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Morphological plasticity in *Cladosporium sphaerospermum*

F.M. Dugan[1], U. Braun[2], J.Z. Groenewald[3], P.W. Crous[3]

Key words

actin
Cladosporium
Davidiella
EF-1α
ITS
morphology
wood-inhabiting

Abstract A morphologically distinct isolate of *Cladosporium sphaerospermum* from a North American patent collection, referenced as *Cladosporium lignicola* in the patent, was examined. Generic affinity was confirmed by scanning electron microscopic examination of conidiogenous loci and conidial hila. Species identity as *C. sphaerospermum* was indicated by DNA sequence data derived from actin and translation elongation factor 1-α genes, and the internal transcribed spacer region. The isolate broadens the morphological limits of *C. sphaerospermum* by production of obclavate, occasionally transversely septate conidia with subrostrate conidiogenous apices ('alternarioid' conidia), and by production of conidia larger than those in prior standard descriptions. Type material of *C. lignicola* was re-examined and compared with the North American fungus, from which it is morphologically distinct. The decision to reduce *C. lignicola* to synonymy under *C. herbarum* was confirmed.

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INTRODUCTION

Cladosporium is one of the largest genera of hyphomycetes, with more than 772 names (Braun et al. 2003, Dugan et al. 2004). *Cladosporium* species are common, widespread fungi, including endophytic, fungicolous, human pathogenic, phytopathogenic and saprobic species (Crous et al. 2007b). Saprobic species occur on various senescing and dead leaves and stems of herbaceous and woody plants, are secondary invaders of necrotic leaf spots, and are frequently isolated from air, soil, foodstuffs, paint, textiles and other organic matter (Riesen & Sieber 1985, Brown et al. 1998, El-Morsy 2000). Furthermore, some *Cladosporium* species, such as *C. bruhnei*, are common contaminants in clinical laboratories and cause allergic lung mycoses (de Hoog et al. 2000, Schubert et al. 2007b).

Because the genus *Cladosporium* is very heterogeneous, David (1997) attempted to circumscribe *Cladosporium* based on scanning electron microscopic examinations of the scar and hilum structure in *Cladosporium* and *Heterosporium*. In so doing, David (1997) demonstrated that the structures of the conidiogenous loci and hila in *Heterosporium* fully agree with those of *Cladosporium*, proving that *Heterosporium* was a synonym of *Cladosporium*. Furthermore, he introduced the term 'coronate' for the *Cladosporium* scar type, which is characterised by having a central convex part (dome), surrounded by a raised periclinal rim (Schubert et al. 2007b, Zalar et al. 2007). Several workers have employed DNA sequence data to prove that *Cladosporium* s.str. is a sister clade to *Mycosphaerella* s.str. (Braun et al. 2003, Crous et al. 2007a, b, Schubert et al. 2007a, b), having teleomorphs in *Davidiella*. Schoch et al. (2006) employed DNA sequence data of four loci (SSU nrDNA, LSU nrDNA, EF-1α, RPB2), revealing species of *Davidiella* to cluster in a separate family (Davidiellaceae) from species of *Mycosphaerella* (Mycosphaerellaceae), with both families residing in the *Capnodiales* (Dothideomycetes).

A cladosporioid hyphomycete, deposited in the patent collection of the United States Department of Agriculture Northern Regional Research Lab (now National Centre for Agricultural Utilization Research) as NRRL 8131 (= ATCC 38493), was referenced in U.S. Patent 4.086.268 as *Cladosporium lignicolum* (sic, without author). The patent does not state the original substratum, but wood is a logical inference from the specific epithet. Ho et al. (1999) described NRRL 8131 from culture, and provided several photomicrographs illustrating the fungus, including 0–1 transversely septate, obclavate conidia with subrostrate apices ('alternarioid' conidia in their description). A comparison of the latter description and illustrations with the original diagnosis of *C. lignicola* revealed obvious discrepancies and called into question the correctness of the identification of the NRRL strain. To help ascertain the identity of NRRL 8131, type material of *C. lignicola* has been re-examined and compared with the North American strain.

We were originally attracted to re-examination of NRRL 8131 not only by the discrepancies referenced above, but by the unique conidial morphology: the 'alternarioid' conidia resembled the beaked conidia of *Alternaria* spp. (Simmons 2007). Scanning electron microscopy (SEM) examination of conidiogenous loci and conidial hila in the NRRL strain of '*C. lignicola*' confirmed generic affinity, and sequence analyses of the actin, translation elongation factor 1-α and the internal transcribed spacer region (ACT, EF, ITS) were used to assign the strain to species.

