Pestalotiopsis—morphology, phylogeny, biochemistry and diversity

**Sajeewa S. N. Maharachchikumbura ·
Liang-Dong Guo · Ekachai Chukeatirote ·
Ali H. Bahkali · Kevin D. Hyde**

Abstract The genus *Pestalotiopsis* has received considerable attention in recent years, not only because of its role as a plant pathogen but also as a commonly isolated endophyte which has been shown to produce a wide range of chemically novel diverse metabolites. Classification in the genus has been previously based on morphology, with conidial characters being considered as important in distinguishing species and closely related genera. In this review, *Pestalotia*, *Pestalotiopsis* and some related genera are evaluated; it is concluded that the large number of described species has resulted from introductions based on host association. We suspect that many of these are probably not good biological species. Recent molecular data have shown that conidial characters can be used to distinguish taxa; however, host association and geographical location is less informative. The taxonomy of the genera complex remains confused. There are only a few type cultures and, therefore, it is impossible to use gene sequences in GenBank to clarify species names reliably. It has not even been established whether *Pestalotia* and *Pestalotiopsis* are distinct genera, as no isolates of the type species of *Pestalotia* have been sequenced, and they are morphologically somewhat similar. When selected GenBank ITS accessions of *Pestalotiopsis clavispora, P. disseminata, P. microspora, P. neglecta, P. photiniae, P. theae, P. virgatula* and *P. vismiae* are aligned, most species cluster throughout any phylogram generated. Since there appears to be no living type strain for any of these species, it is unwise to use GenBank sequences to represent any of these names. Type cultures and sequences are available for the recently described species *P. hainanensis, P. jesteri, P. kunmingensis* and *P. pallidotheae*. It is clear that the important species in *Pestalotia* and *Pestalotiopsis* need to be epitypified so that we can begin to understand the genus/genera. There are numerous reports in the literature that various species produce taxol, while others produce newly discovered compounds with medicinal potential and still others cause disease. The names assigned to these novel compound-producing taxa lack an accurate taxonomic basis, since the taxonomy of the genus is markedly confused. Until the important species have been epitypified with living strains that have been sequenced and deposited in public databases, researchers should refrain from providing the exact name of species.

S. S. N. Maharachchikumbura · L.-D. Guo (✉)
Key Laboratory of Systematic Mycology & Lichenology,
Institute of Microbiology, Chinese Academy of Sciences,
Beijing 100190, People's Republic of China
e-mail: guold@sun.im.ac.cn

S. S. N. Maharachchikumbura · E. Chukeatirote · K. D. Hyde (✉)
School of Science, Mae Fah Luang University,
Thasud, Chiang Rai 57100, Thailand
e-mail: kdhyde3@gmail.com

A. H. Bahkali · K. D. Hyde
College of Science, Botany and Microbiology Department,
King Saud University,
P.O. Box: 2455, Riyadh 1145, Saudi Arabia

Introduction

Pestalotoiopsis Steyaert is an appendage-bearing conidial anamorphic form (coelomycetes) in the family Amphisphaeriaceae (Barr 1975, 1990; Kang et al. 1998, 1999), and molecular studies have shown that *Pestalotiopsis* is monophyletic (Jeewon et al. 2002, 2003, 2004). Species of *Pestalotiopsis* are common in tropical and temperate

ecosystems (Bate-Smith and Metcalfe 1957) and may cause plant disease (Das et al. 2010), are often isolated as endophytes (Liu et al. 2006; Wei et al. 2007; Watanabe et al. 2010), or occur as saprobes (Wu et al. 1982; Agarwal and Chauhan 1988; Yanna et al. 2002; Hu et al. 2007; Liu et al. 2008a). The genus has received much attention from the scientific community. However, this not because of its pathogenic nature (Hyde and Fröhlich 1995; Rivera and Wright 2000; Yasuda et al. 2003), but rather because its species have been shown to produce many important secondary metabolites (Strobel et al. 1996a, 2002; Ding et al. 2008a, b; Aly et al. 2010; Xu et al. 2010). The aim of the present paper on *Pestalotia, Pestalotiopsis* and similar genera is to review (1) historical aspects, (2) morphological and molecular studies, (3) life mode of taxa, (4) species numbers and (5) biochemical production by selected species. The problems of understanding the genus are discussed and the work needed to resolve these problems elaborated. In most cases problems arise due to misidentification of taxa and the review illustrates the importance of the correct identification of strains before they are used in biochemical or other studies.

History

De Notaris (1839) introduced the genus *Pestalotia* De Not. based on the generic type *Pestalotia pezizoides* De Not., which occurred on the leaves of *Vitis vinifera* in Italy. This species is characterized by 6-celled conidia with four deeply olivaceous central cells, distosepta, hyaline terminal cells and simple or branched appendages arising from the apex (Fig. 1.). Steyaert (1949) revised *Pestalotia* and divided the genus into three main groups based on the

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

Fig. 1 *Pestalotia pezizoides* De Not. BPI0406483, **a** Conidia **b** conidiogenous cells. Scale bars: **a**–**b**=20 μm
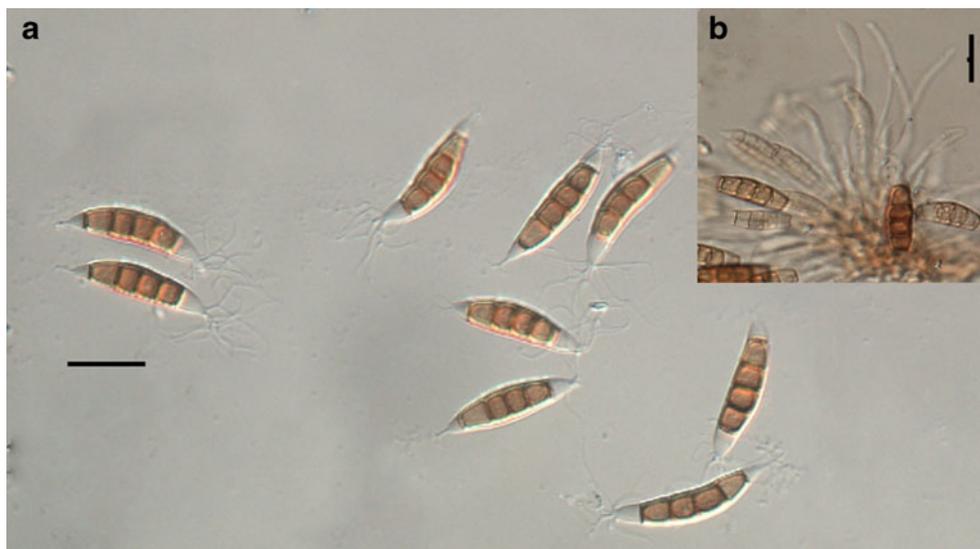
The primary work on *Pestalotia* was carried out by Guba (1961) in his "Monograph of *Monochaetia* and *Pestalotia*". Guba (1961) divided the genus into the sections quadriloculate, quinqueloculatae and sexloculatae for 4-celled conidia, 5-celled conidia and 6-celled conidia respectively. For his sections, Guba (1961) used a simple but very effective system as proposed by Klebahn (1914), which was based on the number conidial cells. Guba (1961) further subdivided the sections into different categories, mainly on the basis of conidial form, colour, and the position, and character of the setulae. *Monochaetia* was retained as a distinct genus based on its single apical appendage, while *Pestalotiopsis* and *Truncatella,* the new genera proposed by Steyaert (1949), were synonymised with *Pestalotia*. Guba (1961) described 258 species of *Pestalotia* in his monograph. Steyaert (1956) argued that the retention of *Monochaetia* as a distinct genus based on a single character, a single apical appendage was incorrect, while other genera (*Pestalotiopsis*, *Truncatella* and *Pestalotia*) were differentiated from each other based on a set of characters.

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

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

Morphological characters used to differentiate species of *Pestalotiopsis* and similar genera are limited (Hu et al. 2007); the morphological characters are plastid and morphological

markers vary between host and environment (Egger 1995). Hu et al. (2007) showed that colony morphology (colour, growth rate and texture) is highly variable within single isolates of *Pestalotiopsis*; this phenomenon can be easily observed through repeated subculturing. Also within a single species, conidial morphology (shape and colour of the median cells), growth rate and fruiting structure, may vary (Jeewon et al. 2003). Satya and Saksena (1984) observed *Pestalotiopsis glandicola* (Castagne) Steyaert and *P. versicolor* var. *polygoni* and found that the intensity of the median cells varied with culture and host and concluded that colour of median cells cannot be used to judge their taxonomic position. Dube and Bilgrami (1965) observed *Pestalotiopsis darjeelingensis* Dube, Bilgrami & H.P. Srivast. and showed morphological variation of conidia in culture (dimension, length of the setulae, shape, number of cells and the colour of the cells). Similar observations were made by Purohit and Bilgrami (1969) when studying more than 100 pathogenic strains. Conidiogenesis is also confusing when used for species separation; Watanabe et al. (1998), showed that *Pestalotiopsis neglecta* (Thüm.) Steyaert and *P. guepinii* having similar acervuli development.

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

culture on autoclaved carnation leaf segments (Liu et al. 2010a).

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

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

## Morphological characters use in the differentiation of species

Conidial morphology (Fig. 2.) is the most widely used taxonomic character for the genus *Pestalotiopsis*. Most species are divided into different groups based on the size of the conidia. The length and width are good taxonomic markers for the genus and stable within the different media and the generations in most cases (Hu et al. 2007). Colour of the median cells is still a widely used character, and all species separate into three groups based on this- concolorous, versicolorous umber olivaceous and versicolorous fuliginous olivaceous. Molecular evidence indicates that it is more precise to group species according to concolorous and versicolorous rather than the above three groups (Jeewon et al. 2003). The length of the apical appendages and the number of the apical appendages are also widely used characters for species identification. Some species can also be identified by the presence of knobbed apical

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

appendages. The apical appendages can arise from the top, middle, bottom or different positions in the apical hyaline cells and such characters are widely used in species identification. Furthermore the apical appendages can be divided into branches; in some species presence or absence of the basal appendages is another character for species diagnosis.

