PESTALOTIOPSIS: PHYLOGENY AND DNA BARCODING

SAJEEWA MAHARACHCHIKUMBURA

DOCTOR OF PHILOSOPHY
IN
BIOSCIENCES

SCHOOL OF SCIENCE
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Sajeewa Maharachchikumbura

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Dissertation Title	*Pestalotiopsis:* Phylogeny and DNA Barcoding
Author	Sajeewa Maharachchikumbura
Degree	Doctor of Philosophy (Biosciences)
Advisor	Assoc. Prof. Dr. Kevin David Hyde
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ABSTRACT

The genus *Pestalotiopsis* has received much attention in recent years, not only because of its role as a plant pathogen, but also as a commonly isolated endophyte which has been shown to produce a wide range of chemically novel diverse metabolites. *Pestalotiopsis* consists of around 230 species, most of which were named according to their host associations. However, Phylogenetic analyses in combination with morphology and review of literatures have shown that the genus needs revision, and many of the traditional species may be spurious. This calls for critical re-examination of the genus, using both phenotypic studies and phylogenetic analyses of sequence data based on ex-type and ex-epitype cultures.

In this study, we have studied the genus *Pestalotiopsis* and tested the use of various genes to resolve species boundaries. The 10 gene regions (ACT, β-tubulin, CAL, GPDH, GS, ITS, LSU, RPB 1, SSU and TEF1) were utilized to resolve cryptic *Pestalotiopsis* species, ITS, β–tubulin and TEF1 proved to be the better markers. The other gene regions were less successful in PCR amplification and/or resolving species.

Out of tested locus, as a single gene TEF1 gave the highest species resolution/ PCR success and combination of ITS, β-tubulin and TEF1 gave the best resolution.

Furthermore, we examined large number of *Pestalotiopsis* strains, which were isolated from various hosts and geographic origins. Phylogenetic relationships between these strains and other genera in the family *Amphisphaeriaceae* were resolved based on LSU sequence data. The phylogeny shows that *Pestalotiopsis* is a distinct clade in *Amphisphaeriaceae* and should be split in three groups; besides *Pestalotiopsis,* the two new genera *Neopestalotiopsis* and *Pseudopestalotiopsis* are proposed. Phenotypic analyses of conidial characters coupled with phylogenetic analyses of sequence data were used to clarify species boundaries in the three genera. Species of *Pestalotiopsis* were assigned to 19 sections, 40 new species were described, one species was epitypified and two ex-types were re-examined. *Neopestalotiopsis protearum* assign as the generic type of the newly proposed *Neopestalotiopsis.* In addition we described 19 new species, two species were epitypified, two ex-type were re-examine and six section names were introduced to the *Neopestalotiopsis*. *Pseudopestalotiopsis theae* placed as the generic type of *Pseudopestalotiopsis*; besides two new species were introduced and one species was epitypified.

Keywords: *Neopestalotiopsis*/ new species/ *Pestalotiopsis*/ phylogeny/ *Pseudopestalotiopsis*

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ABBREVIATIONS AND SYMBOLS

ca.	about, approximately
cf.	compare
cm	centimeter
com.	combination
diam.	diameter
e.g.	for example
et al.	and others
etc.	and so forth
g	gram
gen.	genus (singular) and genera (plural)
i.e.	that is
m.	meter
ml	milliliter
mm	millimeter
nov.	novum (Latin for new thing)
sp.	species
spp.	species (plural)
$\bar{x}$	average
°C	degree centigrade
=	heterotypic (taxonomic, facultative) a synonym
µm	micrometer
µl	microliter
%	percent

CHAPTER 1

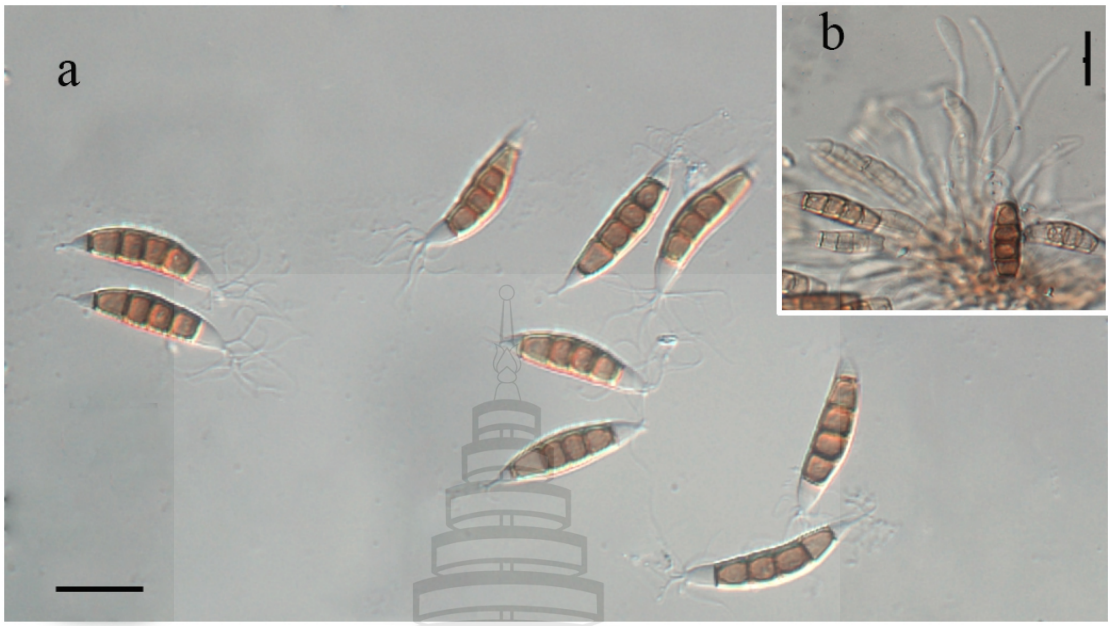
INTRODUCTION

1.1 Introduction

Pestalotoiopsis Steyaert is an appendage-bearing conidial anamorphic form (coelomycetes) in the family Amphisphaeriaceae (Barr, 1975; 1990; Kang, Hyde & Kong, 1998; 1999), and molecular studies have shown that *Pestalotiopsis* is monophyletic (Jeewon, Liew & Hyde, 2002; Jeewon, Liew, Simpson, Hodgkiss & Hyde, 2003; Jeewon, Liew & Hyde, 2004). Species of *Pestalotiopsis* are common in tropical and temperate ecosystems (Bate-Smith & Metcalfe, 1957) and may cause plant disease (Das, Chutia, Das & Jha, 2010), are often isolated as endophytes (Liu, Wu, Xu, Guo & Wei, 2006; Wei et al., 2007; Watanabe, Motohashi & Ono, 2010), or occur as saprobes (Wu, Tseng & Chen, 1982; Agarwal & Chauhan, 1988; Yanna, Ho & Hyde, 2002; Hu, Jeewon, Zhou, Zhou & Hyde, 2007; Liu et al., 2008a). The genus has received much attention from the scientific community. However, this not because of its pathogenic nature (Hyde & Fröhlich, 1995; Rivera & Wright, 2000; Yasuda, Kobayashi, Watanabe & Izawa, 2003), but rather because its species have been shown to produce many important secondary metabolites (Strobel et al., 1996a; 2002; Ding, Liu, Guo, Zhou & Che, 2008a; Ding et al., 2008b; Aly, Debbab, Kjer & Proksch, 2010; Xu, Ebada & Proksch, 2010). The aim of the present chapter on *Pestalotia*, *Pestalotiopsis* and similar genera is to review (1) historical aspects, (2) morphological and molecular studies, (3) life mode of taxa, (4) species numbers and (5) biochemical production by selected species. The problems of understanding the genus are discussed and the work needed to resolve these problems elaborated. In most cases problems arise due to misidentification of taxa and the review illustrates the importance of the correct identification of strains before they are used in biochemical or other studies.

1.2 History

De Notaris (1839) introduced the genus *Pestalotia* De Not. based on the generic type *Pestalotia* pezizoides De Not., which occurred on the leaves of *Vitis vinifera* in Italy. This species is characterized by 6-celled conidia with four deeply olivaceous central cells, distosepta, hyaline terminal cells and simple or branched appendages arising from the apex (Fig. 1.1). Steyaert (1949) revised *Pestalotia* and divided the genus into three main groups based on the conidial forms. Steyaert (1949) also introduced two new genera, *Truncatella* Steyaert for 4-celled conidial forms and *Pestalotiopsis* Steyaert for the 5-celled forms, while the 6- celled forms remained in *Pestalotia*. *Pestalotia* was considered to be a monophyletic genus and Steyaert (1949) suggested that the type species could be distinguished from *Pestalotiopsis* by it cupulate conidiomata and distoseptate median cells. Steyaert (1949) further divided *Pestalotiopsis* into additional sections based on the number of apical appendages. These were the Monosetulatae, Bistulatae, Trisetulatae and Multisetulatae, which were further divided into subdivisions. Conidia with a single setulae (apical appendage) were included in the Monosetulatae, which was further divided into forms with simple and branched setulae. Conidia with two setulae or on average two setulae were included in the Bistulatae. Conidia with three setulae or on average three setulae were included in the Trisetulatae, which was further divided by concolorous or versicolorous conidia, fusiform or claviform conidia and spatulate or nonspatulate setulae. Conidia with more than three setulae were ncluded in the Multisetulatae. Steyaert (1949) reduced Monochaetia (Sacc.) Allesch. from its generic state and placed species with single setula in section Monosetulatae of *Pestalotiopsis* and *Truncatella*. Steyaert (1949) provided descriptions of 46 species and *Pestalotiopsis* guepinii (Desm.) Steyaert was considered to be the type species of the newly introduced genus. *Pestalotiopsis guepinii* is characterized by 4-euseptate and fusiform conidia with a hyaline basal cell. Steyaert's introduction of the genus *Pestalotiopsis* was not supported by Moreau (1949), Servazzi (1953) and Guba (1956; 1961). Steyaert (1953a; 1953b; 1961; 1963), however, published further evidence in support of his new genus with answers to the criticisms made by others.



Notes. a) Conidia b) conidiogenous cells. Scale bars: a-b = 20µm

Figure 1.1 *Pestalotia pezizoides* De Not. BPI0406483

The primary work on *Pestalotia* was carried out by Guba (1961) in his "Monograph of *Monochaetia* and *Pestalotia*". Guba (1961) divided the genus into the sections quadriloculate, quinqueloculatae and sexloculatae for 4-celled conidia, 5-celled conidia and 6-celled conidia respectively. For his sections, Guba (1961) used a simple but very effective system as proposed by Klebahn (1914), which was based on the number conidial cells. Guba (1961) further subdivided the sections into different categories, mainly on the basis of conidial form, colour, and the position, and character of the setulae. *Monochaetia* was retained as a distinct genus based on its single apical appendage, while *Pestalotiopsis* and *Truncatella*, the new genera proposed by Steyaert (1949), were synonymised with *Pestalotia*. Guba (1961) described 258 species of *Pestalotia* in his monograph. Steyaert (1956) argued that the retention of *Monochaetia* as a distinct genus based on a single character, a single apical appendage was incorrect, while other genera (*Pestalotiopsis*, *Truncatella* and *Pestalotia*) were differentiated from each other based on a set of characters.

Sutton (1961; 1980) gave more weight to conidiogenesis when considering *Pestalotia* and *Pestalotiopsis*, and he identified three major problems relating to their taxonomy. According to the Steyaert system, Sutton (1980) concluded that a large number of species that should be included in *Pestalotiopsis* are still placed in *Pestalotia* by some authors. In their studies, Guba (1961), Steyaert (1949; 1953a; 1953b; 1955; 1956; 1961) and most other workers used primarily dried herbarium material. Sutton (1980) pointed out that when species were grown in artificial culture, they show more variability and species limits overlap. Therefore, identification of species from culture and the application of names based on herbarium taxonomy present a confusing situation.

Sutton (1980) used the investigation of Griffiths and Swart (1974a; 1974b), which showed the differentiation of conidial wall development in two species of *Pestalotiopsis*, *P. funerea* (Desm.) Steyaert and *P. triseta* (Moreau & V. Moreau) Steyaert and in *Pestalotia pezizoides* to support Steyaert's opinions. Griffiths and Swart (1974a; 1974b) electron microscopic study was carried out to establish the relationship among *Pestalotia* and *Pestalotiopsis* and other allied generic members of *Monochaetia* and *Seimatosporium* Corda. The minute zonation in conidial wall structure of *P. pezizoides* was thought to separate it from *Pestalotiopsis* (Griffiths & Swart, 1974a; 1974b). Until 1990, phylogenetic understanding of the taxonomy associated with *Pestalotiopsis* and allied genera was based mainly on conidial characters (Steyaert, 1949; Guba, 1961; Nag Rag, 1993), conidiogenesis (Sutton, 1980) and teleomorph association (Barr 1975; 1990; Metz et al., 2000; Zhu, Ge & Xu, 1991).

Morphological characters used to differentiate species of *Pestalotiopsis* and similar genera are limited (Hu et al., 2007); the morphological characters are plastid and morphological markers vary between host and environment (Egger, 1995). Hu et al. (2007) showed that colony morphology (colour, growth rate and texture) is highly variable within single isolates of *Pestalotiopsis*; this phenomenon can be easily observed through repeated subculturing. Also within a single species, conidial morphology (shape and colour of the median cells), growth rate and fruiting structure, may vary (Jeewon et al., 2003). Satya and Saksena (1984) observed *Pestalotiopsis glandicola* (Castagne) Steyaert and *P. versicolor* var. *polygoni* and found that the

intensity of the median cells varied with culture and host and concluded that colour of median cells cannot be used to judge their taxonomic position. Dube and Bilgrami (1965) observed *Pestalotiopsis darjeelingensis* Dube, Bilgrami & H.P. Srivast. and showed morphological variation of conidia in culture (dimension, length of the setulae, shape, number of cells and the colour of the cells). Similar observations were made by Purohit and Bilgrami (1969) when studying more than 100 pathogenic strains. Conidiogenesis is also confusing when used for species separation; Watanabe et al. (1998), showed that *Pestalotiopsis neglecta* (Thüm.) Steyaert and *P. guepinii* having similar acervuli development.

Jeewon et al. (2003) and Tejesvi, Tamhankar, Kini, Ra and Prakash (2009) compared morphology with sequence data and showed that species of *Pestalotiopsis* display considerable diversity in morphology and that isolates grouped together based on similarities in conidial morphology. Hu et al. (2007) found that conidial characters such as conidial length, median cell length, conidial width and colour of median cells were stable characters within *Pestalotiopsis*; however, the length of the apical and basal appendages varied. Jeewon et al. (2003) evaluated the morphological characters that could be used to differentiate species of *Pestalotiopsis*. He suggested that melanin granule deposition within the cell matrix providing pigmentation to the median cells has taxonomic value; this agreed with the findings of Griffiths and Swart (1974a; 1974b). He suggested that the colour of median cells was useful for distinguishing species of *Pestalotiopsis*. Tejesvi et al. (2009) also agreed that species of *Pestalotiopsis* can be distinguished on the basis of morphological characters rather than host-specificity or geographical location. Liu et al. (2010a) proposed that instead of using “concolorous” and “versicolor” as proposed by Steyaert (1949) and Guba (1961), “brown to olivaceous” and “umber to fuliginous” median cells can be a key character in distinguishing species in *Pestalotiopsis*. However the pigmentation can be effected by environmental conditions, different stages of spore maturity and the observer’s expertise (Liu et al., 2010a), hosts, medium, and even different generations through subculturing (Purohit & Bilgrami, 1969; Satya & Saksena, 1984; Hu et al., 2007). The pigmentation of the median cell however, can be stable even within a successive subculture; when using standard conditions and culture on autoclaved carnation leaf segments (Liu et al., 2010a).

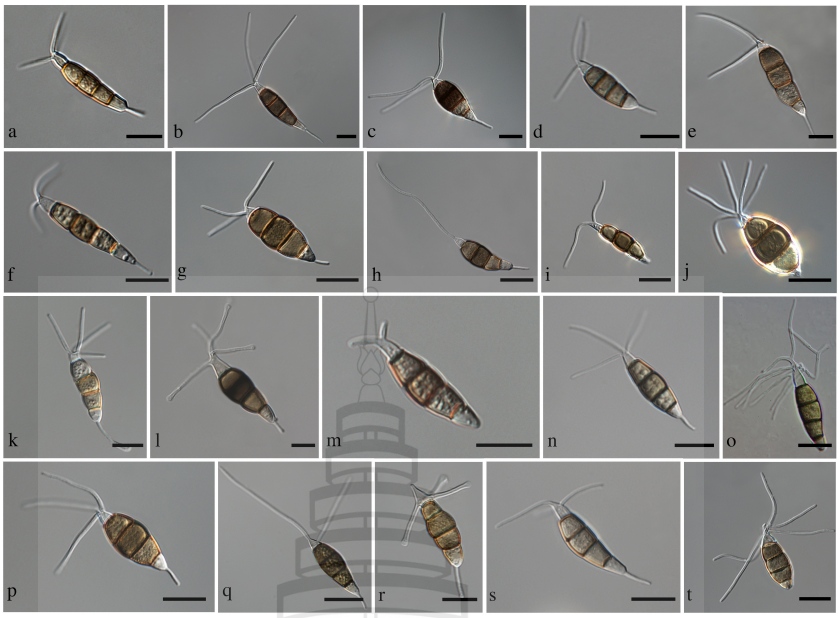
'*The teleomorph of a whole fungus has been traditionally classified and named separately from their anamorphs. Each of the morphs of anamorphosis was also given different binomials as if they were different species. As a result, a whole fungus finds itself in two classification and nomenclature systems against the principle of natural classification*' (Shenoy, Jeewon & Hyde, 2007). The gene responsible for the expression of teleomorph and anamorph evolve at different rates; anamorph characters tend to be morphologically divergent even with the monophyletic groups while teleomorph characters are highly conserved (Chaverri, Castlebury, Overton & Samuels, 2003; Dodd, Lieckfeldt & Samuels, 2003). The teleomorph characters can thus be used as a precise taxonomic marker for *Pestalotiopsis*. However the anamorph of *Pestalotiopsis* is *Pestalosphaeria* M.E. Barr and only twelve species are known as compared to the asexual state (235 species names). *Pestalotiopsis* has been linked to *Neobroomella* Petr. one species and was described by Petrak (1947) and *Pestalosphaeria* (12 species), the genus being described by Barr (1975). As such, the earliest name is *Neobroomella*, but this state has rarely been recorded. *Pestalotia* De Not. has been linked to *Broomella* Sacc. (1883) which has 20 species.

Since *Pestalotiopsis* is the most commonly used name, we therefore suggest that this name be adopted for the anamorph and teleomorph forms. However, if *Pestalotia* is found to incorporate species of *Pestalotiopsis* in future studies, then this name would be used to represent *Broomella*, *Neobroomella* and *Pestalosphaeria*.

1.3 Morphological characters used in the differentiation of species

Conidial morphology (Fig. 1.2) is the most widely used taxonomic character for the genus *Pestalotiopsis*. Most species are divided into different groups based on the size of the conidia. The length and width are good taxonomic markers for the genus and stable within the different media and the generations in most cases (Hu et al., 2007). Colour of the median cells is still a widely used character, and all species separate into three groups based on this- concolorous, versicolorous umber olivaceous and versicolorous fuliginous olivaceous. Molecular evidence indicates that it is more

precise to group species according to concolorous and versicolorous rather than the above three groups (Jeewon et al., 2003). The length of the apical appendages and the number of the apical appendages are also widely used characters for species identification. Some species can also be identified by the presence of knobbed apical appendages. The apical appendages can arise from the top, middle, bottom or different positions in the apical hyaline cells and such characters are widely used in species identification. Furthermore the apical appendages can be divided into branches; in some species presence or absence of the basal appendages is another character for species diagnosis.



Notes. 1) colour of the median cells a) light concolorous b) dark concolorous c) versicolorous 2) size of the conidia d) small conidia e) large conidia f) relatively long conidia g) relatively broad conidia 3) number of apical appendages h) single apical appendages i) three apical appendages j) five apical appendages 4) presence or absence of knobbed apical appendages k) apical appendages without knobbed apical appendages l) apical appendages with knobbed apical appendages 5) length of the apical appendages m) relatively short apical appendages n) relatively large apical appendages 6) branched or unbranched apical appendages o) branched apical appendages 7) position of the apical appendages attached to the apical cell p) attached to the top of the apical appendages q) attached to the middle of the apical appendages r) some attached to the bottom of the apical cell 8) presence or absence of basal appendages s) presence of apical appendages t) absence of apical appendages. Scale bars: a-t = 20µm

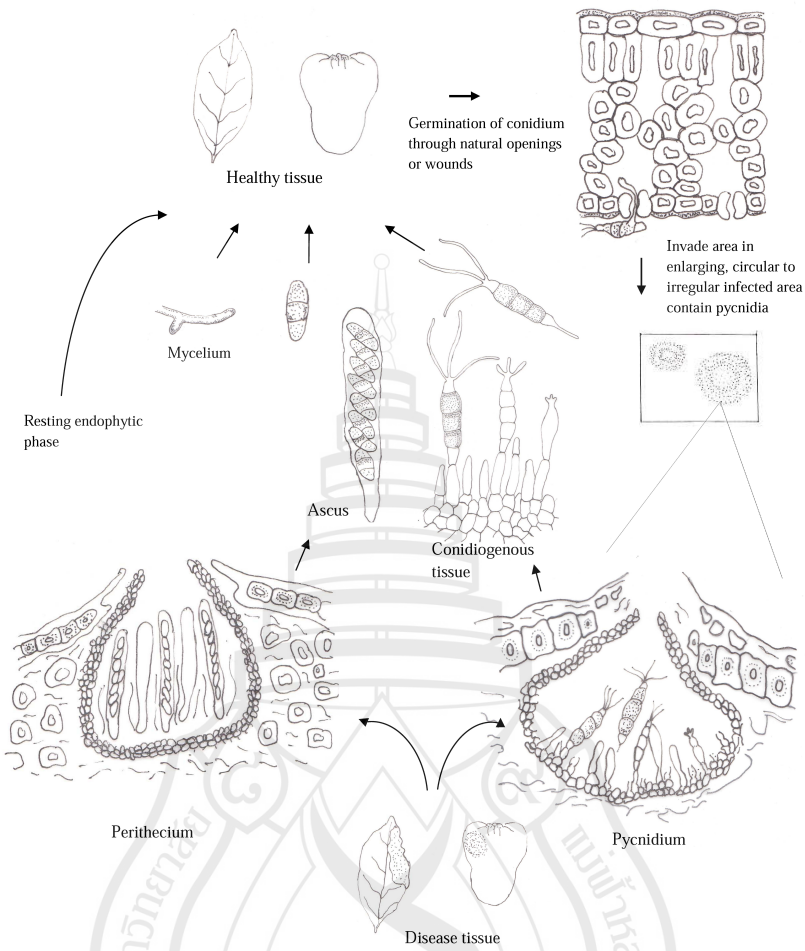
Figure 1.2 Some commonly use conidial characters for *Pestalotiopsis* species identification

1.4 Recent molecular data

Hu et al. (2007) showed that the ITS gene is less informative than the β-tubulin gene in differentiating endophytic species of *Pestalotiopsis* in *Pinus armandii* and *Ribes* spp. When gaps in the ITS region are treated as a missing data, the total number of informative characters is 5% and this results in difficulty in separating taxa and low statistical support. When β-tubulin gene data are used and gaps are treated as missing data, the number of informative characters is about 11%, and when gaps are treated as newstate, it is more than 15%. Thus, Hu et al. (2007) pointed out that the β-tubulin genes resolves *Pestalotiopsis* phylogeny better than the ITS gene. A combination of both the β-tubulin and ITS genes gave better phylogenetic resolution, and they suggested that at least two genes should be used to resolve the phylogeny of species of *Pestalotiopsis*. However, Liu et al. (2010a) disagreed with Hu et al. (2007) concerning the ITS region as being less informative when compared to the β-tubulin region. They indicated that proper analysis and alignment of the ITS region can be a useful character in grouping *Pestalotiopsis* to different types of pigmentation, which can be used as a key character for the phylogeny of the species. Random amplification of polymorphic DNA (RAPD) can also be used to detect genetic diversity in species of *Pestalotiopsis* (Tejesvi, Kini, Prakash, Subbiah & Shetty, 2007a). Tejesvi et al. (2009) showed that the ITS region is more informative than internal transcribed spacer-restriction fragment length polymorphism (ITSRFLP). They used five restriction enzymes (Alu I, Hae III, Ava II, Hpa II and Taq I) in their ITS-RFLP analysis and showed that ITS-RFLP profiles were distinctly different in *P. virgatula* (Kleb.) Steyaert and *P. theae* (Sawada) Steyaert and intraspecific polymorphism highly variable in *P. microspora* (Speg.) G.C. Zhao & N. Li. Based on the ITS sequence, pathogenic and endophytic strains clustered into distinct groups and these clusters were irrespective of the host, parts of the host or location.

1.5 Life cycle in *Pestalotiopsis*

A disease cycle of a pathogen may be closely related to its life cycle, and the former refers to the emergence, development and maintenance of the disease (Agrios, 2005) but is not discussed further here. Species of *Pestalotiopsis* are not highly host-specific and taxa may have the ability to infect a range of hosts (Hopkins & McQuilken, 2000; Keith, Velasquez & Zee, 2006). Species of *Pestalotiopsis* cause a variety of disease in plants, including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and leaf spots (Pirone, 1978; Kwee & Chong, 1990; Xu, Kusakari, Hosomi, Toyoda & Ouchi, 1999; Tagne & Mathur, 2001; Sousa, Tavares, Gerós & Lino-Neto, 2004; Espinoza, Briceno, Keith & Latorre, 2008). Pirone (1978) considered that species of *Pestalotiopsis* are weak or opportunistic pathogens and may cause little damage to ornamental plants; however, Hopkins and McQuilken (2000) pointed out that some species of *Pestalotiopsis* may cause serious damage to pot grown plants and the number of known infected plant species is generally increasing.



Notes. Proposed disease cycle for *Pestalotiopsis* (References: revised and redrawn; Nag Raj, 1993; Kobayashi, Ishihara & Ono, 2001; Von Arx, 1974)

Figure 1.3 Disease cycle of the genus *Pestalotiopsis*

Pathogenic species of *Pestalotiopsis* initially make contact with the host where the infection occurs (inoculum), probably by means of the conidia or fragmented spores (Espinoza et al., 2008). These inocula may survive during harsh weather conditions and may cause primary infections. Secondary inoculum produced on diseased tissue may cause secondary infections and increase the severity of the disease. The source of the inoculum can be wild plantations (Keith et al., 2006),

flowers (Pandey, 1990), crop debris, disease stock plants, used growing media, soil and contaminated nursery tools (McQuilken & Hopkins, 2004), splashed water droplets (Hopkins & McQuilken, 1997; Elliott, Broschat, Uchida & Simone, 2004) and also spores in the air (Xu et al., 1999). Species of *Pestalotiopsis* have constantly been isolated as endophytes from plant tissues (Wei & Xu, 2004; Liu et al., 2006; Wei, Xu, Guo & Pan, 2005; Wei et al., 2007; Tejesvi et al., 2009; Watanabe et al., 2010). We suspect that many endophytic species remain as dormant symptomless inhabitants of plants until the plant is stressed, and then the endophytes become pathogens. This is thought to occur in other pathogenic genera (Gehlot, Bohra & Purohit, 2008). The pathogenic phase may be triggered by a combination of environmental factors, plant susceptibility and the virulence of the pathogen. However, further research is needed to prove the endophytic pathogenic relationship in the genus. *Pestalotiopsis* is also considered to be a weak pathogen (Madar, Solel & Kimchi, 1991), and most weak pathogens penetrate the host through natural openings such as stoma, lenticels and hydathodes (Agrios, 2005). Wright, Rivera & Flynn (1998) stated that species of *Pestalotiopsis* only infect wounded or stressed plants, so pruning wounds or other physical means play important roles in disease development (Elliott et al., 2004; McQuilken & Hopkins, 2004; Keith et al., 2006). Plants may also be stressed due to insect, pesticide or sun damage (Hopkins & McQuilken, 2000). High temperature, high rainfall and human activities may also trigger infections, and this may lead to disease development (Tuset, Hinarejos & Mira, 1999; Hopkins & McQuilken, 2000; Elliott et al., 2004). The anamorph-teleomorph relationships and life cycles are not well known for most species, as the sexual stage does not often develop (Armstrong-Cho & Banniza, 2006). Therefore, conidia therefore appear to play a key role in providing the inocula. A general disease cycle for *Pestalotiopsis* is illustrated in Fig. 1.3.

The spore of *Pestalotiopsis* is considered to be a dry spore. Watanabe, Parbery and Kobayashi (2000) studied conidial adhesion and germination of spores of P. neglecta and showed that infection occurs in four stages. At the beginning, the lower median cell germinates and becomes firmly attached to the substrate. Future successive infections can be achieved by two upper median cells. In the first stage, weak adhesion is achieved by the mucilaginous matrix coating the conidia. A second

weak adhesion occurs at the bases of the pedicel. The next two stages provide a strong attachment by release of fibrillar adhesive substances. In the third stage, fibrillar adhesive substances are produced along the length of the pedicel to the apex of the basal cell and at times a smaller amount of fibrillar material is released from the apical appendages. The fourth stage involves the release of fibrillar material at the point of germ tube emergence. Nag Rag (1993) described conidiomata of the genus as variable, ranging from acervuli to pycnidia. Conidiomata can be immersed to erumpent, unilocular to irregularly plurilocular with the locules occasionally incompletely divided and dehiscence by irregular splitting of the apical wall or overlying host tissue (Nag Rag, 1993). Conidiophores partly or entirely develop inside the conidiomata, and they can be reduced to conidiogenesis cells which are discrete or integrated, cylindrical, smooth, colourless and invested in mucus (Nag Rag, 1993). Pycnidia can mostly be seen with the unaided eye as a black or brown spore masses with copious conidia.

Control strategies are needed for serious *Pestalotiopsis* disease, and therefore, knowledge of the causal agent and the disease cycle is important. Precise knowledge of the plant/ pathogen interaction and its functional variation according to the environmental factors are important for integrated disease management using cultural, biological and chemical methods. Elliott et al. (2004) stated that *Pestalotiopsis* may produce large numbers of spores which are easily dispersed in air or by water splash, thus sanitation and disease management are critical. They suggested that water management strategies, such as elimination of overhead irrigation, decreasing wetness of leaves, increasing the spacing of plants and increasing the air circulation, can reduce disease in palm plantations. Different harvesting factors also directly affected disease development in tea plantations. Sanjay, Ponmurugan and Baby (2008) showed that highest disease incidence occurred in continuously shear-harvested fields and least in hand-plucked plantations, and they evaluated systemic fungicide and biocontrol agents such as a *Trichoderma*, *Gliocladium* and *Pseudomonas* for use in controlling grey blight disease in tea.

1.6 Mode of life

Species of *Pestalotiopsis* commonly cause disease in a variety of plants (Hyde & Fröhlich 1995; Hopkins & McQuilken, 2000; Tagne & Mathur, 2001), are commonly isolated as endophytes (Kumar & Hyde, 2004; Wei & Xu, 2004; Wei et al., 2005; 2007; Liu et al., 2006; Tejesvi et al., 2009; Watanabe et al., 2010) and some species likely have endophytic and pathogenic stages in their life cycle (Wei et al., 2007: Tejesvi et al., 2009). Species have also been recorded as saprobes (Guba, 1961; Wu et al., 1982; Agarwal & Chauhan, 1988; Yanna et al., 2002; Liu et al., 2008a) where they are recyclers of dead plant material (Okane, Nagagiri & Ito, 1998; Osono & Takeda, 1999; Tokumasu & Aoiki, 2002) and even rarely cause disease in humans (Sutton, 1999).

1.6.1 Sexual and asexual forms

One fifth of all known anamorphic fungi lack known sexual states (Shearer, Raja & Schmit, 2007), and out of 2,873 anamorphic genera names, 699 genera and 94 anamorph-like genera are linked to a sexual state (Hyde, McKenzie & KoKo, 2011). The links between sexual and asexual stage are mostly from indirect evidence, with some links known through experimental or molecular data (Kendrick, 1979; Reynolds, 1993; Shenoy et al., 2007; Hyde et al., 2011). *Pestalotiopsis* is a species-rich anamorphic genus with species mostly lacking sexual morphogenesis, unlike the coelomycetous genera *Colletotrichum* and *Phyllosticta* (Armstrong-Cho & Banniza, 2006; Wulandari et al., 2009) and *Penicillium* (Cannon & Kirk, 2000). The sexual states or teleomorphs of *Pestalotiopsis* species have been identified as *Pestalosphaeria* (Barr, 1975) and *Neobroomella* (Kirk, Cannon, Minter & Stalpers, 2008).

The asexual *Pestalotiopsis* state and ascomycetous sexual state have rarely been recorded in the same host plant (Barr, 1975; Nag Raj, 1985; Hyde, 1996). However, it is not always clear that the two stages found are definitely the same biological species and therefore molecular evidence is needed to link them. In the laboratory species of *Pestalotiopsis* rarely develop sexual forms (Metz et al., 2000).

Zhu et al. (1991) induced *Pestalosphaeria accidenta* P.L. Zhu, Q.X. Ge & T. Xu and *P. jinggangensis* P.L. Zhu, Q.X. Ge & T. Xu to form on potato dextrose agar (PDA). However, this took 5 to 6 months of incubation. Metz et al. (2000) obtained the sexual state of *P. microspora*, an endophytic isolate that produced taxol. The asexual stage formed after 3–6 weeks on water agar with dried yew needles when incubated at 16–20 C with 12 h of light per day and was identified as *Pestalosphaeria hansenii* Shoemaker & J.A. Simpson. The twelve sexual states known for species of *Pestalotiopsis* are listed in Table 1.1.

Table 1.1 List of asexual forms with known sexual forms.

Asexual form	**Sexual form**
Pestalotiopsis baarnensis Steyaert	*Pestalosphaeria accidenta*
Pestalotiopsis sp.	*Pestalosphaeria alpiniae* P.K. Chi & S.Q. Chen
Pestalotiopsis sp.	*Pestalosphaeria austroamericana* Nag Raj & DiCosmo
Pestalotiopsis guepinii var *macrotricha* (Kleb.) B. Sutton	*Pestalosphaeria concentrica* M.E. Barr
Pestalotiopsis sp.	*Pestalosphaeria elaeidis* (C. Booth & J.S. Robertson) Aa
Pestalotiopsis eugeniae (Thüm.) S. Kaneko	*Pestalosphaeria eugeniae* P.K. Chi & S.M. Lin
Pestalotiopsis neglecta	*Pestalosphaeria gubae* Tak. Kobay., Ishihara & Yas. Ono
Pestalotiopsis microspora	*Pestalosphaeria hansenii*
Pestalotiopsis podocarpi (Dennis) X.A. Sun & Q.X. Ge	*Pestalosphaeria jinggangensis*
Pestalotiopsis sp.	*Pestalosphaeria leucospermi* Samuels, E. Müll. & Petrini
Pestalotiopsis maculiformans (Guba & Zeller) Steyaert	*Pestalosphaeria maculiformans* Marinc., M.J. Wingf. & Crous
Pestalotiopsis besseyi (Guba) Nag Raj	*Pestalosphaeria varia* Nag Raj

1.6.2 *Pestalotiopsis* Steyaert as a plant pathogen

Pestalotiopsis is a relatively important plant pathogenic genus known mostly from the tropics, where it causing leaf blights (Guba, 1961) in many plant species (Hyde & Fröhlich, 1995; Xu et al., 1999; Das et al., 2010). Species may also cause rots of fruit and other post harvest disease (Ullasa & Rawal, 1989; Korsten et al., 1995; Xu et al., 1999). It has been estimated that in southern India grey blight disease of tea (*Camellia sinensis*) caused by *Pestalotiopsis* has resulted in 17% production loss (Joshi, Sanjay, Baby & Mandal, 2009) and 10–20% yield loss in Japan (Horikawa, 1986). Five species of *Pestalotiopsis* have been recorded from tea (Agnihothrudu, 1964), although *P. longiseta* (Speg.) H.T. Sun & R.B. Cao and *P. theae* are considered to be the major species causing grey blight (Joshi et al., 2009). *Pestalotiopsis sydowiana* (Bres.) B. Sutton causes foliage, root and stem-base browning disease in container-grown ericaceous plants, resulting in plant losses and reduced plant quality (McQuilken & Hopkins, 2004). *Antheraea assamensis*, a silkworm endemic to the north eastern part of India that depends on *Persea bombycina* as the primary food plant, is endangered due to grey blight disease cause by *Pestalotiopsis disseminata* (Thüm.) Steyaert (Das et al., 2010). *Pestalotiopsis funerea* was found to cause leaf spots of *Hakea sericea*, a plant that is considered as an invader of natural habitats in northern Portugal, and this may allow its use in biological control (Sousa et al., 2004). *P. menezesiana* (Bres. & Torrend) Bissett and *P. uvicola* (Speg.) Bissett causes postharvest disease of grape (Xu et al., 1999) and *P. clavispora* (G.F. Atk.) Steyaert, *P. disseminata* and *P. microspora* cause scab in Guava in Hawaii (Keith et al., 2006). The economically important blueberry fruit from Chile is infected by pathogenic *P. clavispora* and *P. neglecta*, which cause canker and twig dieback (Espinoza et al., 2008).

In Sicily, the economically important plant *Laurus nob*ilis is infected by *P. uvicola*, which causes causing leaf spots and stem blights (Vitale & Polizzi, 2005). Chlorosis and reduction of growth were recorded in maize fields in the Cameroons when the plants were infected by *P. neglecta* (Tagne & Mathur, 2001). The medicinally important ornamental shrub *Lindera obtusiloba*, which grows wild in the mountain areas of the Korean Peninsula, is infected by *P. microspora*, and the affected leaves initially have grey or dark brown lesions, surrounded by yellowish

halos; these enlarge, coalesce and become entire at a later stage, finally causing full leaf blight (Jeon, S.G. Kim & YH Kim, 2007). Affected leaves of *Hymenaea courbaril* show symptoms of leaf spots and the pathogen was identified as a *P. subcuticularis* (Guba) J.G Wei & T. Xu (Fail & Langenheim, 1990). Pathogenic *P. funereal* infects conifer species and causes necrosis on infected tissues and sometimes death of the plants involved (Bajo, Santamaria & Diez, 2008). The medicinal and ornamental *Carapa guianensis* is infected by *P. macrochaeta* (Speg.) J. Xiang Zhang & T. Xu, and foliar blight has been observed in the lower canopy of the plants (Halfeld-Vieira & Nechet, 2006). Species of *Pestalotiopsis* also have the potential to cause leaf and/or fruit spots on ginger, rambutan, lychee and orchid (Keith & Zee, 2010).

Pestalotiopsis glandicola is a postharvest pathogen on mango in Bangalore; the disease can be observed on the leaves throughout the year and it provides the inoculum for mature fruits, which develop postharvest decay during storage (Ullasa & Rawal, 1989). Fruit rot of grapevine is caused by *P. menezesiana* and *P. uvicola*, and the pathogens were not only isolated from diseased and healthy fruits but also from the airspora in grape orchards; thus, the authors pointed out that latent infection or conidial attachment to the barriers in the field will lead to postharvest disease in grapes (Xu et al., 1999). *Pestalotiopsis* fruit rot is one of the serious postharvest diseases of rambutan fruit in Thailand (Sangchote, Farungsang U & Farungsang N, 1998). *Pestalotiopsis psidii* (Pat.) Mordue is considered to be the causal agent of scabby fruit canker of guava in India and infection results in rapid yield loss and affects the postharvest quality of the fruits (Kaushik, Thakur & Chand, 1972).

1.6.3 *Pestalotiopsis* as an endophyte

Most resent *Pestalotiopsis* research is based on endophytic isolates (Liu et al., 2006; Wei et al., 2007; Watanabe et al., 2010; Aly et al., 2010) and has resulted in a four new species being described. These are *P. hainanensis* A.R. Liu, T. Xu & L.D. Guo, *P. jesteri* Strobel, J. Yi Li, E.J. Ford & W.M. Hess, *P. kunmingensis* J.G.Wei & T. Xu and *P. pallidotheae* Kyoko Watanabe & Yas. Ono. Most endophytic studies have used morphological characters and either gene sequence data (Hu et al., 2007; Liu, Xu & Guo, 2007; Wei et al., 2007) or RFLP technique (Tejesvi et al., 2007a) or a

combination of gene sequence and RFLP techniques (Tejesvi et al., 2009) to distinguish species. The distribution of the endophytic species of *Pestalotiopsis* is ubiquitous and is not largely influenced by geographical factors (Wei et al., 2007; Tejesvi et al., 2009). Tejesvi et al. (2005) stated that the endophytic species of *Pestalotiopsis* dominant in the winter season and their colonization are comparatively low in the monsoon season. The colonization frequency of species of *Pestalotiopsis* increased with the increasing the age of the host plant and colonization frequency was variable (Wei et al., 2007). Some endophyte studies in which species of *Pestalotiopsis* have been recovered are listed in Table 1.2.

Table 1.2 List of endophytes and associated host

Species	Host	References
P. clavispora	*Camellia oleifera*, *C. sinensis*, *Terminalia arjuna*, *Podocarpus macrophyllus*	Liu et al. (2007); Tejesvi et al. (2007a); Wei et al. (2007); Tejesvi et al. (2009)
P. conigena (Lév.) G.C. Zhao & N. Li	*Lithocarpus glabra*, *C. nitidissima*	Wei et al. (2005); Wei et al. (2007)
P. funerea	*Catharanthus roseus*	Srinivasan & Muthumary (2009)
P. hainanensis	*Podocarpus macrophyllus*	Liu et al. (2007)
P. heterocornis(Guba) Y.X.Chen	*Camellia japonica*, *C. oleifera*, *Castanopsis sclerophylla*, *Cephalotaxus fortunei*, *Podocarpus macrophyllus*, *Lithocarpus glabra*,	Wei et al. (2005); Liu et al. (2007); Wei et al. (2007)
P. jesteri	*Fragraea bodenii*	Strobel et al. (2000)
P. karstenii (Sacc. & P. Syd.) Steyaert	*Camellia japonica*, *C. sasanqua*	Liu et al. (2007); Wei et al. (2007)
P. kunmingensis	*Podocarpus macrophyllus*	Wei et al. (2007)
P. mangifolia (Guba) J. Xiang Zhang & T. Xu	*Camellia japonica*, *C. reticulate*, *C. sasanqua*, *Podocarpus nagi*	Liu et al. (2007); Wei et al. (2007)

Table 1.2 (Continued)

Species	Host	Reference
P. microspora	*Azadirachta indica*, *Camellia sinensis*, *Maytenus ilicifolia*, *Podocarpus macrophyllus*, *Terminalia arjuna*, *T. chebula*, *Taxus wallichiana*, *Taxodium distichum*,	Li, Strobel, Sidhu, Hess & Ford (1996); Strobel et al. (1996a); Wei et al. (2005); Gomes-Figueiredo et al. (2007); Liu et al. (2007); Tejesvi et al. (2007a); Wei et al. (2007); Tejesvi et al. (2009)
P. neglecta	*Camellia sinensis*, *C. nitidissima*, *Podocarpus macrophyllus*, *P. nagi*, *Taxus chinensis*, *T. yunnanensis*	Liu et al. (2007); Wei et al. (2007)
P. olivacea (Guba) G.C. Zhao & J. He	*Camellia sasanqua*, *Podocarpus macrophyllus*,	Liu et al. (2007); Wei et al. (2007)
P. oxyanthi (Thüm.) Steyaert	*Camellia nitidissima*, *Podocarpus macrophyllus*	Liu et al. (2007); Wei et al. (2007)
P. pallidithеae	*Pieris japonica*	Watanabe et al. (2010)
P. photiniae (Thüm.) Y.X. Chen	*Camellia japonica*, *C. sasanqua*, *Podocarpus macrophyllus*, *P. nagi*, *Taxus chinensis*, *Acer palmatum*	Wei et al. (2005); Liu et al. (2007); Wei et al. (2007)
P. subcuticularis	*Camellia sasanqua*, *Taxus yunnanensis*, *T. chinensis*,	Liu et al. (2007); Wei et al. (2007)
P. submersa Sati & N. Tiwari	*Equisetum* sp., *Lyonia ovalifolia*	Sati & Belwal (2005)
P. theae	*Camellia nitidissima*, *C. sinensis*, *Holarrhena antidysenterica*, *Podocarpus macrophyllus*, *Terminalia arjuna*	Liu et al. (2007); Tejesvi et al. (2007a); Wei et al. (2007); Tejesvi et al. (2009)
P. versicolor (Speg.) Steyaert	*Tamarindus indica*	Liu et al. (2007); Liu et al. (2010)

1.6.4 *Pestalotiopsis* as a saprobe

Species of *Pestalotiopsis* have been repeatedly isolated as saprobes from dead leaves, bark and twigs (Guba, 1961). Many species have been isolated from soil, polluted stream water or are associated with the deterioration of wood, paper, fabrics and decay of wool (Guba, 1961). For an example, *P. bicolor* (Ellis & Everh.) A.R. Liu, T. Xu & L.D. Guo, *P. funerea*, *P. monochaetioides* (Doyer) Steyaert, *P. montellica* (Sacc. & Voglino) Tak. Kobay., P. disseminata, *P. foedans* (Sacc. & Ellis) Steyaert, *P. versicolor* and *P. virgatula* are common species recorded either from decaying leaves or bark. Several saprobic species of *Pestalotiopsis* are listed in Table 1.3.

Table 1.3 List of recently recorded saprobes with their host/substrata.

Species	Host/ substrate	References
Pestalotiopsis sydowiana	Dead leaves of *Calluna vulgaris*, *Erica* sp., *Rhododendron ponticum*, *R. hybridum*, *Prunus laurocerasus*	Dennis (1995); M.B. Ellis & J.P. Ellis (1997)
P. funerea	Dead leaves of *Rhododendron* sp, *Chamaecyparis* sp., *Cupressus* sp., *Pinus* sp., *Juniperus* sp.	Dennis (1995); M.B. Ellis & J.P. Ellis (1997)
P. theae	Seeds of *Diospyros crassiflora*	Douanla-Meli & Langer (2009)
P. guepinii	Decaying leaves of *Dracaena loureiri*	Thongkantha, Lumyong, McKenzie & Hyde (2008)
P. palmarum	Dead culms of *Schoenoplectus triqueter*	Wu et al. (1982)

1.6.5 *Pestalotiopsis* as a parasymbiont

Lichen symbiosis is an association between a fungus (the mycobiont) and an alga or a cyanobacterium (the photobiont) (Schwendener, 1868). Most lichens associate with only one fungal species, while some have additional species. In most cases these additional fungal species are parasitic while few are parasymbiont. A

parasymbiont is a secondary fungus present in the lichen thallus, growing in intimate association with the primary symbionts without causing them any apparent harm (Sun, Depriest, Gargas, Rossman & Friedmann, 2002). *Pestalotiopsis maculans* (Corda) Nag Raj is considered to be the dominant parasymbiont in the North American lichen species *Cladonia rangiferina*, *C. subtenuis*, *C. mitis*, *C. leporina*, *Parmotrema perforatum* and *Usnea strigosa* (Sun et al., 2002).

1.6.6 *Pestalotiopsis* as a potential animal and human pathogen

Species of *Pestalotiopsis* are also known to cause human and animal disease. *Pestalotiopsis* has been isolated from the human sinuses, fingernails, a bronchial biopsy, eyes, scalp and feet with corneal abrasions (Sutton, 1999). One isolated from cotton was tested in a toxicity bioassay, which indicated that it caused reduction in weight, pathological abnormalities and even mortality in rats (Diener, Wagener, Morgan-Jones & Davis, 1976).

1.6.7 *Pestalotiopsis* in extreme environments

Some species of *Pestalotiopsis* have also been isolated from extreme environments and these isolates have been shown to produce bioactive metabolites (Tejesvi et al., 2007b). *Pestalotiopsis microspora* isolated from *Taxus* sp. from the foothills of Himalayas produced taxol (Strobel et al., 1996a), *P. microspora* isolated from Sepik River drainage system in Papua New Guinea produced isopestacin (Strobel et al., 2002) and *Pestalotiopsis* sp. obtained from the gut of a grass hopper (*Chondracris rosee*) produced two new phytotoxic g-lactones, pestalotines A and B (Zhang, Ge, Li, Song & Tan, 2008).

1.6.8 Endophyte-pathogen relationship

Lee, Yang, Schwartz, Strobel and Clardy (1995) were able to show that *P. microspora* has an endophyte-pathogen relationship with the North American endangered tree *Torreya taxifolia*. They demonstrated that *P. microspora* inhabits the inner bark of the tree without causing symptoms. However, physiological or environmental factors trigger the fungus to become pathogenic. Typical symptoms include needle spots, needle death and stem cankers. The pathogenic ability of the

fungus depends upon it producing phytotoxins, pestalopyrones, hydroxypestalopyrones and pestalosides. At the same time antifungal activity by the fungus produces exudates of pestaloside; this competes with other fungi. *Pestalotiopsis subcuticularis* naturally inhabits *Hymenaea courbaril* (Leguminosae) and remains dormant until leaves become mature. Fail and Langenheim (1990) stated that when leaves become mature the fungal hyphae spread and enter in to the intracellular spaces of the leaves. When the plant tissues are damaged due to mechanical injury such as insect feeding, active infection by the fungus occurs. The typical symptoms of infected leaves included serious leaf blight.

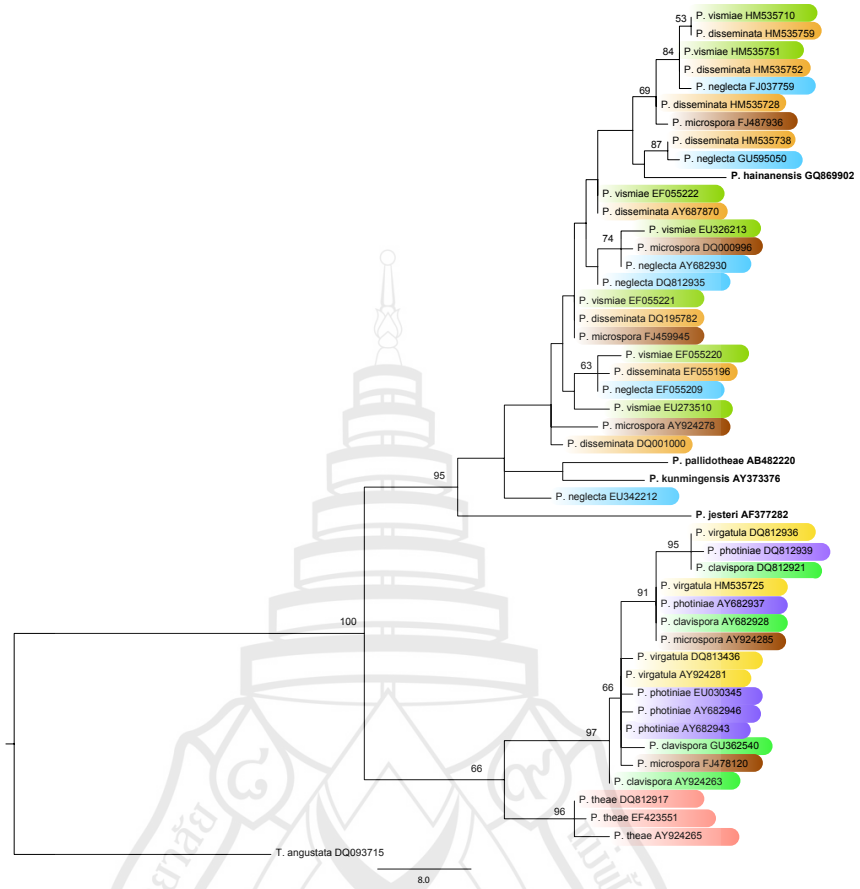
1.7 Phylgenetic analysis of existing data in GenBank

ITS sequences of 48 species of *Pestalotiopsis* were downloaded from GenBank and aligned using Clustal X. The alignment was optimized manually to allow maximum alignment and maximum sequence similarity. Gaps were treated as missing data. Phylogenetic analysis was carried out based on the aligned dataset using PAUP* 4.0b10 (Swofford, 2002). Ambiguously aligned regions were excluded from all analyses. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Trees are figured in Treeview (Page, 1996).

An example of the confusion which results from molecular data is shown in Fig. 1.4 In this phylogram we downloaded 44 selected strains of eight species which have high number of ITS sequences in GenBank plus 4 sequences from ex-type cultures available in GenBank (Table 1.4).

Table 1.4 Isolates and GenBank accession numbers of taxa used to generate the phylogram (Type species are marked in bold).

Species	GenBank accession numbers	Species	GenBank accession numbers
P. clavispora	AY682928	*P. neglecta*	EU342212
P. clavispora	AY924263	*P. neglecta*	FJ037759
P. clavispora	DQ812921	*P. neglecta*	GU595050
P. clavispora	GU362540	***P. pallidotheae***	**AB482220**
P. disseminata	AY687870	*P. photiniae*	AY682937
P. disseminata	DQ001000	*P. photiniae*	AY682943
P. disseminata	DQ195782	*P. photiniae*	AY682946
P. disseminata	EF055196	*P. photiniae*	DQ812939
P. disseminata	HM535728	*P. photiniae*	EU030345
P. disseminata	HM535728	*P. photiniae*	EU030345
P. disseminata	HM535738	*P. virgatula*	AY924281
P. disseminata	HM535752	*P. virgatula*	DQ812936
P. disseminata	HM535759	*P. virgatula*	DQ813436
P.hainanensis	**GQ869902**	*P. virgatula*	HM535725
P. jesteri	**AF377282**	*P. vismiae*	EF055220
P. kunmingensis	**AY373376**	*P. vismiae*	EF055221
P. microspora	AY924278	*P. vismiae*	EF055222
P. microspora	AY924285	*P. vismiae*	EU273510
P. microspora	DQ000996	*P. vismiae*	EU326213
P. microspora	FJ459945	*P. vismiae*	HM535710
P. microspora	FJ478120	*P. vismiae*	HM535751
P. microspora	FJ487936	*P. theae*	AY924265
P. neglecta	AY682930	*P. theae*	DQ812917
P. neglecta	DQ812935	*P. theae*	EF423551
P. neglecta	EF055209	*Truncatella angustata*	DQ093715



Notes. Maximum parsimony phylogram generated from ITS sequence analysis of selected sequences from selected species of *Pestalotiopsis* including *P. clavispora*, *P. disseminata*, *P. microspora*, *P. neglecta*, *P. photiniae*, *P. theae*, *P.virgatula* and *P. vismiae*, downloaded from GenBank with other related taxa. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. Type sequences of *Pestalotiopsis pallidotheae*, *P. hainanensis*, *P. jesteri* and *P. kunmingensis* are in black and bold

Figure 1.4 Maximum parsimony consensus tree for the analyzed *Pestalotiopsis* isolates

According to Jeewon et al. (2003) and Liu et al. (2010a), pigmentation is a highly weighted character in the lineage of species of *Pestalotiopsis* and which can be differentiated into two main groups based on the colour of the median cells. This recent finding was previously supported in the separation of species by Guba (1961) and Steyaert (1949), based on versicolorous median cells as well as those species characterized by concolorous median cells. Jeewon et al. (2003) showed that species such as *P. theae* with dark colored concolorous median cells with knobbed apical appendages should be included in the versicolorous group. Jeewon et al. (2003) argued that the arrangement of Guba (1961) that groups the versicolorous assemblages of species into umber olivaceous and fuliginous olivaceous depends on the color intensity of the median cells. This statements was followed by Liu et al. (2010a) and they proposed the use of "brown to olivaceous" and "umber to fuliginous" colour median cells as valid for the taxonomy of the genus instead of the use of the "concolorous" and "versicolor" median cells grouping system proposed by Steyaert (1949) and Guba (1961).

Pestalotiopsis clavispora, *P. disseminata*, *P. microspora*, *P. neglecta*, *P. photiniae*, *P. theae*, *P. virgatula* and *P. vismiae* can be divided into two groups depending mainly on the colour of the median cells. One group is the versicolorous group, consisting of *P. clavispora*, *P. photiniae* and *P. virgatula*, and dark concolorous median cells with knobbed apical appendages containing the *P. theae* group. The other group consists of species with concolorous median cells (i.e., *P. disseminata*, *P. microspora*, *P. neglecta* and *P. vismiae*. Almost all strains that separate into two main clades depend on the concolorous and versicolor system, and only *P. microspora* strains AY924295 and FJ478120 cluster in the wrong clade. However, within the two main groups, the respective species distributions are scattered and most species overlap with each other. Because of the limitation of characters used to differentiate species (Hu et al., 2007) and many overlapping characters (Sutton, 1980), identification to species in *Pestalotiopsis* is presently difficult. For an example according to Guba (1961), *P. disseminata*, *P. microspora*, *P. neglecta* and *P. vismiae* within the concolorous group have the same conidia size (18–26×5–8 μm). *Pestalotiopsis vismiae* can be differentiated as it has two apical appendages, while *Pestalotiopsis microspora* is differentiated from *P. neglecta* and *P.*

dissementa by the length of the apical appendages. *Pestalotiopsis neglecta* and *P. dissementa* can be distinguished from each other only by the shape of the conidia. Most of above characters vary when in culture and following successive subculturing (Hu et al., 2007). Within the versicolorous group, *P. clavispora* and *P. photiniae* are morphologically very similar (conidia size 19–26×6–8.5 μm), while *P. virgatula* can be differentiated from *P. clavispora* and *P. photiniae* by its relatively small conidia (17–23×6–8 μm). However, these characters overlap and thus identification to these species is rather difficult. For this reason, naming of species is difficult and highly subjective and many sequences for *Pestalotiopsis* deposited in GenBank are likely to be wrongly named.

1.8 Species number

According to Index Fungorum (http://www.indexfungorum.org/names/names.asp; accession date, 2010.10.21) there are 235 *Pestalotiopsis* names, while in MycoBank (www. mycobank.org/mycotaxo.aspx; accession date, 2010.10.21) there are 232 names. The reason for the large number of names is historical and may not reflect the actual number of species (Jeewon et al., 2004). As with other pathogenic genera such as *Colletotrichum* (Cai et al., 2009), species of *Pestalotiopsis* were historically named according to the host from which they were first observed. If a new host occurrence was found a new species was described. For example, Venkatasubbaiah, Grand and Dyke (1991) isolated a species of *Pestalotiopsis* from leaves of *Oenothera laciniata* and described the new species *P. oenotherae* Venkatas., Grand & Van Dyke. The new species was justified because no species of *Pestalotiopsis* had been described previously from *Oenothera* and its morphological characters clearly distinguished it from other species found on any member of the family *Onagraceae* (Venkatasubbaiah, Grand & Dyke, 1991). J. Kohlmeyer and V.B. Kohlmeyer (2001) described *Pestalotiopsis juncestris* Kohlm & Volkm.-Kohlm which was isolated from the host *Juncus roemerianus*; the taxon is morphologically similar to *P. versicolor* and several other species of *Pestalotiopsis*, but the taxon was described as a new species based on the host occurrence. Similarly,

Pal and Purkayastha (1992) and Singh (1981) described the new species *P. agallochae* A.K. Pal bis and Purkay and *P. arborei* N.I. Singh, respectively based on host occurrence. As recently as 2002, Y.X. Chen, Wei and W.P. Chen, (2002) described *P. afinis* Y.X. Chen & G. Wei, *P. alpiniae* Y.X. Chen & G. Wei, *P. antiaris* Y.X. Chen and G. Wei, *P. dilleniae* Y.X. Chen & G. Wei, *P. kuwangsiensis* Y.X. Chen and G.Wei, *P. nelumbinis* Y.X. Chen & G. Wei, *P. schimae* Y.X. Chen & G. Wei and *P. synsepali* Y.X. Chen & G. Wei based on the host association.

More recently, some new species have been introduced based on host occurrence, plus morphological and molecular data. Wei and Xu (2004) isolated an endophytic species of *Pestalotiopsis* (*P. kunmingensis* J.G. Wei & T. Xu) from *Podocarpus macrophyllus* (Thunb.) Sweet and described it as a new species, supported by both morphological and molecular evidence. An endophytic species isolated from the Japanese plant *Pieris japonica* Thunb. L. was named as *Pestalotiopsis pallidotheae* Kyoko Watanabe and Yas. Ono; its conidial morphology is quite similar to *P. theae* but molecular data showed it to be distinct (Watanabe et al., 2010). Similarly, Strobel et al. (2000) and Liu et al. (2007) described *P. jesteri* Strobel, J. Yi Li, E.J. Ford & W.M. Hess and *P. hainanensis* A.R. Liu, T. Xu & L.D. Guo, respectively, using the same considerations.

Species status and host-specificity within the genus *Pestalotiopsis* has been questioned previously or investigated (Zhu, 1989; Jeewon et al., 2004; Wei et al., 2005; 2007; Hu et al., 2007). These authors showed that different species isolated from the same host may not be phylogenetically closely related (Jeewon et al., 2004; Wei et al., 2007). Wei et al. (2007) investigated endophytic species of *Pestalotiopsis* associated with plant species in the families *Podocarpaceae*, *Theaceae* and *Taxaceae*. The endophytic species of *Pestalotiopsis* associated with these host families were not generally host-specific, occurring on a range of hosts. For example, *P. neglecta* (Thüm.) Steyaert and *P. photiniae* were isolated from all the host plants in three plant families. Tejesvi et al. (2007a) isolated endophytic species of *Pestalotiopsis* associated with the medicinal plants *Azadirachta indica*, *Holarrhena antidysenterica*, *Terminalia arjuna* and *T. chebula*. They showed that isolates obtained from a single plant were genetically diverse, while the same species occurred in most plants. According to Guba (1961), most species of the *Pestalotia* were listed from a range of

hosts. For example, *Pestalotia microspora* was listed from several different host plants (i.e., *Ananas comosus*, *Araucaria* sp., *Carya* sp., *Hedera helix*, *Juniperus bermudiana* and *Platanus occidentalis*). Hu et al. (2007) tested the relationships of endophytic *Pestalotiopsis* strains from two tissues of *Pinus armandii* and found that even strains isolated from the same tissue type were not phylogenetically related. Zhu (1989) used artificial cross inoculation studies to show that pathogenic species of *Pestalotiopsis* may not be specific to the single host. Jeewon et al. (2004) pointed out that host-specificity of *Pestalotiopsis* is not supported by the large number of species recorded on one host. They also argued that many taxa used in literature can be misinterpretations or synonyms of species with wide host ranges. Jeewon et al. (2004) used analysis of ITS and 5.8S rDNA to show that isolates taken from the same host were not phylogenetically related and that taxa with similar morphological characters were phylogenetically related.

Up to this time, most phylogenetic research on *Pestalotiopsis* has shown that *Pestalotiopsis* is not highly hostspecific and that species are found on a range of hosts (Jeewon et al., 2004; Wei et al., 2005; 2007; Hu et al., 2007). The diseases caused by species of *Pestalotiopsis* have been recorded in different ecosystems and infect a diverse range of unrelated plant taxa. Isolation of endophytic *Pestalotiopsis* strains for bioprospecting for new biochemical compounds have shown that the same species can be found in a range of hosts. Therefore, most of the species recorded in checklists and the literature may not reflect what actually occurs. As in other related plant pathogenic genera such as *Colletotrichum*, the *Pestalotiopsis* species concept depends mostly on the conidial characteristics. It has been shown that most of the key conidial characters used in species level separation are not stable and vary with host range, generation, culture and other environmental conditions (Hu et al., 2007). The arrangement of species by Steyaert (1949) and Guba (1961) in various coloured groupings is problematic because this character has been shown to be variable within a species (Liu et al., 2010a). Thus, most species in the above arrangements may be confused and many species are probably synonyms. Due to the fact that (1) species of *Pestalotiopsis* are generally not host-specific, (2) conidial characters vary and species limits overlap, and (3) species arrangements in Steyaert (1949) and Guba (1961) are problematic, then the actual number of species in *Pestalotiopsis* is likely to be much

lower than presently recorded in databases (e.g., Index Fungorum, MycoBank) and the literature (Kirk et al., 2008).

For example, according to Guba (1961), *Pestalotiopsis breviseta* (Sacc.) Steyaert, *P. eugeniae*, *P. ilicicola* T., *P. microspora*, *P. podocarpi* and *P. sinensis* (C.I. Chen) P.L. Zhu, Q.X. Ge & T. Xu have very similar, overlapping morphological characters and these species were justified mainly according to the host association. Also the above six species vary from *P. carissae* Guba, *P. disseminata*, *P. neglecta*, names are synonyms of a single biological species. Furthermore, the versicolorous umber olivaceous group which comprises 40 species and versicolorous fuliginous olivaceous group comprising 56 species. These groups are differentiated depending on the intensities of the median cells, while most species have similar conidial measurements and thus are likely to be synonyms. We suspect that the actual number of biological species may be fewer than 50. The scientific community, however, uses many more names when diagnosing disease and in phylogenetic studies and biochemical studies. Therefore, modern research approaches are needed for species of *Pestalotiopsis* in order to establish the acceptable names.

1.8.1 Species number and accepted species

When species are morphologically distinct and molecular evidence shows they are monophyletic, then such species can be considered as a distinct and valid species in a particular genus. Based on their distinct morphological characters, we suggest that the 20 species listed in Table 1.5 can be considered as good species in the genus at this time. Furthermore some other species (Table 1.6) which have considerable value because of their economic roles (in bioactive metabolites production, frequent pathogens, or frequently isolated endophytes) are possibly good species. We suggest that type material of these species should be reexamine and epitypified with fresh collections. With the help of ex-type living cultures and sequence data, a robust species concept can be developed for the genus *Pestalotiopsis*.

Table 1.5 Morphologically distinct *Pestalotiopsis* species with their host and location

Species with distinct morphological characters	Host and location
Pestalotia gaurae Guba	On stem of *Gaura parviflora* in *Hays*, Kansas, United States
Pestalotia multiseta (Speg.) Guba	On fallen leaves of *Iris germanica* in Conegliano, Italy
P. trevoae Speg.	On dead decaying branches of *Trevoa trinervia* in Santiago, Chile
Pestalotiopsis bicolor	Isolated from the dead leaves of *Salix* sp. in *Tuskegee*, Alabama, United States
P. distincta (Guba) K. Yokoy.	On leaves of *Castanopsis cuspidate* in Japan
P. funerea	On dead leaves of *Thuja* sp. in Paris, France
P. guepinii	On stem and leaves of *Camellia japonica* in France.
P. hughesii Steyaert	On stems of *Cyperus articulate* in Gold Coasts in West Africa
P. karstenii	On leaves of *Camellia japonica* in United States
P. leucopogonis Nag Raj	On leaves of *Leucopogan lanceolatus* in Australia
P. macrospora (Ces.) Steyaert	On fronds of *Pteridium aquilinum* in Italy
P. maculans	On leaves of *Camellia japonica* and *Camellia* sp. in Czechoslovakia, France, Germany and United States
P. monochaetioides	On dead twig of *Chamaecyparis lawsoniana* in Naarden, Holland
P. montellica	On dead leaves of *Quercus rubra* in Canada
P. palustris Nag Raj	On *Euphorbia palustris* in Italy
P. perseae Nag Raj	On leaves of *Persea borbonea* in United States
P. pseudomontellica Nag Raj	On leaves of *Lithocarpus densiflora* in United States

Table 1.5 (Continued)

Species with distinct morphological characters	Host and location
P. smilacis (Schwein.) B. Sutton	On stem of *Smilax rotundifolia* in United Sates
P. tecomicola Nag Raj	On *Tecoma radicans* in United States
P. trichocladi (Laughton) Steyaert	On leaves of *Trichocladus crinitus* in South Africa

Table 1.6 Economically important *Pestalotiopsis* species with their host and location

Economically important species	Host and location	Economically importance	Reference
Pestalotiopsis adusta (Ellis & Everh.) Steyaert	On leaves of *Prunus cerasus* in Newfield, New Jersey, United States	Bioactive metabolites	Li, Jiang, Guo, Zhang & Che (2008b)
P. clavispora	On leaves of *Quercus* sp. in Auburn, Alabama, United States	Plant pathogen, Common endophyte	Keith et al. (2006); Espinoza et al. (2008); Wei et al. (2007); Liu et al. (2007)
P. disseminata	On dead leaves of *Eucalyptus globules* in Coimbra, Portugal	Plant pathogen, Bioactive metabolites	Das et al. (2010); Keith et al. (2006); Deyrup, Swenson, Gloer & Wicklow (2006)
P. fici Steyaert	On *Ficus* sp. in Kiagwe, Uganda	Bio active metabolites,	Liu et al. (2008a); Liu et al. (2008b)

Table 1.6 (Continued)

Economically important species	Host and location	Economically importance	Reference
P. foedan (Sacc. & Ellis) Steyaert	On decaying bark of *Thuja occidentalis* in Newfield, New Jersey, United States	Bio active metabolites	Ding et al. (2008a)
P. heterocornis	On leaves of *Anarcardium occidentale* in Cantanduva, São Paulo, Brazil	Common endophyte	Wei et al. (2007); Liu et al. (2007)
P. longiseta	On leaves of *Rubus caesius* in Susegana, Conegliano, Italy	Plant pathogen, Bioactive metabolites	Joshi et al. (2009); Nagata & Ando (1989); Nagata, Ando & Hirota (1992); Xu et al. (2010)
P. microspora	On leaves of *Hedera helix* in Botanical garden, College of Argentina, Buenos Aires, Argentina	Plant pathogen, Common endophyte, Bioactive metabolites	Stroble et al. (1996a); Strobel, Hess, Ford, Siduhu & Yang (1996b); Stroble et al. (2002); Metz et al. (2000); Keith et al. (2006); Jeon et al. (2007); Womersley (1995); Harper, Barich, Hu, Stroble & Grant (2003); Kai et al. (2003)

Table 1.6 (Continued)

Economically important species	Host and location	Economically importance	Reference
P. neglecta	On leaves of *Euonymus japonicas* in Coimbra, portugal	Plant pathogen, Endophyte	Tagne & Mathur (2001); Espinoza et al. (2008);
P. pauciseta (Sacc.) Y.X. Chen	On leaves of *Litsea glutinosa* in Mount Makiling, near Los Banos, Laguna province, Philippine	Bioactive metabolites	Gangadevi, Murugan & Muthumary (2008)
P. photiniae	On leaves of *Photinia serrulata* in Istria, Australia	Bioactive metabolites	Ding et al. (2009)
P. theae	On leaves of *Camellia sinensis* in Japan	Plant pathogen, Endopyte, Bioactive metabolites	Li et al. (2008a); Nagata et al. (1992); Shimada, Takahashi, Kawano & Kimura (2001); Tuset et al. (1999); Worapong, Inthararaungsom, Stroble & Hess (2003); Joshi et al. (2009); Muraleedharan & Chen (1997); Ding et al. (2008a);
P. uvicola	On *Gaura parviflora* and *Vitis vinifera* in Italy	Plant pathogen	Vitale & Polizzi (2005); Xu et al. (1999)

1.9 Novel *Pestalotiopsis* biochemistry

Species of *Pestalotiopsis* have been well-studied because of the diverse array of novel compounds that they have been shown to produce. As such, they are thought to be a rich source for bioprospecting when compared to those of other fungal genera (Aly et al., 2010; Xu et al., 2010). Strobel and Long (1998) described *Pestalotiopsis* as the '*E. coli* of the temperate and tropical rainforest systems'. Species of *Pestalotiopsis* may have an important role in forest ecosystems; they have a cosmopolitan geographical distribution and are found almost everywhere (Tejesvi et al., 2007a). Moreover, species of *Pestalotiopsis* have been found to produce an enormous number of secondary metabolites that may have medicinal, agricultural and industrial applications. The majority of compounds have been discovered from endophytic strains of *Pestalotiopsis* (Lee, Strobel, Lobkovsky & Clardy, 1996; Strobel et al., 1996a; 1996b; Li & Strobel, 2001) plus some pathogenic strains (Kwon et al., 1996).

Species of *Pestalotiopsis* have been shown to produce bioactive alkaloids, terpenoids, isocoumarin derivatives, coumarins, chromones, quinones, semiquinones, peptides, xanthones, xanthone derivatives, phenols, phenolic acids, and lactones with a range of antifungal, antimicrobial, and antitumor activities (Xu et al., 2010). Xu et al. (2010) reviewed 130 different compounds isolated from species of *Pestalotiopsis*. In the present review, we discuss some selected species and their bioactive potential.

Pestalotiopsis microspora is a common species present in tropical and subtropical plants and is a widespread saprobe of bark and decaying plant material (Metz et al., 2000). The species has most commonly been isolated as an endophyte associated with rainforest plants (Strobel et al., 2002) or as a pathogen (Keith et al., 2006). Pathogen associations include scab disease on *Psidium guajava* (Keith et al., 2006), leaf blight of *Lindera obtusiloba* (Jeon et al., 2007) and as an endophyte on *Terminalia morobensis* (Womersley, 1995). *Pestalotiopsis microspora* has the potential to be a model organism for biological and biochemical studies in the laboratory (Metz et al., 2000). Isolates of this species (or possibly species complex) show diverse genetic variation and thus each individual isolate is generally unique in

the substances that it produces (Harper et al., 2003). Long, Smidansky, Archer and Strobel (1998) have shown that under laboratory conditions it can take up heterologous DNA, add telomeric DNA, express heterologous DNA and can replicate independently of chromosomal DNA.

Such genetic diversity would be useful to the species in nature, helping it adapt to a new plant by incorporating plant DNA into its own genome (Strobel et al. 1996a; Li, Strobel, Sidhu, Hess & Ford, 1996). Bioactive compounds such as the anti-cancer drug taxol, jesterone, ambuic acid, torreyanic acid, pestaloside, pestalotiopsins and 2-a hydroxydimeniol (Strobel et al. 2002), hetero-polysaccharides (Kai et al., 2003) have been obtained from *P. microspora*. The multimillion dollar anti-cancer drug, taxol was obtained from an endophytic strain of *P. microspora* isolated from *Taxus wallachiana* (Strobel et al., 1996a) and *Taxodium distichurn* (Strobel et al., 1996b). Kai et al. (2003) found that *P. microspora* can metabolize various monosaccharides and the composition of hetero-polysaccharides depends on the type of monosaccharide in the media. Harper et al. (2003) investigated the production of pestacin, a 1,3-dihydro isobenzofuran with moderate anti-fungal properties and high anti-oxidant activity when compared with the vitamin E derivative trolox from endophytic strains of *P. microspora*. The anti-oxidant activity works mainly by cleavage of an unusually reactive C–H bond. Lee et al. (1995) obtained several anti-fungal compounds such as pestaloside, an aromatic glucoside, and two pyrones (pestalopyrone and hydroxypestalopyrone) from a strain of *P. microspora* isolated from the endangered North American tree *Torreya taxifolia*. When *Pestalotiopsis microspora* is cultured on media containing various monosaccharides as a carbon source, different polysaccharides are produced and this mainly depends on the monosaccharide used as the carbon source (Kai et al., 2003). Whether all these strains were in fact *P. microspora* is yet to be determined, since the identifications were based on morphology or comparison with GenBank sequence data, which itself may be erroneously named. This species is in need of epitypification.

Pestalotiopsis theae is an economically important species that has been reported from all major tea growing countries of the world (Muraleedharan & Chen, 1997) and also as an endophyte (Worapong et al., 2003). Pestalotheols A–D, four new metabolites isolated from endophytic *Pestalotiopsis theae*, and pestalotheol C showed

an inhibitory effect against HIV-1LAI replication in C8166 cells (Li et al., 2008a). Three new compounds, pestalamides A–C and two known metabolites, aspernigrin A and carbonarone A, were obtained from the same fungus isolated from the branches of tea (Ding et al., 2008b). The newly isolated pestalamide B inhibited HIV-1 replication in C8166 cells with EC50 of 64.2 μM and antifungal activity against *Aspergillus fumigatus*. Chloroisosulochrin and chloroisosulochri dehydrate were obtained from the culture filtrate of *P. theae*, and these compounds can be used as plant growth regulators (Shimada et al., 2001). This species is obviously important as a producer of novel medicinal metabolites.

The generic type of *Pestalotiopsis* is *P. guepinii*, a plant pathogen that causes disease in important crop plants (Karaca & Erper, 2001). Strains of *Pestalotiopsis guepinii* isolated as an endophyte from the plant families *Anacardiaceae*, *Apocynaceae*, *Leguminosae* and *Palmae* were tested for their in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity, using Ellman's colorimetric method adapted for thin layer chromatography (Rodrigues, Costa, Carvalho & Epifanio, 2005). *Pestalotiopsis guepinii* from *Anacardium giganteum* inhibited both enzymes in the TLC polar region and a strain isolated from *Myracroduon urundeuva* and *Spondias mombin* showed selective inhibition of AChE. Parshikov et al. (2001) suggested that *P. guepinii* may be a useful model for the mammalian transformation of fluoroquinolones. They obtained the metabolites N-acetylciprofloxacin (52%), desethylene- N acetylciprofloxacin (9.2%), Nformylciprofloxacin (4.2%), and 7-amino-1-cyclopropyl- 6-fluoro- 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2.3%) by specific culture of P. guepinii dosed with ciprofloxacin (300 μM). In addition, by dosing with norfloxacin (313 μM) and the metabolites N-acetylnorfloxacin (55.4%), desethylene-N-acetylnorfloxacin (8.8%), N-formylnorfloxacin (3.6%), and 7-amino 1-ethyl-6- fluoro- 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2.1%) were obtained.

Liu et al. (2008b) isolated five new cyclohexanone derivatives, pestalofones A–E, with the known compounds isosulochrin, isosulochrin dehydrate, and iso-A82775C, from cultures of the plant endophytic fungus *Pestalotiopsis fici*. Pestalofones A and B were inhibitory against HIV-1 replication in C8166 cells, pestalofones C showed antifungal activity against *Aspergillus fumigatus* while

pestalofones E showed both the above effects. Chloropestolide A extracted from the scale-up fermentation extract of *Pestalotiopsis fici* showed significant inhibitory effects on growth of two human cancer cell lines, HeLa and HT29 (Liu et al., 2009). Liu et al., (2010b) obtained chloropupukeanolides A and B (unprecedented spiroketal peroxide) and chloropupukeanone A (three highly functionalized metabolites featuring a chlorinated pupukeanane core) from an endophytic strain of *Pestalotiopsis fici*. The compound chloropupukeanolide A showed significant anti-HIV-1 and cytotoxic effects.

