Mycoscience (2010) 51:182–188
DOI 10.1007/s10267-009-0025-z

FULL PAPER

Description of *Pestalotiopsis pallidotheae*: a new species from Japan

Kyoko Watanabe · Keiichi Motohashi · Yasunori Ono

Received: 7 April 2009 / Accepted: 29 September 2009 / Published online: 5 February 2010

Abstract A new coelomycetes fungal species, *Pestalotiopsis pallidotheae*, is described. This endophytic fungus, isolated from a leaf of Japanese andromeda (*Pieris japonica*), has knob-tipped appendages on the apical and basal cells of the conidia. The conidial morphology is similar to that of *Pestalotiopsis theae* except that the color of the median cells is paler in *P. pallidotheae*. Molecular analyses of the ITS1-5.8S rRNA-ITS2 site placed *P. pallidotheae* in a group different from *P. theae*.

Keywords Coelomycetes · Endophyte · Taxonomy

Introduction

The genus *Pestalotiopsis* contains fungi that are endophytic and/or pathogenic to many plants. The genus is separated from *Pestalotia* on the basis of morphological characteristics of the conidia (Steyaert 1949, 1953, 1954, 1961; Guba 1961). Using internal transcribed spacer (ITS)1-5.8S rRNA-ITS2 sequences, Jeewon et al. (2002) demonstrated that the genus *Pestalotiopsis* was a monophyletic group, and reclassified *Pestalotiopsis* and allied genera using morphological characters.

Pestalotiopsis species have a long tradition of being described as a new species occurring on different host plants, even though the new "species" had no distinctive morphological features. Consequently, this genus currently contains numerous species, and more than 230 have been recorded to date (Index Fungorum; http://www.indexfungorum.org/Names/Names.asp). However, no host-specific fungal relationships have been observed (Jeewon et al. 2004), and Wei et al. (2007) confirmed that *Pestalotiopsis* is not host specific. Thus, to clarify the taxonomic affiliations within this group, reclassification of the fungi at species level is required (Hawksworth 2005; Wei et al. 2005). Using ITS1-5.8S rRNA-ITS2 to identify species, Jeewon et al. (2003) constructed a phylogenetic tree and showed that the most heavily weighted morphological characters were the pigmentation of median cells and the morphology of the tips of appendages. Liu et al. (2007) described a new species, using morphology and ITS1-5.8S rRNA-ITS2 and β-tubulin 2 gene sequences, and constructed a similar phylogenetic tree.

We have isolated a new endophytic species, *Pestalotiopsis pallidotheae*, from the leaf of a Japanese andromeda [*Pieris japonica* (Thunb.) D. Don ex G. Don ssp. *japonica*]. This fungus has conidia with knob-tipped appendages on the apical and basal cells. Although this character and the size of the conidia are similar to those of *P. theae* (Sawada) Steyaert, the pigmentation of the three median cells is paler in *P. pallidotheae*. The comparison of morphological characteristics and the molecular characteristics of ITS1-5.8S rRNA-ITS2, which generally reflect differences at the species level, supported the classification of *P. pallidotheae* as a separate species.

K. Watanabe (✉)
College of Agriculture, Tamagawa University,
Machida, Tokyo 194-8610, Japan
e-mail: wkyoko@agr.tamagawa.ac.jp

K. Motohashi
River Basin Research Center, Gifu University,
Yanagito 1-1, Gifu, Gifu 501-1193, Japan

Y. Ono
Exploratory Research Laboratories I,
Daiichi Sankyo Co. Ltd., Hiromachi,
Shinagawa-ku, Tokyo 140-8710, Japan

Materials and methods

Observation of morphological characters

Conidia were produced in a monoculture derived from a single conidium placed on the boiled leaves of *Hydrangea macrophylla* f. *macrophylla* (Wilson) Hara on water agar, following the method of Kishi (1994). Morphological characters for identification were measured and examined under a light microscope. These characters included conidial length and width, appendage length and number, and the pigmentation of the three median cells of the conidium. Acervuli produced on the leaf were fixed in 4% glutaraldehyde and 0.5 M potassium phosphate (pH 7.4) for 24 h at 4°C, washed in 0.5 M potassium phosphate buffer, dehydrated in a graded ethanol series, dried to critical point, and then mounted on stubs sputter coated with gold (JFC-1100; JEOL, Tokyo, Japan) for examination under a scanning electric microscope (JSM-5200; JEOL).