Recent molecular data

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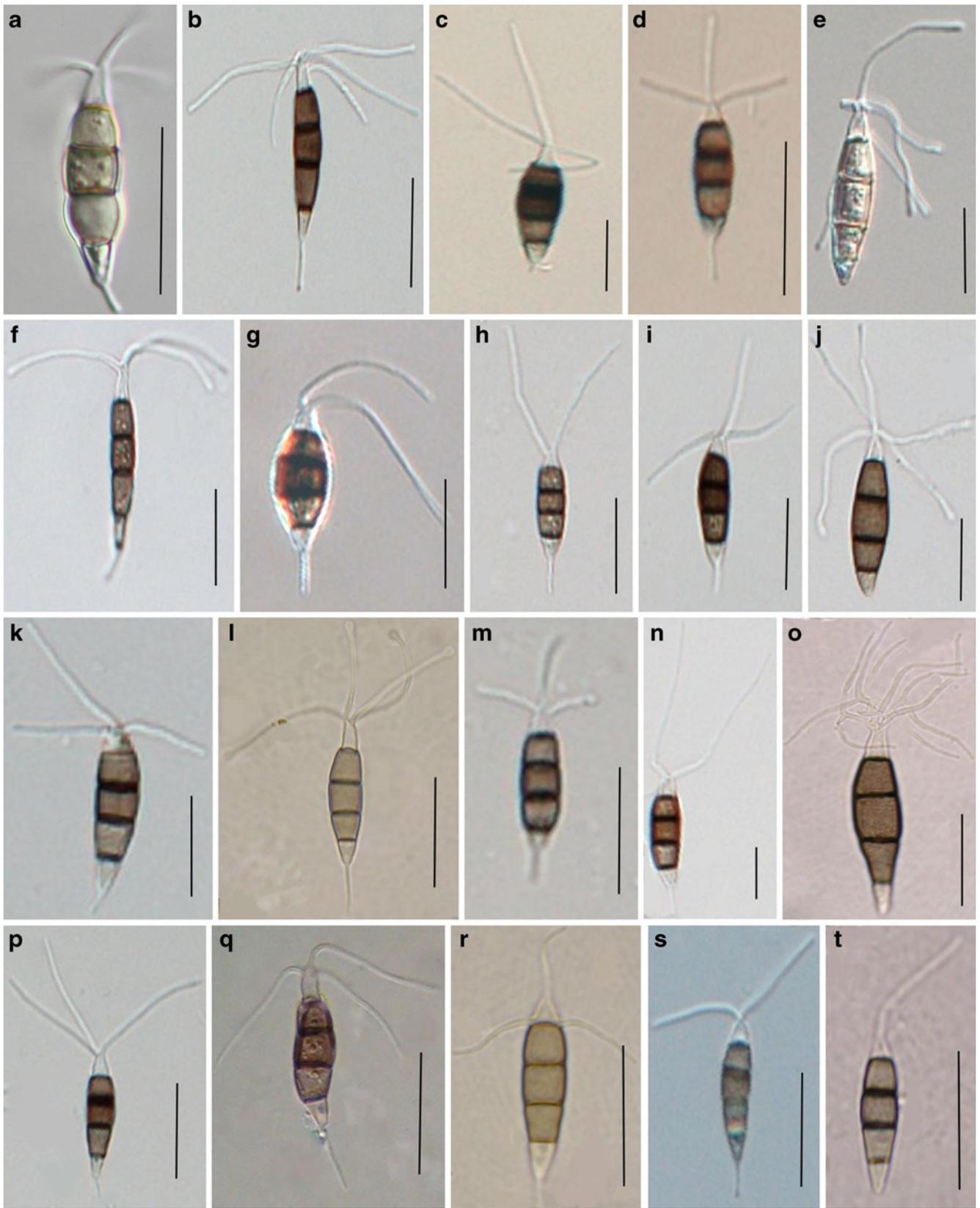
ITS region is more informative than internal transcribed spacer—restriction fragment length polymorphism (ITS-RFLP). They used five restriction enzymes (Alu I, Hae III, Ava II, Hpa II and Taq I) in their ITS-RFLP analysis and showed that ITS-RFLP profiles were distinctly different in *P. virgatula* (Kleb.)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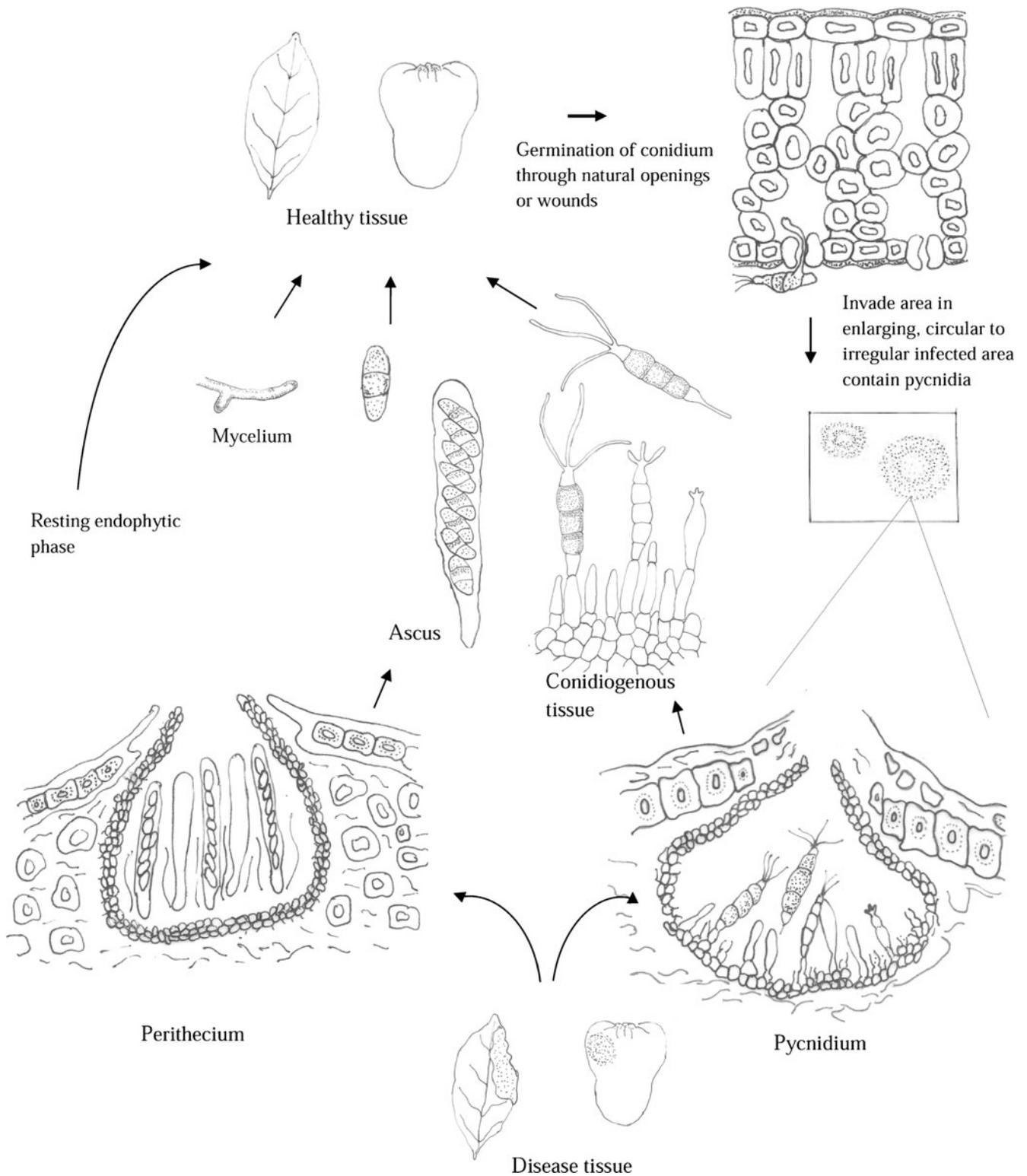
Life cycle in *Pestalotiopsis*

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

Pathogenic species of *Pestalotiopsis* initially make contact with the host where the infection occurs (inoculum), probably by means of the conidia or fragmented spores (Espinoza et al. 2008). These inocula may survive during harsh weather conditions and may cause primary infections. Secondary inoculum produced on diseased tissue may cause secondary infections and increase the severity of the disease. The source of the inoculum can be wild plantations (Keith et al. 2006), flowers (Pandey 1990), crop debris, disease stock plants, used growing media, soil and contaminated nursery tools (McQuilken and Hopkins 2004), splashed water droplets (Hopkins and McQuilken 1997; Elliott et al. 2004) and also spores in the air (Xu et al. 1999). Species of *Pestalotiopsis* have constantly been isolated as endophytes from plant tissues (Wei and Xu 2004; Liu et al. 2006; Wei et al. 2005, 2007; Tejesvi et al. 2009; Watanabe et al. 2010). We suspect that many endophytic species remain as dormant symptomless inhabitants of plants until the plant is stressed, and then the endophytes become pathogens. This is thought to occur in other pathogenic genera (Gehlot et al. 2008). The pathogenic phase may be triggered by a combination of environmental factors, plant susceptibility and the virulence of the pathogen. However, further research is needed to prove the endophytic pathogenic relationship in the genus. *Pestalotiopsis* is also considered to be a weak pathogen (Madar et al. 1991), and most weak pathogens penetrate the host through natural openings such as stoma, lenticels and hydathodes (Agrios 2005). Wright et al. (1998) stated that species of *Pestalotiopsis* only infect wounded or stressed plants, so pruning wounds or other physical means play important roles in disease development (Elliott et al. 2004; McQuilken and Hopkins 2004; Keith et al. 2006). Plants may also be stressed due to insect, pesticide or sun damage (Hopkins and McQuilken 2000). High temperature, high rainfall and human activities may also trigger infections, and this may lead to disease development (Tuset et al. 1999; Hopkins and McQuilken 2000; Elliott et al. 2004). The anamorph-teleomorph relationships and life cycles are not well known for most species, as the sexual stage does not often develop (Armstrong-Cho and Banniza 2006). Therefore, conidia therefore appear to play a key role in providing the inocula. A general disease cycle for *Pestalotiopsis* is illustrated in Fig. 3.