These findings will most likely trigger further studies on total synthesis. Whether *Pestalotiopsis* is unique amongst endophytes or coelomycetes in producing large numbers of secondary metabolites with medicinal and pathogenic control significance has yet to be established.

1.10 Objectives of the research

Pestalotiopsis is taxonomically poorly understood both at the inter- as well as the intraspecific level. It is not clear whether *Pestalotia* is really distinct from *Pestalotiopsis*, since strains of the type of the former have not been sequenced. Nomenclature of the genus is confusing and most host based names in databases may be synonyms. Molecular data have still not been successfully applied for species-level differentiation and names applied to data in GenBank are doubtful, as they are not linked to any type materials. Epitypification with molecular work is therefore needed to understand the species and what distinguishes them. Re-examination of type materials and establishment of epitypes with living cultures is essential for real progress (Hyde & Zhang, 2008), and sequence data are needed to develop a strong species-based taxonomic system for the genus *Pestalotiopsis*.

It is only then that plant pathologists can confidently name disease causal agents, quarantine can put in effective measures to prevent entry of unwanted species of *Pestalotiopsis* and biochemists can confidently put names to species producing novel chemicals and use an understanding of species relationships to aid in bioprospecting.

The present study was conceived with several primary objectives.

First, this investigation was to document the diversity of *Pestalotiopsis* species and conservation in culture collections. The samples of living and decaying plants in forests and other habitats were collected and used to screen for *Pestalotiopsis* species. Endophytes were also isolated.

A second objective of this research was to screen DNA barcodes for the identification of *Pestalotiopsis* species. The use of ribosomal RNA genes and several other protein-coding markers were assessed for suitability in resolving species in *Pestalotiopsis.*

Finally, polyphasic approaches were used to stabilize the nomenclature of *Pestalotiopsis* species and elucidate their species concepts. Towards this aim conidial morphology, conidiogenesis, substrate, geographic influence and host range were determined linking with molecular data to resolve species boundaries.

1.11 Organization of the thesis

This thesis is divided into five chapters. Following this introductory chapter the rest of this dissertation is organized as follows.

Chapter 2 presents the use of various gene regions to resolve the species boundaries in *Pestalotiopsis.* Several gene regions were selected based on their success in PCR amplification and/or in their ability to delimitate species.

Chapter 3 includes the use of polyphasic approaches to analyze cryptic taxa belonging to the genus *Pestalotiopsis.* Based on conidial morphology, sequence data, geographical influence and host occurrence several section names have been proposed to the *Pestalotiopsis*. Another purpose is to epitypify several economically important species, to introduce new taxa, to re-examine ex-types cultures and thereby to strengthen the backbone tree for *Pestalotiopsis* at the species level. In addition genera belongs to the family *Amphisphaeriaceae* and segregate *Pestalotiopsis* and two new genera are proposed.

Chapter 4 formally introduces two novel genera *Neopestalotiopsis* and *Pseudopestalotiopsis.* Several section names were put forward to *Neopestalotiopsis.*

Species belongs to *Neopestalotiopsis* and *Pseudopestalotiopsis* were characterized using polyphasic approaches.

Chapter 5 provides the summary of main findings of this thesis. Overall conclusion of this thesis is drawn and recommendations for future work are also presented in this chapter.

CHAPTER 2

MULTI-GENE ANALYSIS TO RESOLVE *Pestalotiopsis*

2.1 Introduction

The use of molecular data in resolving *Pestalotiopsis* species has been reviewed by Hu et al. (2007), Tejesvi et al. (2007a), Liu et al. (2010a) and Maharachchikumbura, Guo, Chukeatirote, Bahkali and Hyde (2011). These studies have suggested that multi-locus phylogenetic analysis is needed to resolve the cryptic species in the genus. We have been studying the genus *Pestalotiopsis* and testing the use of various genes to resolve species boundaries. In this study, we report on 28 isolates sourced from plant material from Yunnan Province in China. All isolated species were first morphologically characterised and then sequenced using ITS, β-tubulin and TEF1 genes. In order to select suitable gene regions for better species resolution, we analyzed nuclear ribosomal large subunit rDNA (LSU), nuclear ribosomal small subunit rDNA (SSU), partial actin (ACT), glutamine synthase (GS), glyceraldehyde-3-phosphate dehydrogenase (GPDH), RNA polymerase II (RPB1) and calmodulin (CAL) gene regions for 15 isolates of *Pestalotiopsis*. We compared the morphological data versus the sequence data from single and combined genes to establish which characters satisfactorily resolve the species.

2.2 Materials and Methods

2.2.1 Isolation and identification of isolates

Dead plant tissues were collected from different sites in China. The samples were placed in separate plastic bags lined with tissue paper, sprayed with sterile water

to create humid conditions and incubated at room temperature. The fungi present on the samples were isolated by single spore culture technique (Chomnunti et al., 2011). In short, a conidiomata was immersed in 300 µl of sterile distilled water on a slide and left a few minutes so that the conidia were discharged. A conidial suspension was made, small drops were placed on water agar (WA) in Petri dishes and kept at room temperature for 8–12 h for conidia to germinate; single germinating conidia were transferred to potato dextrose agar (PDA) plates. The plates were incubated at 25 °C for 7 to 10 days. Colonies grown on PDA were transferred to PDA slants, and stored at 4 °C for further study. Sporulation was induced by placing sterilized carnation leaves on the surface of PDA with growing mycelia. The morphology of fungal colonies was recorded following the method of Hu et al. (2007). Fungal mycelia and spores were observed under a light microscope and photographed. All microscopic measurements were done with Tarosoft image framework (v. 0.9.0.7) and 30 conidial measurements were taken for each isolate. Isolates were deposited in Novozymes, Beijing and were also transferred to MFLUCC from Novozymes by Material Transfer Agreement and cannot be distributed to a third party. All other cultures dealt with in this study were obtained from China General Microbiological Culture Collection (CGMCC) and the International Collection of Microorganisms from Plants (ICMP).

2.2.2 DNA extraction and PCR condition

Total genomic DNA was extracted from fresh mycelium using a modified protocol of Doyle and Doyle (1987) and Lee and Taylor (1990). Fresh fungal mycelia (500 mg) was scraped from the margin of a PDA plate incubated at 25°C for 7 to 10 d and transferred into a 1.5 ml centrifuge tube with 100 µl of preheated (60°C) 2X 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 2X 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).

2.2.3 PCR amplification

The ITS and 5.8S region of rDNA fragment was amplified using primer pairs ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990), partial 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'-GTCGTYGTYATY GGHCAYGT-3') and EF1-1567R (5'-ACHGTRCCRATACCACCRATCTT-3') (Rehner 2001). In addition to above three gene regions selected LSU, SSU, ACT, GS, GPDH, RPB1 and CAL regions were amplified using primer pair/s listed in Table 2.1.

PCR was performed with the 25 µl reaction system containing 19.5 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 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 LSU, SSU, ACT, GS, GPDH, RPB1and CAL regions were tested under different optimal conditions (not shown). The PCR products were verified by staining with Goldview (Guangzhou Geneshun Biotech, China) on 1% agarose electrophoresis gels.

2.2.4 Phylogenetic analysis

DNAStar and SeqMan were used to obtain consensus sequences from sequences generated from forward and reverse primers. Single locus dataset and combination of multi-locus dataset of three gene regions were aligned using CLUSTALX (v. 1.83) (Thompson et al., 1997). The sequences were further aligned using default settings of MAFFTv6 (Katoh & Toh, 2008) and manually adjusted using BioEdit (Hall, 1999) to allow maximum alignment and minimum gaps. 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 1,000 random sequence additions. Maxtrees were set up to 5,000, 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 1000 bootstrap replications resulting from maximum parsimony analysis, each with 10 replicates of random stemwise addition of taxa (Felsenstein, 1985). The Kishino–Hasegawa tests (Kishino & Hasegawa, 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different. Trees were viewed in Treeview (Page, 1996). Sequences derived from ITS, β-tubulin and TEF1 were deposited.

Table 2.1 Primers used in this study to test different genes

Region	Primer	References
LSU	LR OR /5	Rehner & Samuels (1994); Moriya et al. (2005)
SSU	NS 1/4	White at al. (1999)
ACT	ACT 512F/783R	Carbone & Kohn (1999)
GS	GS F1/R1	Stephenson et al. (1997); Guerber et al. (2003)
GPDH	GDF1/GPD2LM	Myllys et al. (2002); Guerber et al. (2003)
RPB1	RPB1 Af/Ac/Cr	Rehner (2001)
CAL	CL 1/2; CAL 228F/737R	Carbone & Kohn (1999); O'Donnell, Nirenberg, Aoki & Cigelnik (2000)

Table 2.2 Isolates used in this study

Taxon	Isolates*	GenBank Accession Number		
		ITS	β-tubulin	TEF1
P. adusta (Ellis & Everh.) Steyaert	ICMP6088	JX399006	JX399037	JX399070
P. adusta	MFLUCC10-146	JX399007	JX399038	JX399071
P. asiatica Maharachch & K.D. Hyde	MFLUCC 12-0286/ NN047638	JX398983	JX399018	JX399049
P. camelliae Y. M. Zhang, Maharachch & K.D. Hyde	MFLUCC12-0277	JX399010	JX399041	JX399074
P. camelliae	MFLUCC 12-0278	JX399011	JX399042	JX399075
P. chinensis Maharachch & K.D. Hyde	MFLUCC 12-0273/ NN047218	JX398995	-	-
P. chinensis Maharachch & K.D. Hyde	MFLUCC 12-0273/ NN047218	JX398995	-	-
P. chrysea Maharachch & K.D. Hyde	MFLUCC 12-0261/ NN042855	JX398985	JX399020	JX399051
P. chrysea	MFLUCC 12-0262/	JX398986	JX399021	JX399052

Table 2.2 (Continued)

Taxon	Isolates*	GenBank Accession Number		
		ITS	β-tubulin	TEF1
P. clavata Maharachch & K.D. Hyde	MFLUCC 12-0269/ NN047005	JX398991	JX399026	JX399057
P. clavispora	MFLUCC 12-0280/ NN043011	JX398978	JX399013	JX399044
P. clavispora	MFLUCC 12-0281/ NN043133	JX398979	JX399014	JX399045
P. diversiseta Maharachch & K.D. Hyde	MFLUCC 12-0287/ NN047261	JX399009	JX399040	JX399073
P. ellipsospora Maharachch & K.D. Hyde	MFLUCC 12-0283	JX399016	JX399016	JX399047
P. ellipsospora	MFLUCC 12-0284	JX399015	JX399015	JX399046
P. foedans (Sacc. & Ellis) Steyaert	CGMCC 3.9178	JX398989	JX399024	JX399055
P. foedans	CGMCC 3.9123	JX398987	JX399022	JX399053
P. foedans	CGMCC 3.9202	JX398988	JX399023	JX399054
P. furcata Maharachch & K.D. Hyde	MFLUCC 12-0054	JQ683724	JQ683708	JQ683740
P. hainanensis	-	GQ869902	-	-
P. inflexa Maharachch & K.D. Hyde	MFLUCC 12-0270/ NN047098	JX399008	JX399039	JX399072
P. intermedia Maharachch & K.D. Hyde	MFLUCC 12-0259/ NN047642	JX398993	JX399028	JX399059
P. intermedia	MFLUCC 12-0260/ NN047073	JX398997	JX399019	JX399062
P. jesteri Strobel, J.Yi Li, E.J. Ford & W.M. Hess	-	AF377282	-	-
P. jesteri	MFLUCC 12-0279/ NN042849	JX399012	JX399043	JX399076
P. kunmingensis J.G. Wei & T. Xu	-	AY373376	-	-
P. linearis Maharachch & K.D. Hyde	MFLUCC 12-0271/NN047190	JX398992	JX399027	JX399058

Table 2.2 (Continued)

Taxon	Isolates*	GenBank Accession Number		
		ITS	β-tubulin	TEF1
P. linearis	MFLUCC 12-0272/ NN047141	JX398994	-	JX399060
P. pallidotheae Kyoto Watan. & Yas. ono	-	AB482220	-	-
P. samarangensis Maharachch & K.D. Hyde	MFLUCC 12-0233	JQ968609	JQ968610	JQ968611
P. saprophyta Maharachch & K.D. Hyde	MFLUCC 12-0282/ NN047136	JX398982	JX399017	JX399048
P. theae	MFLUCC12-0055	JQ683727	JQ683711	JQ683743
P. theae	SC011	JQ683726	JQ683710	JQ683742
P. trachicarpicola Y. M. Zhang & K.D. Hyde	MFLUCC 12-0263/ NN047072	JX399000	JX399031	JX399064
P. trachicarpicola	MFLUCC 12-0264/ NN047196	JX399004	JX399035	JX399068
P. trachicarpicola	MFLUCC 12-0265/ NN046983	JX399003	JX399034	JX399067
P. trachicarpicola	MFLUCC 12-0266/ NN046978	JX399002	JX399033	JX399066
P. trachicarpicola	MFLUCC 12-0267/ NN047099	JX399001	JX399032	JX399065
P. trachicarpicola	OP068	JQ845947	JQ845945	JQ845946
P. umberspora Maharachch & K.D. Hyde	MFLUCC 12-0285/ NN042986	JX398984	JX399019	JX399050
P. unicolor Maharachch & K.D. Hyde	MFLUCC 12-0275/ NN047308	JX398998	JX399029	JX399063
P. unicolor	MFLUCC 12-0276/ NN046974	JX398999	JX399030	-
P. verruculosa Maharachch & K.D. Hyde	MFLUCC 12-0274/ NN047309	JX398996	-	JX399061
Seiridium sp.	SD096	JQ683725	JQ683709	JQ683741

*Acronyms: NN = Novozymes

- = data not available

2.3 Results and Discussion

Phylogenetic trees were constructed using individual and combined ITS, β-tubulin and TEF1 sequences for our 40 isolates of *Pestalotiopsis* with a *Seiridium* species as the outgroup taxon and other sequences downloaded from GenBank (Table 2.2). We tested 10 genes in PCR amplification, alignment and the species delimitation in *Pestalotiopsis* (Tables 2.3 and 2.4) and found that β-tubulin and TEF1 were the optimal genes, while ITS is included as it is the accepted barcode for fungi (Schoch et al., 2012). We used the available type ITS sequences from other studies (*Pestalotiopsis pallidotheae*, *P. hainanensis*, *P. jesteri* and *P. kunmingensis*), for comparison.

2.3.1 PCR success rate

The results showed that PCR amplifications of ribosomal RNA genes were more reliable across the *Pestalotipsis* than the protein-coding markers. PCR amplification and sequence success rate for ITS, LSU and SSU were 100%. The PCR product for ITS ranged from 450-500 bp and for LSU and SSU amplicons were 1100 bp, 1200 bp respectively. The initial alignment results showed the informative characters were decreases with ITS, LSU and SSU respectively (the sequence data for ITS was discussed in Section 2.3.2). PCR amplification and sequences success rate for protein coding genes were ranged from 95% to 50%. RPB1 and CAL consistently yielded high levels of species discrimination data, even though, their PCR and sequence success rate were very low. Most of the time RPB1 PCR product consist of multiple bands. Thus it can be not use for the phylogenetic studies. Using the primer pair GS F1/R1 unable to get PCR product for GS gene. ACT and GPDH gene region showed a fairly good amplification and sequence success rate. However those two protein markers had less species resolving power. The use of β-tubulin and TEF1 regions were discussed in Section 2.3.3 and 2.3.4.

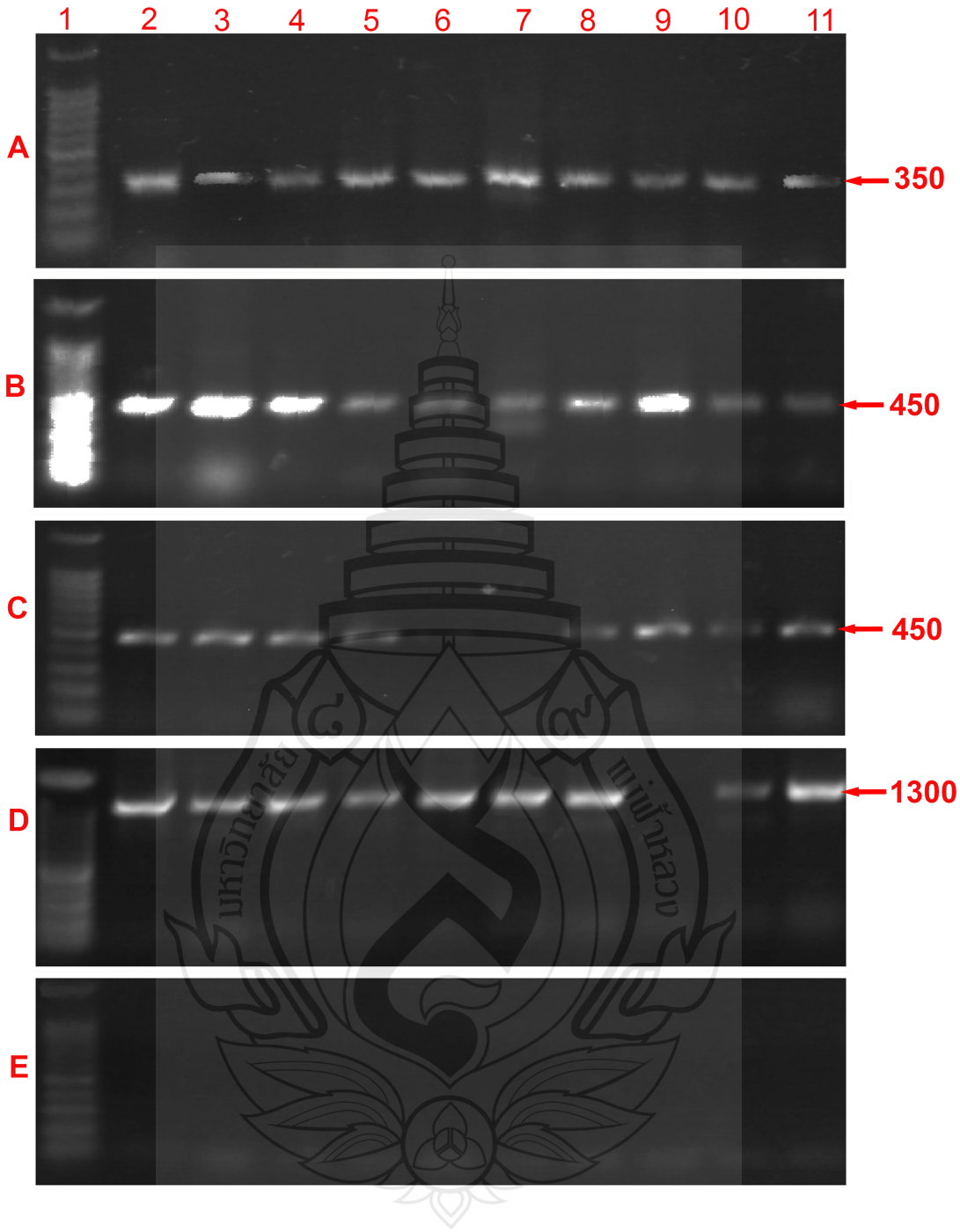
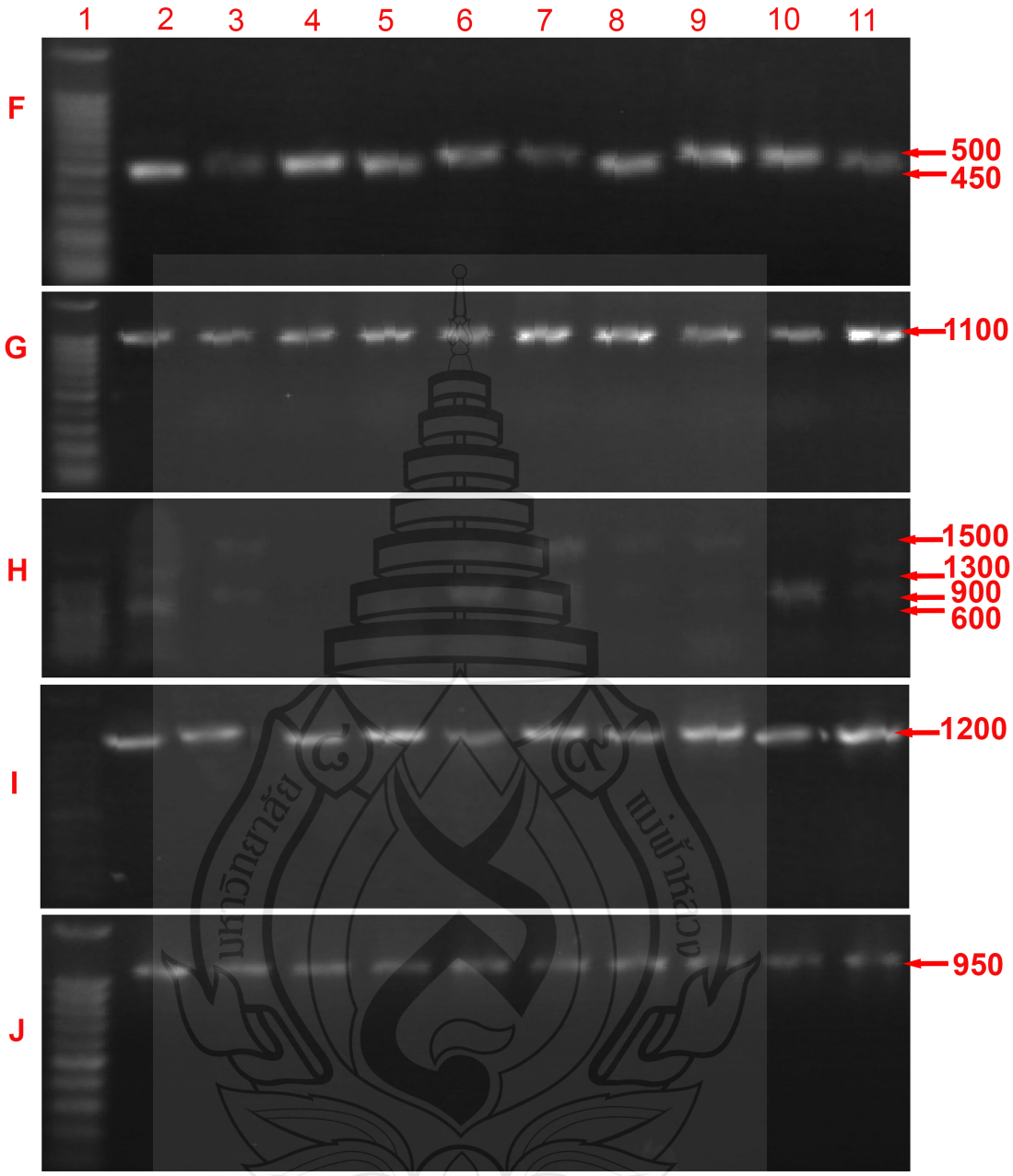


Figure 2.1 Amplification of the ACT (A), β-tub (B), CAL (C), GPDH (D), GS (E) ITS (F), LSU (G), RPB1 (H), SSU (I) and TEF1 (J) gene regions



Note. Amplification of the DNA sequences used in this study. Lane 1, 100 bp DNA marker; lane 2-11 representatives of *Pestalotiopsis* isolates

Figure 2.1 (Continued)

Table 2.3 Comparison of gene regions tested but not used in the final phylogenetic studies

Region	Product length (bp)	PCR success (%)	Sequence success (%)	Species resolution
LSU	1100	100	100	Very low
SSU	1200	100	100	Very low
ACT	350	95	100	Low
GS	-	0	-	-
GPDH	1300	95	100	Low
CAL	450	70	90	High
RPB1	600, 900, 1300, 1500	60	50	High

2.3.2 Sequence analysis of ITS from *Pestalotiopsis* strains

ITS sequences from the types (*Pestalotiopsis pallidotheae*, *P. hainanensis*, *P. jesteri* and *P. kunmingensis*) for *Pestalotiopsis* were analysed with our isolates used in this study. The alignment comprised 45 taxa and 527 characters (including gaps) (Figure 2.2). Parsimony analysis indicates that 398 characters were constant, 41 variable characters parsimony-uninformative and 88 characters are parsimony-informative. The parsimony analysis of the data matrix resulted in two equally parsimonious trees and the first tree (TL= 243, CI= 0.683, RI= 0.910, HI= 0.317, RC= 0.622) is shown in Figure 2.2.

In the ITS phylogram, the *Pestalotiopsis* strains separated into three major clades. The species within each group were not well resolved at the terminal clades. Specifically, all taxa in Clade B did not separate into distinct species but clustered in two subclades. Species resolution was higher in Clade A, although a few species are not well resolved at the terminal ends. Thus, ITS had lower inter-specific variation and, therefore, further gene sequences are needed to determine genetic variation within each biological species.

Figure 2.2 Maximum parsimony phylogram generated from ITS dataset. Data were analyzed with random addition sequences, unweighted parsimony and treating gaps as missing data. A *Seiridium* sp. was used as outgroup. Ex-type and ex-epitype sequences are in bold

2.3.3 Sequence analysis of β-tubulin gene data from *Pestalotiopsis* strains

The aligned dataset for β -tubulin sequences comprised 37 taxa and 487 characters (including gaps). Parsimony analysis indicated that 285 characters were constant, 48 variable characters parsimony-uninformative and 154 characters parsimony-informative. The parsimony analysis of the data matrix resulted in two equally parsimonious trees and the first tree (TL= 410 steps, CI= 0.702, RI= 0.912, HI= 0.298 and RC= 0.640) was shown here (Figure 2.3).

Analysis of the β-tubulin gene sequences resulted in a phylogram (Figure 2.3) in which the *Pestalotiopsis* species separated into three major clades, A, B and C with high bootstrap support. Clade A comprised twelve well-resolved species. There was no PCR products from *P. chinensis* (MFLUCC 12-0273), *P. intermedia* (MFLUCC 12-0260), *P. linearis* (MFLUCC 12-0272) and *P. verruculosa* (MFLUCC 12-0274) using primer pair BT2A and BT2B. Although most of the species were well-resolved in the β-tubulin tree, the success rate of PCR has been low for this gene (Table 2.4). Therefore, further molecular loci were needed to resolve the species in this genus.

Figure 2.3 The maximum parsimony phylogram generated from β-tubulin dataset. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. A *Seiridium* sp. was used as the outgroup. Ex-type and ex-epitype sequences are in bold

2.3.4 Sequence analysis of TEF1 gene data from of *Pestalotiopsis* strains

The aligned dataset for TEF1 sequence data comprised 39 taxa and 1005 characters (including gaps). Among these, 723 characters were constant, 87 variable characters parsimony-uninformative and 195 characters parsimony-informative. The parsimony analysis resulted in six equally parsimonious trees and the first tree (TL= 606 steps, CI= 0.670, RI= 0.896, HI= 0.330 and RC= 0.600) is shown in Figure 2.4.

In the TEF1 phylogram (Fig. 2.4), the *Pestalotiopsis* strains separated into three major Clades, A, B and C with high bootstrap support. In comparison to ITS and β-tubulin, the TEF1 gene clearly separated all species used in this study at the species level, with high bootstrap support. The branch lengths of neighboring clades are longest in the TEF1 gene region and thus signifies speciation in *Pestalotiopsis*. It should be noted that no PCR product were obtained from *P. chinensis* (MFLUCC 12-0273) and *P. unicolor* (MFLUCC 12-0276).

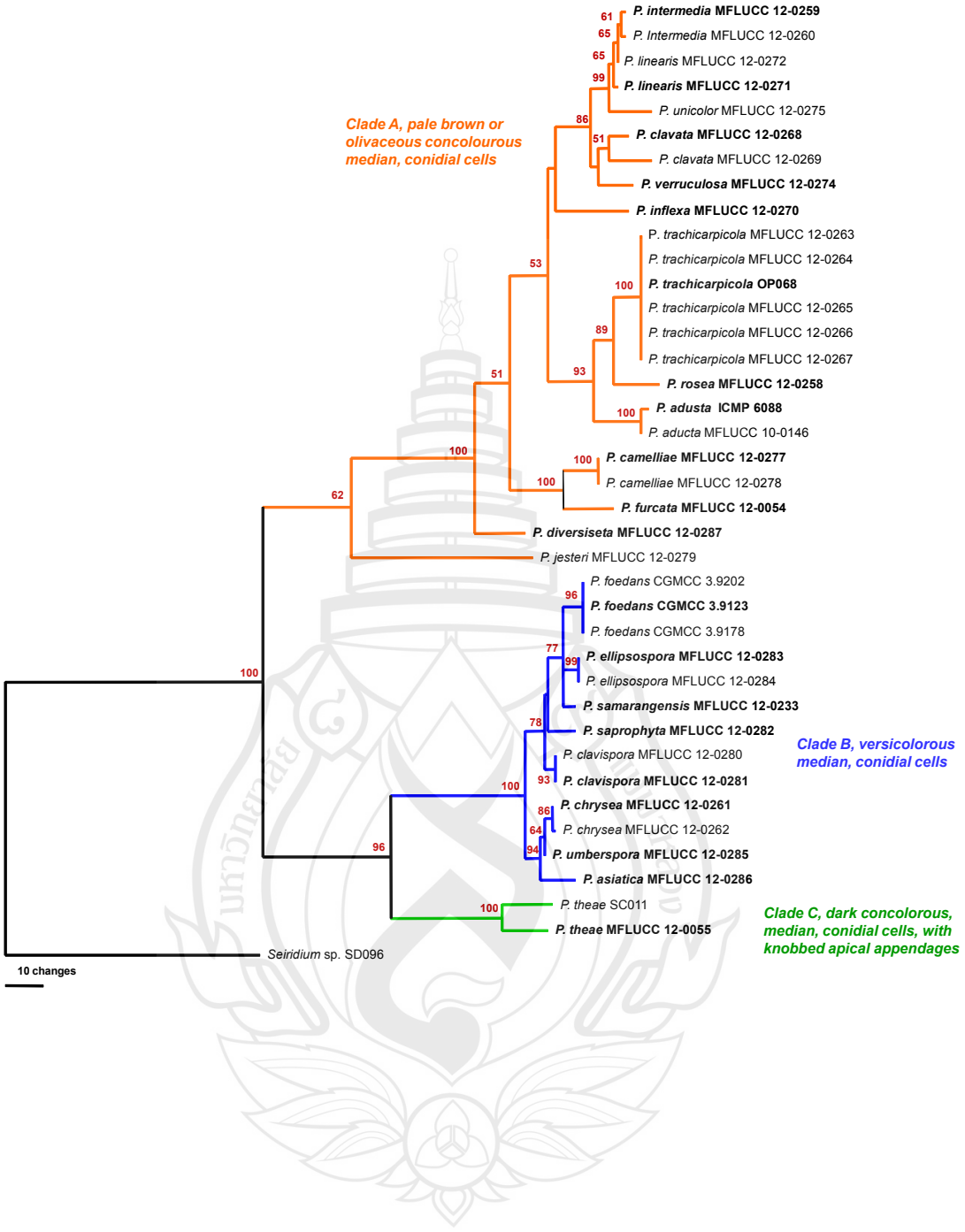


Figure 2.4 Maximum parsimony phylogram generated from TEF1 dataset. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. *Seiridium* spp. was used as the outgroup. Ex-type and ex-epitype sequences are in bold

2.3.5 Combined sequence analysis of ITS, β-tubulin and TEF1 genes sequence data from *Pestalotiopsis* strains

The aligned data matrix for combined ITS, β-tubulin and TEF1 sequences consisted of 41 taxa and 2047 characters (including gaps). Parsimony analysis indicate that 1450 characters were constant, 170 variable characters parsimony-uninformative and 427 characters parsimony-informative. The parsimony analysis of the data matrix resulted in a single parsimonious tree (TL= 1193 steps, CI= 0.685, RI= 0.907, HI= 0.315, RC= 0.621) (Figure 2.5).

In the analysis of the combined dataset from ITS, β-tubulin and TEF1 genes, all species separated into three major clades A, B and C with high bootstrap support. Combined sequence analysis successfully resolved most of the *Pestalotiopsis* species used in this study with high bootstrap supports. The bootstrap support value of terminal and internal node has been increased as compared to the single gene phylogenetic trees.

Table 2.4 Comparison of gene regions used in our study

	ITS	**β-tub**	**TEF1**	**Combined**
PCR success/sequencing success	100%	90%	95%	-
Characters in aligned dataset	546	487	1005	2038
Parsimony-informative characters	78 (14.3 %)	154 (31.6 %)	195 (13 %)	427 (21 %)
Number of bootstrap support >50%	16	24	28	34

Figure 2.5 Maximum parsimony phylogram generated from generated from combine ITS, β -tubulin and TEF1 analysis. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. A *Seiridium* sp. is used as outgroup. Ex-type and ex-epitype sequences are in bold

In this study we attempted to obtain sequence data from 10 genes. In contrast to the other genes, ITS, β-tubulin and TEF1 were relatively easy to amplify, sequence and align. β-tubulin and TEF1 also contained considerably more phylogenetic informative characters. ITS sequence data has relatively poor species resolution for the genus *Pestalotiopsis*, even though it is now standardized as the universal DNA barcode marker for the fungi (Schoch et al., 2012). Therefore, ITS can be used as rough identification guide for some species in *Pestalotiopsis*. β-tubulin and TEF1 successfully resolved most of the strains analyzed in this study to species within *Pestalotiopsis*, although TEF1 had a higher PCR success rate when compared to β-tubulin. Thus, due to its better species resolution and PCR success rate, we suggest that TEF1 is an additional barcode for *Pestalotiopsis* species. At the terminal ends of the clades, most species can be differentiated from closely related species in the β-tubulin and TEF1 and combined ITS, β-tubulin and TEF1 phylograms.

2.3.6 ITS, β-tubulin and TEF1 genes were potential in resolving *Pestalotiopsis* species

In our study, we tested 10 gene regions for suitability in resolving species in *Pestalotiopsis,* but narrowed this down to three most applicable regions, which were tested individually and in combination, to evaluate the differences between species. The ITS is the universal barcode for fungi (Schoch et al., 2012). It has a higher sequence success and PCR success rate with higher resolving power within most fungal lineages (Bridge, Spooner & Roberts, 2005; Schoch et al., 2012). The species of *Pestalotiopsis* sequenced with ITS in this study had a higher sequence success rate. However, ITS could not fulfill the role as candidate gene for species discrimination, as the data did not have high variation between species. Thus, possible cryptic taxa could not be discriminated from one another.

A gene-by-gene assessment of phylogenetic resolution yielded higher levels with protein genes as compared to ribosomal regions (Schoch et al., 2009). Cryptic taxa can be better resolved using slow evolving protein coding genes (Liu, Whelen & Hall, 1999; Liu & Hall 2004). Hu et al. (2007) and Liu et al. (2010) used a β-tubulin fragment to study species relationships within *Pestalotiopsis.* This region has also been shown to resolve species in other genera in groups such as *Aspergillus* (Geiser et

al., 2007), *Discosia* (Tanaka et al., 2011), *Fusarium* (O'Donnell & Cigelnik, 1997), *Nectriaceae* (Zhao et al., 2011), *Seimatosporium* (Tanaka et al., 2011) and *Seiridium* (Barnes, Roux, Coetzee & Wingfield, 2001). TEF1 is a widely used taxonomic marker and this has been successfully utilized to investigate the kingdom-level phylogeny of eukaryotes (Roger et al., 1999; Baldauf, Roger, Wenk-Siefert & Doolittle, 2000) and species in fungal genera such as *Diaporthe* (Santos et al., 2010; Udayanga et al., 2012), *Fusarium* (Geiser et al., 2004; O'Donnell et al., 2010) and *Trichoderma* and *Hypocrea* (Druzhinina et al., 2005). In the present study, β-tubulin and TEF1 gene regions proved to be favorable taxonomic markers for *Pestalotiopsis* since they resolved the taxonomic relationships of most species studied. Further, TEF1 had better PCR amplification success rates (95%) and was found to be superior to β-tubulin (90%). TEF1 is therefore a powerful tool to resolve lineages within *Pestalotiopsis*. Because of the better PCR and sequencing success rate and fewer difficulties with alignment, editing and better resolution, the TEF1 gene appears to be a very good molecular marker for phylogenetic investigation of *Pestalotiopsis.*

Combined sequence analysis of ITS, β-tubulin and TEF1 genes successfully resolved most of the *Pestalotiopsis* species used in this study with high bootstrap support. Hu et al. (2007) and Liu et al. (2010) have previously shown that a combination of β-tubulin and ITS genes gave better species resolution than a single gene and they suggested that at least two genes should be used to resolve species in *Pestalotiopsis*. Similar results have been shown in *Fusarium* (Summerell, Laurence, Liew & Leslie, 2010), *Calonectria* (Lombard, Crous, B.D. Wingfield & M.J. Wingfield, 2010), *Phyllosticta* (Wikee et al., 2011), and *Colletotrichum* (Phoulivong et al., 2010), however the genes best suited for each genus differed. In addition, we tested LSU, SSU, ACT, GPDH, GS, RPB1 and CAL. However, these genes appeared to be inappropriate for use in species differentiation of *Pestalotiopsis* due to low resolution (LSU, SSU, ACT and GPDH) and low PCR success rate (GS, RPB1 and CAL) (see Table 2.3 and Figure 2.1).

2.4 Conclusion

In this study 40 isolates of *Pestalotiopsis*, comprised of 28 strains collected from living and dead plant material of various host plants from China were studied by means of morphology and analysis of 10 gene regions (ACT, β-tubulin, CAL, GPDH, GS, ITS, LSU, RPB1, SSU and TEF1). Of the 10 gene regions (ACT, β-tubulin, CAL, GPDH, GS, ITS, LSU, RPB1, SSU and TEF1) utilized to resolve cryptic *Pestalotiopsis* species, ITS, β–tubulin and TEF1 proved to be the better markers. The other gene regions were less useful due to poor success in PCR amplification and/or in their ability to resolve species boundaries. As a single gene TEF1 met the requirements for an ideal candidate and functions well for species delimitation due to its better species resolution and PCR success. Although β-tubulin showed fairly good differences among species, a combination of ITS, β-tubulin and TEF1 gene data gave the best resolution as compared to single gene analysis.

CHAPTER 3

Pestalotiopsis CRYPTIC SPECIES

3.1. Introduction

Pestalotiopsis Steyaert is an appendage bearing conidial asexual form (coelomycetes) in the family *Amphisphaeriaceae* (Barr, 1975; 1990). Species of *Pestalotiopsis* are common in tropical and temperate ecosystems (Bate–Smith & Metcalfe, 1957) and may cause plant disease (Das et al., 2010; Ko Ko, Stephenson, Bahkali & Hyde, 2011; Zhang et al., 2012). They are also often isolated as endophytes (Wei et al., 2007; Xu et al., 2010), or occur as saprobes (Wu et al., 1982; Yanna et al., 2002).

Maharachchikumbura et al. (2011) reviewed the genus and noted there were only four DNA sequences of the type strains available. Identification of *Pestalotiopsis* to species level is presently difficult and many sequences for *Pestalotiopsis* spp. deposited in GenBank are likely to be wrongly named. There are more than 230 *Pestalotiopsis* names in the literature, most of them described on host association. Recent molecular data have shown that conidial characters of *Pestalotiopsis* can be used to distinguish taxa, however, host association and geographical location is less informative. Thus, names assigned to many *Pestalotiopsis* species lack any accurate taxonomic basis and the taxonomy of the genus is markedly confused.

Phylogenetic studies showed *Pestalotiopsis* strains separated into three strongly supported clades (see Chapter 2). These clades corresponded to three conidial types: i) pale brown or olivaceous concolorous median cells; ii) versicolorous median cells; and iii) dark-coloured concolorous median cells (Jeewon et al., 2003; Maharachchikumbura et al., 2011; Maharachchikumbura, Guo, Chukeatirote,

McKenzie & Hyde, 2012). Steyaert (1949) and Guba (1961) had previously grouped species with versicolorous conidia into two groups based on the intensity of colour of the median cells (umber olivaceous and fuliginous olivaceous). However, sequence data showed division of the "versicolor group" based on colour intensities of the median conidial cell is not a taxonomically good character (Liu et al., 2010a; Maharachchikumbura et al., 2011).

In the present Chapter, phylogenetic relationships between the strains and other genera in the family *Amphisphaeriaceae* are resolved based on analysis of 18S rRNA gene (LSU) sequence data. The phylogeny shows that *Pestalotiopsis* is a distinct clade in *Amphisphaeriaceae* and should be split in to three groups; besides *Pestalotiopsis,* the two new genera *Neopestalotiopsis* and *Pseudopestalotiopsis* are proposed (see Chapter 4). Phylogenetic analyses of combined sequence data of internal transcribed spacer (ITS), β -tubulin gene region and partial translation elongation factor 1-alpha (TEF1) genes plus conidial characters clarify species boundaries in the *Pestalotiopsis*. Furthermore, section names were assigned to species groups upon conidial morphology, host occarance, geographical influvance and sequence data. In addition, the current Chapter describe the distinct morphological and molecular characters of the *Pestalotiopsis* species and placed them in a backbone tree for *Pestalotiopsis*.

3.2. Materials and Methods

3.2.1 Isolation, identification, PCR and sequencing

The specimens were characterised morphologically as described and illustrated in Chapter 2 (see Section 2.1.1). Single spore isolates were done as previously explained in Section 2.2.2.

For nucleotide sequence comparisons, nuclear rDNA operon spanning the 3' end of the 28S rRNA gene (LSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene, β-tubulin gene region (β-tubulin) and partial translation elongation factor 1-alpha (TEF1) genes were amplified using primer pairs LR5/LR0R (Rehner & Samuels,

1994; Vilgalys & Hester, 1990), ITS4/ITS5 (White et al., 1990), T1/BT2B (Glass & Donaldson 1995; O'Donnell & Cigelnik, 1997), and EF728F/EF2 (Carbone and Kohn 1999; O'Donnell et al., 1998). Amplification conditions for LSU, ITS and TEF1 followed Crous et al. (2013) and for β-tubulin followed Lee, Groenewald and Crous, (2004).

Sequencing of the PCR amplicons was conducted using the same primer combinations. The sequence products were purified using Sephadex columns (Sephadex G-50 Superfine, Amersham Biosciences, Roosendaal, Netherlands) and analysed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the manufacturer's instructions. DNASTAR Lasergene SeqMan Pro was used to obtain consensus sequences from sequences generated from forward and reverse primers. All sequences of reference isolates included in this study were obtained from GenBank (Table 3.1).

3.2.3 Phylogenetic analyses

Multiple sequence alignments were generated with MAFFT v. 7 (Katoh & Satandley, 2013), the alignment was visually improved with Mesquite v. 2.75 (Maddison & Maddison, 2011) and Mega 5.2.2 (Kumar, Stecher, Peterson & Tamura, 2012) or Bioedit (Hall, 1999). Three different datasets were used to estimate three phylogenies; an *Amphisphaeriaceae* family tree, *Pestalotiopsis* species tree, and *Neopestalotiopsis* and *Pseudopestalotiopsis* species tree. The first tree focuses on the placement and division of *Pestalotiopsis* in to two new genera in *Amphisphaeriaceae* by using the LSU region, the second and third were produced to show the species relationship in *Pestalotiopsis, Neopestalotiopsis* and *Pseudopestalotiopsis* using combined dataset (ITS, β-tubulin and TEF1). Phylogenetic analyses of the sequence data consisted of Bayesian, Maximum Likelihood and Maximum Parsimony analyses of both the individual data partitions as well as the combined aligned dataset. Ambiguously aligned regions were excluded and gaps were treated as 'fifth character state' in the analysis. Suitable models were first selected using models of nucleotide substitution for each gene, as determined using MrModeltest (Nylander, 2004), and included for each gene partition. The analyses of four Markov Chain Monte Carlo (MCMC) chains were run from random

trees for 100,000,000 generations and sampled every 1,000 generations. The temperature value was lowered to 0.15, burn-in was set to 0.25, and the run was automatically stopped as soon as the average standard deviation of split frequencies reached below 0.01. A maximum likelihood analyses was performed with an Apple-Mac computer using user-friendly, graphical, front-end software, raxmlGUI version 1.3 (Silvestro & Michalak, 2011). The optimal ML tree search was conducted with 100 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model. A maximum parsimony analysis was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002) as previously explained in section 2.2.4. The resulting trees were printed with FigTree v1.4.0 (Rambaut, 2009) and layout with Adobe Illustrator CS v. 6.

Table 3.1 Isolates used in Chapter 3 and 4

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Amphisphaeria umbrina	HKUCC 994	-	-	-	AF452029	-	-	-
Arecophila bambusae	HKUCC 4794	-	-	-	AF452038	-	-	-
Bartalinia bischofiae	HKUCC 6534	-	-	-	AF382367	-	-	-
Bartalinia lateripes	HKUCC 6654	-	-	-	AF382368	-	-	-
Bartalinia laurina	HKUCC 6537	-	-	-	AF382369	-	-	-
Discosia artocreas	NBRC 8975	*Poa pratensis*	*Poaceae*	-	AB593705	-	-	-
Discosia pini	MAFF 410149	*Pinus densiflora*	*Pinaceae*	Japan	AB593708	-	-	-
Discosia sp.	MAFF 238070	*Fallopia japonica*	*Polygonaceae*	Japan	AB593720	-	-	-
Discosia sp.	KT2109	-	-	Japan	AB593712	-	-	-
Discostroma fuscellum	NBRC 32680	*Ribes* sp.	*Grossulariaceae*	-	AB593739	-	-	-
Discostroma fuscellum	NBRC 32625	*Rosa canina*	*Rosaceae*	-	AB593726	-	-	-
Discostroma tostum	NBRC 32626	-	-	-	AB593727	-	-	-

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBankaccession			
					LSU	ITS	β-tubulin	TEF1
Dyrithiopsis lakefuxianensis	HKUCC 7303	-	-	-	AF452047	-	-	-
Funiliomyces biseptatus	CBS 100373	-	*Bromeliaceae*	Brazil	AY772015	-	-	-
Lanceispora sp.	HKUCC 7284	-	-	-	AF452035	-	-	-
Lanceispora sp.	BCP 3574	-	-	-	AF452032	-	-	-
Monochaetia kansensis		*Castanea henryl*	*Fagaceae*	China	DQ534037	-	-	-
Monochaetia kansensis	-	*Quercusaliena Blume*	*Fagaceae*	China	DQ534036	-	-	-
Monochaetia kansensis	-	*Cyclobalaopsis* sp.	*Fagaceae*	China	DQ534035	-	-	-
Neopestalotiopsis aotearoa	CBS 367.54; ATCC 1176; QM 381	canvas	-	New Zealand	Un. Sub.	Un. Sub	Un. Sum.	Un. Sum.
Neopestalotiopsis asiatica	MFLUCC 12-0286	unidentified tree	-	China	-	JX398983	JX399018	JX399049

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis chrysea	MFLUCC 12-0262	dead plant	-	China	-	JX398986	JX399021	JX399052
Neopestalotiopsis chrysea	MFLUCC 12-0261	dead plant	-	China	-	JX398985	JX399020	JX399051
Neopestalotiopsis clavispora	MFLUCC 12-0280	*Magnolia* sp.	*Magnoliaceae*	China	-	JX398978	JX399013	JX399044
Neopestalotiopsis clavispora	MFLUCC 12-0281	*Magnolia* sp.	*Magnoliaceae*	China	-	JX398979	JX399014	JX399045
Neopestalotiopsis clavispora	IFRDCC 2391	*Camellia japonica*	*Theaceae*	China	-	KC537808	KC537822	KC537815
Neopestalotiopsis coffeae-arabicae	HGUP4015	*Coffeae arabica*	*Rubiaceae*	China	-	KF412647	KF412641	KF412644
Neopestalotiopsis coffeae-arabicae	HGUP4019	*Coffeae arabica*	*Rubiaceae*	China	-	KF412649	KF412643	KF412646

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis cubana	CBS 600.96;INIFAT C96/44-4	leaf litter	-	Cuba	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis ellipsospora	MFLUCC 12-0284	dead plant materials	-	China	-	JX399015	JX399015	JX399046
Neopestalotiopsis eucalypticola	CBS 264.37;BBA 5300	*Eucalyptus globulus*	*Myrtaceae*	-	Un. Sub.	Un. Sub.	Un. Sub.	
Neopestalotiopsis foedans	CGMCC 3.9123	Mangrove leaves	-	China	-	JX398987	JX399022	JX399053
Neopestalotiopsis formicarum	CBS 115.83	plant debries	-	Cuba	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis formicarum	CBS 362.72	dead Formicidae (Ant)	-	Ghana	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis honoluluana	CBS 111535;STE-U 2078	*Telopea* sp.	*Proteaceae*	USA	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis honoluluana	CBS 114495;STE-U 2076	*Telopea* sp.	*Proteaceae*	Hawaii	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis javaensis	CBS 257.31	*Cocos nucifera*	*Arecaceae*	Java	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis mesopotamicum	CBS 299.74	*Eucalyptus* sp.	*Myrtaceae*	Turkey	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis mesopotamicum	CBS 464.69	*Achras sapota*	*Sapotaceae*	India	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis piceana	CBS 225.30	*Mangifera indica*	*Anacardiaceae*	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis piceana	CBS 254.32	*Cocos nucifera*	*Arecaceae*	Sulavesi	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis piceana	CBS 394.48	*Picea* sp.	*Pinaceae*	UK	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis australis	CBS 114159;STE-U 3017	*Telopea* sp.	*Proteaceae*	New South Wales	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis protearum	CBS 114178;STE-U 1813	*Leucospermum cuneiforme* cv. 'Sunbird'	*Proteaceae*	Zimbabwe	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis rosa	CBS 101057	*Rosa* sp.	*Rosaceae*	New Zealand	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis rosa	CBS 124745	*Paeonia suffruticosa*	*Paeoniaceae*	USA				
Neopestalotiopsis samarangensis	MFLUCC 12-0233	*Syzygium samarangense*	*Myrtaceae*	Thailand	-	JQ968609	JQ968610	JQ968611

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis saprophyta	CBS 115452;HKUCC 8684	*Litsea rotundifolia*	*Lauraceae*	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis saprophyta	MFLUCC 12-0282	*Magnolia* sp.	*Magnoliaceae*	China	-	JX398982	JX399017	JX399048
Neopestalotiopsis sp.	CBS 110.20							
Neopestalotiopsis sp.	CBS 111494;STE-U 1779	*Protea eximia*	*Proteaceae*	Zimbabwe	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 115113;HKUCC 9136	*Ardisia crenata*	*Myrsinaceae*	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 115451;HKUCC 9095	leaf	-	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis sp.	CBS 119.75	*Achras sapota*	*Sapotaceae*	India	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 164.42	dune sand		France	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 177.25	*Dalbergia* sp.	*Fabaceae*		Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 233.79	*Crotalaria juncea*	*Fabaceae*	India	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 266.37;BBA 5087;IMI 083708	*Erica* sp.	*Ericaceae*	Germany	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 266.80	*Vitis vinifera*	*Vitaceae*	India	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 274.29	*Cocos nucifera*	*Arecaceae*	Java	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 322.76	*Camellia* sp.	*Theaceae*	France	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 323.76	*Erica gracilis*	*Ericaceae*	France	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 360.61	Cinchona sp.	*Rubiaceae*	Guinea	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 361.61	*Cissus*	*Vitaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis sp.	CBS 447.73	decaying wood	-	Sri Lanka	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Neopestalotiopsis sp.	CBS 664.94	*Cocos nucifera*	*Arecaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis steyaertii	IMI 192475	*Eucalyptus viminalis*	*Myrtaceae*	Australia	Un. Sub.	KF582796	KF582794	KF582792
Neopestalotiopsis surinamensis	CBS 450.74	*Elaeis guineensis*	*Arecaceae*	Suriname	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Neopestalotiopsis umberspora	MFLUCC 12-0285	unidentified tree	-	China	-	JX398984	JX399019	JX399050
Neopestalotiopsis zimbabwana	CBS 111495;STE-U 1777	*Leucospermum cunciforme* cv. 'Sunbird'	*Proteaceae*	Zimbabwe	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis adusta	MFLUCC10-146	*Syzygium* sp.	*Myrtaceae*	Thailand	-	JX399007	JX399038	JX399071
Pestalotiopsis adusta	ICMP 6088	on refrigerator door PVC gasket	-	Fiji	-	JX399006	JX399037	JX399070

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis anacardiacearum	IFRDCC 2397	*Mangifera indica*	*Anacardiaceae*	China	-	KC247154	KC247155	KC247156
Pestalotiopsis arenga	CBS 331.92	*Arenga undulatifolia*	*Arecaceae*	Singapore	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis australasia	CBS 114126;STE-U 2896	*Knightia* sp.	*Proteaceae*	New Zealand	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis australasia	CBS 114141;STE-U 2949	*Protea* sp.	*Proteaceae*	New South Wales	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis australis	CBS 111503;STE-U 1770	*Protea susanne*	*Proteaceae*	South Africa	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis australis	CBS 114193;STE-U 3011	*Grevillea* sp.	*Proteaceae*	New South Wales	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis australis	CBS 119350;CMW 20013	*Brabejum stellatifolium*	*Proteaceae*	South Africa	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis biciliata	CBS 124463	*Platanus x hispanica*	*Platanaceae*	Slovakia	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis biciliata	CBS 236.38	*Paeonia* sp.	*Proteaceae*	Italy	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis biciliata	CBS 790.68	*Taxus baccata*	*Taxaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis brassicae	CBS 170.26	*Brassica napus*	*Brassicaceae*	New Zealand	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis camelliae	MFLUCC 12-0278	*Camellia japonica*	*Theaceae*	China	-	JX399011	JX399042	JX399075
Pestalotiopsis camelliae	CBS 443.62	*Camellia sinensis*	*Theaceae*	Turkey	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis camelliae	MFLUCC 12-0277	*Camellia japonica*	*Theaceae*	China	-	JX399010	JX399041	JX399074
Pestalotiopsis chamaeropis	CBS 186.71	*Chamaerops humilis*	*Arecaceae*	Italy	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis chamaeropis	CBS 237.38	-		Italy	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis chinensis	MFLUCC 12-0273	-		China	-	JX398995	-	-
Pestalotiopsis clavata	MFLUCC 12-0268	*Buxus* sp.	*Buxaceae*	China	-	JX398990	JX399025	JX399056
Pestalotiopsis colombiensis	CBS 118553	*Eucalyptus eurograndis*	*Myrtaceae*	Colombia	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis diploclisia	CBS 115449;HKUCC 9103	*Psychotria tutcheri*	*Rubiaceae*	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis diploclisia	CBS 115585;HKUCC 8394	*Diploclisia glaucescens*	*Menispermaceae*	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis diploclisia	CBS 115587;HKUCC 10130	*Diploclisia glaucescens*	*Menispermaceae*	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis ericacearum	IFRDCC 2439	*Rhododendron delavayi*	*Ericaceae*	China	-	KC537807	KC537821	KC537814
Pestalotiopsis foedans	CGMCC 3.9178	*Neodypsis decaryi*	*Arecaceae*	China	-	JX398989	JX399024	JX399055
Pestalotiopsis furcata	MFLUCC 12-0054	*Camellia sinensis*	*Theaceae*	Thailand	-	JQ683724	JQ683708	JQ683740
Pestalotiopsis gaultheria	IFRD 411-014	*Gaultheria forrestii*	*Ericaceae*	China	-	KC537805	KC537819	KC537812
Pestalotiopsis grevillea	CBS 114127;STE-U 2919	-		-	-	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis hawaiiensis	CBS 114491;STE-U 2215	*Leucospermum* sp.	*Myrtaceae*	Hawaii	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis humus	CBS 115450;HKUCC 9100	*Ilex cinerea*	*Aquifoliaceae*	Hong Kong	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis humus	CBS 336.97	soil in tropical forest	-	Papua New Guinea	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis inflexa	MFLUCC 12-0270	unidentified tree	-	China	-	JX399008	JX399039	JX399072
Pestalotiopsis intermedia	MFLUCC 12-0259	unidentified tree	-	China	-	JX398993	JX399028	JX399059
Pestalotiopsis jesteri	CBS 109350	*Fragraea bodenii*	*Gentianaceae*	Papua New Guinea	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis kenyana	CBS 442.67	*Coffea* sp.	*Rubiaceae*	Kenya	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis kenyana	CBS 911.96	-	-	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis knightia	CBS 111963;STE-U 2905	*Knightia* sp.	*Proteaceae*	New Zealand	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis knightia	CBS 114138;STE-U 2906	*Knightia* sp.	*Proteaceae*	New Zealand	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis licualacola	HGUP4057	*Licuala grandis*	*Arecaceae*	China	-	KC436006	KC481683	KC481684
Pestalotiopsis linearis	MFLUCC 12-0271	*Trachelospermum* sp.	*Apocynaceae*	China	-	JX398992	JX399027	JX399058
Pestalotiopsis magna	MFLUCC 12-652	*Pteridium* sp.	*Dennstaedtiaceae*	France	-	KF582795	KF582793	KF582791
Pestalotiopsis malayana	CBS 102220	*Macaranga* triloba	*Euphorbiaceae*	Malaysia	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis monochaeta	CBS 144.97	*Quercus robur*	*Fagaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis monochaeta	CBS 440.83;IFO 32686	*Taxus baccata*	*Taxaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis natalensis	CBS 138.41	*Acacia mollissima*	*Fabaceae*	South Africa	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis novaehollandiae	CBS 130973	*Banksia grandis*	*Proteaceae*	Australia	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis papuana	CBS 331.96	soil along the coast	-	Papua New Guinea	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis papuana	CBS 887.96	*Cocos nucifera*	*Arecaceae*	Papua New Guinea	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis parva	CBS 265.37;BBA 2820	*Delonix regia*	*Fabaceae*	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	**ITS**	**β-tubulin**	**TEF1**
Pestalotiopsis parva	CBS 278.35	*Leucothoe fontanesiana*	*Ericaceae*	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis portugalica	CBS 393.48	-	-	Portugal	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis proteacearum	CBS 111522;STE-U 2083	*Telopea* sp.	*Proteaceae*	USA	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis proteacearum	CBS 353.69	*Oryza sativa*	*Poaceae*	Denmark				
Pestalotiopsis rhododendri	IFRDCC 2399	*Rhododendron sinogrande*	*Ericaceae*	China	-	KC537804	KC537818	KC537811
Pestalotiopsis rhodomyrtus	HGUP4230	-	-	China	-	KF412648	KF412642	KF412645
Pestalotiopsis rosea	MFLUCC12-0258	*Pinus* sp.	*Pinaceae*	China	-	JX399005	JX399036	JX399069

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis sp.	CBS 100567	*Taxus baccata*	*Taxaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis sp.	CBS 113604;STE-U 3078	-	-	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis sp.	CBS 113607;STE-U 3080	-	-	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis sp.	CBS 114137;STE-U 2952	*Protea* sp.	*Proteaceae*	New South Wales	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis sp.	CBS 263.33	*Rhododendron ponticum*	*Ericaceae*	Netherlands	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis sp.	CBS 264.33	*cocos nucifera*	*Arecaceae*	Sulavesi	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis sp.	CBS 543.95	soil under *Araucaria angustifolia*	-	Brazil	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pestalotiopsis spathulata	CBS 356.86	*Gevuina avellana*	*Proteaceae*	Chile	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis telopea	CBS 113606;STE-U 3082	-	-	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis telopea	CBS 114161;STE-U 3083	-	-	-	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pestalotiopsis trachicarpicola	MFLUCC 12-0264	*Chrysophyllum* sp.	*Sapotaceae*	China	-	JX399004	JX399035	JX399068
Pestalotiopsis trachicarpicola	MFLUCC 12-0265	*Schima* sp.	*Theaceae*	China	-	JX399003	JX399034	JX399067
Pestalotiopsis trachicarpicola	MFLUCC 12-0266	*Sympolocos* sp.	*Symplocaceae*	China	-	JX399002	JX399033	JX399066
Pestalotiopsis trachicarpicola	MFLUCC 12-0267	unidentified tree		China	-	JX399001	JX399032	JX399065

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	**ITS**	**β-tubulin**	**TEF1**
Pestalotiopsis trachicarpicola	IFRDCC 2403	*Podocarpus macrophyllus*	*Podocarpaceae*	China	-	KC537809	KC537823	KC537816
Pestalotiopsis trachicarpicola	MFLUCC 12-0263	unidentified tree	-	China	-	JX399000	JX399031	JX399064
Pestalotiopsis unicolor	MFLUCC 12-0276	*Rhododendron* sp.	*Ericaceae*	China	-	JX398999	JX399030	-
Pestalotiopsis unicolor	MFLUCC 12-0275	unidentified tree	-	China	-	JX398998	JX399029	JX399063
Pestalotiopsis verruculosa	MFLUCC 12-0274	*Rhododendron* sp.	*Ericaceae*	China	-	JX398996	-	JX399061
Pseudopestalotiopsis sp.	CBS 387.77	man, skin	-	Finland	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pseudopestalotiopsis sp.	CBS 387.97	soil	-	Papua New Guinea	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pseudopestalotiopsis cocos	CBS 272.29	*Cocos nucifera*	*Arecaceae*	Java	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Pseudopestalotiopsis indica	CBS 459.78	*Rosa sinensis*	*Malvaceae*	India	Un. Sub.	Un. Sub.	Un. Sub.	Un. Sub.
Pseudopestalotiopsis theae	SC011	*Camellia sinensis*	*Theaceae*	Thailand	-	JQ683726	JQ683710	JQ683742
Pseudopestalotiopsis theae	MFLUCC12-0055	*Camellia sinensis*	*Theaceae*	Thailand	-	JQ683727	JQ683711	JQ683743
Seimatosporium elegans	NBRC 32674	*Melaleuca ericifolia*	*Myrtaceae*		AB593733	-	-	-
Seimatosporium eucalypti	CBS 114876	*Eucalyptus smithii*	*Myrtaceae*	South Africa	JN871212	-	-	-
Seimatosporium eucalypti	CBS 115131	*Eucalyptus smithii*	*Myrtaceae*	South Africa	JN871209	-	-	-
Seimatosporium glandigenum	NBRC 32677	*Fagus sylvatica*	*Fagaceae*	-	AB593735	-	-	-
Seimatosporium hypericinum	NBRC 32647	*Hypericum* sp.	*Hypericaceae*	-	AB593737	-	-	-

Table 3.1 (Continued)

Species name	Culture collections	Host/Substrate	Family	Location	GenBank accession			
					LSU	ITS	β-tubulin	TEF1
Seiridium cardinale	ICMP 7323	-	-	-	AF382377	-	-	-
Seiridium cardinale	CBS 172.56	-	-	-	AF382376	-	-	-
Seiridium papillatua	CBS 340.97	-	-	-	DQ414531	-	-	-
Seiridium phylicae	CPC 19970	*Phylica arborea*	*Rhamnaceae*	UK	KC005810	-	-	-
Seiridium phylicae	CPC 19965	*Phylica arborea*	*Rhamnaceae*	UK	KC005809	-	-	-
Seridium sp.	MFLUCC 13030	-	-	Italy		-	-	-
Truncatella hartigii	CBS 118148	-	-	-	DQ278928	-	-	-
Truncatella laurocerasi	ICMP 11214	-	-	-	AF382385	-	-	-
Truncatella restionacearum	CMW 18755	-	-	-	DQ278929	-	-	-
Truncatella sp.	HKUCC 7987	-	-	-	AF382382	-	-	-
Xylaria hypoxylon	HKUCC 3716	-	-	-	AF132333	-	-	-

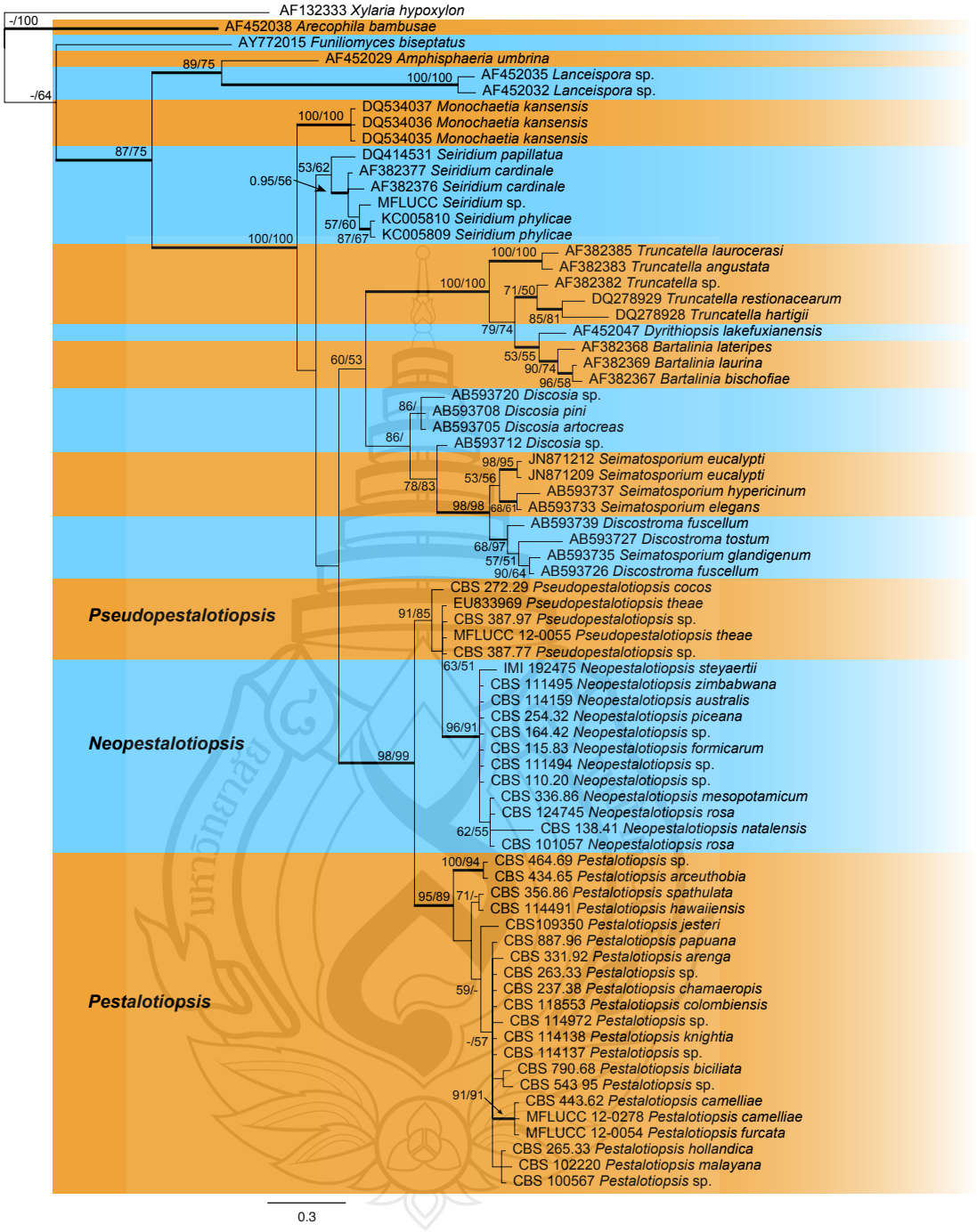
Un. Sub = under submission

3.3 Results and Discussion

3.3.1 Phylogeny

The LSU alignment was used to resolve the generic placement of *Neopestalotiopsis*, *Pseudopestalotiopsis* and *Pestalotiopsis* in *Amphisphaeriaceae* family tree (Figure 3.1). LSU alignment contained 74 sequences (including the outgroup taxon *Xylaria hypoxylon*) and in manually adjust dataset contains 807 characters including gaps. Dirichlet base frequencies and the GTR+I+G model with inverse gamma-distributed rate was resulted for the MrModeltest and set in MrBayes. Parsimony analysis indicated that 617 characters were constant, 73 variable characters parsimony-uninformative and 117 characters parsimony-informative. After a heuristic search using PAUP, 125 most parsimonious trees were retained and in best tree; length = 408 steps, CI = 0.591, RI = 0.871, RC = 0.514 and HI = 0.409. Bayesian analysis resulted in a tree with largely the same topology and clades as the ML and MP trees. The BI, ML and MP analyses of LSU indicated that *Pestalotiopsis* comprises three major monophyletic clades and are supported with high bootstrap confidence. The species possessing similar morphology to the type species of *Pestalotiopsis* (*P. guepini*) clusters in to a one clade and presently remain in *Pestalotiopsis*. In all analyses *Pseudopestalotiopsis* was always sister to the *Pestalotiopsis* and clustered as a basal sister clade to *Neopestalotiopsis*. The species contains versicolours median cells form a monophyletic clade and clusters in *Neopestalotiopsis*, and appear to have evolved from concolorous *Pseudopestalotiopsis*.

To clarify species boundaries within *Pestalotiopsis*, a combined alignment of ITS, β-tubulin and TEF1 sequences from 75 strains and 1519 characters including alignment gaps of *Pestalotiopsis*, and *Neopestalotiopsis saprophyta* (MFLUCC 12-0282) as outgroup taxon was selected (Figure 3.2). Dirichlet base frequencies and the GTR+I+G model with inverse gamma-distributed rate for ITS and HKY+I+G model with inverse gamma-distributed rate for β-tubulin and TEF1 were resulted for the MrModeltest and set in MrBayes. Of the 1519 characters, 890 were constant, 250

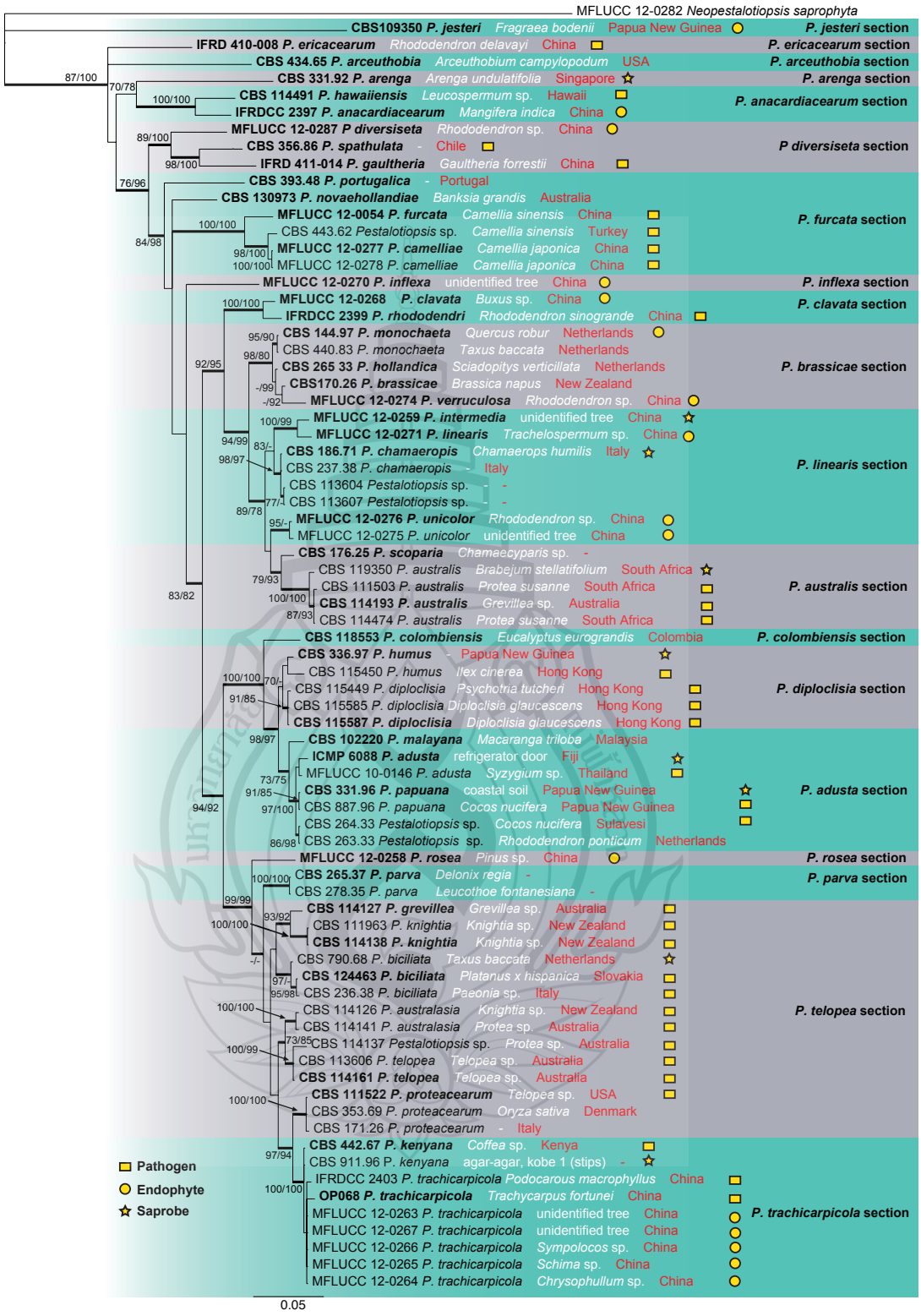


Note. Strict consensus LSU tree from Bayesian analysis of the analyzed *Neoestalotiopsis*, *Pestalotiopsis*, *Pseudopestalotiopsis* and other genera in family *Amphisphaeriaceae*. Genera are indicated in coloured blocks and thickened lines indicate Bayesian posterior probabilities (PP) above 95%. RAxML bootstrap support values (ML) and maximum parsimony bostrap

supports are given at the nodes (ML/MP). The scale bar represents the expected changes per site. The tree was rooted to *Xylaria hypoxylon*

Figure 3.1 Strict consensus LSU tree from Bayesian analysis of the analyzed *Neoestalotiopsis*, *Pestalotiopsis*, *Pseudopestalotiopsis* and other genera in family *Amphisphaeriaceae*

variable characters parsimony uninformative and 379 characters parsimony-informative. An MP analysis yielded 96 equally trees and in first tree; length = 1628 steps, CI = 0.596, RI = 0.808, RC = 0.482 and HI = 0.404. Bayesian analysis resulted in a tree with largely the same topology and terminal clades as the ML and MP trees. The result of the analysis is shown in Figure 3.2 and indicates that *Pestalotiopsis* can be divided into 19 subclades and considering their morphology, host range and geogophy these 19 clades assign in to 19 species complex within genus *Pestalotiopsis*.



Note. Strict consensus combined (ITS+ β-tubulin + TEF1) tree from Bayesian

analysis of the analyzed *Pestalotiopsis* isolates. Species complex are indicated in coloured blocks and thickened lines indicate Bayesian posterior probabilities (PP) above 95%. RAxML bootstrap support values (ML) and maximum parsimony bostrap supports (MP) are given at the nodes (ML/MP). Strain accession numbers are followed by the original species name (ex-type are in bold), the isolation source (white) and country of origin (red). The scale bar represents the expected changes per site. The tree was rooted to *Neopestalotiopsis saprophyta* (MFLUCC 12-0282)

Figure 3.2 Strict consensus combined (ITS+ β-tubulin + TEF1) tree from Bayesian analysis of the analyzed *Pestalotiopsis* isolates

3.3.2 Taxonomy

Species of *Pestalotiopsis* were assigned to 19 sections upon conidial morphology, sequence data, geographical influence and host occurrence. Brief description of each section was provided according to the alphabetical order. Finally the descriptions of the *Pestalotiopsis* are provided.

P. adusta section

P. adusta section is characterized by the species having two apical appendages. *P. adusta*, *P. malayana* and *P. papuana* are the three species belongs to this section.

P. anacardiacearum section

This section consists of two species. *P. anecardiecirum* isolated from mango in China and *P. hawaiiensis* isolated from *Leucospermum* sp. in Hawaii. This section is characterized by its large conidial size and longer apical appendages when compare to sister sections.

P. arceuthobia section

The described section consists of single species; *Pestalotiopsis arceuthobium* isolated from *Arceuthobium campylopodum* in USA. It is distinguished from nearing sections by its narrow conidia as well as short apical appendages.

P. arenga section

This section consists of a single species; *Pestalotiopsis arenga*, isolated from dead leaves of *Arenga undulatifolia* in Singapore. The species section characterized by larger conidia with shorter apical appendages.

P. australis section

P. australis section is sitting next to the *P. linearis* section and characterized by having larger conidia. *Pestalotiopsis australis* and *P. scoparia* are the two species of the section.

P. brassicae section

This section is characterized by larger conidia. Species in this section are distinguished each other by the number and the attachment of apical appendages to the apical cell. *Pestalotiopsis brassicae, P. hollandica*, *P. monochaeta* and *P. verruculosa* are the four species in this section.

P. clavata section

Pestalotiopsis clavata and *P. rhododendri* belongs to this section, which are isolated from China from *Euonymus* sp. and *Rhododendron sinogrande* respectively. This section is characterized by smaller conidia compared to sister, *P. brassicae* section.

P. colombiensis section

Pestalotiopsis colombiensis is an outlying single species in *P. colombiensis* section. It is clearly distinct from sister *P. diploclisia* section by geography and sequence data.

P. diploclisia section

Pestalotiopsis diploclisia and *P. humus* are the two species belongs to this section. It is clearly separated from neighboring *P. adusta* section by having more than two apical appendages (see notes under *P. adusta* section).

P. diversiseta section

This species section is characterized by the characters in the apical appendages. This section consists of the species baring 2–5 appendages that are knobbed and arising from the different parts of the apical cell. *P diversiseta* section contains three species *P. spathulata*, *P. diversiseta* and *P. gaultheria* which were

isolated from leaf spot on *Guevina avellana* in Chile, leaves of *Rhododendron* sp. in China and leaf spots of *Gaultheria forrestii* in China, respectively.