DNA extraction

Twenty-two strains of *Pestalotiopsis* and one strain of *Seiridium* sp. isolated from substrata, soil, and the ocean, including those preserved in MAFF (National Institute of Agrobiological Science, Genebank), were used for DNA extraction and molecular analysis (Table 1). DNA from each fungal strain was extracted from 7-day-old cultures on potato dextrose agar (PDA) (Eiken, Tokyo, Japan) using a QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan).

ITS1-5.8S rRNA-ITS2 amplification and phylogenetic analysis

ITS1-5.8S rRNA-ITS2 was amplified using the ITS5 and ITS4 primer pair (White et al. 1990). PCR was performed with 1 cycle of 94°C for 1 min and 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by 72°C for 10 min. The PCR product was purified with ExoSAP-IT (GE Healthcare Japan, Tokyo, Japan) directly,

Table 1 Strains used in this study with source, location, and accession number

Species	Strain number[a]	Source	Location	Accession number ITS1-5.8S rRNA-ITS2
Pestalotiopsis acaciae	TAP31O086	*Musa* sp.	Tokyo	AB482207
Pestalotiopsis adusta	TAP32O089	*Smilax nipponica*	Tokyo	AB482212
Pestalotiopsis crassiuscula 1	TAP23O074	*Sasakia charonda*	Tokyo	AB482222
Pestalotiopsis crassiuscula 2	TAP07O037	*Bruguiera gymnorhiza*	Okinawa	AB482208
Pestalotiopsis disseminata 1	TAP29O082	Unknown	Ibaraki	AB482213
Pestalotiopsis disseminata 2	MAFF 238347	*Pinus pentaphylla*	Saitama	AB482214
Pestalotiopsis fici 1	TAP34O099	Ocean	Unknown	AB482201
Pestalotiopsis fici 2	TAP39O129	*Bischofia javanica*	Okinawa	AB482200
Pestalotiopsis glandicola	TAP12O048	*Machilus thunbergii*	Tokyo	AB482205
Pestalotiopsis japonica	TAP11O047	*Phoenix roebelenii*	Tokyo	AB482204
Pestalotiopsis longiseta	MAFF 752008	*Thea sinensis*	Shizuoka	AB482206
Pestalotiopsis maculans 1	TAP17O055	*Camellia japonica*	Okinawa	AB482217
Pestalotiopsis neglecta 1	TAP99M112	*Pieris japonica*	Tokyo	AB482211
Pestalotiopsis neglecta 2	TAP08O038	*Phyllanthus flexuosus*	Hiroshima	AB482216
Pestalotiopsis neglecta 3	TAP20O063	*Cupressus macrocarpa*	Saitama	AB482209
Pestalotiopsis palustris	TAP99M106	*Pieris japonica*	Tokyo	AB482215
Pestalotiopsis theae 1	MAFF 752011	*Thea sinensis*	Tokushima	AB482210
Pestalotiopsis theae 2	MAFF 238515	*Rhaphiolepis umbellata*	Okinawa	AB482203
Pestalotiopsis theae 3	TAP36O105	Soil	Okinawa	AB482202
Pestalotiopsis pallidotheae	MAFF 240993	*Pieris japonica*	Tokyo	AB482220
Pestalotiopsis sp. 1	TAP06O030	*Distylium racemosum*	Kagoshima	AB482218
Pestalotiopsis sp. 2	MAFF 238514	Broadleaf tree	Okinawa	AB482219
Seiridium sp.	MAFF 238468	*Eriobotrya japonica*	Okinawa	AB482221

[a] MAFF strains preserved in National Institute of Agrobiological Science Genebank; TAP strains preserved in Tamagawa University

Table 2 Accession numbers of sequences obtained from GenBank for molecular analysis