The spore of *Pestalotiopsis* is considered to be a dry spore. Watanabe et al. (2000) studied conidial adhesion and germination of spores of *P. neglecta* and showed that infection occurs in four stages. At the beginning, the lower median cell germinates and becomes firmly attached to the substrate. Future successive infections can be achieved by two upper median cells. In the first stage, weak adhesion is achieved by the mucilaginous matrix coating the conidia. A second weak adhesion occurs at the bases of the pedicel. The next two stages provide a strong attachment by release of fibrillar adhesive substances. In the third stage, fibrillar adhesive substances are produced along the length of the pedicel to the apex of the basal cell and at times a smaller amount of fibrillar material is released from the apical appendages. The fourth stage involves the release of fibrillar material at the point of germ tube emergence. Nag Rag (1993) described conidiomata of the genus as variable, ranging from acervuli to pycnidia. Conidiomata can be immersed to erumpent, unilocular to irregularly plurilocular with the locules occasionally incompletely divided and dehiscence by irregular splitting of the apical wall or overlying host tissue (Nag Rag 1993). Conidiophores partly or entirely develop inside the conidiomata, and they can be reduced to conidiogenesis cells which are discrete or integrated, cylindrical, smooth, colourless and invested in mucus (Nag Rag 1993). Pycnidia can mostly be seen with the unaided eye as a black or brown spore masses with copious conidia.

Fig. 3 Disease cycle of the genus *Pestalotiopsis* (References: revised and redrawn; Von Arx 1974; Nag Rag 1993; Kobayashi et al. 2001)

Control strategies are needed for serious *Pestalotiopsis* disease, and therefore, knowledge of the causal agent and the disease cycle is important. Precise knowledge of the plant/ pathogen interaction and its functional variation according to the environmental factors are important for integrated disease management using cultural, biological and chemical methods. Elliott et al. (2004) stated that *Pestalotiopsis* may produce large numbers of spores which

are easily dispersed in air or by water splash, thus sanitation and disease management are critical. They suggested that water management strategies, such as elimination of overhead irrigation, decreasing wetness of leaves, increasing the spacing of plants and increasing the air circulation, can reduce disease in palm plantations. Different harvesting factors also directly affected disease development in tea plantations. Sanjay et al. (2008) showed that highest disease incidence occurred in continuously shear-harvested fields and least in hand-plucked plantations, and they evaluated systemic fungicide and biocontrol agents such as a *Trichoderma, Gliocladium* and *Pseudomonas* for use in controlling grey blight disease in tea.

Mode of life

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

Sexual and asexual forms

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

The asexual *Pestalotiopsis* state and ascomycetous sexual state have rarely been recorded in the same host plant (Barr 1975; Nag Raj 1985; Hyde 1996). However, it is not always clear that the two stages found are definitely the same biological species and therefore molecular evidence is needed to link them. In the laboratory species of *Pestalotiopsis* rarely develop sexual forms (Metz et al. 2000). Zhu et al. (1991) induced *Pestalosphaeria accidenta* P.L. Zhu, Q.X. Ge & T. Xu and *P. jinggangensis* P.L. Zhu, Q.X. Ge & T. Xu to form on potato dextrose agar (PDA). However, this took 5 to 6 months of incubation. Metz et al. (2000) obtained the sexual state of *P. microspora,* an endophytic isolate that produced taxol. The asexual stage formed after 3–6 weeks on water agar with dried yew needles when incubated at 16–20 C with 12 h of light per day and was identified as *Pestalosphaeria hansenii* Shoemaker & J.A. Simpson. The twelve sexual states known for species of *Pestalotiopsis* are listed in Table 1.

Pestalotiopsis Steyaert as a plant pathogen

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

Table 1 List of anamorphs with known teleomorphs

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

In Sicily, the economically important plant *Laurus nobilis* is infected by *P. uvicola*, which causes causing leaf spots and stem blights (Vitale and Polizzi 2005). Chlorosis and reduction of growth were recorded in maize fields in the Cameroons when the plants were infected by *P. neglecta* (Tagne and Mathur 2001). The medicinally important ornamental shrub *Lindera obtusiloba*, which grows wild in the mountain areas of the Korean Peninsula, is infected by *P. microspora*, and the affected leaves initially have grey or dark brown lesions, surrounded by yellowish halos; these enlarge, coalesce and become entire at a later stage, finally causing full leaf blight (Jeon et al. 2007). Affected leaves of *Hymenaea courbaril* show symptoms of leaf spots and the pathogen was identified as a *P. subcuticularis* (Guba) J.G Wei & T. Xu (Fail and Langenheim 1990). Pathogenic *P. funerea* infects conifer species and causes necrosis on infected tissues and sometimes death of the plants involved (Bajo et al. 2008). The medicinal and ornamental *Carapa guianensis* is infected by *P. macrochaeta* (Speg.) J. Xiang Zhang & T. Xu, and foliar blight has been observed in the lower canopy of the plants (Halfeld-Vieira and Nechet 2006). Species of *Pestalotiopsis* also have the potential to cause leaf and/or fruit spots on ginger, rambutan, lychee and orchid (Keith and Zee 2010)

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

Pestalotiopsis as an endophyte

Most resent *Pestalotiopsis* research is based on endophytic isolates (Liu et al. 2006; Wei et al. 2007; Watanabe et al. 2010; Aly et al. 2010) and has resulted in a four new species being described. These are *P. hainanensis* A.R. Liu, T. Xu & L.D. Guo, *P. jesteri* Strobel, J. Yi Li, E.J. Ford & W.M. Hess, *P. kunmingensis* J.G. Wei & T. Xu and *P. pallidotheae* Kyoko Watanabe & Yas. Ono. Most endophytic studies have used morphological characters and either gene sequence data (Hu et al. 2007; Liu et al. 2007; Wei et al 2007) or RFLP technique (Tejesvi et al. 2007a) or a combination of gene sequence and RFLP techniques (Tejesvi et al. 2009) to distinguish species. The distribution of the endophytic species of *Pestalotiopsis* is ubiquitous and is not largely influenced by geographical factors (Wei et al. 2007; Tejesvi et al. 2009). Tejesvi et al. (2005) stated that the endophytic species of *Pestalotiopsis* dominant in the winter season and their colonization are comparatively low in the monsoon season. The colonization frequency of species of *Pestalotiopsis* increased with the increasing the age of the host plant and colonization frequency was variable (Wei et al. 2007).

Some endophyte studies in which species of *Pestalotiopsis* have been recovered are listed in Table 2.

Pestalotiopsis as a saprobe

Species of *Pestalotiopsis* have been repeatedly isolated as saprobes from dead leaves, bark and twigs (Guba 1961).

Table 2 List of endophytes and associated host

Species	Host	References
P. clavispora	Camellia oleifera, C. sinensis, Terminalia arjuna, Podocarpus macrophyllus	Liu et al. 2007; Tejesvi et al. 2007a, 2009; Wei et al. 2007
P. conigena (Lév.) G.C. Zhao & N. Li	Lithocarpus glabra, C. nitidissima	Wei et al. 2005, 2007
P. funerea	Catharanthus roseus	Srinivasan and Muthumary 2009
P. hainanensis	Podocarpus macrophyllus	Liu et al. 2007
P. heterocornis (Guba) Y.X.Chen	Camellia japonica, C. oleifera, Castanopsis sclerophylla, Cephalotaxus fortunei, Podocarpus macrophyllus, Lithocarpus glabra,	Wei et al. 2005, 2007; Liu et al. 2007
P. jesteri	Fragraea bodenii	Strobel et al. 2000
P. karstenii (Sacc. & P. Syd.) Steyaert	Camellia japonica, C. sasanqua	Liu et al. 2007; Wei et al. 2007
P. kunmingensis	Podocarpus macrophyllus	Wei et al. 2007
P. mangifolia (Guba) J. Xiang Zhang & T. Xu	Camellia japonica, C. reticulate, C. sasanqua, Podocarpus nagi	Liu et al. 2007; Wei et al. 2007
P. microspora	Azadirachta indica, Camellia sinensis, Maytenus ilicifolia, Podocarpus macrophyllus Terminalia arjuna, T. chebula, Taxus wallichiana, Taxodium distichum,	Li et al. 1996; Strobel et al. 1996a, b; Wei et al. 2005, 2007; Gomes-Figueiredo et al. 2007; Liu et al. 2007; Tejesvi et al. 2007a, 2009
P. neglecta	Camellia sinensis, C. nitidissima, Podocarpus macrophyllus, P. nagi, Taxus chinensis, T. yunnanensis	Liu et al. 2007; Wei et al. 2007
P. olivacea (Guba) G.C. Zhao & J. He	Camellia sasanqua, Podocarpus macrophyllus,	Liu et al. 2007; Wei et al. 2007
P. oxyanthi (Thüm.) Steyaert	Camellia nitidissima, Podocarpus macrophyllus	Liu et al. 2007; Wei et al. 2007
P. paeoniae (Servazzi) Steyaert	Camellia sasanqua, Cephalotaxus fortune, Ginkgo biloba, Podocarpus macrophyllus, Taxus yunnanensis	Wei et al. 2005, 2007 Liu et al. 2007
P. palliditheae	Pieris japonica	Watanabe et al. 2010
P. photiniae (Thüm.) Y.X. Chen	Camellia japonica, C. sasanqua, Podocarpus macrophyllus P. nagi, Taxus chinensis, Acer palmatum	Wei et al. 2005, 2007; Liu et al. 2007
P. subcuticularis	Camellia sasanqua, Taxus yunnanensis, T. chinensis,	Liu et al. 2007; Wei et al. 2007
P. submersa Sati & N. Tiwari	Equisetum sp., Lyonia ovalifolia	Sati and Belwal 2005
P. theae	Camellia nitidissima, C. sinensis, Holarrhena antidysenterica, Podocarpus macrophyllus, Terminalia arjuna	Liu et al. 2007; Tejesvi et al. 2007a, 2009; Wei et al. 2007
P. versicolor (Speg.) Steyaert	Tamarindus indica	Liu et al. 2007, 2010a

Many species have been isolated from soil, polluted stream water or are associated with the deterioration of wood, paper, fabrics and decay of wool (Guba 1961). For an example, *P. bicolor* (Ellis & Everh.) A.R. Liu, T. Xu & L.D. Guo, *P. funerea*, *P. monochaetioides* (Doyer) Steyaert, *P. montellica* (Sacc. & Voglino) Tak. Kobay., *P. disseminata*, *P. foedans* (Sacc. & Ellis) Steyaert, *P. versicolor* and *P. virgatula* are common species recorded either from decaying leaves or bark. Several saprobic species of *Pestalotiopsis* are listed in Table 3.

Pestalotiopsis as a parasymbiont

Lichen symbiosis is an association between a fungus (the mycobiont) and an alga or a cyanobacterium (the photobiont) (Schwendener 1868). Most lichens associate with only one fungal species, while some have additional species. In most cases these additional fungal species are parasitic while few are parasymbiont. A parasymbiont is a secondary fungus present in the lichen thallus, growing in intimate association with the primary symbionts without causing them any apparent harm (Sun et al. 2002). *Pestalotiopsis maculans* (Corda) Nag Raj is considered to be the dominant parasymbiont in the North American lichen species *Cladonia rangiferina*, *C. subtenuis*, *C. mitis*, *C. leporina*, *Parmotrema perforatum* and *Usnea strigosa* (Sun et al. 2002).