P. ericacearum section

Pestalotiopsis ericacearum is a single species in *P. ericacearum* section, which was isolated from leaf spot on *Rhododendron delavayi* in China. This section is characterized by relatively short conidia, olivaceous median cells and having long and knobbed apical appendages.

P. furcata section

This section is characterized by the species often lack basal appendages and high number of apical appendages. *Pestalotiopsis camelliae*, *P. furcata*, *P. novaehollandiae* and *P. portugalica* are the four species in this section.

P. inflexa section

This section contains a single species *P. inflexa,* which was isolated from unidentified tree in China. The section is characterized by 2–5 tubular appendages, which arise subapically, each inserted at a different locus in upper half of the apical cell.

P. jesteri section

P. jesteri section is well characterized and easily recognizable by the unique arrangement of knobbed apical appendages. This section contains two species and only *P. jester* has sequence data. However, *P. jesteri* differs from *P. montellica* by the presence of knobbed apical appendages. Furthermore, this section is an outlying group in the genus, and forms a distinct lineage apart from all other section in *Pestalotiopsis*.

P. linearis section

Pestalotiopsis chamaeropis, *P. intermedia*, *P. linearis* and *P. unicolor* are the four species belongs to this section. Except *P. chamaeropis*, which is isolated from Italy, all other species are recorded from China. This section lies between the *P. brassicae* and *P. australis* sections, which consist species with longer apical appendages and larger conidia.

P. parva section

P. parva is the single species in this section and characterized by smaller conidia (see notes under *P. parva*).

P. rosea section

Phylogenetically *P. rosea* section is a distinct section sister to the *P. parva* section. It consist single species *P. rosea* that has reddish colony and this colour is unique to the species and can be found even in conidiogenous cells and in conidia.

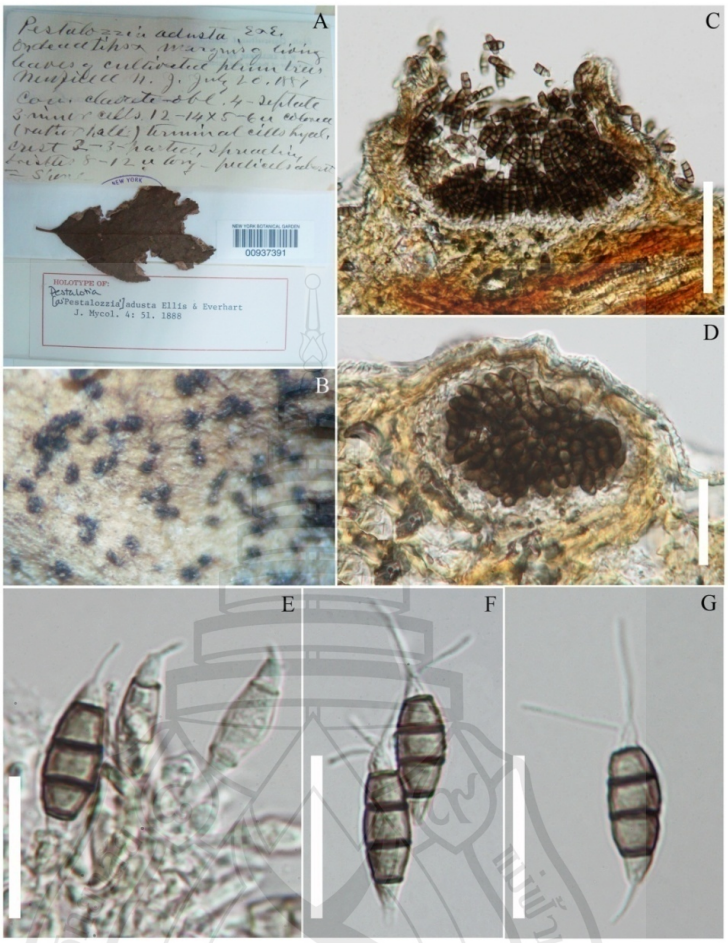
P. telopea section

Pestalotiopsis australasia, *P. biciliata*, *P. grevillea*, *P. knightia*, *P. proteacearum, Pestalotiopsis* sp. (CBS 114137) and *P. telopea* are the species belongs to this section and they all recorded from plant family *Proteaceae*. Due to their distinct in phylogeny and host occurrence, *P. telopea* section is introduced.

P. trachicarpicola section

Species in this section have two basal appendages. *P. kenyana* and *P. trachicarpicola* are the two species in this section and they often have two basal appendages.

Pestalotiopsis adusta (Ellis & Everh.) Steyaert, Trans. Br. mycol. Soc. 36: 82 (1953) MycoBank: MB302600 (Figure 3.3 A–G). Basionym: *Pestalotia adusta* Ellis & Everh., J. Mycol. 4(6): 51 (1888)



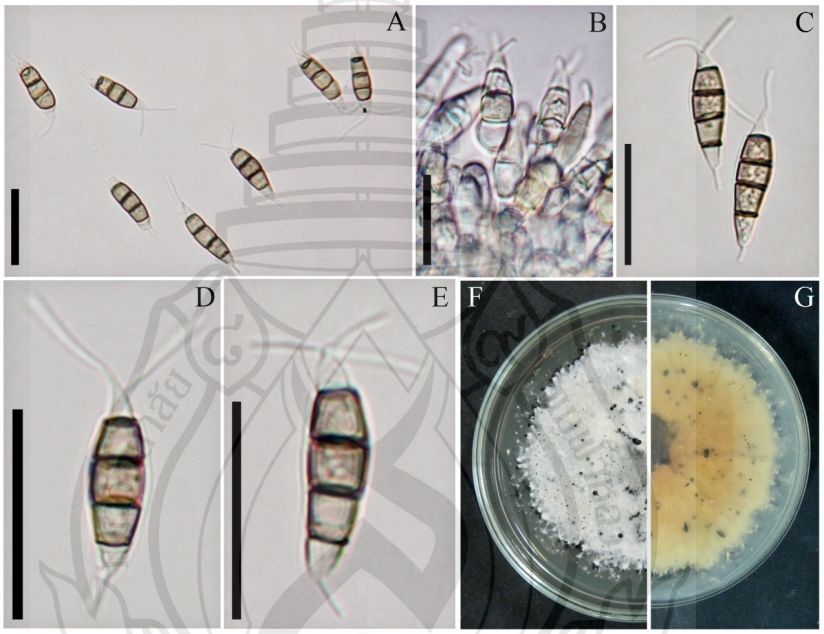
Note. A. Herbarium material – leaves of *Prunus cerasus*. B. Conidiomata, split irregularly. C–D. Section of conidiomata. E. Conidiogenous cells F–G. Conidia with concolorous median cells. Scale Bars: E = 50 µm, F– H = 20 µm

Figure 3.3 *Pestalotiopsis adusta* (holotype)

Description from holotype. (Figure 3.3 A–G)

Conidiomata 80–150 µm diam., acervulus, subepidermal in origin, with basal stroma, with lateral wall 2–4 cells thick comprising hyaline to pale brown cells of *textura angularis*. *Conidiophores* indistinct. *Conidiogenous cells* discrete, simple, short, filiform. *Conidia* 16–20 × 5–7 µm (mean = 18.7 × 6.2 µm), fusiform to ellipsoid, straight to slightly curved, 4-septate, with short basal cell, obtuse, hyaline,

thin-walled and verruculose, 2.7–3.8 µm long (mean = 3.2 µm); with three median cells, doliiform to subcylindrical, concolorous, olivaceous, with septa and periclinal walls darker than the rest of the cell, together 12.4–13.8 µm long (mean = 13.2 µm) (second cell from base 4.3–5.3 µm (mean = 4.8 µm); third cell 4–4.7 µm (mean = 4.2 µm); fourth cell 3.8–4.4 µm (mean = 4 µm); apical cell hyaline, conic, 2.4-3.4 µm long (mean = 3 µm); with two to three appendages, 7–15 µm long (mean = 10 µm), arising from the apex of the apical cell; filiform basal appendage (Figure 3.3).



Note. B. Conidiogenous cells C–E. Conidia with concolorous median cells. F–G. Colony on PDA, F. from above, G. from below. Scale Bars: A– E= 20 µm

Figure 3.4 *Pestalotiopsis adusta* (epitype)

Description from epitype (Figure 3.4 A–G)

Conidiophores indistinct. *Conidiogenous cells* discrete, simple, short, filiform. *Conidia* 17–20 × 5.2–6.6 µm (mean = 19 × 6 µm), fusiform to ellipsoid, straight to slightly curved, 4-septate, basal cell short, obtuse, hyaline, thin-walled and

verruculose, 3–3.8 µm long (mean = 3.3 µm); with three median cells, doliiform to subcylindrical, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, together 12.5–14.2 µm long (mean = 13.6 µm) (second cell from base 4.4–5.5 µm (mean = 4.9 µm); third cell 4.3–5 µm (mean = 4.5 µm); fourth cell 4–4.8 µm (mean = 4.3 µm); apical cell hyaline, conic, 2.7–3.7 µm long (mean = 3.2 µm); with two to three appendages 6–14 (mean = 10 µm) µm long, arising from the apex of the apical cell; filiform basal appendage (Figure 3.4).

Cultural characteristics: Colonies on PDA attaining 7 cm diam. after 7 days at 25^{o}C, with undulate edge, whitish, with dense, aerial mycelium on surface; fruiting bodies black, gregarious; reverse of the colony yellowish.

Material examined: USA, Newfield, New Jersey, on leaves of *Prunus cerasus* L., cultivated plum, 20 July 1887 (NY 00937391, holotype); FIJI, on refrigerator door PVC gasket, 1 June 1978, E.H.C. McKenzie (MFLU12-0425, epitype designated here; ex-type living culture ICMP 6088 = PDDCC 6088).

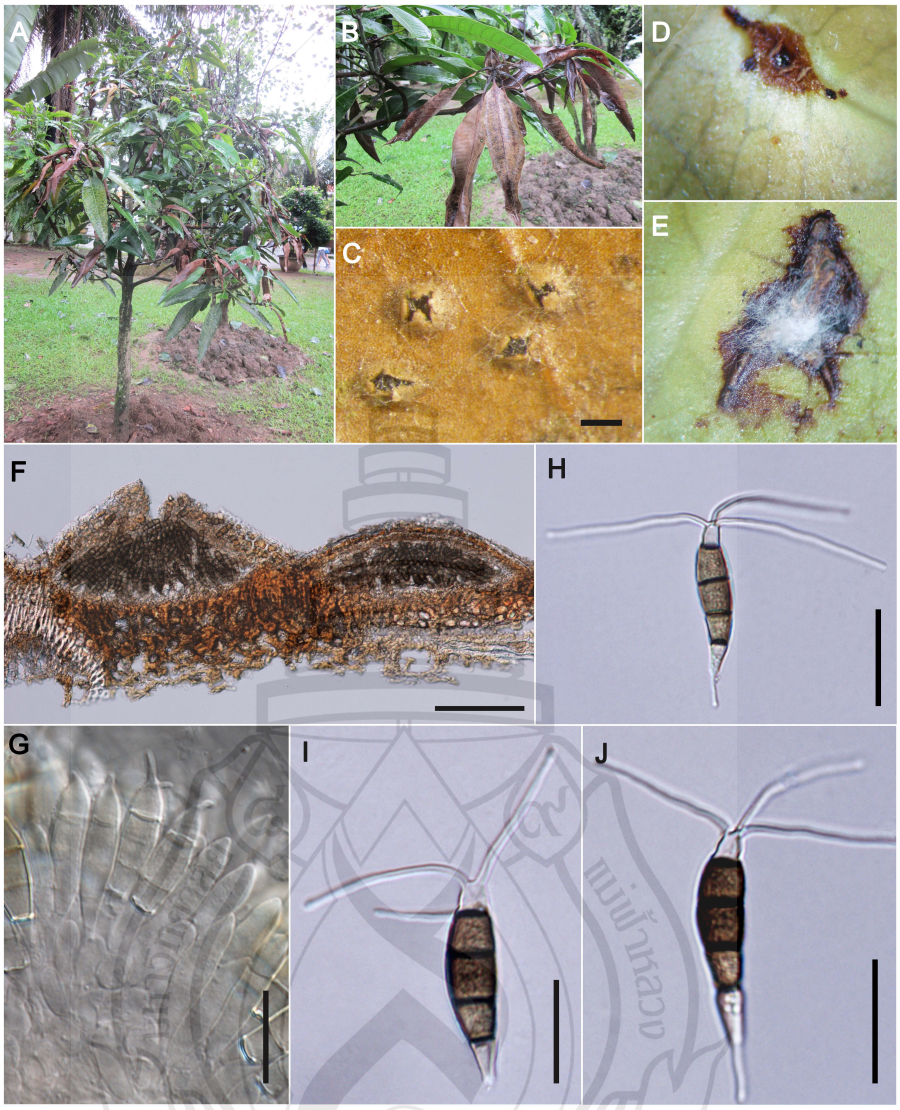
Additional culture examined: Thailand, Chiang Rai, on leaves of *Syzygium* sp., 06 Febuary 2010, S.S.N. Maharachchikumbura SS008 (MFLUCC 10-0146).

Notes: *Pestalotiopsis adusta* was described from cultivated plum in New Jersey (Steyaert, 1949) and recently phenolic compounds isolated from one putative isolate of *P. adusta* showed antimicrobial activity against *Fusarium culmorum*, *Gibberella zeae* and *Verticillium aiboatrum* (Li et al., 2008b). *Pestalotiopsis adusta* is characterized by its small conidia (16–20 × 5–7 µm) and two to three relatively short apical appendages (7–15 µm) (Figure 2.1 E–G). According to Guba (1961), *P. adusta* occurs on various hosts and has a cosmopolitan distribution. Guba (1961) listed it from *Acer platanoides* in Point Pleasant, New Jersey; on stems of *Barringtonia speciosa* in Bermuda; from circular spots on leaves of *Bischofia javanica* in Taiwan; on leaves of *Carpinus betulus* in Italy; as causing fruit rot and grey leaf spot in *Eriobotrya japonica* in Japan; on leaves of *Homalomena philippinensis* in the Philippines; and on spots and dead areas of leaves of *Pavonia multiflora* in Brazil. Living specimens from cultivated plum or from the USA would have been desirable when epitypifying this taxon. The sample collected from Fiji, however, is characteristic of *P. adusta,* a distinct species in the genus. The epitype has identical conidiogenous cells and morphology, including three apical appendages and a spore

size fitting that of the holotype. As we want to advance the understanding of this poorly defined species rich genus, the Fiji collection is designated here as an epitype of *P. adusta.*

Pestalotiopsis anacardiacearum Y.M. Zhang, Maharachch. & K.D. Hyde, Phytotaxa 99 (2): 49–57 (2013), MycoBank: MB802354 (Figure 3.5).

Etymology: *anacardiacearum*, derived from the family name (Anacardiaceae) of the host plant.



Note. *Pestalotiopsis anacardiacearum*. A–B. leaf blight on mango leaves damaged by mango tip borer. C. Acervuli, splitting irregularly through host surface. D. non pin-pricked mango leaf artificially inoculated with the fungus. E. pin-pricked mango leaf artificially inoculated with the fungus. F. Section of acervuli. G. Conidiogenous cells. H–J. Conidia. Scale bars: C = 200 µm, F = 50 µm, G–J = 20 µm

Figure 3.5 *Pestalotiopsis anacardiacearum* (holotype)

Acervuli 180–380 × 180–310 µm (mean = 286 × 232 µm), brown, epidermal to subepidermal, separate or confluent, dehiscence irregularly. Acervuli in section 270–350 µm diam, 85–160 µm high (mean = 300 × 130) (Figure 3.5). *Conidiophores* most often reduced to conidiogenous cells, simple or branched, hyaline, smooth-walled. *Conidiogenous cells* discrete, hyaline, 1-celled, branched or separate at the base, formed from the upper cells of the pseudoparenchymata. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4–septate, 27–39 × 7–10 µm (mean = 32 × 9 µm); basal cell conic to obconic, hyaline or slightly olivaceous, thin-walled and verruculose, 5–7.5 µm long (mean = 6.3 µm); with 3 median cells, doliiform to cylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, with septa and periclinal walls darker than the rest of the cell, together 19–22 µm long (mean = 20 µm) (second cell from base 6.5–8.5 µm (mean = 7.4 µm); third cell 6.8–7.5 µm (mean = 7.3 µm); fourth cell 6.7–8.5 µm (mean = 7.4 µm); apical cell hyaline, subcylindrical to cylindrical, 4–5.3 µm long (mean = 4.8 µm); 2–3 tubular apical appendages (mostly 3), arising from the apex, 20–45 µm long (mean = 33 µm); basal appendage present, 5–9 µm long (mean = 7 µm), rarely absent.

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 14 days at 25°C, edge entire, whitish, with dense, aerial mycelium on surface, with black, gregarious fruiting bodies; reverse of the culture white.

Habitat/Distribution: Endophytes on living leaves of *Mangifera indica*, Mangshi, Dehong, Yunnan Province, China.

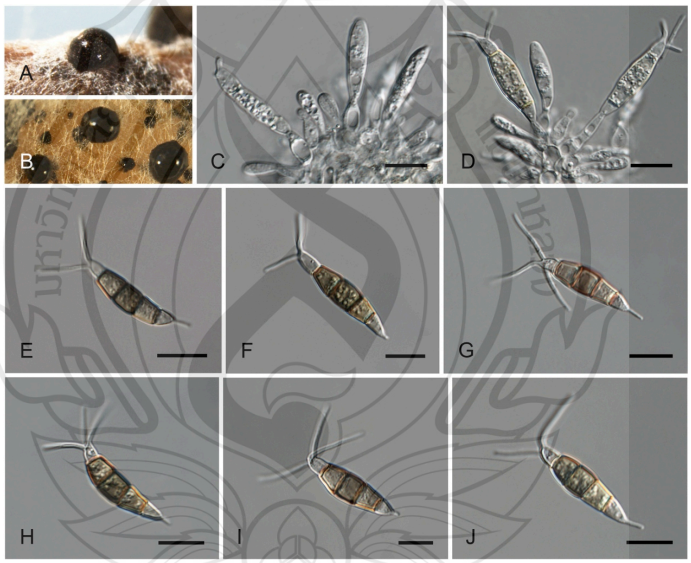
Material examine: CHINA. Yunnan Province: Mangshi, Dehong, living leaf of *Mangifera indica*, September 2011, Zhang Yanmin OP0139, (holotype IFRD 411-015, ex-type living culture IFRDCC 2397).

Notes: *Pestalotiopsis anacardiacearum* can be differentiated from its close relatives in the combined phylogram (Figure 3.2). Furthermore, the branch lengths of *P. anacardiacearum* separating it from neighboring clades are longest in the phylogram and this signifies speciation. The characteristic morphology of *P. anacardiacearum* is due to its large conidial size and three, long, apical appendages. Morphologically similar species to *P. anacardiacearum* in conidial size are *P. hughessii* (35–45 × 7–11 µm), *P. kunmingensis* (33.8–46.8 × 7.5–10 µm) and *P. macrospora* (30–40 × 9–12 µm) (Table 3.2) (Guba, 1961; Wei, 2004). However, *P.*

anacardiacearum can be distinguished from *P. hughessii* and *P. macrospora* by its relatively narrow conidia and also by its long apical appendages (in *P. hughessii* 10–30 µm and in *P. macrospora* 10–20 µm). *P. kunmingensis* differs from *P. anacardiacearum* in having knobbed apical appendages and versicolorous median cells. *Pestalotiopsis anacardiacearum* can clearly be distinguished from the previous *Pestalotiopsis* species recorded on mango, by having larger conidia (27–39 × 7–10 µm) than *P. mangiferae* (20–24 × 4–6 µm) and *P. glandicola* (20–26 × 7–9.5 µm).

Pestalotiopsis arceuthobia Maharachch. & Crous, sp. nov. (Figure 3.6 A–J).

Etymology: Named after the host genus from which it was isolated, *Arceuthobium.*



Note. *Pestalotiopsis arceuthobia* CBS 434.65 ^{T}. A. Conidiomata sporulating on PNA (pine needle agar). B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm

Figure 3.6 *Pestalotiopsis arceuthobia* (holotype)

Conidiomata pycnidial in culture on PDA, globose to clavate, solitary or aggregated in clusters, brown to black, semi-immersed, 100–500 µm diam; exuding dark brown conidia in a slimy, globose mass. *Conidiophores* mostly reduced to conidiogenous cells, branched or unbranched, 0–2 septate, hyaline and smooth, up to 10 µm long. *Conidiogenous cells* discrete, subcylindrical (3–12 × 1–3 µm) or ampulliform to lageniform (3–10 × 2–6 µm), hyaline, smooth, thin-walled, proliferating up to 4 times percurrently, collarette present and not flared. *Conidia* ellipsoid, straight to slightly curved, somewhat constricted at septa, 4-septate, (21–)22–25.5(–26) × 6.5–8(–8.5) µm, mean ± SD = 24.4 ± 1.3 × 7.2 ± 0.5 µm, basal cell obconic with truncate base, thin-walled, rugose, 5–6 µm long; three median cells (14–)15–16.5 µm long, mean ± SD = 15.6 ± 0.9, doliiform, verruculose, concolorous, brown, (second cell from base 5–6 µm long; third cell 5.5–6.5 µm long; fourth cell 4.5–6 µm long); apical cell cylindrical, hyaline, thin and smooth-walled, 4–5 µm long; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, flexuous, unbranched, (10–)11–14.5(–16) µm long, mean ± SD = 12.8 ± 1.0; basal appendage single, tubular, unbranched, centric, 3–6 µm long (Figure 3.6).

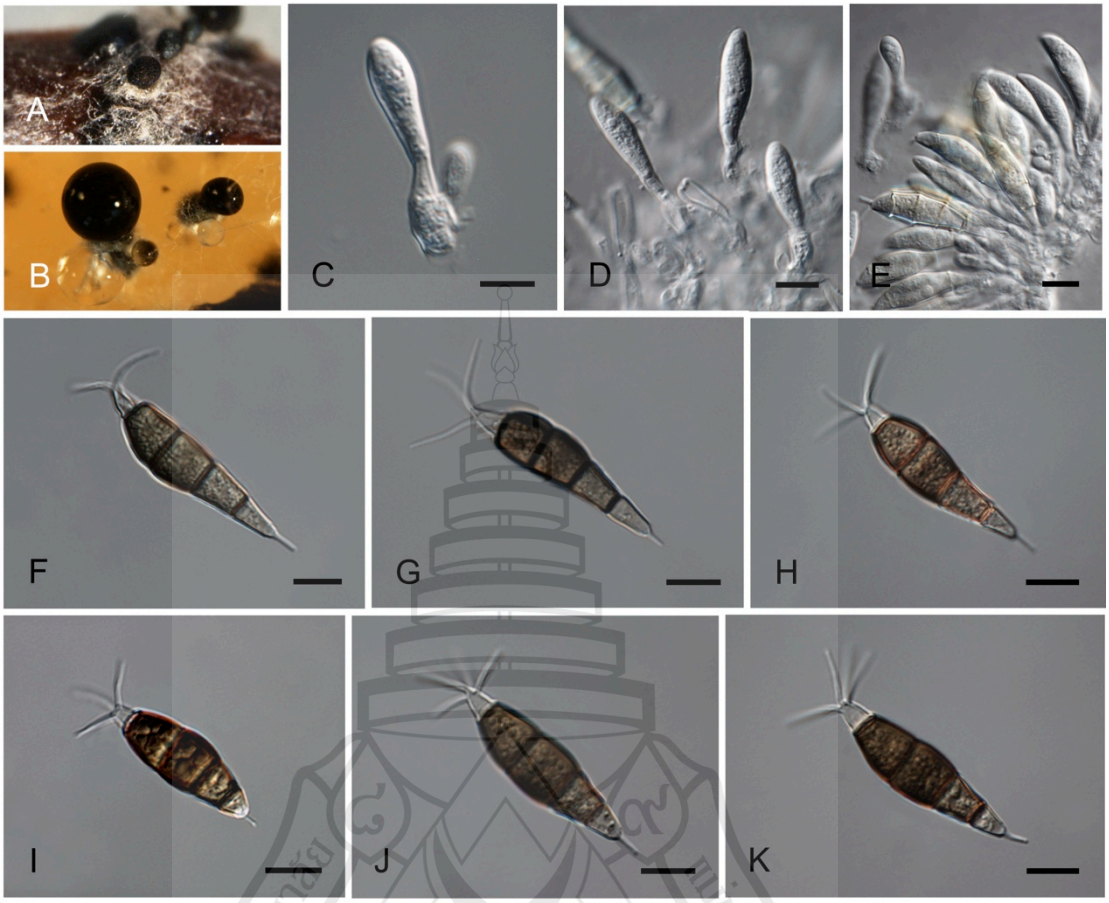
Culture characteristics: Colonies on PDA reaching 60–70 mm diam after 7 days at 25°C, edge entire, whitish to pale honey-coloured, with aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: USA, Washington, King County, North Bend, from *Arceuthobium campylopodum*, Aug 1965, E.F. Wicker (CBS H-15695 holotype, culture ex-type CBS 434.65).

Notes: *Pestalotiopsis arceuthobia* is single species in the *P. arceuthobia* section. It formed a well-supported sister clade to the species complex *P. ericacearum* which also consist of single species *P. ericacearum*. *Pestalotiopsis arceuthobia* distinguished from *P. ericacearum* (15–21 × 5–9 µm) by its narrow conidia (21–26 × 6.5–8.5 µm) as well as short apical appendages (10–16 µm). In *P.ericacearum* the apical appendages are longer (19–45 µm), and knobbed.

Pestalotiopsis arenga Maharachch. & Crous sp. nov. (Figure3.7 A–K).

Etymology: Named after the host genus from which it was isolated, *Arenga.*



Note. *Pestalotiopsis arenga* CBS 331.92 [T]. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.7 *Pestalotiopsis arenga* (holotype)

Conidiomata (on PDA) pycnidial, globose or clavate, solitary or aggregated, semi-immersed, dark brown to black, 200–400 µm diam; exuding dark brown conidial masses. *Conidiophores* most often reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth, thin-walled, 3–15 × 3–10 µm, proliferating several times percurrently, with minute periclinal thickenings. *Conidia* ellipsoid, straight to slightly curved, slightly constricted at septa, 4-septate, (24–)25–32(–33) × 7–9.5(–10) µm, mean ± SD = 27.6 ± 2 × 8 ± 0.4 µm; basal cell

conic with a truncate base, thin-walled and rugose, 4–7 µm long; three median cells (17–)17.5–21.5(–22) µm long, mean ± SD = 19 ± 1.3, doliiform, verruculose, concolorous, brown, septa darker than the rest of the cell, (second cell from base 5.5–7 µm long; third cell 5.5–8 µm long; fourth cell 6–7.5 µm long); apical cell subcylindrical, hyaline, thin and smooth-walled, 2.5–4.5 µm long; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (4–)4.5–11(–12) µm long, mean ± SD = 7.3 ± 1.3; basal appendage single, tubular, unbranched, centric, 1.5–3 µm long (Figure3.7).

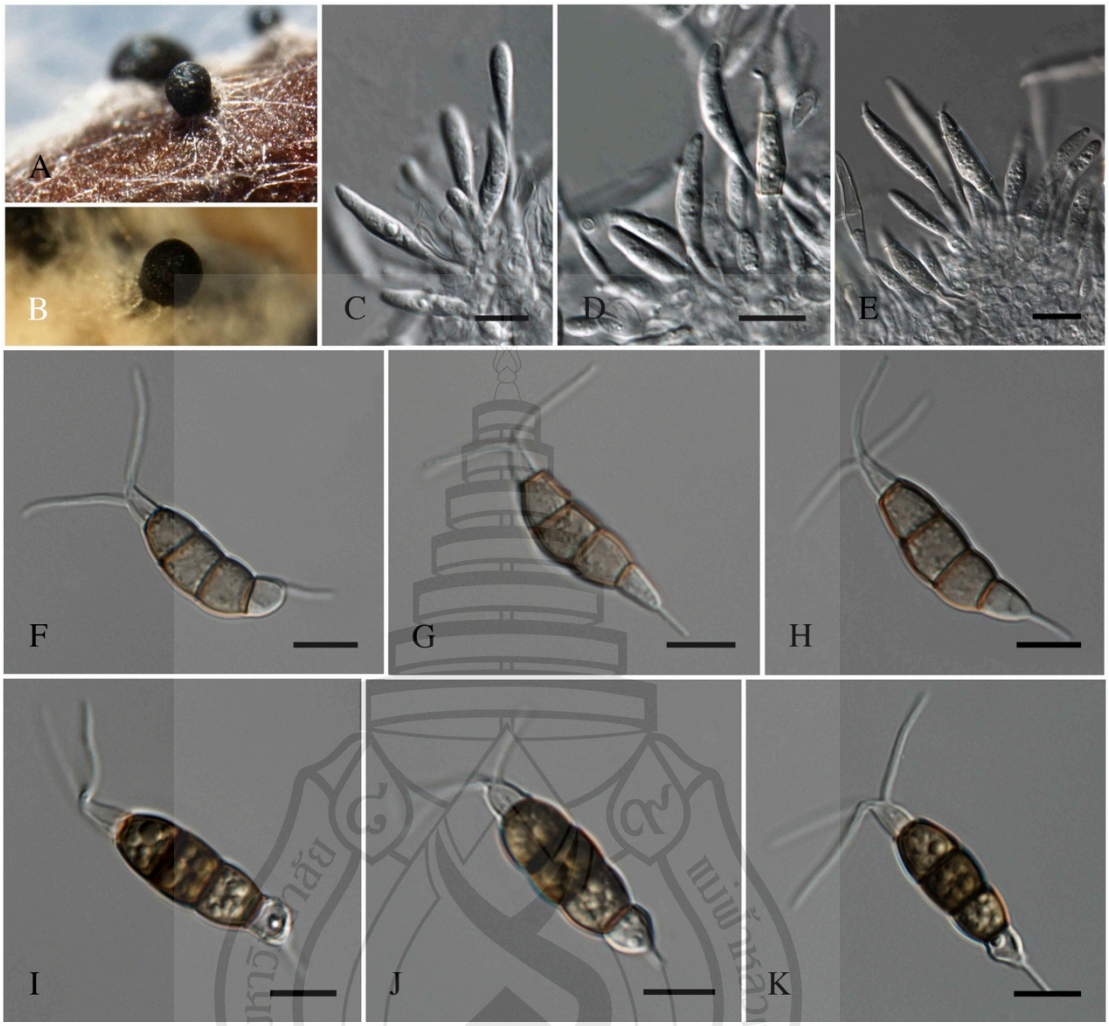
Culture characteristics: Colonies on PDA reaching 70–80 mm diam after 7 days at 25°C, undulate at the margin, white to pale luteous-coloured, with moderate aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: SINGAPORE, Botanical Gardens, from dead leaves of *Arenga undulatifolia*, Nov. 1991, W. Gams (CBS H holotype, culture ex-type CBS 331.92).

Notes: *Pestalotiopsis arenga* forms a separate cluster in the combined gene phylogeny as sister to a *P. anacardiacearum* section including *P. anacardiacearum* and *P. hawaiiensis*, which was isolated on mango from China and *Leucospermum* sp. from Hawaii, respectively. In morphology, *P. arenga* differs from *P. anacardiacearum* and *P. hawaiiensis* by smaller conidia and shorter apical appendages.

Pestalotiopsis australasia Maharachch. & Crous sp. nov. (Figure 3.8 A–H).

Etymology: Refers to the geographical region (Australia and New Zealand), in where fungus was isolated.



Note. *Pestalotiopsis australasia* CBS 114126^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.8 *Pestalotiopsis australasia* (holotype)

Conidiomata pycnidial in culture on PDA, globose, scattered, semi-immersed, up to 200 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete or intergrated, ampulliform or cylindrical, hyaline, minutely verruculose, proliferating 2-4 times percurrently, tapering to a long, thin neck, 15–50 × 3–9 µm,

with flaring collarettes. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (23–)24.5–29(–31) × (6–)6.5–8(–8.5) µm, mean ± SD = 26 ± 1.4 × 7.5 ± 0.2 µm; basal cell obconic to hemispherical, hyaline, thin and verruculose, 5–6.5 µm long; three median cells doliiform, (15–)15.5–18(–18.5) µm long, mean ± SD = 16.7 ± 0.7, wall verruculose, concolorous, brown, septa darker than the rest of the cell (second cell from the base 5–6.5 µm long; third cell 5.5–7 µm long; fourth cell 5.5–7 µm long); apical cell 3.5–5 µm long, hyaline, cylindrical to subcylindrical; with 2–3 tubular apical appendages, arising from an apical crest, unbranched, filiform, flexuous (9–)10–15(–16) µm long, mean ± SD = 12.6 ± 1.7; basal appendage single, tubular, unbranched, centric, 2.5–4.5 µm long (Figure 3.8).

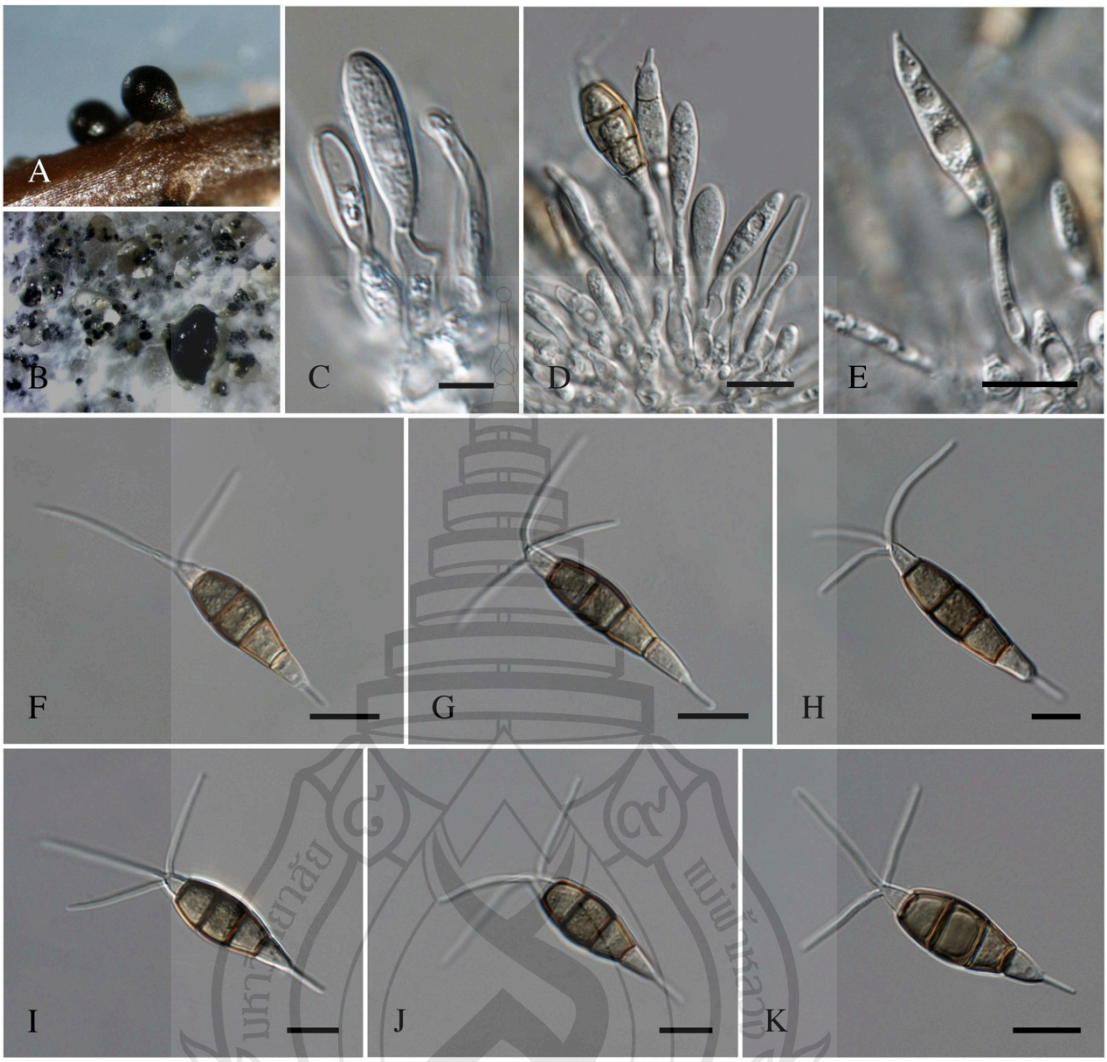
Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, flat with entire edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: NEW ZEALAND, from *Knightia* sp., unknown collection date and collector, (CBS H holotype, culture ex-type CBS 114126= STE-U 2896); Australia, New South Wales, from *Protea* ''Pink Ice'', 12 Oct. 1999, P.W. Crous, culture CBS 114141= STE-U 2949.

Notes: Morphologically *P. australasia* is comparable with *P. knightia, P. parvus* and *P. grevillea*, but differs in having larger conidia compared to *P. parvus,* and shorter apical appendages compared to *P. knightia* and *P. grevillea*. It has an overlapping conidial size with *P. telopea* which cause leaf spot on *Telopea* sp. Since the two species are genetically distinct, we prefer to maintain two separate species (see notes under *P. telopea*).

Pestalotiopsis australis Maharachch. & Crous, sp. nov. (Figure 3.9 A–K).

Etymology: Named after the country where it was collected, Australia.



Note. *Pestalotiopsis australis* CBS 114193^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 μm.

Figure 3.9 *Pestalotiopsis australis* (holotype)

Conidiomata pycnidial in culture on PDA, globose or clavate, aggregated or scattered, semi-immersed or partly erumpent, dark brown to black, up to 400 μm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* 1-3 septate, sparsely branched at the base, subcylindrical, hyaline, verruculose, up to 25

µm. *Conidiogenous cells* discrete or integrated, ampulliform or cylindrical, hyaline, smooth, proliferating 2-4 times percurrently, 20–60 × 2–6 µm, collarette present and slightly flared. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (26–)27–34(–36) × 7–8.5 µm, mean ± SD = 30.8 ± 2.1 × 7.7 ± 0.3 µm; basal cell conic to obconic with a truncate base, hyaline, thin and minutely verruculose, 6–10 × µm long; three median cells doliiform, (16–)17–21(–21.5) µm long, mean ± SD = 19.1 ± 1.2, wall minutely verruculose, concolorous, brown, septa darker than the rest of the cell, (second cell from the base 5.5–7.5 µm long; third cell 5.5–7.5 µm long; fourth cell 6–8 µm long); apical cell 4–6.5 × µm long, hyaline, cylindrical to subcylindrical, thin and smooth walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (11–)12–20(–22) µm long, mean ± SD = 15.5 ± 2.7; basal appendage single, tubular, unbranched, centric, 3–7 µm long (Figure 3.9).

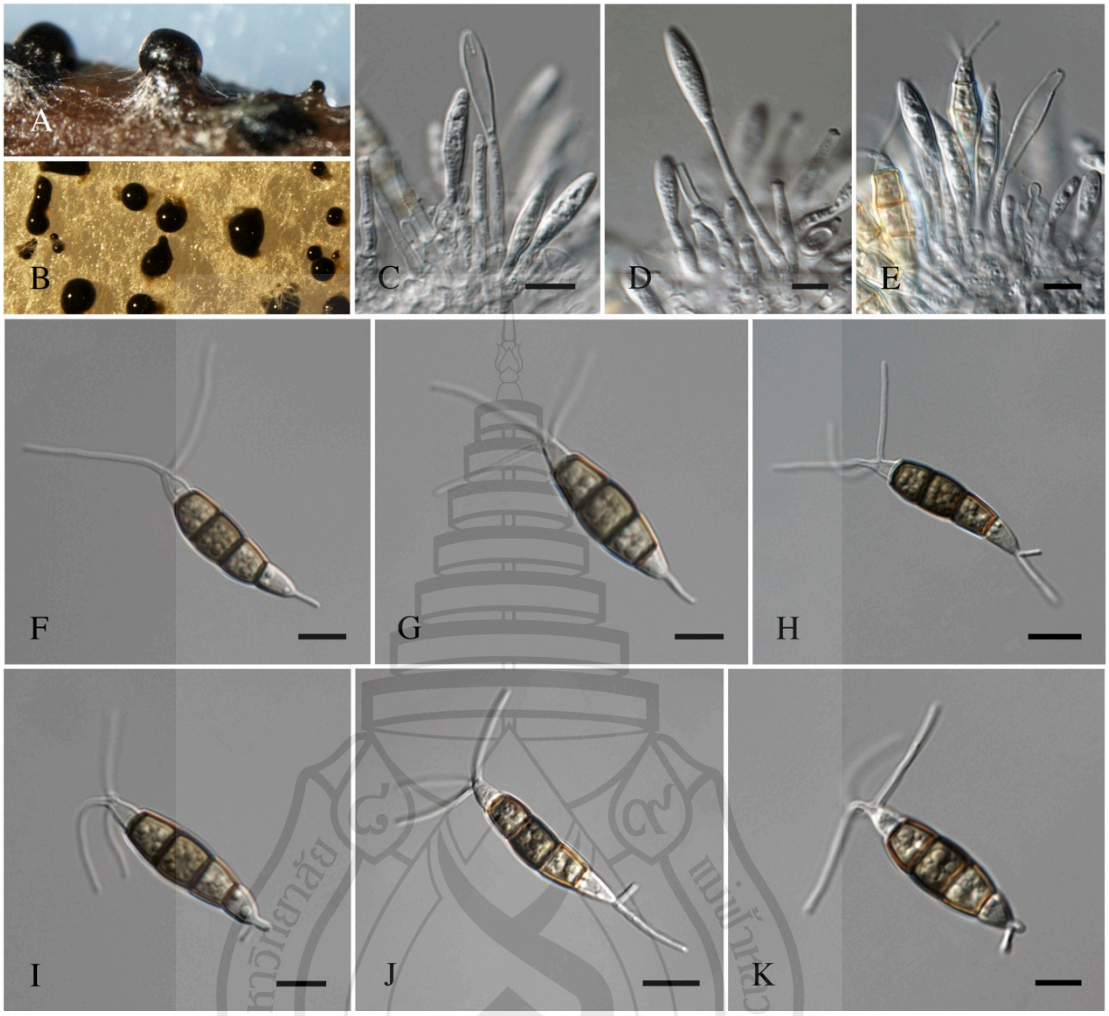
Culture characteristics: Colonies on PDA attaining 35–45 mm diam after 7 days at 25°C, with smooth edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: AUSTRALIA, New South Wales, from *Grevillea* sp. 12 October 1999, P.W. Crous, (CBS H holotype, culture ex-type CBS 114193 = STE-U 3011); South Africa, from *Protea susanne*, ‘’Pink Ice’’, 15 May 1998, L. Swart, culture CBS 114474= STE-U 1769; *ibid*., 15 May 1998, L. Swart, culture CBS 111503=STE-U 1770.

Notes: *Pestalotiopsis australis* is an outlying species which isolated from the plants on family *Proteaceae* in the *P. australis* species complex neighbour to *P. scoparia*, and is distinguished from related species by its large conidia.

Pestalotiopsis biciliata Maharachch. & Crous, sp. nov. (Figure 3.10 A–K).

Etymology: Name referred to having two basal appendages.



Note. *Pestalotiopsis biciliata* CBS 124463^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.10 *Pestalotiopsis biciliata* (holotype)

Conidiomata pycnidial in culture on PDA, globose to clavate, aggregated or scattered, semi-immersed, dark brown to black, up to 300 µm diam; exuding globose, slimy, dark brown conidial droplets. *Conidiophores* sparsely septate and unbranched or irregularly branched at the base, up to 40 µm, or reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical to subcylindrical, hyaline, smooth, tapering

to a long, thin neck, 10–45 × 2–5 µm, proliferating several times percurrently near apex, with flaring collarettes. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (21–)22–28.5(–30) × (5.5–)6–7.5(–8) µm, mean ± SD = 25.3 ± 2 × 6.7 ± 0.3 µm; basal cell obconic to hemispherical with a truncate base, hyaline, thin and wall verruculose, 4–7 µm long; three median cells doliiform, (13.5–)14.5–17.5(–18.5) µm long, mean ± SD = 16 ± 1.1, wall verruculose, concolorous, olivaceous, septa darker than the rest of the cell, (second cell from the base 4–6.5 µm long; third cell 4–7 µm long; fourth cell 4–6.5 µm long); apical cell 3–4.5 µm long, hyaline, subcylindrical, thin and wall rugose; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (6–)8–18(–20) µm long, mean ± SD = 13.3 ± 3.2; two basal appendages; centric appendage tubular, 3–8 µm long and ex-centric appendage tubular, 1–3 µm long (Figure 3.10).

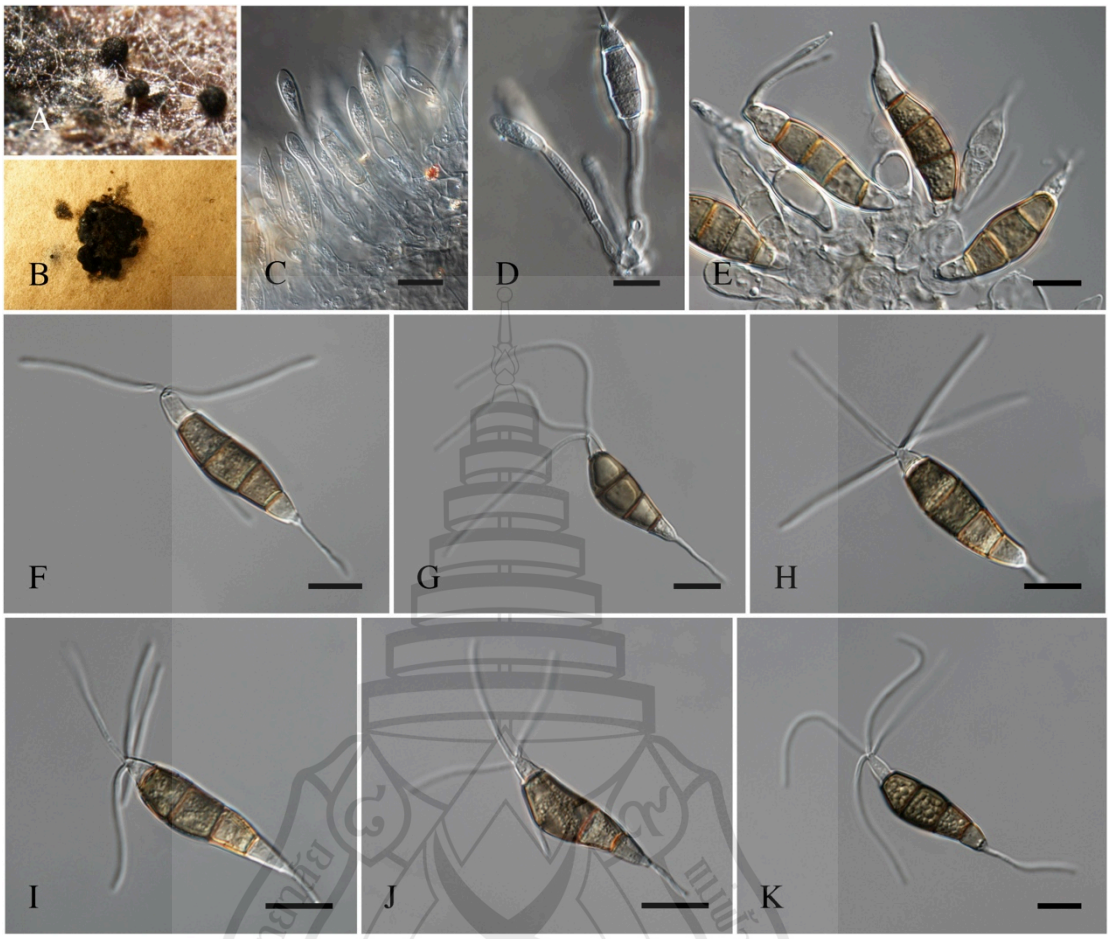
Culture characteristics: Colonies on PDA attaining 40–50 mm diam after 7 days at 25°C, with lobate edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse pale honey-coloured.

Material examined: Slovakia, Giraltovce, from bark of *Platanus x hispanica*, unknown collection date, M. Pastircak, (CBS H holotype, culture ex-type CBS 124463); Italy, from *Paeonia* sp., June 1938, O. Servazzi, culture CBS 236.38; Netherlands, from *Taxus baccata* dry needles attached to the tree, 23 October 1968, H.A. van der Aa, culture CBS 790.68.

Notes: *Pestalotiopsis biciliata* is a species often having two basal appendages and is belongs to the *P. telopea* species complex. *Pestalotiopsis biciliata* overlaps morphologically with *P. trachicarpicola* and *P. kenyana.* However, in phylogenetic analyses it formed a distinct lineage apart from *Pestalotiopsis kenyana* (which has wider conidia) and *P. trachicarpicola.*

Pestalotiopsis brassicae (Guba) Steyert, comb. nov. (Figure 3.11 A–K).

Basionym: *Pestalotia brassicae* Guba, Monograph of *Monochaetia* and *Pestalotia*: 245 (1961) [MB335952].



Note. *Pestalotiopsis brassicae* CBS 170.26. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.11 *Pestalotiopsis brassicae* (isotype)

Conidiomata acervular to pycnidial in culture on PDA, globose, scattered or gregarious and confluent, semi-immersed or erumpent, dark brown to black, up to 500 µm diam; exuding globose, black conidial masses. *Conidiophores* septate near base, branched, subcylindrical, hyaline, up to 10 µm long. *Conidiogenous cells* discrete, cylindrical 20–70 × 2–10 µm or ampulliform to lageniform 4–10 × 3–8 µm, hyaline, smooth-walled, proliferating 2-4 times percurrently, wide at base, collarette present

and not flared, with prominent periclinal thickening. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (29–)30–37(–40) × (8–)8.5–11(–11.5) µm, mean ± SD = 34 ± 2.1 × 9.7 ± 0.7 µm; basal cell obconic with a truncate base, hyaline, thin, minutely verruculose, 5–8.5 × µm long; three median cells doliiform to subcylindrical, (20–)20.5–24.5(–25) µm long, mean ± SD = 22.6 ± 1.5, wall verruculose, concolorous, but occasionally the two upper median cells slightly darker than the lower median cell, brown to olivaceous, septa darker than the rest of the cell, (second cell from the base 5.5–9 µm long; third cell 7–9.5 µm; fourth cell 6–9 µm); apical cell 3.5–7 × µm long, hyaline, cylindrical to subcylindrical, thin and smooth walled; with 3–5 tubular apical appendages (mostly 4), arising from the apical crest, unbranched, filiform, flexuous, (27–)28.5–48(–50) µm long, mean ± SD = 37 ± 5; basal appendage single, tubular, unbranched, centric, 10–25 µm long (Figure 3.11).

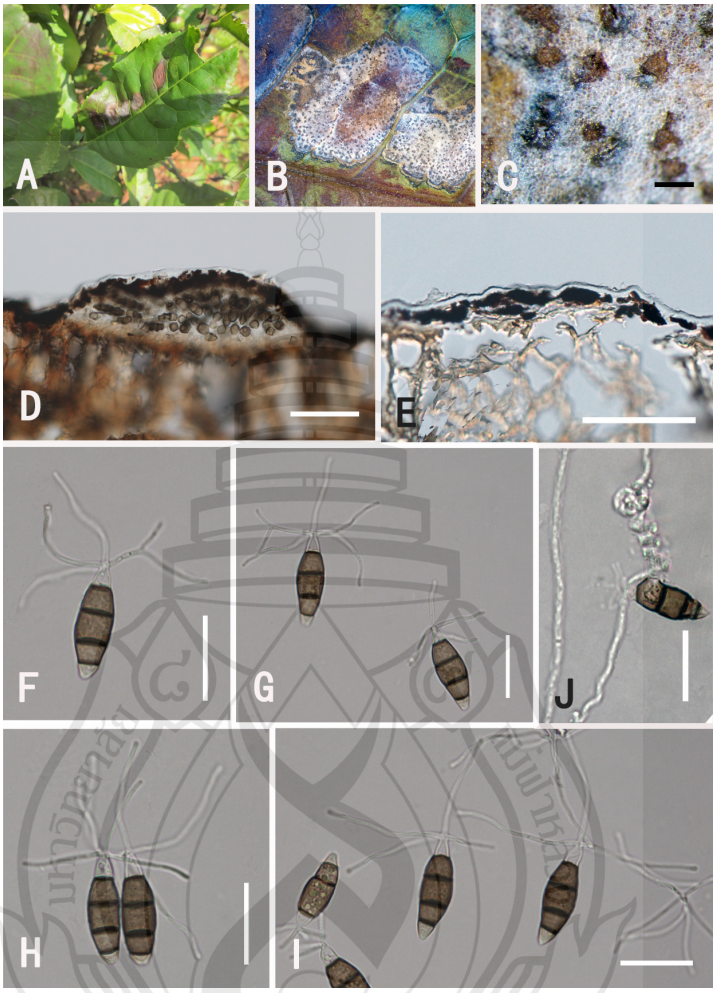
Culture characteristics: Colonies on PDA attaining 25–40 mm diam after 7 days at 25°C, with smooth edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: NEW ZEALAND, from seeds of *Brassica napus,* May 1926, G.H. Cunningham, CBS H-7542, culture ex-isotype CBS 170.26).

Notes: According to the original description of Guba (1961), conidia of *P. brassicae* are somewhat smaller (25–32 × 8.5–9.5 µm) and the apical appendages are shorter in length (20–35 µm) than in present observations. In his monograph Guba placed this species in a group where species having versicolored median cells. However sequence data shows *P. brassicae is* not cluster within the versicolored group (genus *Neopestalotiopsis*). *Pestalotiopsis brassicae*, formed a sister group to *P. hollandica*, which was isolated from *Sciadopitys verticillata* in Netherlands. The latter species is clearly discriminated from *P. brassicae* by having wider conidia, and branched, sub-apically attached apical appendages. Furthermore, *P. brassicae* is distinguished from *P. verruculosa* (28–35×9–11 µm) in the complex by its larger conidia.

Pestalotiopsis camelliae Y.M. Zhang, Maharachch. & K.D. Hyde, Sydowia 64(2):337 (2012)**,** MycoBank: MB 800980 **(**Figure 3.12 A-I).

Etymology: *camelliae*, in reference to the host genus *Camellia.*



Note. *Pestalotiopsis camelliae* (holotype) from host. A. Blight on leaf of *Camellia japonica.* B, C. Conidiomata, splitting irregularly. D. Section of conidiomata. E. Conidiophores/ conidiogenous cells. F–I. Conidia with 3–6 appendages. J. Germination of the conidia. Bars: C 200 µm; D, E 50 µm; F–J 20 µm

Figure 3.12 *Pestalotiopsis camelliae* (holotype)

Associated with grey blight on leaves of *Camellia japonica*, initially producing small, rounded, yellow-green spots on the leaves, spots becoming brown to

grey, with concentric rings and producing black, scattered acervuli (Figure 3.12). Acervuli grey to black, epidermal to subepidermal, separate or confluent, dehiscence irregular, 100–220 μm wide, 76 – 150 μm high, unilocular, glabrous; wall tissue (stroma and parietal cells) only a few cells thick, cell walls thick, outermost layer colourless, inner layers pale brown to brown, encrusted (Figure 3.12). Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, lageniform, smooth, thin-walled, colourless. Conidia 27–33 × 7.5–9.3 μm (mean = 28.5 × 8.5 μm), fusoid, straight to slightly curved, 4–septate, basal cell obconic, colourless, thin- and smooth-walled, 4–7 μm long (mean = 5.4 μm), with three 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 19–22 μm long (mean = 20 μm) (second cell from base 5.6–7.4 μm (mean = 6.1 μm); third cell 5– 8.3 μm (mean = 6.7 μm); fourth cell 6– 8.3 μm (mean = 7.2 μm); apical cell colorless, conic to cylindrical 3–6.4 μm long (mean = 4.7 μm); 3–6 tubular apical appendages, rarely branched, arising from the upper portion of the apical cell, 13.5–34 μm long (mean = 23.8 μm); basal appendages absent.

Culture characteristics: Colonies relatively fast growing 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: On leaf spots of living leaves of *Camellia japonica*, Shuangbai, Chuxiong, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Chuxiong, Shuangbai, on leaf spots of living leaves of *Camellia japonica* L., July 2011, Y.M. Zhang OP111 (IFRD OP111 holotype, ex-type culture MFLUCC = MFLUCC12-0277); *ibid*., August 2011, Y.M. Zhang OP131 (IFRD OP131), MFLUCC = MFLUCC 12-0278.

Pestalotiopsis camelliae is similar to *P. furcata* but is a distinct species in terms of its characteristic morphology and DNA phylogeny. It has relatively small conidia (26.8–33 × 7.5–9.3 μm), although these overlapping in size with those of *P. furcata* (29–39 × 8.5–10.5 μm). *Pestalotiopsis furcata* has more apical appendages (5–9) than *P. camelliae* (3–6). The apical appendages of *P. furcata* consistently divide into branches while this is a rare character in *P. camelliae*. In addition, *P. camelliae* separates from *P. furcata* with high bootstrap suppport (100%) (Figure 3.2) The

conidia of *P. camelliae* (27–33 × 7.5–9.3 µm) lacks basal appendages when comparing those of morphologically similar species such 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. nattrassi* (27–33 × 8–9 µm).

Table 3.2 Synopsis of *Pestalotiopsis camelliae* and related species.

Species	*P. camelliae*	*P. furcata*[a]	*P. nattrassi*[b]	*P. leucopogonis*[c]	*P. hainanensi*[d]
Conidia size (µm)	27–33 × 7.5–9.3	29–39 × 8.5–10.5	27–33 × 8–9	27–32 × 7.5–9.5	19–22 × 5–6
Median cells	Concolorous, olivaceous	Concolorous, olivaceous	Concolorous, brown	Concolorous, brown	Concolorous, brown to olivaceous
Apical appendages:	3–6	5–9	1–4	7–11	1–3
Length (µm)	13.5–33.7	20–35	25–44	12–19	1–10
Branching	Sometimes	Branched	No	No	No
Position	Apex	Apex	Apex	3 rows (top, middle and bottom)	Apex
Basal appendages	Lacking	Lacking	Lacking or present	Lacking or present	Lacking

[a] Maharachchikumbura et al. (2012)

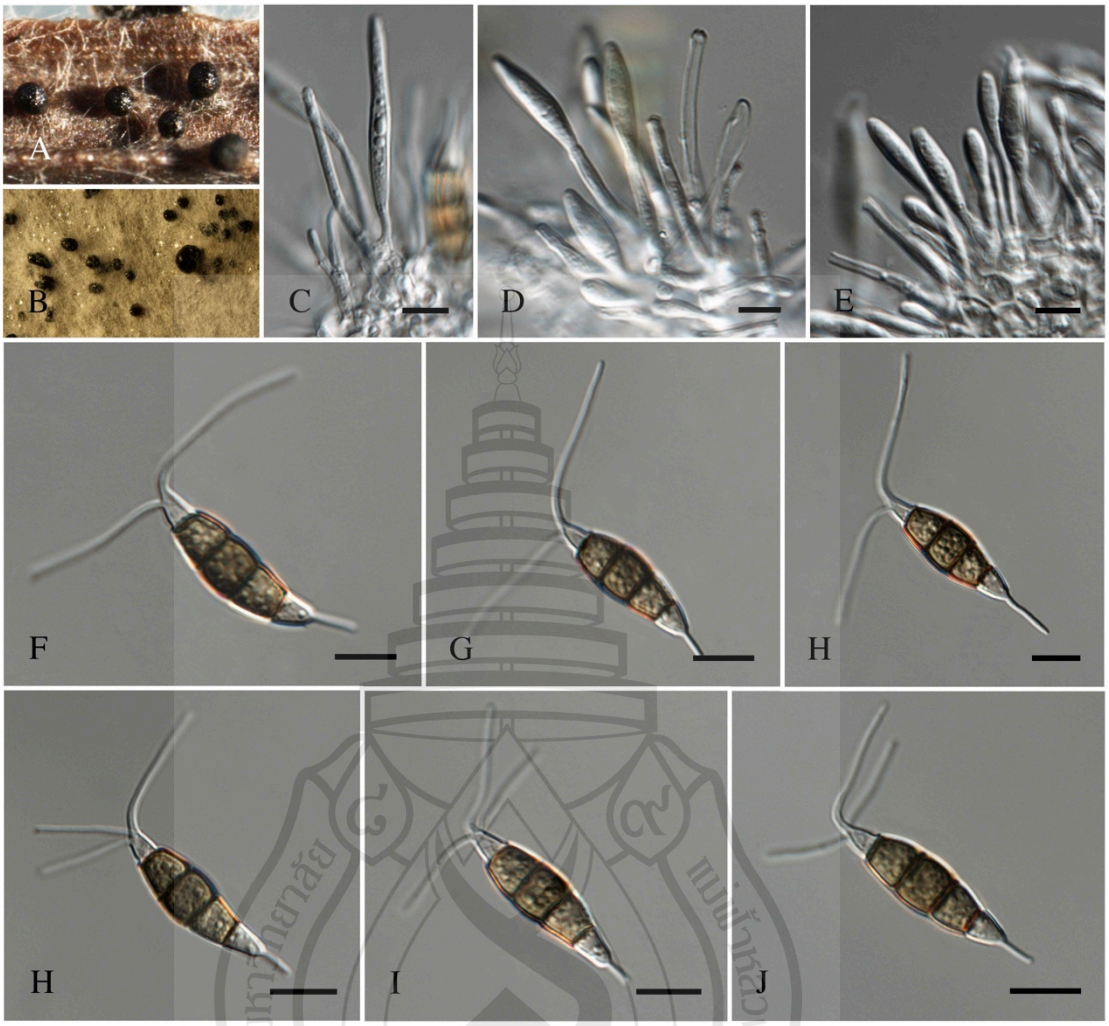
[b] Guba (1961)

[c] Nag Raj (1993)

[d] Liu (2007)

Pestalotiopsis chamaeropis Maharachch. & Crous, sp. nov. (Figure 3.13 A–J).

Etymology: Named after the host genus, *Chamaeropis.*



Note. *Pestalotiopsis chamaeropis* CBS 186.71^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–J. Conidia. Scale bars = 10 µm

Figure 3.13 *Pestalotiopsis chamaeropis* (holotype)

Conidiomata pycnidial in culture on PDA, globose, semi-immersed or partly erumpent, aggregated or scattered, up to 250 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* 1–3 septate, branched, subcylindrical, hyaline, verruculose, up to 25 µm. *Conidiogenous cells* discrete, cylindrical, hyaline, smooth-walled, proliferating 2–4 times percurrently, 20–50 × 2–5 µm, collarette

present and not flared, with prominent periclinal thickening. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (21–)22.5–27(–28) × (6–)7–9(–9.5) µm, mean ± SD = 25.2 ± 1.3 × 8 ± 0.4 µm; basal cell obconic with a truncate base, hyaline, thin and minutely verruculose, 5–6.5 µm long; three median cells doliiform to subcylindrical, (15–)16–17.5(–18.5) µm long, mean ± SD = 16.7 ± 0.8, wall verruculose, concolorous, but occasionally the two upper median cells slightly darker than the lower median cell, brown, septa darker than the rest of the cell, (second cell from the base 4.5–6.5 µm long; third cell 4.5–6.5 µ m long; fourth cell 4.5–6 µm long); apical cell 4–6 × µm long, hyaline, subcylindrical, thin and smooth walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (13–)14.5–23(–24) µm long, mean ± SD = 18 ± 3.1; basal appendage single, tubular, unbranched, centric, 4–8.5 µm long (Figure 3.13).

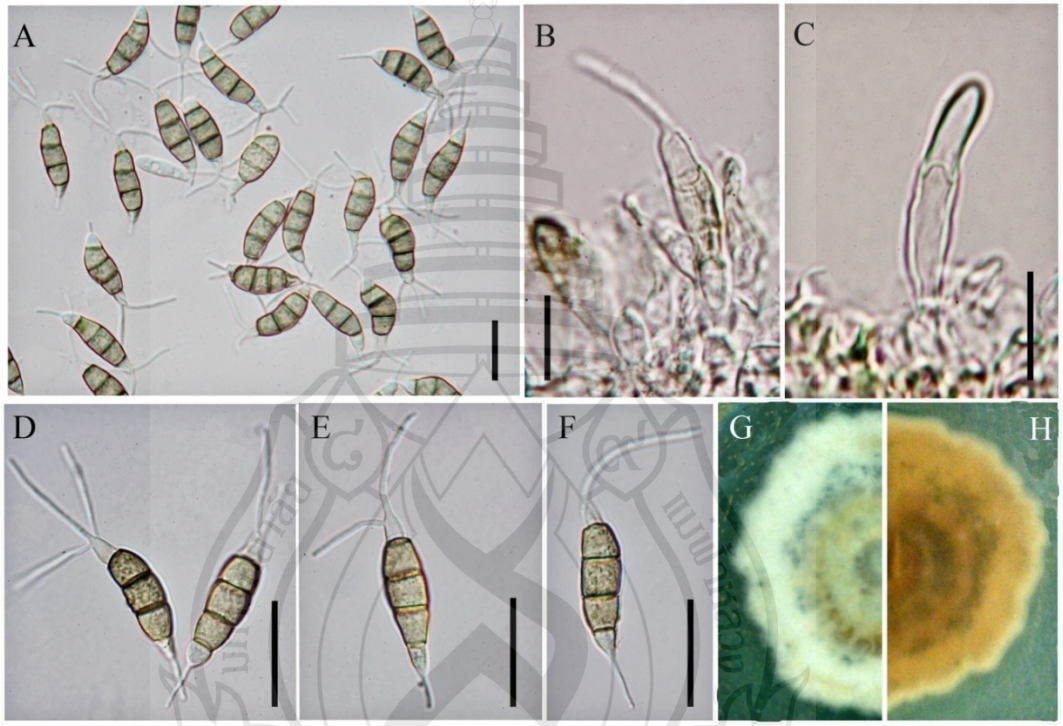
Culture characteristics: Colonies on PDA attaining 35–45 mm diam after 7 days at 25°C, with smooth edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: ITALY, Sardegna, Dorgali, from leaf of *Chamaerops humilis,* Feb. 1971, H.A. van der Aa, (CBS H-15702 holotype, culture ex-type CBS 186.71), Italy, unknown host, unknown collection date (Jun. 1938 deposited in CBS collection), O. Servazzi, culture CBS 237.38.

Notes: *Pestalotiopsis chamaeropis* belongs to the *P. linearis* section and differs from other species in having distinctly wider conidia. *P. chamaeropis* forms a separate cluster in the combined gene phylogeny, as sister to a group including *P. intermedia* and *P. linearis* which were isolated on dead leave of unidentified tree and endophytes from *Trachelospermum* sp. respectively, collected in China. In 1938, O. Servazzi deposited two isolates (CBS 237.38 and CBS 236.38) in CBS as authentic strains of *Pestalotia paeoniae* Servazzi. Even though, these two isolates having overlapping conidial size, the deposited two isolates clusters in genetically clearly distinct two sections (*P. linearis* and *P. telopea* sections) with species having concolorous median cells. According to the description of Guba (1961), *P. paeoniae* belongs to the species with versicoloured median cells (present *Neopestalotiopsis*). So the reliability of two authentic strains are doubtful, and in present paper CBS 237.78 placed in current species (*P. chamaeropis*) and CBS 236.38 in *P. biciliata*.

Pestalotiopsis chinensis Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 107 (2012) MycoBank: MB 800522 (Figure 3.14 A–H.).

Etymology: The specific epithet is referring to China, the country from where the taxon was isolated.



Note. A. Conidia. B–C. Conidiophores/ conidiogenous cells. D–F. Conidia. G. H. Colony on PDA, G from above, I from below. Scale Bars: A– F= 20 µm

Figure 3.14 *Pestalotiopsis chinensis* (holotype)

Conidiophores most often indistinct, septate, hyaline, smooth, rarely branched. *Conidiogenous cells* discrete, ampulliform to lageniform, smooth, thin-walled, hyaline or pale brown, with 2–3 proliferations. *Conidia* fusoid to ellipsoid, straight to slightly

curved, 4–septate, 23–32 × 7–9 µm (mean = 29 × 8.3 µm), basal cell conic to obconic, hyaline or slightly olivaceous, thin-walled and verruculose, 5–7 µm long (mean = 5.7 µm), with three median cells, doliiform to cylindrical, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 20–22 µm long (mean = 20.2 µm) (second cell from base 6–7 µm (mean = 6.5 µm); third cell 7–7.5 µm (mean = 7.1 µm); fourth cell 6–7.5 µm (mean = 6.8 µm); apical cell hyaline, conic to subcylindrical, 3-6 µm long (mean = 4.3 µm); with 1–3 tubular apical appendages (mostly 3), arising from the apex of the apical cell, 25–30 µm long (mean = 28 µm), unequal; basal appendage present 7–11 µm (mean = 8.7 µm) (Figure 3.14).

Cultural characteristics: Colonies on PDA reaching 7 cm diam. after 13 days at 25^{o}C, with edge crenate, whitish to pale yellow, with dense aerial mycelium on surface, fruiting bodies black, developing in concentric circles; reverse of culture yellow to pale orange.

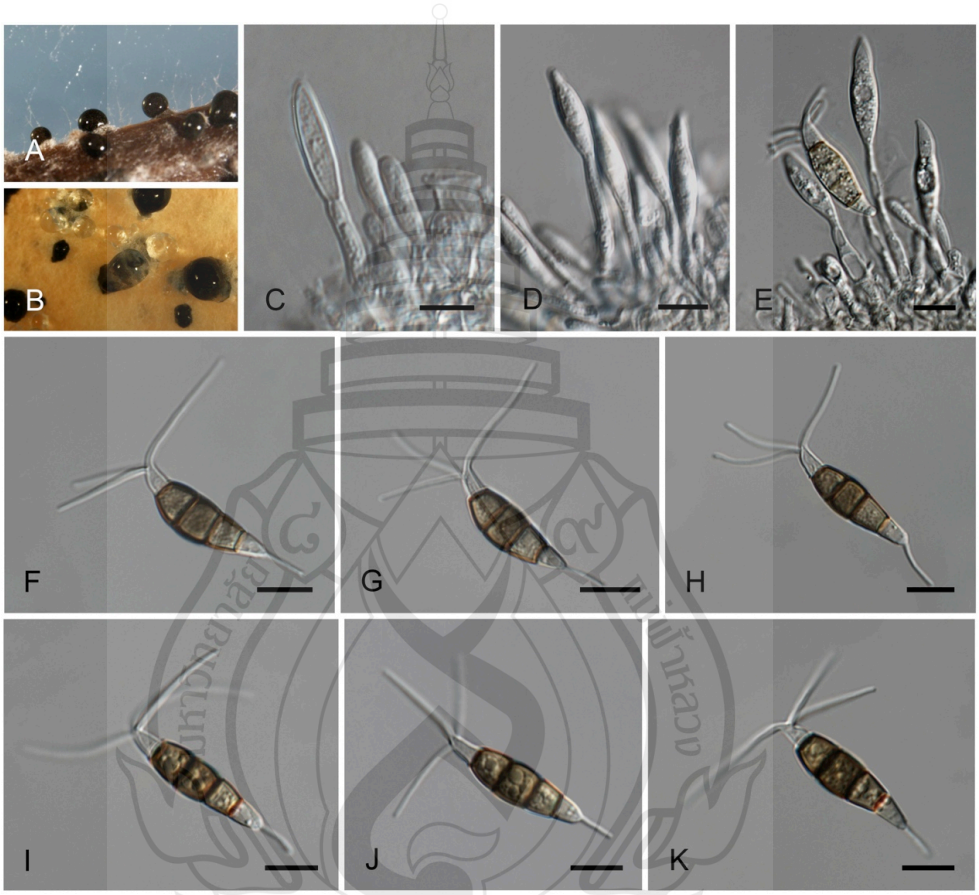
Habitat/Distribution: Endophyte in leaves of *Taxus* sp., Kunming, Yunnan Province China.

Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Taxus* sp., 19 March 2002, Wenping Wu KBG13-9 (HMAS047218, holotype; MFLU12-0415, isotype; ex-type living culture NN047218 = MFLUCC 12-0273).

Notes: The conidial size of *Pestalotiopsis chinensis* overlaps with *P. funerea* (Desm.) Steyaert (21–29 × 7–9.5 µm) (Steyaert, 1949), *P. macrochaeta* (Speg.) J. Xiang Zhang & T. Xu (22–31 × 8–10 µm) (Zhang et al., 2002), *P. mayumbensis* (Steyaert) Steyaert (22–28 × 6.5–8.5 µm) (Steyaert 1949) and *P. osyridis* (Thüm.) H.T. Sun & R.B. Cao (22–28 × 5–7 µm) (Guba, 1961). However, *P. chinensis* can be distinguished from *P. mayumbensis* and *P. osyridis* by its relatively large conidial size and also by its long apical appendages (in *P. mayumbensis* 8–15 µm and in *P. osyridis* up to 14 µm). *Pestalotiopsis chinensis* (1–3 tubular apical appendages) can be differentiated by the number of apical appendages (3–6 apical appendages (mostly 4–5) in *P. funerea* and three apical appendages in *P. macrochaeta*).

Pestalotiopsis colombiensis Maharachch. & Crous, sp. nov. (Figure 3.15 A–K).

Etymology: Named after the country where it was collected, Colombia.



Note. *Pestalotiopsis colombiensis* CBS 118553 [T]. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.15 *Pestalotiopsis colombiensis* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary or aggregated, semi-immersed, dark brown, 200–400 µm diam; exuding globose, dark brown,

glistening conidial masses. *Conidiophores* reduced to conidiogenous cells; when present, septate, unbranched, or irregularly branched, hyaline, thin-walled 5–12 × 2–6 µm. *Conidiogenous cells* discrete, cylindrical, proliferating 2–5 times percurrently, tapering to a long, thin neck, 10–50 × 2–8 µm, prominent periclinal thickening, collarette present and not flared. *Conidia* ellipsoid, straight to slightly curved, 4-septate, slightly constricted at septa, (19–)21–27(–28.5) × 5.5–7.5(–8) µm, mean ± SD = 24 ± 1.5 × 6.3 ± 0.5 µm; basal cell conic to acute with truncate base, thin-walled and minutely verruculose, 5–7.5 µm long; three median cells (13–)13.5–16.5(–17) µm, mean ± SD = 15.2 ± 0.8 µm long, doliiform, thick-walled, verruculose, concolorous, brown, (second cell from base 5–6.5 µm long; third cell 4.5–6 µm long; fourth cell 5–6.5 µm long); apical cell cylindrical to subcylindrical, hyaline, thin and smooth-walled, 3.5–5 µm long; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (11–)13–25(–28) µm, mean ± SD = 17.5 ± 3 µm; basal appendage single, tubular, unbranched, centric, 2–5 µm long (Figure 3.15).

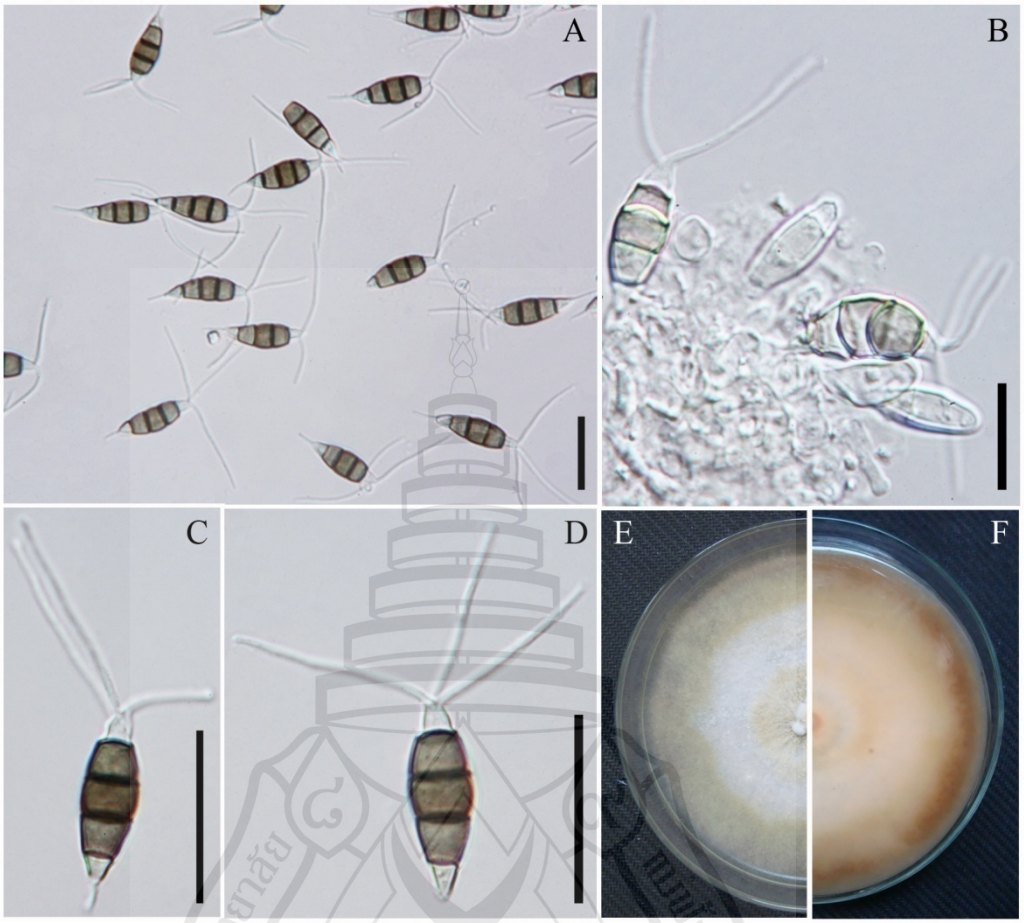
Culture characteristics: Colonies on PDA reaching 70–80 mm diam after 7 days at 25°C, entire at the edge, whitish to pale grey-coloured, with dense aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: COLOMBIA, from living leaves of *Eucalyptus eurograndis*, 2004, M.J. Wingfield (CBS H holotype, culture ex-type 118553=CPC 10969).

