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Pestalotiopsis species associated with *Camellia sinensis* (tea)

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Abstract — We describe a new species *Pestalotiopsis furcata* isolated from *Camellia sinensis* (tea), which is distinguished morphologically by its relatively large conidia (29–39 × 8.5–10.5 μm) and 5–9 apical appendages, some of which are branched and lack basal appendages. A phylogenetic tree based on a combination of ITS, β-tubulin and tef1 clearly distinguishes *P. furcata* from other species in the genus. We examined syntype material of *P. theae,* which we designate as lectotype, and a fresh collection from Thailand, which we designate as epitype.

Key words — *Amphisphaeriaceae,* grey blight, phylogeny, systematics, tea disease

Introduction

Camellia sinensis (L.) Kuntze (*Theaceae*) is widely planted in the tropics and subtropics and probably originated at the point of confluence of the lands of northeast India, north Burma, southwest China and Tibet (Wight 1959; Mondal et al. 2004). Commonly known as tea, it is prepared as a beverage with a cooling, slightly bitter, astringent flavor and is the most widely consumed liquid in the world after water (Mondal et al. 2004). Several fungi are known to cause diseases of foliage, stems and roots of *C. sinensis*. Brown blight (*Colletotrichum camelliae* Massee), leaf blotch (*Colletotrichum* sp.), grey blight (*Pestalotiopsis longiseta* (Speg.) K. Dai & Tak. Kobay. and *P. theae*, blister blight (*Exobasidium vexans* Massee), twig die-back and stem canker (*Macrophoma theicola* Petch), and horse hair blight (*Marasmius crinis-equi* F. Muell. ex Kalchbr.) are common fungal diseases affecting tea plantations (Gadd 1949; Hainsworth 1952; Chen et al. 1982).

The genus *Pestalotiopsis* contains several species responsible for plant diseases. *Pestalotiopsis* species have been isolated as both endophytes and pathogens in *Camellia sinensis* (Wei et al. 2007; Joshi et al. 2009). *Pestalotiopsis* is an anamorphic genus in *Amphisphaeriaceae* (Barr 1975, 1990; Kang et al. 1998, 1999; Maharachchikumbura et al. 2011, 2012). In southern India grey blight disease of tea caused by *Pestalotiopsis* spp. resulted in 17% production loss (Joshi et al. 2009) and 10–20% yield loss in Japan (Horikawa 1986). Five *Pestalotiopsis* species have been recorded from tea (Agnihothrudu 1964), although *P. longiseta* and *P. theae* are considered to be the major species causing grey blight (Joshi et al. 2009).

The taxonomic status of species in *Pestalotiopsis* is unresolved, as many species are generally not host-specific, conidial characters vary, and species limits overlap. The species concepts adopted by Steyaert (1949) and Guba (1961) are problematic (Maharachchikumbura et al. 2011). Epitypification of important species using molecular data and analyses is needed to resolve the species and to establish their important diagnostic characters, as has been done for several other plant pathogenic genera such as *Colletotrichum* (Hyde et al. 2009), *Phomopsis (*Udayanga et al. 2011), *Cochliobolus* (Manamgoda et al. 2011), and *Phyllosticta* (Wikee et al. 2011). Re-examination of type material and establishment of epitypes from fresh collections relying on sequence analyses from living cultures and full descriptions are needed to resolve species in *Pestalotiopsis* and other important pathogenic genera (Hyde et al. 2010; Cai et al. 2011; Koko et al. 2011).

In order to re-evaluate *Pestalotiopsis* species associated with tea (*C. sinensis*), we examined syntype material of *P. theae*, which we here describe, illustrate, and designate as lectotype. We also epitypify this economically important taxon with a fresh collection from tea in Thailand and fully describe the new collection. Sequence data from the epitype allows us to distinguish *P. theae* from other *Pestalotiopsis* collections from tea. We also describe *P. furcata,* a new *Pestalotiopsis* species from tea in Thailand, which is differentiated by morphological and molecular differences.

