

## Pestalotiopsis revisited

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**Abstract:** Species of *Pestalotiopsis* occur commonly as plant pathogens, and represent a fungal group known to produce a wide range of chemically novel, diverse metabolites. In the present study, we investigated 91 *Pestalotiopsis* isolates from the CBS-KNAW Fungal Biodiversity Centre (CBS) culture collection. The phylogeny of the *Amphisphaeriaceae* was constructed based on analysis of 28S nrRNA gene (LSU) sequence data, and taxonomic changes are proposed to reflect more natural groupings. We combined morphological and DNA data, and segregated two novel genera from *Pestalotiopsis*, namely *Neopestalotiopsis* and *Pseudopestalotiopsis*. The three genera are easily distinguishable on the basis of their conidiogenous cells and colour of their median conidial cells. We coupled morphological and combined sequence data of internal transcribed spacer (ITS), partial  $\beta$ -tubulin (*TUB*) and partial translation elongation factor 1- $\alpha$  (*TEF*) gene regions, which revealed 30 clades in *Neopestalotiopsis* and 43 clades in *Pestalotiopsis*. Based on these data, 11 new species are introduced in *Neopestalotiopsis*, 24 in *Pestalotiopsis*, and two in *Pseudopestalotiopsis*. Several new combinations are proposed to emend monophyly of *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis*.

**Key words:** *Amphisphaeriaceae*, New species, *Pestalotia*, *Pestalotiopsis*, Phylogeny, Taxonomy.

**Taxonomic novelties: New genera:** *Neopestalotiopsis* Maharachch., K.D. Hyde & Crous, *Pseudopestalotiopsis* Maharachch., K.D. Hyde & Crous; **New species:** *Neopestalotiopsis aotearoa* Maharachch., K.D. Hyde & Crous, *N. australis* Maharachch., K.D. Hyde & Crous, *N. cubana* Maharachch., K.D. Hyde & Crous, *N. eucalypticola* Maharachch., K.D. Hyde & Crous, *N. formicarum* Maharachch., K.D. Hyde & Crous, *N. honoluluana* Maharachch., K.D. Hyde & Crous, *N. javaensis* Maharachch., K.D. Hyde & Crous, *N. mesopotamica* Maharachch., K.D. Hyde & Crous, *N. piceana* Maharachch., K.D. Hyde & Crous, *N. surinamensis* Maharachch., K.D. Hyde & Crous, *N. zimbabwana* Maharachch., K.D. Hyde & Crous, *Pestalotiopsis arceuthobii* Maharachch., K.D. Hyde & Crous, *P. arengae* Maharachch., K.D. Hyde & Crous, *P. australasiae* Maharachch., K.D. Hyde & Crous, *P. australis* Maharachch., K.D. Hyde & Crous, *P. biciliata* Maharachch., K.D. Hyde & Crous, *P. chamaeropsis* Maharachch., K.D. Hyde & Crous, *P. colombiensis* Maharachch., K.D. Hyde & Crous, *P. diplocislae* Maharachch., K.D. Hyde & Crous, *P. grevilleae* Maharachch., K.D. Hyde & Crous, *P. hawaiiensis* Maharachch., K.D. Hyde & Crous, *P. hollandica* Maharachch., K.D. Hyde & Crous, *P. humus* Maharachch., K.D. Hyde & Crous, *P. kenyaensis* Maharachch., K.D. Hyde & Crous, *P. knightiae* Maharachch., K.D. Hyde & Crous, *P. malayana* Maharachch., K.D. Hyde & Crous, *P. monochaeta* Maharachch., K.D. Hyde & Crous, *P. novae-hollandiae* Maharachch., K.D. Hyde & Crous, *P. oryzae* Maharachch., K.D. Hyde & Crous, *P. papuana* Maharachch., K.D. Hyde & Crous, *P. parva* Maharachch., K.D. Hyde & Crous, *P. portugalica* Maharachch., K.D. Hyde & Crous, *P. scoparia* Maharachch., K.D. Hyde & Crous, *P. spathulata* Maharachch., K.D. Hyde & Crous, *P. telopeae* Maharachch., K.D. Hyde & Crous, *Pseudopestalotiopsis cocos* Maharachch., K.D. Hyde & Crous, *P. indica* Maharachch., K.D. Hyde & Crous; **New combinations:** *Neopestalotiopsis asiatica* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *N. chrysea* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *N. clavispora* (G.F. Atk.) Maharachch., K.D. Hyde & Crous, *N. ellipsospora* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *N. foedans* (Sacc. & Ellis) Maharachch., K.D. Hyde & Crous, *N. magna* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *N. natalensis* (J.F.H. Beyma) Maharachch., K.D. Hyde & Crous, *N. protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, *N. samarangensis* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *N. saprophytica* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *N. steyaertii* (Mordue) Maharachch., K.D. Hyde & Crous, *N. umbrinospora* (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, *Pestalotiopsis brassicae* (Guba) Maharachch., K.D. Hyde & Crous, *Pseudopestalotiopsis theae* (Sawada) Maharachch., K.D. Hyde & Crous.

Published online 29 October 2014; <http://dx.doi.org/10.1016/j.simyco.2014.09.005>. Hard copy: September 2014.

## INTRODUCTION

### History of *Pestalotia*, *Pestalotiopsis* and *Truncatella*

Based on the conidial forms, Steyaert (1949) split *Pestalotia* into three genera, namely *Pestalotia*, *Pestalotiopsis* and *Truncatella*. *Pestalotia pezizoides* is the generic type of *Pestalotia*, which was described from leaves and stems of *Vitis vinifera* collected in Italy, and is presently not known from culture nor DNA sequence. Characteristics of the species include 6-celled conidia with four olivaceous-brown median cells, distoseptate, hyaline terminal cells and simple or branched appendages arising from the apex of the apical cell (Fig. 1). *Pestalotiopsis*

was introduced for species with 5-celled conidia, and *Truncatella* for those with 4-celled conidia. *Pestalotia* was retained as a monotypic genus with a single 6-celled species, *P. pezizoides*. Steyaert (1949) subsequently divided *Pestalotiopsis* into additional sections, namely Monosetulatae, Bisetulatae, Trisetulatae and Multisetulatae, based on the number of apical appendages. These sections were further divided into subdivisions based on concolourous (for those possessing equally pigmented median cells) or versicolourous conidia (two upper median cells darker than lowest median cell), fusoid or claviform conidia, branched or unbranched apical appendages and spatulate or non-spatulate apical appendages. Steyaert (1949) did not retain *Monochaetia* as a distinct genus, and placed species with single apical appendages in section Monosetulatae of *Pestalotiopsis*, or in *Truncatella*. Steyaert (1949) provided

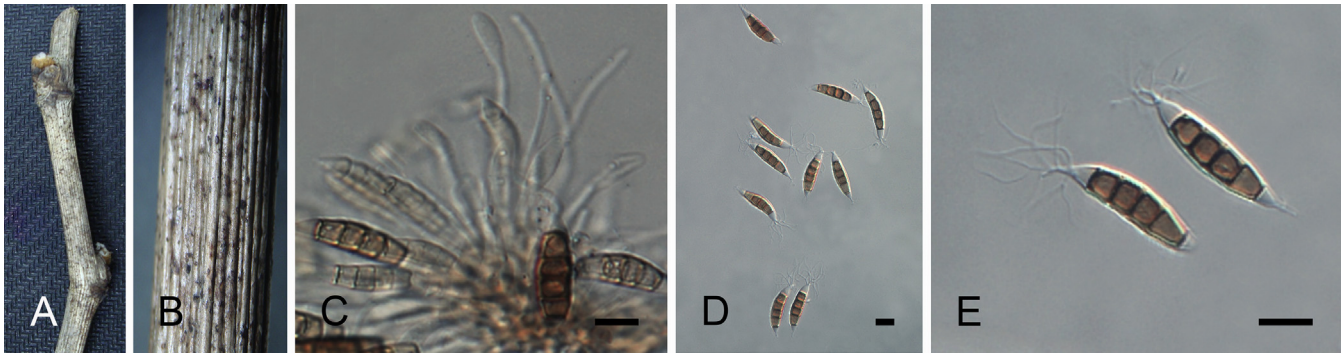


Fig. 1. *Pestalotia pezizoides* (BPI0406483). A–B. Conidiomata on stems of *Vitis vinifera*. C. Conidiogenous cells. D–E. Conidia. Scale bars = 10 µm.

descriptions of 46 species and *Pestalotiopsis guepinii* was considered to be the type species of the newly introduced genus. Steyaert's (1949) introduction of the genus *Pestalotiopsis* to accommodate the 5-celled conidial forms of *Pestalotia* resulted in appreciable controversy from Moreau (1949) and Guba (1956, 1961). All expressed disapproval of Steyaert's classification, which resulted in three different genera instead of the single genus *Pestalotia*.

A major revision of *Pestalotia sensu lato* was published by Guba (1961) in his "Monograph of *Monochaetia* and *Pestalotia*" in which he described 220 species. Guba (1961) separated *Pestalotia* into the sections quadriloculatae (4-celled conidia), quinqueloculatae (5-celled conidia) and sexloculatae (6-celled conidia). He further subdivided the sections into different categories, mainly on the basis of conidial form, colour, and the position, and nature of the setulae. *Monochaetia* was retained as a distinct genus, but the two novel genera (*Pestalotiopsis* and *Truncatella*) proposed by Steyaert (1949) were synonymised under *Pestalotia*. In his support of a single genus Guba (1956) emphasised that there is no justification for other genera based on fruiting structure and there was no point in assembling species with similar numbers of conidial septa into distinct genera. These characters might be useful only for defining species. Furthermore, Dube & Bilgrami (1965) favoured Guba's opinion and pointed out that there is no clear differentiation in conidial morphology of *Pestalotia*, *Pestalotiopsis* and *Truncatella*. Therefore, Dube & Bilgrami (1965) considered it to be more reasonable to retain all species in *Pestalotia*, instead of three different genera, which were introduced mainly on the basis of cell number.

Steyaert (1953a,b, 1961, 1963), however, provided further evidence in support of splitting *Pestalotia*, arguing that retention of *Monochaetia* as a separate genus based on a solitary character, a single apical appendage, was unwise, while *Pestalotiopsis*, *Truncatella* and *Pestalotia* were distinguished from each other based on a set of characters. Steyaert (1963) opined that *Monochaetia* was an artificial genus, which is incompatible with modern views of fungal systematics. Sutton (1980) accepted most of the genera discussed here (*Pestalotia*, *Pestalotiopsis*, *Truncatella*) which fitted into fairly well-defined groups and are characterised by acervuli, most with pigmented conidia, with annellidic conidiogenous cells. Sutton (1980) cited the electron microscope investigation of Griffiths & Swart (1974a,b), which examined the conidial wall of *Pestalotia pezizoides* and two species of *Pestalotiopsis* (*P. funerea* and *P. trisetata*) to support Steyaert's division of *Pestalotiopsis*. Griffiths & Swart (1974a,b) regarded the conidial wall of *P. pezizoides* as being composed of three zones (based on electron density and melanisation) and

in *Pestalotiopsis* of 2-layered zones. Until an evaluation of the 5-celled *Pestalotia* species in culture is made, Sutton (1969) preferred to regard *Pestalotia* as a monotypic genus. According to the revisions of Steyaert (1949) and Sutton (1969, 1980), all earlier designated *Pestalotia* species, except *P. pezizoides*, have been transferred to other genera, many to *Pestalotiopsis*. *Pestalotia valdiviana*, *P. cornu-cervae*, and *P. corni* were also included in *Pestalotia* section sexloculatae (Guba 1961). In his revision of *Pestalotia*, Sutton (1969) considered *P. valdiviana* as a *nomen dubium*, *P. cornu-cervae* was maintained as the type and only species of *Labridella*, and *P. corni* was transferred to *Seiridium*. Sutton (1980) identified several problems with the taxonomy of *Pestalotiopsis*. Although Steyaert (1949) treated *Pestalotia* as a monotypic genus, more than 600 species still remain in the genus and need reassignment to *Monochaetia*, *Pestalotiopsis* or *Truncatella* (Sutton 1980). Furthermore, identification of species from culture and the application of names based on herbarium material as designated by Guba (1961) and Steyaert (1949, 1953a,b, 1955, 1956, 1961), present a confusing situation.

Nag Raj (1985, 1993) found it necessary to reassign many species described in *Pestalotia* to other genera. However Nag Raj (1985, 1993) preferred to adopt a broader concept for *Pestalotiopsis* to include 3-septate conidial forms. *Pestalotiopsis besseyi*, *P. casuarinae*, *P. citrina*, *P. eupyrena*, *P. gastrolobi*, *P. jacksoniae*, *P. moorie*, *P. pestalozzioides*, *P. puyae*, *P. stevensonii* and *P. torrendii* are 3-celled conidial forms Nag Raj (1993) placed in *Pestalotiopsis* but which actually belong in *Truncatella*. Therefore, his view of *Pestalotiopsis* was far broader than the actual concept of Steyaert (1949) (Jeewon et al. 2003). *Pestalotiopsis guepinii*, the type species of *Pestalotiopsis*, was described from stems and leaves of *Camellia japonica* collected in France, and is characterised by 5-celled conidia with three concolourous median cells, hyaline terminal cells and simple or unbranched appendages arising from the apex of the apical cell (Steyaert 1949). However, Nag Raj (1985) pointed out that it is essential to re-examine the type material of *Pestalotiopsis* and related genera and also consider the contentious placement of *P. guepinii* as the generic type of *Pestalotiopsis*. Nag Raj (1985) redescribed *Pestalotiopsis maculans* and considered it as the generic type of *Pestalotiopsis*, with *P. guepinii* as synonym. Hughes (1958) introduced a new combination for *P. maculans*, which was originally described by Corda (1839) as *Sporocadus maculans*. However, the new combination introduced by Hughes (1958) lacked a detailed description of the fungus. Furthermore, there was no reference to this binomial in the monograph of Guba (1961), other than reference to a collection of *S. maculans* listed under

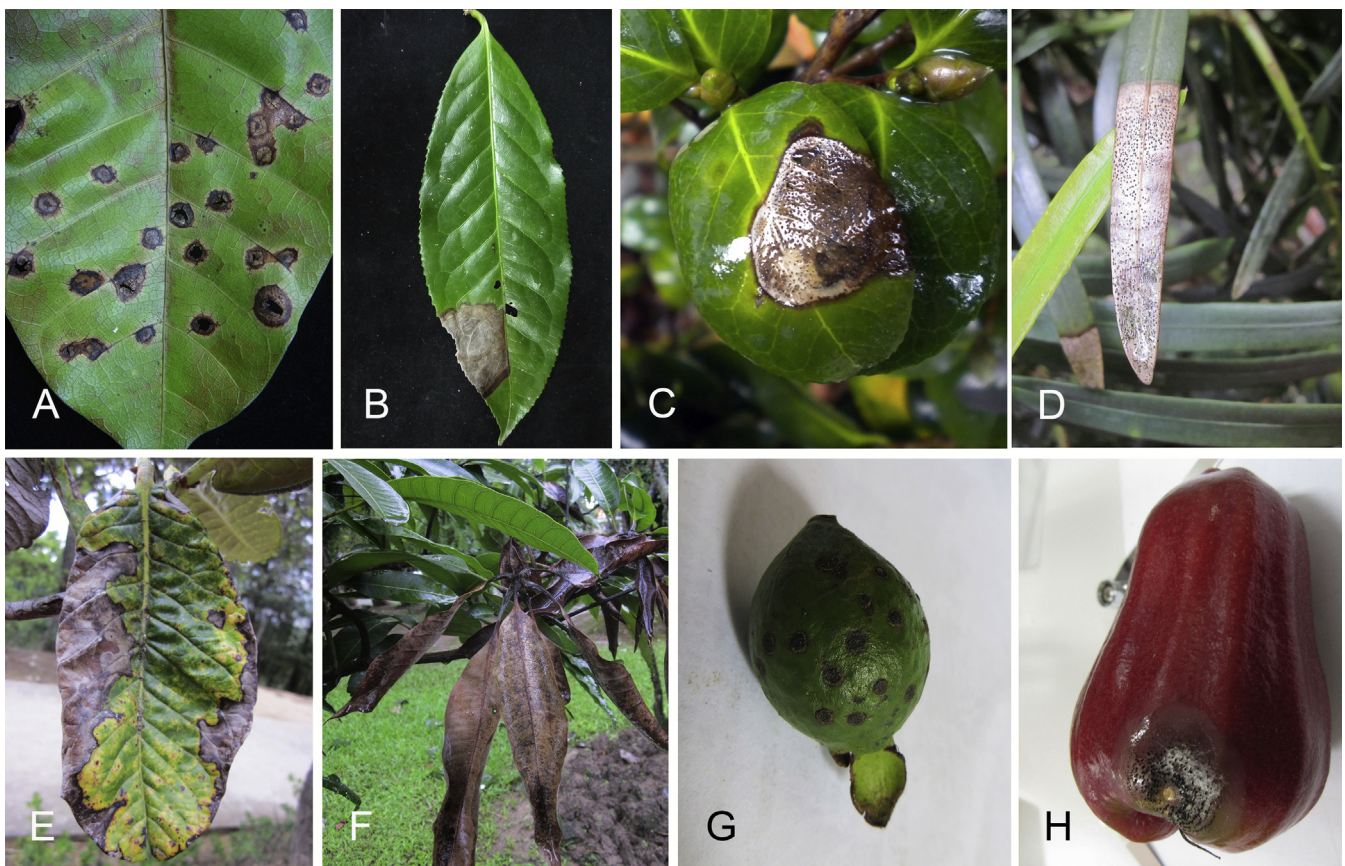
*P. guepinii*. Nag Raj (1985) observed the holotype specimen of *S. maculans* (PR 155665), which was isolated from *Camellia japonica* in Prague, Czech Republic, and clarified that the morphology of the fungus exactly matched the generic concept of *Pestalotiopsis*. Furthermore he observed the isotype specimen of *P. guepinii* in BPI, which he compared with *S. maculans* and found them to be identical. Therefore Nag Raj (1985) regarded *P. maculans* as the correct, older name for *P. guepinii*, and the type species of *Pestalotiopsis*. Based on morphology and phylogeny, Jeewon *et al.* (2003) also pointed out that (based on ITS sequences) *P. maculans* clusters with species having concolourous median cells, and that *P. karstenii* might be a synonym of *P. maculans*.

### Biology of *Pestalotiopsis* species

*Pestalotiopsis* is a species-rich asexual genus with appendage-bearing conidia in the *Amphisphaeriaceae* (Barr 1975, 1990, Kang *et al.* 1999, Lee *et al.* 2006), and is widely distributed throughout tropical and temperate regions (Bate-Smith & Metcalfe 1957). Most species in the genus lack sexual morphs, and presently only 13 sexual morphs have been recorded in literature, which were previously treated as species of *Pestalospaeria* (Maharachchikumbura *et al.* 2011). *Pestalotiopsis* species are common phytopathogens that cause a variety of diseases, including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and various post-harvest diseases (Fig. 2) (Crous *et al.* 2011, Maharachchikumbura *et al.* 2012, 2013a,b,

Zhang *et al.* 2012a, 2013). *Pestalotiopsis* species also reduce production and cause economic loss in apple, blueberry, coconut, chestnut, ginger, grapevine, guava, hazelnut, lychee, mango, orchid, peach, rambutan, tea and wax apple due to disease (Sun & Cao 1990, Sangchote *et al.* 1998, Xu *et al.* 1999, Keith *et al.* 2006, Joshi *et al.* 2009, Keith & Zee 2010, Chen *et al.* 2011, Evidente *et al.* 2012, Ismail *et al.* 2013, Maharachchikumbura *et al.* 2013a,b,c, Ren *et al.* 2013).

*Pestalotiopsis* species are also commonly isolated as endophytes (Watanabe *et al.* 2010, Maharachchikumbura *et al.* 2012, Debbab *et al.* 2013) and there are numerous reports that these endophytes produce novel compounds with medicinal, agricultural and industrial applications (Aly *et al.* 2010, Xu *et al.* 2010, 2014). Species of *Pestalotiopsis* are thought to be a rich source for bioprospecting compared to other fungal genera, and Xu *et al.* (2010, 2014) reviewed 130 and 160 different compounds respectively, isolated from species of *Pestalotiopsis*. Due to their ability to switch nutritional-modes, many endophytic and plant pathogenic *Pestalotiopsis* species persist as saprobes (Hu *et al.* 2007, Maharachchikumbura *et al.* 2012), and have been isolated from dead leaves, bark and twigs (Ellis & Ellis 1997, Maharachchikumbura *et al.* 2013d). Several species have been recovered from soil, polluted stream water, wood, paper, fabrics, and wool (Guba 1961). Some species have been associated with human and animal infections (Sutton 1999, Monden *et al.* 2013) and others (e.g. *Pestalotiopsis guepinii* and *P. microspora*) have also been isolated from extreme environments (Strobel *et al.* 1996, Tejesvi *et al.* 2007).



**Fig. 2.** Disease symptoms associated with various species of *Pestalotiopsis*. A. Leaf spots on *Mangifera indica*. B. Grey blight on *camellia sinensis*. C. Leaf blight on *camellia japonica*. D. Tip blight on *Podocarpus macrophyllus*. E. Leaf blotch on *Rhododendron sinogrande*. F. Shoot dieback on *Mangifera indica*. G. Guava scab on *Psidium guajava*. H. Fruit rot on *Syzygium samarangense*.

## Naming *Pestalotiopsis* species

*Pestalotiopsis* species were historically named according to the host from which they were first observed. In spite of this practise, many argued that *Pestalotiopsis* species are generally not host-specific and are found on a wide range of hosts and substrates (Jeewon *et al.* 2004, Lee *et al.* 2006). Therefore, many of the traditional host-based species may be spurious. However, species of *Pestalotiopsis* display considerable diversity in phenotype, and group together based on similarities in conidial morphology (Jeewon *et al.* 2003, Maharachchikumbura *et al.* 2012, 2013d). Conidial characters such as conidial length, width, median cell length, colour of median cells and length of the apical appendages appear to be stable characters within *Pestalotiopsis* (Jeewon *et al.* 2003, Hu *et al.* 2007). Previous phylogenetic studies revealed *Pestalotiopsis* strains to cluster in three strongly supported clades. These clades corresponded to three conidial types: those with pale brown or olivaceous concolourous median cells, those with versicolourous median cells and those with dark-coloured concolourous median cells (Jeewon *et al.* 2003, Liu *et al.* 2010, Maharachchikumbura *et al.* 2011, 2012). Steyaert (1949) and Guba (1961) had previously grouped species with versicolourous conidia into two groups based on the intensity of colour of the median cells, namely umber-olivaceous (two upper median cells umber and lowest median cell yellow-brown) and fuliginous-olivaceous (two upper median cells fuliginous, usually opaque, and lowest median cell pale brown). However, based on multi-locus DNA sequence analysis, the division of the versicolourous group based on colour intensities of the median conidial cell proved to not be a taxonomically reliable character (Liu *et al.* 2010, Maharachchikumbura *et al.* 2011, 2012).

The sexual state of *Pestalotiopsis* is *Pestalospaeria*, which was introduced by Barr (1975) with the type species *Pestalospaeria concentrica*. This species was isolated from the grey-brown spots on living leaves of *Rhododendron maximum* growing on North Carolina, USA. *Pestalospaeria concentrica* is characterised by immersed, subglobose ascomata and unitunicate, cylindrical asci with a J+ apical ring; ascospores uniseriate in the ascus, ellipsoid, pale dull brown and 2-septate. The germinated ascospores of *Pestalospaeria concentrica* give rise to the *Pestalotiopsis* conidial state, *P. guepini* var. *macrotricha*, which contains three median concolourous conidial cells.

## Objectives of study

In the present study we examined 91 *Pestalotiopsis* strains from the culture collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS), which were isolated from various hosts and geographic origins. Phylogenetic relationships between the strains and other genera in the *Amphisphaeriaceae* are resolved based on analysis of 28S nrRNA gene (LSU) sequence data. The phylogeny resolved *Pestalotiopsis* as a distinct clade in *Amphisphaeriaceae*, with three well-supported groups that correlated with morphology; besides *Pestalotiopsis*, two new genera, *Neopestalotiopsis* and *Pseudopestalotiopsis* are proposed. Various *Pestalotiopsis* species known from culture are therefore allocated to *Neopestalotiopsis* and *Pseudopestalotiopsis*. Phylogenetic analyses of combined sequence data of the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS), partial

$\beta$ -tubulin (*TUB*) and translation elongation factor 1-alpha (*TEF*) gene regions supplemented with conidial morphology clarify species boundaries in the three genera.

## MATERIALS AND METHODS

### Isolates

A total of 91 strains were obtained from the CBS culture collection. Freeze-dried strains were revived in 2 mL malt/peptone (50 % / 50 %) and subsequently transferred to Petri dishes containing oatmeal agar (OA) (Crous *et al.* 2009). Isolates of the CBS collection stored in liquid nitrogen at  $-80^{\circ}\text{C}$  were transferred directly to Petri dishes containing OA.

### Morphological analysis

Morphological descriptions were made for isolates grown on 2 % potato dextrose agar (PDA; Crous *et al.* 2009) under moderate temperatures ( $\sim 22^{\circ}\text{C}$ ) at 12 h daylight. Autoclaved pine needles were placed on synthetic nutrient-poor agar (PNA) (Crous *et al.* 2009) to observe conidiomatal development. Colony colour on PDA was determined with the colour charts of Rayner (1970). Microscopic preparations were made in distilled water, with 30 measurements per structure as observed under a Nikon SMZ1000 dissecting microscope (DM) or with a Nikon Eclipse 80i compound microscope using differential interference contrast (DIC) illumination. Taxonomic descriptions and nomenclature were deposited in MycoBank (Crous *et al.* 2004).

### PCR and sequencing

The UltraClean Microbial DNA Isolation Kit (MoBio laboratories, Carlsbad, CA, USA) was used to extract genomic DNA from fungal mycelia. For nucleotide sequence comparisons, the nuclear rDNA operon spanning the 3' end of the 18S nrRNA gene, the first internal transcribed spacer region, the 5.8S nrRNA gene, the second internal transcribed spacer region and the 5' end of the 28S nrRNA gene (ITS), and the partial  $\beta$ -tubulin (*TUB*) and partial translation elongation factor 1-alpha (*TEF*) genes were amplified using primer pairs LR0R/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994), ITS5/ITS4 (White *et al.* 1990), T1/Bt-2b (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997), and EF1-728F/EF-2 (O'Donnell *et al.* 1998, Carbone & Kohn 1999). Amplification conditions for LSU, ITS and *TEF* followed Crous *et al.* (2013) and for *TUB*, Lee *et al.* (2004).

Sequencing of the PCR amplicons was conducted using the same primers as those used for the amplification reactions. 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 v. 8.1.3 was used to obtain consensus sequences from sequences generated from forward and reverse primers and these were subsequently lodged with GenBank (Table 1).

**Table 1.** Collection details and GenBank accession numbers of isolates included in this study.

Species	Culture accession No. <sup>1</sup>	Host/Substrate	Family	Location	GenBank accession <sup>2</sup>			
					LSU	ITS	TUB	TEF
<i>Neopestalotiopsis aotearoa</i>	CBS 367.54; ATCC 11763; QM 381*	Canvas	—	New Zealand	KM116247	KM199369	KM199454	KM199526
<i>N. asiatica</i>	MFLUCC 12-0286; NN0476380*	Unidentified tree	—	China	—	JX398983	JX399018	JX399049
<i>N. australis</i>	CBS 114159; STE-U 3017*	<i>Telopea</i> sp.	Proteaceae	Australia: New South Wales	KM116252	KM199348	KM199432	KM199537
<i>N. chrysea</i>	MFLUCC 12-0261; NN042855*	Dead leaves	—	China	—	JX398985	JX399020	JX399051
	MFLUCC 12-0262; NN047037	Dead plant	—	China	—	JX398986	JX399021	JX399052
<i>N. clavispota</i>	CBS 447.73	Decaying wood	—	Sri Lanka	KM116275	KM199374	KM199443	KM199539
	MFLUCC 12-0280; NN043011	<i>Magnolia</i> sp.	Magnoliaceae	China	—	JX398978	JX399013	JX399044
	MFLUCC 12-0281; NN043133*	<i>Magnolia</i> sp.	Magnoliaceae	China	—	JX398979	JX399014	JX399045
<i>N. cubana</i>	CBS 600.96; INIFAT C96/44-4*	Leaf litter	—	Cuba	KM116253	KM199347	KM199438	KM199521
<i>N. ellipsospora</i>	CBS 115113; HKUCC 9136	<i>Ardisia crenata</i>	Myrsinaceae	Hong Kong	KM116269	KM199343	KM199450	KM199544
	MFLUCC 12-0283*	Dead plant materials	—	China	—	JX398980	JX399016	JX399047
	MFLUCC 12-0284	Dead plant materials	—	Thailand	—	JX398981	JX399015	JX399046
<i>N. eucalypticola</i>	CBS 264.37; BBA 5300*	<i>Eucalyptus globulus</i>	Myrtaceae	—	KM116256	KM199376	KM199431	KM199551
<i>N. foedans</i>	CGMCC 3.9123*	Mangrove plant	—	China	—	JX398987	JX399022	JX399053
	CGMCC 3.9178	<i>Neodypsis decaryi</i>	Areaceae	China	—	JX398989	JX399024	JX399055
	CGMCC 3.9202	<i>Calliandra haematocephala</i>	Fabaceae	China	—	JX398988	JX399023	JX399054
<i>N. formicarum</i>	CBS 115.83	Plant debris	—	Cuba	KM116255	KM199344	KM199444	KM199519
	CBS 362.72*	Dead <i>Formicidae</i> (ant)	—	Ghana	KM116248	KM199358	KM199455	KM199517
<i>N. honoluluana</i>	CBS 111535; STE-U 2078	<i>Telopea</i> sp.	Proteaceae	USA: Hawaii	KM116263	KM199363	KM199461	KM199546
	CBS 114495; STE-U 2076*	<i>Telopea</i> sp.	Proteaceae	USA: Hawaii	—	KM199364	KM199457	KM199548
<i>N. javaensis</i>	CBS 257.31*	<i>Cocos nucifera</i>	Areaceae	Indonesia: Java	—	KM199357	KM199437	KM199543
<i>N. magna</i>	MFLUCC 12-652; ICMP 20011*	<i>Pteridium</i> sp.	Dennstaedtiaceae	France	—	KF582795	KF582793	KF582791
<i>N. mesopotamica</i>	CBS 299.74	<i>Eucalyptus</i> sp.	Myrtaceae	Turkey	KM116257	KM199361	KM199435	KM199541
	CBS 336.86*	<i>Pinus brutia</i>	Pinaceae	Iraq	KM116271	KM199362	KM199441	KM199555
	CBS 464.69	<i>Achras sapota</i>	Sapotaceae	India	—	KM199353	KM199436	—
<i>N. natalensis</i>	CBS 138.41*	<i>Acacia mollissima</i>	Fabaceae	South Africa	KM116279	KM199377	KM199466	KM199552
<i>N. piceana</i>	CBS 225.30	<i>Mangifera indica</i>	Anacardiaceae	—	KM116270	KM199371	KM199451	KM199535
	CBS 254.32	<i>Cocos nucifera</i>	Areaceae	Indonesia: Sulawesi	KM116267	KM199372	KM199452	KM199529
	CBS 394.48*	<i>Picea</i> sp.	Pinaceae	UK	KM116266	KM199368	KM199453	KM199527
<i>N. protearum</i>	CBS 114178; STE-U 1765*	<i>Leucospermum cuneiforme</i> cv. 'Sunbird'	Proteaceae	Zimbabwe	JN712564	JN712498	KM199463	KM199542

(continued on next page)

Table 1. (Continued)

Species	Culture accession No. <sup>1</sup>	Host/Substrate	Family	Location	GenBank accession <sup>2</sup>			
					LSU	ITS	TUB	TEF
<i>N. rosae</i>	CBS 101057*	<i>Rosa</i> sp.	<i>Rosaceae</i>	New Zealand	KM116245	KM199359	KM199429	KM199523
	CBS 124745	<i>Paeonia suffruticosa</i>	<i>Paeoniaceae</i>	USA	KM116272	KM199360	KM199430	KM199524
<i>N. samarangensis</i>	CBS 115451; HKUCC 9095	Unidentified tree	—	Hong Kong	—	KM199365	KM199447	KM199556
	MFLUCC 12-0233*	<i>Syzygium samarangense</i>	<i>Myrtaceae</i>	Thailand	—	JQ968609	JQ968610	JQ968611
<i>N. saprophytica</i>	CBS 115452; HKUCC 8684	<i>Litsea rotundifolia</i>	<i>Lauraceae</i>	Hong Kong	KM116251	KM199345	KM199433	KM199538
	MFLUCC 12-0282; NN047136*	<i>Magnolia</i> sp.	<i>Magnoliaceae</i>	China	—	JX398982	JX399017	JX399048
<i>Neopestalotiopsis</i> sp. Clade 4	CBS 233.79	<i>Crotalaria juncea</i>	<i>Fabaceae</i>	India	KM116249	KM199373	KM199464	KM199528
<i>Neopestalotiopsis</i> sp. Clade 10	CBS 110.20	—	—	—	KM116250	KM199342	KM199442	KM199540
<i>Neopestalotiopsis</i> sp. Clade 15	CBS 177.25	<i>Dalbergia</i> sp.	<i>Fabaceae</i>	—	KM116246	KM199370	KM199445	KM199533
	CBS 274.29	<i>Cocos nucifera</i>	<i>Areaceae</i>	Indonesia: Java	KM116261	KM199375	KM199448	KM199534
	CBS 322.76	<i>Camellia</i> sp.	<i>Theaceae</i>	France	KM116259	KM199366	KM199446	KM199536
	CBS 664.94	<i>Cocos nucifera</i>	<i>Areaceae</i>	Netherlands	KM116254	KM199354	KM199449	KM199525
<i>Neopestalotiopsis</i> sp. Clade 20	CBS 164.42	Dune sand	—	France	KM116268	KM199367	KM199434	KM199520
	CBS 360.61	<i>Cinchona</i> sp.	<i>Rubiaceae</i>	Guinea	KM116260	KM199346	KM199440	KM199522
<i>Neopestalotiopsis</i> sp. Clade 22	CBS 119.75	<i>Achras sapota</i>	<i>Sapotaceae</i>	India	KM116265	KM199356	KM199439	KM199531
	CBS 266.80	<i>Vitis vinifera</i>	<i>Vitaceae</i>	India	KM116264	KM199352	—	KM199532
<i>Neopestalotiopsis</i> sp. Clade 26	CBS 266.37; BBA 5087; IMI 083708	<i>Erica</i> sp.	<i>Ericaceae</i>	Germany	KM116273	KM199349	KM199459	KM199547
	CBS 323.76	<i>Erica gracilis</i>	<i>Ericaceae</i>	France	KM116262	KM199350	KM199458	KM199550
	CBS 361.61	<i>Cissus</i> sp.	<i>Vitaceae</i>	Netherlands	KM116274	KM199355	KM199460	KM199549
<i>N. steyaertii</i>	IMI 192475*	<i>Eucalyptus viminalis</i>	<i>Myrtaceae</i>	Australia	KM116285	KF582796	KF582794	KF582792
<i>N. surinamensis</i>	CBS 111494; STE-U 1779	<i>Protea eximia</i>	<i>Proteaceae</i>	Zimbabwe	JX556250	JX556232	KM199462	KM199530
	CBS 450.74*	Soil under <i>Elaeis guineensis</i>	<i>Areaceae</i>	Suriname	KM116258	KM199351	KM199465	KM199518
<i>N. umbrinospora</i>	MFLUCC 12-0285; NN042986*	Unidentified plant	—	China	—	JX398984	JX399019	JX399050
<i>N. zimbabwana</i>	CBS 111495; STE-U 1777*	<i>Leucospermum cunciforme</i> cv. 'Sunbird'	<i>Proteaceae</i>	Zimbabwe	JX556249	JX556231	KM199456	KM199545
<i>Pestalotiopsis adusta</i>	ICMP 6088*	On refrigerator door PVC gasket	—	Fiji	—	JX399006	JX399037	JX399070
	MFLUCC 10-146	<i>Syzygium</i> sp.	<i>Myrtaceae</i>	Thailand	—	JX399007	JX399038	JX399071
<i>P. anacardiacearum</i>	IFRDCC 2397*	<i>Mangifera indica</i>	<i>Anacardiaceae</i>	China	—	KC247154	KC247155	KC247156
<i>P. arceuthobii</i>	CBS 434.65*	<i>Arceuthobium campylopodum</i>	<i>Santalaceae</i>	USA	KM116243	KM199341	KM199427	KM199516
<i>P. arengae</i>	CBS 331.92*	<i>Arenga undulatifolia</i>	<i>Areaceae</i>	Singapore	KM116207	KM199340	KM199426	KM199515

Table 1. (Continued)

Species	Culture accession No. <sup>1</sup>	Host/Substrate	Family	Location	GenBank accession <sup>2</sup>			
					LSU	ITS	TUB	TEF
<i>P. australasiae</i>	CBS 114126; STE-U 2896*	<i>Knightia</i> sp.	<i>Proteaceae</i>	New Zealand	KM116218	KM199297	KM199409	KM199499
	CBS 114141; STE-U 2949	<i>Protea</i> sp.	<i>Proteaceae</i>	Australia: New South Wales	KM116203	KM199298	KM199410	KM199501
<i>P. australis</i>	CBS 111503; STE-U 1770	<i>Protea neriifolia</i> × <i>susannae</i> cv. 'Pink Ice'	<i>Proteaceae</i>	South Africa	KM116200	KM199331	KM199382	KM199557
	CBS 114193; STE-U 3011*	<i>Grevillea</i> sp.	<i>Proteaceae</i>	Australia: New South Wales	KM116197	KM199332	KM199383	KM199475
	CBS 114474; STE-U 1769	<i>Protea neriifolia</i> × <i>susannae</i> cv. 'Pink Ice'	<i>Proteaceae</i>	South Africa	KM116220	KM199334	KM199385	KM199477
	CBS 119350; CMW 20013	<i>Brabejum stellatifolium</i>	<i>Proteaceae</i>	South Africa	KM116209	KM199333	KM199384	KM199476
<i>P. biciliata</i>	CBS 124463*	<i>Platanus</i> × <i>hispanica</i>	<i>Platanaceae</i>	Slovakia	KM116224	KM199308	KM199399	KM199505
	CBS 236.38	<i>Paeonia</i> sp.	<i>Proteaceae</i>	Italy	KM116214	KM199309	KM199401	KM199506
	CBS 790.68	<i>Taxus baccata</i>	<i>Taxaceae</i>	Netherlands	KM116235	KM199305	KM199400	KM199507
<i>P. brassicae</i>	CBS 170.26*	<i>Brassica napus</i>	<i>Brassicaceae</i>	New Zealand	—	KM199379	—	KM199558
<i>P. camelliae</i>	CBS 443.62	<i>Camellia sinensis</i>	<i>Theaceae</i>	Turkey	KM116225	KM199336	KM199424	KM199512
	MFLUCC 12-0277*	<i>Camellia japonica</i>	<i>Theaceae</i>	China	—	JX399010	JX399041	JX399074
	MFLUCC 12-0278	<i>Camellia japonica</i>	<i>Theaceae</i>	China	KM116284	JX399011	JX399042	JX399075
<i>P. chamaeropsis</i>	CBS 113604; STE-U 3078	—	—	—	KM116201	KM199323	KM199389	KM199471
	CBS 113607; STE-U 3080	—	—	—	KM116211	KM199325	KM199390	KM199472
	CBS 186.71*	<i>Chamaerops humilis</i>	<i>Arecaceae</i>	Italy	KM116210	KM199326	KM199391	KM199473
	CBS 237.38	—	—	Italy	KM116217	KM199324	KM199392	KM199474
<i>P. clavata</i>	MFLUCC 12-0268; NN0471340*	<i>Buxus</i> sp.	<i>Buxaceae</i>	China	—	JX398990	JX399025	JX399056
<i>P. colombiensis</i>	CBS 118553; CPC 10969*	<i>Eucalyptus eurograndis</i>	<i>Myrtaceae</i>	Colombia	KM116222	KM199307	KM199421	KM199488
<i>P. diploclisiae</i>	CBS 115449; HKUCC 9103	<i>Psychotria tutcheri</i>	<i>Rubiaceae</i>	Hong Kong	KM116215	KM199314	KM199416	KM199485
	CBS 115585; HKUCC 8394	<i>Diploclisia glaucescens</i>	<i>Menispermaceae</i>	Hong Kong	KM116213	KM199315	KM199417	KM199483
	CBS 115587; HKUCC 10130*	<i>Diploclisia glaucescens</i>	<i>Menispermaceae</i>	Hong Kong	KM116242	KM199320	KM199419	KM199486
<i>P. diversiseta</i>	MFLUCC 12-0287; NN0472610*	<i>Rhododendron</i> sp.	<i>Ericaceae</i>	China	—	JX399009	JX399040	JX399073
<i>P. ericacearum</i>	IFRDCC 2439*	<i>Rhododendron delavayi</i>	<i>Ericaceae</i>	China	—	KC537807	KC537821	KC537814
<i>P. furcata</i>	MFLUCC 12-0054; CPC 20280*	<i>Camellia sinensis</i>	<i>Theaceae</i>	Thailand	KM116283	JQ683724	JQ683708	JQ683740
<i>P. gaultheria</i>	IFRD 411-014*	<i>Gaultheria forrestii</i>	<i>Ericaceae</i>	China	—	KC537805	KC537819	KC537812
<i>P. grevilleae</i>	CBS 114127; STE-U 2919*	<i>Grevillea</i> sp.	<i>Proteaceae</i>	Australia	KM116212	KM199300	KM199407	KM199504
<i>P. hawaiiensis</i>	CBS 114491; STE-U 2215*	<i>Leucospermum</i> sp. cv. 'Coral'	<i>Myrtaceae</i>	USA: Hawaii	KM116239	KM199339	KM199428	KM199514
<i>P. hollandica</i>	CBS 265.33*	<i>Sciadopitys verticillata</i>	<i>Sciadopityaceae</i>	Netherlands	KM116228	KM199328	KM199388	KM199481

(continued on next page)

Table 1. (Continued)