Notes: *Pestalotiopsis colombiensis* is an outlying single species in species complex *P. colombiensis*. It differs from *P. diploclisia* and *P. humus* in *P. diploclisia* species complex by its longer apical appendages. Furthermore *P. colombiensis* is geographically clearly distinct from *P. diploclisia* and *P. humus*, which were isolated from Hong Kong and Papua New Guinea, respectively.

Pestalotiopsis clavata Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 108 (2012), MycoBank: MB 800524 (Figure 3.16 A–F).

Etymology: In Latin, *clavatus* refers to the clavate conidia.



Note. A. Conidia. B. Conidiophores/ conidiogenous cells. C. D. Conidia. E–F. Colony on PDA, E. from above, F. from below. Scale Bars: A– G= 20 µm

Figure 3.16 *Pestalotiopsis clavata* (holotype)

Conidiophores most often indistinct. *Conidiogenous cells* discrete ampulliform to lageniform, smooth, thin-walled, hyaline, short. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4-septate, 20–27 × 6.5–8 µm (mean = 22.6 × 7.3 µm), basal cell conic to obconic with obtuse end, hyaline, thin-walled and verruculose, 4–5 µm long (mean = 4.6 µm), with three median cells, doliiform, concolorous, olivaceous to brown, septa and periclinal walls darker than the rest of the cell; wall rugose, together 15–16 µm long (mean = 15.2 µm) (second cell from

base 5–6 µm (mean = 5.2 µm); third cell 4–5 µm (mean = 4.8 µm); fourth cell 5–5.5 µm (mean = 5.2 µm); apical cell hyaline, conic to cylindrical 3-5 µm long (mean = 3.75 µm), with 2– 3 tubular apical appendages (mostly 3) arising from the apex of the apical cell, 20–25 µm long (mean = 23 µm); basal appendage mostly present, 7–9 µm long (mean = 7.8 µm).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 8 days at 25^{o}C, with edge entire, whitish to pale brown, with dense, aerial mycelium on the surface, with black fruiting bodies; reverse of culture pale brown to brown.

Habitat/Distribution: Endophyte in living leaves of *Buxus* sp. and *Euonymus* sp., Hunan and Yunnan provinces, China.

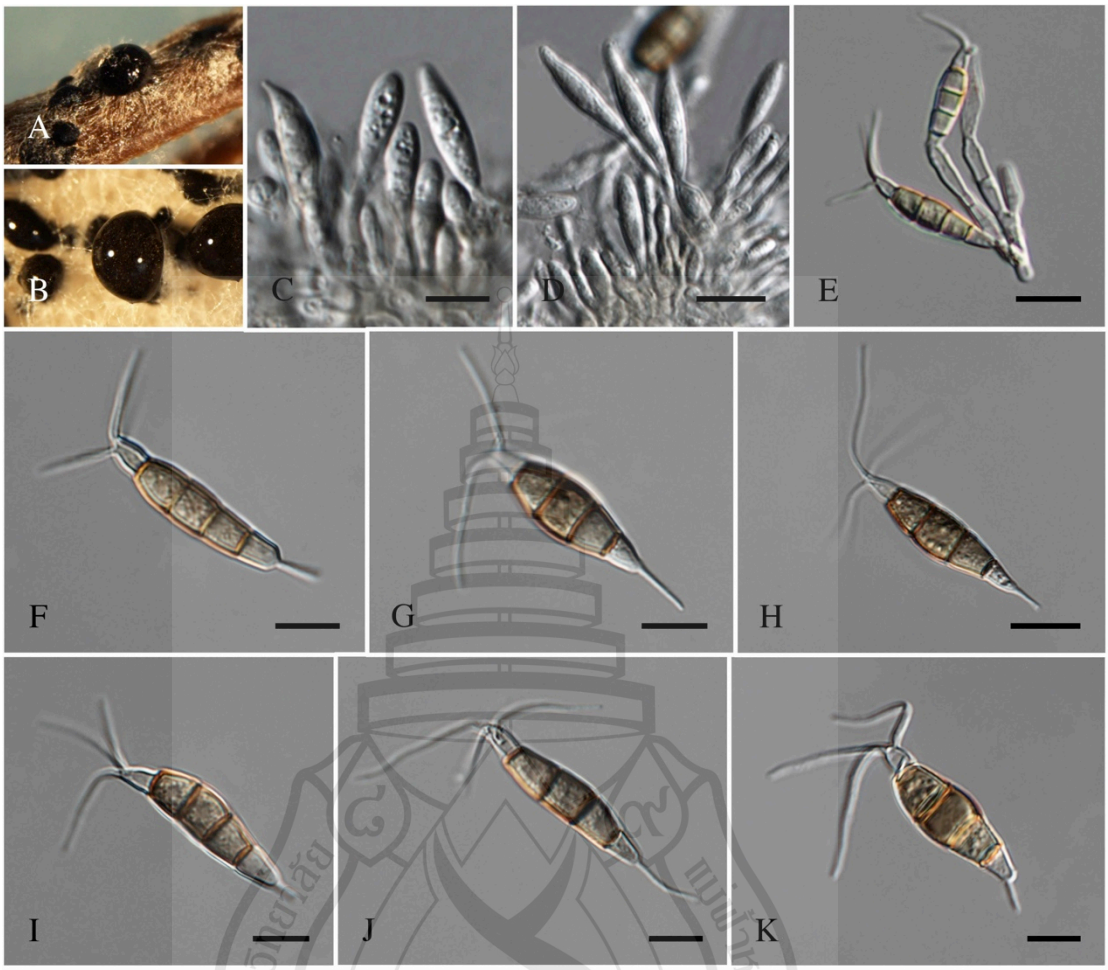
Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, living leaf of *Buxus* sp., 19 March 2002, Wenping Wu KBG26-5 (HMAS047134, holotype; MFLU12-0412, isotype; ex-type living culture NN047134 = MFLUCC 12-0268).

Additional culture examined: CHINA, Hunan Province, Yizhang County, Mangshan, living leaf of *Euonymus* sp., 12 April 2002, Wenping Wu HN49-6 (NN047005 = MFLUCC 12-0269).

Notes: *Pestalotiopsis clavata* is a distinct species recognized based on its morphology and phylogeny. It has similar sized conidia to *P. heterocornis* (Guba) Y.X. Chen (18–26 × 6.5–8 µm) (Guba, 1961). However, these species are distinct in the length and number of their apical appendages. *P. clavata* has conidia with 2–3 apical appendages (mostly 3) which are 20–25 µm long, while in *P. heterocornis* the apical appendages are unequal in length being 9–21 µm long (Guba, 1961). *Pestalotiopsis carveri* (Guba) P.L. Zhu, Q.X. Ge & T. Xu (20–26 × 6–7 µm) (Guba, 1961) also has a somewhat similar conidial morphology with *P. clavata,* but they differ in the length and number of their apical appendages. In *P. carveri* the two apical appendages are unequal in length being 12–26 µm long.

Pestalotiopsis diploclisia Maharachch. & Crous, sp. nov. (Figure 3.17 A–K).

Etymology: Named after the host genus from which it was isolated, *Diploclisia.*



Note. *Pestalotiopsis diploclisia* CBS 115587^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.17 *Pestalotiopsis diploclisia* (holotype)

Conidiomata pycnidial in culture on PDA, globose, solitary or aggregated, semi-immersed, black, up to 500 µm diam; exuding globose, slimy, dark brown, conidial droplets. *Conidiophores* often reduced to conidiogenous cells, sparsely septate at the base and unbranched or branched, up to 20 µm long. *Conidiogenous cells* discrete, cylindrical to subcylindrical, hyaline, smooth, simple, proliferating 2–3 times percurrently, 6–20 × 2–5 µm. *Conidia* fusiform, ellipsoid, straight to slightly

curved, 4-euseptate, (20–)22–26.5(–28) × 5–6.5(–7) µm, mean ± SD = 24 ± 1.3 × 5.7 ± 0.4 µm; basal cell obconic to subcylindrical with a truncate base, hyaline, thin and wall rugose, 4–6.5 µm long; three median cells doliiform, (13.5–)14–16(–17) µm long, mean ± SD = 15.4 ± 0.9, wall minutely verruculose, concolorous, pale brown, septa darker than the rest of the cell, (second cell from the base 4.5–6 µm; third cell 4.5–7 µm; fourth cell 4.5–6.5 µm); apical cell 3.5–6 µm long, hyaline, subcylindrical, thin and smooth-walled; with 2–4 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (10–)13–19(–22) µm long, mean ± SD = 16.6 ± 2.1; basal appendage single, tubular, unbranched, centric, 3–8 µm long (Figure 3.17).

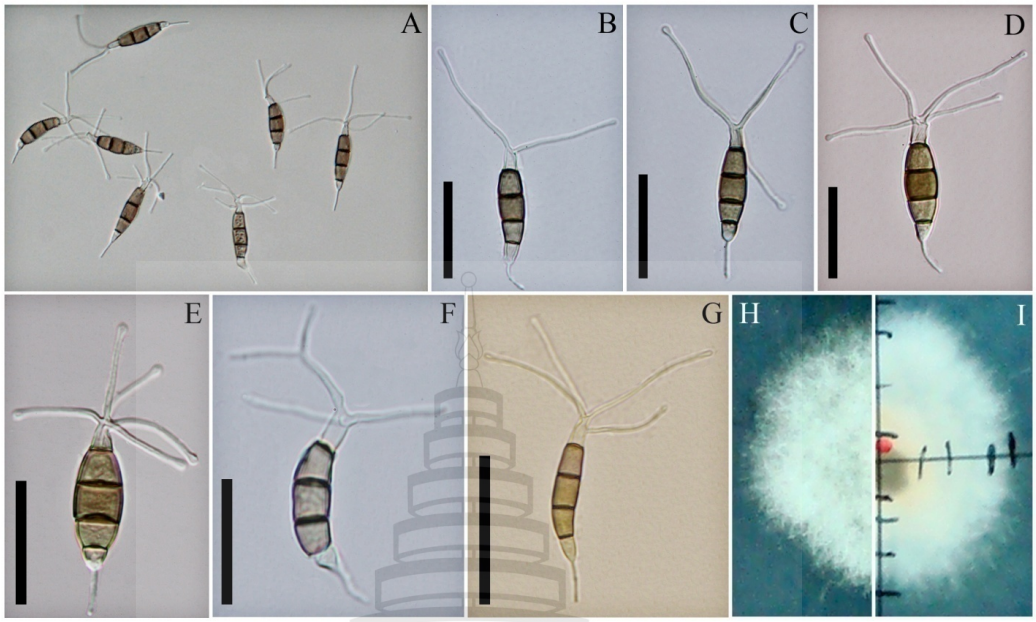
Culture characteristics: Colonies on PDA attaining 35–45 mm diam after 7 days at 25°C, with smooth edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: HONG KONG, Lamma Island, from fruit of *Diploclisia glaucescens*, 5 July 2001, K.D. Hyde, (CBS H holotype, culture ex-type CBS 115587= HKUCC 10130); Hong Kong, Lamma Island, from fruit of *Diploclisia glaucescens*, 5 July 2001, K.D. Hyde, culture CBS 115585=HKUCC 8394; Hong Kong, Mount Nicholson, from fruit of *Psychotria tutcheri*, 15 February 2002, K.D. Hyde, culture CBS 115449 = HKUCC 9103.

Notes: *Pestalotiopsis diploclisia* pertains to the *P. diploclisia* section and is morphologically very close to the *P. colombiensis*, but genetically clearly distinct, forming a well separate clade. *P. diploclisia* is genetically close to the *P. humus*, which was isolated from soil in Papua New Guinea, but well distinguished by having narrow conidia and longer apical appendages.

Pestalotiopsis diversiseta Maharachch & K.D. Hyde, Fungal Diversity 56(1): 111 (2012), MycoBank: MB 800526 (Figure 3.18 A–I).

Etymology: The specific epithet is based on the diverse arrangement, Latin= *diversisetae* of the apical appendages.



Note. A–G. Conidia. H–I. Colony on PDA, H. from above, I. from below. Scale Bars: A– G= 20 µm

Figure 3.18 *Pestalotiopis diversiseta* (holotype)

Conidia fusoid to ellipsoid, straight to slightly curved, 4–septate, 27–34 × 5.5–8 µm (mean = 29.7 × 6.3 µm), with basal cell obconic and obtuse at the base, hyaline, thin-walled and verruculose, 3–6 µm long (mean = 4.5 µm), with three median cells, doliiform, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 17–21 µm long (mean = 19 µm) (second cell from base 5–7 µm (mean = 5.8 µm); third cell 6–8 µm (mean = 6.8 µm); fourth cell 6–7 µm (mean = 6.3 µm); apical cell hyaline, cylindrical 4–7 µm long (mean = 6 µm); with 3–5 tubular appendages (rarely 2); some appendages branched, slightly swollen at the tip, arising from the apex of the apical cell and sometimes arising from the different parts of the apical cell, 22–30 µm long (mean = 26 µm); with basal appendage 5–9 µm long, rarely absent (Figure 3.18).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 8 days at 25°C, edge fimbriate, whitish, with dense, aerial mycelium on surface, with black fruiting bodies, gregarious; reverse of the culture white.

Habitat/Distribution: Endophyte on living leaf of *Rhododendron* sp., Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, living leaves of *Rhododendron* sp., 19 March 2002, Wenping Wu HN26-5 (HMAS047261, holotype; MFLU12-0423, isotype; ex-type living culture NN047261 = MFLUCC 12-0287).

Table 3.3 Synopsis of *Pestalotiopsis diversiseta* and related species.

Species	***P. diversiseta***	***P. theae*** [a]	***P. leucopogonis*** [b]	***P. perseae*** [b]
Conidia size (μm)	27–34 × 5.5–8	22–32 × 5–8	27–32×7.5–9.5	24–36×7–8
Median cells	Concolorous, olivaceous	Concolorous, dark brown to olivaceous	Concolorous, brown	Versicolorous
Apical appendages:	3–5 (sometimes branched)	2–4 (not branched)	7–11(not branched)	2–4(not branched)
Apical appendage length (μm)	22–30, unequal	25–50	12–19	10–23
Appendage tip	Knobbed	Knobbed	Not knobbed	Knobbed
Position of appendage on apical cell	Top to middle	Apex only	3 rows (top, middle and bottom)	Top row and subapical row

[a] Guba (1961)

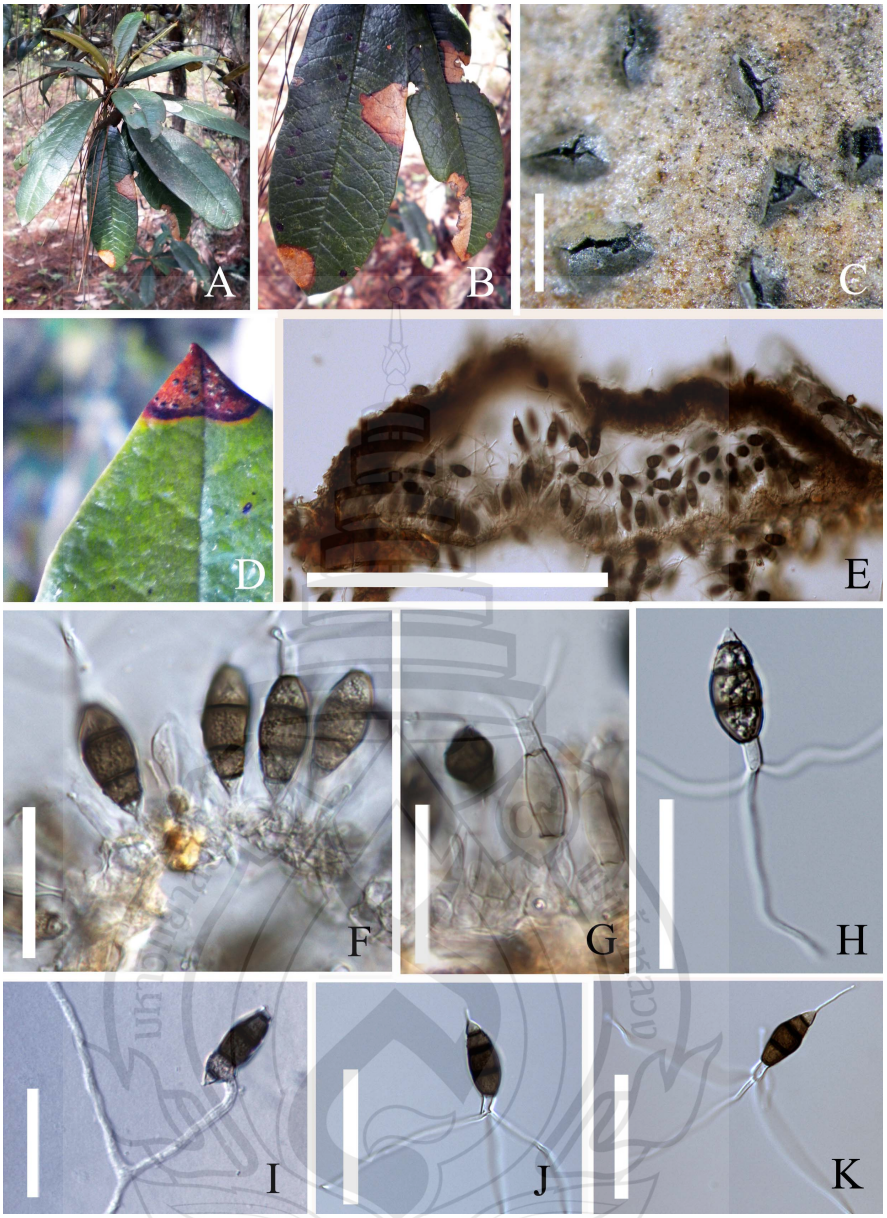
[b] Nag Raj (1993)

Notes: *Pestalotiopsis diversiseta* is a morphologically distinct species, also shown in its DNA phylogeny. However, it has an overlapping conidial size with *P. leucopogonis, P. perseae* and *P. theae* (Guba, 1961; Nag Raj, 1993). *Pestalotiopsis*

diversiseta can be differentiated from all these species by its morphological distinction. *P. diversiseta* has 3–5 apical appendages which differ from *P. leucopogonis* (7–11 apical appendages), *P. perseae* (2–4 apical appendages) and *P. theae* (7–11 apical appendages) (Guba, 1961; Nag Raj, 1993). Its apical appendages are also knobbed unlike those in *P. leucopogonis* (Nag Raj, 1993). Although *P. diversiseta* and *P. perseae* have knobbed apical appendages with similar attachment to the apical cell, and length (22-30 vs 10-23 µm), the species can be distinguished by its concolorous median cells which in *P. perseae* are versicoloured with irregular longitudinal ridges (Guba, 1961; Nag Raj, 1993).

Pestalotiopsis ericacearum Y. M. Zhang, Maharachch. & K.D. Hyde, sp. nov. MycoBank: MB 803235. (Figure 3.19 A–K).

Etymology. The specific epithet is based on the host family *Ericaceae*, from which the fungus was isolated.



Note. A–D. *Pestalotiopsis ericacearum* associated with leaf blotch on leaves of *Rhododendron delavayi*. C. Acervuli, splitting irregularly. E. Section of acervulus. F–G. Conidiogenous cells. H–K. Conidia with knobbed apical appendages. I. Germination of spore. Scale Bars: C = 1000 µm, E = 100 µm, F–K = 20 µm

Figure 3.19 *Pestalotiopsis ericacearum* (holotype)

Associated with leaf blotch on living leaves of *Rhododendron arboretum* subsp. *delavayi*; initially black and rounded and later expanding to form a brown blotch. Sexual state not observed. Asexualstate: *Acervuli* (200)250–500(600) μm in diam, black, epidermal to subepidermal in origin, separate or confluent, dehiscence irregular. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* hyaline, branched or unbranched at the base or above, cylindrical, lageniform or claviform. *Conidia* (15)16–20(21) × 5–9 μm (mean = 18 × 6.5 μm), fusiform, straight to slightly curved, 4-septate; basal cell conical, hyaline, thin and smooth walled, 2–3 μm long (mean = 2.6 μm); with three median cells, doliiform to cylindrical, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, together (9)10–14(15) μm long (mean = 12 μm); second cell from base 4–6 μm (mean = 5 μm); third cell 4.5–6.5 μm (mean = 5.8 μm); fourth cell 4–6 μm (mean = 5 μm); apical cell hyaline, cylindrical, 4–6 μm long (mean = 5 μm); with 3–4 tubular apical appendages (mostly 3), arising from the apex of the apical cell, knobbed at the end, (19)20–43(45) μm long (mean = 32 μm), unequal in length; basal appendage present 2–9 μm (mean = 4 μm).

Material examined: CHINA, Yunnan Province, Chuxiong, Zixishan, on leaf spots on living leaves of *Rhododendron delavayi*, February 2011, leg. Y. Zhang OP023, holotype IFRD 410-008.

Table 3.4 Synopsis of *Pestalotiopsis ericacearum* and related species.

Species	***P. ericacearum***	***P. pallidotheae*** [a]	***P. theae*** [b]	***P. kunmingensis*** [c]	***P. tecomicola*** [d]
Conidia size (μm)	16–20 ×5 –9	21.5–30.7× 5.4–7.7	22–32 × 5–8	33.8–46.8 × 7.5–10	23–31.5 × 7.5–8.5
Median cells	Concolorous, dark brown	Concolorous, pale (light) brown	Concolorous, dark brown	Concolorous, brown	Concolorous, pale brown to brown
Apical appendages:	3	2–4	2–4	2–4	3

Table 3.4 (Continnued)

Species	*P. ericacearum*	*P. pallidotheae* [a]	*P. theae* [b]	*P. kunmingensis* [c]	*P. tecomicola* [d]
Length (μm)	17–40	12.3–39.2	25–50	14.3–52.7	11–16
Tip	Knobbed	Knobbed	Knobbed	Knobbed	Knobbed
Basal appendages	Present	10–20	Present	Present, branched	Present

[a]Watanabe et al. (2010)

[b]Guba (1961)

[c]Wei and Xu (2004)

[d]Nag Raj (1993)

Notes: *Pestalotiopsis ericacearum* is a morphologically characteristic species and its distinctness is supported by molecular phylogeny (Figure 3.2, Table 3.4). It has relatively short conidia (16–20 μm) compared to other species that have knobbed apical appendages; *P. kunmingensis* (33.8–46.8 μm), *P. pallidotheae* (21.5–30.7 μm), *P. theae* (22–32 μm) and *P. tecomicola* (23–31.5 μm). In the phylogram it is clearly distinguished from *P. anacardiacearum* and *P. karstenii* with high bootstrap support. No cultures were obtained for this species and thus DNA was directly extracted from the acervuli.

Pestalotiopsis furcata Maharachch. & K.D. Hyde, sp. nov. MycoBank. MB564563. (Figure 3.20 A–I).

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

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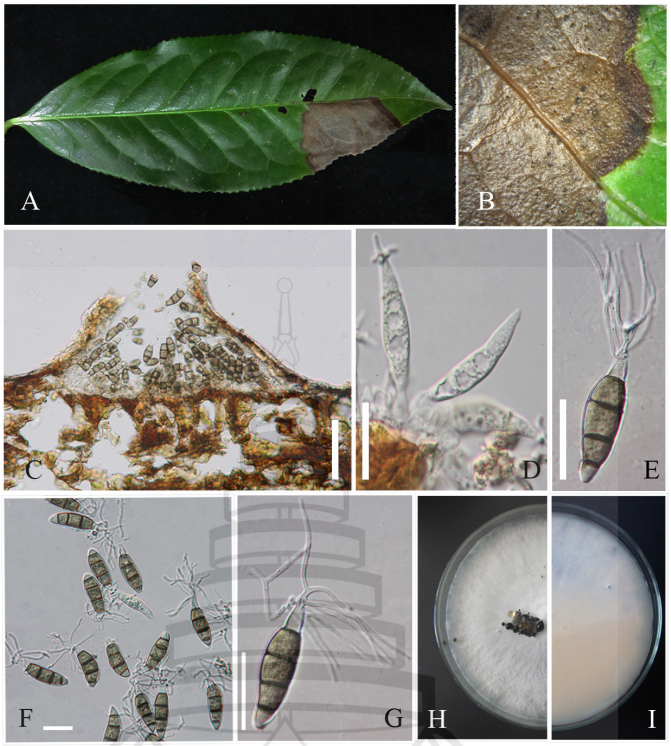


Note. A. Blight on leaf of *Camellia sinensis*. B. Conidiomata, split irregularly. C. Section of conidiomata. D. Conidiophores/conidiogenous cells. E–G. Conidia with branched appendages. H–I. Colony on PDA, H. from above, I. from below. Scale Bars: C = 50 µm, D– G= 20 µm

Figure 3.20 *Pestalotiopsis furcata* (holotype)

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 (Figure 3.20). *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 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.

Culture characteristics: 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.

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*, 20 January 2010, S.S.N. Maharachchikumbura S200110 (Holotype, MFLU 12-0112; ex-holotype culture MFLUCC 12-0054 = CPC 20280; GenBank, JQ683724, JQ683708, JQ683740).

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).

Notes: *Pestalotiopsis furcata* is a characteristic, distinct species in terms of morphology, and its DNA phylogeny (Figure 3.2). It has relatively large conidia (29–39 × 8.5–10.5 µm) compared with other species in the genus. Conidia of *P. furcata* are also wider than the morphologically similar species such 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). The most characteristic feature of *P. furcata* 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 are 12–19 µm long and thus shorter than those of *P. furcata* (20–35 µm). *P. macrospora* has branched apical appendages, 15–22 µm long, which are shorter than those of *P. furcata*. *Pestalotiopsis furcata* also lacks basal appendages, which are otherwise present in most species of *Pestalotiopsis*.

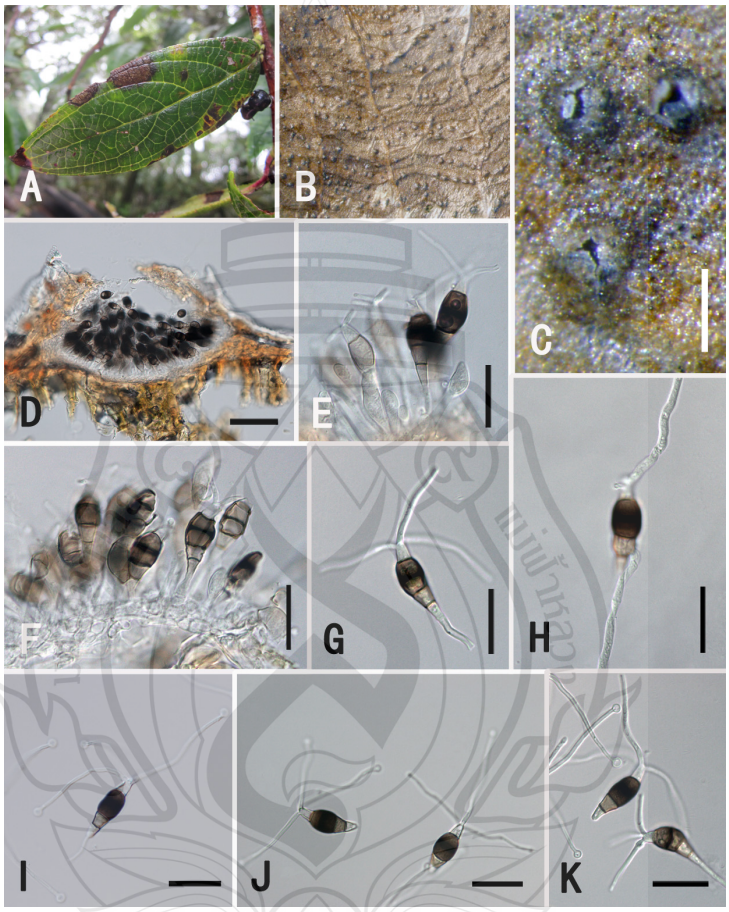
Table 3.5 Synopsis of *Pestalotiopsis furcata* and related species.

	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	Concolorou, 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
Branch	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] Guba (1961); [b] Nag Raj (1993); [c] Liu (2007a)

Pestalotiopsis gaultheria Y. M. Zhang, Maharachch. & K. D. Hyde, sp. nov. MycoBank: MB 803236 (Figure 3.21 A–K).

Etymology: The specific epithet is based on the host genus *Gaultheria*, which is the fungus was isolated.



Note. A. *Pestalotiopsis gaultheria* associated with leaf blight on leaves of *Gaultheria forrestii*. B–C.Acervuli. D. Section of acervular. E–F. Conidiogenous cells. H. Germination of spore. G–K. Conidia with knobbed apical appendages. Scale Bars: C = 200 µm, D = 50 µm, E–K = 20 µm

Figure 3.21 *Pestalotiopsis gaultheria* (holotype)

Associated with brown leaf spots on living leaves of *Gaultheria forrestii*. *Acervuli* 100–310 μm in diam., grey to black, epidermal to subepidermal, separate or confluent, dehiscence irregularly. *Conidiophores* indistinct. *Conidiogenous cells* hyaline, branched or separate at the base and the above. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4–septate, 23–31×7–9.5 μm (mean = 26.4 × 8.6 μm), with basal cell obconic, hyaline, thin-walled and verruculose, 3–5 μm long (mean = 5 μm), with three median cells, doliiform, concolorous and versicolours when mature, septa and periclinal walls darker than the rest of the cell, wall rugose, together 15–20 μm long (mean = 17 μm) second cell from base 4–6 μm (mean = 4.8 μm); third cell 5–7 μm (mean = 5.8 μm); fourth cell 4–6 μm (mean = 5 μm); apical cell hyaline, cylindrical 4–5 μm long (mean = 4.5 μm); with 3 tubular appendages, swollen at the tip, arising from the apex of the apical cell 15–50 μm long (mean = 35 μm); with basal appendage 2.5–4 μm long, rarely absent (Figure 3.21).

Habitat/Distribution: Associated with leaf spots on leaf spots on living leaves of *Gaultheria forrestii,* Yunnan provinces, China.

Material examined: CHINA, Yunnan Province, Dehong, Mangshi, on leaf spots on living leaves of *Gaultheria forrestii*, September 2011, leg. Y. M. Zhang OP 137, holotype IFRD 411-014.

Table 3.6 Synopsis of *Pestlotiopsis gaultheria* and related species.

Species	***P. gaultheria***	***P. diversiseta*** [a]	***P. jesteri*** [b]	***P. pallidotheae*** [c]	***P. theae*** [a]
Conidia size (μm)	23–31 × 7–9.5	27–34 × 5.5–8	19– 23×5– 7	21.5–30.7× 5.4–7.7	22–32 × 5–8
Median cells	Versicolorous, dark brown	Concolorous, olivaceous	Concolorous, pale brown	Concolorous, pale (light) brown	Concolorous, dark brown
Apical appendages:	3	3–5 (sometimes branched)	3–4	2– 4	2– 4

Table 3.6 (Continued)

Species	***P. gaultheria***	***P. diversiseta*** [a]	***P. jesteri*** [b]	***P. pallidotheae*** [c]	***P. theae*** [a]
Tip	Knobbed	Knobbed	Knobbed	Knobbed	Knobbed
Position	Apex	Top to middle	Arising from juncture apical cell	Apex	Apex
Basal appendages	2.5– 4	Present	Present	Present	Present

[a] Maharachchikumbura et al. (2012)

[b] Strobel et al. (2000)

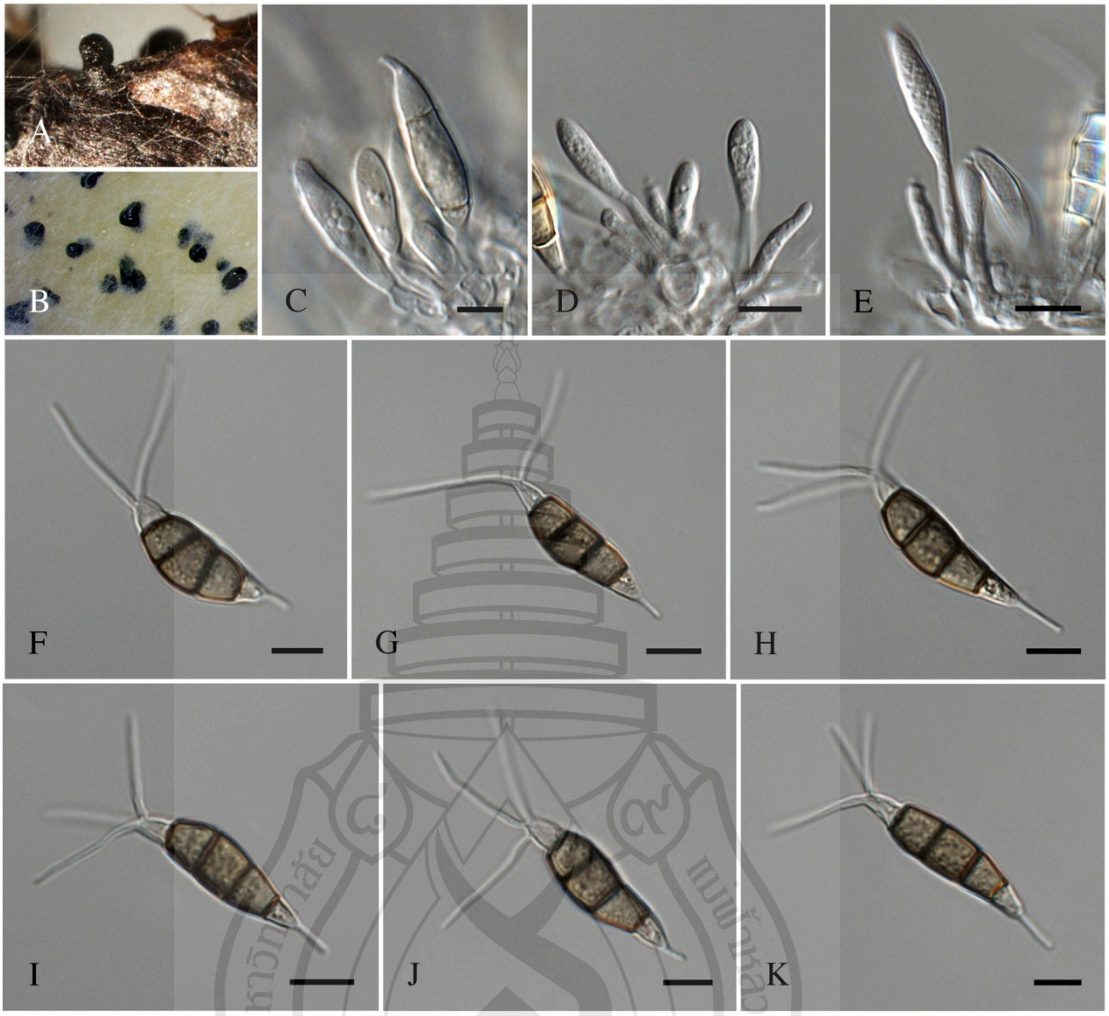
[c] Watanabe et al. (2010)

[d] Guba (1961)

Notes: *Pestalotiopsis gaultheria* is a morphologically distinct species, also shown in its DNA phylogeny. Species belongs to the clade that consist *P. gaultheria* are concolorous. However most interestingly, when mature, the two upper median cells in *P. gaultheria* become darker than the lower median cell. *Pestalotiopsis gaultheria* has a very long apical appendages (15–50 µm) when compared with species having similar conidial size, such as *P. diversiseta* (22–30 µm) *P. jesteri* (11–28 µm) and *P. pallidotheae* (12–40 µm). In phylogenetic tree it forms a sister clade with *P. diversiseta,* however, *P. gaultheria* is clearly distinguished from *P. diversiseta* by having lesser number of apical appendages. Furthermore in *P. diversiseta* some apical appendages branched and sometimes arising from the different parts of the apical cell and this cannot see in *P. gaultheria.*

Pestalotiopsis grevillea Maharachch. & Crous, sp. nov. (Figure 3.22 A–K).

Etymology: Named after the host genus from which it was isolated, *Grevillea.*



Note. *Pestalotiopsis grevillea* CBS 114127^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.22 *Pestalotiopsis grevillea* (holotype)

Conidiomata pycnidial in culture on PDA, globose, aggregated or scattered, semi-immersed, dark brown to black, up to 200 µm diam; releasing globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical to subcylindrical, hyaline, smooth, proliferating 2-3 times percurrently, flared collarette, with prominent

periclinal thickening. 5–25 × 2–8 µm. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (21–)22.5–28(–29) × (7–)7.5–9(–9.5) µm, mean ± SD = 25.2 ± 1.2 × 8.2 ± 0.5 µm; basal cell conic with a truncate base, hyaline, thin and wall rugose, 3.5–5.5 µm long; three median cells doliiform, (12.5–)13–17(–17.5) µm long, mean ± SD = 15 ± 1.2, wall verruculose, concolorous, olivaceous, septa darker than the rest of the cell, (second cell from the base 4.5–6.5 µm; third cell 4.5–6.5 µm; fourth cell 4–6.5 µm); apical cell 3.5–5.5 µm long, hyaline, cylindrical to subcylindrical, thin and wall rugose; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (12–)14–26.5(–29) µm long, mean ± SD = 19 ± 3; basal appendage single, tubular, unbranched, centric, 3–8 µm long (Figure 3.22).

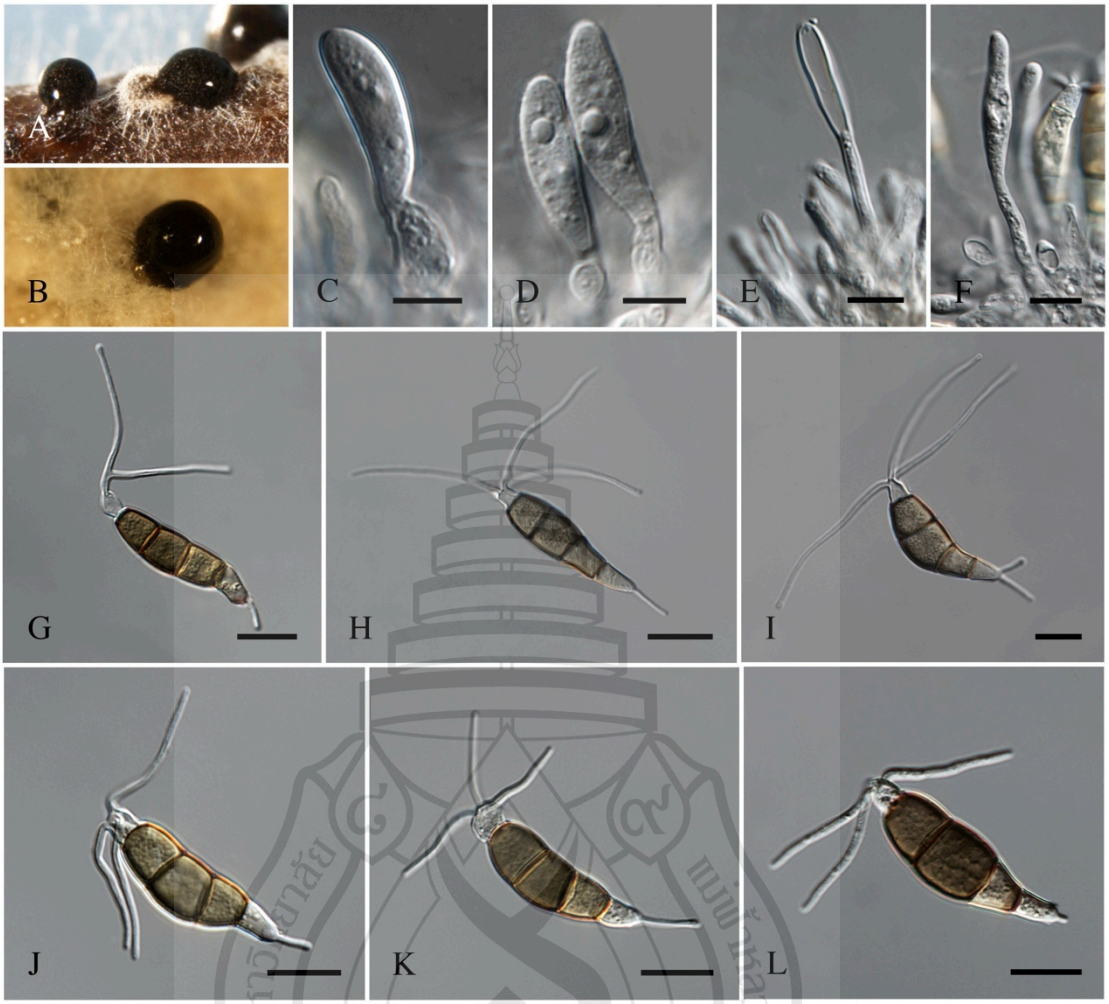
Culture characteristics: Colonies on PDA attaining 35–45 mm diam after 7 days at 25°C, with smooth edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: AUSTRALIA, *Grevillea* sp., 1999, P.W. Crous, (CBS H holotype, culture ex-type CBS 114127= STE-U 2919).

Notes: *Pestalotiopsis grevillea* forms a sister clade to *P. knightia* in the *P. telopea* section, being distinct from the latter species in having narrow conidia. *P. grevillea* has an overlapping conidial measurements with *P. australasia*, even though their basal cells are quit distinct. In *P. grevillea* the basal cells are conic, while in *P. australasia* they are obconic to hemispherical. Furthermore, DNA sequence data revealed that the two species are genetically distinct.

Pestalotiopsis hawaiiensis Maharachch. & Crous, sp. nov. (Figure 3.23 A–L).

Etymology: Named after the country where it was collected, Hawaii.



Note. *Pestalotiopsis hawaiiensis* CBS 114491^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–F. Conidiogenous cells. G–L. Conidia. Scale bars = 10 µm

Figure 3.23 *Pestalotiopsis hawaiiensis* (holotype)

Conidiomata (on PDA) pycnidial, globose, solitary, semi-immersed, dark brown to black, 200–600 µm diam; exuding globose, brown to black conidial masses. *Conidiophores* simple or branched, hyaline, subcylindrical, smooth-walled, 5–15 × 3–8 µm. *Conidiogenous cells* discrete, cylindrical, hyaline, smooth-walled, proliferating 2-4 times percurrently near apex, 20–50 × 3–6 µm, collarette present and not flared,

with prominent periclinal thickening. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (26–)27–34.5(–37) × (7–)7.5–10(–10.5) µm, mean ± SD = 31.6 ± 2 × 8.7 ± 0.6 µm; basal cell conic to obconic with a truncate base, hyaline, thin, minutely verruculose, 4–8 × µm long; three median cells doliiform to subcylindrical, (19–)19.5–23(–25) µm long, mean ± SD = 21.4 ± 1.2, wall verruculose, concolorous brown, septa darker than the rest of the cell, (second cell from the base 5–8.5 µm; third cell 6.5–9.5 µm; fourth cell 6–9 µm); apical cell 4–7 × µm long, hyaline, cylindrical to subcylindrical, thin and smooth walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (14–)19–33(–36) µm long, mean ± SD = 25.3 ± 4.1; basal appendage single, tubular, unbranched, centric, 5–11 µm long (Figure 3.23).

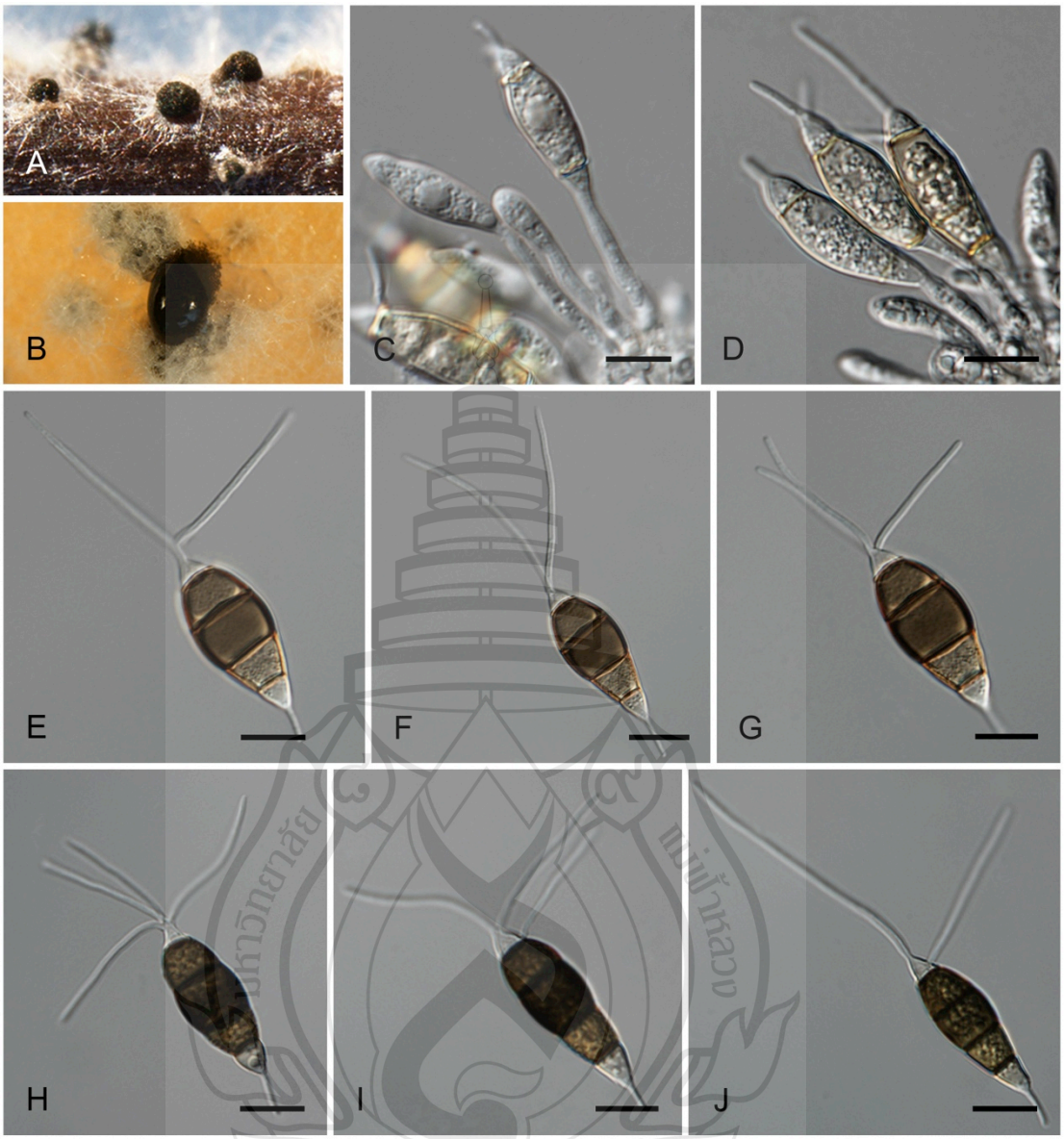
Culture characteristics: Colonies on PDA attaining 30–45 mm diam after 7 days at 25°C, with undulate edge, whitish, sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: HAWAII, from *Leucospermum* sp. (Coral), 9 Dec. 1999, P.W. Crous (CBS H holotype, culture ex-type CBS 114491= STE-U 2215).

Notes: *Pestalotiopsis hawaiiensis,* known from Hawaii, on *Leucospermum* sp., and its conidial length and width overlapping with *P. anacardiacearum* (27–39 × 7–10 µm), which was isolated from leaves of *Mangifera indica* in China. However, *P. anacardiacearum* (20–45 µm) differ from *P. hawaiiensis* by having longer apical appendages. Furthermore, the two species are genetically, geographically and ecologically clearly distinct, we prefer to maintain two separate species.

Pestalotiopsis hollandica Maharachch. & Crous sp. nov. (Figure 3.24 A–J).

Etymology: Named after the country where it was collected, Holland.



Note. *Pestalotiopsis hollandica* CBS 265.33^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 μm

Figure 3.24 *Pestalotiopsis hollandica* (holotype)

Conidiomata (on PDA) pycnidial, 200–350 μm diam, globose or clavate, solitary or aggregated, semi-immersed, dark brown to black; exuding dark brown

conidial masses. *Conidiophores* septate, branched at base, sometimes reduced to conidiogenous cells, hyaline, smooth-walled, up to 30 µm. *Conidiogenous cells* discrete, cylindrical, proliferating 2–5 times percurrently near apex, tapering to a long, thin neck, collarette present and not flared. *Conidia* ellipsoid, straight to slightly curved, 4-septate, slightly constricted at septa, (25–)25.5–33(–34) × 8.5–10(–10.5) µm, mean ± SD = 28 ± 2 × 9.4 ± 0.3 µm; basal cell conic to obconic with truncate base, thin-walled 5–7.5 µm long; three median cells (16.5–)17–23(–24) µm long; mean ± SD = 28 ± 2 × 9.4 ± 0.3 µm, doliiform, thick-walled, verruculose, concolorous, but occasionally the two upper median cells slightly darker than the lower median cell, wall rugose, (second cell from base 5–8.5 µm; third cell 6–9 µm; fourth cell 6–8 µm); apical cell conic, hyaline, thin and smooth-walled, 3.5–5 µm long; with 1–4 tubular apical appendages, with some branched appendages, arising from the apex of the apical cell and sometimes from just above the septum separating the apical and subapical cell, 20–40 µm long (mean = 27 µm); basal appendage single, tubular, unbranched, centric, 3–9 µm (mean = 4.7 µm) (Figure 3.24).

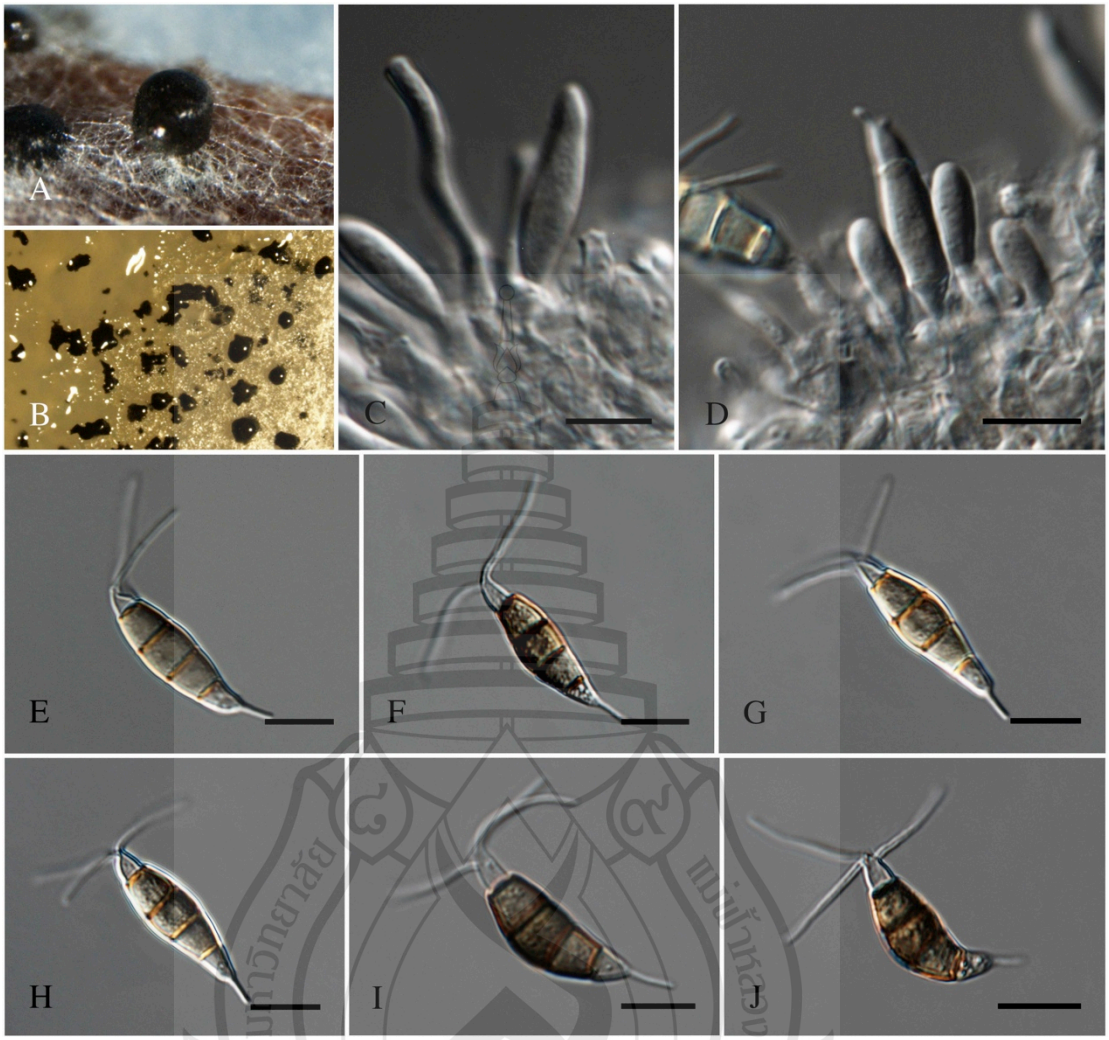
Culture characteristics: Colonies on PDA reaching 60–70 mm diam. after 7 days at 25°C, with an undulate edge, whitish to pale grey-coloured, with dense aerial mycelium on surface, and black, gregarious fruiting bodies; reverse similar in colour.

Material examined: NETHERLANDS, Baarn, from *Sciadopitys verticillata*, July 1933, A. Punt (CBS H- 15703 holotype, culture ex-type CBS 265.33).

Notes: *Pestalotiopsis hollandica* differs from all other species in this section by having some appendages that arise from different parts of the apical cell. *P. hollandica* differs from *P. monochaetioides* (22–30 × 5–10 µm), which was isolated from a dead twig of *Chamaecyparis lawsoniana* in the Netherlands, and by its branched, and subapically attached apical appendages.

Pestalotiopsis humus Maharachch. & Crous, sp. nov. (Figure 3.25 A–J).

Etymology: Name refers to the substrate from which it was isolated, soil.



Note. *Pestalotiopsis humus* CBS 336.97^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm

Figure 3.25 *Pestalotiopsis humus* (holotype)

Conidiomata pycnidial in culture on PDA, globose, semi-immersed, aggregated or scattered, up to 400 µm diam; exuding dark brown to black, globose, conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical, hyaline, smooth-walled, simple, proliferating up to 3 times

percurrently, 8–28 × 2–5 µm, apex 1–2 µm diam. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, constricted at septum, (17–)18.5–22(–23) × 5–7(–7.5) µm, mean ± SD = 20 ± 1.4 × 6 ± 0.4 µm; basal cell obconic to conic with a truncate base, hyaline, thin and minutely verruculose, 3.5–5.5 µm long; three median cells subcylindrical, (11.5–)12–14(–14.5) µm long, mean ± SD = 12.8 ± 0.8, wall rugose, concolorous, brown, septa darker than the rest of the cell, (second cell from the base 3.5–5.5 µm long; third cell 3.5–6 µm long; fourth cell 3.5–5.5 µm long); apical cell 3.5–4.5 × µm long, hyaline, subcylindrical; with 2–3 tubular apical appendages, arising from an apical crest, unbranched, filiform, flexuous, (6–)6.5–12(–13) µm long, mean ± SD = 9.0 ± 1.5; basal appendage single, tubular, unbranched, centric, 2–5 µm long (Figure 3.25).

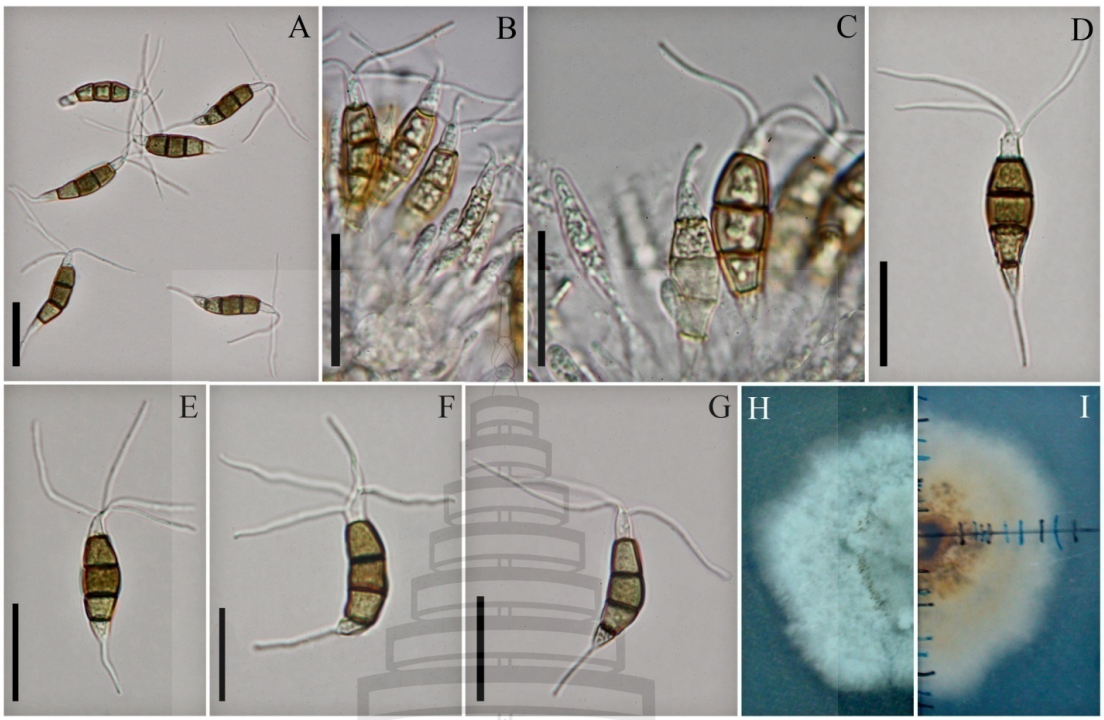
Culture characteristics: Colonies on PDA attaining 45–50 mm diam after 7 days at 25°C, with smooth edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: PAPUA NEW GUINEA, from soil in tropical rain forest, Nov. 1995, A. Aptroot, (CBS H holotype, culture ex-type CBS 336.97); Hong Kong, from fruit of *Ilex cinerea*, 20 January 2002, collector unknown, culture CBS 115450 = HKUCC 9100.

Notes: Sequences of *Pestalotiopsis humus* form a sister clade to *P. diploclisia* in the *P. diploclisia* section. *P. diploclisia* differs from *P. humus* in conidial morphology, in that conidia are narrower (20–28 × 5–7 µm), and have longer apical appendages (10–22 µm).

Pestalotiopis inflexa Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 114 (2012), MycoBank: MB 800530 (Figure3.26 A–I).

Etymology: From the Latin, inflexus in reference to the curved nature of the conidia.



Note. A. Conidia. B–C. Conidiophores/ conidiogenous cells. D–G. Conidia. H. I. Colony on PDA, H. from above, I. from below. Scale Bars: A– G= 20 µm

Figure 3.26 *Pestalotiopsis inflexa* (holotype)

Conidiophores most often reduced to conidiogenous cells, simple, hyaline, smooth-walled. *Conidiogenous cells* discrete, ampulliform to lageniform, smooth, thin-walled, hyaline or pale olivaceous. Conidia fusoid to ellipsoid, straight to slightly curved, 4–septate, 24–31 × 6–9 µm (mean = 27 × 7.6 µm), basal cell conic to obconic, hyaline or slightly olivaceous, thin-walled and verruculose, 5–7 µm long (mean = 5.7 µm), with 3 median cells, doliiform to cylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, with septa and periclinal walls darker than the rest of the cell, wall rugose, together 15–19 µm long (mean = 17.1 µm) (second cell from base 5–7 µm (mean = 5.7 µm); third cell 5–7 µm (mean = 5.8 µm); fourth cell 4.5–6 µm (mean = 5.3 µm); apical cell hyaline, subcylindrical to cylindrical 4-5 µm long (mean = 4.6 µm); 2–5 tubular apical appendages (mostly 3-

4), often arising from the apex of the apical cell or rar3.ly arising from just below the apex of apical cell, 20–30 µm long (mean = 24 µm), unequal, rarely branched; basal appendage present, relatively long 9–15 µm (mean = 12 µm).

Culture characteristic: Colonies on PDA reaching 7 cm diam. after 18 days at 25°C, edge undulate, whitish, with dense, aerial mycelium on surface, with black, gregarious fruiting bodies; reverse of the culture yellowish.

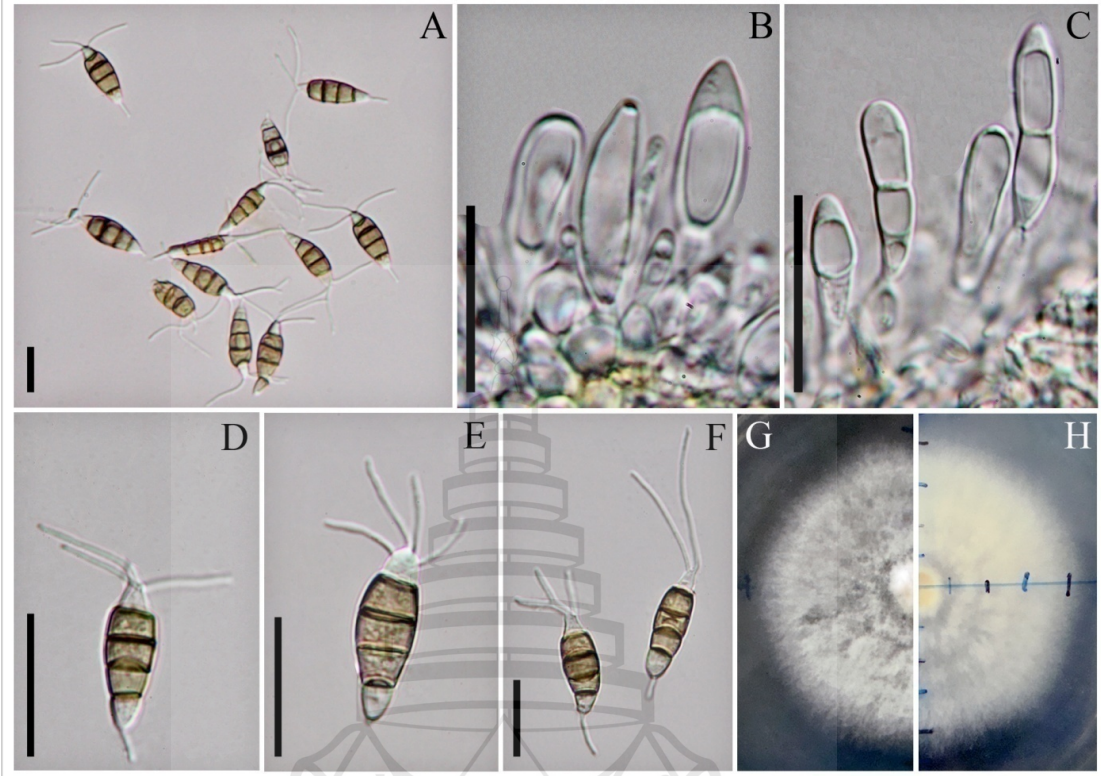
Habitat/Distribution: Endophyte in living leaves of unidentified plant, Hunan Province, China.

Material examined: CHINA, Hunan Province, Yizhang County, Mangshan, living leaf of unidentified tree, 12 April 2002, Wenping Wu HN14-2 (HMAS047098, holotype; MFLU12-0413, isotype; ex-type living culture NN047098 = MFLUCC 12-0270).

Notes: *Pestalotiopsis inflexa* can be differentiated from its close relatives in the β-tubulin, TEF1 and combined phylogram. The characteristic morphology of *P. inflexa* is due to its divergent, 2 to 5 apical appendages, sometimes arising from the middle of apical cell and by a relatively long basal appendage (9–15 µm). Morphologically similar species to *P. inflexa* in conidial size is *P. thujicola* (J.L. Maas) Y. Suto & Tak. Kobay (25–31 × 6.5–10 µm) (Maas, 1971). However, *P. thujicola* can be differentiated by its 3–6 apical appendages radiating from different parts of the apical cell. In *P. inflexa,* the appendages usually arise from the tip of the apical cell and rarely from the middle.

Pestalotiopsis intermedia Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 115 (2012), MycoBank: MB 800532 (Figure 3.27 A–H).

Etymology: From Latin, intermediate pertaining to the intermediate size of the conidia.



Note. A. Conidia. B–C. Conidiophores/ conidiogenous cells. D–F. Conidia. G. H. Colony on PDA, G. from above, H. from below. Scale Bars: A– F= 20 µm

Figure 3.27 *Pestalotiopsis intermedia* (holotype)

Conidiophores indistinct: *Conidiogenous* cells discrete, simple, filiform, smooth, thin-walled, hyaline, and short. Conidia fusoid to ellipsoid, straight to slightly curved, 4–septate, 24–28 × 5.5–6.5 µm (mean = 25.7 × 6 µm), basal cell conic to obconic with obtuse end, hyaline, thin- and verruculose, 4–5 µm long (mean = 4.8 µm), with three median cells, doliiform, concolorous, olivaceous to brown, septa *and* periclinal walls darker than the rest of the cell, wall rugose, together 15–19 µm long (mean = 17 µm) (second cell from base 5–6 µm (mean = 5.7 µm); third cell 5–6 µm (mean = 5.7 µm); fourth cell 5–6.5 µm (mean = 5.2 µm); apical cell hyaline, conic to cylindrical 4-5 µm long (mean = 4.5 µm); with 2–3 tubular apical appendages (rarely 4), arising from the apex of the apical cell, 10–28 µm long (mean = 18.5 µm),

unequal; basal appendage present 6–10 µm (mean = 7.5 µm), rarely absent (Figure 3.27).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25°C, edge entire, whitish, with dense, aerial mycelium on surface, fruiting bodies black; reverse of culture whitish to pale yellow.

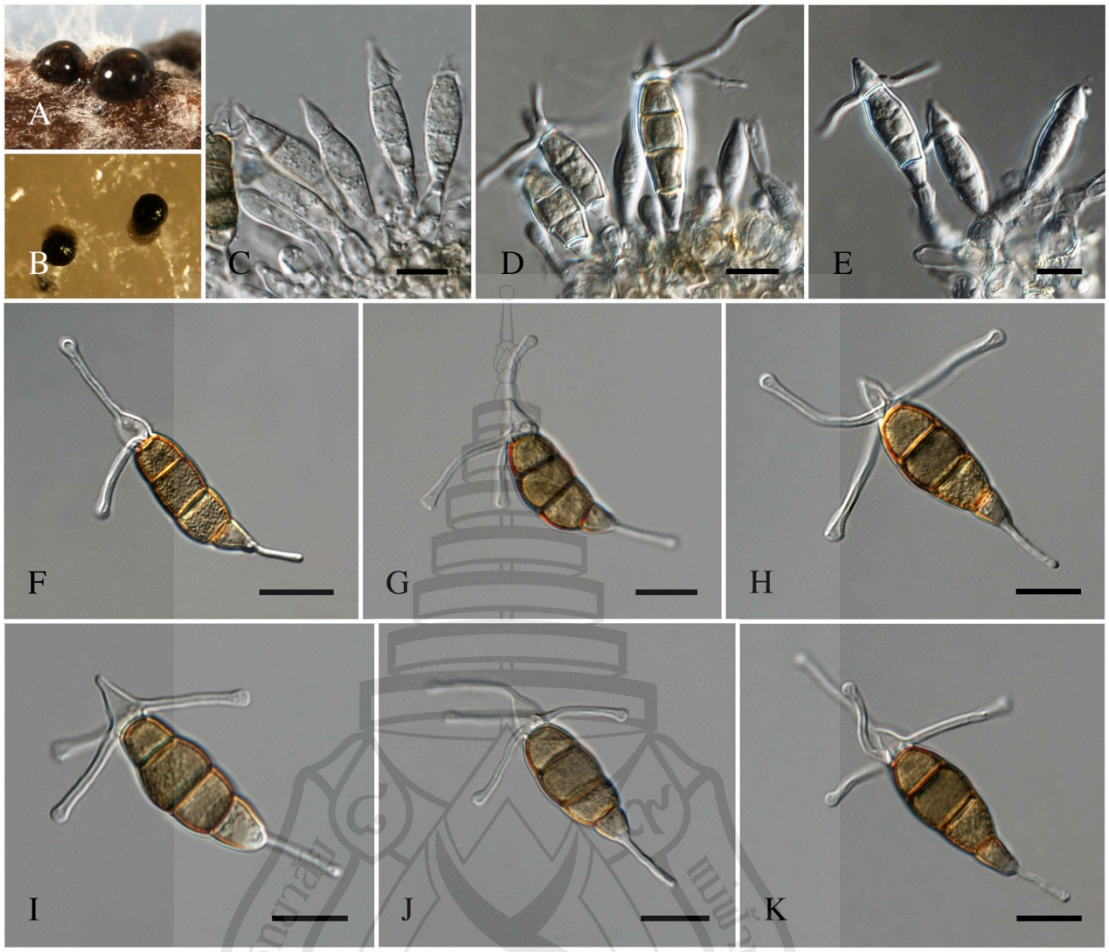
Habitat/Distribution: Saprobe/endophyte on unidentified trees, Hubei and Yunnan provinces, China.

Material examined: CHINA, Hubei Province, Shengnongjia, on dead leave of unidentified tree, 24 March 2003, Wenping Wu WUFH7033 (HMAS047642, holotype; MFLU12-0410, isotype; ex-type living culture NN047642 = MFLUCC 12-0259).

Additional culture examined: CHINA, Yunnan Hunan Province, Yizhang County, Mangshan, on living leaf of unidentified plant, 12 April 2002, Wenping Wu HN28-16 (NN047073 = MFLUCC 12-0260).

Notes: The morphologically similar species to *P. intermedia* (24–28 × 5.5–6.5 µm) in conidial size are *P. lespedezae* (Syd.) Bilgrami (20–25 × 7–9 µm) (Guba 1961), *P. osyridis* (Thüm.) H.T. Sun & R.B. Cao (22–28 × 5–7 µm) and *P.* cocculi (Guba) G.C. Zhao & N. Li (22–29 × 5.5–7 µm) (Guba, 1961). *Pestalotiopsis intermedia* can be differentiated from *P. lespedezae* by its long and thin conidia; and from *P. osyridis* and *P. cocculi* by its long apical appendages (*P.* osyridis usually has 3 apical appendages (rarely 2) measuring up to 14 µm long and in *P. cocculi* there are three apical appendages (sometimes 2), up to 11–12 µm long).

Pestalotiopsis jesteri Strobel, J.Yi Li, E.J. Ford & W.M. Hess, in Strobel, Li, Ford, Worapong, Baird & Hess, Mycotaxon 76: 260 (2000). MycoBank: MB 466231. (Figure 3.28 A–K).



Note. *Pestalotiopsis jesteri* CBS 109350. A. Conidioma sporulating on PNA. B. Conidioma on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 μm

Figure 3.28 *Pestalotiopsis jesteri* (holotype)

Conidiomata (on PDA) pycnidial, globose, solitary or aggregated, immersed, medium to dark brown, 100–450 μm diam; releasing globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, lageniform to subcylindrical, hyaline, smooth, proliferating once or twice, 5–20 × 3–7 μm; collarette flared, opening 2–5 μm diam. *Conidia* fusiform, ellipsoid to subcylindrical, straight to slightly curved, 4-euseptate,

(21–)22.5–31(–34.5) × 7–9 µm, mean ± SD = 26.8 ± 3 × 8.2 ± 0.2 µm; basal cell narrowly obconic with a truncate base, hyaline, thin and smooth-walled, 4.5–6.5 µm long; three median cells doliiform to subcylindrical, (15.5–)16–20(–21) µm long, mean ± SD = 17.5 ± 1.4, wall rugose, concolorous, golden brown, septa darker than the rest of the cell, (second cell from the base 4.5–7 µm long; third cell 5.5–7.5 µm long; fourth cell 5.5–7.5 µm long); apical cell 3.5–7.5 µm long, hyaline, obconic with an acute apex, thin and smooth-walled; appendages tubular, attenuated; apical appendage single, 14–25 long; lateral appendages 2–4, arising just above the septum separating the apical cell and upper median cell, unbranched, 14–25 long; basal appendage single, tubular, unbranched, centric, 4–14 µm long (Figure 3.28).

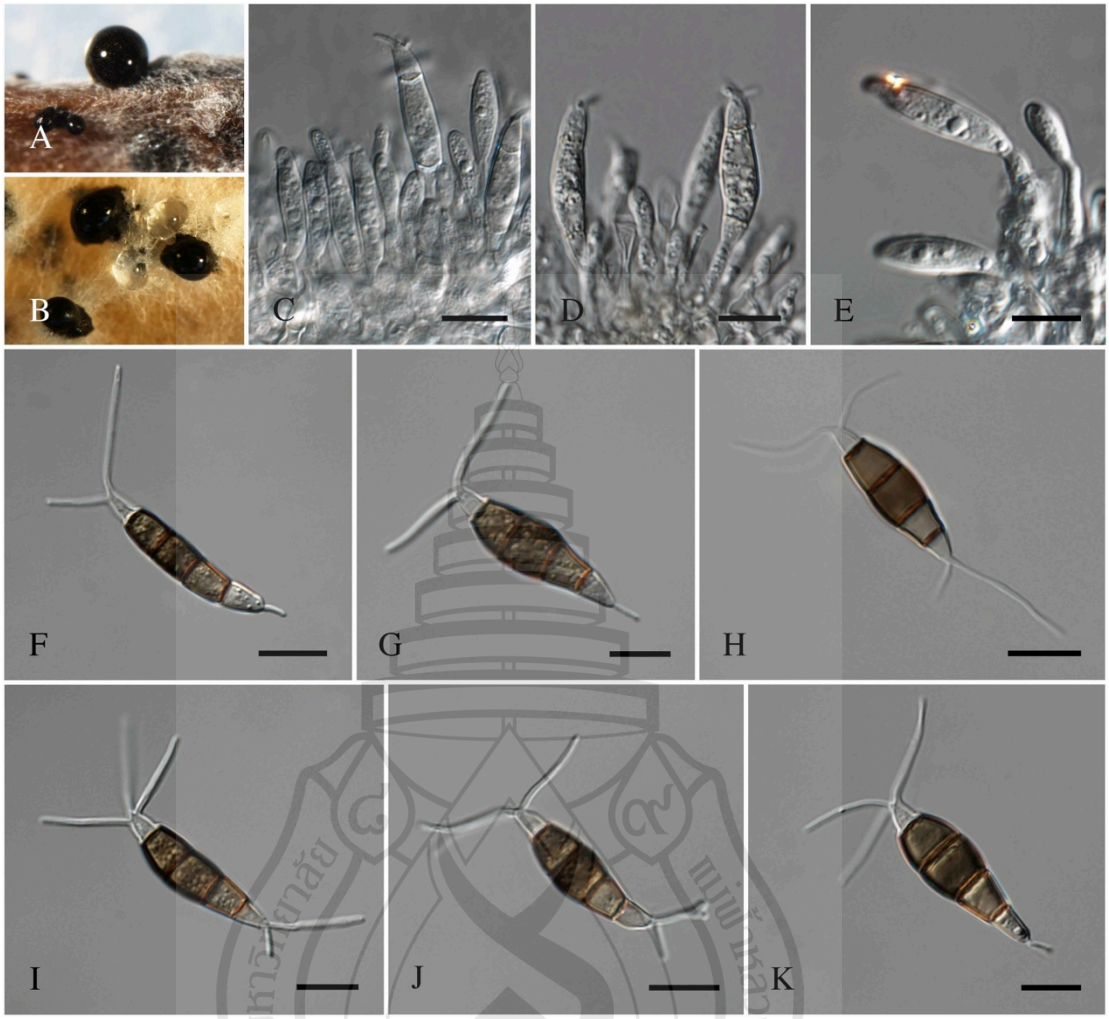
Culture characteristics: Colonies on PDA attaining 20–30 mm diam after 7 days at 25°C, with undulate edge, whitish, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: Papua New Guinea, Southern Highlands, Aluak ambe village, from bark of *Fragraea bodenii*, E. Eroli & G. Strobel (deposited in CBS collection Mar 2001 by G. Strobel), culture CBS 109350= MONT 6 M3.

Notes: *Pestalotiopsis jesteri* is well characterized and easily recognizable by the unique apical appendages attachment to the apical cell. The arrangement of apical appendages in *P. jesteri* is comparable with *Pestalotia montellica* (Guba, 1961). However, *P. jesteri* differs from *Pestalotia montellica* by the presence of knobbed apical appendages. Furthermore, *P. jester* is an outlying species in the genus, and forms a distinct lineage apart from all other species.

Pestalotiopsis kenyana Maharachch. & Crous, sp. nov. (Figure 3.29 A–K).

Etymology: Named after the country where it was collected, Kenya.



Note. *Pestalotiopsis kenyana* CBS 442.67^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.29 *Pestalotiopsis kenyana* (holotype)

Conidiomata pycnidial in culture on PDA, globose, scattered, semi-immersed, black, up to 400 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* sparsely septate at base, branched or unbranched, subcylindrical, hyaline, smooth, up to 15 µm or reduced to *Conidiogenous cells*. *Conidiogenous cells* discrete, lageniform to subcylindrical, hyaline, smooth, proliferating 1-3 times

percurrently, 10–25 × 2–5 µm, apex with minute periclinal thickening and collarette. *Conidia* fusiform, ellipsoid to subcylindrical, straight to slightly curved, 4-euseptate, (22–)23–28(–29) × 7–9 µm, mean ± SD = 25.5 ± 1.2 × 8 ± 0.4 µm; basal cell conic to obconic with a truncate base, hyaline, thin and wall minutely verruculose, 4–6 µm long; three median cells doliiform, (15–)15.5–18.5(–19) µm long, mean ± SD = 17 ± 0.7, wall verruculose concolorous, brown, septa darker than the rest of the cell, (second cell from the base 4.5–6 µm long; third cell 5.5–7.5 µm long; fourth cell 3.5–4.5 µm long); apical cell 3.5–5.5 µm long, hyaline, subcylindrical, thin and wall rugose; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (8–)9–18(–20) µm long, mean ± SD = 14 ± 3; two basal appendages; centric appendage tubular, flexuous, 3–20 µm long and ex-centric appendage tubular, 1–4 µm long (Figure 3.29).