Species	Accession number ITS1-5.8SrRNA-ITS2
Pestalotiopsis funerea	AF405299
Pestalotiopsis heterocornis 1	AY681489
Pestalotiopsis heterocornis 2	AY681492
Pestalotiopsis karstenii	AY681476
Pestalotiopsis maculans 2	AF405296
Pestalotiopsis microspora 1	AY681484
Pestalotiopsis microspora 2	AY687882
Pestalotiopsis olivacea	DQ417182
Pestalotiopsis kunmingensis	AY373376
Seiridium cardinale	AF409995

or after subcloning. For subcloning, a TOPO(R) cloning kit (Invitrogen, Tokyo, Japan) was used in accordance with the manufacturer's instructions. The purified product was sequenced using an ABI 310 DNA sequencer (ABI, Tokyo, Japan).

For the molecular analysis, sequence data from nine isolates of *Pestalotiopsis* species, and for the outgroup *Seiridium cardinale* (W.W. Wagener) B. Sutton and I.A.S. Gibson (Jeewon et al. 2003), were obtained from GeneBank (Table 2), and were added to our obtained 23 sequences (see Table 1). All sequences were assembled and aligned (TreeBase no. S2595, M4953) using BioEdit version 7.0.9 (Hall 1999) and optimized by eye. Phylogenetic analyses of sequences were performed by the neighbor-joining (NJ) (Saitou and Nei 1987) and maximum-parsimony (MP) methods using PAUP* version 4.0b10 (Swofford 2002). The best-fit evolutionary model was determined for the dataset by comparing different evolutionary models using Modeltest version 3.7 (Posada and Crandall 1998) for NJ and Kakusan3 (Tanabe 2007) for MP. NJ and MP analyses with the selected evolutionary model were done in PAUP*. For NJ analysis, evolutionary distance was measured with the maximum-likelihood model (TVM + I + G). MP analysis was performed for 1000 replications with different random starting points using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. Alignment gaps were treated as missing data, and characters were unordered and had equal weight or were weighted by transition/transversion (ti/tv) ratio estimated by the Kishino–Hasegawa likelihood test (Kishino and Hasegawa 1989). The branch-swapping algorithm was tree bisection and reconstruction (TBR). Branches of zero length were collapsed, and all multiple, equally parsimonious trees were saved. The best tree topology of MP trees was conducted using the Kishino–Hasegawa likelihood test (Kishino and Hasegawa 1989) with the selected evolutionary model (J1 + G). Tree length (TL), consistency index (CI), retention index (RI), homoplasy index (HI), and rescaled consistency index (RC) were calculated. The strength of the internal branches from the resulting tree was tested by bootstrap (BS) analysis (Felsenstein 1985) using 1000 replications in both distance and parsimony analysis.

Results

Molecular phylogenetics

A total of 33 ITS1-5.8S rRNA-ITS2 sequences were used to construct the phylogenetic trees. Of them, *Seiridium* sp. and *S. cardinale* were used as outgroup taxa. The aligned data matrix of 33 sequences consisted of 539 characters, of which 120 characters were variable and 82 characters were phylogenetically informative for parsimony analysis. The equally weighted MP (eMP) analysis using PAUP* generated 1241 equally parsimonious trees with 179 steps (CI = 0.7654, RI = 0.9132, HI = 0.2346, RC = 0.6989). The weighted MP (wMP) analysis generated 120 equally parsimonious trees with 181.19875 steps (CI = 0.7187, RI = 0.9026, HI = 0.2813, RC = 0.6487). These 1361 trees were very similar in topology excepting the terminal branch order (data not shown). Also, the best tree topology of eMP (Fig. 1) was similar to that of wMP trees and the NJ tree. Moreover, *Pestalotiopsis pallidotheae* and *P. kunmingensis* J.G. Wei & T. Xu, which have knobbed conidial appendages and pale median cells, were placed in completely different subclades.

Taxonomy

Pestalotiopsis pallidotheae Kyoko Watan. &Yas. Ono, sp. nov. Figs. 2–4.

Mycobank no.: MB 513396.