Species	Culture accession No. <sup>1</sup>	Host/Substrate	Family	Location	GenBank accession <sup>2</sup>			
					LSU	ITS	TUB	TEF
<i>P. humus</i>	CBS 115450; HKUCC 9100	<i>Ilex cinerea</i>	<i>Aquifoliaceae</i>	Hong Kong	KM116208	KM199319	KM199418	KM199487
	CBS 336.97*	Soil	—	Papua New Guinea	KM116230	KM199317	KM199420	KM199484
<i>P. inflexa</i>	MFLUCC 12-0270; NN0470980*	Unidentified tree	—	China	—	JX399008	JX399039	JX399072
<i>P. intermedia</i>	MFLUCC 12-0259; NN0476420*	Unidentified tree	—	China	—	JX398993	JX399028	JX399059
<i>P. jesteri</i>	CBS 109350 = MONT 6M-B-3*	<i>Fragraea bodenii</i>	<i>Gentianaceae</i>	Papua New Guinea	KM116281	KM199380	KM199468	KM199554
<i>P. kenya</i>	CBS 442.67*	<i>Coffea</i> sp.	<i>Rubiaceae</i>	Kenya	KM116234	KM199302	KM199395	KM199502
	CBS 911.96	Raw material from agar-agar	—	—	KM116204	KM199303	KM199396	KM199503
<i>P. knightiae</i>	CBS 111963; STE-U 2905	<i>Knightia</i> sp.	<i>Proteaceae</i>	New Zealand	KM116241	KM199311	KM199406	KM199495
	CBS 114138; STE-U 2906*	<i>Knightia</i> sp.	<i>Proteaceae</i>	New Zealand	KM116227	KM199310	KM199408	KM199497
<i>P. linearis</i>	MFLUCC 12-0271; NN0471900*	<i>Trachelospermum</i> sp.	<i>Apocynaceae</i>	China	—	JX398992	JX399027	JX399058
<i>P. malayana</i>	CBS 102220*	<i>Macaranga triloba</i>	<i>Euphorbiaceae</i>	Malaysia	KM116238	KM199306	KM199411	KM199482
<i>P. monochaeta</i>	CBS 144.97*	<i>Quercus robur</i>	<i>Fagaceae</i>	Netherlands	KM116229	KM199327	KM199386	KM199479
	CBS 440.83; IFO 32686	<i>Taxus baccata</i>	<i>Taxaceae</i>	Netherlands	KM116196	KM199329	KM199387	KM199480
<i>P. novae-hollandiae</i>	CBS 130973*	<i>Banksia grandis</i>	<i>Proteaceae</i>	Australia	KM116232	KM199337	KM199425	KM199511
<i>P. oryzae</i>	CBS 111522; STE-U 2083	<i>Telopea</i> sp.	<i>Proteaceae</i>	USA: Hawaii	—	KM199294	KM199394	KM199493
	CBS 171.26	—	—	Italy	KM116206	KM199304	KM199397	KM199494
	CBS 353.69*	<i>Oryza sativa</i>	<i>Poaceae</i>	Denmark	KM116221	KM199299	KM199398	KM199496
<i>P. papuana</i>	CBS 331.96*	Coastal soil	—	Papua New Guinea	KM116240	KM199321	KM199413	KM199491
	CBS 887.96	<i>Cocos nucifera</i>	<i>Areaceae</i>	Papua New Guinea	KM116231	KM199318	KM199415	KM199492
<i>P. parva</i>	CBS 265.37; BBA 2820*	<i>Delonix regia</i>	<i>Fabaceae</i>	—	KM116226	KM199312	KM199404	KM199508
	CBS 278.35	<i>Leucothoe fontanesiana</i>	<i>Ericaceae</i>	—	KM116205	KM199313	KM199405	KM199509
<i>P. portugolica</i>	CBS 393.48*	—	—	Portugal	KM116233	KM199335	KM199422	KM199510
<i>P. rhododendri</i>	IFRDCC 2399*	<i>Rhododendron sinogrande</i>	<i>Ericaceae</i>	China	—	KC537804	KC537818	KC537811
<i>P. rosea</i>	MFLUCC 12-0258; NN0471350*	<i>Pinus</i> sp.	<i>Pinaceae</i>	China	—	JX399005	JX399036	JX399069
<i>P. scoparia</i>	CBS 176.25*	<i>Chamaecyparis</i> sp.	<i>Cupressaceae</i>	—	KM116216	KM199330	KM199393	KM199478
<i>Pestalotiopsis</i> sp. Clade 33	CBS 263.33	<i>Rhododendron ponticum</i>	<i>Ericaceae</i>	Netherlands	KM116198	KM199316	KM199414	KM199489
	CBS 264.33	<i>Cocos</i> sp.	<i>Areaceae</i>	Indonesia: Sulawesi	KM116199	KM199322	KM199412	KM199490
<i>P. spathulata</i>	CBS 356.86*	<i>Gevuina avellana</i>	<i>Proteaceae</i>	Chile	KM116236	KM199338	KM199423	KM199513
<i>P. telopeae</i>	CBS 113606; STE-U 3082	<i>Telopea</i> sp.	<i>Proteaceae</i>	Australia	KM116202	KM199295	KM199402	KM199498
	CBS 114137; STE-U 2952	<i>Protea neriifolia</i> × <i>susannae</i> cv. 'Pink Ice'	<i>Proteaceae</i>	Australia	KM116219	KM199301	KM199469	KM199559
	CBS 114161; STE-U 3083*	<i>Telopea</i> sp.	<i>Proteaceae</i>	Australia	—	KM199296	KM199403	KM199500



Table 1. (Continued)

Species	Culture accession No. <sup>1</sup>	Host/Substrate	Family	Location	GenBank accession <sup>2</sup>			
					LSU	ITS	TUB	TEF
<i>P. trachicarpicola</i>	IFRDCC 2403	<i>Podocarpus macrophyllus</i>	<i>Podocarpaceae</i>	China	—	KC537809	KC537823	KC537816
	MFLUCC 12-0263; NN0470720	Unidentified tree	—	China	—	JX399000	JX399031	JX399064
	MFLUCC 12-0264; NN0471960	<i>Chrysophyllum</i> sp.	<i>Sapotaceae</i>	China	—	JX399004	JX399035	JX399068
	MFLUCC 12-0265; NN0469830	<i>Schima</i> sp.	<i>Theaceae</i>	China	—	JX399003	JX399034	JX399067
	MFLUCC 12-0266; NN0469780	<i>Symplocos</i> sp.	<i>Symplocaceae</i>	China	—	JX399002	JX399033	JX399066
	MFLUCC 12-0267; NN0470990	Unidentified tree	—	China	—	JX399001	JX399032	JX399065
	OP068; IFRDCC 2440*	<i>Trachycarpus fortunei</i>	<i>Arecaceae</i>	China	—	JQ845947	JQ845945	JQ845946
<i>P. unicolor</i>	MFLUCC 12-0275; NN0473080	Unidentified tree	—	China	—	JX398998	JX399029	JX399063
	MFLUCC 12-0276; NN0469740*	<i>Rhododendron</i> sp.	<i>Ericaceae</i>	China	—	JX398999	JX399030	—
<i>P. verruculosa</i>	MFLUCC 12-0274; NN0473090*	<i>Rhododendron</i> sp.	<i>Ericaceae</i>	China	—	JX398996	—	JX399061
<i>Pseudopezalotiopsis cocos</i>	CBS 272.29*	<i>Cocos nucifera</i>	<i>Arecaceae</i>	Indonesia: Java	KM116276	KM199378	KM199467	KM199553
<i>Ps. indica</i>	CBS 459.78*	<i>Hibiscus rosa-sinensis</i>	<i>Malvaceae</i>	India	—	KM199381	KM199470	KM199560
<i>Ps. theae</i>	MFLUCC 12-0055; CPC 20281*	<i>Camellia sinensis</i>	<i>Theaceae</i>	Thailand	KM116282	JQ683727	JQ683711	JQ683743
	SC011	<i>Camellia sinensis</i>	<i>Theaceae</i>	Thailand	—	JQ683726	JQ683710	JQ683742

<sup>1</sup> ATCC: American Type Culture Collection, Virginia, USA; BBA: Institute for Plant Virology, Microbiology and Biosafety, Federal Biological Research Centre for Agriculture and Forestry (BBA), Germany; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC: Culture collection of Pedro Crous, housed at CBS; HKUCC: The University of Hong Kong Culture Collection, Hong Kong, China; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IFRDCC: International Fungal Research & Development Centre Culture Collection, China; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; INIFAT: Alexander Humboldt Institute for Basic Research in Tropical Agriculture, Ciudad de La Habana, Cuba; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NN: Novozymes, Beijing, China; QM: Quarter Master Culture Collection, Amherst, MA, USA; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa. \* = ex-holotype or ex-epitype culture.

<sup>2</sup> LSU: large subunit (28S) of the nrRNA gene operon; ITS: internal transcribed spacers and intervening 5.8S nrDNA; TUB: partial beta-tubulin gene; TEF: partial translation elongation factor 1-alpha gene.

## Phylogenetic analyses

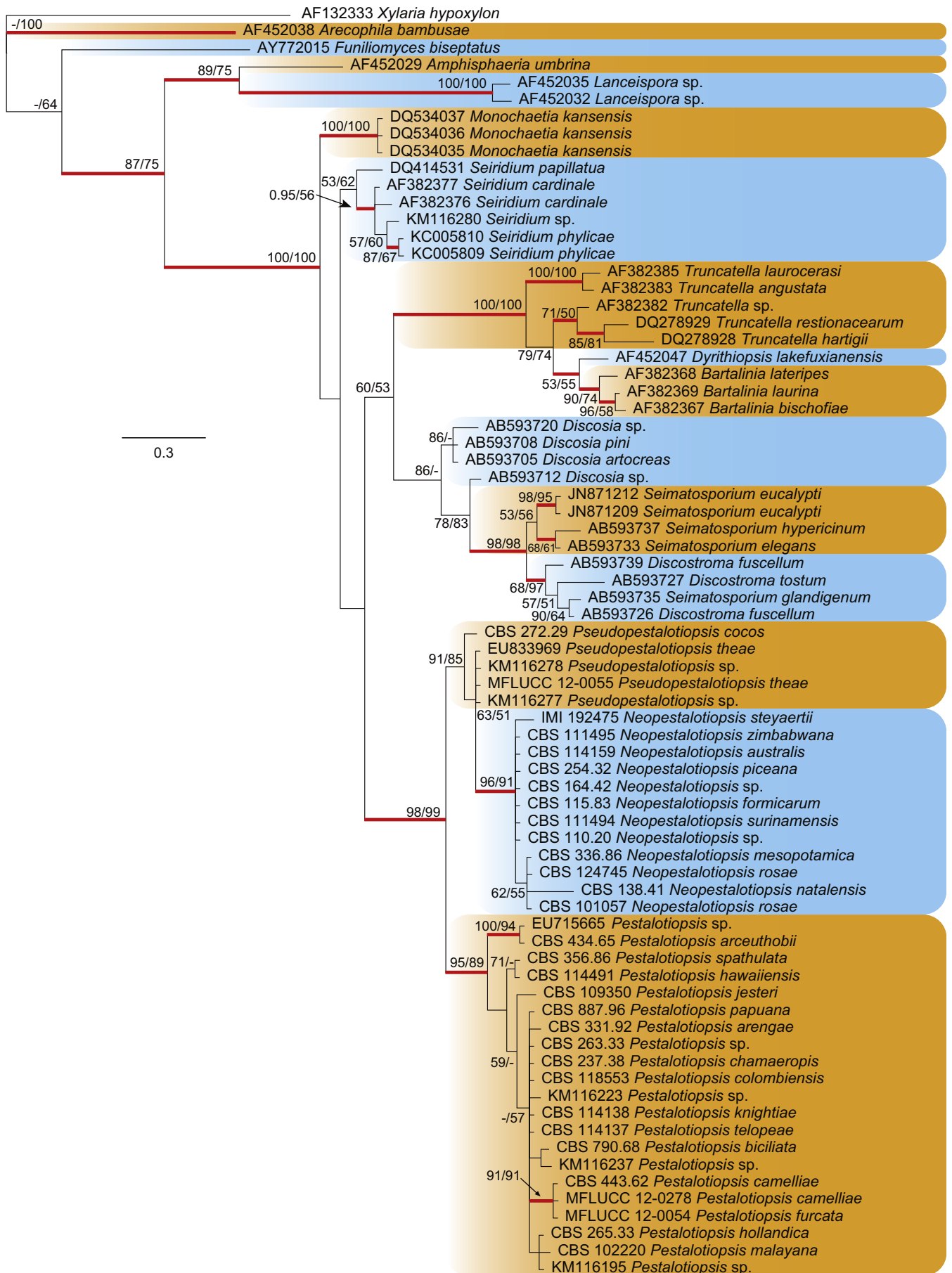
The sequences generated in this study were supplemented with additional sequences obtained from GenBank (Table 1) based on blast searches and literature. Multiple sequence alignments were generated with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>); the alignment was visually improved with Mesquite v. 2.75 (Maddison & Maddison 2011) and MEGA v. 5.2.2 (Kumar et al. 2012) or BioEdit v. 7.0.5.2 (Hall 1999). Three different datasets were used to estimate three phylogenies: an *Amphisphaeriaceae* family tree, a combined *Neopestalotiopsis* and *Pseudopestalotiopsis* species tree, and a *Pestalotiopsis* species tree. The first tree focuses on the placement and further division of *Pestalotiopsis* into two new genera in *Amphisphaeriaceae* by using the LSU region. The second and third phylogenetic analyses were produced to show species relationships in *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis* based on the combined datasets (ITS, *TUB* and *TEF*). The combined alignments were split between the genera to improve the robustness of the alignment across the three loci. Phylogenetic analyses of the sequence data consisted of Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses of both the individual data partitions as well as the combined aligned dataset. Ambiguously aligned regions were excluded from all analyses and gaps were treated as “fifth character state” in the parsimony analysis. Suitable models for the Bayesian analysis were first selected using models of nucleotide substitution for each gene, as determined using MrModeltest v. 2.2 (Nylander 2004), and included for each gene partition. The Bayesian analyses (MrBayes v. 3.2.1; Ronquist et al. 2012) of four simultaneous Markov Chain Monte Carlo (MCMC) chains were run from random trees for 10 000 000 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 analysis was performed using raxmlGUI v. 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 GTR+GAMMA substitution model. The MP analysis was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). Trees were inferred by using the heuristic search option with TBR branch swapping and 1 000 random sequence additions. The maximum number of retained trees were limited to 5 000, branches of zero length were collapsed and all multiple equally most 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) values were calculated. The robustness of the equally most parsimonious trees was evaluated by 1 000 bootstrap replications (Felsenstein 1985) resulting from a maximum parsimony analysis, each with 10 replicates of random stepwise addition of taxa. The Kishino-Hasegawa tests (Kishino & Hasegawa 1989) were performed to determine whether the trees inferred under different optimality criteria were significantly different. The resulting trees were printed with FigTree v. 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>) and the layout was done with Adobe Illustrator CS v. 6. The alignments and trees were deposited in TreeBASE ([www.treebase.org/treebase/index.html](http://www.treebase.org/treebase/index.html)).

## RESULTS

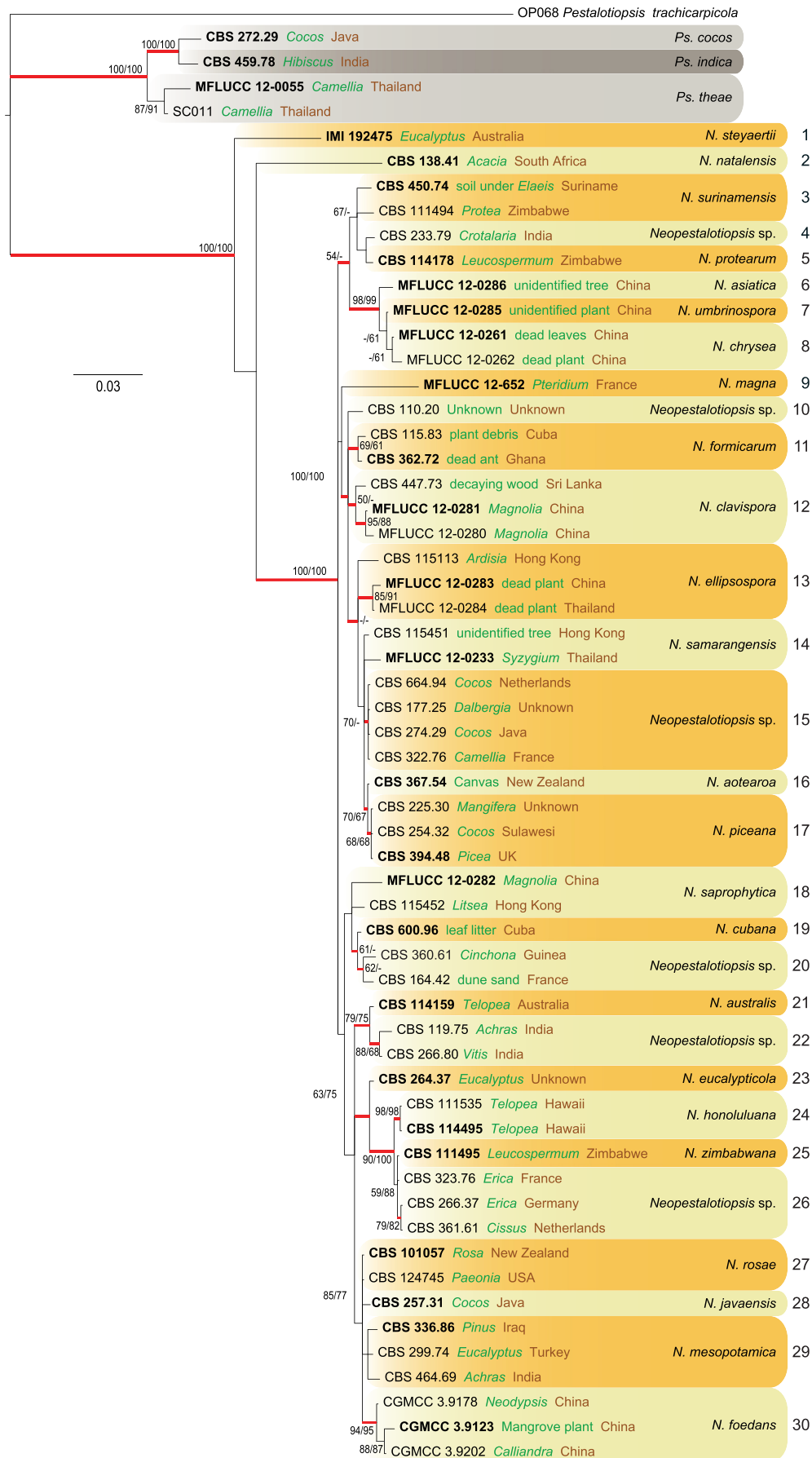
### Phylogeny

The LSU alignment was used to resolve the generic placement of *Pestalotiopsis* strains in the *Amphisphaeriaceae* (Fig. 3). The alignment comprised 74 strains (including the outgroup taxon *Xylaria hypoxylon*) and the manually adjusted dataset comprised 807 characters including gaps; the data partition contained 173 unique site patterns. Dirichlet base frequencies and the GTR+I+G model with inverse gamma-distributed rate were recommended by the MrModeltest analysis and used in the Bayesian analysis. The Bayesian analysis lasted 1 435 000 generations and the 50 % consensus trees and posterior probabilities were calculated from the 2 154 trees left after discarding 718 trees (the first 25 % of generations) for burn-in (Fig. 3). The 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 equally most parsimonious trees were obtained (tree length = 408 steps, CI = 0.591, RI = 0.871, RC = 0.514, HI = 0.409). The Bayesian analysis resulted in a tree with 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, each supported with high bootstrap confidence or posterior probability. Species possessing morphology similar to the type species of *Pestalotiopsis* (*P. maculans*) clustered in one clade designated as *Pestalotiopsis* s. str. Two well-supported clades clustered outside *Pestalotiopsis* s. str., for which two new genera, *Neopestalotiopsis* and *Pseudopestalotiopsis* are introduced. In all analyses, *Pseudopestalotiopsis* was always sister to *Pestalotiopsis* and clustered as a basal sister clade to *Neopestalotiopsis*. The species containing versicolourous median cells form a monophyletic clade named *Neopestalotiopsis* and appear to have evolved from the *Pseudopestalotiopsis* lineage, whose members have concolourous median cells.

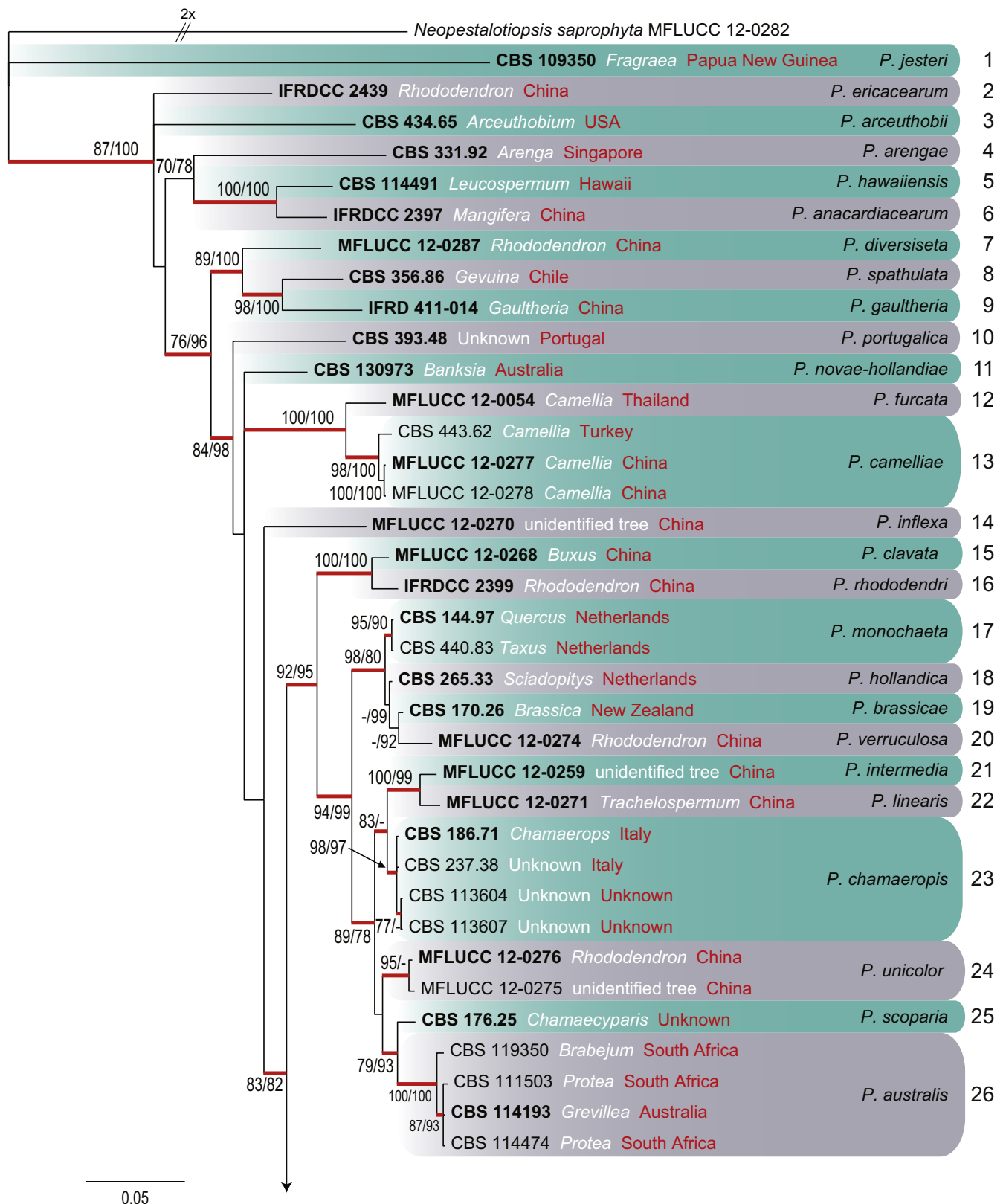
Species relationships in *Neopestalotiopsis* and *Pseudopestalotiopsis* are shown in Fig. 4. For the combined genes, BI, ML, and MP consensus trees revealed the same phylogenetic relationships between the significantly supported clades. The combined ITS, *TUB* and *TEF* alignment comprises 59 strains (including 24 ex-type / ex-epitype strains for species of *Neopestalotiopsis*, three ex-type / ex-epitype strains for species of *Pseudopestalotiopsis*, and *Pestalotiopsis trachicarpicola* as the outgroup taxon) and 1 418 characters including gaps with 66, 145 and 180 unique site patterns for ITS, *TUB* and *TEF*, respectively. Suitable models were selected using models of nucleotide substitution for each gene, as determined using MrModeltest. The GTR+I model with a proportion of invariable sites for ITS and the HKY+G model with gamma-distributed rate model for *TUB* and the GTR+I+G model with inverse gamma rate were selected for *TEF* and included for each gene partition. The Bayesian analysis lasted 2 585 000 generations and the 50 % consensus trees and posterior probabilities were calculated from the 3 880 trees left after discarding 1 293 trees (the first 25 % of generations) for burn-in (Fig. 4). Among these 1 418 characters (ITS = 491, *TUB* = 442 and *TEF* = 485), 990 were constant, 172 variable characters parsimony uninformative and 256 characters parsimony-informative. The parsimony analysis resulted in 108 equally most parsimonious trees (tree length = 805 steps,



**Fig. 3.** Consensus phylogramme (50 % majority rule) of 2154 trees resulting from a Bayesian analysis of the LSU sequence alignment of *Neopestalotiopsis*, *Pestalotiopsis*, *Pseudopestalotiopsis* and other genera in family *Amphisphaeriaceae*. Genera are indicated in coloured blocks and red-thickened lines indicate Bayesian posterior probabilities (PP) above 95 %. RAxML bootstrap support values (MLB) and maximum parsimony bootstrap support values (MPB) are given at the nodes (MLB/MPB). The scale bar represents the expected number of changes per site. The tree was rooted to *Xylaria hypoxylon* (GenBank AF132333).



**Fig. 4.** Consensus phylogramme (50 % majority rule) of 3880 trees resulting from a Bayesian analysis of the combined (ITS+TUB+TEF) alignment of the analysed *Neopestalotiopsis* and *Pseudopestalotiopsis* sequences. *Pseudopestalotiopsis* is indicated in grey shades and *Neopestalotiopsis* clades are indicated in yellow and orange coloured blocks. Clades are numbered to the right of the blocks (1–30). Red-thickened lines indicate Bayesian posterior probabilities (PP) above 95 %. RAxML bootstrap support values (MLB) and maximum parsimony bootstrap supports (MPB) are given at the nodes (MLB/MPB). Strain accession numbers (sequences derived from ex-type are printed in bold) are followed by the isolation source (green) and country of origin (brown). The correct species name is indicated to the right of the clade. The scale bar represents the expected number of changes per site. The tree was rooted to *Pestalotiopsis trachicarpicola* (OP068).



**Fig. 5.** Consensus phylogramme (50 % majority rule) of 1120 trees resulting from a Bayesian analysis of the combined (ITS+TUB+TEF) alignment of the analysed *Pestalotiopsis* isolates. Clades are indicated in coloured blocks. Clades are numbered to the right of the boxes (1–43). Red-thickened lines indicate Bayesian posterior probabilities (PP) above 95 %. RAxML bootstrap support values (MLB) and maximum parsimony bootstrap supports (MPB) are given at the nodes (MLB/MPB). Strain accession numbers (sequences derived from ex-type are printed in bold) are followed by the isolation source (white) and country of origin (red). The correct species name is indicated to the right of the clade. The scale bar represents the expected number of changes per site. The tree is rooted to *Neopestalotiopsis saprophytica* (MFLUCC 12-0282).

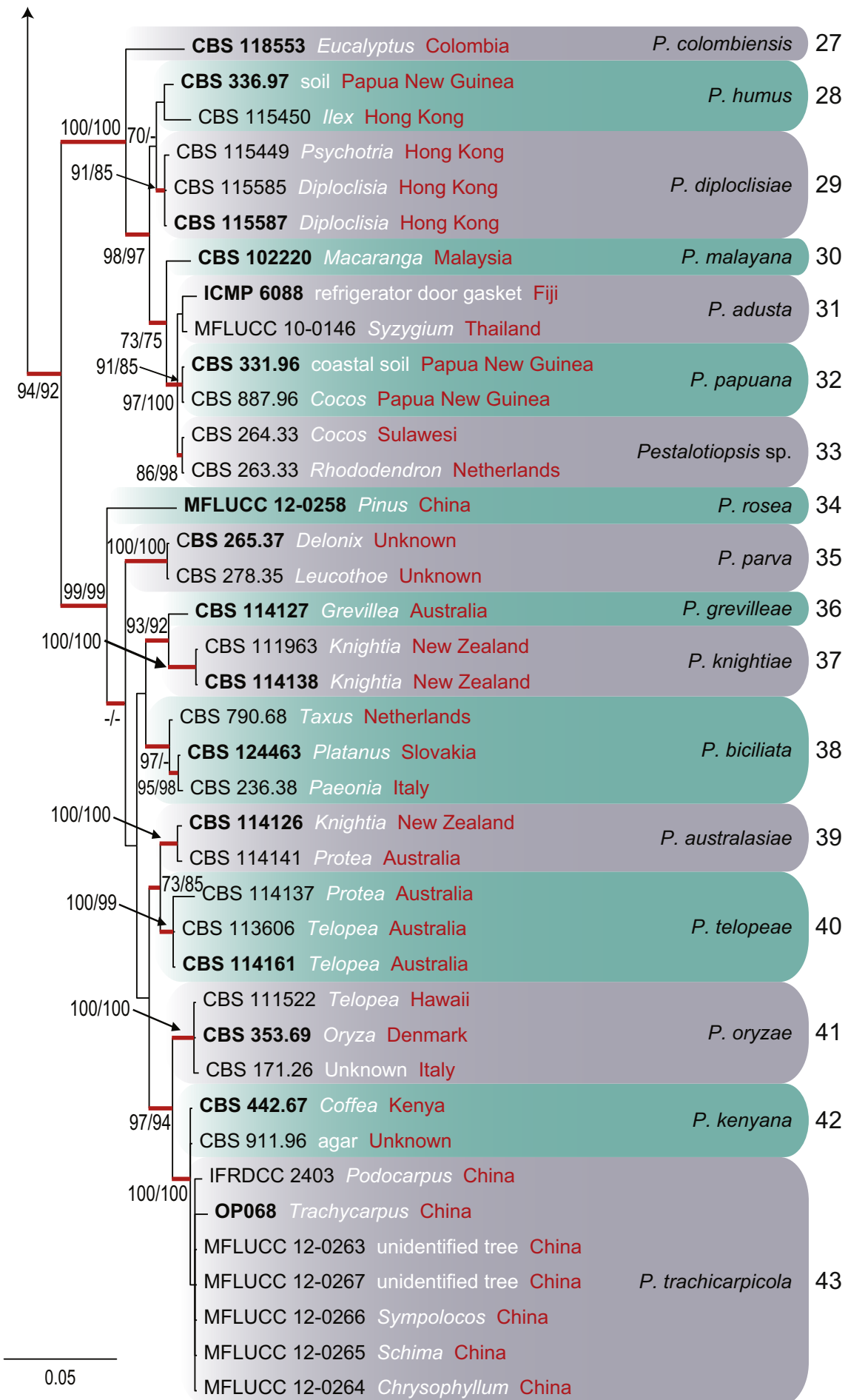


Fig. 5. (Continued).

CI = 0.688, RI = 0.810, RC = 0.557, HI = 0.312). *Neopestalotiopsis* and *Pseudopestalotiopsis* isolates clustered into two well-supported clades (BI = 1, ML = 100 and MP = 100). Furthermore, thirty clades are recognised in *Neopestalotiopsis* and discussed here (Fig. 4).

To clarify species boundaries within *Pestalotiopsis*, a combined alignment of ITS, *TUB* and *TEF* contained 96 sequences (including the outgroup *Neopestalotiopsis saprophytica*; MFLUCC 12-0282), and 1519 characters including alignment gaps with 101, 213 and 268 unique site patterns for ITS, *TUB* and *TEF*, respectively (Fig. 5). 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 were selected for *TUB* and *TEF* and set in MrBayes. The Bayesian analysis lasted 745 000 generations and the 50 % consensus trees and posterior probabilities were calculated from the 1120 trees left after discarding 373 trees (the first 25 % of generations) for burn-in (Fig. 5). Of the 1519 characters (ITS = 552, *TUB* = 463 and *TEF* = 504), 890 were constant, 250 variable characters parsimony uninformative and 379 characters parsimony-informative. A MP analysis yielded 96 equally most parsimonious trees (tree length = 1628 steps, CI = 0.596, RI = 0.808, RC = 0.482, HI = 0.404). The Bayesian analysis resulted in a tree with the same topology and terminal clades as the ML and MP trees. Forty-three clades are recognised and discussed here (Fig. 5).

## Taxonomy

Phylogenetic analyses based on the LSU alignment, together with an appraisal of the literature and morphology, resulted in the proposal of two novel genera in *Amphisphaeriaceae*. The new genera *Neopestalotiopsis* and *Pseudopestalotiopsis*, which segregate off *Pestalotiopsis*, are proposed based on the types *Neopestalotiopsis protearum* and *Pseudopestalotiopsis theae*, respectively. Descriptions of the new genera *Neopestalotiopsis* and *Pseudopestalotiopsis* are provided. Based on the results of ITS, *TUB* and *TEF* sequence analyses, 30 internal clades (clades 1–30; Fig. 4) can be distinguished in *Neopestalotiopsis*; three clades in *Pseudopestalotiopsis* (Fig. 4) and 43 clades in *Pestalotiopsis* (clades 1–43; Fig. 5). Several *Pestalotiopsis* species are transferred to *Neopestalotiopsis* and *Pseudopestalotiopsis*. Eleven new species of *Neopestalotiopsis* are described and one ex-type re-examined. Two novel species are introduced in *Pseudopestalotiopsis*. Twenty-four new species of *Pestalotiopsis* are described and illustrated here and two ex-types are re-examined. Based on the molecular phylogeny, several remaining isolates represent unnamed species; these are not treated further as most of these isolates did not sporulate, or due to lack of ecological diversity.

***Neopestalotiopsis*** Maharachch., K.D. Hyde & Crous, **gen. nov.** MycoBank MB809759.

*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, becoming percurrent to produce additional conidia at slightly higher levels. *Conidia* fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate; basal cell conic to subcylindrical, with a truncate base, hyaline or pale brown to olivaceous, thin and rugose to smooth-walled; three median cells doliform, 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.

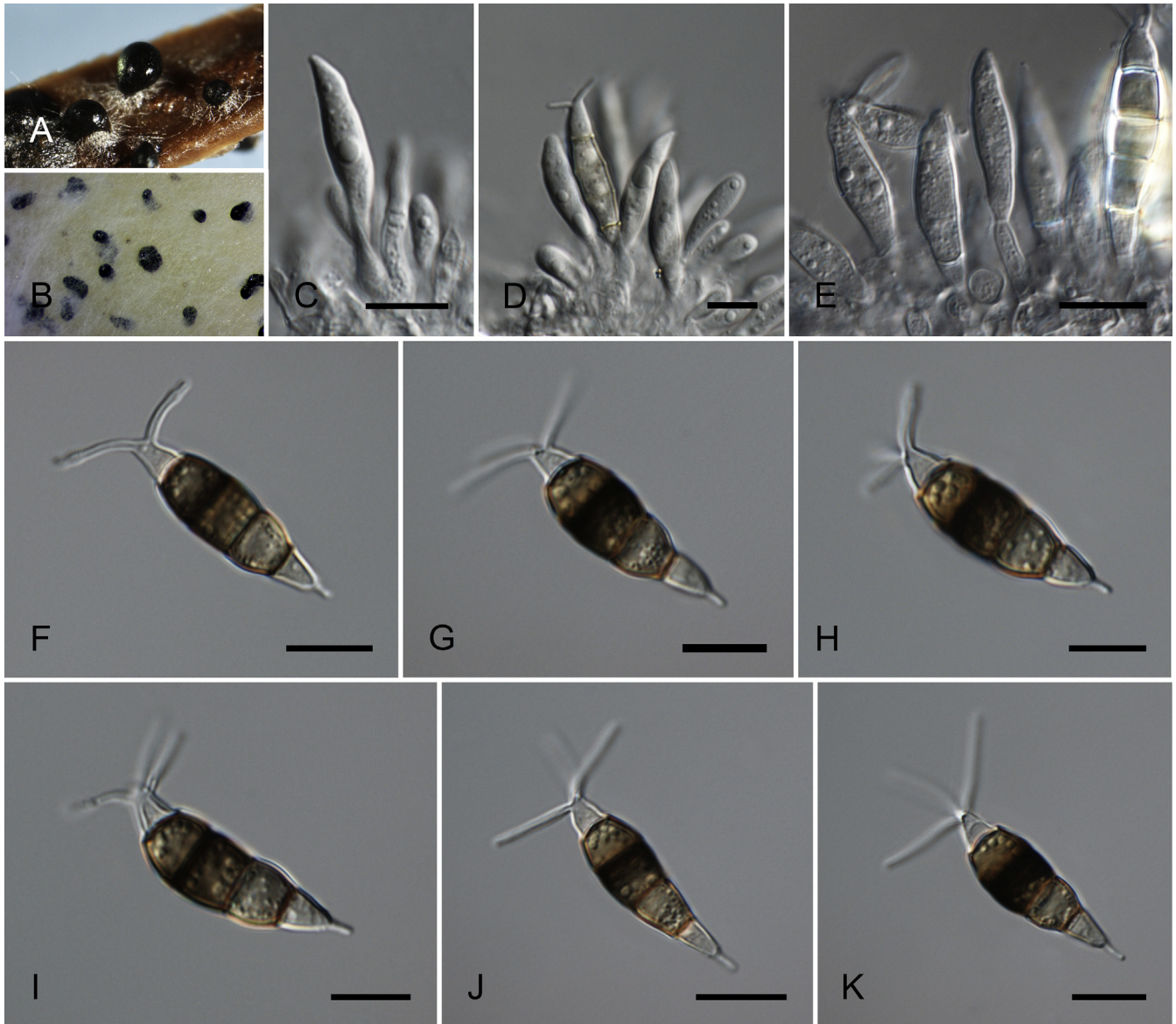
*Type species*: *Neopestalotiopsis protearum* (Crous & L. Swart) Maharachch., K.D. Hyde & Crous (see below).

*Notes*: Based on LSU sequence data (Fig. 3), *Neopestalotiopsis* clusters in *Amphisphaeriaceae* and is distinct from *Pseudopestalotiopsis* and *Pestalotiopsis*, and is best treated as a separate genus. Liu *et al.* (2010), based on the length of the ITS alignment, also revealed that species of *Pestalotiopsis* cluster in three groups. The ITS sequence lengths in groups A, B, and C (i.e. *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis*) were 480–484 bp, 489–495 bp and 536–540 bp, respectively. Morphologically *Neopestalotiopsis* can also be easily distinguished from *Pseudopestalotiopsis* and *Pestalotiopsis* by its versicolourous median cells. Furthermore, in *Neopestalotiopsis* conidiophores are indistinct and often reduced to conidiogenous cells. In the key provided by Guba (1961) and Steyaert (1949) the species in the versicolourous group divided into two subgroups: umber-olivaceous (two upper median cells umber and lowest median cell yellow-brown) and fuliginous-olivaceous (two upper median cells fuliginous, usually opaque, and lowest median cell pale brown). In his monograph Guba (1961) treated the versicolourous umber-olivaceous group, which comprised 40 species and the versicolourous fuliginous-olivaceous group, which comprised 56 species. The two groups were 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 versicolourous group based on colour intensities of the median conidial cell is not a taxonomically good character. Instead of using two groups, we propose *Neopestalotiopsis* as a new genus for the versicolourous group.

***Neopestalotiopsis aotearoa*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809760. Fig. 6.

*Etymology*: Named after the Maori name (= Aotearoa) for the country where it was collected, New Zealand.

*Conidiomata* (on PDA) pycnidial, globose to clavate, solitary or confluent, embedded 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, apex 2–5 µm diam. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate, (19.5–)21–28(–29) × (6–)6.5–8.5(–9) µm,  $\bar{x} \pm SD = 24.8 \pm 1.6 \times 7.7 \pm 0.5$  µm; basal cell conic with a truncate base, hyaline, rugose and thin-walled,



**Fig. 6.** *Neopestalotiopsis aotearoa* CBS 367.54<sup>T</sup>. A. Conidiomata sporulating on PNA (pine needle agar). B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

4–6.5  $\mu$ m long; three median cells doliiform, (13–)14–18(–18.5)  $\mu$ m long,  $\bar{x} \pm SD = 15.9 \pm 1.1$   $\mu$ m, wall verruculose, versicoloured, septa darker than the rest of the cell (second cell from the base pale brown, 4–6  $\mu$ m long; third cell honey-brown, 3.5–7  $\mu$ m long; fourth cell brown, 4–6.5  $\mu$ m long); apical cell 3.5–5.5  $\mu$ m 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)  $\mu$ m long,  $\bar{x} \pm SD = 8.1 \pm 1.2$   $\mu$ m; basal appendage single, tubular, unbranched, centric, 1.5–4  $\mu$ m long.

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

**Habitat:** Saprobe on canvas.

**Known distribution:** New Zealand.

**Material examined:** New Zealand, from canvas, Sep. 1954, G.C. Wade (CBS H-15765, **holotype**, ex-type culture CBS 367.54 = ATCC 11763 = QM 381).

**Notes:** *Neopestalotiopsis aotearoa* (clade 16; Fig. 4) is described from a canvas in New Zealand. In the phylogenetic analyses, *N. aotearoa* proved to be sister to *N. piceana* (clade 17; Fig. 4), but the two species are morphologically easily distinguishable. *Neopestalotiopsis piceana* is distinct from *N. aotearoa* by its clavate conidia, longer basal, and apical appendages.

***Neopestalotiopsis asiatica*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809761.

**Basionym:** *Pestalotiopsis asiatica* Maharachch. & K.D. Hyde, Fungal Divers. 56: 104. 2012.

**Material examined:** China, Hunan Province, Yizhang County, Mangshan, from living leaves of unidentified tree, 12 Apr. 2002, W.P. Wu (HMAS047638, **holotype**; MFLU 12-0422, **isotype**, ex-type culture NN0476380 = MFLUCC 12-0286).

**Note:** This species (clade 6; Fig. 4) was treated in detail by Maharachchikumbura et al. (2012).



***Neopestalotiopsis australis*** Maharachch., K.D. Hyde & Crous, *sp. nov.* MycoBank MB809762. Fig. 7.

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

*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, apex 1–2 µm diam. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate, (19–) 21–27(–28) × (7–)7.5–9(–9.5) µm,  $\bar{x} \pm SD = 24.6 \pm 1.8 \times 8 \pm 0.4$  µm; basal cell conic with a truncate base, hyaline, rugose and thin-walled, 3.5–5.5 µm long; three median cells doliiiform, (13–)14–18(–18.5) µm long,  $\bar{x} \pm SD = 16.1 \pm 1$  µm, 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, rugose and thin-walled; with 3–4 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous, (19–) 21–32(–34) µm long,  $\bar{x} \pm SD = 26.6 \pm 3$  µm; basal appendage single, tubular, unbranched, centric, 3–7 µm long.

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

*Habitat:* On *Telopea* sp.

*Known distribution:* Australia.

*Material examined:* **Australia**, New South Wales, from *Telopea* sp., 12 Oct. 1999, P.W. Crous (CBS H-21773, **holotype**, ex-type culture CBS 114159 = STE-U 3017).

