesia and on *Wisteria japonica* (syn. *Milletia japonica* (Siebold & Zucc.) A. Gray, Fabaceae, in Japan. Another is *Erysiphe sidae* Siahaan & S. Takam. on *Sida rhombifolia* L., Malvaceae, in Indonesia.

Chapter 4 provides information of a new powdery mildew species, *Erysiphe aucubae* S. Takam. & Siahaan on *Aucuba japonica* Thunb., Garryaceae, in Japan.

Chapter 5 provides information on additional hosts of *Erysiphe quercicola* from the tropics, especially from Indonesia and how this fungus expands its host range to tropical trees. This fungus was collected from *Bauhinia purpurea* Wall., Fabaceae, *Durio zibethinus* Rumph. ex Murray, Malvaceae and *Nephelium lappaceum* L., Sapindaceae.

Chapter 6 provides information of a new powdery mildew fungus, *Podosphaera perseae-americanae* Siahaan & S. Takam. on *Persea americana* Mill., Lauraceae in Indonesia. In addition, this chapter also describes on how this fungus is phylogenetically unique and why the fungus is temporarily included in the genus *Podosphaera*.

Chapter 7 provides information on all powdery mildews isolates collected in Indonesia from 2013-2017, summarized in this chapter, including those that have been published. Each fungus is identified by morphological characteristics and/or molecular sequences and host ranges.

Chapter 8 discusses the important findings of this dissertation. It summarizes how the data obtained in this study significantly contributed to the powdery mildew research in the tropics, especially in Indonesia, through (i) providing a better way to identify fungal

species by combination of fungal taxonomy and molecular tools, and (ii) emphasizing that Indonesia as a country with high plants biodiversity has many unexplored powdery mildew fungi.

Appendix 1 is about a new species, *Erysiphe desmodiicola,* on soybean powdery mildews (*Desmodium* spp.) from East Asia. This fungus was previously considered as *E. glycines*, however, the phylogenetic analysis clearly showed that *E. desmodiicola* formed a separate clade from *E. glycines*. Morphological observation data also confirms that this fungus is morphologically differed from that of *E. glycines* on *Amphicarpaea* spp. and on *Glycine max*.

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Chapter 2

Phyllactinia poinsettiae **sp. nov.: A new species of powdery mildew on poinsettia from Indonesia.**

Siska A.S. Siahaan[1], Hidayat I[2], Kramadibrata K[2], Meeboon J[3], and Takamatsu S[1].

[1] Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan

[2] Research Centre for Biology, Indonesian Institute of Sciences-LIPI, Jl. Raya Jakarta-Bogor KM 46, Cibinong 16911 West Java, Indonesia

[3] Department of Agriculture, Ministry of Agriculture and Cooperatives, 50 Phaholyothin Rd., Ladyao, Chatuchak, Bangkok, Thailand

Abstract

Poinsettia (*Euphorbia pulcherrima*, Euphorbiaceae) is a flowering plant indigenous to Mexico and CentralAmerica. The poinsettia is largely known for its red bracts and green foliage, and is widely used in winter festivities around temperate regions such as Christmas floral displays. A powdery mildew was found on this plant species in Java and Bali Islands of Indonesia in 2013. Morphological and molecular analyses revealed that this species is new to science. It is an asexual morph belonging to the genus *Phyllactinia* (syn. *Ovulariopsis*). The new species is described and illustrated as *Phyllactinia poinsettiae* sp. nov.

Key words: Anamorph, Erysiphaceae, *Euphorbia pulcherrima*, Molecular phylogeny, *Ovulariopsis*

1. Introduction

The poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch), indigenous to Mexico and Central America, is a typical flowering shrub or small tree, highly favored due to its beautiful color of bracts. In Indonesia, this plant is economically important and generally cultivated as an ornamental plant due to its magnificent red floral display. During a collection trip to Java and Bali Islands, Indonesia, a powdery mildew was found on poinsettia. This fungus appears as subevanescent white patches on the lower leaf surface. Morphological and molecular analyses revealed that this asexual morph represents a new, undescribed species belonging to the genus *Phyllactinia* (syn. *Ovulariopsis*).

2. Materials and methods

Morphological examinations and DNA sequencing were conducted according to the procedure described by Meeboon and Takamatsu (2015). The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and internal transcribed spacer (ITS) regions including the 5.8S rRNA gene were determined in this study. New sequences determined in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC036593–LC036595. These sequences were aligned with other sequences of *Phyllactinia* using MUSCLE (Edgar 2004) implemented in the MEGA 5 program (Tamura et al. 2011). Alignments were further manually refined and deposited in TreeBASE (http://www.treebase.org/) under the accession number S17332. Maximum likelihood(ML) tree was obtained by MEGA 5 using the Kimura 2-parameter+ I model (Kimura 1980) that was determined as the best evolutionary model for the current data set. The strength of internal branches of the resulting tree was tested with bootstrap analysis using 1000 replications (Felsenstein 1985).

3. Results and discussion

Phyllactinia poinsettiae S.A.S. Siahaan and S. Takam., sp. nov.   Fig.1.

MycoBank, MB811962

Similar to asexual morphs of *Phyllactinia kakicola* and *P. enkianthi*, but differs in having slightly curved or curved foot-cells of conidiophores, clavate conidia, and its host being *Euphorbia pulcherrima.*

Type: On *Euphorbia pulcherrima* (Euphorbiaceae), Indonesia, Bali, Kintamani, S 08°09′57.6″ E 115°15′05.2″, 16 Sep. 2013, S.A.S. Siahaan, I. Hidayat, J. Meeboon, and S. Takamatsu,TNS-F-61900 (holotype), BO 22734, MUMH 5709 (isotype).

Etymology: Epithet derived from the common name of the host plant "poinsettia".

Gene sequences (holotype): LC036595 (ITS+28S).

Mycelium hypophyllous, ectophytic and endophytic white mycelium, subevanescent. Hyphae hyaline, appressoria solitary or in pairs, lobed, sometimes multilobed or irregularly branched. Conidiophores emerge from superficial hyphae, erect, up to 380 µm arising from the upper part of mother cells. Foot-cell cylindrical, slightly curved to curved, 69–224 × 4–8 µm, followed by 1–3 cells, basal septum 10–50 µm away from the branching point. Conidia solitary, clavate with papillate tips, (50–)56–73(–77) × (16–)17.5–23(–26) µm. Conidial germ tube terminal to subterminal, sometimes lateral. Sexual morph not observed.

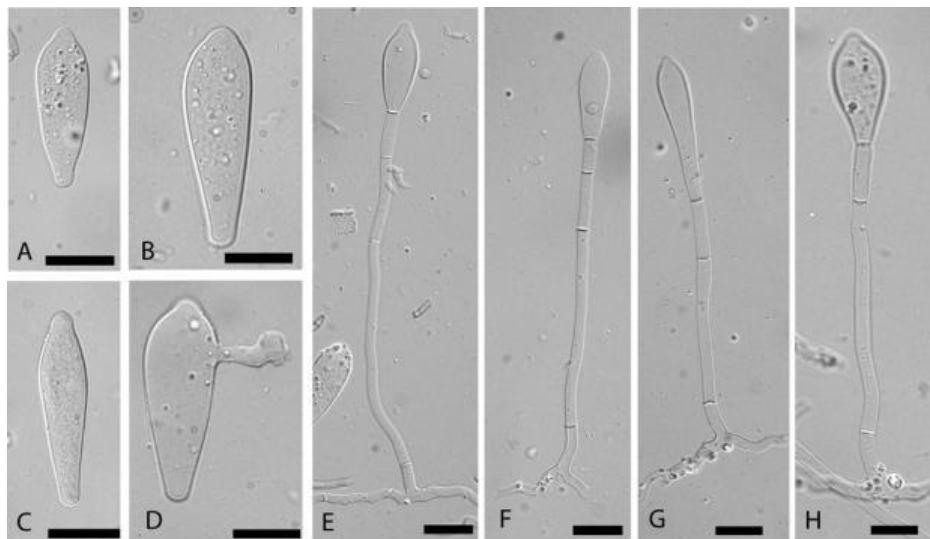

Fig.1. *Phyllactinia poinsettiae* on *Euphorbia pulcherrima*. A–C: Conidia. D: Germ tube. E–H: Conidiophores. Bars: A–D 20µm; E–H 50µm.
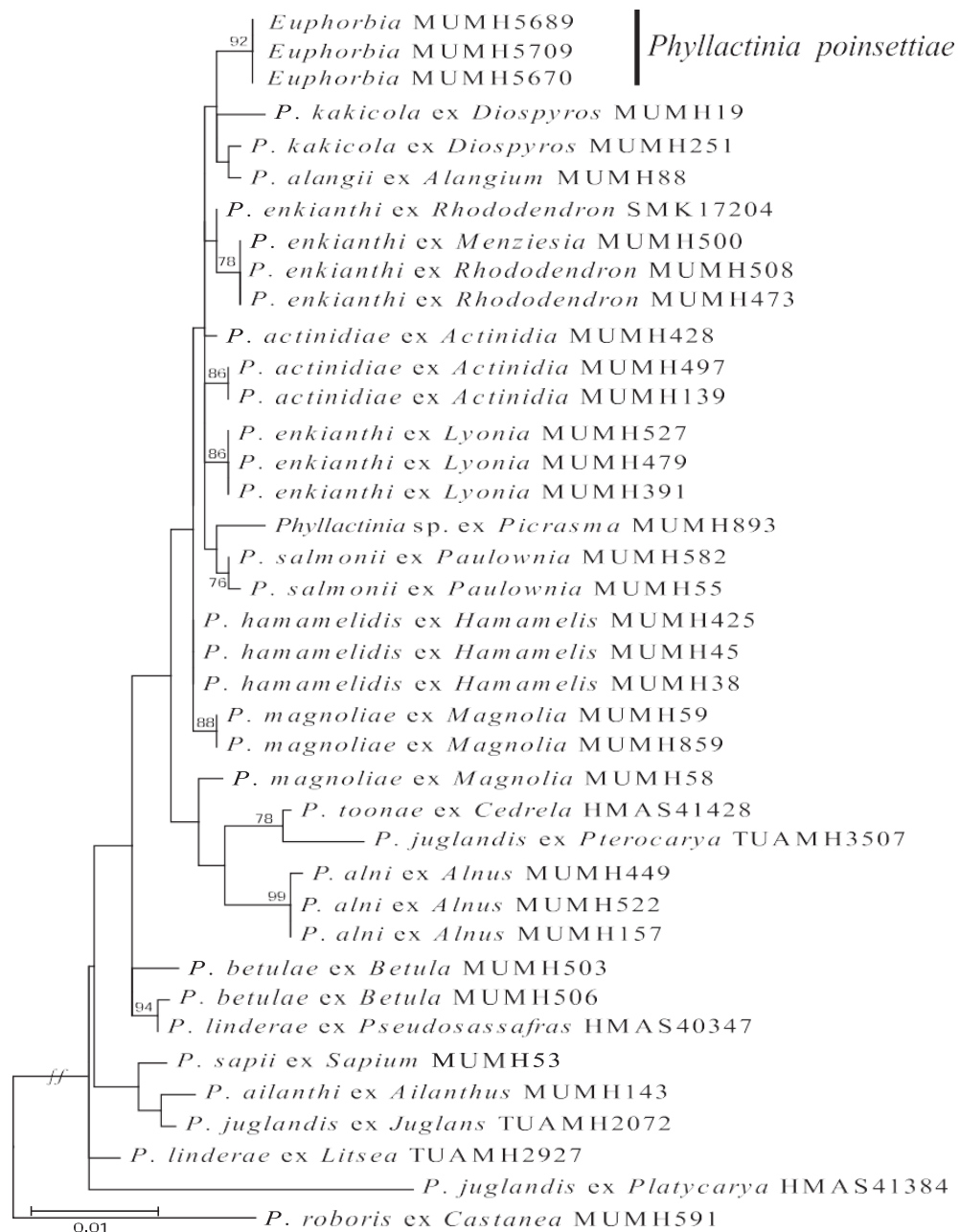
Fig. 2 – A maximum likelihood tree inferred froma combined data set of ITS and 28S rRNA gene sequences for 39 sequences from the genus *Phyllactinia* and *P. poinsettiae*. The highest log likelihood tree with percentage bootstrap support (1000 replications; ≥ 70%) are shown.

Additional specimens examined: On *Euphorbia pulcherrima* (Euphorbiaceae), Indonesia, West Java, Bogor, Curug Cigamea, S 06°39′38.9″ E 106°43′47.1″, 10 Sep. 2013, S.A.S. Siahaan, J. Meeboon, and S. Takamatsu, BO 22696, MUMH 5670, GenBank accession number: LC036593 (ITS+28S); Indonesia, West Java, Bandung, Rawa Upas, S

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07°08′52.2″ E 107°30′56.8″, 14 Sep. 2013, S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon, and S. Takamatsu, BO 22715, MUMH 5689, GenBank accession number: LC036594 (ITS+28S).

Host range and distribution: On *E. pulcherrima,* (Indonesia).

Note: The ITS and 28S rRNA gene nucleotide sequences obtained from three specimens of *P. poinsettiae* were determined in order to clarify the phylogenetic placement of this species. The result showed that the ITS and 28S sequences of the three specimens were identical to each other. Because a preliminary phylogenetic analysis (data not shown) revealed that this fungus belongs to the clade 3 of *Phyllactinia* (Takamatsu et al. 2008), further phylogenetic analysis was carried out using an ITS+28S rRNA combined data set. The data set consists of 39 sequences of *Phyllactinia*, viz, 35 from *Phyllactinia* clade 3 (Takamatsu et al. 2008), three from *P. poinsettiae*, and one outgroup [*P. roboris* (Gachet) S. Blumer ex *Castanea*]. The ML tree (Fig. 2) showed that the three sequences from *P. poinsettiae* formed an independent clade with 92% BS (bootstrap support). *Phyllactinia kakicola* Sawada and *Ph. alangii* Y.N. Yu & Y.Q. Laiwere were sisters to *P. poinsettiae*, but this was not supported by BS analysis. In the maximum parsimony analysis, *Ph. enkianthi* was sister to *P. poinsettiae*, but this was also not supported by the BS analysis (data not shown). Consequently, *P. poinsettiae* is a conidial form of *Phyllactinia*, belonging to the clade 3 of Takamatsu et al. (2008). The phylogenetically confirmed position of this asexual morph in the *Phyllactinia* clade allows the application of ICN, Art. 59.1, i.e. the new species, although its sexual morph is still unknown, is assignable to *Phyllactinia* which has priority over the anamorph-typified genus *Ovulariopsis*. The latter genus is now a heterotypic synonym of *Phyllactinia.*

Pseudoidium poinsettiae (U. Braun, Minnis & Yáñez-Morales) U. Braun, Minnis & Yáñez-Morales and *Leveilulla clavata* Nour have been reported as powdery mildews of poinsettia (Celio and Hausbeck 1998; Braun and Cook 2012). Of these, *Ps. poinsettiae* differs from *P. poinsettiae* by its ectoparasitic nature. *Leveillula clavata* was firstly

described from Africa, and in Asia, this species is distributed in India and Indonesia (Braun and Cook 2012). In 2005, a powdery mildew occurred on poinsettia in Italy and Japan, and the causal agent in Italy was identified as *L. clavata* based on anamorph (Garibaldi et al. 2006; Horie et al. 2006). The fungus that occurred in Japan may also belong to this species because of its morphological similarity. The ITS and 28S rRNA gene sequences of this fungus that occurred in Japan clearly differed from those of *P. poinsettiae* (unpublished data). Thus, it is clear that two powdery mildews species with (partially) endoparasitic nature occur on poinsettia. The morphology of the conidia between *P. poinsettiae* and the fungus that occurred in Tokyo were also distinct. Taxonomic reexamination of the fungus from Tokyo collected in 2005 is now in progress and corresponding results will be reported elsewhere. *Ovulariopsis erysiphoides* Pat. & Har. was also reported on poinsettia in Venezuela (R. Urtiaga, personal communication). However, identification of this fungus is obscure because of lacking morphological data.

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Chapter 3

***Erysiphe baliensis* and *E. sidae,* two new species of anamorphic *Erysiphe* (powdery mildew) from Indonesia.**

Siska A.S. Siahaan[1], Kramadibrata K[2], Hidayat I[2,], Meeboon J[3], and Takamatsu S[1].

[1] Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan

[2] Research Centre for Biology, Indonesian Institute of Sciences-LIPI, Jl. Raya Jakarta-Bogor KM 46, Cibinong 16911 West Java, Indonesia

[3] Department of Agriculture, Ministry of Agriculture and Cooperatives, 50 Phaholyothin Rd., Ladyao, Chatuchak, Bangkok, Thailand

Abstract

Two new anamorphic species belonging to the genus *Erysiphe*, viz. *E. baliensis* and *E. sidae*, are described in this study. *Erysiphe baliensis* was found on *Gliricidia sepium* on the Island of Bali in Indonesia, and on *Wisteria japonica* (syn. *Millettia japonica*) in Japan. Internal transcribed spacer sequences and the anamorph characters of the collections on the hosts of the two plant genera collected in Indonesia and Japan were identical to each other and considered to be conspecific. *Erysiphe sidae* found on *Sida rhombifolia* is an isolated species in the genus *Erysiphe* based on molecular phylogenetic data.

Key words: Erysiphaceae, *Gliricidia sepium*, *Pseudoidium*, *Sida rhombifolia*, *Wisteria japonica.*

1. Introduction

Of the 873 species of the Erysiphales described in Braun and Cook (2012), only four species are listed from Indonesia (Farr and Rossman 2015). The degree of exploration in other SoutheastAsian countries is comparably low, undoubtedly due to a relatively small number of mycologists dealing with powdery mildews compared with Europe, North America and Eastern Asia, and also because the sexual morph of the powdery mildews, necessary for species reliable identifications, are mostly lacking in subtropical and tropical areas. However, recent surveys of powdery mildews in Southeast Asia using combined molecular and morphological analyses suggest that many undescribed species of this fungal group are to be expected in this area (Divarangkoon et al. 2011; Monkhung et al. 2011, 2013; Meeboon et al. 2012a, b; Meeboon et al. 2013a, b, c; Hidayat et al. 2014; Siahaan et al. 2015). Therefore, a comprehensive survey of powdery mildews in these areas is required to clarify the biodiversity and evolution of this important plant pathgenic fungal group. During collection trips on the Islands of Java and Bali in Indonesia, in 2011 and 2013, new, undescribed powdery mildew species with *Pseudoidium* anamorphs were found on *Gliricidia sepium* (Jacq.) Kunth ex Walp.and *Sida rhombifolia* L., both of which belong to the genus *Erysiphe* in molecular phylogenetic analyses. These fungi are described here as new species of the genus *Erysiphe* based on morphological and molecular data.

2. Materials and methods

2.1. Molecular phylogeny

The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and the internal transcribed spacer (ITS) regions were determined according to the procedure described by Meeboon and Takamatsu (2014). Representative new sequences determined in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC06023–LC06027. New sequences were aligned with other sequences of the Erysiphaceae using MUSCLE (Edgar 2004) implemented in MEGA 6

(Tamura et al. 2013). Alignments were further manually refined using the MEGA6 program and were deposited in TreeBASE (http://www.treebase.org/) under the accession number S17749. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) method in PAUP 4.0a144 with heuristic search option using 'tree bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analyses using 1K replications with step-wise addition option set as simple (Felsenstein 1985). BS value higher than 70% were given. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

2.2. Morphology

Morphological examinations were carried out as outlined in Meeboon and Takamatsu (2015a). All the specimens were examined using a light microscope with phase contrast objectives. Thirty conidiophores and conidia were measured per sample. The size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of conidiophores, e.g. size and shape of foot-cells, position of basal septa, shape and position of hyphal appressoria; position of germ tubes of conidia; and shape of appressoria on germ tubes of conidia (if found) were documented. Specimens were deposited at the National Museum of Nature and Science (TNS), Japan, Mie University Mycological Herbarium (MUMH), Japan, and Herbarium Bogoriense (BO), Indonesia.

3. Results

3.1. The fungus on *Gliricidia sepium*

ITS and 28S rRNA gene (D1/D2 region) sequences were determined for two collections on *G. sepium* in this study. A preliminary analysis suggested that these sequences are closely related to *E. cruciferarum* Opiz ex L. Junell. We thus combined the two sequences

with a part of data set of the *Microsphaera*-lineage of the genus *Erysiphe* (Meeboon and Takamatsu 2015b) used in Takamatsu et al. (2015a). This data set of combined ITS and 28S rRNA gene sequences consisted of 46 sequences and 1366 characters, of which 127 (9.3%) characters were variable and 94 (4.7%) characters were informative for parsimony analysis. Three sequences from *E. astragali* DC. were used as outgroup based on Takamatsu et al. (2015a). A total of 157,851 equally parsimonious trees with 178 steps were constructd by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch length. One of the trees is shown in Fig. 1. Surprisingly, the sequences from the collections on *G. sepium* collected in Indonesia were identical to the sequences from collections on *Wisteria japonica* Siebold et Zucc.[syn. *Millettia japonica* (Siebold & Zucc.) A.Gray] from Japan, that were reported as *Pseudoidium* sp. in Takamatsu et al. (2015a). We conducted morphological examinations for the collections on *W. Japonica* to confirm the morphological similarity. These four sequences formed an independent clade with 100% BS support. This clade was sister to the clades of *E. oehrensii and E. cruciferarum*, but this was not supported by BS analysis.

3.2. The fungus on *Sida rhombifolia*

ITS and 28S rRNA gene (D1/D2 region) sequences were determined for three collections on *S. rhombifolia* in this study. These sequences were identical to each other in both ITS and 28S rRNA gene sequences. BLAST search with ITS sequence resulted in 89–90% similarity with *Erysiphe* and *Neoerysiphe* species. However, the alignments covered only 5.8S rRNA gene and partial ITS2 regions. BLAST search with ITS1 sequences hit no sequence in GenBank DNA database. BLAST search with 28S rRNA gene sequence resulted in 93% similarity with several *Erysiphe* species. Based on these results, we used only 28S rRNA sequences for the following phylogenetic analysis. The three sequences determined in this study were combined with the partial data set of *Erysiphe* used in Takamatsu et al. (2015b). This data set consisted of 70 sequences and 819 characters,
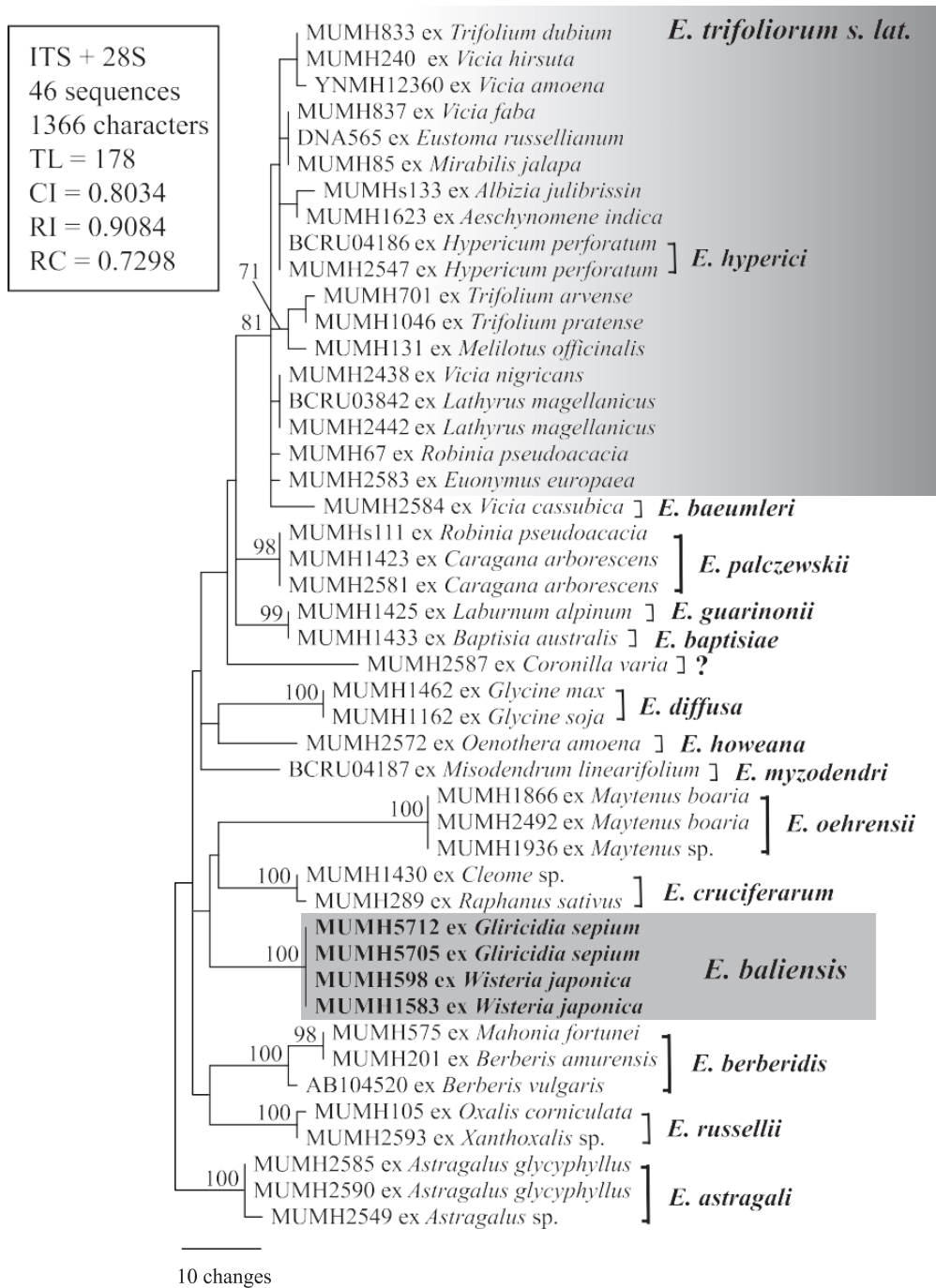
Fig.1–Phylogenetic analysis of combined data of the divergent domains D1 and D2 sequences of the 28S rRNA gene and ITS region for 46 sequences from the *Microsphaera*-lineage of the genus *Erysiphe*. This tree is a phylogram of one of the 157,851 most parsimonious trees with 178 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS (>70%) values with 1000 replicates were shown on the respective branch. Sequences from *E. baliensis* are shown in boldface.

of which 195 (23.8%) characters were variable and 142 (17.3%) characters were informative for parsimony analysis. *Erysiphe australiana* (McAlp.) U. Braun & S. Takam. was used as outgroup based on Takamatsu et al. (2015b). A total of 8260 equally parsimonious trees with 564 steps were constructd by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal branches and branch length. One of the trees is shown in Fig. 2. The three sequences from the collections on *S. rhombifolia* formed an independent clade with 100% BS support. This clade was sister to *E. carpini-cordatae* (Tanda & Y. Nomura) U. Braun & S. Takam., but this was not supported by BS analysis. The unusual long branch leading to the clade of the collections on *S. rhombifolia* supports the isolated position of this fungus. Based on the present phylogenetic analysis, it is clear that this fungus is an anamorph of the genus *Erysiphe*, but the exact phylogenetic position in this genus is still unclear.

4. Taxonomy

Erysiphe baliensis Siahaan & S. Takam., sp. nov.   Fig.3.

MycoBank no.: MB 812860.

Genetically clearly distinct from all other species of *Erysiphe*. Morphologically close to but readily distinguishable from *E. robiniae* Grev.in having smaller conidia.

Type: On *Gliricidia sepium* (Fabaceae), INDONESIA, Bali Island, Banyar Belok Village, S 08°17′29.1″ E 115°14′25.7″, 16 Sep 2013, S.A.S. Siahaan, I. Hidayat, J. Meeboon, and S. Takamatsu, TNS-F-61949 (holotype), BO 22736 (isotype), MUMH 5712 (isotype).

Gene sequences (holotype): LC06027 (ITS+28S).

Colonies on leaves epiphyllous, forming irregular white patches on host surface. Hyphae branched, septate, hyaline, superficial, 4–8 μm wide. Hyphal appressoria well-developed, lobed to multilobed, single or occasionally in pairs. Conidiophores arising from the upper part of mother cells, position mostly central, erect, (48–)53.5–75(–84) ×5–

8.5 µm. Foot-cells moderate flexuous to sinuous, width uniform throughout, 14–37 × 4–6 µm, followed by 1–2 shorter cells. Conidia produced solitarily, ellipsoid or doliiform, without conspicuous fibrosin bodies, (20–)22–28.5(–31) ×10–14 µm. Germ tubes perihilar, moderately long to long. Sexual morph not found.

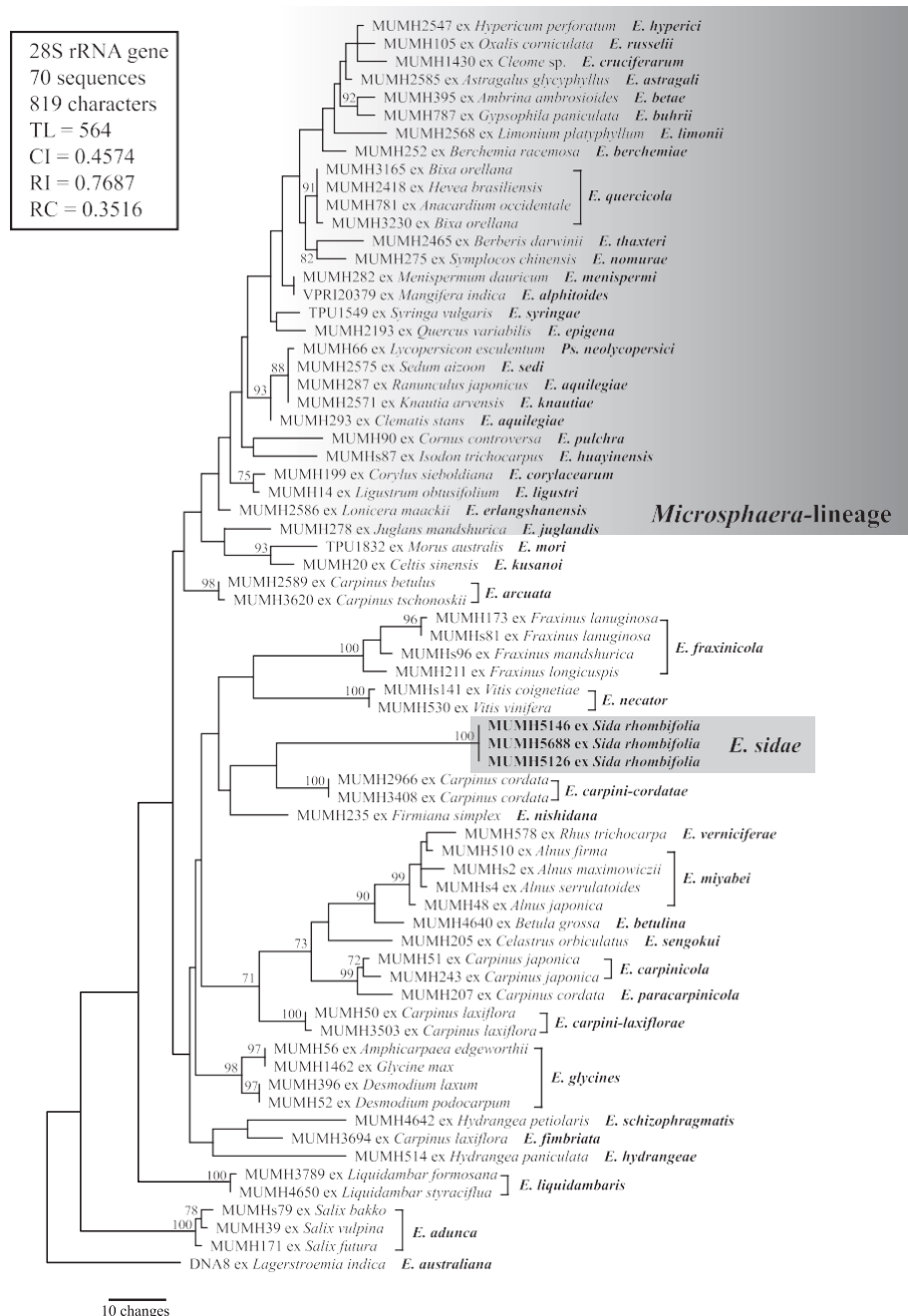Fig. 2 – Phylogenetic analysis of combined data of the divergent domains D1 and D2 sequences of the 28S rRNA gene for 70 sequences from the genus *Erysiphe*. This tree is a phylogram of one of the 8260 most parsimonious trees with 564 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch

of the tree. BS (>70%) values with 1000 replicates were shown on the respective branch. Sequences from *E. sidae* are shown in boldface.

Etymology: Epithet derived from the location of the type collection "Bali Island".

Additional specimens examined: On *Gliricidia sepium* (Fabaceae), INDONESIA, Bali Island, Gitgit waterfall area, S 08°12′13.7″E 115°08′21.9″,16 Sep 2013,S.A.S. Siahaan, I. Hidayat, J. Meeboon, and S. Takamatsu, BO 22730, MUMH 5705, GenBank accession number: LC060726 (ITS+28S); On *Wisteria japonica* (syn. *Millettia japonica*, Fabaceae), JAPAN, Mie Prefecture, Owase-shi, Mt. Tengukura, 22 Nov 1998, S. Takamatsu, MUMH 598, GenBank accession number: LC009952 (ITS); Japan, Mie Prefecture, Nanto-cho, 2 Nov 2001, S. Takamatsu, MUMH 1583, GenBank accession number: LC009996 (ITS).

Host range and distribution – On *Gliricidia sepium*, *Wisteria japonica* (Fabaceae); Indonesia, Japan.
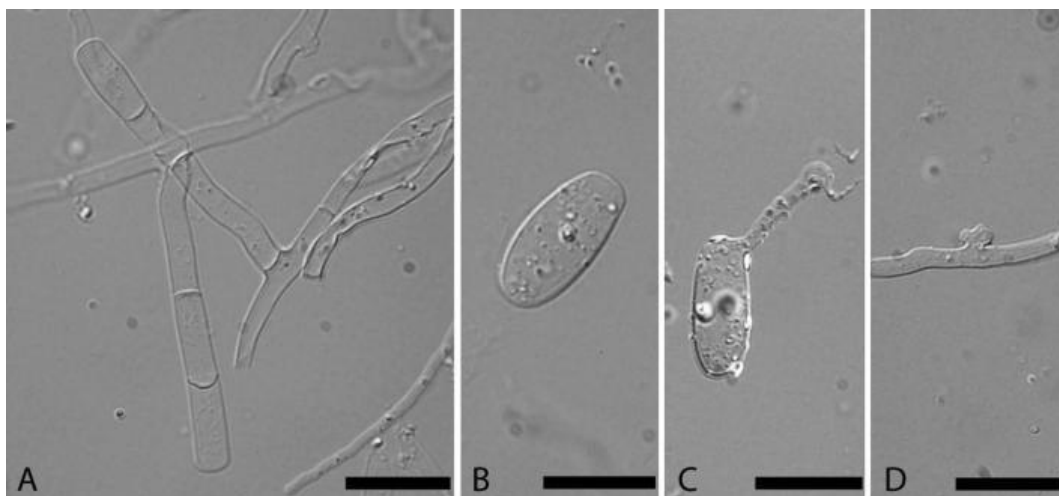
Fig. 3 – *Erysiphe baliensis* ex *Gliricidia sepium*. A: Conidiophores. B: Conidium. C:Germ tube. D: Hyphal appressorium. *Bars*: 20 μm.

Notes: *Gliricidia sepium* is a leguminous tree native to Meso-America, and it is now cultivated throughout the tropics (Dawson et al. 1995). *Oidium* sp. has been reported on this plant species from Guatemala, Honduras, Jamaica, and Venezuela (Farr and Rossman 2015), but its morphology is unknown. Braun and Cook (2012) listed *G. sepium*as a host of *E. robiniae* Grev. The present fungus is similar to *E. robiniae* in having

non-catenate conidia and foot-cells with moderate flexuous base (Shin 2000), but readily distinguishable in having smaller conidia. In addition, ITS sequences from this fungus differ from the sequence (LC009913) from a collection ex *Robinia pseudoacacia* L. (putative *E. robiniae*) (Fig. 1). It was unexpected that the sequences of the present fungus were identical to ITS sequences from collections ex *W. japonica* that were reported as *Pseudoidium* sp. in Takamatsu et al. (2015a). Examinations of the collections ex *W. japonica* revealed that the morphology is identical among the collections from the two host species. This result is acceptable when considering that *Wisteria* is closely related to *Gliricidia* in the Fabaceae in molecular phylogeny (Wojciechowski et al. 2004). Occurrence of *E. alphitoides* (Griff. &Maubl.) U. Braun & S. Takam.was recently reported on *Wisteria* spp. in the United Kingdom (Henricot and Cook 2008, Cook et al. 2015). However, the DNA sequences from the present collections clearly differ from the sequences from *E. alphitoides* ex *Wisteria* spp. Consequently, *E. baliensis* is a conidial form of *Erysiphe*, belonging to the *Microsphaera*-lineage (Takamatsu et al. 2015a).The phylogenetically confirmed position of this asexual morph in the *Erysiphe* clade allows the application of ICN (International Code of Nomenclature for algae, fungi, and plants), Art. 59.1, i.e. the new species, although its sexual morph is still unknown, is assignable to *Erysiphe* which has priority over the anamorph-typified genus *Pseudoidium*. The latter genus is now a synonym of *Erysiphe*.

Erysiphe sidae Siahaan & S. Takam., sp. nov.   Fig. 4.
MycoBank no.: MB 812861

Morphologically similar to *Pseudoidium pavoniae* (Bagyan. & U. Braun) U.Braun & R.T.A. Cook, but differ in having shorter foot-cells of conidiophores and in its deviating conidial shape.

Type: On *Sida rhombifolia* (Malvaceae), INDONESIA, West Java, Bandung, Taman Hutan Raya Ir. H. Djuanda, S 06°51′25.5″ E 107°38′00.0″, 12Mar 2011, S.A.S. Siahaan, I. Hidayat, J. Meeboon, K. Kramadibrata and S. Takamatsu, TNS-F-61950 (holotype),

BO 22680 (isotype), MUMH 5126 (isotype).

Etymology: Epithet derived from the host genus name "*Sida*".

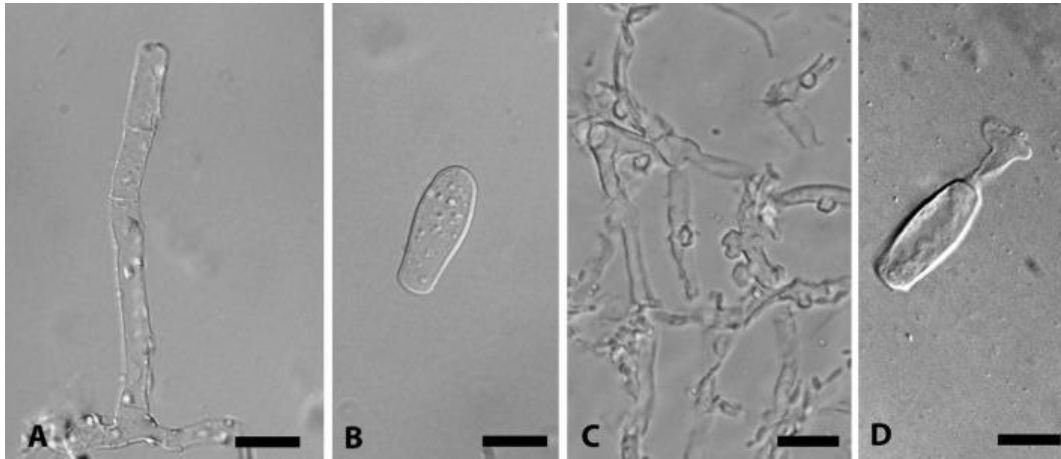Gene sequences (holotype): LC060723 (ITS+28S).

Fig.4– *Erysiphe sidae* ex *Sida rhombifolia*. A: Conidiophore. B: Conidium. C: Hyphal appressoria. D: Germ tube. *Bars*: 20 µm.

Colonies hypophyllous, forming irregular patches, white, sub-evanescent. Hyphae branched, septate, hyaline, almost straight to somewhat wavy, 2–6 µm wide. Hyphal appressoria well-developed, nipple-shape to lobed (multilobed), single or occasionally in pairs. Conidiophores erect, (70–)75–113(–136) ×4.5–8(–10) µm, arising from the upper part of mother cells, centrally. Foot-cells straight, cylindrical,33–69×4.5–10 µm, followed by 1–2 shorter cells. Conidia produced solitarily, ellipsoid to ovoid, somewhat doliiform, 30–37(–43) ×10–16(–20), without conspicuous fibrosin bodies. Sexual morph not found.