Conidiomata in foliis sterilifactis *Hydrangeae macrophyllae*, acervulata, lentiformia, 200–500 μm diametro. Conidiophora circum cavitatem conidiomatis enascentia, aseptata or septata. Cellulae conidiogenae integratae, holoblasticae, lageniformes vel ampulliformes vel subcylindricae, hyalinae, laeves. Conidia mucigera, 4-septata, fusiformia, subcylindrica vel doliiformes, recta vel leviter curvata, 21.5–30.7 × 5.4–7.7 μm; cellulae medianae tres, pallide brunneae, laeves, 13.8–19.2 μm; cellula basalis hyalina, obconica, cellula apicalis hyalina, conica; appendices super cellula apicali 2–4(–5), plerumque 3, 12.3–39.2 μm, tubulares, ad apicem gibbosae (spathulatae); appendices basalis, singularis, polaris, apice gibbosa (spathulata), 7.7–30.8 μm.

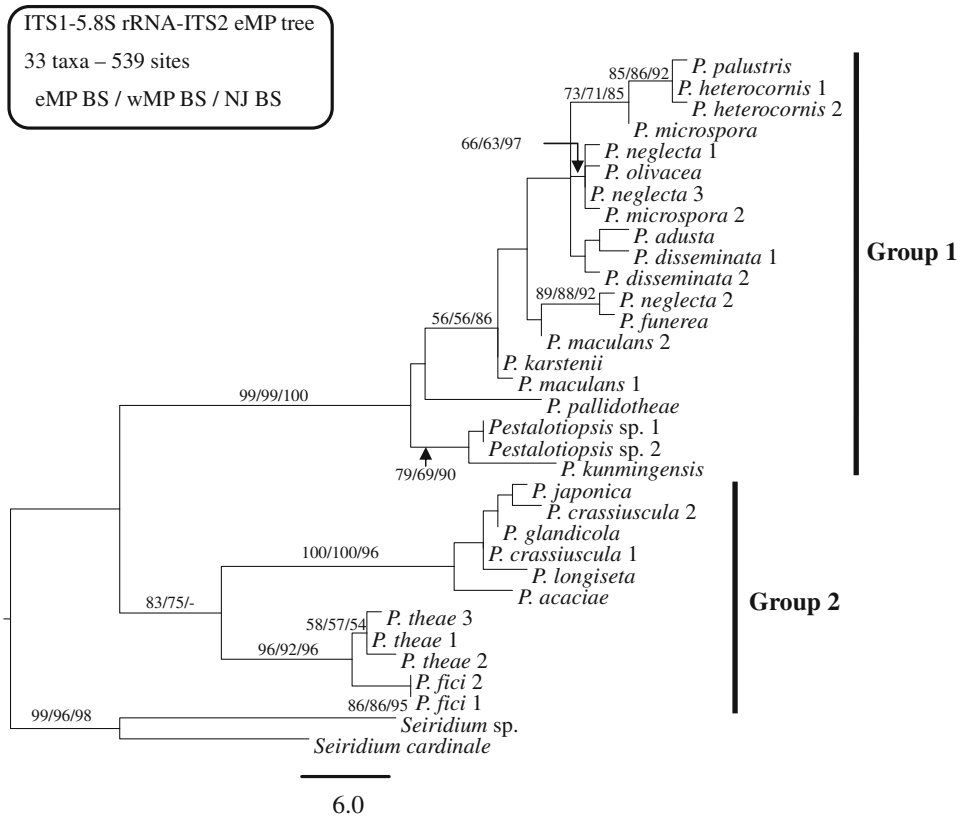


Fig. 1 Phylogenetic analysis of the nucleotide sequence of ITS1-5.8S rRNA-ITS2 region for 33 sequences from *Pestalotiopsis* and *Seiridium*. The phylogram represented is the resulting best equally weighted MP (*eMP*) tree generated by the Kishino–Hasegawa likelihood test. Percentage bootstrap values (*BS*) are shown for equally weighted maximum parsimony (*eMP*), weighted maximum parsimony (*wMP*), and neighbor-joining (*NJ*) analysis. BS values higher than 50% are indicated *above* the branches. DNA and sequence data sources are listed in Tables 1 and 2. Tree length (TL) = 179 steps; consistency index (CI) = 0.7654; retention index (RI) = 0.9132; homoplasy index (HI) = 0.2346, rescaled consistency index (RC) = 0.6989

Habitat: In foliis vivis of *Pieris japonica* ssp. *japonica*.