*Notes:* *Neopestalotiopsis australis* (clade 21; Fig. 4) was isolated from *Telopea* sp. in New South Wales, Australia. The conidiogenous cells and conidia of *N. australis* resemble those of the

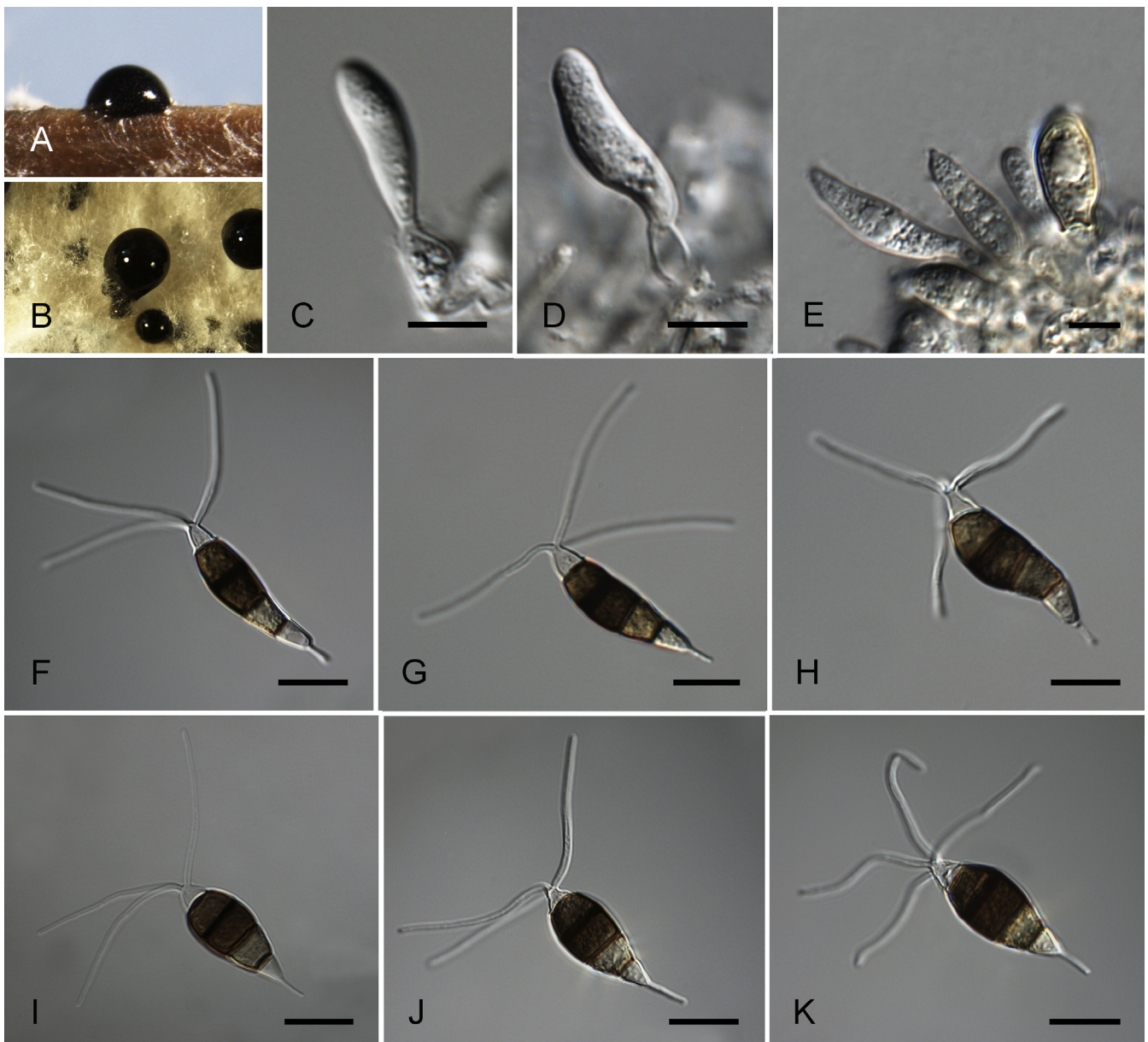


Fig. 7. *Neopestalotiopsis australis* CBS 114159<sup>T</sup>. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

two Indian isolates, CBS 266.80 and CBS 119.75 (clade 22; Fig. 4), which were isolated from *Vitis vinifera* and *Eucalyptus globulus*, respectively. Since there is geographical variation of the two Indian isolates and a slight distinction in phylogeny, they are tentatively maintained as *Neopestalotiopsis* sp. Clade 22 until additional collections and cultures become 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* have subsequently been isolated from several *Protea* and *Leucospermum* hosts (Swart et al. 1999), and intercepted at quarantine inspection points (Taylor 2001). *Neopestalotiopsis australis*, *N. honoluluana*, *N. protearum* and *N. zimbabweana* are recorded from *Proteaceae* plants. Most of these species cause leaf spots and tip dieback, and can be easily identified based on diagnostic morphology and phylogeny.

***Neopestalotiopsis chrysea*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809763.

**Basionym:** *Pestalotiopsis chrysea* Maharachch. & K.D. Hyde, Fungal Divers. 56: 107. 2012.

**Materials examined:** China, Guangxi Province, Shangsi, Shiwandashan, Wangle, dead leaves of unidentified plant, 2 Jan. 1997, W.P. Wu (HMAS042855, **holotype**; MFLU 12-0411, **isotype**, ex-type culture NN042855 = MFLUCC 12-0261); Hunan Province, Yizhang County, Mangshanon, dead plant material, 12 Apr. 2002, W.P. Wu, culture NN047037 = MFLUCC 12-0262.

**Note:** This species (clade 8; Fig. 4) was treated in detail by Maharachchikumbura et al. (2012).

***Neopestalotiopsis clavispora*** (G.F. Atk.) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809764.

**Basionym:** *Pestalotia clavispora* G.F. Atk., Bull. Cornell Univ. 3: 37. 1897.

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

**Materials examined:** China, Guangxi Province, Shiwandashan, on dead leaves of *Magnolia* sp., 28 Dec. 1997, W.P. Wu (HMAS043133 = MFLU 12-0418, **epitype**, ex-epitype culture NN043133 = MFLUCC 12-0281); Guangxi Province, Yunnan, Shiwandashan, on dead leaves of *Magnolia* sp., 28 Dec. 1997, W.P. Wu, culture NN043011 = MFLUCC 12-0280. **Sri Lanka**, decaying wood, 23 Jan. 1973, W. Gams, culture CBS 447.73. **USA**, Auburn, Alabama, on fallen leaves of *Quercus rubra*, 10 Mar. 1891, F. Atkinson (CUP-A-032389, **holotype**).

**Note:** This species (clade 12; Fig. 4) was treated in detail by Maharachchikumbura et al. (2012).

***Neopestalotiopsis cubana*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809765. Fig. 8.

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

**Conidiomata** pycnidial in culture on PDA, globose, solitary or aggregated, embedded or semi-immersed, dark brown to black, up to 250 µm diam; exuding globose, brown to black conidial masses. **Conidiophores** reduced to conidiogenous cells. **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** fusoid, ellipsoid, straight to slightly curved, somewhat constricted at septa, 4-septate, (19–) 20–25(–27) × (7.5–)8–9.5(–10) µm,  $\bar{x} \pm SD = 23.4 \pm 1.4 \times 8.8 \pm 0.4$  µm; basal cell obconic to conic with a truncate base, hyaline, rugose and thin-walled, 3–5 µm long; three median cells doliform, (13.5–)14–16.5(–17.5) µm long,  $\bar{x} \pm SD = 15.5 \pm 0.9$  µm, 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,  $\bar{x} \pm SD = 24 \pm 2$  µm; basal appendage single, tubular, unbranched, centric, 4–7 µm long.

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

**Habitat:** On leaf litter.

**Known distribution:** Cuba.

**Material examined:** Cuba, from leaf litter, Jun. 1996, R.F. Castañeda (CBS H-21772, **holotype**, ex-type culture CBS 600.96 = INIFAT C96/44-4).

**Notes:** *Neopestalotiopsis cubana* (clade 19; Fig. 4) is from leaf litter isolated in Cuba, and forms a sister clade to CBS 164.42 and CBS 360.61, which were isolated from sand dunes in France and *Cinchona* sp. in Guinea, respectively. The latter isolates are morphologically somewhat similar to *N. cubana*, even though, due to clear ecological differences we prefer to maintain them as *Neopestalotiopsis* sp. Clade 20 until we have obtained more cultures and collections. *Neopestalotiopsis cubana* is distinguished from the sister *N. saprophytica* (clade 18; Fig. 4) (22–30 × 5–6 µm) by its wider conidia.

***Neopestalotiopsis ellipsospora*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809766.

**Basionym:** *Pestalotiopsis ellipsospora* Maharachch. & K.D. Hyde, Fungal Divers. 56: 112. 2012.

**Materials examined:** China, Yunnan Province, on dead plant materials, L.D. Guo (MFLU 12-0420, **holotype**, ex-type culture MFLUCC 12-0283); Hong Kong, on fruits of *Ardisia crenata*, 1 Jan. 2002, unknown collector, culture CBS 115113 = HKUCC 9136. **Thailand**, Chiang Rai, Tool Kwan, Huay Mesak waterfall, on dead plant material, 12 Jan. 2010, S.S.N. Maharachchikumbura, culture MFLUCC 12-0284.

**Note:** This species (clade 13; Fig. 4) was treated in detail by Maharachchikumbura et al. (2012).

***Neopestalotiopsis eucalypticola*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809767. Fig. 9.

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

**Conidiomata** (on PDA) pycnidial, globose, solitary or aggregated in clusters, semi-immersed, brown to black, 100–400 µm diam;

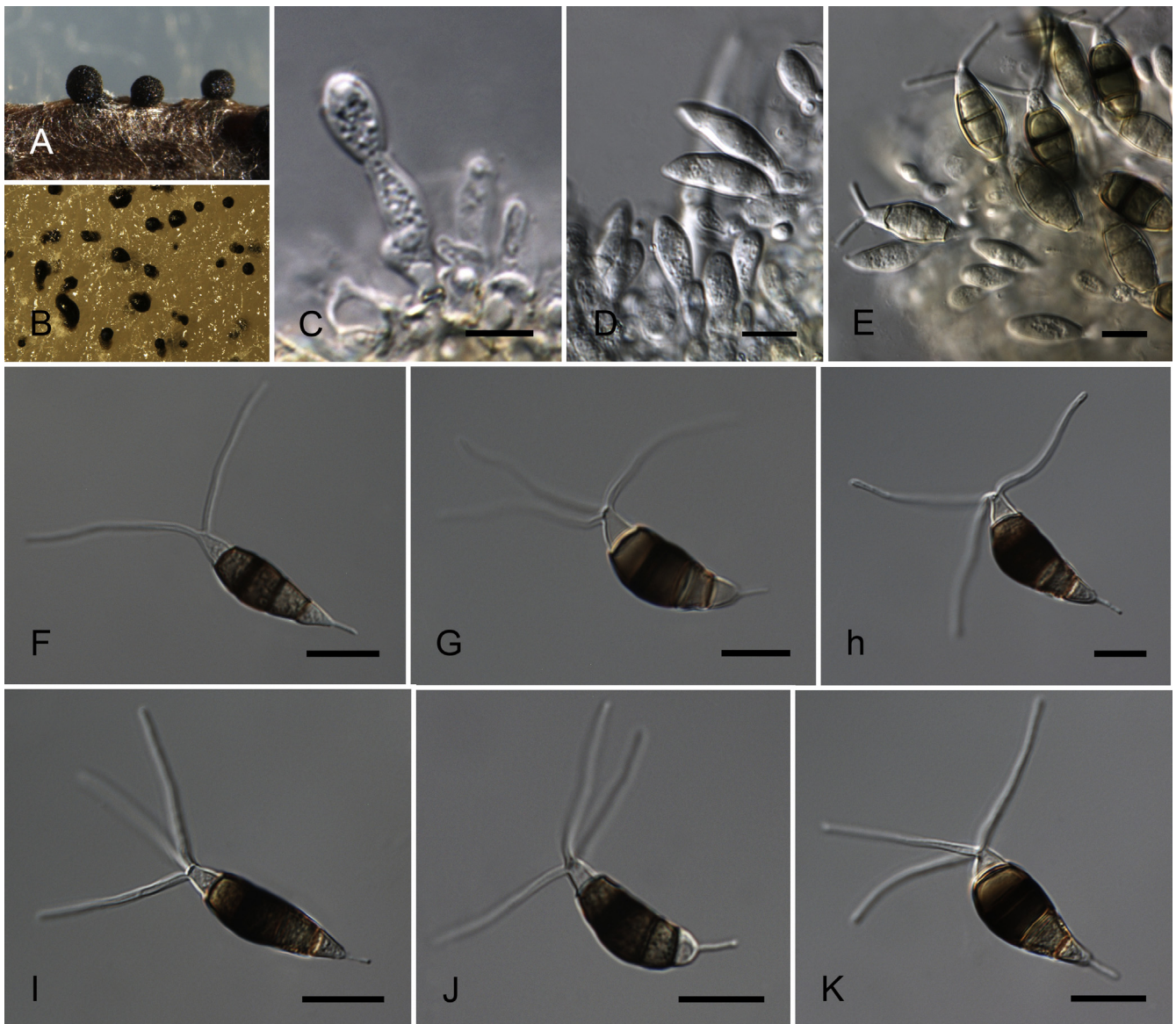


Fig. 8. *Neopestalotiopsis cubana* CBS 600.96<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

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,  $3\text{--}10 \times 2\text{--}8 \mu\text{m}$ , opening  $2\text{--}6 \mu\text{m}$  diam. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate,  $(22\text{--})23\text{--}30\text{--}(31) \times (9\text{--})7.5\text{--}9\text{--}(9.5) \mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 26.7 \pm 1.3 \times 8.3 \pm 0.4 \mu\text{m}$ ; basal cell conic to obconic with a truncate base, hyaline, rugose and thin-walled,  $5\text{--}7 \mu\text{m}$  long; three median cells doliform,  $(15.5\text{--})16\text{--}19.5\text{--}(20) \mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 17.6 \pm 1.1 \mu\text{m}$ , wall rugose, versicoloured, septa darker than the rest of the cell (second cell from the base pale brown,  $5\text{--}7 \mu\text{m}$  long; third cell darker brown,  $4.5\text{--}7.5 \mu\text{m}$  long; fourth cell darker brown,  $5\text{--}7 \mu\text{m}$  long); apical cell  $4.5\text{--}7.5 \mu\text{m}$  long, hyaline, cylindrical to subcylindrical, rugose and thin-walled; with 1–2 tubular apical appendages, arising as an extension of the apical cell, unbranched, attenuated, flexuous,  $(20\text{--})32\text{--}55\text{--}(66) \mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 43 \pm 6 \mu\text{m}$ ; basal appendage single, tubular, unbranched, centric,  $6\text{--}11 \mu\text{m}$  long.

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

**Habitat:** On *Eucalyptus globulus*.

**Known distribution:** Unknown.

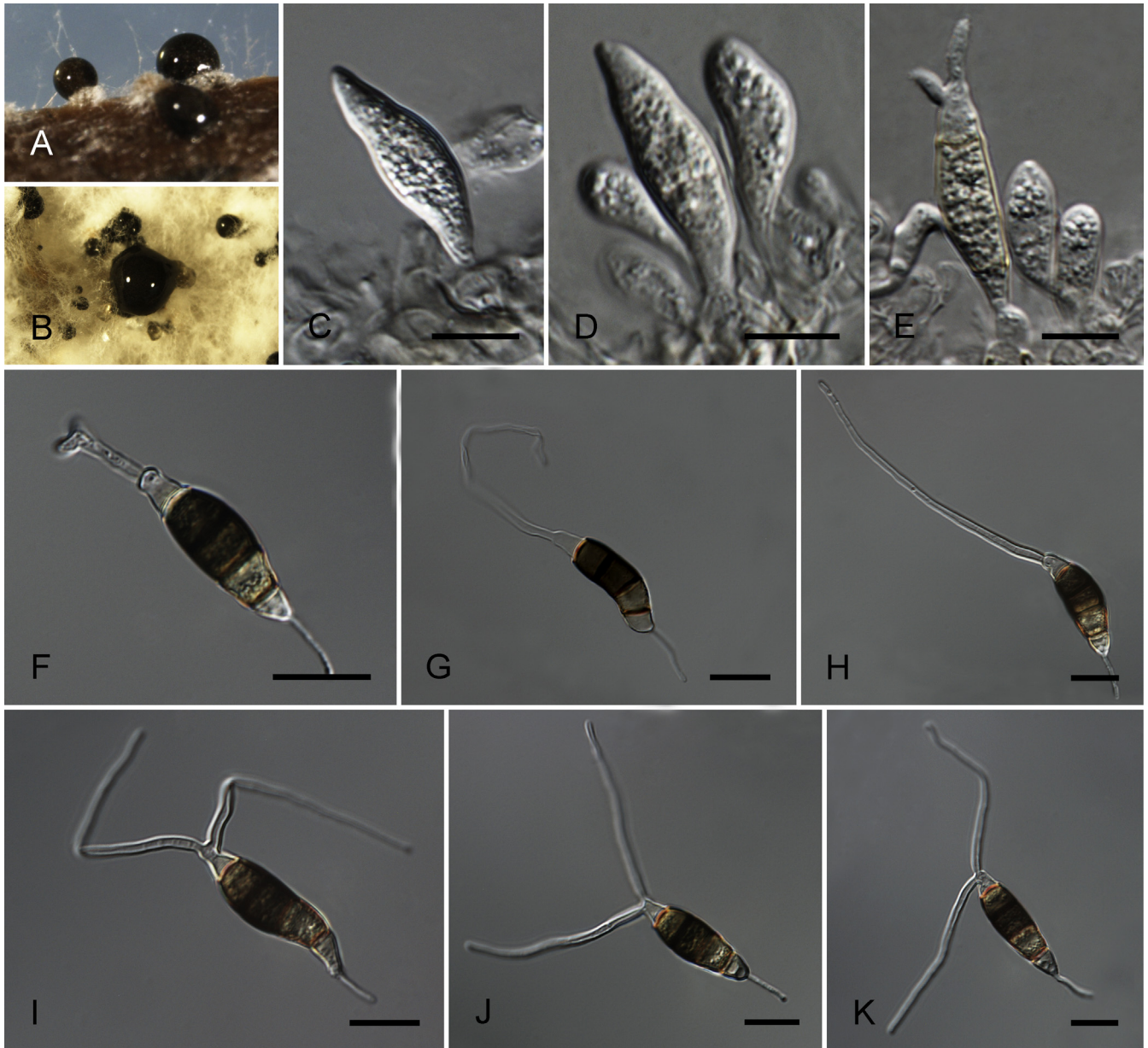
**Material examined:** Unknown country, from *Eucalyptus globulus*, Jun. 1937, H.W. Wollenweber (CBS H-15658, holotype, ex-type culture CBS 264.37 = BBA 5300).

**Notes:** *Neopestalotiopsis eucalypticola* (clade 23; Fig. 4), which was isolated from *Eucalyptus globulus*, is phylogenetically and morphologically well distinguished from all other species in the genus. The 1–2, long tubular apical appendages, which are sometimes branched, attenuated, arising as an extension of the apical cell, notably distinguish *N. eucalypticola* from other species.

***Neopestalotiopsis foedans*** (Sacc. & Ellis) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809768.

**Basionym:** *Pestalotia foedans* Sacc. & Ellis, *Michelia* 2: 575. 1882.  
 $\equiv$  *Pestalotiopsis foedans* (Sacc. & Ellis) Steyaert, *Bull. Jard. bot. État Brux.* 14: 329. 1949.

**Materials examined:** China, Xinglong, Hainan, on mangrove plant leaves, Apr. 2005, A.R. Liu (MFLU 12-0424, epitype, ex-epitype culture CGMCC 3.9123);



**Fig. 9.** *Neopestalotiopsis eucalypticola* CBS 264.37<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

Xinglong, Hainan, on leaves of *Calliandra haematocephala*, May 2004, A.R. Liu, culture CGMCC 3.9202; Xinglong, Hainan, on leaves of *Neodypsis decaryi*, May 2004, A.R. Liu, culture CGMCC 3.9178. **USA**, Newfield, New Jersey, on decaying bark of white cedar, *Thuja occidentalis*, Oct. 1880, Ellis & Harkness (BPI 0405695, **holotype**).

**Note:** This species (clade 30; Fig. 4) was treated in detail by Maharachchikumbura *et al.* (2012).

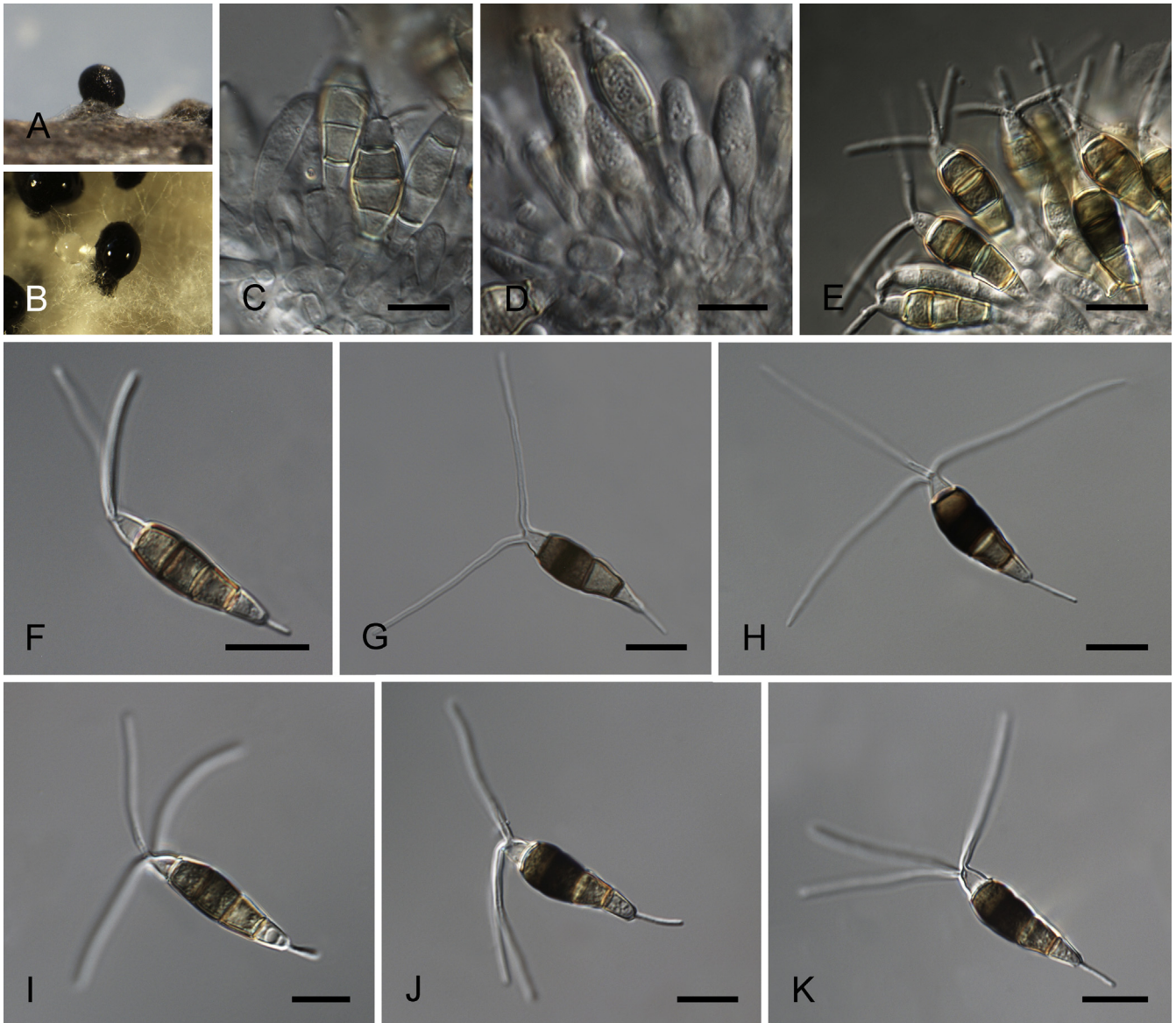
***Neopestalotiopsis formicarum*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809769. Fig. 10.

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

**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, apex 1–3 µm diam. **Conidia** ellipsoid, straight to slightly curved, 4-septate, (20–)21–28(–29) × 7.5–9.5 µm,  $\bar{x} \pm SD = 24.6 \pm 1.4 \times 8.6 \pm 0.4$  µm; somewhat constricted at septa; basal cell conic to acute with truncate base, rugose and thin-walled, 4.5–6 µm long; three median cells (14–)15–16.5(–17) µm long,  $\bar{x} \pm SD = 15.1 \pm 1$  µm, 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 dark 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,  $\bar{x} \pm SD = 27 \pm 4$  µm; basal appendage single, tubular, unbranched, centric, 4–8 µm long.

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



**Fig. 10.** *Neopestalotiopsis formicarum* CBS 362.72<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

**Habitat:** On dead ants and plant debris.

**Known distribution:** Cuba and Ghana.

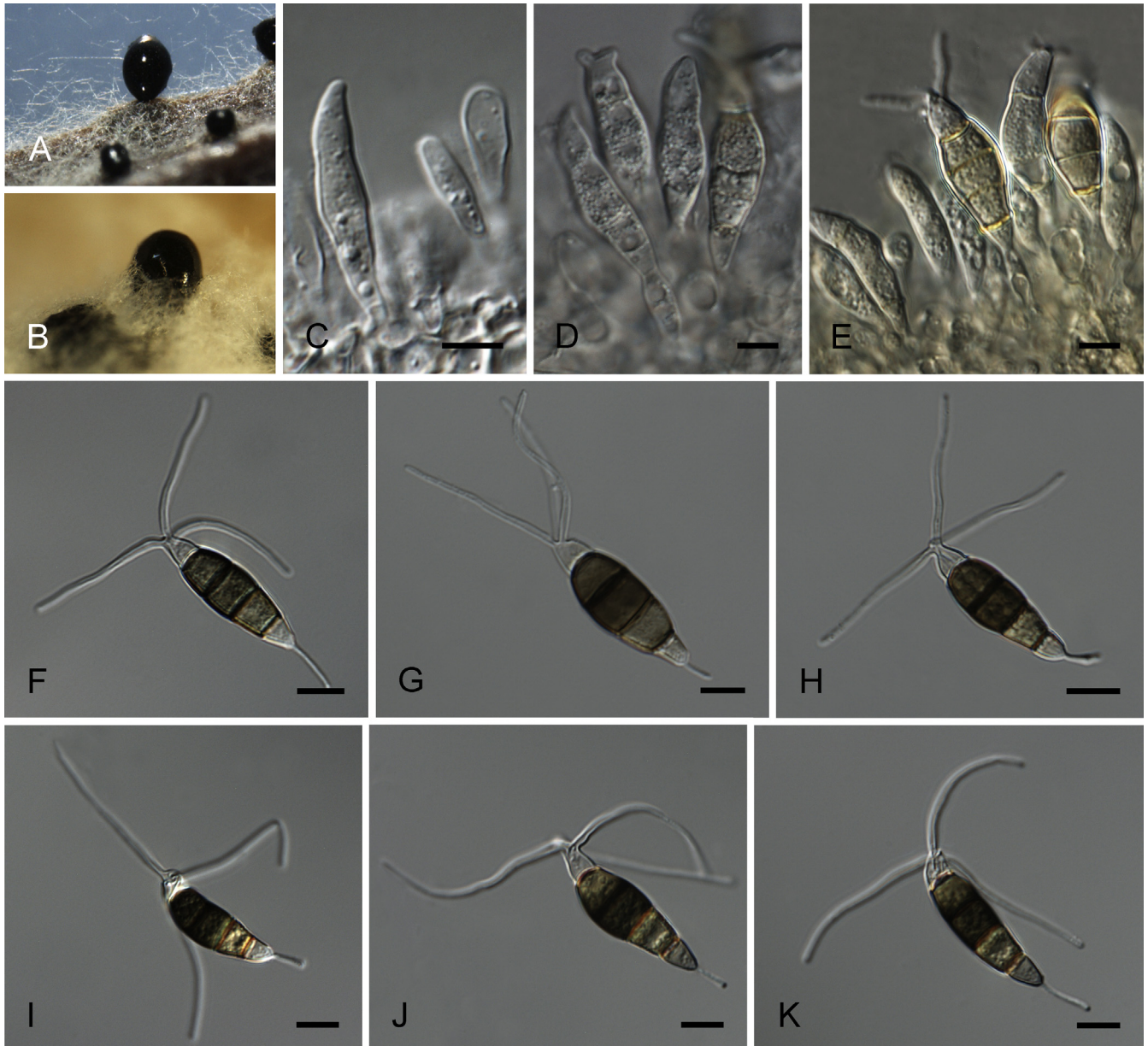
**Materials examined:** **Cuba**, from plant debris, 1982, sent to CBS for ident. by G. Arnold (via W. Gams), CBS H-15752, culture CBS 115.83. **Ghana**, from dead ant (*Formicidae*), Nov. 1971, H.C. Evans (CBS H-15661, **holotype**, ex-type culture CBS 362.72).

**Notes:** *Neopestalotiopsis formicarum* (clade 11; Fig. 4) is a saprobic species collected from dead ants in Ghana and plant debris from Cuba. This species is a sister taxon to *N. clavispora* and *Neopestalotiopsis* sp. Clade 10 (clades 12 and clade 10, respectively; Fig. 4). It differs from *N. clavispora* in having larger conidia and longer apical appendages.

***Neopestalotiopsis honoluluana*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809770. Fig. 11.

**Etymology:** Named after the city where it was collected, Honolulu in Hawaii.

*Conidiomata* pycnidial in culture on PDA, globose to clavate, solitary or aggregated in clusters, semi-immersed, brown to black, 100–400  $\mu$ m 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  $\times$  2–6  $\mu$ m, opening 1–3  $\mu$ m diam. *Conidia* ellipsoid, straight to slightly curved, somewhat constricted at septa, 4-septate, (21–)24–34(–35)  $\times$  (7–)7.5–9.5(–10)  $\mu$ m,  $\bar{x} \pm SD = 28 \pm 2.3 \times 8.3 \pm 0.6$   $\mu$ m, basal cell obconic with truncate base, rugose and thin-walled, 4.5–7  $\mu$ m long; three median cells (14.5–)15–20(–21)  $\mu$ m long,  $\bar{x} \pm SD = 17.3 \pm 1.6$   $\mu$ m, doliform, rugose, versicoloured, brown to olivaceous (second cell from base pale brown, 4.5–7  $\mu$ m long; third cell darker brown, 4–6.5  $\mu$ m long; fourth cell brown, 5.5–7.5  $\mu$ m long); apical cell subcylindrical, hyaline, thin- and smooth-walled, 4–7.5  $\mu$ m long; with 3 tubular apical appendages, arising from the apical crest, flexuous, unbranched, (22–)23–40(–47)  $\mu$ m long,  $\bar{x} \pm SD = 32 \pm 6.0$   $\mu$ m; basal appendage single, unbranched, centric, 2.5–10  $\mu$ m long.



**Fig. 11.** *Neopestalotiopsis honoluluana* CBS 114495<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

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

**Habitat:** On *Telopea* sp.

**Known distribution:** USA (Hawaii).

**Materials examined:** USA, Hawaii, Honolulu, from *Telopea* sp., 8 Dec. 1998, P.W. Crous & M.E. Palm (CBS H-21771, **holotype**, ex-type culture CBS 114495 = STE-U 2076); Waimea, *Telopea* sp., 8 Dec. 1998, P.W. Crous & M.E. Palm, culture CBS 111535 = STE-U 2078.

**Notes:** *Neopestalotiopsis honoluluana* (clade 24; Fig. 4) is confined to *Telopea* sp. in Hawaii, and is a sister taxon to *N. eucalypticola* and *N. zimbabwana*. *Neopestalotiopsis eucalypticola* differs from *N. honoluluana* in its longer and fewer apical appendages. The conidia of *N. zimbabwana* are smaller and apical appendages are shorter than those in *N. honoluluana*.

*Neopestalotiopsis australis* was isolated from the same host genus *Telopea*, in Australia. Morphologically, however, conidia of *N. australis* are smaller and apical appendages are somewhat shorter.

***Neopestalotiopsis javaensis*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809771. Fig. 12.

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

**Conidiomata** pycnidial in culture on PDA, globose to clavate, solitary, semi-immersed, dark brown to black, up to 250  $\mu$ m diam; exuding dark brown to black conidial masses. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** discrete, ampulliform to lageniform, hyaline, rugose-walled, proliferating 2–3 times percurrently, 5–25  $\times$  3–10  $\mu$ m, apex 2–4  $\mu$ m diam. **Conidia** fusoid, ellipsoid, straight to slightly curved, 4-septate, (24–)25–30(–31)  $\times$  (6.5–)7–8.5(–9)  $\mu$ m,  $\bar{x} \pm SD = 27.3 \pm 1.6 \times 7.6 \pm 0.3 \mu$ m; basal cell conic to obconic with a truncate base,

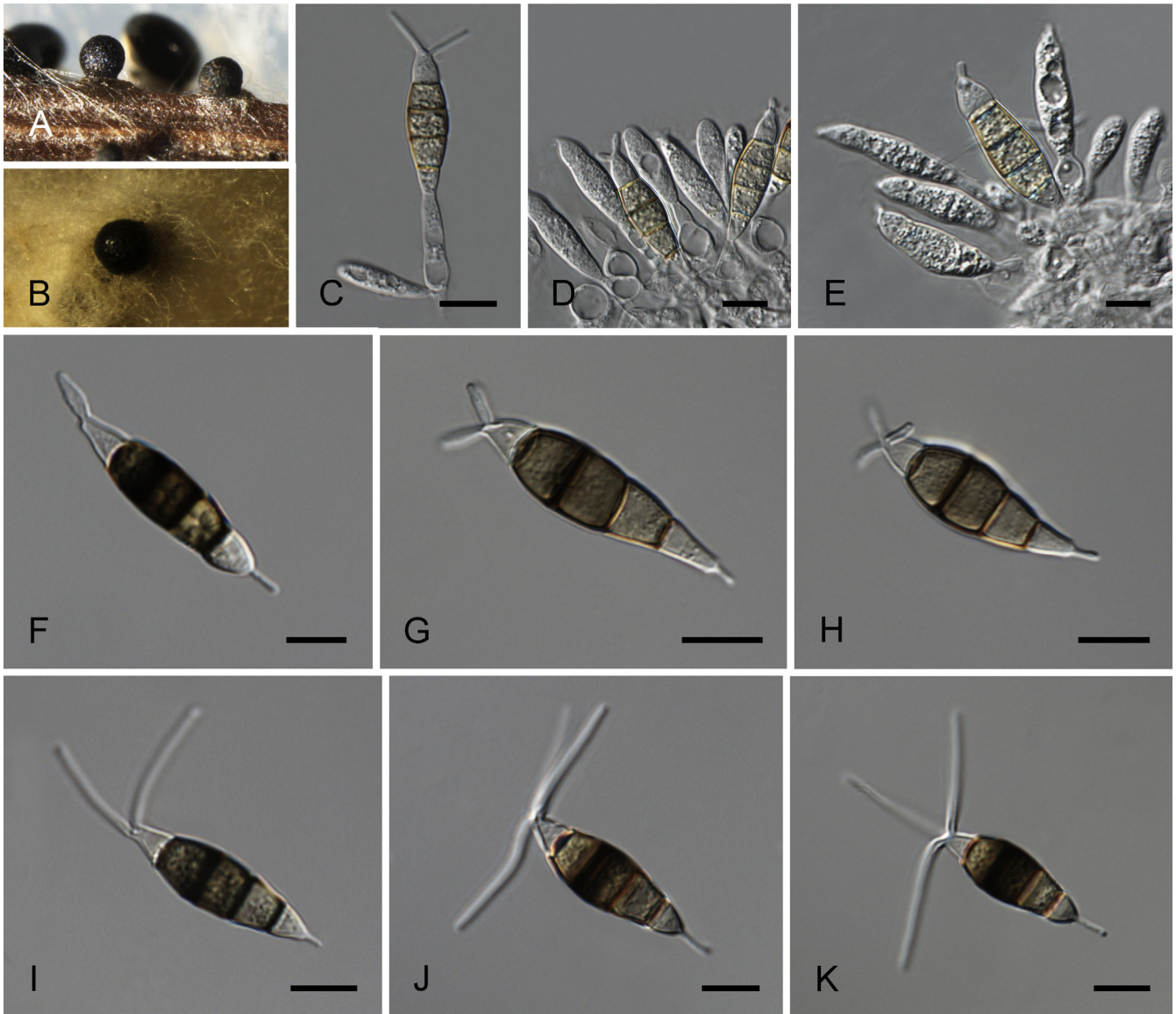


Fig. 12. *Neopestalotiopsis javaensis* CBS 257.31<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidioma on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

hyaline, rugose and thin-walled, 4.5–6.5 µm long; three median cells doliiform, (14.5–)15–18.5(–19) µm long,  $\bar{x} \pm \text{SD} = 17.1 \pm 1.2$  µm, 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,  $\bar{x} \pm \text{SD} = 5.7 \pm 3$  µm; basal appendage single, tubular, unbranched, centric, 2–4 µm long.

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

**Habitat:** On leaves of *Cocos nucifera*.

**Known distribution:** Java.

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

**Notes:** *Neopestalotiopsis javaensis* (clade 28; Fig. 4) was isolated from leaves of coconut in Java. It forms a separate cluster in the DNA phylogeny, as sister to a species assemblage including *N. foedans*, *N. mesopotamica* and *N. rosae*. *Nestalotiopsis javaensis* has relatively larger conidial dimensions when compared with *N. foedans* (19–23.5 × 5.5–7 µm) (Maharachchikumbura *et al.* 2012). *Nestalotiopsis javaensis* differs from *N. mesopotamica* and *N. rosae* in having notably shorter apical appendages (see notes under *N. rosae*).

***Neopestalotiopsis magna*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809772.

**Basionym:** *Pestalotiopsis magna* Maharachch. & K.D. Hyde, Mycol. Prog. 13: 618. 2013.

**Material examined:** France, Ariège, Rimont, on decaying leaves of *Pteridium* sp., Aug. 2011, K.D. Hyde (MFLU 13-0594, **holotype**, ex-type culture MFLUCC 12-0652 = ICMP 20011).

**Note:** This species (clade 9; Fig. 4) was treated in detail by Maharachchikumbura *et al.* (2013d).

***Neopestalotiopsis mesopotamica*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809773. Fig. 13.

**Etymology:** Named after the country where the type specimen was collected, Iraq, hence Mesopotamia.

**Conidiomata** (on PDA) pycnidial, globose or clavate, aggregated or confluent, embedded 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, collarete present and not flared, with prominent periclinal thickening. **Conidia** fusoid, ellipsoid, straight to slightly curved, 4-septate, (25–)26–32(–34) × (7–)7.5–9(–9.5) µm,  $\bar{x} \pm SD = 29.6 \pm 1.1 \times 8 \pm 0.4$  µm; basal cell conic with a truncate base, hyaline, rugose and thin-walled, 6–7.5 µm long; three median cells doliform, (17–)17.5–20(–21) µm long,  $\bar{x} \pm SD = 18.5 \pm 1.2$  µm, 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

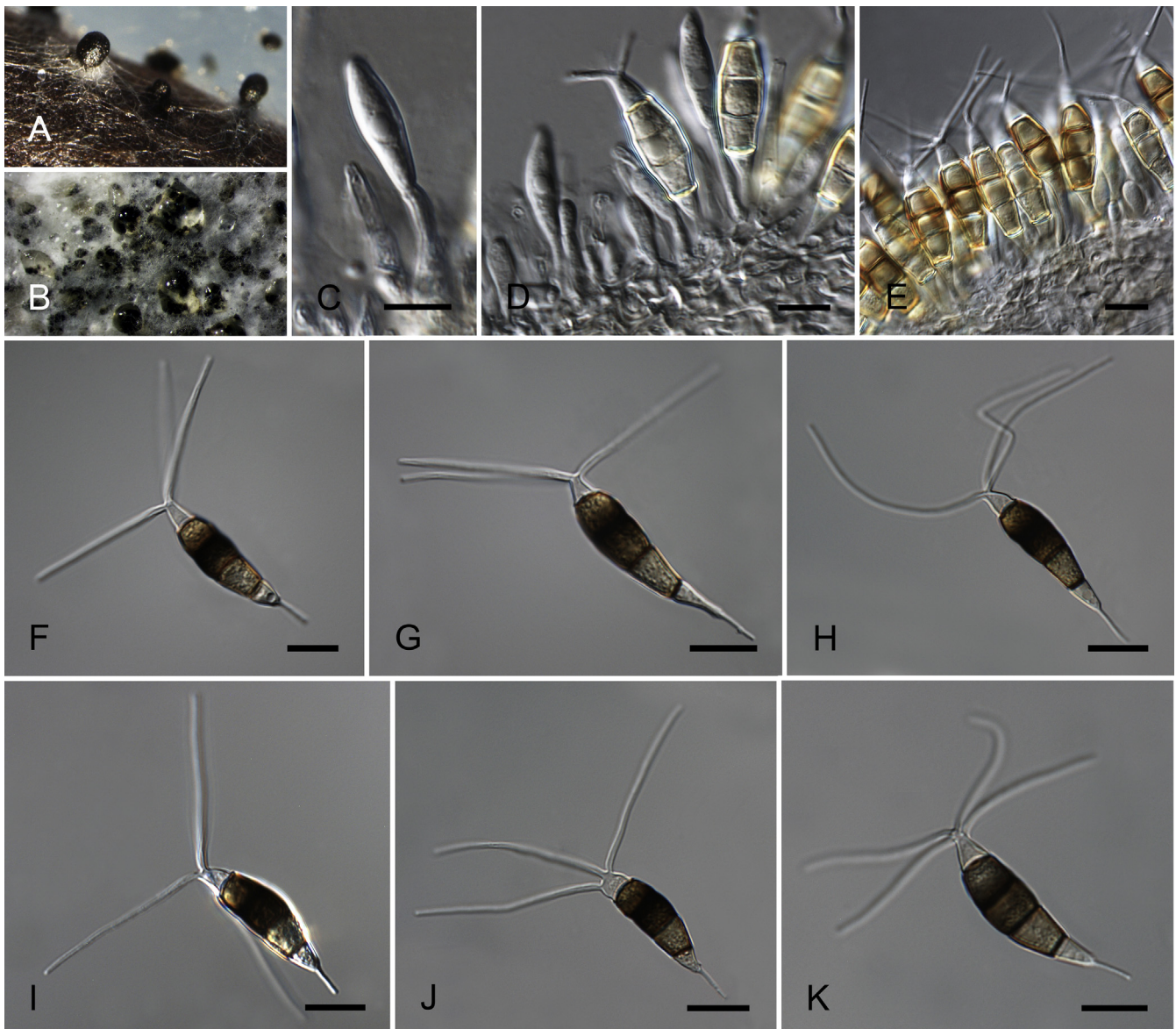
cell honey brown, 6.5–7.5 µm long); apical cell 4.5–6 µm long, hyaline, cylindrical to subcylindrical, thin- and smooth-walled; with 3–4 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (25–)28–38(–41) µm long,  $\bar{x} \pm SD = 33.3 \pm 3.2$  µm; basal appendage single, tubular, unbranched, centric, 4–6.5 µm long.

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

**Habitat:** On *Achras sapota*, *Eucalyptus* sp. and *Pinus brutia*.

**Known distribution:** India, Iraq and Turkey.

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



**Fig. 13.** *Neopestalotiopsis mesopotamica* CBS 336.86<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.



Notes: *Neopestalotiopsis mesopotamica* (clade 29; Fig. 4) forms a sister group to *N. javaensis* and *N. rosae*, and deviates in having larger conidia and longer apical appendages (see notes under *N. rosae*).

***Neopestalotiopsis natalensis*** (J.F.H. Beyma) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809774. Fig. 14.

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

=*Pestalotiopsis natalensis* (J.F.H. Beyma) Steyaert, *Bull. Jard. bot. État Brux.* 19: 344. 1949.

**Conidiomata** (on PDA) pycnidial, globose, solitary or aggregated, immersed or semi-immersed, dark brown, 50–150 µm diam. ***α*-conidiophores** indistinct, often reduced to conidiogenous cells. ***α*-conidiogenous cells** discrete, hyaline, rugose, simple, ampuliform, sometimes slightly wide at the base, truncate at apex, proliferating once or twice, 4–10 × 3–9 µm. ***α*-conidia** fusoid,

ellipsoid, straight to slightly curved, 4-septate, (21–) 23–28(–29) × (7.5–)8–10(–10.5) µm,  $\bar{x} \pm SD = 25.0 \pm 1.6 \times 9 \pm 0.4$  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,  $\bar{x} \pm SD = 17.5 \pm 0.8$  µm, concolourous 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,  $\bar{x} \pm SD = 25 \pm 4$  µm; lacking basal appendages, when present unbranched, centric, 2–8 µm long. ***β*-conidiophores** 1–2 septate, subcylindrical, hyaline, smooth, up to 12 µm long or often reduced to conidiogenous cells. ***β*-conidiogenous cells** discrete, hyaline, smooth, cylindrical, terminated in an apex with 1–2 loci which gave rise to *β*-conidia in a sympodial arrangement. 5–15 × 2–6 µm. ***β*-conidia** (20–) 22–29(–31) × 1–3 µm,  $\bar{x} \pm SD = 25.6 \pm 2 \times 1.9 \pm 0.2$  µm, widest in the middle, curved, hyaline, apex subobtuse, base truncate.