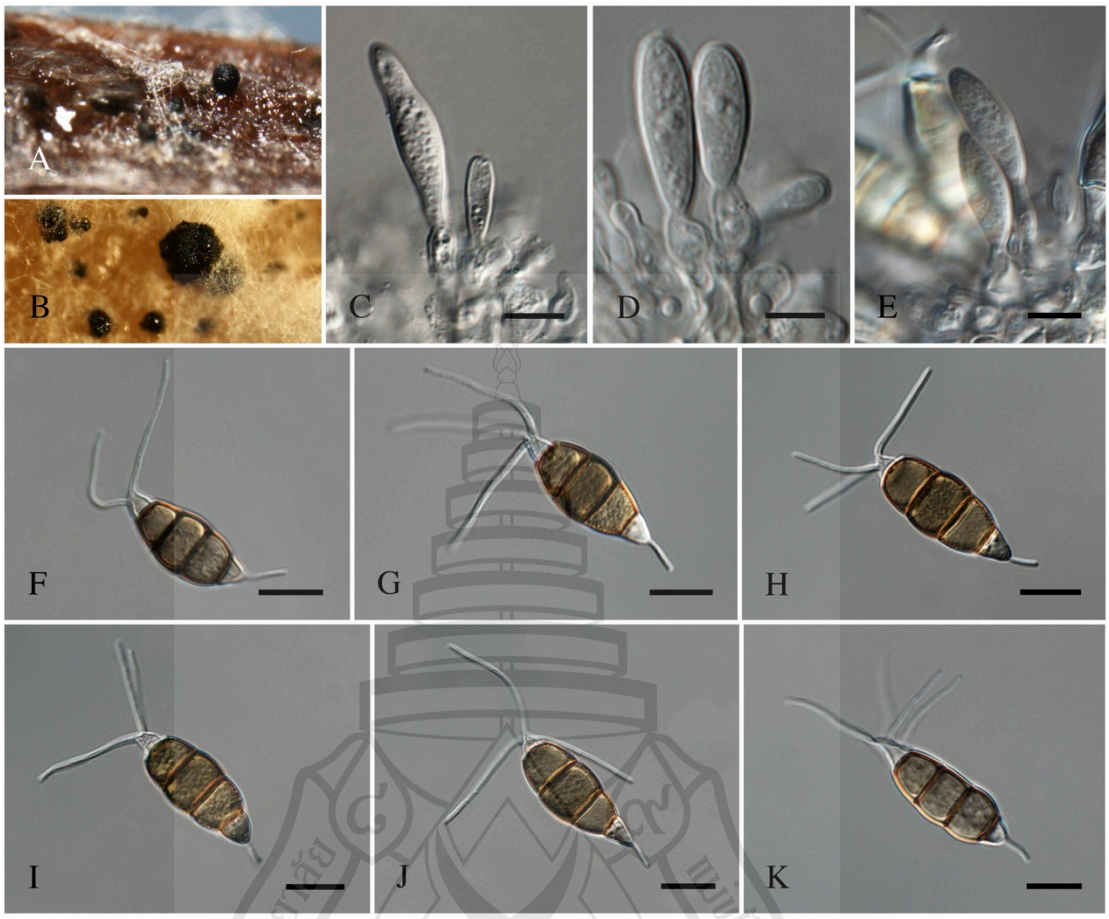
Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with undulate edge, whitish, with medium dense aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: KENYA, from *Coffea* branch, October 1967, H. Vermeulen (CBS H-15657 holotype, culture ex-type CBS 442.67); unknown country, from raw material from agar-agar, kobe 1 (stips), Sept. 1996, A.K. Johansen, culture CBS 911.96.

Notes: *Pestalotiopsis kenyana*, which formed a separate clade in phylogenetic analyses as sister to *P. trachicarpicola*, is a member of the *P. trachicarpicola* section and belongs to a group of species with two apical appendages. *Pestalotiopsis kenyana* differs from *P. trachicarpicola* and *P. biciliata* by having wider conidia (see comparison under *P. biciliata*).

Pestalotiopsis knightia Maharachch. & Crous, sp. nov. (Figure 3.30 A–K).

Etymology: Named after the host genus from which it was isolated, *Knightia.*



Note. *Pestalotiopsis knightia* CBS 114138[T]. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.30 *Pestalotiopsis knightia* (holotype)

Conidiomata pycnidial, globose, aggregated or scattered, semi-immersed to erumpent on PDA, dark brown to black, 100–200 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform or lageniform, hyaline, smooth, simple, proliferating once or twice, wide at the base, 10–30 × 2–10 µm. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (21–)22–27(–29) × (8–)8.5–10.5(–11) µm, mean ± SD = 24.8 ± 1.3 × 9.6 ± 0.4 µm; basal cell

obconic to conic with a truncate base, hyaline, thin and smooth-walled, 3–6.5 µm long; three median cells doliiform, (15.5–)16–18.5(–19.5) µm long, mean ± SD = 17.4 ± 1.2, wall minutely rugose, concolorous, pale brown, septa darker than the rest of the cell, (second cell from the base 5.5–7 µm long; third cell 6–7.5 µm long; fourth cell 5.5–7 µm long); apical cell 3–4.5(–5) µm long, hyaline, cylindrical to subcylindrical; with 2–4 tubular apical appendages (mostly 3), not arising from the apical crest, but each inserted at a different locus in the upper half of the cell, unbranched, filiform, (8–)12–20(–23) µm long, mean ± SD = 15 ± 3.9; basal appendage single, tubular, unbranched, centric, 2.5–7.5 µm long (Figure 3.30).

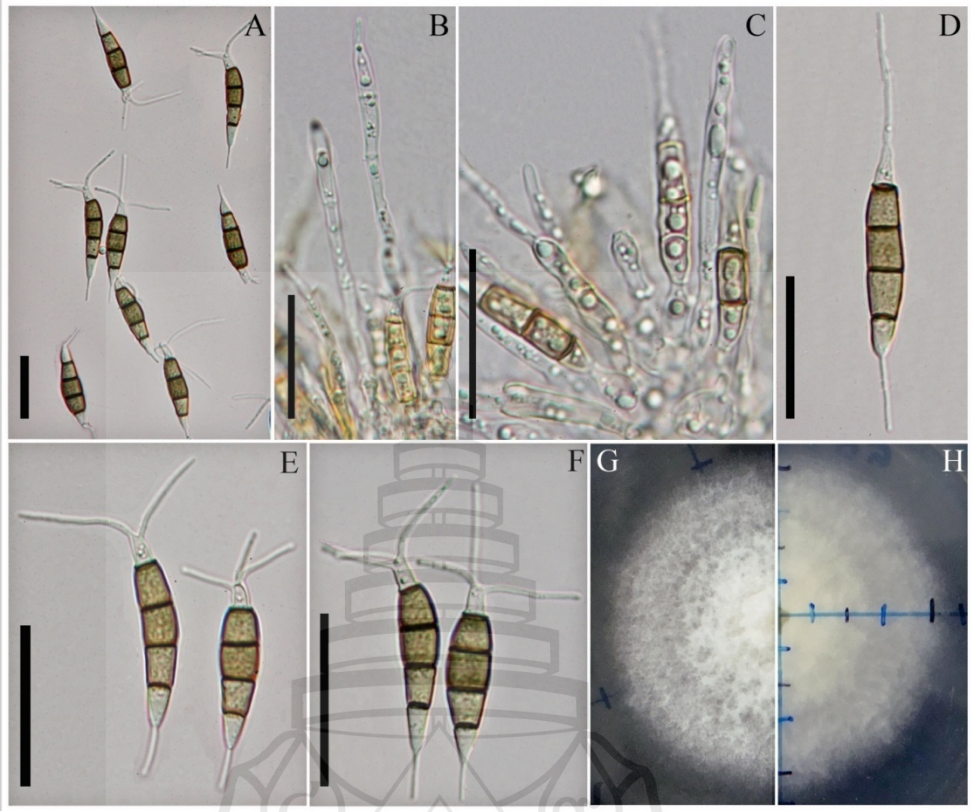
Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with lobate edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: NEW ZEALAND, from *Knightia* sp., unknown collection date and collector, (CBS H holotype, culture ex-type CBS 114138= STE-U 2906); New Zealand, Tamaki, Maori Village, from *Knightia* sp., 1999, P.W. Crous, culture CBS 111963= STE-U 2905.

Notes: *Pestalotiopsis knightia* is a species occurring on *Knightia* sp. in New Zealand, and well distinguished from other *Pestalotiopsis* species in the section based on its DNA phylogeny. It forms a sister clade to *P. grevillea*, and is distinguishable from other species in the complex by its wider conidia.

Pestalotiopis linearis Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 117 (2012), MycoBank: MB 800531 (Figure 3.31 A–H).

Etymology: The specific epithet is based on the linear shape of the conidia and in Latin, linear is *linearis*.



Note. A. Conidia. B–C. Conidiophores/ conidiogenous cells. D–F. Conidia. G. H. Colony on PDA, G. from above, H. from below. Scale Bars: A– F= 20 µm

Figure 3.31 *Pestalotiopsis linearis* (holotype)

Conidiophores often reduced to conidiogenous cells, sometimes sparsely septate at the base and unbranched or branched, hyaline, smooth. Conidiogenous cells discrete ampulliform to lageniform, smooth, thin-walled, hyaline, with 1–2 proliferations, sometimes remain vegetative. Conidia fusiform, straight to slightly curved, 4-septate, 24–33 × 4.7–6 µm (mean = 29 × 5.5 µm), basal cell conic to obconic, hyaline or slightly olivaceous, thin- and verruculose, 3.5–5.5 µm long (mean = 4.4 µm), with three median cells, doliiform to cylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, together 17–21 µm long (mean = 19 µm) (second cell

from base 5–6.2 µm (mean = 5.5 µm); third cell 6–7 µm (mean = 6.3 µm); fourth cell 6–8 µm (mean = 6.6 µm); apical cell hyaline, cylindrical to subcylindrical 4-5 µm long (mean = 4.2 µm); with 2–3 tubular apical appendages (rarely 1), arising from the apex of the apical cell, 10–20 µm long (mean = 15 µm), unequal in length; basal appendage present, rarely two, 4–7 µm long (mean = 5.7 µm).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25oC, edge entire, whitish, with dense, aerial mycelium on surface, with black, gregarious fruiting bodies; reverse of culture white.

Habitat/Distribution: Endophytes on living leaves of *Trachelospermum* sp. and *Tsuga* sp., Yunnan Province, China.

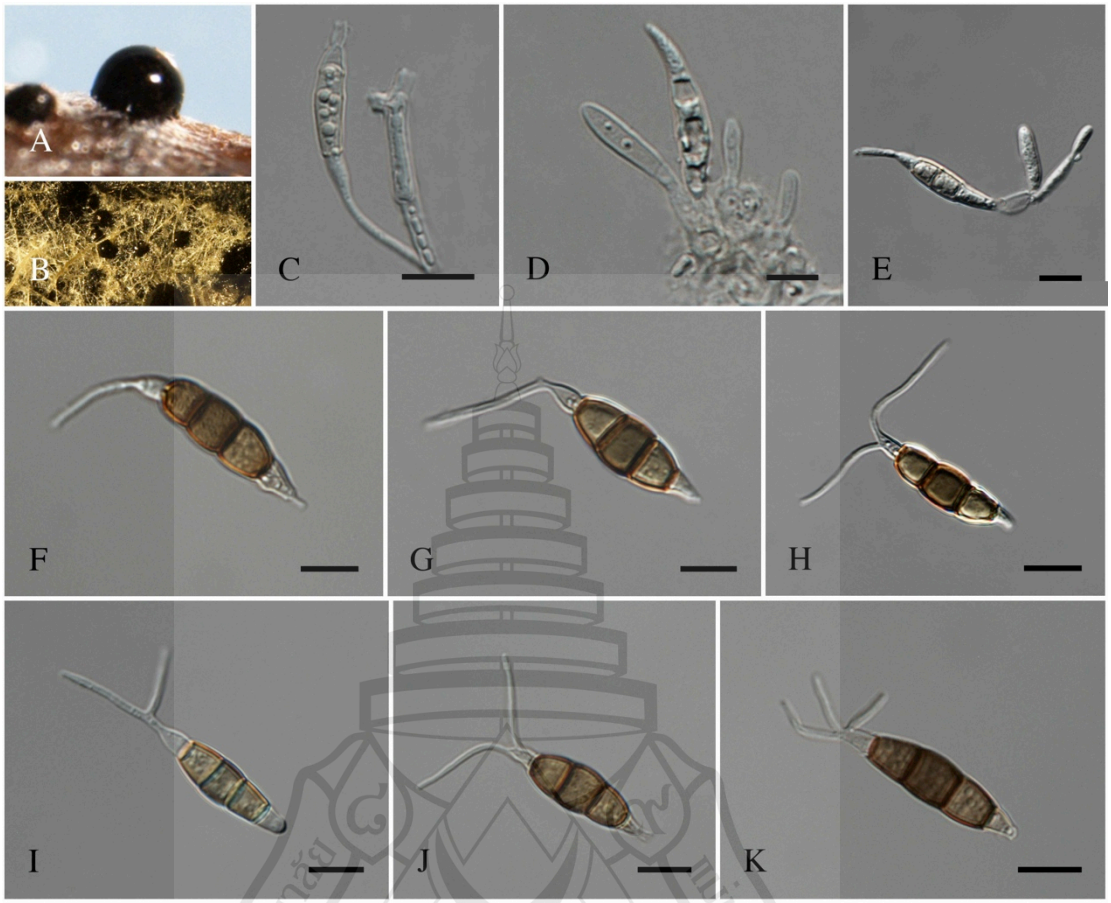
Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of Trachelospermum sp., 19 March 2002, Wenping Wu KBG14-3 (HMAS047190 holotype; MFLU12-0414, isotype; ex-type living culture NN047190 = MFLUCC 12-0271).

Additional culture examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Tsuga sp.,* 19 March 2002, Wenping Wu KBG16-7 (NN047141 = MFLUCC 12-0272).

Notes: *Pestalotiopsis linearis* is a distinct species both in conidial morphology and phylogeny. It can be easily differentiated from its phylogenetically related species *P. intermedia* in combined trees (Figure 3.2) and morphologically related species in the concolorous groups such as *P. macrochaeta* (Speg.) J. Xiang Zhang & T. Xu (22–31 × 8–10 µm) and *P. caudata* (Syd.) B. Sutton (22–31 × 8–10 µm) (Saccardo 1902). In *P. linearis* (24–33 × 4.7–6 µm) conidia are much thinner than these two species.

Pestalotiopsis malayana Maharachch. & Crous, sp. nov. (Figure 3.32 A–K).

Etymology: Named after the country where it was collected, Malaysia.



Note. *Pestalotiopsis malayana* CBS 102220^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm

Figure 3.32 *Pestalotiopsis malayana* (holotype)

Conidiomata (on PDA) pycnidial, globose, scattered or aggregated, semi-immersed, dark brown to black, up to 400 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* 2–5-septate, irregular branched, cylindrical, hyaline, verruculose-walled, up to 50 µm, sometimes reduced to conidiogenous cells. *Conidiogenous cells* discrete, subcylindrical to ampulliform, hyaline, smooth, tapering to a long, thin neck, 6–18 × 2–4 µm, proliferating several times percurrently near apex, with flaring collarettes. *Conidia* fusiform, ellipsoid, straight to slightly curved,

slightly constricted at septa, 4-euseptate, (20.5–)22–29.5(–31) × 5–7.5 µm, mean ± SD = 25.6 ± 2 × 6.3 ± 0.4 µm; basal cell obconic to conic with a truncate base, hyaline, thin and minutely verruculose, 3.5–7.5 µm long; three median cells doliiform, 15–18 µm long, mean ± SD = 16.5 ± 0.8, wall minutely verruculose, concolorous, pale brown, septa darker than the rest of the cell, (second cell from the base 4.5–7 µm long; third cell 4.5–6.5 µm long; fourth cell 5–7 µm long); apical cell 3–6 µm long, hyaline, cylindrical to subcylindrical; with 1–3 tubular apical appendages (mostly 2), arising as an extension of the apical cell, unbranched, filiform, (11–)11.5–18.5(–19) µm long, mean ± SD = 15.1 ± 1.4; basal appendage single, tubular, unbranched, centric, 2–5 µm long (Figure 3.32).

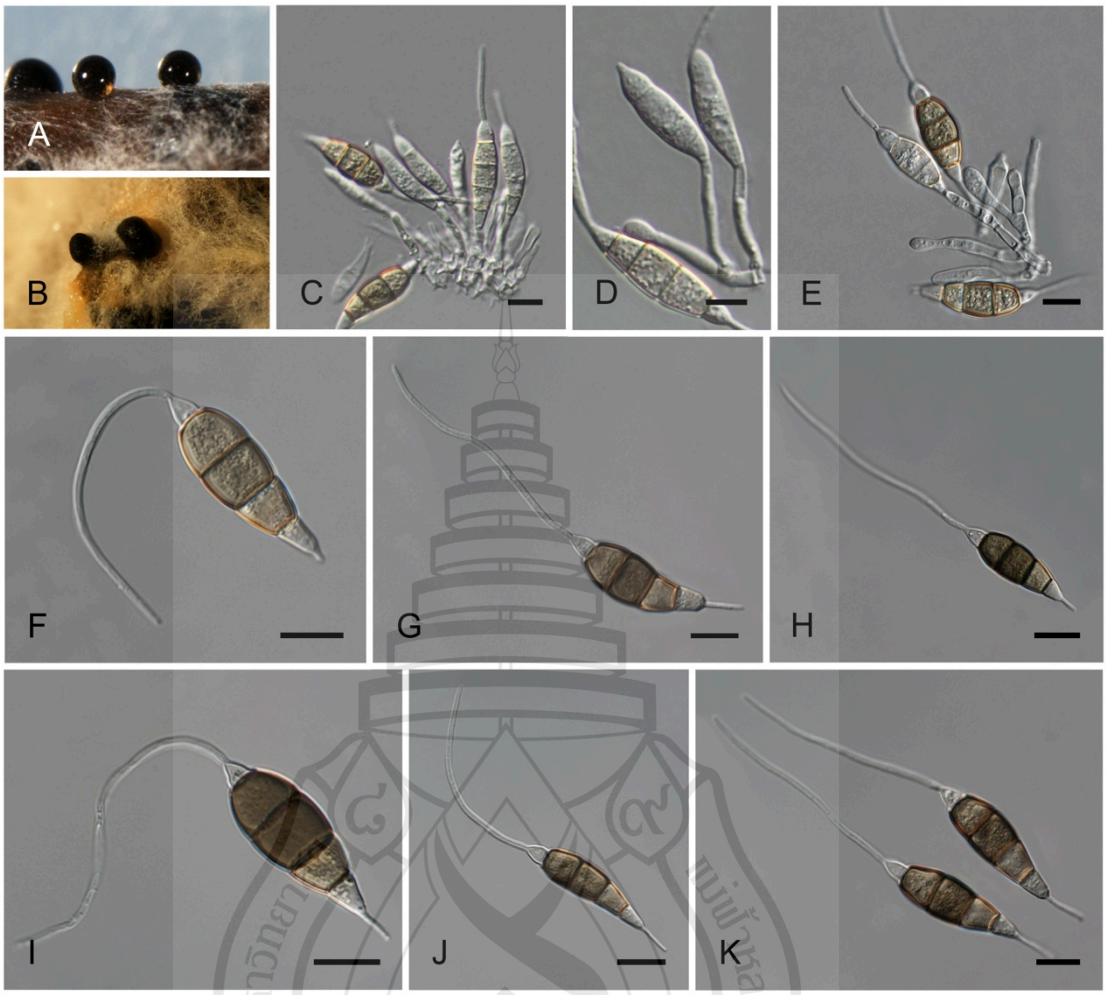
Culture characteristics: Colonies on PDA reaching 22–30 mm after 7 days at 25°C, edge rhisoid, white to pale honey-coloured, fruiting bodies black, gregarious; reverse of culture same colours.

Material examined: MALAYSIA, from stem of *Macaranga triloba* colonized by ants, Sept. 1999, W. Federle, (CBS H holotype, culture ex-type CBS 102220).

Notes: *Pestalotiopsis malayana* belongs to the *P. adusta* section, which is characterized by having two apical appendages. *Pestalotiopsis malayana* formed a distinct lineage in phylogenetic analyses in this complex. Furthermore, morphologically *P. malayana* is well distinguished from allied species by its larger conidia and longer apical appendages.

Pestalotiopsis monochaeta Maharachch. & Crous, sp. nov. (Figure 3.33 A–K).

Etymology: The name refers to the unique single apical appendage.



Note. *Pestalotiopsis monochaeta* CBS 144.97 [T]. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.33 *Pestalotiopsis monochaeta* (holotype)

Conidiomata pycnidial in culture on PDA, globose or clavate, aggregated or scattered, semi-immersed or partly erumpent, 250–500 µm diam; exuding a globose, dark brown to black conidial masses. *Conidiophores* septate, sparsely branched and sometimes reduced to conidiogenous cells, hyaline, smooth-walled, up to 50 µm long. *Conidiogenous cells* discrete or integrated, ampulliform to lageniform (4–12 × 2–4

µm) or cylindrical (10–60 × 2–8 µm), proliferating 2–4 times percurrently near apex, tapering to a long, thin neck, collarette present and not flared. *Conidia* ellipsoid, straight to slightly curved, 4-septate, slightly constricted at septa, (25–)27–40(–42) × 7–11(–11.5) µm, mean ± SD = 32.8 ± 3.5 × 9.6 ± 0.6 µm; basal cell conic to obconic with a truncate base, thin-walled, rugose, 5.5–9.5 µm long; three median cells (17–)18–25(–26) µm, mean ± SD = 21 ± 2 µm, doliiform, verruculose, concolorous, but occasionally the two upper median cells slightly darker than the lower median cell, (second cell from base 5–8.5 µm long; third cell 7–9 µm long; fourth cell 7–9 µm long); apical cell conic, hyaline, thin and smooth-walled, 4–6.5 µm long; with single, central, tubular apical appendage, unbranched, filiform, (40–)43–67(–75) µm, mean ± SD = 51 ± 6 µm; basal appendage single, tubular, unbranched, centric, 6–14 µm long.

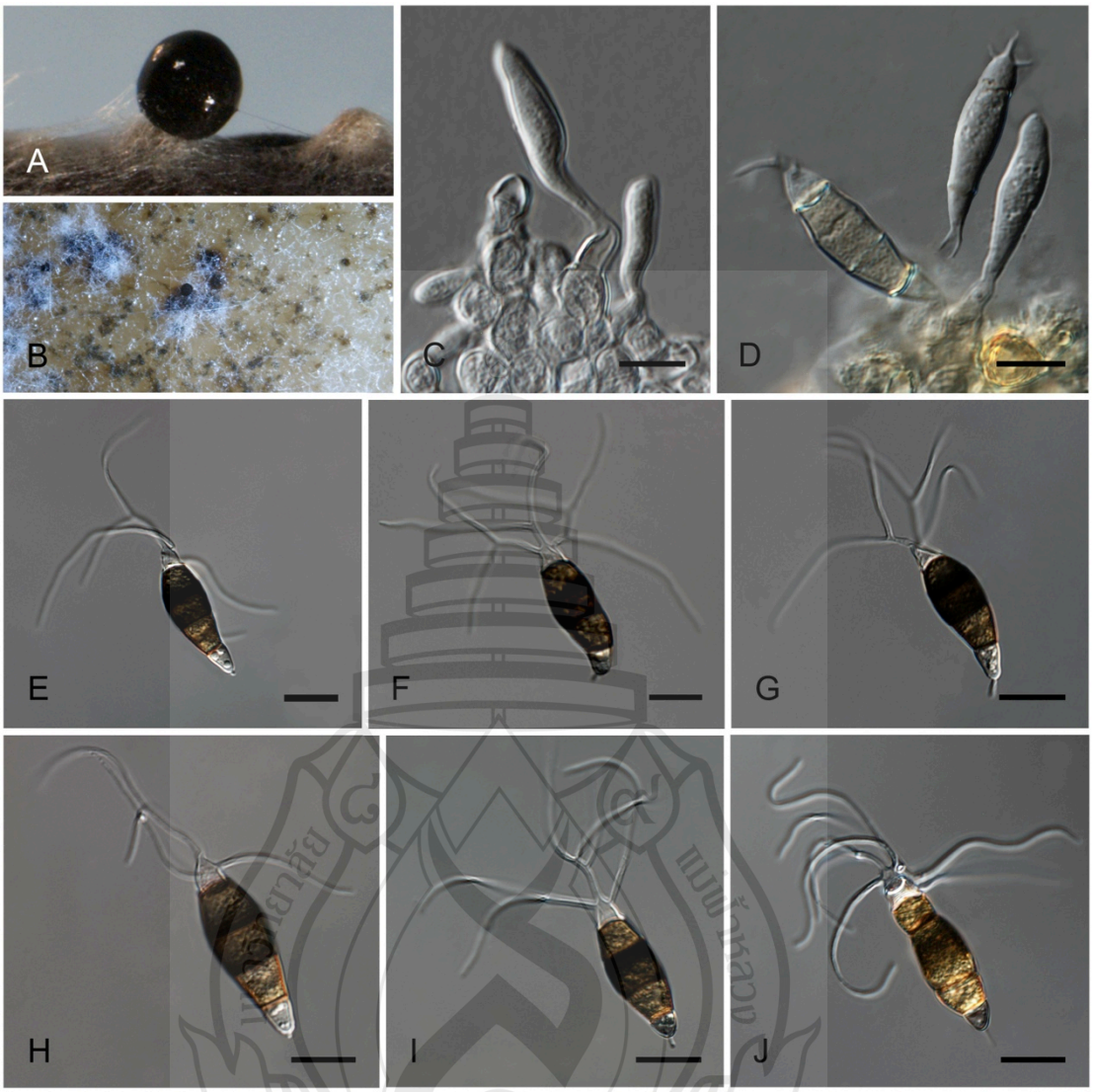
Culture characteristics: Colonies on PDA reaching 50–60 mm diam after 7 days at 25°C, with undulate edge, whitish to pale yellow-coloured, with dense, with aerial mycelium on surface, with black, gregarious fruiting bodies; reverse of colony same colours.

Material examined: NETHERLANDS, Baarn, Eemnesserweg, endophytes on branches of *Quercus robur*, July 1996, H.A. van der Aa (CBS H holotype, culture ex-type CBS 144.97); Netherlands, Baarn, Eemnesserweg 90, from *Taxus baccata,* 14 Apr. 1983, H.A. van der Aa, CBS H-14560, culture CBS 440.83=IFO 32686.

Notes: *Pestalotiopsis monochaeta* differs from all other species in the genus by having a single apical appendage. All other species in this section (*Pestalotiopsis brassicae, P. hollandica* and *P. verruculosa*) consist of more than two apical appendages. This species can easily be misidentified as *Monochaetia,* since it has borderline morphological features of both genera.

Pestalotiopsis novaehollandiae Maharachch. & Crous, sp. nov. (Figure 3.34 A–J).

Etymology: Named after the country where it was collected, New Holland, hence Australia.



Note. *Pestalotiopsis novaehollandiae* CBS 130973 ^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm

Figure 3.34 *Pestalotiopsis novaehollandiae* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary to aggregated, imbedded or semi-immersed, dark brown, 200–450 µm diam, exuding a globose, dark brown, glistening conidial masses. *Conidiophores* reduced to conidiogenous cells.

Conidiogenous cells discrete, simple, straight to curved, lageniform, smooth, thin-walled, hyaline, 5–20 × 5–10 µm. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4-septate, (24–)25–31(–32) × (7.5–)8–10(–10.5) µm, mean ± SD = 28.1 ± 1.6 × 9 ± 0.7 µm; basal cell obconic with truncate base, hyaline or slightly olivaceous, thin-walled and rugose, 4–7 µm long; three median cells (16–)16.5–20.5(–21) µm long, mean ± SD = 19 ± 1.3, doliiform to subcylindrical, verruculose, concolorous, olivaceous, constricted at the septa, (second cell from base 6–8 µm long; third cell 6–7 µm long; fourth cell 5–7 µm long); apical cell hyaline, conic to cylindrical, hyaline, thin and smooth-walled, 3–5 µm long; with 3–9 tubular apical appendages, arising not in an apical crest, but each inserted at a different locus in the upper half of the cell, unequal in length, some appendages branched, filiform, flexuous, (20–)22–44(–50) µm long, mean ± SD = 31 ± 9 µm; basal appendage single, tubular, unbranched, centric, 2–5 µm (Figure 3.34).

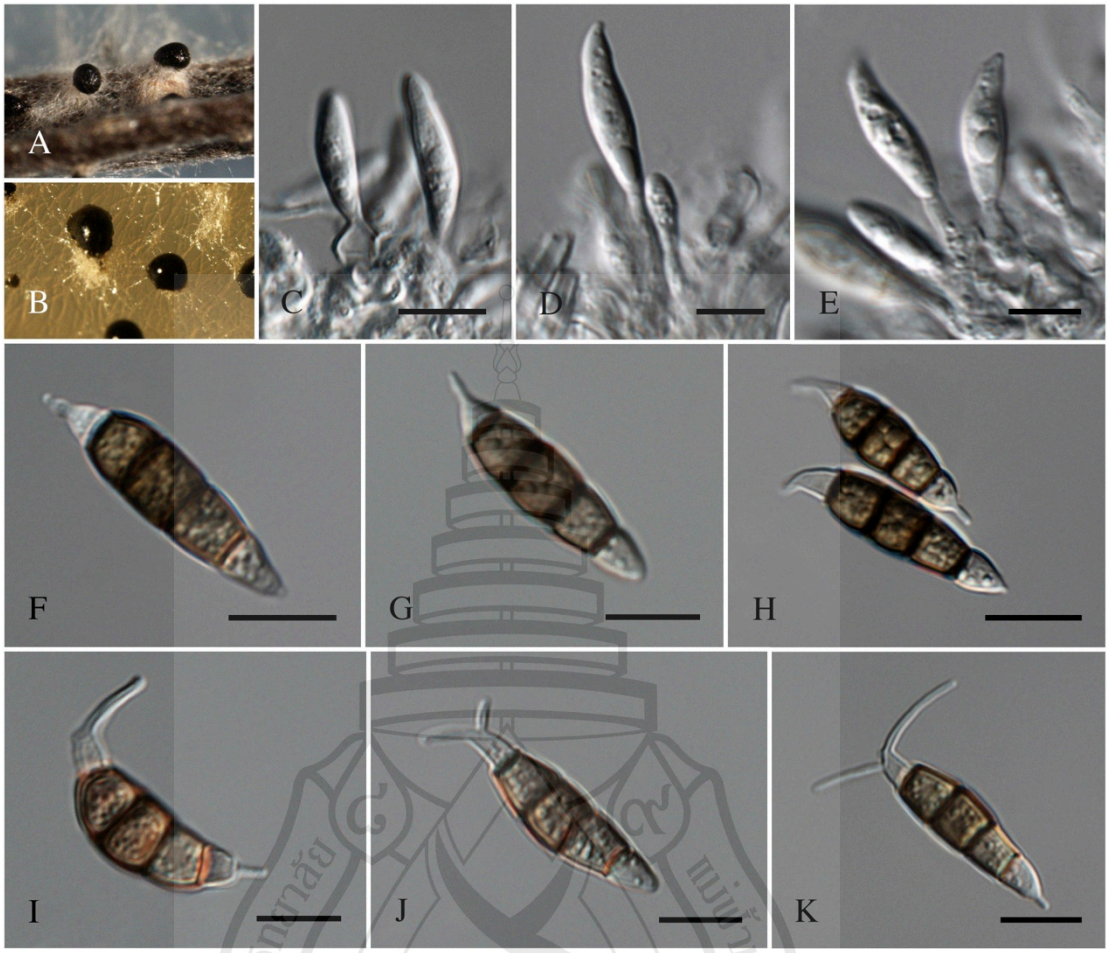
Culture characteristics: Colonies on PDA reaching 50–80 mm diam after 7 days at 25°C, undulated at the edge, whitish to pale yellow-coloured, with dense aerial mycelium on surface, forming black, gregarious fruiting bodies; reverse similar in colour.

Material examined: AUSTRALIA, Perth, Jarrah Forest, from old inflorescence of *Banksia grandis*, 2010, W. Gams (CBS H holotype, culture ex-type CBS 130973).

Notes: This species is characterised species in *P. furcata* section by a large number of apical appendages and having a short basal appendage. Species such as *P. camelliae* and *P. furcata* have higher number of apical appendages like those in *P. novaehollandiae,* but they lack a basal appendage. *P. novaehollandiae* is sister to *P. portugalica*, which has rather smaller conidia (15–21 × 5–7 µm), and a lower number of apical appendages (1–3).

Pestalotiopsis papuana Maharachch. & Crous, sp. nov. (Figure 3.35 A–K).

Etymology: Named after the country where it was collected, Papua New Guinea.



Note. *Pestalotiopsis papuana* CBS 331.96^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.35 *Pestalotiopsis papuana* (holotype)

Conidiomata pycnidial, globose to clavate, aggregated or scattered, semi-immersed on PDA, dark brown to black, 100–500 µm diam; exuding globose, dark brown conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, lageniform to subcylindrical, hyaline, smooth, proliferating once or twice, 4–20 × 2–4 µm; apex with minute periclinal thickening and flaring collarettes. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-

euseptate, (17–)18–22(–24) × 6–7.5 µm, mean ± SD = 20.5 ± 1.5 × 6.7 ± 0.3 µm; basal cell obconic with a truncate base, hyaline, thin and wall verruculose, 3–5 µm long; three median cells doliiform, 12–15 µm long, mean ± SD = 13.6 ± 0.7, wall verruculose, concolorous, brown, septa darker than the rest of the cell, (second cell from the base 3.5–5.5 µm; third cell 4.5–5.5 µm; fourth cell 4.5–6 µm); apical cell 2–4 µm long, hyaline, cylindrical to subcylindrical, thin and wall rugose; with 1–2 tubular apical appendages, arising from the apical crest, unbranched, filiform, 1.5–7 µm long, mean ± SD = 4.1 ± 1; basal appendage single, tubular, unbranched, centric, 0.5–2 µm long (Figure 3.35).

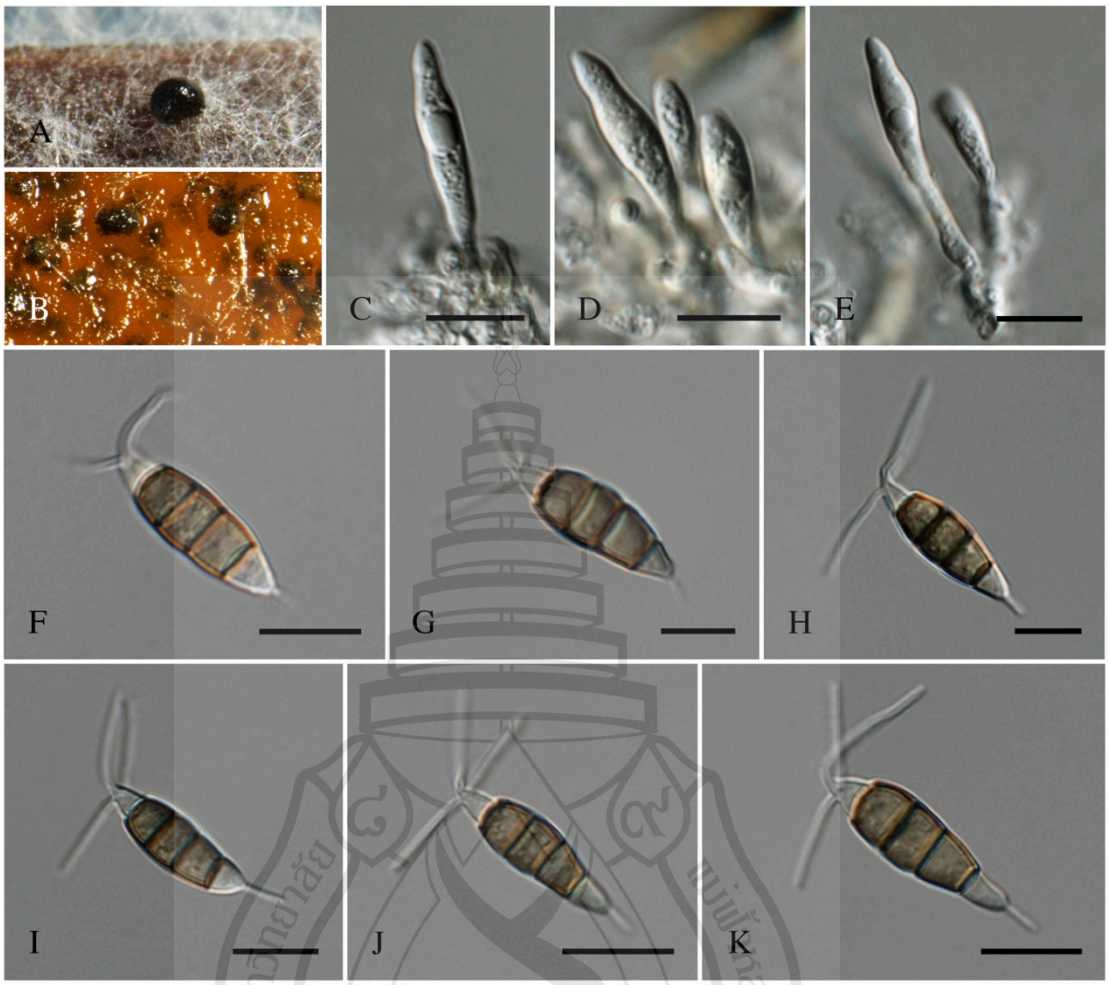
Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with undulate edge, pale honey-coloured, with medium sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: PAPUA NEW GUINEA, from soil along the coast, November 1995, A. Aptroot, (CBS H holotype, culture ex-type CBS 331.96); Papua New Guinea, from leaves of *Cocos nucifera* (coastal primary forest), 27 October 1995, A. Aptroot, culture CBS 887.96.

Notes: *Pestalotiopsis papuana* is genetically close to *P. adusta* and *Pestalotiopsis* spp. isolates CBS 263.33 (*Rhododendron ponticum* in Netherlands) and 264.33 (*Cocus nucifera* in Sulawesi). The latter two isolates were maintained as *Pestalotiopsis* spp. since both cultures were sterile, making morphological comparisons impossible. Morphologically, however, *P. papuana* is quite distinct from *P. adusta* by having a larger conidia and shorter apical appendages.

Pestalotiopsis parva Maharachch. & Crous, sp. nov. (Figure 3.36 A–K).

Etymology: The epithet *parva* refers to the smaller conidial size of this species.



Note. *Pestalotiopsis parva* CBS 278.35^{T}. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–I. Conidia. Scale bars = 10 µm

Figure 3.36 *Pestalotiopsis parva* (holotype)

Conidiomata pycnidial, globose, aggregated or scattered, dark brown to black, semi-immersed on PDA, 100–200 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical to subcylindrical, hyaline, smooth-walled, simple, proliferating 2–3 times percurrently, 5–18 × 2–4 µm, apex 1–1.5 µm diam.

Conidia fusiform, straight to slightly curved, 4-septate, (16–)16.5–20(–21) × 5–7(–7.5) µm, mean ± SD = 18.3 ± 1.2 × 6.2 ± 0.5 µm; basal cell obconic to conic with a truncate base, hyaline, thin and smooth-walled, 3–5 µm long; three median cells doliiform, (10–)10.5–13(–13.5) µm long, mean ± SD = 12.1 ± 1.0, wall minutely verruculose, concolorous, pale brown, septa darker than the rest of the cell, (second cell from the base 3.5–5 µm long; third cell 3.5–4.5 µm long; fourth cell 4–5 µm long); apical cell (2–)2.5–4 µm long, hyaline, subcylindrical; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, (6–)6.5–12(–13) µm long, mean ± SD = 9.0 ± 1.9; basal appendage single, tubular, unbranched, centric, 2–4 µm long (Figure 3.36).

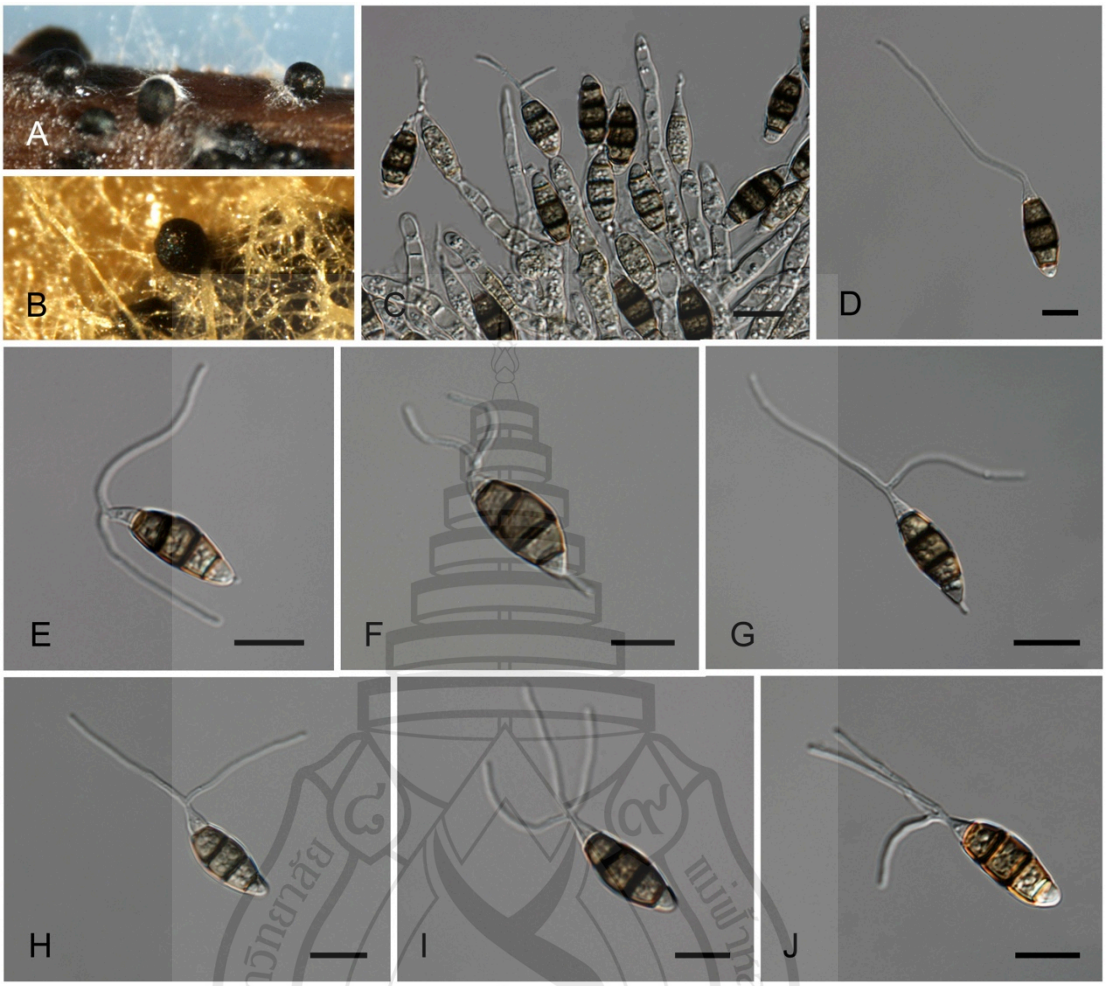
Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with smooth edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: UNKNOWN COUNTRY, from *Leucothoe fontanesiana*, 1935, R.P. White, (CBS H- 15694 holotype, culture ex-type CBS 278.35); unknown country, from *Delonix regia*, H.W. Wollenweber, CBS H-15659, culture CBS 265.37=BBA 2820.

Notes: *Pestalotiopsis parva* is a single species in the *P. parva* section. *P. rosea* is the sister species belongs to the *P. rosea* section, which isolated as endophytes from living leaves of *Pinus* sp. collected in China. *P. parva* is morphologically close to *P. rosea,* but later species differ in having distantly longer apical appendages which are some times branched. Furthermore, the reddish colony is unique to the *P. rosea* and this can be found even in conidiogenous cells and some conidia.

Pestalotiopsis portugalica Maharachch. & Crous, sp. nov. (Figure 3.37 A–J).

Etymology: Named after the country where it was collected, Portugal.



Note. *Pestalotiopsis portugalica* CBS 393.48^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C. Conidiogenous cells. D–J. Conidia. Scale bars = 10 µm

Figure 3.37 *Pestalotiopsis portugalica* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary or aggregated, black, semi-immersed, 200–400 µm diam; releasing brown to black, slimy, globose conidial masses. *Conidiophores* hyaline, septate, irregularly branched, up to 100 µm long. *Conidiogenous* cells cylindrical, hyaline, smooth, proliferating 2–6 times percurrently, 10–60 × 4–12 µm, collarette present and not flared, with prominent

periclinal thickening. *Conidia* fusoid, straight to slightly curved, 4–septate, (14.5–)15.5–20(–21.5) × 5–7 µm, mean ± SD = 17.9 ± 1.6 × 6.0 ± 0.5 µm; basal cell obconic with a truncate base, hyaline, thin- and smooth-walled, 2.5–4 µm long; three median cells (9–)9.5–13.5(–14) µm long, mean ± SD = 11.7 ± 1 µm, doliiform to subcylindrical, with thick verruculose walls, constricted at the septa, concolorous, pale brown, (second cell from base 3–5 µm long; third cell 3.0–5 µm long; fourth cell 3.5–5 µm long); apical cell conic to cylindrical, hyaline, thin and smooth-walled, 2–5 µm long; 1–3 tubular apical appendages arising from an apical crest or branched irregular along their length resulting 2–3 branched, filiform, (8–)9–18(–20) µm long, mean ± SD = 14 ± 3 µm; basal appendage lack or when present single, tubular, unbranched, centric, 1–4 µm long (Figure 3.37).

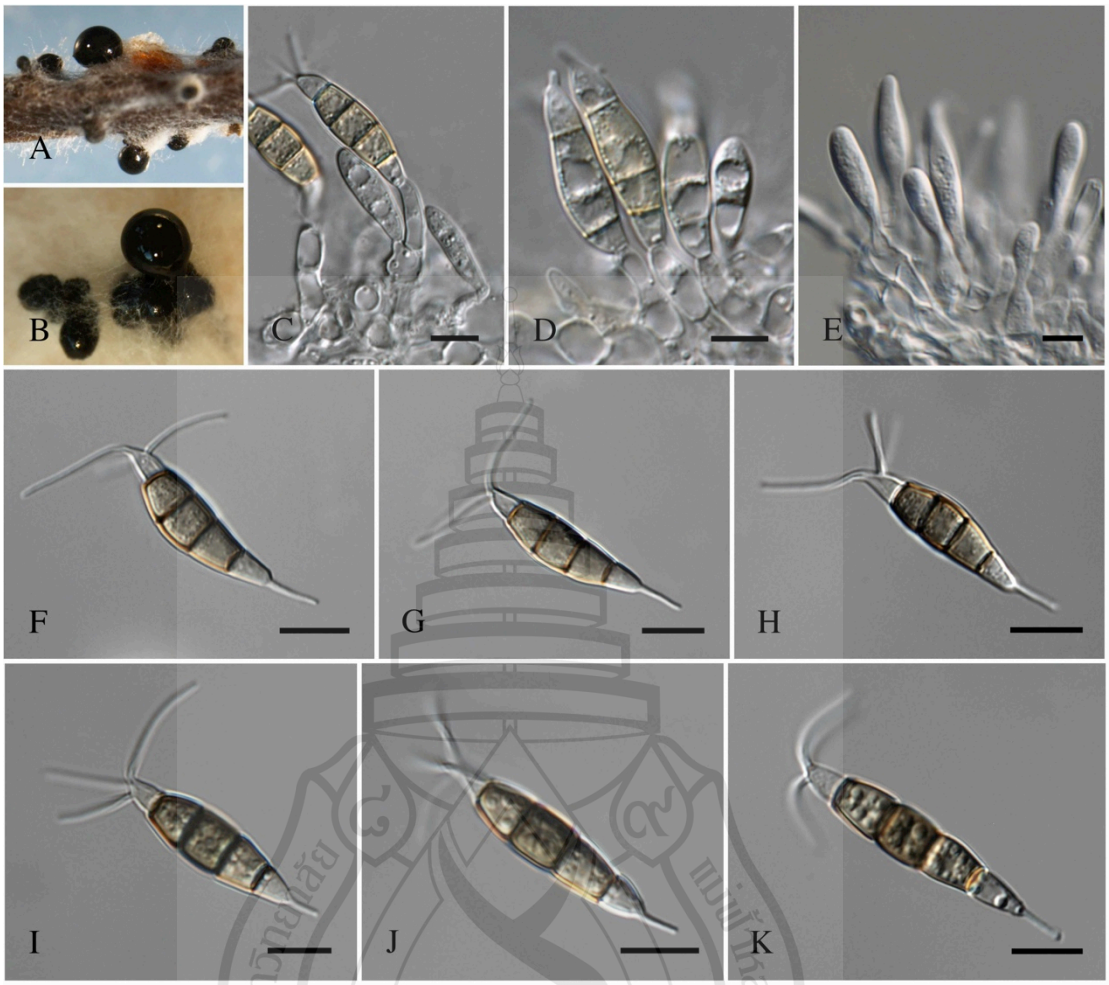
Culture characteristics: Colonies on PDA reaching 60–70 mm diam after 7 days at 25°C, edge entire, whitish to pale honey-coloured, aerial mycelium on surface, fruiting bodies black, gregarious; reverse similar in colour.

Material examined: PORTUGAL, unknown host, June 1948, collector unknown (CBS H holotype, culture ex-type CBS 393.48).

Notes: *Pestalotiopsis portugalica* is a distinct species in terms of morphology and phylogeny. It differs from its phylogenetically related species *P. camelliae*, *P. furcata* and *P.novaehollandiae* by smaller conidia and lower number of apical appendages. Its conidial size overlaps with *P. rosea* (17.5–21.8 × 5.7–7 µm), but those two species cluster in two distinct sections.

Pestalotiopsis proteacearum Maharachch. & Crous, sp. nov. (Figure 3.38 A–K).

Etymology: Named after the host family from which it was isolated, *Proteaceae.*



Note. *Pestalotiopsis proteacearum* CBS 111522^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.38 *Pestalotiopsis proteacearum* (holotype)

Conidiomata pycnidial in culture on PDA, globose to clavate, aggregated or scattered, dark brown to black, semi-immersed or partially erumpent, up to 300 µm diam; releasing globose, dark brown to black conidial masses. *Conidiophores* sparsely septate at base, branched or unbranched, subcylindrical, hyaline, smooth, up to 20 µm. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth, proliferating 2-5 times percurrently, 10–25 × 3–7 µm. *Conidia* fusiform, ellipsoid to

subcylindrical, straight to slightly curved, 4-euseptate, (23–)24.5–29(–30) × 6–8 µm, mean ± SD = 26.9 ± 1.4 × 7 ± 0.2 µm; basal cell obconic to conic with a truncate base, hyaline, thin and wall verruculose, 4.5–6.5 µm long; three median cells doliiform, (14–)16–18.5(–19) µm long, mean ± SD = 17 ± 1.3, wall minutely verruculose, concolorous or middle median cell is much darker than other cell, olivaceous, septa darker than the rest of the cell, (second cell from the base 5–7 µm; third cell 5.5–7 µm; fourth cell 5–6.5 µm); apical cell 3.5–5 µm long, hyaline, cylindri cal to subcylindrical, thin and smooth-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (9–)18–27(–17) µm long, mean ± SD = 12.9 ± 1.7; basal appendage single, tubular, unbranched, centric, 3–6 µm long (Figure 3.38).

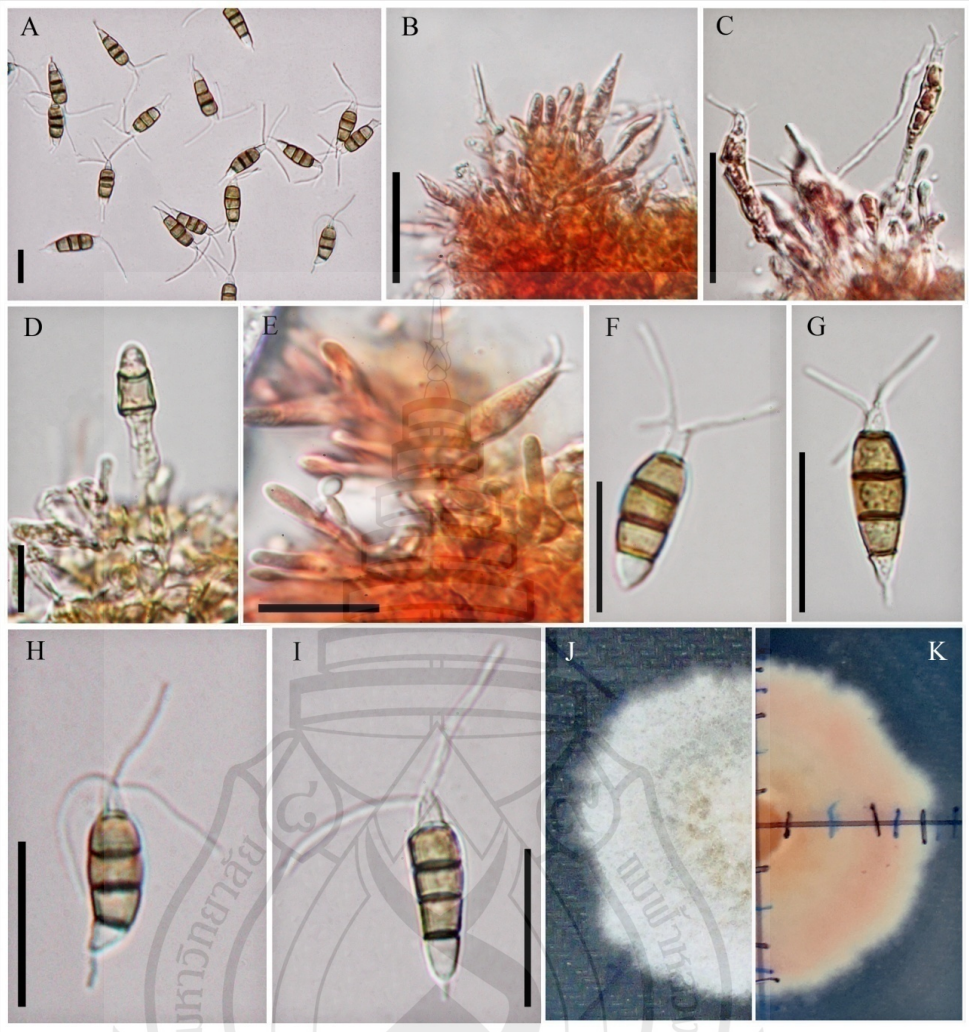
Culture characteristics: Colonies on PDA attaining 35–45 mm diam after 7 days at 25°C, with undulate edge, convex with papilate surface, hyaline to pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse pale honey-coloured.

Material examined: USA, Hawaii, from *Telopea* sp. (introduced from Australia), 8 December 1998, P.W. Crous & M.E. Palm, (CBS H holotype, culture ex-type CBS 111522 = STE-U 2083); Denmark, from seeds of *Oryza sativa,* S.B. Mathur, culture CBS 353.69; Italy, Dec. 1926, R. Ciferri, culture CBS 171.26.

Notes: *Pestalotiopsis proteacearum* has overlapping conidial morphology with species in the *P. trachicarpicola* section, which is characterized by having conidia with two basal appendages. However, *P. proteacearum* is genetically and geographically clearly distinct species from *P. trachicarpicola* section.

Pestalotiopsis rosea Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 118 (2012), MycoBank: MB 800521 (Figure 3.39 A– K).

Etymology: The specific epithet is based on the Latin roseus in reference to the rose-colored, colony of this species.



Note. A. Conidia. B–E. Conidiophores/ conidiogenous cells. F–I Conidia. J. K. Colony on PDA, J from above, K from below. Scale Bars: A– I= 20 µm

Figure 3.39 *Pestalotiopsis rosea* (holotype)

Conidiophores septate, unbranched, up to 20 µm long, often reduced to conidiogenous cells, smooth walled; Conidiogenous cells discrete, ampulliform to lageniform, smooth, thin-walled, slightly red, rarely hyaline, with 2–3 proliferations. Conidia fusoid to ellipsoid, straight to slightly curved, 4–septate, 17.5–21.8 × 5.7–7 µm (mean = 19.2 × 6.2 µm), basal cell obconic, hyaline, thin- and verruculose, 3.1–4

µm long (mean = 3.6 µm), with three median cells, doliiform to subcylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous with slightly red, septa and periclinal walls darker than the rest of the cell, wall rugose, together 11.8–13.8 µm long (mean = 12.9 µm) (second cell from base 4–5.3 µm (mean = 4.5 µm); third cell 3.3–5.1 µm (mean = 4.3 µm); fourth cell 4.2–5.4 µm (mean = 4.7 µm); apical cell hyaline, conic to cylindrical 2.6-4.2 µm long (mean = 3.3 µm); with 1–3 tubular apical appendages, some appendages branched, arising from the apex of the apical cell, 14–22 µm long (mean = 16.5 µm); basal appendage present 2–5.7 µm (mean = 4.1 µm), rarely absent (Figure 3.39).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 27 days at 25°C, edge undulate, whitish or pale red, with dense, aerial mycelium on surface, with black to reddish brown fruiting bodies, gregarious; reverse of culture white or slightly red to red.

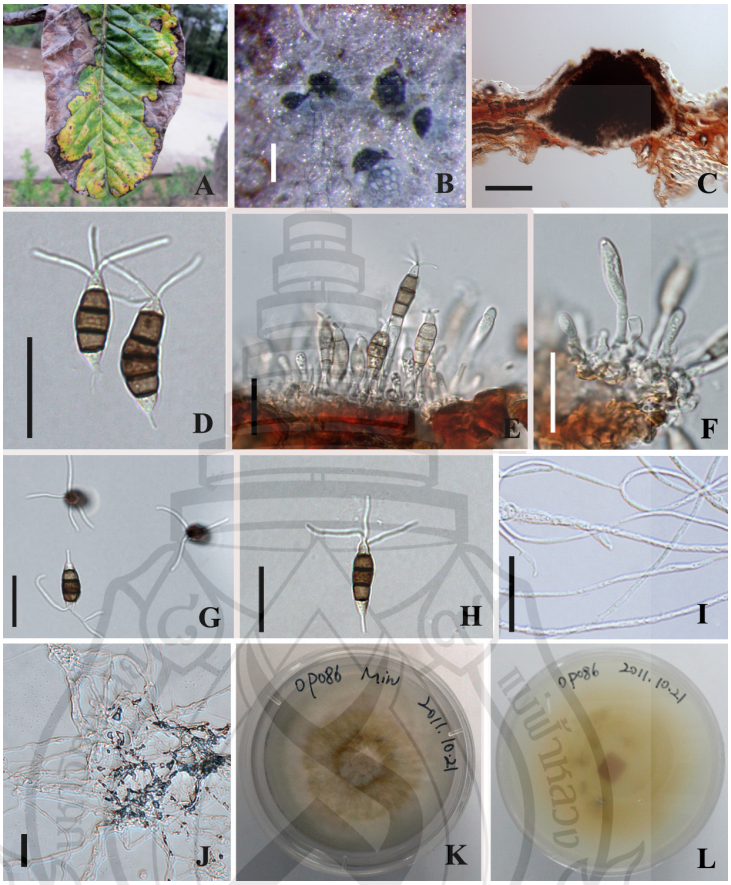
Habitat/Distribution: Endophyte on living leaves of *Pinus* sp.*,* Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of Pinus sp., 19 March 2002, Wenping Wu KBG25-3 (HMAS047135, holotype; MFLU12-0409, isotype; ex-type living culture NN047135 = MFLUCC 12-0258).

Notes: *P. rosea is* a distinct species in the genus. The reddish colony is unique to the species and this can be found even in conidiogenous cells and in some conidia. This species was quite similar to the type species of the genus *Pestalotiopsis*; *P. guepinii* (Desm.) Steyaert (Guba, 1961; Nag Raj, 1993) isolated from *Camellia japonica*. In *P. guepini,* conidia are 14–21 × 5.5–6.6 µm, with 1–3 apical appendages that are sometimes knobbed at their apices. However, in *P. rosea* apical appendages are not knobbed and according to Guba (1961) *P. guepinii* is restricted to *Camellia* specie.

Pestalotiopsis rhododendri Y. M. Zhang, Maharachch. & K. D. Hyde, sp. nov. MycoBank: MB 803237 (Figure 3.40 A–L).

Etymology: The specific epithet is based on the host genus *Rhododendron*, which the fungus was isolated.



Note. A. *Pestalotiopsis rhododendri* associated with leaf blight on leaves of *Rhododendron sinogrande*. B. Acervuli, splitting irregularly. C. Section of acervulus. E, F. Conidiogenous cells D, G, H. Conidia. I. Mycelium, hyaline, no septum. J. The black mucilage with a mass of conidia. K. *Pestalotiopsis rhododendri* colony on PDA from above. L. *Pestalotiopsis rhododendri* colony on PDA from below. Scale Bars: B. – C. = 100 µm, D. – J. = 20 µm

Figure 3.40 *Pestalotiopsis rhododendri* (holotype)

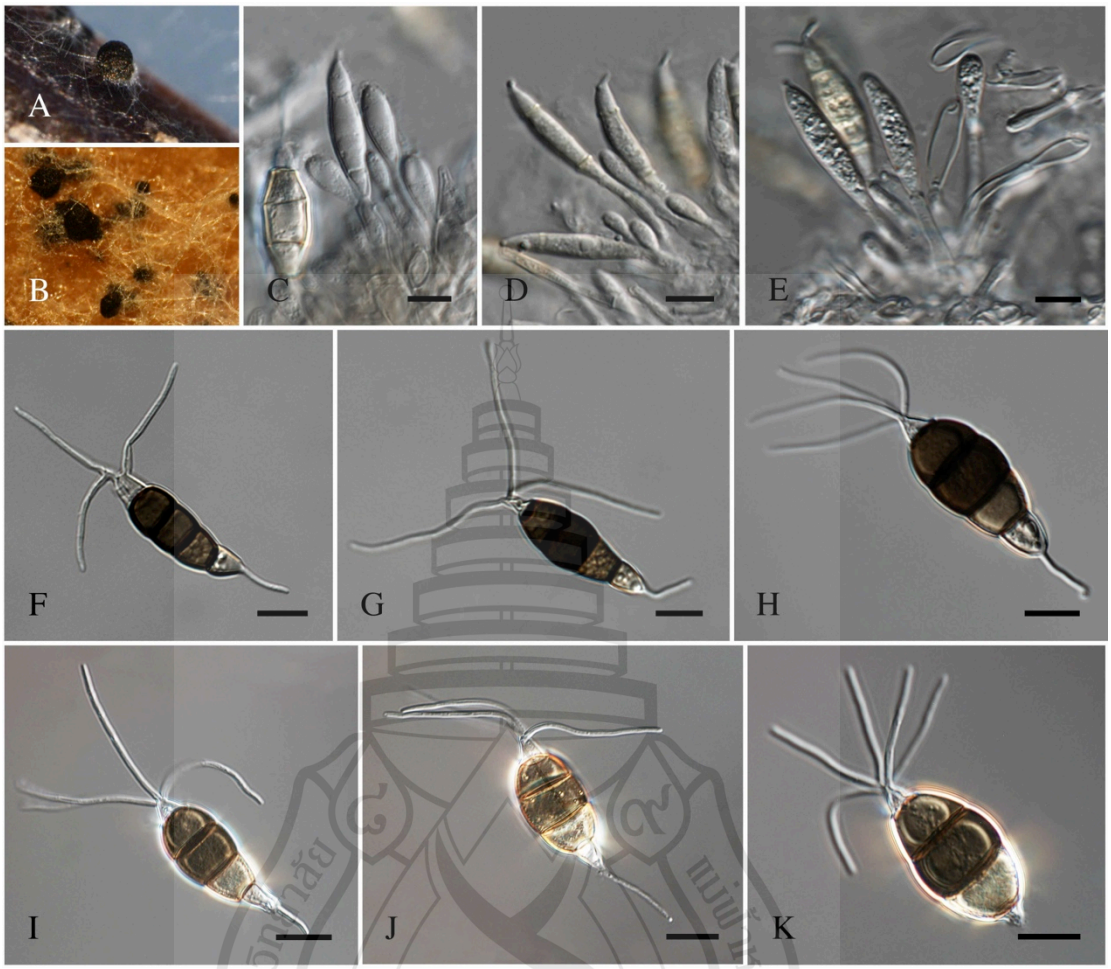
Associated with dead parts of living leaves of *Rhododendron sinogrande*. Sexual state not observed. Asexual state: *Acervuli* 50–190 × 50–140 μm in diam, black, epidermal to subepidermal, separate or confluent, dehiscence irregularly. *Conidiophores* indistinct. *Conidiogenous cells* discrete, simple, short, filiform. *Conidia* 18–27 × 5–8 μm (mean = 21 × 7 μm), fusiform, straight to slightly curved, 4-septate; basal cell conical to acute, hyaline, thin and smooth-walled, 3–5 μm long (mean = 4 μm), with three median cells, doliiform to cylindrical, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 12–18 μm long (mean = 15 μm) (second cell from base 4–6 μm (mean = 4.7 μm); third cell 4–6 μm (mean = 4.6 μm); fourth cell 4–6 μm (mean = 4.6 μm); apical cell hyaline, conical, 3-5 μm long (mean = 4 μm); with 3 tubular apical appendages, arising from the apex of the apical cell, knobbed at the end, 7–21 μm long (mean = 14 μm), unequal; basal appendage present 2–6 μm (mean = 4 μm) (Figure 3.40).

Colonies on PDA reaching 7 cm diam. after 5 days at 25 °C, with entire edge, whitish and with time change in to pink, with aerial mycelium on surface; fruiting bodies black, gregarious; reverse of culture whitish to pale yellow.

Notes: *Pestalotiopsis rhododendri* (18–27 × 5–8 μm) has an overlapping conidial size with *P. clavata* (20–27 × 6.5–8 μm), but it has shorter apical appendages (7–15 μm) than *P. clavata* (20–25 μm) and it is also separated in DNA phylogeny.

Pestalotiopsis scoparia Maharachch. & Crous, sp. nov. (Figure 3.41 A–K).

Etymology: The epithet *scoparia* refers to the broom-shaped apical appendages of this species.



Note. *Pestalotiopsis scoparia* CBS 176.25^{T}. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.41 *Pestalotiopsis scoparia* (holotype)

Conidiomata pycnidial, globose, aggregated or scattered, semi-immersed on PDA, dark brown to black, 100–400 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical to subcylindrical, hyaline, smooth, proliferating up to 3 times, 10–30 × 2–4 µm, with visible periclinal thickening; collarette slightly flared, up to 3 µm long when present. *Conidia* fusiform, ellipsoid,

straight to slightly curved, 4-euseptate, (22–)23.5–29(–31) × 6–8.5 µm, mean ± SD = 26.3 ± 2 × 7.4 ± 0.3 µm; basal cell hemispherical to obconic with a truncate base, hyaline, thin and wall verruculose, 4–6 µm long; three median cells doliiform, 15.5–19.5 µm long, mean ± SD = 17 ± 1, wall verruculose, concolorous, but occasionally the two upper median cells darker than the lower median cell, brown, septa darker than the rest of the cell, (second cell from the base 5–6.5 µm long; third cell 5–7 µm long; fourth cell 5.5–7.5 µmlong); apical cell 4.5–6 µm long, hyaline, subcylindrical, thin and wall rugose; with 3–5 tubular apical appendages, arising from the apical crest, unbranched, filiform, (20–)23–35(–42) µm long, mean ± SD = 29.6 ± 4; basal appendage single, tubular, unbranched, centric, 9–25 µm long (Figure 3.41).

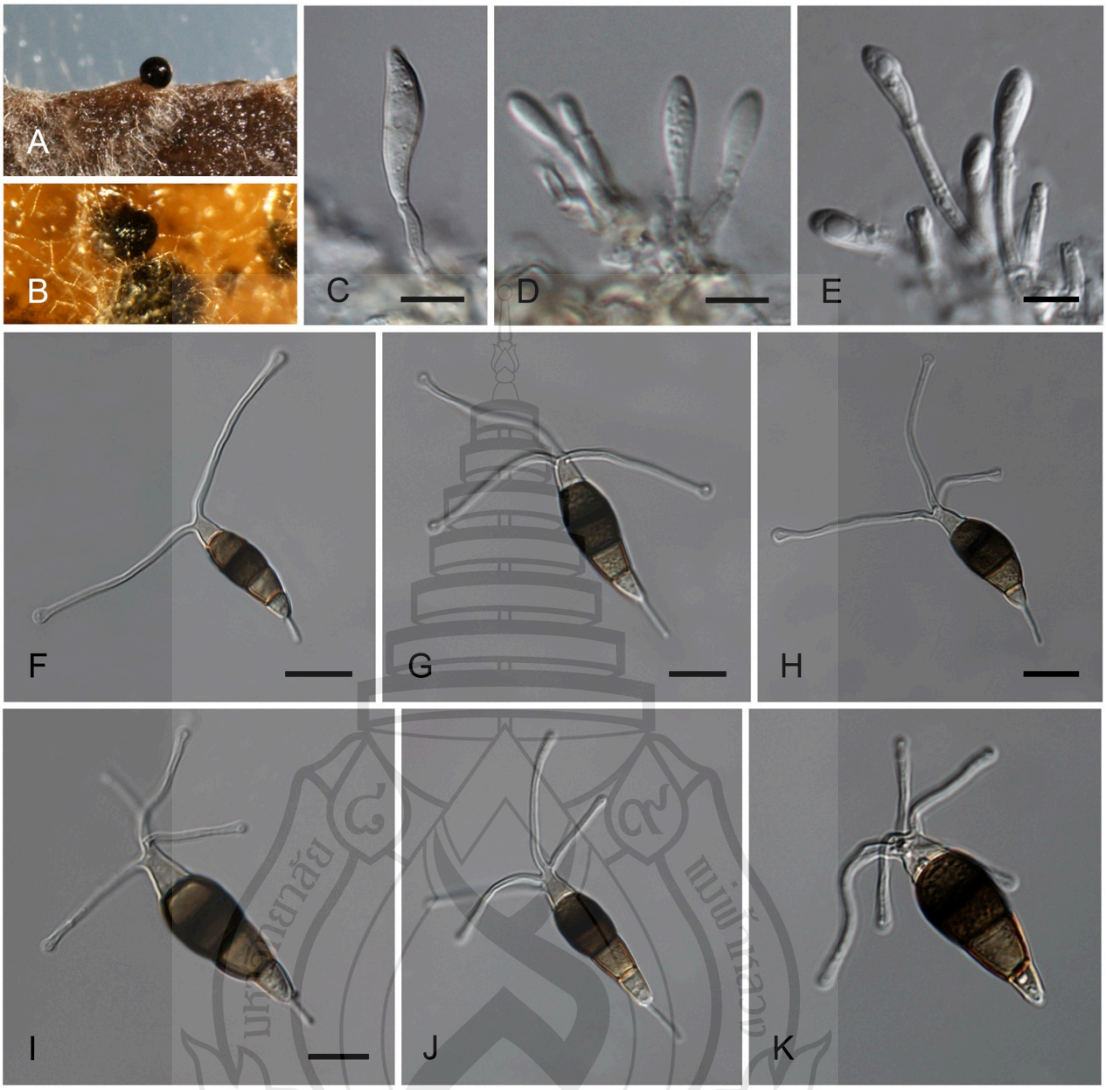
Culture characteristics: Colonies on PDA attaining 35–45 mm diam after 7 days at 25°C, with smooth edge, pale honey-coloured, with medium dense aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: UNKNOWN COUNTRY, from young *Chamaecyparis* sp., 'Retinospora', May 1925, C.M. Doyer, (CBS H holotype, culture ex-type CBS 176.25).

Notes: *Pestalotiopsis scoparia* is genetically a clearly distinct species in *P. australis* section, forming a separate clade in sister position to *P. australis* and *P. unicolor* in *P. linearis* section. It is well characterized by forming usually rather long broom-shaped, 3 to 5 apical appendages, long basal appendages and occasionally by having versicoloured median cells.

Pestalotiopsis spathulata Maharachch. & Crous, sp. nov. (Figure 3.42 A–K).

Etymology: the species epithet refers to the knobbed nature of the apical appendages.



Note. *Pestalotiopsis spathulata* CBS 356.86 [T]. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K Conidia. Scale bars = 10 µm

Figure 3.42 *Pestalotiopsis spathulata* (holotype)

Conidiomata pycnidial, globose, aggregated or scattered, semi-immersed to erumpent or imbedded on PDA, dark brown to black, 100–400 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* 0–2 septate, branched at base, subcylindrical, often reduced to conidiogenous cells, hyaline, smooth-walled up

to 20 µm long. *Conidiogenous cells* discrete, ampulliform to lageniform or cylindrical, proliferating 2–5 times percurrently, wide at the base, tapering to a long, thin neck, 5–40 × 2–8 µm, prominent periclinal thickening with flaring collarettes. *Conidia* fusoid, straight to slightly curved, 4-septate, (24–)25–31(–32) × 7.5–9.5 µm, mean ± SD = 27.7 ± 2 × 8.6 ± 0.3 µm, slightly constricted at septa; basal cell conic to obconic with a truncate base, thin-walled and rugose, 5–7.5 µm long; three median cells, (13–)14–19.5(–20) µm, mean ± SD = 17.1 ± 1.8 µm, doliiform, verruculose, dark brown to olivaceous, versicoloured, (second cell from base pale brown to olivaceous, 4.5–7 µm, third cell honey brown, 4.5–6 µm long; fourth cell honey brown, 5.5–7 µm long); apical cell cylindrical, hyaline, thin and smooth-walled, 5–6 µm long; with 2–5 tubular apical appendages, arising not in an apical crest, but each inserted at a different locus in the upper half of the cell, swollen at the tip, filiform, flexuous, some appendages branched, (17–)18–24(–25) µm, mean ± SD = 21.1 ± 1.7 µm; basal appendage single, tubular, unbranched, centric, 4–7 µm long (Figure 3.42).

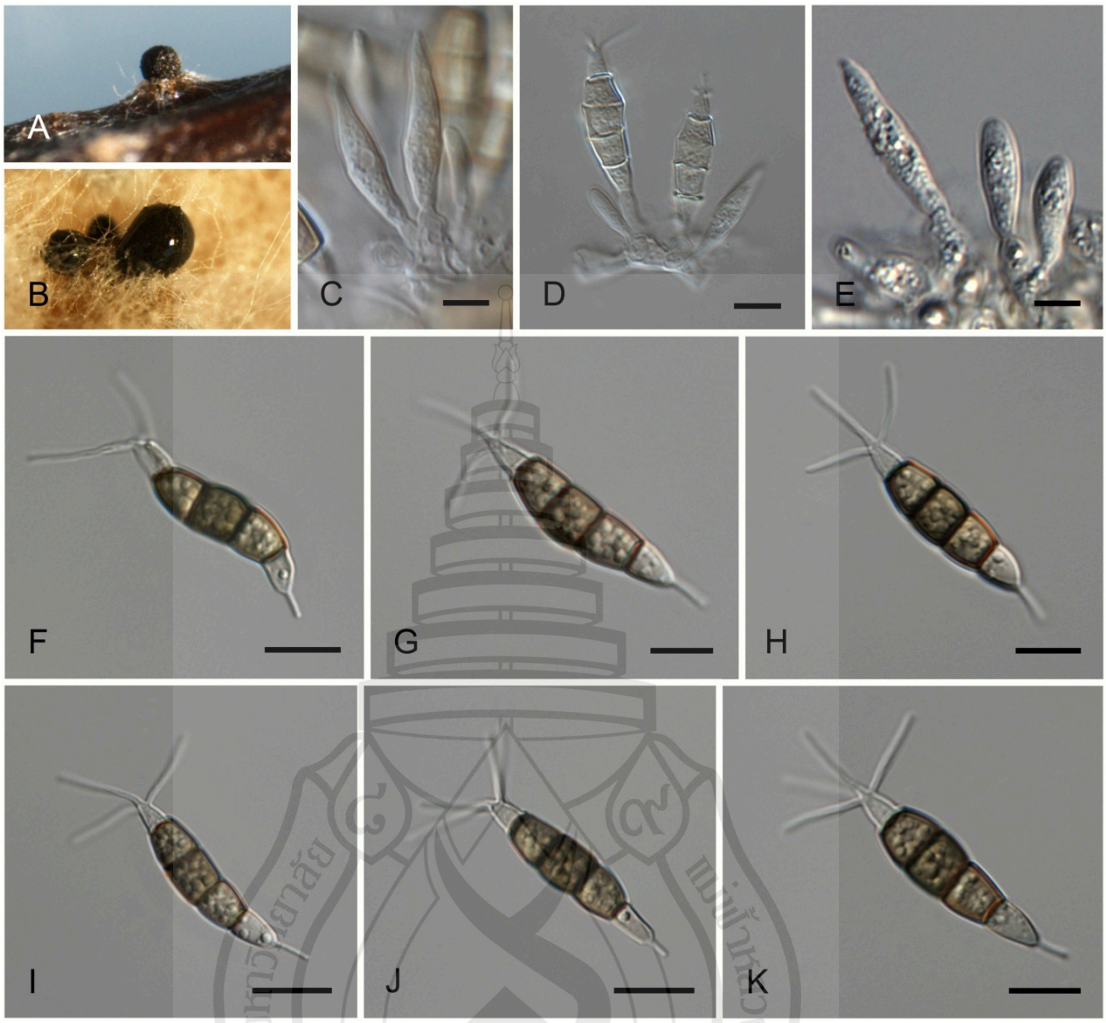
Culture characteristics: Colonies on PDA reaching 50–60 mm diam after 7 days at 25°C, with undulate edge, whitish, with dense, aerial mycelium on surface, fruiting bodies black, gregarious; reverse similar in colour.

Material examined: CHILE, leaf spot on *Guevina avellana*, Sept. 1961, unknown collector (CBS H holotype, culture ex-type CBS 356.86).

Notes: *Pestalotiopsis spathulata* is morphologically distinct and the distinction is also shown in its DNA phylogeny. Especially, the two upper median cells in *P. spathulata* are darker than the lower median cell. This is also seen in its sister species *P. gaultheria*. *P. gaultheria* however differs from *P. spathulata* by having fewer (–3), and long apical appendages (13–54 µm).

Pestalotiopsis telopea Maharachch. & Crous sp. nov. (Figure 3.43 A–K).

Etymology: Named after the host genus, *Telopea.*



Note. *Pestalotiopsis telopea* CBS 114161^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 3.43 *Pestalotiopsis telopea* (holotype)

Leaf spots on *Telopea* sp. circular to subcircular, up to 2 cm diam, amphigenous, pale to medium brown with a broad, dark brown border, which can be conspicuously raised in some leaf spots. *Conidiomata* pycnidial in culture on PDA, globose, aggregated or scattered, semi-immersed, dark brown to black, up to 500 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores*

indistinct, often reduced to conidiogenous cells. *Conidiogenous* cells discrete, ampulliform or lageniform, hyaline, smooth, proliferating 2-4 times percurrently, 5–15 × 2–9 µm, collarette present and not flared. Conidia fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (24–)24.5–31(–32) × 6–8 µm, mean ± SD = 27 ± 1.5 × 7 ± 0.3 µm; basal cell obconic, hyaline, thin and verruculose, 4.5–7 µm long; three median cells doliiform, (15–)16–18.5(–19) µm long, mean ± SD = 17.1 ± 1, wall verruculose, concolorous, brown to olivaceous, (second cell from the base 4.5–7 µm long; third cell 5–7.5 µm long; fourth cell 5–7 µm long); apical cell 3.5–5.5 µm long, hyaline, subcylindrical; with 2–4 tubular apical appendages (mostly 3), arising from an apical crest, unbranched, filiform, (7–)8–15(–16) µm long, mean ± SD = 12.6 ± 1.7; basal appendage single, tubular, unbranched, centric, 3.5–7 µm long (Figure 3.43).

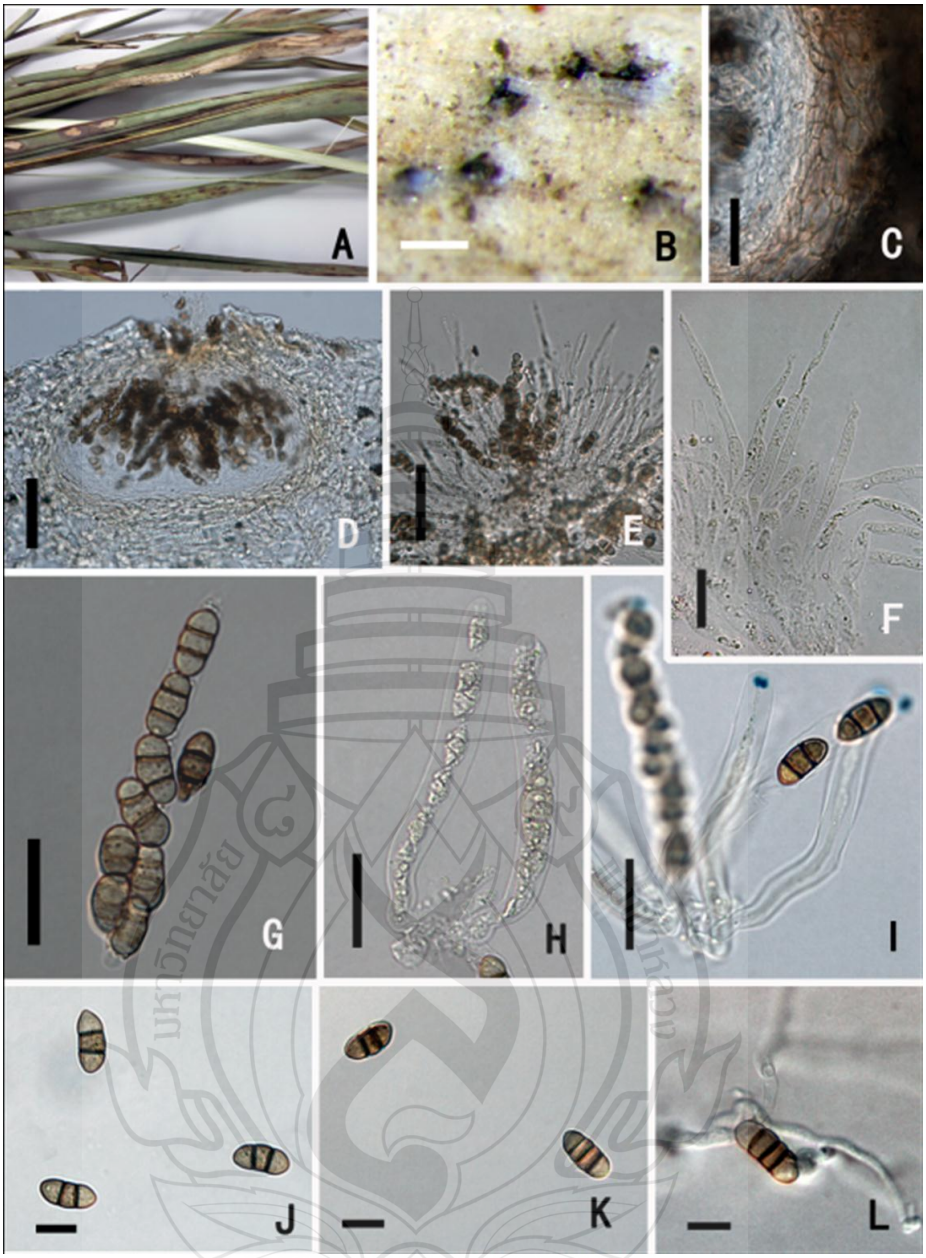
Culture characteristics: Colonies on PDA reaching 40–50 mm diam after 7 days at 25°C, with entire edge, whitish, with dense, aerial mycelium on surface, fruiting bodies black, gregarious; reverse similar in colour.

Material examined: AUSTRALIA, New South Wales, Mount Annan, on leaves of *Telopea* sp., Aug. 1999, P.W. Crous, JT 975, (CBS H holotype, culture ex-type CBS 114161= STE-U 3083); ditto, JT 975, culture CBS 113606= STE-U 3082.

Notes: The two collections of *P. telopea* are morphologically most similar to *P. australasia*, but differ in having shorter conidiogenous cells. Furthermore, in phylogenetic analyses, *P. telopea* form a distinct clade apart from *P. australasia*. *Pestalotiopsis telopea* is an important pathogen of *Telopea* sp. in Australia. We didn't prove the pathogenicity test, however, all the *Telopea* sp. in Mount Annan had a prominent leaf spot disease associated with *P. telopea.*

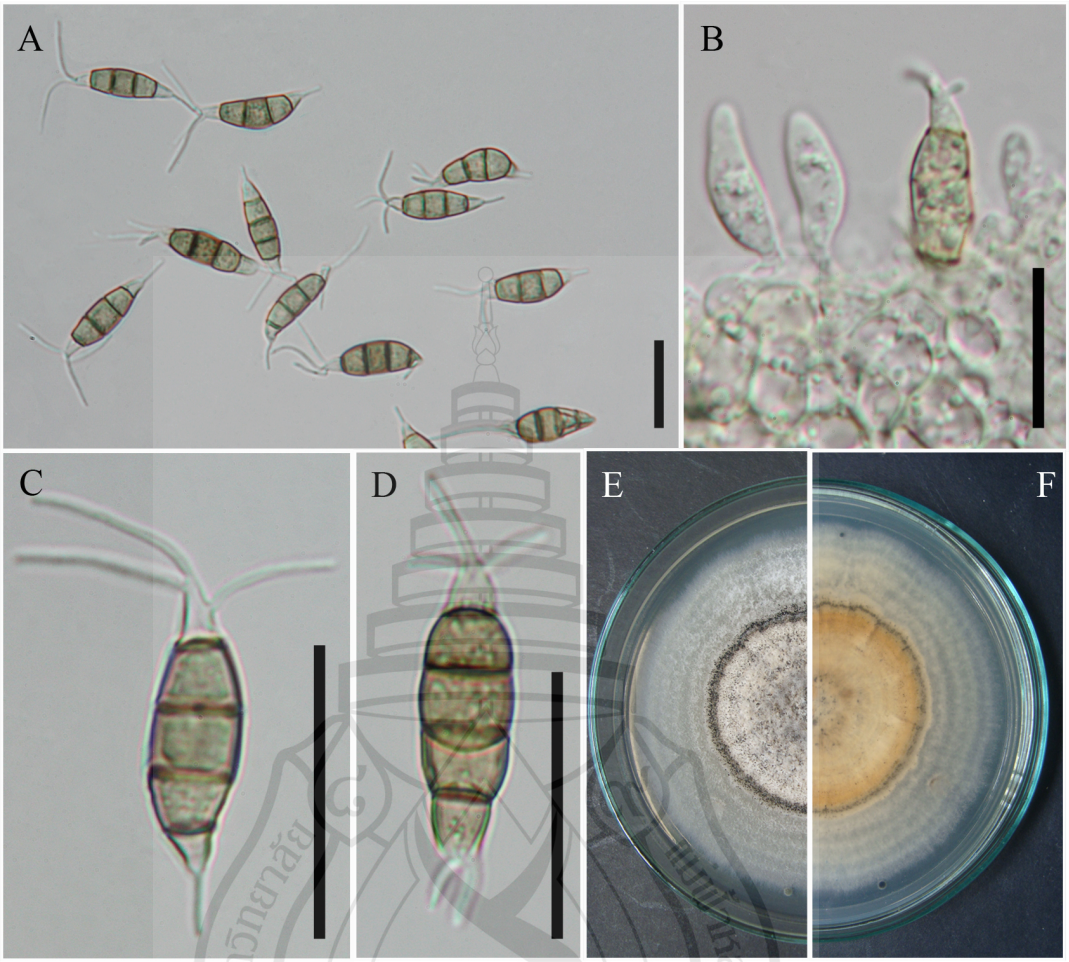
Pestalotiopsis trachicarpicola Y. M. Zhang & K.D. Hyde, Cryptog. Mycol. 33: 315 (2012) MycoBank: MB 564879 (Figure3.44 A-L, Figure 3.45 A-F).

Etymology: In reference to its occrruence on the host *Trachycarpus.*



Note. A. B. Leaf spot on living leaves. C. Peridium with five cell layers. D. Section of ascomata. E. Asci and paraphyses F. Hyaline paraphyses with septum. G. H. Mature and immature unitunicate asci. I. Asci in Melzer's reagent, note the distinct J+ apical ring. J. K. Ascospores. L. Ascospore germination. Scale Bars: B = 200 µm, C. F. G. H. I = 20 µm, D = 50 µm, J. K. L = 10 µm

Figure 3.44 *Pestalotiopsis trachicarpicola* (holotype)



Note. A. Colonies producing black slimy masses of conidia. B. Conidiogenous cells discrete and lageniform C-D. Conidia. E. F. Colony on PDA, E from above, F from below. Scale Bars: A-D = 20 µm

Figure 3.45 *Pestalotiopsis trachycarpicola* anamorph

Forming leaf spots on *Trachycarpus fortunei*. Ascomata 115–215 µm diam × 140–185 µm high (mean = 177 × 157 µm, n=10), scattered or gregarious, immersed under slightly raised areas of host epidermis, subglobose to globose, with central black irregular ostioles (Figure 3.44 B, D). Peridium 20–26 µm wide, comprising 3–5 layers of brown, relatively thick-walled cells of *textura angularis*, inner cells flattened

and thin-walled. Paraphyses 3–5 µm wide, with few septa, base relatively wide and tapering to free ends (Figs. 3.44 C, E, F). Asci 65–76 × 5–14 µm (mean= 73.6 × 9.3 µm, n=10), 8–spored, unitunicate, cylindrical, pedicel short, 3.8–5.8 µm long, with a distinct J+, 3–5 µm in diam, amyloid apical ring in ascus apex, Ascospores 12–16 × 5–8 µm (mean = 14.1 × 6.5 µm, n=30), uniseriate, or 1–seriate in the upper part and 2–seriate at the base, oblong to ellipsoidal or fusiform, smooth or verrucose, pale yellowish brown, 2–3-transversely septate and constricted at the septa, with obconic or semicircular end cells, sometimes the colour of the end cells lighter than cells in the middle cells, cells fairly uniform in size (Figure3.44).

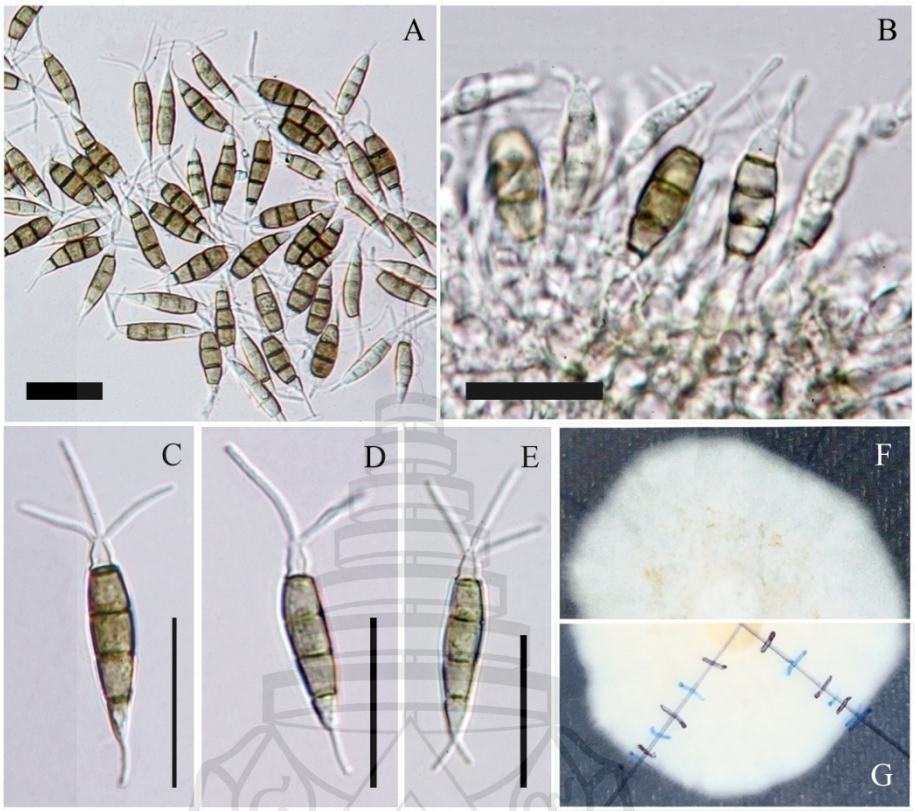
Culture characteristics: Colonies on PDA white, thin, with entire edge, growth determined after 3 days at 25ºC (4.6 cm in 3 days, 1.52 cm/day). After a few weeks, black slimy conidial masses produced on the white colonies, agar changing colour to orange to deep brown. Mycelium hyaline, sparsely septate, with small guttules, 1.3–6.4 µm diam. *Conidiophores* reduced to conidiogenous cells lining the inner wall of the conidiomatal cavity. *Conidiogenous cells* discrete, lageniform, smooth, thin-walled, colorless, with 2–4 proliferations. *Conidia* fusoid, straight to slightly curved, 4–septate, 19–24.9 × 5.3–6.3 µm (mean = 22 × 6.0 µm, n=30), basal cell obconic with truncate base, hyaline, thin- and smooth-walled, 3–5 µm long (mean = 4 µm), with 3 median cells, doliiform, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, together 11.7–13.7 µm long (mean = 12.4 µm) (second cell from base 3.7–5.7 µm (mean = 4.7 µm); third cell 3.75–4.7 µm (mean = 4.2 µm); fourth cell 4–4.7 µm (mean = 4.4 µm); apical cell hyaline, conic to cylindrical 2.2–4.4 µm long (mean = 3.2 µm); 3 tubular apical appendages, arising from the upper portion of the apical cell, 9.4-17.8 µm long; basal appendage present, 2.7–5.5 µm long (Figure 3.45 A-D).

Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Gardens, on leaf spots on living leaves of *Trachycarpus fortunei*, March 2011, K.D. Hyde OP068 (IFRD 9026，holotype), extype living culture IFRDCC 2440; CHINA, Yunnan Province, Kunming, Kunming Botanical Gardens, on leaf spots on living leaves of *Trachycarpus fortunei*, September 2011, Y.M. Zhang OP145 (IFRD 411-019).

Notes: *Pestalotiopsis palmarum* (Cooke) Steyaert has previously been reported to cause leaf spots on *Cocos nucifera* L. (Guba, 1961), however this taxon differs from *P. trachycarpicola* because in *P. palmarum* the three median cells of the conidia are versicolorous while in *P. trachycarpicola* they are concolorous. The sexual morph of *Pestalotiopsis trachycarpicola* is morphologically most similar to *Pestalosphaeria accidenta* P.L. Zhu, T. Xu & Q.X. Ge (Zhu et al., 1991). *P. accidenta* was recorded on *Rhododendron latoncheae* Franch, and ascomata are 315–420 μm diam, which is considerably larger than those of *P. trachycarpicola* (116–214 μm in diam). The asci and ascospores of both species are similar, but the ends of the ascospores of *P. trachicarpicola* are more rounded and some are verrucose. There are presently 12 species of *Pestalosphaeria* (and these will all need transferring to *Pestalotiopsis*, which is both the oldest and more commonly used name (Maharachchikumbura et al., 2011).

Pestalotiopsis unicolor Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 122 (2012), MycoBank: MB 800523 (Figure 3.46 A–G).

Etymology: Specific epithet in reference to concolorous median cells.



Note. A. Conidia. B. Conidiophores/conidiogenous cells. C–E. Conidia. F–G. Colony on PDA, F. from above, G. from below. Scale Bars: A– E= 20 µm

Figure 3.46 *Pestalotiopsis unicolor* (holotype)

Conidiophores indistinct. *Conidiogenous cells* discrete ampulliform to lageniform, smooth, thin-walled, hyaline, with 1–2 proliferations. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4–septate, 20–24.5 × 4–6 µm (mean = 22 × 5.1 µm), basal cell conic to obconic, hyaline or slightly olivaceous, thin- and verruculose, 4–5.5 µm long (mean = 4.9 µm), with three median cells, doliiform to cylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 13–16 µm long (mean = 14.7 µm) (second cell from base 4–5 µm (mean = 4.8 µm); third cell 4–5 µm (mean = 4.8 µm); fourth cell 4–6 µm (mean = 5 µm); apical cell hyaline, conic

to subcylindrical 3-5 µm long (mean = 4.2 µm); with 2–3 tubular apical appendages, arising from the apex of the apical cell, 11–20 µm long (mean = 17.5 µm), of unequal length; basal appendages present, rarely two, 4–10 µm (mean = 6.9 µm) long.

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25 °C, edge entire, whitish, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture pale yellow.

Habitat/Distribution: Endophyte on *Rhododendron* sp. and unidentified plant, Hunan Province, China.

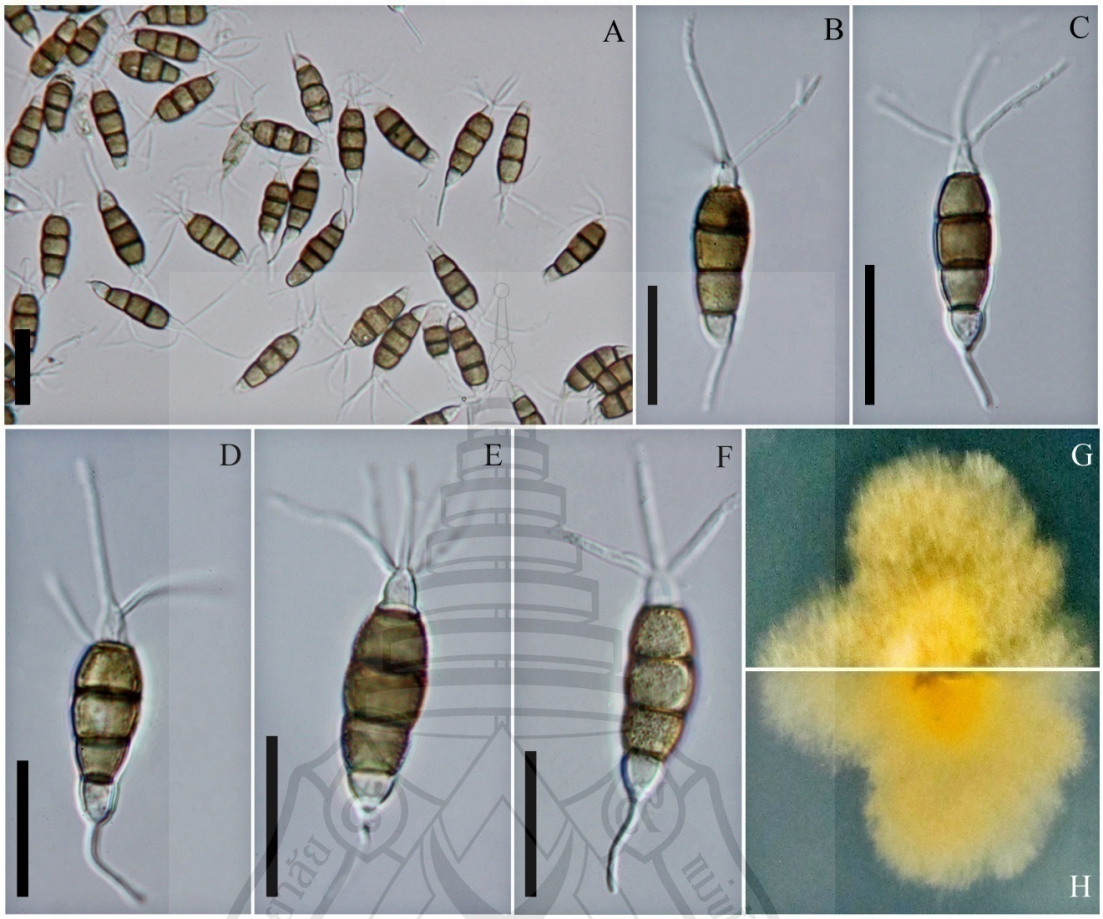
Material examined: CHINA, Hunan Province, Yizhang County, Mangshan, on living leaf of *Rhododendron* sp., 12 April 2002, Wenping Wu HN42-1 (HMAS046974, holotype; MFLU12-0417, isotype; ex-type living culture NN046974 = MFLUCC 12-0276).

Additional culture examined: CHINA, Hunan Province, Yizhang County, Mangshan, on living leaf of unidentified tree, 12 April 2002, Wenping Wu HN51-1 (NN047308 = MFLUCC 12-0275).

Notes: *Pestalotiopsis unicolor* is a distinct species in the genus from molecular and morphological characters. The morphologically similar species in conidial size are *P. kawakamii* Sawada (20–24 × 5–7 µm) (Guba 1961) and *P. algeriensis* (Sacc. & Berl.) W.P. Wu. (17–23 × 5–7 µm) (Guba, 1961). However, 2–3 tubular apical appendages (11–20 µm long) of *P. unicolor* are longer than in *Pestalotia kawakamii* (3 apical appendages; 5–10 µm long). The conidial width of *P. algeriensis* is similar to *P. unicolor* but the length of conidia and apical appendages are smaller in *P. algeriensis* and length (up to 16 µm) is shorter than in *P. unicolor*.

Pestalotiopsis verruculosa Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 123 (2012), MycoBank: MB 800527 (Figure 3.47 A–H).

Etymology: The specific epithet is based on the Latin *verruculose* in reference to the verrucose pattern in walls of three median cells.



Note. A. Conidia. A–F. Conidia. G–H. Colony on PDA, G. from above, H. from below. Scale Bars: A– F= 20 µm

Figure 3.47 *Pestalotiopsis verruculosa* (holotype)

Conidia ellipsoid, straight to slightly curved, 4–septate, 28–35 × 9–11 µm (mean = 30.6 × 10.3 µm), basal cell conic with obtuse end, hyaline, thin-walled and verruculose, 5–7 µm long (mean = 5.7 µm), with three median cells, doliiform to cylindrical, with thick verruculose walls, constricted at the septa, concolorous, olivaceous, septa and periclinal walls darker than the rest of the cell, wall rugose, together 18–26 µm long (mean = 21.6 µm) (second cell from base 6–9 µm (mean = 6.8 µm); third cell 9–9 µm (mean = 7.5 µm); fourth cell 6–9 µm (mean = 7.3 µm);

apical cell hyaline, conic to subcylindrical 4-6 µm long (mean = 4.8 µm); with 2–6 tubular apical appendages (mostly 3–4), arising from the apex of the apical cell (rarely 1 appendage arising from just above the septum separating upper median and apical cell, 25–40 µm long (mean = 34 µm); basal appendage present 8–12 µm (mean = 9 µm).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 15 days at 25°C, edge undulate, whitish to pale yellow, with dense, aerial mycelium on surface, with black, gregarious fruiting bodies; reverse of culture yellow to pale orange.

Habitat/Distribution: Endophytes on living leaf of *Rhododendron* sp., Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaf of *Rhododendron* sp., 19 March 2002, Wenping Wu KBG25-8 (HMAS047309, holotype; MFLU12-0416, isotype; ex-type culture NN047309 = MFLUCC 12-0274).

Table 3.7 Synopsis of *P. verruculosa* and related species.

Species	***P. verruculosa***	***P. funerea***[a]	***P. multiseta***[a]	***P. macrospora***[a]
Conidia size (µm)	28–35 × 9–11	21–29 × 7–9.5	22–26×7.5–9.5	30–40×7–9
Median cells	Olivaceous, concolorous	Umber or olivaceous, concolorous	Umber, concolorous	Olivaceous, versicolour
Apical appendages:	2–6 (mostly 3–4, rarely branched)	3–6 (unbranched)	3–5 (branched)	4–5 (arising separately or pairs and often branched)
Length (µm)	25–40	5–20	9–16	15–22
Tip	Not knobbed	Not knobbed	Not knobbed	Not knobbed

[a] Guba (1961)

Notes: *Pestalotiopsis verruculosa* is a distinct species in terms of morphology, and its molecular phylogeny. It has a relatively large conidial size (28–35 × 9–11 µm) when compared with morphologically similar species (*P. funereal*, 21–29 × 7–9.5 µm); *P. multiseta*, 22–26×7.5–9.5 µm). It also has a long apical appendage (25–40 µm) when compared to *P. funerea* (5–20 µm), *P. multiseta* (9–16 µm) and *P. macrospora* (15–22 µm) (Nag Raj, 1993).

3.4 Conclusion

In this study, we include 75 sequences of *Pestalotiopsis* to provide a backbone tree for the genus *Pestalotiopsis*. Based on morphological and molecular data, we determined that the 75 sequenced isolates comprise 43 species of *Pestalotiopsis*. Based on molecular and morphological data we describe 40 new species (*P. anacardiacearum, P. arceuthobia, P. arenga, P. australasia, P. australis*, *P. biciliata, P. camelliae, P. chamaeropis, P. chinensis, P. clavata, P. colombiensis, P. diploclisia, P. diversiseta, P. ericacearum, P. furcata, P. gaultheria, P. grevillea, P. hawaiiensis, P. hollandica, P. humus, P. inflexa, P. intermedia, P. kenyana, P. knightia, P. linearis, P. malayana, P. monochaeta, P. novaehollandiae, P. papuana, P. parva, P. portugalica, P. proteacearum, P. rhododendri, P. rosea, P. scoparia, P. spathulata, P. telopea, P. trachicarpicola, P. unicolor* and *P. verruculosa*). *P. adusta* is epitypified and ex-type of *P. brassicae* and *P. jesteri* are re-examineed. This work provides a backbone tree for 43 ex-type/epitypified species of *Pestalotiopsis* and can be used in future studies of the genus. This backbone tree needs expanding by re-examining type materials of some of the important species described in *Pestalotiopsis* and using multi-locus analysis to establish extypes.

CHAPTER 4

Neopestalotiopsis AND *Pseudopestalotiopsis* gen. nov.

4.1. Introduction

Several recent studies showed that *Pestalotiopsis* remarkably highly diverse in morphologically, genetically and contains three distinct lineages (Jeewon et al., 2003; Maharachchikumbura et al., 2012). Based on these findings, *Pestalotiopsis* is complex and thus; *Neopestalotiopsi*s and *Pseudopestalotiopsis* are introduced to accommodate three distinct lineages in *Pestalotiopsis*. In the present chapter, two new genera *Neopestalotiopsis* and *Pseudopestalotiopsis* were proposed. In addition various species were placed in synonymy, and new combinations in *Neopestalotiopsis* and *Pseudopestalotiopsis* were made for the species previously belonging to the genus *Pestalotiopsis*. Phenotypic analyses of conidial characters and phylogenetic analyses of combined sequence data of internal transcribed spacer, β-tubulin gene region and partial translation elongation factor 1-alpha genes were used to clarify species boundaries of species in *Neopestalotiopsis* and *Pseudopestalotiopsis*. Furthermore, complex names were assigned to species upon conidial morphology and sequence data.

4.2. Materials and Methods

The specimens were characterized morphologically as described in Section 2.1.1. Single spore isolates were done as previously explained in Section 2.2.2. The ITS, β-tubulin gene region and partial translation elongation factor 1-alpha region were amplified and sequenced by the primer pairs and conditions as mentioned in

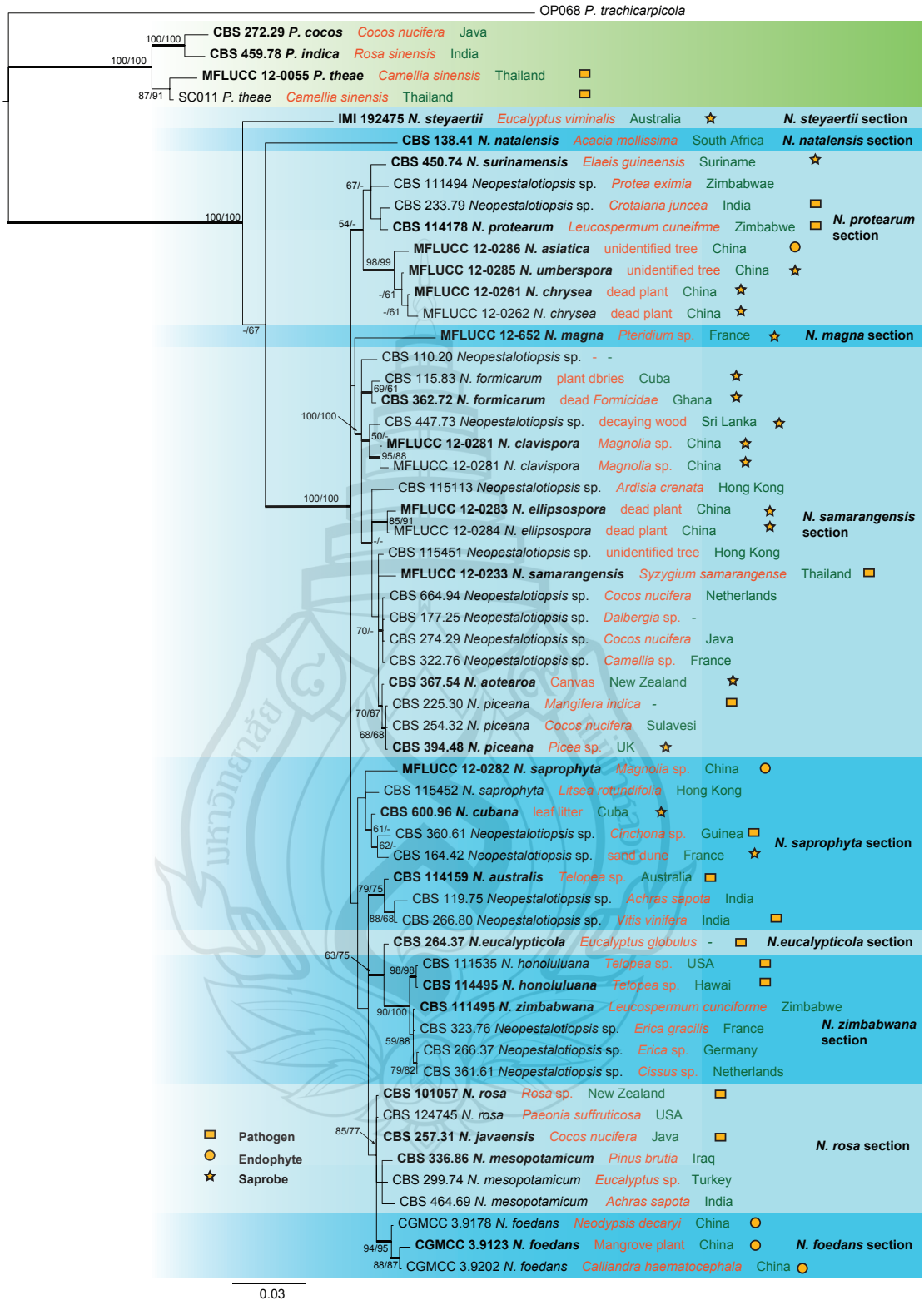
Section 3.1.1. Phylogenetic methods were carried out based on the approaches described in Section 3.1.2.

4.3 Results and Discussion

4.3.1 Phylogeny

The BI, ML and MP analyses of LSU indicated that *Pestalotiopsis* comprises three major monophyletic clades and are supported with high bootstrap confidence (in Chapter 3 discussed this, Figure 3.1) and two new genera *Neopestalotiopsis* and *Pseudopestalotiopsis* were introduced here in.

Information on species relationship in *Neopestalotiopsis* and *Pseudopestalotiopsis* phylogenetic trees is shown in Figure 4.1. For combined gene, BI, ML, and MP consensus trees revealed the same relationships between the significantly supported clades. Combined ITS, β-tubulin and TEF1 alignment contained 61 sequences (including 55 sequences of *Neopestalotiopsis*, four sequences of *Pseudopestalotiopsis*, and *Pestalotiopsis trachicarpicola* as outgroup taxon) and 1418 characters including gaps. Suitable models were selected using models of nucleotide substitution for each gene, as determined using MrModeltest. The GTR+I model with proportion of invariable sites for ITS and the HKY+G model with gamma-distributed rate model for β-tubulin and the GTR+I+G model with invgamma rate was selected for TEF1 and included for each gene partition. Among these 1418 characters, 990 were constant, 172 variable characters parsimony uninformative and 256 characters parsimony-informative. The parsimony analysis resulted in 108 equally parsimonious trees and the first tree; length = 805 steps, CI = 0.688, RI = 0.810, RC = 0.557 and HI = 0.312. *Neopestalotiopsis* and *Pseudopestalotiopsis* isolates clustered into two well-supported clades (BI= 1 ML=100 and MP=100). In these phylogenetic analyses six clades were recognized in *Neopestalotiopsis*. Phylogeny, morphology, geography and host occrance take in to account, these six clades were assign in to six sections in *Neopestalotiopsis* (Figure 4.1)



Note. Strict consensus combined (ITS+ β-tubulin+ TEF1) tree from Bayesian analysis of the analyzed *Neopestalotiopsis* and *Pseudopestalotiopsis*. Species complex

are indicated in coloured blocks and thickened lines indicate Bayesian posterior probabilities (PP) above 95%. RAxML bootstrap support values (ML) and maximum parsimony bostrap supports (MP) are given at the nodes (ML/MP). Strain accession numbers are followed by the original species name (ex-type are in bold), the isolation source (orange) and country of origin (green). The scale bar represents the expected changes per site. The tree was rooted to *Pestalotiopsis trachicarpicola* (OP068)

Figure 4.1 Strict consensus combined (ITS+ β-tubulin + TEF1) tree from Bayesian analysis of the analyzed *Neopestalotiopsis* and *Pseudopestalotiopsis*

4.3.2 Taxonomy

Neopestalotiopsis Maharachch., K.D. Hyde & Crous, gen. nov.

Type species: *Neopestalotiopsis protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous (see below).

Etymology. Named after its morphological similarity to *Pestalotiopsis*.

Conidiomata acervular or pycnidial, subglobose, globose, clavate, solitary or aggregated, dark brown to black, immersed to erumpent, unilocular or irregularly plurilocular; exuding dark brown to black conidia in a slimy, globose mass. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical, ampulliform to lageniform, hyaline, smooth, thin-walled; conidiogenesis initially holoblastic, percurrent proliferations to produce additional conidia at slightly higher levels. *Conidia* fusiform, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell with a conic to subcylindrical with a truncate base, hyaline or pale brown to olivaceous, thin and rugose to smooth-walled; three median cells doliiform, wall rugose to verruculose, versicoloured, septa darker than the rest of the cell; apical cell, hyaline, conic to cylindrical, thin and smooth-walled; with tubular apical appendages, one to many, filiform or attenuated, flexuous, branched or unbranched; basal appendage single, tubular, unbranched, centric.

Notes: Based on LSU sequence (Chapter 3, Figure 3.1), the *Neopestalotiopsis* clusters in *Amphisphaeriaceae* distinct from the *Pseudoestalotiopsis* and *Pestalotiopsis*, and therefore it would be best to treat this as a separate genus. *Neopestalotiopsis* can be easily distinguished from *Pseudoestalotiopsis* and *Pestalotiopsis* by its two upper median cells are darker than the lower median cells (versicolorous). Furthermore, conidiophores in *Neopestalotiopsis* are indistinct and often reduced to conidiogenous cells. Guba (1961) treated that groups the versicolorous assemblages of species into umber olivaceous and fuliginous olivaceous. In his monograph Guba (1961) placed versicolorous umber olivaceous group which comprises 40 species and versicolorous fuliginous olivaceous group comprising 56 species. These groups are differentiated depending on the intensities of the median cells, while most species have similar conidial measurements. Jeewon et al. (2003), Liu et al. (2010) and Maharachchikumbura et al. (2011) concluded that the division of the "versicolor group" based on colour intensities of the median conidial cell is not a taxonomically good character. Instead of using two groups here we proposed *Neopestalotiopsis* as a new genus for the versicolorous group. Based on the length of the ITS alignment Liu et al. (2010a) group *Pestalotiopsis* in to 3 groups. The sequences of ITS regions in group A, B, and C were 480–484 bp, 489–495 bp and 536–540 bp, respectively (presently *Neopestalotiopsis*, *Pseudpestalotiopsis* and *Pestalotiopsis*) and thereby, *Pestalotiopsis* strain would be assigned to groups.

Species of *Neopestalotiopsis* were assigned to 6 sections, upon conidial morphology, sequence data, geographical influence and host occurrence. Brief description of each section was provided according to the alphabetical order. Finally the descriptions of the *Pestalotiopsis* species belong to the sections are provided.

N. magna section

The described section consists of single species *Neopestalotiopsis magna* which is characterised by larger conidia. *Neopestalotiopsis magna* isolated from decaying leaves of *Pteridium* sp. collected in France. It forms a distinct clade apart from *N. protearum* and *N. samarangensis* sections.

N. protearum section

Our sequence data showed this is a phylogenetically clearly distant from sister sections. *Neopestalotiopsis protearum* section comprises five species including the generic type of *Neopestalotiopsis*, *N. protearum*. The other four species assigned to this section are *N. asiatica*, *N. chrysea*, *N. surinamensis* and *N. umberspora.*

N. natalensis section

Our study places a monotypic *N. natalensis* in this section. *Neopestalotiopsis natalensis* which presence a synanamorph and forms a sister clade to *N. steyaertii* section.

N. samarangensis section

Six known species, *N. aotearoa, N. clavispora*, *N. ellipsospora*, *N. formicarum*, and *N. samarangensis* have placed in the section. *N. magna* section form a basal sister clade to this section.

N. saprophyta section

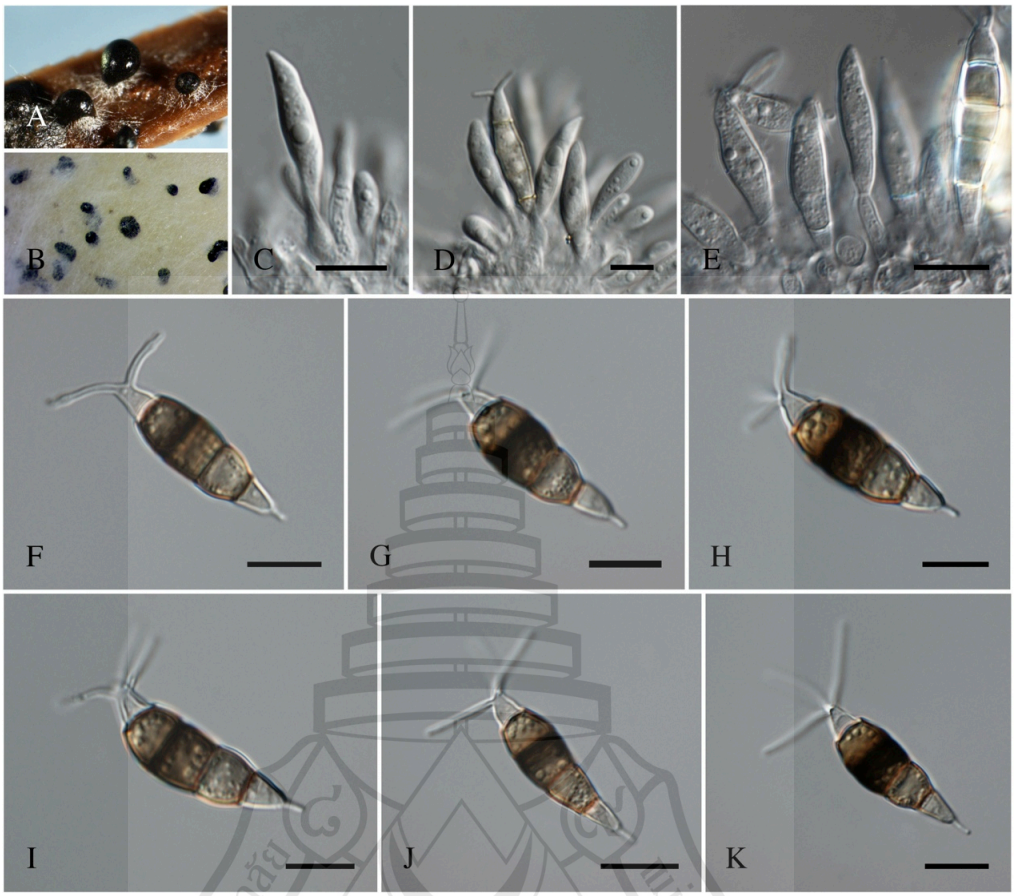
N. saprophyta section comprises 10 known species (*N. australis*, *N. cubana*, *N. eucalypticola*, *N. foedans*, *N. honoluluana*, *N. javaensis*, *N. mesopotamicum*, *N. rosa*, *N. saprophyta* and *N. zimbabwana*) including unidentified *Neopestalotiopsis* species. Phylogenetically it forms separate clade apart from other sections in the genus. Morphological and molecular variations within this section is high.

N. steyaertii section

Neoestalotiopsis steyaertii is the single species belongs to the section. This species is characterised by its unusual conidial shape. Furthermore it lacks apical and basal appendages. The section displays a higher level of genetic variation apart from other section in the genus.

Neopestalotiopsis aotearoa Maharachch. & Crous, sp. nov. (Figure 4.2 A-K)

Etymology: Named after the country where it was collected, New Zeeland; Maori name for New Zealand is Aotearoa.



Note. *Neopestalotiopsis aotearoa* CBS 367.54^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.2 *Pestalotiopsis aotearoa* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary or confluent, imbedded or semi-immersed to erumpent, dark brown, 200–450 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, subcylindrical to ampulliform, hyaline, proliferating 2–4 times percurrently, 5–20 × 2–10 µm, wide at the base, opening 2–5 µm diam. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (19.5–)21–28(–29) × (6–)6.5–8.5(–9) µm, mean ± SD = 24.8 ± 1.6 × 7.7 ± 0.5

µm; basal cell conic with a truncate base, hyaline, thin and wall rugose, 4–6.5 µm long; three median cells doliiform, (13–)14–18(–18.5) µm long, mean ± SD = 15.9 ± 1.1, wall verruculose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 4–6 µm long; third cell honey brown, 3.5–7 µm long; fourth cell brown, 4–6.5 µm long); apical cell 3.5–5.5 µm long, hyaline, cylindrical to subcylindrical, thin and smooth-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (3–)5–12(–13) µm long, mean ± SD = 8.1 ± 1.2; basal appendage single, tubular, unbranched, centric, 1.5–4 µm long (Figure 4.2).

Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with undulate edge, pale honey-coloured, sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

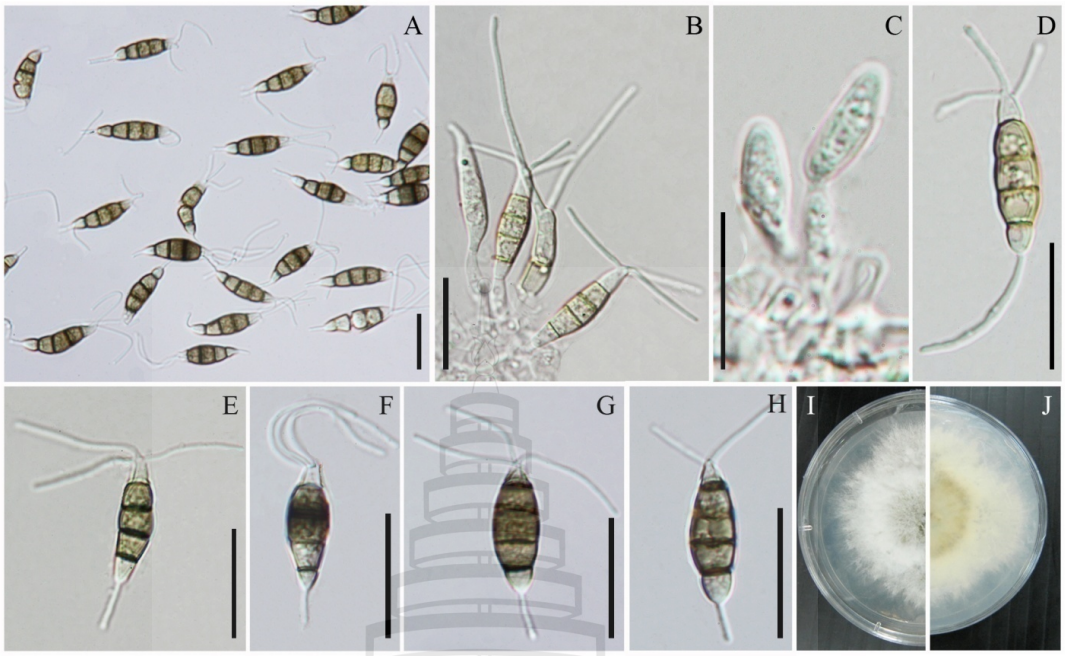
Material examined: NEW ZEALAND, from canvas, Sept. 1954, G.C. Wade (CBS H-15765 holotype, culture ex-type CBS 367.54= ATCC 11763=QM 381).

Notes: Neopestalotiopsis aotearoa described from a canvas in New Zealand. In phylogenetic analyses, *N. aotearoa* proved to be sister to *N. piceana*, but two species are morphologically easily distinguishable. The latter species is quite distinct from *N. aotearoa* by its clavate shaped conidia, longer basal appendage, and longer apical appendages.

Neopestalotiopsis asiatica (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.3 A- J).

Basionym: *Pestalotiopsis asiatica* Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 104 (2012).

Etymology: The specific epithet is based on the geographical region (Asia), In reference to where fungus was isolated.



Note. A. Conidia B–C. Conidiophores/ conidiogenous cells. D. E. Immature conidia. F–H. Mature conidia. I. J. Colony on PDA, I. from above, J. from below. Scale Bars: A– H= 20 µm

Figure 4.3 *Neopestalotiopsis asiatica* (holotype)

Conidiophores indistinct. *Conidiogenous cells* hyaline, simple, filiform, 3–12 µm long. *Conidia* 20–26 × 5–7 µm (mean = 22.6 × 6.25 µm), fusiform, straight to slightly curved, 4-septate; basal cell conical, hyaline, thin and verruculose, 3–5 µm long (mean = 4µm); three median cells 13–15.5 µm long (mean = 14 µm), dark brown, verruculose, septa and periclinal walls darker than the rest of the cell, versicoloured, second cell from base pale brown, 4–5.5 µm (mean = 4.5 µm); third cell darker brown, 4–5 µm (mean = 4.8 µm; fourth cell darker, 4–5 µm (mean = 4.7 µm); apical cell 3.5–5 µm long (mean = 3.35 µm), hyaline, conical to cylindrical, comprising 2-4 appendages (mainly 3); apical appendages 20–30 µm long (mean = 25.6 µm), tubular, arising from the apex of the apical cell; basal appendage, 4–8 µm long (mean = 5.65 µm), filiform (Figure 4.3).

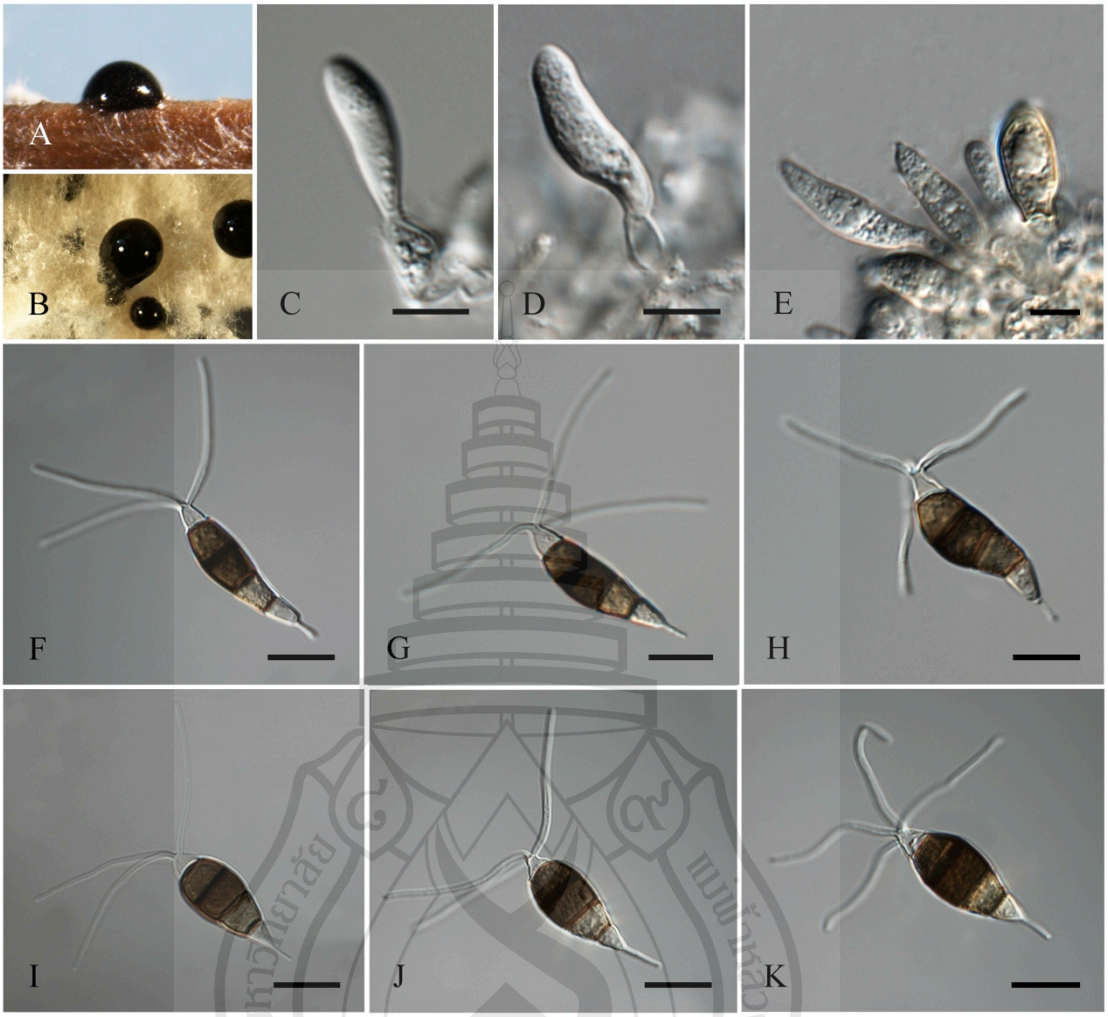
Cultural characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25^{o}C, with crenate edge, whitish, with aerial mycelium on surface; fruiting bodies black, gregarious; reverse of culture whitish to pale yellow.

Material examined: CHINA, Hunan Province, Yizhang County, Mangshan, isolated from living leaves of unidentified tree, 12 April 2002, Wenping Wu HN51-1 (HMAS047638, holotype; MFLU12-0422, isotype; ex-type living culture NN047638 = MFLUCC 12-0286).

Notes: *Neopestalotiopsis asiatica* is a distinct species in the versicolour group and clearly distinguishable from *P. chrysea* and *N. umberspora* in the β-tubulin, TEF1 and combined gene phylogram. *Neopestalotiopsis asiatica* (20–26 × 5–7 µm) is morphologically similar to *P. pauciseta* (Sacc.) Y.X. Chen (conidia 20–24 × 4.5–5 µm) (Saccardo, 1914) and *P. gracilis* (Kleb.) Steyaert (conidia 19–23 × 6–7 µm) (Saccardo, 1931). However, *N. asiatica* differs from *P. pauciseta* by its wider conidia and from *P. gracilis* in having long apical appendages (in *P. gracilis* 10–26 µm).

Neopestalotiopsis australis Maharachch. & Crous, sp. nov. (Figure 4.4 A-K).

Etymology: Named after the country where it was collected, Australia.



Note. *Neopestalotiopsis australis* CBS 114159^{T}. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.4 *Neopestalotiopsis australis* (holotype)

Conidiomata pycnidial in culture on PDA, globose to clavate, solitary or aggregated in clusters, semi-immersed, brown to black, 100–500 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, rugose-walled, simple, proliferating 1–3 times percurrently, 5–12 × 2–7 µm,

wide at the base, opening 1–2 µm diam. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (19–)21–27(–28) × (7–)7.5–9(–9.5) µm, mean ± SD = 24.6 ± 1.8 × 8 ± 0.4 µm; basal cell conic with a truncate base, hyaline, thin and wall rugose, 3.5–5.5 µm long; three median cells doliiform, (13–)14–18(–18.5) µm long, mean ± SD = 16.1 ± 1, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 3.5–6.5 µm long; third cell darker brown, 4–7 µm long; fourth cell brown, 5–6.5 µm long); apical cell 3–6 µm long, hyaline, subcylindrical to obconic, thin and wall rugose; with 3–4 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous, (19–)21–32(–34) µm long, mean ± SD = 26.6 ± 3; basal appendage single, tubular, unbranched, centric, 3–7 µm long (Figure 4.4).

Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with lobate edge, pale honey-coloured, with dance aerial mycelium on the surface with black, concentric fruiting bodies; reverse similar in colour.

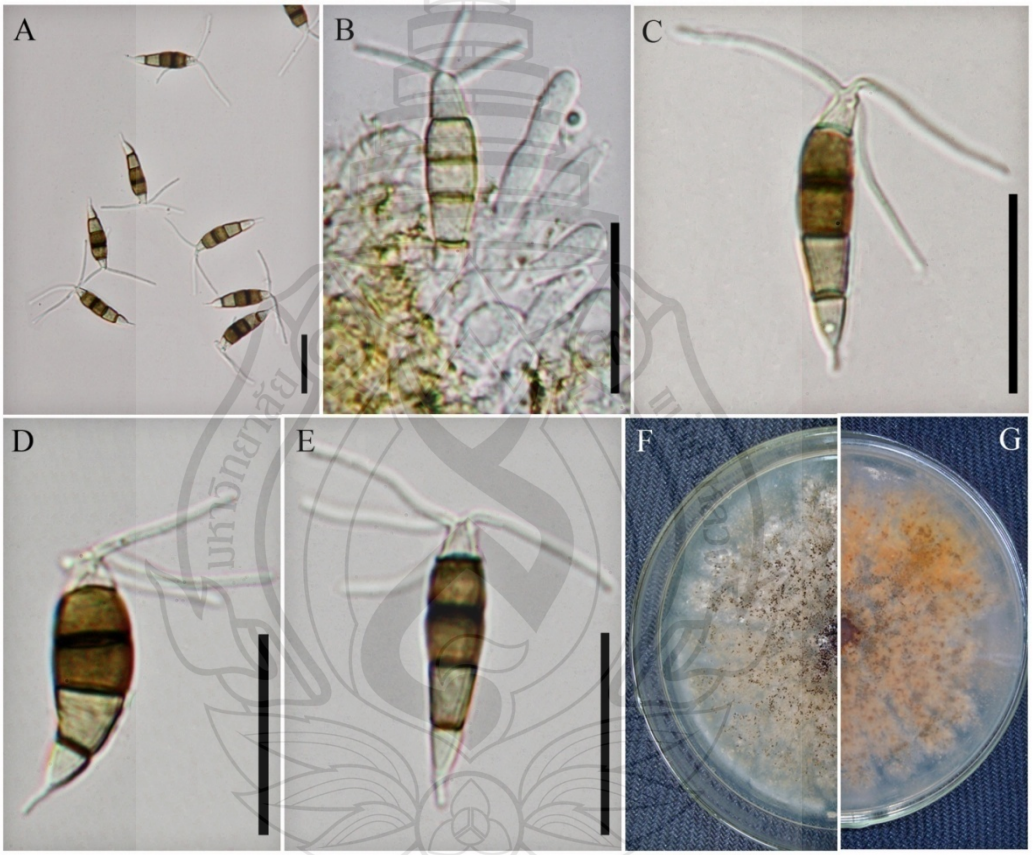
Material examined: AUSTRALIA, New South Wales, from *Telopea* sp., 12 Oct. 1999, P.W. Crous (CBS H holotype, culture ex-type CBS 114159= STE-U 3017).

Notes: Neopestalotiopsis australis belongs to the *N. saprophyta* complex, which is isolated from *Telopea* sp. in New South Wales, Australia. The Conidiogenous cells and conidia of *Neopestalotiopsis australis* resemble those of the two Indian isolates, CBS 266.80 and CBS 119.75, which are isolated from *Vitis vinifera* and *Eucalyptus globulus*, respectively. Since the clear geographical variation and slightly distinction in phylogeny of two Indian isolates, tentatively maintained as a *Neopestalotiopsis* spp. until additional collections and cultures are available. There are various fungal pathogens recorded from *Proteaceae*, which is an important plant family in world floriculture markets (Crous et al., 2011). *Neopestalotiopsis* and *Pestalotiopsis* subsequently been isolated from several *Protea* and *Leucospermum* hosts (Swart, Taylor, Crous & Percival, 1999) and may intercepted at quarantine inspection points (Taylor, 2001). *Neopestalotiopsis australis*, *N. honoluluana*, *N. protearum* and *N. zimbabwana* are fours species recorded from plants in *Proteaceae*. Most of these species cause leaf spots and may causing tip dieback and can be resolved easily based on their diagnostic morphological and phylogeny.

Neopestalotiopsis chrysea (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.5 A-G).

Basionym: *Pestalotiopsis chrysea* Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 107 (2012).

Etymology: The specific refers to the golden yellow colour of the colony (Latin- *chryseus*) of this species.



Note. A. Conidia. B. Conidiophores/ conidiogenous cells. C–E. Conidia. F. G. Colony on PDA, F. from above, G. from below. Scale Bars: A– E= 20 µm

Figure 4.5 *Neopestalotiopsis chrysea* (holotype)

Conidiophores indistinct. *Conidiogenous cells* discrete or integrated, lageniform, hyaline, smooth-walled. *Conidia* 20–24 × 5.5–7 µm (mean = 22.3 × 6.1 µm), fusiform, straight to slightly curved, 4-septate; basal cell obconic to conic, hyaline, thin and smooth-walled, 3–5 µm long (mean = 4.3 µm); three median cells 14–16 µm long (mean = 14.8 µm), dark brown to olivaceous, septa and periclinal walls darker than the rest of the cell, versicoloured, verruculose, second cell from base pale brown, 4–5 µm (mean = 4.6 µm); third cell darker brown, 4–5 µm (mean = 4.6 µm; fourth cell darker, 4–5 µm (mean = 4.5 µm); apical cell 3.5–4.5 µm long (mean = 4 µm), hyaline, conic to obconic; apical appendages 22–30 µm long (mean = 26.8 µm), 3, tubular, arising from the apex; basal appendage, 3 –6 µm long (mean = 4.4 µm), filiform (Figure 4.5).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 10 days at 25^{o}C, edge irregular, yellowish to pale brown, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of the colony orange to brown.

Habitat/Distribution: Saprobe on dead plant material, Guangxi and Hunan provinces, China.

Material examined: CHINA, Guangxi Province, Shangsi, Shiwandashan, Wangle, dead leaves of unidentified plant, 2 January 1997, Wenping Wu WUFH1303a (HMAS042855, holotype; MFLU12-0411, isotype; ex-type living culture NN042855 = MFLUCC 12-0261).

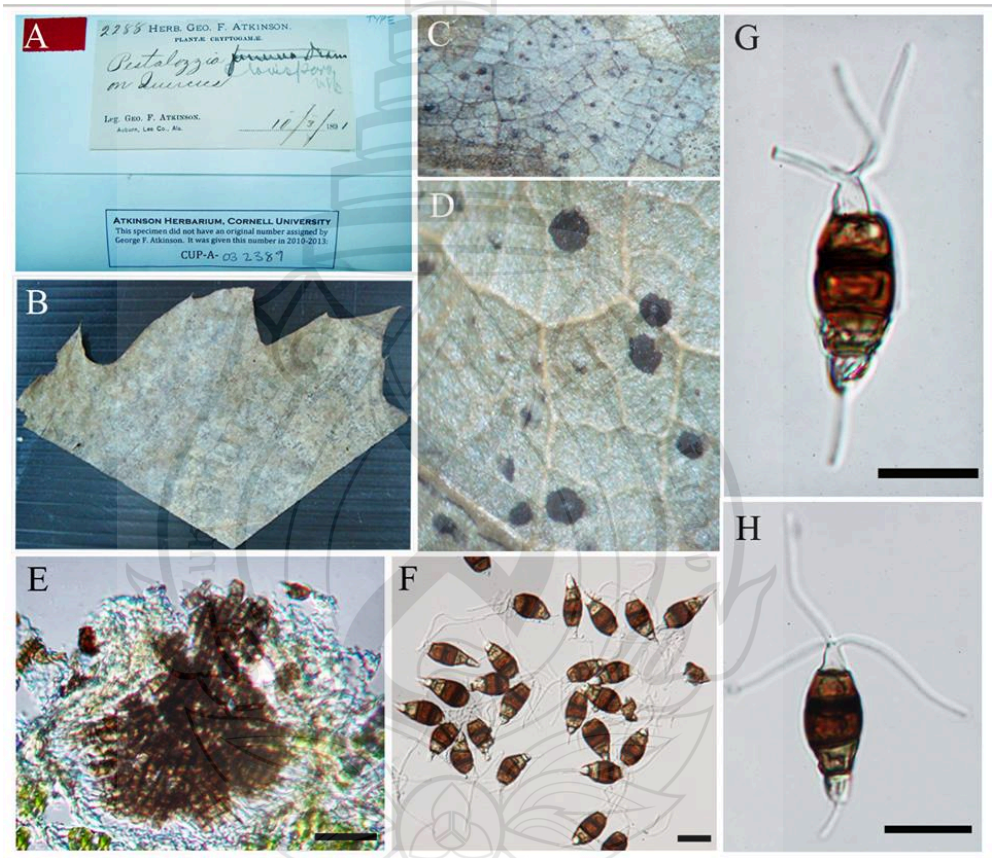
Additional culture examined: CHINA, Hunan Province, Yizhang County, Mangshanon dead plant material, 12 April 2002, Wenping Wu HN27-10 (NN047037 = MFLUCC 12-0262).

Notes: *Neopestalotiopsis chrysea* is a morphologically distinct species in the genus with its yellowish colony; its conidiogenous cells and conidia are also slightly yellowish. It can clearly be differentiated from its phylogenetically related sibling species, *N. umberspora* (19–29 × 6–8 µm) in having relatively narrow conidia (20–24 × 5.5–7 µm) and also in combined gene phylogenetic trees (Figure 4.1).

Neopestalotiopsis clavispora (G.F. Atk.) Maharachch., K.D. Hyde & Crous comb. nov.

Basionym: *Pestalotia clavispora* G.F. Atk., Bulletin of Cornell University 3: 37 (1897).

≡*Pestalotiopsis clavispora* (G.F. Atk.) Steyaert, Bull. Jard. bot. État Brux. 19: 335 (1949).

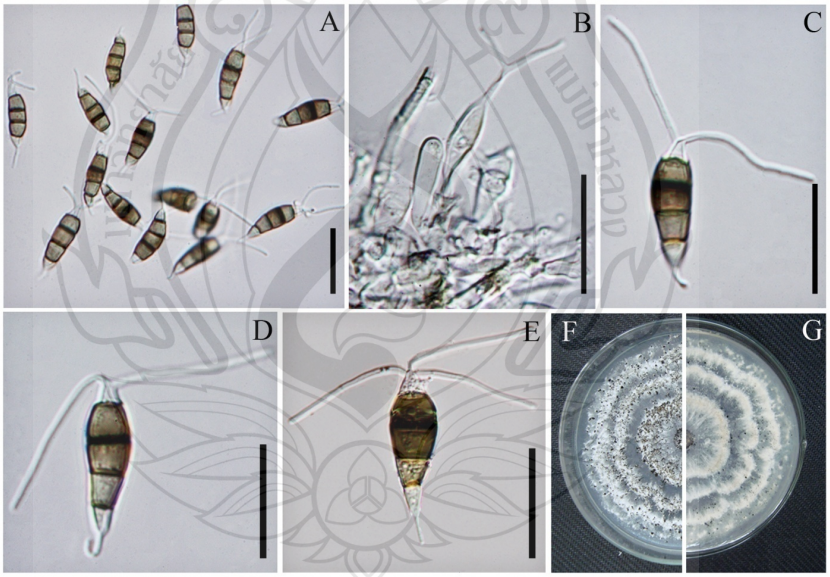


Note. A. *Neopestalotiopsis clavispora* (holotype). B. Fallen leaves of *Quercus* sp.. C-D. Conidiomata, split irregularly. E. Section of conidiomata. F–H. Conidia with versicolorous median cells. Scale Bars: E = 50 µm, F–H = 15 µm

Figure 4.6 *Neopestalotiopsis clavispora* (holotype)

Description from holotype (Figure 4.6 A–H).

Conidiomata 150-250 µm in diam., black, numerous, scattered, rupturing the epidermis and dehiscing irregularly. *Conidia* 18– 26 × 6.5– 8.5 µm (mean= 21 × 7.5 µm), fusiform, 4-septate, straight or slightly curved and clavate-fusiform; basal cell long and conic, hyaline, thin and verruculose, 4–5 µm long (mean = 4.2 µm); with three median cells 13.7–15.3 µm long (mean = 14.7 µm), dark brown to olivaceous, septa and periclinal walls darker than the rest of the cell, versicolored, verruculose, second cell from base pale brown, 4.3–5.3 µm (mean = 4.8 µm); third cell darker brown, 5.5–6.4 µm (mean = 5.8 µm; fourth cell darker, 4.5–5.8 µm (mean = 5 µm); apical cell 3.3–4.2 µm long (mean = 3.7 µm), short, broad conic, hyaline, subcylindric; with apical appendages 19–30 µm long (mean = 24.5 µm), tubular, 2-3 (rarely 2), arising from the apex of the apical cell; with basal appendage present, filiform.



Note. A. Conidia. B. Conidiophores/ conidiogenous cells. C–D. Conidia. E. Mature conidia F–G. Colony on PDA, F. from above, G. from below. Scale Bars: A–E= 20 µm

Figure 4.7 *Neopestalotiopsis clavispora* (epitype)

Description from epitype (Figure 4.7 A–G)

Conidiophores indistinct. *Conidiogenous cells* hyaline, simple, short or relatively long, filiform, 4–10 um long. *Conidia* 20–24 × 6–8 µm (mean = 22 × 7.2 µm), fusiform, straight to slightly curved, 4-septate, clavate-fusiform when mature; basal cell conical, hyaline, thin and verruculose, 3–5 µm long (mean = 3.8 µm); three median cells 13–15 µm long (mean = 13.9 µm), dark brown to olivaceous, verruculose-walled, septa and periclinal walls darker than the rest of the cell, versicoloured, second cell from base pale brown, 4–5 µm (mean = 4.5 µm); third cell darker brown, 4–5 µm (mean = 4.6 µm; fourth cell darker, 4–5 µm (mean = 4.5 µm); apical cell 3–5 µm long (mean = 4.3 µm), hyaline, subcylindric; with apical appendages 22–32 µm long (mean = 26.5 µm), tubular, 2- 3 (rarely 2), arising from apex of the apical cell; with basal appendage, 3–5.5 µm (mean = 4 µm), filiform.

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 7 days at 25°C, edge undulate, whitish, aerial mycelium on surface, fruiting bodies black, concentric; reverse of culture pale luteous.

Habitat/Distribution: Known to inhabit in *Quercus rubra* in USA and *Magnolia* sp. in China.

Material examined: USA, Auburn, Alabama, on fallen leaves of *Quercus rubra* L., 10 March 1891, F. Atkinson (CUP-A-032389, holotype); CHINA, Guangxi Province, Shiwandashan, on dead leaves of *Magnolia* sp., 28 Dec 1997, Wenping Wu WUFH1486c (HMAS043133 = MFLU12-0418, epitype designated here; ex-type living culture NN043133 = MFLUCC 12-0281).

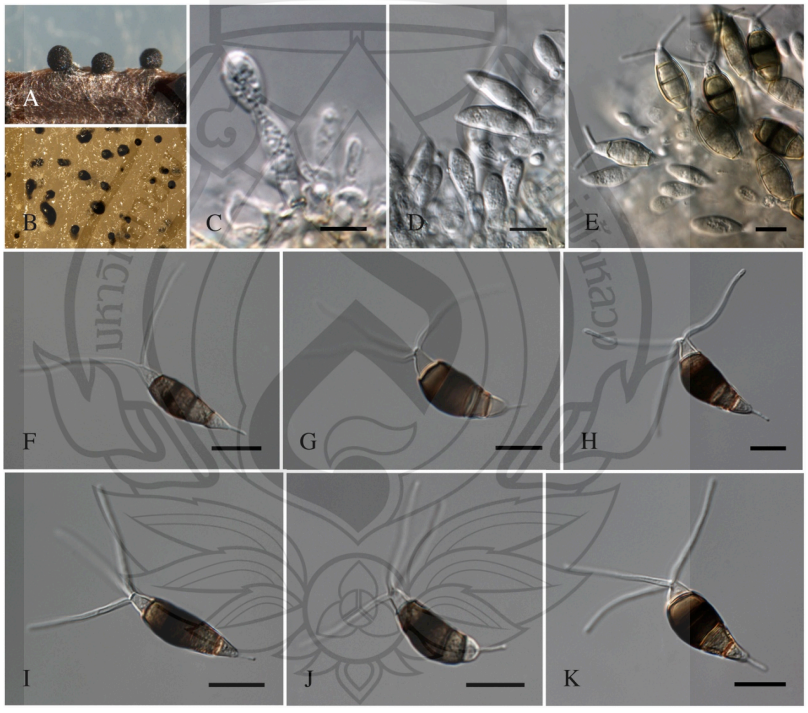
Additional culture examined: CHINA, Yunnan Guangxi Province, Shiwandashan, on dead leaves of *Magnolia* sp., 28 December 1997, Wenping Wu (NN043011 = MFLUCC 12-0280)

Notes: Neopestalotiopsis clavispora is known as a plant pathogen but has been isolated as a common endophyte in recent studies (Keith et al., 2006; Espinoza et al., 2008; Liu et al., 2007; Wei et al., 2007). The holotype of *N. clavispora* was recorded from fallen leaves of *Quercus rubra*, in Auburn, Alabama, USA. In addition, *N. clavispora* has been recorded from leaves of black oak, *Quercus minima* and on fruit husks and leaves of *Aleurites fordii* grows in different parts of USA and on living leaves of *Bruchellia bubalina* in South Africa (Guba, 1961). Thus, *N. clavispora*

appears to have a wide host range and distribution. Since no ex-type culture is available for this species, an epitype with a living culture is designated from a sample collected in Guangxi Province, China. We would prefer to choose an epitype from USA and the original host however, in order to expedite the understanding of this poorly resolved genus, we preferred to designate an epitype which has conidial characters (length, width and length of apical appendages) fitting that of the holotype. The present material is a good match for *N. clavispora.*

Neopestalotiopsis cubana Maharachch. & Crous, sp. nov. (Figure 4.8 A-K).

Etymology: Named after the country where it was collected, Cuba.



Note. *Neopestalotiopsis cubana* CBS 600.96^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.8 *Neopestalotiopsis cubana* (holotype)

Conidiomata pycnidial in culture on PDA, globose, solitary or aggregated, imbedded or semi-immersed, dark brown to black, up to 250 µm diam; exuding globose, brown to black conidial masses. *Conidiophores* reduced to conidiogenous cell. *Conidiogenous cells* discrete, cylindrical to subcylindrical 5–12 × 2–4 µm, or ampulliform to lageniform 3–8 × 1–4 µm, hyaline, smooth-walled, proliferating 2–4 times percurrently, 5–15 × 2–5 µm, collarette present and not flared. *Conidia* fusiform, ellipsoid, straight to slightly curved, somewhat constricted at septa, 4-septate, (19–)20–25(–27) × (7.5–)8–9.5(–10) µm, mean ± SD = 23.4 ± 1.4 × 8.8 ± 0.4 µm; basal cell obconic to conic with a truncate base, hyaline, thin and wall rugose, 3–5 µm long; three median cells doliiform, (13.5–)14–16.5(–17.5) µm long, mean ± SD = 15.5 ± 0.9, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 4.5–6 µm long; third cell honey brown, 4.5–6.5 µm long; fourth cell brown, 4–5.5 µm long); apical cell 4–5 µm long, hyaline, subcylindrical, thin and smooth-walled; with 2–4 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous, (19–)21–27(–28) µm long, mean ± SD = 24 ± 2; basal appendage single, tubular, unbranched, centric, 4–7 µm long (Figure 4.8).

Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with lobate edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

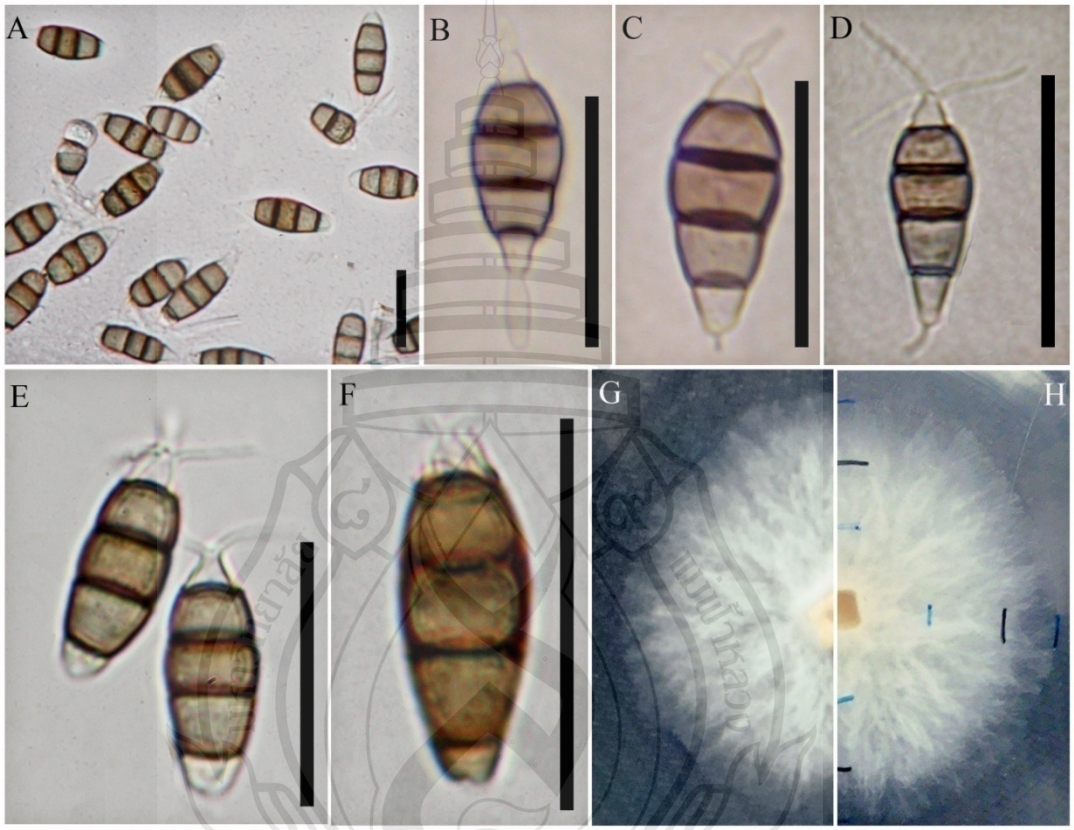
Material examined: CUBA, from leaf litter, June 1996, R.F. Castaneda (CBS H holotype, culture ex-type CBS 600.96=INIFAT C96/44-4).

Notes: *Neopestalotiopsis cubana* is a leaf litter fungus isolated from Cuba, and from a sister clade to CBS 164.42 and CBS 360.61, which are isolated from sand dune in France and *Cinchon*a sp. in Guinea, respectively. The later two isolates are morphologically somewhat similar to *N. cubana*, even though, due to clear ecological differences we prefer to maintain them as *Neopestalotiopsis* spp. until we obtained more cultures and collections. *Neopestalotiopsis cubana* is distinguished from *N. saprophyta* (22–30×5–6 µm) in the section by its wider conidia.