Pestalotiopsis as potential human and animal pathogens

Species of *Pestalotiopsis* are also known to cause human and animal disease. *Pestalotiopsis* has been isolated from

Table 3 List of recently recorded saprobes with their host/substrata

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

the human sinuses, fingernails, a bronchial biopsy, eyes, scalp and feet with corneal abrasions (Sutton 1999). One isolated from cotton was tested in a toxicity bioassay, which indicated that it caused reduction in weight, pathological abnormalities and even mortality in rats (Diener et al. 1976)

Pestalotiopsis in extreme environments

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

Endophyte-pathogen relationships

Lee et al. (1995) was able to show that *P. microspora* has an endophyte-pathogen relationship with the North American endangered tree *Torreya taxifolia*. They demonstrated that *P. microspora* inhabits the inner bark of the tree without causing symptoms. However, physiological or environmental factors trigger the fungus to become pathogenic. Typical symptoms include needle spots, needle death and stem cankers. The pathogenic ability of the fungus depends upon it producing phytotoxins, pestalopyrones, hydroxypestalopyrones and pestalosides. At the same time antifungal activity by the fungus produces exudates of pestaloside; this competes with other fungi. *Pestalotiopsis subcuticularis* naturally inhabits *Hymenaea courbaril* (Leguminosae) and remains dormant until leaves become mature. Fail and Langenheim (1990) stated that when leaves become mature the fungal hyphae spread and enter in to the intracellular spaces of the leaves. When the plant tissues are damaged due to

mechanical injury such as insect feeding, active infection by the fungus occurs. The typical symptoms of infected leaves included serious leaf blight.

Phylogenetic analysis of existing data in GenBank

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

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

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

Fig. 4 Maximum parsimony phylogram generated from ITS sequence analysis of selected sequences from selected species of *Pestalotiopsis* including *P. clavispora, P. disseminata, P. microspora, P. neglecta, P. photiniae, P. theae, P.virgatula* and *P. vismiae* downloaded from GenBank with other related taxa. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. Type sequences of *Pestalotiopsis pallidotheae, P. hainanensis, P. jesteri* and *P. kunmingensis* are in *black* and **bold**
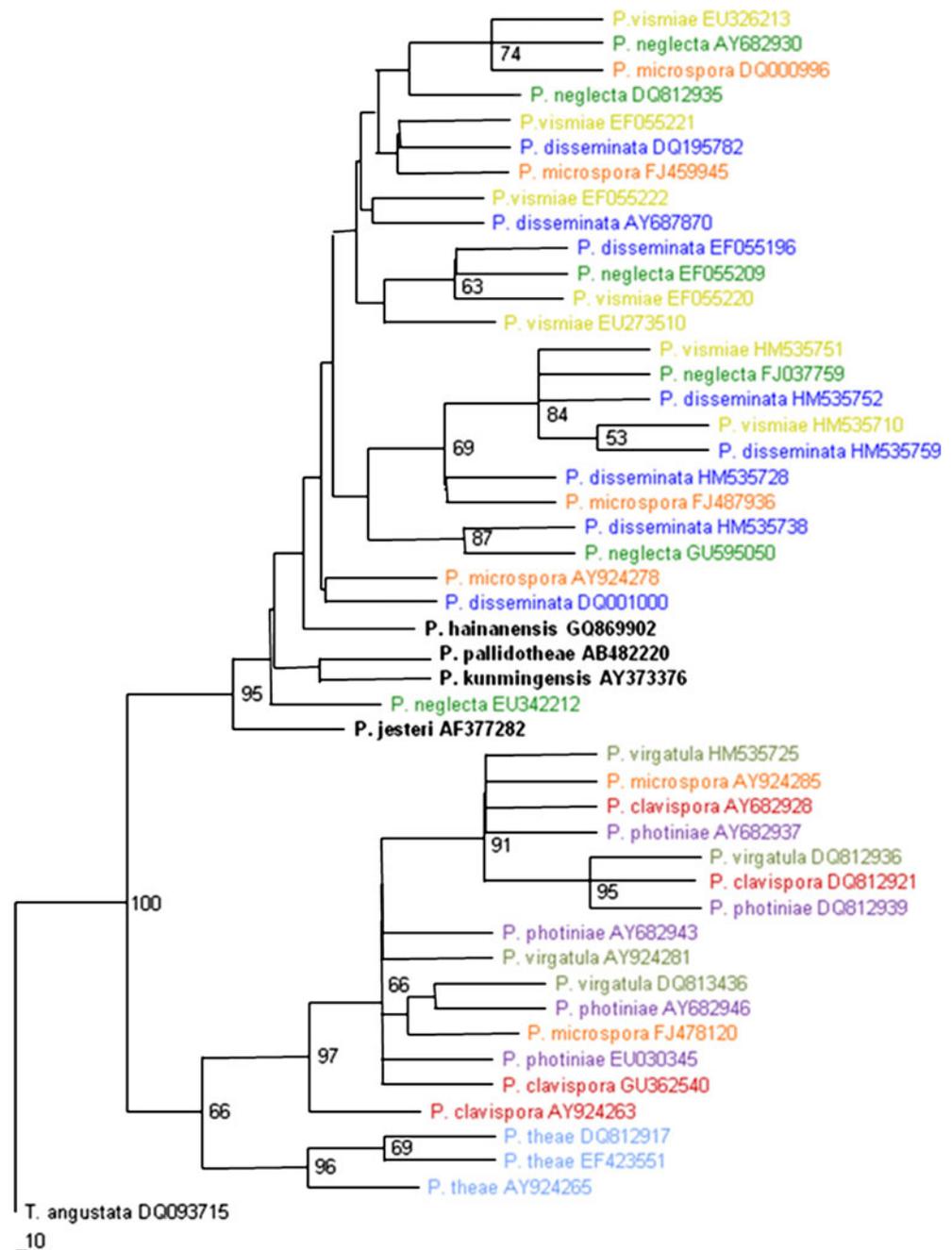
olivaceous" and "umber to fuliginous" colour median cells as valid for the taxonomy of the genus instead of the use of the "concolorous" and "versicolor" median cells grouping system proposed by Steyaert (1949) and Guba (1961).

Pestalotiopsis clavispora, P. disseminata, P. microspora, P. neglecta, P. photiniae, P. theae, P. virgatula and *P. vismiae* can be divided into two groups depending mainly on the colour of the median cells. One group is the versicolorous group, consisting of *P. clavispora, P. photiniae* and *P. virgatula*, and dark concolorous median cells with knobbed apical appendages containing the *P. theae* group. The other group consists of species with concolorous median cells (i.e.,

P. disseminata, P. microspora, P. neglecta and *P. vismiae*. Almost all strains that separate into two main clades depend on the concolorous and versicolor system, and only *P. microspora* strains AY924295 and FJ478120 cluster in the wrong clade. However, within the two main groups, the respective species distributions are scattered and most species overlap with each other. Because of the limitation of characters used to differentiate species (Hu et al. 2007) and many overlapping characters (Sutton 1980), identification to species in *Pestalotiopsis* is presently difficult. For an example according to Guba (1961), *P. disseminata, P. microspora, P. neglecta* and *P. vismiae* within the concolo-

Table 4 Isolates and GenBank accession numbers of taxa used to generate the phylogram. Type species are marked in bold

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

rous group have the same conidia size (18–26×5–8 μm). *Pestalotiopsis vismiae* can be differentiated as it has two apical appendages, while *Pestalotiopsis microspora* is differentiated from *P. neglecta* and *P. dissementa* by the length of the apical appendages. *Pestalotiopsis neglecta* and *P. dissementa* can be distinguished from each other only by the shape of the conidia. Most of above characters vary when in culture and following successive subculturing (Hu et al. 2007). Within the versicolorous group, *P. clavispora* and *P. photiniae* are morphologically very similar (conidia size 19–26×6–8.5 μm), while *P. virgatula* can be differentiated from *P. clavispora* and *P. photiniae* by its relatively small conidia (17–23×6–8 μm). However, these characters overlap and thus identification to these species is rather difficult. For this reason, naming of species is difficult and highly subjective and many sequences for *Pestalotiopsis* deposited in GenBank are likely to be wrongly named.

Species numbers

According to *Index Fungorum* (http://www.indexfungorum. org/names/names.asp; accession date, 2010.10.21) there are 235 *Pestalotiopsis* names, while in MycoBank (www. mycobank.org/mycotaxo.aspx; accession date, 2010.10.21)

there are 232 names. The reason for the large number of names is historical and may not reflect the actual number of species (Jeewon et al. 2004). As with other pathogenic genera such as *Colletotrichum* (Cai et al. 2009), species of *Pestalotiopsis* were historically named according to the host from which they were first observed. If a new host occurrence was found a new species was described. For example, Venkatasubbaiah et al. (1991) isolated a species of *Pestalotiopsis* from leaves of *Oenothera laciniata* and described the new species *P. oenotherae* Venkatas., Grand & Van Dyke. The new species was justified because no species of *Pestalotiopsis* had been described previously from *Oenothera* and its morphological characters clearly distinguished it from other species found on any member of the family Onagraceae (Venkatasubbaiah et al. 1991). Kohlmeyer and Kohlmeyer (2001) described *Pestalotiopsis juncestris* Kohlm & Volkm.-Kohlm which was isolated from the host *Juncus roemerianus*; the taxon is morphologically similar to *P. versicolor* and several other species of *Pestalotiopsis*, but the taxon was described as a new species based on the host occurrence. Similarly, Pal and Purkayastha (1992) and Singh (1981) described the new species *P. agallochae* A.K. Pal bis and Purkay and *P. arborei* N.I. Singh, respectively based on host occurrence. As recently as 2002, Chen et al. (2002) described *P. afinis* Y.X. Chen & G.

Wei, *P. alpiniae* Y.X. Chen & G. Wei, *P. antiaris* Y.X. Chen and G. Wei, *P. dilleniae* Y.X. Chen & G. Wei, *P. kuwangsiensis* Y.X. Chen and G. Wei, *P. nelumbinis* Y.X. Chen & G. Wei, *P. schimae* Y.X. Chen & G. Wei and *P. synsepali* Y.X. Chen & G. Wei based on the host association.

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

Species status and host-specificity within the genus *Pestalotiopsis* has been questioned previously or investigated (Zhu 1989; Jeewon et al. 2004; Wei et al. 2005, 2007; Hu et al. 2007). These authors showed that different species isolated from the same host may not be phylogenetically closely related (Jeewon et al. 2004; Wei et al. 2007). Wei et al. 2007 investigated endophytic species of *Pestalotiopsis* associated with plant species in the families Podocarpaceae, Theaceae and Taxaceae. The endophytic species of *Pestalotiopsis* associated with these host families were not generally host-specific, occurring on a range of hosts. For example, *P. neglecta* (Thüm.) Steyaert and *P. photiniae* were isolated from all the host plants in three plant families. Tejesvi et al. (2007a) isolated endophytic species of *Pestalotiopsis* associated with the medicinal plants *Azadirachta indica*, *Holarrhena antidysenterica*, *Terminalia arjuna* and *T. chebula*. They showed that isolates obtained from a single plant were genetically diverse, while the same species occurred in most plants. According to Guba (1961), most species of the *Pestalotia* were listed from a range of hosts. For example, *Pestalotia microspora* was listed from several different host plants (i.e., *Ananas comosus*, *Araucaria* sp., *Carya* sp., *Hedera helix*, *Juniperus bermudiana* and *Platanus occidentalis*). Hu et al. (2007) tested the relationships of endophytic *Pestalotiopsis* strains from two tissues of *Pinus armandii* and found that even strains isolated from the same tissue type were not phylogenetically related. Zhu (1989) used artificial cross inoculation studies to show that pathogenic species of *Pestalotiopsis* may not be specific to the single host. Jeewon et al. (2004) pointed out that host-specificity of *Pestalotiopsis* is not supported by the large number of species recorded on one host. They also argued that many taxa used in literature can be misinterpretations or synonyms

of species with wide host ranges. Jeewon et al. (2004) used analysis of ITS and 5.8S rDNA to show that isolates taken from the same host were not phylogenetically related and that taxa with similar morphological characters were phylogenetically related.

Up to this time, most phylogenetic research on *Pestalotiopsis* has shown that *Pestalotiopsis* is not highly host-specific and that species are found on a range of hosts (Jeewon et al. 2004; Wei et al. 2005, 2007; Hu et al. 2007). The diseases caused by species of *Pestalotiopsis* have been recorded in different ecosystems and infect a diverse range of unrelated plant taxa. Isolation of endophytic *Pestalotiopsis* strains for bioprospecting for new biochemical compounds have shown that the same species can be found in a range of hosts. Therefore, most of the species recorded in checklists and the literature may not reflect what actually occurs. As in other related plant pathogenic genera such as *Colletotrichum*, the *Pestalotiopsis* species concept depends mostly on the conidial characteristics. It has been shown that most of the key conidial characters used in species level separation are not stable and vary with host range, generation, culture and other environmental conditions (Hu et al. 2007). The arrangement of species by Steyaert (1949) and Guba (1961) in various coloured groupings is problematic because this character has been shown to be variable within a species (Liu et al. 2010a). Thus, most species in the above arrangements may be confused and many species are probably synonyms. Due to the fact that (1) species of *Pestalotiopsis* are generally not host-specific, (2) conidial characters vary and species limits overlap, and (3) species arrangements in Steyaert (1949) and Guba (1961) are problematic, then the actual number of species in *Pestalotiopsis* is likely to be much lower than presently recorded in databases (e.g., Index Fungorum, MycoBank) and the literature (Kirk et al. 2008).

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

studies. Therefore, modern research approaches are needed for species of *Pestalotiopsis* in order to establish the acceptable names.

Species numbers and accepted species

When species are morphologically distinct and molecular evidence shows they are monophyletic, then such species can be considered as a distinct and valid species in a particular genus. Based on their distinct morphological characters, we suggest that the 20 species listed in Table 5 can be considered as good species in the genus at this time. Furthermore some other species (Table 6) which have considerable value because of their economic roles (in bioactive metabolites production, frequent pathogens, or frequently isolated endophytes) are possibly good species. We suggest that type material of these species should be reexamine and epitypified with fresh collections. With the help of ex-type living cultures and sequence data, a robust species concept can be developed for the genus *Pestalotiopsis*.

Novel *Pestalotiopsis* biochemistry

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

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

Pestalotiopsis microspora is a common species present in tropical and subtropical plants and is a widespread saprobe of bark and decaying plant material (Metz et al.

Table 5 Morphologically distinct *Pestalotiopsis* species with their host and location

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

Table 6 Economically important *Pestalotiopsis* species with their host and location

Economically important species	Host and location	Economically importance	
Pestalotiopsis adusta (Ellis & Everh.) Steyaert	On leaves of *Prunus cerasus* in Newfield, New Jersey, United States	Bioactive metabolites	Li et al. 2008b
P. clavispora	On leaves of *Quercus* sp. in Auburn, Alabama, United States	Plant pathogen, Common endophyte	Keith et al. 2006; Espinoza et al. 2008; Wei et al. 2007; Liu et al. 2007
P. disseminata	On dead leaves of *Eucalyptus globules* in Coimbra, Portugal	Plant pathogen, Bioactive metabolites	Das et al. 2010; Keith et al. 2006; Deyrup et al. 2006
P. fici Steyaert	On *Ficus* sp. in Kiagwe, Uganda	Bio active metabolites	Liu et al. 2008a, b, 2009b
P. foedan (Sacc. & Ellis) Steyaert	On decaying bark of *Thuja occidentalis* in Newfield, New Jersey, United States	Bio active metabolites	Ding et al. 2008a
P. heterocornis	On leaves of *Anarcardium occidentale* in Cantanduva, São Paulo, Brazil	Common endophyte	Wei et al. 2007; Liu et al. 2007
P. longiseta	On leaves of *Rubus caesius* in Susegana, Conegliano, Italy	Plant pathogen, Bioactive metabolites	Joshi et al. 2009; Nagata and Ando 1989; Nagata et al. 1992; Xu et al. 2010
P. microspora	On leaves of *Hedera helix* in Botanical garden, College of Argentina, Buenos Aires, Argentina	Plant pathogen, Common endophyte, Bioactive metabolites	Strobel et al. 1996a, b, 2000; Metz et al. 2000; Keith et al. 2006; Jeon et al. 2007; Womersley (1995); Harper et al. 2003; Lee et al. 1995; Kai et al. 2003
P. neglecta	On leaves of *Euonymus japonicas* in Coimbra, Portugal	Plant pathogen, Endophyte	Tagne and Mathur 2001; Espinoza et al. 2008; Wei et al. 2007; Liu et al. 2007
P. pauciseta (Sacc.) Y.X. Chen	On leaves of *Litsea glutinosa* in Mount Makiling, near Los Banos, Laguna province, Philippine	Bioactive metabolites	Gangadevi et al. 2008
P. photiniae	On leaves of *Photinia serrulata* in Istria, Australia	Bioactive metabolites	Ding et al. 2009
P. theae	On leaves of *Camellia sinensis* in Japan	Plant pathogen, Endopyte, Bioactive metabolites	Li et al 2008a; Nagata et al. 1992; Shimada et al. 2001; Tuset et al. 1999; Worapong et al. 2003; Joshi et al. 2009; Muraleedharan and Chen 1997; Ding et al. 2008b; Shimada et al. 2001
P. uvicola	On *Gaura parviflora* and *Vitis vinifera* in Italy	Plant pathogen	Vitale and Polizzi 2005; Xu et al. 1999

2000). The species has most commonly been isolated as an endophyte associated with rainforest plants (Strobel et al. 2002) or as a pathogen (Keith et al. 2006). Pathogen associations include scab disease on *Psidium guajava* (Keith et al. 2006), leaf blight of *Lindera obtusiloba* (Jeon et al. 2007) and as an endophyte on *Terminalia morobensis* (Womersley 1995). *Pestalotiopsis microspora* has the potential to be a model organism for biological and biochemical studies in the laboratory (Metz et al. 2000). Isolates of this species (or possibly species complex) show diverse genetic variation and thus each individual isolate is generally unique in the substances that it produces (Harper et al. 2003). Long et al. (1998) have shown that under laboratory conditions it can take up heterologous DNA, add telomeric DNA, express heterologous DNA and can replicate independently of chromosomal DNA.

Such genetic diversity would be useful to the species in nature, helping it adapt to a new plant by incorporating plant DNA into its own genome (Strobel et al. 1996a; Li et al. 1996). Bioactive compounds such as the anti-cancer drug taxol, jesterone, ambuic acid, torreyanic acid, pestaloside, pestalotiopsins and 2-a hydroxydimeniol (Strobel et al. 2002), hetero-polysaccharides (Kai et al. 2003) have been obtained from *P. microspora*. The multimillion dollar anti-cancer drug, taxol was obtained from an endophytic strain of *P. microspora* isolated from *Taxus wallachiana* (Strobel et al. 1996a) and *Taxodium distichurn* (Strobel et al. 1996b). Kai et al. (2003) found that *P. microspora* can metabolize various monosaccharides and the composition of hetero-polysaccharides depends on the type of monosaccharide in the media. Harper et al. (2003) investigated the production of pestacin, a 1,3-dihydro isobenzofuran with moderate anti-fungal properties and high anti-oxidant activity when compared with the vitamin E derivative trolox from endophytic strains of *P. microspora*. The anti-oxidant activity works mainly by cleavage of an unusually reactive C–H bond. Lee et al. (1995) obtained several anti-fungal compounds such as pestaloside, an aromatic glucoside, and two pyrones (pestalopyrone and hydroxypestalopyrone)

from a strain of *P. microspora* isolated from the endangered North American tree *Torreya taxifolia*. When *Pestalotiopsis microspora* is cultured on media containing various monosaccharides as a carbon source, different polysaccharides are produced and this mainly depends on the monosaccharide used as the carbon source (Kai et al. 2003). Whether all these strains were in fact *P. microspora* is yet to be determined, since the identifications were based on morphology or comparison with GenBank sequence data, which itself may be erroneously named. This species is in need of epitypification.

Pestalotiopsis theae is an economically important species that has been reported from all major tea growing countries of the world (Muraleedharan and Chen 1997) and also as an endophyte (Worapong et al. 2003). Pestalotheols A–D, four new metabolites isolated from endophytic *Pestalotiopsis theae,* and pestalotheol C showed an inhibitory effect against HIV-1$_{LAI}$ replication in C8166 cells (Li et al. 2008a). Three new compounds, pestalamides A–C and two known metabolites, aspernigrin A and carbonarone A, were obtained from the same fungus isolated from the branches of tea (Ding et al. 2008b). The newly isolated pestalamide B inhibited HIV-1 replication in C8166 cells with EC$_{50}$ of 64.2 μM and antifungal activity against *Aspergillus fumigatus*. Chloroisosulochrin and chloroisosulochrin dehydrate were obtained from the culture filtrate of *Pestalotiopsis theae,* and these compounds can be used as plant growth regulators (Shimada et al. 2001). This species is obviously important as a producer of novel medicinal metabolites.