Additional specimens examined: On *Sida rhombifolia* (Malvaceae), INDONESIA, West Java, Bandung, Situ Lembang, S 06°47′02.3″ E 107°34′46.2″, 13 Mar 2011,S.A.S. Siahaan, I. Hidayat, J. Meeboon,K. Kramadibrata and S. Takamatsu, BO 22684, MUMH 5146, GenBank accession number: LC060724 (ITS+28S); Indonesia, West Java, Bandung, Rawa Upas, S 07°08′52.2″ E 107°30′56.8″, 14 Sep 2013, S.A.S. Siahaan, I. Hidayat, J. Meeboon,K. Kramadibrata and S. Takamatsu, BO 22714, MUMH 5688, GenBank accession number: LC060725 (ITS+28S).
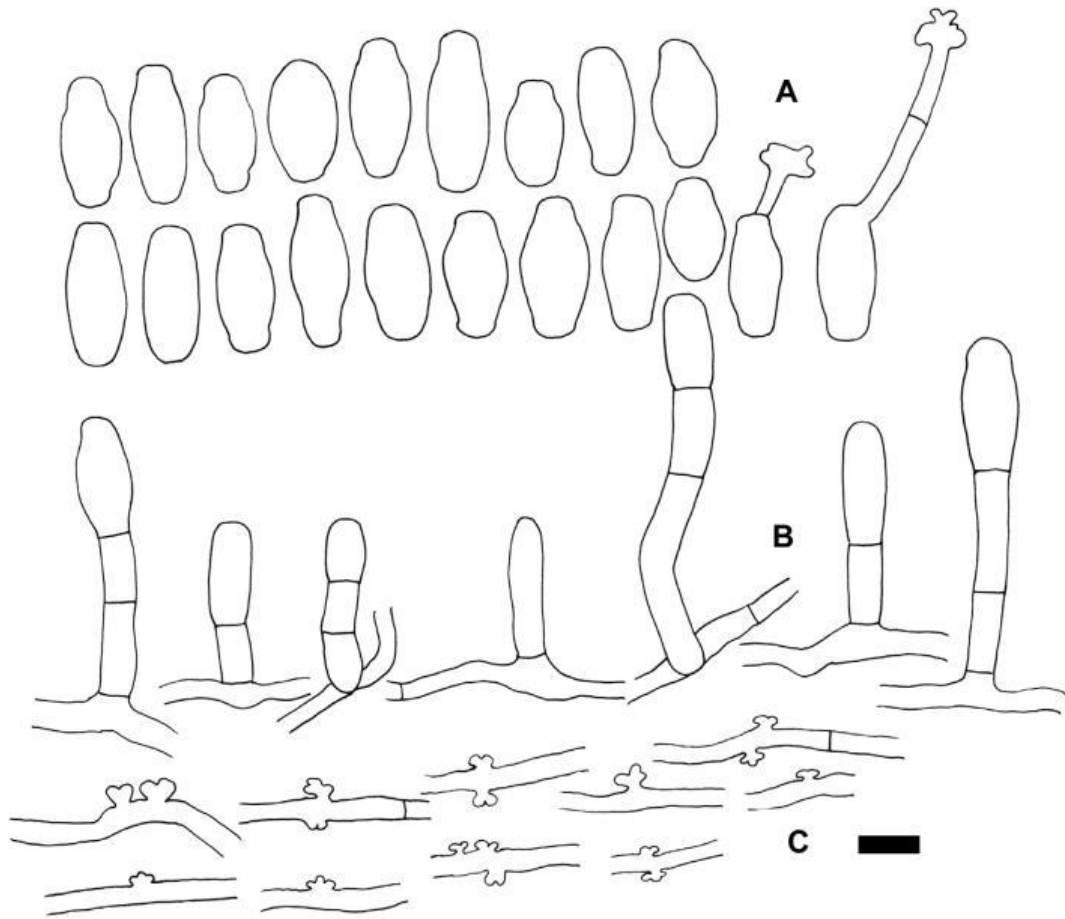
Fig.5– *Erysiphe sidae* ex *Sida rhombifolia* (MUMH 5126) A: Conidia and conidial germ tubes. B: Conidiophores. C: Hyphal appressoria. *Bars*: 15 μm.

Notes: *Sida rhombifolia* is a perennial or sometimes annual plant in the family Malvaceae, native to the New World tropics and subtropics, and now widely distributed in tropical and subtropical regions including Southeast Asia (Dzoyem et al. 2010). *Oidium* sp. has been recorded on this plant species from Central and South America, and New Guinea (Farr and Rossman 2015), but its morphology is unknown. This plant species was also listed as a host of *Pseudoidium pavoniae* (Bagyan. & U. Braun) U. Braun & R.T.A. Cook (Bagyanarayana and Braun 1986; Braun and Cook 2012). We thus conducted molecular and morphological comparisons of the holotype of *Ps. Pavoniae* on *Pavonia*

zeylanica Cav. with the present collections. Unfortunately, specimens of *Ps. Pavoniae* on *S. rhombifolia* were not available. Although DNA sequencing of the type material failed, we were able to observe the anamorph. The present fungus is similar to *Ps. pavoniae*, but differs in having shorter foot-cells of conidiophores and deviates in the conidial shape. The molecular phylogenetic analysis indicated that *E. sidae* is placed in the clade of the genus *Erysiphe*. However, due to the unusual long-branch leading to the *E. sidae* clade, the exact phylogenetic position of this species within *Erysiphe* remains unclear. Blast search result by the ITS sequence also suggests an isolated position of this species.

Disclosure

The authors declare no conflicts of interest. All experiments undertaken in this study comply with the current laws of the country where they were performed.

Acknowledgement

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Chapter 4

***Erysiphe aucubae* sp. nov., a new powdery mildew species on Aucuba japonica from Japan.**

Siska A.S. Siahaan, Takamatsu S.

Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan

Abstract

Aucuba japonica (Japanese aucuba), native to Japan, is an evergreen shrub distributed in the Japanese Archipelago and cultivated worldwide as a garden plant. A powdery mildew with *Pseudoidium*-type asexual morph commonly occurs onthis species, but because of lacking sexual stage, taxonomic placement of this fungus has long been unclear. A new species, *Erysipheaucubae,* is proposed for this fungus with molecular phylogeny and morphological descriptions of asexual morph.

Key words: Anamorph, Erysiphaceae, Molecular phylogeny, Morphology, *Pseudoidium*

1. Introduction

The genus *Aucuba* (Garryaceae) consists of species that are evergreen shrubs distributed in the Himalayas, southern China and Japan. *Aucuba japonica* Thunb. (Japanese aucuba), native to Japan, is distributed from Miyagi prefecture and westward of the main island, Shikoku, Kyushu, and Ryukyu islands of the Japanese Archipelago. This species was introduced into England in 1783 and cultivated worldwide as a garden plant ever since. A powdery mildew with *Pseudoidium*-type asexual morph commonly occurs on *A. japonica* var. *japonica* and var. *borealis* in Japan, but because of the absence of sexual morph (chasmothecia) the taxonomic identity of this fungus has been unclear for a long time (Nomura et al. 1976;Amano 1986; Sato and Eto 2014). Although Amano (1986) recorded *Microsphaera* sp. (present name *Erysiphe* sect. *Microsphaera*) on *A. japonica* var. *japonica* and var. *borealis*, this affiliation was based on asexual morph and host plant, and thus should be re-examined. Molecular phylogenetic analysis and morphological observations revealed that this fungus is an undescribed species belonging to the genus *Erysiphe*. *Erysiphe aucubae* S. Takam. & Siahaan is proposed for this fungus with morphological descriptions of asexual morph in this study.

2. Materials and methods

Morphological examinations and DNA sequencing were conducted according to the procedure described by Meeboon and Takamatsu (2015). The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and internal transcribed spacer (ITS) regions including the 5.8S rRNA gene were determined in this study. New sequences determined were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC121919–LC121922. These sequences were aligned with closely related sequences of *Erysiphe* spp. retrieved from DNA databases using MUSCLE (Edgar

2004) implemented in the MEGA 6 program (Tamura et al. 2013). Alignments were further manually refined and deposited in TreeBASE (http://www.treebase.org/) under the accession number S18825. Phylogenetic trees were obtained from the data using maximum parsimony (MP) and maximum likelihood (ML) methods as described in Meeboon and Takamatsu (2014). Gaps were treated as the 5th character in the MP analysis.

3. **Results and discussion**

Erysiphe aucubae S. Takam. & Siahaan, sp. nov.   Fig.1.

MycoBank no.: MB815276

Similar to asexual morphs of *Erysiphe alphitoides* (Griff. & Maubl.) U. Braun & S. Takam., but genetically different and distinguished in having longer conidia with l/w ratio higher than 2.0, and *A. japonica* as host, belonging to the Garryaceae.

Type: On *Aucuba japonica* var. *japonica* (Garryaceae), JAPAN, Shiga Prefecture, Maibara-shi, Ikesita, Green Park Santo, 35°22′28.93″N 136°21′37.47″E, 14 Oct 2015, S. Takamatsu and S.A.S. Siahaan, TNS-F-65454 (holotype), MUMH 6474 (isotypes), HAL 2984 F (isotypes).

Etymology: Epithet derived from the genus name of the host plant, *Aucuba*.

Gene sequences (holotype): LC121921 (ITS+28S).

Mycelium hypophyllous, effuse, persistent, forming irregular white patches; hyphae substraight to somewhat wavy, 4–7 μm wide; hyphal appressoria solitary or in opposite pairs, lobed; conidiophores on top of mother cell, erect, 61–94 μm long; foot-cells cylindrical, straight or somewhat curved at the base, 21–40 × 7–10 μm, followed by 1–2 mostly shorter cells, forming conidia singly; conidia ellipsoid-ovoid, subcylindrical, 39–53 × 15–20 μm (l/w = 2.1–3.1), producing germ tubes on shoulder, germ tubes terminating in multilobed appressoria; chasmothecia not found.

Additional specimens examined: On *Aucuba japonica* var. *japonica* (Garryaceae), Japan, Nara Prefecture, Uda-shi, Mt. Kuroso, 30 Oct 1994, S. Takamatsu, MUMH 57, GenBank accession number: LC009911 (ITS+28S); Shiga Pref., Hikone-shi, Mt.

Sawayama, 1 Oct 1997, S. Takamatsu, MUMH 392; Mie Pref., Tsu-shi, Mt. Kyogamine, 20 Jun 1999, S. Takamatsu, MUMH 832; Mie Pref., Inabe-shi, Mt. Fujiwara, 15 Oct 2002, S. Takamatsu, MUMH 2121; Shiga Pref., Maibara-shi, Mt. Ibuki, 2 Nov 2003, S. Takamatsu, MUMH 2726; 6 Nov 2004, S. Takamatsu, MUMH 3660; Nara Pref., Sakurai-shi, Mt. Torimi, 11 Nov 2007, S. Takamatsu, MUMH 4814; Nara Pref., Gose-shi, Takamahiko Jinja, 25 Nov 2007, S. Takamatsu, MUMH 4846; Shiga Pref., Maibara-shi, Ikesita, Green Park Santo, 35°22′27.20″N 136°21′32.46″E, 14 Oct 2015, S. Takamatsu and S.A.S. Siahaan, MUMH 6468, HAL 2978 F; 35°22′29.00″N 136°21′34.74″E, MUMH 6469, HAL 2979 F, GenBank accession number: LC121919 (ITS+28S); MUMH 6470, HAL 2980 F; MUMH 6471, HAL 2981 F; MUMH 6472 HAL 2982 F, GenBank accession number: LC121920 (ITS+28S); MUMH 6473, HAL 2983 F; 35°22′28.93″N 136°21′37.47″E, MUMH 6475, HAL 2985 F, GenBank accession number: LC121922 (ITS+28S); On *A. japonica* var. *borealis* Miyabe & Kudô (Garryaceae), Japan, Fukui Prefecture, Nanjo-cho, Mt. Somayama, Sep 1996, S. Takamatsu, MUMH 132.

Host range and distribution: On *Aucuba japonica* var. *japonica*, *A. japonica* var. *borealis* (Japan, endemic).


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Fig.1 –*Erysiphe aucubae* ex *Aucuba japonica*. A: Leaves of the host plant affected by the fungus. B: Germ tube. C: Hyphal appressoria. D: Conidia. E, F: Conidiophores. *Bars*: 20 μm.

Note: The ITS and 28S rRNA gene nucleotide sequences obtained from four specimens of *E. aucubae* were determined in this study to clarify the phylogenetic placement of this species. These sequences were aligned with a sequence of this species and 22 sequences from closely related species reported in Takamatsu et al. (2015). The 5'-end of the 28S rRNA gene (692 bp) was identical among the five *E. aucubae* specimens and there were only two base variations in the ITS region. The combination data set of ITS and 28S rRNA gene sequences consisted of 27 sequences and 1251 characters, of which 29 (2.3%) characters were variable and 20 (1.6%) characters were informative for parsimony analysis. Three sequences from *E. hypophylla* (Nevod.) U. Braun & Cunnington were used as outgroup based on Takamatsu et al. (2015). Two equally parsimonious trees with 32 steps were constructed by the MP analysis. One of the trees with higher likelihood value is shown in Fig. 2. ML analysis generated a tree topology almost identical to the MP tree. Thus, only BS values of ML analysis were shown on Fig. 2. The five sequences of *E. aucubae* formed a monophyletic group with strong bootstrap (BS) supports (MP = 98%; ML = 100%). There were 5–7 base differences from the sequences of *E. alphitoides*.

The present phylogenetic analysis as well as the previous report (Takamatsu et al. 2015) revealed that *E. aucubae* is closely related to *E. alphitoides* occurring on deciduous *Quercus* species. Both species share conidia formed singly, conidiophores with straight or sometimes curved foot cells, and conidial germ tubes with complicated lobed appressoria. On the other hand, conidia of *E. aucubae* is distinctly longer than those of *E. alphitoides*, and l/w ratio is 2.1–3.1 in the former species and less than 2.0 in the latter species. Thus, besides clear phylogenetic differences, *E. aucubae* is also distinguished from *E. alphitoides* based on morphology of conidia. The phylogenetically confirmed position of this asexual morph in the *Erysiphe* clade allows the application of ICN

(International Code of Nomenclature for algae, fungi, and plants), Art. 59.1, i.e. the new species, although its sexual morph is still unknown, is assignable to *Erysiphe* which has priority over the anamorph-typified genus *Pseudoidium*. The latter genus is now a heterotypic synonym of *Erysiphe*.
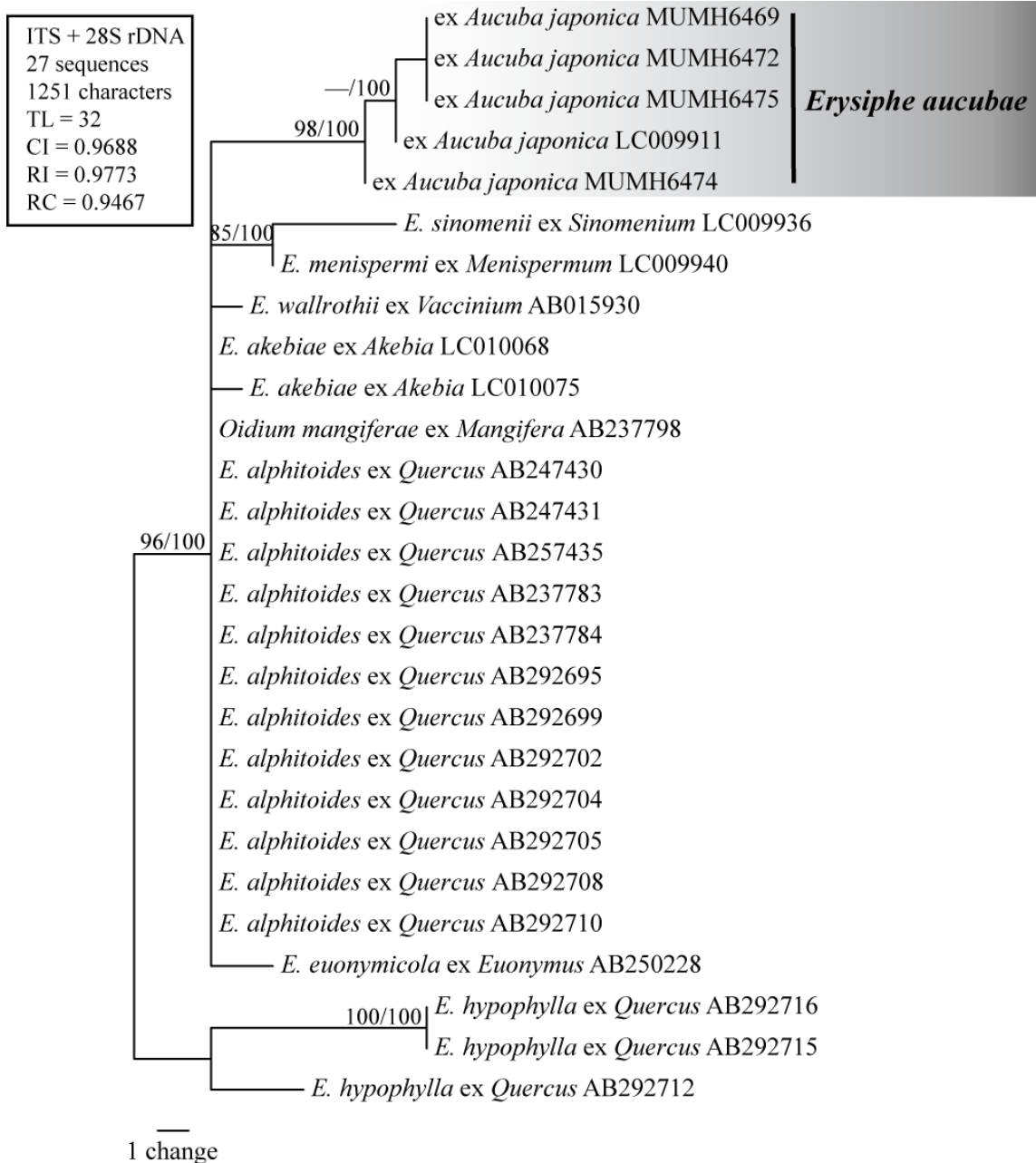
Fig. 2 – Phylogenetic analysis of combined data of the divergent domains D1 and D2 sequences of the 28S rRNA gene and ITS region for 27 sequences from the genus *Erysiphe*. This tree is a phylogram of one of the two equally parsimonious trees with 32 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS (>70%) values with 1000 replicates were shown on the respective branch.

Takamatsu et al. (2015) found a homogenous clade (*E. alphitoides* clade) consisting of *E. alphitoides* and several *Erysiphe* species occurring on a wide range of plant families. This clade includes *E. akebiae* (Sawada) U. Braun & S. Takam. On *Akebia* spp. (Lardizabalaceae), *E. euonymicola* U. Braun on *Euonymus* spp. (Celastraceae), *E. menispermi* (Howe) U. Braun & S. Takam. on *Menispermum* spp. (Menispermaceae), *E. pseudolonicerae* (E.S. Salmon) U. Braun & S. Takam. on *Cocculus* spp. (Menispermaceae), *E. sinomenii* (Howe) U. Braun & S. Takam.on *Sinomenium* spp. (Menispermaceae), and *E. wallrothi* (U. Braun & Tanda) U. Braun & S. Takam. On *Vaccinium* spp. (Ericaceae) as well as *E. aucubae* (Takamatsu et al. 2015). Powdery mildews on *Paeonia* spp. (Paeoniaceae), *Wisteria sinensis* (Sims) Sweet (Fabaceae), *Sorbaria sorbifolia* (L.) A. Braun (Rosaceae) also have ITS sequences identical to *E. alphitoides* (Takamatsu et al. 2006; Henricot and Cook 2008; Denton et al. 2013; Cook et al. 2015). "*Oidium mangiferae*" occurring on *Mangifera indica* L. (mango), a tropical fruit tree, is divided into two genetic groups (Limkaisang et al. 2006). One group has rRNA gene sequence identical to *E. quercicola* S. Takam. & U. Braun, another powdery mildew species occurring on deciduous oaks. The second group that were collected from Australia has a sequence identical to *E. alphitoides.* Pairwise sequence similarities of ITS region were higher than 99% in the "*E. alphitoides* clade". Because of obligate biotrophic nature, host relationships of powdery mildews have long been considered as conservative, that is, host range of a single powdery mildews have long been considered as conservative, that is, the host range of a single powdery mildew species has been supposed to be restricted in a single family, closely related genera or even a species (reviewed in Braun 1987). However, recent molecular phylogenetic analyses revealed that there are several homogenous groups consisting of many powdery mildew species occurring on a wide range of host plants (Khodaparast et al. 2001; Voytyuk et al. 2009; Ito and Takamatsu 2010; Takamatsu et al. 2013, 2015). The "*E. alphitoides* clade" is one of the groups. Deciduous *Quercus* species are a major component of temperate forests worldwide, on which *E. alphitoides* commonly occurs. The other host plants including in the "*E.

alphitoides clade" are also components in temperate forests. This kind of niche overlapping might have increasedfrequency of attacks by spores of *E. alphitoides* and triggered host expansion.

Acknowledgments

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Chapter 5

Bauhinia purpurea, Durio zibethinus, **and** *Nephelium lappaceum*: **Additional hosts of the asexual morph of** *Erysiphe quercicola.*

Siska A.S. Siahaan[1], Kramadibrata K[2], Hidayat I[2,], Meeboon J[1], and Takamatsu S[1].

[1] Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan

[2] Research Centre for Biology, Indonesian Institute of Sciences-LIPI, Jl. Raya Jakarta-Bogor KM 46, Cibinong 16911 West Java, Indonesia

Abstract

Because of the obligate biotrophic nature, host relationships of powdery mildews have long been considered as conservative. Especially, no tree-parasitic powdery mildew species having wide host ranges have been known so far. However, previous reports revealed that powdery mildews occurring on eight genera of tropical fruits trees and other woody plants covering five families belong to a single species, *Erysiphe quercicola*. The authors found in Indonesia three additional tropical trees infected by *E. quercicola*, viz. *Bauhinia purpurea*, *Durio zibethinus*, and *Nephelium lappaceum*, which are cultivated widely in tropical regions as fruit trees, flower trees, or industrial crops. This result suggests that these tree species play important roles as alternative hosts for powdery mildew epidemics caused by *E. quercicola* in tropical regions. Investigations and surveys

of the exact host range of *E. quercicola* are important for the management of this powdery mildew.

Key words: Erysiphaceae, Host range, Molecular phylogeny, Powdery mildew, *Quercus phillyreoides*

1. Introduction

Powdery mildews are a fungal group belonging to the Erysiphaceae (Ascomycota: Erysiphales), all of which are exclusively obligate biotrophs of plants. Their host range reaches ca 10,000 species of angiosperms, but there are no records of any gymnospermes and ferns as hosts. Because of the obligate biotrophic nature, host relationships of powdery mildews have long been considered as conservative, that is, the host range of a single powdery mildew species has been supposed to be restricted to a single family, closely related genera or even a single species (reviewed in Braun 1987). However, inoculation tests revealed that there are some powdery mildew species having wide host ranges. For example, Hammarlund (1945) and Blumer (1952) reported that *Erysiphe polyphaga* Hammarl. [present name: *Golovinomyces orontii* (Castagne) V.P. Heluta] infects ca 100 plant species covering a wide range of families. *Leveillula taurica* (Lév.) G. Arnaud and *Sphaerotheca fuliginea* (Schltdl.) Pollacci [present name: *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff] also have wide host ranges (Abiko 1978, 1982a, 1982b; Palti 1988). Most of these inoculation tests were supported by the molecular phylogenetic analyses conducted subsequently (Khodaparast et al. 2001; Voytyuk et al. 2009; Ito and Takamatsu 2010; Takamatsu et al. 2013). All three species are herb-parasitic having a relatively recent origin (Takamatsu 2013). On the other hand, species parasitic on woody plants, generally having an older origin compared with herb parasitic species, have mostly narrow host ranges. For example, *Erysiphe* (section *Uncinula*) on *Carpinus* spp. was previously regarded as a single species, *E. carpinicola* (Hara) U. Braun & S. Takam., but has been separated into three species along with host specialization based on molecular and morphological analyses (Braun et al. 2006). A total of seven *Erysiphe*

species have been so far described on *Carpinus* (Takamatsu et al. 2008b; Braun and Cook 2012; Meeboon and Takamatsu 2013). *Phyllactinia*, a genus of the Erysiphaceae, is exclusively a pathogen of woody plants, and *Ph. guttata* (Wallr. : Fr.) Lév. has been regarded as a widespread species with very wide host range (Braun 1987). Molecular phylogenetic analyses revealed that this species represents a complex composed of numerous species with much narrower host and distribution ranges (Takamatsu et al. 2008a) and was divided into various species confined to certain host families or genera (Braun and Cook 2012). No tree-parasitic powdery mildew species having wide host ranges previously known.

Limkaisang et al. (2005) first reported that *Oidium heveae* B.A. Steinm. on para rubber tree [*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (Euphorbiaceae)] has an internal transcribed spacer (ITS) sequence of rRNA gene identical to that of *Erysiphe* sp. on *Quercus phillyreoides* A. Gray (Fagaceae). Para rubber tree is cultivated in tropical areas worldwide as the most important source of natural rubber for the manufacture of rubber products and latex coagulates, whereas *Q. phillyreoides* is an evergreen oak distributed in Japan and warm-temperate region of China. Thus, the distributions of para rubber tree may not be overlapped with *Q. phillyreoides.* It was very curious that two powdery mildew species occurring on distantly related plants with no niche overlapping have identical ITS sequences. Limkaisang et al. (2006) further reported that *Oidium mangiferae* Berthet on mango (*Mangifera indica* L., Anacardiaceae), *O. anacardii* F. Noack on cashew nuts (*Anacardium occidentale* L., Anacardiaceae), *O. bixae* Viégas on *Bixa orellana* L. (Bixaceae), *O. citri* (J.M. Yen) U. Braun on *Citrus* spp. (Rutaceae), and *Oidium* sp. on *Acacia* spp. (Fabaceae) also have ITS sequences identical or very similar to that of *Erysiphe* sp. on *Q. phillyreoides*, which was later classified as *E. quercicola* S. Takam. & U. Braun in Takamatsu et al. (2007). Although the powdery mildews on these tropical fruit trees and other woody plants have been classified as separate species by their hosts, they were re-classified as asexual morph of *E. quercicola* by Takamatsu et al. (2007). In addition, powdery mildews on *Cinnamomum camphora* (L.) J. Presl.

(Lauraceae), *Murrata paniculata* (L.) Jack (Rutaceae), and *Citrus reticulata* Blanco (Rutaceae) were also reported as asexual morph of *E. quercicola* (Kirschner and Liu 2014; Baiswar et al. 2015). In total, powdery mildews occurring on eight genera of tropical fruit trees and other woody plants covering five angiosperm families belong to the asexual morph of *E. quercicola*. Because surveys on the biodiversity of powdery mildews in tropical regions are still far from being adequate, additional hosts of the asexual morph of *E. quercicola* are expected to be found in the course of further surveys of tropical regions.

The authors have investigated the diversity of powdery mildews in Indonesia since 2011 and found three additional tropical trees infected by the asexual morph of *E. quercicola*. Detailed morphological descriptions and molecular analyses of these fungi are presented in this report.

2. Materials and methods

2.1. Molecular phylogeny

The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and ITS regions were determined according to the procedure described by Meeboon and Takamatsu (2014). Representative new sequences retrieved in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC128423–LC128429. New sequences were aligned with other sequences of the Erysiphaceae using MUSCLE (Edgar 2004) implemented in MEGA 6 (Tamura et al. 2013). Alignments were further manually refined using the MEGA 6 program and were deposited in TreeBASE (http://www.treebase.org/) under the accession number S18959. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) method in PAUP 4.0a167 (Swofford 2002) with heuristic search option using 'tree bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analyses using 1000

replications with step-wise addition option set as simple (Felsenstein 1985). BS value higher than 70% were given. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated.

2.2. Morphology

Morphological examinations were carried out as outlined in Meeboon and Takamatsu (2015). All the specimens were examined using a light microscope with phase contrast 10×, 20× and 40× objectives. Thirty conidiophores and conidia were measured per sample. The size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of conidiophores, e.g. size and shape of foot-cells, position of basal septa, shape and position of hyphal appressoria; position of germ tubes of conidia; and shape of appressoria on germ tubes of conidia (if found) were documented. For dried conidia in herbarium samples, conidia width was multiplied by the factor 1.2 and the length by 1.15 according to Braun and Cook (2012). Specimens were deposited at the Mie University Mycological Herbarium (MUMH), Japan, and Herbarium Bogoriense (BO), Indonesia.

3. Results

3.1. Molecular phylogenetic study

ITS and 28S rRNA gene (D1/D2 region) sequences were determine for the samples collected in this study. The combined data set of ITS and 28S rRNA gene sequences consisted of 33 sequences and 1324 characters, of which 30 (2.2%) characters were variable and 24 (1.8%) characters were informative for parsimony analysis (Supplementary table S1). Two sequences of *E. alphitoides* (Griff. & Maubl.) U. Braun & S. Takam. and one sequence of *E. euonymicola* U. Braun were used as outgroup based on Limkaisang et al. (2006). A total of 91 equally parsimonious trees with 32 steps were constructed by MP analysis. Tree topologies were almost consistent among the trees, but

the placements of the two sequences from the samples on *Cin. camphora* (KC857653) and *Mu. paniculata* (KC85765) reported by Kirschner and Liu (2014) were changeable due to lacking 28S rRNA gene sequence. One of the trees is shown in Fig. 1. The ingroup was divided into 3 subgroups, in which they had only 1 base difference. The grouping of the two sequences from the samples on *Cin. camphora* (KC857653) and *Mu. paniculata* (KC85765) was uncertain. All the new sequences obtained in this study were placed in subgroup I. The sequences from the fungi on *Bauhinia purpurea* L. and *Nephelium lappaceum* L. were identical to *E. quercicola* on *Q. phillyreoides*. Two sequences from the fungus on *Durio zibethinus* Murray differed in three bases from those of *B. purpurea*
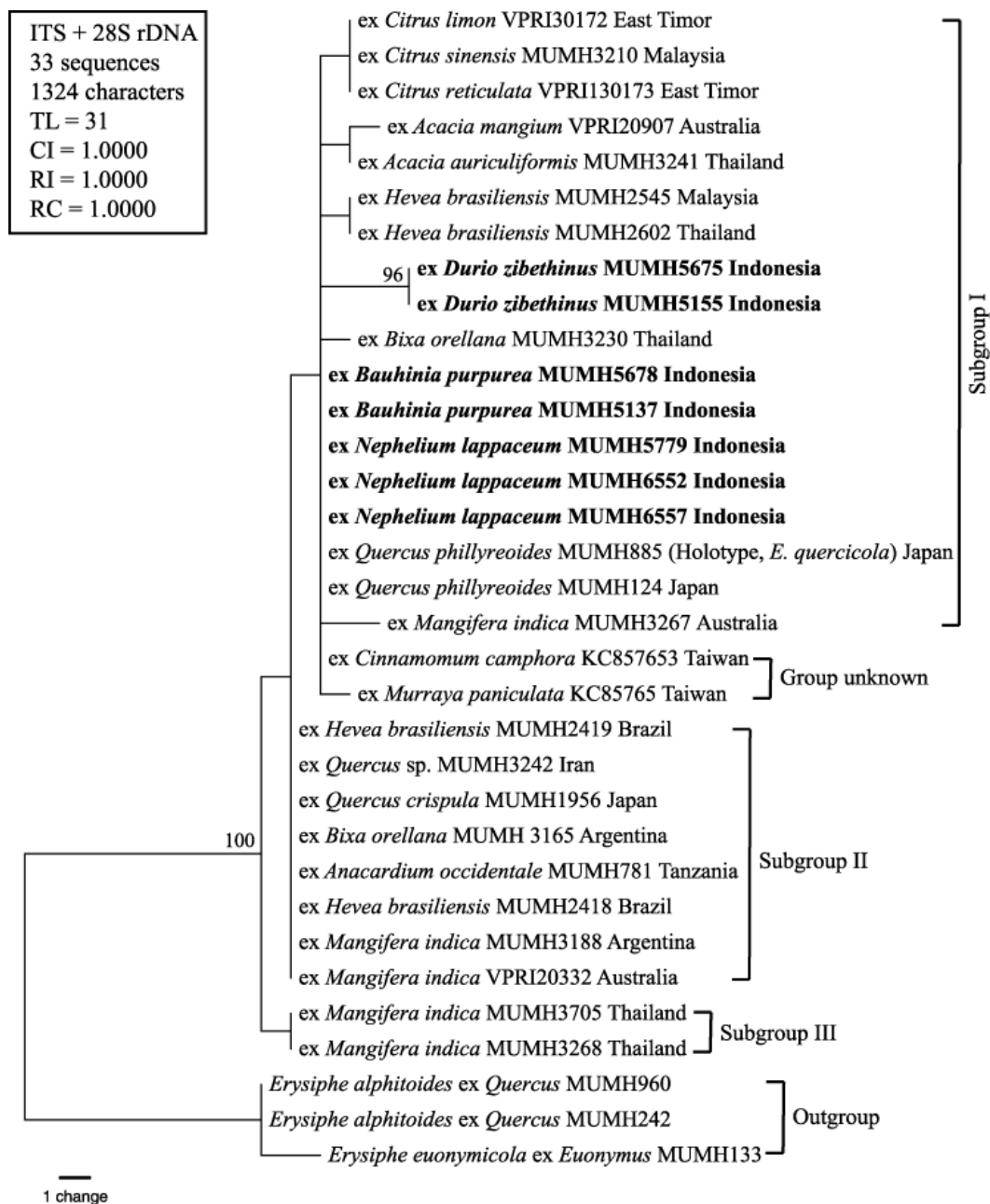
Fig. 1– Phylogenetic analysis of combined data of the divergent domains D1 and D2 sequences of the 28S rRNA gene and ITS region for 30 sequences from *Erysiphe quercicola* and 3 outgroup sequences. This tree is a phylogram of one of the 91 equally parsimonious trees with 32 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS (>70%) values with 1000 replicates were shown on the respective branch. Sequences determined in this study were shown in boldface.

and *N. lappaceum*, and formed a clade with strong bootstrap support (96%).

3.2. Morphological study

Erysiphe quercicola S. Takam. & U. Braun, in Takamatsu et al., Mycol. Res. 111: 819 (2007)

= *Oidium bauhiniae* G.J.M. Gorter & Eicker, Mycotaxon 22:39 (1985).

≡ *Pseudoidium bauhiniae* (G.J.M. Gorter & Eicker) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiphales (Powdery Mildews) (2012): 598.

= *Oidium nephelii* Hadiw. ex U. Braun, Mycotaxon 25: 267 (1986).

≡ *Pseudoidium nephelii* (Hadiw. ex U. Braun) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiphales (Powdery Mildews) (2012): 613.

= *Oidium nephelii* Hadiw., Landbouw (Landbouwkundig maandblad voor Indonesié) 22(5–6): 253 (1950)

≡ *Oidium erysiphoides* f. *nephelii* (Hadiw.) J.M. Yen, Rev. Mycol. 31(4): 286 (1966).

Asexual morph of *Erysiphe quercicola* on *Bauhinia purpurea*.   Fig. 2.

Specimens: On living leaves of *Bauhinia purpurea* (Fabaceae), INDONESIA, Bogor, Botani Square, 06°36'12.8"S 106°48'22.3″E, 11 Sep 2013, S.A.S. Siahaan and J. Meeboon, BO 22704, MUMH 5678. GenBank accession number: LC128425 (ITS + 28S); West Bandung, Parongpong, 06°48'24.7"S 107°35'20.2″E, 13 Mar 2011, S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon and S. Takamatsu, MUMH5137, GenBank accession number: LC128426 (ITS + 28S); Bogor, Botani Square, 06°36'12.8"S 106°48'22.3″E, 14 Mar 2011, S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon

and S. Takamatsu, MUMH 5154.

Notes: The genus *Bauhinia* comprises trees and shrubs that grow in warm climate. *Bauhinia purpurea* is native to South China including Hongkong and Southeast Asia, and it is now often planted in gardens along roadside for its large purple beat flowers (Daksha et al. 2012). Two species of *Pseudoidium* spp., i.e. *Ps. bauhiniae* (G.J.M. Gorter & Eicker) U Braun & R.T.A. Cook and *Ps. Caesalpiniacearum* (Hosag. & U. Braun) U. Braun & R.T.A. Cook, were recorded on *B. purpurea* (Braun and Cook 2012). *Pseudoidium caesalpinacearum* differs from *Ps. bauhiniae* by having nipple shaped rather than multilobed hyphal appressoria. The asexual morph of the fungus on *B. purpurea* is in good agreement with the morphology of *Ps. bauhiniae*. However, the latter species was described from South Africa on *B. galpinii* N.E.Br. Therefore, this species can only tentatively be considered a synonym of *E. quercicola*. Sequences based on South African material are required to confirm the identity, above all since legumes are hosts of a wide range of powdery mildews with similar asexual morphs.
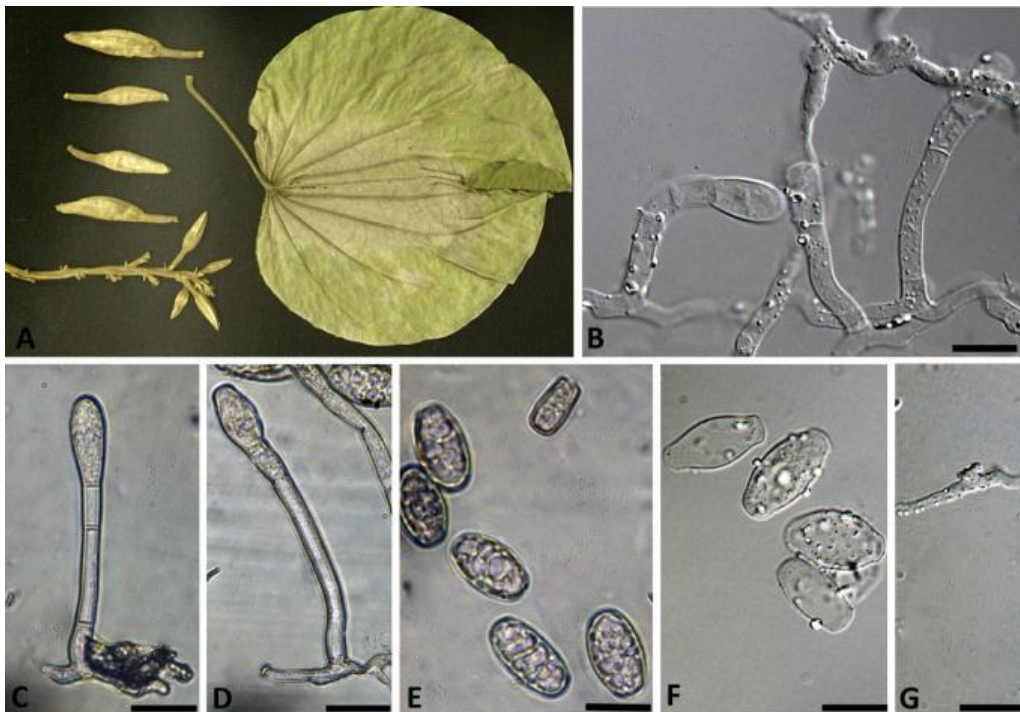
Fig. 2–Asexual morph of *Erysiphe quercicola* on *Bauhinia purpurea*. A: Symptoms. B–D: Conidiophores. E, F: Conidia. G: Hyphal appressorium. *Bars*: 20 μm

Asexual morph of *Erysiphe quercicola* on *Durio zibethinus*   Fig. 3.

Specimens: On living leaves of *Durio zibethinus* (Malvaeae), INDONESIA, Bogor, Curug Cigamea, 06°41'37.9"S 106°40'54.7″E, 11 Sep 2013, S.A.S. Siahaan and J. Meeboon, BO 22701, MUMH 5675. GenBank accession number: LC128423 (ITS + 28S); Palembang, 10 Mar 2011, S.A.S. Siahaan, Suparman, A. Umayah, J. Meeboon and S. Takamatsu, MUMH 5155, GenBank accession number: LC128424 (ITS + 28S).

