

Spadicoides camelliae and *Diplococcium livistonae*, two new hyphomycetes on dead branches from Fujian Province, China

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Received: 13 August 2010 / Accepted: 14 June 2011 / Published online: 6 July 2011
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Abstract Two new dematiaceous hyphomycetes, *Spadicoides camelliae* and *Diplococcium livistonae*, are described and illustrated based on specimens collected from the subtropical forests in Fujian Province, southeast China. They were collected on dead branches of *Camellia japonica* and *Livistona chinensis*, respectively. *Spadicoides camelliae* is characterized by polytretic, terminal conidiogenous cells with solitary, ovoid, versicolored, and mainly 2-septate conidia (15.0–22.0 × 7.0–10.0 µm). *Diplococcium livistonae* is distinguished by polytretic, terminal, and intercalary conidiogenous cells with catenate, cylindrical, concolored, and mainly 2-septate conidia (15.0–28.0 × 5.0–7.0 µm). They are compared with other similar species by their morphological characteristics.

Keywords Anamorphic fungi · Subtropical forests · Taxonomy

Introduction

The genus *Spadicoides*, typified by *S. bina* (Corda) S. Hughes, was established by Hughes (1958). It has similar conidial ontogeny to the most closely related genus *Diplococcium* Grove (1885), of which the type species is *D. spicatum* Grove. Both genera have macronematous, mononematous conidiophores with polytretic, terminal, or intercalary conidiogenous cells producing acropleurogenous, euseptate conidia (Ellis 1971). The differences between these two genera are that conidiophores are

generally unbranched in *Spadicoides* while those of *Diplococcium* are normally branched, and the conidia are solitary in *Spadicoides* whereas those of *Diplococcium* are catenate. The branching of conidiophores and the catenation of conidia, together, have been used to separate the two genera. However, the distinction of these two genera on the basis of catenate conidia has been questioned by Wang and Sutton (1982). Sinclair et al. (1985) emended the generic descriptions and pointed out that both genera may have branched or unbranched conidiophores and the catenation of conidia is the sole diagnostic character separating *Diplococcium* from *Spadicoides*. In accordance with criteria adopted by Sinclair et al. (1985), Goh and Hyde (1996, 1998a) reviewed *Spadicoides* and *Diplococcium*, respectively, and recognized 21 species in each genus. Species were assigned in the respective genera based on conidial characteristics and the branching of conidiophores. Thus far, 33 species have been accepted in *Spadicoides*, 6 of which were described from China (Zhou et al. 1999; Wong et al. 2002; Ho et al. 2002; Cai et al. 2004; Li et al. 2010; Ma et al. 2010); 24 species have been accepted in *Diplococcium*. Most described species of *Spadicoides* and *Diplococcium* are saprobes on rotten leaves or dead branches. *Spadicoides heterocolorata* (R.F. Castañeda, Guarro & Cano) Goh & K.D. Hyde, *S. obclavata* Kuthub. & Nawawi, *S. wufengensis* D.W. Li, J.Y. Chen & Y.X. Wang, and *D. hughesii* C.J.K. Wang & B. Sutton were reported to have a *Selenosporella* synanamorph (Kuthubutheen and Nawawi 1991; Goh and Hyde 1998b; Wang and Sutton 1998; Li et al. 2010). The *Selenosporella* synanamorph was found on the host fungi. It grows from conidia of *Spadicoides heterocolorata*, *S. obclavata*, and *S. wufengensis* and from hyphae and conidiophores of *D. hughesii*.