Neopestalotiopsis ellipsospora (Maharachch. & K.D. Hyde) Maharachch, K.D. Hyde & Crous comb. nov. (Figure 4.9 A-H).

Basionym: *Pestalotiopsis ellipsospora* Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 112 (2012).

Etymology: The specific epithet is based on the ellipsoid shape, Latin = *ellipsospora*, of the conidia.



Note. A–F. Conidia. G–H. Colony on PDA, G. from above, H. from below. Scale Bars: A– G= 20 µm

Figure 4.9 *Neopestalotiopsis ellipsospora* (holotype)

Conidia 19–25 × 5–6.5 µm (mean = 21.7 × 6 µm), fusiform, straight to slightly curved, 4-septate; with basal cell conical with obtuse end, hyaline, thin and smooth-walled, 4–5 µm long (mean = 4.3µm); with three median cells 13–15 µm long (mean = 14.1 µm), dark brown, septa and periclinal walls darker than the rest of the

cell, versicoloured, second cell from base pale brown, 4–5 µm (mean = 4.8 µm); third cell darker brown, 4–5 µm (mean = 4.7 µm; fourth cell darker, 4–5 µm (mean = 4.5 µm); apical cell 3–4 µm long (mean = 3.8 µm), hyaline, conical; with apical appendages 5–12 µm long (mean = 8 µm), tubular, 1-3, arising from the apex of the apical cell; basal appendage small or absent, 3–4 µm long (mean = 3.4 µm), filiform (Figure 4.9).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25°C, edge crenate, whitish, with aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse of the culture white.

Habitat/Distribution: Saprobe on dead plant material in Yunnan Province, China and Chiang Rai Province Thailand.

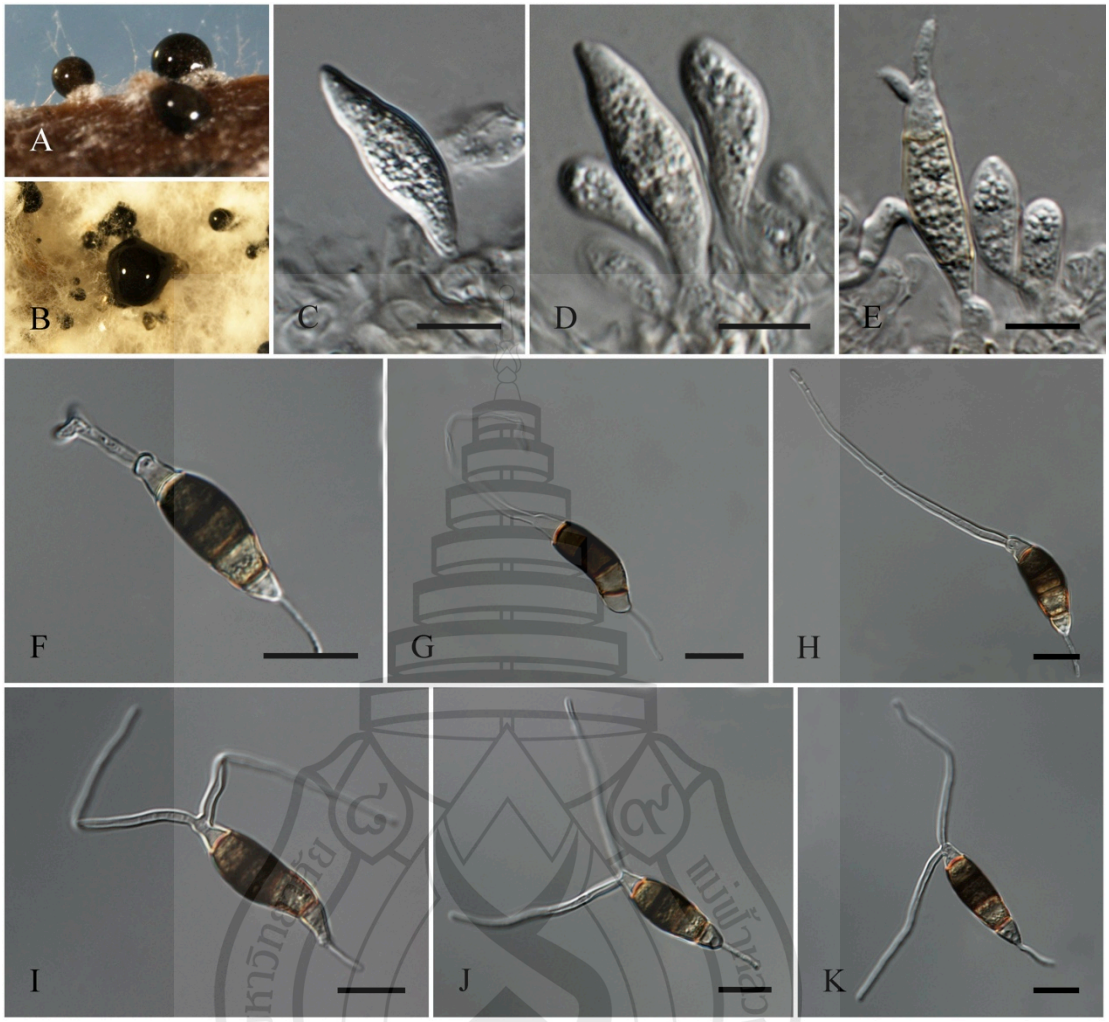
Material examined: CHINA, Yunnan Province, on dead plant materials, Guo Liang-Dong Guo986 (MFLU12-0420; holotype; ex-type living culture MFLUCC 12-0283).

Additional culture examined: THAILAND, Chiang Rai, Tool Kwan, Huay Mesak waterfall, on dead plant material, 12 January 2010, S.S.N Maharachchikumbura (MFLUCC 12-0280)

Notes: Neopestalotiopsis ellipsospora (conidia 19–25 × 5–6.5 µm) can be morphologically distinguished from its phylogenetically closely related species, *N. samarangensis* (conidia 18–21 × 6.5–7.5 µm) (Maharachchikumbura et al. 2012). *Neopestalotiopsis samarangensis* has three long apical appendages (12–18 µm long) whereas in *N. ellipsospora* the 1–3 appendages are shorter (5–12 µm).

Neopestalotiopsis eucalypticola Maharachch. & Crous, sp. nov. (Figure 4.10 A-K).

Etymology: Named after the host genus from which it was isolated, *Eucalyptus*.



Note. *Neopestalotiopsis eucalypticola* CBS 264.37^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.10 *Neopestalotiopsis eucalypticola* (holotype)

Conidiomata (on PDA) pycnidial, globose, solitary or aggregated in clusters, semi-immersed, brown to black, 100–400 µm diam; exuding globose, dark brown conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth, thin-walled, simple, proliferating up to several times percurrently, 3–10 × 2–8 µm, opening 2–6 µm diam.

Conidia fusiform, ellipsoid, straight to slightly curved, 4-euseptate, (22–)23–30(–31) × (9–)7.5–9(–9.5) µm, mean ± SD = 26.7 ± 1.3 × 8.3 ± 0.4 µm; basal cell conic to obconic with a truncate base, hyaline, thin and wall rugose, 5–7 µm long; three median cells doliiform, (15.5–)16–19.5(–20) µm long, mean ± SD = 17.6 ± 1.1, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 5–7 µm long; third cell darker brown, 4.5–7.5 µm long; fourth cell darker brown, 5–7 µm long); apical cell 4.5–7.5 µm long, hyaline, cylindrical to subcylindrical, thin and wall rugose; with 1–2 tubular apical appendages, arising an an extension of the apical cell, unbranched, attenuated, flexuous, (20–)32–55(–66) µm long, mean ± SD = 43 ± 6; basal appendage single, tubular, unbranched, centric, 6–11 µm long (Figure 4.10).

Culture characteristics: Colonies on PDA attaining 30–50 mm diam after 7 days at 25°C, with smooth edge, white to pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: UNKNOWN COUNTRY, from *Eucalyptus globulus*, June 1937, H.W. Wollenweber (CBS H- 15658 holotype, culture ex-type CBS 264.37= BBA 5300).

Notes: Neopestalotiopsis eucalypticola, which is isolated from *Eucalyptus globulus*, phylogenetically and morphologically well distinguished from all other species in the section. The 1–2 tubular, long apical appendages, which are sometimes branched, attenuated, arising an extension of the apical cell notably distinguish *N. eucalypticola* from other species.

Neopestalotiopsis foedans (Sacc. & Ellis) Maharachch., K.D. Hyde & Crous comb. nov.

Basionym: *Pestalotia foedans* Sacc. & Ellis, Michelia 2(no. 8): 575 (1882).

≡*Pestalotiopsis foedans* (Sacc. & Ellis) Steyaert, Bull. Jard. bot. État Brux. 14: 329 (1949).

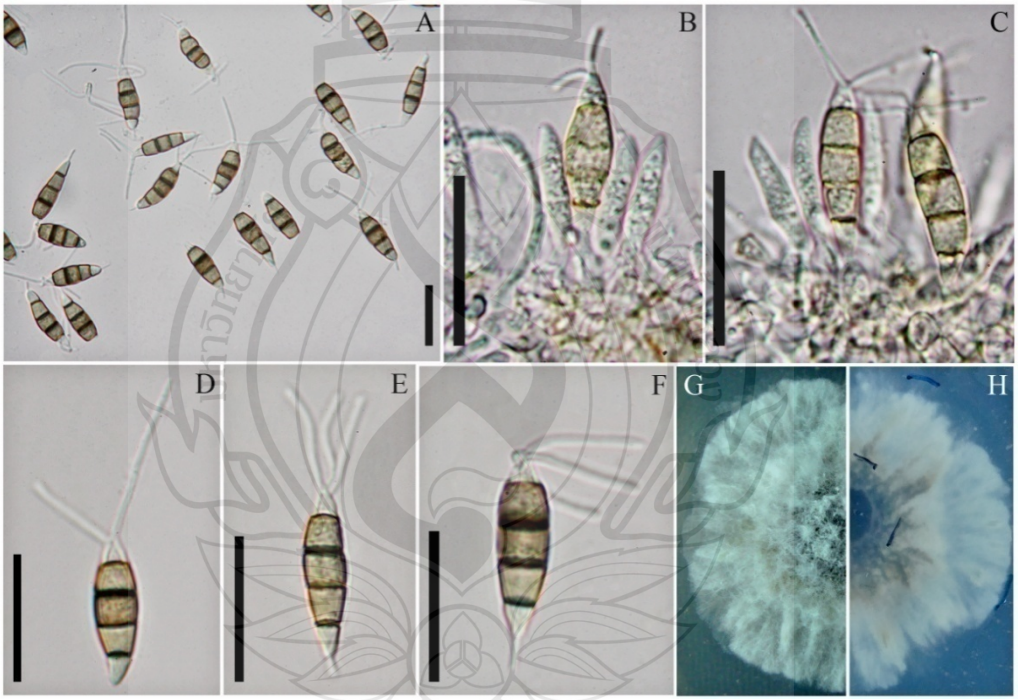
Note. A. Herbarium materials. B. on decaying bark of white cedar *Thuja occidentalis* C. Conidiomata, split irregularly. D. Section of conidiomata. E. Conidiogenous cells F–H. Conidia with versicolorous median cells. Scale Bars: D = 50 µm, E–H= 20 µm

Figure 4.11 *Neopestalotiopsis foedans* (holotype)

Description from holotype (Figure 4.11 A–H).

Conidiomata acervuli, with basal stroma and lateral wall 1–3 cells thick; the wall cells pale brown, *textura angularis*, 200– 400 × 150– 300 µm. *Conidiophores* reduced to conidiogenous cells arising in the concavity of acervuli. *Conidiogenous*

cells discrete, simple, short, filiform. *Conidia* 19–24 × 5.7–6.9 µm (mean = 20.7 × 6.4 µm), fusiform to ellipsoid, straight to slightly curved, 4-septate; basal cell conic, hyaline, thin and smooth-walled, 3.2–4.5 µm long (mean = 4 µm); three median cells 12.5–14.6 µm long (mean = 14 µm), hyaline, versicoloured, verruculose; second cell from base pale brown to olivaceous, 4.3–5.7 µm (mean = 4.9 µm); third cell darker brown to olivaceous, 4.7–6 µm (mean = 5 µm); fourth cell darker, 4.5–5 µm (mean = 4.7 µm); apical cell 4–5 µm long (mean = 4.3 µm), hyaline, cylindric to subcylindric; apical appendages 6–18 µm long (mean = 13.3 µm), 2-3 (mostly 3), arising from the apex of the apical cell; basal appendage present (rarely absent), filiform 3–5 µm (mean = 4 µm).



Note. A. Conidia. B–C. Conidiogenous cells D–F. Conidia with versicolorous median cells. G–H. Colony on PDA, G. from above, H. from below. Scale Bars: A– F= 20 µm

Figure 4.12 *Neopestalotiopsis foedans* (epitype)

Description from epitype (Figure 4.12 A–H).

Conidiophores indistinct, arising in the concavity of acervuli. *Conidiogenous cells* discrete, simple, short, filiform, 2–4 um. *Conidia* 19.2–23.4 × 5.5–7 µm (mean = 20.6 × 6.7 µm), fusiform to ellipsoid, straight to slightly curved, 4-septate; basal cell conic, hyaline, thin and smooth-walled, 3.2–5 µm long (mean = 4.4 µm), three median cells hyaline, versicoloured, verruculose, 12.7–15.3 µm long (mean = 13.7 µm); second cell from base pale brown to olivaceous, 4.1–5.2 µm (mean = 4.8 µm); third cell darker brown to olivaceous, 4.7–5.3 µm (mean = 5 µm); fourth cell darker, 4.9–5.7 µm (mean = 5.3 µm); apical cell 4–5 µm long (mean = 4.3 µm), hyaline, cylindric to subcylindric; apical cell hyaline, subcylindric to conic 3.3–4.4 µm (mean = 3.7 µm); apical appendages 8–15 µm long (mean = 12.6 µm), 2–3 (mostly 3), arising from the apex of the apical cell; basal appendage present (rarely absent), filiform, 36 µm long (mean = 4.3 µm).

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25°C, edge undulate, whitish, aerial mycelium on surface, with black fruiting bodies, gregarious; reverse of culture whitish (rarely pale luteous).

Habitat/Distribution: Known to inhabit in *Thuja occidentalis* in USA and mangroves, *Calliandra haematocephala* and *Neodypsis decaryi* in China.

Material examined: USA, Newfield, New Jersey, on decaying bark of white cedar, *Thuja occidentalis* L., October 1880, Ellis and Harkness (BPI 0405695, holotype); CHINA, Xinglong, Hainan, on mangrove leaves, April 2005, A.R. Liu L443 (MFLU 12-0424, epitype designated here; extype living culture-CGMCC 3.9123).

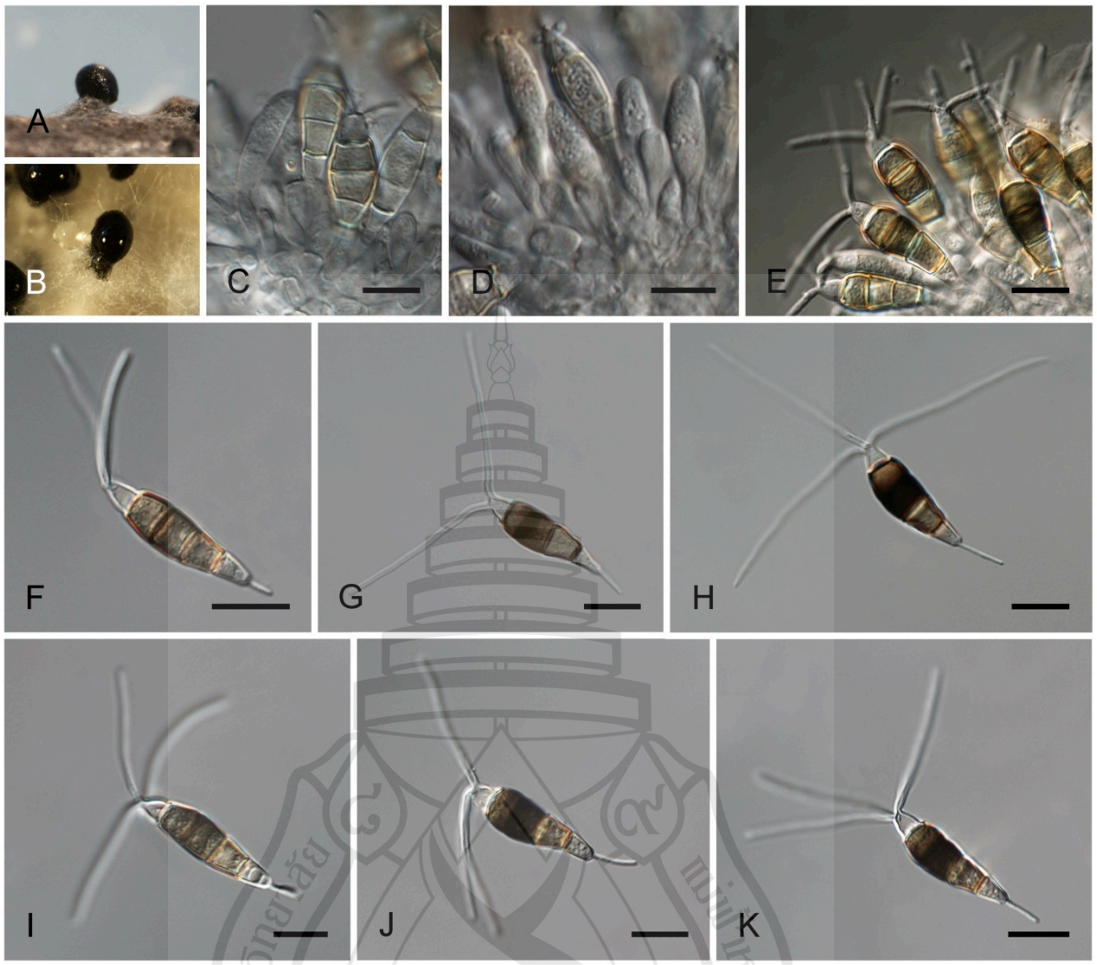
Additional culture examined: CHINA, Xinglong, Hainan, on leaves of *Calliandra haematocephala*, May 2004, A.R. Liu L101 (CGMCC 3.9202); CHINA, Xinglong, Hainan, on leaves of *Neodypsis decaryi*, May 2004, A.R. Liu L96 (CGMCC 3.9178).

Notes: The holotype of *N. foedans* was recorded from decaying bark of white cedar, in New Jersey, USA. In addition, *N. foedans* was recorded from *Cupressus thyoides* in New Jersey, USA; on *Cryptomeria japonica* in Philadelphia and Japan; leaves and twigs of *C. japonica* in Princeton and on needles of *Pinus mugo* in Pennington (Guba, 1961). Thus, *N. foedans* appears to have a wide host range and

distribution. Recently, *N. foedans* was discovered as a source of bioactive metabolites of high economic importance (Ding et al., 2008a). Since no ex-type culture is available for this species, an epitype with a living culture is designated from a sample collected in Hainan Province, China. We would prefer to choose an epitype from USA and the original host however, in order to expedite the understanding of this poorly resolved genus we choose to be pragmatic and designated an epitype which has conidial characters (length, width and length of apical appendages) similar to that of the holotype and hence this is a good match for *N. foedans*.

Neopestalotiopsis formicarum Maharachch. & Crous, sp. nov. (Figure 4.13 A-K).

Etymology: Named after the host family from which it was isolated, *Formicidae*.



Note. *Neopestalotiopsis formicarum* CBS 362.72^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.13 *Neopestalotiopsis formicarum* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary or aggregated in clusters, semi-immersed, brown to black, 200–500 µm diam; exuding globose, dark brown conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth, thin-walled, simple, proliferating several times percurrently, 3–10 × 2–5 µm, opening 1–3 µm diam. *Conidia* ellipsoid, straight to slightly curved, 4-septate, (20–)21–28(–29) ×

7.5–9.5 µm, mean ± SD = 24.6 ± 1.4 × 8.6 ± 0.4 µm; somewhat constricted at septa; basal cell conic to acute with truncate base, thin-walled, rugose, 4.5–6 µm long; three median cells (14–)15–16.5(–17) µm long, mean ± SD = 15.1 ± 1, doliiform, verruculose, versicoloured, brown, septa darker than the rest of the cell, (second cell from base pale brown, 4–6.5 µm long; third cell darke brown, 4–6 µm long; fourth cell brown, 4.5–6.5 µm long); apical cell subcylindrical, hyaline, thin and smooth-walled, 4–5.5 µm long; with 2–3 tubular apical appendages, arising from the apical crest, flexuous, unbranched, (20–)23–33(–36) µm long, mean ± SD = 27 ± 4; basal appendage single, tubular, unbranched, centric, 4–8 µm long (Figure 4.13).

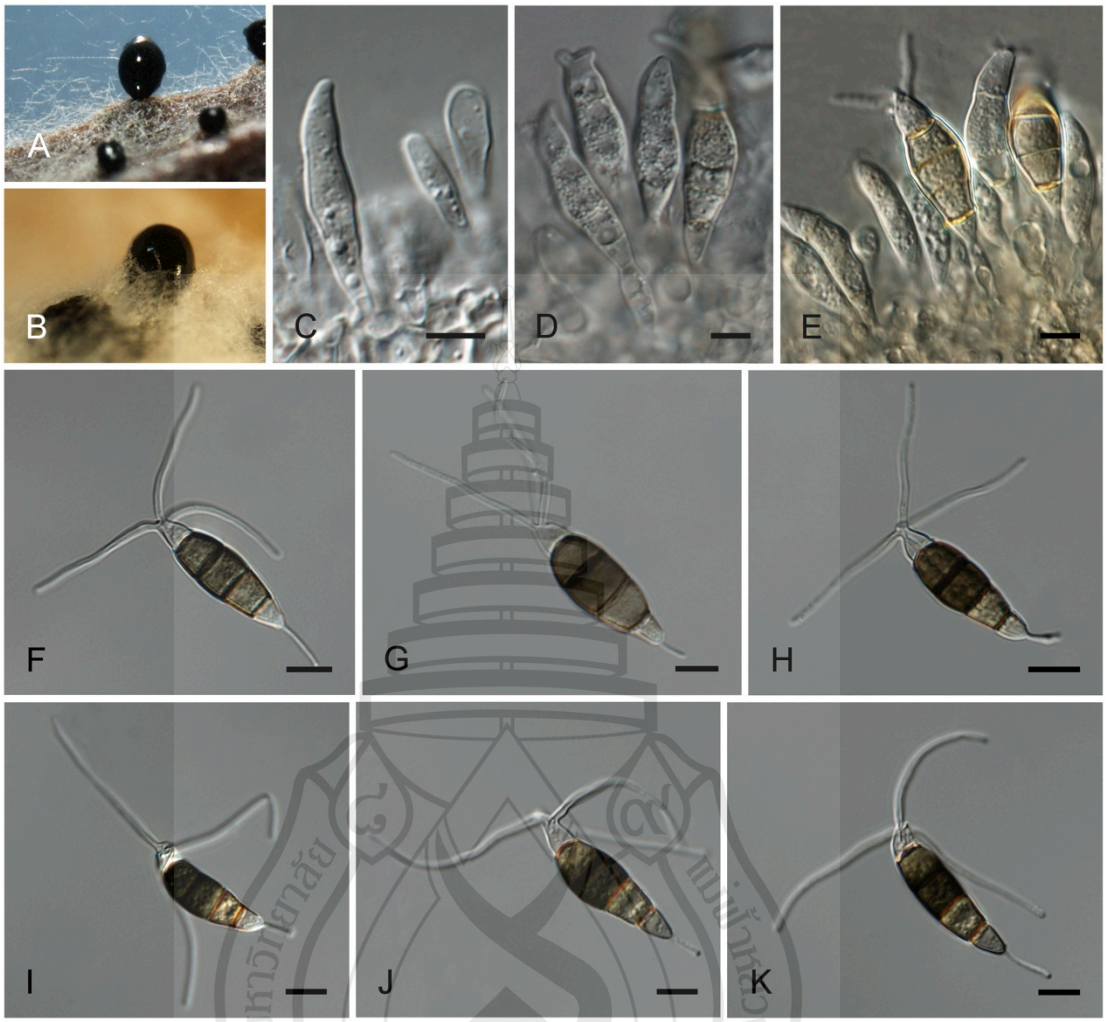
Culture characteristics: Colonies on PDA reaching 30–40 mm diam after 7 days at 25°C, edge undulate, whitish to pale honey-coloured, with moderate aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: GHANA, from dead ant (*Formicidae*), Nov 1971, H.C. Evans, (CBS H-15661 holotype, culture ex-type CBS 362.72); Cuba, from plant debris, 1982, sent to CBS for ident. by G. Arnold (via W. Gams), CBS H-15752, culture CBS 115.83.

Notes: Neopestalotiopsis formicarum is a saprobic species recorded from dead ant in Ghana and plant debris from Cuba. It belongs to the *N. samarangensis* section and phylogenetically clearly separable by all other related taxa.

Neopestalotiopsis honoluluana Maharachch. & Crous, sp. nov. (Figure 4.14 A-K).

Etymology: Named after the capital of the country where it was collected, Honolulu in Hawaii.



Note. *Neopestalotiopsis honoluluana* CBS 114495^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.14 *Neopestalotiopsis honoluluana* (holotype)

Conidiomata pycnidial in culture on PDA, globose to clavate, solitary or aggregated in clusters, semi-immersed, brown to black, 100–400 µm diam; exuding globose, dark brown conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, subcylindrical to ampulliform, hyaline, smooth, thin-walled, simple, proliferating up to 3 times percurrently, 5–20 × 2–6 µm, opening 1–3

µm diam. *Conidia* ellipsoid, straight to slightly curved, somewhat constricted at septa, 4-septate, (21–)24–34(–35) × (7–)7.5–9.5(–10) µm, mean ± SD = 28 ± 2.3 × 8.3 ± 0.6 µm, basal cell obconic with truncate base, thin-walled, rugose, 4.5–7 µm long; three median cells (14.5–)15–20(–21) µm long, mean ± SD = 17.3 ± 1.6, doliiform, rugose, versicoloured, brown to olivaceous, (second cell from base pale brown, 4.5–7 µm long; third cell darker brown, 4–6.5 µm long; fourth cell brown, 5.5–7.5 µm long); apical cell subcylindrical, hyaline, thin and smooth-walled, 4–7.5 µm long; with 3 tubular apical appendages, arising from the apical crest, flexuous, unbranched, (22–)23–40(–47) µm long, mean ± SD = 32 ± 6.0; basal appendage single, unbranched, centric, 2.5–10 µm long (Figure 4.14).

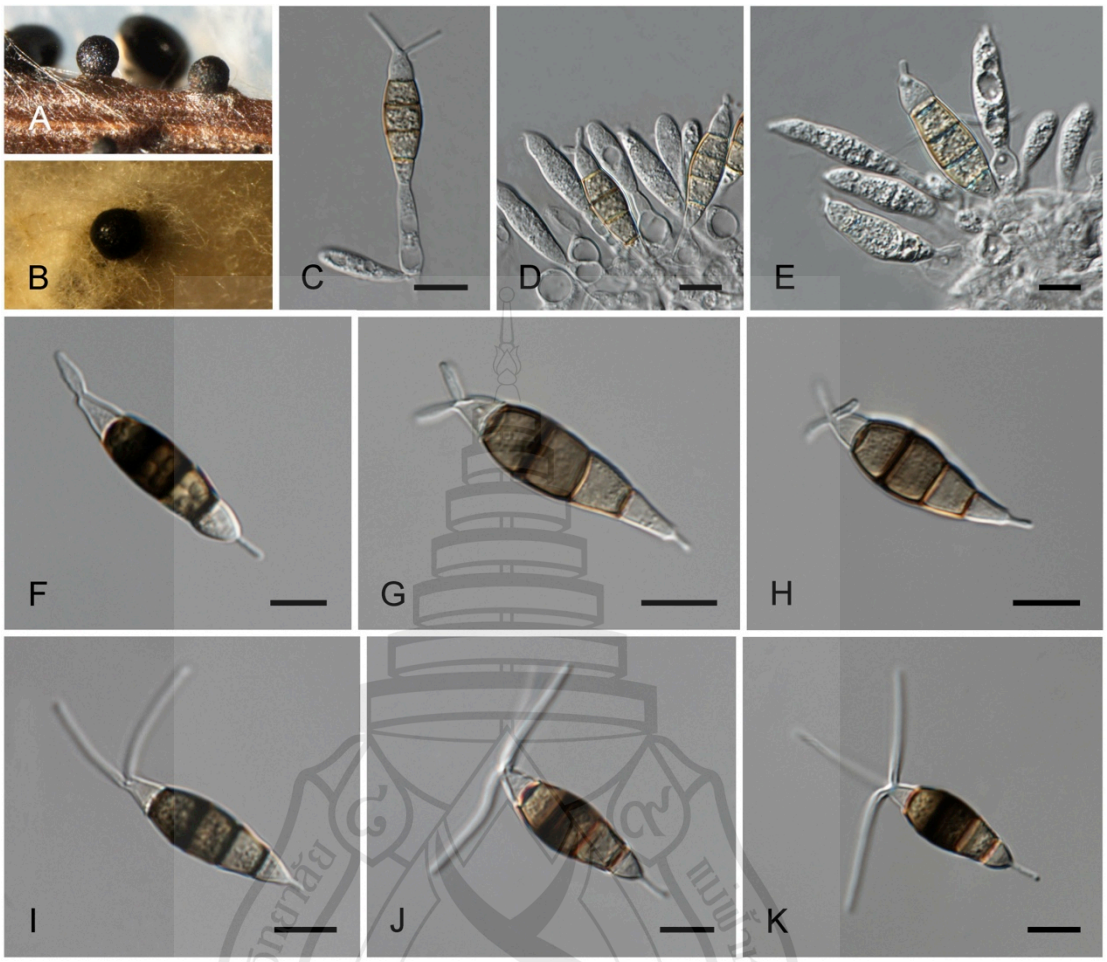
Culture characteristics: Colonies on PDA reaching 30–50 mm diam after 7 days at 25°C, edge entire, whitish to pale honey-coloured, with moderate aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: HAWAII, from *Telopea* sp., 8 Dec 1998, P.W. Crous & M.E. Palm (CBS H holotype, culture ex-type CBS 114495= STE-U 2076).

Notes: Neopestalotiopsis honoluluana confined to *Telopea* sp. in Hawaii and USA, which is a sister taxon to *N. eucalypticola* and *N. zimbabwana*. *Neopestalotiopsis eucalypticola* differ from *N. honoluluana* by its longer and lesser number of apical appendages. The conidia of *N. zimbabwana* are smaller and apical appendages are shorter than those in *N. honoluluana. Neopestalotiopsis australis* is another disease causal agent in the section, which isolated from the same host genus *Telopea*, but in Australia. Morphologically, however, conidia of *N. australis* are smaller in size and apical appendages are somewhat shorter.

Neopestalotiopsis javaensis Maharachch. & Crous, sp. nov. (Figure 4.15 A-K).

Etymology: Named after the island where it was collected, Java.



Note. *Neopestalotiopsis javaensis* CBS 257.31^{T}. A. Conidiomata sporulating on PNA. B. Conidioma on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 μm

Figure 4.15 *Neopestalotiopsis javaensis* (holotype)

Conidiomata pycnidial in culture on PDA, globose to clavate, solitary, semi-immersed, dark brown to black, up to 250 μm diam; exuding dark brown to black conidial masses. *Conidiophores* reduced to conidiogenous cell. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, rugose-walled, proliferating 2–3 times percurrently, 5–25 × 3–10 μm, wide at the base, opening 2–4 μm diam. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (24–)25–30(–31) × (6.5–)7–

8.5(–9) µm, mean ± SD = 27.3 ± 1.6 × 7.6 ± 0.3 µm; basal cell conic to obconic with a truncate base, hyaline, thin and wall rugose, 4.5–6.5 µm long; three median cells doliiform, (14.5–)15–18.5(–19) µm long, mean ± SD = 17.1 ± 1.2, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 5–7 µm long; third cell brown, 5–7 µm long; fourth cell brown, 5.5–7.5 µm long); apical cell subcylindrical, hyaline, thin and smooth-walled, 3.5–5.5 µm long; with 1–3 tubular apical appendages, arising from the apical crest, unbranched, filiform, 2–10(–18) µm long, mean ± SD = 5.7 ± 3; basal appendage single, tubular, unbranched, centric, 2–4 µm long (Figure 4.15).

Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with lobate edge, pale honey-coloured, sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

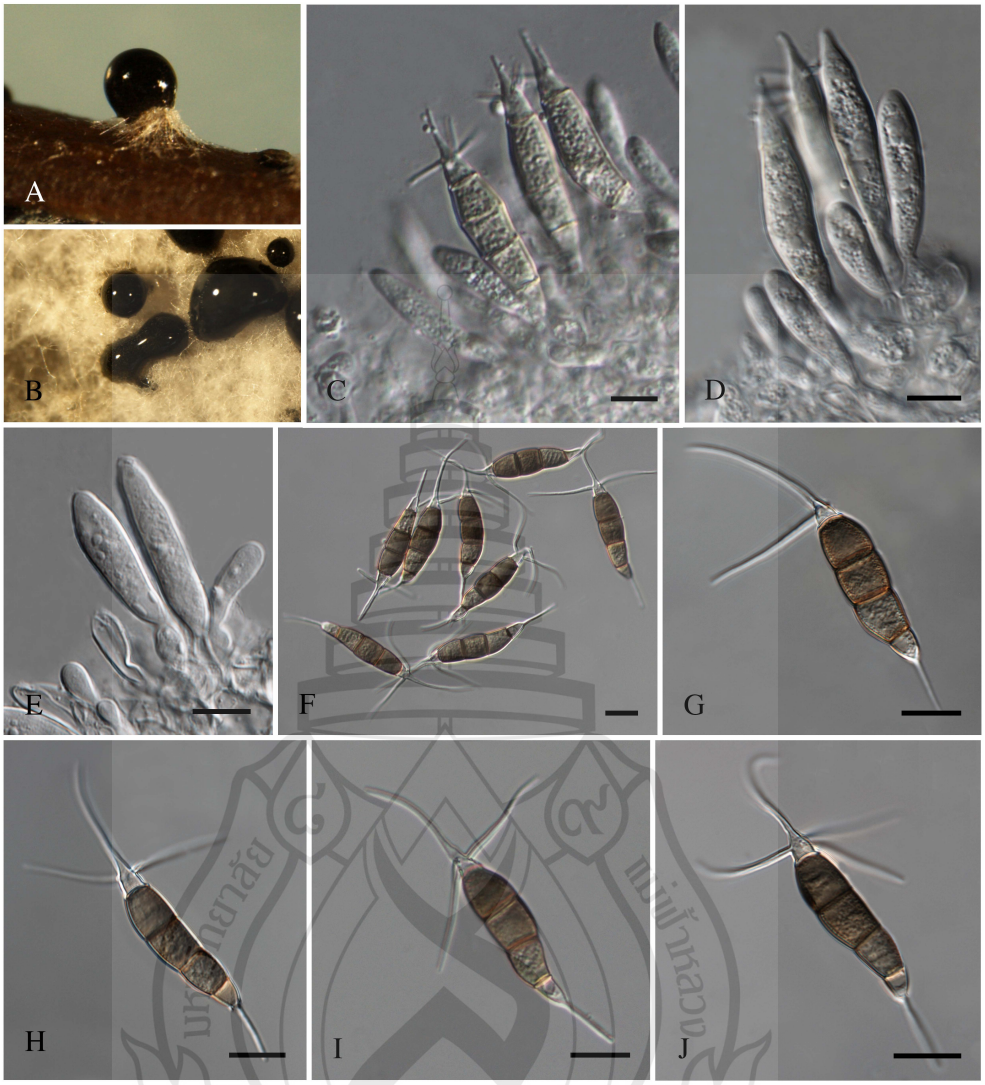
Material examined: JAVA, Manado, from leaf of *Cocos nucifera,* collection date unknown, R.L. Steyaert (CBS H-15764 holotype, culture ex-type CBS 257.31).

Notes: *Neopestalotiopsis javaensis* belongs to the *N. saprophyta* section, which is isolated from leaves of coconut in Java. It forms a separate cluster in DNA phylogeny, as sister to a species including *N. foedans*, *N. mesopotamicum* and *N. rosa. Nestalotiopsis javaensis* has a relatively larger conidial dimensions when compared with *N. foedans* (19–23.5 × 5.5–7 µm) (Maharachchikumbura et al. 2012). *Nestalotiopsis javaensis* differ from *N. mesopotamicum* and *N. rosa* by having notable shorter apical appendages (see notes under *N. rosa*).

Neopestalotiopsis magna (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.16 A-J).

Basionym: *Pestalotiopsis magna* Maharachch. & K.D. Hyde, Mycol. Prog. 56(1): 121 (2013).

Etymology: The specific epithet is based on the larger size of the conidia compare to most species in versicolour clade and in Latin, large is *magnus*.



Note. A. Conidioma on Water Agar with sterile pine needles. B. Conidiomata on PDA. C-E. Conidiogenous cells and developing conidia. F-J. Conidia. Scale bars = 10 µm

Figure 4.16 *Neopestalotiopsis magna* (holotype)

Saprobic on decaying leaves. Sexual state: Unknown. Asexual state: *Conidiomata* 200–400 µm diam, pycnidial, globose, brown, semi-immersed on PDA releasing black conidia in a slimy, globose, glistening mass. *Conidiophores* indistinct. *Conidiogenous cells* discrete to lageniform, hyaline, smooth and thin-walled, 3–8 ×

2–6 µm, proliferating 1-2 times percurrently, collarette present and not flared. *Conidia* (40)42–46(47)× (9)9.5–12 µm (mean ± SD = 44.1 ± 1.4 × 11.0 ± 0.6 µm), fusiform to clavate, straight to slightly curved, 4-septate; basal cell obconic with a truncate base, hyaline or sometimes pale brown, thin- and smooth-walled, 8.5–9 µm long; three median cells (30)31–33.5(34) µm long (mean ± SD = 31.8 ± 1.4 µm), brown, septa and periclinal walls darker than rest of the cell, versicoloured, wall rugose; second cell from base pale brown, 9.5–11.5 µm long; third cell brown, 9.5–11 µm long; fourth cell brown, 10.5–12 µm long; apical cell 5–8 µm long, hyaline, conic to acute; with 2–4 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (10)16–26(30) µm long (mean ± SD = 23.2 ± 4.2 µm); single basal appendage, tubular, unbranched, centric, 11–15 µm long.

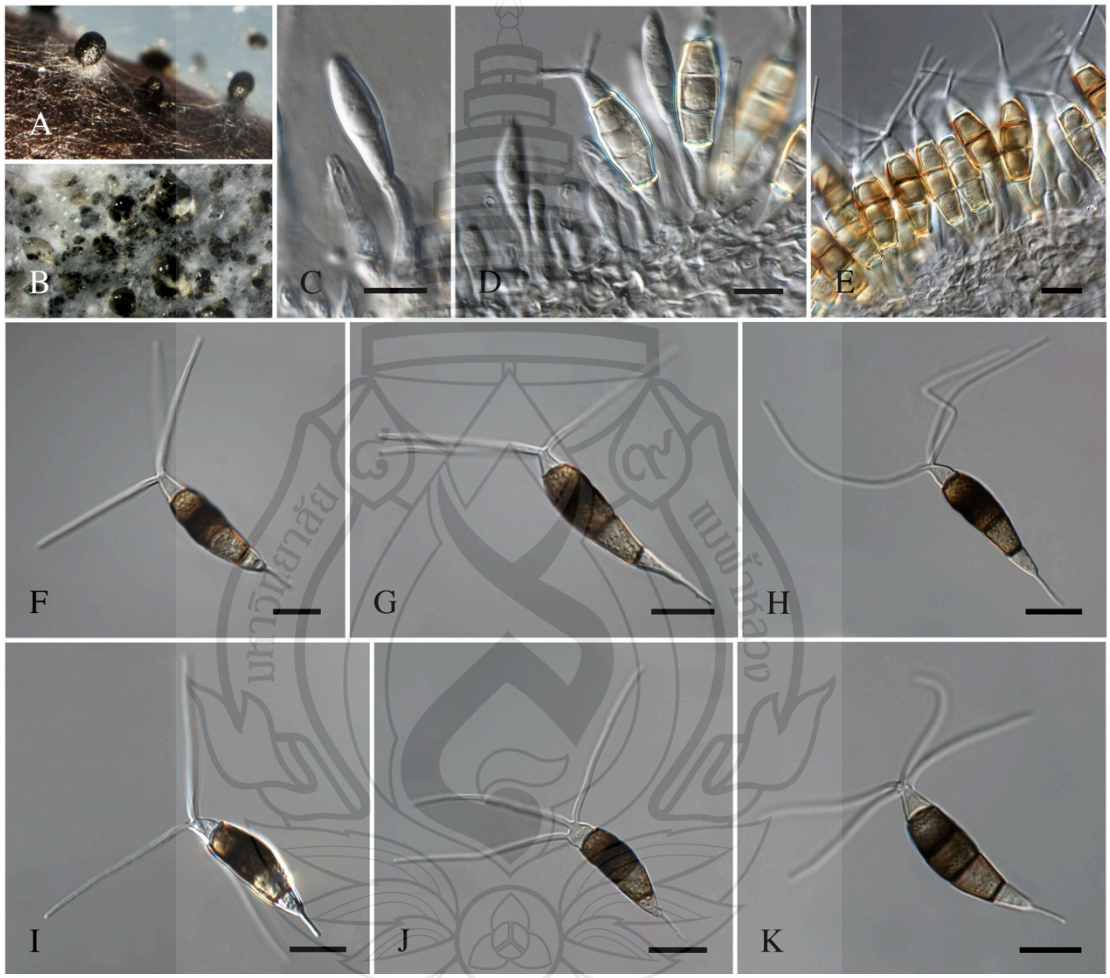
Culture characteristics: Colonies fast growing on PDA attaining 50-70 mm diam after 7 days at 25 °C, edge entire, yellowish white, dense, aerial mycelium on surface, fruiting bodies black; reverse similar in colour.

Material examined: FRANCE, Ariège, Rimont, on decaying leaves of *Pteridium* sp., Aug 2011, coll. K.D. Hyde, isol. S.S.N. Maharachchikumbura, (MFLU 13-0594 holotype, culture ex-type = MFLUCC 12-0652)

Notes: Neopestalotiopsis magna is an outlying species in the *Neopestalotiopsis* and is distinguished from related species by its larger conidia. The morphologically overlapping species in conidial size are *Pestalotiopsis. grandis* Dube & Bilgrami (26–48 × 7–8 µm), *P. hughessii* Steyaert (34–45 × 7–11 µm), *P. kunmingensis* J.G. Wei & T. Xu (33–47 × 7.5–10 µm), *P. macrospora* (Ces.) Steyaert (30–45 × 9–12 µm) and *P. montellicoides* (Doyer) Steyaert (35–48 7.5–10.6 µm) (Steyaert 1949; 1953; Guba 1961; Dube and Bilgrami 1966). However, with the exception of *P. kunmingensis* the three median cells in all of the above species are concolorous (*Pestalotiopsis*) contrast to versicolorous (present in *Neopestalotiopsis*) in *N. magna*. Molecular data shows that *P. kunmingensis* clusters in the concolorous (*Pestalotiopsis*) group (Maharacchchikumbura et al. 2012; 2013a) and apical appendages in *N. magna* are not knobbed, like those in *P. kunmingensis*.

Neopestalotiopsis mesopotamicum Maharachch. & Crous, sp. nov. (Figure 4.17 A-K).

Etymology: Named after the country where it was collected, Mesopotamia, hence Iraq.



Note. *Neopestalotiopsis mesopotamicum* CBS 336.86^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 μm

Figure 4.17 *Neopestalotiopsis mesopotamicum* (holotype)

Conidiomata (on PDA) pycnidial, globose or clavate, aggregated or confluent, imbedded or semi-immersed, black, up to 250 µm diam; exuding brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical to subcylindrical 8–20 × 2–7 µm, hyaline, smooth-walled, proliferating 2–3 times percurrently, 5–18 × 2–4 µm, collarette present and not flared, with prominent periclinal thickening. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (25–)26–32(–34) × (7–)7.5–9(–9.5) µm, mean ± SD = 29.6 ± 1.1 × 8 ± 0.4 µm; basal cell conic with a truncate base, hyaline, thin and wall rugose, 6–7.5 µm long; three median cells doliiform, (17–)17.5–20(–21) µm long, mean ± SD = 18.5 ± 1.2, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 5–7.5 µm long; third cell honey brown, 5.5–7.5 µm long; fourth cell honey brown, 6.5–7.5 µm long); apical cell 4.5–6 µm long, hyaline, cylindrical to subcylindrical, thin and smooth-walled; with 3–4 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (25–)28–38(–41) µm long, mean ± SD = 33.3 ± 3.2; basal appendage single, tubular, unbranched, centric, 4–6.5 µm long (Figure 4.17).

Culture characteristics: Colonies on PDA attaining 30–50 mm diam after 7 days at 25°C, with lobate edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, concentric fruiting bodies; reverse similar in colour.

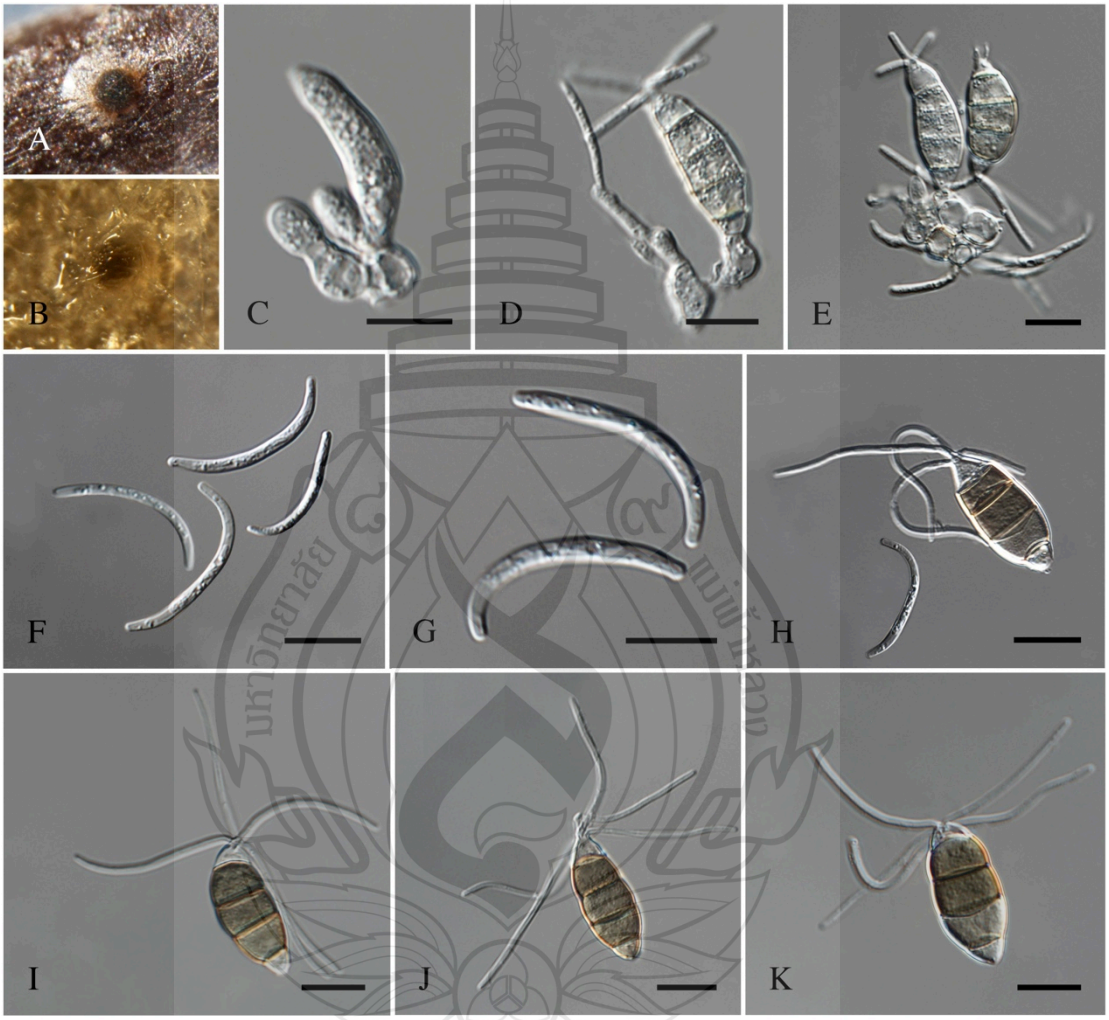
Material examined: IRAQ, from *Pinus brutia*, 23 June 1986, sent to CBS for ident. by A. I. Al-Kinany, Mosul University, Mosul, Iraq (CBS H-15782 holotype, culture ex-type CBS 336.86); Turkey, from *Eucalyptus* sp., 2 Apr. 1974, G. Turhan, herbarium CBS H-15739; CBS H-15741, culture CBS 299.74; India, New Delhi, from *Achras sapota,* May 1969, unknown collector, culture CBS 464.69.

Notes: *Neopestalotiopsis mesopotamicum* pertains to the *N. rosa* complex and forms a sister group to *N. javaensis* and *N. rosa*, and deviates by having larger conidia and longer apical appendages (see notes under *N. rosa*).

Neoestalotiopsis natalensis (J.F.H. Beyma) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.18 A-K).

Basionym: *Pestalotia natalensis* J.F.H. Beyma, Antonie van Leeuwenhoek, 6: 288, 1940. MycoBank 289181.

≡*Pestalotiopsis natalensis* (J.F.H. Beyma) Steyaert, Bulletin du Jardin Botanique de l'État à Bruxelles, 19 (3): 344, 1949. MycoBank 289212.



Note. *Neoestalotiopsis natalensis* CBS 138.41^{T}. A. Conidioma sporulating on PNA. B. Conidioma on PDA. C–E. Conidiogenous cells. F–G. Beta conidia. H. Beta and alpha conidia. I–K. Alpha conidia. Scale bars = 10 µm

Figure 4.18 *Neoestalotiopsis natalensis* (holotype)

Conidiomata (on PDA) pycnidial, globose, solitary or aggregated, immersed or semi-immersed, dark brown, 50–150 µm diam. *Alpha Conidiophores* indistinct, often reduced to conidiogenous cells. *Alpha Conidiogenous cells* discrete, hyaline, rugose, simple, ampulliform, sometimes slightly wide at the base, truncate at apex, proliferating once or twice, 4–10 × 3–9 µm. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (21–)23–28(–29) × (7.5–)8–10(–10.5) µm, mean ± SD = 25.0 ± 1.6 × 9 ± 0.4 µm; basal cell hemispherical, hyaline or slightly brown, thin and smooth-walled, 4–7 µm long; three median cells (15.5–)16–19(–19.5) µm long, mean ± SD = 17.5 ± 0.8, concolorous or two upper median cells slightly darker than the lower median cell, brown, septa darker than the rest of the cell, and conidium constricted at septum (second cell from the base 5.5–8 µm long; third cell 5.5–8 µm long; fourth cell 5–7 µm long); apical cell 4–6.5 µm long, hyaline, conic; with 3–5 tubular apical appendages, arising from the apical crest, unbranched, (15–)18–32(–35) µm long, mean ± SD = 25 ± 4; lack basal appendages, when present unbranched, centric, 2–8 µm long. *beta Conidiophores* 1-2 septate, subcylindrical, hyaline, smooth, up to 12 µm long or often reduced to conidiogenous cells. *beta Conidiogenous cells* discrete, hyaline, smooth, cylindrical, terminated in an apex with 1–2 loci which gave rise to *beta* conidia in a sympodial arrangement. 5–15 × 2–6 µm. *beta conidia* (20–)22–29(–31) × 1–3 µm, mean ± SD = 25.6 ± 2 × 1.9 ± 0.2, widest in the middle, curved, hyaline, apex subobtuse, base truncate.

Culture characteristics: Colonies on PDA attaining 25–35 mm diam after 7 days at 25°C, with smooth edge, whitish, with sparse aerial mycelium on the surface; reverse similar in colour. Cultures hardly sporulate on PDA, only few conidiomata can be seen upon 4 month of incubation.

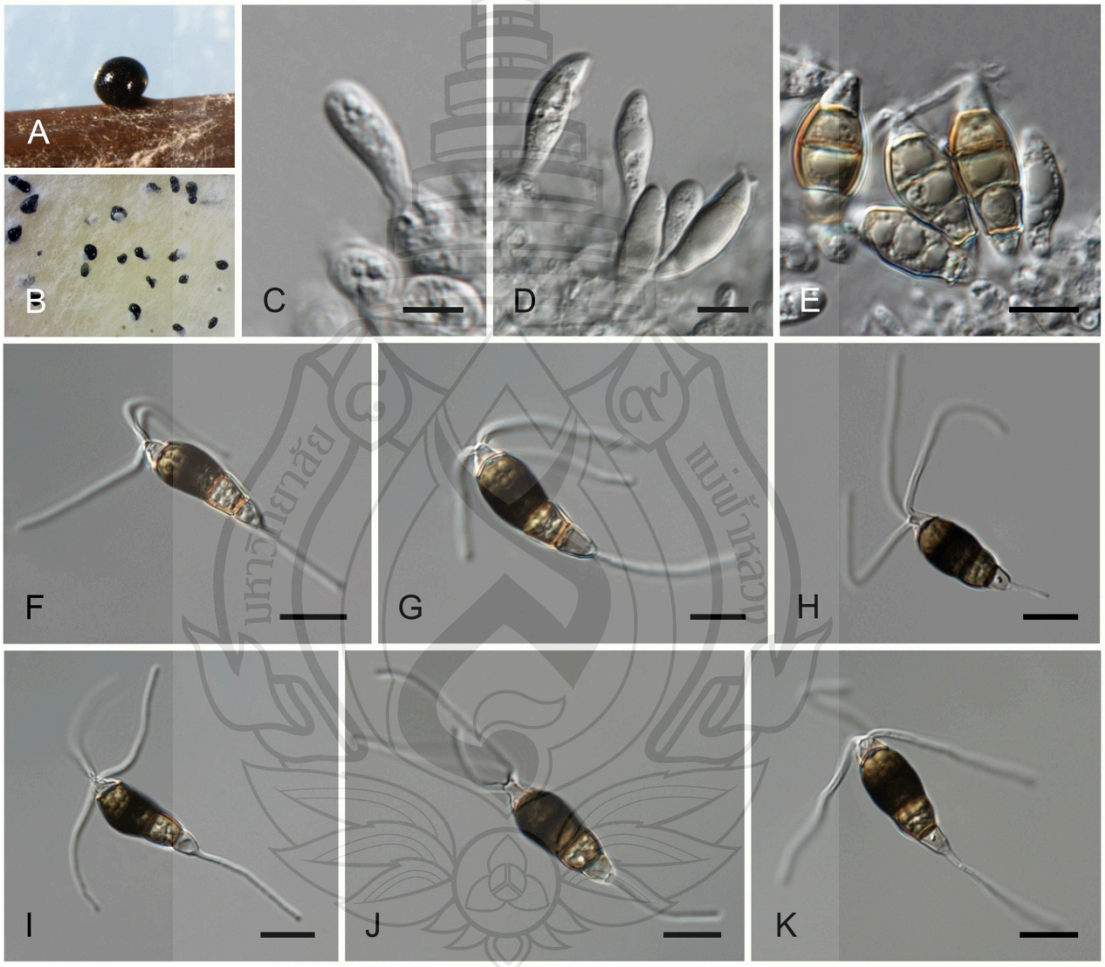
Material examined: SOUTH AFRICA, Natal, from *Acacia mollissima* (black wattle), Jan. 1941, M.S.J. Ledeboer, culture ex-type CBS 138.41.

Notes: An unusual feature of the *N. natalensis* is the presence of a synanamorph in culture. Most species seem to form them on the host tissue. Crous et al. (2006) observed alpha and beta conidia in *Pestalotiopsis disseminata* isolated from *Eucalyptus eurograndis* in Colombia. However, alpha and beta conidia only seen in the original host substrate and not in the culture. According to the original description,

the conidia of *N. natalensis* are narrow (25–33 × 6–9 µm) and apical appendages are longer (30–40 µm) than in present observations.

Neopestalotiopsis piceana Maharachch. & Crous, sp. nov. (Figure 4.19 A-K).

Etymology: Named after the host genus from which it was isolated, *Picea*.



Note. *Neopestalotiopsis piceana* CBS 394.48^{T}. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.19 *Neopestalotiopsis piceana* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary, semi-immersed, brown to black, 100–300 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth, thin-walled, simple, (4–12 × 2–10 µm), wide at base, opening 2–5 µm. *Conidia* ellipsoid to clavate, straight to slightly curved, 4-septate, (19–)19.5–25(–26) × (7–)7.5–9(–9.5) µm, mean ± SD = 22.1 ± 0.8 × 8.1 ± 0.6 µm; somewhat constricted at septa; basal cell obconic with truncate base, thin-walled, rugose, 3.5–5.5 µm long; three median cells (13–)13.5–16(–16.5) µm long, mean ± SD = 15 ± 0.9, doliiform, verruculose, versicoloured, septa darker than the rest of the cell, (second cell from base pale brown, 4–6 µm long; third cell dark brown, 4.5–6.5 µm long; fourth cell brown, 5–7 µm long); apical cell obconic, hyaline, thin and smooth-walled, 3–6 µm long; with 3 tubular apical appendages, arising from the apical crest, flexuous, unbranched, (19–)21–31(–33) µm long, mean ± SD = 24.8 ± 3; basal appendage single, tubular, unbranched, centric, 6–23 µm long .

Culture characteristics: Colonies on PDA reaching 40–50 mm diam after 7 days at 25°C, edge entire, whitish to pale honey-coloured, with spares aerial mycelium on the surface, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: UK, from wood of *Picea* sp., Aug. 1948, S.M. Hasan, (CBS H-15705 holotype, culture ex-type CBS 394.48); Sulavesi, from *Cocos nucifera*, unknown collection date and collector, CBS H-15645, culture CBS 254.72; unknown country, from fruit of *Mangifera indica*, Apr. 1930, Levie, CBS H-15688, culture CBS 225.30.

Notes: Neopestalotiopsis piceana is characteried by clavate shape conidia with a long basal appendage. *Neopestalotiopsis piceana* is genetically sister position to *N. aotearoa*, which has been described from a canvas in New Zealand. Two species are differing from each other by shape of the conidia and length of the apical appendage (see notes under *N. aotearoa*).

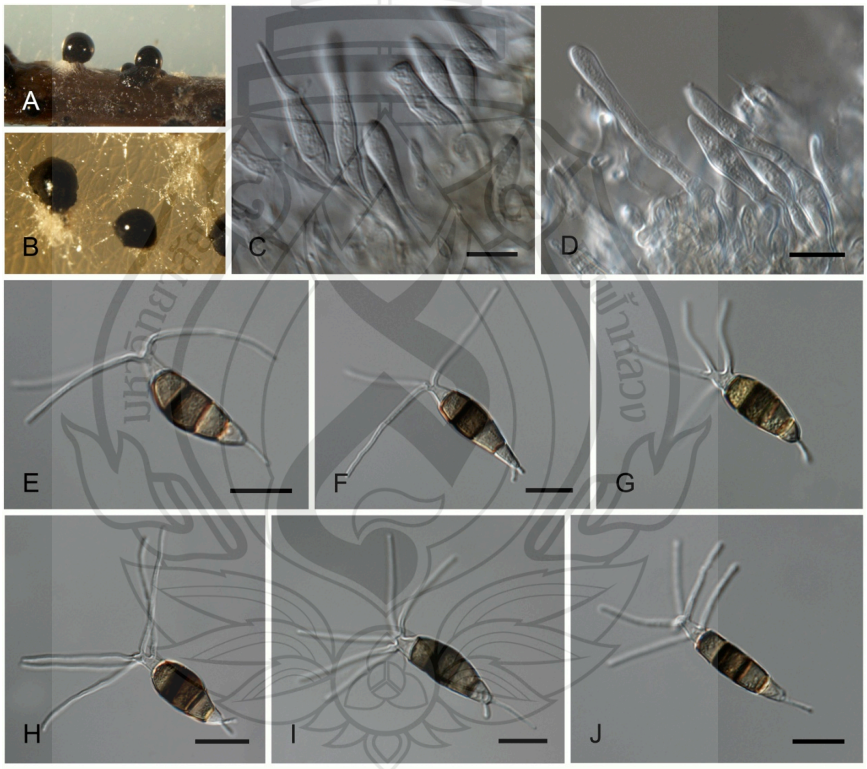
Neopestalotiopsis protearum (Crous & L. Swart) Maharachch., K.D. Hyde & Crous comb. nov.

Basionym: *Pestalotiopsis protearum* (Crous & L. Swart), Persoonia, Mol. Phyl. Evol. Fungi 27: 34 (2011).

Material examined: Zimbabwe, Harare, Aveley Farm, on living leaves of *Leucospermum cuneiforme* cv. 'Sunbird', 6 Mar. 1998, L. Swart, (PREM 56186 holotype, culture ex-type CPC 1765 = CBS 114178).

Neopestalotiopsis rosa Maharachch. & Crous, sp. nov. (Figure 4.20 A-J).

Etymology: Named after the host genus from which it was isolated, *Rosa.*



Note. *Neopestalotiopsis rosa* CBS 101057^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 μm

Figure 4.20 *Neopestalotiopsis rosa* (holotype)

Conidiomata (on PDA) pycnidial, globose, solitary, semi-immersed, dark brown to black, 100–300 µm diam; exuding a globose, dark brown, glistening conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical, hyaline, smooth-walled, simple, proliferating 2–4 times percurrently, tapering towards a truncate apex with visible periclinal thickening, 5–20 × 2–8 µm. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (20–)22–37(–29) × (7–)7.5–9.5(–10.5) µm, mean ± SD = 24.8 ± 1.5 × 8.5 ± 0.6 µm; basal cell conic to obconic with a truncate base, hyaline, thin and wall rugose, 3.5–6 µm long, often with a short oblique appendage projecting from the base adjoining the point of attachment of the basal appendage; three median cells doliiform, (14–)14.5–18(–18.5) µm long, mean ± SD = 16 ± 1.1, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 4.5–6.5 µm long; third cell honey brown, 5–7 µm long; fourth cell brown, 5–7 µm long); apical cell 3.5–5.5 µm long, hyaline, cylindrical, thin and smooth-walled; with 3–5 tubular apical appendages, not arising from the apical crest, but each inserted at a different locus in the upper half of the apical cell, unbranched, filiform, (22–)24–31(–33) µm long, mean ± SD = 27 ± 2.1; basal appendage single, tubular, unbranched, centric, 5–8 µm long (Figure 4.20).

Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with lobate edge, pale yellow-coloured, with moderate aerial mycelium on the surface with black, concentric fruiting bodies; reverse similar in colour.

Material examined: NEW ZEALAND, from stem lesion in *Rosa* sp., July 1998, J. Reeve (CBS H holotype, culture ex-type CBS 101057); USA, Connecticut, Torrington, from stem of *Paeonia suffruticosa,* 17 May 2007, R. E. Marra, culture CBS 124745.

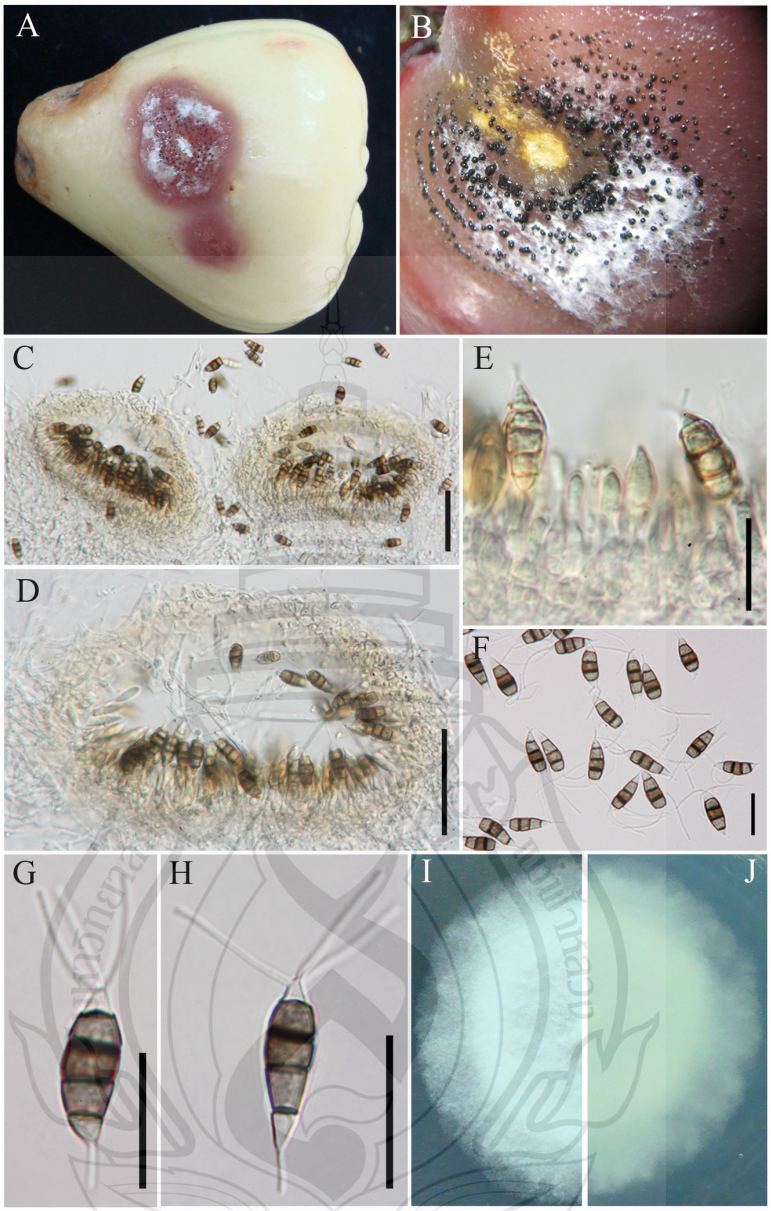
Notes: *Neopestalotiopsis rosa* is isolated from stem lesion in *Rosa* sp. in New Zealand and stem of *Paeonia suffruticosa* in USA, and it is morphologically quite distinct from other taxa described in the genus. It has 3–5 tubular apical appendages, which are not arising from the apical crest, instead arising at a different locus in the upper half of the apical cell. Sequences of *N. rosa* form a sister group to *N. javaensis* and *N. mesopotamicum*. *Neopestalotiopsis javaensis* can be differentiated from *N. rosa* by its long and thin conidia, and shorter apical appendages. The conidial of *N.*

rosa is wider to *N. mesopotamicum* and the length of conidia and length of apical appendages are short.

Neopestalotiopsis samarangensis (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.21 A-J).

Basionym: *Pestalotiopsis samarangensis* Maharachch. & K.D. Hyde, Tropical Plant Pathology 38(3): 229 (2013).

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



Note. A–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 µm, E–H = 20 µm

Figure 4.21 *Neopestalotiopsis samarangensis* (holotype)

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 4.21 B–C). *Conidiophores* correspond to conidiogenous cells arising within the acervuli. *Conidiogenous cells* discrete, simple, short, filiform (Figure 4.21 E). *Conidia* 18–21 × 6.5–7.5 µm (mean = 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 (mean = 4 µm); apical cell 2.5–4.6 µm long (mean = 3.4 µm), conical, hyaline, thin- and smooth-walled; three median cells together 12.8–13.8 µm long (mean = 13.5 µm), with thick verruculose walls, dark brown, the second cell from base pale brown, 4.3–5.3 µm long (mean = 4.8 µm); third cell darker brown, 3.7–5 µm long (mean = 4.1 µm; the fourth cell darkest, 4.5–5.3 µm (mean = 4.9 µm); three apical appendages 12–18 µm long (mean = 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 4.21 F–H). Colonies on PDA reaching 7 cm diam after 6 days at 25ºC, edge entire, whitish aerial mycelium, fruiting-bodies black, gregarious; reverse of culture white.

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, culture ex-type 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).

Notes: Previously, *Pestalotiopsis eugeniae* (Thüm.) S. Kaneko has been described from wax apple fruit (Lan, 2001). *Pestalotiopsis eugeniae* differs from *Neopestalotiopsis. samarangensis* by concolourous (present *Pestalotiopsis*) conidia. *N. samarangensis* also has overlapping morphology with *Pestalotiopsis versicolor* and *P. virgatula* (Table 4.1). However, *Neopestalotiopsis samarangensis* differs from *P. versicolor* and *P. virgatula* by molecular data and by shorter apical appendages

(Guba, 1961; Nag Raj, 1993). *Neopestalotiopsis 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 × 5 µm, whereas in *N. samarangensis* conidia measure on average 20 × 7 µm, thus definitely different. According to Guba (1961), the conidia of *P. palmarum* measure 16–22 × 5–7 µm, overlapping with our fungus (Table 4.1). In pathological consideration, *Pestalotiopsis* or *Neopestalotiopsis* 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 *N. 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 & 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 (*Pestalotiopsis*). *Neopestalotiopsis samarangensis* is distinct from all these species by versicoloured median cells. It is distinct from *N. clavispora* (conidia 18–26 × 6.5– 8.5 µm; apical appendages 17–31 µm) and *P. mangiferae* (conidia 22–26 × 8–11 µm; apical appendages 17–31 µm) by smaller conidia (18–21 × 6.5–7.5 µm) and shorter apical appendages (12–18 µm) (Guba, 1961; Nag Raj, 1993). We therefore introduce a new species to accommodate this taxon, which causes a disease of wax apple.

Table 4.1 Comparison of conidia of *Pestalotiopsis samarangensis* and other similar species.

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

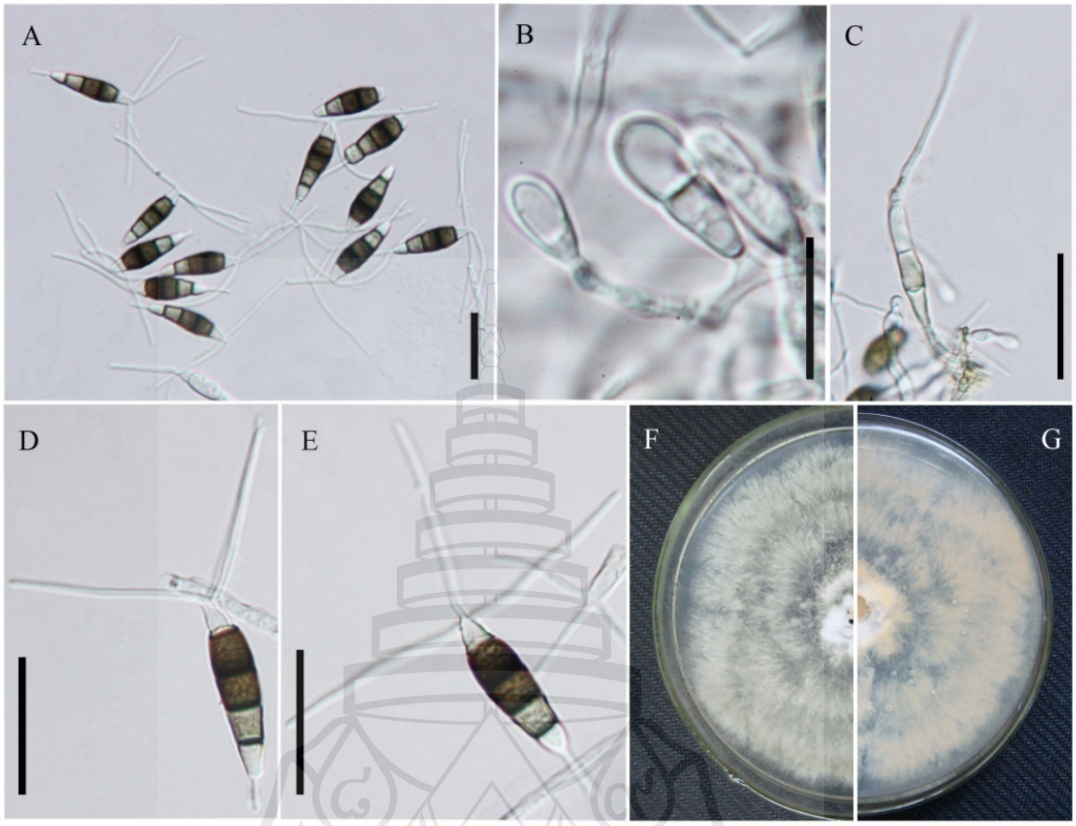
a BPI 406804

b Guba (1961)

Neopestalotiopsis saprophyta (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.22 A-G).

Basionym: *Pestalotiopsis saprophyta* Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 119 (2012).

Etymology: From the Latin *saprophyta.*



Note. A. Conidia. B–C. Conidiophores/ conidiogenous cells. D–E. Conidia. F. G. Colony on PDA, F. from above, G. from below. Scale Bars: A– E= 20 µm

Figure 4.22 *Neopestalotiopsis saprophyta* (holotype)

Conidiophores 0–1-septate, unbranched or irregularly branched, colorless, smooth-walled. *Conidiogenous cells* discrete or integrated, lageniform, subcylindric to cylindric, hyaline. Conidia 22–30 × 5–6 µm (mean = 24.9 × 5.7 µm), fusiform, straight to slightly curved, 4-septate; basal cell conical to obtuse, hyaline, thin and smooth-walled, 4–7 µm long (mean = 5 µm); three median cells 14–20 µm long (mean = 15.5 µm), dark brown to olivaceous, septa and periclinal walls darker than the rest of the cell, versicoloured, verruculose, second cell from base pale brown to olivaceous, 4.5–7 µm (mean = 5.3 µm); third cell darker brown to dark olivaceous, 4–5 µm (mean = 4.7 µm; fourth cell darker, 4–6 µm (mean = 5 µm); apical cell 4–5 µm

long (mean = 4.3 μm), hyaline, cylindric to subcylindric; apical appendages 23–35 μm long (mean = 27.3 μm), tubular, 2-4 (often 3), arising from the apex of the apical cell; basal appendage, 4 –7 μm (mean = 6 μm), filiform.

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 7 days at 25°C, edge crenate, off white, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture off white.

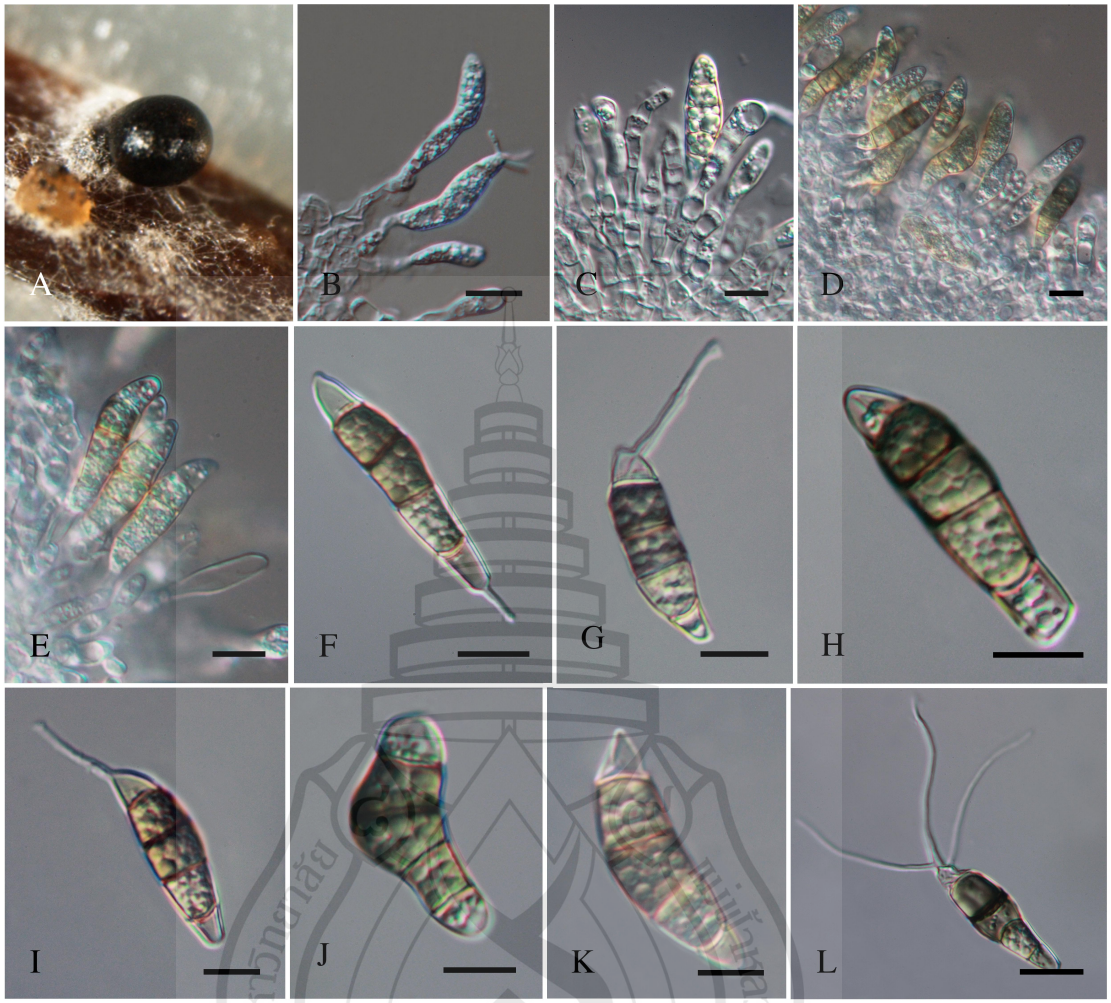
Habitat/Distribution: Saprobes on leaves of *Magnolia* sp., Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Kunming, Kunming Botanical Garden, on leaves of *Magnolia* sp., 19 March 2002, Wenping Wu KBG29-2 (HMAS047136, holotype; MFLU12-0419, isotype; ex-type living culture NN047136 = MFLUCC 12-0282).