Colonies on leaves epiphyllous, especially along the veins, effuse and persistent. In addition, fungal colonies were also noticeable on the fruit surface, located among the sidelines of thorns. Hyphae branched, septate, hyaline, 3–6 µm wide. Hyphal appressoria well developed, lobed to multilobe, single or in pairs. Conidiophores arising from the upper part of mother cells, erect, 32–72 × 5–8 µm. Foot-cells straight, sometimes slightly curved, width uniform throughout, 8–30 × 5–8 µm, followed by 1–2 shorter cells. Conidia produced solitary, without fibrosin bodies, ellipsoid-doliiform, 25–41 × 14–20 µm (l/w ratio = 1.7–2.1).
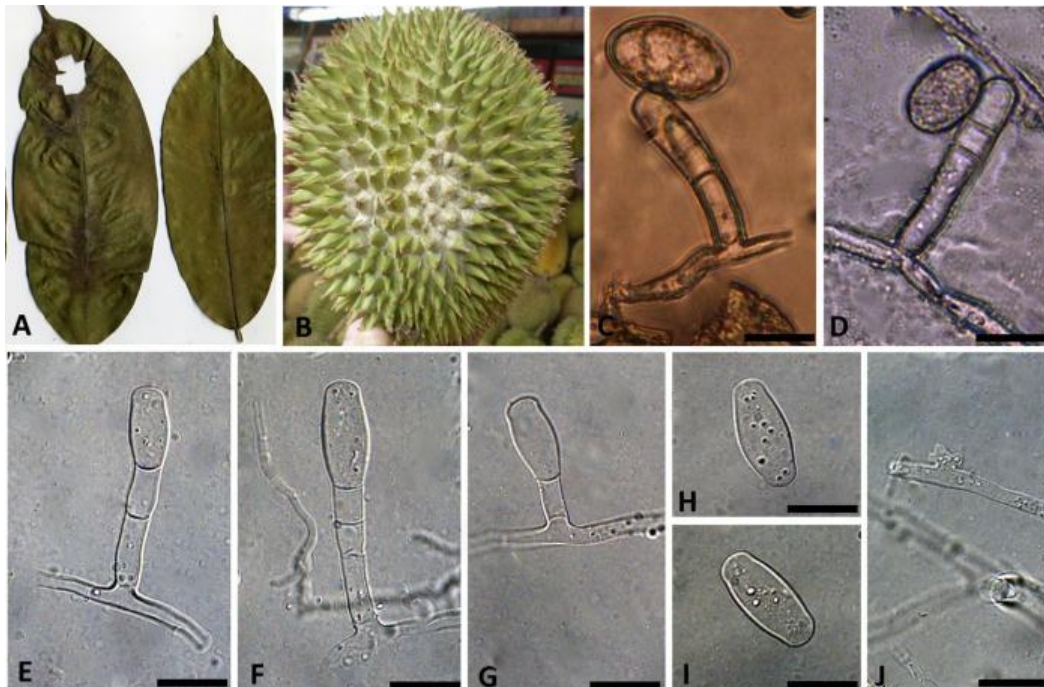
Fig. 3 –Asexual morph of *Erysiphe quercicola* on *Durio zibethinus*. A, B: Symptoms. C–G: Conidiophores. H, I: Conidia. J: Hyphal appressorium. *Bars*: 20 µm

Notes: *Durio zibethinus* (Durian) is a tropical fruit tree, belonging to the family Malvaceae, originating from the Malay Peninsula. It is distinctive for its large size, unique odour and thorn-covered husk and is extensively grown as one of the agriculturally most important economic fruits in the Southeast Asian countries (Vanijajiva 2011). The asexual morph of powdery mildew on *D. zibethinus* and its molecular data are described here in detail for the first time. Two sequences of powdery mildew isolates of *D. zibethinus* are identical to each other, but exhibit three base nucleotide differences in the ITS region from the sequences of the samples on *B. purpurea*, *N. lappaceum*, and *Q. phillyreoides.*

Asexual morph of *Erysiphe quercicola* on *Nephelium lappaceum* Fig.4.

Specimens: On living leaves of *Nephelium lappaceum* (Sapindaceae), INDONESIA, Pematangsiantar, 02°96'63.1"S 99°08'22.3″E, 15 Jan 2015, S.A.S. Siahaan, MUMH 5779. GenBank accession number: LC128427 (ITS + 28S); INDONESIA, Samosir Island, Tuk tuk, 02°40'25"S 98°54'30″E, 5 Jan 2016, S.A.S. Siahaan, MUMH 6552, GenBank accession number: LC128428 (ITS + 28S); INDONESIA, Samosir Island, Tuk tuk, 02°45'08"S 98°43'18″E, 5 Jan 2016, S.A.S. Siahaan, MUMH 6557, GenBank accession number: LC128429 (ITS + 28S).
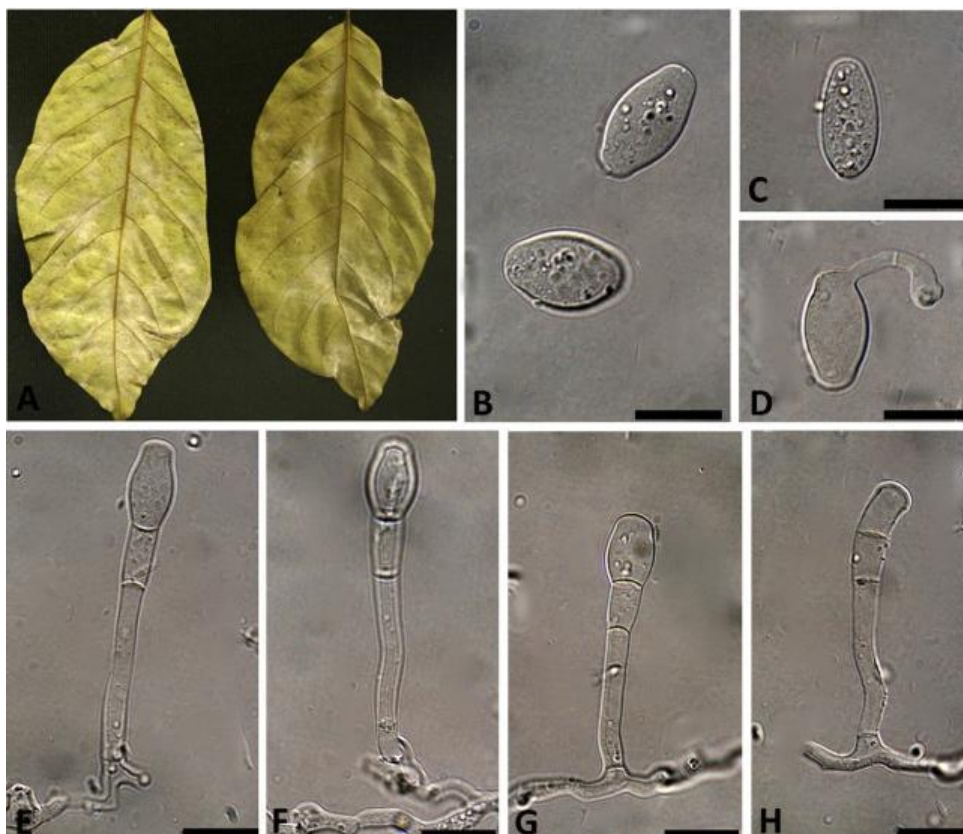
Fig. 4 –Asexual morph of *Erysiphe quercicola* on *Nephelium lappaceum*. A: Symptoms. B, C: Conidia. D: Germ tube. E–H: Conidiophores. *Bars*: 20 µm

Colonies hypophyllous on young leaves, effuse and persistent, causing yellowish discoloration leading to early defoliation of young leaves. Hyphae branched, septate, hyaline, 3–6 µm wide. Hyphal appressoria well developed, lobed to multilobe, single or in pairs. Conidiophores arising from the upper part of mother cells, erect, 48–89 × 4–8 µm. Foot-cells sometimes straight, but usually slightly curved at the base, (10–)22–56 × 3–7 µm, followed by 1(–2) shorter cells. Conidia produced solitary, without fibrosin bodies, ellipsoid-doliiform, 24–37 × 12–21 µm (l/w ratio = 1.7–2.0), germ tubes *Pseudoidium* type.

Notes: *Nephelium lappaceum*, commonly known as rambutan and belonging to the Sapindaceae, is an attractive tropical fruit tree extensively grown and widely distributed in Southeast Asian countries. The fruits are ovoid, with a red or orange-yellowish pericarp covered with soft spines that vary in coloring from green, yellow and red (Minh 2014). There was only one powdery mildew species recorded on *N. lappaceum*, *Ps. nephelii*. The asexual morph of this fungus is morphologically similar to that of *E. quercicola*. Since there was no evidence of teleomorph and molecular sequence, Braun and Cook (2012) preferred to retain the fungus as a separate species from *E. quercicola*, at least tentatively. This study provides not only reliable anamorphic features but also molecular data, showing that the fungus on *N. lappaceum* is an asexual morph of *E. quercicola*. To compare the present fungus with type specimen of *Ps. nephelii*, we tried to obtain the type specimen from Herbarium Bogoriense (BO), Bogor, Indonesia. However, type specimen was not deposited at BO. According to personal communication with Uwe Braun, "The type was in the possession of the original author, and if later not deposited in BO, the holotype is probably not preserved."

4. Discussion

When the world was divided into several regions and the numbers of host species of powdery mildews were counted by the regions, the Northern Hemisphere had the highest number of hosts and those in tropical regions and the Southern Hemisphere were much lower compared to the Northern Hemisphere (Amano 1986). One of the reasons why the host numbers of powdery mildews are lower in tropical regions may be attributed to the small number of mycologists in these areas. Actually, many new powdery mildew species or new hosts have been reported from Southeast Asia based on surveys of powdery mildews combined with molecular analyses (Divarangkoon et al. 2011; Monkhung et al. 2011, 2013; Meeboon et al. 2012a, b, 2013a, b, c; Hidayat et al. 2014; Siahaan et al. 2015, 2016). However, despite taking this factor into account, the Northern Hemisphere may still have the highest number of hosts in the world. Takamatsu (2013) thought that the first divergence of powdery mildews occurred in the early Paleogen Period in high latitude areas of the Northern Hemisphere. Subsequently climatic deterioration occurred, involving a decrease in world temperature, which may have caused migration of powdery mildews and their hosts southward and triggered further speciation. Therefore, ancestors of powdery mildews distributed in tropical regions might have migrated from northern parts and adapted to tropical environments. *Erysiphe quercicola* produces chasmothecia (the sexual fruiting bodies of powdery mildews) on *Q. phillyreoides* in Japan, but chasmothecia of this species have never been found on hosts of tropical fruit trees and other woody plants. In addition, five *Erysiphe* species (including *E. quercicola*) on Asian *Quercus* species (*E. alphitoides* s. lat.) form a monophyletic group, suggesting that these species diverged on *Quercus* species (Takamatsu et al. 2015). These evidences suggest that *Q. phillyreoides* may be the original host of this species, from which this fungus expanded its host range to tropical tree species.

Although the *E. quercicola* clade was strongly supported (100%) by BS analysis, there are some sequence variations within the clade, i.e. six base substitutions (99.5%

similarity) in maximum. The *E. quercicola* clade was divided into three subgroups each with one base substition (Fig. 1). Subgroups I and III were occupied by samples collected in Southeast Asia, excepting for two sample from Australia and two samples on *Q. phillyreoides* (collected in Japan). On the other hand, subgroup II consisted of samples collected in South America, Africa and Australia excepting for two samples on *Quercus* spp. The placement of the two samples reported from Taiwan was uncertain due to lacking 28S rRNA gene sequence. The single base substitution between subgroups I and II is located in the D1/D2 region of the 28S rRNA gene, i.e. "C" in subgroup II and "G" in subgroup I. Because it was "C" in the outgroup taxa, subgroup II might be ancestral and the substitution from "C" to "G" occurred in the common ancestor of the subgroup I (Southeast Asia group). However, this sequence variation was also found among samples on *Quercus* species. Thus, an alternative and more likely interpretation might be that *E. quercicola* on *Quercus* already had this variation and only a partial haplotype of the mother population was inherited to tropical fruit trees and other woody plants in South America and Southeast Asia, separately.

Boesewinkel (1980) was the first author who pointed out the similarity between *Erysiphe* species on *Quercus* and powdery mildews on tropical fruit trees. He conducted cross-inoculation test between powdery mildews on *Quercus robur* L. and mango to show that these fungi can infect each other. This result was later confirmed by Takushi et al. (2014). In addition, *O. heveae* can infect *Jatropha curcas* L. (Ramakrishnan and Radhakrishna Pillay 1963), *B. orellana* (Thankamma 1968), *Urena lobata* L., and *Alchornea davidii* Franch. (Zhuotong et al. 1996), suggesting a polyphagous nature of this species. However, all of these reports are only partial experiments. Comprehensive cross-inoculation tests are urgently required to investigate the exact host range of this species. Especially, because samples on *Durio zibethinus*, *Citrus* spp., *Acacia* spp., and *H. brasiliensis* form each separate clade with 1–3 base substitutions, it is possible that these fungi have already specialized in narrower host ranges, although the question arises whether potential specializations already exceeded beyond the level of formae speciales.

This as well as previous reports (Limkansang et al. 2005, 2006; Kirschner and Liu 2014; Baiswar et al. 2015) revealed that the asexual morph of *E. quercicola* occurs on tropical fruit trees and other woody plants covering 11 genera and seven plant families. Characteristics of asexual morph of the fungi on these hosts were shown in Supplementary Table S2. All these plants are cultivated widely in tropical regions as fruit trees, flower trees, or industrial crops. This result suggests that these plant species play an important role as alternative hosts for powdery mildew epidemics of the powdery mildew concerned in tropical regions. Therefore, the investigation and survey of the exact host range of this species is important for management purposes of powdery mildews in tropical regions. However, because the investigation of the biodiversity of powdery mildews in tropical regions is still far from being adequate, additional host plants of this species will probably be found in future. Further comprehensive investigations are necessary. No tree-parasitic powdery mildew with such a wide host ranges has hitherto been found in temperate regions. Is this kind of polyphagous nature of *E. quercicola* a special case of adaptation to tropical environments? Or, are similar case to be found also in temperate regions? Further investigations are required to address the question.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.myc.2016.06.001.

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Supplementary Table S1. Source of *Erysiphe quercicola* materials and DNA database accession numbers of sequences used in this study

Host plants		Herbarium accession no.[a]	Location	Database accession no.[b]	
Species	Family			ITS	28S
Acacia auriculiformis	Fabaceae	MUMH 3241	Thailand	AB237805	AB237832
Acacia mangium	Fabaceae	VPRI 20907	Australia	AB237808	AB237833
Anacardium occidentale	Anacardiaceae	MUMH 781	Tanzania	AB237786	AB237814
Bauhinia purpurea	Fabaceae	MUMH 5137; BO 22673	Indonesia	LC128426	LC128426
Bauhinia purpurea	Fabaceae	MUMH 5678; BO 22704	Indonesia	LC128425	LC128425
Bixa orellana	Bixaceae	MUMH 3165	Argentina	AB237787	AB237815
Bixa orellana	Bixaceae	MUMH 3230	Thailand	AB237789	AB237816
Cinnamomum camphora	Lauraceae	TNM F0026772	Taiwan	KC857653	—
Citrus limon	Rutaceae	VPRI 30172	East Timor	AB237791	AB237818
Citrus reticulata	Rutaceae	VPRI 130173	East Timor	AB237792	AB237819
Citrus sinensis	Rutaceae	MUMH 3210	Malaysia	AB237793	AB237820
Durio zibethinus	Malvaceae	MUMH 5155	Indonesia	LC128424	LC128424
Durio zibethinus	Malvaceae	MUMH 5675; BO 22701	Indonesia	LC128423	LC128423
Hevea brasiliensis	Euphorbiaceae	MUMH 2418	Brazil	AB193606	AB197133
Hevea brasiliensis	Euphorbiaceae	MUMH 2419	Brazil	AB193607	AB197134
Hevea brasiliensis	Euphorbiaceae	MUMH 2545	Malaysia	AB193588	AB197132
Hevea brasiliensis	Euphorbiaceae	MUMH 2602	Thailand	AB193589	AB197136
Mangifera indica	Anacardiaceae	MUMH 3188	Argentina	AB237794	AB237821
Mangifera indica	Anacardiaceae	MUMH 3705	Thailand	AB237802	AB237829
Mangifera indica	Anacardiaceae	MUMH 3267	Australia	AB237800	AB237827

Mangifera indica	Anacardiaceae	MUMH 3268	Thailand	AB237801	AB237828
Mangifera indica	Anacardiaceae	VPRI 20332	Australia	AB237797	AB237823
Murraya paniculata	Rutaceae	TNM F0026774	Taiwan	KC 857652	—
Nephelium lappaceum	Sapindaceae	MUMH 5779	Indonesia	LC128427	LC128427
Nephelium lappaceum	Sapindaceae	MUMH 6552	Indonesia	LC128428	LC128428
Nephelium lappaceum	Sapindaceae	MUMH 6557	Indonesia	LC128429	LC128429
Quercus sp.	Fagaceae	MUMH 3242	Iran	AB292693	AB292693
Quercus crispula	Fagaceae	MUMH 1956	Japan	AB292691	AB292691
Quercus phyllireoides	Fagaceae	MUMH 124	Japan	AB193590	AB197135
Quercus phyllireoides	Fagaceae	MUMH 885	Japan	AB193591	AB237813

[a]MUMH, Mie University Mycological Herbarium, Japan; BO, Herbarium Bogoriense, Indonesia; VPRI, Plant Disease Herbarium, Institute for Horticultural Development, Victoria, Australia; TNM, Herbarium of National Museum of Natural Science, Taichung, Taiwan.

[b]DDBJ, EMBL, and GenBank database accession number of the nucleotide sequence data AB292691.

Supplementary Table S2. Morphological characteristics of asexual morphs of *Erysiphe quercicola* on various hosts

	Conidia			Conidial germ tube	Foot cell		No. of additional cells	Hyphal appressoria	Reference
	Shape	Size (µm)	l/w ratio		Size (µm)	Base			
Quercus phyllireoides (MUMH 885: holotype)	Primary conidia obovoid-ellipsoid, apex rounded, base subtruncate, secondary conidia doliiform when mature, ends truncate or subtruncate, immature ones sometimes ellipsoid-cylindrical	25–40(–45) × 12–22	1.5–2.3 mostly ≤2	—	20–40 × 7–11	Straight or curved at the base	1–2	Solitary or in opposite pairs	Takamatsu et al. 2007
Anacardium occidentale (MUMH781)	Ellipsoid-cylindrical	25.3–36.3 × 14.1–19.1	1.5–3.3	—	24–66.2 × 5–7.7	Straight	1–3	Lobed	Limkaisang et al. 2006
Bixa orellana (MUMH2606)	Ellipsoid-cylindrical	28.8–40 × 15–20.5	1.7–2.4	Polygoni-type	11.3–43.8 × 6.3–8.8	Straight	1–3	Lobed	Limkaisang et al. 2006
Cinnamomum camphora	Ellipsoid, lemon-shape, short-cylindrical	23–39 × 12–20	—	—	23–39 × 6–9	Straight to somewhat curved	1–2	Lobed	Kirschner and Liu, 2014
Citrus sinensis (MUMH3210)	Ellipsoid-cylindrical	24.8–41.3 × 13.1–17.4	1.7–2.9	Polygoni-type	10.4–45.2 × 6.5–8.7	Straight	1–3	Lobed	Limkaisang et al. 2006
Hevea brasiliensis	Ellipsoid-cylindrical	25.1–54 × 14.2–25.7	1.4–2.4	Polygoni-type	13.4–61.6 × 7–9.7	Straight	1–3	Lobed	Limkaisang et al. 2005; 2006
Mangifera indica (MUMH3267, VPRI20332)	Ellipsoid-cylindrical	21.4–54 × 14.2–25.7	1.4–2.4	Polygoni-type	25.7–120.9 × 5.8–7.8	Straight	1–3	Lobed	Limkaisang et al. 2006

Murraya paniculata	Ellipsoid, lemon-shape, short-cylindrical	25–41 × 13–22.5	—	Subterminal, longitubus pattern	23–73 × 6–10	Straight to somewhat curved	1–2	Lobed	Kirschner and Liu, 2014
Acacia spp. (MUMH1183, MUMH1805)	Ellipsoid-ovoid	24–38 × 13–19	1.5–2.5	Polygoni-type	32–64 × 7.5	Straight	1–3	Lobed	Limkaisang et al. 2006
Bauhinia purpurea (MUMH5678)	Ellipsoid-doliiform	23.3–32.5 × 11–16.3	1.6–2.3	—	11–42 × 4–7	Straight to somewhat curved	1–2	Lobed	This study
Durio zibethinus (MUMH5675)	Ellipsoid-doliiform	22–35 × 11–17	1.5–2.3	Subterminal, longitubus pattern	8–30 × 5–8	Straight to somewhat curved	1–2	Lobed	This study
Nephelium lappaceum (MUMH5779)	Ellipsoid-doliiform	21–32 × 10–18	1.4–2.4		(10–)22–56 × 3–7	Straight to somewhat curved	1(–2)	Lobed	This study

Chapter 6

***Podosphaera perseae-americanae*, a new powdery mildew species on *Persea americana* (avocado) from Indonesia.**

Siska A.S. Siahaan[1], Hidayat I[2,], Kramadibrata K[2], Meeboon J[1], and Takamatsu S[1].

[1] Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan

[2] Research Centre for Biology, Indonesian Institute of Sciences-LIPI, Jl. Raya Jakarta-Bogor KM 46, Cibinong 16911 West Java, Indonesia

Abstract

An asexual stage of powdery mildew was found on *Persea americana* in West Java and North Sumatera, Indonesia. Based on phylogenetic analyses of the 28S rRNA gene and ITS region as well as morphological investigations, a new species, *Podosphaera perseae-americanae*, is proposed for this fungus including detailed descriptions and illustrations of morphological and a discussion of its generic affinity and phylogeny.

1. Introduction

Avocado (*Persea americana* Mill., Lauraceae) is an evergreen subtropical fruit tree, native to Central America and Mexico, and now commercially cultivated worldwide (Evans et al. 2010; Rodríguez-Carpena et al. 2011). In September 2013, we collected young leaves of avocado heavily infected by powdery mildew in West Java, Indonesia (Fig. 1A). An additional collection was carried out in January 2015 in North Sumatra, Indonesia. Morphological observations and molecular analyses revealed that this fungus is an undescribed species belonging to tribe Cystotheceae.

2. Materials and methods

Morphological examinations and DNA sequencing were conducted according to the procedure described by Meeboon and Takamatsu (2015). The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and internal transcribed spacer (ITS) regions including the 5.8S rRNA gene were determined in this study. Newly determined sequences were deposited in DNA Database of Japan (DDBJ). These sequences were aligned with other sequences of the Erysiphaceae using MUSCLE (Edgar 2004) implemented in MEGA 6 (Tamura et al. 2013). Alignments were further manually refined using the MEGA6 program and were deposited in TreeBASE (http://www.treebase.org/) under the accession number S19366. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods as described in Meeboon and Takamatsu (2015).

3. Results and discussion

Podosphaera perseae-americanae Siahaan & S. Takam., sp. nov.   Fig. 1.

MycoBank, MB817227

      Characterized by having conidia produced in chains (catenescent) with crenate edge line, *Fibroidium*-type conidial germ tubes, *Persea americana* (Lauraceae) as host, and a unique rRNA sequence.
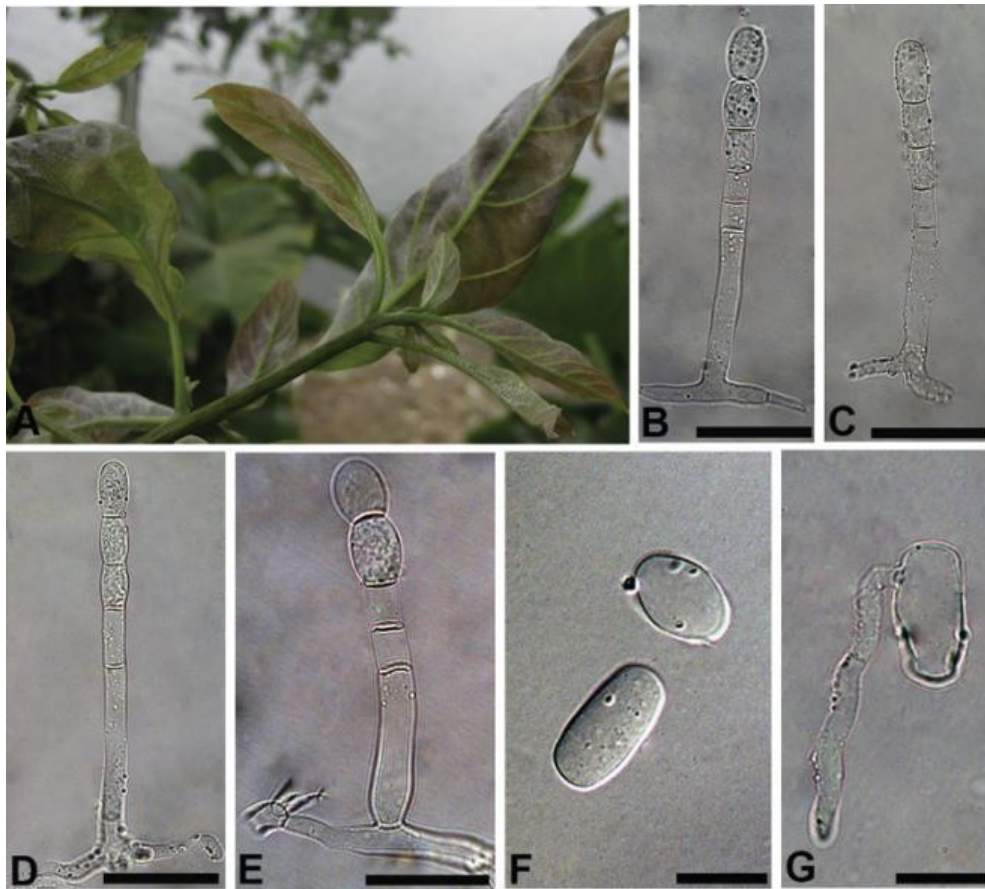
Fig. 1 – *Podosphaera perseae-americanae* on *Persea americana* A: Symptoms. B–E: Conidiophores. F: Conidia. H: Germ tube. *Bars:* B–E 50μm; F–H 20 μm

Type : On *Persea americana* (Lauraceae), INDONESIA, West Java, Ciwidey, Situ Patenggang Village, 07°49'48.8"S 107°21'22.3"E, 13 Sep 2013, S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon, and S. Takamatsu. BO 22708 (holotype), MUMH 5682 (isotype).

Etymology: Epithet derived from the host plant.

Gene sequences (isotype): LC155953 (ITS + 28S).

Mycelium hypophyllous, ectophytic, white, persistent to subevanescent. Hyphae hyaline, hyphal appressoria indistinct to nipple shape. Conidiophores erect (50–)77–153 × 9.5–15.5 μm. Foot-cells cylindrical, straight to slightly curved, 22–63 × 9.5–12 μm, followed by 1–4 (–5) shorter cells, basal septum at the junction with the supporting hyphae or raised up to 20 μm above the branching point, sometimes slightly constricted at the base. Conidia produced in chains (catenescent), cylindrical-doliiform, 24.5–33.5 ×

84

28S rRNA
43 sequences
809 characters
TL = 673
CI = 0.4414
RI = 0.7230
RC = 0.3191

100/100 — *A. mougeotii* ex *Lycium* AB022379
91/92 — *A. mougeotii* ex *Lycium* AB329690   *Arthrocladiella*

G. cichoracearum ex *Solidago* AB077650
99/100 — *G. orontii* ex *Physalis* AB077646   *Golovinomyces*

E. abbreviata ex *Quercus* AB271785
72/70 — *E. alphitoides* ex *Quercus* AB257431
91/95 — *E. quercicola* ex *Quercus* AB292694
E. pulchra ex *Benthamidia* AB015935
72/— *E. aquilegiae* ex *Cimicifuga* AB022405
E. vanbruntina ex *Sambucus* AB015925   *Erysiphe*
95/89 — *E. pulchra* ex *Swida* AB022389
E. japonica ex *Quercus* AB022415
95/93 — *E. gracilis* ex *Quercus* AB022357
E. australiana ex *Lagerstroemia* AB022407

Pl. shiraiana ex *Celtis* AB022403   *Pleochaeta*

L. taurica ex *Artemisia* AB080470
L. taurica ex *Helianthus* AB080472   *Leveilulla*

91/99
100/100 — *Ph. moricola* ex *Morus* AB022401
Ph. ailanthi ex *Ailanthus* AB080380   *Phyllactinia*
100/100 *Ph. linderae* ex *Litsea* AB080421

N. cumminsiana ex *Cacalia* AB329669
100/100 — *N. galii* ex *Galium* AB329681   *Neoerysiphe*
100/100 *N. galii* ex *Galium* AB329682

Pa. septata ex *Quercus* AB022420
100/100 — *Pa. septata* ex *Quercus* AB183533   *Parauncinula*

Ca. forestalis ex *Schinopsis* AB193467   *Caespitotheca*

99/99 — *Cy. lanestris* ex *Quercus* AB022353
Cy. wrightii ex *Quercus* AB022355   *Cystotheca*
Se. castanopsidis ex *Castanopsis* MUMH5123
96/100 *Se. castanopsidis* ex *Castanopsis* MUMH5147

78/86 — *Sa. polyfida* ex *Acer* AB022364
Sa. tulasnei ex *Acer* AB022366   *Sawadaea*

89/99 — *Po. filipendulae* ex *Filipendula* AB022384
Po. pannosa ex *Rosa* AB022347
94/99 — *Po. longiseta* ex *Prunus* AB022423   *Podosphaera*
93/94 — *Po. tridactyla* ex *Prunus* AB022393
Po. xanthii ex *Melothria* AB022410

100/99

Persea americana LC155953
100/100 **Persea americana LC155954**   *Podosphaera perseae-americanae*

Tribe Cystotheceae

B. graminis ex *Bromus* AB022362
100/100 — *B. graminis* ex *Hordeum* AB022399   *Blumeria*
B. graminis ex *Triticum* AB022376

Byssoascus striatisporus U17912

— 5 changes

Fig. 2 – Phylogeny *Podosphaera perseae-americanae* inferred from 28S rRNA gene for 42 sequences from the Erysiphales and a sequence from *Byssoascus striatisporus* used as an outgroup taxon. The percentage bootstrap support (1K replications; ≥ 70%) values are shown on/under the respective branch. Sequences determined in this study are shown in boldface.

Fig. 3 – Phylogeny of *Podosphaera perseae-americanae* inferred from ITS sequences for 20 sequences from the tribe Cystotheceae and two sequences from *Blumeria graminis* used as an outgroup taxon. The percentage bootstrap support (1K replications; ≥ 70%) values are shown on/under the respective branch. Sequences determined in this study are shown in boldface.

13.5–20 μm (l/w ratio 1.6–2.2) (using Blumer's factors). Fibrosin bodies were obscure because of dried materials. Conidial germ tubes rise from lateral side, short to moderately long, *Fibroidium* type.

Additional specimen examined: On *P. americana* (Lauraceae), INDONESIA, North Sumatera, Berastagi, 3°18'61.11"S 98°50'86.42"E, 18 Jan 2015, S.A.S. Siahaan, MUMH 5775, GenBank accession number: LC155954 (ITS + 28S).

Host range and distribution: On *P. americana*, Indonesia.

Note: The ITS and 28S rRNA gene (D1/D2 region) sequences obtained from two collections on *P. americana* were identical to each other. The two 28S rRNA gene sequences were combined with 40 sequences of the Erysiphales and one sequence from *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx (U17912) that was used as an

outgroup based on Mori et al. (2000). This data set consists of 43 sequences and 809 characters, of which 216 (26.7%) characters were variable and 169 (20.9%) were informative for parsimony analysis. A total of eight equally parsimonious trees with 673 steps were constructed by the MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. One of the trees with the highest likelihood value is shown in Fig. 2. ML analysis generated a tree topology almost identical to the MP tree. Thus, only BS values of the ML analysis were shown on Fig. 2. The two sequences from *Podosphaera perseae-americanae* belonged to a clade corresponding with tribe Cystotheceae with strong BS supports (MP = 94%, ML = 99%) and formed an independent clade (MP = 100%, ML = 100%) within this tribe. Further phylogenetic analysis using ITS sequences was carried out to confirm the 28S rRNA gene tree. The ITS data set consists of 22 sequences and 514 characters, of which 196 (38.1%) characters were variable and 168 (32.7%) characters were informative for parsimony analysis. *Blumeria graminis* (DC.) Speer was used as an outgroup taxon. A total of 60 equally parsimonious trees with 438 steps were constructed by the MP analysis. One of the trees with the highest likelihood value is shown in Fig. 3. Again, *P. perseae-americanae* formed an independent clade in tribe Cystotheceae (MP = 100%, ML = 100%).

Both 28S rRNA gene and ITS sequences indicate that this fungus forms an independent lineage within tribe Cystotheceae, suggesting that this fungus may belong to an undescribed genus. Based on the crenate-type edge line of conidiophore, this fungus is distinguished from Cystotheca having sinuate-type edge line (Shin and La 1993). The present fungus also differs from *Sawadaea* in lacking micro-conidia. On the other hand, this fungus is morphologically indistinguishable from asexual morphs of *Podosphaera* (=*Fibroidium*). Its phylogenetic position is also close to *Podosphaera* clade. Based on these results and without sexual morph, a final conclusion with regard to generic affinity of the avocado mildew is not feasible. Therefore, we currently refrain from introducing a new genus for this fungus and assign it tentatively to *Podosphaera* until additional

collections with fruiting bodies will be available.

Fungal disease of avocado plant have been relatively well studied (McMillan 1976; Prusky 1996). A few species of powdery mildew have been reported on the genus *Persea*. *Pseudoidium perseae-americanae* (Liberato & R.W. Barreto) Liberato & R.W. Barreto (Liberato and Barreto 2006) was described as a powdery mildew occurring on *P. americana* in Braun and Cook (2012). *Erysiphe machiliana* U. Braun & Y.S. Paul (sect. *Uncinula*) was described on *P. odoratissima* (Nees) Kosterm. (Braun and Cook 2012). These two species having non-catenate conidia distinctly differ from the present fungus having conidia produced in chains (catenate-type). *Sphaerotheca* sp. (presently *Podosphaera* sect. *Sphaerotheca*) was recorded as a powdery mildew belonging to tribe Cystotheceae occurring on avocado in Cuba (Roseñada 1973; Amano 1986). However, due to lacking morphological descriptions of the Cuban records, a comparison with Indonesian collections was impossible.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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Chapter 7

Biodiversity of powdery mildews in Indonesia.

Siska A.S. Siahaan[1], Nakashima C[1,], Kramadibrata K[2], Oktavia G.A.E.[1], and Takamatsu S[1].

[1] Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu 514-8507, Japan

[2] Research Centre for Biology, Indonesian Institute of Sciences-LIPI, Jl. Raya Jakarta-Bogor KM 46, Cibinong 16911 West Java, Indonesia

[3] Eka Karya Bali Botanic Garden, Indonesian Institute of Science-LIPI, Indonesia

Abstract

Powdery mildews (Erysiphales, Erysiphaceae) is one of the most common, conspicuous widespread and easily recognizable pant disease, but not much yet explored in the tropics and subtropics, especially in Indonesia. In an attempt to explore the diversity of the Indonesia powdery mildews, we collected 105 symptomatic samples from Indonesia during 2013-2017. To determine the fungal specimens to the species level, we combine both morphology and molecular sequence of ITS, 28S and/or 18S regions.

Key words: Anamorph, Erysiphaceae, Molecular phylogeny, Taxonomy

1. Introduction

Powdery mildews is one of the most recognizable plant disease in the nature by its white powdery growth on leaves, flowers, fruit and stems. Due to their obligate nature, researchers have not had the advantage of routinely cultivating these fungi on artificial media, although many powdery mildews have been grown on detached leaves of their hosts (Cook et al. 2015). In the nature, they are often conspicuous white powdery masses owing to the profuse production of conidia that give them their common name.

In general, the magnitude of fungal diversity is estimated to be 1.5 million species, but only approximately 5% of them have been described (Hawksworth 2001). He also noted that half of the newly described fungal species come from the tropics (Blackwell 2011, Hawksworth 1993, 2001, 2004). Furthermore, Hawksworth and Rossman (1997) hypothesized that the remaining undescribed fungi could lie in the tropics due to its high plant richness diversity. Meanwhile, the exploration of powdery mildews in the tropics especially in Indonesia are underresearched despite of the biodiversity of the plants. There were very few reports on the occurrence of powdery mildews in Indonesia. If any, those reports were mainly written based on the traditional taxonomical system, i.e. based only morphological and/or host range data. These methods are not sufficient for a precise identification to species level, because most powdery mildews in the tropics lack sexual morph, essential for identification. In addition, the anamorphic features are often unreliable since they are almost similar, especially to those closely related species. Thus, our main objective in this study is to identify the powdery mildews species on the respective host plants collected in Indonesia during 2013-2017 by combining morphological, molecular and host range data.

2. Material and methods

2.1. Samples collection

A total of 109 symptomatic samples of powdery mildews were collected from 2013 to 2017 in Bali, North Sumatra and West Java provinces, Indonesia (Supplementary Table

1). Specimens were deposited at the Herbarium of Bogoriense (BO, Indonesia), Mie University Mycological Herbarium (MUMH) and at the Museum of Nature and Science (TNS), Japan.

2.2. Morphological examination

To examine the asexual morph on a fresh sample, mycelial colonies were stripped off from the leaf surfaces with clear adhesive tape, mounted on a glass slide with the fungal mycelium uppermost, and examined in water using a standard light microscope (Axio Imager; Carl Zeiss, Göttingen, Germany) and differential interference contrast optical instruments and devices. In order to examine the asexual morph on the herbarium specimens, mycelial colonies on a small piece of infected leaf were rehydrated by the method described by Shin & La (1993). Thirty conidiophores and conidia were measured for each specimen examined. For the rehydrated samples, the width of conidia was multiplied by Blumer's factor according to Braun & Cook (2012).

2.3. Molecular phylogeny

The nucleotide sequences of the 5'-end of the 28S rRNA gene (including domains D1 and D2) and ITS regions were determined according to Meeboon and Takamatsu (2014). All primers used in this study are provided in Table 2. Newly determined sequences were aligned with other sequences of the Erysiphaceae retrieved from DNA databases (DDBJ, EMBL, NCBI) using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html). Alignments were further manually refined using the MEGA7 program (Kumar et al. 2016) were deposited in TreeBASE (http://www.treebase.org/) under the accession number S00000. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods. MP analysis was performed in PAUP* v. 4.0b10 (Swofford 2003) with heuristic search option using the tree bisection reconnection (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as

Table 1. PCR primers used to amplify and sequencing the 28S, ITS and 18S rDNA regions.

Primer name	Nucleotide primers
28S	
PM3	5'-GKG CTY TMC GCG TAG T-3'
PM3 Sida	5'- CAG GCT CTA CGC GTA GTA C-3' (specific for *Sida* PM)
PM 8	5'-CTC GTG TGC TAC GCG TAG T-3' (specific for *Persea americana* PM)
NLP2	5'-GGT CCC AAC AGC TAT GCT CT-3'
ITS	
ITS 5	5'-GGA AGT AAA AGT CGT AAC AAG G-3'
ITS 4	5'-TCC TCC GCT TAT TGA TAT GC-3'
ITS p3	5'-GCC GCT TCA CTC GCC GTT AC-3'
PH5	5'-TTG CTT TGG YAG GCC GGG-3' (*Phyllactinia* specific)
PH7	5'-TGT TGC TTT GGY AGG CCG-3' (*Phyllactinia* specific)
PH8	5'-GCC CCA AGA CCA AGC C-3' (*Phyllactinia* specific)
PM5	5'-TTG CTT TGG CGG GCC GGG-3'
PM5 Golovinomyces	5'-GAC CCT CCA CCC GTG T-3' (*Golovinomyces* specific)
PM6	5'-GYC RCY CTG TCG CGA G-3'
PM6 Avocado	5'CGT ACA CTG TCG CGA G-3' (specific for *Persea americana* PM)
PM6 Golovinomyces	5'-CGA GCC CCA ACA CCA A-3' (*Golovinomyces* specific)
PM6 Sida	5'-GTT GCC GCT CTG TCG CGA T-3' (specific for *Sida* PM)
18S	
NS1	5'-GTA GTC ATA TGC TTG TCT C-3'
P3	5'-TTT TGT TGG TTT CTA GGA CC-3'
P6	5'-CTT CCG TCA ATT TCT TTA AG-3'
PM4	5'-CCG GCC CGC CAA AGC AAC-3'

unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analysis using 1000 replications (Hillis & Bull 1993). Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. The ML analysis was done using the gamma model of rate heterogeneity using the RAxML BlackBox online server (http://embnet.vital-it.ch/raxml-bb/). The maximum likelihood search option after rapid bootstrap analyses of 100 replicates (Stamatakis et al. 2008) was applied to find the best-scoring tree. Trees from both analyses were visualized in FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree). The generated trees and those alignments were deposited into TreeBASE at www.treebase.org (http://purl.org/phylo/treebase/phylows/study/TB2:S******). Bayes Kakusan 4 (Tanabe 2011) was used to determine the best nucleotide substitution model settings for each data partition in order to perform a model-optimised Bayesian phylogenetic reconstruction using MrBayes v. 3.2.6 (Ronquist et al. 2011). The heating chain was set at 0.1, and Markov Chain Monte Carlo (MCMC) analyses of four chains were performed in parallel from a random tree topology, terminating when the the average standard deviation of split frequencies reached a value of 0.01.