MATERIALS AND METHODS

Materials examined

Several specimens deposited at PRM (Herbarium, Department of Mycology, National Museum, Prague, Czech Republic) under *C. lignicola* were examined: on rotten wood, Czechia, near

[1] Western Regional Plant Introduction Germplasm Testing and Research Station, USDA-ARS, Washington State University, Pullman, WA 99164-6402, USA; corresponding author e-mail: fdugan@wsu.edu.
[2] Martin-Luther-Universität, Institut für Biologie, Geobotanik und Botanischer Garten, Herbarium, Neuwerk 21, D-06099 Halle (Saale), Germany.
[3] CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.

Table 1 *Cladosporium* isolates used for sequence analysis.

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	Source	GenBank numbers[2] (ITS, EF, ACT)
C. antarcticum	–	CBS 690.92	*Caloplaca regalis*	Antarctica	C. Möller	Schubert et al. 2007	EF679334, EF679405, EF679484
C. bruhnei	*Davidiella allicina*	CBS 157.82	*Quercus robur*	Belgium	–	Schubert et al. 2007	EF679336, EF679407, EF679486
		CBS 161.55	Man, sputum	The Netherlands	–	Schubert et al. 2007	EF679338, EF679409, EF679488
		CBS 121624; CPC 12211	*Hordeum vulgare*	Belgium	J.Z. Groenewald	Schubert et al. 2007	EF679350, EF679425, EF679502
C. cladosporioides complex	–	CBS 673.69	Air	The Netherlands	–	Schubert et al. 2007	EF679353, EF679428, EF679505
	Davidiella sp.	CBS 109082	*Silene maritima*	United Kingdom	A. Aptroot	Schubert et al. 2007	EF679354, EF679429, EF679506
	–	CPC 11606	*Musa* sp.	India	M. Arzanlou	Schubert et al. 2007	EF679355, EF679430, EF679507
C. herbaroides	–	CBS 121626; CPC 12052; EXF-1733	Hypersaline water from salterns	Israel	P. Zalar	Schubert et al. 2007	EF679357, EF679432, EF679509
C. herbarum	*Davidiella tassiana*	CBS 121621; CPC 12177	*Hordeum vulgare*	The Netherlands	–	Schubert et al. 2007	EF679363, EF679440, EF679516
		CPC 12181	*Hordeum vulgare*	The Netherlands	–	Schubert et al. 2007	EF679367, EF679444, EF679520
		CPC 12183	*Hordeum vulgare*	The Netherlands	–	Schubert et al. 2007	EF679368, EF679445, EF679521
C. iridis	*Davidiella macrospora*	CBS 107.20	*Iris* sp.	–	–	Schubert et al. 2007	EF679369, EF679446, EF679522
		CBS 138.40	*Iris* sp.	The Netherlands	–	Schubert et al. 2007	EF679370, EF679447, EF679523
C. macrocarpum	*Davidiella macrocarpa*	CBS 299.67	*Triticum aestivum*	Turkey	–	Schubert et al. 2007	EF679372, EF679450, EF679526
		CBS 121811; CPC 12755	*Spinacia oleracea*	USA	–	Schubert et al. 2007	EF679376, EF679454, EF679530
		CPC 12756	Spinacia oleracea	USA	–	Schubert et al. 2007	EF679377, EF679455, EF679531
C. ossifragi	–	CBS 842.91	*Narthecium ossifragum*	Norway	M. di Menna	Schubert et al. 2007	EF679381, EF679459, EF679535
		CBS 843.91	*Narthecium ossifragum*	Norway	M. di Menna	Schubert et al. 2007	EF679382, EF679460, EF679536
C. pseudiridis	–	CBS 116463; ICMP 15579	*Iris* sp.	New Zealand	C.F. Hill	Schubert et al. 2007	EF679383, EF679461, EF679537
C. ramotenellum	–	CBS 121628; CPC 12043; EXF-454	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007	EF679384, EF679462, EF679538
		CPC 12047; EXF-967	Air conditioning system	Slovenia	P. Zalar	Schubert et al. 2007	EF679385, EF679463, EF679539
C. sinuosum	–	CBS 121629; CPC 11839; ICMP 15819	*Fuchsia excorticata*	New Zealand	A. Blouin	Schubert et al. 2007	EF679386, EF679464, EF679540
Cladosporium sp.	–	CBS 300.96	Soil along coral reef coast	Papua New Guinea	A. Aptroot	Zalar et al. 2007	DQ780352, EU570259, EF101385
C. sphaerospermum	–	CBS 109.14; ATCC 36950	*Carya illinoensis* leaf scale	USA	–	Zalar et al. 2007	DQ780350, EU570260, EF101384
		CBS 193.54; ATCC 11289; IMI 49637	Human nails	The Netherlands	G.A. de Vries	Zalar et al. 2007	DQ780343, EU570261, EU570269
		CBS 102045; EXF-2524; MZKI B-1066	Hypersaline water	Spain	P. Zalar	Zalar et al. 2007	DQ780351, EU570262, EF101378
		CPC 11822	*Phyllactinia guttata* on *Corylus* sp.	USA	D. Glawe	This study	EU570254, EU570263, EU570270
		CPC 12476	*Ambrosia artemisiifolia*	Germany	J. Nitzsche	This study	EU570255, EU570264, EU570271
		CPC 13368	*Phaseolus lunatus*	Germany	N. Ale-Agha	This study	EU570256, EU570265, EU570272
		CPC 13995; CAMS 000750	Thatch	South Africa	–	This study	EU570257, EU570266, EU570273
		CPC 14016; MRC 10263	*Triticum aestivum*	South Africa	–	This study	EU570258, EU570267, EU570274
		CBS 117728; ATCC 38493; CPC 12098; NRRC 8131	–	–	–	This study	AF393709, EU570268, EU570275

