



# A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangensis*

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## ABSTRACT

A new fungal species, *Pestalotiopsis samarangensis*, was isolated from fruit rot in *Syzygium samarangense* from markets in Chiang Mai and Chiang Rai provinces, Thailand. Initially small, circular, black, slightly sunken spots developed on fruits. Later, the spots enlarged rapidly, became sunken, and resulted in a soft decay of the fruit flesh. Molecular analysis of the ITS,  $\beta$ -tubulin, and *tefl* combined gene sequences distinguish *P. samarangensis* from other species in the genus. Pathogenicity testing proved that wounding triggers the disease symptoms and thus careful handling of fruits during transport and storage helps to prevent the disease.

**Key words:**  $\beta$ -tubulin, fruit rot, ITS, neighbor-joining, phylogenetic, *tefl*.

## INTRODUCTION

*Syzygium samarangense* Merr. (Myrtaceae) is widely grown for its fruits throughout Cambodia, Laos, India, Philippines, Samoa, Sri Lanka, Taiwan, Thailand, and Vietnam (Srisaard, 2003; Vara-Ubol et al., 2006). In Thailand, the wax apple fruit is commonly known as chomphu and harvested the year round with a peak during January to March from the cultivars 'Dang Indo', 'Phet Ban Plew', 'Phet Jin Da', 'Phet Nam Pueng', 'Phet Sai Rung', 'Phet Sam Phran', 'Thub Thim Chan', and 'Thun Klao'. In 2004, Thailand earned US\$ 26.5 million from 69,608 tons of wax apple planted over 10,240 ha (Shü et al., 2008). The fruits have a thin, delicate skin and are thus easily susceptible to pest and disease attack. Diseases of wax apple include *Pestalotiopsis* fruit rot (*Pestalotiopsis eugeniae* (Thüm.) S. Kaneko), shoot dieback (*Fusarium* sp.), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.), *Phytophthora* fruit rot (*Phytophthora palmivora* (E.J. Butler) E.J. Butler), *Dothiorella* fruit rot (*Dothiorella* sp.), and *Pseudocercospora* fruit rot (*Pseudocercospora* sp.) (Janick & Paull, 2008; Lan, 2001), although these species need to be confirmed by molecular data (Phoulivong et al., 2010; Ko Ko et al., 2011).

*Pestalotiopsis* spp. are important plant-pathogenic species known mostly from the tropics, where they cause leaf blights (Guba, 1961) in many plant species (Hyde

& Fröhlich, 1995; Xu et al., 1999; Das et al., 2010; Maharachchikumbura et al., 2011). Species may also cause rots of fruit and other post-harvest diseases (Ullasa & Rawal, 1989; Korsten et al., 1995; Xu et al., 1999). Several post-harvest diseases are caused by species of *Pestalotiopsis*, e.g., postharvest decay of mangos by *P. glandicola* (Castagne) Steyaert (Ullasa & Rawal, 1989), fruit rot of grapevine by *P. menezesiana* (Bres. & Torrend) Bissett as well as *P. uvicola* (Speg.) Bissett (Xu et al., 1999), and fruit rot of rambutan by *Pestalotiopsis* sp. (Sangchote et al., 1998). Scabby fruit canker of guava is caused by *P. psidii* (Pat.) Mordue (Kaushik et al., 1972).

We surveyed market fruit disease of wax apple in Chiang Mai and Chiang Rai provinces in Thailand in 2010 and 2011, and constantly observed a distinctive fruit rot disease. In this study, we introduce this new *Pestalotiopsis* fruit rot disease of wax apple, with a description of morphological and molecular characteristics of the fruit rot agent.

## MATERIALS AND METHODS

### Symptoms and sample collection

Surveys of post-harvest disease of wax apples were conducted in markets of Chiang Mai and Chiang Rai provinces from January to August in 2010 and from March to December in 2011. Wax apple fruits with disease symptoms were carried to the laboratory and photographed.

Samples were sorted by symptoms and the presence of wounds and incubated in a clean polythene bag to promote fungal development. The type herbarium specimen (BPI 406804) of *P. eugeniae*, a pathogen previously recorded from wax apple was borrowed and studied.