3. Results

3.1. Taxonomic part

Tribe Cystotheceae

Subtribe Cystothecinae

Genus *Cystotheca*

Cystotheca tjibodensis (Gäum.) Katum., Rep. Tottori Mycol. Inst. 10:443. 1973.

≡ *Lanomyces tjibodensis* Gäum., Ann. Jard. Bot. Buitenzorg 32: 46. 1922.

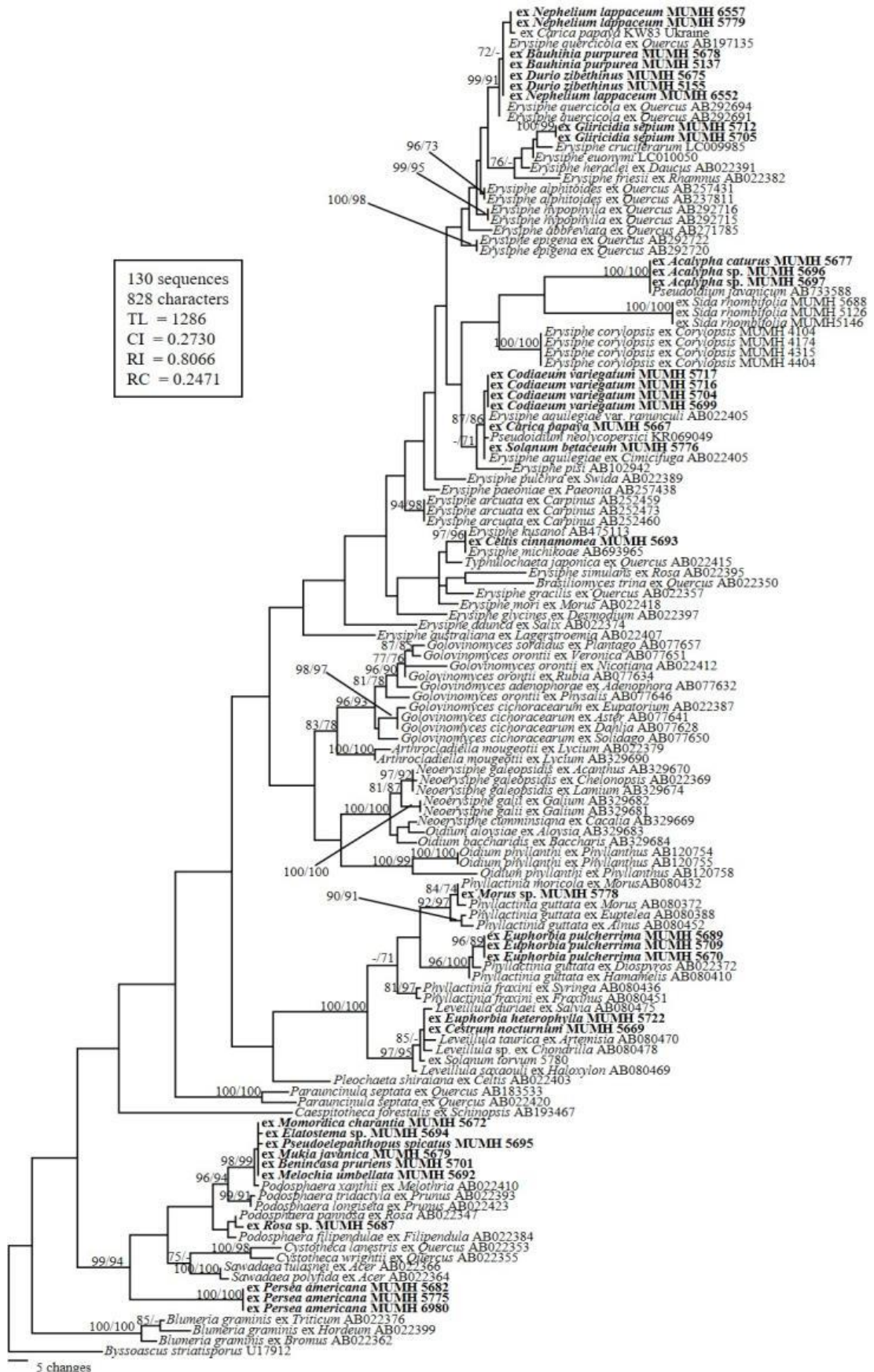Description and illustration — See Meeboon et al. (2012a).

Fig.1A. – Phylogenetic analysis of the 28S rRNA gene (including D1/D2 domains) for 130 sequences from Erysiphaceae. This tree is one of the 558,652 equally parsimonious trees with 1,069 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

96
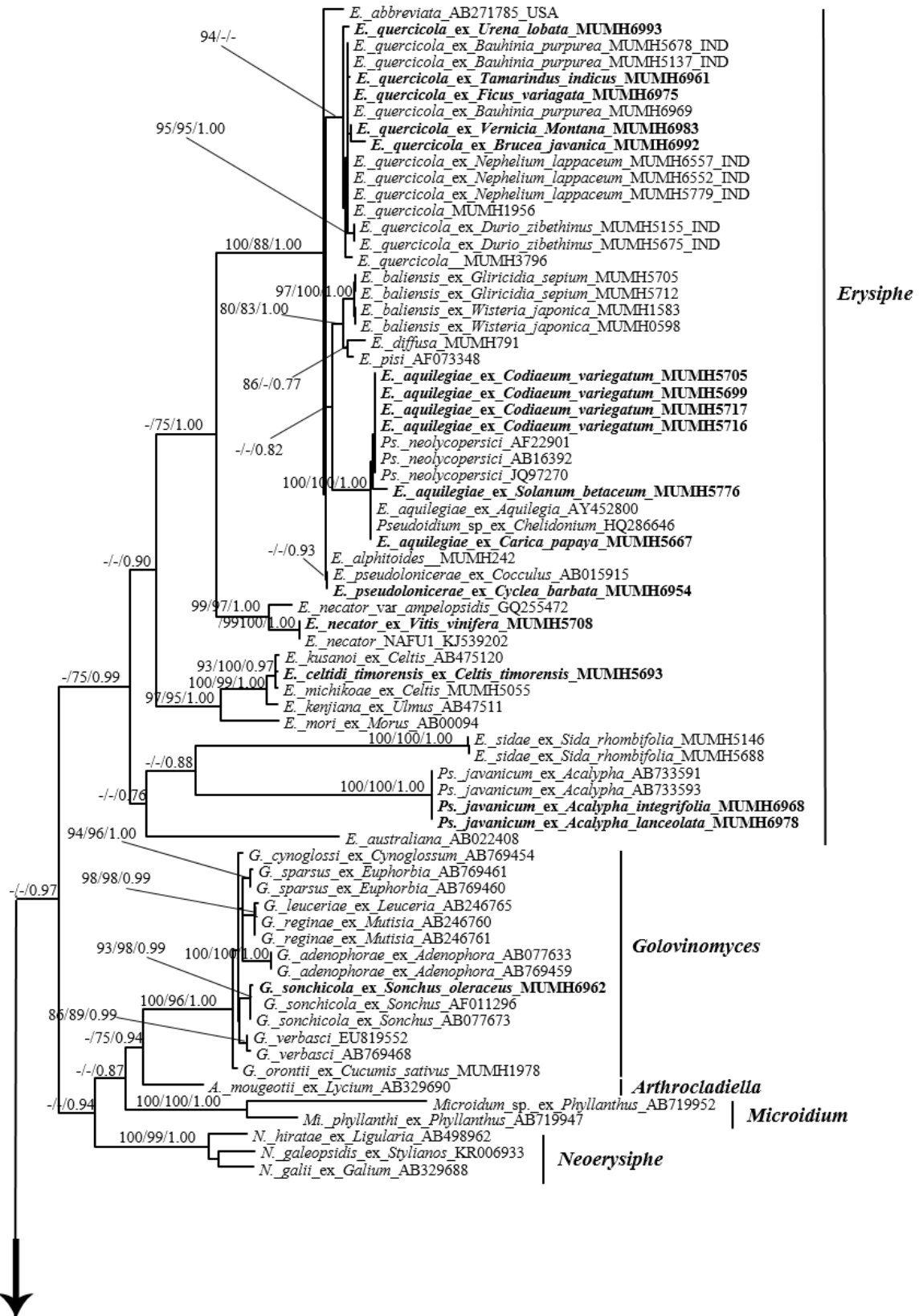
Fig.1B. – Phylogenetic analysis of the ITS rRNA gene for 155 sequences from Erysiphaceae and one sequence of *Byssoascus striatosporus* as an outgroup taxon. The BS (bootstrap values) and PP (posterior probability) with ≥ 70% values were written on respective branches.
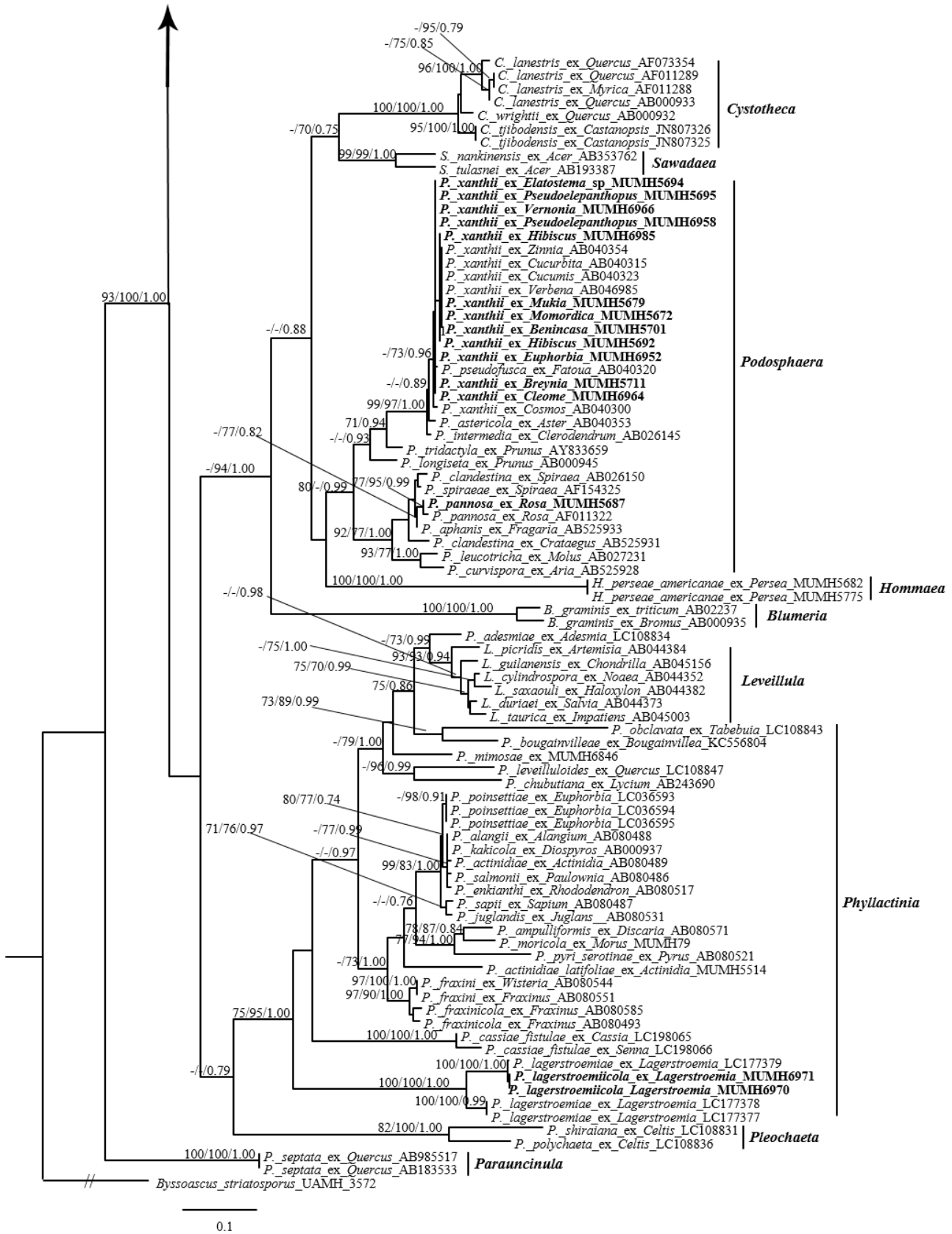
Fig.1B. (continued).

Lectotype (designated by Braun 1987) — on *Castanea argentea*, INDONESIA. Java, Tjibodas, Feb. 1920, *E. Gäumann*, Syd., Fungi Exot. Exs.503 (S).

Specimens examined. on *Castanopsis argentea*, INDONESIA, West Java, Cibodas, Cibodas Botanical Garden, 14 Mar. 2011, *J. Meeboon, I. Hidayat, S.A.S. Siahaan & S. Takamatsu* (Epitype TSU-MUMH5164: MBJN807325, Iso-epitype TSU-MUMH5148).

Notes — Newly designated epitype is topotypic materials, rediscovered for the first time in 90 years.

Setoidium castanopsidis Meeboon & S. Takam., Mycoscience 54: 275. 2013 — Fig. 2. *Colonies on leave*s amphigenous, mostly hypophyllous effuse, persistent to subevanescent. *Hyphae* hyaline 3–7 μm wide; *hyphal appressoria* indistinct to nipple shaped. *Conidiophores* erect, arising from the top of mother cell, 97–185 x 10–13 um, *foot-cells* cylindrical, slightly curved, 39.4–110 x 6.5–11 um, followed by 0–2 shorter cells, forming catenescent conidia with sinuate outline, containing fibrosin bodies. *Conidia* broadly ellipsoid–ovoid, or drum-shape-like 27.5–40.5 × 17.5–26 μm, length/width ratio 1.2–2.1. Germ tube subterminal, alobatus, very long up to 87 μm.

Host in Indonesia — *Castanopsis argentea* (Blume) A. DC., *C. javanica* (Blume) A.DC. (Fagaceae).

Specimens examined. on *Castanopsis argentea*, INDONESIA, West Java, Bogor, Talaga Warna Puncak, 11 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22702, TSU-MUMH 5676); West Java, Cibodas, Cibodas Botanical Garden, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22845,TSU-MUMH 6987, BO 22846,TSU-MUMH 6988); on *C. javanica*, West Java, Cianjur, Cibodas Botanical Garden, 14 Mar. 2011, *I. Hidayat, J. Meeboon & S. Takamatsu* (Isotype, TSU-MUMH 5147).
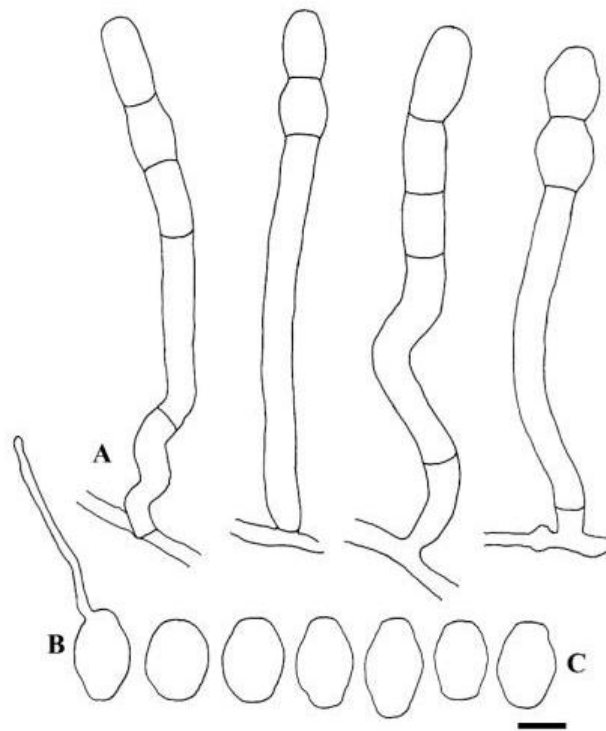
Fig.2. – *Setoidium castanopsidis* on *Castanopsis argentea*. A: Conidiophores. B: Germ tube. C: Conidia. *Bars*: 20 μm.

Notes — *Castanopsis argentea* is newly added as a host plant of present species in this study. According to Meeboon et al. (2013c), present species on *C. javanica* was described that the foot-cell was straight. However, in our observation of isotype material, the foot-cells are sometimes curved or even somewhat flexuous.

Hommaea **S.A.S.** Siahaan, 2017, gen. nov. MycoBank no.: MB000000

Etymology — Name composed of Homma (Y. Homma, the first Japanese woman mycologist working on mycogeography of powdery mildew).

Morphologically distinct from the other genera *Cystotheca, Podosphaera* and *Sawadaea* within tribe Cystotheceae in having lobed to elongated and forked hyphal appressoria, foot-cells followed by a longer cell, directly or after shorter cells, and dimorphic conidia.

Type species — *Hommaea perseae-americanae* (S.A.S. Siahaan & S. Takam.) S.A.S. Siahaan.

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Notes — The new genus *Hommaea* has intermediate morphological characteristics of generic character of tribe Cystotheceae. Especially its characteristics are same as that of the genus *Podosphaera*. From the results of observation using scanning electron microscope, the end wall of *Hommaea* is whorled and its outer wall is smooth (Fig. 2). The germination pattern is *Fibroidium* type, *Orthotubus* subtype. Moreover, this genus forms a well-supported clade within tribe Cystoteceae and is recognized as a sister clade of the genus *Podosphaera*.On the other hands, it differs in having well developed and bifurcated hyphal appressoria, which has not been known as the morphological character of tribe Cystotheceae.

Hommae perseae-americanae (Siahaan & S. Takam.) Siahaan, comb. nov. — Fig. 3.

Basionym – *Podosphaera perseae-americanae* Siahaan & S. Takam., Mycoscience 57: 418. 2016.

Mycelium hypophyllous, forming white patches, effuse to dense, evanescent or persistent, causing yellowish discoloration on infected areas. *Hyphae* hyaline, 5–10 µm wide; *hyphal appressoria* well-developed, lobed to elongated, rarely forked, solitary, up to ±10 µm. *Conidiophores* arising from external hyphae, on upper surface of mother cells, erect, (50–)65–150 × 8–13 µm, with basal septum at the junction with the supporting hyphae or elevated up to 20 µm; *foot-cells* straight to slightly curved, 20–60 × 8.5–12 µm, directly followed by a longer cell, 1.2–2 times longer than the attached conidia, or by 0–1(–2) shorter cells then followed by a longer cell, forming catenescent conidia with a crenate line of immature conidia. *Conidia* catenescent, broadly cylindrical to doliiform, subtruncate at the both ends, dimorphic in size; *major conidia* 28–50(–70) × 12–19 µm, length/width ratio 1.6–3.7, smaller conidia 19–27 × 12–19.5 µm , length/width ratio 1.2–2; *germ tubes* terminal, subterminal or lateral, *Fibroidium* type, *Orthotubus* subtype; *conidial end walls* under SEM observation smooth to whorled towards the center.

Host — *Persea americana* Mill. (Lauraceae).
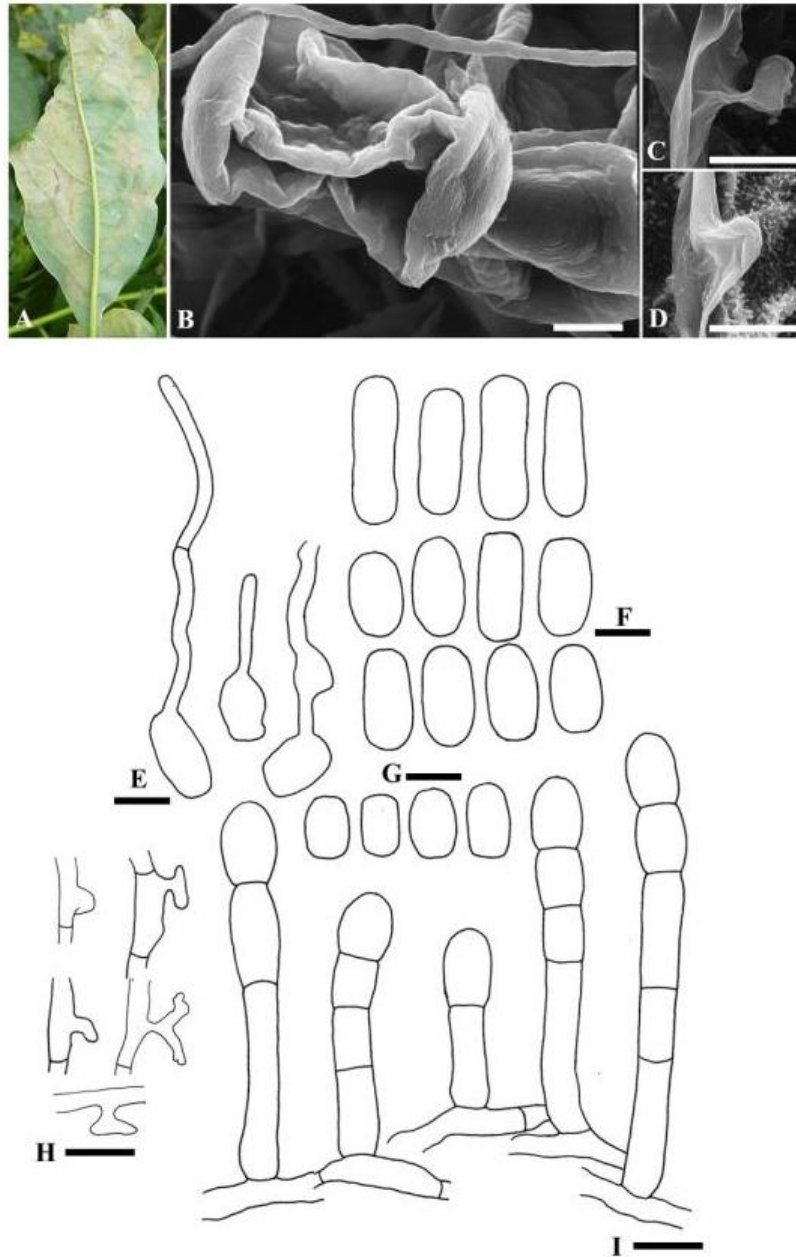
Fig.3. – *Hommae perseae-americanae* on *Persea americana.* A: Symptoms. B: Dried conidia under SEM. C–D: Appressoria under SEM. E: Germ tube. F: Conidia. G: Conidia. H: Appressoria. I: Conidiophores. *Bars*: B–D =5 μm; E–G, I=20 μm; H= 10 μm.
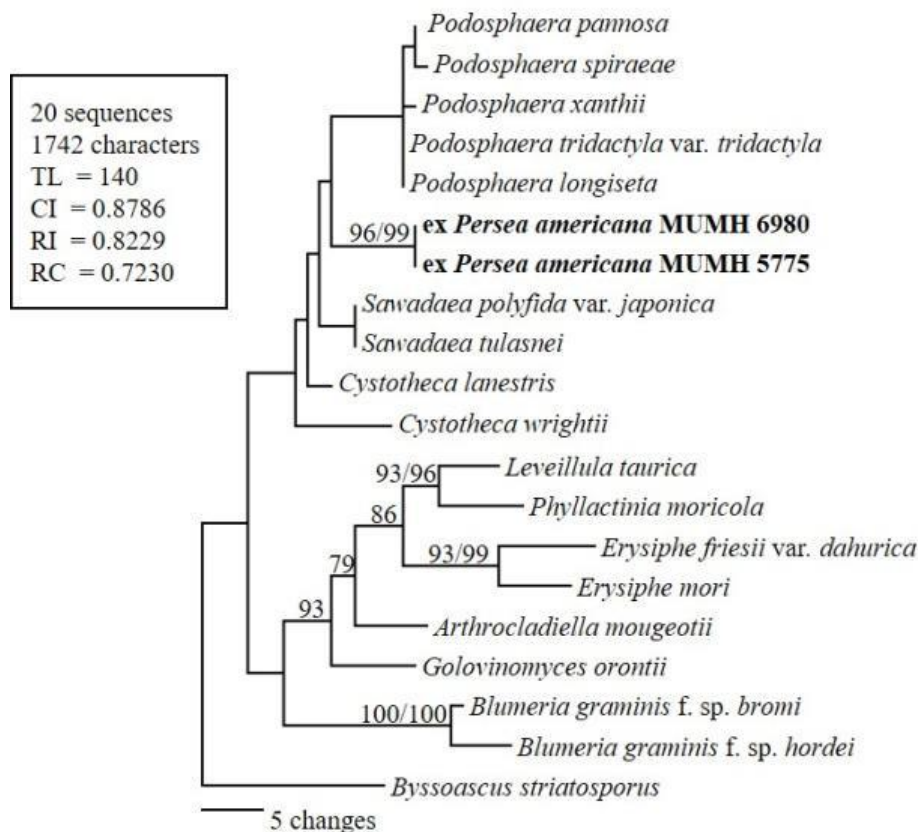
Fig.4. – Phylogenetic analysis of the 18S rRNA gene for 20 sequences of the *Erysiphaceae*, including a sequence of *Byssoascus striatosporus* as an outgroup taxon. This tree is one of the 305 equally parsimonious trees with 138 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

Specimens examined. on *Persea americana* Mill. (Lauraceae), INDONESIA, West Java, Bandung, Ciwidey, Situ Patenggang, 13 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, Meeboon J & S. Takamatsu* (holotype of *Podosphaera perseae-americanae* BO 22708, TSU-MUMH 5682, isotype of *P. perseae-americanae* TNS-F-65542); North Sumatra, Berastagi, 18 Jan. 2015, *S.A.S. Siahaan* (MUMH 5775); West Java, Bandung, Cisarua, 22 Oct 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22830,TSU-MUMH 6972); West Java, Bandung, Situ Patenggang, 23 Oct 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22834,TSU-MUMH 6976, BO 22834, TSU-MUMH 6977); West Java, Bandung, Jalan Raya Ciwidey-Ranca Bali II, 23 Oct 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22838,TSU-MUMH 6980).

Genus *Podosphaera*

Podosphaera pannosa (Wallr. : Fr.) de Bary, Abh. Senkenb. Naturf. Ges. 7: 408. 1870 — Fig. 5.

≡ *Alphitomorpha pannosa* Wallr., Verh. Ges. Naturf. Freunde Berlin 1: 43. 1819.

= *Oidium leucoconium* Desm., Ann. Sci. nat., Ser. 1, 13: 102. 1829.

Other synonyms — See Braun & Cook (2012).
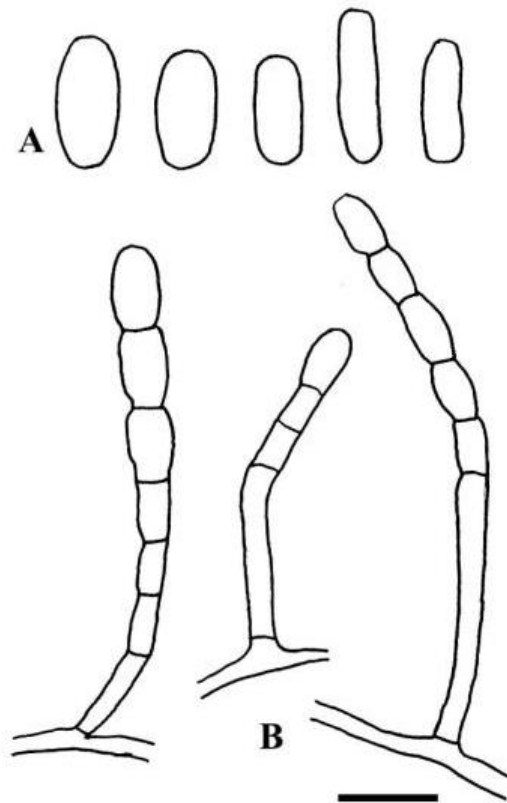
Fig.5. – *Podosphaera pannosa* on *Rosa* sp. A: Conidia. B: Conidiophores. *Bar*: 20 μm.

*Colonies on leave*s amphigenous, forming white patches, effuse, persistent to subevanescent. *Hyphae* hyaline 3–6 μm wide; *hyphal appressoria* indistinct to nipple shaped. *Conidiophores* erect, arising from the top of mother cell, 71.5–196.5 x 6–11 um, *foot-cells* cylindrical, straight or slightly curved, (28.5–)37.5–82.5 x 6–9 um, followed by 1–2(–3) shorter cells, forming catenescent conidia with crenate edge. *Conidia* cylindrical to ellipsoid, 24–34.5(–43.5) × 10.5–16 μm, length/width ratio 1.7–3(–3.4). Germ tubes

not observed.

Host in Indonesia — *Rosa* sp. (Rosaceae).

Specimen examined. on *Rosa* sp., INDONESIA, West Java, Bandung, Rawa Upas, 14 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22712, TSU-MUMH 5687).
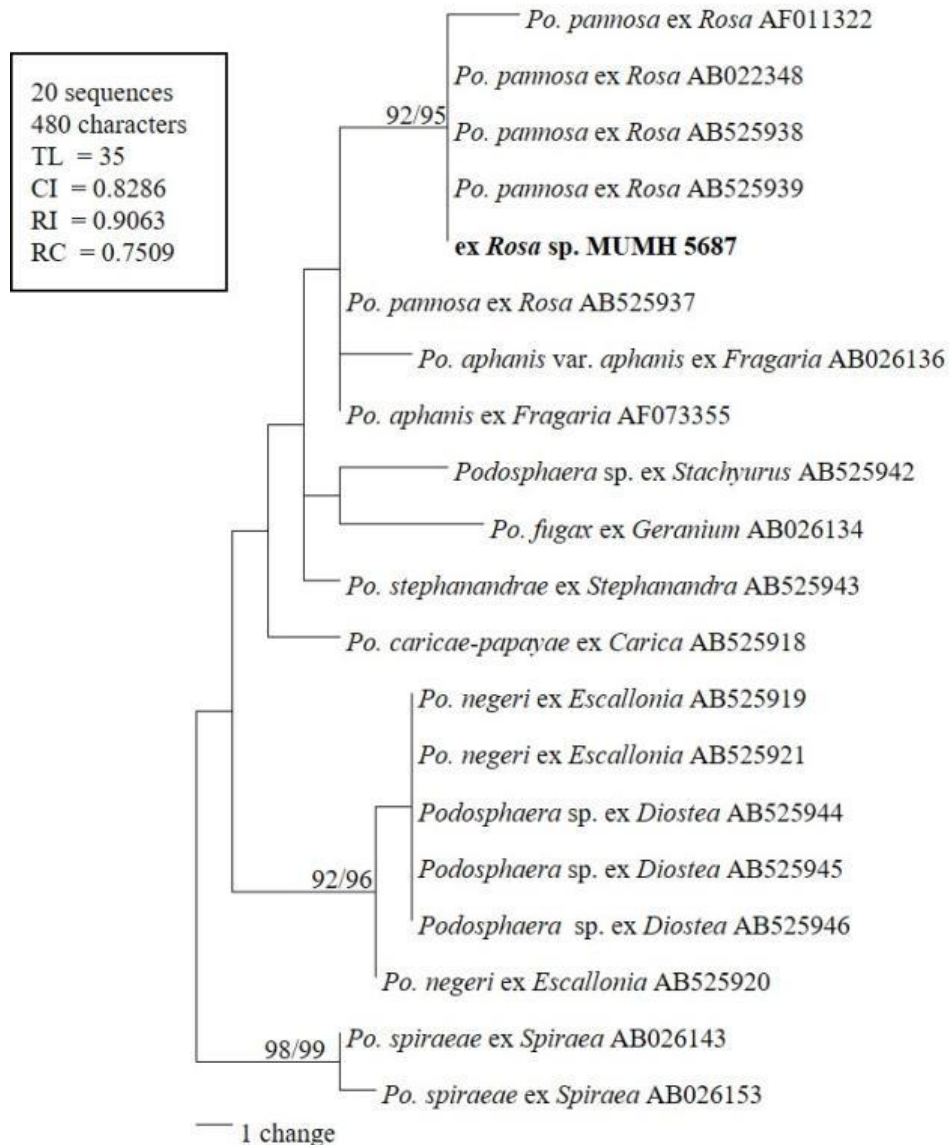
Fig.6. – Phylogenetic analysis of the ITS rRNA gene for 20 sequences of the *Podosphaera* spp. This tree is one of the four equally parsimonious trees with 32 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

Podosphaera xanthii (Castagne) U. Braun & Shishkoff, Schlechtendalia 4: 31. 2000 —
Fig. 7.

≡ *Erysiphe xanthii* Castagne, Cat. pl. Marseille: 188. 1845.

≡ *Sohaerotheca xanthii* (Castagne) L. Junell, Svensk Bot. Tidskr. 60(3): 382. 1966.

= *Oidium balsaminae* Rajd., Mycopathol. Mycol. Appl. 28: 150. 1966.

Other synonyms — See Braun & Cook (2012).

*Colonies on leave*s amphigenous, forming white patches, effuse, persistent to subevanescent. *Hyphae* hyaline 3–5 μm wide; *hyphal appressoria* indistinct to nipple shaped. *Conidiophores* erect, arising from the top of mother cell, 69–213 x 8.5–13 um, *foot-cells* cylindrical, straight or occasionally slightly curved, sometimes constricted and swollen at the base and 26.5–69 x 7–11.5 um, followed by 1–3 shorter cells, forming catenescent conidia with crenate edge. *Conidia* ellipsoid-doliiform, with fibrosin bodies, 24–42.5(–55.5) × 13–19 μm, length/width ratio 1.5– 2.6(–3.0). Germ tubes lateral with *Fibroidium* type, *brevitubus* subtype, occasionally from terminal, alobatus and very long.
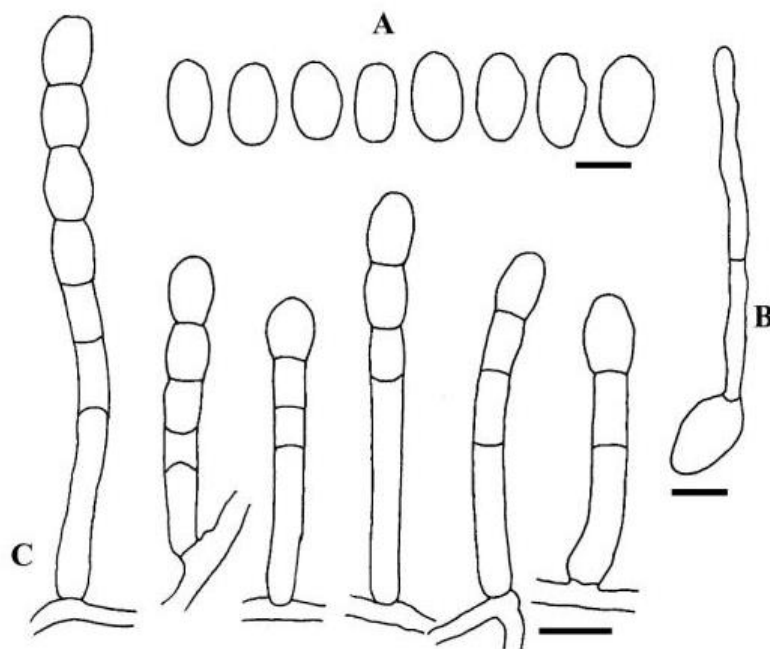
Fig.7. – *Podosphaera xanthii*. A: Conidia. B: Germ tube. C: Conidiophores. *Bars*: 20 μm.

Hosts in Indonesia — *Ageratum conyzoides* L. (Asteraceae), *Benincasa pruriens*

(Sol. ex Seem.) W.J. de Wilde (Cucurbitaceae), *Breynia virgata* (Blume) Müll. Arg. (Phyllanthaceae), *Cleome viscosa* L. (Capparidaceae), *Cucurbita maxima* Duchesne (Cucurbitaceae), *Cyanthilium cinereum* (L). H. Rob. (Asteraceae), *Euphorbia hirta* L. (Euphorbiaceae), *Elatostema* sp. J.R. Forst. & G. Forst. (Urticaceae), *Hibiscus indicus* (Burm.f.) Hochr. (Malvaceae), *Momordica charantia* L. (Cucurbitaceae), *Mukia javanica* (Miq.) C. Jeffrey (Cucurbitaceae), *Pseudoelephantopus spicatus* (Juss. Ex Aubl.)Rohr. (Asteraceae), *Vernonia cinerea* (L.) Less. (Asteraceae).

Specimens examined. on *Ageratum conyzoides*, INDONESIA, West Java, Bandung, Situ Patenggang, Ciwidey, 13 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22706, TSU-MUMH 5680); West Java, Bandung, Rawa Upas, 14 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22710, TSU-MUMH 5685); Bali, Gitgit waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22732, TSU-MUMH 5707); on *Benincasa pruriens*, Bali, Gitgit waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22726, TSU-MUMH 5701); on *Breynia virgata*, Bali, Kintamani, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22735, TSU-MUMH 5711); on *Cleome viscosa*, Bali, 20 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22823, TSU-MUMH 6964); on *Cucurbita maxima*, Bali, 17 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22814, TSU-MUMH 6955); on *Cyanthilium cinereum*, Bali, 18 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22816, TSU-MUMH 6957); on *Euphorbia hirta*, Bali, 17 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22811, TSU-MUMH 6952); on *Elatostema* sp., Bali, Gitgit waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22720, TSU-MUMH 5694); on *Hibiscus indicus*, West Java, Bandung, Rawa Upas, 14 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22718, TSU-MUMH 5692); West Java, Cibodas, Cibodas Botanical Garden, 25 Oct. 2013, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22843, TSU-MUMH 6985); on *Momordica charantia*, West Java, Bogor, Curug Cigamea, 10 Sept. 2013, *S.A.S. Siahaan & J.*
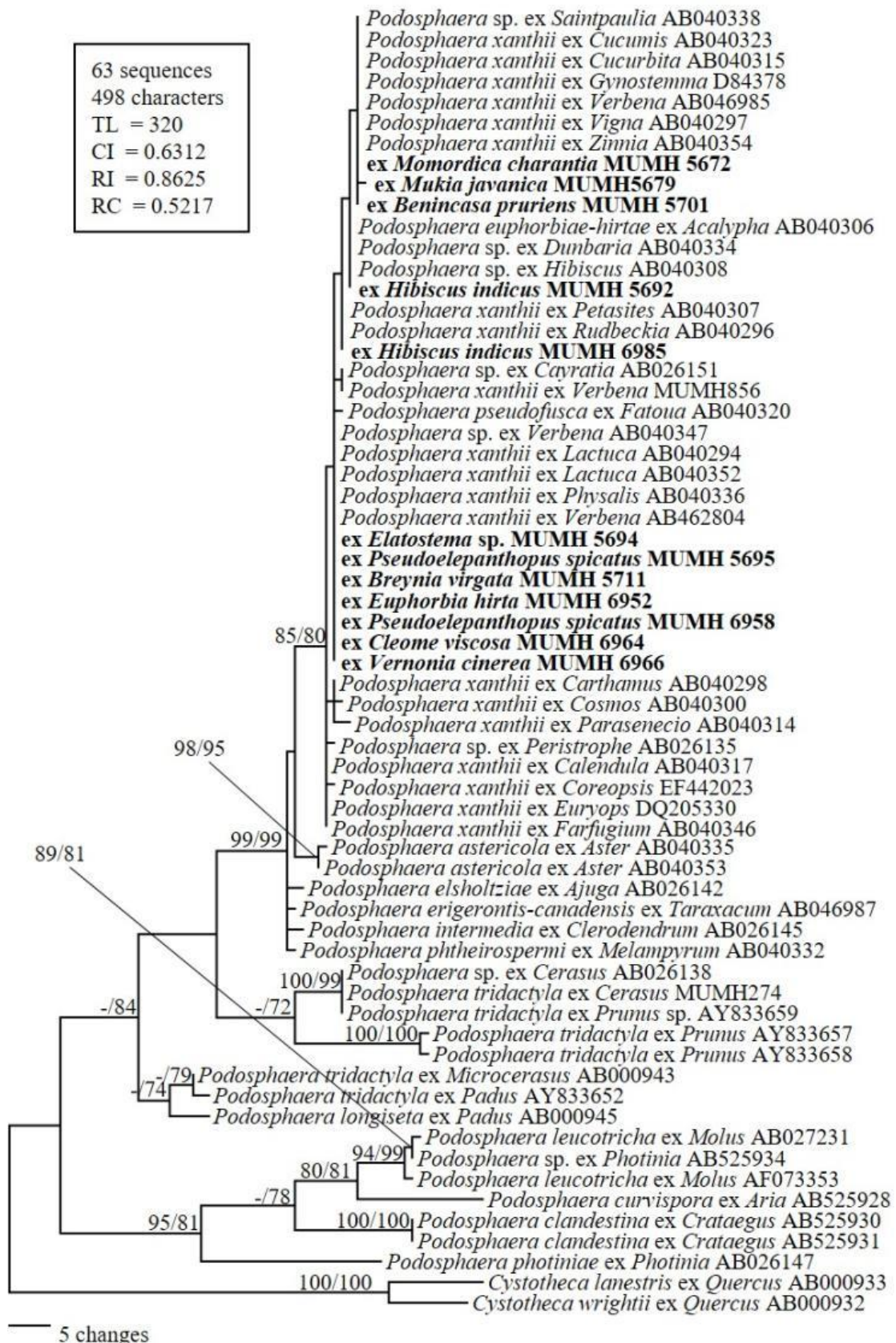
Fig.8. – Phylogenetic analysis of the ITS rRNA gene for 63 sequences of the *Podosphaera* spp. This tree is one of the 12,082 equally parsimonious trees with 283 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

Meeboon (BO 22698, TSU-MUMH 5672); Bali, 20 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22822, TSU-MUMH 6963); on *Mukia javanica*, West Java, Bandung, Situ Patenggang, Ciwidey, 13 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22705, TSU-MUMH 5679); on *Pseudoelephantopus spicatus*, Bali, Gitgit waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22721, TSU-MUMH 5695); Bali, Ubud Monkey Forest, 18 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22817, TSU-MUMH 6958); on *Vernonia cinerea*, Bali, Ubud Monkey Forest, 20 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (MUMH 6966).