Materials and methods

Study site and plant material

Diseased leaves of tea were collected from Chiang Mai Province, northern Thailand, near the Mushroom Research Centre, at an elevation of 900 m at 19°17.123′N 98°44.009′E. The region has a mean annual temperature of 24°C and mean annual precipitation of 2121 mm (http://www.tmd.go.th/en/). Leaf samples were placed in clean polythene bags and symptoms were recorded.

Isolation and identification of pathogen

A single conidium culture technique was used to obtain pure fungal colonies according to Chomnunti et al. (2011). The colonies were transferred to 2% potato-

dextrose agar (PDA) medium and incubated at room temperature (25°C). Sporulation was induced on sterilized carnation leaves that were aseptically placed on the surface of PDA 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. Microtome sections were made with a Leica CM1850 freezing microtome. All the microscopic measurements were measured with Tarosoft image framework (v. 0.9.0.7) and 30 conidial measurements were taken for each isolates.

All other cultures were obtained from China General Microbiological Culture Collection (CGMCC) and a syntype herbarium specimen of *P. theae* (BPI 406804) was borrowed from BPI and studied. Methods of examination, photography, and isolation followed Boonmee et al. (2011).

DNA extraction and PCR condition

Total genomic DNA was extracted from fresh cultures using a modified protocol of Doyle & Doyle (1987) and Lee & Taylor (1990). Fresh fungal mycelia (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. Mycelia were 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 5M KOAc 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 with 70% ethanol twice and dried using SpeedVac® (AES 1010; Savant, Holbrook, NY, USA) until dry. 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 rDNA molecule was amplified using primer pairs ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (White et al. 1990), β-tubulin gene region was amplified with primer pairs BT2A (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and BT2B (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) (Glass & Donaldson 1995; O'Donnell & Cigelnik 1997) and TEF1 was amplified using the primer pairs EF1-526F (5′-GTCGTYGTYATYGGHCAYGT-3′) and EF1-1567R (5′-ACHGTRCCRATACCACCRATCTT-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 $MgCl_2$, 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 was as follows:

For ITS an initial denaturing step of 95°C for 3 min, followed by 35 amplification cycles of 95°C for 30 s, 52°C for 45 s, and 72°C for 90 s, and a final extension step of 72°C

Table 1. *Pestalotiopsis* isolates (and *Seiridium* outgroup) considered in the phylogenetic study

	Isolate	GenBank accession number		
		ITS	β-tubulin	tef1
P. cf. *algeriensis*	sd077	jq683718	jq683702	jq683734
P. cf. *disseminata*	sd034	jq683716	jq683700	jq683732
P. cf. *menezesiana*	sg064	jq683719	jq683703	jq683735
	sd072	jq683713	jq683697	jq683729
P. cf. *microspora*	sd056	jq683722	jq683706	jq683738
P. cf. *versicolor*	sg100	jq683712	jq683696	jq683728
	sd047	jq683715	jq683699	jq683731
	sd091	jq683714	jq683698	jq683730
	sd040	jq683717	jq683701	jq683733
P. cf. *virgatula*	sd004	jq683723	jq683707	jq683739
P. furcata (ex-holotype)	mflucc12-0054	jq683724	jq683708	jq683740
P. hainanensis (ex-type)	—	gq869902	—	—
P. jesteri (ex-type)	—	af377282	—	—
P. kunmingensis (ex-type)	—	ay373376	—	—
P. pallidotheae (ex-type)	—	ab482220	—	—
P. theae (ex-epitype)	mflucc12-0055	jq683727	jq683711	jq683743
	sc011	jq683726	jq683710	jq683742
Pestalotiopsis sp.	sd012	jq683720	jq683704	jq683736
	sd072	jq683713	jq683697	jq683729
Seiridium sp.	sd096	jq683725	jq683709	jq683741

for 10 min. For β-tubulin PCR conditions were an initial step of 3 min at 95°C, 35 cycles of 1 min at 94°C , 50 s at 55°C , and 1 min at 72°C, followed by 10 min at 72°C. For tef1, an initial step of 5 min at 94°C, 10 cycles of 30 s at 94°C, 55 s at 63°C or 66°C (decreasing 1°C per cycle), 90 s at 72°C, plus 36 cycles of 30 s at 94°C, 55 s at 53°C or 56°C, 90 s at 72°C, followed by 7 min at 72°C. The PCR products were verified by staining with Goldview (Guangzhou Geneshun Biotech, China) on 1% agarose electrophoresis gels.