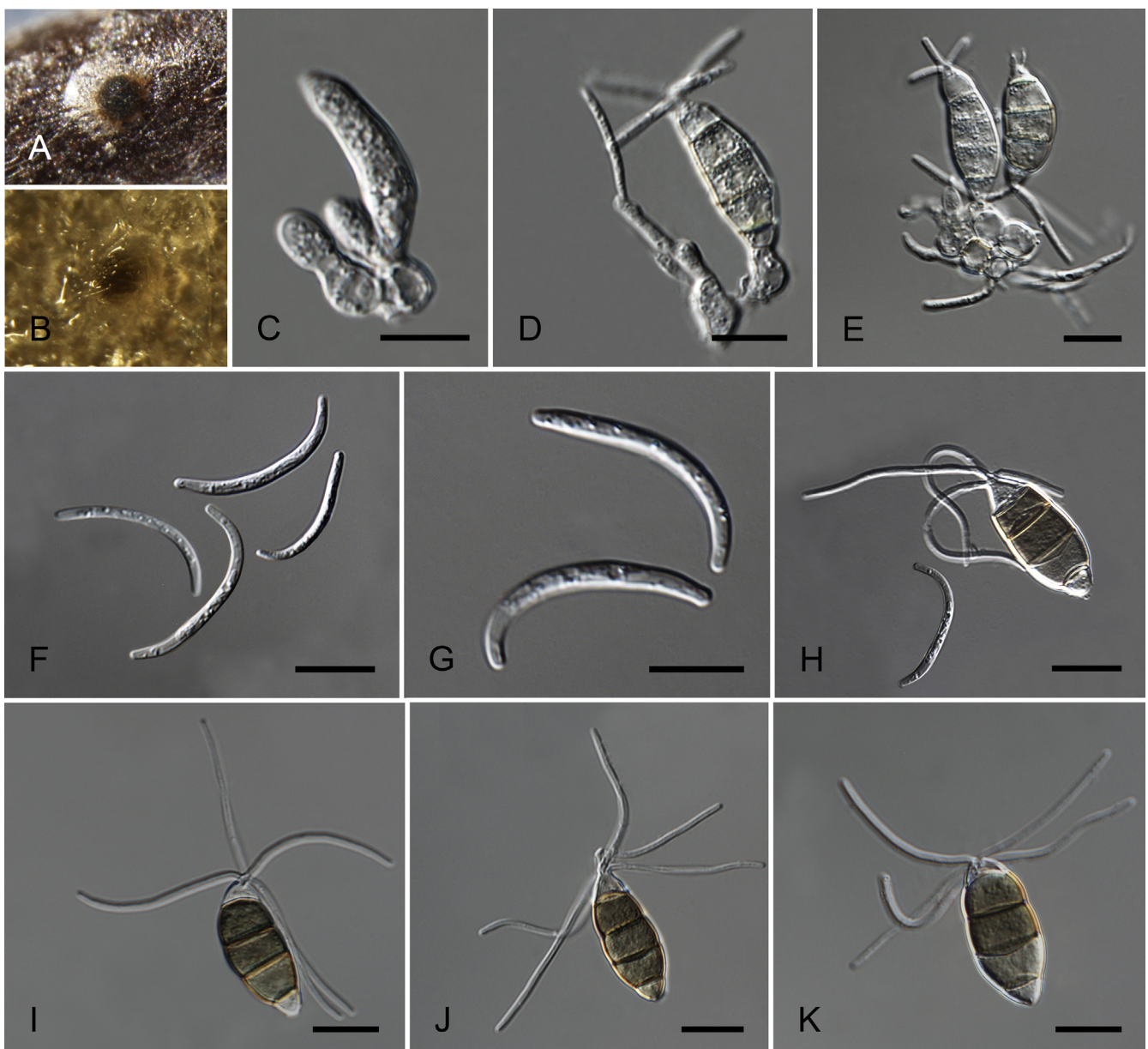


Fig. 14. *Neopestalotiopsis natalensis* CBS 138.41<sup>T</sup>. A. Conidioma sporulating on PNA. B. Conidioma on PDA. C–E. Conidiogenous cells. F–G. *β*-conidia. H. Beta and alpha conidia. I–K. *α*-conidia. Scale bars = 10 µm.

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

**Habitat:** On *Acacia mollissima*.

**Known distribution:** South Africa.

**Material examined:** South Africa, KwaZulu-Natal, from *Acacia mollissima* (black wattle), Jan. 1941, M.S.J. Ledebøer, **ex-type** culture CBS 138.41.

**Notes:** An unusual feature of *N. natalensis* (clade 2; Fig. 4) is the presence of a synanamorph in culture. Most species form  $\beta$ -conidia 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 were only observed on the original host substrate and not in culture. According to the original description of Van Beyma (1940), the conidia of *N. natalensis* are narrower ( $25\text{--}33 \times 6\text{--}9 \mu\text{m}$ ) and apical appendages are longer ( $30\text{--}40 \mu\text{m}$ ) than observed here.

***Neopestalotiopsis piceana*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809775. Fig. 15.

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

**Conidiomata** (on PDA) pycnidial, globose to clavate, solitary, semi-immersed, brown to black, 100–300  $\mu\text{m}$  diam; exuding globose, dark brown to black conidial masses. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** discrete, ampulliform to lageniform, hyaline, smooth- and thin-walled, simple, ( $4\text{--}12 \times 2\text{--}10 \mu\text{m}$ ), apex 2–5  $\mu\text{m}$  diam. **Conidia** ellipsoid to clavate, straight to slightly curved, 4-septate, ( $19\text{--}19.5\text{--}25\text{--}26 \times 7\text{--}7.5\text{--}9\text{--}9.5 \mu\text{m}$ ),  $\bar{x} \pm \text{SD} = 22.1 \pm 0.8 \times 8.1 \pm 0.6 \mu\text{m}$ ; somewhat constricted at septa; basal cell obconic with truncate base, rugose and thin-walled, 3.5–5.5  $\mu\text{m}$  long; three median cells ( $13\text{--}13.5\text{--}16\text{--}16.5 \mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 15 \pm 0.9 \mu\text{m}$ ), doliiform, verruculose, versicoloured, septa darker than the rest of the cell (second cell from base pale brown, 4–6  $\mu\text{m}$  long; third cell dark brown, 4.5–6.5  $\mu\text{m}$  long; fourth cell brown, 5–7  $\mu\text{m}$  long); apical cell obconic, hyaline, thin- and smooth-walled, 3–6  $\mu\text{m}$  long; with 3 tubular apical

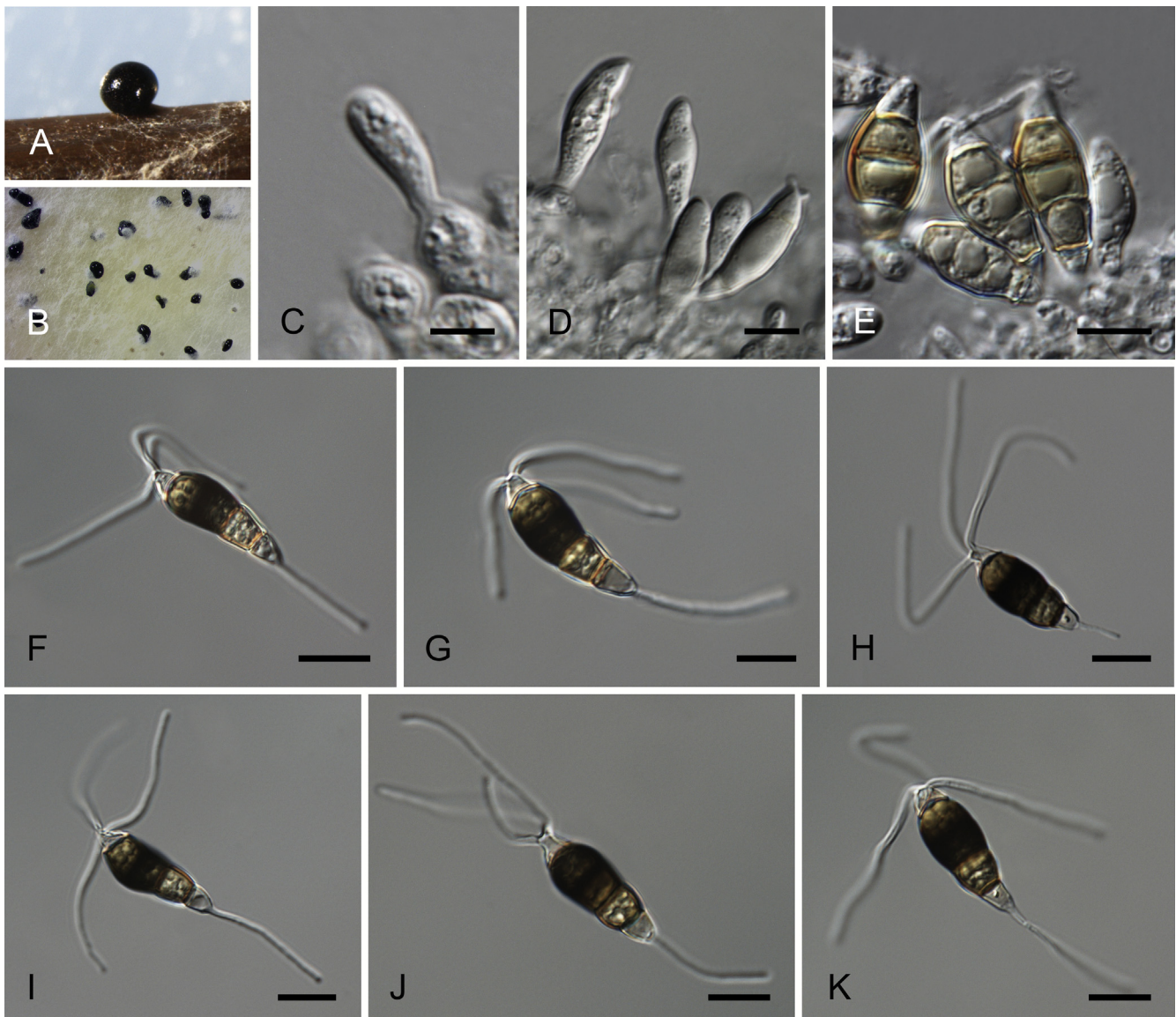


Fig. 15. *Neopestalotiopsis piceana* CBS 394.48<sup>T</sup>. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu\text{m}$ .

appendages, arising from the apical crest, flexuous, unbranched, (19–)21–31(–33)  $\mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 24.8 \pm 3 \mu\text{m}$ ; basal appendage single, tubular, unbranched, centric, 6–23  $\mu\text{m}$  long.

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

**Habitat:** On wood of *Picea* sp., *Cocos nucifera* and fruit of *Mangifera indica*.

**Known distribution:** Indonesia (Sulawesi) and UK.

**Materials examined:** **Indonesia**, Sulawesi, from *Cocos nucifera*, unknown collection date and collector, CBS H-15645, culture CBS 254.32. **UK**, from wood of *Picea* sp., Aug. 1948, S.M. Hasan (CBS H-15705, **holotype**, ex-type culture CBS 394.48). **Unknown country**, from fruit of *Mangifera indica*, Apr. 1930, Levie, CBS H-15688, culture CBS 225.30.

**Notes:** *Neopestalotiopsis piceana* (clade 17; Fig. 4) is characterised by clavate conidia with a long basal appendage. *Neopestalotiopsis piceana* is sister to *N. aotearoa* (clade 16; Fig. 4), which has been described from a canvas in New Zealand. The two species are distinguishable by *TEF* (3 bp) sequence data and not by their ITS and *TUB* sequences. The species differ by shape of their conidia and length of their apical appendages (see notes under *N. aotearoa*).

***Neopestalotiopsis protearum*** (Crous & L. Swart) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809776.

**Basionym:** *Pestalotiopsis protearum* Crous & L. Swart, *Persoonia* 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**, ex-type culture CBS 114178 = STE-U 1765).

**Note:** This species (clade 5; Fig. 4) was treated in detail by Crous *et al.* (2011).

***Neopestalotiopsis rosae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809777. Fig. 16.

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

**Conidiomata** (on PDA) pycnidial, globose, solitary, semi-immersed, dark brown to black, 100–300  $\mu\text{m}$  diam; exuding 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  $\times$  2–8  $\mu\text{m}$ . **Conidia** fusoid, ellipsoid, straight to slightly curved, 4-septate, (20–) 22–37(–29)  $\times$  (7–)7.5–9.5(–10.5)  $\mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 24.8 \pm 1.5 \times 8.5 \pm 0.6 \mu\text{m}$ ; basal cell conic to obconic with a truncate base, hyaline, rugose and thin-walled, 3.5–6  $\mu\text{m}$  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)  $\mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 16 \pm 1.1 \mu\text{m}$ , wall rugose, versicoloured, septa darker than the rest of the cell (second cell from the base pale brown, 4.5–6.5  $\mu\text{m}$  long; third cell honey brown, 5–7  $\mu\text{m}$  long; fourth cell brown, 5–7  $\mu\text{m}$  long); apical cell 3.5–5.5  $\mu\text{m}$  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)  $\mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 27 \pm 2.1 \mu\text{m}$ ; basal appendage single, tubular, unbranched, centric, 5–8  $\mu\text{m}$  long.

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

**Habitat:** On stem of *Paeonia suffruticosa* and stem lesion in *Rosa* sp.

**Known distribution:** New Zealand and USA.

**Materials examined:** **New Zealand**, from stem lesion in *Rosa* sp., Jul. 1998, J. Reeve (CBS H-21770, **holotype**, ex-type culture CBS 101057). **USA**, Connecticut, Torrington, from stem of *Paeonia suffruticosa*, 17 May 2007, R.E. Marra, culture CBS 124745.

**Notes:** *Neopestalotiopsis rosae* (clade 27; Fig. 4) was isolated from a stem lesion in *Rosa* sp. in New Zealand and stem of *Paeonia suffruticosa* in USA, and is morphologically quite distinct from other taxa in the genus. It has 3–5 tubular apical appendages, which do not arise from the apical crest; instead they arise at different regions in the upper half of the apical cell. Sequences of *N. rosae* form a sister group to *N. javaensis* (clade 28; Fig. 4) and *N. mesopotamica* (clade 29; Fig. 4), but *N. rosae* could be separated from *N. javaensis* by Bayesian analysis. However the two clades were supported in the ML and MP analyses. The two species are separable by *TEF* (5 bp) sequence data. There is only a 2-bp difference in ITS sequence between *N. javaensis* and *N. rosae*. *Neopestalotiopsis javaensis* can be differentiated morphologically from *N. rosae* by its long and thin conidia, and shorter apical appendages. The conidia of *N. rosae* are wider than those of *N. mesopotamica*, and the conidia and apical appendages are shorter.

***Neopestalotiopsis samarangensis*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809778.

**Basionym:** *Pestalotiopsis samarangensis* Maharachch. & K.D. Hyde, *Trop. Plant Pathol.* 38: 229. 2013.

**Materials examined:** **China**, Hong Kong, leaf of unidentified tree, 6 Mar. 2002, unknown collector, culture CBS 115451 = HKUCC 9095. **Thailand**, Chiang Mai Province, Chiang Mai, on fruits of *Syzygium samarangense*, 20 Jan. 2010, S.S.N. Maharachchikumbura (MFLU 12-0133, **holotype**, ex-type culture MFLUCC 12-0233); *ibid.*, 15 May 2011, S.S.N. Maharachchikumbura, MFLU 12-0134; Chiang Rai Province, Chiang Rai, 15 Sep. 2011, S.S.N. Maharachchikumbura, MFLU 12-0135.

**Note:** This species (clade 14; Fig. 4) was treated in detail by Maharachchikumbura *et al.* (2013b).

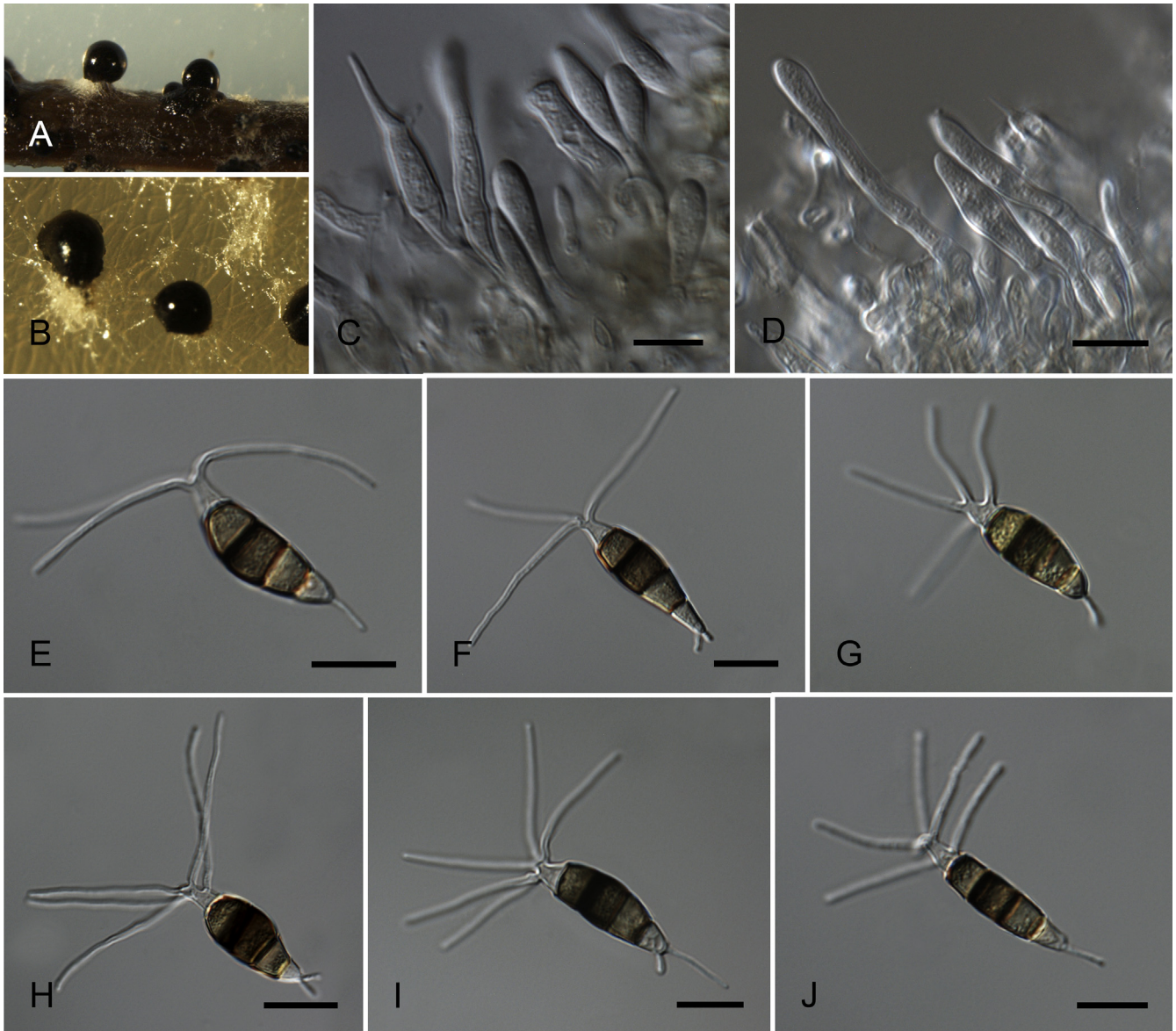


Fig. 16. *Neopestalotiopsis rosae* CBS 101057<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10  $\mu$ m.

***Neopestalotiopsis saprophytica*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809780.

*Basionym:* *Pestalotiopsis saprophyta* Maharachch. & K.D. Hyde, Fungal Divers. 56: 119. 2012.

*Materials examined:* **China**, Hong Kong, on fruits of *Litsea rotundifolia*, 19 Nov. 2001, unknown collector, culture CBS 115452 = HKUCC 8684; Yunnan Province, Kunming, Kunming Botanical Garden, on leaves of *Magnolia* sp., 19 Mar. 2002, W.P. Wu (HMAS047136, **holotype**: MFLU 12-0419, **isotype**, ex-type culture NN047136 = MFLUCC 12-0282).

*Note:* This species (clade 18; Fig. 4) was treated in detail by Maharachchikumbura et al. (2012).

#### ***Neopestalotiopsis* sp. Clade 4**

*Material examined:* **India**, from leaf *Crotalaria juncea*, Feb. 1979, M. Mathur, culture CBS 233.79.

*Notes:* Culture CBS 233.79 (clade 4; Fig. 4) represents a *Neopestalotiopsis* sp. that was isolated from a leaf of *Crotalaria*

*juncea* in India. Sequences of this taxon form a sister group to *N. protearum* (clade 5; Fig. 4). However, due to clear ecological differences, we retain this isolate as *Neopestalotiopsis* sp. until we obtain more collections and cultures for further study.

#### ***Neopestalotiopsis* sp. Clade 10**

*Material examined:* **Unknown country**, unknown host, Dec. 1920, N.A. Brown, culture CBS 110.20.

*Note:* Although phylogenetically slightly distinct (clade 10; Fig. 4), this culture proved to be sterile, and thus is not treated further.

#### ***Neopestalotiopsis* sp. Clade 15**

*Materials examined:* **France**, from twig of *Camellia* sp., Apr. 1976, J. Vegh, culture CBS 322.76. **Indonesia**, Java, *Cocos nucifera*, C.M. Doyer, culture CBS 274.29. **Netherlands**, from commercial *Cocos nucifera* imported from Africa, Jan. 1995, A. Aptroot, culture CBS 664.94. **Unknown country**, from *Dalbergia* sp., unknown collector and collection date, culture CBS 177.25.

*Notes:* Although these isolates (clade 15; Fig. 4) appear to represent an undescribed species based on phylogenetic data,

due to clear ecological differences of the isolates, we maintain this clade as *Neopestalotiopsis* sp. until more cultures and collections are obtained.

### *Neopestalotiopsis* sp. Clade 20

*Materials examined:* **France**, on dune sand, Mar. 1942, F. Moreau, culture CBS 164.42. **Guinea**, from young shoot of *Cinchona* sp. (attacked by *Phytophthora* canker), Nov. 1961, J. Chevaugon, culture CBS 360.61.

*Note:* Although phylogenetically distinct (clade 20; Fig. 4), both cultures of this species proved to be sterile, and thus are not treated further.

### *Neopestalotiopsis* sp. Clade 22

*Materials examined:* **India**, from *Achras sapota*, Feb. 1975, H.S. Sohi, culture CBS 119.75; from berries, leaves and canes of *Vitis vinifera*, Apr. 1980, H.R. Reddy, culture CBS 266.80.

*Notes:* Although phylogenetically and ecologically distinct, these two isolates (clade 22; Fig. 4) are morphologically similar to *N. australis* (clade 21; Fig. 4). Therefore, until more cultures and collections become available, we prefer to maintain this as *Neopestalotiopsis* sp. Clade 22.

### *Neopestalotiopsis* sp. Clade 26

*Materials examined:* **France**, from *Erica gracilis*, Aug. 1975, sent to CBS for ident. by J. Vegh, culture CBS 323.76. **Germany**, from *Erica* sp., unknown date, H.W. Wollenweber, culture CBS 266.37 = BBA 5087 = IMI 083708. **Netherlands**, from *Cissus* sp., unknown collector and collection date, culture CBS 361.61.

*Note:* See notes under *N. zimbabwana*.

***Neopestalotiopsis steyaertii*** (Mordue) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809779.

*Basionym:* *Pestalotiopsis steyaertii* Mordue, Trans Brit. Mycol. Soc. 85: 379. 1985.

*Material examined:* **Australia**, Australian Capital Territory, Brindabella mountains, from roots of *Eucalyptus viminalis* grown in soil, 24 Mar. 1975, G.C. Johnson (**ex-type** culture IMI 192475).

*Note:* This species (clade 1; Fig. 4) was treated in detail by Maharachchikumbura *et al.* (2013d).

***Neopestalotiopsis surinamensis*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809781. Fig. 17.

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

*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* fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate, (23–) 24–28(–29) × (7–)7.5–9(–9.5) µm,  $\bar{x} \pm SD = 27.7 \pm 1 \times 8.1$

± 0.4 µm; basal cell obconic to subcylindrical with a truncate base, hyaline, rugose and thin-walled, 5–7.5 µm long; three median cells doliform, (14.5–)15–17(–17.5) µm long,  $\bar{x} \pm SD = 16.5 \pm 0.6$  µm, 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,  $\bar{x} \pm SD = 21.6 \pm 3$  µm; basal appendage single, tubular, unbranched, centric, 5–7 µm long.

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

*Habitat:* On soil under *Elaeis guineensis* and leaves of *Protea eximia*.

*Known distribution:* Suriname and Zimbabwe.

*Materials examined:* **Suriname**, Brokobaka, from soil under *Elaeis guineensis*, Mar. 1974, J.H. van Emden (CBS H-15730, **holotype**, ex-type culture CBS 450.74). **Zimbabwe**, Karoi, Glenellen Farm, on living leaves of *Protea eximia*, 10 Mar. 1998, L. Swart, PREM 56190, culture CBS 111494 = STE-U 1779.

*Notes:* *Neopestalotiopsis surinamensis* (clade 3; Fig. 4) was isolated from soil under *Elaeis guineensis* (African oil palm) in Suriname, which is the principal source of palm oil and leaves of *Protea eximia* in Zimbabwe. Although phylogenetically closely related to *N. protearum* (clade 5; Fig. 4) (Crous *et al.* 2011), the two species can be distinguished by their ITS (4 bp) and *TEF* (9 bp) sequences, and less easily by their *TUB* (1 bp) sequences. In morphology, *N. surinamensis* differs from *N. protearum* in having wider conidia, as well as longer and fewer apical appendages.

***Neopestalotiopsis umbrinospora*** (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809782.

*Basionym:* *Pestalotiopsis umberspora* Maharachch. & K.D. Hyde, Fungal Divers. 56: 121. 2012.

*Material examined:* **China**, Guangxi Province, Shiwandashan, on dead leaves of unidentified plant, 30 Dec. 1997, W.P. Wu (HMAS042986, **holotype**; MFLU 12-0421, **isotype**, ex-type culture NN042986 = MFLUCC 12-0285).

*Note:* This species (clade 7; Fig. 4) was treated in detail by Maharachchikumbura *et al.* (2012).

***Neopestalotiopsis zimbabwana*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809783. Fig. 18.

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

*Conidiomata* (on PDA) pycnidial, globose, aggregated or scattered, semi-immersed, black, 150–400 µm diam; exuding globose, dark brown to black conidial masses. *Conidiophores*

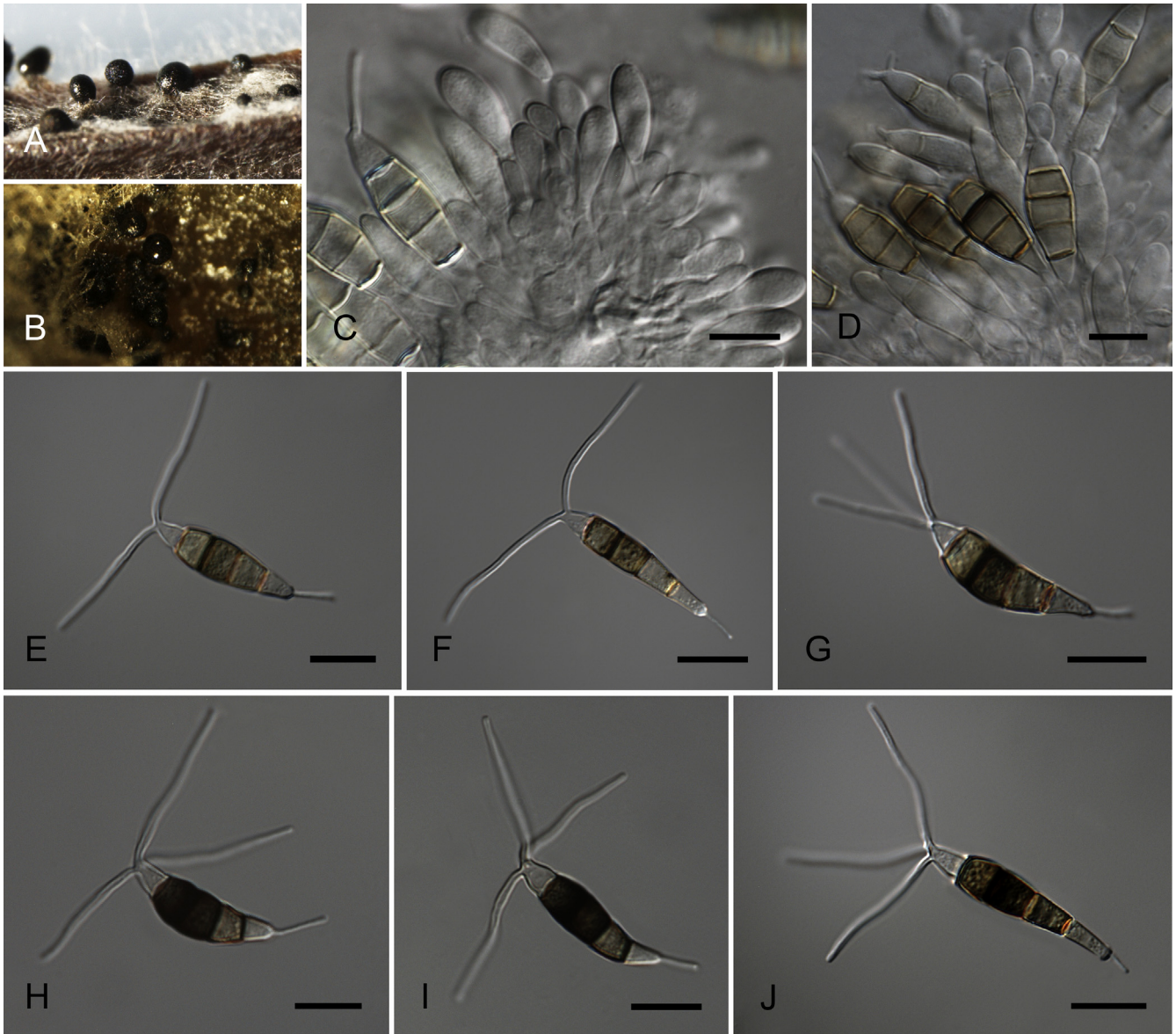


Fig. 17. *Neopestalotiopsis surinamensis* CBS 450.74<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10  $\mu$ m.

indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete, ampulliform, hyaline, smooth-walled, simple, proliferating several times percurrently, 5–15  $\times$  3–8  $\mu$ m, apex 2–5  $\mu$ m diam. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate, (22–)23–29(–30)  $\times$  (6.5–)7–8.5(–9)  $\mu$ m,  $\bar{x} \pm SD = 25.3 \pm 1.2 \times 7.7 \pm 0.3 \mu$ m; basal cell conic to obconic with a truncate base, hyaline, rugose and thin-walled, 3.5–5.5  $\mu$ m long; three median cells doliiform, (15–)15.5–17.5(–18)  $\mu$ m long,  $\bar{x} \pm SD = 16.5 \pm 0.6 \mu$ m, 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  $\mu$ m long; third cell brown to olivaceous, 4.5–6.5  $\mu$ m long; fourth cell brown to olivaceous, 5–7  $\mu$ m long); apical cell 4–6.5  $\mu$ m long, hyaline, cylindrical to subcylindrical, rugose and thin-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (18–)23–35(–41)  $\mu$ m long,  $\bar{x} \pm SD = 28.6 \pm 4 \mu$ m; basal appendage single, tubular, unbranched, centric, 3–9.5  $\mu$ m long.

*Culture characteristics*: Colonies on PDA attaining 30–45 mm diam after 7 d at 25  $^{\circ}$ C, with smooth edge, pale honey-coloured,

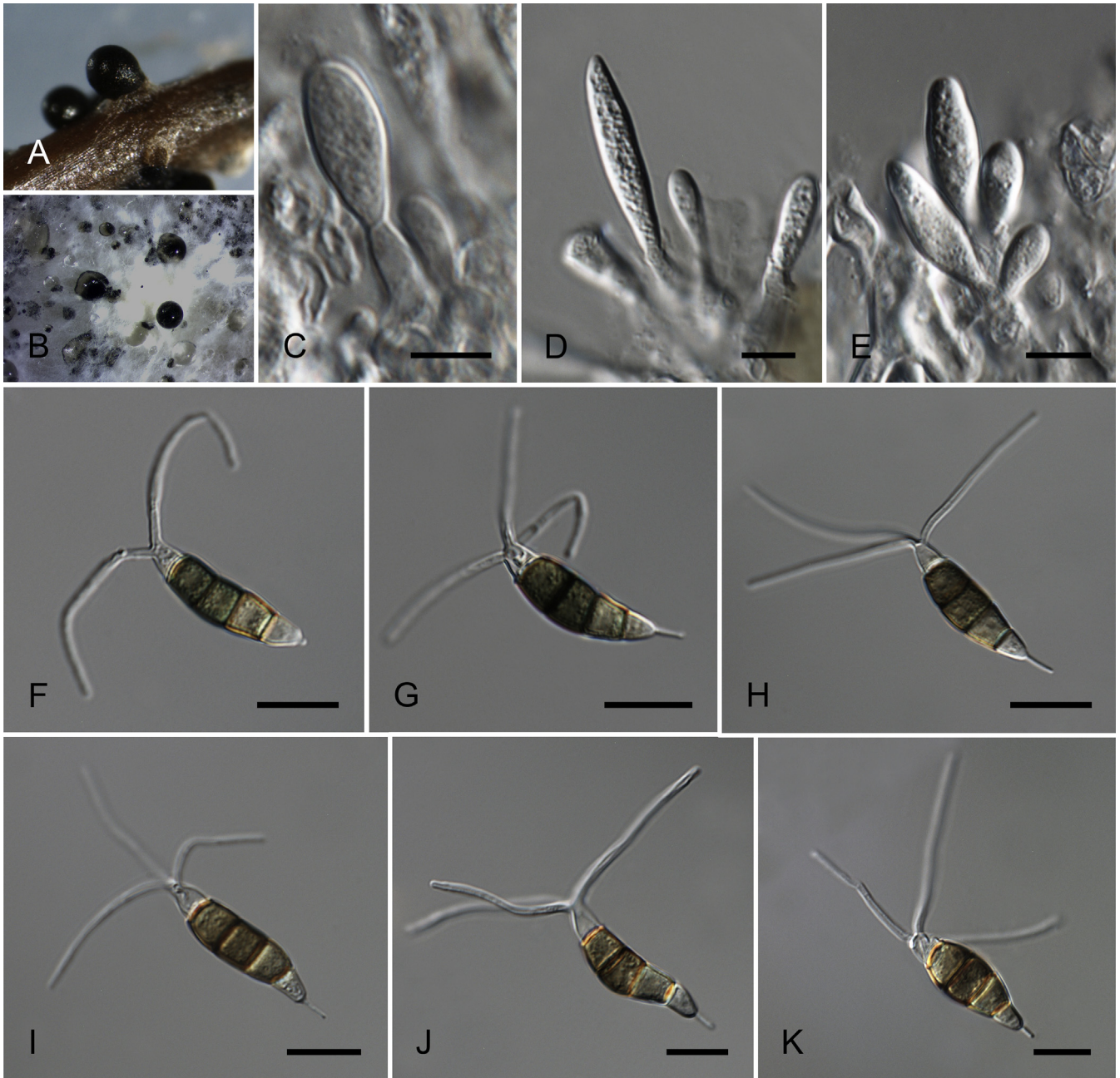
with sparse aerial mycelium on the surface with black, gregarious conidiomata; reverse similar in colour.

*Habitat*: On *Leucospermum cuneiforme*.

*Known distribution*: Zimbabwe.

*Material examined*: Zimbabwe, Banket, Mariondale Farm, on living leaves of *Leucospermum cuneiforme* cv. 'Sunbird', 9 Mar. 1998, L. Swart (CBS H-21769, **holotype**; PREM 56188, **isotype**, ex-type culture CBS 111495 = STE-U 1777).

*Notes*: *Neopestalotiopsis zimbabwana* (clade 25; Fig. 4) occurs on leaf spots of *Leucospermum cuneiforme* in Zimbabwe. In our phylogenetic analyses, *N. zimbabwana* proved to be allied to CBS 266.37, CBS 361.61 and CBS 323.76 (clade 26; Fig. 4), which were isolated from *Erica* sp. in Germany, *Cissus* sp. in Netherlands and *Erica gracilis* in France, respectively. Even though the latter isolates have overlapping morphological characters with *N. zimbabwana*, due to clear geographical differences, we maintain these isolates as *Neopestalotiopsis* sp. Clade 26 until we have obtained more collections and cultures. *Neopestalotiopsis protearum* (clade 5; Fig. 4) was also identified



**Fig. 18.** *Neopestalotiopsis zimbabwana* CBS 111495<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

as a pathogen on *Leucospermum cuneiforme* in Zimbabwe. However, *N. protearum* and *N. zimbabwana* are phylogenetically distinct.

#### ***Pestalotiopsis adusta* (Ellis & Everh.) Steyaert**

*Materials examined:* Fiji, on refrigerator door PVC gasket, 1 Jun. 1978, E.H.C. McKenzie (MFLU 12-0425, **epitype**, ex-epitype culture ICMP 6088 = PDDCC 6088). Thailand, Chiang Rai, on living leaves of *Syzygium* sp., 6 Feb. 2010, S.S.N. Maharachchikumbura, culture MFLUCC 10-0146.

*Note:* This species (clade 31; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

***Pestalotiopsis anacardiacearum*** Y. M. Zhang, Maharachchikumbura & K. D. Hyde

*Material examined:* China, Yunnan Province, Mangshi, Dehong, on living leaves of *Mangifera indica*, Sep. 2011, Y.M. Zhang (IFRD 411-015, **holotype**, ex-type culture IFRDCC 2397).

*Note:* This species (clade 6; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2013c).

***Pestalotiopsis arceuthobii*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809728. Fig. 19.

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

*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

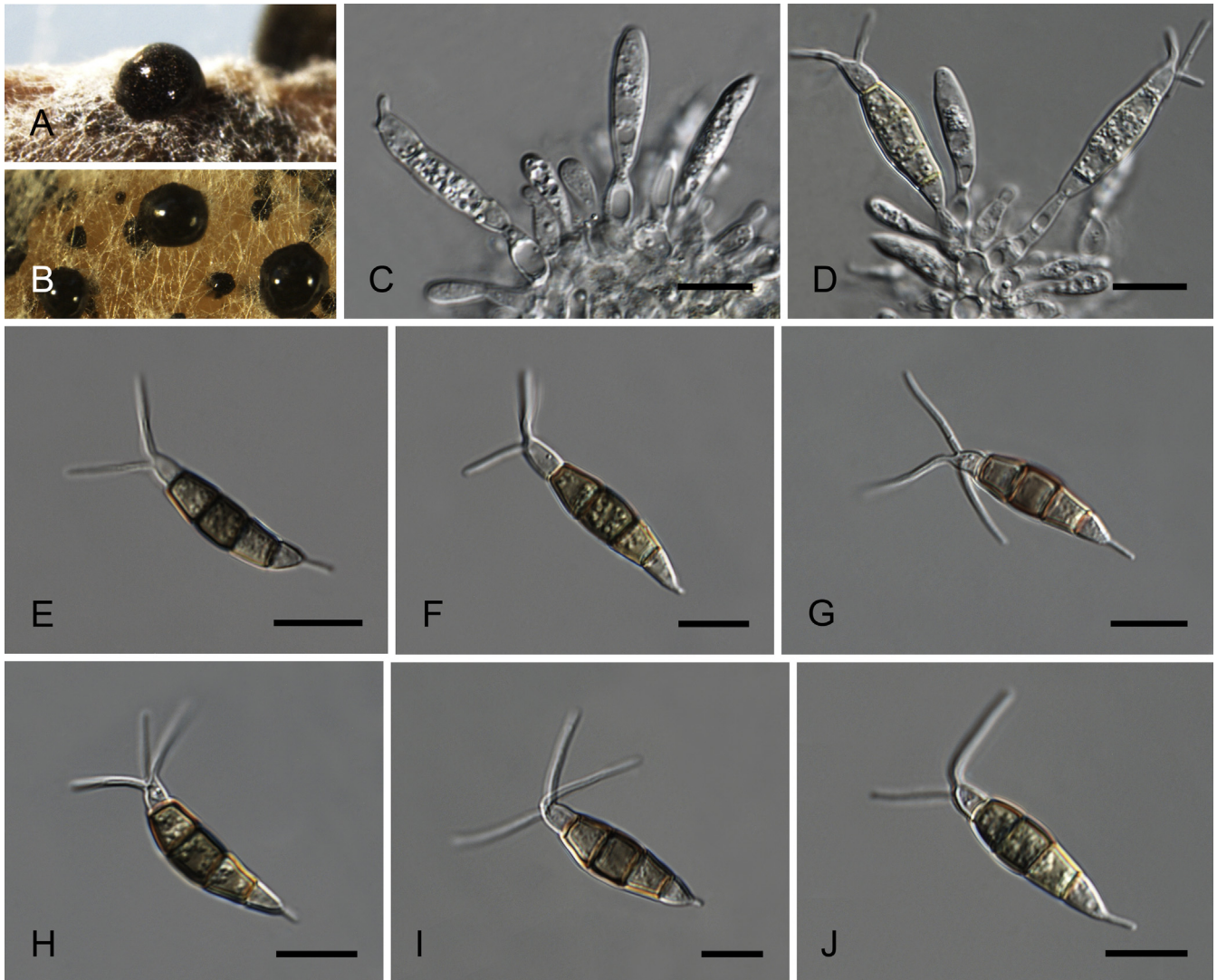


Fig. 19. *Pestalotiopsis arceuthobii* CBS 434.65<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10  $\mu$ m.

mass. *Conidiophores* mostly reduced to conidiogenous cells, branched or unbranched, 0–2-septate, hyaline and smooth, up to 10  $\mu$ m long. *Conidiogenous cells* discrete, subcylindrical (3–12  $\times$  1–3  $\mu$ m) or ampulliform to lageniform (3–10  $\times$  2–6  $\mu$ m), 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)  $\times$  6.5–8(–8.5)  $\mu$ m,  $\bar{x} \pm SD = 24.4 \pm 1.3 \times 7.2 \pm 0.5 \mu$ m, basal cell obconic with truncate base, rugose and thin-walled, 5–6  $\mu$ m long; three median cells (14–) 15–16.5  $\mu$ m long,  $\bar{x} \pm SD = 15.6 \pm 0.9 \mu$ m, doliiform, verruculose, concolourous, brown (second cell from base 5–6  $\mu$ m long; third cell 5.5–6.5  $\mu$ m long; fourth cell 4.5–6  $\mu$ m long); apical cell cylindrical, hyaline, thin- and smooth-walled, 4–5  $\mu$ m long; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, flexuous, unbranched, (10–) 11–14.5(–16)  $\mu$ m long,  $\bar{x} \pm SD = 12.8 \pm 1.0 \mu$ m; basal appendage single, tubular, unbranched, centric, 3–6  $\mu$ m long.

*Culture characteristics*: Colonies on PDA reaching 60–70 mm diam after 7 d at 25  $^{\circ}$ C, edge entire, whitish to pale honey-coloured, with aerial mycelium on the surface, with black, gregarious conidiomata; reverse similar in colour.

*Habitat*: On *Arceuthobium campylopodum*.

*Known distribution*: USA.

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

*Notes*: *Pestalotiopsis arceuthobii* is a distinct species represented by a single isolate (clade 3; Fig. 5), sister to *P. ericacearum* (clade 2; Fig. 5). *Pestalotiopsis arceuthobii* can be distinguished from *P. ericacearum* (conidia size = 15–21  $\times$  5–9  $\mu$ m) by its narrow conidia (size = 21–26  $\times$  6.5–8.5  $\mu$ m) as well as short apical appendages (10–16  $\mu$ m). In *P. ericacearum* the apical appendages are longer (19–45  $\mu$ m), and knobbed.

***Pestalotiopsis arengae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809729. Fig. 20.

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

*Conidiomata* (on PDA) pycnidial, globose or clavate, solitary or aggregated, semi-immersed, dark brown to black, 200–400  $\mu$ m



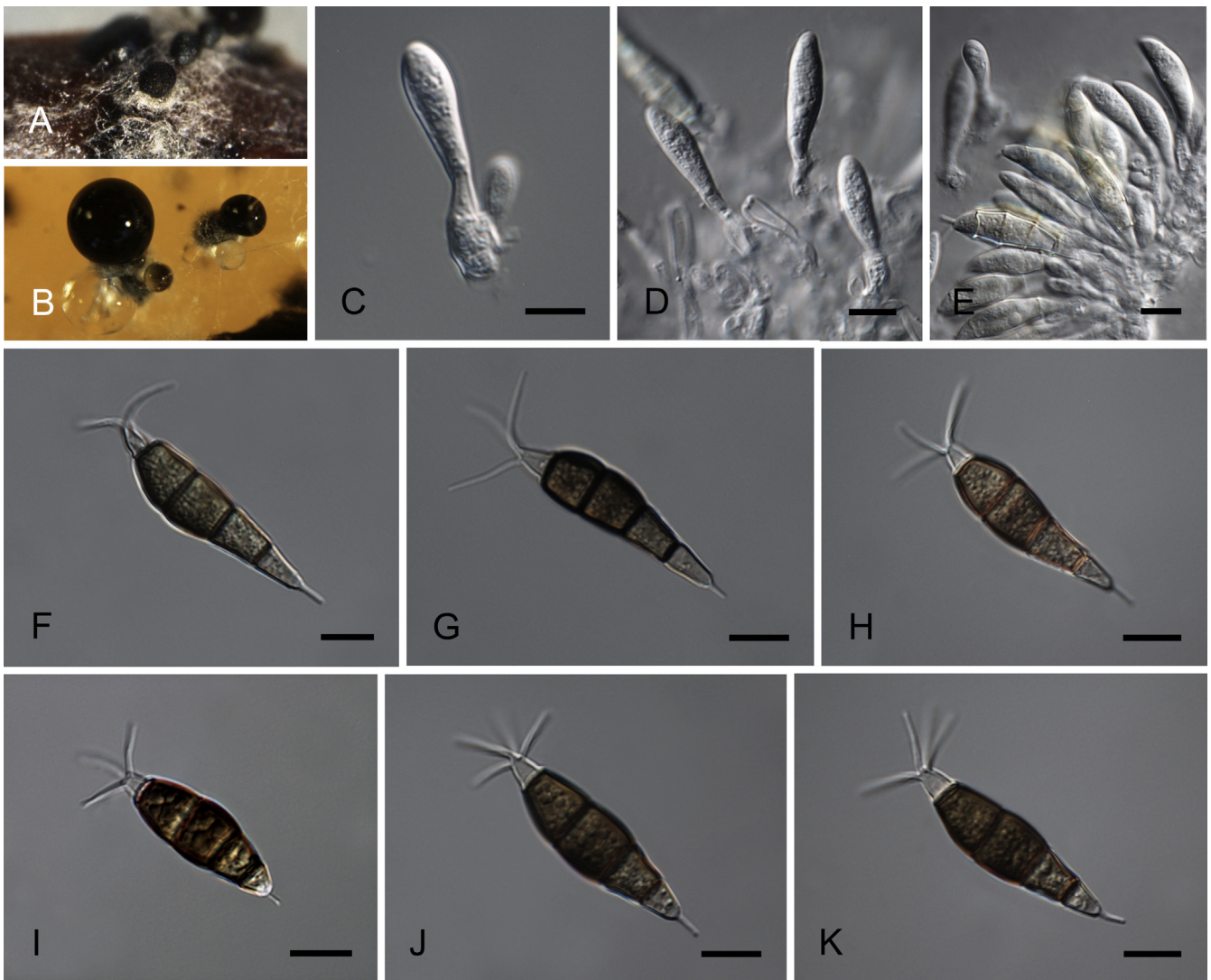


Fig. 20. *Pestalotiopsis arengae* CBS 331.92<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

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  $\times$  3–10  $\mu$ m, proliferating several times percurrently, with minute periclinal thickenings. *Conidia* ellipsoid, straight to slightly curved, slightly constricted at septa, 4-septate, (24–) 25–32(–33)  $\times$  7–9.5(–10)  $\mu$ m,  $\bar{x} \pm SD = 27.6 \pm 2 \times 8 \pm 0.4 \mu$ m; basal cell conic with a truncate base, rugose and thin-walled, 4–7  $\mu$ m long; three median cells (17–) 17.5–21.5(–22)  $\mu$ m long,  $\bar{x} \pm SD = 19 \pm 1.3 \mu$ m, doliiform, verruculose, concolourous, brown, septa darker than the rest of the cell (second cell from base 5.5–7  $\mu$ m long; third cell 5.5–8  $\mu$ m long; fourth cell 6–7.5  $\mu$ m long); apical cell sub-cylindrical, hyaline, thin- and smooth-walled, 2.5–4.5  $\mu$ m long; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (4–)4.5–11(–12)  $\mu$ m long,  $\bar{x} \pm SD = 7.3 \pm 1.3 \mu$ m; basal appendage single, tubular, unbranched, centric, 1.5–3  $\mu$ m long.

**Culture characteristics:** Colonies on PDA reaching 70–80 mm diam after 7 d at 25  $^{\circ}$ C, undulate at the margin, white to pale luteous-coloured, with moderate aerial mycelium on the surface, with black, gregarious conidiomata; reverse similar in colour.

**Habitat:** On dead leaves of *Arenga undulatifolia*.

**Known distribution:** Singapore.

**Material examined:** Singapore, Botanical Gardens, from dead leaves of *Arenga undulatifolia*, Nov. 1991, W. Gams (CBS H-21768, **holotype**, ex-type culture CBS 331.92).

**Notes:** *Pestalotiopsis arengae* (clade 4; Fig. 5) forms a separate cluster in the combined phylogeny as basal sister to *P. anacardiacearum* (clade 6; Fig. 5) and *P. hawaiiensis* (clade 5; Fig. 5), which were isolated from mango from China and *Leucospermum* sp. from Hawaii, respectively. In morphology, *P. arengae* differs from *P. anacardiacearum* and *P. hawaiiensis* by its smaller conidia and shorter apical appendages.

***Pestalotiopsis australasiae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809730. Fig. 21.

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

*Conidiomata* pycnidial in culture on PDA, globose, scattered, semi-immersed, up to 200  $\mu$ m diam; exuding globose, dark

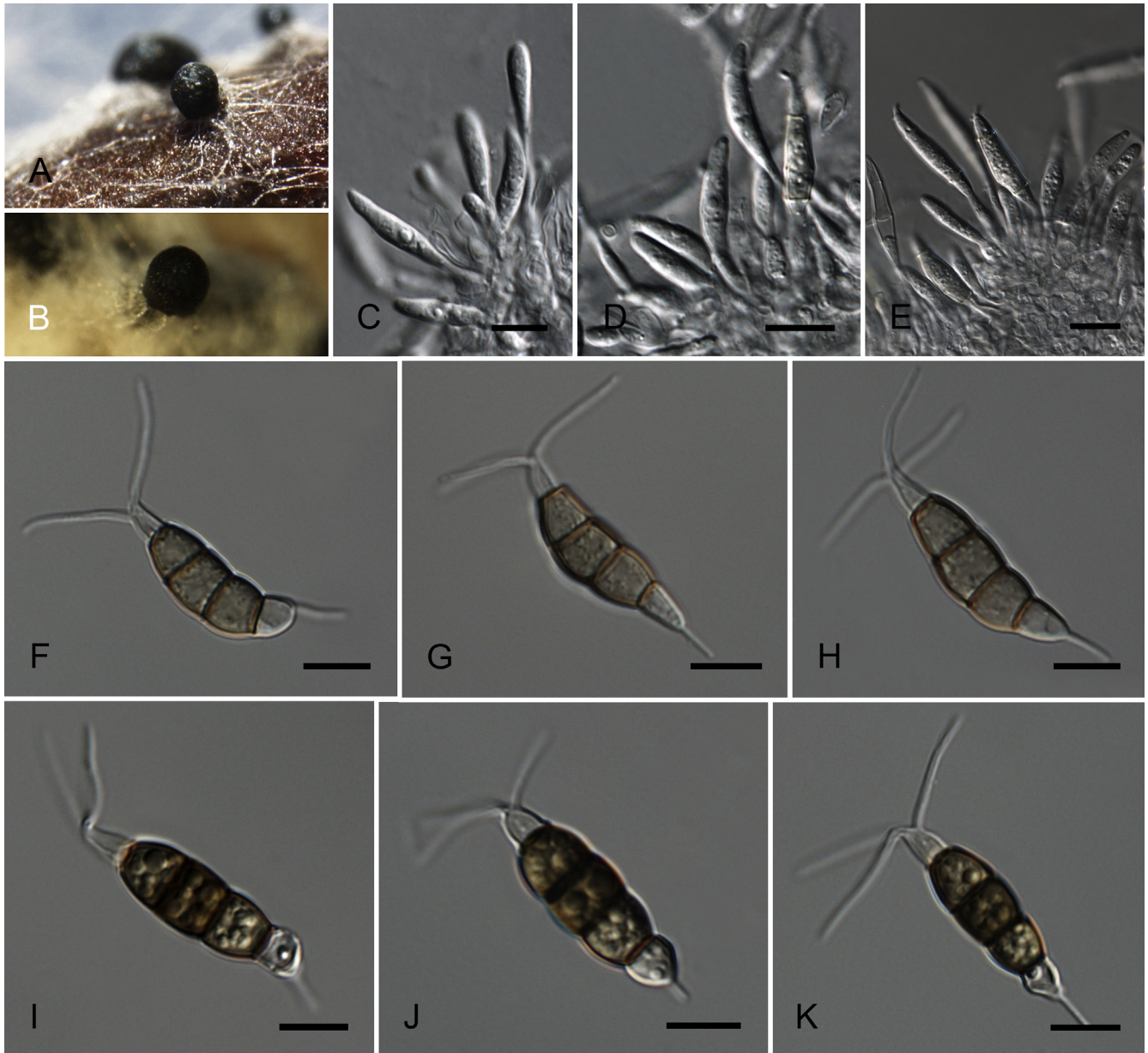


Fig. 21. *Pestalotiopsis australasiae* CBS 114126<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

brown to black conidial masses. *Conidiophores* indistinct, often reduced to conidiogenous cells. *Conidiogenous cells* discrete or integrated, 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* fusoid, ellipsoid, straight to slightly curved, 4-septate, (23–)24.5–29(–31) × (6–)6.5–8(–8.5) µm,  $\bar{x} \pm SD = 26 \pm 1.4 \times 7.5 \pm 0.2$  µm; basal cell obconic to hemispherical, hyaline, verruculose and thin-walled, 5–6.5 µm long; three median cells doliiform, (15–)15.5–18(–18.5) µm long,  $\bar{x} \pm SD = 16.7 \pm 0.7$  µm, wall verruculose, concolourous, 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,  $\bar{x} \pm SD = 12.6 \pm 1.7$  µm; basal appendage single, tubular, unbranched, centric, 2.5–4.5 µm long.

**Culture characteristics:** Colonies on PDA attaining 30–40 mm diam after 7 d at 25 °C, flat with entire edge, whitish, with sparse

aerial mycelium on the surface with black, gregarious conidiomata; reverse similar in colour.

**Habitat:** On *Knightia* sp. and *Protea* sp.

**Known distribution:** Australia and New Zealand.

**Materials examined:** **Australia**, New South Wales, from *Protea neriifolia* × *susannae* cv. 'Pink Ice', 12 Oct. 1999, P.W. Crous, culture CBS 114141 = STE-U 2949. **New Zealand**, from *Knightia* sp., 2002, P.W. Crous (CBS H-21767, **holotype**, ex-type culture CBS 114126 = STE-U 2896).

**Notes:** Morphologically *P. australasiae* (clade 39; Fig. 5) is comparable to *P. knightiae* (clade 37; Fig. 5), *P. parva* (clade 35; Fig. 5) and *P. grevilleae* (clade 36; Fig. 5), but differs in having larger conidia when compared to *P. parva*, and shorter apical appendages when compared to *P. knightiae* and *P. grevilleae*. It has an overlapping conidial size with *P. telopeae* (clade 40; Fig. 5), which causes a leaf spot disease on *Telopea* spp. Since the two species are genetically distinct, we maintain them as two separate species (see notes under *P. telopeae*).

*Pestalotiopsis australis* Maharachch., K.D. Hyde & Crous, sp. nov. MycoBank MB809731. Fig. 22.

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

*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 long. *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* fusoid, ellipsoid, straight to slightly curved, 4-septate, (26–) 27–34(–36) × 7–8.5 µm,  $\bar{x} \pm SD = 30.8 \pm 2.1 \times 7.7 \pm 0.3$  µm; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 6–10 × µm long; three median cells dolliiform, (16–)17–21(–21.5) µm long,  $\bar{x} \pm SD = 19.1 \pm 1.2$  µm,

wall minutely verruculose, concolourous, 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,  $\bar{x} \pm SD = 15.5 \pm 2.7$  µm; basal appendage single, tubular, unbranched, centric, 3–7 µm long.

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

*Habitat*: On *Brabejum stellatifolium*, *Grevillea* sp. and *Protea neriifolia* × *susannae*.

*Known distribution*: Australia and South Africa.

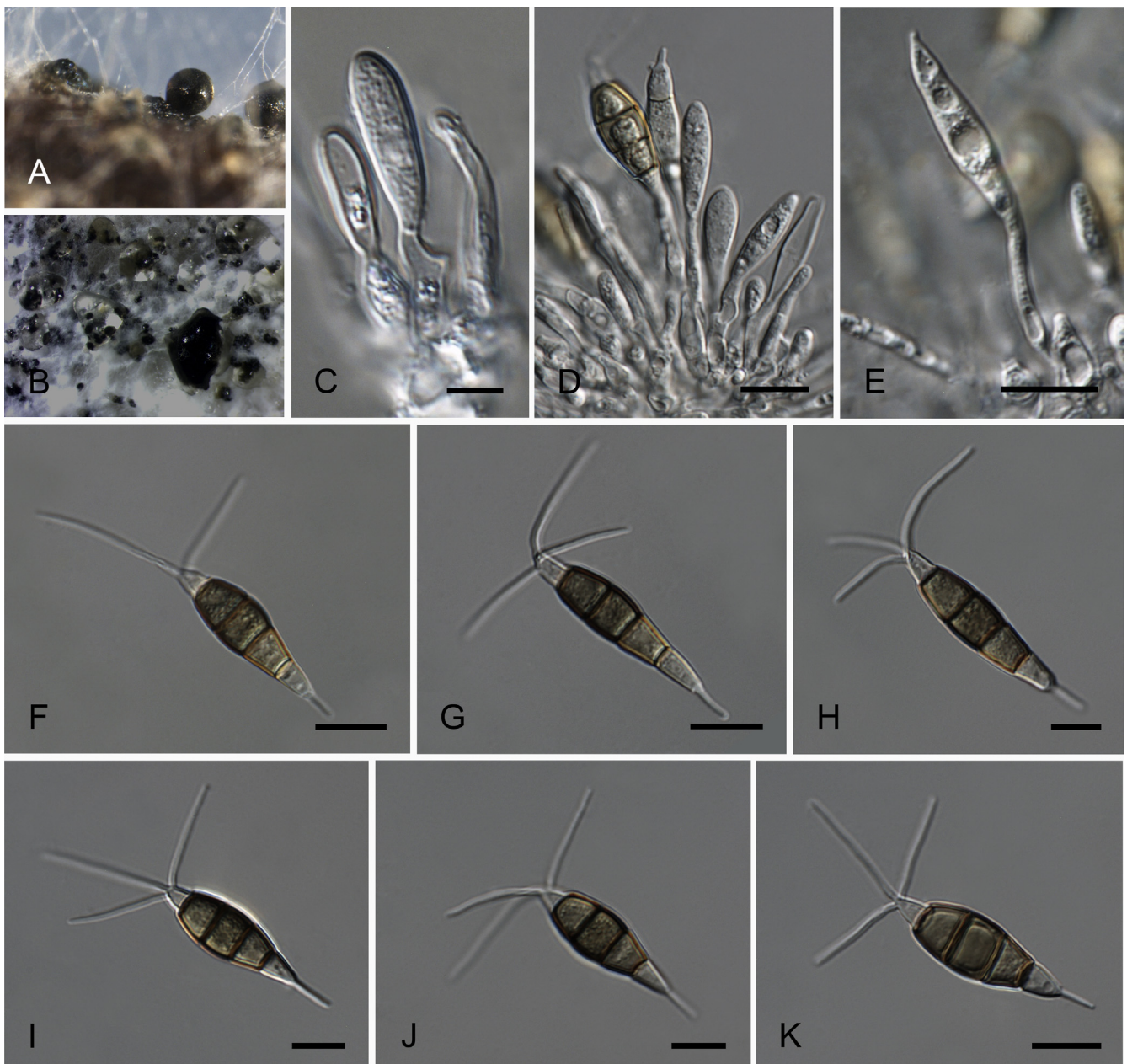


Fig. 22. *Pestalotiopsis australis* CBS 114193<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

**Materials examined:** **Australia**, New South Wales, from *Grevillea* sp. 12 Oct. 1999, P.W. Crous (CBS H-21766, **holotype**, ex-type culture CBS 114193 = STE-U 3011). **South Africa**, KwaZulu-Natal, from *Protea neriifolia* × *susannae* cv. '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; on dead leaves of *Brabejum stellatifolium*, 3 Nov. 2000, S. Lee, PREM 59519, culture CBS 119350 = CMW 20013.

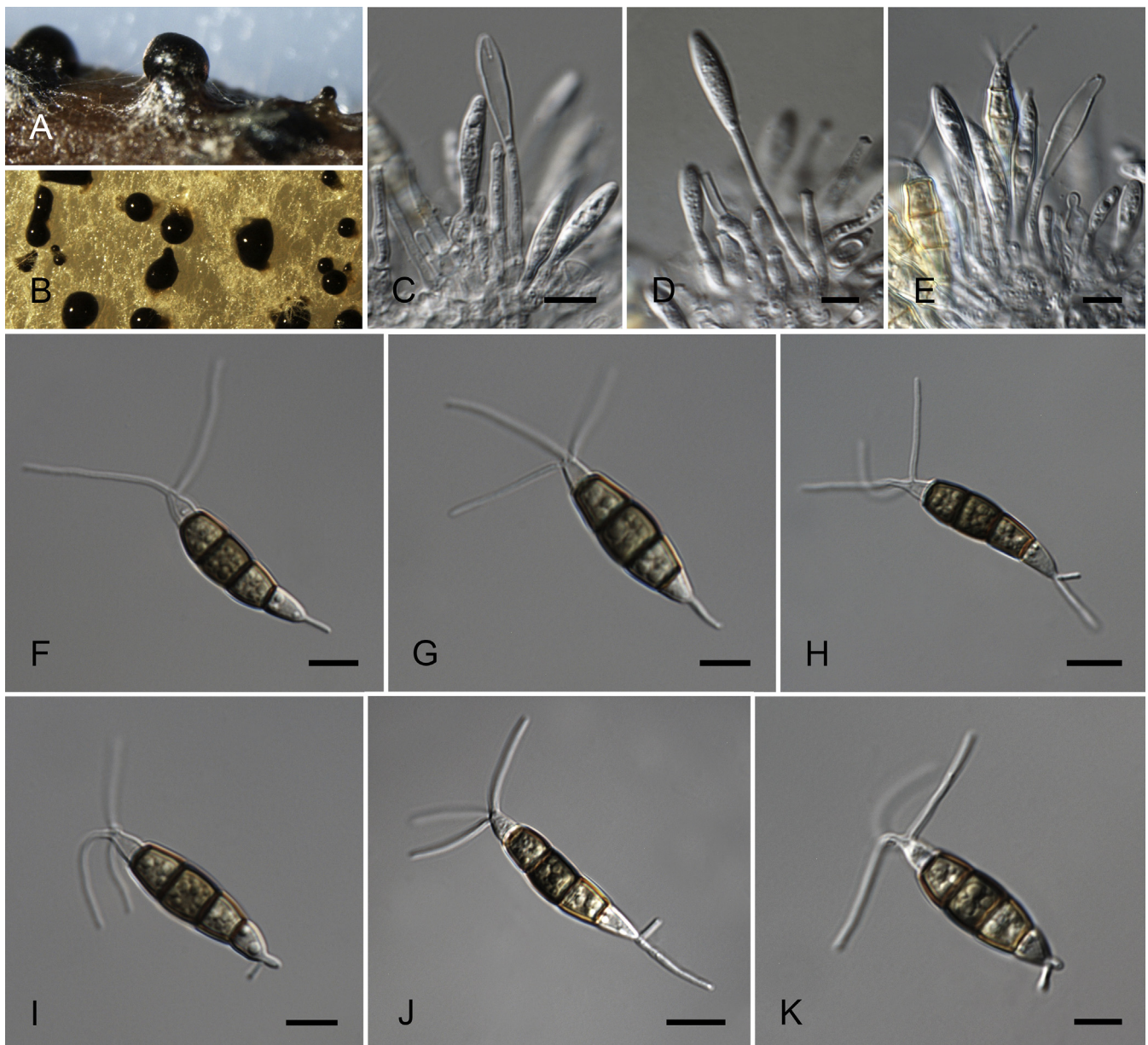
**Notes:** *Pestalotiopsis australis* (clade 26; Fig. 5) is a distinct species, which can be isolated from members of *Proteaceae*. *Pestalotiopsis australis* is closely related to *P. scoparia* (clade 25; Fig. 5), and is distinguished morphologically from related species by its large conidia.

***Pestalotiopsis biciliata*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809732. Fig. 23.

**Etymology:** Name refers to its two basal appendages.

*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 long, 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* fusoid, ellipsoid, straight to slightly curved, 4-septate, (21–)22–28.5(–30) × (5.5–)6–7.5(–8) µm,  $\bar{x} \pm SD = 25.3 \pm 2 \times 6.7 \pm 0.3$  µm; basal cell obconic to hemispherical with a truncate base, hyaline, verruculose and thin-walled, 4–7 µm long; three median cells dolii-form, (13.5–)14.5–17.5(–18.5) µm long,  $\bar{x} \pm SD = 16 \pm 1.1$  µm, wall verruculose, concolourous, 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, rugose and thin-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, (6–)8–18(–20) µm long,  $\bar{x} \pm SD = 13.3 \pm 3.2$  µm; two basal appendages; centric appendage tubular, 3–8 µm long and excentric appendage tubular, 1–3 µm long.



**Fig. 23.** *Pestalotiopsis biciliata* CBS 124463<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

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

**Habitat:** On *Paeonia* sp., bark of *Platanus* × *hispanica* and *Taxus baccata* dry needles.

**Known distribution:** Italy, Netherlands and Slovakia.

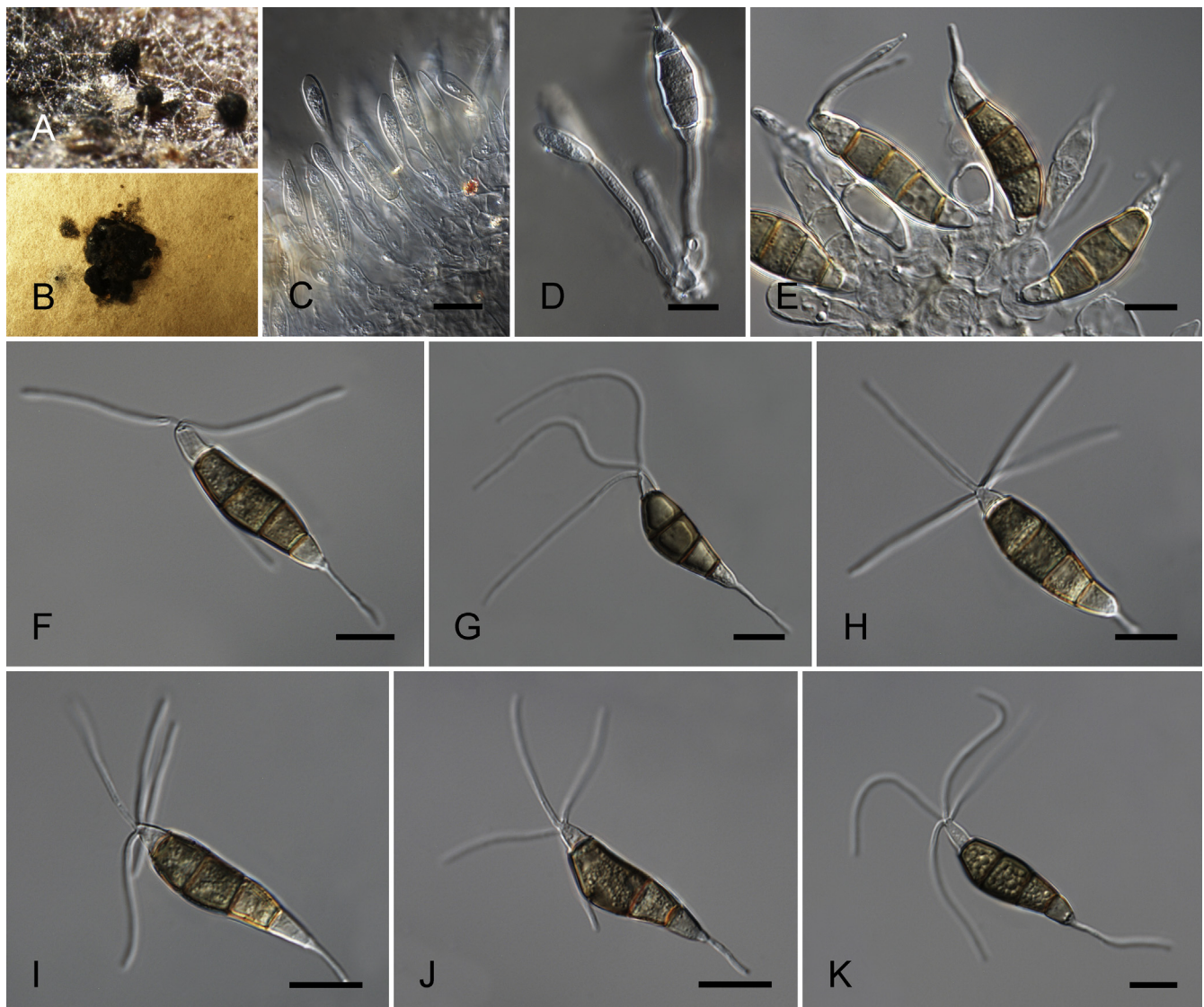
**Materials examined:** **Italy**, from *Paeonia* sp., Jun. 1938, O. Servazzi, culture CBS 236.38. **Netherlands**, from *Taxus baccata* dry needles attached to the tree, 23 Oct. 1968, H.A. van der Aa, culture CBS 790.68. **Slovakia**, Giraltovce, from bark of *Platanus* × *hispanica*, unknown collection date, M. Pastircak (CBS H-21765, **holotype**, ex-type culture CBS 124463).

**Notes:** *Pestalotiopsis biciliata* (clade 38; Fig. 5) is a species often having two basal appendages. *Pestalotiopsis biciliata* overlaps morphologically with *P. trachicarpicola* (clade 43; Fig. 5). However, in the phylogenetic analyses it formed a distinct lineage apart from *Pestalotiopsis kenyana* (which has wider conidia; clade 42; Fig. 5) and *P. trachicarpicola* (clade 43; Fig. 5).

***Pestalotiopsis brassicae*** (Guba) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809734. Fig. 24.

**Basionym:** *Pestalotia brassicae* Guba, Monograph of *Monochaetia* and *Pestalotia*: 245. 1961.

*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* fusoid, ellipsoid, straight to slightly curved, 4-septate, (29–)30–37(–40) × (8–)8.5–11(–11.5) µm,  $\bar{x} \pm SD = 34 \pm 2.1 \times 9.7 \pm 0.7$  µm; basal cell obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 5–8.5 × µm long; three median cells doliform to subcylindrical, (20–)20.5–24.5(–25) µm long,  $\bar{x} \pm SD = 22.6 \pm 1.5$  µm, wall verruculose, concolourous, 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,



**Fig. 24.** *Pestalotiopsis brassicae* CBS 170.26<sup>isoT</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

filiform, flexuous, (27–)28.5–48(–50)  $\mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 37 \pm 5 \mu\text{m}$ ; basal appendage single, tubular, unbranched, centric, 10–25  $\mu\text{m}$  long.

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

**Habitat:** On seeds of *Brassica napus*.

**Known distribution:** New Zealand.

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

**Notes:** According to the original description of Guba (1961), conidia of *P. brassicae* are somewhat smaller (25–32  $\times$  8.5–9.5  $\mu\text{m}$ ) and the apical appendages are shorter (20–35  $\mu\text{m}$ ) than in the present observation. In his monograph Guba placed this species in a group with species having versicoloured median cells. However, our phylogenetic analyses (Fig. 5) do not support placing *P. brassicae* (clade 19; Fig. 5) within the versicoloured group (genus *Neopestalotiopsis*; Fig. 4). *Pestalotiopsis brassicae* formed a sister group to *P. hollandica* (clade 18; Fig. 5), which was isolated from *Sciadopitys verticillata* in the Netherlands. The latter species is clearly distinguished from *P. brassicae* by having wider conidia, and branched, sub-apically attached apical appendages. Furthermore, *P. brassicae* is distinguished from its other closest phylogenetic neighbour, *P. verruculosa* (clade 20; Fig. 5) (28–35  $\times$  9–11  $\mu\text{m}$ ) by its larger conidia.

***Pestalotiopsis camelliae*** Y.M. Zhang, Maharachch. & K.D. Hyde

**Materials examined:** **China**, Yunnan Province, Chuxiong, Shuangbai, on living leaves of *Camellia japonica*, Jul. 2011, Y.M. Zhang (IFRD OP111, **holotype**, ex-type culture MFLUCC 12-0277); *ibid.*, Aug. 2011, IFRD OP131, culture MFLUCC 12-0278. **Turkey**, Samsun, on leaf of *Camellia sinensis*, collection date unknown, O. Orbas, culture CBS 443.62.

**Note:** This species (clade 13; Fig. 5) was treated in detail by Zhang *et al.* (2012b).

***Pestalotiopsis chamaeropsis*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809735. Fig. 25.

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

**Conidiomata** pycnidial in culture on PDA, globose, semi-immersed or partly erumpent, aggregated or scattered, up to 250  $\mu\text{m}$  diam; exuding globose, dark brown to black conidial masses. **Conidiophores** 1–3-septate, branched, subcylindrical, hyaline, verruculose, up to 25  $\mu\text{m}$  long. **Conidiogenous cells** discrete, cylindrical, hyaline, smooth-walled, proliferating 2–4 times percurrently, 20–50  $\times$  2–5  $\mu\text{m}$ , collarette present and not flared, with prominent periclinal thickening. **Conidia** fusoid, ellipsoid, straight to slightly curved, 4-septate, (21–)22.5–27(–28)  $\times$  (6–)7–9(–9.5)  $\mu\text{m}$ ,  $\bar{x} \pm \text{SD} = 25.2 \pm 1.3 \times 8 \pm 0.4 \mu\text{m}$ ; basal cell obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 5–6.5  $\mu\text{m}$  long; three median cells doliform to subcylindrical, (15–)16–17.5(–18.5)  $\mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 16.7 \pm 0.8 \mu\text{m}$ , wall verruculose,

concolourous, but occasionally the two upper median cells are 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  $\mu\text{m}$  long; third cell 4.5–6.5  $\mu\text{m}$  long; fourth cell 4.5–6  $\mu\text{m}$  long); apical cell 4–6  $\mu\text{m}$  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)  $\mu\text{m}$  long,  $\bar{x} \pm \text{SD} = 18 \pm 3.1 \mu\text{m}$ ; basal appendage single, tubular, unbranched, centric, 4–8.5  $\mu\text{m}$  long.

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

**Habitat:** On leaves of *Chamaerops humilis*.

**Known distribution:** Italy.

**Materials examined:** **Italy**, Sardinia, Dorgali, from leaf of *Chamaerops humilis*, Feb. 1971, H.A. van der Aa (CBS H-15702, **holotype**, ex-type culture CBS 186.71); unknown collection details (June 1938 deposited in CBS collection), O. Servazzi, culture CBS 237.38. **Unknown locality**, unknown collection details, culture CBS 113604 = STE-U 3078, CBS 113607 = STE-U 3080.

**Notes:** Clade 23 (Fig. 5) is represented by four isolates of *P. chamaeropsis*. It differs from related species in having distinctly wider conidia. *Pestalotiopsis chamaeropsis* forms a separate cluster in the combined phylogeny, as sister to a group including *P. intermedia* (clade 21; Fig. 5) and *P. linearis* (clade 22; Fig. 5), which were isolated from dead leaves of an unidentified tree, and as an endophyte of *Trachelospermum* sp. respectively, both 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*. Even though these two isolates had overlapping conidial dimensions, the deposited isolates cluster in distinct clades (CBS 237.38 in clade 23 and CBS 236.38 in clade 38; Fig. 5) with species having concolourous median cells. According to the description of Guba (1961), *P. paeoniae* belongs to the species with versicoloured median cells (presently *Neopestalotiopsis*; Fig. 4). The reliability of these two “authentic” strains is thus doubtful, and CBS 237.78 is placed in *P. chamaeropsis*, and CBS 236.38 in *P. biciliata* (clade 38; Fig. 5).

***Pestalotiopsis clavata*** Maharachch. & K.D. Hyde

**Material examined:** **China**, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Buxus* sp., 19 Mar. 2002, W.P. Wu (HMAS047134, **holotype**, MFLU 12-0412, **isotype**, ex-type culture NN0471340 = MFLUCC 12-0268).

**Note:** This species (clade 15; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

***Pestalotiopsis colombiensis*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809736. Fig. 26.

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

**Conidiomata** (on PDA) pycnidial, globose to clavate, solitary or aggregated, semi-immersed, dark brown, 200–400  $\mu\text{m}$  diam; exuding globose, dark brown, glistening conidial masses. **Conidiophores** reduced to conidiogenous cells; when present,

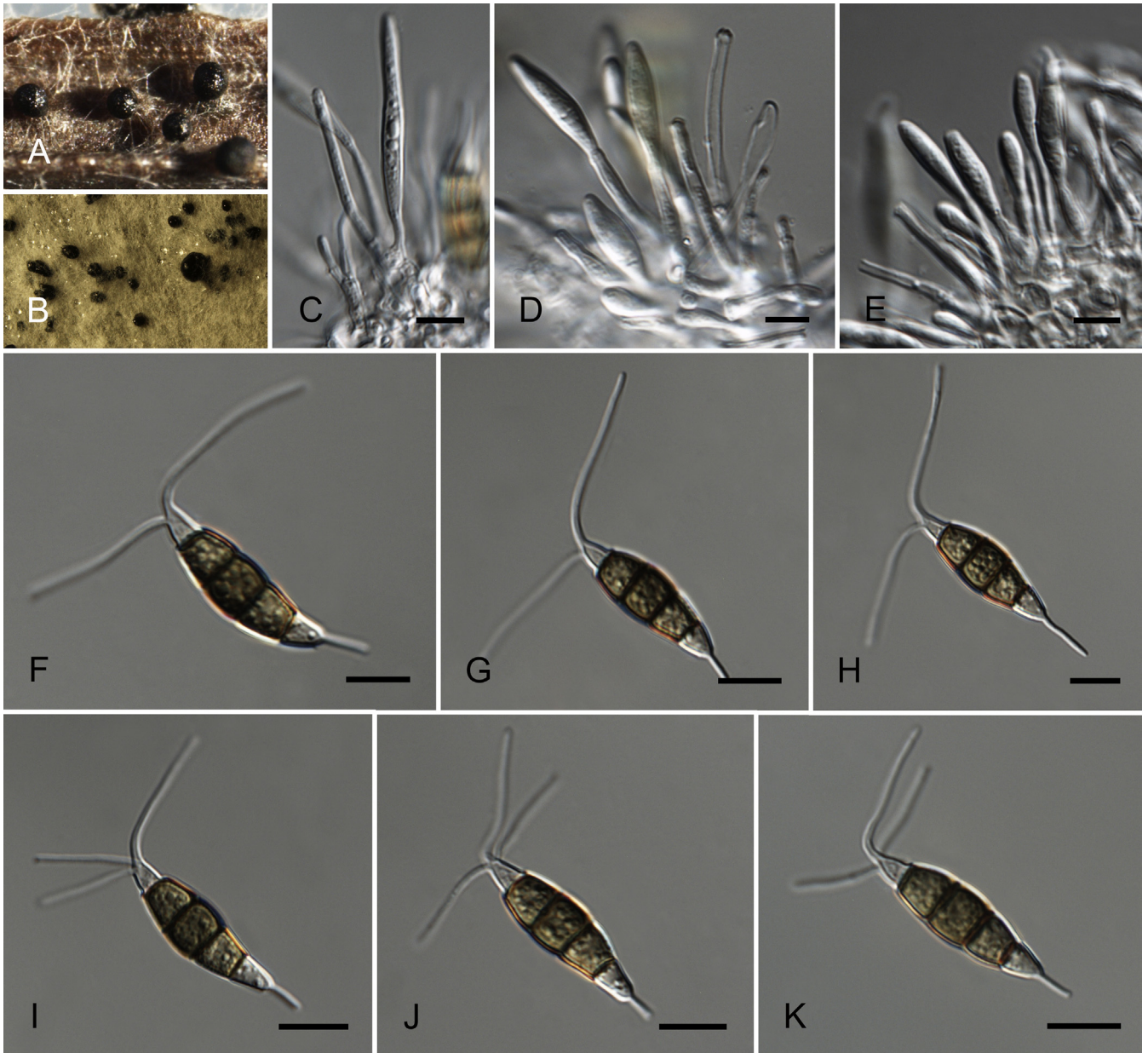


Fig. 25. *Pestalotiopsis chamaeropsis* CBS 186.71<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

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

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

*Habitat*: On living leaves of *Eucalyptus eurograndis*.

*Known distribution*: Colombia.

*Material examined*: Colombia, from living leaves of *Eucalyptus eurograndis*, 2004, M.J. Wingfield (CBS H-21764, holotype, ex-type culture CBS 118553 = CPC 10969).

*Notes*: *Pestalotiopsis colombiensis* (clade 27; Fig. 5) is a distinct species represented by a Colombian isolate from *Eucalyptus*. It differs from its closest phylogenetic neighbours, *P. diploclisiae* (clade 29; Fig. 5) and *P. humus* (clade 28; Fig. 5) by its longer apical appendages. Furthermore *P. colombiensis* is geographically

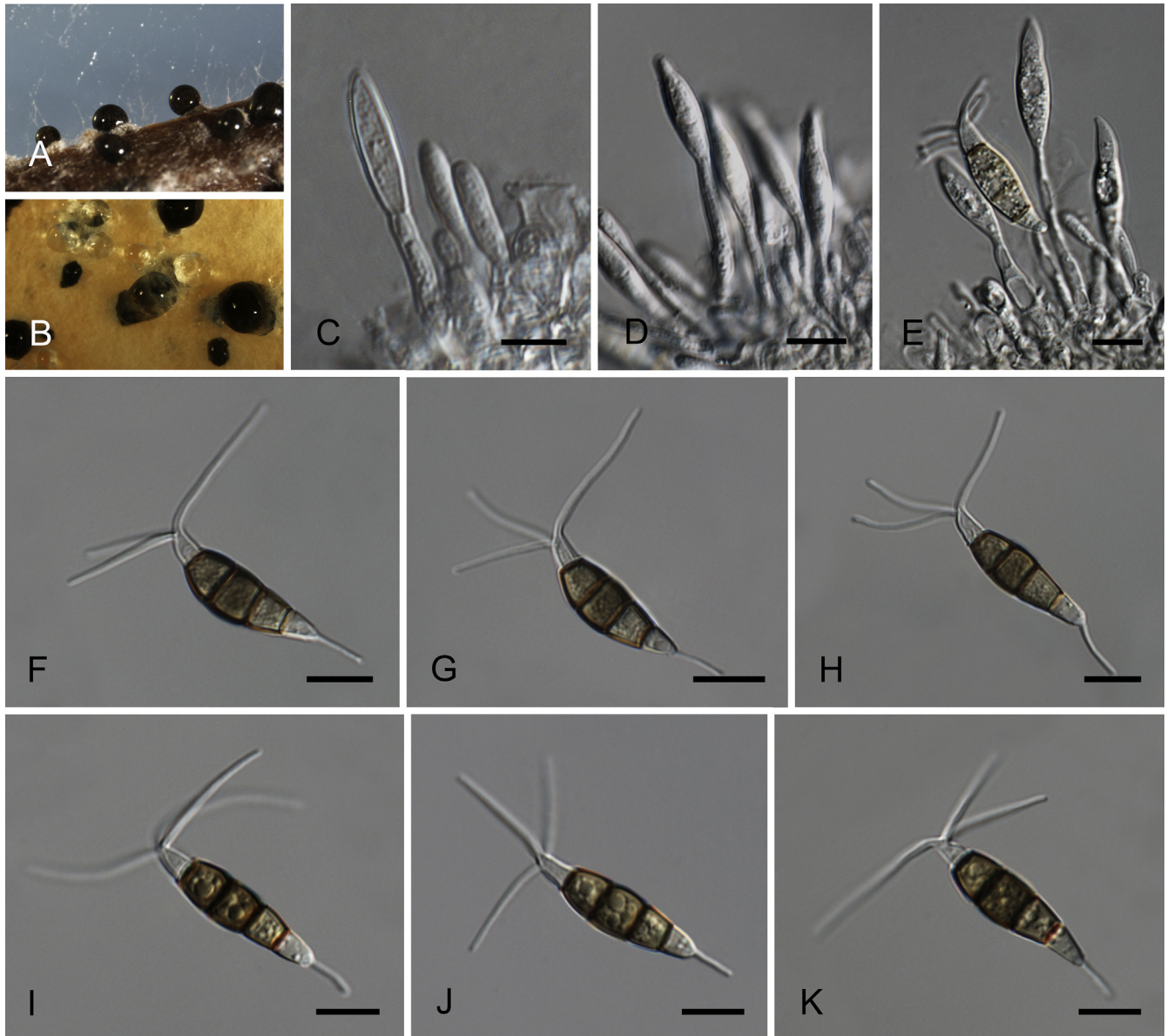


Fig. 26. *Pestalotiopsis colombiensis* CBS 118553<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

distinct from *P. diploclisiae* and *P. humus*, which were isolated from Hong Kong and Papua New Guinea, respectively.

***Pestalotiopsis diploclisiae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809737. Fig. 27.

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

**Conidiomata** pycnidial in culture on PDA, globose, solitary or aggregated, semi-immersed, black, up to 500  $\mu$ m 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  $\mu$ m long. **Conidiogenous cells** discrete, cylindrical to subcylindrical, hyaline, smooth, simple, proliferating 2–3 times percurrently, 6–20  $\times$  2–5  $\mu$ m. **Conidia** fusoid, ellipsoid, straight to slightly curved, 4-septate, (20–)22–26.5(–28)  $\times$  5–6.5(–7)  $\mu$ m,  $\bar{x} \pm SD = 24 \pm 1.3 \times 5.7 \pm 0.4 \mu$ m; basal cell obconic to subcylindrical with a truncate base, hyaline, rugose and thin-walled,

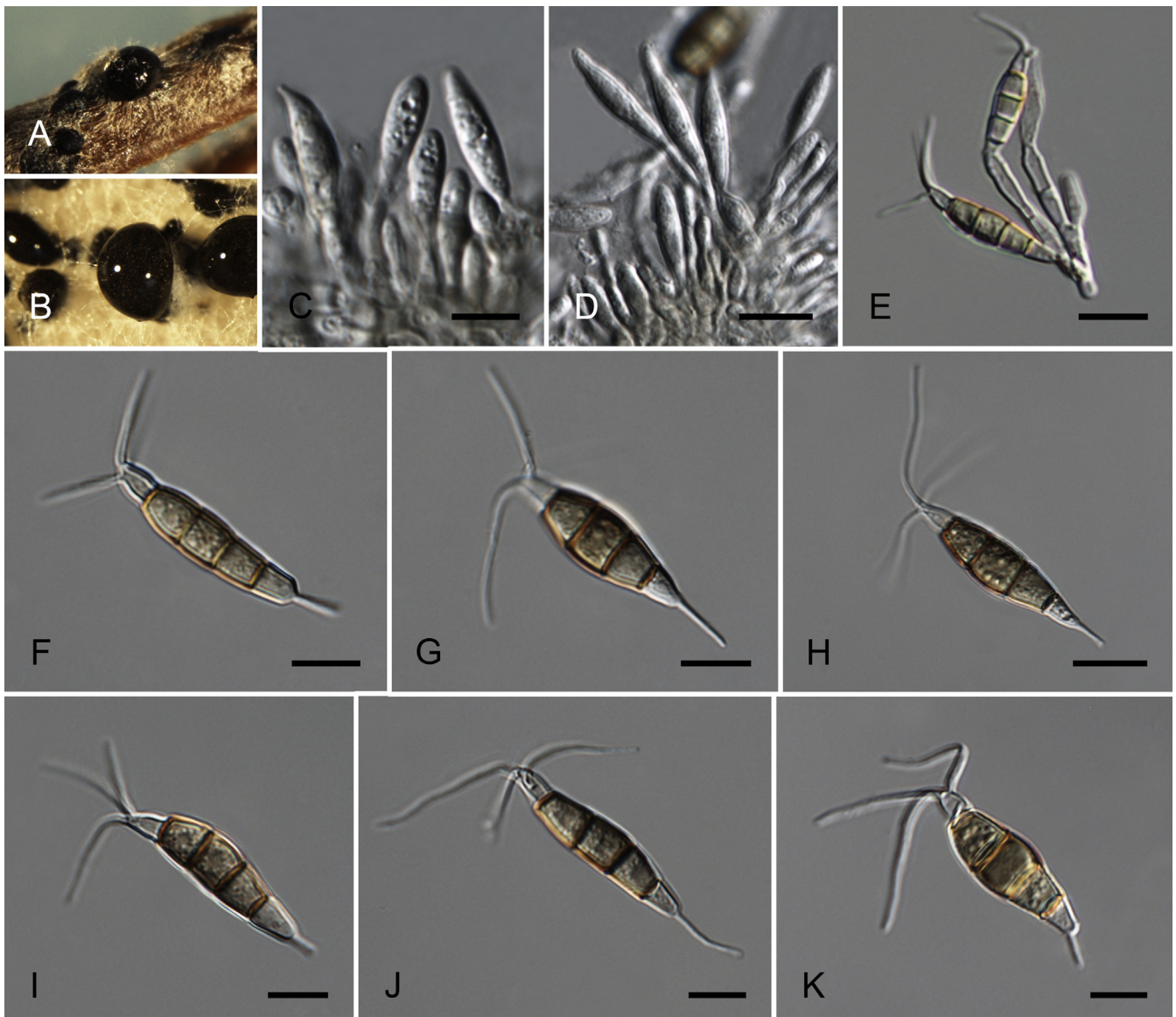
4–6.5  $\mu$ m long; three median cells doliiform, (13.5–)14–16(–17)  $\mu$ m long,  $\bar{x} \pm SD = 15.4 \pm 0.9 \mu$ m, wall minutely verruculose, concolourous, pale brown, septa darker than the rest of the cell (second cell from the base 4.5–6  $\mu$ m; third cell 4.5–7  $\mu$ m; fourth cell 4.5–6.5  $\mu$ m); apical cell 3.5–6  $\mu$ m 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)  $\mu$ m long,  $\bar{x} \pm SD = 16.6 \pm 2.1 \mu$ m; basal appendage single, tubular, unbranched, centric, 3–8  $\mu$ m long.

**Culture characteristics:** Colonies on PDA attaining 35–45 mm diam after 7 d at 25  $^{\circ}$ C, with smooth edge, whitish, with sparse aerial mycelium on the surface with black, gregarious conidiomata; reverse similar in colour.

**Habitat:** On fruit of *Diploclisia glaucescens* and *Psychotria tutcheri*.

**Known distribution:** China (Hong Kong).





**Fig. 27.** *Pestalotiopsis diploclisiae* CBS 115587<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

**Materials examined:** **China**, Hong Kong, Lamma Island, from fruit of *Diploclisia glaucescens*, 5 Jul. 2001, K.D. Hyde (CBS H-21763, **holotype**, ex-type culture CBS 115587 = HKUCC 10130); *ibid.*, culture CBS 115585 = HKUCC 8394; Mount Nicholson, from fruit of *Psychotria tutcheri*, 15 Feb. 2002, K.D. Hyde, culture CBS 115449 = HKUCC 9103.

**Notes:** *Pestalotiopsis diploclisiae* (clade 29; Fig. 5) comprises three isolates originating from Hong Kong. *Pestalotiopsis diploclisiae* is morphologically very similar to *P. colombiensis* (clade 27; Fig. 5), but genetically clearly distinct, forming a well-separated clade. *Pestalotiopsis diploclisiae* is genetically close to *P. humus* (clade 28; Fig. 5), which was isolated from soil in Papua New Guinea, but can be distinguished by its narrow conidia and longer apical appendages.

#### ***Pestalotiopsis diversiseta* Maharachch. & K.D. Hyde**

**Material examined:** **China**, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Rhododendron* sp., 19 Mar. 2002, W.P. Wu (HMAS047261, **holotype**, MFLU 12-0423, **isotype**, ex-type culture NN0472610 = MFLUCC 12-0287).

**Note:** This species (clade 7; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

#### ***Pestalotiopsis ericacearum* Y. M. Zhang, Maharachch. & K. D. Hyde**

**Material examined:** **China**, Yunnan Province, Chuxiong, Zixishan, leaf spots on *Rhododendron delavayi*, Feb. 2011, Y.M. Zhang (IFRD 410-008, **holotype**, ex-type culture IFRDCC 2439).

**Note:** This species (clade 2; Fig. 5) was treated in detail by Zhang *et al.* (2013).

#### ***Pestalotiopsis furcata* Maharachch. & K.D. Hyde**

**Material examined:** **Thailand**, Chiang Mai Province, Mae Taeng District, Ban Pha Deng, Mushroom Research Centre, 19°17.123'N 98°44.009'E, on living leaves of *Camellia sinensis*, 20 Jan. 2010, S.S.N. Maharachchikumbura (MFLU 12-0112, **holotype**, ex-type culture MFLUCC 12-0054 = CPC 20280).

**Note:** This species (clade 12; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2013a).

#### ***Pestalotiopsis gaultheria* Y. M. Zhang, Maharachch. & K. D. Hyde**

*Material examined:* China, Yunnan Province, Dehong, Mangshi, leaf spots on *Gaultheria forrestii*, Sep. 2011, Y.M. Zhang (IFRD 411-014, **holotype**).

*Note:* This species (clade 9; Fig. 5) was treated in detail by Zhang et al. (2013).

***Pestalotiopsis grevilleae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809738. Fig. 28.

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

*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 collarete, with prominent periclinal thickening, 5–25 × 2–8 µm. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate, (21–)22.5–28(–29) × (7–)7.5–9(–9.5) µm,  $\bar{x} \pm SD = 25.2 \pm 1.2 \times 8.2$

± 0.5 µm; basal cell conic with a truncate base, hyaline, rugose and thin-walled, 3.5–5.5 µm long; three median cells doliiform, (12.5–)13–17(–17.5) µm long,  $\bar{x} \pm SD = 15 \pm 1.2$  µm, wall verruculose, concolourous, 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, rugose and thin-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform, flexuous (12–)14–26.5(–29) µm long,  $\bar{x} \pm SD = 19 \pm 3$  µm; basal appendage single, tubular, unbranched, centric, 3–8 µm long.

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

*Habitat:* On *Grevillea* sp.

*Known distribution:* Australia.

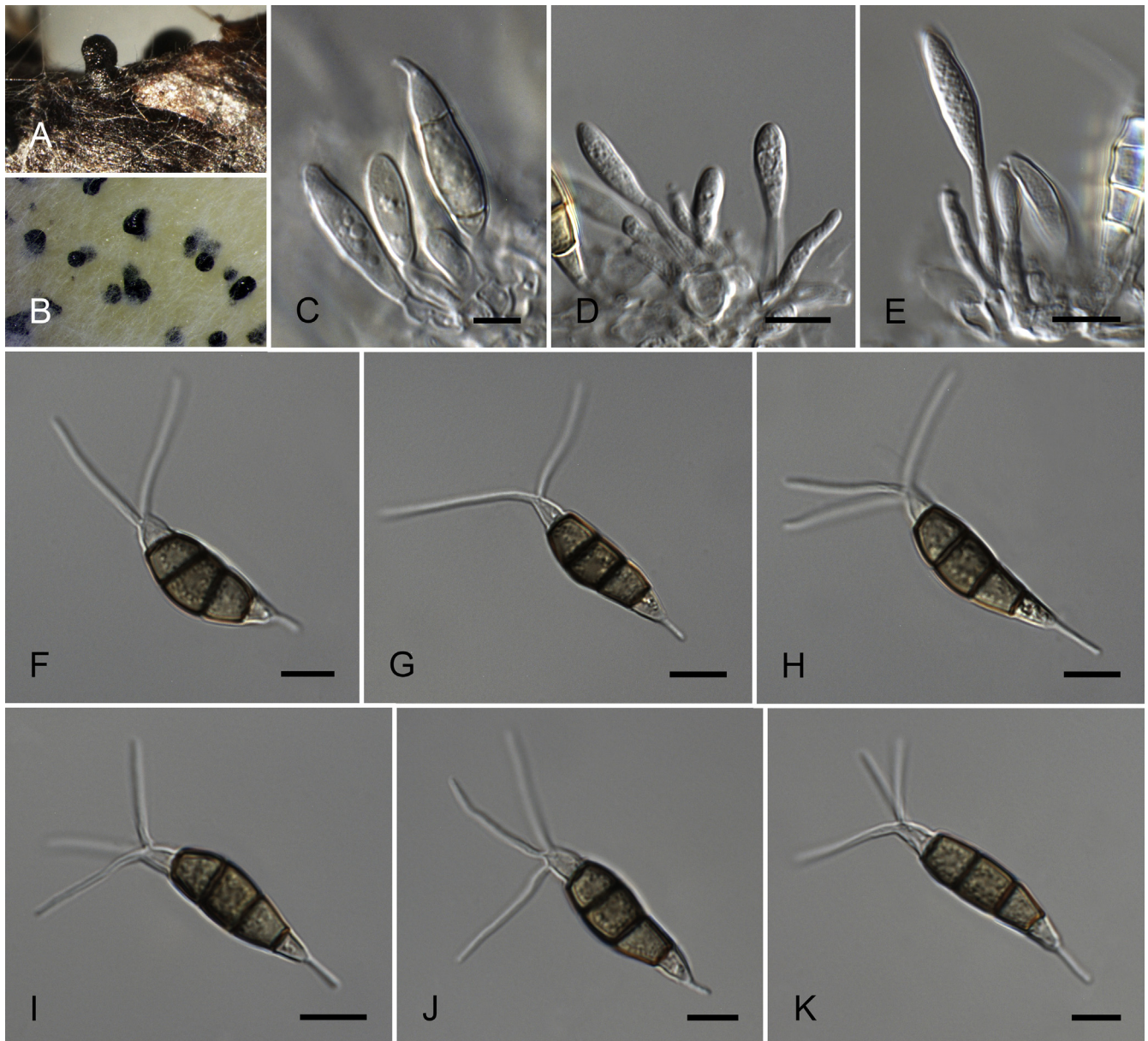


Fig. 28. *Pestalotiopsis grevilleae* CBS 114127<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

*Material examined:* Australia, New South Wales, Sydney, *Grevillea* sp., 1999, P.W. Crous (CBS H-21762, holotype, ex-type culture CBS 114127 = STE-U 2919).

*Notes:* *Pestalotiopsis grevilleae* (clade 36; Fig. 5) forms a sister clade to *P. knightiae* (clade 37; Fig. 5), being distinct from the latter species in having narrower conidia. *Pestalotiopsis grevilleae* has overlapping conidial dimensions with *P. australasiae* (clade 39; Fig. 5), although their basal cells are distinct. In *P. grevilleae* the basal cells are conic, while in *P. australasiae* they are obconic to hemispherical. Furthermore, phylogenetic analyses (Fig. 5) indicate that the two species are genetically distinct.

***Pestalotiopsis hawaiiensis*** Maharachch., K.D. Hyde & Crous, sp. nov. MycoBank MB809739. Fig. 29.

*Etymology:* Named after the island from where it was collected, Hawaii.

*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* fusoid, ellipsoid, straight to slightly curved, 4-septate, (26–) 27–34.5(–37) × (7–)7.5–10(–10.5) µm,  $\bar{x} \pm SD = 31.6 \pm 2 \times 8.7 \pm 0.6$  µm; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 4–8 µm long; three median cells doliform to subcylindrical, (19–)19.5–23(–25) µm long,  $\bar{x} \pm SD = 21.4 \pm 1.2$  µm, wall verruculose, concolourous 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,  $\bar{x} \pm SD = 25.3 \pm 4.1$  µm; basal appendage single, tubular, unbranched, centric, 5–11 µm long.

*Culture characteristics:* Colonies on PDA attaining 30–45 mm diam after 7 d at 25 °C, with undulate edge, whitish, sparse aerial

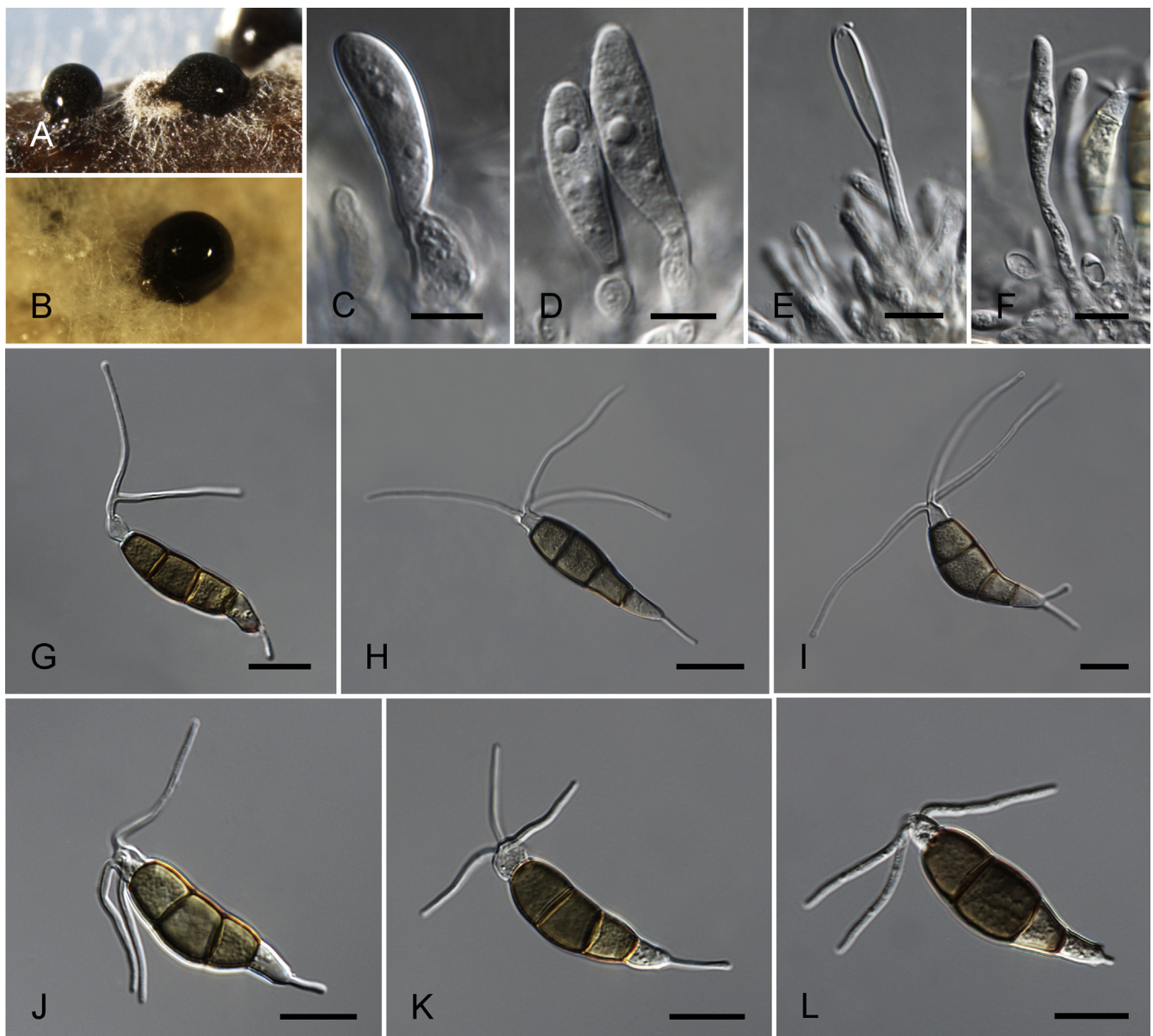


Fig. 29. *Pestalotiopsis hawaiiensis* CBS 114491<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–F. Conidiogenous cells. G–L. Conidia. Scale bars = 10 µm.

mycelium on the surface with black, gregarious conidiomata; reverse similar in colour.

*Habitat:* On *Leucospermum* sp.

*Known distribution:* USA (Hawaii).

*Material examined:* USA, Hawaii, from *Leucospermum* sp. cv. 'Coral', 9 Dec. 1999, P.W. Crous (CBS H-21761, **holotype**, ex-type culture CBS 114491 = STE-U 2215).

*Notes:* *Pestalotiopsis hawaiiensis* (clade 5; Fig. 5), known from Hawaii on *Leucospermum* sp., has overlapping conidial dimensions with *P. anacardiacearum* (27–39 × 7–10 µm; clade 6; Fig. 5), which was isolated from leaves of *Mangifera indica* in

China (Maharachchikumbura et al. 2013c). However, *P. anacardiacearum* differs from *P. hawaiiensis* by having longer apical appendages (20–45 µm). Furthermore, the two species are genetically, geographically and ecologically distinct, and thus we maintain them as two separate species.

***Pestalotiopsis hollandica*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809740. Fig. 30.

*Etymology:* Named after the *pars pro toto* name "Holland" for the country where it was collected, the Netherlands.

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

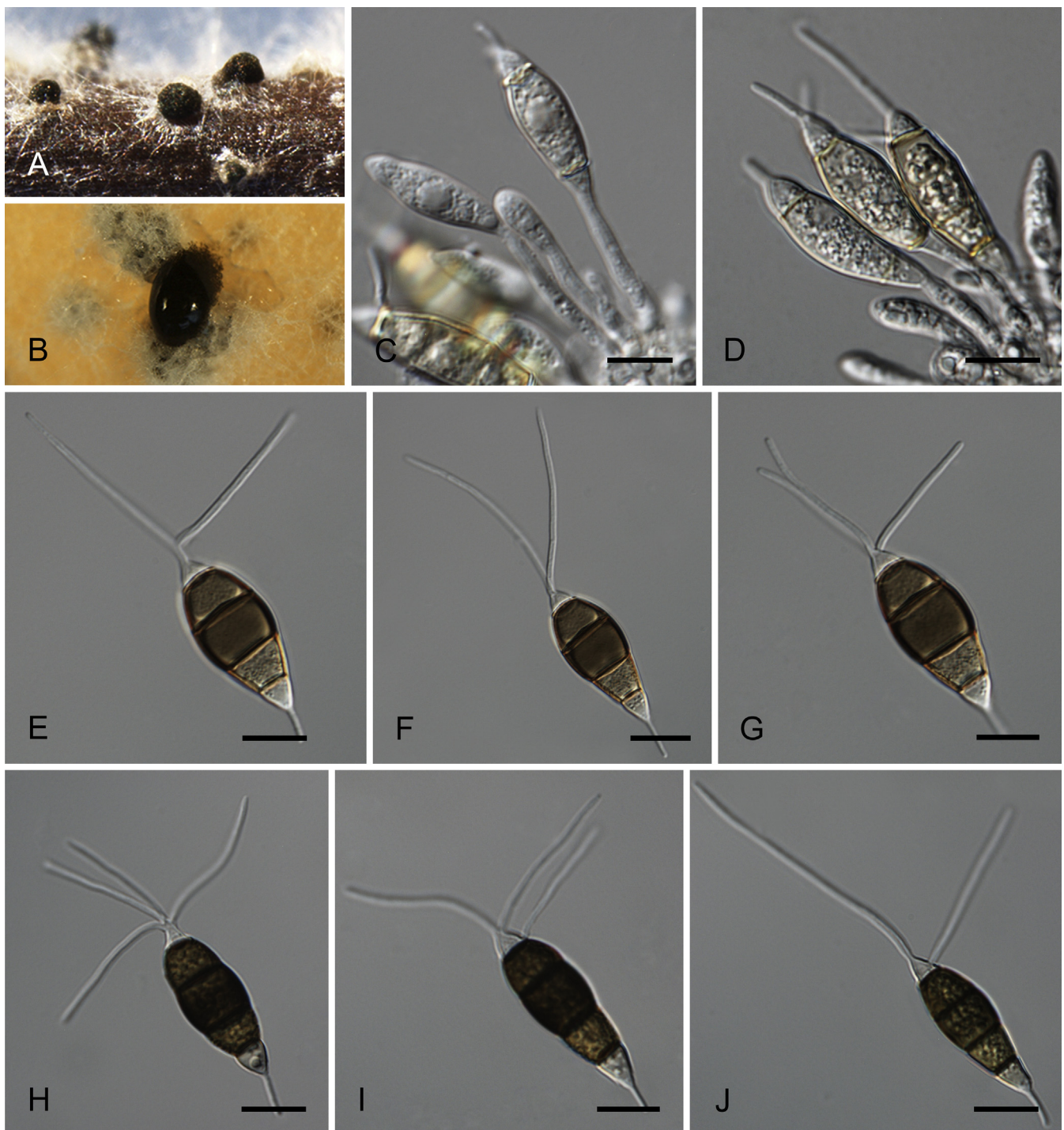


Fig. 30. *Pestalotiopsis hollandica* CBS 265.33<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm.

black; exuding dark brown conidial masses. *Conidiophores* septate, branched at base, sometimes reduced to conidiogenous cells, hyaline, smooth-walled, up to 30 µm long. *Conidiogenous cells* discrete, cylindrical, proliferating 2–5 times percurrently near apex, tapering to a long, thin neck, collarete 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,  $\bar{x} \pm SD = 28 \pm 2 \times 9.4 \pm 0.3$  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;  $\bar{x} \pm SD = 28 \pm 2 \times 9.4 \pm 0.3$  µm, doliform, thick-walled, verruculose, concolourous, 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,  $\bar{x} \pm SD = 27 \pm 1.5$  µm; basal appendage single, tubular, unbranched, centric, 3–9 µm long.

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

**Habitat:** On *Sciadopitys verticillata*.

**Known distribution:** Netherlands.

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

**Notes:** *Pestalotiopsis hollandica* (clade 18; Fig. 5) differs from all other related species (clades 17, 19 and 20; Fig. 5) in having some appendages that arise from different parts of the apical cell. *Pestalotiopsis hollandica* differs from *P. monochaetioides* (22–30 × 5–10 µm; no culture available for molecular study), which was isolated from a dead twig of *Chamaecyparis lawsoniana* in the Netherlands (Guba 1961), by its branched, subapically attached apical appendages.

***Pestalotiopsis humus*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809727. Fig. 31.

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

*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* fusoid, ellipsoid, straight to slightly curved, 4-septate, constricted at septum, (17–)18.5–22(–23) × 5–7(–7.5) µm,  $\bar{x} \pm SD = 20 \pm 1.4 \times 6 \pm 0.4$  µm; basal cell obconic to conic with a truncate base, hyaline, minutely verruculose and thin-walled, 3.5–5.5 µm long; three median cells subcylindrical, (11.5–)12–14(–14.5) µm long,  $\bar{x} \pm SD = 12.8 \pm 0.8$  µm, wall rugose, concolourous, 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,  $\bar{x} \pm SD = 9.0 \pm 1.5$  µm; basal appendage single, tubular, unbranched, centric, 2–5 µm long.

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

**Habitat:** On fruits of *Ilex cinerea* and soil.

**Known distribution:** China (Hong Kong) and Papua New Guinea.

**Materials examined:** China, Hong Kong, from fruit of *Ilex cinerea*, 20 Jan. 2002, K.D. Hyde, culture CBS 115450 = HKUCC 9100. Papua New Guinea, from soil in tropical rain forest, Nov. 1995, A. Aptroot (CBS H-21760, **holotype**, ex-type culture CBS 336.97).

**Notes:** Clade 28 (Fig. 5) comprises *P. humus*, isolated from rain forest soil in Papua New Guinea and fruit of *Ilex cinerea* in Hong Kong. Sequences of *Pestalotiopsis humus* form a sister clade to *P. diploclisiae* (clade 29; Fig. 5). *Pestalotiopsis diploclisiae* differs from *P. humus* in conidial morphology, in having narrower conidia (20–28 × 5–7 µm), and longer apical appendages (10–22 µm).

### ***Pestalotiopsis inflexa*** Maharachch. & K.D. Hyde

**Material examined:** China, Hunan Province, Yizhang County, Mangshan, on living leaves of unidentified tree, 12 Apr. 2002, W.P. Wu (HMAS047098, **holotype**, MFLU 12-0413, **isotype**, ex-type culture NN0470980 = MFLUCC 12-0270).

**Note:** This species (clade 14; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

### ***Pestalotiopsis intermedia*** Maharachch. & K.D. Hyde

**Material examined:** China, Hubei Province, Shengnongjia, on dead leaves of unidentified tree, 24 Mar. 2003, W.P. Wu (HMAS047642, **holotype**, MFLU 12-0410, **isotype**, ex-type culture NN0476420 = MFLUCC 12-0259).

**Note:** This species (clade 21; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

***Pestalotiopsis jesteri*** Strobel, J. Yi Li, E.J. Ford & W.M. Hess, *Mycotaxon* 76: 260. 2000. Fig. 32.

*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; collarete flared, apex 2–5 µm diam. *Conidia* fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate, (21–)22.5–31(–34.5) × 7–9 µm,  $\bar{x} \pm SD = 26.8 \pm 3 \times 8.2 \pm 0.2$  µm; basal cell narrowly obconic with a truncate base, hyaline, thin- and smooth-walled, 4.5–6.5 µm long; three median cells doliform to subcylindrical, (15.5–)16–20(–21) µm long,  $\bar{x} \pm SD = 17.5 \pm 1.4$  µm, wall rugose, concolourous, 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,

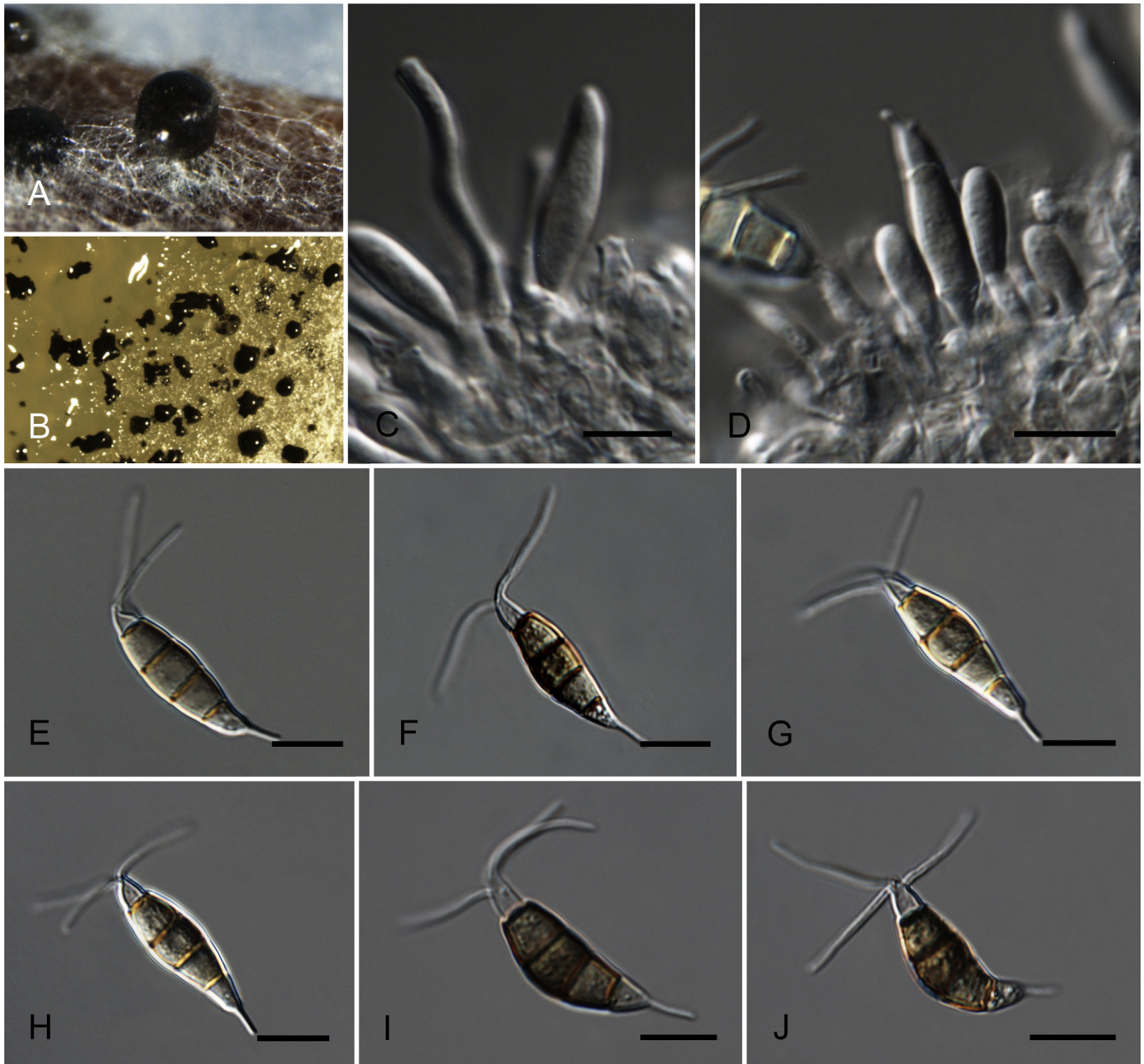


Fig. 31. *Pestalotiopsis humus* CBS 336.97<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10  $\mu$ m.

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  $\mu$ m long.

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

**Habitat:** On bark of *Fragraea bodenii*.

**Known distribution:** Papua New Guinea.

**Material examined:** Papua New Guinea, Southern Highlands, Aluak ambe village, from bark of *Fragraea bodenii*, E. Erol & G. Strobel (deposited in CBS collection Mar. 2001 by G. Strobel) (MONT Strobel 6T-L-3, **holotype**, MONT

Strobel 6M-B-3 and MONT Strobel 6B-S-4, **isotypes**, ex-type culture CBS 109350 = MONT 6M-B-3).

**Notes:** *Pestalotiopsis jesteri* (clade 1; Fig. 5) is described from bark of *Fragraea bodenii* in Papua New Guinea and is well-characterised and easily recognisable by the unique appendages attached 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. jesteri* is a basal species in the species phylogeny (Fig. 5), and forms a lineage distinct from all other species.

***Pestalotiopsis kenyana*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809741. Fig. 33.

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

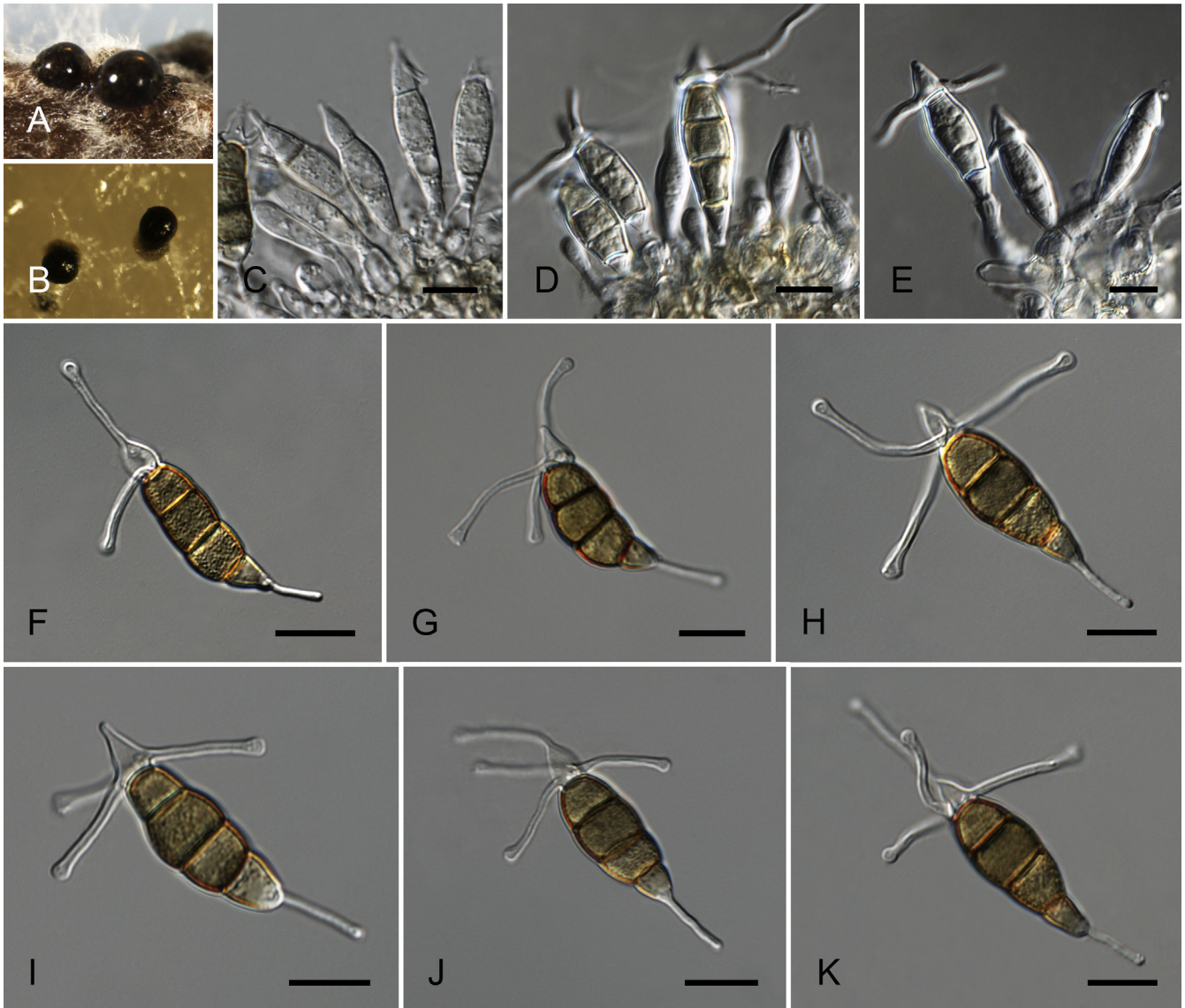


Fig. 32. *Pestalotiopsis jesteri* CBS 109350. A. Conidioma sporulating on PNA. B. Conidioma on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

*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,  $10\text{--}25 \times 2\text{--}5$  µm, apex with minute periclinal thickening and collarette. *Conidia* fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate,  $(22\text{--})23\text{--}28(\text{--}29) \times 7\text{--}9$  µm,  $\bar{x} \pm \text{SD} = 25.5 \pm 1.2 \times 8 \pm 0.4$  µm; basal cell conic to obconic with a truncate base, hyaline, minutely verruculose and thin-walled, 4–6 µm long; three median cells dolii-form,  $(15\text{--})15.5\text{--}18.5(\text{--}19)$  µm long,  $\bar{x} \pm \text{SD} = 17 \pm 0.7$  µm, wall verruculose concolourous, 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, rugose and thin-walled; with 2–3 tubular apical appendages (mostly 3), arising from the apical crest, unbranched, filiform,  $(8\text{--})9\text{--}18(\text{--}20)$  µm long,  $\bar{x} \pm \text{SD} = 14 \pm 3$  µm; two basal appendages; centric appendage tubular, flexuous, 3–20 µm long and eccentric appendage tubular, 1–4 µm long.

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

*Habitat*: On branches of *Coffea* sp., and raw material from agar-agar, kobe 1.

*Known distribution*: Kenya.

*Materials examined*: Kenya, from *Coffea* sp. branch, Oct. 1967, H. Vermeulen (CBS H-15657, **holotype**, ex-type culture CBS 442.67). **Unknown country**, from raw material from agar-agar, kobe 1 (stips), Sep. 1996, A.K. Johansen, culture CBS 911.96.

*Notes*: *Pestalotiopsis kenyana* (clade 42; Fig. 5) formed a separate clade in the phylogenetic analyses as sister to *P. trachicarpicola* (clade 43; Fig. 5). Both *P. kenyana* and *P. trachicarpicola* often have two basal appendages. *Pestalotiopsis kenyana* differs from both *P. trachicarpicola* and *P. biciliata* (clade 38; Fig. 5) in having wider conidia (see comparison under *P. biciliata*).

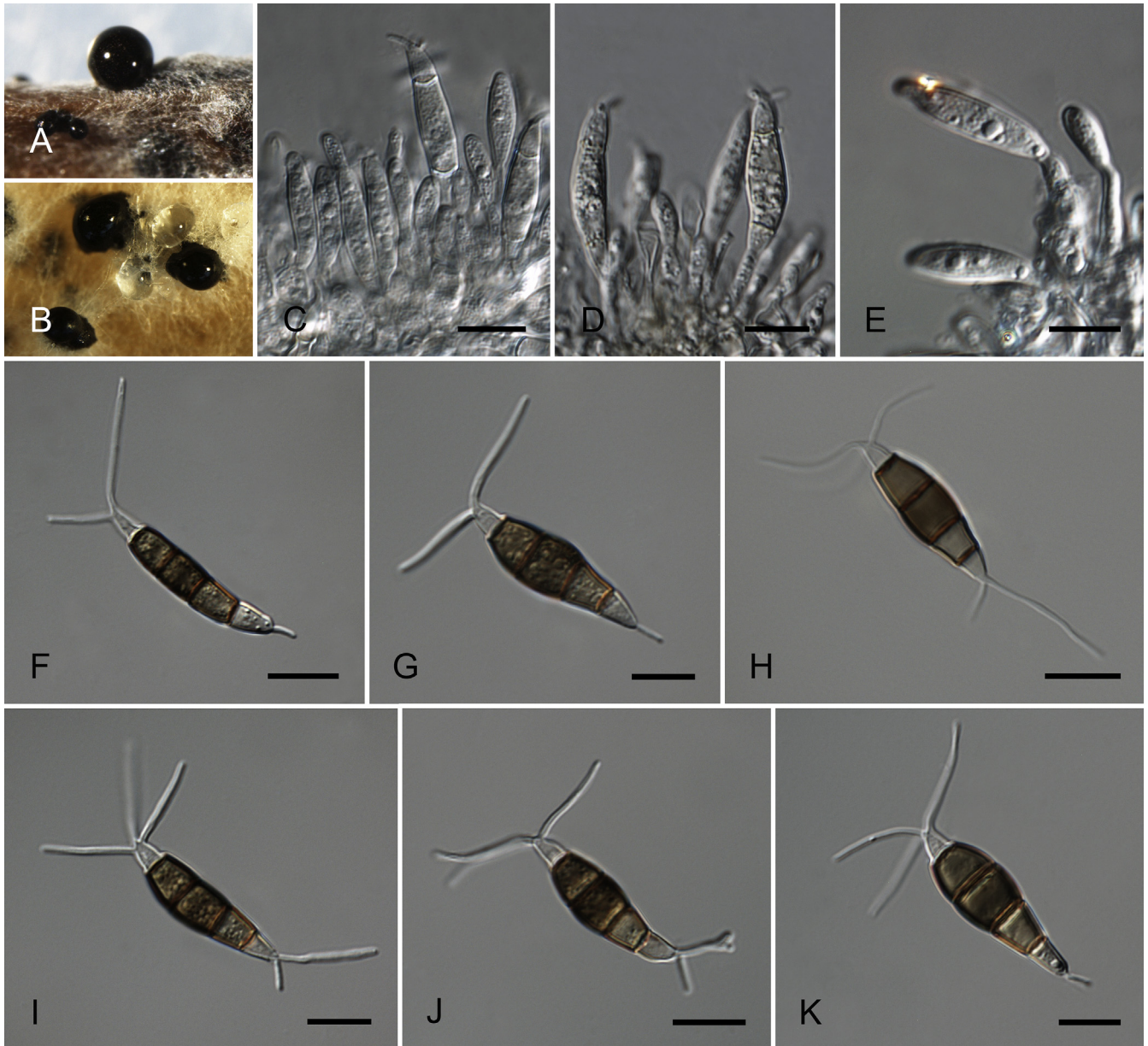


Fig. 33. *Pestalotiopsis kenyana* CBS 442.67<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

***Pestalotiopsis knightiae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809742. Fig. 34.

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

**Conidiomata** pycnidial, globose, aggregated or scattered, semi-immersed to erumpent on PDA, dark brown to black, 100–200  $\mu$ m 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  $\times$  2–10  $\mu$ m. **Conidia** fusoid, ellipsoid, straight to slightly curved, 4-septate, (21–)22–27(–29)  $\times$  (8–)8.5–10.5(–11)  $\mu$ m,  $\bar{x} \pm SD = 24.8 \pm 1.3 \times 9.6 \pm 0.4 \mu$ m; basal cell obconic to conic with a truncate base, hyaline, thin- and smooth-walled, 3–6.5  $\mu$ m long; three median cells doliform, (15.5–)16–18.5(–19.5)  $\mu$ m long,  $\bar{x} \pm SD = 17.4 \pm 1.2 \mu$ m, wall minutely rugose, concolourous, pale brown, septa darker than the rest of the cell (second cell from the base 5.5–7  $\mu$ m long; third cell

6–7.5  $\mu$ m long; fourth cell 5.5–7  $\mu$ m long); apical cell 3–4.5(–5)  $\mu$ m 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)  $\mu$ m long,  $\bar{x} \pm SD = 15 \pm 3.9 \mu$ m; basal appendage single, tubular, unbranched, centric, 2.5–7.5  $\mu$ m long.

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

**Habitat:** On *Knightia* sp.

**Known distribution:** New Zealand.

**Materials examined:** **New Zealand**, from *Knightia* sp., 1999, P.W. Crous (CBS H-21759, **holotype**, ex-type culture CBS 114138 = STE-U 2906); Tamaki, Maori Village, from *Knightia* sp., 1999, P.W. Crous, culture CBS 111963 = STE-U 2905.



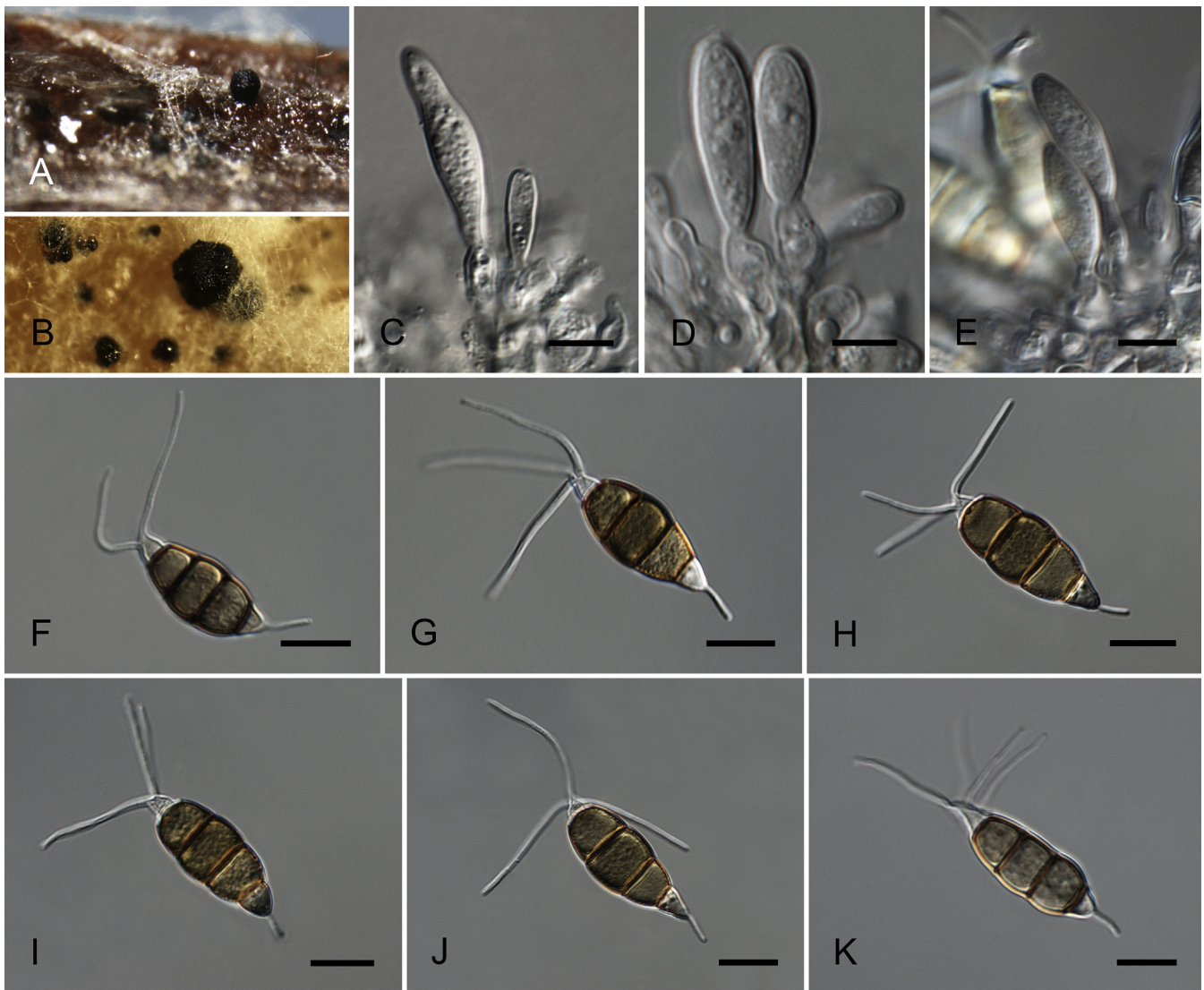


Fig. 34. *Pestalotiopsis knightiae* CBS 114138<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

*Notes:* *Pestalotiopsis knightiae* (clade 37; Fig. 5) is a species occurring on *Knightsia* sp. in New Zealand, and is distinct from other morphologically closely related species (clades 36 and 38; Fig. 5) based on its DNA phylogeny. It forms a sister clade with *P. grevilleae* (clade 36; Fig. 5), and is distinguishable from other phylogenetically closely related species by its wider conidia.

