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

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Wei, J.G. and Xu, T. (2004). *Pestalotiopsis kunmingensis* sp. nov., an endophyte from *Podocarpus macrophyllus*. *Fungal Diversity* 15: 247-254.

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 *Fragraea bodenii* (*Gentianaceae*) in Papua New Guinea (Strobel *et al.*, 2000). Endophytic species of *Pestalotiopsis* are, however, frequently found in plants (Okane *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.

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

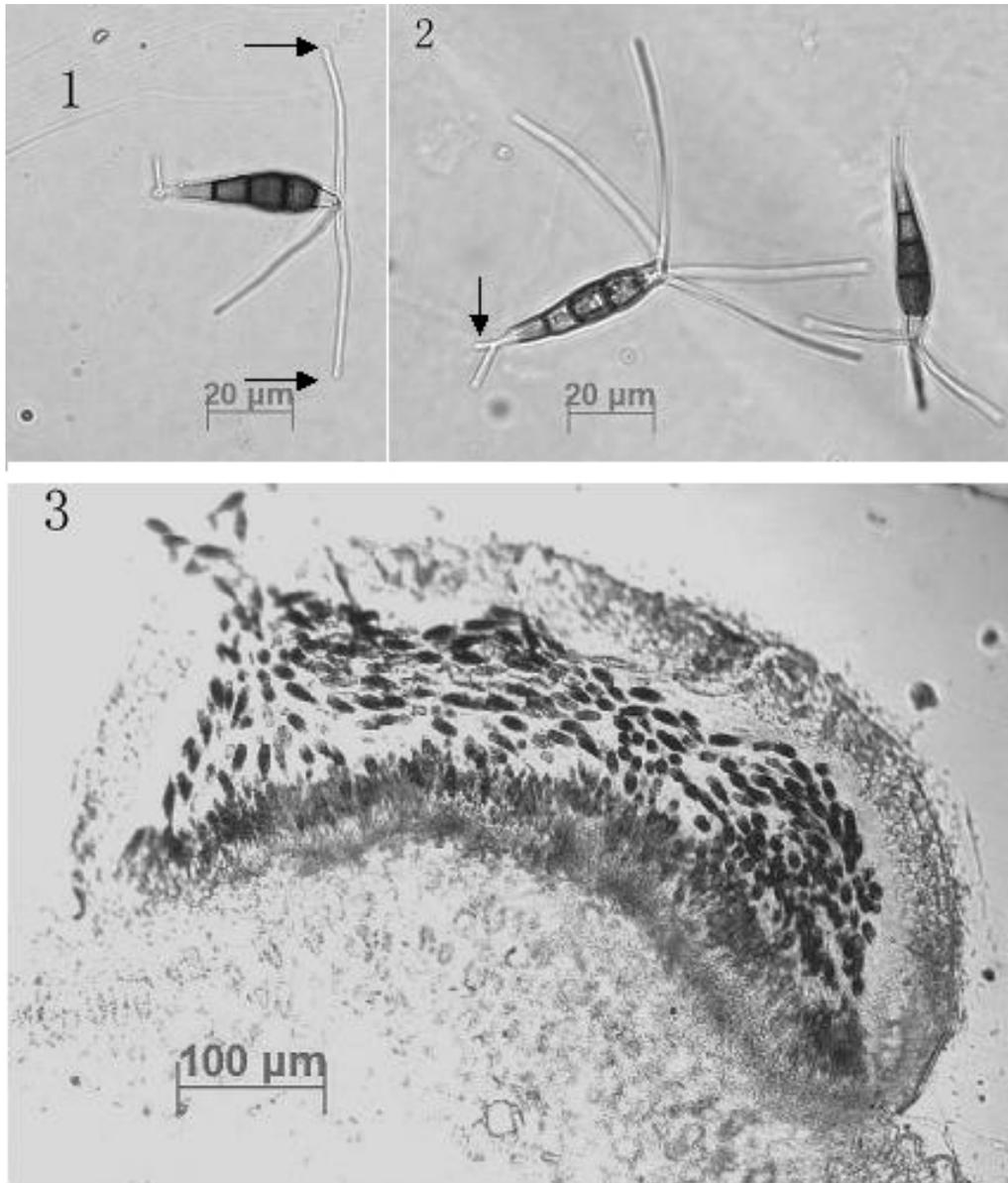
The surface of leaves samples was washed with running tap water and sterilized with 75% ethanol (60 seconds), 1:3 dilution of water and NaClO (5 minutes) and 75% ethanol (30 seconds) (Zheng and Jiang, 1995). Samples were washed three times with sterilized water, cut into about 0.5-0.6 cm segments and transferred to PDA medium in Petri-dishes. Plates were incubated at 25C 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 . *Cellulae conidiogenae* discretatae vel integratae, lageniformes vel ampuliformes vel subcylindraceae, hyalinae, laeves, 13-23.4 \times 2.3-5.2 μm (medio 18.2 \times 3.4 μm) semel vel bis prolificantes. *Conidia* fusiformia, quadrisepata, 33.8-46.8 \times 7.5-10 μm (medio 41 \times 8.6 μm); cellulae medianae tres, subcylindraceae vel doliiformes, crassitunicatae, versicolores, laevia, simul 20.8-28.6 μm (medio 25.6 μm) long. [cellula secunda a basi olivaceae, 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, nonramosae, plerumque tribus, subinde duabus vel quattuor, apice spathulatis (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 ramosae, 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.



Figs. 1-3. Conidia and acervulus of *Pestalotiopsis kunmingensis* (from holotype). **1.** Conidium with knobbed tips of apical appendages (arrowed) **2.** Conidia showing a branched basal appendage (arrowed). **3.** Vertical section of an acervulus on an autoclaved segment of carnation leaf.

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 \times 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 \times 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 cell umber brown, 7.8-10.4 μm (\bar{x} = 9.3 μm) and fourth cell umber 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 versicolorous 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 phylogenetically 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.

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

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

Acknowledgments

The authors would like to thank R. Jeewon for pre-submission review. This project was supported by the National Natural Science Foundation of China (No. 30270015) and Systematic Mycology & Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences.

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(Received 5 August 2003; accepted 8 December 2003)