Phylogenetic analysis

DNAStar, SeqMan was used to obtain consensus sequences from sequences generated from β-tubulin and tef1, forward and reverse primers. Combination sequence data obtained from three gene regions were aligned using CLUSTALX (v. 1.83) (Thompson et al. 1997). The sequences were manually adjusted using BioEdit (Hall 1999), to allow maximum alignment and maximum sequence similarity. A maximum parsimony analysis (MP) was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Ambiguously aligned regions were excluded and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were set up to 5000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], homoplasy index [HI], and log likelihood [–ln L] (HKY model) were calculated for trees generated under different optimality criteria. The robustness of the

most parsimonious trees was evaluated by 100 bootstrap replications resulting from maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino–Hasegawa tests (Kishino & Hasegawa 1989) were performed in order to determine whether the trees inferred under different optimality criteria were significantly different. Trees were viewed in Treeview (Page 1996).

The same procedure was used for the ITS gene for the 16 *Pestalotiopsis* isolates and the type sequences of *P. hainanensis*, *P. jesteri*, *P. kunmingensis,* and *P. pallidotheae* were downloaded from GenBank.

Results

A phylogenetic tree was constructed using combined ITS, β-tubulin and TEF1 sequences of 16 isolates of *Pestalotiopsis,* with *Seiridium* sp. as the outgroup (TABLE 1). The aligned data matrix comprised 2019 characters of which 1490 were constant, 199 were variable and parsimony-uninformative, and 330 were parsimony-informative. The Kishino-Hasegawa (KH) test showed that the two trees generated from parsimonious analysis did not differ significantly (length = 768 steps, CI = 0.848, RI = 0.905, HI = 0.152, RC = 0.767). The aligned data matrix of ITS sequences consisted of 528 characters of which 395 were constant, 62 were variable and parsimony-uninformative, and 71 were parsimony-informative. Kishino-Hasegawa (KH) test showed length = 178 steps, CI = 0.826, RI = 0.913, HI = 0.174, RC = 0.754).

Taxonomy

Pestalotiopsis theae (Sawada) Steyaert, Bull. Jard. bot. État Brux. 19(3): 327 (1949)

FIGS 1, 2

≡ *Pestalotia theae* Sawada, Spec. Report Agric. Exp. Station Formosa 11: 113 (1915), as "*Pestalozzia*"

TYPE: Taiwan, Taipei [Taihokuchô, Rigyokutsu (Tanaka 1917)], on living leaves of *Camellia sinensis*, 13 July 1908, coll. Y. Fujikiro, det. K. Sawada (**Lectotype designated here**, BPI 406804). Thailand, Chiang Mai Prov., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, 900 m, rainforest, on living leaves of *Camellia sinensis*, January 20, 2010, S.S.N. Maharachchikumbura St200110 (**Epitype designated here**, MFLU 12-0116; ex-epitype culture, MFLUCC 12-0055 = CPC 20281; GenBank, JQ683727, JQ683711, JQ683743).

LECTOTYPE

LEAF SPOTS initially brown on leaves of tea, becoming 1 cm in diam., and grey with brown margins when mature, or covering up to half of the leaf; dotted with acervuli. ACERVULI initially subepidermal, later erumpent, finally exposed. CONIDIOPHORES in clusters, simple, short, filiform, fugacious. CONIDIA fusiform, slightly constricted at septa, 4-septate, 24–28 × 6.6–8.3 μm (mean = 26.5 × 7.4 μm); basal cell obconic, hyaline, thin and smooth-walled, 4.3–5.6 μm long (mean = 5 μm); 3 median cells, with thick verruculose walls,

dark brown, septa and periclinal walls darker than the rest of the cell, together 15–20 µm long (mean = 18.5 µm) second cell from base 6.5–7 µm (mean = 6.8 µm); third cell 4.8-5.5 µm (mean = 5.3 µm; fourth cell 5.5–6.0 µm (mean = 5.7 µm); apical cell hyaline, conic to cylindrical 4.2–5.8 µm long (mean =