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

N-formylnorfloxacin (3.6%), and 7-amino-1-ethyl-6-fluoro- 4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2.1%) were obtained.

Liu et al. (2008b) isolated five new cyclohexanone derivatives, pestalofones A–E, with the known compounds isosulochrin, isosulochrin dehydrate, and iso-A82775C, from cultures of the plant endophytic fungus *Pestalotiopsis fici*. Pestalofones A and B were inhibitory against HIV-1 replication in C8166 cells, pestalofones C showed antifungal activity against *Aspergillus fumigatus* while pestalofones E showed both the above effects. Chloropestolide A extracted from the scale-up fermentation extract of *Pestalotiopsis fici* showed significant inhibitory effects on growth of two human cancer cell lines, HeLa and HT29 (Liu et al. 2009). Liu et al. (2010b) obtained chloropupukeanolides A and B (unprecedented spiroketal peroxide) and chloropupukeanone A (three highly functionalized metabolites featuring a chlorinated pupukeanane core) from an endophytic strain of *Pestalotiopsis fici*. The compound chloropupukeanolide A showed significant anti-HIV-1 and cytotoxic effects.

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

Taxonomic confusion and way forward

Pestalotiopsis is taxonomically poorly understood both at the inter- as well as the intraspecific level. It is not clear whether *Pestalotia* is really distinct from *Pestalotiopsis,* since stains of the type of the former have not been sequenced. Nomenclature of the genus is confusing and most host based names in databases may be synonyms. Molecular data have still not been successfully applied for species-level differentiation and names applied to data in GenBank are doubtful, as they are not linked to any type materials. Epitypification with molecular work is therefore needed to understand the species and what distinguishes them. Re-examination of type materials and establishment of epitypes with living cultures is essential for real progress (Hyde and Zhang 2008), and sequence data are needed to develop a strong species-based taxonomic system for the genus *Pestalotiopsis*. It is only then that plant pathologists can confidently name disease causal agents, quarantine can put in effective measures to prevent entry of unwanted species of *Pestalotiopsis,* plant breeders can breed resistance against pathogenic species and biochemists can confidently put names to species producing novel chemicals and use an understanding of species relationships to aid in bioprospecting.

References

Agarwal AK, Chauhan S (1988) A new species of the genus *Pestalotiopsis* from Indian soil. Indian Phytopathol 41:625–627

Agnihothrudu V (1964) A world list of fungi reported on tea. J Madras University 34:155–271

Agrios GN (2005) Plant pathology, 5th edn. Elsevier Academic, USA

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41(1):1–16

Armstrong-Cho CL, Banniza S (2006) *Glomerella truncata* sp. nov., the teleomorph of *Colletotrichum truncatum*. Mycol Res 110:951–956

Bajo J, Santamaria O, Diez J (2008) Cultural characteristics and pathogenicity of *Pestalotiopsis funerea* on *Cupressus arizonica*. For Pathol 38:263–274

Barr ME (1975) *Pestalosphaeria*, a new genus in the Amphisphaeriaceae. Mycologia 67:187–194

Barr ME (1990) Prodromus to nonlichenized, pyrenomycetous members of class Hymenoascomycetes. Mycotaxon 39:43–184

Bate-Smith EC, Metcalfe CR (1957) Leucanthocyanins .3. The nature and systematic distribution of tannin in dicotyledonous plants. J Linn Soc (Bot) 55:669–705

Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller J, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Divers 39:183–204

Cannon PF, Kirk PM (2000) The philosophy and practicalities of amalgamating anamorph and teleomorph concepts. Stud Mycol 45:19–25

Chaverri P, Castlebury LA, Overton BE, Samuels GJ (2003) *Hypocrea/Trichoderma*: species with conidiophore elongations and green conidia. Mycologia 95:1100–1140

Chen YX, Wei G, Chen WP (2002) New species of *Pestalotiopsis*. Mycosystema 21:316–323

Das Ranjana, Chutia M, Das K, Jha DK (2010) Factors affecting sporulation of *Pestalotiopsis disseminata* causing grey blight disease of *Persea bombycina* Kost., the primary food plant of muga silkworm. Crop Prot 29:963–968

De Notaris G (1839) Micromycetes italiei Dec II. Mere R Acad Sci Torino II 3:80–81

Dennis RWG (1995) Fungi of the South East England. Royal Botanic Gardens, Kew

Deyrup ST, Swenson DC, Gloer JB, Wicklow DT (2006) Caryophyllene sesquiterpenoids from a fungicolous isolate of *Pestalotiopsis disseminata*. J Nat Prod 69:608–611

Diener UL, Wagener RE, Morgan-Jones G, Davis ND (1976) Toxigenic fungi from cotton. Phytopathology 66:514–516

Ding G, Liu S, Guo L, Zhou Y, Che Y (2008a) Antifungal metabolites from the plant endophytic fungus *Pestalotiopsis foedan*. J Nat Prod 71(4):615–618

Ding G, Jiang L, Guo L, Chen X, Zhang H, Che Y (2008b) Pestalazines and pestalamides, bioactive metabolites from the plant pathogenic fungus *Pestalotiopsis theae*. J Nat Prod 71 (11):1861–1865

Ding G, Zheng Z, Liu S, Zheng H, Guo L, Che Y (2009) Photinides A-F, cytotoxic benzofuranone-derived γ-lactones from the plant endophytic fungus *Pestalotiopsis photiniae*. J Nat Prod 72:942–945

Dodd SL, Lieckfeldt E, Samuels GJ (2003) *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. Mycologia 95:27–40

Douanla-Meli C, Langer E (2009) *Pestalotiopsis theae* (*Ascomycota*, *Amphisphaeriaceae*) on seeds of *Diospyros crassiflora* (*Ebenaceae*). Mycotaxon 107:441–448

Dube HC, Bilgrami KS (1965) Variations in the conidial morphology of *Pestalotiopsis darjeelingensis* in culture. Curr Sci 34:487

Egger KN (1995) Molecular analysis of ectomycorrhizal fungal communities. Can J Bot 73:1415–1422

Elliott ML, Broschat TK, Uchida JY, Simone GW (eds) (2004) Diseases and disorders of ornamental palms. American Phytopathological Society, St. Paul

Ellis MB, Ellis JP (1997) Microfungi on land plants: an identification handbook, 2nd edn (New Enlarged). The Richmond Publishing Co. Ltd

Espinoza JG, Briceno EX, Keith LM, Latorre BA (2008) Canker and Twig Dieback of blueberry caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. Plant Dis 92:1407–1414

Fail GL, Langenheim JH (1990) Infection process of *Pestalotia subcuticularis* on leaves of *Hymenaea courbaril*. Phytopathology 80:1259–1265

Gangadevi V, Murugan M, Muthumary J (2008) Taxol determination from *Pestalotiopsis pauciseta*, a fungal endophyte of a medicinal plant. Chin J Biotechnol 24(8):1433–1438

Gehlot P, Bohra NK, Purohit DK (2008) Endophytic mycoflora of inner bark of *Prosopis cineraria*—a key stone tree species of Indian desert. Am-Eur J Bot 1(1):01–04

Gomes-Figueiredo J, Pimentel IC, Vicente VA, Pie MR, Kava-Cordeiro V, Galli-Terasawa L, Pereira JO, de Souza AQ, Glienke C (2007) Bioprospecting highly diverse endophytic *Pestalotiopsis* spp. with antibacterial properties from *Maytenus ilicifolia*, a medicinal plant from Brazil. Can J Microbiol 53 (10):1123–1132

Griffiths DA, Swart HJ (1974a) Conidial structure in two species of *Pestalotiopsis*. Trans Br Mycol Soc 62:295–304

Griffiths DA, Swart HJ (1974b) Conidial structure in *Pestalotia pezizoides*. Trans Br Mycol Soc 63:169–173

Guba EF (1956) *Monochaetia* and *Pestalotia* vs. *Truncatella*, *Pestalotiopsis* and *Pestalotia*. Ann Microb Enzymol Milan 7:74–76

Guba EF (1961) Monograph of *Pestalotia* and *Monochaetia*. Harvard University Press, Cambridge

Halfeld-Vieira BA, Nechet KA (2006) First report of *Pestalotiopsis macrochaeta* on *Carapa guianensis*. Plant Pathol 55:304

Harper JK, Barich DH, Hu JZ, Strobel GA, Grant DM (2003) Stereochemical analysis by solid-state NMR: structural predictions in ambuic acid. J Org Chem 68:4609–4614

Hopkins KE, McQuilken MP (1997) *Pestalotiopsis* on nursery stock, in HDC Project News No 39. Horticultural Development Council, East Malling

Hopkins KE, McQuilken MP (2000) Characteristics of *Pestalotiopsis* associated with hardy ornamental plants in the UK. Eur J Plant Pathol 106:77–85

Horikawa T (1986) Yield loss of new tea shoots due to grey blight caused by *Pestalotia longiseta* Spegazzini. Bull Shizuoka Tea Exp Stn 12:1–8

Hu HL, Jeewon R, Zhou DQ, Zhou TX, Hyde KD (2007) Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: evidence from rDNA and β-tubulin gene phylogenies. Fungal Divers 24:1–22

Hyde KD (1996) Fungi from palms. XXV. Pestalosphaeria elaeidis. Mycotaxon 57:353–357

Hyde KD, Fröhlich J (1995) Mycosphaerella palmicola associated with leaf spots of Cocos nucifera in Australia Iran Jaya and Papua New Guinea. Mycol Res 99:704–706

Hyde KD, Zhang Y (2008) Epitypification: should we epitypify?. Journal of Zhejiang University Science B 9:842–846.

Hyde KD, McKenzie EHC, KoKo TW (2011) Towards incorporating anamorphic fungi in a natural classification—checklist and notes for 2010. Mycosphere 2(1):1–88

Jeewon R, Liew ECY, Hyde KD (2002) Phylogenetic relationships of Pestalotiopsis and allied genera inferred from ribosomal DNA sequences and morphological characters. Mol Phylogenet Evol 25:378–392

Jeewon R, Liew ECY, Simpson JA, Hodgkiss IJ, Hyde KD (2003) Phylogenetic significance of morphological characters in the taxonomy of Pestalotiopsis species. Mol Phylogenet Evol 27:372–383

Jeewon R, Liew ECY, Hyde KD (2004) Phylogenetic evaluation of species nomenclature of Pestalotiopsis in relation to host association. Fungal Divers 17:39–55

Jeon YH, Kim SG, Kim YH (2007) First report on leaf blight of Lindera obtusiloba caused by Pestalotiopsis microspora in Korea. Plant Pathol 56:349

Joshi SD, Sanjay R, Baby UI, Mandal AKA (2009) Molecular characterization of Pestalotiopsis spp. associated with tea (Camellia sinensis) in southern India using RAPD and ISSR markers. Indian J Biotechnol 8(4):377–383

Kai A, Kikawa M, Hatanaka K, Matsuzaki K, Mimura T, Kaneko Y (2003) Biosynthesis of hetero-polysaccharides by Pestalotiopsis microspora from various monosaccharides as carbon source. Carbohydr Polym 54:381–383

Kang JC, Kong RYC, Hyde KD (1998) Studies on the Amphisphaeriales I. Amphisphaeriaceae (sensu stricto) and its phylogenetic relationships inferred from 5.8S rDNA and ITS2 sequences. Fungal Divers 1:147–157

Kang JC, Hyde KD, Kong RYC (1999) Studies on the Amphisphaeriales. The Amphisphaeriaceae (sensu stricto). Mycol Res 103:53–64

Karaca GH, Erper I (2001) First report of Pestalotiopsis guepinii causing twig blight on hazelnut and walnut in Turkey. Plant Pathol 50:415

Kaushik CD, Thakur DP, Chand JN (1972) Parasitism and control of Pestalotia psidii causing cankerous disease of ripe guava fruits. Indian Phytopath 25:61–64

Keith LM, Zee FT (2010) Guava disease in Hawaii and the characterization of Pestalotiopsis spp. affecting guava. Acta Horticulturae (ISHS) 849:269–276

Keith LM, Velasquez ME, Zee FT (2006) Identification and characterization of Pestalotiopsis spp. causing scab disease of guava, Psidium guajava in Hawaii. Plant Dis 90:16–23

Kendrick B (ed) (1979) The whole fungus: the sexual-asexual synthesis. Volume 1–2. National Museums of Canada, Ottawa

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi, 10th edn. CABI, Wallingford

Klebahn H (1914) Beitrage zur Kenntnis der Fungi Imperfecti. Zur Kritik einiger Pestalozzia-Arten. Mykol Zbl 4:1–19

Kobayashi T, Ishihara M, Ono Y (2001) A new species of Pestalosphaeria, the telomorph of Pestalotiopsis neglecta. Mycoscience 42:211–216

Kohlmeyer J, Kohlmeyer VB (2001) Fungi on Juncus roemerianus 16. More new coelomycetes, including Tetranacriella gen. nov. Bot Mar 44:147–156

Korsten L, De Jager ES, De Villers EE, Lourens A, Kotze JM, Wehner FC (1995) Evaluation of bacterial epiphytes isolated from avocado leaf and fruit surfaces for biocontrol of avocado postharvest diseases. Plant Dis 79:1149

Kumar DSS, Hyde KD (2004) Biodiversity and tissue-recurrence of endophytic fungi in Tripterygium wilfordii. Fungal Divers 17:69–90

Kwee LT, Chong KK (1990) Guava in Malaysia: production, pests and diseases. Tropical Press SDN. BHD, Kuala Lumpur

Kwon GS, Moon SH, Hong SD, Lee HM, Kim HS, Oh HM, Yoon BD (1996) A novel flocculant biopolymer produced by Pestalotiopsis sp. KCTC 8637P. Biotechnol Lett 18(12):1459–1464

Lee JC, Yang X, Schwartz M, Strobel G, Clardy J (1995) The relationship between an endangered North American tree and an endophytic fungus. Chem Biol 2:721–727

Lee JC, Strobel GA, Lobkovsky E, Clardy JC (1996) Torreyanic acid: a selectively cytotoxic quinone dimer from the endophytic fungus Pestalotiopsis microspora. J Org Chem 61:3232–3233

Li JY, Strobel GA (2001) Jesterone and hydroxy-jesterone antioomy-cetcyclohexenenone epoxides from the endophytic fungus Pestalotiopsis jesteri. Phytochemistry 57:261–265

Li JY, Strobel GA, Sidhu RS, Hess WM, Ford EJ (1996) Endophytic taxol-producing fungi from bald cypress, Taxodium distichum. Microbiology 142:2223–2226

Li E, Tian R, Liu S, Chen X, Guo L, Che Y (2008a) Pestalotheols A–D, bioactive metabolites from the plant endophytic fungus Pestalotiopsis theae. J Nat Prod 71(4):664–668

Li E, Jiang L, Guo L, Zhang H, Che Y (2008b) Pestalachlorides A–C, antifungal metabolites from the plant endophytic fungus Pestalotiopsis adusta. Bioorg Med Chem 16:7894–7899

Liu AR, Wu XP, Xu T, Guo LD, Wei JG (2006) Notes on endophytic Pestalotiopsis from Hainan, China. Mycosystema 25:389–397

Liu AR, Xu T, Guo LD (2007) Molecular and morphological description of Pestalotiopsis hainanensis sp. nov., a new endophyte from a tropical region of China. Fungal Divers 24:23–36

Liu L, Liu S, Jiang L, Chen X, Guo L, Che Y (2008a) Chloropupukeananin, the first chlorinated pupukeanane derivative, and its precursors from Pestalotiopsis fici. Org Lett 10:1397–1400

Liu L, Tian RR, Liu SC, Chen XL, Guo LD, Che YS (2008b) Pestaloficiols A–E, bioactive cyclopropane derivatives from the plant endophytic fungus Pestalotiopsis fici. Bioorg Med Chem 16:6021–6026

Liu L, Li Y, Liu SC, Zheng ZH, Chen XL, Zhang H, Guo LD, Che YS (2009) Chloropestolide A, an antitumor metabolite with an unprecedented spiroketal skeleton from Pestalotiopsis fici. Org Lett 11:2836–2839

Liu AR, Chen SC, Wu SY, Xu T, Guo LD, Jeewon R, Wei JG (2010a) Cultural studies coupled with DNA based sequence analyses and its implication on pigmentation as a phylogenetic marker in Pestalotiopsis taxonomy. Mol Phylogenet Evol 57:528–535

Liu L, Niu S, Lu X, Chen X, Zhang H, Guo L, Che Y (2010b) Unique metabolites of Pestalotiopsis fici suggest a biosynthetic hypothesis involving a Diels-Alder reaction and then mechanistic diversification. Chem Comm 46:460–462

Long DM, Smidansky ED, Archer A, Strobel GA (1998) In vivo addition of telomeric repeats to foreign DNA generates extra-chromosomal DNAs in the taxol-producing fungus Pestalotiopsis microspora. Fungal Genet Biol 24:335–344

Madar Z, Solel Z, Kimchi M (1991) Pestalotiopsis canker of Cypress in Israel. Phytoparasitica 19(1):79–81

McQuilken MP, Hopkins KE (2004) Biology and integrated control of Pestalotiopsis on container-grown ericaceous crops. Pest Manag Sci 60:135–142

Metz AM, Haddad A, Worapong J, Long DM, Ford EJ, Hess WM, Strobel GA (2000) Induction of the sexual stage of Pestalotiopsis microspora, a taxol-producing fungus. Microbiology 146:2079–2089

Moreau C (1949) Micomycetes africains. I. Rev Mycol, Suppl Colon (Paris) 14:15–22

Muraleedharan N, Chen ZM (1997) Pests and diseases of tea and their management. J Plant Crop 25:15–43

Nag Rag TR (1993) Coelomycetous anamorphs with appendage bearing conidia. Mycologue, Waterloo

Nag Raj TR (1985) Redisposals and redescriptions in the *Monochaetia.—Seiridium*, *Pestalotia—Pestalotiopsis* complexes. II. *Pestalotiopsis besseyii* (Guba) comb. nov. and *Pestalosphaeria varia* sp. nov. Mycotaxon 22:52–63

Nagata T, Ando Y (1989) Oxysporone, a phytotoxin isolated from the tea gray blight fungus *Pestalotia longiseta*. Agric Biol Chem 53:2811

Nagata T, Ando Y, Hirota A (1992) Phytotoxins from tea gray blight fungi, *Pestalotiopsis longiseta* and *Pestalotiopsis theae*. Biosci Biotechnol Biochem 56:810–811

Okane I, Nagagiri A, Ito T (1998) Endophytic fungi in leaves of ericaceous plants. Can J Bot 76:657–663

Osono T, Takeda H (1999) Decomposing ability of interior and surface fungal colonizers of beech leaves with reference to lignin decomposition. Eur J Soil Biol 35:51–56

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Pal AK, Purkayastha RP (1992) New parasitic fungi from Indian mangrove. J Mycopathol Res 30:173–176

Pandey RR (1990) Mycoflora associated with floral parts of guava (*Psidium guajava* L.). Acta Bot Sin 18:59–63

Parshikov IA, Heinze TM, Moody JD, Freeman JP, Williams AF, Sutherland JB (2001) The fungus *Pestalotiopsis guepinii* as a model for biotransformation of ciprofloxacin and norfloxacin. Appl Microbiol Biotechnol 56:474–477

Petrak VF (1947) *Neobroomella* n. gen., eine neue Gattung der Sphaeriales. Sydowia 1(1–3):5

Pirone PP (1978) Diseases and pests of ornamental plants. Wiley Interscience, New York

Purohit DK, Bilgrami KS (1969) Variations in the conidial morphology of genus *Pestalotiopsis*. Indian Phytopathology 22:275–279

Reynolds DR (1993) The fungal holomorph: an overview. In: Reynolds DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. CAB International, Oxon, pp 15–25

Rivera MC, Wright ER (2000) First report of azalea petal blight caused by *Pestalotiopsis guepinii* in Argentina. Plant Dis 84:100

Rodrigues KF, Costa GL, Carvalho MP, Epifanio RA (2005) Evaluation of extracts produced by some tropical fungi as potential cholinesterase inhibitors. World J Microbiol Biotechnol 21:1617–1621

Sangchote S, Farungsang U, Farungsang N (1998) Pre and postharvest infection of rambutan by pathogens and effect on postharvest treatments. In: Coates LM, Hofman PJ, Johnson GI (eds) Disease control and storage life extension in fruits. Proceeding of an international workshop held at Chaing Mai, Thailand May 22–23, 1997. ACIAR Proceeding No. 81: 87–91