#### *Pestalotiopsis linearis* Maharachch. & K.D. Hyde

*Material examined:* China, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Trachelospermum* sp., 19 Mar. 2002, W.P. Wu (HMAS047190, holotype, MFLU 12-0414, isotype, ex-type culture NN0471900 = MFLUCC 12-0271).

*Note:* This species (clade 22; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

*Pestalotiopsis malayana* Maharachch., K.D. Hyde & Crous, *sp. nov.* MycoBank MB809743. Fig. 35.

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

*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* fusoid, ellipsoid, straight to slightly curved, slightly constricted at septa, 4-septate, (20.5–) 22–29.5(–31) × 5–7.5 µm,  $\bar{x} \pm SD = 25.6 \pm 2 \times 6.3 \pm 0.4$  µm; basal cell obconic to conic with a truncate base, hyaline, minutely verruculose and thin-walled, 3.5–7.5 µm long; three median cells doliiform, 15–18 µm long,  $\bar{x} \pm SD = 16.5 \pm 0.8$  µm, wall minutely verruculose, concolourous, 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,  $\bar{x} \pm SD = 15.1 \pm 1.4$  µm; basal appendage single, tubular, unbranched, centric, 2–5 µm long.

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

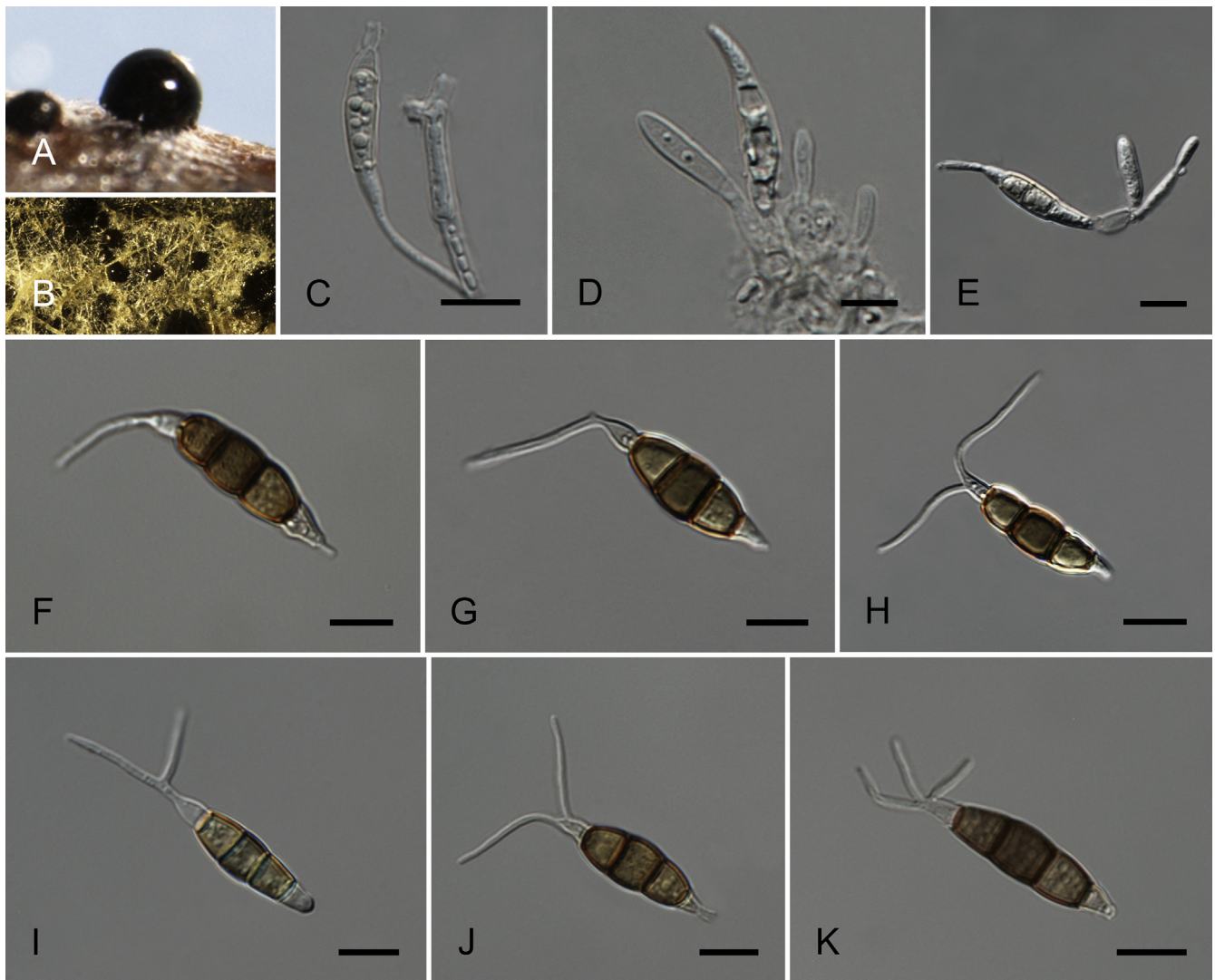


Fig. 35. *Pestalotiopsis malayana* CBS 102220<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10  $\mu$ m.

**Habitat:** On stem of *Macaranga triloba* colonised by ants.

**Known distribution:** Malaysia.

**Material examined:** Malaysia, from stem of *Macaranga triloba* colonised by ants, Sep. 1999, W. Federle (CBS H-21758, **holotype**, ex-type culture CBS 102220).

**Notes:** Clade 30 (Fig. 5) represents *Pestalotiopsis malayana* (CBS 102220), which is characterised by having two apical appendages. *Pestalotiopsis malayana* formed a distinct lineage in the phylogenetic analyses from its closely related species *P. adusta* (clade 31; Fig. 5), *P. papuana* (clade 32; Fig. 5) and *Pestalotiopsis* sp. Clade 33 (clade 33; Fig. 5). Furthermore, morphologically *P. malayana* is well distinguished from allied species by its larger conidia and longer apical appendages.

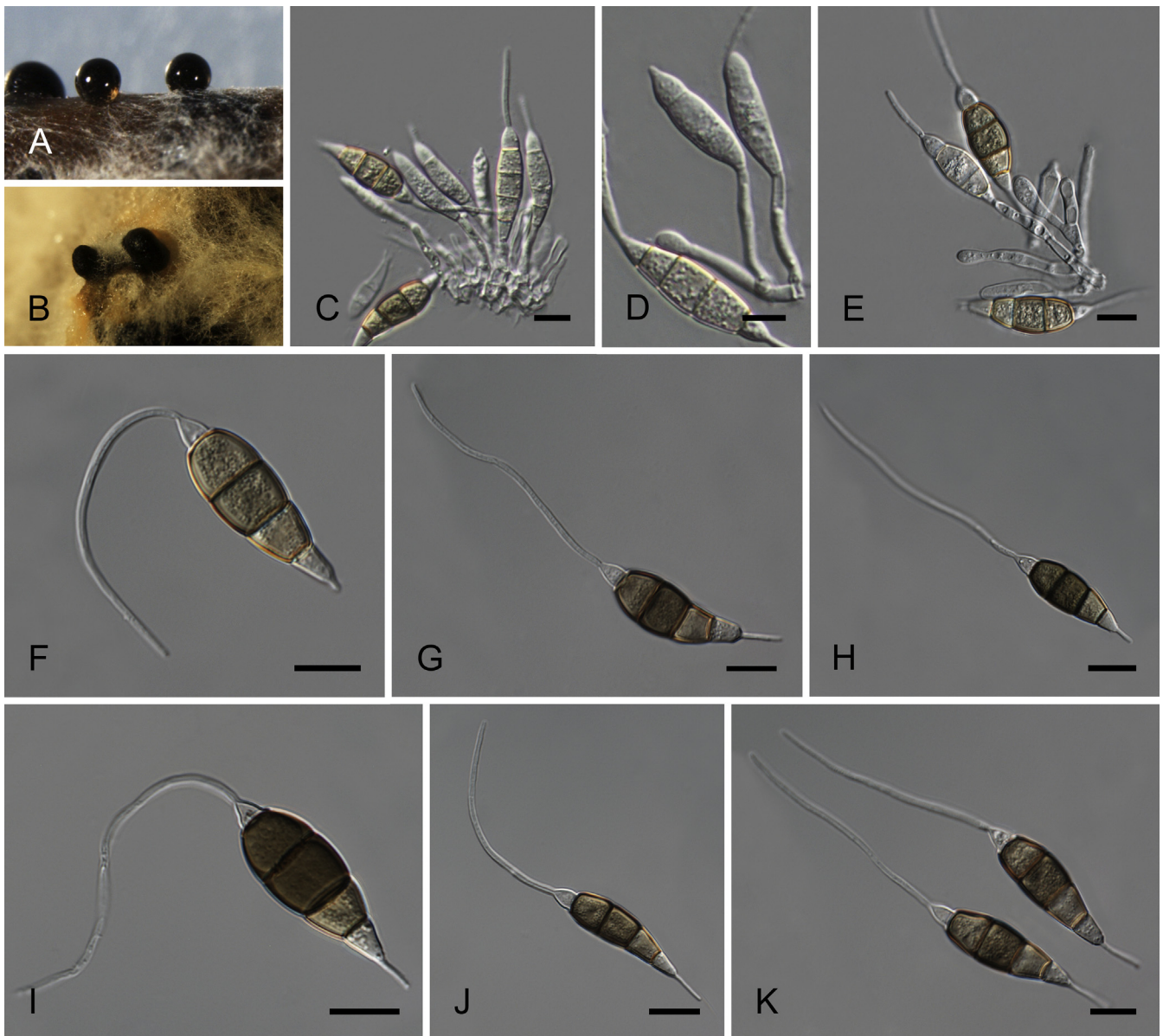
***Pestalotiopsis monochaeta*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809744. Fig. 36.

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

**Conidiomata** pycnidial in culture on PDA, globose or clavate, aggregated or scattered, semi-immersed or partly erumpent, 250–500  $\mu$ m 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  $\mu$ m long. **Conidiogenous cells** discrete or integrated, ampulliform to lageniform (4–12  $\times$  2–4  $\mu$ m) or cylindrical (10–60  $\times$  2–8  $\mu$ m), 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)  $\times$  7–11 (–11.5)  $\mu$ m,  $\bar{x} \pm SD = 32.8 \pm 3.5 \times 9.6 \pm 0.6 \mu$ m; basal cell conic to obconic with a truncate base, rugose and thin-walled, 5.5–9.5  $\mu$ m long; three median cells (17–)18–25(–26)  $\mu$ m,  $\bar{x} \pm SD = 21 \pm 2 \mu$ m, doliiform, verruculose, concolourous, but occasionally the two upper median cells slightly darker than the lower median cell (second cell from base 5–8.5  $\mu$ m long; third cell 7–9  $\mu$ m long; fourth cell 7–9  $\mu$ m long); apical cell conic, hyaline, thin- and smooth-walled, 4–6.5  $\mu$ m long; with single, central, tubular apical appendage, unbranched, filiform, (40–)43–67(–75)  $\mu$ m,  $\bar{x} \pm SD = 51 \pm 6 \mu$ m; basal appendage single, tubular, unbranched, centric, 6–14  $\mu$ m long.

**Culture characteristics:** Colonies on PDA reaching 50–60 mm diam after 7 d at 25  $^{\circ}$ C, with undulate edge, whitish to pale yellow-coloured, with dense, with aerial mycelium on surface, with black, gregarious conidiomata; reverse similar in colour.



**Fig. 36.** *Pestalotiopsis monochaeta* CBS 144.97<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

**Habitat:** On *Taxus baccata* and endophytic in branches of *Quercus robur*.

**Known distribution:** Netherlands.

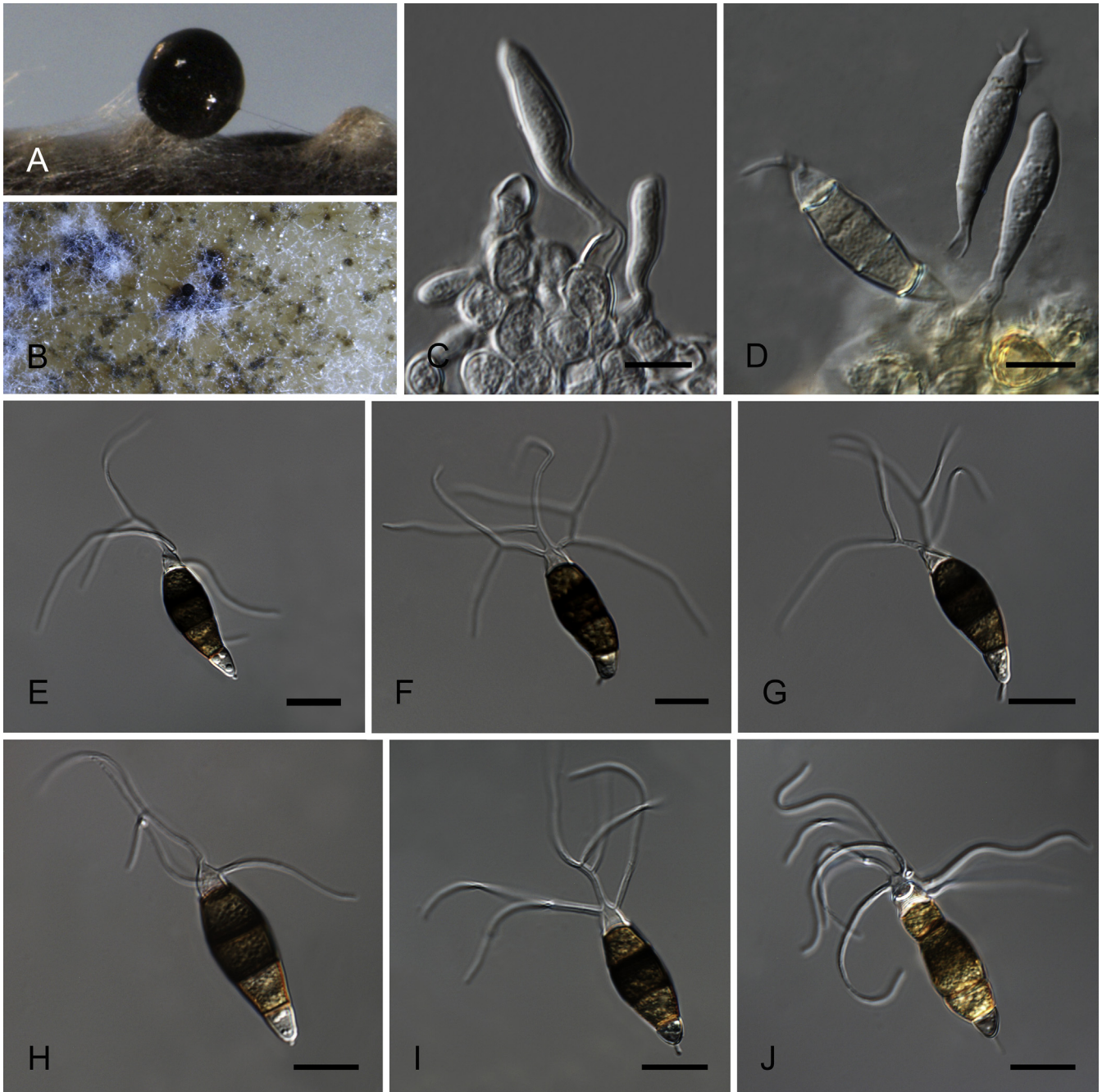
**Materials examined:** Netherlands, Baarn, Eemnesserweg, endophytes on branches of *Quercus robur*, Jul. 1996, H.A. van der Aa (CBS H-21757, **holotype**, ex-type culture CBS 144.97); Baarn, Eemnesserweg 90, from *Taxus baccata*, 14 Apr. 1983, H.A. van der Aa, CBS H-14560, culture CBS 440.83 = IFO 32686.

**Notes:** *Pestalotiopsis monochaeta* (clade 17; Fig. 5) differs from all other species in the genus in having a single apical appendage. *Pestalotiopsis brassicae* (clade 19; Fig. 5), *P. hollandica* (clade 18; Fig. 5) and *P. verruculosa* (clade 20; Fig. 5) are closely related species, but have conidia with more than two apical appendages. This species can easily be misidentified as *Monochaetia*, since it has borderline morphological features of both genera.

***Pestalotiopsis novae-hollandiae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809745. Fig. 37.

**Etymology:** Named after the historical European name, New Holland or Hollandia Nova, for the country where it was collected, Australia.

**Conidiomata** (on PDA) pycnidial, globose to clavate, solitary to aggregated, embedded 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,  $\bar{x} \pm SD = 28.1 \pm 1.6 \times 9 \pm 0.7$  µm; basal cell obconic with truncate base, hyaline or slightly olivaceous, rugose and thin-walled, 4–7 µm long; three median cells (16–)16.5–20.5(–21) µm long,  $\bar{x} \pm SD = 19 \pm 1.3$  µm, doliform to subcylindrical, verruculose, concolourous, 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



**Fig. 37.** *Pestalotiopsis novae-hollandiae* CBS 130973<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–J. Conidia. Scale bars = 10 µm.

locus in the upper half of the cell, unequal in length, some appendages branched, filiform, flexuous, (20–)22–44(–50) µm long,  $\bar{x} \pm SD = 31 \pm 9$  µm; basal appendage single, tubular, unbranched, centric, 2–5 µm long.

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

**Habitat:** On old inflorescence of *Banksia grandis*.

**Known distribution:** Australia.

**Material examined:** Australia, Perth, Jarrah Forest, from old inflorescence of *Banksia grandis*, 2010, W. Gams (CBS H-21756, **holotype**, ex-type culture CBS 130973).

**Notes:** This species (clade 11; Fig. 5) is characterised by a large number of apical appendages and in having a short basal appendage. Species such as *P. camelliae* (clade 13; Fig. 5) and *P. furcata* (clade 12; Fig. 5) have as large a number of apical appendages as *P. novae-hollandiae*, but they lack a basal appendage. *Pestalotiopsis novae-hollandiae* is sister to *P. portugalica* (clade 10; Fig. 5), which has rather smaller conidia (15–21 × 5–7 µm), and few apical appendages (1–3).

***Pestalotiopsis oryzae* Maharachch., K.D. Hyde & Crous, sp. nov.** MycoBank MB809746. Fig. 38.

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

**Conidiomata** pycnidial in culture on PDA, globose to clavate, aggregated or scattered, dark brown to black, semi-immersed or

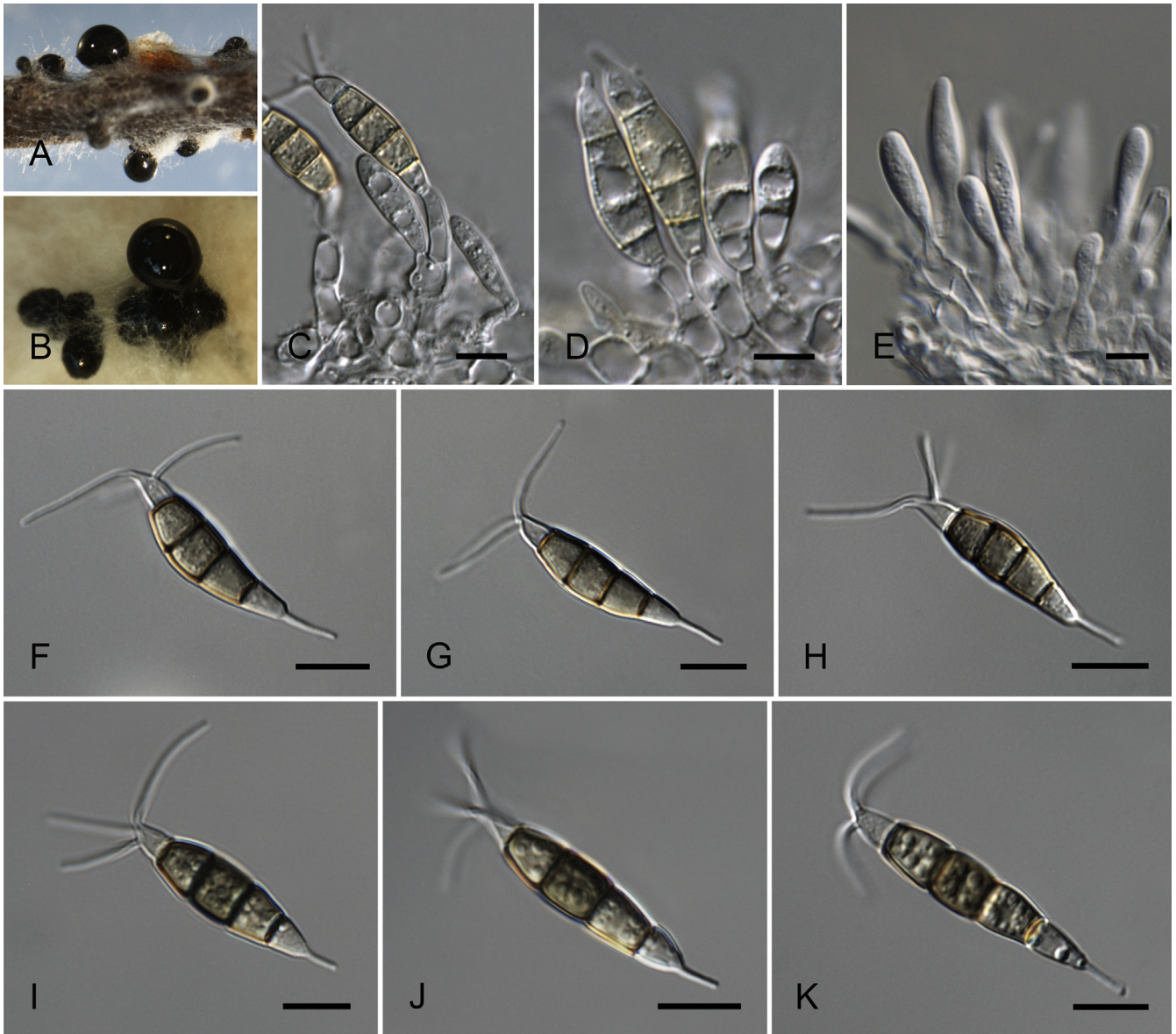


Fig. 38. *Pestalotiopsis oryzae* CBS 353.69<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

partially erumpent, up to 300  $\mu$ m diam; releasing globose, dark brown to black conidial masses. *Conidiophores* sparsely septate at base, branched or unbranched, subcylindrical, hyaline, smooth, up to 20  $\mu$ m. *Conidiogenous cells* discrete, ampulliform to lageniform, hyaline, smooth, proliferating 2–5 times percurrently, 10–25  $\times$  3–7  $\mu$ m. *Conidia* fusoid, ellipsoid to subcylindrical, straight to slightly curved, 4-septate, (23–) 24.5–29(–30)  $\times$  6–8  $\mu$ m,  $\bar{x} \pm SD = 26.9 \pm 1.4 \times 7 \pm 0.2 \mu$ m; basal cell obconic to conic with a truncate base, hyaline, verruculose and thin-walled, 4.5–6.5  $\mu$ m long; three median cells doliiform, (14–)16–18.5(–19)  $\mu$ m long,  $\bar{x} \pm SD = 17 \pm 1.3 \mu$ m, wall minutely verruculose, concolourous 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  $\mu$ m; third cell 5.5–7  $\mu$ m; fourth cell 5–6.5  $\mu$ m); apical cell 3.5–5  $\mu$ m 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 (9–) 18–27(–17)  $\mu$ m long,  $\bar{x} \pm SD = 12.9 \pm 1.7 \mu$ m; basal appendage single, tubular, unbranched, centric, 3–6  $\mu$ m long.

*Culture characteristics*: Colonies on PDA attaining 35–45 mm diam after 7 d at 25  $^{\circ}$ C, with undulate edge, convex with papillate surface, hyaline to pale honey-coloured, with sparse aerial mycelium on the surface with black, gregarious conidiomata; reverse pale honey-coloured.

*Habitat*: On *Telopea* sp. and seeds of *Oryza sativa*.

*Known distribution*: Denmark, Italy and USA.

*Materials examined*: **Denmark**, from seeds of *Oryza sativa*, S.B. Mathur (CBS H-15697, **holotype**, ex-type culture CBS 353.69). **Italy**, unknown substrate, Dec. 1926, R. Ciferri, culture CBS 171.26. **USA**, Hawaii, from *Telopea* sp. (introduced from Australia), 8 Dec. 1998, P.W. Crous & M.E. Palm, CBS H-21753, culture CBS 111522 = STE-U 2083.

*Notes*: Clade 41 (Fig. 5) consists of three isolates of *P. oryzae*, including the ex-type strain (CBS 353.69) isolated from seeds of *Oryza sativa* from Denmark. *Pestalotiopsis oryzae* has overlapping conidial characters with *P. kenyana* (clade 42; Fig. 5) and *P. trachicarpicola* (clade 43; Fig. 5). However, *P. oryzae* is

genetically distinct and has a different geographic distribution from these two species.

***Pestalotiopsis papuana*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809747. Fig. 39.

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

**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** fusoid, ellipsoid, straight to slightly curved, 4-septate, (17–)18–22(–24) × 6–7.5 µm,  $\bar{x} \pm SD = 20.5 \pm 1.5 \times 6.7 \pm 0.3$  µm; basal cell obconic with a truncate base, hyaline, verruculose and thin-walled, 3–5 µm long; three median cells doliiform, 12–15 µm long,  $\bar{x} \pm SD = 13.6 \pm 0.7$  µm, wall verruculose, concolourous, 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, rugose and thin-walled; with 1–2 tubular apical appendages, arising from the apical crest, unbranched, filiform, 1.5–7 µm long,  $\bar{x} \pm SD = 4.1 \pm 1$  µm; basal appendage single, tubular, unbranched, centric, 0.5–2 µm long.

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

**Habitat:** On coastal soil and leaves of *Cocos nucifera*.

**Known distribution:** Papua New Guinea.

**Materials examined:** **Papua New Guinea**, from soil along the coast, Nov. 1995, A. Aptroot (CBS H-21755, **holotype**, ex-type culture CBS 331.96); from leaves of *Cocos nucifera* (coastal primary forest), 27 Oct. 1995, A. Aptroot, culture CBS 887.96.

**Notes:** *Pestalotiopsis papuana* (clade 32; Fig. 5) is genetically close to *P. adusta* (clade 31; Fig. 5) and two isolates representing *Pestalotiopsis* sp. Clade 33 (clade 33; Fig. 5). The latter two isolates are unnamed for the present since both cultures were

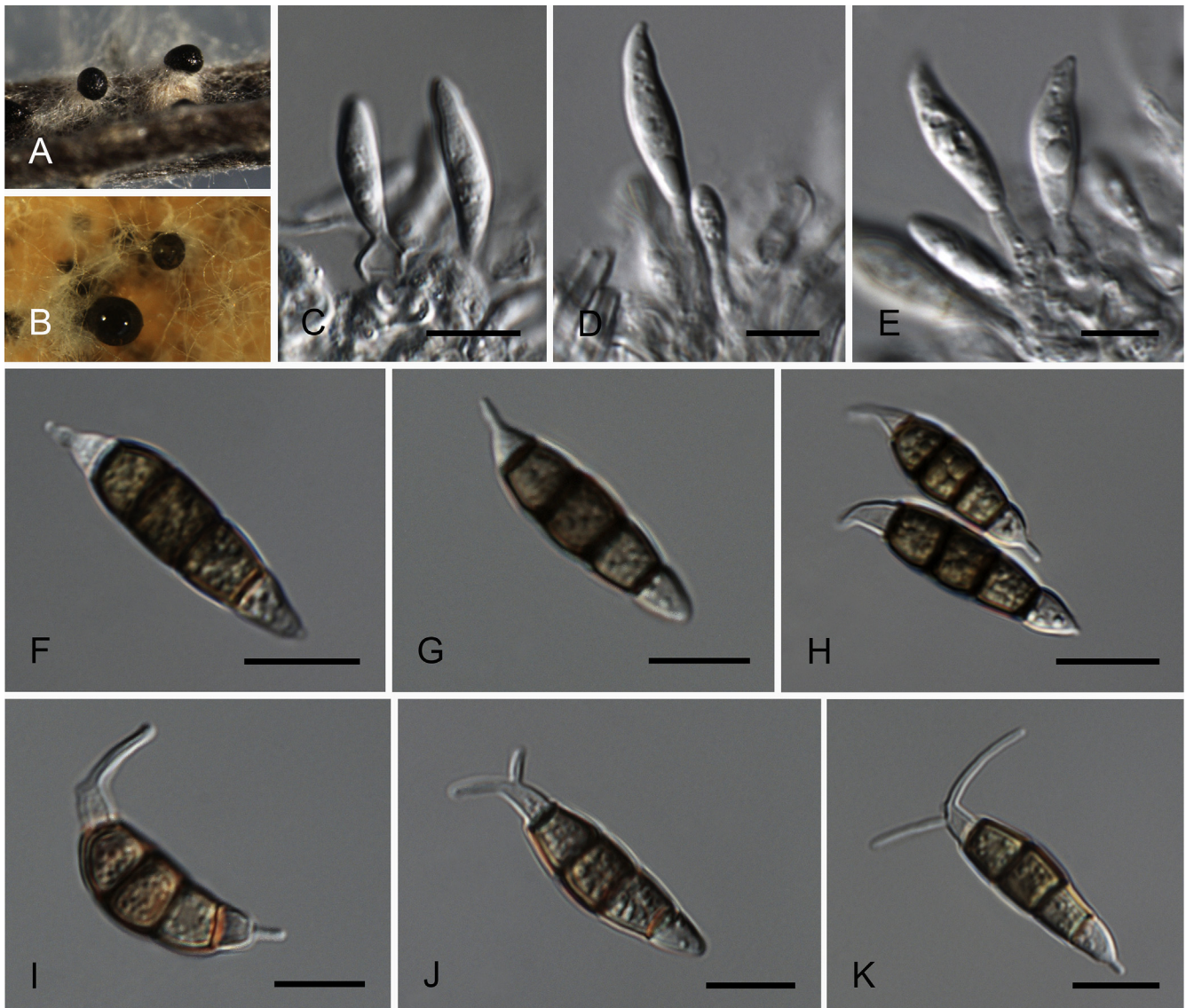


Fig. 39. *Pestalotiopsis papuana* CBS 331.96<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

sterile, making morphological comparisons impossible (see notes under *Pestalotiopsis* sp. Clade 33). Morphologically, however, *P. papuana* is quite distinct from *P. adusta* in having larger conidia and shorter apical appendages.

***Pestalotiopsis parva*** Maharachch., K.D. Hyde & Crous, sp. nov. MycoBank MB809748. Fig. 40.

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

**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** fusoid, straight to slightly curved, 4-septate, (16–)16.5–20(–21) × 5–7(–7.5) µm,  $\bar{x} \pm SD = 18.3 \pm 1.2 \times 6.2 \pm 0.5$  µm; basal cell obconic to conic with a truncate base, hyaline, thin- and smooth-walled, 3–5 µm

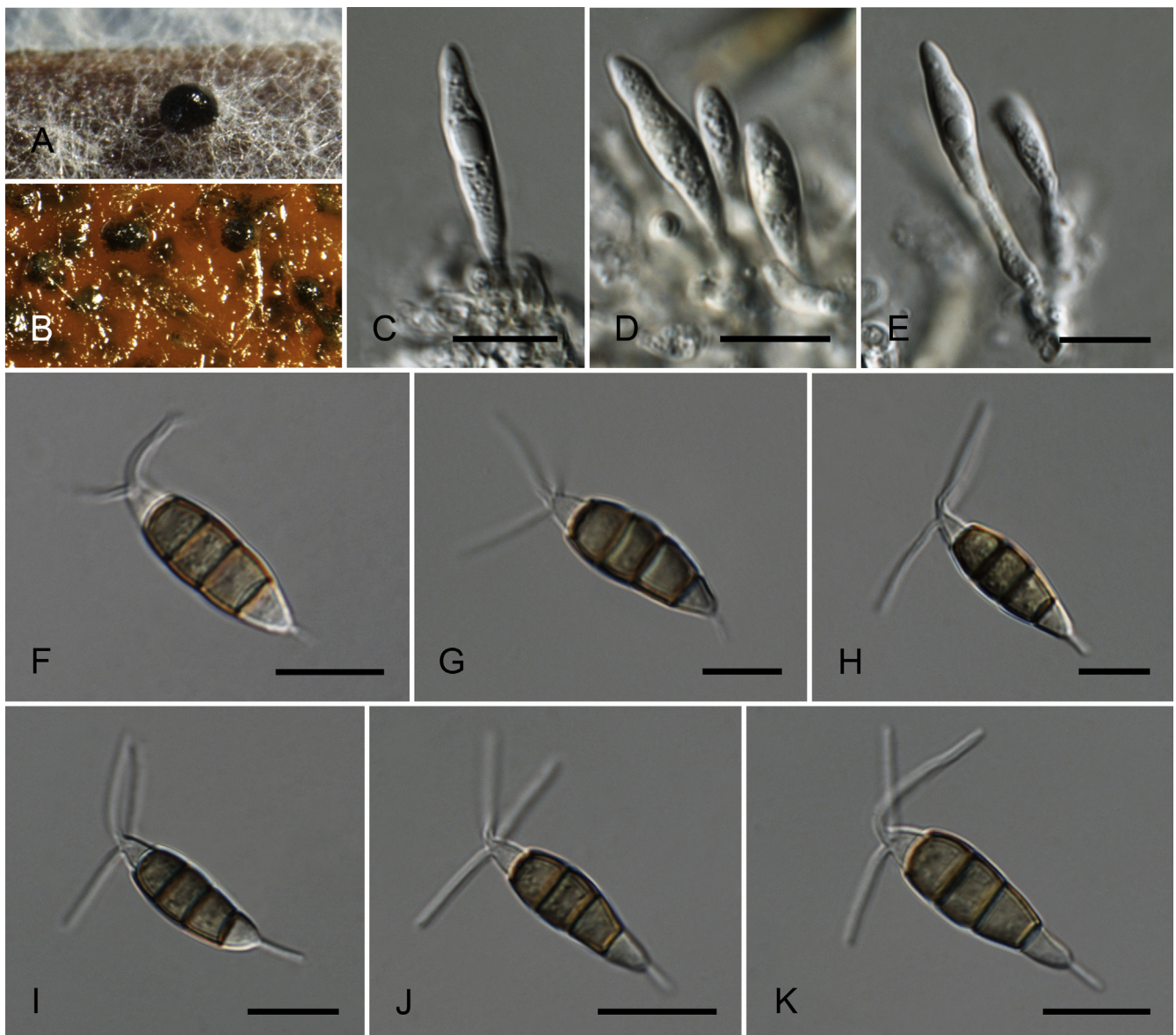
long; three median cells doliform, (10–)10.5–13(–13.5) µm long,  $\bar{x} \pm SD = 12.1 \pm 1.0$  µm, wall minutely verruculose, concolourous, pale brown, septa darker than the rest of the cell (second cell from the base 3.5–5 µm long; third cell 3.5–4.5 µm long; fourth cell 4–5 µm long); apical cell (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,  $\bar{x} \pm SD = 9.0 \pm 1.9$  µm; basal appendage single, tubular, unbranched, centric, 2–4 µm long.

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

**Habitat:** On *Delonix regia* and *Leucothoe fontanesiana*.

**Known distribution:** Unknown.

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



**Fig. 40.** *Pestalotiopsis parva* CBS 278.35<sup>T</sup>. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–D. Conidiogenous cells. E–I. Conidia. Scale bars = 10 µm.

*Notes:* *Pestalotiopsis parva* is a distinct species represented by two isolates (clade 35; Fig. 5). *Pestalotiopsis rosea* (clade 34; Fig. 5), which is an endophyte isolated from living leaves of *Pinus* sp. in China, is a sister species. Although these two species are morphologically similar, they differ in having distinctly longer apical appendages, which are sometimes branched. Furthermore, the reddish colony is unique to *P. rosea* and this reddish colour can be seen even in conidiogenous cells and some conidia.

***Pestalotiopsis portugalica*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809749. Fig. 41.

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

*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 in 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,  $\bar{x} \pm SD = 17.9 \pm 1.6 \times 6.0 \pm 0.5$  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,  $\bar{x} \pm SD = 11.7 \pm 1$  µm, doliiform to subcylindrical, with thick verruculose walls, constricted at the septa, concolourous, 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,  $\bar{x} \pm SD = 14 \pm 3$  µm; basal appendage lack or when present single, tubular, unbranched, centric, 1–4 µm long.

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

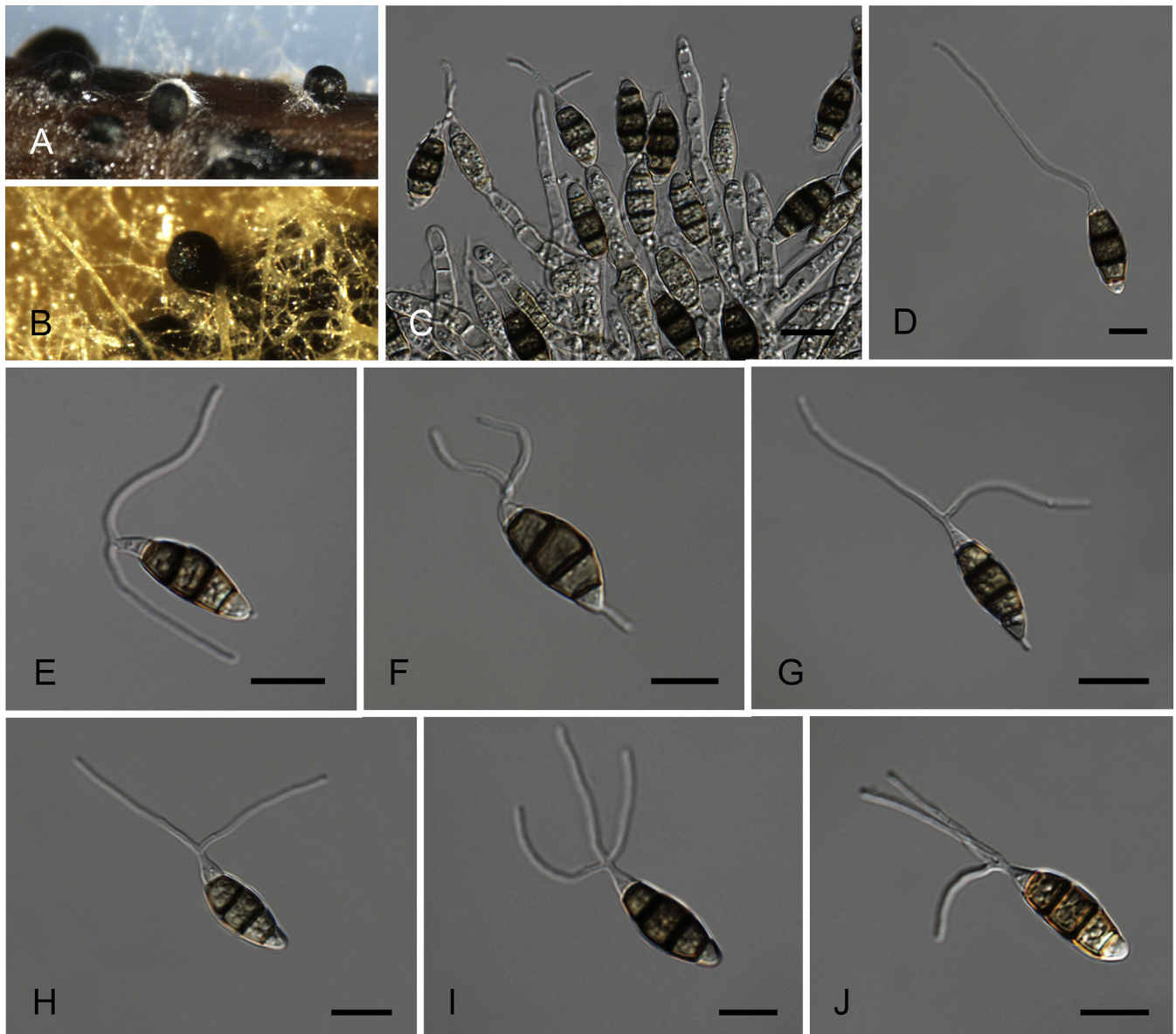


Fig. 41. *Pestalotiopsis portugalica* CBS 393.48<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C. Conidiogenous cells. D–J. Conidia. Scale bars = 10 µm.



*Habitat:* Unknown.

*Known distribution:* Portugal.

*Material examined:* Portugal, unknown host, Jun. 1948, collector unknown (CBS H-21754, **holotype**, ex-type culture CBS 393.48).

*Notes:* *Pestalotiopsis portugalica* (clade 10; Fig. 5) is a distinct species in terms of morphology and phylogeny. It differs from its phylogenetically related species *P. camelliae* (clade 13; Fig. 5), *P. furcata* (clade 12; Fig. 5) and *P. novae-hollandiae* (clade 11; Fig. 5) by smaller conidia and fewer apical appendages. Its conidial size overlaps with *P. rosea* (17.5–21.8 × 5.7–7 µm; clade 34; Fig. 5), but those two species are phylogenetically distinct.

***Pestalotiopsis rhododendri*** Y.M. Zhang, Maharachch. & K.D. Hyde

*Material examined:* China, Yunnan Province, Chuxiong, Zixishan, leaf spots on *Rhododendron sinogrande*, May 2011, Y.M. Zhang (IFRD 410-018, **holotype**, ex-type culture IFRDCC 2399).

*Note:* This species (clade 16; Fig. 5) was treated in detail by Zhang *et al.* (2013).

***Pestalotiopsis rosea*** Maharachch. & K.D. Hyde

*Material examined:* China, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Pinus* sp., 19 Mar. 2002, W.P. Wu (HMAS047135, **holotype**, MFLU12 0409, **isotype**, ex-type culture NN0471350 = MFLUCC 12-0258).