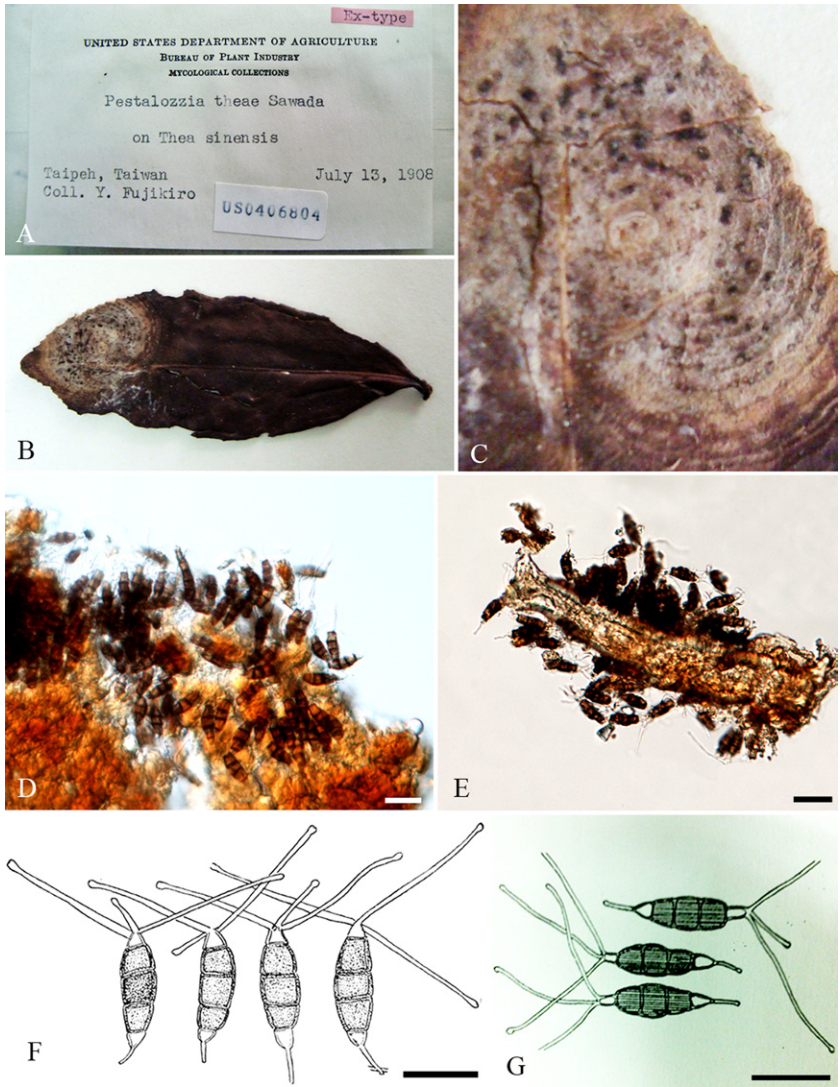

Fig. 1. *Pestalotiopsis theae* (lectotype, BPI 406804). A. Lectotype herbarium material. B,C. Leaf blight symptoms on leaf of *Camellia sinensis*. D,E. Conidia. F. Conidia (drawing from Steyaert 1943) G. Conidia (drawing from Guba 1961). Scale bars: D–G= 20 µm.

4.9 µm); apical appendages tubular, 3–4, arising from the upper portion of the apical cell, 23–33 µm long (mean = 27 µm), slightly swollen at the apex; basal appendages, filiform, 5–9 µm, sometimes knobbed.

