**Pestalotiopsis kunmingensis** sp. nov., an endophyte from *Podocarpus macrophyllus*

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A study on the fungal diversity of *Pestalotiopsis* species occurring on *Podocarpaceae* yielded an unknown species, which is described here as *Pestalotiopsis kunmingensis*. This species that occurs as an endophyte, was isolated from *Podocarpus macrophyllus*. It shares similar morphological characters to other *Pestalotiopsis* species, but possesses long apical appendages that are knobbed (spathulate) at the tip, a single branched basal appendage and versicolorous median cells.

**Key words:** Pestalotiopsis, endophyte, morphology, taxonomy

**Introduction**

The genus *Pestalotiopsis* was established by Steyaert, following a taxonomic amendment to the genus *Pestalotia* (Steyaert, 1949, 1953a,b, 1961). Steyaert (1949) restricted *Pestalotia* to a single species and reassigned some species formerly disposed in *Pestalotia* to new anamorphic genera, *Pestalotiopsis* and *Truncatella*, but a majority of the species remained unstudied. Guba (1961) preferred to adopt a broader generic concept and reduced *Pestalotiopsis* and *Truncatella* to synonymy with *Pestalotia* and accepted 220 species in *Pestalotia*. Sutton (1980) and Nag Raj (1993) favoured Steyaert’s opinion. Molecular studies have shown that *Pestalotiopsis* is a monophyletic genus (Jeewon et al., 2002). The conspicuous character of this genus is that relatively fusiform conidia formed within compact acervuli and the conidia are usually 5-celled with 3 coloured median cells and colourless end cells, and with two to more apical appendages arising from the apical cell.

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Pestalotiopsis species are anamorphic members of the family Amphisphaeriaceae (Zhu et al., 1991; Kang et al., 1998, 1999).

Pestalotiopsis is a complex genus and consists of members difficult to identified at the species level. Keys to the genus have been provided by Steyaert and Guba, respectively. At present, inter-specific delimitation is still obscure (Morgan et al., 1998; McPartland and Cubeta, 1997). A specimen may be identified and given various species names by different workers due to the overlap of characters, such as size of conidia, length of appendages and darkness of median coloured cells in the genus. Some species have also been identified based on their host occurrence (Venkatasubbaiah et al., 1991; Kohlmeyer and Volkmann-Kohlmeyer, 2001; Chen et al., 2002) although it has been concluded that there is a general lack of host-specificity in the genus. Molecular studies have shown that the genus contains two distinct lineages based on pigmentation of median cells and four distinct groupings based on morphology of apical appendages, but the reliability of other phenotypic characters of this genus was not supported (Jeewon et al., 2003).

Pestalotiopsis species are ubiquitous in distribution, occurring on a wide range of substrata and 206 species are listed in Index Fungorum http://www.indexfungorum.org/Names/Names.asp. Most species are plant pathogens (Dube and Bilgrami, 1966; Zhu et al., 1991; Zhang et al., 2003) and some are saprobes in soil (Agarwal and Chauhan, 1988), degraders of plant materials (Osono and Takeda, 1999; Tokumasu and Aoiki, 2002) or grow on decaying wild fruits (Tang et al., 2003). Several species that had previously been reported as plant pathogens (e.g. Pestalotiopsis funerea, P. microspora, P. maculans, P. palmarum and P. besseyi) have also been recorded as endophytes (Espinosa-Garcia et al., 1990; Strobel et al., 1996, 1997; Ramos-Mariano et al., 1997; Guo, 2002; Kumaresan and Suryanarayanan, 2002), while Pestalotiopsis jesteri has recently been reported as an endophyte from Fragaria bodenii (Gentianaceae) in Papua New Guinea (Strobel et al., 2000). Endophytic species of Pestalotiopsis are, however, frequently found in plants (Okan et al., 1997, 1998; Wei and Xu, 2003). Some species of endophytic Pestalotiopsis produce secondary metabolites with potential use on medical application and control of plant diseases (Li et al., 2001; Strobel et al., 1996).