#### Isolation and identification of the causal agent of the disease

A single-conidium culture technique was applied to obtain pure colonies of the fungi following a modified method as outlined by Chomnunti et al. (2011). In short, a small piece of diseased fruit bearing acervuli was immersed in 300 µL of sterile distilled water and left a few minutes so that conidia liberate from the conidiomata. A suspension was made and small drops of this suspension were dispersed on water agar (WA) in Petri dishes and kept at room temperature in the laboratory. Conidia were left for 8-12 hours to germinate and single germinating conidia were transferred to fresh PDA plates. The plates were incubated at 25°C for 7 to 10 days. Colonies were grown on PDA plates, later transferred to PDA slants, and stored at 4 °C for further study. Sporulation was induced using sterilized (autoclaved) carnation leaves, which were aseptically placed on the surface of the PDA medium with growing mycelium. The morphology of fungal colonies was recorded following the method of Hu et al. (2007). Fungal mycelium and spores were observed under the light microscope and photographed. All microscopic measurements were made with Tarosoft image framework (v. 0.9.0.7).

#### Pathogenicity tests

Ten-days-old sporulating cultures of *Pestalotiopsis* strains on PDA (25°C, day light) were flooded with Czapek-Dox nutrient solution (Lingappa & Lingappa, 1965), and the mycelia were scraped using a spatula. The conidiomata were crushed using a glass rod. The mycelial suspension was passed through glass wool to remove hyphal fragments and the filtrate containing conidia was collected. The filtrate was centrifuged at 3000 rpm for 3 min and a suspension of 10<sup>3</sup> mL<sup>-1</sup> conidia was prepared. *In vitro* pathogenicity testing was carried out on 15 healthy fruits of wax apple bought from the market. The inoculations were carried out in April 2010 as well as September and November 2011. The surface of the fruits was wiped with 70% ethanol and allowed to air-dry. Fruits were separated into three groups of five fruits each. One group was wounded by pin-pricking. A conidial suspension (20 µL) was placed on the surface of the five unwounded fruits and on five wounded fruits. The remaining five fruits were maintained as controls and inoculated with drops of sterile deionized water. The fruits were placed in polythene bags, labeled, and the moisture was maintained for 10 days by the addition of wet sterile cotton wool. Daily observations were made on the development of disease symptoms. Fruits showing symptoms were removed and the pathogen in the diseased tissue was re-isolated on PDA as described above.

#### DNA extraction and PCR conditions

Total genomic DNA was extracted by the modified protocol of Guo et al. (2000). Fresh fungal mycelium (500 mg) was scraped from the margin of a PDA plate incubated at 25°C for 7 to 10 days and transferred into a 1.5 mL centrifuge tube with 100 µL of preheated (60°C) 2× CTAB extraction buffer (2% (w/v) CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, pH 8.0) and 200 mg sterilized quartz sand. Mycelium was ground using a glass pestle for 5 min, and an extra 500 µL 2× CTAB preheated (60°C) was added and incubated in a 65°C water bath for 30 min with occasional shaking. 500 µL of phenol:chloroform (1:1) was added to each tube and shaken thoroughly to form an emulsion. The mixture was spun at 11900 g for 15 min at 25°C in a microcentrifuge and the supernatant phase decanted into a fresh 1.5 mL tube. Supernatant containing DNA was re-extracted with phenol:chloroform (1:1) at 4°C until no interface was visible. 50 µL of 5 M KOAc (KO(C<sub>2</sub>H<sub>3</sub>O)) was added into the supernatant followed by 400 µL of isopropanol and inverted gently to mix. The genomic DNA was precipitated at 9200 g for 2 min at 4°C in a microcentrifuge. The DNA pellet was washed twice with 70% ethanol and dried using SpeedVac® (AES 1010; Savant, Holbrook, NY, USA). The DNA pellet was then resuspended in 100 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA).

#### PCR amplification

The ITS and 5.8S region of the rDNA molecule was amplified using primer pairs ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') (White et al., 1990), the β-tubulin gene region was amplified with primer pairs BT2A (5'-GGT AAC CAA ATC GGT GCT GCT TTC-3') and BT2B (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') (Glass & Donaldson, 1995; O'Donnell & Cigelnik, 1997), and *tef1* was amplified using the primer pairs EF1-526F (5'-GTC GTY GTY ATY GGH CAY GT-3') and EF1-1567R (5'-ACH GTR CCR ATA CCA CCR ATC TT-3') (Rehner, 2001). PCR was performed with the 25 µL reaction system consisting of 19.75 µL of double-distilled water, 2.5 µL of 10× Taq buffer with MgCl<sub>2</sub>, 0.5 µL of dNTP (10 mM each), 0.5 µL of each primer (10 µM), 0.25 µL Taq DNA polymerase (5 U/µL), 1.0 µL of DNA template. The thermal cycling program followed the methods used by Maharachchikumbura et al. (2012). The PCR products were verified by staining with Goldview (Guangzhou Geneshun Biotech, China) on 1% agarose electrophoresis gels.