Etymology: *Pestalotiopsis pallidotheae*: *pallido* is from *pallidulus* (Latin, an adjective meaning pale); *theae* is from *P. theae*.

Acervuli on leaves of *Hydrangea macrophylla* treated by the leaf-agar method, pateriform, 200–500 μm (Fig. 2). Conidiophores line the cavity of the acervuli entirely, aseptate or septate. Conidiogenous cells lageniform, ampliform or subcylindrical, colorless, smooth-walled (Fig. 3). Conidia mucilaged, holoblastic, fusiform, straight and slightly curved, 4-septate, 21.5–30.7 × 5.4–7.7 μm (mean 25.7 × 8.6 μm, $n = 40$); three median cells subcylindrical to doliiform, pale brown, 13.8–19.2 μm (Fig. 4); the apical and basal cells conical in shape, colorless. Apical appendages knobbed (spathulate) 2 to 4 (–5), mostly 3, 12.3–39.2 μm long; basal appendage single, centric, with knobbed tip, 7.7–30.8 μm long.

Holotypus: TNS-F-18615, Dried culture specimen (from MAFF 240993) grown on leaves of *Hydrangea macrophylla*, deposited in the National Museum of Nature and Science, Tokyo.

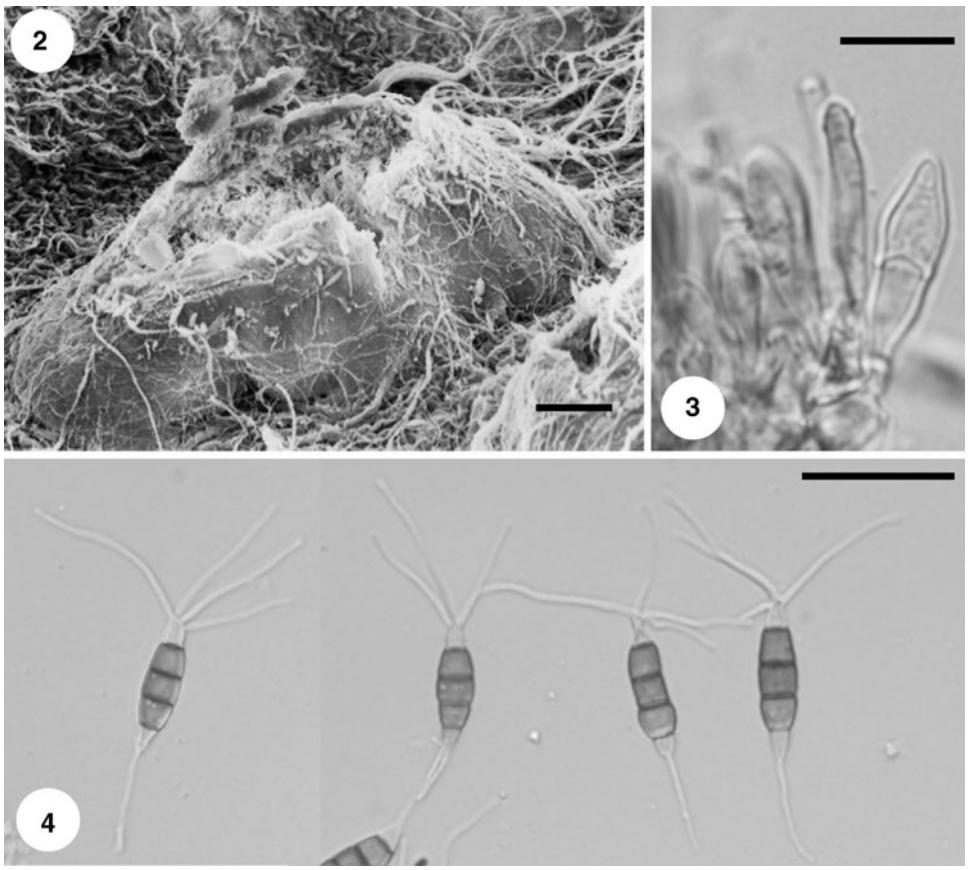
Ex holotype cultures: MAFF 240993 (NIAS, National Institute of Agrobiological Science), TAP99M110 (Tamagawa University). This fungus was collected in Japan, Tokyo, Machida, Tamagawa University, N 35°33′37″, E 139°28′21″, from a leaf of *Pieris japonica* spp. *japonica* as an endophyte, 10 July 1999, collected by S. Matsuda.