Molecular data from 28S rRNA gene phylogenetic studies (Shenoy et al. 2010) suggest that *Diplococcium*

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species have phylogenetic affinities with the Helotiales (Leotiomycetes) and Pleosporales (Dothideomycetes), whereas *Spadicoides* species are phylogenetically clustered with the Pleosporales (Dothideomycetes) and Sordariomycetes. Although several *Helminthosphaeria* species have the anamorph–teleomorph associations with *Diplococcium* anamorphs (e.g., Samuels et al. 1997; Goh and Hyde 1998a), these associations still remains to be confirmed by more molecular data (Shenoy et al. 2010). The connection of *D. pulneyense* with *Othia pulneyensis* (Subramanian and Sekar 1987) has been found to be untenable (Phillips et al. 2005; Crous et al. 2006). The phylogenetic association of *Spadicoides* species with *Tengiomyces* (Réblová 1999) could not be probed without DNA sequences in public DNA databases. The phylogenetic-centric fungal systematics is an ideal approach for classification; however, in view of our failures to obtain cultures in this study, morphological data for classification are used herein.

Fujian Province, located on the southeastern coast of China, has an average annual temperature that ranges from 17° to 21°C with an average annual precipitation between 1,400 and 2,000 mm. Situated in the northwest of Fujian Province, Wuyi Mountain Nature Reserve has a marine monsoon climate that is especially conducive to the developmental growth of various plant and microbial species. In our study on conidial fungi from the subtropical forests in Fujian Province, two undescribed species of *Spadicoides* and *Diplococcium* were collected. These species are compared with morphologically similar species and described here as new to science.

Materials and methods

Samples of decomposed woody debris were collected in Wuyi Mountain Nature Reserve, Fujian Province, China, placed in Zip-lock plastic bags, and taken to the laboratory. The samples were incubated in plastic boxes containing damp tissue paper at 27°C for more than 2 weeks in damp chamber cultures. Samples were examined periodically under an Olympus SZ61 dissecting microscope. Conidia and conidiophores were obtained from the surface of the wood with a needle and transferred into a drop of lactophenol on a slide; the cover slip was sealed with neutral balsam. All microscopic characteristics were determined based on the measurements of 50 mature conidia and 30 conidiophores mounted in lactophenol at 100× magnification. The following abbreviations are used for conidial measurements: SDL = standard deviation of length, SDW = standard deviation of width. The photographs used for these measurements were obtained with a Nikon 90i microscope (Nikon, Japan). Then, several photographs were digitally altered to be a combined photograph.

The type specimens are deposited in Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP), with isotypes in Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS) (<http://hmas.im.ac.cn>).

Taxonomy

Spadicoides camelliae L.G. Ma & X.G. Zhang, sp. nov.
Fig. 1

Mycobank no.: MB 518667

Coloniae effusae in substrato naturali, atro-brunneae. Mycelium partim superficiale, partim immersum, ex hyphis ramosis, septatis, pallide brunneis, laevibus, 1.5–2.0 µm crassis compositum. Conidiophora macronematosa, mononematosa, singula, simplicia, nonramosa, erecta, cylindrica, recta vel leniter flexuosa, laevia, crassitunicata, atro-brunnea, 8–13-septata, 160–280 µm longa, ad basim 10.0–18.0 µm crassa, ad apicem 5.0–7.0 µm crassa. Cellulae conidiogenae polytreticae, in conidiophoris incorporatae, terminales, cylindricae, brunneae, 14.0–20.0 × 5.0–7.0 µm. Conidia solitaria, terminalia, simplicia, ovoidea, utrinque rotundata, crassitunicata, laevia, (1)–2-euseptata, ad septa interdum crassa et atriora, versicoloria, cum cellulis basalibus atro-brunneis, cellulis apicalibus subhyalinis, et cellulis mediis pallide brunneis, 15.0–22.0 × 7.0–10.0 µm, ad apicem 4.0–6.5 µm crassa.