*Note:* This species (clade 34; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

***Pestalotiopsis scoparia*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809750. Fig. 42.

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

*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

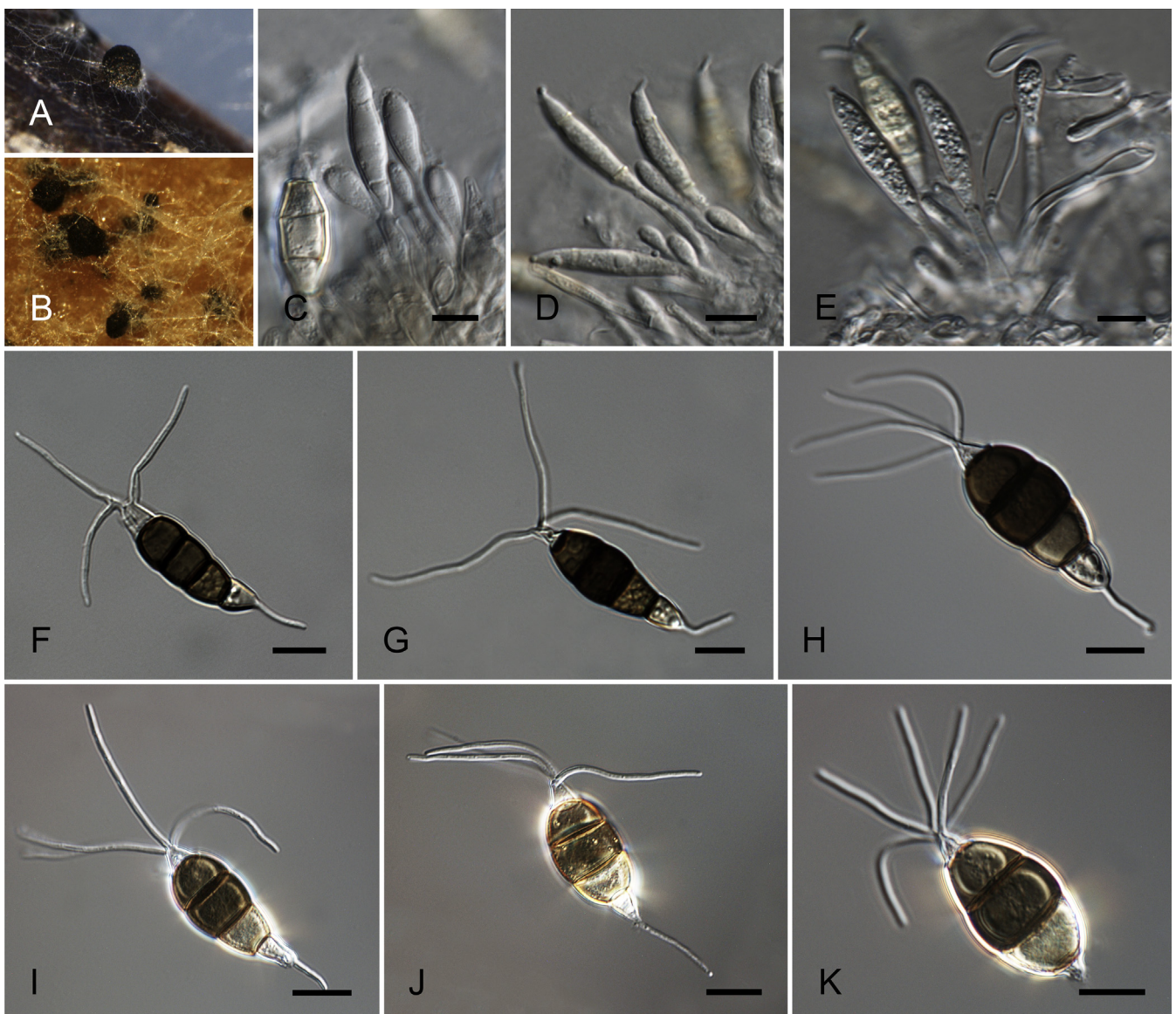


Fig. 42. *Pestalotiopsis scoparia* CBS 176.25<sup>T</sup>. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

present. *Conidia* fusoid, ellipsoid, straight to slightly curved, 4-septate, (22–)23.5–29(–31) × 6–8.5 µm,  $\bar{x} \pm SD = 26.3 \pm 2 \times 7.4 \pm 0.3$  µm; basal cell hemispherical to obconic with a truncate base, hyaline, verruculose and thin-walled, 4–6 µm long; three median cells doliiform, 15.5–19.5 µm long,  $\bar{x} \pm SD = 17 \pm 1$  µm, wall verruculose, concolourous, 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 µm long); apical cell 4.5–6 µm long, hyaline, subcylindrical, rugose and thin-walled; with 3–5 tubular apical appendages, arising from the apical crest, unbranched, filiform, (20–)23–35(–42) µm long,  $\bar{x} \pm SD = 29.6 \pm 4$  µm; basal appendage single, tubular, unbranched, centric, 9–25 µm long.

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

**Habitat:** On *Chamaecyparis* sp.

**Known distribution:** Unknown.

**Material examined:** **Unknown country**, from young *Chamaecyparis* sp. 'Retinospora', May 1925, C.M. Doyer (CBS H-21752, **holotype**, ex-type culture CBS 176.25).

**Notes:** *Pestalotiopsis scoparia* (clade 25; Fig. 5) is genetically a clearly distinct species, forming a separate clade in a sister position to *P. australis* (clade 26; Fig. 5) and *P. unicolor* (clade 24; Fig. 5). It is well characterised by its rather long broom-shaped, 3–5 apical appendages, long basal appendages and occasionally by having versicoloured median cells.

### ***Pestalotiopsis* sp. Clade 33**

**Materials examined:** **Indonesia**, Sulawesi, from leaf spot in bibit of *Cocos* sp., unknown collection date, P.M.L. Tammes, culture CBS 264.33. **Netherlands**, Boskoop, from *Rhododendron ponticum*, Mar. 1933, W.F. van Hell, culture CBS 263.33.

**Note:** Although phylogenetically distinct (clade 33; Fig. 5), both cultures of this species proved to be sterile, and thus are not treated further.

***Pestalotiopsis spathulata*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809751. Fig. 43.

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

**Conidiomata** pycnidial, globose, aggregated or scattered, semi-immersed to erumpent or embedded 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,  $\bar{x} \pm SD = 27.7 \pm 2 \times 8.6 \pm 0.3$  µm, slightly constricted at septa; basal cell conic to obconic with a truncate base, rugose and thin-walled, 5–7.5 µm long; three median cells, (13–)14–19.5(–20) µm long,  $\bar{x} \pm SD = 17.1 \pm 1.8$  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,  $\bar{x} \pm SD = 21.1 \pm 1.7$  µm; basal appendage single, tubular, unbranched, centric, 4–7 µm long.

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

**Habitat:** On leaf spot on *Gevuina avellana*.

**Known distribution:** Chile.

**Material examined:** **Chile**, leaf spot on *Gevuina avellana*, Sep. 1961, unknown collector (CBS H-21751, **holotype**, ex-type culture CBS 356.86).

**Notes:** *Pestalotiopsis spathulata* (clade 8; Fig. 5) is morphologically and phylogenetically distinct (Fig. 5). The two upper median cells in *P. spathulata* are especially darker than the lower median cell. This is also found in its sister species *P. gaultheria* (clade 9; Fig. 5). *Pestalotiopsis gaultheria* differs from *P. spathulata* in having fewer (–3), and longer apical appendages (13–54 µm).

***Pestalotiopsis telopeae*** Maharachch., K.D. Hyde & Crous, **sp. nov.** MycoBank MB809752. Fig. 44.

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

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** fusoid, ellipsoid, straight to slightly curved, 4-septate, (24–)24.5–31(–32) × 6–8 µm,  $\bar{x} \pm SD = 27 \pm 1.5 \times 7 \pm 0.3$  µm; basal cell obconic, hyaline, verruculose and thin-walled, 4.5–7 µm long; three median cells doliiform, (15–)16–18.5(–19) µm long,  $\bar{x} \pm SD = 17.1 \pm 1$  µm, wall verruculose, concolourous, 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,  $\bar{x} \pm SD = 12.6 \pm 1.7$  µm; basal appendage single, tubular, unbranched, centric, 3.5–7 µm long.

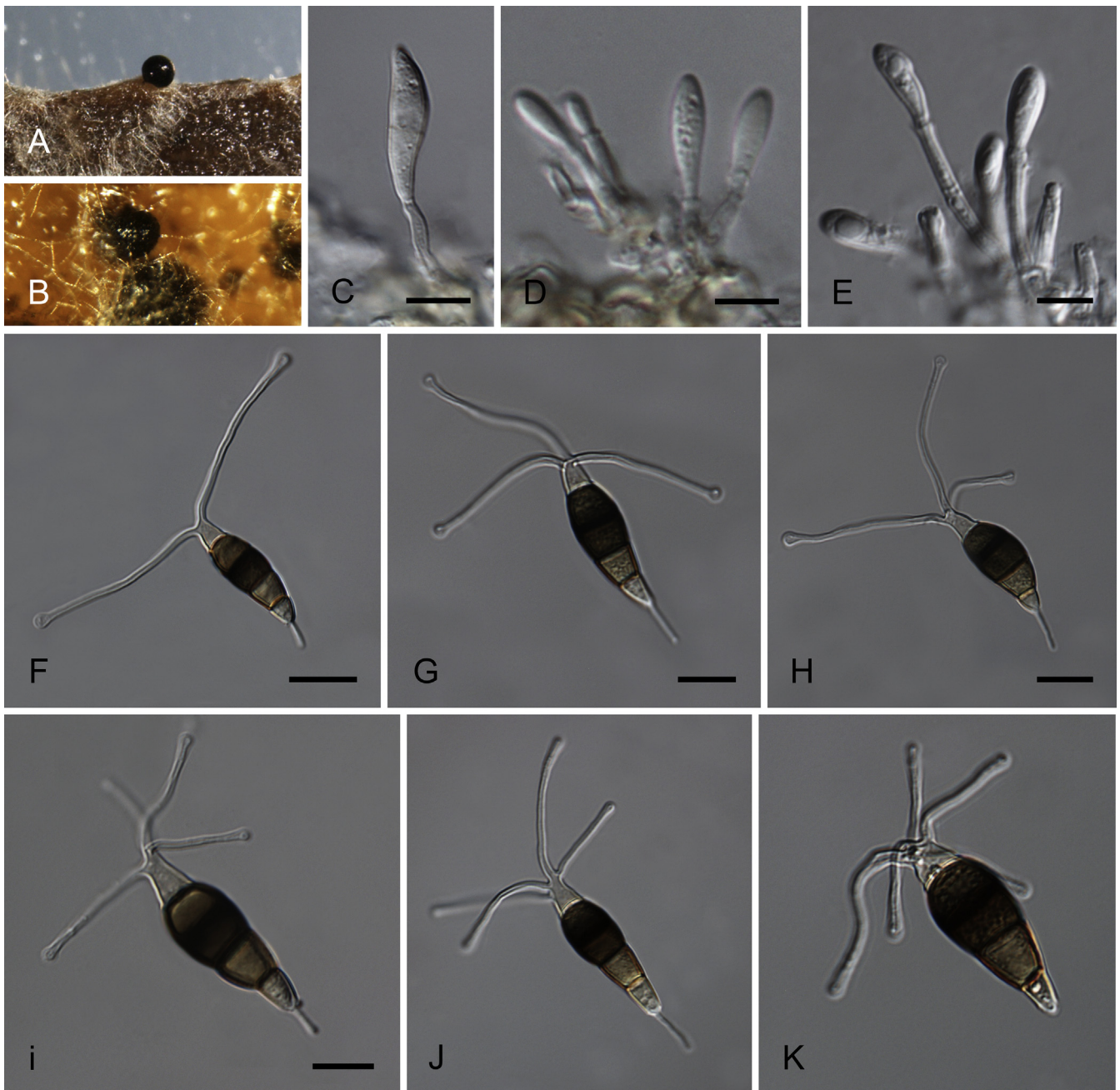


Fig. 43. *Pestalotiopsis spathulata* CBS 356.86<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10  $\mu$ m.

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

**Habitat:** On leaves of *Telopea* sp.

**Known distribution:** Australia.

**Materials examined:** **Australia**, New South Wales, Mount Annan, on leaves of *Telopea* sp., Aug. 1999, P.W. Crous, JT 975 (CBS H-21750, **holotype**, ex-type culture CBS 114161 = STE-U 3083); *ibid.*, JT 975, culture CBS 113606 = STE-U 3082; *Protea neriifolia* × *susannae* cv. 'Pink Ice', 12 Oct. 1999, P.W. Crous, culture CBS 114137 = STE-U 2952.

**Notes:** The two collections of *P. telopeae* (clade 40; Fig. 5) are morphologically most similar to *P. australasiae* (clade 39; Fig. 5), but differ in having shorter conidiogenous cells. Furthermore, in the phylogenetic analyses, *P. telopeae* represents a distinct

clade. Although no pathogenicity tests were conducted, *P. telopeae* is consistently associated with a prominent leaf spot disease of *Telopea* in Australia.

#### *Pestalotiopsis trachicarpicola* Y.M. Zhang & K.D. Hyde

**Materials examined:** **China**, Hunan Province, Yizhang County, Mangshan, on living leaves of *Schima* sp., 12 Apr. 2002, W.P. Wu, culture NN0469830 = MFLUCC 12-0265; Hunan Province, Yizhang County, Mangshan, on living leaves of *Symplocos* sp., 12 Apr. 2002, W.P. Wu, culture NN0469780 = MFLUCC 12-0266; Hunan Province, Yizhang County, Mangshan, on living leaves of unidentified tree, 12 Apr. 2002, W.P. Wu, cultures NN0470990 = MFLUCC 12-0267, NN0470720 = MFLUCC 12-0263; Yunnan Province, Dehong, Mangshi, leaf spots on *Podocarpus macrophyllus*, Sep. 2011, Y.M. Zhang, IFRD 411-018, culture IFRDCC 2403; Yunnan Province, Kunming, Kunming Botanical Gardens, leaf spots on *Trachycarpus fortunei*, Mar. 2011, K.D. Hyde OP068 (IFRD 9026, **holotype**, ex-type culture IFRDCC 2440); Yunnan Province, Kunming, on living leaves of *Chrysothallum* sp., 19 Mar. 2002, W.P. Wu, culture NN0471960 = MFLUCC 12-0264.

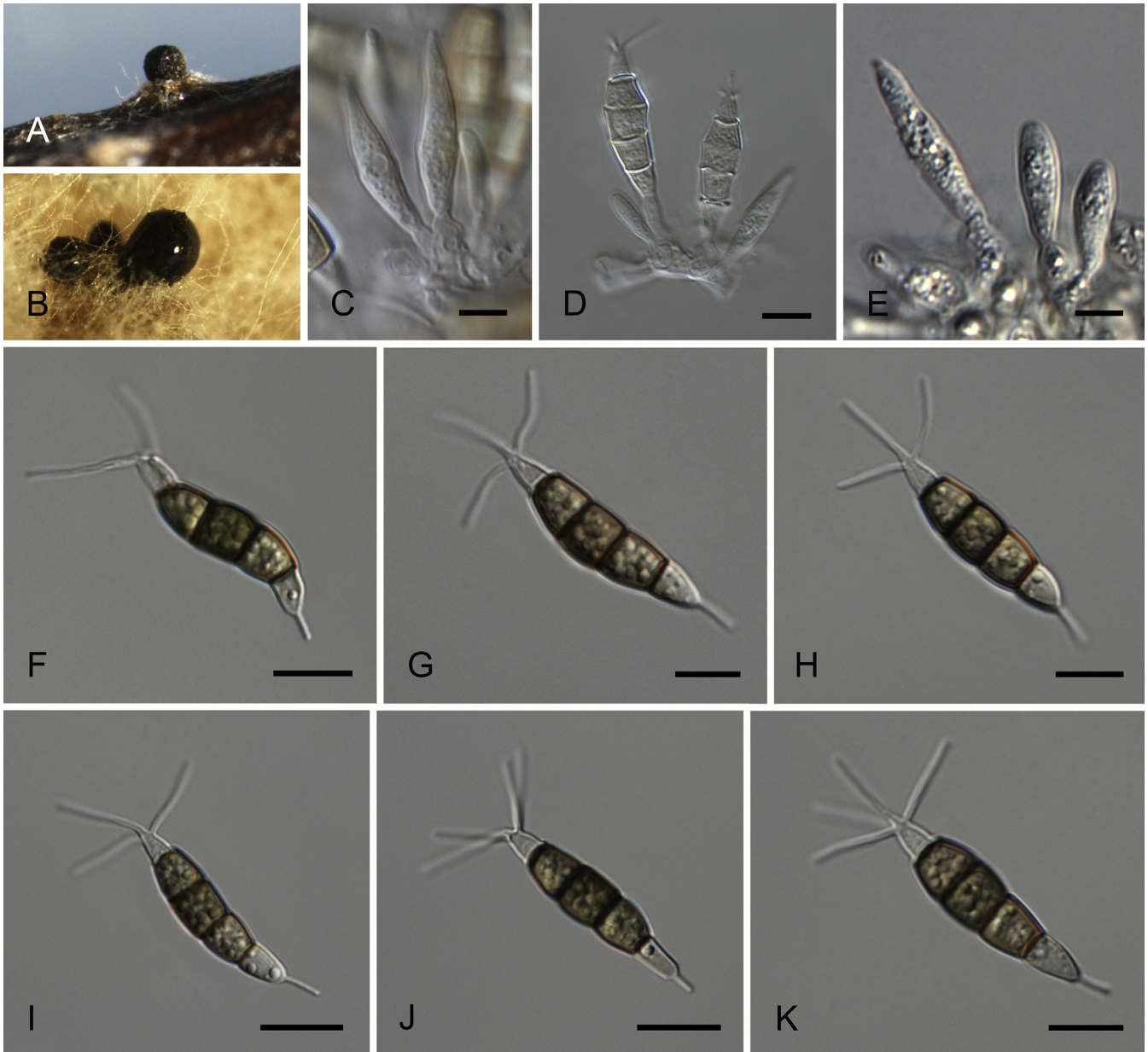


Fig. 44. *Pestalotiopsis telopeae* CBS 114161<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

Note: This species (clade 43; Fig. 5) was treated in detail by Zhang *et al.* (2012a).

***Pestalotiopsis unicolor* Maharachch. & K.D. Hyde**

Materials examined: China, Hunan Province, Yizhang County, Mangshan, on living leaves of *Rhododendron* sp., 12 Apr. 2002, W.P. Wu (HMAS046974, holotype, MFLU 12-0417, isotype, ex-type culture NN0469740 = MFLUCC 12-0276); Hunan Province, on living leaves of unidentified tree, 12 Apr. 2002, W.P. Wu, culture NN0473080 = MFLUCC 12-0275.

Note: This species (clade 24; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

***Pestalotiopsis verruculosa* Maharachch. & K.D. Hyde**

Material examined: China, Yunnan Province, Kunming, Kunming Botanical Garden, on living leaves of *Rhododendron* sp., 19 Mar. 2002, W.P. Wu (HMAS047309, holotype, MFLU 12-0416, isotype, ex-type culture NN0473090 = MFLUCC 12-0274).

Note: This species (clade 20; Fig. 5) was treated in detail by Maharachchikumbura *et al.* (2012).

***Pseudopestalotiopsis* Maharachch., K.D. Hyde & Crous, gen. nov. MycoBank MB809753.**

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- and 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 cylindrical with a truncate base; three median cells doliform,

concolourous, 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.

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

*Notes:* In most studies (Jeewon *et al.* 2003, Liu *et al.* 2010, Hu *et al.* 2007, Maharachchikumbura *et al.* 2011, 2012), species with dark concolourous median cells with knobbed apical

appendages formed a distinct clade with high support, which is defined here as a novel genus, *Pseudopestalotiopsis*. Partial LSU sequence data confirm that *Pseudopestalotiopsis* is phylogenetically related to *Neopestalotiopsis* (Fig. 3), but these genera are also morphologically distinct. In *Pseudopestalotiopsis* the three median cells are the same colour (concolourous), whereas in *Neopestalotiopsis* these are versicoloured.

*Pseudopestalotiopsis cocos* Maharachch., K.D. Hyde & Crous, *sp. nov.* MycoBank MB809754. Fig. 45.

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

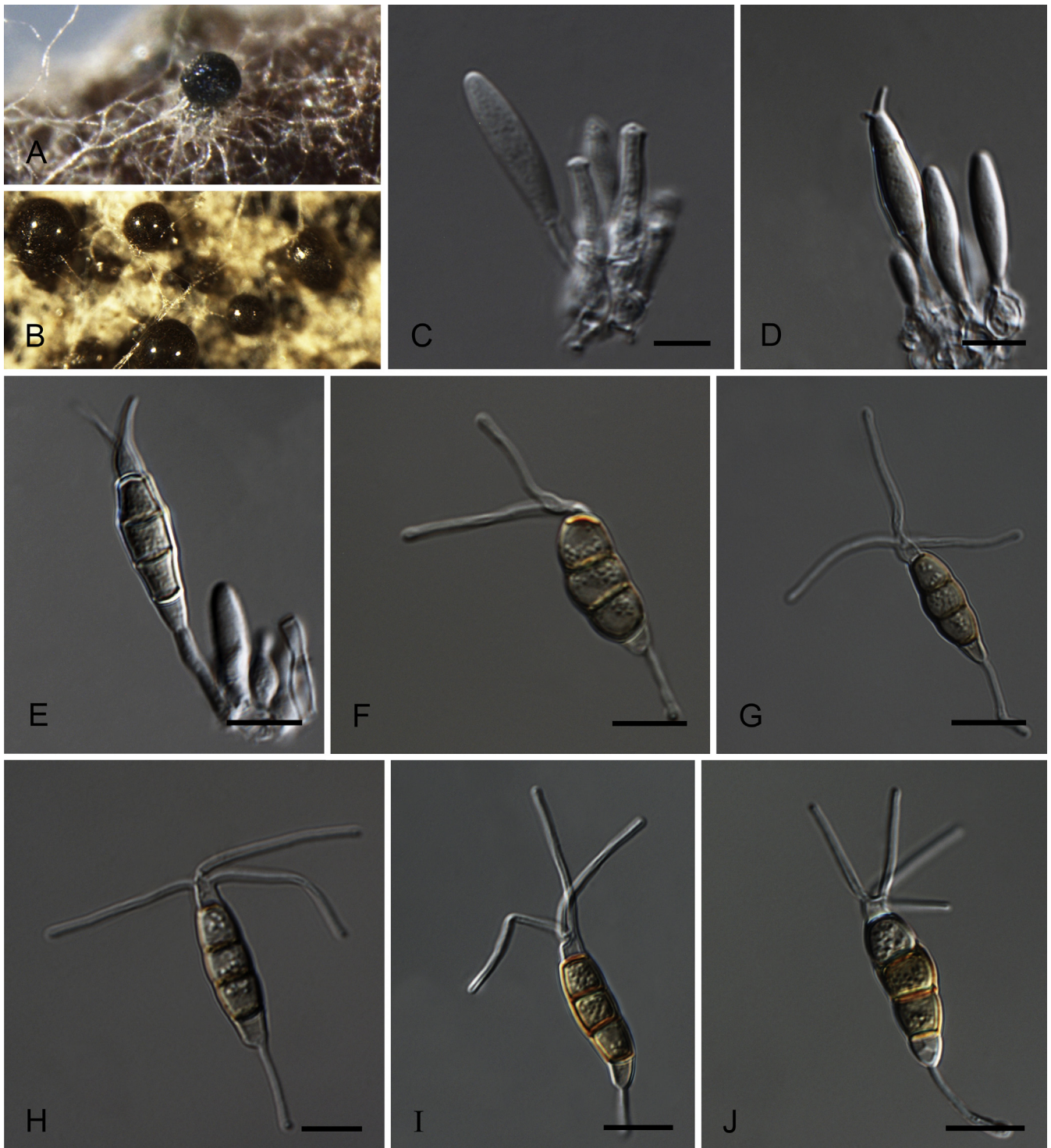


Fig. 45. *Pseudopestalotiopsis cocos* CBS 272.29<sup>T</sup>. A. Conidioma sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–J. Conidia. Scale bars = 10 µm.

*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* fusoid, ellipsoid, straight to slightly curved, 4-septate, constricted at septum, (20–) 21–25(–26.5) × 6–7.5 µm,  $\bar{x} \pm SD = 23.0 \pm 1.6 \times 6.5 \pm 0.4$  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,  $\bar{x} \pm SD = 15.5 \pm 1.2$  µm, concolourous, 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,  $\bar{x} \pm SD = 17.6 \pm 3.2$  µm; basal appendage single, tubular, unbranched, centric, 5–8 µm long.

*Culture characteristics*: Colonies on PDA attaining 50–60 mm diam after 7 d at 25 °C, with smooth edge, whitish to grey, with black, gregarious conidiomata; reverse similar in colour.

*Habitat*: On *Cocos nucifera*.

*Known distribution*: Indonesia (Java).

*Material examined*: Indonesia, Java, Bogor (Buitenzorg), from *Cocos nucifera*, unknown collection date, C.M. Doyer (CBS H-15666, holotype, ex-type culture CBS 272.29).

*Notes*: *Pseudopestalotiopsis cocos* is a distinct species based on its morphology and phylogeny (Figs 3, 4). It can clearly be differentiated from its sibling species, *Ps. indica* (31.5–37 × 6.5–9 µm; Fig. 4) by relatively smaller conidia (20–26.5 × 6–7.5 µm), and shorter apical appendages (12–23 µm), whereas in *Ps. indica* appendages are longer (30–40 µm). Furthermore, the three median cells in *Ps. cocos* are paler in colour than in *Ps. indica*. This species is sister to a clade that contains *Ps. theae* (22–32 × 5–8 µm; Fig. 4) and they have overlapping morphometric characters. However, in *Ps. theae* the apical appendages are knobbed, which is a feature absent in *Ps. cocos*.

***Pseudopestalotiopsis indica*** Maharachch., K.D. Hyde & Crous, *sp. nov.* MycoBank MB809755. Fig. 46.

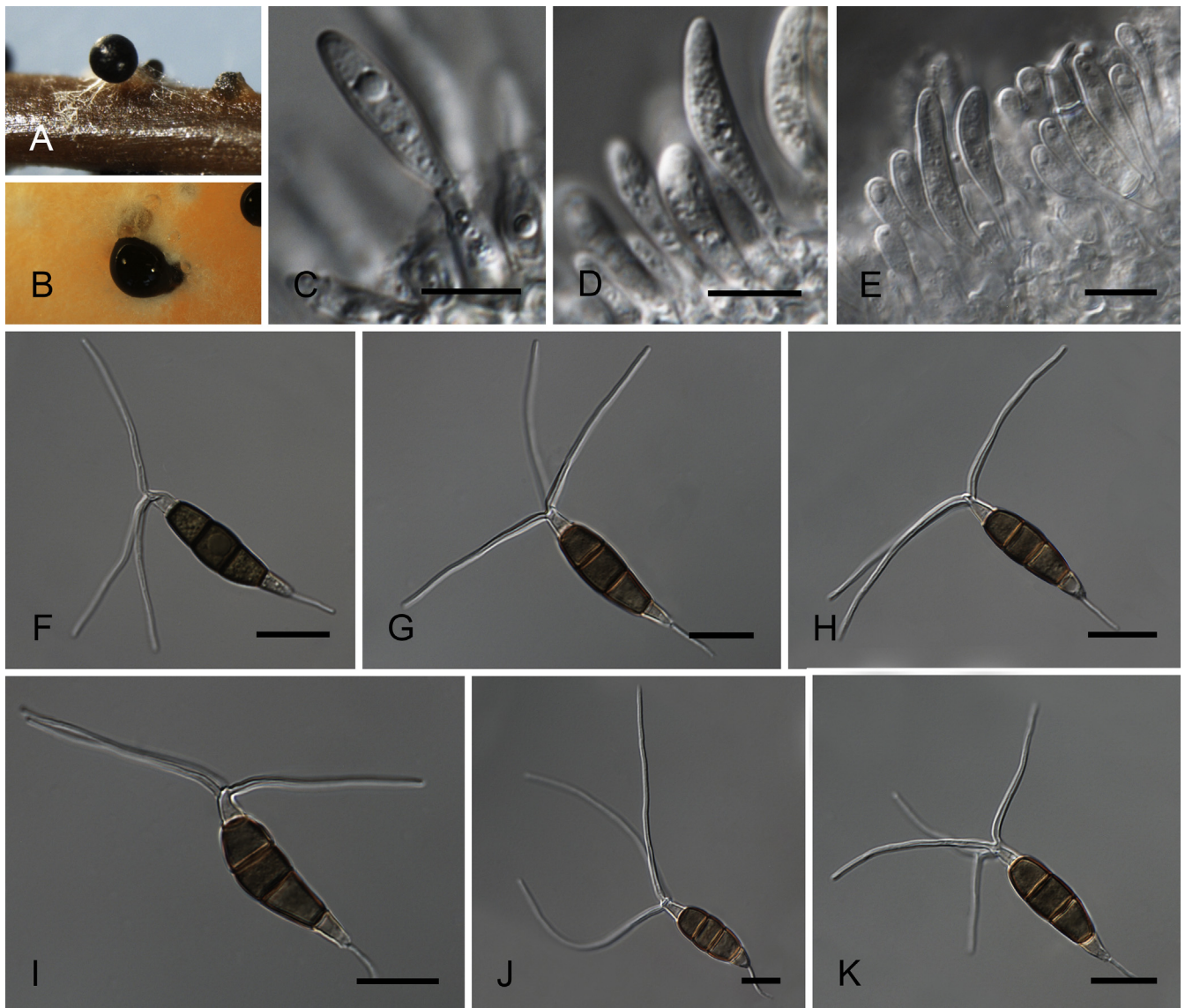


Fig. 46. *Pseudopestalotiopsis indica* CBS 459.78<sup>T</sup>. A. Conidiomata sporulating on PNA. B. Conidiomata on PDA. C–E. Conidiogenous cells. F–K. Conidia. Scale bars = 10 µm.

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

*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- and thin-walled, sometimes percurrently proliferating 1–2 times, periclinal thickening in the apical region, collarete 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,  $\bar{x} \pm SD = 34.5 \pm 1.6 \times 7.5 \pm 0.5$  µm; basal cell conic with truncate base, rugose and thin-walled, 5.5–7 µm long; three median cells (19.5–)20–22(–22.5) µm long,  $\bar{x} \pm SD = 21.6 \pm 1.0$  µm, doliiform, verrucose, concolourous, 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,  $\bar{x} \pm SD = 35 \pm 2.8$  µm; basal appendage single, tubular, unbranched, centric, 6–10 µm long.

**Culture characteristics:** Colonies on PDA reaching 60–80 mm diam after 7 d at 25 °C, undulate at the edge, whitish to pale honey-coloured, with black, gregarious conidiomata; reverse pale honey-coloured.

**Habitat:** On *Hibiscus rosa-sinensis*.

**Known distribution:** India.

**Material examined:** India, Bangalore, on *Hibiscus rosa-sinensis*, Aug. 1978, H.C. Govindu (CBS H-21749, **holotype**, ex-type culture CBS 459.78).

**Notes:** This species is characterised by large conidia (32.5–36 × 7–8.5 µm) with three median cells that are dark in colour. It forms a sister group (Fig. 4) with *Ps. cocos* and *Ps. theae*. *Pseudopestalotiopsis indica* differs from *Ps. cocos* (20–26.5 × 6–7.5 µm) and *Ps. theae* (22–32 × 5–8 µm) in its larger conidia.

***Pseudoestalotiopsis theae*** (Sawada) Maharachch., K.D. Hyde & Crous, **comb. nov.** MycoBank MB809756.

**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: 327. 1949.

**Materials examined:** Taiwan, Republic of China, Taipei, on living leaves of *Camellia sinensis*, 13 Jul. 1908, Y. Fujikuro, det. K. Sawada (BPI 406804, **lectotype**). Thailand, Chiang Mai Prov., Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123'N 98°44.009'E, 900 m, rainforest, on living leaves of *Camellia sinensis*, 20 Jan. 2010, S.S.N. Maharachchikumbura (MFLU 12-0116, **epitype**, ex-epitype culture MFLUCC 12-0055 = CPC 20281); on living leaves of *Camellia sinensis*, unknown collection date and collector, culture SC011.

## DISCUSSION

Winter (1887) established the *Amphisphaeriaceae*, which is characterised by having immersed ascomata in the host and with

dark peridial walls and ascus apices that are usually amyloid (Barr 1975). The *Amphisphaeriaceae* is a large heterogeneous family, which mainly possesses pestalotiopsis-like asexual states (Jeewon *et al.* 2002). These conidial forms are generally characterised by septate conidia with filiform apical appendages (Barr 1990, Nag Raj 1993) and with the exception of *Bartalinia*, *Discosia* and *Monochaetia*, most genera are linked to a sexual morph. Conidial septation appears to be effective in placement of taxa in genera of *Amphisphaeriaceae*. Sequence data generated to date reveal *Truncatella*, *Pestalotiopsis* and *Seiridium* to represent three distinct genera, which are characterised by 4-celled, 5-celled and 6-celled conidia, respectively. However, it has not been established whether, as defined, *Pestalotia* differs from *Pestalotiopsis* based on molecular evidence. Although they are clearly distinct in conidial morphology, *Pestalotiopsis* has 5-celled conidia while *Pestalotia* has 6-celled conidia. From a phenotypic viewpoint, *Pestalotia* species are more similar to *Seiridium* species, as both have 6-celled conidial forms. The type species of *Pestalotia*, *P. pezizoides*, can be distinguished from *Seiridium* species by its more numerous appendages, which are branched, while in *Seiridium* appendages are fewer and generally unbranched. However, branched apical appendages typical of *Pestalotia* are also found in *S. corni* and *S. venetum* (Nag Raj 1993), and thus *Pestalotia* could potentially prove to be congeneric with *Seiridium*. Appendage morphology appears to be highly informative at the species level, even though conidial appendages alone cannot be used as a useful character for generic separation (Crous *et al.* 2012). The monotypic genus *Pestalotia* (1839) may therefore be a synonym of *Seiridium* (1816), since both genera have similar morphologies. However, Guba's (1961) treatment of *Monochaetia* as a distinct genus has proved valid. LSU phylogenetic analyses reveal *Monochaetia* to represent a genus that is distinct from *Pestalotiopsis*, *Seiridium* and *Truncatella* (Fig 3). However, it is essential to incorporate molecular data and more taxon sampling in future analyses as *Monochaetia* includes 3-, 4-, and 6-celled conidial forms.

*Pestalotiopsis* species are morphologically diverse in conidial morphology, and phylogenetic analyses of different gene regions have established that *Pestalotiopsis* comprises three distinct lineages (Jeewon *et al.* 2003, Maharachchikumbura *et al.* 2011, 2012). Based on these findings, we divided *Pestalotiopsis* into three genera: *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis*. However, our phylogenetic analyses disagree with Nag Raj's (1993) broad concept of *Pestalotiopsis*, which included 3-celled, and 4-celled conidial forms. All species within *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis* contain only 4-celled conidial forms. *Pestalotiopsis maculans*, which is the type species of *Pestalotiopsis*, commonly occurs on *Camellia* and provides a stable generic concept for *Pestalotiopsis*. In *P. maculans* conidiophores are septate, unbranched and often reduced to conidiogenous cells; conidiogenous cells are ampulliform to lageniform or cylindrical to subcylindrical phialides, and conidia have concolourous median cells. *Neopestalotiopsis* has versicolourous median cells with indistinct conidiophores, while *Pseudopestalotiopsis* can be distinguished from *Pestalotiopsis* by sequence data and generally dark-coloured concolourous median cells with indistinct conidiophores. The three genera can also be roughly assigned to distinct groups based on the total number of base pairs in the ITS region.

*Pestalotiopsis* is a species-rich asexual morph-typified genus with only 13 known sexual states, as compared to the possible

253 asexual names (Zhang *et al.* 2012a, Maharachchikumbura *et al.* 2013d). Of the 13 sexually reproducing species, nine are linked to named *Pestalotiopsis* species and eight have concolourous median cells typical of *Pestalotiopsis*. *Pestalosphaeria maculiformans* is linked to *Pestalotiopsis maculiformans* (Marincowitz *et al.* 2008), which has versicolourous median cells, hence, belongs in *Neopestalotiopsis*. However, based on a megablast search of NCBI's GenBank nucleotide database for this species (CBS 122683, GenBank EU552147), the closest hits using the ITS sequence had highest similarity to *Pestalotiopsis* (species with concolourous median cells). Therefore, presently the known asexual states of *Pestalosphaeria* are *Pestalotiopsis* species. Because only one name can be applied to any fungal species (Hawksworth *et al.* 2011, Taylor 2011, Wingfield *et al.* 2012) and since *Pestalotiopsis* is the oldest and the most common name; Maharachchikumbura *et al.* (2011) suggested that *Pestalotiopsis* should be adopted for this genus. This has been followed in subsequent publications and is followed in this paper (Maharachchikumbura *et al.* 2012, Zhang *et al.* 2012a).

Conidial morphology is the most widely used taxonomic character for inter-specific delineation of *Pestalotiopsis* (Steyaert 1949, Guba 1961, Nag Raj 1993). However, there are considerable overlapping phenotypic characteristics that make it difficult to segregate morphologically equivocal taxa (Tejesvi *et al.* 2009). Conidial length and width have been emphasised as crucial characters for species identification, and many contemporary researchers have used length and width to segregate taxa (Steyaert 1949, Guba 1961, Mordue 1985). In the present study, however, species sharing similar conidial dimensions did not necessarily group together. As an example, *P. malayana* (clade 30; Fig. 5) and *P. biciliata* (clade 38; Fig. 5) have similar conidial dimensions, but cluster in distinct clades. Therefore, the continued use of conidium length and width in classification for *Pestalotiopsis* species is unwise. A similar observation was made by Dube & Bilgrami (1965) who showed that conidial size is a homoplasious character and species sharing similar spore sizes may not be closely related (Jeewon *et al.* 2003).

Various features/aspects of conidial appendages are taxonomically informative at the species level in many coelomycetous genera (Nag Raj 1993, Crous *et al.* 2012). The function of appendages should not be considered in isolation since appendages usually relate to an ecological function linked to spore dispersal, liberation, deposition and the colonisation of new substrates or niches (Gregory 1952, Crous *et al.* 2012). Watanabe *et al.* (2000) investigated the conidial adhesion and germination of *Pestalotiopsis neglecta* and observed that apical appendages firmly attached conidia to the substrate during the infection process. Generally in *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis* apical appendages arise as tubular extensions and maintain protoplasmic continuity with the conidium body. Appendage morphology has been widely used in *Pestalotiopsis* taxonomy to introduce novel taxa (Steyaert 1949, Guba 1961, Nag Raj 1993, Maharachchikumbura *et al.* 2013a, Zhang *et al.* 2012b). Among the appendage-bearing coelomycetes, *Pestalotiopsis* shows high variation in appendage morphology. These apical appendage characters vary in length of the apical appendage, appendage number, shape, branched or unbranched nature, presence or absence of knobbed tips and the position of the apical appendage attached to the conidial body.

The ecology of species of *Pestalotiopsis* is poorly understood, especially now that species have been circumscribed using

molecular data. There is little data on geographical distribution and even host range. Since our data set is not robust, it is not clear whether the geographic influences or hosts range or allopatry play a key role in species circumscription and delineation. Therefore, much research is needed and it might be useful to account for substrate, geographic influences, host ranges, and morphological characters when incorporating molecular sequence data to define species borders within *Neopestalotiopsis*, *Pestalotiopsis* and *Pseudopestalotiopsis*. This kind of approach has been successfully used in the past to investigate species in for example *Cladosporium* (Bensch *et al.* 2012), *Colletotrichum* (Damm *et al.* 2012), *Diaporthe* (Gomes *et al.* 2013) and *Teratosphaeriaceae* (Quaedvlieg *et al.* 2014).

Common problems in *Pestalotiopsis* taxonomy are that new species (e.g. *P. alpiniae*, *P. oenotherae* and *P. nelumbinis*) have been defined without accompanying sequence data. In fact, in 2011 there were only four ex-type cultures available for this study on *Pestalotiopsis* phylogeny. In the first inclusive phylogenetic study of *Pestalotiopsis*, Jeewon *et al.* (2003) used ITS sequence data to evaluate the phylogenetic significance of *Pestalotiopsis* morphological characters in taxonomy. In differentiating endophytic species of *Pestalotiopsis* in *Pinus armandii* and *Ribes* spp., Hu *et al.* (2007) pointed out that the *TUB* gene better resolved *Pestalotiopsis* phylogeny. A combination of both the *TUB* 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*. Maharachchikumbura *et al.* (2012) tested 10 gene regions to resolve species boundaries in the *Pestalotiopsis* (actin, calmodulin, glutamine synthase, glyceraldehyde-3-phosphate dehydrogenase, ITS, LSU, 18S nrDNA, RNA polymerase II, *TEF* and *TUB*). The authors compared the morphological versus sequence data from each gene to establish which characters satisfactorily resolved species limits and ITS, *TUB* and *TEF* proved to be the better molecular markers. In the present study, phylogenetic species recognition based on combined ITS, *TUB* and *TEF* gene regions gave a high number of strongly supported nodes at the terminal clades. In *Neopestalotiopsis* however, overall branch-length support values were lower, when compared to *Pestalotiopsis*. Future studies of *Neopestalotiopsis* may require additional loci to obtain a better separation of species.

The genus *Pestalotiopsis* has been shown to produce numerous secondary metabolites with diverse structural features, with antitumour, antifungal, antimicrobial and other activities (Xu *et al.* 2010, 2014). Three reviews have been recently published and reveal the chemistry of *Pestalotiopsis* species and related genera. Species belonging to these genera are a rich source for bioprospecting when compared to other fungal genera (Aly *et al.* 2010, Xu *et al.* 2010, 2014). Xu *et al.* (2010) discussed 130 diverse compounds isolated from species of *Pestalotiopsis* in the past 10 years, while Xu *et al.* (2014) discussed a further 160 compounds. These biochemicals may have significance in pharmaceutical, agricultural and industrial applications. The names assigned to *Pestalotiopsis* species producing novel compounds lacked a phylogenetic basis (Maharachchikumbura *et al.* 2012, 2013c). It would be interesting to establish if different species of *Pestalotiopsis* were chemically more creative than others and also to establish if *Neopestalotiopsis* and *Pseudopestalotiopsis* species are different from *Pestalotiopsis* species in this regard.

*Pestalotiopsis* species are important causal agents of plant disease (Keith *et al.* 2006, Joshi *et al.* 2009, Keith & Zee 2010,



Chen *et al.* 2011, Evidente *et al.* 2012, Maharachchikumbura *et al.* 2013a,b,c), chemically highly diverse (Aly *et al.* 2010, Xu *et al.* 2014), extremely common in most habitats (Bate-Smith & Metcalfe 1957, Jeewon *et al.* 2004, Maharachchikumbura *et al.* 2011) and are fascinating because of their distinct conidial morphology (Sutton 1980, Nag Raj 1993); thus they are a remarkable group of fungi that have been well-studied morphologically in the past (Steyaert 1949, Maharachchikumbura *et al.* 2013a,b). In this study we advance the understanding of this group using morphology and multilocus sequence analyses and introduce two new genera, *Neopestalotiopsis* and *Pseudopestalotiopsis*, to accommodate segregates of *Pestalotiopsis*. Phenotypic analyses of conidial characters coupled with phylogenetic analyses of sequence data were used to clarify species boundaries in the three genera. Although genetic differences exist, several isolates were not assigned to species because of sterile cultures and lack of data on geographical differences; thus the data were insufficient to determine species boundaries in those cases. Sequence data are provided for 24 species of *Neopestalotiopsis*, 43 species of *Pestalotiopsis* and three species of *Pseudopestalotiopsis* and can be used in future studies to increase the understanding of this group. We predict that future studies will reveal numerous distinct and new taxa in this generic complex.

## ACKNOWLEDGEMENTS

We thank the Kunming Institute of Botany, Chinese Academy of Sciences for providing facilities and the World Agroforestry Centre, East and Central Asia office for hosting us, we would also like to thank Humidtropics, a CGIAR Research Program that aims to develop new opportunities for improved livelihoods in a sustainable environment, for partially funding this work, and the National Research Council of Thailand (grant for *Pestalotiopsis* No: 55201020008). We thank the CBS-KNAW Fungal Biodiversity Centre for funding, and the technical staff, Arien van Iperen (cultures) and Mieke Starink-Willemse (DNA isolation, amplification, and sequencing) for their invaluable assistance.

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