Fibroidium balsaminae (Rajd.) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiphales (Powdery Mildews): 167. 2012 — Fig. 9.

≡ *Oidium balsaminae* Rajd., Mycopathol. Mycol. Appl. 28: 150. 1966.

= *Sphaerotheca balsaminae* auct. p.p.
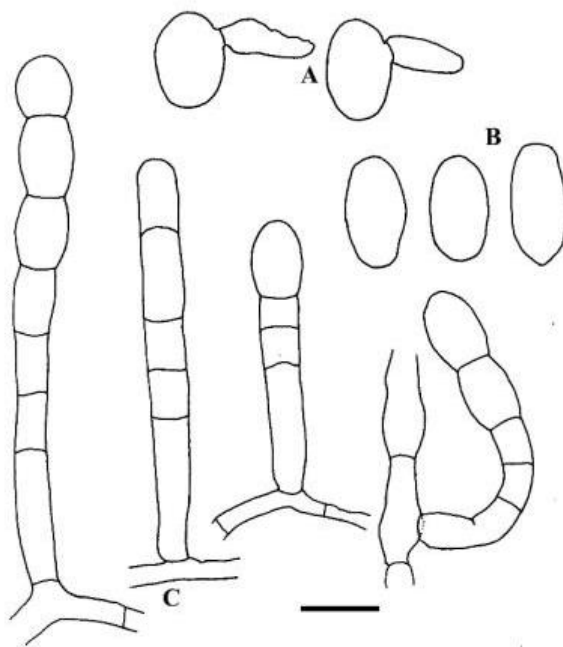
Fig.9. – *Fibroidium balsaminae* on *Impatiens balsamina* A: Germ tubes. B: Conidia. C: Conidiophores. *Bar*: 20 μm.

*Colonies on leave*s amphigenous, effuse, subevanescent. *Hyphae* hyaline 3–5 μm wide; *hyphal appressoria* indistinct. *Conidiophores* erect, arising from the top of mother cell, 67–148.5 x 9–13 um, *foot-cells* cylindrical, straight or slightly curved, (25–)30–53.5 x 8–10.5 um, occasionally slightly constricted at the base, followed by 1–2 shorter cells, forming catenescent conidia with crenate edge, containing fibrosin bodies. *Conidia* ellipsoid–ovoid, 27–38.5 × 13.5–21.5 μm, length/width ratio 1.4–2.2. Germ tubes *Fibroidium* type, almost terminal to lateral, short, alobatus.

Host in Indonesia — *Impatiens balsamina* L. (Balsaminaceae).

Specimens examined. on *Impatiens balsamina* L. (Balsaminaceae), INDONESIA, Bali, Baturiti, 17 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22743, TSU-MUMH 5720); Bali, 18 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22818, TSU-MUMH 6959)

Notes — According to Braun & Cook (2012), the present species is widely distributed throughout the world. It is newly added to Indonesian mycoflora in this study. This fungus is closely related to *P. balsaminae* on *Impatiens noli-tangere* L. Although no DNA sequences obtained in this study, however, based on Ito & Takamatsu (2010) and Braun & Cook (2012), the phylogenetic position of fungus on *I. balsamina* collected in Asia was clustered apart from that of *I. noli-tangere*. Thus, *F. balsaminae* is proposed to facilitate the anamorphs collected on this plant.

Tribe Phyllactinieae

Genus *Leveillula*

Leveillula taurica (Lév.) G. Arnaud, Ann. Épiphyt. 7: 94. 1921 — Fig. 10.

= *Oidiopsis sicula* Scal., Atti. Congr. Bot. Palermo: 396.1902.

Other synonyms — See Braun & Cook (2013).

Colonies on leaves amphigenous, effuse to subevansecent or persistent, *mycelium* internal and external. *Hyphae* hyaline 2–7 μm wide, *hyphal appressoria* nipple shaped, lobed to multilobed, coralloid, solitary, 3–7 μm. *Conidiophores* arise from the top of

mother cells, erect, 138.5–270.5 × 3.5–6.5 μm. *Foot-cells* straight, slightly curved, 42–193 × 3–5.5 μm, with basal septum 9–40 μm away from the branching point, followed by 1–2 shorter cells. *Conidia* solitary, with *primary conidia* ovoid-lanceolate, narrowed towards the apex, tips pointed, base rounded, 43.5–67.5 × 13.5–21 μm. *Secondary conidia* cylindrical to subcylindrical, 38–65.5 × 11.5–22 μm. *Germ tubes* alobate, terminal to subterminal, short to long.

Host in Indonesia — *Capsicum frutescens* L., *Cestrum nocturnum* L., *Solanum torvum* Sw. (Solanaceae), *Euphorbia heterophylla* L. (Euphorbiaceae).
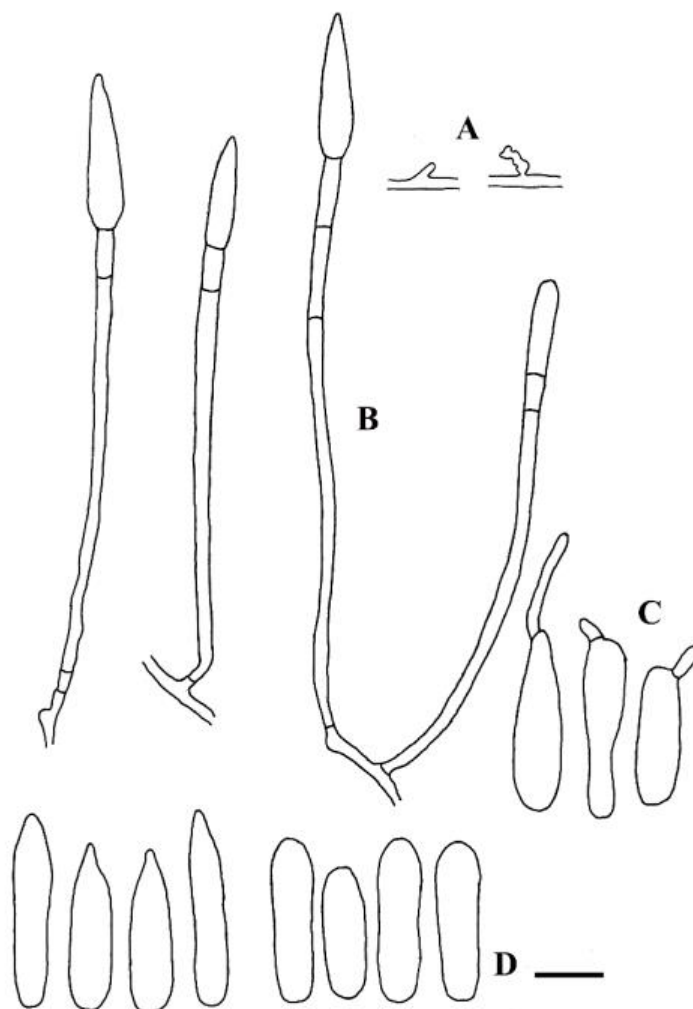
Fig.10. – *Leveilulla taurica*. A: Hyphal appressoria. B: Conidiophores. C: Germ tubes. D: Conidia. *Bar*: 20 μm.

Specimens examined. on *Capsicum fruscens*, INDONESIA, West Java, Bogor,

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Curug Nangka, 10 Sept. 2013, *S.A.S. Siahaan, J. Meeboon* (BO 22693, TSU-MUMH 5668); Bali, Banyar Belok, Belok Village, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22737, TSU-MUMH 5713); on *Cestrum nocturnum*, West Java, Bogor, Curug Nangka, 10 Sept. 2013, *S.A.S. Siahaan & J. Meeboon*, (BO 22695, TSU-MUMH 5669); on *Solanum torvum*, West Java, Bogor, Curug Cigamea, 10 Sept. 2013, *S.A.S. Siahaan & J. Meeboon* (BO 22700, TSU-MUMH 5674); on *S. torvum*, North Sumatra, Pematangsiantar, 15 Jan. 2015, *S.A.S. Siahaan* (MUMH 5780); on *Euphorbia heterophylla*, Bali, Baturiti, 17 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22745, TSU-MUMH 5722); 17 Oct. 2016, *S.A.S. Siahaan, S. Takamatsu* (BO 22812, TSU-MUMH 6953, BO 22815, TSU-MUMH 6956, BO 22824, TSU-MUMH 6965).

Notes — The host plant *Cestrum nocturnum* is newly recorded in this study.

Genus *Phyllactinia*

***Phyllactinia lagerstroemiicola* S.A.S. Siahaan sp. nov.** — Fig. 11.

MycoBank, MB000000

Etymology — Epithet derived from host plant genera.

Similar to asexual morphs of *Phyllactinia lagerstroemiae,* but it differs in having smaller primary conidia and longer foot-cells.

Mycelium internal and external, hypophyllous, forming white patches, effuse, evanescent. *Hyphae* hyaline, 3–6 μm wide; *hyphal appressoria* lobed to multilobed, coral-like, hooked, solitary or in opposite pairs, 6–11 μm. *Conidiophores* arising from external hyphae, on upper part of mother cells, erect, 169–227 × 5–6.5 μm, with basal septum elevated up to 20 μm; *foot-cells* flexuous, sinuous to subhelicoid, 90–173 × 4.5–8 μm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* solitary, dimorphic; *primary conidia* lanceolate, narrowed towards a pointed tip, rounded to almost truncate at the base, 65–83.5 × 15.5–18.5 μm; *secondary conidia* ellipsoid-cylindrical to clavate, subtruncate at the both ends, 64–80.5 × 14.5–20 μm.

Type — on *Lagerstroemia floribunda* Jack. (Lythraceae), INDONESIA, West Java, Bandung, Pendidikan Indonesia University, 21 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (Holotype: BO 22828, Isotype: TSU-MUMH 6970).

Gene sequences (ex-holotype): LC00000 (ITS).

Host — *Lagerstroemia floribunda* Jack., *L. speciosa* (L.) Pers. (Lythraceae).
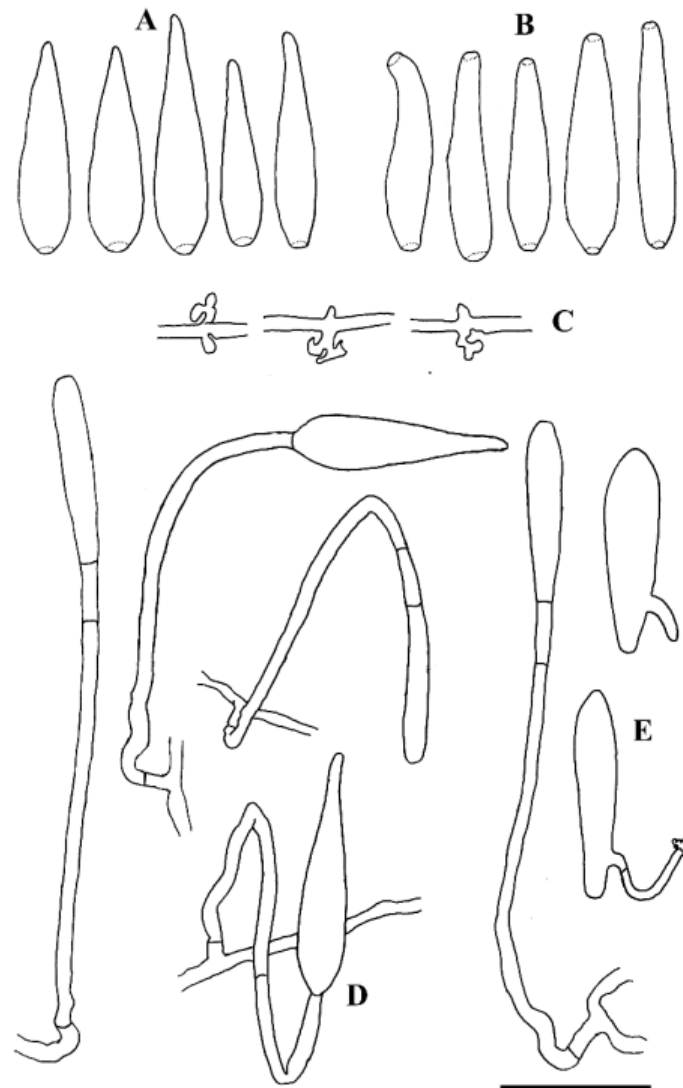
Fig.11. – *Phyllactinia lagerstroemiicola*. on *Lagerstroemia floribunda* and *L. speciosa*. A: Primary conidia. B: Secondary conidia. C: Hyphal appressoria. D: Conidiophores. E: Germ tubes. *Bar*: 50 μm.

Additional specimens examined. on *Lagerstroemia speciosa* (L.) Pers., INDONESIA, West Java, Bandung, Pendidikan Indonesia University, 21 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22829, TSU-MUMH 6971), GenBank
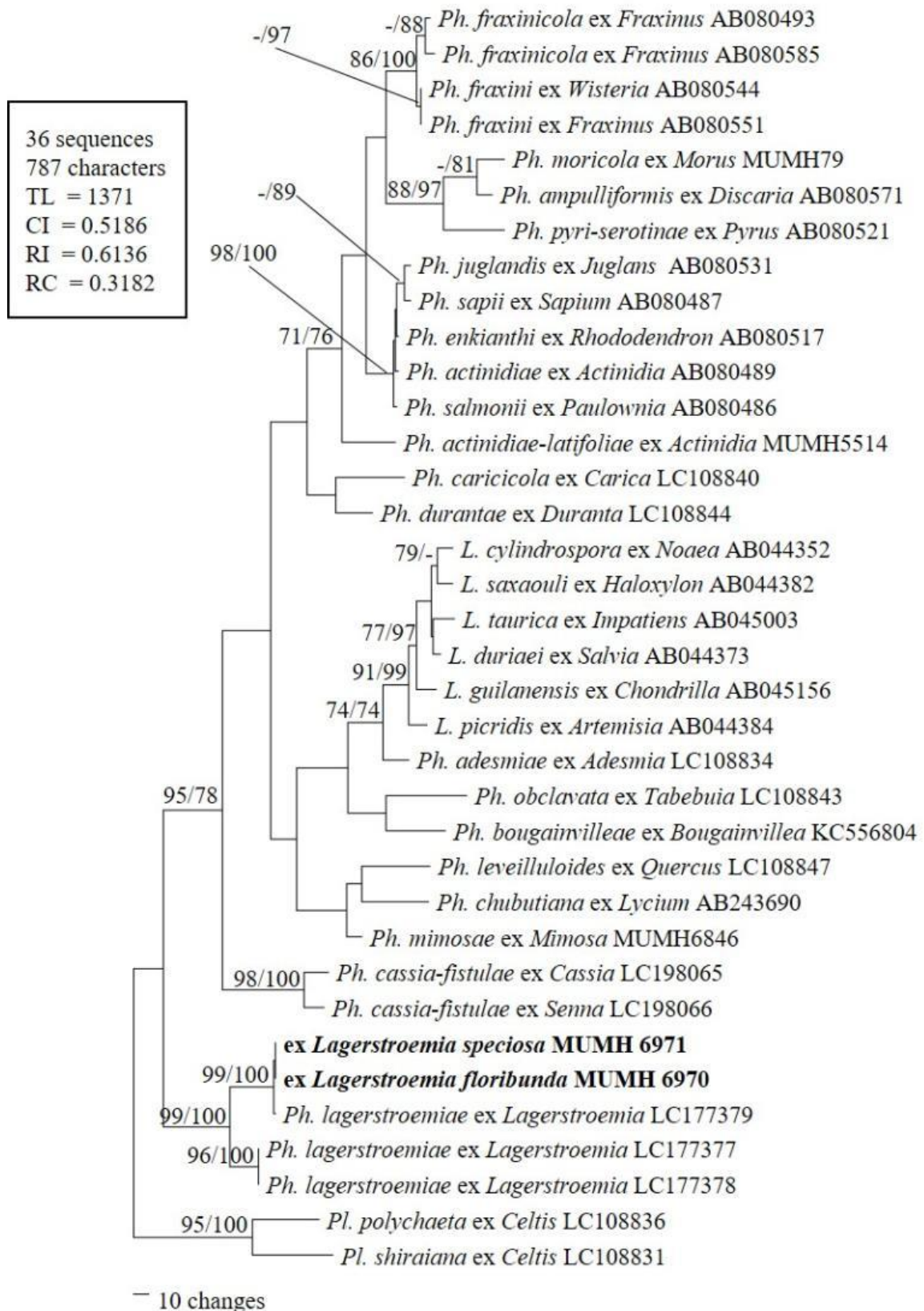
Fig.12. – Phylogenetic analysis of the ITS rRNA gene for 36 sequences of the tribe Phyllactinieae. This tree is one of the 32 equally parsimonious trees with 1,297 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

accession number: LC00000 (ITS). ; THAILAND, Chiang Mai, 20 Jan. 2008, *J. Meeboon* (TSU-MUMH 3342), GenBank accession number: LC 177379 (ITS + 28S) (as *Ph. lagerstroemiae*).

Notes — A morphologically similar species, *Phyllactinia lagerstroemiae*, is proposed by Meeboon and Takamatsu (2017) based on the specimens from Thailand. *Phyllactinia lagerstroemiicola* differs in having shorter primary conidia and longer foot-cells than those of *Ph. lagerstroemiae* (primary conidia (93.5–)99–112.5(–132) × (15.5–)18.5–20.5(–26) μm , foot-cells 110 μm). This result was quite different from the original description by Meebon & Takamatsu (2017) due to incoherent information which appears to be the result of confusement between primary and secondary conidia. In their report, the shape of primary conidia were lanceolate, attenuated towards the tip, while the secondary conidia were clavate and somewhat narrowed at the base. Referring to the drawing of primary and secondary conidia, we hypothesized that the description of both primary and secondary conidia in Meebon & Takamatsu (2017) were swapped. Thus, to confirm our hypothesis, we re-observed the type specimen of *Ph. lagerstromiae,* on *L. macrocarpa* (TSU-MUMH 5750). Our result (data not shown) confirm our hypothesis, that primary conidia of *P. lagerstroemiae* were lanceolate, with attenuated apex and pointed tips with round base, while secondary conidia were marked by its truncated shaped on both edges of conidia, have clavate shape and narrowed at the base. So, based on this result, the revised measurement for *P. lagerstroemiae* is (93.5–)99–112.5(–132) × (15.5–)18.5–20.5(–26) μm for primary conidia, and (64.5–)74–87.5(–92.5) × (26–)29–30(–33.5) μm for secondary conidia. The new measurement result is based on the fact that they observed dried materials, however, did not multiply the measurement results with Blumer's factor (personal communication). Moreover, these two species are phylogenetically distinguishable on the basis of those ITS sequences. See phylogeny part.

Phyllactinia moricola (Henn.) Homma, Trans. Sapporo Nat. Hist. Soc. 11: 174. 1930 — Fig. 13.

≡ *Phyllactinia suffulta* var. *moricola* Henn., Bot. Jahrb. Syst. 28: 271. 1901.

≡ *Phyllactinia moricola* (Henn.) Sawada, Rept. Dept. Agric. Gov. Res. Inst. Formosa 49: 84. 1930.

= *Phyllactinia suffulta* f. *moricola* Jacz. Karmanny opredelitel' gribov. Vyp. 2. Muchnisto-rosyanye griby: 434. 1927.

= *Phyllactinia corylea* auct. p.p.

= *Phyllacitnia guttata* auct. p.p.

= *Phyllactinia suffulta* auct. p.p.

= *Ovulariopsis moricola* Delacr., Bull. Trimestriel Soc. Mycol. France 19:345. 1903.
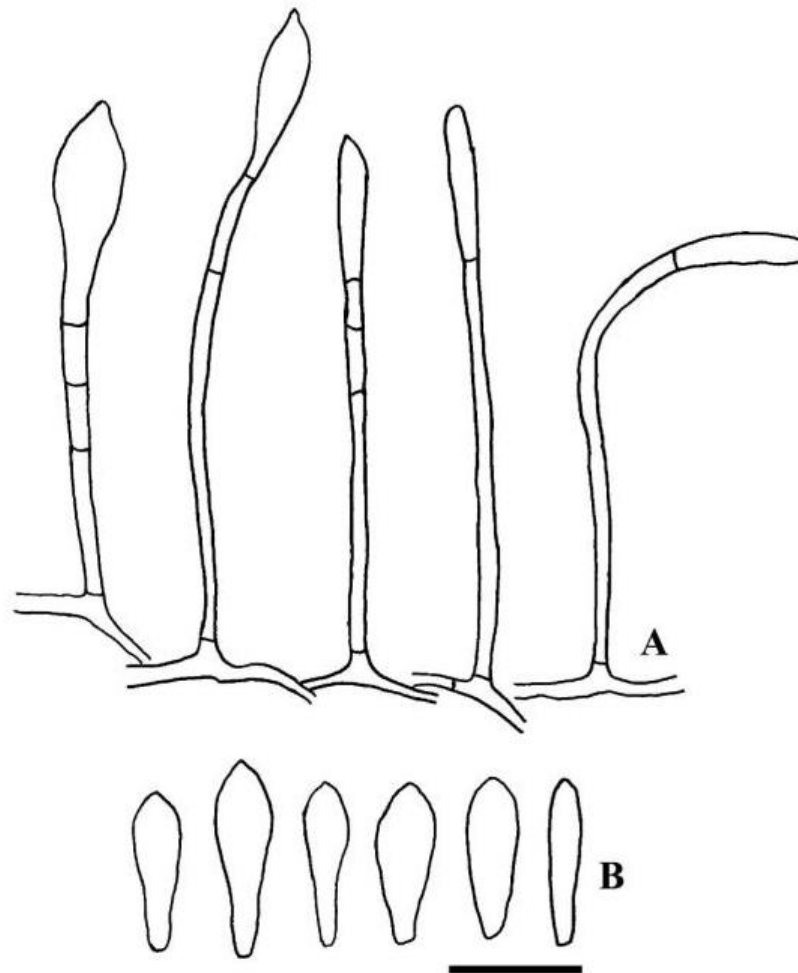
Fig.13. – *Phyllactinia moricola* on *Morus* sp. A: Conidiophores. B: Conidia. *Bar*: 50 μm.

Colonies on leaves epiphyllous, forming thinly effused or occasionally conspicuous thick white patches, *mycelium* internal and external. *Hyphae* hyaline up to 5 μm wide, *hyphal appressoria* nipple to hook-shaped, sometimes lobed, solitary, up to 9 μm. *Conidiophores* arise from the top of mother cells, 1–2 on a single cells, erect, 109–274.5(–320) × 4–6 μm. *Foot-cells* straight, 65–215 × 4–5.5 μm, with basal septum 2–14 μm away from the branching point, followed by 1–3 shorter cells. *Conidia* solitary, clavate non papillate but narrowed at the apex, 62–84.5 × 16.5–23 μm.

Host in Indonesia — *Morus alba* L.*, Morus* sp. L. (Moraceae).

Specimens examined. on *Morus alba*, INDONESIA, West Java, Bandung, Ciwidey, Situ Patenggang, 13 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22709, TSU-MUMH 5683); West Java, Cibodas Botanical Garden, 20 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22852, TSU-MUMH 6994); on *Morus* sp., North Sumatra, Pematangsiantar, 15 Jan. 2015, *S.A.S. Siahaan* (MUMH 5778).

Phyllactinia poinsettiae Siahaan & S. Takam., Mycoscience 56: 581. 2015.

Description and Illustration — See S.A.S. Siahaan et al. (2015)

Host — *Euphorbia pulcherrima* Willd. ex Klotzsch (Euphorbiaceae)

Specimen examined. on *Euphorbia pulcherrima*, INDONESIA, Bali, Kintamani, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (Isotype TSU-MUMH 5709); West Java, Bogor, Curug Cigamea, 10 Sept. 2013, *S.A.S. Siahaan, J. Meeboon & S. Takamatsu* (TSU-MUMH 5670); West Java, Bandung, Rawa Upas, 14 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (TSU-MUMH 5689).

Notes — This is an endemic species of the genus *Phyllactinia* in Indonesia.

Tribe Golovinomyceteae

Genus *Golovinomyces*

Golovinomyces sonchicola U. Braun & R.T.A. Cook, in Cook & Braun, Mycol. Res. 113: 629. 2009 — Fig. 14.

    = *Erysiphe cichoracearum* f. *sonchi* Jacz. Karmanny opredelitel' gribov. Vyp. 2. Muchnisto-rosyanye griby: 210. 1927.

    = *Erysiphe cichoracearum* auct. p.p.

    = *Golovinomyces cichoraearum* auct. p.p.

    = *Oidium sonchi-arvensis* Sawada, Bull. Dept. Agric. Gov. Res. Inst. Formosa 24: 48. 1927.
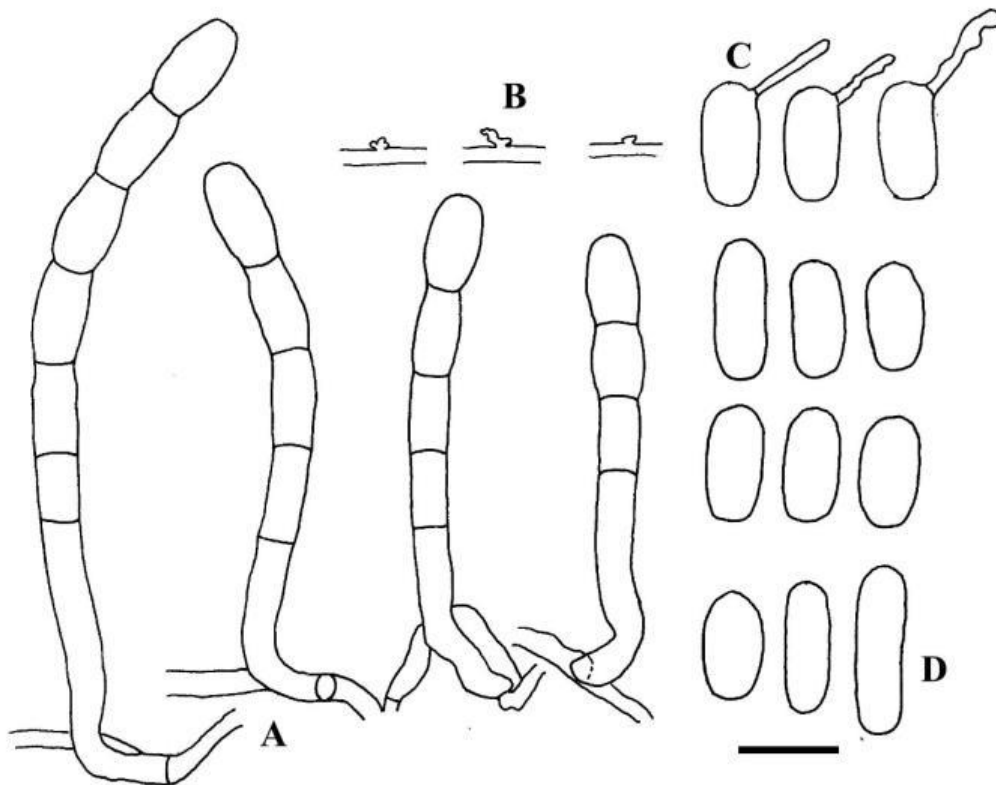
Fig.14. – *Golovinomyces sonchicola* on *Sonchus* sp. A: Conidiophores. B: Hyphal appressoria. C: Germ tubes. D: Conidia. *Bar*: 20 μm.
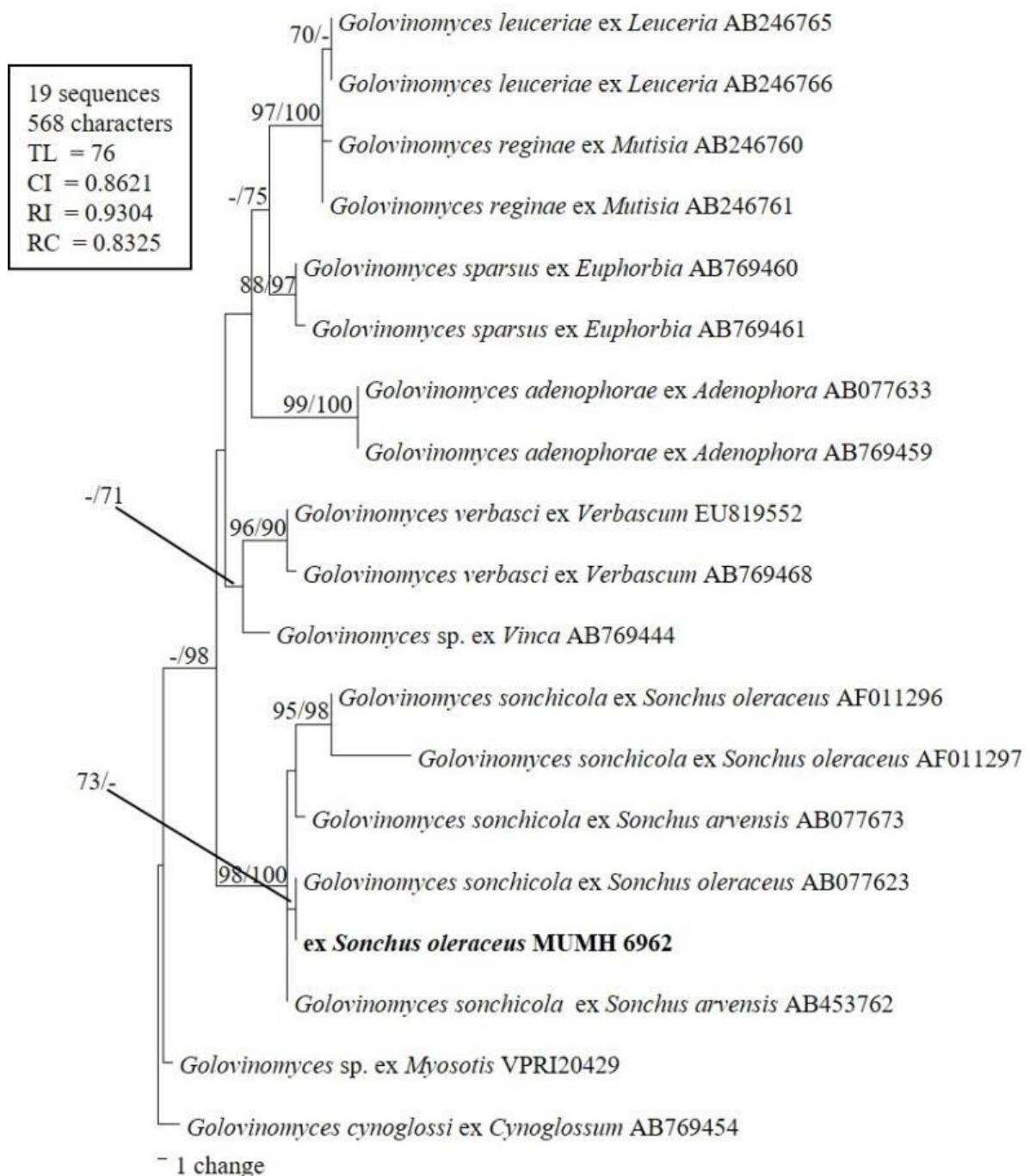
Fig.15. – Phylogenetic analysis of the ITS rRNA gene for 19 sequences of the *Golovinomyces* spp. This tree is one of the 18 equally parsimonious trees with 75 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

Colonies on leaves amphigenous, effuse or patches, subevanescent. *Hyphae* hyaline 3–6 µm wide; *hyphal appressoria* well developed, nipple shaped to simply lobed,

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solitary, up to 5 μm. *Conidiophores* arising from the lateral side of mother cells, bend upwards, 73–141 × 8–13 μm; *foot-cells* curved, 24–58.5 × 6.5–10.5 μm, followed by 1–3 shorter cells, forming conidia singly. *Conidia* ellipsoid-cylindrical, without fibrosin bodies, 24–37 × 11–17 μm, length/width ratio 1.7–2.6.

Host in Indonesia — *Sonchus arvensis* L.*, S. oleraceus* L. (Asteraceae).

Specimens examined. on *Sonchus arvensis*, INDONESIA, Bali, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboom & S. Takamatsu* (BO 22738, TSU-MUMH 5715); on *S. oleraceus*, Bali, 20 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22821,TSU-MUMH 6962).

Notes — The morphological characteristics of this fungus were typical *Euoidium*-type, the anamorph of the genus *Golovinomyces*. The fungus from Indonesia measurement and characteristics were in good agreement with that of *G. sonchicola* in Braun & Cook (2012). To confirm the result, the sequence of rDNA ITS region from a representative specimen was aligned with other *Golovinomyces* sequences retrieved from the DNA database and analysed those phylogenetic relationships. The result showed that the sequence nested with other *G. sonchicola* with strong bootstarp support (98/100 ML/MP values) (Fig. 15). Both morphology and phylogenetic analysis indicate this fungus is *G. sonchicola*. This species *G. sonchicola* is new to South East Asia including Indonesia.

Tribe Erysipheae

Genus *Erysiphe*

Erysiphe baliensis S.A.S. Siahaan and S. Takam., Mycoscience 57: 37. 2016.

Description and illustration — See S.A.S. Siahaan et al. 2016a.

Host — *Gliricidia sepium* (Jacq.) Kunth ex Walp. (Fabaceae), *Wisteria japonica* Siebold & Zucc. (Fabaceae).

Specimens examined. on *Gliricidia sepium*, INDONESIA, Bali, Banyar Belok, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (isotype MUMH

5712); Bali, Gitgit waterfall area, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, & S. Takamatsu* (MUMH 5705).

Notes — The distribution of present species is disjunct distribution, Japan and Indonesia.

Erysiphe caricae-papayae Meeboon & S. Takam. Mycosphere 8(9): 1408. 2017.

On *Carica papaya* — Fig. 16.
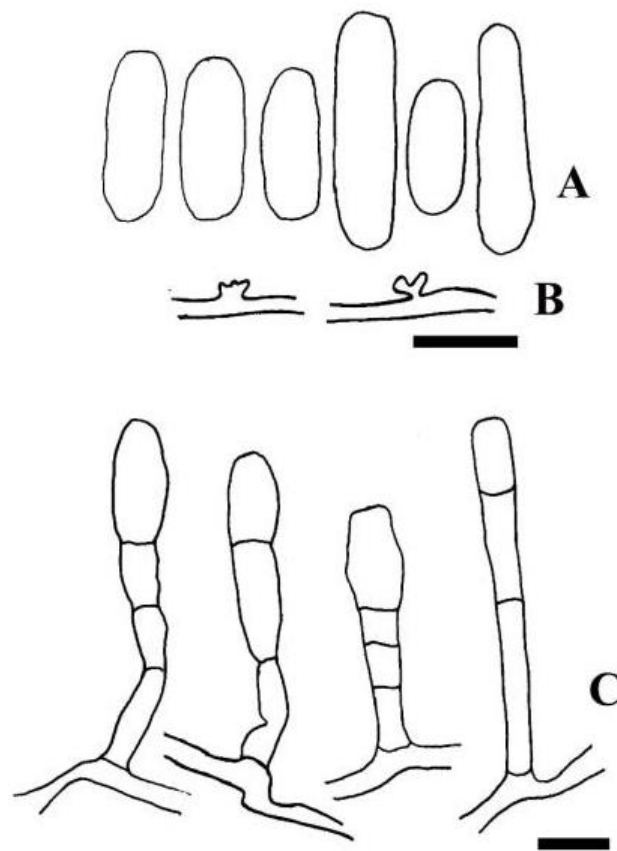
Fig.16. – *Erysiphe aquilegiae* on *Carica papaya*. A: Conidia. B: Hyphal appressoria. C: Conidiophores. *Bars*: 20 μm.

*Colonies on leave*s amphigenous, mostly hypophyllous, visible as irregular white patches, evanescent or persistent. *Hyphae* hyaline, 3–6.5 μm wide; *hyphal appressoria* well-developed, lobed, solitary, 2–6.5 μm. *Conidiophores* erect, arising centrally or slightly laterally from the mother cell, 63.5–140 × 5–8.5 μm; *foot–cells* cylindrical, straight or slightly curved at the base, 13.5–44 × 5–8.5 μm long, followed by 1–2 shorter cells,

ex *Datura* sp. DNA230 Japan
ex *Lycopersicon esculentum* BPI746433 USA
ex *Lycopersicon esculentum* DNA231 Japan
ex *Lycopersicon esculentum* MUMH66 Japan
ex *Codiaeum variegatum* MUMH 5699
ex *Codiaeum varigatum* MUMH 5704
ex *Codiaeum varigatum* MUMH 5703
ex *Nicotiana tabacum* DNA24 Japan
ex *Caltha palustris* MUMH4330 Japan
ex *Caltha palustris* MUMH4353 Japan
ex *Cotyledon orbiculata* EU185640 UK
ex *Crassula* sp. EF434394 UK
ex *Dudleya palmeri* EU185639 UK
ex *Echeveria* sp. EU185636 UK
ex *Echeveria gibbiflora* EU185638 UK
ex *Echeveria gibbiflora* EU185637 UK
ex *Elsholtzia ciliata* MUMH167 Japan
ex *Hydrangea macrophylla* MUMH71 Japan | *Ps. hortensiae*
ex *Hylotelephium erythrostictum* JX173289 Korea
ex *Kalanchoe blossfeldiana* JX173288 Korea
ex *Nierenbergia* sp. MUMH1286 Japan
ex *Pilea pumila* MUMH2987 Japan | *E. pileae*
ex *Saxifraga stolonifera* MUMH4037 JPN
ex *Sedum aizoon* MUMH2575 Russia
ex *Sedum alboroseum* EU185641 Hungary
ex *Sedum pallescens* MUMH2577 Russia | *E. sedi*
ex *Sedum* sp. JX173290 Korea
ex *Sedum* sp. MUMH2576 Russia
ex *Solanum betaceum* MUMH 5776
ex *Solanum betaceum* AB911568
ex *Solanum betaceum* AB473221
ex *Chloranthus serratus* MUMH202 Japan
ex *Cimicifuga simplex* TPU495 Japan
ex *Clematis* sp. DNA558 Japan
ex *Ranunculus japonicus* MUMH287 Japan
ex *Aquilegia* sp. BCRU00359 Argentina
ex *Aquilegia vulgaris* MUMH2456 Argentina
ex *Boronia megastigma* VPRI18337 Tasmania
ex *Chamaesyce nutans* MUMH4646 Japan
ex *Chelidonium majus* MUMH3912 Japan
ex *Circaea lutetiana* AB104517 Iran
ex *Clematis integrifolia* AF154322 Switzerland
ex *Clematis terniflora* MUMH98 Japan
ex *Knautia arvensis* MUMH2571 Ukraine
ex *Laurus nobilis* MUMH2559 Ukraine
ex *Macleaya cordata* TPU1873 Japan
ex *Macleaya microcarpa* JQ681217 Poland
ex *Ranunculus peduncularis* MUMH2513 Argentina
ex *Carica papaya* MUMH 5667
ex *Clematis apiifolia* MUMH277 Japan
ex *Clematis stans* MUMH293 Japan
ex *Delphinium* sp. AY452802 Australia
ex *Delphinium* sp. AY452803 Australia
ex *Baptisia australis* MUMH897 Japan
ex *Sophora flavescens* MUMH89 Japan
ex *Arabidopsis thaliana* FJ548627 Korea
ex *Brassica rapa* EU140958 Italy
ex *Mangifera indica* MUMH3268 Thailand
ex *Magnolia obovata* JX235964 Korea
ex *Magnolia obovata* MUMH5227 Japan

96/93
99/99
100/99
100/100
98/100
94/97
88/91
-/83

60 sequences
524 characters
TL = 106
CI = 0.8396
RI = 0.8982
RC = 0.7542

— 1 change

Fig.17. – Phylogenetic analysis of the ITS rRNA gene for 60 sequences of the *Erysiphe* spp. (including two *E. magnoliae* as outgroup sequences). This tree is a single parsimonious tree with 106 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

forming conidia singly. *Conidia* doliiform–cylindrical, without fibrosin bodies, subtruncate at the both ends, 25.5–42 × 9–16.5μm, length/width ratio 2–4.