#### Phylogenetic analysis

Sequences were optimized manually to allow maximum alignment and maximum sequence similarity as detailed in Maharachchikumbura et al. (2012). Phylogenetic trees were constructed using the neighbor-joining method (Saitou & Nei, 1987) using Mega 4 (Tamura et al., 2007). Evolutionary distances are in the units of the number of

base pair substitutions per site and were computed using the maximum composite likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option in Saitou & Nei, 1987). Branching pattern confidence levels were estimated by the bootstrap resamplings of data based on 1000 random replicates and 64238 random seeds. Gaps were treated as a pairwise deletion and trees were viewed with Mega 4.

## RESULTS

### Symptoms

Initially small, circular, black, slightly sunken spots appear on fruits of *Syzygium samarangense*, which later expand outwards on the surface of the fruit (Figure 1). Following storage these spots enlarge rapidly, become sunken, and result in a soft decay of the fruit flesh. Raised masses of black conidia arranged in concentric bands (up to 1–1.5 cm diam.) develop on the surface of the lesions and are surrounded by white mycelium. In most cases, spots coalesce and form large irregular rotting areas with lesions extending into the pulp. The disease appears the year round.

### Pathogenicity testing

To comply with Koch's postulates, lesions resembling initial symptoms were observed after 8 days on pin-pricked fruits inoculated with a conidial suspension. No symptoms were observed on fruits inoculated with a conidial suspension without pin-pricking. No symptoms were observed in the control fruits. The taxon re-isolated from diseased fruits was identical with the original isolates. The experiment was carried out using five replicates and was repeated three times with the same results.

### Phylogeny

Sequences of nineteen strains of *Pestalotiopsis* are included in the ITS phylogram (Figure 2) including one new sequence generated in this study, six type or epitype strains, and 12 strains used in our previous study. In the ITS phylogram, seven strains (SD004, SD047, SD072, SD091, SG064, SG100 and SS010) cluster in Clade A with strong bootstrap support (100%). However, the strain isolated from wax apple (SS010) is separated from the other six strains. In the combined phylogram (Figure 3), the seven strains again clustered with strong bootstrap support (100%). Three strains of *Pestalotiopsis* cf. *versicolor* form a strongly supported subclade (99%), while the wax apple strain is separated from this subclade. Two strains of *P. cf. meneziana* isolated from tea also cluster in Clade A with 100% bootstrap support. Both, ITS and multigene combined phylograms (Figures 2,3) show that the sequences obtained from *Pestalotiopsis* sp. on wax apple (SS010) are different from sequences obtained from strains SD004, SD047,

SD072, SD091, SG064, and SG100. Therefore, the species represented by the SS010 strain from wax apple apparently is different from the *Pestalotiopsis* species included in the analysis and might correspond to a species new to science.

### Taxonomy

*Pestalotiopsis samarangensis* Maharachchikumbura & K.D. Hyde, **sp. nov.**

Mycobank: MB 800178

FIG 1 A–J

Etymology: The specific epithet is based on the host species, from which the fungus was isolated.

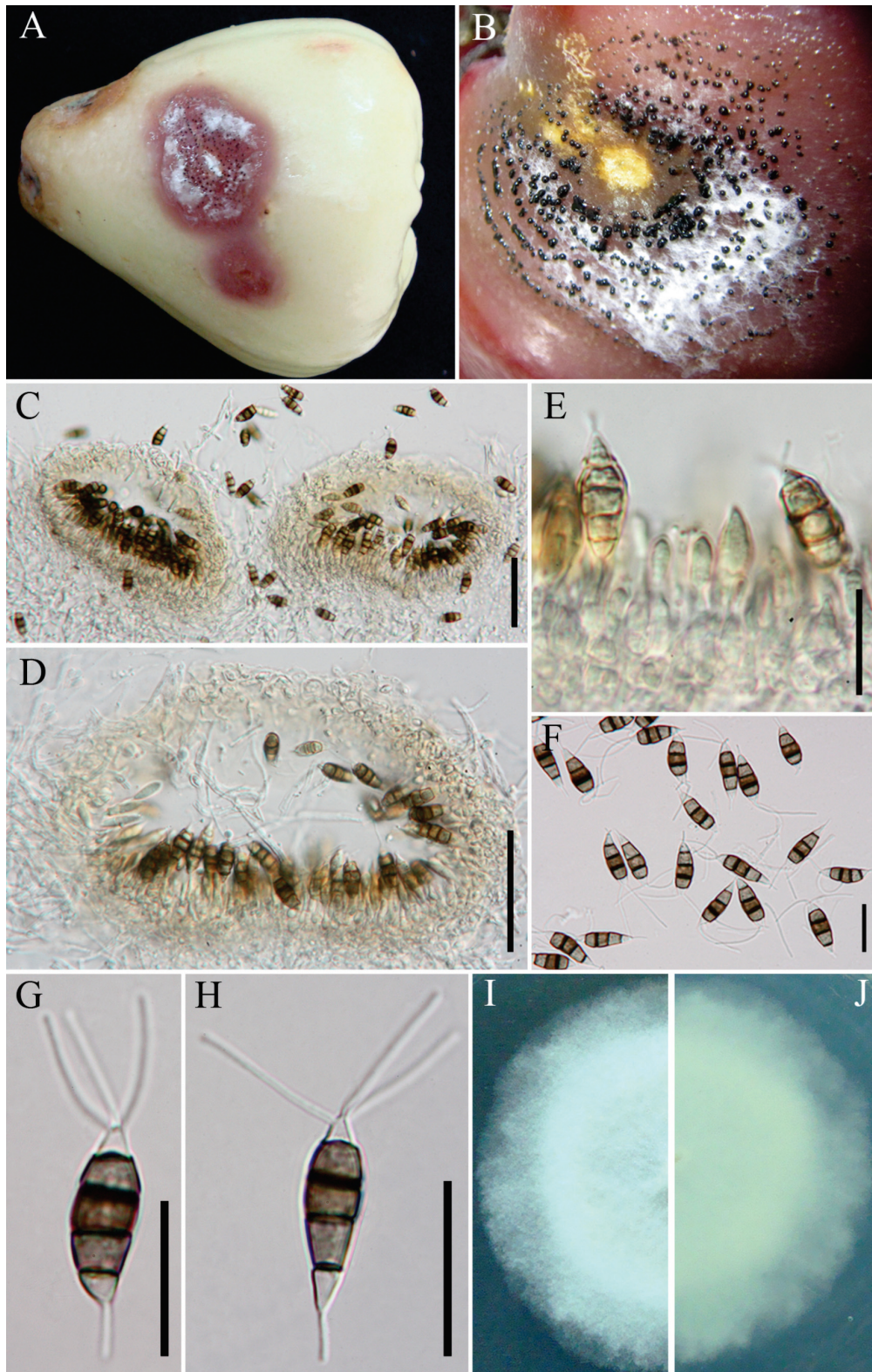
*Conidiomata* acervuli, in concentric bands, confluent, erumpent when mature, rounded to oval in outline, epidermal to superficial in origin, basal stroma and lateral wall 2–4 cells thick; cells hyaline to pale brown, textura angularis, 100–350 µm wide, 80–150 deep (Figure 1 B–C). *Conidiophores* correspond to conidiogenous cells arising within the acervuli. *Conidiogenous cells* discrete, simple, short, filiform (Figure 1 E). *Conidia* 18–21 × 6.5–7.5 µm ( $\bar{x}$  = 20 × 7 µm), fusiform to ellipsoid, broadly clavate, straight to slightly curved, 4-septate, versicoloured; basal cell conical, hyaline, thin and smooth-walled, 3.5–4.8 µm long ( $\bar{x}$  = 4 µm); apical cell 2.5–4.6 µm long ( $\bar{x}$  = 3.4 µm), conical, hyaline, thin- and smooth-walled; three median cells together 12.8–13.8 µm long ( $\bar{x}$  = 13.5 µm), with thick verruculose walls, dark brown, the second cell from base pale brown, 4.3–5.3 µm long ( $\bar{x}$  = 4.8 µm); third cell darker brown, 3.7–5 µm long ( $\bar{x}$  = 4.1 µm); the fourth cell darkest, 4.5–5.3 µm ( $\bar{x}$  = 4.9 µm); three apical appendages 12–18 µm long ( $\bar{x}$  = 15 µm), tubular, without terminal inflation, arising from the upper portion of the apical cell; single basal appendage, 3.5–5.2 µm long, filiform (Figure 1 F–H).

Colonies on PDA reaching 7 cm diam after 6 days at 25°C, edge entire, whitish aerial mycelium, fruiting-bodies black, gregarious; reverse of culture white (Figure 1 I–J).

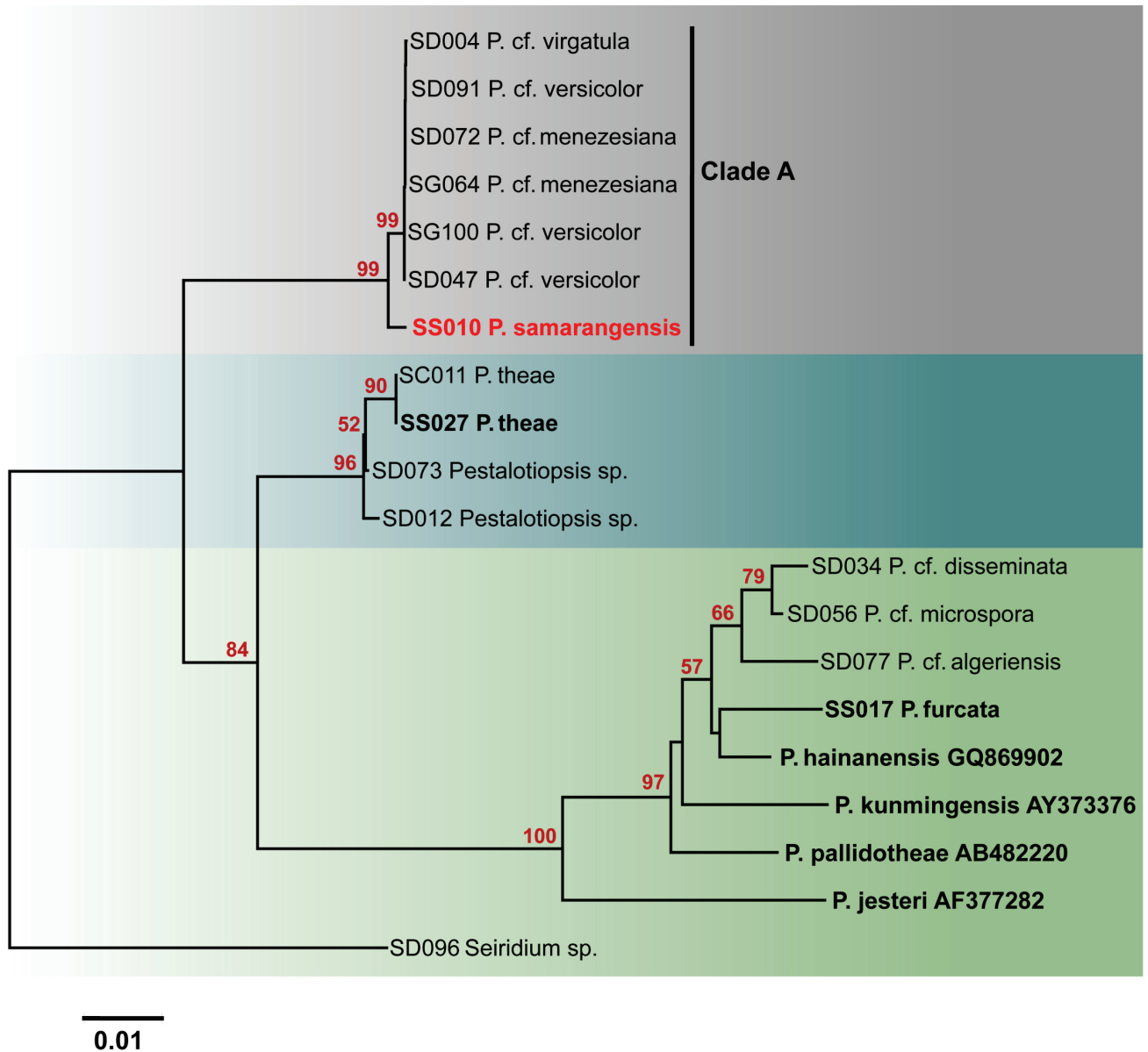
Habitat/Distribution: Known to cause fruit rot on *Syzygium samarangense* in Thailand.

Material examined: **THAILAND**, Chiang Mai Province, Chiang Mai, on fruits of *Syzygium samarangense*, 20 January 2010, S.S.N. Maharachchikumbura S200110b (MFLU 12-0133; **holotype**) - ex-type culture MFLUCC 12-0233; *ibid.*, 15 May 2011, S.S.N. Maharachchikumbura S200511 (MFLU 12-0134); Chiang Rai Province, Chiang Rai, 15 September 2011, S.S.N. Maharachchikumbura S150911 (MFLU 12-0135) (Table 1).

As the names of *Pestalotiopsis* taxa downloaded from GenBank have been shown to be unreliable (Maharachchikumbura et al., 2011) we prefer to label the downloaded taxa as *Pestalotiopsis* cf. *meneziana*, *P. cf. versicolor*, and *P. cf. virgatula* to prevent future confusion. The task of epitypification will however take some time and



**FIGURE 1** - **A.** *Pestalotiopsis samarangensis* (holotype). **B.** Fruit rot of wax apple **C.D.** Acervular conidiomata, epidermal to superficial in origin. **E.** Conidiogenous cells and conidia. **F-H.** Versicoloured conidia. **I-J.** Colony on PDA top (**I**) and reverse (**J**). Scale bars: **C, D** = 50  $\mu\text{m}$ , **E-H** = 20  $\mu\text{m}$ .



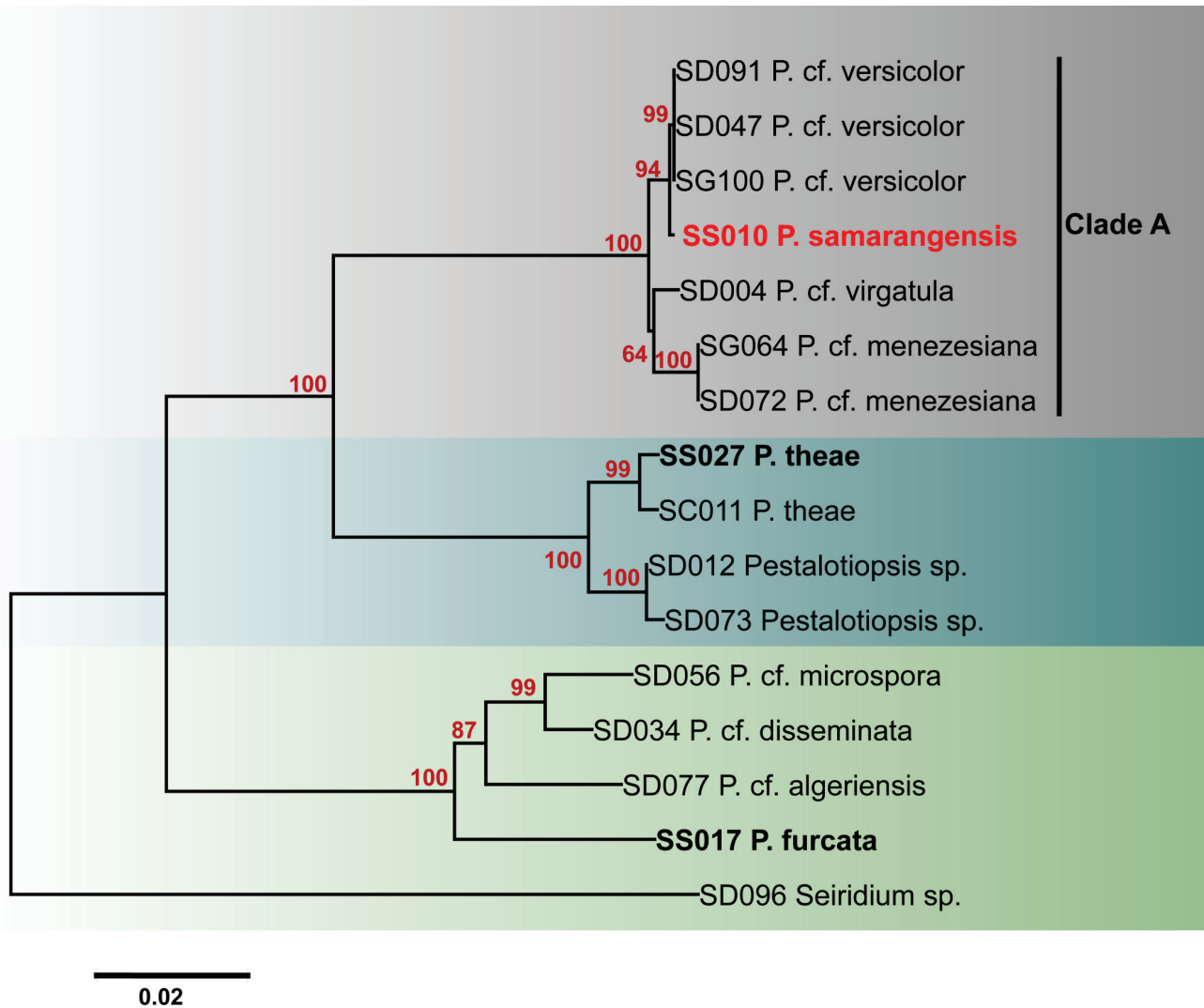
**FIGURE 2** - Neighbor-joining phylogram generated from ITS gene sequence analysis of type and putatively named *Pestalotiopsis* species in GenBank. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* sp. is placed as the outgroup taxon, ex-type sequences are written in bold.

therefore we use cf. (similar to the available species, but not certainly identified as this species = compare with) until more species have been epitypified.

### DISCUSSION

Previously, *Pestalotiopsis eugeniae* (Thüm.) S. Kaneko has been described from wax apple fruit (Lan, 2001). *Pestalotiopsis eugeniae* differs from *P. samarangensis* by concolourous conidia. *Pestalotiopsis samarangensis* also has overlapping morphology with *P. versicolor* and *P. virgatula* (Table 2). However, *P. samarangensis* differs

from *P. versicolor* and *P. virgatula* by molecular data and by shorter apical appendages (Guba, 1961; Nag Raj, 1993). *Pestalotiopsis samarangensis* is somewhat similar in morphology to *P. palmarum* (Cooke) Steyaert), which was isolated in India from coconut. In the original description, Cooke (1876) did not indicate the range of conidial dimensions for *P. palmarum*, but only the average conidial size as  $15 \times 5 \mu\text{m}$ , whereas in *P. samarangensis* conidia measure on average  $20 \times 7 \mu\text{m}$ , thus definitely different. According to Guba (1961), the conidia of *P. palmarum* measure  $16\text{--}22 \times 5\text{--}7 \mu\text{m}$ , overlapping with our fungus (Table 2). In pathological consideration, *Pestalotiopsis*



**FIGURE 3** - Neighbor-joining phylogram of *Pestalotiopsis* spp. generated from combined sequences of ITS,  $\beta$ -tubulin, and *tefl*. *Seiridium* sp. is the outgroup taxon; ex-type sequences are written in bold.

species attacking members of the family Myrtaceae may be specific since the essential oils of the family are likely to exert a selection on pathogens capable to attack these plants (Lee et al., 2008). In this family, scab disease of *Psidium guajava* caused by *P. clavispora* (G.F. Atk.) Steyaert, *P. microspora* (Speg.) G.C. Zhao & N. Li, *P. psidii* (Pat.) Mordue, and *P. disseminata* (Thum.) Steyaert (Keith et al., 2006); bark lesions of *Eucalyptus globulus* stems by *P. guepinii* (Desm.) Steyert (Alonso et al., 2009); a leaf spot of *Anogeissus latifolia* caused by *P. versicolor* (Speg.) Steyaert (Agarwal and Ganguli 1959), and leaf spot of *Eucalyptus camaldulensis* by *P. mangiferae* (Henn.) Steyaert (El-Sayed et al., 1985) are known. *Pestalotiopsis disseminata*, *P. guepinii*, *P. microspora*, and *P. psidii* have concolorous median cells. *P. samarangensis* is distinct from all these species by versicoloured median cells. It is distinct from *P. clavispora* (conidia  $18-26 \times 6.5-8.5 \mu\text{m}$ ; apical appendages  $17-31 \mu\text{m}$ ) and *P. mangiferae*

(conidia  $22-26 \times 8-11 \mu\text{m}$ ; apical appendages  $17-31 \mu\text{m}$ ) by smaller conidia ( $18-21 \times 6.5-7.5 \mu\text{m}$ ) and shorter apical appendages ( $12-18 \mu\text{m}$ ) (Guba, 1961; Nag Raj, 1993). We therefore introduce a new species to accommodate this taxon which causes a disease of wax apple.

Species of *Pestalotiopsis* are not well resolved in the ITS tree, however, ITS,  $\beta$ -tubulin, and *tefl* combined gene analysis successfully resolved the species. The combined phylogenetic tree comprises three ex-type strains and a further twelve putatively named species (due to their economic importance) from our studies. Only for six species of *Pestalotiopsis*, ex-type strains are available; *P. furcata*, *P. hainanensis*, *P. jesteri*, *P. kunmingensis*, *P. pallidotheae* and *P. theae*. Thus there is a need to epitypify other *Pestalotiopsis* species (Maharachchikumbura et al., 2011). Epitypification in *Pestalotiopsis* spp. is, however, somehow problematic as species are not thought to be host-