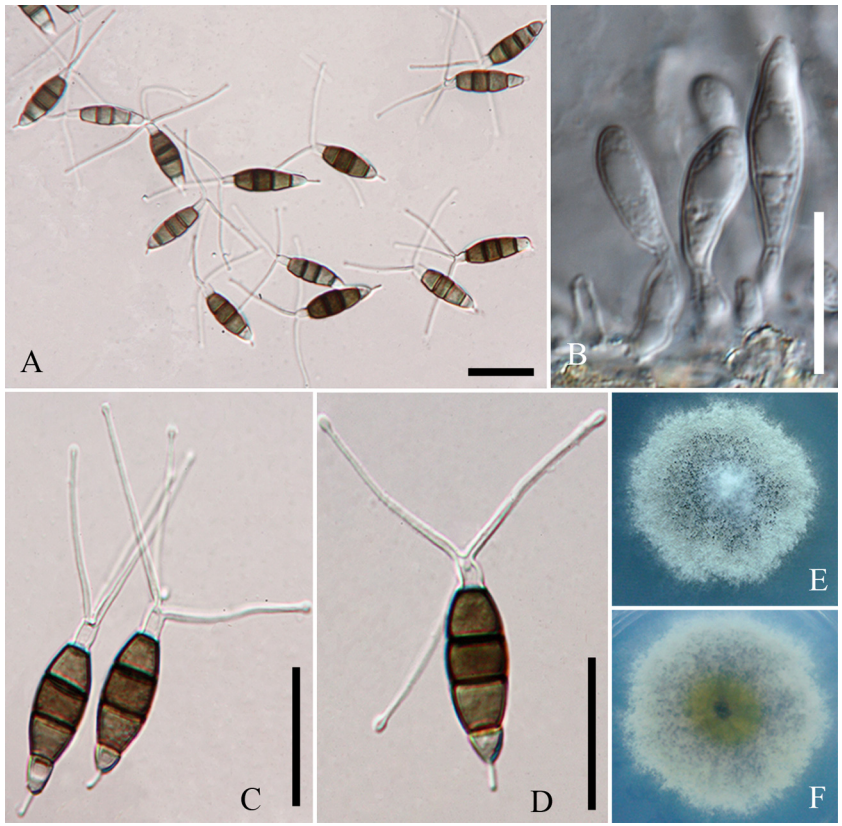

Fig. 2. *Pestalotiopsis theae* (epitype, MFLU 12-0116) A. Conidia in culture. B. Conidiogenous cells. C,D. Conidia. E,F. Colony in culture (E. from above; F. from below). Scale bars: A,B= 20 µm, C,D=15 µm.

Epitype

Conidiophores growing in clusters, simple, short, filiform, fugacious, smooth, thin-walled, hyaline, 4–8 × 1–2 µm (mean = 6 × 1.5 µm). Conidia fusiform to ellipsoid, straight to slightly curved, 4-septate 22.5–28 × 6.7–8.2 µm (mean = 25.5 × 7.6 µm), basal cell conic or obconic, hyaline, thin and smooth walled, 3.9–5.3 µm long (mean = 4.55 µm), with 3 median cells, thick verruculose walls, constricted at the septa, concolorous, dark brown, septa and periclinal walls darker than the rest of the cell, together 14.5–18.5 µm long

(mean = 16.7 µm) (second cell from base 5–7.2 µm (mean = 6.3 µm); third cell 4.8–6 µm (mean = 5.4 µm); fourth cell 5–6.8 µm (mean = 5.7 µm)); apical cell hyaline, cylindrical 4.2–5.9 µm long (mean = 5.2 µm); 3–4 apical appendages, tubular, arising from the upper portion of the apical cell, 22.5–31 µm long (mean = 26.5 µm), slightly swollen at the apex; basal appendages, filiform, 4–7 µm.

Colonies growing relatively fast on PDA, reaching 7 cm after 5 days at 25°C, fimbriate, whitish, dense, aerial mycelium on surface, fruiting bodies black; reverse of the culture yellowish white.

The syntypes of *P. theae* were recorded from diseased leaves of *Camellia sinensis* growing in Taiwan. The specimen from BPI corresponds with one of the collections listed in the translated protologue, and therefore constitutes a syntype specimen (Tanaka 1917, as "Taihokucho, Rigyokutsu, July 13, 1908, Y. Fujikuro"). Since no ex-type culture is available and the lectotype is in poor condition, an epitype with a living culture is designated from a sample collected in Thailand.

Pestalotiopsis furcata Maharachch. & K.D. Hyde, **sp. nov.** Fig. 3

MycoBank MB564563

Differs from other *Pestalotiopsis* species with large conidia by its wider conidia with 5–9 apical, branched appendages.

Type: Thailand, Chiang Mai Prov., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, 20 January 2010, S.S.N. Maharachchikumbura S200110 (**Holotype**, MFLU 12-0112; ex-holotype culture MFLUCC 12-0054 = CPC 20280; GenBank, JQ683724, JQ683708, JQ683740).

Etymology: The specific epithet refers to the branching nature of the apical appendages.

Associated with grey blight on leaves of *Camellia sinensis*, small, rounded, yellow-green spots on the leaves become brown to grey, with concentric rings bearing black, scattered conidiomata (Fig. 3). *Conidiomata* acervuli scattered or gregarious, rarely confluent, subepidermal in origin, erumpent when mature, round to oval in outline, conical to oval in longitudinal section, 180–300 µm wide, 70–160 µm high, unilocular, glabrous; wall tissue (stroma and parietal cells) only a few cells thick (14–22 µm), forming a textura angularis, cell walls thick, outermost layer hyaline, inner layers pale brown to brown, encrusted. *Conidiophores* reduced to conidiogenous cells lining the inner wall of the conidiomatal cavity. *Conidiogenous cells* discrete, lageniform, smooth, thin-walled, hyaline, with 2–3 proliferations. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4-septate, 29–39 × 8.5–10.5 µm (mean = 35.5 × 9.7 µm), basal cell obconic, hyaline or slightly olivaceous, thin- and smooth-walled, 4.9–6.4

Fig. 3. *Pestalotiopsis furcata* (holotype, MFLU 12-0112). A. Blight on leaf of *Camellia sinensis*. B. Conidiomata, split irregularly. C. Section of conidiomata. D. Conidiophores/conidiogenous cells. E–H. Conidia with branched appendages. I,J. Colony on PDA (I from above, J from below). Scale bars: C= 50 µm, D–H= 20 µm.

µm long (mean = 5.8 µm), with 3 median cells, doliiform to subcylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 20.7–25 µm long (mean = 23.4 µm) (second cell from base 7–9 µm (mean = 7.9 µm); third cell 7.5–9.1 µm (mean = 8.2 µm); fourth cell 7.2–9.2 µm (mean = 8.0 µm); apical cell hyaline, conic to cylindrical 6.3-8.44 µm long (mean = 7.48 µm); 5–9 tubular apical appendages, some appendages branched, arising from the upper portion of the apical cell, 20–35 µm long (mean = 27.7 µm), unequal; basal appendages absent.

Colonies on PDA reaching 7 cm after 7 days at 25°C, edge entire, whitish, with dense, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture white.

Habitat/distribution: Known to inhabit living leaves of *Camellia sinensis*, Thailand.

Additional material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, 10 July 2010, S.C. Karunarathna S100710 (MFLU12-0113); 9 September 2011, S.S.N. Maharachchikumbura S110911 (MFLU12-0114); 9 December 2011, S.S.N. Maharachchikumbura S91211 (MFLU12-0115).

Table 2. Synopsis of *Pestalotiopsis furcata* and related species.

	P. furcata	*P. nattrassii* [a]	*P. leucopogonis* [b]	*P. macrospora* [a]	*P. hainanensi* [c]
Conidia size (μm)	29–39 × 8.5–10.5	27–33 × 8–9	27–32 × 7.5–9.5	30–40 × 7–9	19–22 × 5–6
Median cells	Concolorous, olivaceous	Concolorous, brown	Concolorous, brown	Concolorous, olivaceous	Concolorous, Brown to olivaceous
Apical appendages					
–number	5–9	1–4	7–11	3–5	1–3
–length (μm)	20–35	25–44	12–19	15–22	1–10
–branching	Branched	No	No	Branched	No
–position	Apex	Apex	3 rows (top, middle, and bottom)	Apex	Apex
Basal appendages	Lacking	Lacking or short	Lacking or present	Present	Lacking

[a] = from Guba (1961); [b] = from Nag Raj (1993); [c] = from Liu (2007)