Host in Indonesia — *Carica papaya* L. (Caricaceae)

Specimen examined. on *Carica papaya*, INDONESIA, West Java, Bogor, Curug Nangka, 10 Sept. 2013, *S.A.S. Siahaan & J. Meeboon* (BO 22694,TSU-MUMH 5667).

***Erysiphe celtidi-timorensis* S.A.S.** Siahaan *sp. nov.* — Mycobank MB 000000; Fig. 18.

Etymology —Named after the host species from which it was observed, *Celtis timorensis*. Morphologically close to *E. kusanoi* and *E. michikoae*, but readily distinguishable from *E. kusanoi* by having shorter foot-cells and differs from *E. michikoae* by having alobate germ-tubes.

Colonies on leaves amphigenous, appear by forming irregular patches, white, evanescent. *Hyphae* hyaline 2–5 μm wide, *hyphal appressoria* well-developed, lobed to multilobed, solitary or occasionally in opposite pairs, 4–7 μm. *Conidiophores* erect, arising from the upper part of or laterally from the mother cell, 42–74 × 6.5–10 μm; *foot–cells* curved at the base, 17–36 × 5–8 μm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* doliiform–cylindric, without fibrosin bodies*,* subtruncate at the both ends, 29–41 × 11–17 μm, length/width ratio 2–3.4; *germ-tubes* sub-terminal, short, aseptate, alobate.

Host in Indonesia — *Celtis timorensis* Span. (Cannabaceae)

Type — On *Celtis timorensis* Span. (Cannabaceae), INDONESIA, Bali, Gitgit Waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, & S. Takamatsu* (Holotype: BO 22719, Isotype: TSU-MUMH 5693).

Gene sequences (ex-holotype) — LC00000 (ITS), LC000000 (28S).

Specimen examined. See type material.

Notes — The morphology of the fungus on *Celtis timorensis* of Indonesia differs from that of *E. kusanoi*, in which the later fungus has longer foot cell 20–60 × 6–10 μm with conidiophore up to 90 μm (Braun & Cook, 2012). In addition, *E. michikoae* differs from the current fungus by having longer conidiophores (50–) 65–97 (–100) × (5.5–) 6.5–9 (–10.5) μm with 2–3 following cells. And also, its conidial shape were oval, ellipsoid

or cylindric and having germ tube with multi-lobed terminal end (Meeboon et al. 2013a).
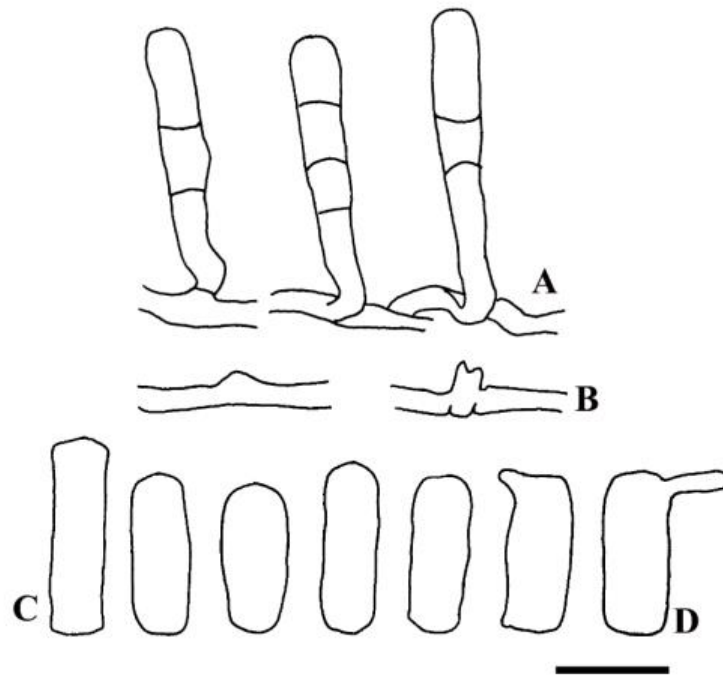
Fig.18. – *Erysiphe celtidi-timorensis* on *Celtis timorensis*. A: Conidiophores. B: Hyphal appressoria. C: Conidia. D: Germ tube. *Bar*: 20μm.
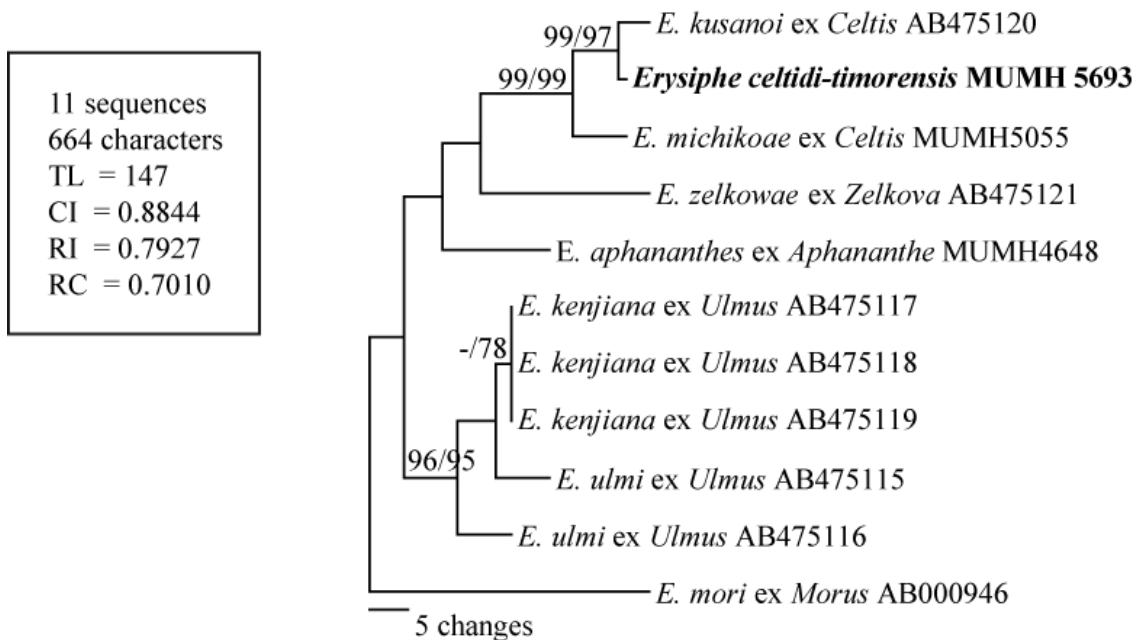
Fig.19. – Phylogenetic analysis of the ITS rRNA gene for 11 sequences of the *Erysiphe* sect. *Uncinula*. This tree is a single parsimonious tree with 145 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

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Erysiphe javanica Meeboon & S. Takam., Mycotaxon 120: 191. 2013

Description & Illustrations — Meeboon et al. (2012b).

Host — *Castanopsis javanica* (Blume) A. DC. (Fagaceae).

Specimen examined. on *Castanopsis javanica*, West Java, Cibodas, Cibodas Botanical Garden, 14 March 2011, *Meeboon J, Hidayat I & Takamatsu S.* (isotype:TSU-MUMH 5153); West Java, Cibodas, Cibodas Botanical Garden, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22847,TSU-MUMH 6989, BO 22854,TSU-MUMH 6996).

Erysiphe necator Schwein, Trans. Amer. Philos. Soc. II, 4: 270. 1834 — Fig. 20.

≡ *Uncinula necator* (Schwein.) Burrill, in Ellis & Everh., North Amer. Pyrenomyc: 15. 1892.

= *Erysiphe tuckeri* Berk., J. Hort. Soc. London 9: 66. 1855.

= *Sphaerotheca castagnei* var. *vitis* Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 79.1870.

= *Uncinula americana* Howe, J. Bot., N.S., 1: 170. 1872.

= *Uncinula spiralis* Berk. & M.A. Curtis, Grevillea 4:159.1876.

= *Uncinula spiralis* var. *racemosum* Thum., Pilze des Weinst.: 12. 1878.

= *Oidium tuckeri* Berk., Gard. Chron. 7: 779. 1847.

≡ Acrosporium tuckeri (Berk.) Sumst., Mycologia 5: 58. 1958.

≡ Acrosporium tuckeri (Berk.) Subram., Hyphomycetes (New Delhi): 840. 1971.

≡ *Pseuoidium tuckeri* (Berk.) Y.S. Paul & J.N. Kapoor, Indian Phytopathol. 38(4):763. 1986.

Colonies on leaves amphigenous, appear by forming irregular patches, effuse, persistent or evanescent. *Hyphae* hyaline *3*–5 μm wide. *Conidiophores* erect, arising from upper part of mother cell, 64–93 × 6.5–7.5 μm; *foot–cells* curved at the base, 24.5–32.5 × 5–7.5 μm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* ovoid–

doliiform, 26–42 × 11.5–15.5 µm, length/width ratio 2–3.3(–3.7); *germ-tubes* terminal or sub-terminal, short to medium, with simple or lobatus apices.
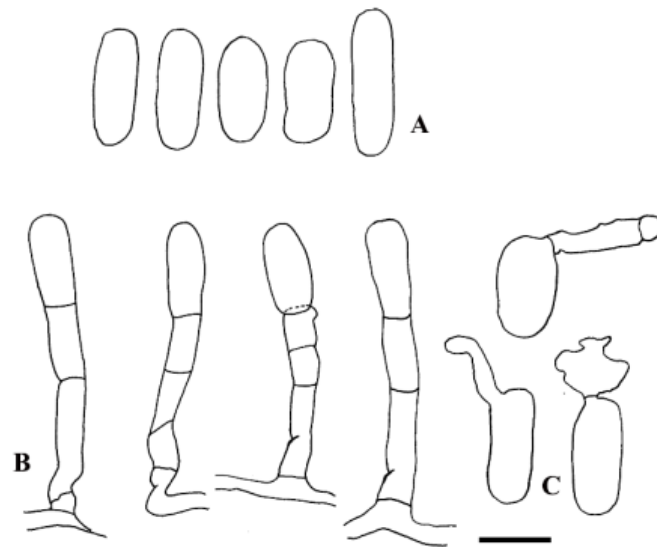
Fig.20. – *Erysiphe necator* on *Vitis vinifera.* A: Conidia. B: Conidiophores. C: Germ tube. *Bar*: 20 µm.
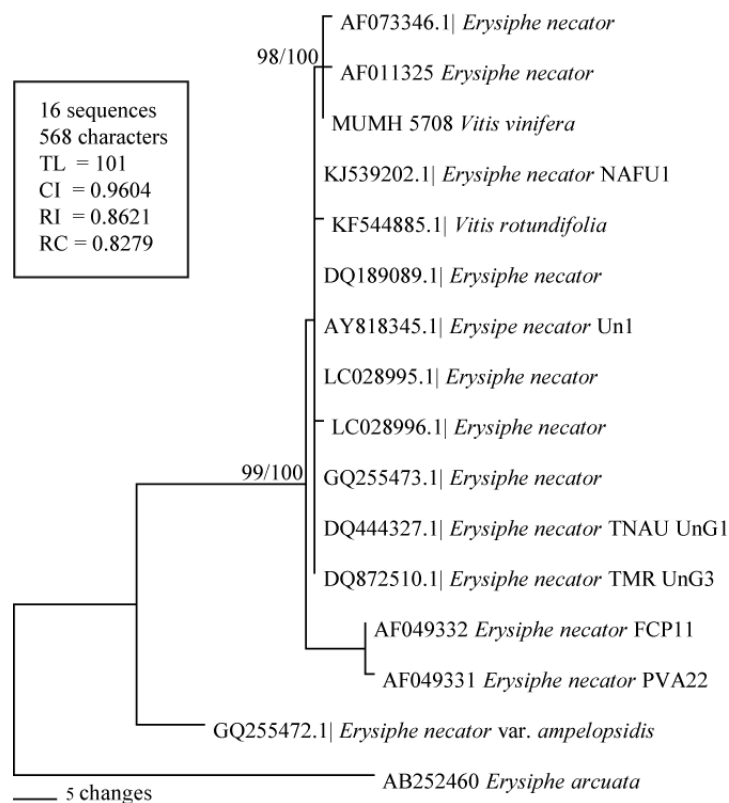
Fig.21. – Phylogenetic analysis of the ITS rRNA gene for 16 sequences of the *Erysiphe necator* s. lat. This tree is one of the seven equally parsimonious trees with 99 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

Erysiphe pseudolonicerae (E. S. Salmon) U. Braun & S. Takam., Schlechtendalia 4: 12. 2000 — Fig. 22.

≡ *Microsphaera alni* var. *pseudolonicerae* E.S. Salmon, Ann. Mycol. 6: 4. 1908.

≡ *Microsphaera pseudolonicerae* (E.S. Salmon) S. Blumer, Beitr. Krypt.–Fl. Schweiz 7(1): 351. 1933.

Colonies on leaves amphigenous, forming white patches, effuse to dense, persistent. *Hyphae* hyaline, 3–6 µm wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in oppiste pairs, 3–8 µm. *Conidiophores* arising from the upper part of mother cells, erect, 50–114 × 6.5–8 µm; *foot-cells* straight or curved, mildly flexuous at the base, uniform throughout in width, 19–54.5 × 5–8 µm, followed by 1–3 shorter cells, forming conidia singly. *Conidia* produced solitary, ellipsoid-ovoid, without fibrosin bodies, rounded to subtruncate at the both ends, 31.5–44 × 14.5–16 µm, length/width ratio 2.3–3.

Host in Indonesia — *Cyclea barbata* Miers (Menispermaceae)

Specimen examined. on *Cyclea barbata*, INDONESIA, Bali, Pandak Badung Village, 17 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22813,TSU-MUMH 6954).

Notes — *Erysiphe pseudolonicerae* had been known on the plant genus *Cocculus* in Far East Asia, Japan and Korea. Recently, the host plant *Cyclea barbata,* which is the same as that of Indonesian *E. pseudolonicerae*, was reported from Thailand (Meeboon & Takamatsu 2017). In this study, this species is newly added to Indonesian mycoflora.

Erysiphe quercicola S. Takam. & U. Braun, in Takamatsu et al., Mycol. Res. 111: 819. 2007

= *Pseudoidium anacardii* (Noack) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiplales (Powdery Mildews): 497. 2012.

= *Oidium bauhiniae* G.J.M. Gorter & Eicker, Mycotaxon 22: 39 (1985).

≡ *Pseudoidium bauhiniae* (G.J.M. Gorter & Eicker) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiphales (Powdery Mildews): 598. 2012.
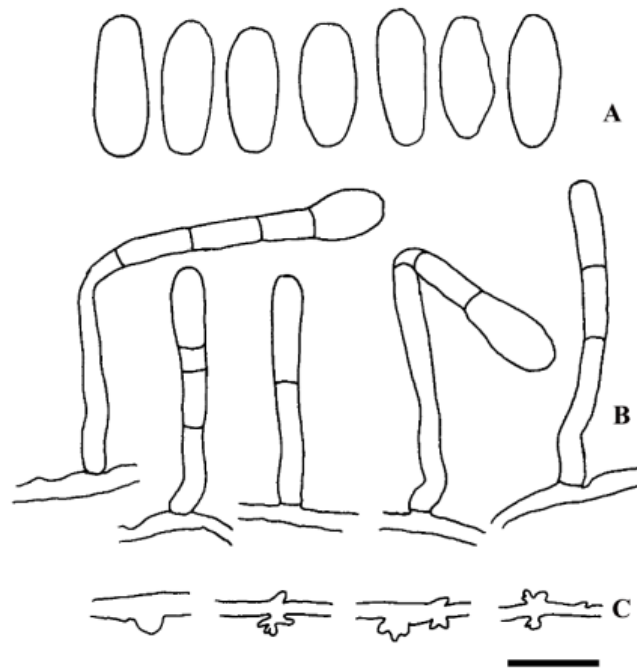
Fig.22. – *Erysiphe pseudolonicerae* on *Cyclea barbata*. A: Conidia. B: Conidiophores. C: Hyphal appressoria. *Bar*: 20 μm.

= *Oidium nephelii* Hadiw. ex U. Braun, Mycotaxon 25: 267. 1986.

≡ *Pseudoidium nephelii* (Hadiw. ex U. Braun) U. Braun & R.T.A. Cook, Taxonomic Manual of the Erysiphales (Powdery Mildews): 613. 2012.

= *Oidium nephelii* Hadiw., Landbouw (Landbouwkundig maandblad voor Indonesie) 22(5–6): 253. 1950.

≡ *Oidium erysiphoides* f. *nephelii* (Hadiw.) J.M. Yen, Rev. Mycol. 31(4): 286 (1966).

on *Bauhinia purpurea* L. (Fabaceae),

Description and illustration — See Siahaan et al. (2016c).

Specimen examined. on *Bauhinia. purpurea* (Fabaceae), INDONESIA, Bogor, Botani Square, 11 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22704,TSU-MUMH 5678); West Bandung, Parongpong, 13 Mar. 2011, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* BO22673,TSU-MUMH5137); Bogor, Botani Square, 14 Mar 2011, *S.A.S. Siahaan, I. Hidayat, K.*

Kramadibrata, J. Meeboon & S. Takamatsu (MUMH 5154).

Fig.23. – Symptoms of *E. quercicola* on several new hosts. A: on upper side leaves of *Brucea javanica*. B: on lower side leaves of *Brucea javanica*. C: on lower side leaves of *Ficus variegata*. D: on stems of *Ficus variegata*. E. on upper side leaves of *Ficus variegata*. F. on upper side leaves of *Vernicia montana*.

on *Brucea javanica javanica* (L.) Merr. (Simaroubaceae) — Fig. 24.

Colonies on leaves amphigenous, forming white patches, effuse to dense, persistent. *Hyphae* hyaline, 2.5–5 µm wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in opposite pairs, 1.5–4 µm. *Conidiophores* arising from the upper part of mother cells, erect, 40–89.5 × 4.5–9 µm; *foot-cells* straight, uniform throughout in width, 17–37.4 × 4–7.5 µm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* produced solitary, ellipsoid-doliiform, without fibrosin bodies, rounded to subtruncate at the both ends, 24–42.5 × 11.5–24µm, length/width ratio 1.6–2.6(–3.5); *germ-tubes* terminal to sub-terminal, showing longitubus pattern, moderate to long, without terminal appressorium.
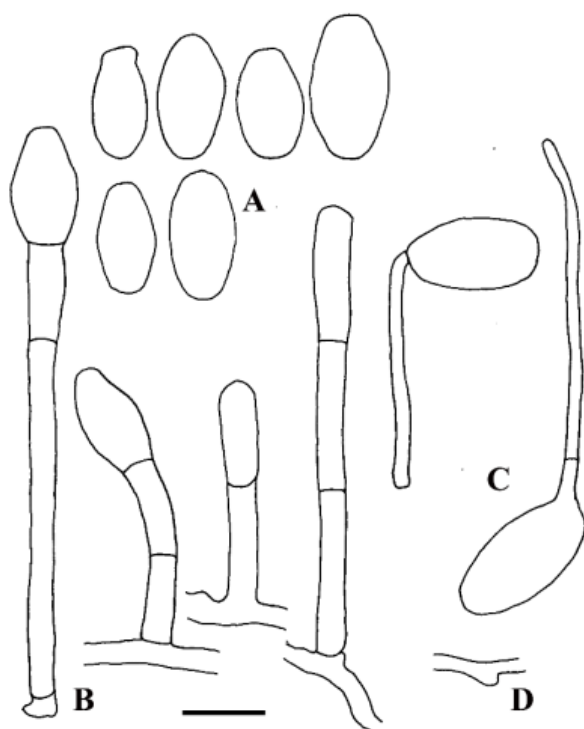
Fig.24. – *Erysiphe quercicola* on *Brucea javanica* A: Conidia. B: Conidiophores. C: Germ tubes. D: Hyphal appressoria. *Bar*: 20 μm.

Specimen examined. on *Brucea javanica*, INDONESIA, West Java, Cibodas, Cibodas Botanical Garden, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22850,TSU-MUMH 6992).

on *Durio zibethinus* L. (Malvaeae)

Description and illustration — See Siahaan et al. (2016c).

Specimen examined. on *Durio zibethinus*, Bogor, Curug Cigamea, 11 Sept. 2013, *S.A.S. Siahaan & J. Meeboon* (BO 22701,TSU-MUMH5675; Palembang, 10 Mar. 2011, *S.A.S. Siahaan, Suparman, A. Umayah, J. Meeboon & S. Takamatsu* (MUMH5155).

on *Ficus variegata* Blume (Moraceae) — Fig. 25.

Colonies on leaves amphigenous, forming white patches, effuse to dense, persistent, causing yellowish discoloration on infected areas; *colonies* on the young stems noticeable. *Hyphae* hyaline, 3–6 μm wide; *hyphal appressoria* well developed, lobed to multilobed,

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single or in opposite pairs, 2.5–6.5 μm. *Conidiophores* arising from the upper part of mother cells, erect, 42.5–118.5 × 5–9.2 μm; *foot-cells* straight, uniform throughout in width, 21–61.5 × 4.5–8 μm, followed by 1–2(–3) shorter cells, forming conidia singly. *Conidia* produced solitary, ellipsoid-doliiform, without fibrosin bodies, subtruncate at the both ends, 30–37 × 12–21.5 μm, length/width ratio 1.7–2.7.

Specimen examined. on *Ficus variegata*, INDONESIA, West Java, Bandung, Padjajaran University, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22833,TSU-MUMH 6975).

on *Nephelium lappaceum* L. (Sapindaceae)

Description and illustration — See Siahaan et al. (2016c).

Specimen examined. on *Nephelium lappaceum,* Pematangsiantar, 15 Jan. 2015, *S.A.S. Siahaan* (TSU-MUMH 5779); Samosir Island, Tuk tuk, 5 Jan. 2016, *S.A.S. Siahaan,* (TSU-MUMH 6552); Samosir Island, Tuk tuk, 5 Jan 2016, *S.A.S. Siahaan* (TSU-MUMH6557).
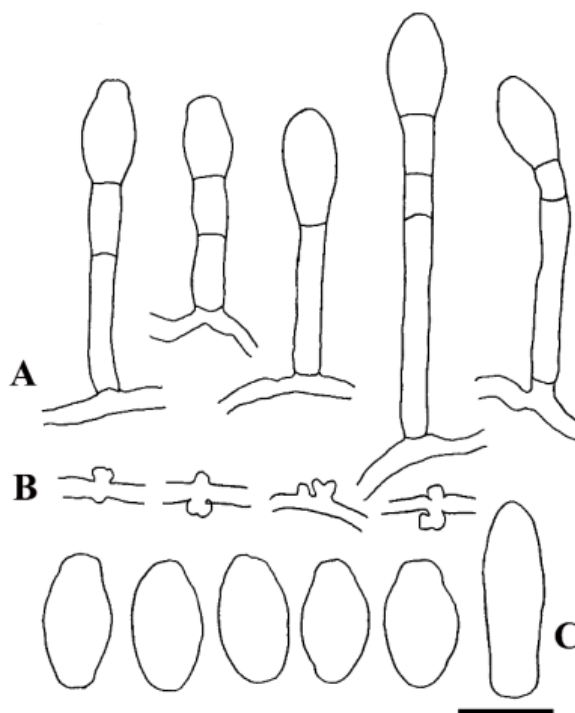
Fig.25. – *Erysiphe quercicola* on *Ficus variegata.* A: Conidiophores. B: Hyphal appressoria. C: Conidia. *Bar*: 20 μm.

on *Tamarindus indica* L. (Fabaceae) — Fig. 26.

Colonies on leaves amphigenous, forming white patches, effuse to dense, persistent. *Hyphae* hyaline, 2–6 μm wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in opposite pairs, 2–6 μm. *Conidiophores* arising from the upper part of mother cells, erect, 44.5–87.5 × 6.5–7 μm; *foot-cells* straight to somewhat curved, uniform throughout in width, 37–51 × 4–6 μm, followed by 1(–2) shorter cells, forming conidia singly. *Conidia* cylindrical-doliiform, without fibrosin bodies, obutuse at the both ends, 27.5–37 × 13.5–16 μm, length/width ratio 1.9–3.

Specimen examined. on *Tamarindus indica*, Bali, 20 Oct. 2016, *S.A.S. Siahaan, G.A.E. Oktavia & S. Takamatsu* (BO 22820,TSU-MUMH 6961).
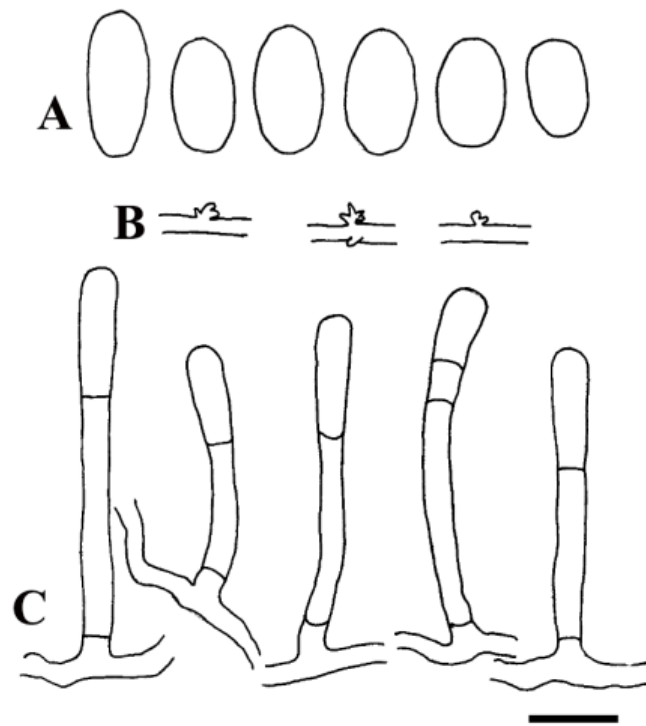
Fig.26. – *Erysiphe quercicola* on *Tamarindus indicus.* A: Conidia. B: Hyphal appressoria. C: Conidiophores. *Bar*: 20 μm.

on *Urena lobata* L. (Malvaceae) — Fig. 27.

Colonies on leaves amphigenous, forming white patches, effuse, evanescent. *Hyphae* hyaline 3–6 μm wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in opposite pairs, 2–5 μm. *Conidiophores* arising from the upper part of mother cells,

erect, 140–198 × 6.5–8 μm; *foot-cells* straight to mildly flexuous, uniform throughout in width, 21.5–75 × 6.5–8 μm, followed by a longer cell or 1–2 cells about the same length, and often followed by another very short cell. *Conidia* produced solitary, without fibrosin bodies, ellipsoid-doliiform, subtruncate to truncate at the both ends, 25–41 × 13–23 μm, length/width ratio 1.5–2.4.

Specimen examined. on *Urena lobata*, West Java, Cibodas, Cibodas Botanical Garden, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22851,TSU-MUMH 6993).
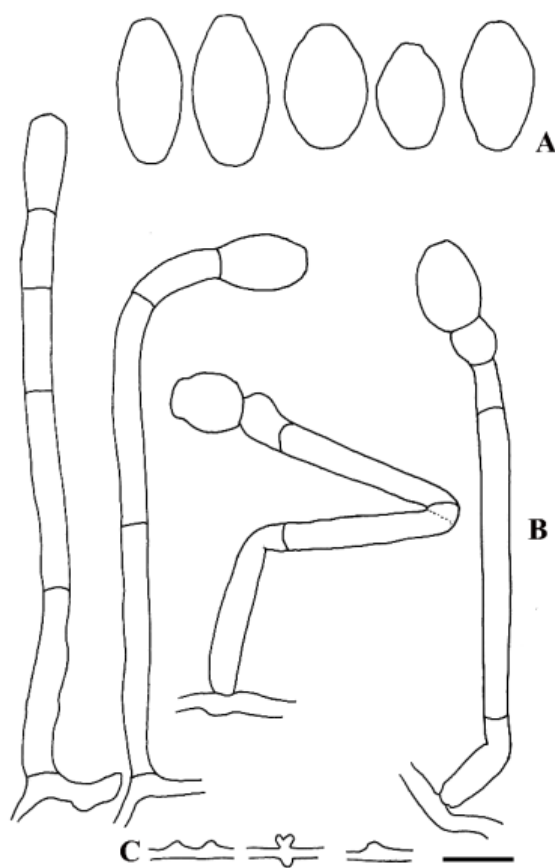
Fig.27. – *Erysiphe quercicola* on *Urena lobata.* A: Conidia. B: Conidiophores. C: Hyphal appressoria. *Bar*: 20 μm.

on *Vernicia montana* Lour. (Euphorbiaceae) — Fig. 28.

Colonies on leaves amphigenous, forming white patches, effuse to dense, persistent, causing yellowish to brownish discoloration on infected areas. *Hyphae* hyaline 3–6μm

wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in opposite pairs, 3.5–7μm. *Conidiophores* arising from the upper part of mother cells, erect, 42.5–74 × 4–7.5 μm; *foot-cells* straight to somewhat curved, uniform throughout in width, 11–32 × 4–6.5 μm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* cylindrical-doliiform, without fibrosin bodies, rounded to subtruncate at the both ends, 26.5–44.5 × 13–17.5 μm, length/width ratio 1.8–2.7.

Specimen examined. on *Vernicia montana*, West Java, Bogor, 24 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22840, 22841,TSU-MUMH 6982, 6983).

Notes —*Brucea javanica* (L.) Merr. (Simaroubaceae), *Ficus variegata* Blume (Moraceae), *Tamarindus indica* L. (Fabaceae), *Urena lobata* L. (Malvaceae), *Vernicia montana* Lour. (Euphorbiaceae) are newly added as host plants of *E. quercicola*.
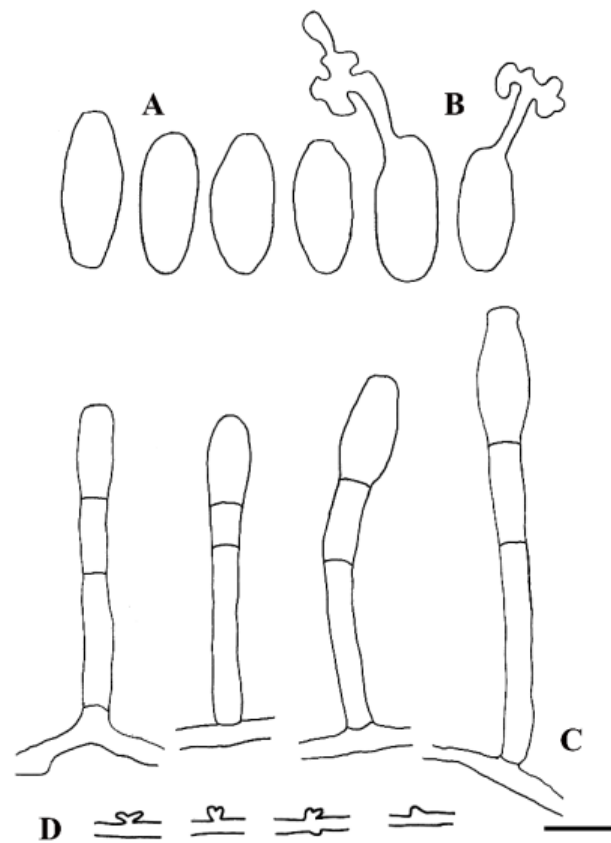
Fig.28. – *Erysiphe quercicola* on *Vernicia montana* A: Conidia. B: Germ tubes. C: Conidiophores. D: Hyphal appressoria. *Bar*: 20 μm.
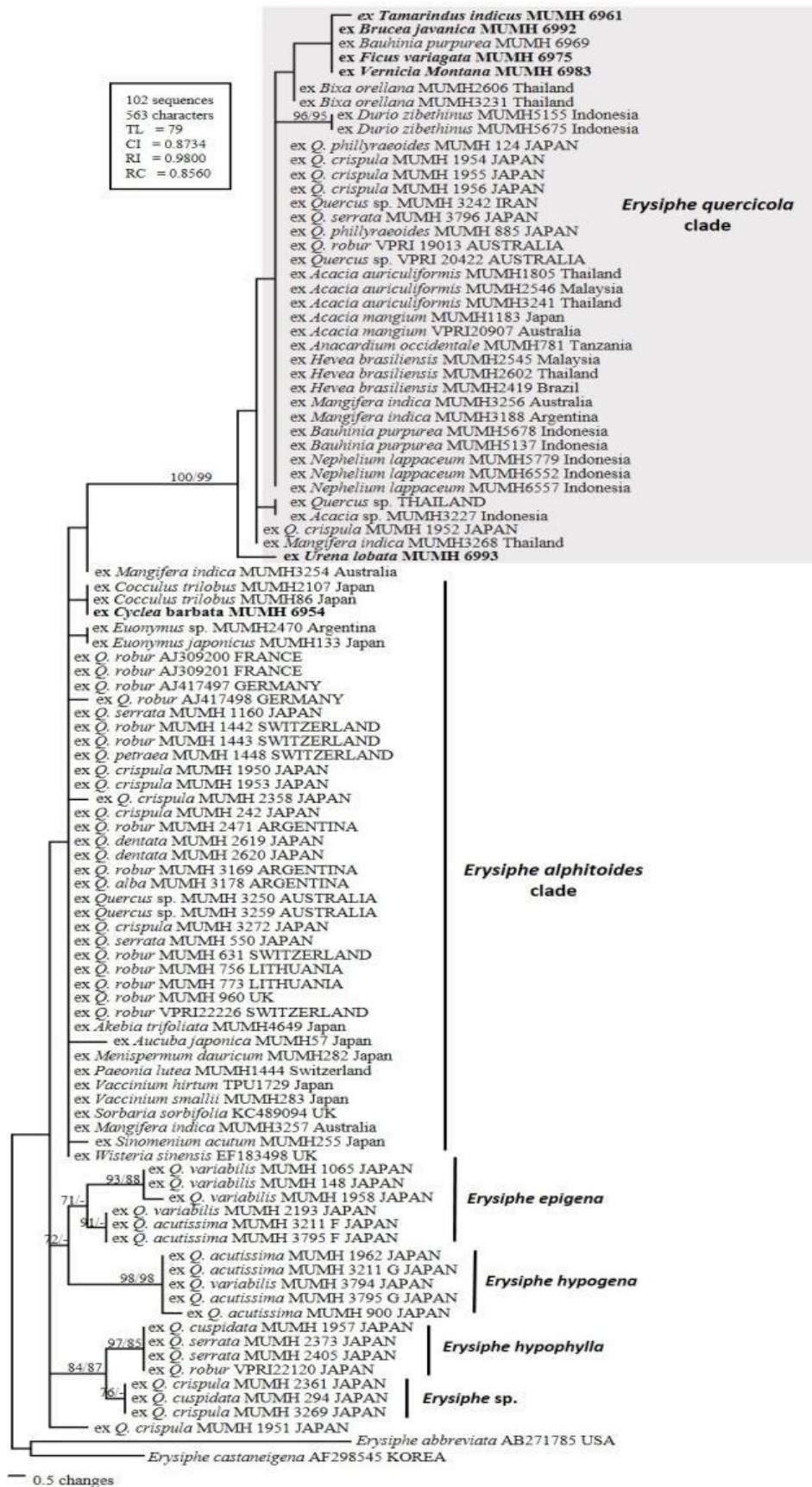
Fig.29. – Phylogenetic analysis of the ITS rRNA gene for 102 sequences of the *Erysiphe* spp. This tree is one of the 214,066 equally parsimonious trees with 76 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

Erysiphe sidae S.A.S. Siahaan & S. Takam. Mycoscience 57: 39. 2016.

Description and illustrations — See S.A.S. Siahaan & S. Takamatsu (2016b).

Host — *Sida rhombifolia* L. (Malvaceae)

Specimens examined. on *Sida rhombifolia*, INDONESIA, West Java, Bandung, Taman Hutan Raya Ir. H. Djuanda, 12 Mar. 2011, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, K. Kramadibrata & S. Takamatsu* (isotype MUMH 5126); West Java, Bandung, Situ Lembang, 13 Mar. 2011, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, K. Kramadibrata & S. Takamatsu* (BO 22684, MUMH 5146); West Java, Bandung, Rawa Upas, 14 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, K. Kramadibrata & S. Takamatsu* (BO 22714, MUMH 5688).

Pseudoidium cf. neolycopersici (L. Kiss) L. Kiss, in Braun & Cook, Taxonomic manual of the Erysiphales (powdery mildews): 612. 2012.

On *Codiaeum variegatum* — Fig. 30.

Colonies on leaves amphigenous, forming irregular white patches, effuse, evanescent. *Hyphae* hyaline, 4–6 μm wide; *hyphal appressoria* well-developed, lobed, solitary or in opposite pairs, 3–8 μm. *Conidiophores* erect, arising centrally or laterally from the mother cell, 68–114 × 7.5–10 μm; *foot-cells* straight or slightly curved at the base, 26.5–43 × 6.5–9 μm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* doliiform–cylindric, without fibrosin bodies, rounded to subtruncate at the both ends, (24.5–)33.5–44.5 × 8.5–16 μm, length/width ratio 2–4; *germ tubes* subterminal, long, simple or *Pseudoidium*-type with lobed conidial appressorium.

Host in Indonesia — *Codiaeum variegatum* (L.) Blume (Euphorbiaceae)

Specimens examined. on *Codiaeum variegatum*, INDONESIA, Bali, Gitgit Waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, S. Takamatsu* (BO 22725,TSU-MUMH 5699, BO 22728,TSU-MUMH 5703; BO 22729,TSU-MUMH 5704; BO 22739-22741,TSU-MUMH 5716-5718); North Sumatra, Samosir Island, Tuk

Tuk, 6 Jan. 2016, *S.A.S. Siahaan* (TSU-MUMH 6561, 6563); West Java, Cianjur District, Cibodas Botanical Garden, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata, & S. Takamatsu* (BO 22842,TSU-MUMH 6984).
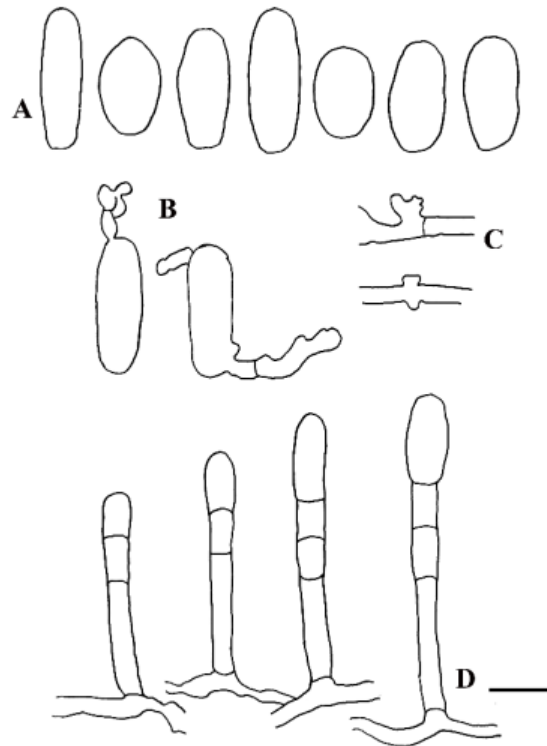
Fig.30. – *Pseudoidium cf. neolycopersici* on *Codiaeum variegatum.* A: Conidia. B: Germ tubes. C: Hyphal appressoria. D: Conidiophores. *Bar*: 20 μm.

On *Solanum betacea* Cav. (Solanaceae)

Description and Illustration —See Hidayat et al. (2014)

Specimen examined. on *Solanum betacea*, INDONESIA, North Sumatra, Berastagi, 1 Jan. 2015, *S.A.S. Siahaan* (BO 22725,TSU-MUMH 5776).

Pseudoidium hortensiae (Jørst.) U. Braun & R.T.A. Cook, Taxonomic manual of the Erysiphales (powdery mildews): 606. 2012 — Fig. 31.

≡ *Oidium hortensiae* Jørst., Skr. Norske Vidensk.-Akad. Oslo, I. Mat. Naturvidensk. Kl., 1925, 10: 106. 1926.

Colonies on leaves amphigenous, effuse or patches, persistent to subevanescent. *Hyphae*

hyaline 3–6 µm wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in opposite pairs, 3–6.5µm. *Conidiophores* arising from the upper part of mother cells, erect, 57.5–85 × 6.5–9.5 µm; *foot-cells* straight sometimes slightly curved, 15–29.5 × 6.5–9 µm, followed by 1–2 shorter cells, forming conidia singly. *Conidia* cylindrical-doliiform, without fibrosin bodies, 23–38 × 14–18 µm, length/width ratio (1.5–)1.8–2.7, *germ tubes* on the shoulder (*Pseudoidium*-type), with lobed terminal.

Host in Indonesia — *Hydrangea hortensis* Sm., *H. macrophylla* (Thunb.) Ser. (Hydrangeaceae).

Specimen examined. on *Hydrangea hortensis*, West Java, Bogor, Curug Cigamea, 10 Sept. 2013, *S.A.S. Siahaan & J. Meeboon* (BO 22699,TSU-MUMH 5673); Bali, Gitgit Waterfall, 10 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon, & S. Takamatsu* (BO 22727,TSU-MUMH 5702); on *H. macrophylla*, West Java, Bandung, Situ Patenggang, 23 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata, & S. Takamatsu* (BO 22837,TSU-MUMH 6979).

Notes — Present species are known as a cosmopolitan species on the plant genus *Hydrangea* spp.
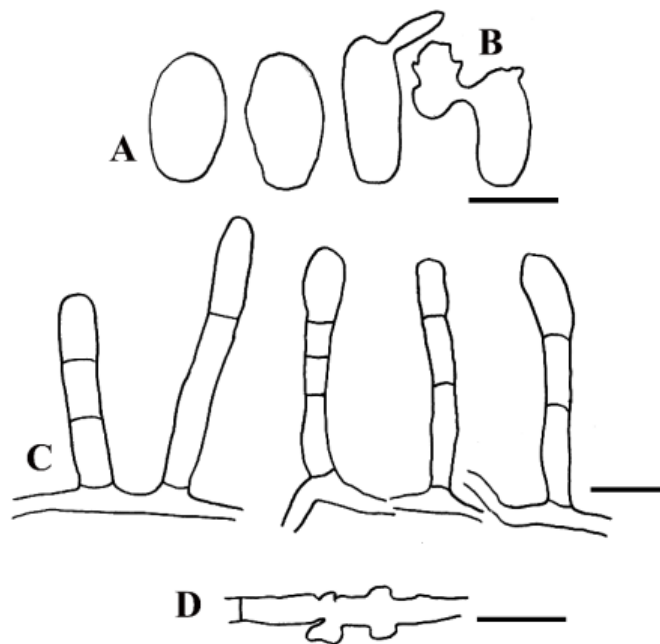
Fig.31. – *Pseudoidium hortensiae* on *Hydrangea* spp. A: Conidia. B: Germ tubes. C: Conidiophores. D: Hyphal appressoria. *Bars*: 20 µm.

Pseudoidium javanicum Meeboon & S. Takam. Mycoscience 54: 184. 2013 — Fig. 32.

Colonies on leaves amphigenous, mostly epiphyllous, effuse or patches, persistent to subevanescent. *Hyphae* hyaline 3–6 µm wide; *hyphal appressoria* well developed, lobed to multilobed, solitary or in opposite pairs, 3–7 µm. *Conidiophores* arising from the upper part of mother cells, erect, 46–72.5 × 6.5–9 µm; *foot-cells* straight, rarely slightly curved, 20–42 × 6.5–8 µm, followed by 1(–2) shorter cells, forming conidia singly. *Conidia* ellipsoid-doliiform, without fibrosin bodies, 26–36.5 × 11–18 µm, length/width ratio 1.7–2.3(–2.7).

Host in Indonesia — *Acalypha caturus* Blume, *Acalypha gracilipes* Baill. (= *A. integrifolia* Willd.), *A. hispida* Burm.f., *A. lanceolata* Willd., *A. paniculata* Miq., *A. wilkesiana* var. *marginata* Mill., *Acalypha* × *cristata* Radcl. –Sm., *Acalypha* sp. (Euphorbiaceae).
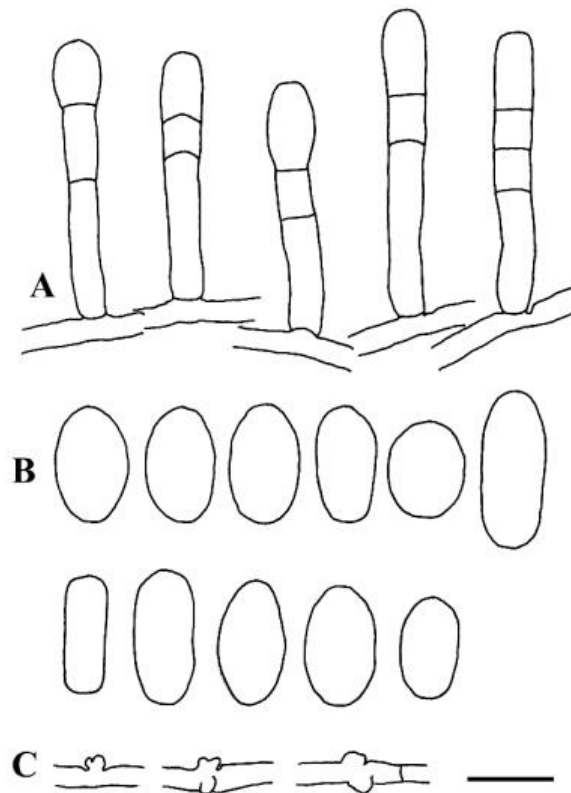
Fig.32. – *Pseudoidium javanicum* on *Acalypha* spp. A: Conidiophores. B: Conidia. C: Hyphal appressoria. *Bar*: 20 µm.

41 sequences
740 characters
TL = 847
CI = 0.5950
RI = 0.7234
RC = 0.4304

86/90 — *Erysiphe heraclei* AB000942
94/99 — *Erysiphe friesii* var. *dahurica* LC010070
Erysiphe pisi AF011306
Erysiphe abbreviata DQ866999
Erysiphe alphitoides AB237783
Erysiphe alphitoides AB257430
100/100 *Erysiphe quercicola* AB193591
Erysiphe quercicola AB193590
87/97 *Erysiphe hypophylla* AB292716
Erysiphe hypophylla AB292715
Erysiphe epigena AB292722
93/- *Erysiphe epigena* AB292721
Erysiphe epigena AB292720
99/99 *Erysiphe epigena* AB292717
Erysiphe hypogena AB292727
Erysiphe hypogena AB292724
99/76 99/100 *Erysiphe macleayae* AB016048
Erysiphe aquilegiae var. *ranunculi* AB000944
Erysiphe aquilegiae LC009944
Erysiphe blasti AB015918
99/100 *Erysiphe weigelae* AB015932
Erysiphe weigelae AB015931
Erysiphe paeoniae AB257437
100/100 *Erysiphe pulchra* var. *japonica* AB015924
76/96 *Erysiphe pulchra* var. *japonica* AB000941
79/93 *Erysiphe staphyleae* AB015922
Erysiphe helwingiae AB015916
100/100 *Erysiphe glycines* AB015934
Erysiphe glycines AB015927
Erysiphe adunca LC028968
99/94 *Erysiphe japonica* var. *japonica* AB701302
Erysiphe japonica var. *crispulae* AB022416
75/- *Erysiphe trinae* AB022351
93/98 *Erysiphe simulans* var. *simulans* AB015926
Erysiphe gracilis var. *gracilis* AB022358
Erysiphe mori AB000946
-/92 **ex *Acalypha lanceolata* MUMH6978**
ex *Acalypha integrifolia* MUMH6968
98/100 *Pseudoidium javanicum* AB733591
Pseudoidium javanicum AB733593
Erysiphe australiana AB022408

— 10 changes

Fig.33. – Phylogenetic analysis of the ITS rRNA gene for 41 sequences of the *Erysiphe* spp. This tree is one of the 36 equally parsimonious trees with 824 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

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Specimens examined. on *Acalypha caturus*, INDONESIA, West Java, Cibodas, Cibodas Botanical Garden, 11 Sept. 2013, *S.A.S. Siahaan & J. Meeboon* (BO 22703,TSU-MUMH 5677); on *A. hispida*, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22844,TSU-MUMH 6986); on *Acalypha × cristata* (BO 22848,TSU-MUMH 6990); on A. gracilipe*s,* Bali, Bedugul, 20 Oct. 2016, *S.A.S. Siahaan & S. Takamatsu* (BO 22826,TSU-MUMH 6968); on *A. lanceolata*, West Java, Bandung, Situ Patenggang, 23 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22836,TSU-MUMH 6978); on *A. paniculata*, West Java, Cibodas Botanical Garden, 25 Oct. 2016, *S.A.S. Siahaan, K. Kramadibrata & S. Takamatsu* (BO 22849,TSU-MUMH 6991); on *A. wilkesiana* var. *marginata* Mill., West Java, Cibodas Botanical Garden, Bogor, 7 Mar. 2012, Hidayat I. (Isotypus TSU-MUMH5559); West Java, Bandung, Rawa Upas, 14 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, K. Kramadibrata, J. Meeboon & S. Takamatsu* (BO 22717,TSU-MUMH 5691); Bali, Gitgit waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22724,TSU-MUMH 5698); on *Acalypha* sp., Bali, Gitgit waterfall, 16 Sept. 2013, *S.A.S. Siahaan, I. Hidayat, J. Meeboon & S. Takamatsu* (BO 22722-22723, TSU-MUMH 5696, 5697).

Notes: *Acalypha caturus*, *A. gracilipes*, *A. hispida*, *A. lanceolata*, and *A. paniculata* are newly recorded as host plant species of this fungus.

3.2. Molecular phylogeny

Phylogeny within the Erysiphaceae tree

A total of 130 of 28S rRNA sequences consisted of isolates collected from Indonesia and sequences retrieved from DNA database were aligned. This alignment consisted of 828 characters, of which 258 (31.3%) characters were variable and 202 (24.4%) characters were informative for parsimony analysis. A sequence of *Bysssoascus striatisporus* (G.L. Barron & C. Booth) was used as outgroup taxon based on Siahaan et al. (2016d). A total of 558,652 equally parsimonious trees with 1069 steps were constructed by MP analysis. Tree topologies were almost consistent among the trees

except for branching orders of the terminal branches and branch length. One of the tree is shown in Fig. 1A. Each fungus nested in the genera clade related to their anamorph characteristics.

For ITS rRNA analysis, a total of 156 sequences of Erysiphaceae including a sequence of *Byssoascus striatosporus* as an outgroup taxon, were used. One of the most likelihood trees is shown in Fig.1B. In the Bayesian analyses, the optimum evolutionary model ITS was selected by KAKUSAN4 (Tanabe 2011). These optimal model was the GTR+I+G. The Bayesian analysis was conducted for 1,500,000 generations. Trees were saved every 1,000 generations, resulting in 1501 trees saved. Of these, 325 trees were considered "burn-in," after which the likelihood values were stationary. The Bayesian 50% majority rule consensus tree of these posterior-sampled trees was generated in MrBayes v. 3.2.6 (tree not shown). The Bayesian posterior probabilities were calculated and indicated on the node of the most parsimonious tree generated in this study (Fig. 1B). Trees from both analyses were visualized in FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree). In the MP analysis, the alignment consisted of 753 characters, of which of which 464 (61.6%) characters were variable and 392 (52%) characters were informative for parsimony analysis. A sequence of *Bysssoascus striatisporus* (G.L. Barron & C. Booth) was used as outgroup taxon based on Siahaan et al. (2016d).The BS value ≥ 70% are shown on the respective branches.

Phylogeny within the genus *Hommae*

Two 18S rRNA sequences obtained from the sample on *Persea americana* Mill. were aligned with other 18S rRNA genes of the Erysiphales retrieved from the DNA Database. The alignment consisted of 20 taxa, with 1742 characters of which 110 (6.3%) characters were variable and 46 (2.6%) characters were informative for parsimony analysis. A sequence of *Byssoascus striatosporus* (AB015776) was used as outgroup taxon based on Mori et al. (2000a, b). A total of 305 parsimonious trees with 138 steps

were generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. A tree with the highest likelihood score is shown in Fig. 4. The sequences obtained from on *P. americana* were nested in the Cystotheceae tribe, however, formed an independent clade separately from the three genera, *Cystotheca, Podosphaera* and *Sawadaea* with strong BS support (ML/MP = 96/99).

Phylogeny within the genus *Podosphaera*

Two phylogenetic analyses were conducted to investigate the phylogenetic analyses of specimens belong in the genus *Erysiphe*. In the first analysis, one rDNA ITS sequence of *Erysiphe* on *Rosa* sp. L. (Rosaceae) were aligned with that of the *Podosphaera* in the Rosoideae group in Takamatsu et al. (2010). The alignment consisted of 20 taxa, with 480 characters of which 27 (5.6%) characters were variable and 15 (3.1%) characters were informative for parsimony analysis. Two sequences of *P. spiraeae* were used as outgroup taxa based on the same paper. A total of four parsimonious trees with 132 steps were generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. A tree with the highest likelihood score is shown in Fig. 6. In the analysis, our sequence nested in the *P. pannosa* clade on *Rosa* spp. with strong BS support (ML/MP = 92/95).

In the second analysis, sequences obtained from colonies on *Benincasa pruriens* (Sol. ex Seem.) W.J. de Wilde (Cucurbitaceae), *Breynia virgata* (Blume) Müll. Arg. (Phyllanthaceae), *Cleome viscosa* L. (Capparidaceae), *Euphorbia hirta* L. (Euphorbiaceae), *Elatostema* sp. J.R. Forst. & G. Forst. (Urticaceae), *Hibiscus indicus* (Burm.f.) Hochr. (Malvaceae), *Momordica charantia* L. (Cucurbitaceae), *Mukia javanica* (Miq.) C. Jeffrey (Cucurbitaceae), *Pseudoelephantopus spicatus* (Juss. Ex Aubl.) Rohr. (Asteraceae); *Vernonia cinerea* (L.) Less. (Asteraceae) were aligned our sequences with that of Meeboon & Takamatsu (2015). The alignment consisted of 63 taxa, with 498 characters of which 149 (29.9%) characters were variable and 116 (23.3%) characters

were informative for parsimony analysis. Two sequences of *Cystotheca* sp., i.e. *C. lanestris* were used as outgroup taxa according to Mori et al. (2000). A total of 12,082 parsimonious tree with 282 steps were generated by MP analysis. One of the most parsimonious tree is shown in Fig. 8. The phylogenetic analysis showed that the all of our sequences nested in the same clade with *P. xanthii* with strong BS support (85/80 ML/MP values).

Phylogeny within the genus *Phyllactinia*

The alignment consisted of 36 taxa, with 787 characters of which 399 (50%) characters were variable and 290 (36.8%) characters were informative for parsimony analysis. A sequence of *G. cynoglossi* (Wallr.) V.P. Heluta was used as outgroup taxon based on the same paper. A total of 32 parsimonious tree with 1297 steps was generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. A tree with the highest likelihood score is shown in Fig. 12. The fungus on *L. floribunda* and *L. speciosa* from Indonesia were partially grouped with *Ph. lagerstroemiae*, especially with the sequence on *L. speciosa* (MUMH 3342) with strong BS support (99/100 ML/MP values). This clade is sister to the other two sequences of *Ph. lagerstroemiae* on *Ph. macrocarpa* (MUMH 5750 (holotype) and TSU-MUMH 3351), also with strong BS support (99/100 ML/MP values). Partial ITS rRNA sequences obtained from *Lagerstroemia floribunda* Jack. and *L. speciosa* (L.) Pers. (Lythraceae) collected in Indonesia were identical to each other. In addition, these sequences formed an obviously-differentiated clade (BS: MP/ML = 99/100) with a sequence of *Phyllactinia lagerstroemiae* collected from Thailand (LC177379) whereas the sequences including the ex-type of *Phyllactinia lagerstroemiae,* which is described Meeboon & Takamatsu (2017) based on the specimens on *L. macrocarpa* and *L. speciosa* from Thailand*,* formed a well-supported sister clade. Moreover, the results of morphological observation reveals that they belong to the genus *Phyllactinia*. From these results, these specimens were an undescribed species of this

genus.

Phylogeny within the genus *Golovinomyces*

A sequence obtained from a specimen on *Sonchus oleraceus* L. (Asteraceae) has highest similarity with *Golovinomyces cichoracearum*. Thus, we aligned the sequence with partial alignment published by Takamatsu et al. (2013). The alignment consisted of 19 taxa, with 568 characters of which 59 (10.4%) characters were variable and 42 (7.4%) characters were informative for parsimony analysis. A sequence of *G. cynoglossi* (AB769454) was used as outgroup taxon based on the same paper. A total of 18 parsimonious tree with 75 steps was generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. A tree with the highest likelihood score is shown in Fig. 15. The fungus on *S. oleraceus* was in group with *G. sonchicola* on other *Sonchus* spp. with strong BS support (98/100 ML/MP values).

Phylogeny within the genus *Erysiphe*

Five phylogenetic analyses using the concatenated alignment of ITS and 28S sequences were conducted to investigate the phylogenetic analyses of specimens belong in the genus *Erysiphe*. In the first analysis, *Erysiphe aquilegiae* clade including three specimens on *Codiaeum variegatum* (L.) Blume (Euphorbiaceae), one specimen on *Solanum betacea* Cav. (Solanaceae), and one specimen from *Carica papaya* L. (Caricaceae) has analyzed. The alignment consisted of 60 taxa, with 524 characters of which 73 (13.9%) characters were variable and 55 (10.5%) characters were informative for parsimony analysis. Two sequences of *E. magnoliae* were used as outgroup taxa based on Takamatsu et al. (2015). A single parsimonious tree with 106 steps was generated by MP analysis. The tree is shown in Fig. 17. In the analysis, the specimens on *C. variegatum* and *S. betacea* were nidentified as *Pseudoidium cf. neolycopersici*. Meanwhile, the other specimen on *Carica papaya* was identified as *E. caricae-papayae* based on the report of

Braun et al. (2017). The phylogenetic position of all specimens within the *E. aquilegiae* clade was strongly supported by bootstrap support (96/93 ML/MP values).

In the second analysis, a sequence obtained from the colony on *Celtis timorensis* Span. (Cannabaceae) has been clustered with *Erysiphe kusanoi* and *E. michikoae*. Thus, we aligned the sequence with alignment of Meeboon et al. (2013a). The alignment consisted of 11 taxa, with 664 characters of which 113 (17%) characters were variable and 35 (5.3%) characters were informative for parsimony analysis. A sequence of *E. mori* was used as outgroup taxon according to the same paper. A single parsimonious tree with 145 steps was generated by MP analysis. The tree is shown in Fig. 19. The phylogenetic analysis showed that the fungus on *C. timorensis* was grouped with *E. kusanoi* on *Celtis,* but form a different clade from the latter fungus with 99/97 (ML/MP) bootstrap support.

In the third, a sequence obtained from a powdery mildew specimen on *Vitis vinifera* L. (Vitaceae) was aligned with other powdery mildew sequences on *Vitis* spp. retrieved from DNA database. The alignment consisted of 16 taxa, with 568 characters of which 90 (15.8%) characters were variable and 26 (4.6%) characters were informative for parsimony analysis. A total of seven equally parsimonious trees with 99 steps were constructed by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. The tree with highest likelihood score is shown in Fig. 21. The sequence obtained from a specimen on *V. vinifera* was nested with *E. necator* var. *necator* with strong bootstrap support (99/100 ML/MP values).

In the fourth analysis, sequences obtained from the colonies on *Brucea javanica,* (L.) Merr. (Simaroubaceae), *Ficus variegata* Blume (Moraceae)*, Tamarindus indica* L. (Fabaceae)*, Urena lobata* L. (Malvaceae), and *Vernicia montana* Lour. (Euphorbiaceae), showed closest similarity with *Erysiphe quercicola,* and one sequence obtained from a specimen on *Cyclea barbata* Miers. (Menispermaceae) showed closest similarity with *E. alphitoides*. Thus, we aligned those sequences with partial alignment published by Takamatsu et al. (2015). The alignment consisted of 102 taxa, with 563 characters of

which 63 (11.2%) characters were variable and 36 (6.4%) characters were informative for parsimony analysis. A sequence of *E. abbreviata* (AB271785) and *E. castaneigena* (AF298545) were used as outgroup taxa based on the same paper. A total of 214,066 parsimonious tree with 76 steps was generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. One of the most parsimonious tree is shown in Fig. 29. The phylogenetic analysis showed that the sequences of fungi on *B. javanica, F. variegata, T. indica, U. lobata* and *V. montana* were grouped with *E. quercicola* clade with strong bootstrap support (100/99 ML/MP values). Meanwhile, the sequence obtained from a specimen on *C. barbata* was group with *E. pseudolonicerae* on *Cocculus* in the big clade of *E. alphitoides*, although this was supported by low BS value (≤70%).

The fifth analysis comprises of *Erysiphe* spp. retrieved from DNA database and two ITS sequences of each of the fungus on *Acalypha integrifolia* Willd. and *A. lanceolata* Willd. The alignment consisted of 41 taxa with 740 characters, which 329 (44.5%) characters were variable and 226 (30.5%) characters were informative for parsimony analysis. A sequence of *E. australiana* (AB022408) was used as outgroup taxon based on Meeboon et al. (2013b). A total of 36 parsimonious tree with 824 steps was generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. One of the most parsimonious tree is shown in Fig. 33. The phylogenetic analysis showed that the sequences of fungi on *A. integrifolia* and *A. lanceolata* nested with *Pseudoidium javanicum* Meeboon & S. Takam. isolated from *Acalypha* spp. from Indonesia.

4. Discussions

In this study, we determined 155 ITS rRNA and 34 28S sequences obtained from powdery mildew specimen from 23 host families. From the overview tree generated with the sequences of rDNA ITS region obtained from Indonesian powdery mildews, the

species have been widely dispersed in various clades from phylogenetically basal to terminal clades within Erysiphales (Fig. 1). Of them, the species having the wide host range and known as tropic powdery mildew such as *Erysipe aquilegiae* and *E. alphitoides* are collected in this survey.

The fungi on *Carica papaya, Codiaeum variegatum* and *Solanum betacea* from Indonesia are nested in the *E. aquilegiae* species complex, including several small taxa such as *E. chloranthi, E. euphorbiae, E. hommae, E. knautiae, E. macleayae, E. pileae, E. sedi, E. takamatsui,* etc (Takamatsu et al. 2015). The morphology of the powdery mildews on the Indonesian specimens are quite similar with that of *E. aquilegiae*, i.e. mycelium is amphigenous, effuse or in patches, evanescent to persistent. Its hyphal appressoria are lobed, solitary or in opposite pairs, 3–7 μm in size. The conidiophores are erect from the top of mother cell. Foot-cells are cylindrical, straight or occasionally slightly curved-sinuous at the base, (15–)20–40 × 7–11 μm, followed by (0–)1–2(–3) shorter cells, forming conidia singly. The conidia are ellipsoid-ovoid to subcylindrical, (25–)28–50 × (12–)16–22(–24) μm, length/width ratio 1.6–2.5. Those germ tubes are usually ± terminal, short to moderately long, sometimes septate, showing *longitubus* pattern, with conidial hyphal appressoria lobed or unlobed, variable in shape according to the hosts (Braun & Cook 2012). Although phylogenetic analysis showed that the three specimens nested in *E. aquilegiae* clade (Fig. 17), other evidence of host range such as inoculation test is urgently required. Based on morphological and molecular data of ITS region, Hidayat et al. (2014) reported a fungal specimen on tamarillo (*S. betacea*) from Indonesia, which had similar anamorph characteristics with *P. neolycopersici.* Inoculation test of this fungus to tomato and other Solanum species was failed, thus they proposed *Pseudoidium aff. neolycopersici* for this specimen. Referring to this report, the fungi on *S. betacea* and *C. variegatum* in this report will be tentatively identified as *Pseudoidium cf. neolycopersici.* Meanwhile, the fungus on *C. papaya* is identified as *Erysiphe caricae-papayae* based on the report of Braun et al. (2017).

Cyclea barbata Miers is newly added as a host plant of powdery mildew in the

world. The partial sequence of ITS obtained from this host plant suggests that it belongs to *Erysiphe alphitoides* (99% similarity in BLAST serch). In the phylogenetic analysis with ITS regions of specimens on *E. alphitoides* clade, the fungus on the genus *Cyclea* was nested with *E. pseudolonicerae* on *Cocculus trilobus* (Menispermaceae) although it was supported by low bootstrap value (Fig. 29). The morphological characteristics of anamorphic state of the current fungus are in a good agreement with that of *E. pseudolonicerae*.

As mentioned above, *Erysiphe quercicola* has wide host range. According to Takamatsu et al. (2007), this species known as a species distributing in the tropical to subtropical area, although the only teleomorphic state of this species was collected in temperate region, Japan. Up to this date, the hosts of this fungus were known on nine host families, i.e. Anacardiaceae, Bixaceae, Euphorbiaceae, Fabaceae, Fagaceae, Lauraceae, Malvaceae, Rutaceae and Sapindaceae (Limkaisang et al. 2006, Takamatsu et al. 2007, Kirschner & Liu 2014, Siahaan et al. 2016c). All those reports also indicated that although *E. quercicola* clade contains some sequence variations, however, due to similarity of morphological characteristics of the anamorphs, the clade is still categorized as a single species.

In this study, five specimens from Indonesia nested in the clade *E. quercicola* with strong bootstrap support (99/99 ML/MP values) (Fig. 29). Among those five host plants, three of them, i.e. *Brucea javanica, Ficus variegata,* and *Vernicia montana* are newly recognized as host plants of *E. quercicola*. According to Braun & Cook (2012), six *Erysiphe* species on the genus *Ficus*, one on the genus *Vernicia* are hitherto known respectively, and also, none of *Erysiphe* species on the genus *Brucea* are recorded. However, all those hitherto known fungi are included into the '*Uncinula*' lineage, which morphologically differ from the Indonesian specimens by having conidiophores arise from the lateral side of the hyphae. Thus this paper provides some additional information regarding new hosts of *E. quercicola* from the tropics, i. e. *Brucea javanica* (Simaroubaceae), *Ficus variegata* (Moraceae), *Tamarindus indica* (Fabaceae), *Urena*

lobata (Malvaceae) and *Vernicia montana* (Euphorbiaceae).

The basionym of *Hommaea perseae-americanae, Podosphaera perseae-americanae* was proposed by Siahaan et al. (2016d) based on fungal collection on *Persea americana*, Indonesia. The phylogeny suggested by sequences of rDNA ITS and 28S genes indicated that this fungus belong to an undescribed genus within the tribe Cystotheceae. In addition, the phylogeny of this fungus using 18S rDNA region sequences were analysed. The topology of generated tree was similar and confirmed that new genus *Hommaea* formed an independent lineage in the Erysiphaceae, more specifically in the Cystotheceae (Fig. 4). Morphologically, *Hommaea* differs from *Cystotheca* having sinuate-type edge line and barrel-like conidia. In addition, *Hommaea* also differs from *Sawadaea* in lacking micro-conidiophores and micro-conidia and shape of conidia. Previously *H. perseae-americanae* (syn.: *Podosphaera perseae-americanae*) was considered morphologically closed to *Podosphaera* by having crenate-type edge immature conidia, however, the fungus differs in having lobed to elongated, forked appressoria. Furthermore, the presence of a longer cell after the foot-cells or short cells is also new important characteristic, which was not mentioned in the original description.

The host plant of *H. perseae-americanae, Persea americana* originated in Central America and is recorded as having been growing for food since 8 000 B.C. And it was introduced to Spain in 1601 (Barwick & van der Schans 2004). On the other hand, the tribe Cystotheceae is located in a basal clades of Erysiphales. In this study, we cannot judge whether the fungus was introduced to Indonesia with its host plant, or has expanded the host range or has changed the host plant in Indonesia. It is quite interesting where a host plant and a fungus meet. However, the fungus is collected from only on the host at type locality in the tropics and subtropics. The additional examination using fresh material collected from all over the world is mandatory.

In this study, several fungal species were newly described from the well-known plants as the host of powdery mildews. Meeboon & Takamatsu (2017) described *Ph. lagerstoemiae* on *L. macrocarpa* and *L. speciosa* from Thailand. Their phylogenetic

analyses results showed that three specimens from *Lagerstroemia* sp. formed two clades. Sequence obtained from a specimen on *L. speciosa* (TSU-MUMH 3342, LC177379) separated from the other two specimens with strong bootstrap support (84% in the 28S and 100% in rDNA ITS analyses respectively). In our study, when we aligned our sequences with alignment of Meeboon & Takamatsu (2017), the Indonesian specimens formed different clade from the two specimens of *Ph. lagerstroemiae* from Thailand, but nested at the same clade with specimen TSU-MUMH 3342 on *L. speciosa* (with strong bootstrap support ML/MP = 99/100 respectively) (Fig. 12). Moreover, those morphological characteristics showed that the fungus on *L. speciosa* from Thailand is identical with the specimens from Indonesia. From these reason, we proposed new species, *Phyllactinia lagerstroemiicola*.

In addition, four *Erysiphe* species were hitherto known on the plant genus *Celtis* in the world, i.e. *Erysiphe celtidis, E. kusanoi, E. parvula* and *E. michikoae*. All of these fungi belong to *Erysiphe* sect. *Uncinula* lineages. *E. celtidis* distributes in Western Asia, while *E. parvula* distributes in North America. These two fungi do not have anamorphic state. The other two species, *E. kusanoi* and *E. michikoae,* distribute in East and South Asia region (Braun & Cook 2012). BLAST search indicates 98% similarity to both *E. kusanoi* and *E. michikoae* with eight bp differences to each of the fungus. In this study, we described a new species as the fifth species on *Celtis, Erysiphe celtidi-timorensis* from Indonesia. Phylogenetic analysis of rDNA ITS sequence showed that *E. celtidi-timorensis* forms a clade with *E. kusanoi* with high bootstrap support (ML/MP = 99/97) (Fig. 19). This result supported that the *E. celtidi-timorensis* is an independent species of *Erysiphe*, and is sister to *E. kusanoi*.

These results suggest that a powdery mildew species in tropic area is not necessarily correspond to the hitherto known species in the same or different geographic area. It means the species diversity of the powdery mildews are enormously rich considering the diversity of the host plants. The similar cases have been known for the plant pathogenic fungi such as Cercosporoid fungi. Identification of those species based

on the host, symptom and morphology is often not warranted even if the same geographic region (Crous et al. 2013, Groenewald et al. 2013, Nakashima et al. 2016). Moreover, these species have led to the host expansion and speciation regardless of the lacking in the teleomorphic state in the tropic area. It means that the agents of these evolutional or ecological event is not teleomorphic state but rather anamorphic state.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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Supplementary Table 1. List of powdery mildew samples collected from Indonesia from 2013-2017

No.	Host name	Host family	Collection place	GPS data			BO No.	MUMH No.	Sequencing		Anamorphic observation
				LatNS	LonWE	Elevation			ITS	28S	
1.	*Carica papaya* L.	Caricaceae	Curug nangka, Bogor	06⁰41'00.0"	106⁰45'16.7"	441	22694	5667	O	O	*Pseudoidium* sp.
2.	*Capsicum frutescents* L.	Solanaceae	Curug nangka, Bogor	06⁰41'00.0"	106⁰45'16.7"	441	22693	5668	-	-	*Oidiopsis* sp.
3.	*Cestrum nocturnum* L.	Solanaceae	Curug cigamea, Bogor	06⁰39'38.9"	106⁰43'47.1"	691	22695	5669	-	O	*Oidiopsis* sp.
4.	*Euphorbia pulcherrima* Willd. Ex Klotzch	Euphorbiaceae	Curug cigamea, Bogor	06⁰39'38.9"	106⁰43'47.1"	691	22696	5670	O	O	*Ovulariopsis* sp.
5.	*Solanum lycopersicum* Lam.	Solanaceae	Curug cigamea, Bogor	06⁰39'38.9"	106⁰43'47.1"	691	22697	5671	-	-	*Pseudoidium* sp.
6.	*Momordica charantia* L.	Cucurbitaceae	Curug cigamea, Bogor	06⁰39'38.9"	106⁰43'47.1"	691	22698	5672	O	O	*Fibroidium* sp.
7.	*Hydrangea hortensis* Sm.	Hydrangeaceae	Curug cigamea, Bogor	06⁰39'38.9"	106⁰43'47.1"	691	22699	5673	-	-	*Pseudoidium* sp.
8.	*Solanum torvum* Sw.	Solanaceae	Curug cigamea, Bogor	06⁰39'38.9"	106⁰43'47.1"	692	22700	5674	-	-	*Oidiopsis* sp.
9.	*Durio zibethinus* Rumph. ex Murray	Malvaceae	Curug cigamea, Bogor	06⁰41'37.9"	106⁰40'54.7"	691	22701	5675	O	O	*Pseudoidium* sp.
10.	*Castanopsis argentea* A.Dc.	Fagaceae	Talaga Warna, Puncak, Bogor	06⁰42'10.9"	106⁰59'45.7"	1466	22702	5676	-	O	*Setoidium* sp.
11.	*Acalypha caturus* Blume	Euphorbiaceae	Kebun Raya, Bogor	06⁰36'09.7"	106⁰47'46.3"	250	22703	5677	-	-	*Pseudoidium* sp.
12.	*Bauhinia purpurea* L.	Fabaceae	Botani Square Mall, Bogor	06⁰36'12.8"	106⁰48'22.3"	250	22704	5678	O	O	*Pseudoidium* sp.
13.	*Mukia javanica* (Miq.) C. Jeffrey	Cucurbitaceae	Desa Situ Patengang, Ciwidey, Bandung	07⁰09'55.7"	107⁰21'38.4"	767	22705	5679	O	O	*Fibroidium* sp.
14.	*Ageratum conyzoides* L.	Asteraceae	Desa Situ Patengang, Ciwidey, Bandung	07⁰09'55.7"	107⁰21'38.4"	767	22706	5680	-	-	*Fibroidium* sp.
15.	*Dahlia* sp. Cav.	Asteraceae	Desa Situ Patengang, Ciwidey, Bandung	07⁰09'37.3"	107⁰21'14.9"	975	22707	5681	-	-	*Euoidium* sp.
16.	*Persea americana* Mill.	Lauraceae	Desa Situ Patengang, Ciwidey, Bandung	07⁰49'48.8"	107⁰21'22.3"	1614	22708	5682	O	O	*Hommaea* sp.

No.	Species	Family	Location	Latitude	Longitude	Elev.					Fungus
17.	*Morus alba* L.	Moraceae	Desa Situ Patengang, Ciwidey, Bandung	07⁰49'48.8"	107⁰21'22.3"	1615	22709	5683	-	-	*Ovulariopsis* sp.
18.	Unidentified host	Asteraceae	Pusat Penelitian T eh dan Kina Gambung, Bandung	07⁰08'42.7"	107⁰30'52.6"	1410	22713	5684	-	-	*Fibroidium* sp.
19.	*Ageratum conyzoides* L.	Asteraceae	Rawa Upas, Bandung	07⁰08'22.0"	107⁰23'25.3"	1801	22710	5685	-	-	*Fibroidium* sp.
20.	*Castanopsis argentea* A.Dc.	Fagaceae	Rawa Upas, Bandung	07⁰08'22.4"	107⁰23'29.5"	1793	22711	5686	-	-	*Setoidium* sp.
21.	*Rosa* sp. L.	Rosaceae	Rawa Upas, Bandung	07⁰08'17.3"	107⁰23'33.0"	1782	22712	5687	O	O	*Fibroidium* sp.
22.	*Sida rhombifolia* L.	Malvaceae	Rawa Upas, Bandung	07⁰08'52.2"	107⁰30'56.8"	1757	22714	5688	O	O	*Pseudoidium* sp.
23.	*Euphorbia pulcherrima* Willd. ex Klotzch	Euphorbiaceae	Rawa Upas, Bandung	07⁰08'52.2"	107⁰30'56.8"	1758	22715	5689	O	O	*Ovulariopsis* sp.
24.	*Citrus* sp. L.	Rutaceae	Rawa Upas, Bandung	07⁰08'41.0"	107⁰30'57.8"	1388	22716	5690	-	-	*Pseudoidium* sp.
25.	*Acalypha wilkesiana* Müll. Arg.	Euphorbiaceae	Rawa Upas, Bandung	07⁰08'41.0"	107⁰30'57.8"	1389	22717	5691	-	-	*Pseudoidium* sp.
26.	*Hibiscus indicus* (Burm.f.) Hochr.	Malvaceae	Rawa Upas, Bandung	07⁰08'39.6"	107⁰30'55.3"	1377	22718	5692	O	O	*Fibroidium* sp.
27.	*Celtis timorensis* Span.	Cannabaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22719	5693	O	O	*Pseudoidium* sp.
28.	*Elatostema* sp. J.R. Forst. & G. Forst.	Urticaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22720	5694	O	O	*Fibroidium* sp.
29.	*Pseudoelephantopus spicatus* (Juss. Ex Aubl.)Rohr	Asteraceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22721	5695	O	O	*Fibroidium* sp.
30.	*Acalypha* sp. L.	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22722	5696	-	O	*Pseudoidium* sp.
31.	*Acalypha* sp. *L.*	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22723	5697	-	O	*Pseudoidium* sp.
32.	*Acalypha wilkesiana* Müll. Arg.	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22724	5698	O	O	*Pseudoidium* sp.
33.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22725	5699	-	-	*Pseudoidium* sp.
34.	Unidentified	Unidentified	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808		5700	-	-	not identified

No.	Species	Family	Location	Latitude	Longitude						
35.	*Benincasa pruriens* (Sol. ex Seem.) W.J. de Wilde & Duyfjes	Cucurbitaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22726	5701	O	O	*Fibroidium* sp.
36.	*Hydrangea hortensis* Sm.	Hydrangeaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22727	5702	-	-	*Pseudoidium* sp.
37.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22728	5703	O	O	*Pseudoidium* sp.
38.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'13.2"	115⁰08'22.8"	786	22729	5704	O	O	*Pseudoidium* sp.
39.	*Gliricidia sepium* Kunth ex Steud.	Fabaceae	Gitgit Waterfall, Bali	08⁰12'13.7"	115⁰08'21.9"	797	22730	5705	O	O	*Pseudoidium* sp.
40.	*Citrus* sp. L.	Rutaceae	Gitgit Waterfall, Bali	08⁰12'13.4"	115⁰08'21.6"	828	22731	5706	-	-	*Pseudoidium* sp.
41.	*Ageratum conyzoides* L.	Asteraceae	Gitgit Waterfall, Bali	08⁰12'13.4"	115⁰08'21.6"	809	22732	5707	-	-	*Fibroidium* sp.
42.	*Vitis vinifera* L.	Vitaceae	Singaraja, Bali	08⁰07'20.8"	115⁰03'55.9"	18	22733	5708	O	-	*Pseudoidium* sp.
43.	*Euphorbia pulcherrima* Willd. ex Klotzch	Euphorbiaceae	Kintamani, Bali	08⁰09'57.6"	115⁰15'05.2"	970	22734	5709	O	O	*Ovulariopsis* sp.
44.	*Unidentified*	Lamiaceae	Kintamani, Bali	08⁰09'57.6"	115⁰15'05.2"	970	-	5710	-	O	Not identified
45.	*Breynia virgata* (Blume) Müll. Arg.	Phyllanthaceae	Kintamani, Bali	08⁰09'57.6"	115⁰15'05.2"	970	22735	5711	O	-	*Fibroidium* sp.
46.	*Gliricidia sepium* Kunth ex Steud.	Fabaceae	Desa Belok, Banyar Belok, Bali	08⁰17'29.1"	115⁰14'25.7"	993	22736	5712	O	O	*Pseudoidium* sp.
47.	*Capsicum frutescens*L.	Solanaceae	Desa Belok, Banyar Belok, Bali	08⁰17'29.1"	115⁰14'25.7"	993	22737	5713	-	-	*Oidiopsis* sp.
48.	Unidentified	Unidentified	Desa Belok, Banyar Belok, Bali	08⁰17'02.0"	115⁰09'42.9"	1285		5714	-	-	not identified
49.	*Sonchus arvensis* L.	Asteraceae	Gerbang kebun raya Eka karya, bali	08⁰17'03.1"	115⁰09'43.6"	1309	22738	5715	-	-	*Euoidium* sp.
50.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22739	5716	O	O	*Pseudoidium* sp.

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No.	Species	Family	Location	Latitude	Longitude	Altitude					Genus
51.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22740	5717	O	O	*Pseudoidium* sp.
52.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Gitgit Waterfall, Bali	08⁰12'06.9"	115⁰08'25.9"	808	22741	5718	-	-	*Pseudoidium* sp.
53.	*Impatiens balsamina* L.	Balsaminaceae	Jl. Raya Baturiti, Bali	08⁰18'50.8"	115⁰10'54.3"	171	22743	5720	-	-	*Fibroidium* sp.
54.	*Euphorbia heterophylla* L.	Euphorbiaceae	Jl. Raya Baturiti, Bali	08⁰31'47.8"	115⁰13'33.6"	170	22745	5722	O		*Oidiopsis* sp.
55.	*Persea americana* Mill.	Lauraceae	Brastagi, North Sumatera	03⁰18'61.11	98⁰50'86.42"			5775	O	O	*Hommaea* sp.
56.	*Solanum betaceum* Cav.	Solanaceae	Brastagi, North Sumatera					5776	O	O	*Pseudoidium* sp.
57.	*Nephelium lappaceum* Poir.	Sapindaceae	BDB Lorong 29, P. siantar	02⁰96'62.7	99⁰08'30.8"			5777	O	O	*Pseudoidium* sp.
58.	*Morus* sp. L.	Moraceae	Jl Rima Raya no. 24 P. siantar					5778	-	O	*Ovulariopsis* sp.
59.	*Nephelium lappaceum* Poir.	Sapindaceae	Jl. Kertas, P. siantar	02⁰96'63.1	99⁰08'22.3"			5779	O	O	*Pseudoidium* sp.
60.	*Solanum torvum* Sw.	Solanaceae	Jl. Danau Toba, P. siantar					5780	-	O	*Oidiopsis* sp.
61.	*Nephelium lappaceum* Poir.	Sapindaceae	Tuk Tuk, Samosir Island, North Sumatra	02°40'25."	98°54'30."	913		6552	O	O	*Pseudoidium* sp.
62.	*Nephelium lappaceum* Poir.	Sapindaceae	Tuk Tuk, Samosir Island, North Sumatra	02°45'08."	98°43'18."	919		6557	O	O	*Pseudoidium* sp.
63.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Tuk Tuk, Samosir Island, North Sumatra	02°33'06."	98°38'21."	1583		6561			*Pseudoidium* sp.
64.	*Codiaeum variegatum* (L.) Blume	Euphorbiaceae	Tuk Tuk, Samosir Island, North Sumatra	02°33'06."	98°38'21."	1583		6563			*Pseudoidium* sp.
65.	*Euphorbia hirta* L.	Euphorbiaceae	Bali	08⁰38'40.84"	115⁰09'29.78"	51	22811	6952	O	-	*Fibroidium* sp.
66.	*Euphorbia heterophylla* L.	Euphorbiaceae	Bali	not recorded			22812	6953	-	-	*Oidiopsis* sp.
67.	*Cyclea barbata* Miers.	Menispermaceae	Bali, Desa Pandak Badung	08⁰34'48.55"	115⁰08'00.72"	101	22813	6954	O	-	*Pseudoidium* sp.
68.	*Cucurbita maxima* Duchesne	Cucurbitaceae	Bali	08⁰29'06.64"	114⁰56'47.07"	14	22814	6955	-	-	*Fibroidium* sp.
69.	*Euphorbia heterophylla* L.	Euphorbiaceae	Bali	08⁰10'36.01"	114⁰27'48.70"	14	22815	6956	-	-	*Oidiopsis* sp.
70.	*Cyanthilium cinereum* (L). H. Rob	Asteraceae	Bali	08⁰38'57.57"	115⁰15'29.70"	34	22816	6957	-	-	*Fibroidium* sp.

No.	Species	Family	Location	Latitude	Longitude						
71.	*Pseudoelephantopus spicatus* (Juss. Ex Aubl.)Rohr.	Asteraceae	Bali, Ubud, Monkey Forest	08⁰31'05.10"	115⁰15'32.87"	182	22817	6958	O	-	*Fibroidium* sp.
72.	*Impatiens balsamina* L.	Balsaminaceae	Bali	08⁰21'34.75"	115⁰18'35.31"	798	22818	6959	-	-	*Fibroidium* sp.
73.	*Euphorbia pulcherrima* Willd. Ex Klotzch	Euphorbiaceae	Bali, Kintamani area	08⁰18'24.43"	115⁰20'07.52"	1100	22819	6960	-	-	*Ovulariopsis* sp.
74.	*Tamarindus indica* L.	Fabaceae	Bali	08⁰31'12.13"	115⁰16'15.47"	114	22820	6961	O	-	*Pseudoidium* sp.
75.	*Sonchus oleraceu*s L.	Asteraceae	Bali	08⁰31'12.13"	115⁰16'15.47"	114	22821	6962	O	-	*Euoidium* sp.
76.	*Momordica charantia* L.	Cucurbitaceae	Bali	08⁰31'12.13"	115⁰16'15.47"	114	22822	6963	O	-	*Fibroidium* sp.
77.	*Cleome viscosa* L.	Capparidaceae	Bali	08⁰32'22.60"	115⁰12'51.32"	154	22823	6964	O	-	*Fibroidium* sp.
78.	*Euphorbia heterophylla* L.	Euphorbiaceae	Bali	08⁰32'22.60"	115⁰12'51.32"	154	22824	6965	O	-	*Oidiopsis* sp.
79.	*Vernonia cinerea* (L.) Less.	Asteraceae	Bali	08⁰32'22.60"	115⁰12'51.32"	154	0	6966	O	-	*Fibroidium* sp.
80.	*Morus alba* L.	Moraceae	Bali	08⁰31'34.48"	115⁰12'41.96"	171	22825	6967	O	-	*Ovulariopsis* sp.
81.	*Acalypha integrifolia* Willd.	Euphorbiaceae	Bali, Bedugul	08⁰17'08.11"	115⁰09'50.21"	1301	22826	6968	O	-	*Pseudoidium* sp.
82.	*Bauhinia purpurea* L.	Fabaceae	Bandung, UPI	06⁰51'38.07"	107⁰35'22.32"	935	22827	6969	O	-	*Pseudoidium* sp.
83.	*Lagerstroemia floribunda* Jack.	Lythraceae	Bandung, UPI	06⁰51'41.74"	107⁰35'21.96"	932	22828	6970	O	-	*Ovulariopsis* sp.
84.	*Lagerstroemia speciosa* (L.) Pers.	Lythraceae	Bandung, UPI	06⁰51'41.74"	107⁰35'21.96"	932	22829	6971	O	-	*Ovulariopsis* sp.
85.	*Persea americana* Mill.	Lauraceae	Bandung Barat, Cisarua	06⁰49'26.83"	107⁰31'47.20"	1066	22830	6972	-	-	*Hommaea* sp.
86.	*Oxalis barreliaeri* L.	Oxalidaceae	Unpad	06⁰55'50.33"	107⁰46'26.25"	742	22831	6973	-	-	*Pseudoidium* sp.
87.	*Bauhinia purpurea* L.	Fabaceae	Unpad	06⁰55'51.15"	107⁰46'24.35"	735	22832	6974	O	-	*Pseudoidium* sp.
88.	*Ficus variegata* Blume	Moraceae	Unpad	06⁰55'51.39"	107⁰46'22.46"	730	22833	6975	O	-	*Pseudoidium* sp.
89.	*Persea americana* Mill.	Lauraceae	Bandung, Situ Patenggang	07⁰09'48.82"	107⁰21'22.06"	1594	22834	6976	-	-	*Hommaea* sp.
90.	*Persea americana* Mill.	Lauraceae	Bandung, Situ Patenggang	07⁰09'48.67"	107⁰21'21.82"	1600	22835	6977	-	-	*Hommaea* sp.

No.	Species	Family	Location	Latitude	Longitude						Fungus
91.	*Acalypha lanceolata* Willd.	Euphorbiaceae	Bandung, Situ Patenggang	07⁰09'37.85"	107⁰21'50.35"	1645	22836	6978	O	-	*Pseudoidium* sp.
92.	*Hydrangea macrophylla* (Thunb.) Ser.	Hydrangeaceae	Bandung, Situ Patenggang	07⁰09'37.85"	107⁰21'50.35"	1645	22837	6979	-	-	*Pseudoidium* sp.
93.	*Persea americana* Mill.	Lauraceae	Kab. Bandung, Jl. Raya Ciwidey-Ranca Bali II	07⁰10'18.18"	107⁰21'58.37"	1680	22838	6980	O	-	*Hommaea* sp.
94.	*Leucaena leucocephala* (Lam.) de Wit	Fabaceae	Kab. Bandung, Kampung Sawah	07⁰02'16.28"	107⁰33'04.14"	692	22839	6981	-	-	*Pseudoidium* sp.
95.	*Vernicia montana* Lour.	Euphorbiaceae	Cibinong, LIPI, Ecology Park	06⁰29'16.82"	106⁰51'21.63"	147	22840	6982	-	-	*Pseudoidium* sp.
96.	*Vernicia montana* Lour.	Euphorbiaceae	Cibinong, LIPI, Ecology Park	06⁰29'32.30"	106⁰51'16.59"	163	22841	6983	O	-	*Pseudoidium* sp.
97.	*Codiaeum variegatum* (L.) Rumph ex A. Juss	Euphorbiaceae	Kebun Raya Cibodas	06⁰44'18.90"	107⁰00'20.25"	1334	22842	6984	-	-	*Pseudoidium* sp.
98.	*Hibiscus indicus* (Burm.f.) Hochr.	Malvaceae	Kebun Raya Cibodas	06⁰44'17.84"	107⁰00'20.92"	1351	22843	6985	O	-	*Fibroidium* sp.
99.	*Acalypha hispida* Burm. f.	Euphorbiaceae	Kebun Raya Cibodas	06⁰44'17.23"	107⁰00'21.86"	1340	22844	6986	-	-	*Pseudoidium* sp.
100.	*Castanopsis argentea* (Blume) A. DC	Fagaceae	Kebun Raya Cibodas	06⁰44'12.70"	107⁰00'28.52"	1334	22845	6987	-	-	*Setoidium* sp.
101.	*Castanopsis argentea* (Blume) A. DC	Fagaceae	Kebun Raya Cibodas	06⁰44'12.73"	107⁰00'28.19"	1336	22846	6988	-	-	*Setoidium* sp.
102.	*Castanopsis javanica* (Blume) A. DC.	Fagaceae	Kebun Raya Cibodas	06⁰44'12.89"	107⁰00'28.25"	1344	22847	6989	-	-	*Pseudoidium* sp.
103.	*Acalypha x cristata* Radcl.-Sm.	Euphorbiaceae	Kebun Raya Cibodas	06⁰44'32.57"	107⁰00'22.19"	1408	22848	6990	-	-	*Pseudoidium* sp.
104.	*Acalypha paniculata* Miq.	Euphorbiaceae	Kebun Raya Cibodas	06⁰44'32.08"	107⁰00'22.10"	1406	22849	6991	-	-	*Pseudoidium* sp.
105.	*Brucea javanica* (L.) Merr.	Simaroubaceae	Kebun Raya Cibodas	06⁰44'14.89"	107⁰00'32.92"	1341	22850	6992	-	-	*Pseudoidium* sp.
106.	*Urena lobata* L.	Malvaceae	Kebun Raya Cibodas	06⁰44'18.92"	107⁰00'41.42"	1298	22851	6993	O	-	*Pseudoidium* sp.
107.	*Morus alba* L.	Moraceae	Kebun Raya Cibodas	06⁰44'18.58"	107⁰00'41.32"	1296	22852	6994	O	-	*Ovulariopsis* sp.

| 108. | *Euphorbia pulcherrima* Willd. Ex Klotzch | Euphorbiaceae | Kebun Raya Cibodas | 06⁰44'19.13" | 107⁰00'41.61" | 1299 | 22853 | 6995 | - | - | *Ovulariopsis* sp. |
| 109. | *Castanopsis javanica* (Blume) A. DC. | Fagaceae | Kebun Raya Cibodas | 06⁰44'17.12" | 107⁰00'47.85" | 1304 | 22854 | 6996 | - | - | *Pseudoidium* sp. |

Chapter 8

General Discussion and Conclusions

General Discussions

Amano's compilation of host and geographical ranges of powdery mildews remain the most comprehensive source in the world. This fungal group is believed to be more diverse in the temperate region, since this area is believed to be the origin of this group (Takamatsu 2013). Among Asian countries, powdery mildews have been recorded abundantly in the East Asia (subtropical to temperate regions) and fewer in the tropical to subtropical regions (including the Southeast Asia) (Amano 1986). He also noted that Eastern Asia region has been recognized to have especially many arboreal host plants, thus making it rich in several tree-parasitic powdery mildew genera, such as: *Microsphaera* and *Uncinula* (now *Erysiphe* sect. *Microsphaera* and sect. *Uncinula*) and *Phyllactinia*. Particularly the *Erysiphe* sect. *Uncinula* and the genus *Phyllactinia*, considered to be the old origin among powdery mildew genera based on phylogenetic analysis (Mori et al. 2000), was abundantly distributed in East Asia compared to Europe and/or North America (Takamatsu 2013).

By comparing the numbers of host species in different regions, Amano (1986) noted that the powdery mildews exploration degree in an area or region is determined by the composition of its plant species and the degree of surveying powdery mildews and their host plants. The latter explains why the powdery mildews exploration and reports mostly came from the Northern hemishphere regions. Meanwhile, in the tropics and subtropics regions, which have warmer climates and more diverse plant species, is lacking in teleomorphs, important characteristics for fungal identification. Although anamorphs are usually present, however, these characteristics are usually similar, especially to closely related species, making the process of delineating powdery mildew species based only anamorph without the help of molecular tools become complicated (Arnold 2001; Limkaisang et al. 2005, 2006).

The increasing of powdery mildews exploration in the tropics and subtropics in recent times is fruitful. Not only several new species or the information on the host range expansion were discovered, but also the discovery of several new genera of the

Erysiphales, showing that this regions still have a lot of powdery mildews genetic resources remain unexplored. For example, the *Oidium* subgenus *Mirooidium* proposed by To-anun et al. (2005) based on fungal specimen collected on *Phyllanthus* spp. in Thailand. This subgenus is created to facilitate those powdery mildews anamorphs having catenate (chain-type) conidia without fibrosin bodies, a character alike the genera *Neoerysiphe* or *Golovinomyces* but showing a *Microidium*-type germination, in which the germ tubes arose from the shoulder or at the end of conidia. Braun and Cook (2012) recorded that currently there are three *Microoidium* species known in the world, i.e. *M. agatidis* (Foex) U Braun, *M. bauhinicola* (U. Braun & Dianese) U Braun & Dianese and *M. phyllanthii* (J.M. Yen) To-anun & S. Takam. Another example is the genus *Bulbomicroidium* on *Bauhinia macranthera*, recently proposed from Mexico by Marmolejo et al. (2017). This fungus is morphologically similar to *Microidium*, but distinguishable by having conidiophores with a distinct basal swelling. In addition, the current fungus is phylogenetically distant from that of *Microidium*. To this date, only one species is known on this genera, namely *Bulbomicroidium bahuniicola* (U. Braun & Dianese) Marm., S. Takam. & U. Braun. Last but not least is the new genus, *Hommaea,* proposed in this study. This genus was previously recognized as *Podosphaera perseae-americanae* on *Persea americana* collected in Indonesia (Siahaan et al. 2016b). The phylogenetic analyses of 18S, ITS and 28S rRNA genes clearly showed that this fungus belongs to the tribe Cystotheceae, consisted of four genera, *Cystotheca, Podosphaera, Sawadaea* and *Takamatsuella.* In all phylogenetic analyses, the fungal sequences from *P. americana* readily distinguishable by forming an independent clade from those known genera within the tribe Cystotheceae. The morphological reasons of why this fungus cannot be grouped with *Cystotheca* and *Sawadaea* have been discussed in Siahaan et al. (2016b). However, in addition to their explanation, these two genera are tree parasitic and have been long known to be host specific with narrow host range. *Cystotheca* is host specific to Fagaceae, and *Sawadaea* is host specific to Aceraceae (Takamatsu et al. 2010). The genus *Takamatsuella* has only one species, *T. circinata* (Cook & Peck) U. Braun &

A. Shi, however, anamorph is not known on this fungus, besides, Blast search result of the fungus on *P. americana* hit highest similarity with the member of the genus *Podosphaera*. The genus *Podosphaera*, which is both tree and herbaceous parasitic, is divided into two sections, *Podosphaera* and *Sphaerotheca*. Of all the tree hosts of *Podosphaera* spp., however, *Persea americana* is not one of them.

There were several new powdery mildew species that have been reported from the East Asian region for the past three years, since 2014, i.e. *E.aucubae* S. Takam., & Siahaan on *Aucuba japonica* (Thunb.) (Siahaan et al. 2016a), *E. baliensis* Siahaan & S. Takam. (although this fungus was reported from Indonesia, however, one of its host plants came from Japanese specimen, *Wisteria japonica* Siebold & Zucc.), *E. kissiana* S. Takam. on *Castanopsis cuspidata* (Thunb.) Schottky (Fagaceae) (Takamatsu et al. 2015), *E. magnoliicola* S.E. Cho, S. Takam., & H.D. Shin on *Magnolia* spp. from Korea (Cho et al. 2014). In addition, several reports showing that sometimes a single species was actually a complex species consisted of several taxa based on molecular and morphological data, such as: *Parauncinula polyspora* Meeboon & S. Takam. and *P. uncinata* Meeboon & S. Takam. on *Quercus* spp., previously included in a single species, *Parauncinula septata*, from Japan (Meeboon et al. 2017) and *Erysiphe desmodiicola* Siahaan & C. Nakash. on *Desmodium* spp., described in another chapter in this study, previously included as *Erysiphe glycines*. These show that despite of its known rich powdery mildews diversity, yet East Asian region still keeps many undesribed powdery mildew species.

The implementation of both taxonomic and phylogenetic data, along with the host range and geographical distribution in species identification, carried out since 2011, has brought significant improvement on powdery mildews research in Indonesia. For example, Aryuti & Rifai (1987) classified the powdery mildews species in their reports based on two general genera keys identification and recognized only three genera in their study. The first key identification was that the conidium matured and detached one by one, with narrowed tips and forming no chain. In this type, two genera were recognized, *Oidiopsis* sp. for fungal specimens with (semi-) endophytic hyphae, and *Ovulariopsis* sp.

for ectophytic hyphae. The second one was that the conidium matured gradually, forming chain type-like, with round tips, with only one genus recognized, i.e. *Oidium* sp. However, in recent study, key species determination is becoming more complex. The use of teleomorph (if available), the presence or absence of fibrosin bodies and other morphological characteristics, molecular sequences and host range data have shown that the genera *Oidium* and *Ovulariopsis* recognized in Aryuti & Rifai (1987) can be each further separated into several powdery mildew genera (Braun & Cook 2012).

The study of taxonomy and phylogeny of powdery mildews in one region may play role as a basic study for this fungal group. However, by knowing and understanding of what species do present in a region, how to identify the species by symptoms, morphology or molecular tools, and their evolution, could lead into preventive application to invasive species. Although invasive species have not been reported from Indonesia, such case has been observed in other regions. Lilac powdery mildews (Seko et al. 2008, 2011) and *Erysiphe kenjiana* (Heluta et al. 2009) in Europe, *Erysiphe salmonii* in Ukraine (Heluta et al. 2017) and other powdery mildew species summarized by Kiss (2005), were several examples of invasive powdery mildews on their new habitats. As the study of powdery mildews in Indonesia are still limited, the biodiversity study of Indonesian powdery mildews is essentially required. Thus, this study could provide useful information as a basis for an ongoing research for biodiversity of powdery mildews in Indonesia.

Conclusions

There are still many undescribed or misidentified species found in the East Asian region waiting to be discovered. In addition, the information contained in this study may provide useful information regarding biodiversity of powdery mildew in the tropics, especially in Indonesia. The information provided in this report will be of interest and utility to other scientists interested in the phylogeny and biogeography of Indonesia powdery mildews, especially in identification, surveillance and prevention of emergence and invasion from other regions. Moreover, precise identification of powdery mildews will allow us to be

able to determine the best step to biologically control the occurrence of the fungus which has not been widely applied in Indonesia.

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Appendix 1

A new powdery mildew species, *Erysiphe desmodiicola,* morphophylogenetically sisters to *E. glycines*.

Siska A.S. Siahaan, Chiharu Nakashima, Susumu Takamatsu,

Department of Bioresources, Graduate School, Mie University, 1577 Kurima-machiya, Tsu, 514-8507, Japan

Abstract

Erysiphe glycines is known as a causal agent of soybean powdery mildews disease throughout the world. The fungus was originally described on *Glycine max*, from China. In this study, we amplified partial ITS sequences of *E. glycines from* several hosts belonging to the genera *Desmodium* and *Amphicarpaea*. Phylogenetic analyses of several previous reports have shown that *E. glycines* was clearly divided into two separate groups, fungus on genus *Amphicarpaea* formed a clade with that of genus *Glycines*, recognized as *E. glycines.* On the other hands, the fungus on genus *Desmodium* formed a clade distinct from that of the first group, and in fact, was a sister to *E. glycines*. Morphologically, *E. desmodiicola* differs from *E. glycines* by its smaller chasmothecial and asci size, and fewer number of asci.

Key words: Erysiphaceae, soybean, *Amphicarpaea, Desmodium*, molecular phylogeny

1. Introduction

Two powdery mildew species were recognized as causal agents for soybean powdery mildews, i.e. *Erysiphe diffusa* and *E. glycines*. *Erysiphe diffusa* is distributed worldwide, while *E. glycines* was originally described in China and reported in Japan. The latest fungus seems to be an endemic species in the East Asia region. Phylogenetic analyses of the Erysiphaceae, especially within the genus *Erysiphe*, often show that *E. glycines* was divided into two phylogenetically distinct clades (Takamatsu et al. 2002, 2015a, b). Those analyses showed enough phylogenetic evidences that *E. glycines* was actually a group that consist of at least two powdery mildew species. However, to determine a new species, not only molecular sequences, but also several other aspects, such as host range and clear morphological information should available. Thus, the main purpose in this study is to examine the morphology of fungus in the *E. glycines* group on different hosts, including on genera *Amphicarpaea*, *Desmodium* and *Glycine*.

Materials and methods

2.1. Molecular phylogeny

Whole-cell DNA was extracted from chasmothecia using the chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The internal transcribed spacer (ITS) regions including the 5.8S rDNA sequences were amplified by polymerase chain reaction (PCR) using ITS5/PM6 (Takamatsu & Kano 2001). KOD FX Neo DNA polymerase (Toyobo, Japan) was used in the PCR reaction according to the manufacturer's protocol. The amplicons were sent to Solgent Co. Ltd. (Daejeon, South Korea) for sequencing using primer PM6. Representative new sequences retrieved in this study were deposited in DNA Data Base of Japan (DDBJ) under the accession numbers LC00000–LC00000.

New sequences were aligned with other sequences of the Erysiphaceae, closely related to *E. glycines* retrieved from DNA databases (DDBJ, EMBL, NCBI) using MUSCLE (Edgar 2004) implemented in MEGA 7 (Kumar et al. 2016). Alignments were further manually refined using the MEGA7 program and were deposited in TreeBASE

(http://www.treebase.org/) under the accession number S00000. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods. MP analysis was performed in PAUP 4.0a152 (Swofford 2002) with heuristic search option using the tree bisection reconnection (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. Kishino-Hasegawa (KH) and Shimodaira-Hasegawa (SH) tests (Kishino & Hasegawa 1989, Shimodaira & Hasegawa 1999) were performed to determine whether a given dataset can significantly reject a constraint tree constructed based on a hypothesis. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analysis using 1000 replications with the step-wise addition option set as simple (Felsenstein 1985). Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. Maximum likelihood (ML) tree was obtained by MEGA7 using the Kimura 2-parameter+ G model (Kimura 1980) that was determined as the best evolutionary model for the current data set. The strength of internal branches of the resulting tree was tested with bootstrap analysis using 1000 replications (Felsenstein 1985).

2.2. Morphological examination

In order to examine the traits of the sexual morphs, chasmothecia were stripped off from the leaf surfaces with a clean needle and mounted on a microscope slide in 3% NaOH using a standard light microscope (Axio Imager; Carl Zeiss, Göttingen, Germany) and differential interference contrast optical instruments and devices. To examine the asexual morph, herbarium samples were rehydrated using Shin & La (1993) methods. Herbarium samples were rehydrated by boiling a small piece of infected leaf with the fungal mycelium downwards in a drop of lactic acid on a slide (Shin and La 1993). After boiling, the rehydrated mycelium was scraped off and mounted in lactic acid using a light microscope. Thirty chasmothecia, conidia, and conidiophores were measured for each

specimen examined.

3. Results

3.1. Molecular phylogenetic studies

Nucleotide sequences of partial ITS rRNA gene were generated for two specimens on each *Amphicarpaea* and *Desmodium* hosts. A total of four sequences newly obtained in this study were aligned with other *E. glycines* sequences retrieved from DNA databases. In the end of analysis, four fungal sequences on *Amphicarpaea* were identical to each other. On the other hand, of the four fungal sequences on *Desmodium*, there was one bp nucleotide difference on specimen MUMH 5644.
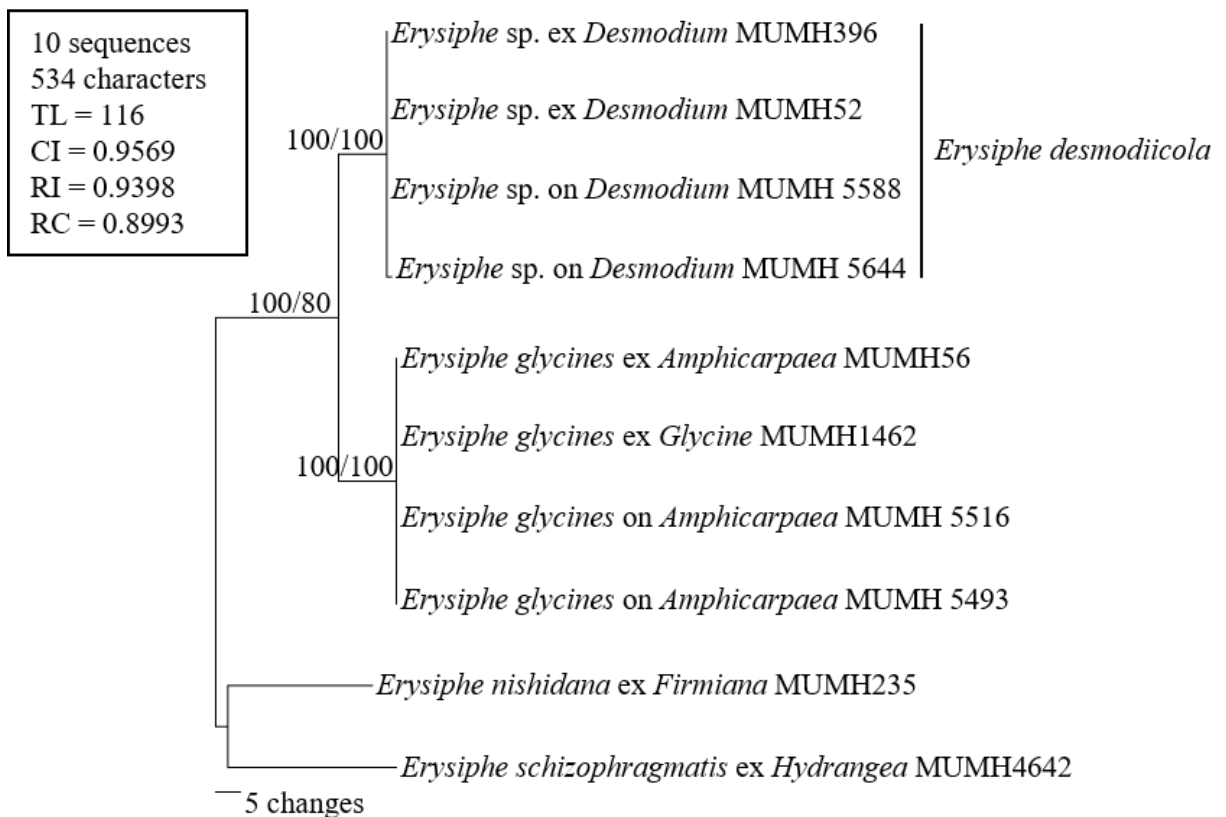
Fig.1 – Phylogenetic analysis of the ITS rRNA gene sequences for 10 sequences of the *Erysiphe*. This tree is one of the six equally parsimonious trees with 116 steps which were found using a heuristic search. BS (≥ 70%) values by the maximum likelihood (ML) and maximum parsimony (MP) methods were shown on the respective branches.

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Our alignment consisted of 10 taxa with 534 characters of which 97 (18.2%) characters were variable and 37 (6.9%) characters were informative for parsimony analysis. A sequence of *E. schizophragmatis* (Tanda & Y. Nomura) U. Braun on *Hydrangea petiolaris* Siebold. & Zucc. & S. Takam. and a sequence of *E. nishidana* (Homma) U. Braun & S. Takam. on *Firmiana simplex* (L.) W. Wight were used as outgroup taxa based on Takamatsu et al. (2015b). A total of six parsimonious tree with 116 steps were generated by MP analysis. Tree topologies were almost consistent among the trees except for branching orders of the terminal branches and branch length. One of the most parsimonious tree is shown in Fig. 1. The phylogenetic analysis clearly showed that the isolates on *Amphicarpaea* spp. and *Glycine max* formed a clade together with 100% BS support (MP/ML values). This clade was sister to isolates on *Desmodium* spp. with 100% / 80% BS values (MP/ML respectively).

3.2. Taxonomy

Erysiphe desmodiicola Siahaan & C. Nakash., sp. nov. — Fig. 2.

Differs from *E. glycines* in having smaller chasmothecia diameter and smaller asci.
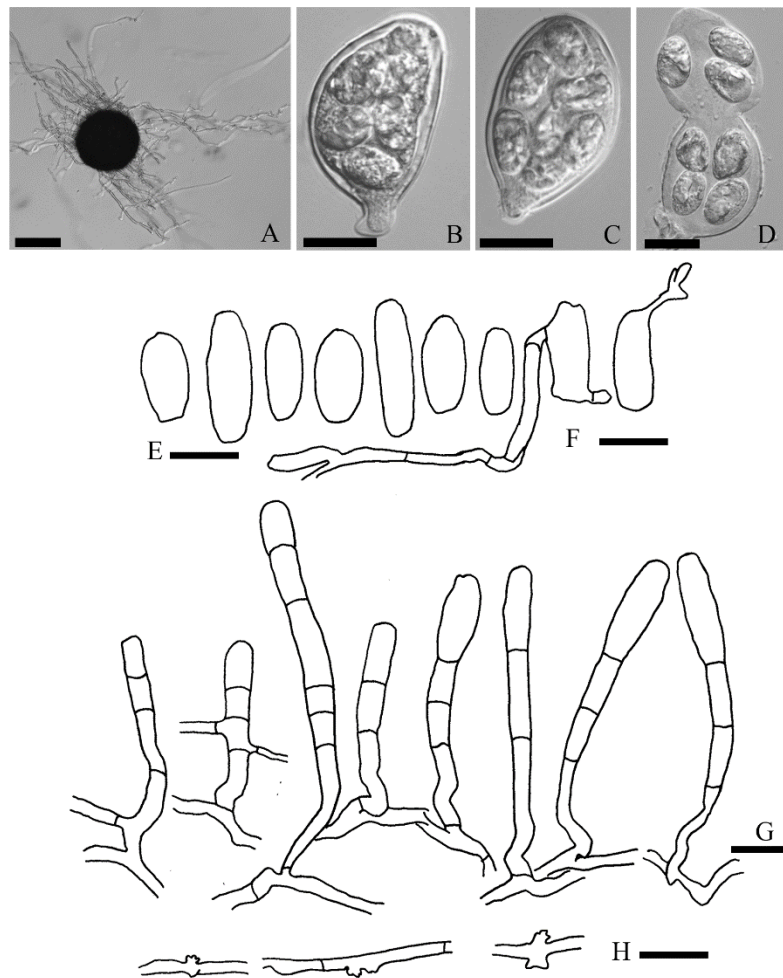
Type: on *Desmodium podocarpum* DC. subsp. *oxyphyllum* (DC.) H. Ohashi var. *japonicum* (Miq.) (Fabaceae), JAPAN, Nara Prefecture, Uda-gun, Mt. Kudoso, 30 Oct. 1994, S. Takamatsu, Tetsuya Hirata, TNS-F-00000 (holotype), TSU-MUMH52 (isotype).

Etymology: The name of the new species is derived from the scientific name of the host genus "*Desmodium*".

Mycelium amphigenous, persistent or evanescent, effuse or thin. *Hyphae* hyaline 3–5.5 μm wide, *hyphal appressoria* nipple shaped to lobed, solitary or in opposite pairs. *Conidiophores* erect, arising from the top of mother cells, up to 110 μm, single or occasionally by twos on a hyphal cell. *Foot-cells* cylindrical, curved to sinuous flexuous, somewhat spiral, sometimes small and slender at the base and becoming wider toward the first following cell, followed by 0–2(–4) shorter cells. *Conidia* ovoid-doliiform, 26.5–

52.5 × 11–20 µm, l/w ratio 1.8–3(–3.5). *Germ tubes* terminal to subterminal, moderate to long, sometimes appear on both terminal, ended with lobed appressorium or with simple apices. New hyphae elongated from the conidiophore cells, but very rare, about five out of 200 conidiophore samples. *Chasmothecia* scattered, 90–137 µm, *appendages* numerous in the lower part, 5–6 times of chasmothecial diam., simple mycelioid or irregularly branched. *Asci* (3–)4–12, broadly ellipsoid-obovoid, sessile or short stalked, 44–57 × 23.5–36(–46.3) µm, 4–7(–8) spored, *ascospores* ellipsoid-ovoid, 15–22.5 × 8.5–12.5µm.

Host range: on *Desmodium* spp., East Asia.

Fig.2. – *Erysiphe desmodiicola* on *D. podocarpum* DC. subsp. *oxyphyllum* (DC.) H. Ohashi var. *japonicum* (Miq.). A. Chasmothecium B–C. Asci. D. Ascospores. E. Conidia. F. Germ tubes. G. Conidiophores. H. Appressoria. *Bars*: A= 50 µm, B–H= 20 µm.
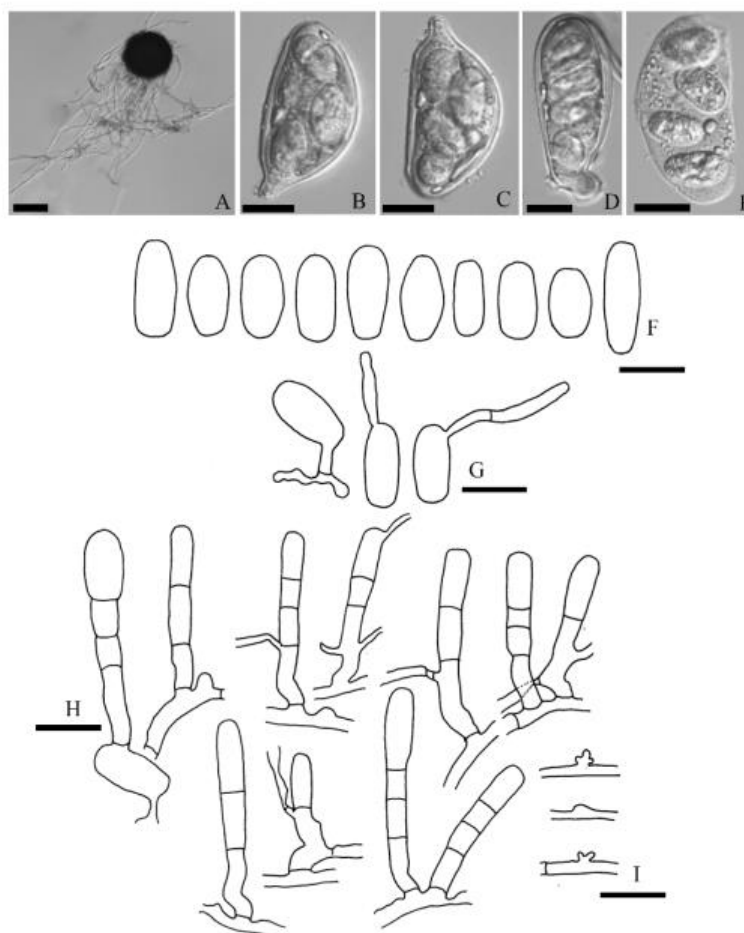
Fig.3. – *Erysiphe glycines* on *A. bracteata* (L.) Fernald. A. Chasmothecium B–D. Asci. E. Ascospores. F. Conidia G. Germ tubes. H. Conidiophores. I. Appressoria. *Bars*: A= 50 µm, B–I= 20 µm.

Additional collections examined: on *Desmodium podocarpum* DC. subsp. *oxyphyllum* (DC.) H. Ohashi var. *japonicum* (Miq.) (Fabaceae), JAPAN, Shiga Pref., Mt. Ibuki, 22 Oct. 2010, TSU-MUMH 5588; Gifu Pref., Tanigumi Village, 31 Oct. 2005, S. Takamatsu, TSU-MUMH 4053; Nara Pref., Ryuugatake, 28 Oct. 2006, S. Takamatsu, TSU-MUMH 4463; Shiga Pref., Mt. Ibuki, 22 Oct. 2010, TSU-MUMH 5580; on *Desmodium podocarpum* DC. subsp. *podocarpum*, 4 Nov. 2010, TSU-MUMH 5644.

Discussion

Erysiphe glycines F.L. Tai was first described on *Glycine* sp. from China (Braun & Cook, 2012). Takamatsu et al. (2002) reported that there are two different *Erysiphe*

species infected soybean in Japan, *E. diffusa* and *E. glycines*. In their phylogenetic analysis, it was shown that *E. glycines* on *Amphicarpaea bracteata* (L.) Fernald subsp. *edgeworthii* (Benth.) Ohashi var. *japonica* (Oliver) Ohashi and on *Glycine max* subsp. *max* formed a clade together with 100% BS and then further grouped with *E. glycines* on *Desmodium podocarpum* DC. subsp. *oxyphyllum* (DC.) Ohashi with high BS support (100%). They also reported that the genetic distance of these two group was relatively high (7.8%). Nevertheless, they maintained the specimen on *Desmodium* as *E. glycines*, probably due to lack of both morphological and molecular evidence as there was only one specimen available for the fungus on *Desmodium* in their report. Eventually, the examination of *E. glycines* group remains unsolved.

In our study, the two fungi in *E. glycines* group could easily distinguished by the differences in their chasmothecial diameter and number of asci in chasmothecia. The fungus on *A. bracteata* was characterized by *chasmothecia* scattered, (96.5–)103–159 μm in diam., appendages numerous in the lower part, up to 5 times of chasmothecial diam.; *asci* (4–)6–12, broadly ellipsoid-obovoid, or oblong ovate-sessile or short-stalked, 51.5–66.5 × (20–)25–37 μm, 4–7 spored; *ascospores* ellipsoid-ovoid, 15–22 × 8.5–12.5μm. Unfortunately, the sexual morphs on *G.max* (AB078807 / TSU-MUMH 1462) were not available due to the scarce chasmothecia. *Mycelium* amphigenous, persistent or evanescent, effuse or in thin, irregular patches; *hyphal appressoria* solitary or in pairs, nipple shaped to lobed, up to 5 μm wide; *conidiophores* erect, arising from the top of mother cell, up to 110 μm, single or occasionally by twos on a hyphal cell. *Foot-cell* cylindrical, curved to sinuous–flexuous, (7–)24–41 × 4.5–7.5 μm; followed by 0–3 cells. *Conidia* ovoid-doliiform, 26.5–39(–47) × 10–20 μm; *germ tube* terminal, moderate to long, ended with a lobed appressorium. New hyphae elongated from the conidiophore cells, very often, especially on samples TSU-MUMH 3032, 5493 (Fig. 3). This character was not mentioned in the original description.

Morphologically, both fungi included in the *E. glycines* group were quite distinct from its original description that came from China. Referring to the original description

in Braun & Cook (2012), the holotype was mostly distinct by its wide range of chasmothecial diam., (75–)85–140(–165) μm, and numerous number of asci, 4–16 with (40–)50– 65(–80) × (20–)25–45(–50) μm in sizes. Thus, to confirm this original morphological description, we re-observed the holotype specimens. Due to limited amount of chasmothecia on the holotype, only 26 chasmothecia were examined. Based on our examination, the holotype has 101–146 um chasmothecial diam., asci 6–13, broadly-ellipsoid, 44–61 × 26.5–36.5 um. Ascospores unobservable. The attempt to obtain the molecular sequence of the holotype was failed because the specimen was too old. So based on those results, the morphology of the holotype were identic with the fungus on *Amphicarpaea*.

Paul & Kapoor (1984) proposed *E. desmodii* based on their fungal collection on *Desmodium* sp. in India. However *E. desmodii* of India is morphologically distinguished from our collection by its smaller chasmothecia diam., which was 85–98 um, fewer asci 3–5, 57–64 × 42–53 um, with 6–8 ascospores. Later, *E. desmodii* from India was reduced to the synonymy *E. glycines*.

Disclosure

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