**TABLE 1** - Isolates and sequences used in the phylogenetic study

Taxon	Collection No.*	Isolate**	GenBank accession numbers		
			ITS	$\beta$ -tubulin	<i>Tef1</i>
<i>P. cf. algeriensis</i> (Sacc. & Berl.) W.P. Wu	CGMCC 3.9164	SD077	JQ683718	JQ683702	JQ683734
<i>P. cf. disseminata</i> (Thüm.) Steyaert	CGMCC 3.9160	SD034	JQ683716	JQ683700	JQ683732
<i>P. cf. menezesiana</i> (Bres. & Torrend) Bissett	CGMCC 3.9181	SG064	JQ683719	JQ683703	JQ683735
<i>P. cf. menezesiana</i> (Bres. & Torrend) Bissett	CGMCC 3.9134	SD072	JQ683713	JQ683697	JQ683729
<i>P. cf. microspora</i> (Speg.) G.C. Zhao & N. Li	CGMCC 3.9199	SD056	JQ683722	JQ683706	JQ683738
<i>P. cf. versicolor</i> (Speg.) Steyaert	CGMCC 3.9107	SG100	JQ683712	JQ683696	JQ683728
<i>P. cf. versicolor</i> (Speg.) Steyaert	CGMCC 3.9159	SD047	JQ683715	JQ683699	JQ683731
<i>P. cf. versicolor</i> (Speg.) Steyaert	CGMCC 3.9135	SD091	JQ683714	JQ683698	JQ683730
<i>P. cf. virgatula</i> (Kleb.) Steyaert	CGMCC 3.9202	SD004	JQ683723	JQ683707	JQ683739
<i>P. furcata</i> Maharachchikumbura & K.D. Hyde	MFLUCC 12-0054	SS017	JQ683724	JQ683708	JQ683740
<i>P. hainanensis</i> A.R. Liu, T. Xu & L.D. Guo	-	-	GQ869902	-	-
<i>P. jesteri</i> Strobel, J.Yi Li, E.J. Ford & W.M. Hess	-	-	AF377282	-	-
<i>P. kunmingensis</i> J.G. Wei & T. Xu	-	-	AY373376	-	-
<i>P. samarangensis</i> Maharachchikumbura & K.D. Hyde	MFLUCC 12-0233	SS010	JQ968609	JQ968610	JQ968611
<i>P. pallidotheae</i> K. Watan. & Yas. Ono	-	-	AB482220	-	-
<i>P. theae</i> (Sawada) Steyaert	MFLUCC 12-0055	SS027	JQ683727	JQ683711	JQ683743
<i>P. theae</i> (Sawada) Steyaert	-	SC011	JQ683726	JQ683710	JQ683742
<i>Pestalotiopsis</i> sp.	CGMCC 3.9188	SD012	JQ683720	JQ683704	JQ683736
<i>Pestalotiopsis</i> sp.	CGMCC 3.9134	SD072	JQ683713	JQ683697	JQ683729
<i>Seiridium</i> sp.	-	SD096	JQ683725	JQ683709	JQ683741

\*Acronyms: CGMCC= China General Microbiological Culture Collection, MFLUCC= Mae Fah Luang University Culture Collection; \*\*SD = sample number 2, SG = sample number 1, SS = sample number 3

**TABLE 2** - Comparison of conidia of *Pestalotiopsis samarangensis* and other similar species

Species	Conidia size ( $\mu$ m) range	Median cells	Number of apical appendages:	Length of apical appendages ( $\mu$ m)	Length of Basal appendages ( $\mu$ m)
<i>P. samarangensis</i>	18–21 $\times$ 6.5–7.5	Versicoloured, brown	3	12–18	Up to 5
<i>P. eugeniae</i> <sup>a</sup>	19–23 $\times$ 6–7	Concolorous, pale brown	3	3–11	short
<i>P. menezesiana</i> <sup>b</sup>	25–27 $\times$ 8–9	Versicoloured, brown	3	28–30	short
<i>P. palmarum</i> <sup>b</sup>	16–22 $\times$ 5–7	Versicoloured, yellow brown	2–3	Up to 16	Up to 6
<i>P. versicolor</i> <sup>b</sup>	19–23 $\times$ 7.5–9.5	Versicoloured, dark brown	3	9–22	Up to 5
<i>P. virgatula</i> <sup>b</sup>	17–23 $\times$ 6–8	Versicoloured, dark brown	2–3	12–26	Up to 3

<sup>a</sup>BPI 406804

<sup>b</sup>Guba (1961)

specific, and material of morphologically similar species taken from the same host and geographical location may actually belong to a different species to what was originally described. Maharachchikumbura et al. (2011) showed that many *Pestalotiopsis* species had been previously named according to host association, and that only a small number of characteristics are available to differentiate species. Therefore, identification of *Pestalotiopsis* to species level is presently difficult and many sequences for *Pestalotiopsis* spp. deposited in GenBank are likely to be wrongly named. Because of this unreliability of sequences in GenBank we are referring these taxa as *Pestalotiopsis* cf. *menezesiana*, *P. cf. versicolor*, and *P. cf. virgatula* for the time being. Correct species identification is essential

in plant-pathogenic genera (Rossman & Palm-Hernández, 2008). Plant pathologists need to be able to confidently name causal agents in *Pestalotiopsis*, so that effective quarantine measures can be put in place to prevent entry of unwanted diseases into a country (Maharachchikumbura et al., 2011). Only wounded or damaged fruits have developed disease symptoms indicating that careful handling of the fruit during transportation and storage can help prevent *P. samarangensis* fruit rot.

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