As part of an ongoing study on the fungal diversity on different hosts of Podocarpaceae in China, we have isolated more than 90 strains of Pestalotiopsis. A particular endophytic strain isolated from healthy leaves of Podocarpus macrophyllus is described here as a new species.
Fungal Diversity

Materials and Methods

Sample collection

Healthy leaves of *Podocarpus macrophyllus* were collected in November, 2002, in Kunming Botanical Garden of Chinese Academy, Kunming, Yunnan Province, China. The Botanic Garden is located at highland of 1990m elevation. The annual average temperature and relative humidity in the Botanic Garden are 17.4°C and 73%, respectively.

Isolation and sporulation

The surface of leaves samples was washed with running tap water and sterilized with 75% ethanol (60 seconds), 1:3 dilution of water and NaClO (5 minutes) and 75% ethanol (30 seconds) (Zheng and Jiang, 1995). Samples were washed three times with sterilized water, cut into about 0.5-0.6 cm segments and transferred to PDA medium in Petri-dishes. Plates were incubated at 25°C for 3-20 days and checked regularly. When mycelial growth and spores were observed, further isolation were carried out by hyphal tipping and single spore isolations following the methods as outlined by Choi et al. (1999) and Lacap et al. (2002). A strain, PSHI2002Endo766 isolated from old leaf (4 years), was grown on autoclaved segments of carnation leaf (*Dianthi caryophylli*) for sporulation (Fisher, 1982; Strobel, 1996) and its morphology was observed.

Taxonomy

**Pestalotiopsis kunmingensis** J.G. Wei & T. Xu, sp. nov.  
(Figs 1-3)

*Fungus*, in foliis *Dianthi caryophylli* cretis, acervulis quibus sunt erumpentibus pustuliformibus unilocularibus et ovalibus ad irregularibus in ambitu. *Acervuli* plerumque 150-450 µm diametro, aliquando ad paene 700 µm. *Cellulæ conidiogenæ* discretæ vel integratæ, lageniformes vel ampuliformes vel subcylindraceæ, hyalinae, laeves, 13-23.4 × 2.3-5.2 µm (medio 18.2 × 3.4 µm) semel vel bis prolificantes. *Conidia* fusiformia, quadriseptata, 33.8-46.8 × 7.5-10 µm (medio 41 × 8.6 µm); *cellulæ medianæ* tres, subcylindraceæ vel doliiformes, crassitunicatae, versicolores, laeves, simul 20.8-28.6 µm (medio 25.6 µm) long. [*cellula secunda* a basi olivaceæ, 5.9-8.5 µm (medio 7.6 µm); *cellula tertia* umberrina-brunnea, 7.8-10.4 µm (medio 9.3 µm) and *cellula quarta* umberrina-brunnea, 7.9-9.8 µm (medio 8.5 µm)]; *cellula apicalis conica*, hyaline, laevis, 3.3-6.5 µm (medio 5.3 µm) long.; *appendices apicalis* 1-1.4 µm diametro, tubulares, nonramosæ, plurumque tribus, subinde duabus vel quattuor, apice spathulatæ (medio 1.8 µm diametro), 14.3-52.7 µm (medio 32.9 µm) long.; *cellula basalis obconica*, laevia, hyalina, 5.9-10.4 µm (medio 8.5 µm) long.; *appendix basalis centrica*, saepe ramose, 6.5-10.4 µm (medio 8.5 µm) long.; *ratione conidii long./lat.* = 4.82:1.

Etymology: The specific epithet is based on the city where this species was obtained.
**Fungus**, grown on carnation leaves, with acervuli forming on carnation which are erumpent, pustule-like, unilocular, oval or irregular in outline. *Acervuli* 150-450 µm in diam., occasionally up to 700 µm. *Conidiogenous cells* discrete or integrated, lageniform to ampulliform or subcylindrical, colourless, smooth-walled, 13-23.4 × 2.3-5.2 µm ($\bar{x} = 18.2 \times 3.4$ µm) with up to 3 proliferations. *Conidia* fusiform, 4-septate, 33.8-46.8 × 7.5-10 µm ($\bar{x} = 41 \times$ 8.6 µm); 3 median cells subcylindrical to doliiform, thick-walled, smooth, pigmented and versicolourous, together 20.8-28.6 µm ($\bar{x} = 25.6$ µm ) long [second cell from the base olivaceous, 5.9-8.5 µm ($\bar{x} = 7.6$ µm ); third cellumber brown, 7.8-10.4 µm ($\bar{x} = 9.3$ µm) and fourth cellumber brown, 7.9-9.8 µm ($\bar{x} = 8.5$ µm )]; apical cell conic, colourless, smooth, 3.3-6.5 µm ($\bar{x} = 5.3$ µm ) long; basal cell obconical, smooth-walled, colourless, 5.9-10.4 µm ($\bar{x} = 8.5$ µm) long; apical appendages 1-1.4 µm in diam., tubular, unbranched, mostly 3 , sometimes 2 or 4, tip spathulate ($\bar{x} = 1.8$ µm wide), 14.3-52.7 µm ($\bar{x} = 32.9$ µm) long; basal appendage centric, often branched, 6.5-10.4 µm ($\bar{x} = 8.5$ µm ) long; mean conidium length/width ratio = 4.82:1.

**Colony** on PDA white, cottony, fruitbodies ink-like, more or less gregarious, reverse of the culture yellow white.

**Habitat/Distribution:** Known to inhabit living leaf of *Podocarpus macrophyllus*, China.

**Holotype:** CHINA, Yunnan, Kunming, endophyte of *Podocarpus macrophyllus*, (PSHI2002 endo766), 11 November 2002, J.G. Wei (deposited in the Department of Plant Protection, College of Agriculture & Biotechnology, Zhejiang University).

*Pestalotiopsis kunmingensis* can be distinguished from *Pestalotiopsis montellicoides*, *P. macrospora* and *P. hughessii* as the conidia have knobbed and longer apical appendages which are often branched basal appendages and its versicolourous median cells (Mordue, 1986; Steyaert, 1949, 1953b). Based on taxonomic literature available, this endophytic species isolated from *Podocarpus macrophyllus* is morphologically distinct from other known species and hence a new species, *Pestalotiopsis kunmingensis* is described.

It is interesting to note that Worapong *et al.* (2002) subjected spores of *Pestalotiopsis* species to UV light and induced biotypes with multiple conidial forms. The implications of this study were that many species in this group of fungi were either closely related or identical. Nucleotide sequences from the ITS regions of the rDNA of *Pestalotiopsis kunmingensis* were aligned with other similar sequences of *Pestalotiopsis* obtained from GenBank. Initial results showed that *P. kunmingensis* is phylogenitically distinct from other *Pestalotiopsis* species, and it similar to *P. jesteri* with similarity of 85.12% in the ITS regions of the rDNA sequences (not including 5.8S rDNA ).
Table 1. *Pestalotiopsis kunmingensis* compared with similar species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pestalotiopsis kunmingensis</th>
<th>Pestalotiopsis grandi</th>
<th>Pestalotiopsis montelicoides</th>
<th>Pestalotiopsis macrospora</th>
<th>Pestalotiopsis hughessii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conidia length</td>
<td>33.8-46.8</td>
<td>26-48</td>
<td>35-48</td>
<td>30-45</td>
<td>34-45</td>
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<tr>
<td>width</td>
<td>7.5-10</td>
<td>7-8</td>
<td>7.5-10.6</td>
<td>9-12</td>
<td>7-11</td>
</tr>
<tr>
<td>median cells</td>
<td>versicolorous</td>
<td>concolorous</td>
<td>concolorous</td>
<td>concolorous</td>
<td>concolorous</td>
</tr>
<tr>
<td>Apical appendages number</td>
<td>2-4</td>
<td>2-4</td>
<td>3-4</td>
<td>2-5</td>
<td>2-3</td>
</tr>
<tr>
<td>position</td>
<td>apical</td>
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<td>apical/subapical</td>
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</tr>
<tr>
<td>tip</td>
<td>knobbed</td>
<td>knobbed</td>
<td>unknobbed</td>
<td>unknobbed</td>
<td>unknobbed</td>
</tr>
<tr>
<td>length</td>
<td>14.3-52.7</td>
<td>10-36</td>
<td>12-22</td>
<td>10-19</td>
<td>9-29</td>
</tr>
<tr>
<td>Basal appendage</td>
<td>often branched</td>
<td>unbranched</td>
<td>unbranched</td>
<td>unbranched</td>
<td>unbranched</td>
</tr>
<tr>
<td>Habit</td>
<td>endophytic</td>
<td>pathogenic</td>
<td>from air</td>
<td>pathogenic</td>
<td>pathogenic</td>
</tr>
</tbody>
</table>

The unit of length and width of conidia and apical appendages is µm.

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**References**


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