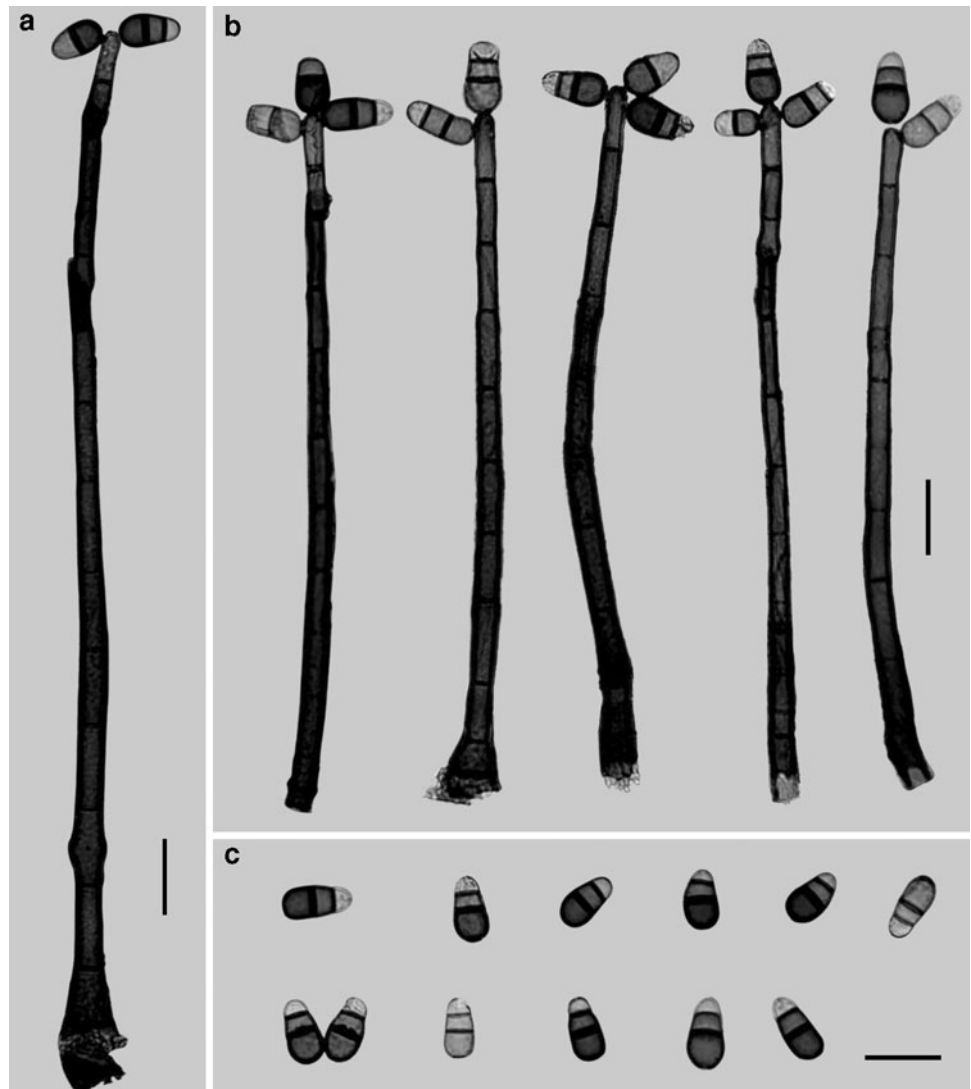
Typus: China, Fujian Province, Wuyi Mountain Nature Reserve (117°37'E, 27°39'N), on dead branches of *Camellia japonica* L. (*Theaceae*), 16 August 2009, leg. L.G. Ma (holotypus, HSAUP-H1028; isotypus, HMAS-146073).

Etymology: *camelliae*, in reference to the host *Camellia japonica* L., where the taxon was found.

Teleomorph: Unknown.

Colonies effuse on natural substratum, dark brown. Mycelium partly superficial, partly immersed, composed of branched, septate, pale brown, smooth-walled hyphae, 1.5–2.0 µm thick. Conidiophores macronematous, mononematous, single, unbranched, erect, cylindrical, straight or slightly flexuous, smooth, thick-walled, dark brown, 8–13-septate, 160–280 µm long, 10.0–18.0 µm wide at the base, 5.0–7.0 µm wide at the apex. Conidiogenous cells polytretic, integrated, terminal, cylindrical, brown, 14.0–20.0 × 5.0–7.0 µm, 17.0 × 6.0 µm on average. Conidia solitary, terminal, unbranched, rounded at both ends, ovoid, thick-walled, smooth-walled, (1)–2-euseptate, mostly 2-euseptate, sometimes thick and darkly pigmented at the septa, versicolored, with basal cell dark brown, apical cell subhyaline,

Fig. 1 *Spadicoides camelliae* (HSAUP-1028). **a, b** Conidiophores with terminal and polytretic conidiogenous cells and conidia. **c** Versicolored, solitary, and ovoid conidia with (1)–2 eusepta. Bars **a–c** 20 μ m



central cell pale brown, $15.0\text{--}22.0 \times 7.0\text{--}10.0 \mu\text{m}$ in the broadest part, $18.5 \times 8.5 \mu\text{m}$ on average, $\text{SDL} = 1.78$, $\text{SDW} = 1.03$, $4.0\text{--}6.5 \mu\text{m}$ wide at the apex.

Diplococcium livistonae L.G. Ma & X.G. Zhang, sp. nov. Fig. 2

Mycobank no.: MB 518668

Coloniae in substrato naturali effusae, atro-brunneae. Mycelium partim superficiale, partim immersum. Hyphae ramosae, septatae, pallide brunneae, laeves. Conidiophora macronematosae, mononematosae, singula, erecta, cylindrica, recta, laevia, aurea, non ramosa, 4–13-septata, usque $250 \mu\text{m}$ longa, $4.0\text{--}7.0 \mu\text{m}$ lata, crassitunicata. Cellulae conidiogenae in conidiophoris incorporatae, polytreticae, terminales et intercalares, cylindricae, post separationem conidiorum poris minutis circularibus remanentibus. Conidia in catenis acropleurogenis formata, usque ad 6–7 per

catenam, catenis saepe ramosis, ramoconidiis septatis, ramis infra septa cellularum subapicalium, cylindrica, 2–(3)-eu-septata, ad septa leniter constricta, utrinque rotundata, concoloria, aurea, simplicia, laevia, crassitunicata, illa biseptata $15.0\text{--}23.0 \times 5.0\text{--}6.5 \mu\text{m}$, illa triseptata $23.0\text{--}28.0 \times 5.0\text{--}7.0 \mu\text{m}$, ad septa crassa et atriora.

Typus: China, Fujian Province, Wuyi Mountain Nature Reserve ($117^{\circ}42'\text{E}$, $27^{\circ}41'\text{N}$), on dead branches of *Livistona chinensis* (Jacq.) R. Br. (*Arecaceae*), 17 August 2009, leg. L.G. Ma (holotypus, HSAUP-H1030; isotypus, HMAS-146074).

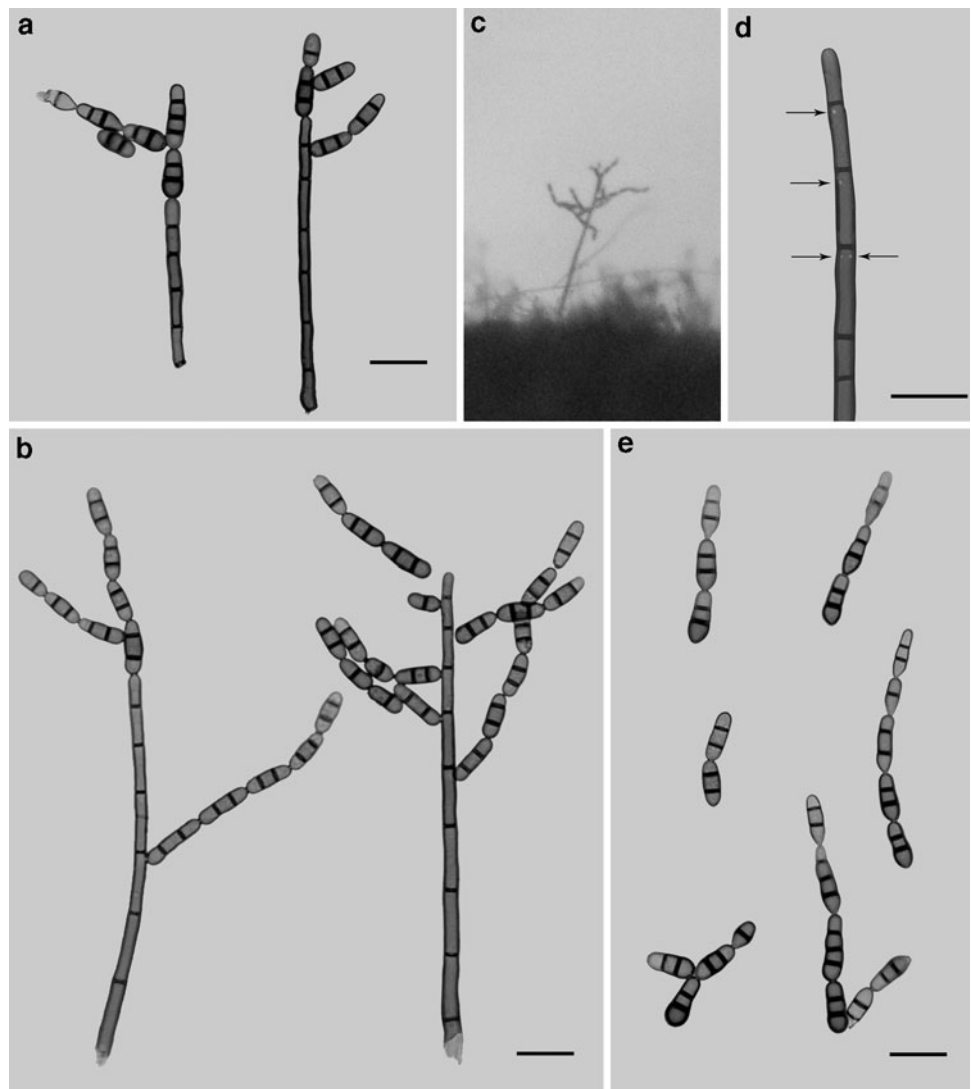
Etymology: *livistonae*, in reference to the host *Livistona chinensis* (Jacq.) R. Br., where the taxon was found.

Teleomorph: Unknown.

Colonies on natural substratum effuse, dark brown. Mycelium partly superficial, partly immersed. Hyphae branched,

Fig. 2 *Diplococcium livistonae* (HSAUP-1030).

a, b Conidiophores with terminal and intercalary, polytretic conidiogenous cells and conidia. **c** Conidiophore arising from wood. **d** Conidiogenous pores shown by arrows. **e** Concolored, catenate, and cylindrical conidia with 2–(3) eusepta. Bars **a, b, d, e** 20 μ m



septate, pale brown, smooth. Conidiophores macronematous, mononematous, singular, erect, cylindrical, straight, smooth, golden yellow, unbranched, 4–13-septate, up to 250 μ m long, 4.0–7.0 μ m wide, thick-walled. Conidiogenous cells integrated, polytretic, terminal and intercalary, cylindrical, borne in the top 1–7 cells of conidiophores, with minute circular pores visible after secession of conidia. Conidia in acropleurogenous chains, up to 6–7 in number, chains often branched, ramoconidia septate, with branches below septa of subapical cells, cylindrical, 2–(3)-euseptate, mostly 2-euseptate, slightly constricted at the septa, rounded at both sides, concolored, golden-yellow, unbranched, smooth, thick-walled; 2-septate conidia 15.0–23.0 \times 5.0–6.5 μ m, 19.0 \times 6.0 μ m on average, SDL = 2.05, SDW = 0.5, 3-septate conidia 23.0–28.0 \times 5.0–7.0 μ m, 25.5 \times 6.0 μ m on average, SDL = 1.64, SDW = 1.09, thick and darkly pigmented at the septa.

Discussion

Spadicoides and *Diplococcium* are closely related genera, sharing similar conidial ontogeny. *Spadicoides* and *Diplococcium* are most similar to three other genera: *Helminthosporium* Link, *Polytretophora* Mercado, and *Paliphora* Sivan. & B. Sutton (Link 1809; Mercado 1983; Sivanesan and Sutton 1985). These five genera all have polytretic conidiogenesis and macronematous conidiophores. However, conidia of *Helminthosporium* are distoseptate whereas conidia in *Spadicoides* and *Diplococcium* are either euseptate or with both eusepta and distosepta. Conidia are 1-septate and broadly ellipsoidal in *Polytretophora*, whereas those of *Spadicoides* and *Diplococcium* are variable in septation and shape. Furthermore, *Paliphora* can be separated from *Spadicoides* and *Diplococcium* by its setiform conidiophores and hyaline conidia.

Diplococcium also resembles five other genera—*Dendryphion* Wallr., *Didymobotryum* Sacc., *Dendryphiella* Bubák & Ranoj., *Corynesporella* Munjal & H.S. Gill, and *Corynesporopsis* P.M. Kirk (Wallroth 1833; Saccardo 1886; Ranojevic 1914; Munjal and Gill 1961; Kirk 1981)—in conidial morphology and production of conidia in chains. *Corynesporella*, *Corynesporopsis*, and *Didymobotryum* possess monotretic conidiogenous cells. Moreover, *Corynesporella* differs from *Diplococcium* in its distoseptate conidia and all branched conidiophores. *Didymobotryum* can be separated from *Diplococcium* by its synnematus conidiophores and 1-septate conidia. Furthermore, the conidiogenous cells of *Dendryphiella* and *Dendryphion* are sympodially proliferating, cicatrized, and with conspicuous scars formed after the dehiscence of conidia.

Spadicoides camelliae resembles *S. klotzschii* S. Hughes (Hughes 1973), *S. macrobovata* Matsush. (Matsushima 1995), *S. obclavata* Kuthub. & Nawawi (Kuthubutheen and Nawawi 1991), and *S. obovata* (Cooke & Ellis) S. Hughes (Hughes 1958) in versicolored, mainly 2-septate and smooth-walled conidia. However, conidia of *S. camelliae* differ in their ovoid shape from those of *S. klotzschii* (clavate, ellipsoidal), *S. macrobovata* (obovate), *S. obclavata* (obclavate), and *S. obovata* (obovate, clavate). Moreover, the conidia of *S. camelliae* are much larger than those of *S. klotzschii* (10.0–13.0 × 4.0–5.5 µm), although much smaller than those of *S. macrobovata* (16.0–37.0 × 11.0–22.0 µm). In addition, the conidia of *S. camelliae* are wider than those of *S. obclavata* (4.0–6.0 µm) and longer than those of *S. obovata* (12.5–16.0 µm).

Among the known species of *Diplococcium*, *D. livistonae* is most similar to *D. dendrocalami* Goh, K.D. Hyde & Umali (Goh et al. 1998) and *D. peruamazonicum* Matsush. (Matsushima 1993) based on their unbranched conidiophores and concolored, 1–2-septate conidia. *Diplococcium dendrocalami* differs from *D. livistonae* by wider (6.0–9.0 µm), oblong or ellipsoidal, and mostly 1-septate conidia. *Diplococcium peruamazonicum* can be distinguished from *D. livistonae* by wider (7.0–10.5 µm), broadly fusiform or ovate, and mainly 1-septate conidia.

Names of herbaria. The type specimens studied are deposited in Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP) with isotypes in Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS) (<http://hmas.im.ac.cn>).

Acknowledgments The authors are grateful to Dr. Shaun Pennycook (Landcare Research, Auckland, New Zealand) for improving the Latin descriptions. This project was supported by the National Natural Science Foundation of China (Nos. 30770015, 30499340, 2006FY120100). The experiments comply with current Chinese laws.

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