Note: The colony of *P. pallidotheae* on PDA was white, cottony, and the reverse of the culture was pale orange.

Discussion

There are many problems with the morphological classification systems that have traditionally been used to identify species of *Pestalotiopsis*. The main problem is that the morphological criteria create trivial differentiations among species. The current number of species in *Pestalotiopsis* is more than 230, but that number must be reduced by a reclassification using additional criteria. Molecular analysis is a useful method for identification of species in *Pestalotiopsis* and other genera, although some problems remain with the classifications.

The morphological characteristics of *P. pallidotheae* clearly differentiate it from hitherto known species of the genus *Pestalotiopsis*. On traditional identification criteria, *P. pallidotheae* has morphological features similar to only *P. theae*, which has appendages with knobbed tips and similar conidial size. The conidial size of *P. pallidotheae* is 21.5–30.7 × 5.4–7.7 μm ($n = 40$) with appendages 12.3–39.2 μm in length, and the conidial size of *P. theae* is 22–32 × 5–8 μm with appendages 25–50 μm in length (Guba 1961). The main difference between *P. pallidotheae* and *P. theae* is the density of pigmentation in the three median



Figs. 2–4 *Pestalotiopsis pallidotheae*. **2** Scanning electron micrograph of acervulus under the epidermis of a leaf of *Hydrangea macrophylla* f. *macrophylla*. Conidiogenous cells are shown lining the inner layer of the acervuli. **3** Light micrograph of aneroid of conidiogenous cells. **4** Light micrograph of conidia. *Bars* **2** 100 μm; **3**, **4** 20 μm

conidial cells (Table 3). *Pestalotiopsis pallidotheae* has pale median conidial cells compared to *P. theae*.

Jeewon et al. (2003) showed that pigmentation of the median cells of conidia, appendage morphology, and phylogeny of the ITS1-5.8S rRNA-ITS2 region should be highly weighted criteria for species separation. They concluded that the phylogenetic tree consisted of three groups that were characterized by conidial morphology features, i.e., concolorous (brown) median cells, concolorous median cells with knobbed conidial appendages, and versicolorous median cells. The group of concolorous median cells with conidial knobbed appendages has the same ancestor as the versicolorous group. Similar phylogenetic trees have been derived from ITS1-5.8S rRNA-ITS2 and/or β-tubulin sequences (Jeewon et al. 2004; Lee et al. 2006; Liu et al. 2007). Our results with NJ and MP trees obtained from the molecular analyses using ITS1-5.8S rRNA-ITS2 also indicate that the genus *Pestalotiopsis* separates into two major clades, distinguished by median cells, the color tone of which is concolorous (group 1) or versicolorous (group 2) against other pigmented cells. Subclades were distinguished by the presence or absence of knobbed appendages (Fig. 4). Many molecular analyses have indicated that the pigmentation of the three median cells is the most important morphological character for classification. With this background, molecular analyses of ITS1-5.8S rRNA-ITS2 in the new species *P. pallidotheae* revealed that it is distinct from *P. theae*.

According to Wei and Xu (2004), *P. kunmingensis*, which has knobbed appendage tips, also has versicolorous median cells. However, there are some contradictions here. Liu et al. (2007) showed that this is not consistent with molecular analysis based on ITS1-5.8SrRNA-ITS2, and that *P. kunmingensis* belongs in the concolorous group. Our results are consistent with Liu et al. (2007), with the subgroup containing *P. kunmingensis* in the concolorous group, and *P. kunmingensis* not closely related to *P. theae*, which has versicolorous median cells. Our molecular analysis confirms that *P. kunmingensis* is different from *P. theae* and is in the concolorous group. The color of the conidial median cells in *P. pallidotheae* is similar to that of *P. kunmingensis*, which has appendages with knobbed tips (Wei and Xu 2004). However, the conidia in *P. pallidotheae* are substantially smaller than those in *P. kunmingensis* (33.8–46.8 × 7.5–10 μm), and our molecular analyses also indicate that *P. pallidotheae* is distinguishable from *P. kunmingensis* within the concolorous clade.