Notes: *Pestalotiopsis furcata* is both morphologically (Table 2) and phylogenetically (Figs. 4–5) distinct. Its conidia (29–39 × 8.5–10.5 μm) are larger than those of other *Pestalotiopsis* species as well as wider than such morphologically similar species as *P. hainanensis* (19–22 × 5–6 μm), *P. leucopogonis* (27–32 × 7.5–9.5 μm), *P. macrospora* (30–40 × 7–9 μm), and *P. nattrassii* (27–33 × 8–9 μm). Its most characteristic feature is its 5–9 apical, branched appendages. Although *P. leucopogonis* also has 7–11 apical appendages, they arise from three levels attached to the apical cell (apex, middle, and base) and at 12–19 μm long are shorter than those of *P. furcata* (20–35 μm). *Pestalotiopsis macrospora* has shorter (15–22 μm) branched apical appendages. *Pestalotiopsis furcata* also lacks basal appendages, which are otherwise present in most *Pestalotiopsis* species.

Discussion

In the combined dataset *Pestalotiopsis* species cluster in two main clades with high (100%) bootstrap support (Fig. 5). These two clades can be

differentiated by the color of the median cells, confirming earlier reports (Jeewon et al. 2003; Liu et al. 2010; Maharachchikumbura et al. 2011, 2012). In the multigene analysis (Fig. 5), clade A (*P. theae*) was separate from clade B with high bootstrap support. When additional ITS sequences downloaded from GenBank were analyzed (results not shown), putatively named *P. theae* strains clustered in both clades, indicating that many have misapplied names. The median conidial cells of clade B strains are olivaceous, while clade A strains have brown median conidial cells similar to the type (Fig. 5). We blasted the

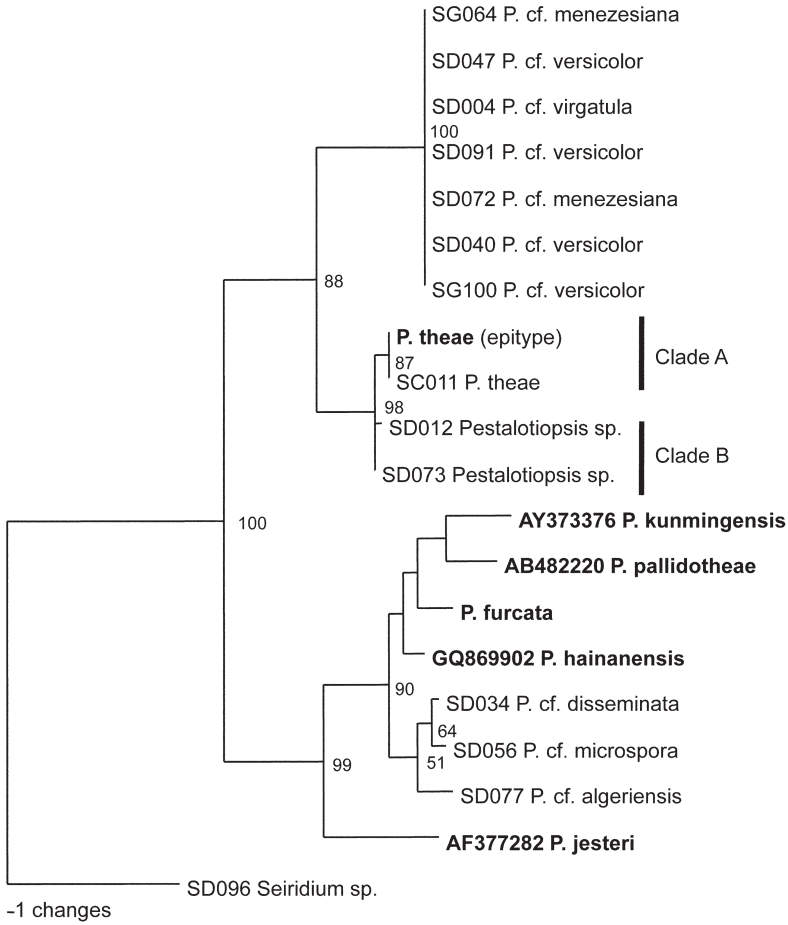

Fig. 4. Maximum parsimony phylogram generated from ITS gene region analysis of species of *Pestalotiopsis* recorded from tea and other available *Pestalotiopsis* spp. type sequences. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* sp. is placed as outgroup, and all ex-type sequences are set in bold font.

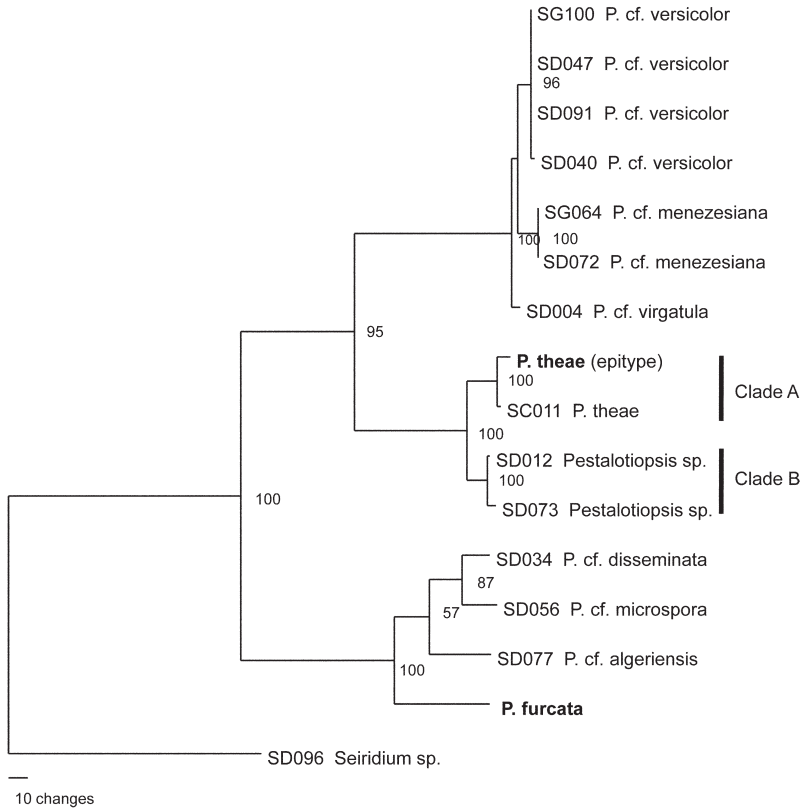

Fig. 5. Maximum parsimony phylogram generated from combine three genes (ITS, β-tubulin and tef1) analysis of species of *Pestalotiopsis* recorded from tea. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* sp. is placed as outgroup, and all ex-type sequences are set in bold font.

isolates from tea plants at the Mushroom Research Centre with ITS sequences in GenBank and there was one similar species. The type strain of *P. hainanensis* is similar to the ITS sequence of the Thailand tea isolate, although morphological characters differentiate these species. In the phylogram (Fig. 5) the Thailand tea species forms a monophyletic subclade and is distinguished as the new species, *P. furcata*. Our analyses confirm the observations by Hu et. al. (2007) and Liu et al. (2010) that a combined multigene dataset better resolves the taxonomy of *Pestalotiopsis*. Hu et al. (2007) showed that the ITS gene is less informative than the β-tubulin gene in differentiating *Pestalotiopsis* species. Liu et al. (2010), however, disagreed and indicated that proper analysis and alignment of the ITS

region can usefully group *Pestalotiopsis* species according to different types of conidial pigmentation, which can be used as a key character for the phylogeny of the genus. We constructed phylogenetic trees separately for each gene region (results not shown for β-tubulin and TEF1) with ITS < β-tubulin < TEF1 in their degree of species resolution, but best resolution results were with all three genes combined.

Most DNA sequences deposited for *Pestalotiopsis* species in GenBank are problematic (Maharachchikumbura et al. 2011), as they are likely to have been wrongly named. There are very few ex-type sequences and therefore the resolution of species and their authentic naming are difficult and confused. Re-examination of type materials and establishment of epitypes with sequenced living cultures are needed to advance understanding of species in this genus.

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