Notes: *Neopestalotiopsis saprophyta* is a distinct species in the *Neopestalotiopsis* with a higher conidial length to width ratio compared with other species. In β-tubulin and TEF1 phylograms, it separates well with other species in the *Neopestalotiopsis*. *N. saprophyta* separates from its phylogenetic relative, *N. foedans* (19–25 × 5.5–7 μm) by having larger conidia (22–30 × 5–6 μm) and longer apical appendages (23–35 μm in *N. saprophyta* and 6-18 um in *N. foedans*). Other morphologically related species are *Pestalotiopsis batatas* (Ellis & Everh.) G.C. Zhao & N. Li (23–28 × 7–8 μm) (Zhao & Li, 1995), *P. matildae* (Richatt) S.J. Lee & Crous (22–32 × 6–8 μm) (Lee et al. 2006), and *P. paeoniae* (Servazzi) Steyaert (20–28 × 6–8 μm) (Guba, 1961). However, in *Neopestalotiopsis saprophyta* conidia are thinner and apical appendages are longer.

Neopestalotiopsis steyaertii (Mordue) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.23 A-L).

Basionym: *Pestalotiopsis steyaertii* Mordue, Trans. Br. mycol. Soc. 85(2): 379 (1985).



Note. A. Conidioma on Water Agar with sterile pine needles. B. Conidiomata on PDA. C-F. Conidiogenous cells and developing conidia. G-M. Conidia. Scale bars = 10 µm

Figure 4.23 *Neopestalotiopsis steyaertii* (holotype)

Saprobic on soil. Sexual state: Unknown. Asexual state: *Conidiomata* 300–500 µm diam, pycnidial, globose, brown, semi-immersed on PDA releasing black conidia in a slimy, globose, glistening mass. *Conidiophores* septate at base, branched, colorless, smooth-walled. *Conidiogenous cells* discrete or integrated, short cylindric, hyaline, 5–12 × 2–4 µm. *Conidia* (25)27–34 × 7–9.5(10) µm, mean ± SD = 30.1 ± 2.2

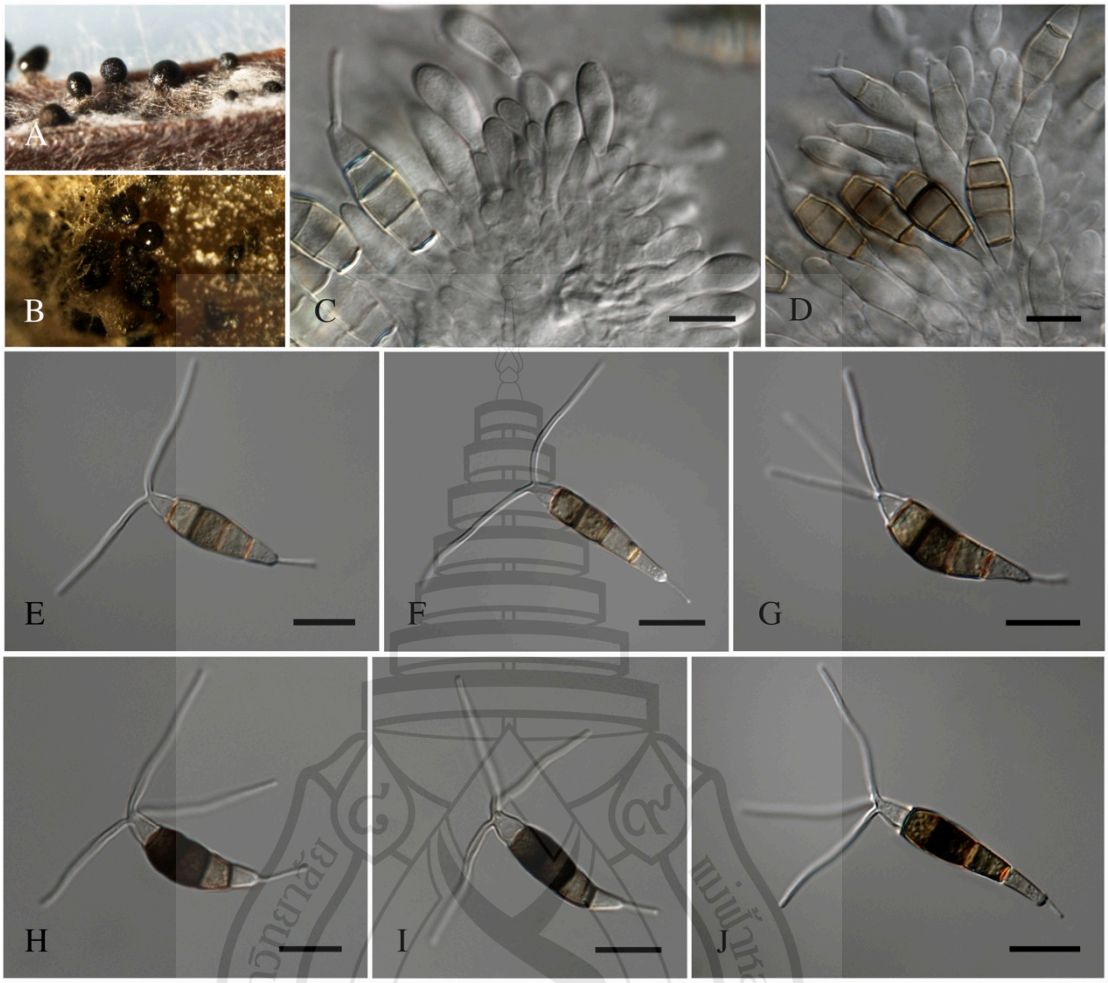
× 8.0 ± 0.5 µm, fusiform to clavate, straight to curved, 4-septate; basal cell conical to cylindric, hyaline or pale olivaceous, thin and walled-verruculose, 6–8 µm long; three median cells (16)18–23(25) µm long, mean ± SD = 22.1 ± 2.1 µm olivaceous, septa and periclinal walls darker than rest of the cell, versicoloured, walled-verruculose; second cell from base pale olivaceous, 6–8 µm long; third cell dark olivaceous, 7–9 µm; fourth cell darker, 6–9 µm; apical cell 6–8 µm long, hyaline or pale olivaceous, conic to hemispherical; apical appendages mostly absent, when present 1–5 tubular appendages on apical cell, inserted at different loci but in a crest at the apex of the apical cell, unbranched, flexuous, (17)20–31(34) µm long, mean ± SD = 25.2 ± 3.4 µm; basal appendage mostly absent, when present single, tubular, unbranched, centric, 2–6 µm long.

Colonies fast growing on PDA attaining 50-60 mm diam after 7 days at 25 °C, edge entire, white, dense aerial mycelium on surface, fruiting bodies black, concentric; reverse similar in colour.

Notes: Neopestalotiopsis steyaertii is a distinct species in terms of its morphology and DNA phylogeny. This species is characterised by its unusual conidial shape. According to Mordue's (1985) observations, most of the isolates of *N. steyaertii* lack apical appendages in conidia. We observed this in the ex-type culture. *N. steyaertii* forms a sister group to species having versicolorous (*Neopestalotiopsis*) median cells and dark concolorous median cells with knobbed apical appendages (*Pseudopestalotiopsis*).

Neopestalotiopsis surinamensis Maharachch. & Crous, sp. nov. (Figure 4.24 A- J).

Etymology: Named after the country where it was collected, Suriname.



Note. *Neopestalotiopsis surinamensis* CBS 450.74^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm

Figure 4.24 *Neopestalotiopsis surinamensis* (holotype)

Conidiomata (on PDA) pycnidial, globose, mostly aggregated in clusters, semi-immersed or erumpent, black, up to 350 µm diam; exuding globose, brown conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform 4–10 × 2–6 µm, hyaline, smooth-walled, simple, proliferating 2–3 times percurrently, wide at the base,

opening 1–2 µm diam. *Conidia* fusiform, ellipsoid to subcylindrical, straight to slightly curved, 4-septate, (23–)24–28(–29) × (7–)7.5–9(–9.5) µm, mean ± SD = 27.7 ± 1 × 8.1 ± 0.4 µm; basal cell obconic to subcylindrical with a truncate base, hyaline, thin and wall rugose, 5–7.5 µm long; three median cells doliiform, (14.5–)15–17(–17.5) µm long, mean ± SD = 16.5 ± 0.6, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown, 5.5–6.5 µm long; third cell honey brown, 5–6.5 µm long; fourth cell brown, 4.5–6 µm long); apical cell 4–5.5 µm long, hyaline, cylindrical to subcylindrical, thin and smooth-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (15–)18–27(–28) µm long, mean ± SD = 21.6 ± 3; basal appendage single, tubular, unbranched, centric, 5–7 µm long.

Culture characteristics: Colonies on PDA attaining 30–40 mm diam after 7 days at 25°C, with lobate edge, pale honey-coloured, with dence aerial mycelium on the surface with black, concentric fruiting bodies; reverse similar in colour.

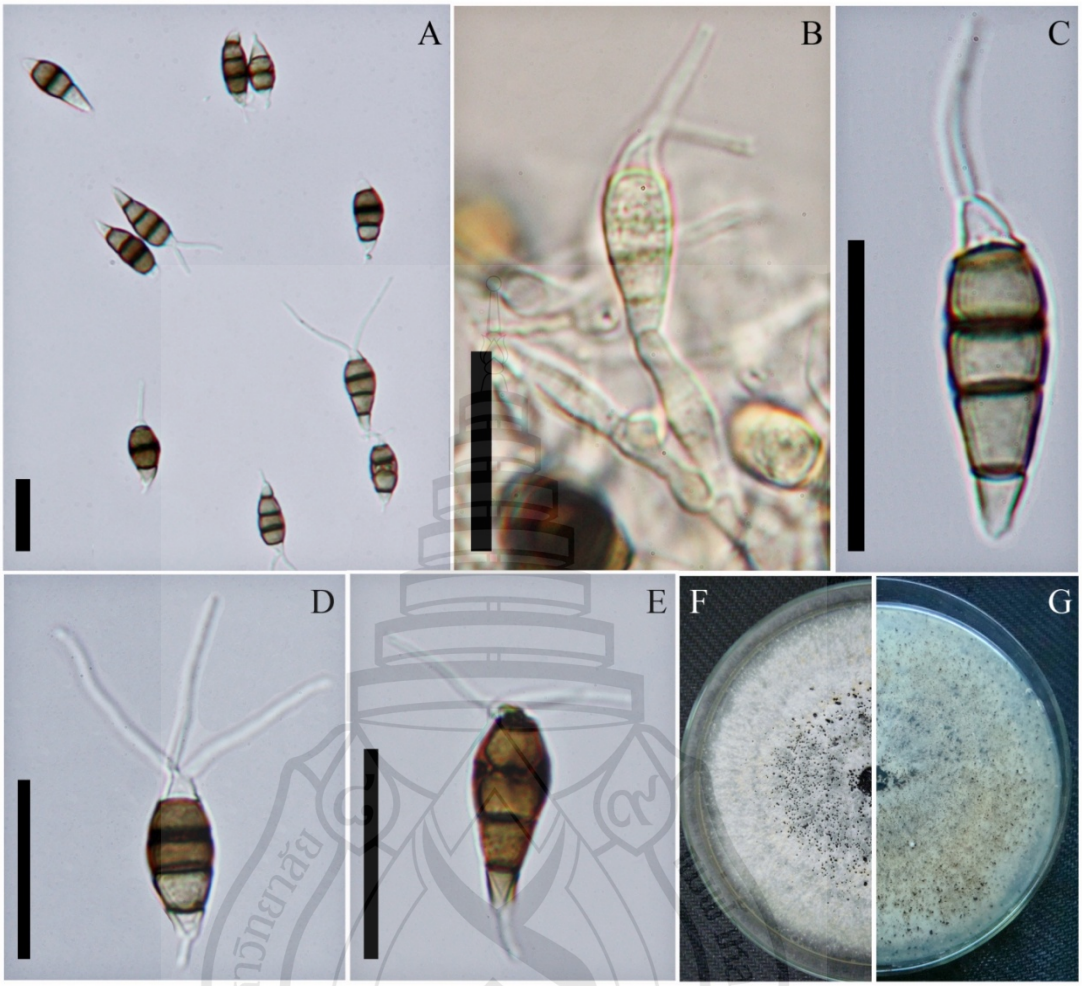
Material examined: SURINAME, Brokobaka, from soil under *Elaeis guineensis*, Mar. 1974, J.H. van Emden (CBS H-15730 holotype, culture ex-type CBS 450.74).

Notes: *Neopestalotiopsis surinamensis* is isolated from soil under *Elaeis guineensis* (African oil palm) in Suriname, which is the principal source of palm oil. *Neopestalotiopsis surinamensis* belongs to the *N. protearum* complex, but differs from *N. protearum* (Crous et al., 2011) by having wider conidia, longer and lesser number of apical appendages.

Neopestalotiopsis umberspora (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous comb. nov. (Figure 4.25 A-G).

Basionym: *Pestalotiopsis umberspora* Maharachch. & K.D. Hyde, Fungal Diversity 56(1): 121 (2012).

Etymology: The specific epithet is based on the Latin = umber, in reference to the umber earth brown colour of the median cells of the conidia.



Note. A. Conidia. B. Conidiophores/ conidiogenous cells. C–E. Conidia. F. G. Colony on PDA, F. from above, G. from below. Scale Bars: A– E= 20 µm

Figure 4.25 *Neopestalotiopsis umberspora* (holotype)

Conidiophores reduced to conidiogenous cells. *Conidiogenous cells* discrete or integrated, lageniform, hyaline, smooth walled, sometimes septate. *Conidia* 19–25 × 6–8 µm (mean = 21.3 × 6.5 µm), fusiform, straight to slightly curved, 4-septate; basal cell obconic to conic, hyaline or pale brown, thin and verruculose, 3–4.5 µm long (mean = 3.8 µm); three median cells 12–14 µm long (mean = 13.1 µm), umber brown to olivaceous, septa and periclinal walls darker than the rest of the cell,

versicoloured, verruculose, second cell from base pale brown, 3–4.5 µm (mean = 3.9 µm); third cell darker brown, 3.5–5 µm (mean = 4.3 µm; fourth cell darker, 3.5–4.5 µm (mean = 4.2 µm); apical cell 3–4.5 µm long (mean = 3.9 µm), hyaline, conic to obconic; with apical appendages 22–35 µm long (mean = 27.7 µm), tubular, 1–3 (mainly 3), arising from the upper portion of the apical cell; basal appendage, 5 –7 µm (mean = 5.9 µm), filiform.

Culture characteristics: Colonies on PDA reaching 7 cm diam. after 6 days at 25°C, edge entire, whitish, aerial mycelium on surface, fruiting bodies black, gregarious; reverse of culture pale yellow.

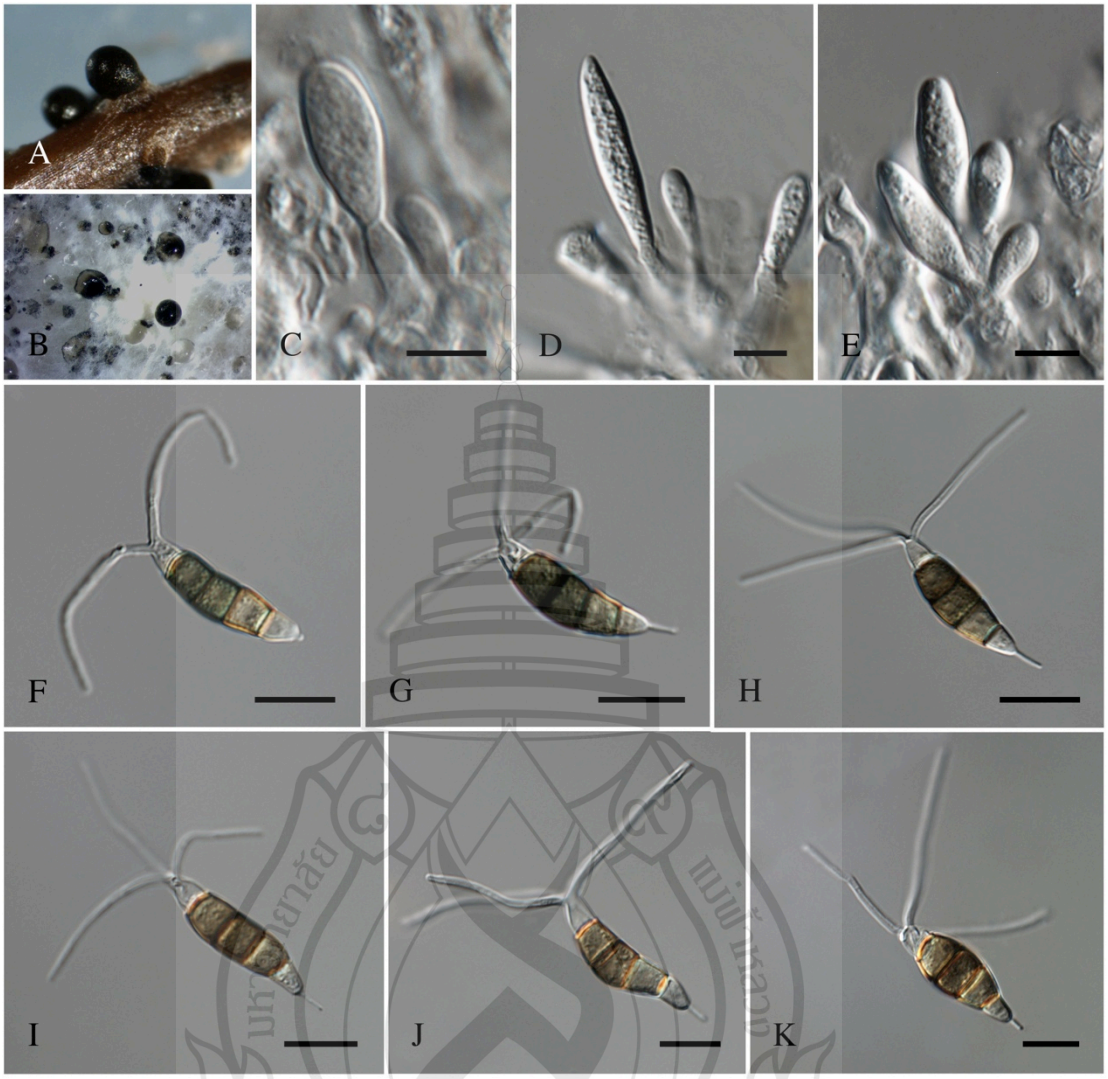
Habitat/Distribution: Saprobe on dead plant material, Guangxi Province, China.

Material examined: CHINA, Guangxi Province, Shiwandashan, on dead leaves of unidentified plant, 30 December 1997, Wenping Wu WU1554j (HMAS042986, holotype; MFLU12-0421, isotype; ex-type living culture NN042986 = MFLUCC 12-0285).

Notes: *Neopestalotiopsis umberspora* is a phylogenetically distinct species in the genus and separates well in combined multi-locus tree with its phylogenetically related species *N. crysea*. Its umber coloured and relatively wider mature conidia are characteristic to the species.

Neopestalotiopsis zimbabwana Maharachch., K.D. Hyde & Crous, sp. nov. (Figure 4.26 A-K).

Etymology: Named after the country where it was collected, Zimbabwe.



Note. *Neopestalotiopsis zimbabwana* CBS 111495^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.26 *Neopestalotiopsis zimbabwana* (holotype)

Conidiomata (on PDA) pycnidial, globose, aggregated or scattered, semi-immersed, black, 150–400 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform, hyaline, smooth-walled, simple,

proliferating several times percurrently, 5–15 × 3–8 µm, wide at the base, opening 2–5 µm diam. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, (22–)23–29(–30) × (6.5–)7–8.5(–9) µm, mean ± SD = 25.3 ± 1.2 × 7.7 ± 0.3 µm; basal cell conic to obconic with a truncate base, hyaline, thin and wall rugose, 3.5–5.5 µm long; three median cells doliiform, (15–)15.5–17.5(–18) µm long, mean ± SD = 16.5 ± 0.6, wall rugose, versicoloured, septa darker than the rest of the cell, (second cell from the base pale brown to pale olivaceous, 4.5–6.5 µm long; third cell brown to olivaceous, 4.5–6.5 µm long; fourth cell brown to olivaceous, 5–7 µm long); apical cell 4–6.5 µm long, hyaline, cylindrical to subcylindrical, thin and wall rugose; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (18–)23–35(–41) µm long, mean ± SD = 28.6 ± 4; basal appendage single, tubular, unbranched, centric, 3–9.5 µm long (Figure 4.26).

Culture characteristics: Colonies on PDA attaining 30–45 mm diam after 7 days at 25°C, with smooth edge, pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: ZIMBABWE, from *Leucospermum cuneiforme*, 15 May 1998, L. Swart (CBS H holotype, culture ex-type CBS 111495= STE-U 1777).

Notes: *Neopestalotiopsis zimbabwana* occurs on *Leucospermum cuneiforme* in Zimbabwe. In phylogenetic analyses, *N. zimbabwana* proved to be allied to CBS 266.37, CBS 361.61, CBS 323.76, which were isolated from Erica sp. in Germany, *Cissus* sp. in Netherlands and *Erica gracilis* in France, respectively. Even though, later isolates have overlapping morphology with *N. zimbabwana*, due to clear ecological differences, we prefer to maintain these isolates as *Neopestalotiopsis* spp. until we obtained more collections and cultures. *Neopestalotiopsis protearum* previously identified as a pathogen on *Leucospermum cuneiforme* in Zimbabwe. However both *N. protearum* and *N. zimbabwana* are found in genetically clearly distinct species complexes.

Pseudopestalotiopsis Maharachch., K.D. Hyde & Crous, gen. nov.

Type species: *Pseudoestalotiopsis theae* (Sawada) Maharachch., K.D. Hyde & Crous (see below).

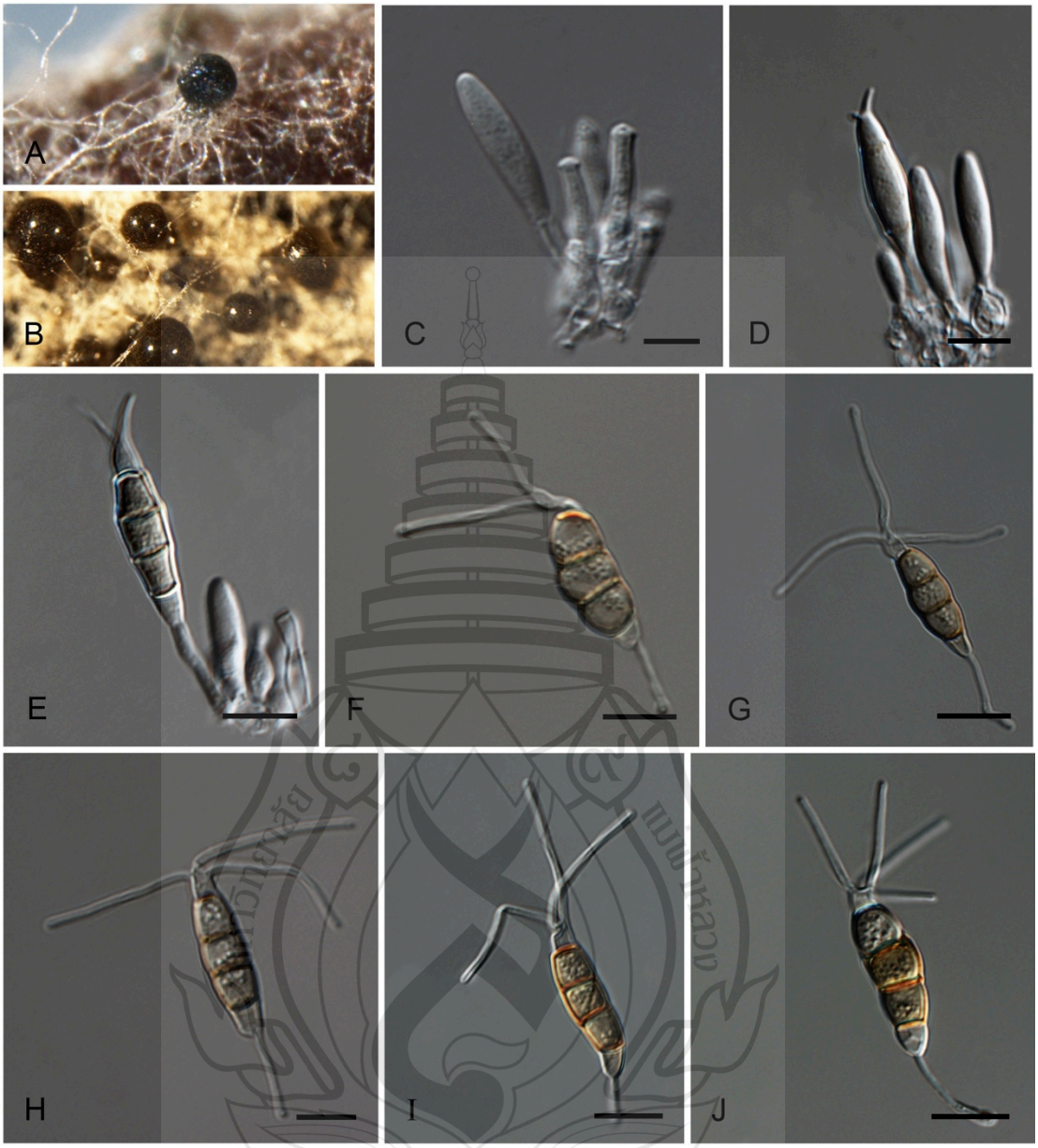
Etymology: Named after its morphological similarity to *Pestalotiopsis*.

Conidiomata acervular or pycnidial, subglobose, globose, clavate, solitary or aggregated, dark brown to black, immersed to erumpent, unilocular; exuding dark brown to black conidia in a slimy, globose mass. *Conidiophores* indistinct, reduced to conidiogenous cells. *Conidiogenous cells* discrete, cylindrical, ampulliform to lageniform, hyaline, smooth, thin-walled; conidiogenesis initially holoblastic, percurrent proliferations to produce additional conidia at slightly higher levels. *Conidia* fusoid, ellipsoid, subcylindrical, straight to slightly curved, 4-septate, slightly constricted at septa; basal cell conical to cylindric with a truncate base; three median cells doliiform, concolorous, brown to dark brown or olivaceous, wall rugose to verruculose, septa darker than the rest of the cell; apical cell conic to cylindrical, thin and smooth-walled; with tubular apical appendages, one to many, filiform or attenuated, flexuous, branched or unbranched, with or without spatulate tips; basal appendage single, tubular, unbranched, centric.

Notes: The majority of the studies (Jeewon et al., 2003; Liu et al., 2010a; Hu et al.; 2007, Maharachchikumbura et al., 2011; 2012), species with dark concolorous median cells with knobbed apical appendages form a clade with higher support and this clade is defined here as a novel genus, *Pseudoestalotiopsis*. Based on LSU data of the present study we confirmed its placement. *Psedopestalotiopsis* is phylogenetically related to *Neopestalotiopsis*. These genera are morphologically distinct. The three median cells of *Psedopestalotiopsis* are same in colour (concolorous) while in *Neopestalotiopsis* these are versicolour.

Pseudopestalotiopsis cocos Maharachch. & Crous, sp. nov. (Figure 4.27 A-J).

Etymology: Named after the host genus from which it was isolated, *Cocos*.



Note. *Pseudopestalotiopsis cocos* CBS 272.29^{T}. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–J. Conidia. Scale bars = 10 µm

Figure 4.27 *Pseudopestalotiopsis cocos* (holotype)

Conidiomata pycnidial, 100–300 µm diam, globose, dark brown, semi-immersed on host substrate on PDA; exuding black conidia in a slimy, globose, glistening mass. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, hyaline, smooth-walled, simple, filiform, sometimes slightly wide at the base, truncate at apex, proliferating 2–3 times percurrently, 12–15 × 1–3 µm. *Conidia* fusiform, ellipsoid, straight to slightly curved, 4-septate, constricted at septum, (20–)21–25(–26.5) × 6–7.5 µm, mean ± SD = 23.0 ± 1.6 × 6.5 ± 0.4 µm; basal cell obconic with a truncate base, hyaline, thin and smooth-walled, granular, 3.5–5 µm long; three median cells (13.5–)14–16.5(–17.5) µm long, mean ± SD = 15.5 ± 1.2, concolorous, pale brown, septa darker than the rest of the cell, (second cell from the base 5.5–6.5 µm long; third cell 4.5–5.5 µm long; fourth cell 5.5–6 µm long); apical cell 3.5–5 µm long, hyaline, cylindrical; with 2–4 tubular apical appendages (mostly 3), arising in an apical crest, but each inserted at a different locus, flexuous, unbranched, (12–)14–21(–23) µm long, mean ± SD = 17.6 ± 3.2; basal appendage single, tubular, unbranched, centric, 5–8 µm long (Figure 4.27).

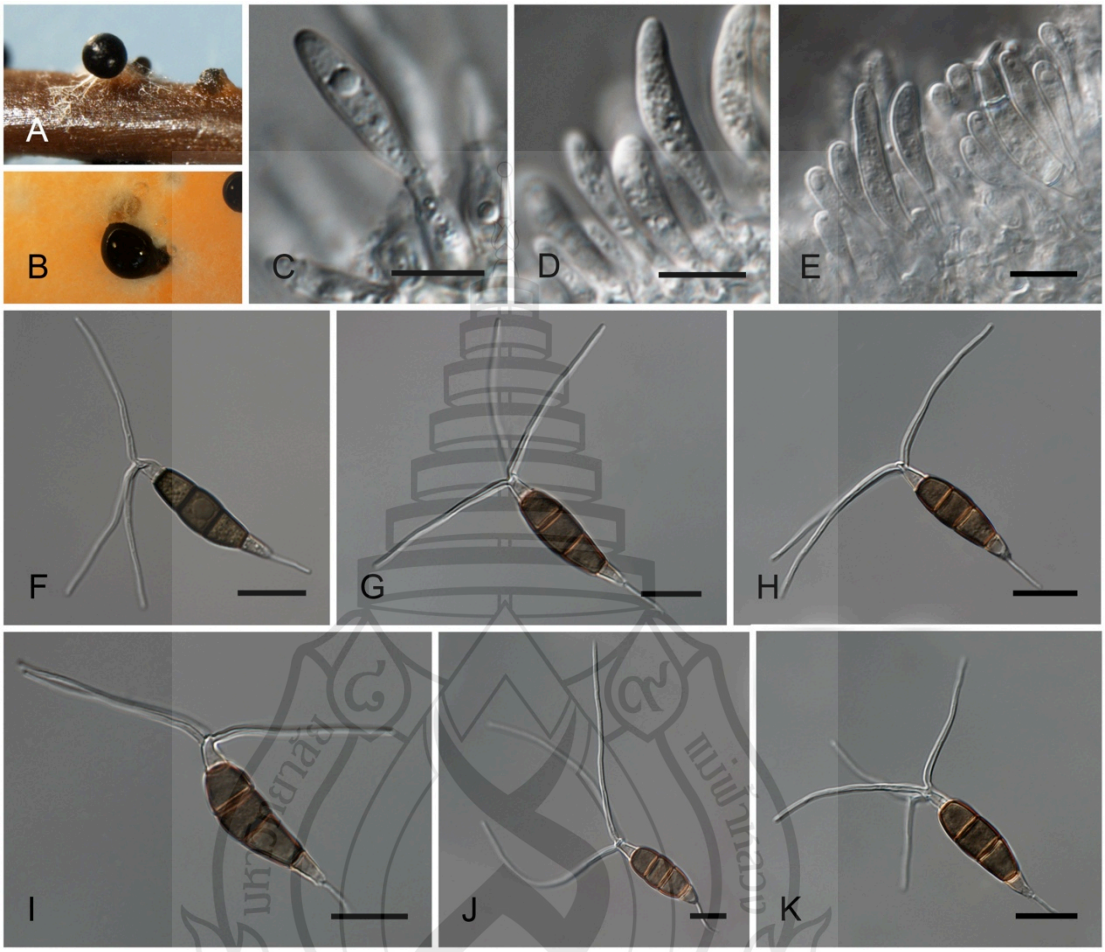
Culture characteristics: Colonies on PDA attaining 50–60 mm diam. after 7 days at 25°C, with smooth edge, whitish to grey, with black, gregarious fruiting bodies; reverse similar in colour.

Material examined: JAVA, Buitenzorg, from *Cocos nucifera*, unknown collection date, C.M. Doyer (CBS H-15666 holotype, culture ex-type CBS 272.29).

Notes: *Pseudopestalotiopsis cocos* is a distinct species, recognized based on its morphology and phylogeny. It can clearly be differentiated from its phylogenetically related sibling species, *P. indica* (31.5–37 × 6.5–9 µm) by relatively smaller conidia (20–26.5 × 6–7.5 µm), and shorter apical appendages (12–23 µm), whereas in *P. indica* appendages are longer (30–40 µm). Further the three median cells in *P. cocos* are paler in colour than in *P. indica.* This species is sister to a clade that contains *P. theae* (22–32 × 5–8 µm) and they have overlapping morphology. However, in *P. theae* the apical appendages are knobbed, which is a feature absent in *P. cocos.*

Pseudopestalotiopsis indica Maharachch. & Crous, sp. nov. (Figure 4.28 A-K).

Etymology: Named after the country where it was collected, India.



Note. *Pseudopestalotiopsis indica* CBS 459.78^{T}. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm

Figure 4.28 *Pseudopestalotiopsis indica* (holotype)

Conidiomata (on PDA) pycnidial, globose to clavate, solitary or aggregated, dark brown, semi-immersed or partly erumpent, 200–500 µm diam; exuding brown to black conidial masses. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform to lageniform, 5–18 × 2–7 µm, hyaline, smooth, thin-

walled, sometimes proliferating 1–2 times percurrently, periclinal thickening in the apical region, collarette present and flared. *Conidia* fusoid to ellipsoid, straight to slightly curved, 4-septate, slightly constricted at septa, (31.5–)32.5–36(–37) × 6.5–9 µm, mean ± SD = 34.5 ± 1.6 × 7.5 ± 0.5 µm; basal cell conic with truncate base, with thin, rugose wall, 5.5–7 µm long; three median cells (19.5–)20–22(–22.5) µm long, mean ± SD = 21.6 ± 1.0, doliiform, verrucose, concolorous, dark brown, septa darker than the rest of the cell, (second cell from base 6.5–8.5 long; third cell 5.5–8 µm long; fourth cell 6.5–8.5 µm long); apical cell subcylindrical, hyaline, thin and smooth-walled, 5.5–7 µm long; with 3–4 tubular apical appendages (mostly 3) arising from the apical crest, flexuous, unbranched, (30–)33–39(–40) µm long, mean ± SD = 35 ± 2.8; basal appendage single, tubular, unbranched, centric, 6–10 µm long (Figure 4.28).

Culture characteristics: Colonies on PDA reaching 60–80 mm diam after 7 days at 25°C, undulate at the edge, whitish to pale honey-coloured, with black, gregarious fruiting bodies; reverse pale honey-coloured.

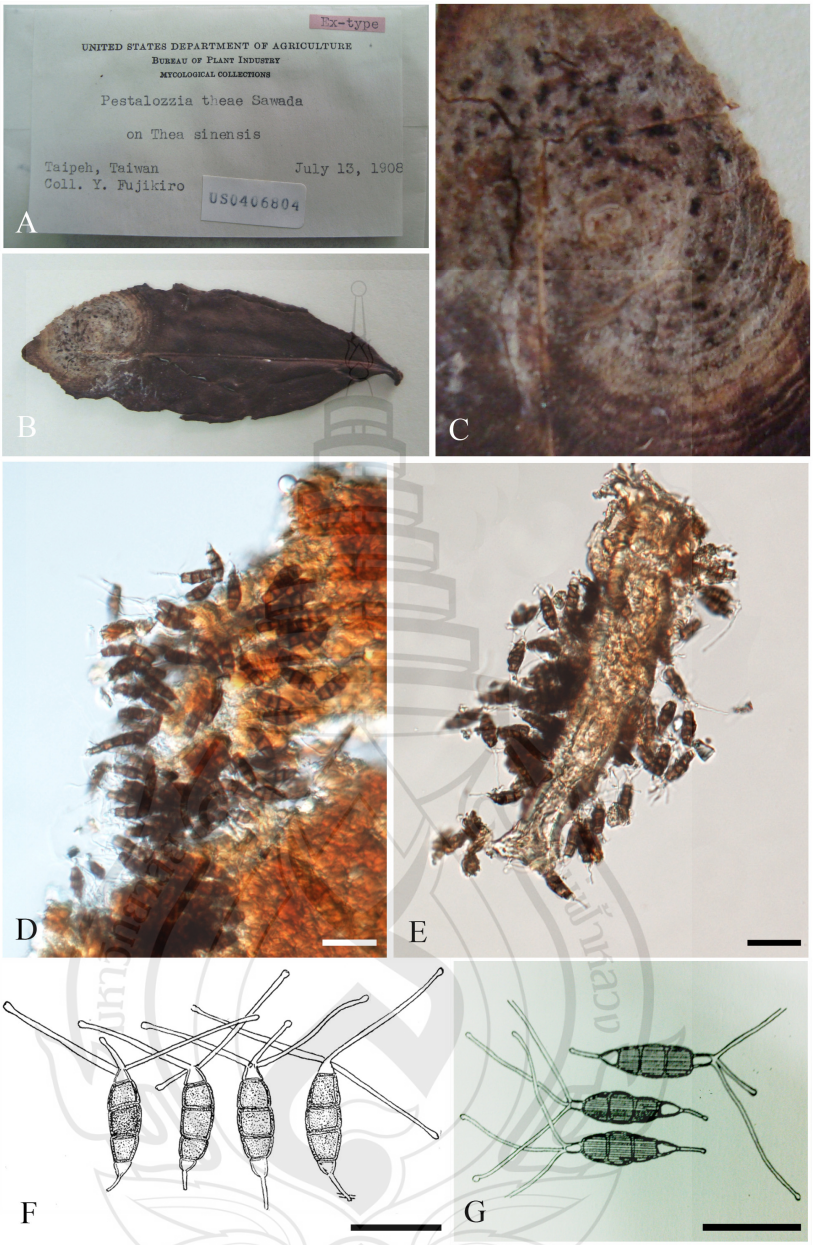
Material examined: INDIA, Bangalore, from *Hibiscus rosa-sinensis,* Aug. 1978, H.C. Govindu (CBS H holotype, culture ex-type CBS 459.78).

Notes: This species is characterised by large conidia (32.5–36 × 7–8.5 µm) with three median cells that are darker in colour. It forms a sister group to *P. cocos* and *P. theae*. *P. indica* differs from *P. cocos* (20–26.5 × 6–7.5 µm) and *P. theae* (22–32 × 5–8 µm) in morphology of its large conidia.

Pseudoestalotiopsis theae (Sawada) Maharachch, K.D. Hyde & Crous, comb. nov. (Figure 4.29 A-G).

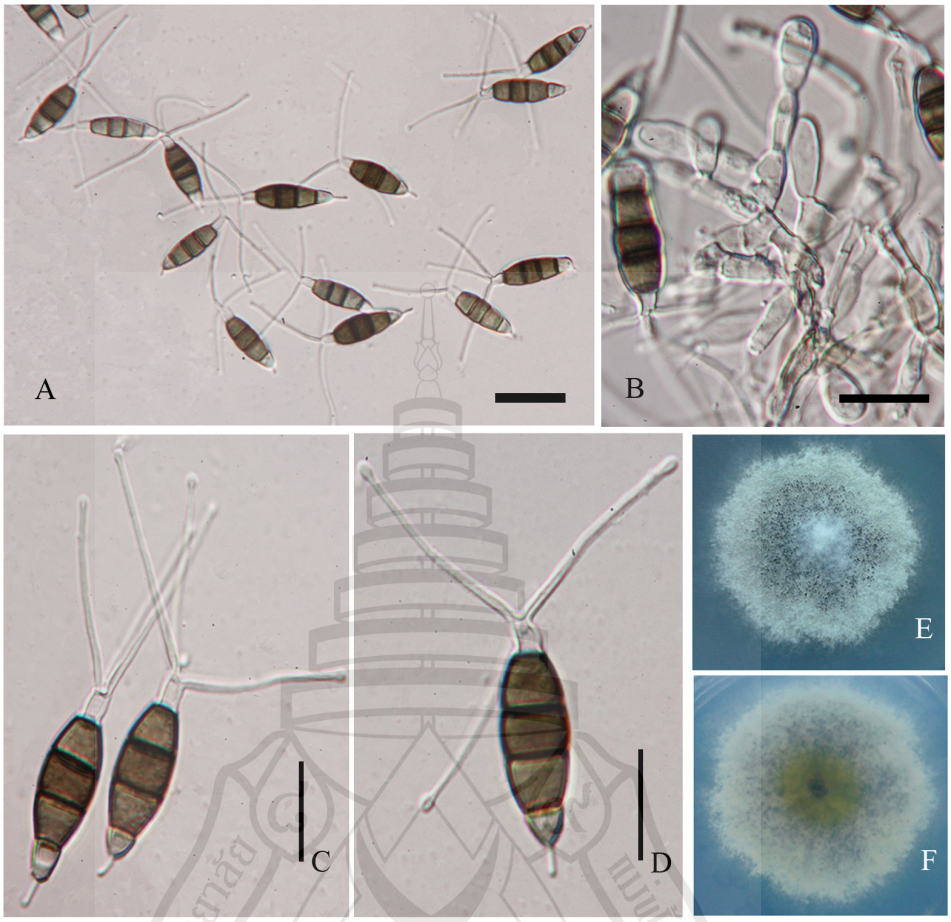
Basionym: *Pestalotia theae* Sawada, Spec. Report Agric. Exp. Station Formosa 11: 113 (1915), as "*Pestalozzia*".

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



Note. 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

Figure 4.29 *Pseudopestalotiopsis theae* (lectotype)



Note. *Pseudopestalotiopsis 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

Figure 4.30 *Pseudopestalotiopsis theae* (epitype)

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 = 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.

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^{o}C, fimbriate, whitish, dense, aerial mycelium on surface, fruiting bodies black; reverse of the culture yellowish white.

Material examined: 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, elevation 900 m, rainforest, on living leaves of *Camellia sinensis*, January 20, 2010, S.S.N. Maharachchikumbura St200110 (MFLU 12-0116 epitype; culture ex-epitype MFLUCC 12-0055 = CPC 20281).

Notes: 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.

4.4 Conclusion

In this present study, we proposed that *Pestalotiopsis* genus is cryptic and can be divided into two new genera *Neopestalotiopsi*s and *Pseudopestalotiopsis* based on morphology and molecular data. Morphology of *Pestalotiopsis guepini*, which was known to occur commonly on *Camellia* fit the generic concept of *Pestalotiopsis*. In *Pestalotiopsis guepini* conidipores are sepatate, unbranched and often reduced to conidiogenous cells; conideogenous cells phialides, ampulliform to lageniform or cylindrical to subcylindrical; conidia 5-celled with three concolours median cells and with 1–4 apical appendages which are sometimes minutely knobbed and branched. However, these morphological characters only confined to species in *Pestalotiopsis*, do not match with those of *Neopestalotiopsi*s and *Pseudopestalotiopsis*. The sequence of 55 *Neopestalotiopsi*s and four *Pseudopestalotiopsis* were studied and used to provide a backbone tree for both *Neopestalotiopsi*s and *Pseudopestalotiopsis*. Based on molecular and morphological data we describe 19 new species of *Neopestalotiopsi*s (*N. aotearoa, N. asiatica, N. chrysea, N. cubana, N. ellipsospora, N. eucalypticola, N. formicarum, N. honoluluana, N. javaensis, N. magna, N. mesopotamicum, N. piceana, N. rosa, N. samarangensis, N. saprophyta, N. steyaertii, N. surinamensis, N. umberspora, N. zimbabwana*), *N. clavispora* and *N. foedans* are epitypified and ex-type of *N. natalensis* and *N. steyaertii* are re-examined. Four *Pseudopestalotiopsis* sequences comprised two new species (*P. cocus* and *P. indica*) and *P. theae* is epitypified.

CHAPTER 5

OVERALL CONCLUSIONS

5.1 *Pestalotia* or *Pestalotiopsis*?

Based on the conidial forms, Steyaert (1949) introduced the genus *Pestalotoiopsis* by splitting *Pestalotia* De Not. in to three genera. Septation is very effective system use in segregation of taxa in to genera in *Amphisphaeriaceae*. Sequence data show *Truncatella*, *Pestalotiopsis* and *Seridium* to be three distinct genera, which were characterised by 4-celled conidia, 5-celled conidia and 6-celled conidia respectively. However, it has not been established whether *Pestalotia* differs from *Neopestalotiopsi*s, *Pseudopestalotiopsis* and *Pestalotiopsis* based on the molecular data. Although, they are clearly distinct from the conidial forms, *Neopestalotiopsi*s, *Pseudopestalotiopsis* and *Pestalotiopsis* have five cell forms while *Pestalotia* has six cell forms. From a phenotypic view, *Pestalotia* species are more similar to *Seridium* species, as both have 6-celled conidial forms. The type species of *Pestalotia, Pestalotia pezizoides,* can be distinguished from *Seridium* species by its branched appendages, which arise from an apical crest of the apical cell, while in type of *Seridium*, *S. marginatum* appendages are singular and unbranched. However, branched apical appendages typical of *Pestalotia* are found in *Seridium corni* and *S. venetum* (Nag Rag, 1993) and thus *Pestalotia* species and *Seridium* species are probably congeneric. Therefore monotypic *Pestalotia* (1839) might be a synonym of *Seridium* (1816), since both genera consist similar morphology.

5.2 A multi-locus data to resolve species

In order to select suitable gene regions for better species resolution, we analyzed ACT, β-tubulin, CAL, GPDH, GS, ITS, LSU, RPB 1, SSU and TEF1 gene regions for several isolates of *Neopestalotiopsis*, *Pseudopestalotiopsis* and *Pestalotiopsis.* We compared the morphological data versus the sequence data from each gene to establish which characters satisfactorily resolve the species. We narrowed down tested 10 gene regions to three most applicable regions (ITS, β-tubulin and TEF1) which were tested individually and in combination, to evaluate the differences between species. The ITS is the universal barcode for fungi (Schoch et al., 2012). The species of sequenced with ITS in this study had a high PCR and sequence success rate and β-tubulin and TEF1 gene regions proved to be favorable taxonomic markers for *Neopestalotiopsis*, *Pseudopestalotiopsis* and *Pestalotiopsis* since they resolved the taxonomic relationships of most species studied. Further, TEF1 had better PCR amplification success rates (95 %) and was found to be superior to β-tubulin (90 %). TEF1 is therefore a powerful tool to resolve lineages within three genera. Because of the better PCR and sequencing success rate and fewer difficulties with alignment, editing and better resolution, the TEF1 gene appears to be a very good molecular marker for phylogenetic investigation of three genera. Furthermore, combination of ITS, β-tubulin and TEF1 gene data gave the best resolution as compared to single gene analysis. In addition to the above three genes, we tested LSU, SSU, ACT and GPDH (low resolution), GS and RPB1 (cannot be synthesized using available primers or multiple copies) and Calmodulin (species resolution is high, PCR success rate low) and these were less successful in PCR amplification and/or resolving species.

5.3 Segregation *Pestalotiopsis* in to *Pseudopestalotiopsis* and *Neopestalotiopsis* gen. nov.

Recent studies have shown that *Pestalotiopsis* species are morphologically diverse in, and phylogenetic analyses show species to comprize three distinct lineages (Jeewon et al., 2003; Maharachchikumbura et al., 2011; 2012a). Based on these findings, we separate *Pestalotiopsis* into three genera, *Pestalotiopsis*, *Neopestalotiopsi*s and *Pseudopestalotiopsis*. *Pestalotiopsis guepini*, which is the type species of *Pestalotiopsis,* commonly occurs on *Camellia* and provides a typical generic concept for *Pestalotiopsis*. In *Pestalotiopsis guepini* conidiophores are septate, unbranched and often reduced to conidiogenous cells; conideogenous cells are ampulliform to lageniform or cylindrical to subcylindrical phialides, and conidia are 5-celled with three concolourous median cells and with 1–4 apical appendages which are sometimes minutely knobbed and branched. *Neopestalotiopsi*s has indistinct conidiophores and versicolorous median cells, while *Pseudopestalotiopsisi* can be distinguished by phylogeny and reduced conidiophores.

5.4 *Pestalotiopsis*

Pestalotiopsis, species-rich, appendage-bearing, conidial anamorphic genus in the family *Amphisphaeriaceae* (Barr, 1975; 1990; Kang et al., 1999; Lee et al., 2006), is widely distributed throughout tropical and temperate regions (Bate-Smith & Metcalfe, 1957). Species belonging to the genus *Pestalotiopsis* are thought to be a rich source for bio prospecting when compared to other fungal genera and Xu et al. (2010). In the present study, we have collected *Pestalotiopsis* isolates from different habitats, hosts and also have been sourced from different culture collections. Based on morphological and molecular data, we described 40 new species, one species is epitypified, two ex-type are re-examine and furthermore, for each species complete morphological and molecular characterization is provide. *Pestalotiopsis* displays a higher level of phenotypic and genetic variation and upon conidial morphology, host

occurrence, geographical influence and sequence data we introduced 19 sections for the *Pestalotiopsis*. There are many unusual species in the genus that need re-examination, and we believe that many distinct, well-separated sections will arise from such studies.

5.5 *Neopestalotiopsis*

Based on sequence data, the *Neopestalotiopsis* clusters in *Amphisphaeriaceae* distinct from the *Pesudoestalotiopsis* and *Pestalotiopsis*. Furthermore, *Neopestalotiopsis* can be easily distinguished from *Pesudoestalotiopsis* and *Pestalotiopsis* by its versicolorous median cells. Furthermore, in *Neopestalotiopsis* conidiophores are indistinct and often reduced to conidiogenous cells unlikely those in well develop in *Pestalotiopsis*. *Neopestalotiopsis protearum* which was isolated from disease leaves of leaves of *Leucospermum cuneiforme* from Zimbabwe assign as the generic type of the genus. Furthermore, based on molecular and morphological data we described 19 new species, two species are epitypified and two ex-type are re-examine and six section names were introduced to the *Neopestalotiopsis*.

5.6 *Pseudopestalotiopsis*

Based on conidial morphology and sequence data *Pseudopestalotiopsis* gen. nov. is introduced. *Pseudopestalotiopsis theae* which was isolated from tea placed as the generic type. Two new species were introduced and one species is epitypified. Further collections of *Pseudopestalotiopsis* are needed from different geographically origin and host plants to understand their taxonomy and ecology.

5.7 Morphological characters, host occurrence and geography influence use in species delineation

Conidial morphology is the most widely used taxonomic character for the genus *Pestalotiopsis*. Those morphological important characters include colour of the median cells, the size (length and width) of conidia and the characters within the apical appendages. The character within apical appendages include length, number, branch or unbranch, presence or absence of knobbed tips and position of apical appendages attached to the apical cell. Previous researchers showed conidiogenesis is confusing when used for species separation (Purohit & Bilgrami, 1969, Watanabe et al., 1998). However we argue that it may has taxonomic value. Both *Neopestalotiopsis* and *Pseudopestalotiopsis* conidiophores are indistinct and often reduced to conidiogenous cells however it is much vary within the species of *Pestalotiopsis* and most species section has similar conidiogenous development.

So far there were only few studies with taxon sampling from major geographical regions, which focused on geographic distribution of *Pestalotiopsis* and related genera. Therefore knowledge about actual distribution of *Pestalotiopsis,* and how biogeographical distribution patterns affect in species circumscription is lacking. *Pestalotiopsis furcata* and *P. camelliae* are species which characterised by lack of basal appendages and so far recorded only from plant genus *Camellia*. *Pestalotiopsis australasia*, *P. biciliata*, *P. grevillea*, *P. knightia*, *P. proteacearum*, *Pestalotiopsis* sp. (CBS 114137) and *P. telopea* are sister species recorded from plant family *Proteaceae* (except *P. biciliata* isolate CBS 790.68; CBS 236.38 and *P. proteacearum* isolate CBS 353.69). *Pestalotiopsis australis* is another species recorded from three different genera in *Proteaceae* (*Brabejum*, *Grevillea* and *Protea*). *Pestalotiopsis adusta* (Fiji and Thailand) *P. malayana* (Malaysia), *P. papuana* (Papua New Guinea) and *Pestalotiopsis* sp. CBS 264.33 (Sulavesi) belongs to the *P. adusta* section, which is characterized by having two apical appendages mostly recorded Asian and Melanesia regions (except *Pestalotiopsis* sp. strain CBS 264.33 isolated from *Rhododendron ponticum* in Netherlands). *Pestalotiopsis diploclisia* and *P. humus* are two species form a sister clade to the *P. adusta* species complex, which recorded from

Hong Kong and Papua New Guinea. *Neopestalotiopsis asiatica*, *N. chrysea* and *N. umberspora* are three sister species recorded from China. In present study we treated three species under genus *Pseudopestalotiopsis* and all of them derived from Asia. In some cases samples collected from the same region but in different host plants were genetically much closer. For an example, *Pestalotiopsis diploclisia* derived from two different hosts in Hong Kong, *P. monochaeta* comes from two different hosts in Nederland and *P. trachicarpicola* from five different hosts in China. Since our data set is not robust, it is not clear whether the geographic influences or hosts range or allopatry play a key role superior to host association in species circumscription and delineation. However, there may be species with wide host ranges, groups of species specialized on different hosts with restricted gene flow, species those with cosmopolitan distribution and reproductive isolated species due to geographical influences. Therefore, it is essential to account substrate, geographic influences, host ranges, morphological characters with incorporating molecular sequence data are necessary to define species borders within *Neopestalotiopsi*s, *Pesudopestalotiopsis* and *Pestalotiopsis*.

5.9 Future works

This thesis raises a number of new avenues for future research. Besides ITS, β-tubulin and TEF1; CAL showed a higher species resolution. Thus, design a new primer pairs for CAL can help to extending the resolution belongs to species in three genera. This work has laid a foundation for the importance of host and geography influence for discriminate species and sections in *Neopestalotiopsi*s, *Pesudopestalotiopsis* and *Pestalotiopsis*. Therefore we suggest sampling efforts on different host, habitat and geographically regions are essential. Another interesting area for research is the study of chemotaxonomy of *Pestalotiopsis* and related genera, since they are highly creative among fungal kingdom. Furthermore, species resulted in this study may used to screen novel secondary metabolites.

5.10 Significance and Publications Resulting from This Thesis

During this study, I collected 150 specimens from different habitats and hosts. Most of the *Pestalotiopsis* isolates were obtained from economically important cash crops. I manage to isolate 60 *Pestalotiopsis* strains from those 150 specimens. I also examined 900 isolates sourced from CBS, CGMCC, ICMP and other collaborating institutes. All isolates were first morphologically observed, and in order to better species resolution, selected 500 strains were sequenced using ITS, β-tubulin and TEF1 gene. Most of these isolates are not included in the thesis, but will be written up and published in future work. During this study I also examined herbaria materials of 20 species. In this study besides *Pestalotiopsis,* the two new genera *Neopestalotiopsis* and *Pseudopestalotiopsis* are proposed. This work provides a backbone trees for 70 ex-type/epitypified species of *Neopestalotiopsis*, *Pseudopestalotiopsis* and *Pestalotiopsis* which can be, used in future studies. The results of my study have been published in six papers in SCI journals as first author (including one important review paper and one large backbone tree for identifying species in the genus) four publications as co-author and four papers are in preparation.

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APPENDIX

APPENDIX A

MEDIA

1. Water Agar (WA) used for single spore isolation of fungi

Agar 15 g

Dissolve 15 g agar in distilled water then mixed and add volume to 1000 ml of water. Heath until dissolved and autoclave at 121°C for 15 minutes.

2. Potato Dextrose Agar (PDA) used for fungal cultivation

Potatoes	200 g
Oxoid Agar N^o 3	20 g
Dextrose	15 g
Tap water	1 l

Scrub potatoes clean and cut into 12 mm cubes (do not peel). Weigh out 200 g rinse rapidly under a running tap, and drop into 1000 ml of tap water in a saucepan. Boil until potatoes are soft (about 1 h) then put through blender. Add 20 g of agar, and heat in a double saucepan until dissolved. Then add 20 g of dextrose and stir until dissolved. Make up to 1000 ml. Pour into bottles, stiring occasionally to ensure that each bottle has a percentage of solid matter. Autoclave at 121 ^{0}C for 20 min.

2. Pine Needle Agar (PDA) used for fungal sporulation

Preparation of water agar (WA) as above. place sterilised pine needles as substratum on WA.

APPENDIX B

PUBLICATIONS AND ABSTRACT PRESENTED AT CONFERENCE

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Pestalotiopsis—morphology, phylogeny, biochemistry and diversity

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Abstract The genus *Pestalotiopsis* has received considerable attention in recent years, not only because of its role as a plant pathogen but also as a commonly isolated endophyte which has been shown to produce a wide range of chemically novel diverse metabolites. Classification in the genus has been previously based on morphology, with conidial characters being considered as important in distinguishing species and closely related genera. In this review, *Pestalotia*, *Pestalotiopsis* and some related genera are evaluated; it is concluded that the large number of described species has resulted from introductions based on host association. We suspect that many of these are probably not good biological species. Recent molecular data have shown that conidial characters can be used to distinguish taxa; however, host association and geographical location is less informative. The taxonomy of the genera complex remains confused. There are only a few type cultures and, therefore, it is impossible to use gene sequences in GenBank to clarify species names reliably. It has not even been established whether *Pestalotia* and *Pestalotiopsis* are distinct genera, as no isolates of the type species of *Pestalotia* have been sequenced, and they are morphologically somewhat similar. When selected GenBank ITS accessions of *Pestalotiopsis clavispora, P. disseminata, P. microspora*, *P. neglecta*, *P. photiniae, P. theae, P. virgatula* and *P. vismiae* are aligned, most species cluster throughout any phylogram generated. Since there appears to be no living type strain for any of these species, it is unwise to use GenBank sequences to represent any of these names. Type cultures and sequences are available for the recently described species *P. hainanensis, P. jesteri*, *P. kunmingensis* and *P. pallidotheae*. It is clear that the important species in *Pestalotia* and *Pestalotiopsis* need to be epitypified so that we can begin to understand the genus/genera. There are numerous reports in the literature that various species produce taxol, while others produce newly discovered compounds with medicinal potential and still others cause disease. The names assigned to these novel compound-producing taxa lack an accurate taxonomic basis, since the taxonomy of the genus is markedly confused. Until the important species have been epitypified with living strains that have been sequenced and deposited in public databases, researchers should refrain from providing the exact name of species.

Keywords Epitypify · Host occurrence · *Pestalotia* · *Pestalosphaeria* · Pigmentation · Secondary metabolites · Taxol

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Introduction

Pestalotoiopsis Steyaert is an appendage-bearing conidial anamorphic form (coelomycetes) in the family Amphisphaeriaceae (Barr 1975, 1990; Kang et al. 1998, 1999), and molecular studies have shown that *Pestalotiopsis* is monophyletic (Jeewon et al. 2002, 2003, 2004). Species of *Pestalotiopsis* are common in tropical and temperate

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A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species

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Abstract *Pestalotiopsis* is a taxonomically confused, pathogenic and chemically creative genus requiring a critical re-examination using a multi-gene phylogeny based on ex-type and ex-epitype cultures. In this study 40 isolates of *Pestalotiopsis*, comprised of 28 strains collected from living and dead plant material of various host plants from China were studied by means of morphology and analysis of ITS, β–tubulin and *tef1* gene sequence data. Based on molecular and morphological data we describe 14 new species (*Pestalotiopsis asiatica*, *P. chinensis*, *P. chrysea*, *P. clavata*, *P. diversiseta*, *P. ellipsospora*, *P. inflexa*, *P. intermedia*, *P. linearis*, *P. rosea*, *P. saprophyta*, *P. umberspora*, *P. unicolor* and *P. verruculosa*) and three species are epitypified (*P. adusta*, *P. clavispora* and *P. foedans*). Of the 10 gene regions (ACT, β-tubulin, CAL, GPDH, GS, ITS, LSU, RPB 1, SSU and *tef1*) utilized to resolve cryptic *Pestalotiopsis* species, ITS, β–tubulin and *tef1* proved to be the better markers. The other gene regions were less useful due to poor success in PCR amplification and/or in their ability to resolve species boundaries. As a single gene *tef1* met the requirements for an ideal candidate and functions well for species delimitation due to its better species resolution and PCR success. Although β-tubulin showed fairly good differences among species, a combination of ITS, β-tubulin and *tef1* gene data gave the best resolution as compared to single gene analysis. This work provides a backbone tree for 22 ex-type/epitypified species of *Pestalotiopsis* and can be used in future studies of the genus.

Keywords β-tubulin · Epitype · ITS · Phylogeny · Saprobe · *tef1*

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A novel species of *Pestalotiopsis* causing leaf spots of *Trachycarpus fortunei*

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Abstract – Specimens of a new *Pestalotiopsis* species were obtained from leaves of *Trachycarpus fortunei* from Kunming Botany Garden, Kunming, Yunnan Province, China, where it caused serious leaf blotch and defoliation in the garden. Single ascospore isolates from the teleomorph produced identical colonies with black slimy conidial masses. Morphological characteristics of the conidia produced in culture accorded well with the genus *Pestalotiopsis*. Based on morphological characters and molecular analysis, *Pestalotiopsis trachicarpicola* sp. nov. is introduced and both its asexual and sexual forms are described.

Coelomycetes / new species / holomorph / *Pestalosphaeria*

INTRODUCTION

We are in the process of studying the pathogens of ornamental plants in Yunnan Province. The study involves collecting fresh specimens, isolation, and molecular analysis, and reporting the known and the novel pathogens, so as to strengthen plant quarantine, integrated pest management and diagnosis of fungal diseases of these plants. In this paper we address a species of *Pestalotiopsis* causing serious leaf spotting disease of *Trachycarpus fortunei* (Chinese windmill palm, Arecaceae).

Pestalotiopsis is a confused genus with 234 names (http://www.index fungorum. org/names/names.asp; accession date, 2012.02.25), which is in need of molecular characterization. Maharachchikumbura *et al.* (2011) reviewed the genus and noted there were only four sequenced type or epitype strains available and

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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).

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Pestalotiopsis anacardiacearum sp. nov. (*Amphisphaeriaceae*) has an intricate relationship with *Penicillaria jocosatrix*, the mango tip borer

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Abstract

Pestalotiopsis anacardiacearum sp. nov. is described from leaves of *Mangifera indica* from Yunnan Province, China. The taxon can clearly be distinguished from all known species of *Pestalotiopsis* by its morphology. Phylogenetic analysis based on combined multi-locus alignment of the internal transcribed spacer (ITS), partial β-tubulin and partial translation elongation factor 1-alpha (*tef1*) also distinguishes this taxon. It can be distinguished from previously recorded *Pestalotiopsis* pathogens on mango by having larger conidia. The species occurs on leaves of mango following death associated with the mango tip borer (*Penicillaria jocosatrix*).

Key words: leaf blight, new species, phylogeny

Introduction

Mangifera indica (mango) is a major cash crop in the subtropics and tropics (Yogisha & Raveesha 2010) and China was the second largest producer of mango worldwide in 2005 (Evans 2008). Several fungal diseases infect different parts of the trees and fruits, and cause serious damage and yield reduction (Okigbo & Osuinde 2003). Diseases of mango include fruit anthracnose, wilt, leaf blight and fruit rots, which are listed as caused by *Alternaria alternata*, *A. tenuissima, Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Dothiorella* sp. and *Phoma mangiferae* (Okigbo & Osuinde 2003, Karunanayake *et al*. 2011, Rizwana *et al*. 2012). Many of these names are likely, however, to be erroneous as molecular data is showing that these names need revision (Cai *et al*. 2011, Ko Ko *et al*. 2011a, b, Maharachchikumbura *et al*. 2011). For instance, in a survey of *Colletotrichum* species infecting tropical fruits it was shown that *Colletotrichum gloeosporioides* was not a causal agent (Phouvilong *et al*. 2010).

Pestalotiopsis is an appendage-bearing conidial asexual coelomycetous fungus in the family *Amphisphaeriaceae* (Barr 1975, 1990, Kang *et al*. 1998, 1999) that is common in tropical and temperate ecosystems (Maharachchikumbura *et al*. 2011, 2012). The genus is well known for its ability to produce novel medicinal compounds (Aly *et al*. 2010, Xu *et al*. 2010, Debbab *et al*. 2011). Species of *Pestalotiopsis* cause a variety of disease in plants (Tagne & Mathur 2001, Sousa *et al*. 2004, Espinoza *et al*. 2008) and are often isolated as endophytes (Botella & Diez 2011, Rocha *et al*. 2011, Debbab *et al*. 2012). The accurate identification of *Pestalotiopsis* species is, however, difficult as shown by recent studies (Maharachchikumbura *et al*. 2011, 2012) and therefore the *Pestalotiopsis* species infecting mango need to be re-established.

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Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*

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Six DNA regions were evaluated as potential DNA barcodes for *Fungi*, the second largest kingdom of eukaryotic life, by a multinational, multilaboratory consortium. The region of the mitochondrial cytochrome *c* oxidase subunit 1 used as the animal barcode was excluded as a potential marker, because it is difficult to amplify in fungi, often includes large introns, and can be insufficiently variable. Three subunits from the nuclear ribosomal RNA cistron were compared together with regions of three representative protein-coding genes (largest subunit of RNA polymerase II, second largest subunit of RNA polymerase II, and minichromosome maintenance protein). Although the protein-coding gene regions often had a higher percent of correct identification compared with ribosomal markers, low PCR amplification and sequencing success eliminated them as candidates for a universal fungal barcode. Among the regions of the ribosomal cistron, the internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode gap between inter- and intraspecific variation. The nuclear ribosomal large subunit, a popular phylogenetic marker in certain groups, had superior species resolution in some taxonomic groups, such as the early diverging lineages and the ascomycete yeasts, but was otherwise slightly inferior to the ITS. The nuclear ribosomal small subunit has poor species-level resolution in fungi. ITS will be formally proposed for adoption as the primary fungal barcode marker to the Consortium for the Barcode of Life, with the possibility that supplementary barcodes may be developed for particular narrowly circumscribed taxonomic groups.

DNA barcoding | fungal biodiversity

The absence of a universally accepted DNA barcode for *Fungi*, the second most speciose eukaryotic kingdom (1, 2), is a serious limitation for multitaxon ecological and biodiversity studies. DNA barcoding uses standardized 500- to 800-bp sequences to identify species of all eukaryotic kingdoms using primers that are applicable for the broadest possible taxonomic group. Reference barcodes must be derived from expertly identified vouchers deposited in biological collections with online metadata and validated by available online sequence chromatograms. Interspecific variation should exceed intraspecific variation (the barcode gap), and barcoding is optimal when a sequence is constant and unique to one species (3, 4). Ideally, the barcode locus would be the same for all kingdoms. A region of the mitochondrial gene encoding the cytochrome *c* oxidase subunit 1 (*CO1*) is the barcode for animals (3, 4) and the default marker adopted by the Consortium for the Barcode of Life for all groups of organisms, including fungi (5). In *Oomycota*, part of the kingdom *Stramenopila* historically studied by mycologists, the de facto barcode internal transcribed spacer (ITS) region is suitable for identification, but the default *CO1* marker is more reliable in a few clades of closely related species (6). In plants, *CO1* has limited value for differentiating species, and a two-marker system of chloroplast genes was adopted (7, 8) based on portions of the ribulose 1-5-biphosphate carboxylase/oxygenase large subunit gene and a maturase-encoding gene from the intron of the *tm*K gene. This system sets a precedent for reconsidering *CO1* as the default fungal barcode.

CO1 functions reasonably well as a barcode in some fungal genera, such as *Penicillium*, with reliable primers and adequate species resolution (67% in this young lineage) (9); however, results in the few other groups examined experimentally are inconsistent, and cloning is often required (10). The degenerate primers applicable to many *Ascomycota* (11) are difficult to assess, because amplification failures may not reflect priming mismatches. Extreme length variation occurs because of multiple introns (9, 12–14), which are not consistently present in a species. Multiple copies of different lengths and variable sequences occur, with identical sequences sometimes shared by several species (11). Some fungal clades, such as *Neocallimastigomycota* (an early diverging lineage of obligately anaerobic, zoosporic gut fungi), lack mitochondria (15). Finally, because most fungi are microscopic and inconspicuous and many are unculturable, robust, universal primers must be available to detect a truly representative profile. This availability seems impossible with *CO1*.

The nuclear rRNA cistron has been used for fungal diagnostics and phylogenetics for more than 20 y (16), and its components are most frequently discussed as alternatives to *CO1* (13, 17). The eukaryotic rRNA cistron consists of the 18S, 5.8S, and 28S rRNA genes transcribed as a unit by RNA polymerase I. Posttranscriptional processes split the cistron, removing two internal transcribed spacers. These two spacers, including the 5.8S gene, are usually referred to as the ITS region. The 18S nuclear ribosomal small subunit rRNA gene (SSU) is commonly used in phylogenetics, and although its homolog (16S) is often used as a species diagnostic for bacteria (18), it has fewer hypervariable

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Data deposition: The sequences reported in this paper have been deposited in GenBank. Sequences are listed in Dataset S1.

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MICROBIOLOGY

Pestalotiopsis camelliae, a new species associated with grey blight of *Camellia japonica* in China

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We have been surveying diseases of ornamental plants in Yunnan Province, China and discovered a previously undescribed species of *Pestalotiopsis* causing grey blight disease on leaves of *Camellia japonica*. The associated causal agent of the disease is introduced as a new species, *Pestalotiopsis camelliae*, in this paper. The taxon can clearly be distinguished from all known species of *Pestalotiopsis* by its morphology and DNA (combined ITS, β–tubulin and *tef1* gene regions) phylogeny. Its most similar relative, *P. furcata* has conidia with more apical appendages (5–9) than in *P. camelliae* (3–6) and the appendages of *P. furcata* consistently divide into branches, that is rare in *P. camelliae*.

Keywords: asexual taxon, leaf spot, *Pestalotiopsis furcata* phylogeny, sp. nov.

During a survey of diseases of ornamental plants in Yunnan Province, China, we constantly observed grey blight of *Camellia japonica* L. (Japanese camellia) and primary identification found a *Pestalotiopsis* species associated with the disease symptom. *Pestalotiopsis* Steyaert is an appendage–bearing conidial asexual form (coelomycetes) in the family Amphisphaeriaceae (Barr 1975, 1990). Species of *Pestalotiopsis* are common in tropical and temperate ecosystems (Bate–Smith & Metcalfe 1957) and may cause plant disease (Das *et al.* 2010, Ko Ko *et al.* 2011, Zhang *et al.* 2012). They are also often isolated as endophytes (Wei *et al.* 2007, Xu *et al.* 2010), or occur as saprobes (Wu *et al.* 1982, Yanna *et al.* 2002).

The taxonomic status of species within the genus is confused and species identification using only molecular or morphological data is problem-

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RESEARCH ARTICLE / *ARTIGO*

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, β-tubulin, and *tef1* 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: β-tubulin, fruit rot, ITS, neighbor-joining, phylogenetic, *tef1.*

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.

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Pestalotiopsis species on ornamental plants in Yunnan Province, China

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Zhang Y. M., Maharachchikumbura S. S. N., Tian Q. & Hyde K. D. (2013) *Pestalotiopsis* species on ornamental plants in Yunnan Province, China– Sydowia 65 (1): 59–74.

Pestalotiopsis species were obtained from diseased leaves of ornamental plants collected in Yunnan Province, China. Morphological comparison and phylogenetic analysis of combined sequence data of the internal transcribed spacer (ITS), partial β-tubulin and partial translation elongation factor 1-alpha (*tef1*) showed that the isolates comprised seven species of *Pestalotiopsis*. Three species, *Pestalotiopsis ericacearum, P. gaultheria* and *P. rhododendri,* are new to science and described herein.

Keywords: leaf blight, new species, pathogen, phylogeny.

Pestalotiopsis, an appendage-bearing conidial asexual form in the family Amphisphaeriaceae (Barr 1975, 1990, Kang *et al.* 1998, 1999), is widely distributed throughout tropical and temperate ecosystems (Maharachchikumbura *et al.* 2011). It is an important plant pathogenic genus (Yasuda *et al.* 2003; Das *et al.* 2010; Maharachchikumbura *et al.* 2011, 2013 a) with about 250 species, traditionally named according to their host associations (Guba 1961, Steyaert 1949, Venkatasubbaiah *et al.* 1991, Kohlmeyer & Volkmann-Kohlmeyer 2001). Many of these names are likely, however, to be erroneous as molecular data has shown that the genus needs revision (Cai *et al.* 2011; KoKo *et al.* 2011; Maharachchikumbura *et al.* 2011, 2012). *Pestalotiopsis* species have been also often isolated as endophytes (Liu *et al.* 2006; Hu *et al.* 2007; Wei *et al.* 2007; Watanabe *et al.* 2010; Botella & Diez 2011; Rocha *et al.* 2011, Debbab *et al.* 2011, 2012; Maharachchikumbura *et al.* 2012), or occur as saprobes (Wu *et al.* 1982, Agarwal & Chauhan 1988, Yanna *et al.* 2002, Hu *et*

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ORIGINAL ARTICLE

Improving the backbone tree for the genus *Pestalotiopsis*; addition of *P. steyaertii* and *P. magna* sp. nov.

Sajeewa S. N. Maharachchikumbura · Liang-Dong Guo · Ekachai Chukeatirote · Kevin D. Hyde

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Abstract A novel, saprobic *Pestalotiopsis* species isolated from the decaying leaves of *Pteridium* sp. collected in France is described as *Pestalotiopsis magna*. The novelty of the species is confirmed based on phenotypic analyses of conidial characters and phylogenetic analyses of sequence data. *Pestalotiopsis magna* can also be distinguished from similar and related species by its larger conidia. Phylogenetic species recognition, based on combined, multilocus alignment of the internal transcribed spacer (ITS), partial β-tubulin, and partial translation elongation factor 1-alpha (*tef1*), strongly supported the monophyly of *P. magna* with relation to other versicolorous species. The ex-type culture of *P. steyaertii* was also sequenced and placed in the backbone tree for *Pestalotiopsis*.

Keywords New species · Phylogeny · Saprobe

Introduction

Pestalotiopsis Steyaert (1949) is an appendage-bearing, conidial, asexual fungus (coelomycetes) in the family *Amphisphaeriaceae* (Barr 1975, 1990; Kang et al. 1998), and is common in tropical and temperate ecosystems (Bate-Smith and Metcalfe 1957). Species of *Pestalotiopsis* cause a variety of diseases in plants (Maharachchikumbura et al. 2013a, b, c; Zhang et al. 2012a, b) and are also often isolated as endophytes (Xu et al. 2010; Maharachchikumbura et al. 2012; Debbab et al. 2013). They are not highly host-specific, and their taxa may have the ability to infect a range of hosts (Hopkins and McQuilken 2000). Due to their ability to switch life-modes, many endophytic and pathogenic *Pestalotiopsis* species persist as saprobes (Hu et al. 2007; Maharachchikumbura et al. 2012) and have been isolated from dead leaves, bark and twigs (Guba 1961; Maharachchikumbura et al. 2012). Several species have been recovered from soil, polluted stream water, wood, paper, fabrics, and wool (Guba 1961).

Pestalotiopsis consists of around 250 species, most of which were named according to their host associations (Guba 1961; Steyaert 1949; Kohlmeyer and Kohlmeyer 2001). However, molecular data has shown that the genus needs revision (Maharachchikumbura et al. 2011, 2012; Zhang et al. 2013), and many of the traditional species may be spurious. This calls for critical re-examination of the genus, using both morphological studies and a multigene phylogeny based on ex-type and ex-epitype cultures (Maharachchikumbura et al. 2012, 2013a).

The current paper aims to provide a complete morphological and molecular characterization of *P. magna*, a new *Pestalotiopsis* species isolated as a saprobe from dead fern leaves in France. We also re-examined and sequenced an ex-type culture of *P. steyaertii* Mordue, and provide here a description and sequence data for this species, thereby strengthening the backbone tree for *Pestalotiopsis* at the species level.

Materials and methods

Isolation and identification

Decaying Bracken (*Pteridium* sp.) leaves were collected from Rimont village, France in August 2011. The isolation of *P.*

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[O_D05]

Diversity of *Pestalotiopsis* spp. in China and Thailand

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Pestalotiopsis is a chemically highly diverse genus traditionally naming according to their host association. Recent molecular data have shown that Conidial characters are a decisive character in distinguishing *Pestalotiopsis* species; however, host association and geographical location is less informative. As a result, many *Pestalotiopsis* species described in literature are probably not good biological species. Re-examination of type materials and establishment of epitypes with living cultures is thus essential for progress and multi gene analysis with distinct morphological characters are needed to develop a strong species base taxonomic system for the genus *Pestalotiopsis.* At present, we are in the process of studying *Pestalotiopsis* in various host plants from China and Thailand by means of morphology and analysis of sequence data. Based on molecular and morphological data we describe 22 new species (*Pestalotiopsis anacardiacearum*, *P. asiatica*, *P. camelliae*, *P. chinensis*, *P. chrysea*, *P. clavata*, *P. diversiseta*, *P. ellipsospora*, *P. ericacearum*, *P. furcata*, *P. gaultheria*, *P. inflexa*, *P. intermedia*, *P. linearis*, *P. rhododendri*, *P. rosea*, *P. samarangensis*, *P. saprophyta*, *P. trachicarpicola*, *P. umberspora*, *P. unicolor* and *P. verruculosa*) and four species are epitypified (*P. adusta*, *P. clavispora*, *P. foedans* and *P. theae*). Of the 10 gene regions (ACT, β-tubulin, CAL, GPDH, GS, ITS, LSU, RPB 1, SSU and *tef1*) utilized to resolve cryptic *Pestalotiopsis* species, ITS, β–tubulin and *tef1* proved to be the better markers. This work provides a backbone tree for 25 ex-type/epitypified species of *Pestalotiopsis* and can be used in future study of the genus.

Key words: Endophytes, new species, pathogens, phylogeny, saprobes

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