Pestalotiopsis perseae Nag Raj, *P. smilacis* (Schwein.) B. Sutton, and *P. tecomicola* Nag Raj all have knobbed appendage tips, but there are other clear morphological

Table 3 Morphological characteristics of *Pestalotiopsis pallidotheae* and five congeneric species

Characteristics	Species					
	P. pallidotheae (MAFF240993)	*P. theae*[a]	*P. kunmingensis*[b]	*P. perseae*[c]	*P. smilacis*[c]	*P. tecomicola*[c]
Conidial length (μm)	21.5–30.7	22–32	33.8–46.8	24–36	23–37	23–31.5
Width (μm)	5.4–7.7	5–8	7.5–10	7–8 (–9)	7–9 (–9.5)	7.5–8.5 (–9)
Color of median cells	Pale (light) brown	Brown, yellow-brown, concolorous	Versicolorous	Mostly versicolorous, occasionally concolorous	Versicolorous	Pale brown to brown, mostly concolorous
Appendage morphology						
Tips	Knobbed	Knobbed	Knobbed	Knobbed	Knobbed	Knobbed
Apical branching	Unbranched	Unbranched	Unbranched	Unbranched	Unbranched	Unbranched
Apical number	2–4 (–5)	2–4	2–4	2–4	3–4	(2–)3(–4)
Apical length (μm)	12.3–39.2	25–50	14.3–52.7	10–23 (–30)	5–21	11–16
Basal branching	Mostly unbranched	Unbranched	Branched	Unbranched	Unbranched	Unbranched
Basal length (μm)	(8–) 10–20 (–25)			2–6 (–8)	Up to 7	2–5

[a] Guba (1961)

[b] Wei and Xu (2004)

[c] Nag Raj (1993)

differences between them and *P. pallidotheae* (Table 3). *Pestalotiopsis perseae* and *P. smilacis* are broadly distinguished by the color of the three median cells and conidial length. Both species have versicolorous median cells and slightly larger conidia than *P. pallidotheae*. Also, the conidial appendages of *P. pallidotheae* are substantially longer than those of *P. perseae* or *P. smilacis*. *Pestalotiopsis pallidotheae* and *P. tecomicola* are distinguished with respect to median cell color (pale brown to brown), and *P. tecomicola* has wider conidia with shorter appendages than *P. pallidotheae*. Thus, *P. pallidotheae* is described as a new species based on both morphological and molecular analyses.

Acknowledgments The authors thank Dr. Kobayashi for presubmission review. This study was supported by National Institute of Agrobiological Science Genebank to K.W.

References

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

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

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hawksworth DL (2005) Host specificity in *Pestalotiopsis*. Mycol Res 109:5

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

Kishi K (1994) Production of pycnidia and acervuli by agar-leaf disk method (in Japanese). Ann Phytopathol Soc Jpn 60:345

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J Mol Evol 29:170–179

Lee S, Crous PW, Wingfield MJ (2006) Pestalotioid fungi from *Restionaceae* in the Cape Floral Kingdom. Stud Mycol 55:175–187

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

Nag Raj TR (1993) *Coelomycetous anamorphs* with appendage-bearing conidia. Mycologue Publications, Waterloo, Ontario

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

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

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

Steyaert RL (1954) Concerning some South African *Pestalotiopsis* Steyaert. Bothalia 6:379–383

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

Swofford DL (2002) Phylogenetic analysis using parsimony (PAUP). Version 4.0b. Sinauer Associates, Sunderland

Tanabe AS (2007) Kakusan: a computer program to automate the selection of a nucleotide substitution model and the configuration of a mixed model on multilocus data. Mol Ecol Notes 7:962–964

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, Liu AR, Pan XH, Zhang JC, Yuan GQ (2005) Delimitation of *Pestalotiopsis* species based on morphological and molecular phylogenetic characters (in Chinese). J Guangxi Agric Biol Sci 24:304–313

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

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego