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Cladosporium leaf-blotch and stem rot of *Paeonia* spp. caused by *Dichocladosporium chlorocephalum* gen. nov.

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Abstract: *Cladosporium chlorocephalum* (= *C. paeoniae*) is a common, widespread leaf-spotting hyphomycete of peony (*Paeonia* spp.), characterised by having dimorphic conidiophores. During the season, one stage of this fungus causes distinct, necrotic leaf-blotch symptoms on living leaves of *Paeonia* spp. In late autumn, winter or after overwintering, a second morphologically distinct conidiophore type occurs on dead, blackish, rotting stems. Conspecificity of the two morphs, previously proposed on the basis of observations in culture, was supported by DNA sequence data from the ITS and LSU gene regions, using cultures obtained from leaf-blotch symptoms on living leaves, as well as from dead stems of *Paeonia* spp. Sequence data were identical, indicating a single species with two morphs. On account of its distinct conidiogenous loci and conidial hila, as well as its sequence-based phylogenetic position separate from the *Davidiella/Cladosporium* clade, the peony fungus has to be excluded from *Cladosporium s. str.*, but still belongs to the *Davidiellaceae* (*Capnodiales*). The leaf-blotching (cladosporioid) morph of this fungus morphologically resembles species of *Fusicladium*, but differs in having dimorphic fruiting, and is phylogenetically distant from the *Venturiaceae*. The macronematous (periconioid) morph resembles *Metulocladosporiella* (*Chaetothyriales*), but lacks rhizoid conidiophore hyphae, and has 0–5-septate conidia. Hence, *C. chlorocephalum* is assigned to the new genus *Dichocladosporium*.

Taxonomic novelties: *Dichocladosporium* K. Schub., U. Braun & Crous, gen. nov., *Dichocladosporium chlorocephalum* (Fresen.) K. Schub., U. Braun & Crous, comb. nov.
Key words: Anamorphic fungi, *Cladosporium chlorocephalum*, *C. paeoniae*, hyphomycetes, new genus, phylogeny, taxonomy.

INTRODUCTION

Fresenius (1850) described *Periconia chlorocephala* Fresen. from Germany on dead stems of *Paeonia* sp. Mason & Ellis (1953) examined this species *in vitro* and *in vivo* and stated that it only occurred on dead stems of *Paeonia* spp. They described, illustrated and discussed this species in detail, and reallocated it to the genus *Cladosporium* Link.

A second, cladosporioid hyphomycete on *Paeonia* spp., *Cladosporium paeoniae* Pass., was collected by Passerini on living leaves of *P. albiflora* (as *P. edulis*) in Italy, and distributed in Thümen, Herbarium mycologicum oeconomicum, Fasc. IX, No. 416 (1876), together with the first valid description, which was repeated by Passerini (1876). Later, Passerini collected this fungus on *Paeonia officinalis* at Parma in Italy and distributed it in Thümen, Mycotheca universalis, No. 670 (1877). Saccardo (1882) listed a collection of this species on *Paeonia anomala* from Russia, Siberia, which he later described as *Cladosporium paeoniae* var. *paeoniae-anomalae* Sacc. (Saccardo 1886). A first examination of *C. paeoniae* in culture was accomplished by Meuli (1937), followed by a treatment *in vitro* by de Vries (1952). Mason & Ellis (1953) described and illustrated in their treatment of *C. chlorocephalum* macroconidiophores, agreeing with those of the original diagnosis and illustration of *Periconia chlorocephala*, as well as semi-macronematous conidiophores concurring with those of *C. paeoniae*, although no mention was made of the latter name. McKemy & Morgan-Jones (1991) carried out comprehensive studies on *Cladosporium* on *Paeonia* spp. *in vitro* and *in vivo*, including detailed discussions of the history of the taxa concerned, taxonomic implications and comprehensive descriptions and illustrations. They concluded that *Cladosporium paeoniae*, found in culture together with *C. chlorocephalum*, was a semi-macronematous form (synanamorph) of the latter species, and reduced *C. paeoniae* to synonymy with the latter species.

In the present study, re-examination and reassessment of morphological characters, conidiogenesis, and DNA sequence data of the ITS and 28S nrDNA were used to confirm the identity of *Cladosporium chlorocephalum* (the periconioid morph) and *C. paeoniae* (the cladosporioid morph), and clarify their relation to *Cladosporium s. str.* (*Davidiellaceae*) (*emend.* David 1997, Braun *et al.* 2003).

MATERIALS AND METHODS

Isolates

Single-conidial isolates were obtained from symptomatic leaves and dead stems, and cultured as detailed in Crous (1998). Cultural characteristics and morphology of isolates (Table 1) were recorded from plates containing either 2 % potato-dextrose agar (PDA) or synthetic nutrient-poor agar (SNA) (Gams *et al*. 2007). Plates were incubated at 25 °C under continuous near-UV light to promote sporulation.

DNA isolation, amplification and sequencing

Fungal colonies were established on agar plates, and genomic DNA was isolated following the protocol of Lee & Taylor (1990). The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et al*. 1990), LR0R (Rehner & Samuels 1994), LR3R (www.biology.duke.edu/fungi/mycolab/primers.htm) and LR16 (Moncalvo *et al.* 1993) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous *et al*. (2006b). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Morphology

Morphological examinations were made from herbarium samples, fresh symptomatic leaves and stems, as well as cultures sporulating on SNA. Structures were mounted in water or Shear's solution (Dhingra & Sinclair 1985), and 30 measurements at × 1 000 magnification were made of each structure under an Olympus BX 50 microscope (Hamburg, Germany). The 95 % confidence levels were determined and the extremes of spore measurements given in parentheses. Scanning electron microscopic examinations were conducted at the Institute of Zoology, Martin-Luther-University, Halle (Saale), Germany, using a Hitachi S-2400. Samples were coated with a thin layer of gold applied with a sputter coater SCD 004 (200 s in an argon atmosphere of 20 mA, 30 mm distant from the electrode). Colony colours were noted after 2 wk growth on PDA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures studied were deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1). Taxonomic novelties were lodged with MycoBank (www.MycoBank.org).

RESULTS

DNA phylogeny

Amplification products of approximately 1 700 bases were obtained for the isolates listed in Table 1. The ITS region of the sequences was used to obtain additional sequences from GenBank which were added to the alignment. The manually adjusted alignment contained 26 sequences (including the two outgroup sequences) and 518 characters including alignment gaps. Of the 518 characters used in the phylogenetic analysis, 226 were parsimony-informative, 33 were variable and parsimony-uninformative, and 259 were constant. Neighbour-joining analysis using three substitution models on the sequence data yielded trees supporting the same clades but with a different arrangement at the deeper nodes. These nodes were supported poorly in the bootstrap analyses (the highest value observed for one of these nodes was 64 %; data not shown).

Two equally most parsimonious trees (TL = 585 steps; CI = 0.761; RI = 0.902; RC = 0.686), one of which is shown in Fig. 1, was obtained from the parsimony analysis of the ITS region. The *Dichocladosporium* K. Schub., U. Braun & Crous isolates formed a well-supported clade (100 % bootstrap support), distinct from clades containing species of *Davidiella* Crous & U. Braun, *Mycosphaerella* Johanson and *Teratosphaeria* Syd. & P. Syd. This placement was also supported by analyses of the first part of the 28S rRNA gene (see Crous *et al.* 2007 – this volume).

Taxonomy

Because conidia formed holoblastically in simple or branched acropetal chains, *Cladosporium chlorocephalum* and *C. paeoniae* coincided with previous concepts of *Cladosporium s. lat.* (Braun *et al.* 2003, Schubert 2005), belonging to a wide assemblage of genera classified by Kiffer & Morelet (1999) as "*Acroblastosporae*". Previous studies conducted *in vitro* concluded that *Cladosporium chlorocephalum* and *C. paeoniae* represent two developmental stages (morphs) of a single species, a result confirmed here by DNA sequence analyses. A detailed analysis of conidiogenesis, structure of the conidiogenous loci and conidial hila, and a comparison with *Cladosporium s. str.*, typified by *C. herbarum* (Pers. : Fr.) Link, revealed obvious differences: The conidiogenous loci and conidial hila of *C. chlorocephalum* are quite distinct from those of *Cladosporium s. str.* by being denticulate or subdenticulate, apically broadly truncate, unthickened or slightly thickened, but somewhat darkened-refractive. The scars in *Cladosporium s. str.* are, however, characteristically coronate, i.e., with a central convex dome surrounded by a raised periclinal rim (David 1997, Braun *et al.* 2003, Schubert 2005). Hence, the peony fungus has to be excluded from *Cladosporium s. str.* A comparison with phaeoblastic hyphomycetous genera revealed a close similarity of this fungus with the genus *Metulocladosporiella* Crous, Schroers, J.Z. Groenew., U. Braun & K. Schub. recently introduced for the Cladosporium speckle disease of banana (Crous *et al.* 2006a). Both fungi have dimorphic fruiting, pigmented macronematous conidiophores often with distinct basal swellings and densely branched terminal heads composed of short branchlets and ramoconidia, denticulate or subdenticulate unthickened, but somewhat darkened-refractive conidiogenous loci, as well as phaeoblastic conidia, formed in simple or branched acropetal chains. The semi-macronematous leaf-blotching morph is close to and barely distinguishable from *Fusicladium* Bonord. However, unlike *Metulocladosporiella*, the peony fungus does not form rhizoid hyphae at the base of conidiophore swellings and the conidia are amero- to phragmosporous [0–5-septate *versus* 0(–1)-septate in *Metulocladosporiella*]. Furthermore, the peony fungus neither clusters within the *Chaetothyriales* (with *Metulocladosporiella*) nor within the *Venturiaceae* (with *Fusicladium*), but clusters basal to the *Davidiellaceae* (see also Crous *et al.* 2007 – this volume). Hence, we propose to place *C. chlorocephalum* in the new genus *Dichocladosporium*.

Dichocladosporium K. Schub., U. Braun & Crous, **gen. nov.** MycoBank MB504428. Figs 2–5.

Etymology: *dicha* in Greek = twofold.

Differt a *Metulocladosporiella* conidiophoris cum cellulis basalibus saepe inflatis, sed sine hyphis rhizoidibus, conidiis amero- ad phragmosporis (0–5-septatis).

Type species: Dichocladosporium chlorocephalum (Fresen.) K. Schub., U. Braun & Crous, comb. nov.

Dichocladosporium chlorocephalum (Fresen.) K. Schub., U. Braun & Crous, **comb. nov.** MycoBank MB504429. Figs 2–5.

Basionym: *Periconia chlorocephala* Fresen., Beiträge zur Mykologie 1: 21. 1850.

≡ *Haplographium chlorocephalum* (Fresen.) Grove, Sci. Gossip 21: 198. 1885.

≡ *Graphiopsis chlorocephala* (Fresen.) Trail, Scott. Naturalist (Perth) 10: 75. 1889.

≡ *Cladosporium chlorocephalum* (Fresen.) E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 123. 1953.

= *Cladosporium paeoniae* Pass., in Thümen, Herb. Mycol. Oecon., Fasc. IX, No. 416. 1876, and in Just´s Bot. Jahresber. 4: 235. 1876.

= *Periconia ellipsospora* Penz. & Sacc., Atti Reale Ist. Veneto Sci. Lett. Arti, Ser. 6, 2: 596. 1884.

= *Cladosporium paeoniae* var. *paeoniae-anomalae* Sacc., Syll. Fung. 4: 351. 1886.

= *Haplographium chlorocephalum* var. *ovalisporum* Ferraris, Fl. Ital. Cryptog., Hyphales: 875. 1914.

Descriptions: Mason & Ellis (1953: 123–126), McKemy & Morgan-Jones (1991: 140–144), Schubert (2005: 216).

Illustrations: Fresenius (1850: Pl. IV, figs 10–15), Mason & Ellis (1953: 124–125, figs 42–43), McKemy & Morgan-Jones (1991: 137, fig. 1; 141, fig. 2; 139, pl. 1; 143, pl. 2), Schubert (2005: 217, fig. 113; 275, pl. 34).

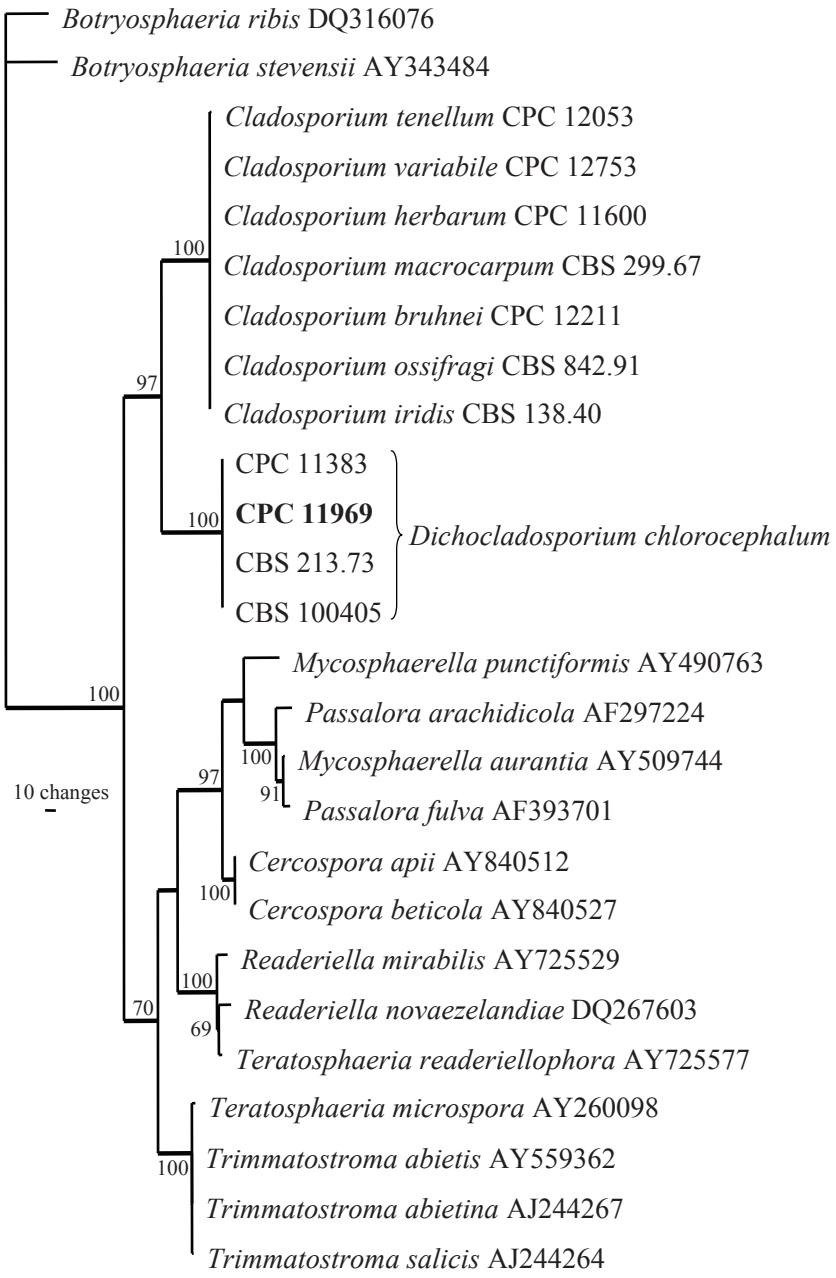

Fig. 1. One of two equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches. The tree was rooted to two *Botryosphaeria* species.

Characters of the cladosporioid morph: *Leaf-blotch symptoms* on living leaves amphigenous, variable in shape and size, subcircular-oval to irregular, broad, oblong to expanded, up to 30 mm long and 20 mm wide, at times covering the entire leaf surface, forming olivaceous-brown to blackish brown patches, rarely violet-brown, margin usually indefinite, attacked areas turning dry with age, also occurring on young, green stems. *Colonies* amphigenous, punctiform to effuse, loose to dense, caespitose, brown, villose. *Mycelium* immersed, subcuticular to intraepidermal; hyphae sparingly branched, 4–7(–10) µm wide, septate, sometimes with swellings and constrictions, swollen cells up to 13 µm diam, subhyaline to pale brown, smooth, walls thickened, hyphae sometimes aggregated; *in vitro* mycelium at first mainly immersed, later also superficial, branched, 1–5(–7) µm wide, pluriseptate, often constricted at septa and with swellings and constrictions, therefore irregular in outline, smooth to verruculose or irregularly rough-walled, loosely verruculose with distinct large warts. *Semi-macronematous conidiophores* formed on leaf-blotches solitary or in small, loose groups, arising from internal hyphae or swollen hyphal cells, erumpent through the cuticle, occasionally emerging through stomata, erect, straight to somewhat flexuous, oblong-cylindrical, usually unbranched or occasionally branched, 13–80 (–120) × (4–)5–8(–10) µm, slightly attenuated towards the apex, septate, septa often dense, unconstricted, pale to medium brown, sometimes paler towards the apex, smooth, thick-walled, wall often with two distinct layers, often somewhat inflated at the very base, up to 14 µm diam, occasionally proliferating enteroblastically; *in vitro* conidiophores arising laterally from plagiotropous hyphae or terminally from ascending hyphae, the latter usually appearing more filiform than those arising laterally from plagiotropous hyphae, erect, straight to slightly flexuous, cylindrical-oblong, not geniculate, usually unbranched, rarely with a short lateral prolongation near the apex, 18–60(–100) × 3–6 µm, slightly attenuated towards the apex, septate, pale to medium brown or olivaceous-brown, smooth to asperulate, walls somewhat thickened. *Conidiogenous cells* integrated, terminal or intercalary, subcylindrical, 7–45 µm

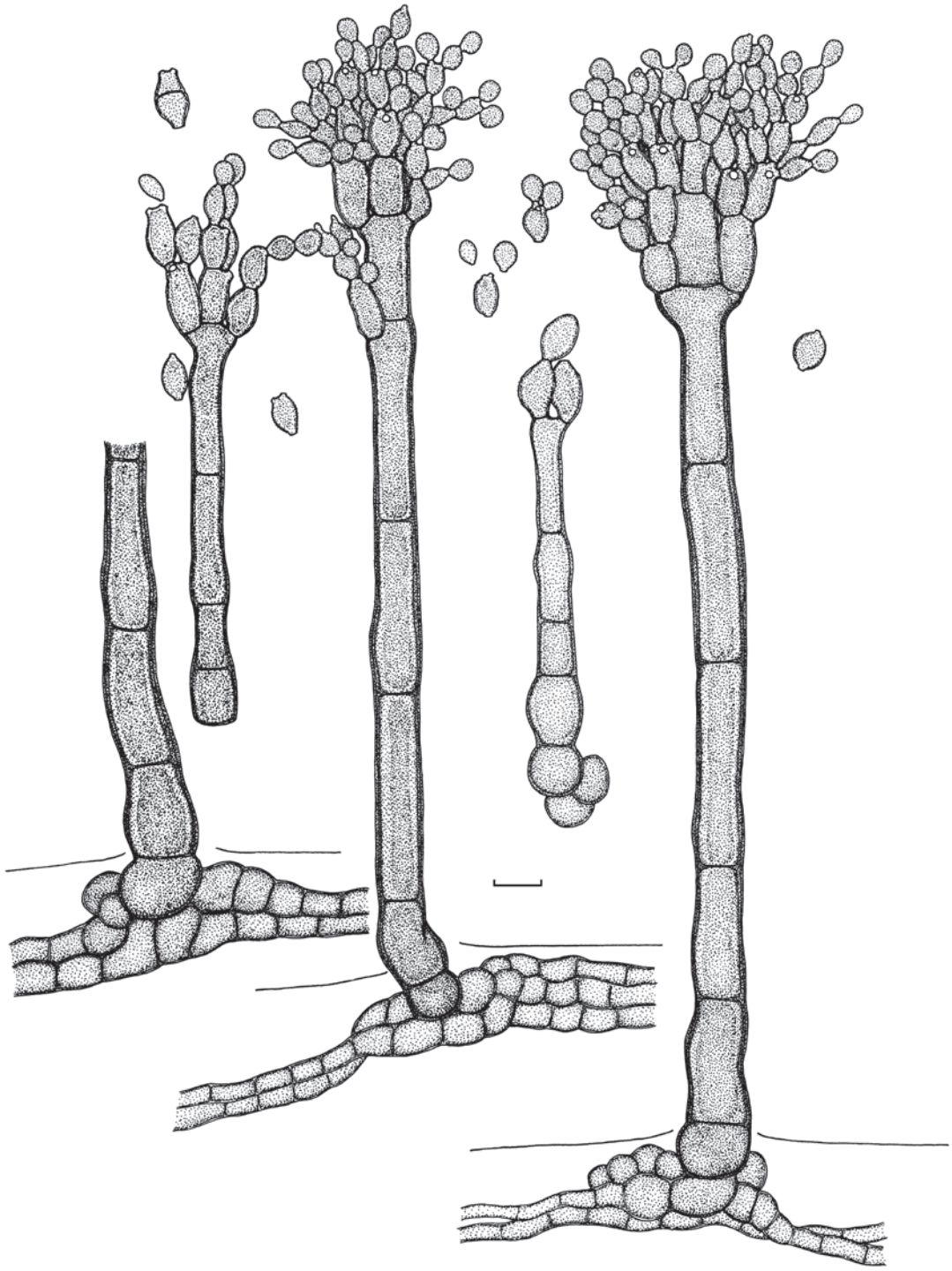

Fig. 2. *Dichocladosporium chlorocephalum* (HAL 1924 F), periconioid, stem rotting morph. Conidiophores and conidia. Scale bar = 10 µm.

Table 1. Isolates subjected to DNA analysis and morphological examination.

Species	Accession number[1]	Host	Country	Collector	GenBank accession number
Dichocladosporium chlorocephalum	CBS 213.73; IMI 048108a	*Paeonia* sp.	United Kingdom	F. Rilstone	EU009455
	CBS 100405	*Paeonia* sp.	New Zealand	M. Braithwaite	EU009456
	CBS 121522; CPC 11383	*Paeonia delavayi*	Germany	K. Schubert	EU009457
	CBS 121523*; CPC 11969	*Paeonia officinalis*	Germany	K. Schubert	EU009458

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.

*Ex-type cultures.

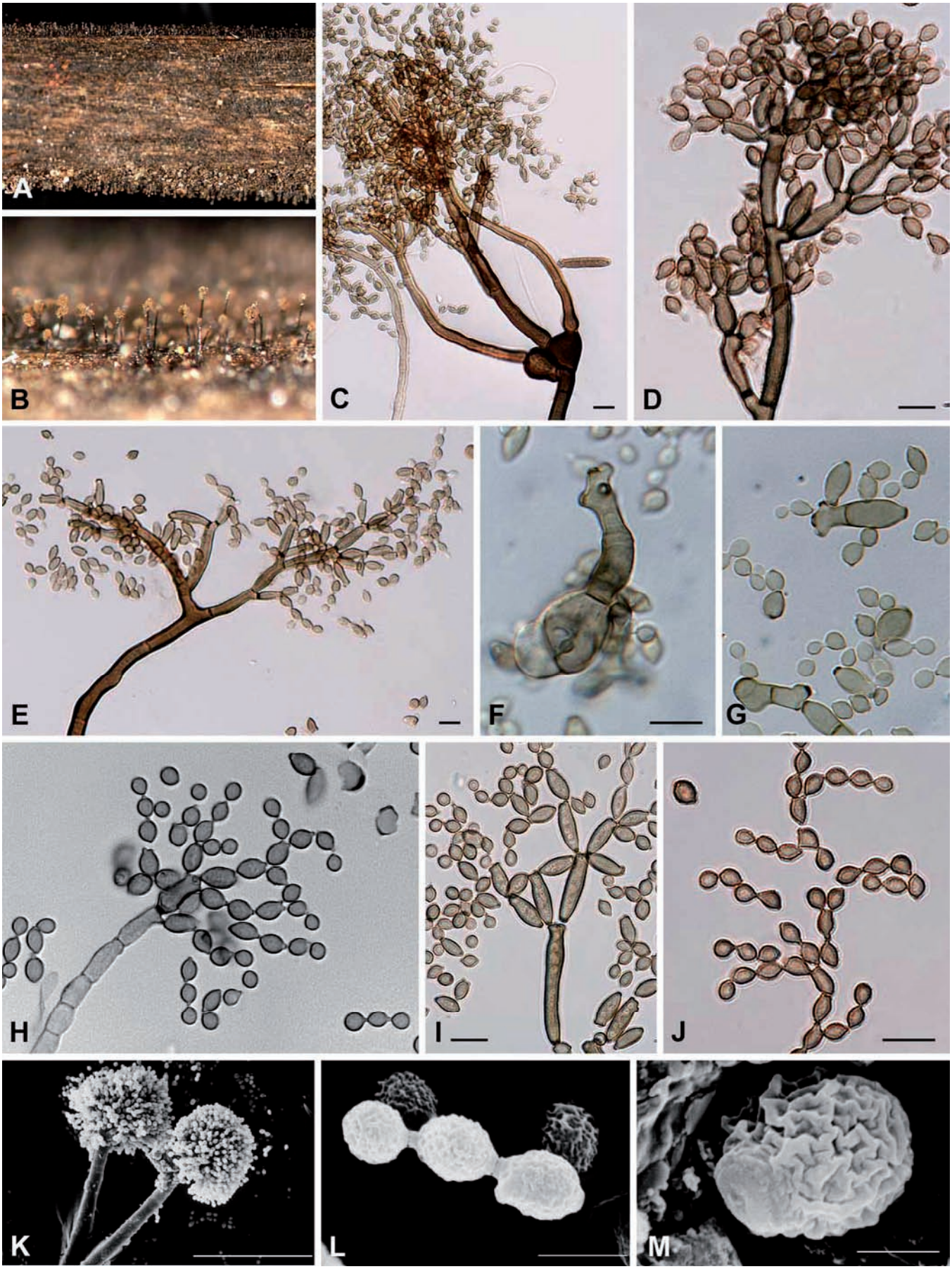

Fig. 3. *Dichocladosporium chlorocephalum* (CBS 121523 = CPC 11969). A–B. Symptoms of the periconioid, stem rotting morph. C–E, H. Macroconidiophores and conidia. F–G. Semi-macronematous conidiophores and conidia. I–J. Ramoconidia and conidia. K–M. Scanning electron microscopic photographs. K. Conidiophores. L. Conidial chain. M. Single conidium showing the surface ornamentation and scar structure. Scale bars: C–J, L = 10 µm; K = 100 µm; M = 2 µm.

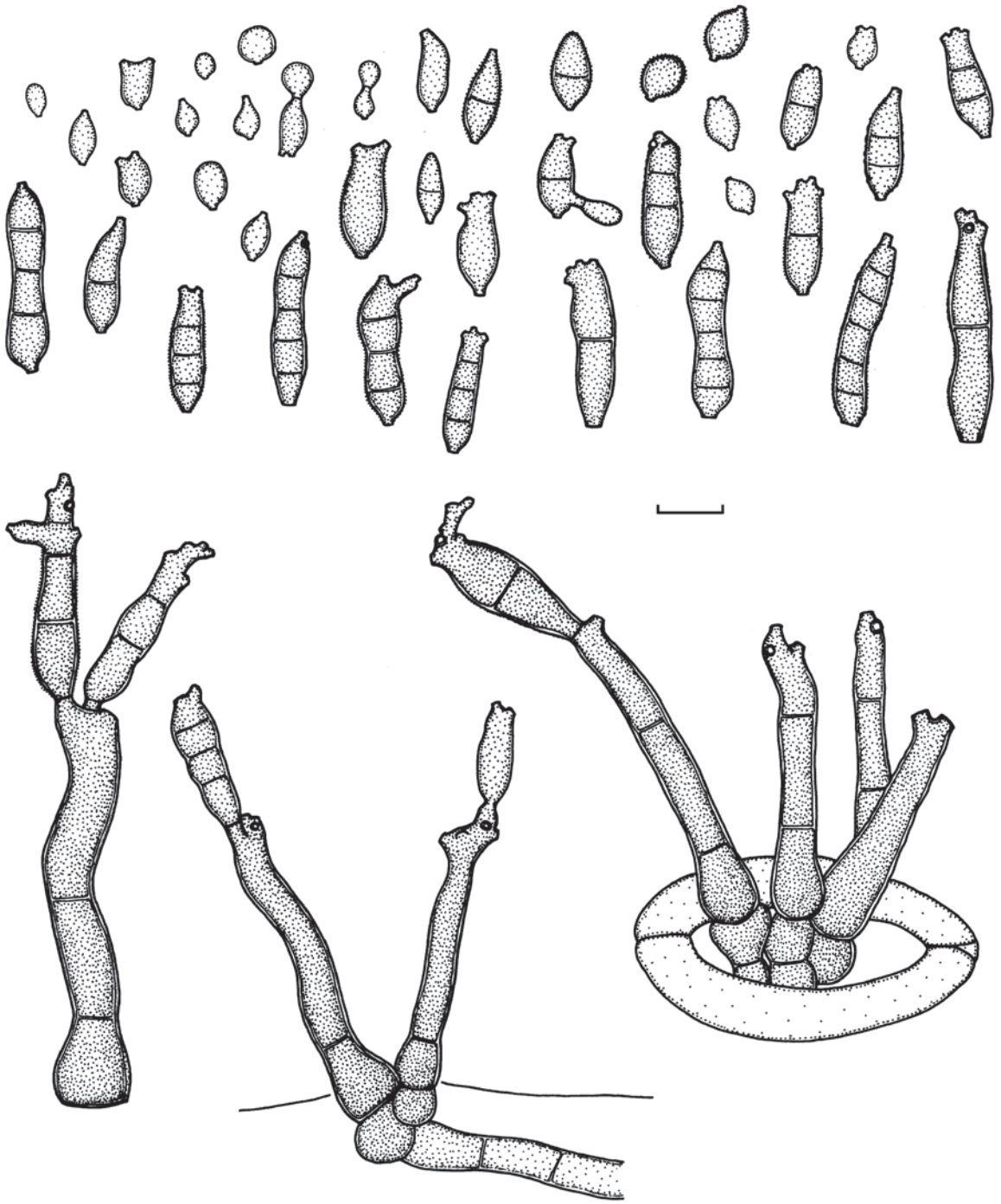

Fig. 4. *Dichocladosporium chlorocephalum* (HAL 2011 F), cladosporioid, leaf-spotting morph. Conidiophores and conidia. Scale bar = 10 µm.

long, proliferation sympodial, with one to several conidiogenous loci, subdenticulate or denticulate, protuberant, terminally broadly truncate, 1.5–3 µm wide, unthickened or almost so, somewhat darkened-refractive. *Conidia* catenate, in simple or branched chains, polymorphous, small conidia globose, subglobose, broadly obovoid, 3–9 × 3–5 µm, aseptate, pale to medium brown, smooth, intercalary conidia limoniform, ellipsoid-fusiform, oblong, 5–23 × 3.5–6.5 µm, 0–2-septate, medium brown, smooth to minutely verruculose or irregularly rough-walled, large conidia ellipsoid, oblong-cylindrical, ampulliform, 22–45(–52) × (4.5–)5–8 µm, 0–5-septate, medium brown, smooth to minutely verruculose or irregularly rough-walled, walls somewhat thickened, hila truncate, 1–3 µm wide, unthickened or almost so, somewhat darkened-refractive; occasionally with microcyclic conidiogenesis; *in vitro* numerous, polymorphous, catenate, in loosely branched chains, small conidia globose, subglobose, or obovoid, 3–8 × 3–4 µm, aseptate, intercalary conidia limoniform to ellipsoid-fusiform, 9–18 × 3.5–4.5 µm, 0–1-septate, large conidia ellipsoid to cylindrical-oblong, 14–30(–38) × 3–6(–7) µm, 0–3-septate, pale to medium brown, asperulate, minutely verruculose to irregularly rough-walled, walls thickened, hila usually short denticle-like, protuberant, truncate, in smaller conidia 0.5–1.8 µm wide, in larger conidia (1.5–)2–3 µm wide, unthickened or almost so but usually darkened-refractive; with occasional microcyclic conidiogenesis.

Characters of the periconioid morph: *Macronematous conidiophores* formed on faded or dead stems in late autumn, winter or after overwintering; colonies at first visible as reddish brown streaks, later turning olivaceous-brown to black, sometimes linear, sometimes encircling the stems, often occupying large stem segments, effuse, densely caespitose, velvety. *Mycelium* immersed, subcuticular

to intraepidermal; hyphae at first sparsely branched, 3–7 µm wide, septate, not constricted at the septa, becoming swollen and wider, up to 11 µm wide, often branched, pale to medium olivaceous-brown, walls thickened, forming loose to dense hyphal aggregations; *in vitro* mycelium immersed to superficial, loosely branched, 2–6(–7) µm wide, pluriseptate, usually without swellings and constrictions, subhyaline to medium brown or olivaceous-brown, almost smooth to asperulate or irregularly rough-walled, in older colonies on PDA up to 10 µm wide, sometimes single hyphal cells distinctly swollen, up to 16(–20) µm wide, mainly at the base of conidiophores, sometimes covered by a slime coat or enveloped in a polysaccharide-like layer. *Stromata* well-developed, large and expanded, up to about 50–320 µm in length, 15–30 µm deep, composed of a single to several layers of swollen pale to medium brown stromatic cells, 5–18 µm diam, thick-walled. *Conidiophores* solitary or in loose groups, arising from swollen hyphal cells or stromata, erumpent through the cuticle, erect, straight, rigid to slightly flexuous, 150–680 µm long, composed of a subcylindrical stipe, 13–24 µm wide at the base, slightly attenuated towards the apex, 5–15 µm just below the branched head, pluriseptate, not constricted at the septa, young conidiophores pale medium olivaceous-brown, later medium to usually dark brown, sometimes slightly paler at the distal end, smooth or almost so, often appearing somewhat granular, roughened, walls distinctly thickened, 1.5–3(–4) µm wide; apex with a roughly subglobose to ovoid head, about 35–70 µm diam, composed of dense branchlets and ramoconidia, primary branchlets close to the apex and below the first and sometimes second and third septa, solitary, in pairs or small verticils, appressed against the stipe or somewhat divergent, subcylindrical to ellipsoid-oval, aseptate, rarely 1-septate, pale olivaceous to dark brown, 10–20 × 5–8.5 µm; *in vitro* conidiophores initially micro- and semimacronematous, then progressively macronematous as colonies age, arising laterally from plagiotropous hyphae or terminally from ascending hyphae, sometimes also from swollen hyphal cells; micronematous conidiophores filiform, narrowly cylindrical-oblong, unbranched, up to 150 µm long, 2–3.5 µm wide, septate, septa often appear to be darkened, pale to pale medium olivaceous-brown, asperulate, walls slightly thickened; semi-macronematous conidiophores often resembling those formed by the leaf-blotching (cladosporioid) morph on the natural host, subcylindrical to cylindrical-oblong, straight to slightly flexuous, unbranched, rarely branched, (10–)15–120 × 3–5(–6) µm, slightly attenuated towards the apex, septate, medium brown, minutely verruculose to irregularly rough-walled, walls more or less thickened; macronematous conidiophores formed in older cultures on SNA, PDA and also MEA (according to McKemy & Morgan-Jones 1991), but more prominent on PDA and MEA, resembling those formed by the stem-rotting morph (i.e., the periconioid morph, in planta), consisting of a long unbranched stipe and a subglobose head, but in culture the heads are often more loosely branched than on the natural substratum, not always forming a compact head, up to 580 µm long, 5–13 µm wide, attenuated towards the apex, 4–8 µm just below the branched upper part, somewhat swollen at the base, septate, medium to very dark brown, minutely verruculose, walls distinctly thickened, two distinct wall layers visible, 1–2 µm thick. *Conidiogenous cells* holoblastic, integrated, terminal, intercalary or even discrete, ellipsoid to cylindrical or doliiform, subdenticulate, proliferation sympodial, multilocal, conidiogenous loci truncate, flat, unthickened, 1–3 µm wide, somewhat darkened-refractive; in culture conidiogenous loci appearing to be somewhat thickened and distinctly darkened-refractive, 1–2.5(–3) µm wide. *Conidia* catenate, in long, branched chains, straight, subglobose, aseptate, 3.5–7 µm diam, or ellipsoid-ovoid, 6–15 × 4–9 µm, 0(–1)-septate, pale olivaceous to olivaceous-brown, smooth to verruculose (under the light microscope), hila flat, truncate, unthickened, (0.5–)1–2(–2.5) µm wide, not darkened, but somewhat refractive; *in vitro* conidia numerous, catenate, formed in long, branched chains, small conidia globose to subglobose, (2–)3–7 × (2–)3–4 µm, aseptate, intercalary ones ellipsoid-ovoid, 6–16 × 3.5–5 µm, 0(–1)-septate, *secondary ramoconidia* ellipsoid to cylindrical-oblong, (13–)15–34(–47) × (3–)4–6(–7) µm, 0–2-septate, sometimes slightly constricted at the septa, medium olivaceous-brown, verruculose or irregularly rough-walled, walls slightly to distinctly thickened, hila more or less protuberant, subdenticulate to denticulate, in small and intercalary conidia 0.5–1(–1.5) µm, in secondary ramoconidia 1–2.5(–3) µm, unthickened or somewhat thickened, darkened-refractive; occasional microcyclic conidiogenesis.

Cultural characteristics: Colonies on PDA at first whitish or smoke grey, reverse smoke-grey to olivaceous-grey, with age smoke-grey to olivaceous or olivaceous-grey, sometimes even dark mouse-grey, reverse iron-grey to dark mouse-grey or black, felty; margin white to smoke-grey, narrow to more or less broad, regular to slightly undulate, glabrous to somewhat feathery; aerial mycelium at first mainly in the colony centre, with age abundantly formed, covering almost the whole colony, whitish, smoke-grey to olivaceous, felty; growth low convex to raised; numerous small exudates formed, sometimes becoming prominent; fertile.

Specimens examined: **Czechoslovakia**, Bohemia, Turnau, on leaves of *Paeonia arborea*, 15 Sep. 1905, J.E. Kabát, Kabát & Bubák, Fungi Imperf. Exs. 396, B 70-6669. **France**, on dead stems of *Paeonia* sp., 1901, ex Herbario Musei Parisiensis, ex herb. Magnus, exs. Desmazières, Pl. Crypt. N. France, Ed. 2, Ser. 1, 1621, HBG, as "*Periconia atra*"; Chailly-en-Biere, Seine-et-Marne, Feuilleaubois, on stems of *P. officinalis*, 27 Mar. 1881, Roumeguère, Fungi Sel. Gall. Exs. 1803, HBG, as "*Periconia atra*". **Germany**, Baden-Würtemberg, Kreis Tübingen, Drusslingen, on leaves of *P. officinalis*, Jun. 1935, Raabe, B 70-6670; Bayern, Freising, on leaves of *P. officinalis*, Sep. 1918, Prof. Dr. J.E. Weiß, Herbarium pathologicum, B 70-6663; Brandenburg, Schloßpark zu Tamsel, on leaves of *P. officinalis*, 15 Aug. 1924, P. Vogel, Sydow, Mycoth. Germ. 2447, M-57751, PH; Triglitz, on leaves of *P. officinalis*, 3 Oct. 1909, Jaap, B 70-6668; Hessen, Frankfurt am Main, botanical garden, on leaves of *P. potaninii*, 7 Oct. 2004, R. Kirschner, HAL, RoKi 2222; Kreis Kassel, Hofgeismar, Garten von Prof. Grupe, on leaves of *P. officinalis*, 3 Sep. 1947, Schulz, B 70-6658; Mecklenburg-Vorpommern, Rostock, neuer botanischer Garten, on leaves of *P. corallina* (= *P. mascula*), 27 Aug. 1950, Becker, B 70-6662; Nordrhein-Westfalen, Duisburg, Dinslake, private garden, on leaves of *P. anomala*, 9 Aug. 2005, N. Ale-Agha, HAL 2014 F; Hamborn, botanical garden, on leaves of *P. obovata*, 10 Aug. 2005, N. Ale-Agha, HAL 2017 F; Essen, botanical garden of the university of Essen, on leaves of *P. mlokosewitschii*, 10 Aug. 2005, N. Ale-Agha, HAL 2013 F; on leaves of *P. officinalis* and *P. suffruticosa*, 11 Aug. 2005, N. Ale-Agha, HAL 2016, 2017 F; Sachsen, Königstein, in Gärten, verbreitet, on leaves of *P. officinalis*, Aug. 1896, W. Krieger, Krieger, Fungi Saxon. Exs. 1545, M-57749; Aug., Sep. 1896, 1915, W. Krieger, Krieger, Schädliche Pilze, B 70-6666, 70-6667; Sachsen-Anhalt, Halle (Saale), Botanical Garden, on leaves of *P. delavayi*, 22 Jun. 2004, K. Schubert, HAL 2011 F, culture deposited at the CBS, CBS 121522 = CPC 11383; on leaves of *P. officinalis*, 22 Jun. 2004, K. Schubert, HAL 2012 F; on stems of *P. officinalis*, 16 Mar. 2005, K. Schubert, **neotype of *Dichocladosporium chlorocephalum* designated here** HAL 1924 F, isoneotype CBS-H 19869, culture ex-neotype CBS 121523 = CPC 11969; on dead stems of *Paeonia* sp., Jan. 1873, G. Winter, Rabenhorst, Fungi Eur. Exs. 1661, HBG, as "*Periconia chlorocephala*"; Thüringen, Fürstlicher Park zu Sondershausen, on leaves of *P. arborea*, 20 Aug. 1903, G. Oertel, Sydow, Mycoth. Germ. 196, PH. **Italy**, Thümen, Herb. Mycol. Oecon. 416, on living leaves of *Paeonia lactiflora* [= *P. edulis*] (M-57753), **lectotype of *"Cladosporium paeoniae"* designated here**; *isolectotypes*: Thümen, Herb. Mycol. Oecon. 416; Padova, on leaves of *P. officinalis*, Aug. 1902, P.A. Saccardo, Saccardo, Mycoth. Ital. 1186, B 70-6660, SIENA; Parma, on leaves of *P. officinalis*, Jul. 1876, Prof. Passerini, Thümen, Mycoth. Univ. 670, B 70-6654, 70-6655, M-57752; Pavia, botanical garden, on leaves of *P. officinalis*, summer 1889, Briosi & Cavara, Fung. Paras. Piante Colt. Utili Ess. 78, M-57748; F. Cavara, Roumeguère, Fungi Sel. Gall. Exs. 5193, mixed infection with *Cladosporium herbarum*, B 70-6656; Siena, Hort. Bot., on leaves of *Paeonia* sp., Nov. 1899, SIENA. **Latvia**, prov. Vidzeme, Kreis Riga, Riga, in a garden, on leaves of *P. foemina* [= *P. officinalis*], 28 Aug. 1936, J. Smarods, Fungi Lat. Exs. 799, M-57747. **New Zealand**, isolated from

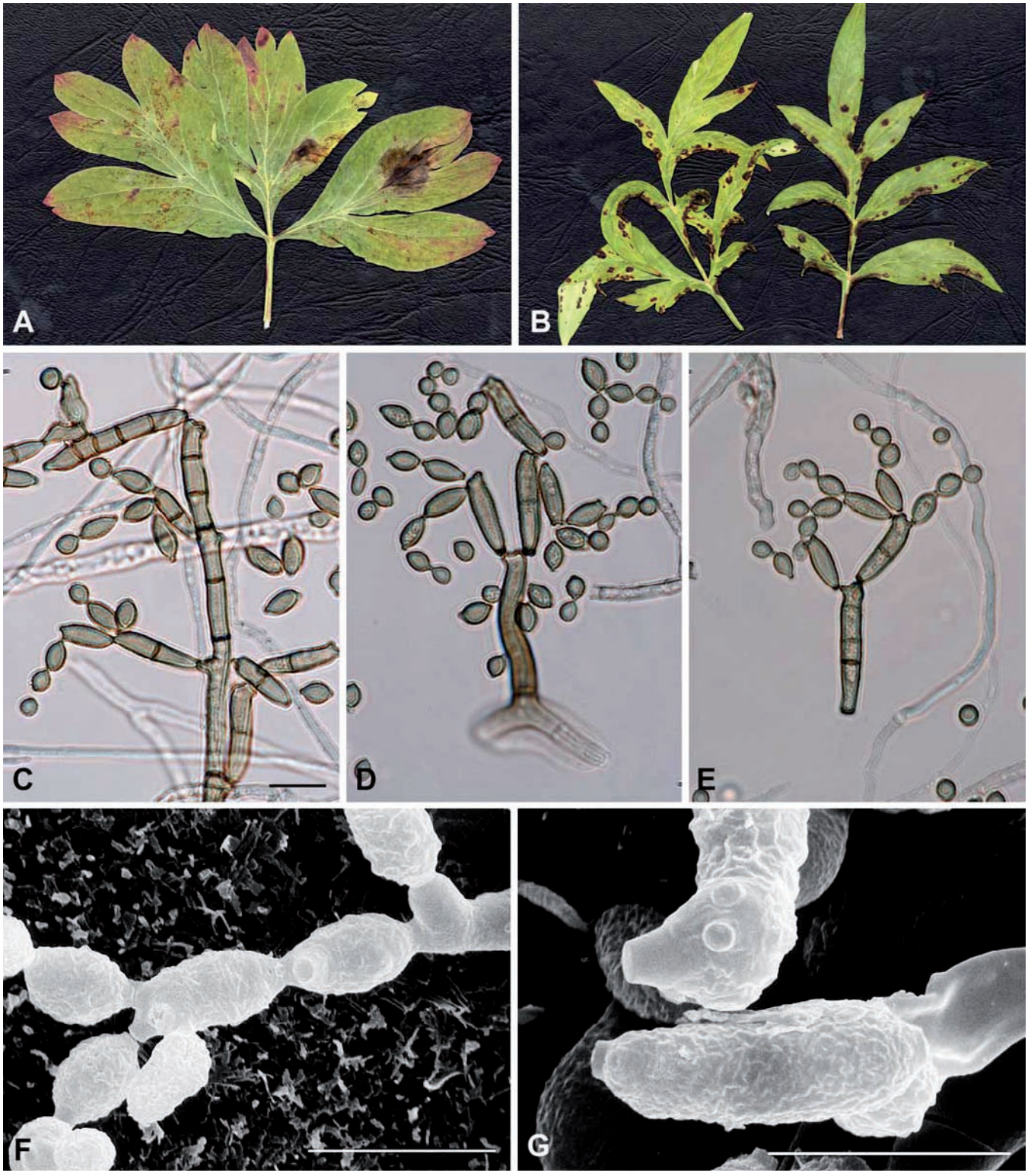

Fig. 5. *Dichocladosporium chlorocephalum* (CBS 121522 = CPC 11383). A–B. Symptoms on leaves of *Paeonia officinalis* and *P. delavayi* caused by the cladosporioid, leaf spotting morph. C–D. Conidiophores and conidia. E. Ramoconidia and conidia. F–G. Scanning electron microscopic photographs. F. Conidial chain still attached to a conidiophore. G. Conidia showing surface ornamentation and scar structure. Scale bars: C–E, G = 10 µm; F = 5 µm.

red leaf and stem lesions on *Paeonia* sp., M. Braithwaite, CBS 100405. **Romania**, Râmnicu-Vâlcea, distr. Vâlcea, Oltenia, on leaves of *P. officinalis*, 17 Aug. 1930, Tr. Săvulescu & C. Sandu, Săvulescu, Herb. Mycol. Roman. 298, M-57742. **U.K.**, England, Cornwall, Lambounce Hill, Perranzubuloe, isolated from dead stems of *Paeonia* sp., isol. F. Rilstone, CBS 213.73 = IMI 048108a. **U.S.A.**, on leaves of *Paeonia* sp., Sep. 1878, Ellis, N. Amer. Fungi 543, B 70-6659, M-57744, PH; Illinois, Cobden, on leaves of *Paeonia* sp., 8 Aug. 1882, F.S. Earle, No. 91, B 70-6657; Kansas, Topeka, on leaves of *P. officinalis*, 7 Jul. 1922, C.F. Menninger, US Dept. Agric., Pathol. Mycol. Coll. 60085, B 70-6661, F; Montana, Columbia, on leaves of *P. officinalis*, Aug. 1886, B.T. Galloway, Ellis & Everh., N. Amer. Fungi Ser. II, 1991, PH; on leaves of *Paeonia* sp., 18 Oct. 1931, W.E. Maneval, F.

Host range and distribution: On *Paeonia anomala, P. arborea, P. delavayi, P. hybrida, P. lactiflora, P. mascula, P. mlokosewitschii, P. moutan, P. obovata, P. obovata* var. *willmotiae, P. officinalis, P. potaninii, P. suffruticosa, Paeonia* spp. (*Paeoniaceae*), Asia (Armenia, China, Georgia, Kazakhstan, Russia), Europe (Belgium,

Czechoslovakia, Denmark, France, Germany, Italy, Latvia, Moldova, Poland, Romania, Switzerland, U.K., Ukraine), North America (Canada, U.S.A.), New Zealand.

Notes: Type material of *Periconia chlorocephala* is not preserved in the herbarium of G. Fresenius at FR (Forschungsinstitut Senkenberg, Frankfurt a. M., Germany). Hence, a new specimen collected in the Botanical Garden of the Martin-Luther-University Halle (Saale), Germany, is proposed to serve as neotype. A culture derived from this collection is deposited at the CBS, Utrecht, the Netherlands as ex-neotype culture. A leaf-blotch sample, also collected in the Botanical Garden at Halle (Saale), from which we also derived a living culture, is designated as representative of the synanamorph, *Cladosporium paeoniae*. Both cultures have been used to generate DNA sequence data.

The two stages (morphs) of this fungus are usually ecologically and seasonally separated, but sometimes conidiophores of the leaf-blotching (cladosporioid) morph also occur on dead stems of peony intermixed with the macronematous conidiophores of the periconioid morph. In culture conidiophore and conidial width tends to be narrower than on the natural substratum, and the conidia are not as frequently septate.

DISCUSSION

Cultural studies by ourselves and McKemy & Morgan-Jones (1991), and molecular sequence analyses documented herein clearly demonstrate that *Cladosporium chlorocephalum*, occurring on necrotic stems, and *C. paeoniae*, causing leaf-blotch symptoms on living leaves of *Paeonia* spp., are two synanamorphs of a single species, which has to be excluded from *Cladosporium s. str.* since the conidiogenous loci are quite distinct from the characteristically coronate scars in the latter genus and because ITS sequences indicate clear separation from *Cladosporium s. str*.

Analysis of the morphology and conidiogenesis showed that the macronematous stage of this fungus (*C. chlorocephalum*, the periconioid morph) closely resembles *Metulocladosporiella*, recently introduced for the Cladosporium speckle disease of banana. There are, however, some differences. In *Metulocladosporiella musae* (E.W. Mason) Crous *et al.*, the type species, micronematous conidiophores occur *in vitro* and *in vivo*, and macronematous conidiophores occur on leaf-spots, whereas in *C. chlorocephalum* the semi-macronematous conidiophores usually accompany leaf-blotch symptoms on living leaves and the macronematous conidiophores occur in saprobic growth on old necrotic stems. Rhizoid hyphae arising from the swollen basal cells of the macronematous conidiophores are characteristic for *M. musae*, but lacking in *C. chlorocephalum*, and the conidia in the latter species are 0–5-septate, but only 0(–1)-septate in *M. musae*. The semi-macronematous, leaf-blotching stage (the cladosporioid morph) is barely distinguishable from the present concept of *Fusicladium*, which includes species with catenate conidia (Schubert *et al.* 2003). However, the peony fungus does not cluster within the *Venturiaceae*. Since *C. chlorocephalum* clusters apart of the *Chaetothyriales*, the clade to which *Metulocladosporiella* belongs, the differences observed here seem to be sufficient to place this fungus in a new genus (also see Crous *et al.* 2007 – this volume). Crous *et al.* (2006a) discussed differences between *Metulocladosporiella* and allied dematiaceous hyphomycete genera and provided a key to the latter genus and morphologically similar genera. Using this key, attempts to determine the macronematous morph of *Cladosporium chlorocephalum* lead to *Metulocladosporiella*. Differences between morphologically similar genera have been discussed in the paper by Crous *et al.* (2006a) and are also valid for the new genus *Dichocladosporium*. *Parapericoniella* U. Braun, Heuchert & K. Schub., a fungicolous genus recently introduced to accommodate *Cladosporium asterinae* Deighton, is also morphologically similar in having apically, densely branched conidiophores and truncate, unthickened conidiogenous loci and hila, but is quite distinct in not having micronematous conidiophores (Heuchert *et al.* 2005).

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