Sanjay R, Ponmurugan P, Baby UI (2008) Evaluation of fungicides and biocontrol agents against grey blight disease of tea in the field. Crop Prot 27:689–694

Sati SC, Belwal M (2005) Aquatic hyphomycetes as endophytes of riparian plant roots. Mycologia 97:45–49

Satya HN, Saksena SB (1984) Some aspects of taxonomy of the genus *Pestalotia* I-Color intensities of intermediate cells of spores. In: Subramanian CV (ed) Proceedings of the International Symposium on Taxonomy of Fungi

Schwendener S (1868) Ueber die Beziehungen zwischen Algen und Flechtengonidien. Bot Zeitung 26:289–292

Servazzi O (1953) Nuovo Giorn. Bot. Ital. 60 (n.s.) 4:943–947

Shearer CA, Raja HA, Schmit JP (2007) Freshwater ascomycetes and their anamorphs—website available online at http://www.life.uiuc.edu/fungi/

Shenoy BD, Jeewon R, Hyde KD (2007) Impact of DNA sequence-data on the taxonomy of anamorphic fungi. Fungal Divers 26:1–54

Shimada A, Takahashi I, Kawano T, Kimura YZ (2001) Chloroisosulochrin, chloroisosulochrin dehydrate, and pestheic acid, plant growth regulators, produced by *Pestalotiopsis theae*. J Biosci (Z Naturforsch) 56b:797–803

Singh NI (1981) Some new host records for India. Indian Phytopathology 34:233–234

Sousa MF, Tavares RM, Gerós H, Lino-Neto T (2004) First report of *Hakea sericea* leaf infection caused by *Pestalotiopsis funerea* in Portugal. Plant Pathol 53:535

Srinivasan K, Muthumary J (2009) Taxol production from *Pestalotiopsis* sp an endophytic fungus isolated from *Catharanthus roseus*. J Ecobiotechnology 1(1):28–31

Steyaert RL (1949) Contributions à l'étude monographique de *Pestalotia* de Not. et *Monochaetia* Sacc. (*Truncatella* gen. nov. et *Pestalotiopsis* gen. nov.). Bull. Jard. Bot. Bruxelles 19:285–354

Steyaert RL (1953a) New and old species of *Pestalotiopsis*. Trans Br Mycol Soc 36:81–89

Steyaert RL (1953b) *Pestalotiopsis* from the Gold Coast and Togoland. Trans Br Mycol Soc 36:235–242

Steyaert RL (1955) *Pestalotia*, *Pestalotiopsis* et *Truncatella*. Bull. Jard. Bot. Bruxelles 25:191–199

Steyaert RL (1956) A reply and an appeal to Professor Guba. Mycologia 48:767–768

Steyaert RL (1961) Type specimens of Spegazzini's collections in the *Pestalotiopsis* and related genera (Fungi Imperfecti: Melanconiales). Darwinia (Buenos Aires) 12:157–190

Strobel GA, Long DM (1998) Endophytic microbes embody pharmaceutical potential. Am Soc Microbiol News 64:263–268

Strobel G, Yang XS, Sears J, Kramer R, Sidhu RS, Hess WM (1996a) Taxol from *Pestalotiopsis microspora* of *Taxus wallachiana*. Microbiology 142:435–440

Strobel GA, Hess WM, Ford EJ, Siduhu RS, Yang XJ (1996b) Taxol from fungal endophytes and the issue of biodiversity. Indian Microbiol 17:417–423

Strobel G, Li JY, Ford E, Worapong J, Gary IB, Hess WM (2000) *Pestalotiopsis jesteri*, sp. nov. an endophyte from *Fragraea bodenii* Wernh, a common plant in the southern highlands of Papua New Guinea. Mycotaxon 76:257–266

Strobel G, Ford E, Worapong J, Harper JK, Arif AM, Grant DM, Fung PC, Chau MW (2002) Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. Phytochemistry 60:179–183

Sun HJ, Depriest PT, Gargas A, Rossman AY, Friedmann EI (2002) *Pestalotiopsis maculans*: a dominant parasymbiont in North American lichens. Symbiosis 33:215–226

Sutton BC (1961) Coelomycetes. I. Mycol. Pap. 80:1–16

Sutton BC (1980) The coelomycetes: fungi imperfecti with pycnidia, acervular and stromata. Commonwealth Mycological Institute, Kew

Sutton DA (1999) Coelomycetous fungi in human disease. A review: clinical entities, pathogenesis, identification and therapy. Rev Iberoam Mycol 16:171–179

Swofford DL (2002) PAUP* 4.0: phylogenetic analysis using parsimony (* and other methods). Sinauer Associates, Sunderland

Tagne A, Mathur SB (2001) First report of chlorotic spot of maize caused by *Pestalotiopsis neglecta*. Plant Pathol 50:791

Tejesvi MV, Mahesh B, Nalini MS, Prakash HS, Kini KR, Subbiah V, Shetty HS (2005) Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W and A. (Combretaceae). World J Microbiol Biotechnol 21:1535–1540

Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS (2007a) Genetic diversity and antifungal activity of species of *Pestalo-*

tiopsis isolated as endophytes from medicinal plants. Fungal Divers 24:37–54

Tejesvi MV, Nalini MS, Mahesh B, Prakash HS, Kini KR, Shetty HS, Subbiah V (2007b) New hopes from endophytic fungal secondary metabolites. Bol Soc Quím de Méx 1(1):19–26

Tejesvi MV, Tamhankar SA, Kini KR, Rao VS, Prakash HS (2009) Phylogenetic analysis of endophytic *Pestalotiopsis* species from ethnopharmaceutically important medicinal trees. Fungal Divers 38:167–183

Thongkantha S, Lumyong S, McKenzie EHC, Hyde KD (2008) Fungal saprobes and pathogens occurring on tissues of *Dracaena lourieri* and *Pandanus* spp. in Thailand. Fungal Divers 30:149–169

Tokumasu S, Aoiki T (2002) A new approach to studying microfungal succession on decaying pine needles in an oceanic subtropical region in Japan. Fungal Divers 10:167–183

Tuset JJ, Hinarejos C, Mira JL (1999) First report of leaf blight on sweet persimmon tree by *Pestalotiopsis theae* in Spain. Plant Dis 83:1070

Ullasa BA, Rawal RD (1989) Occurrence of a new post-harvest disease of mango due to *Pestalotiopsis glandicola*. Acta Horticulturae (ISHS) 231:540–543

Venkatasubbaiah P, Grand LF, Dyke CGV (1991) A new species of *Pestalotiopsis* on Oenothera. Mycologia 83(4):511–513

Vitale A, Polizzi G (2005) Occurrence of *Pestalotiopsis uvicola* causing leaf spots and stem blight on bay laurel (*Laurus nobilis*) in Sicily. Plant Dis 89(12):1362

Von Arx JA (1974) The genera of fungi sporulating in pure culture. In: Cramer J (ed) The genera of fungi sporulating in pure culture. A. R.Gantner Veriag Kommanditgesellschaft, Vaduz

Watanabe K, Doi Y, Kobayashi T (1998) Conidiomatal development of *Pestalotiopsis guepinii* and *P. neglecta* on leaves of *Gardenia jasminoides*. Mycoscience 39:71–75

Watanabe K, Parbery DG, Kobayashi T, Doi Y (2000) Conidial adhesion and germination of *Pestalotiopsis neglecta*. Mycol Res 104(8):962–968

Watanabe K, Motohashi K, Ono Y (2010) Description of *Pestalotiopsis pallidotheae*: a new species from Japan. Mycoscience 51:182–188

Wei JG, Xu T (2004) *Pestalotiopsis kunmingensis*, sp. nov., an endophyte from *Podocarpus macrophyllus*. Fungal Divers 15:247–254

Wei JG, Xu T, Guo LD, Pan XH (2005) Endophytic Pestalotiopsis species from southern China. Mycosystema 24:481–493

Wei JG, Xu T, Guo LD, Liu AR, Zhang Y, Pan XH (2007) Endophytic *Pestalotiopsis* species associated with plants of *Podocarpaceae*, *Theaceae* and *Taxaceae* in southern China. Fungal Divers 24:55–74

Womersley JS (1995) Handbooks of the flora of Papua New Guinea. Melbourne University Press, Melbourne

Worapong J, Inthararaungsom S, Stroble GA, Hess WM (2003) A new record of *Pestalotiopsis theae*, existing as an endophyte on *Cinnamomum iners* in Thailand. Mycotaxon 88:365–372

Wright ER, Rivera MC, Flynn MJ (1998) First report of *Pestalotiopsis guepinii* and *Glomerella cingulata* on blueberry in Buenos Aires (Argentina). Boletín 28:219–220

Wu CG, Tseng HY, Chen ZC (1982) Fungi inhabiting on *Schoenoplectus triqueter* (L.) Palla (I). Taiwania 27:35–38

Wulandari NF, To-anun C, Hyde KD, Duong LM, de Gruyter J, Meffert JP, Groenewald JZ, Crous PW (2009) *Phyllosticta citriasiana* sp. nov., the cause of Citrus tan spot of *Citrus maxima* in Asia. Fungal Divers 34:23–39

Xu L, Kusakari S, Hosomi A, Toyoda H, Ouchi A (1999) Postharvest disease of grape caused by *Pestalotiopsis* species. Ann Phytopathol Soc Jpn 65:305–311

Xu J, Ebada SS, Proksch P (2010) *Pestalotiopsis* a highly creative genus: chemistry and bioactivity of secondary metabolites. Fungal Divers 44(1):15–31

Yanna, Ho WH, Hyde KD (2002) Fungal succession on fronds of *Phoenix hanceana* in Hong Kong. Fungal Divers 10:185–211

Yasuda F, Kobayashi T, Watanabe H, Izawa H (2003) Addition of *Pestalotiopsis* spp. to leaf spot pathogens of Japanese persimmon. J Gen Plant Pathol 69:29–32

Zhang YL, Ge HM, Li F, Song YC, Tan RX (2008) New phytotoxic metabolites from *Pestalotiopsis* sp. HC02, a fungus residing in chondracris rosee gut. Chem Biodivers 5(11):2402–2407

Zhu PL (1989) Study on identification and taxonomy of *Pestalotiopsis* spp. from common ornamental plants. Ms. Thesis. Zhejiang Agricultural University, China

Zhu P, Ge Q, Xu T (1991) The perfect stage of *Pestalosphaeria* from China. Mycotaxon 40:129–140