C. spinulosum	–	CBS 102044	Hypersaline water from salterns	Slovenia	S. Soujak	Schubert et al. 2007	EF679387, EF679465, EF679541
		CBS 119907; CPC 12040; EXF-334	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007	EF679388, EF679466, EF679542
C. subinflatum	–	CBS 121630; CPC 12041; EXF-343	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007	EF679389, EF679467, EF679543
C. subtilissimum	–	CBS 113753	Bing cherry fruits	USA	–	Schubert et al. 2007	EF679396, EF679474, EF679550
		CBS 113754	Grape berry	USA	–	Schubert et al. 2007	EF679397, EF679475, EF679551
		CPC 12044; EXF-462	Hypersaline water from salterns	Slovenia	P. Zalar	Schubert et al. 2007	EF679398, EF679476, EF679552
C. tenellum	–	CBS 121634; CPC 12053; EXF-1735	Hypersaline water from salterns	Israel	P. Zalar	Schubert et al. 2007	EF679401, EF679479, EF679555
		CPC 11813	*Phyllactinia* sp. on *Corylus* sp.	USA	D. Glawe	Schubert et al. 2007	EF679399, EF679477, EF679553
C. variabile	*Davidiella variabile*	CBS 121636; CPC 12751	*Spinacia oleracea*	USA	–	Schubert et al. 2007	EF679402, EF679480, EF679556
		CPC 12753	*Spinacia oleracea*	USA	–	Schubert et al. 2007	EF679403, EF679481, EF679557

[1] ATCC: American Type Culture Collection, Virginia, USA; CAMS: SERA's Centre for Applied Mycological Studies, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; EXF: Extremophilic Fungi Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; MRC: Culture collection of PROMEC, Medical Research Council, Cape Town, South Africa; MZKI: Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia; NRRC: Agricultural Research Culture Collection, Peoria, Illinois, USA.

[2] ACT: partial actin gene; EF: partial elongation factor 1-α gene; ITS: internal transcribed spacer region.

Prague (PRM 155424, holotype!); on bark, Bohemia, castle Kačina at Nové Dovory, near Kutná Hora, 1856, *J. Peyl* (PRM 657824); Germany, Saxony, 'ad lignam putrida agri Dresdensis', Rabenhorst, Fungi Eur. 1271 (PRM 657821); ex herb. 'Verein der Naturfreunde in Reichenberg' (PRM 657823). These specimens were compared to the type material of *C. herbarum* (CBS-H 19853), with which *C. lignicola* has been considered synonymous in the past (Hughes 1958). Furthermore, they were also compared to materials of the U.S. patent strain NRRL 8131 = ATCC 38493 = CBS 117728 = CPC 12098, which had previously been identified as *C. lignicola*.

DNA isolation and phylogenetic analysis

Fungal colonies were established on agar plates, and genomic DNA was isolated as described in Gams et al. (2007). Partial gene sequences were determined as described by Crous et al. (2006) for actin (ACT), translation elongation factor 1-α (EF), and part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer, the 5.8S rRNA gene, the second internal transcribed spacer and the 5' end of the 28S rRNA gene (ITS). The nucleotide sequences were generated using both forward and reverse PCR primers to ensure good quality sequences over the entire length of the amplicon. Sequence data obtained from Schubert et al. (2007b) and Zalar et al. (2007) were used as reference data for the alignments (Table 1). Subsequent sequence alignment and phylogenetic analysis followed the methods of Crous et al. (2006). 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 the alignment and tree in TreeBASE (www.treebase.org).

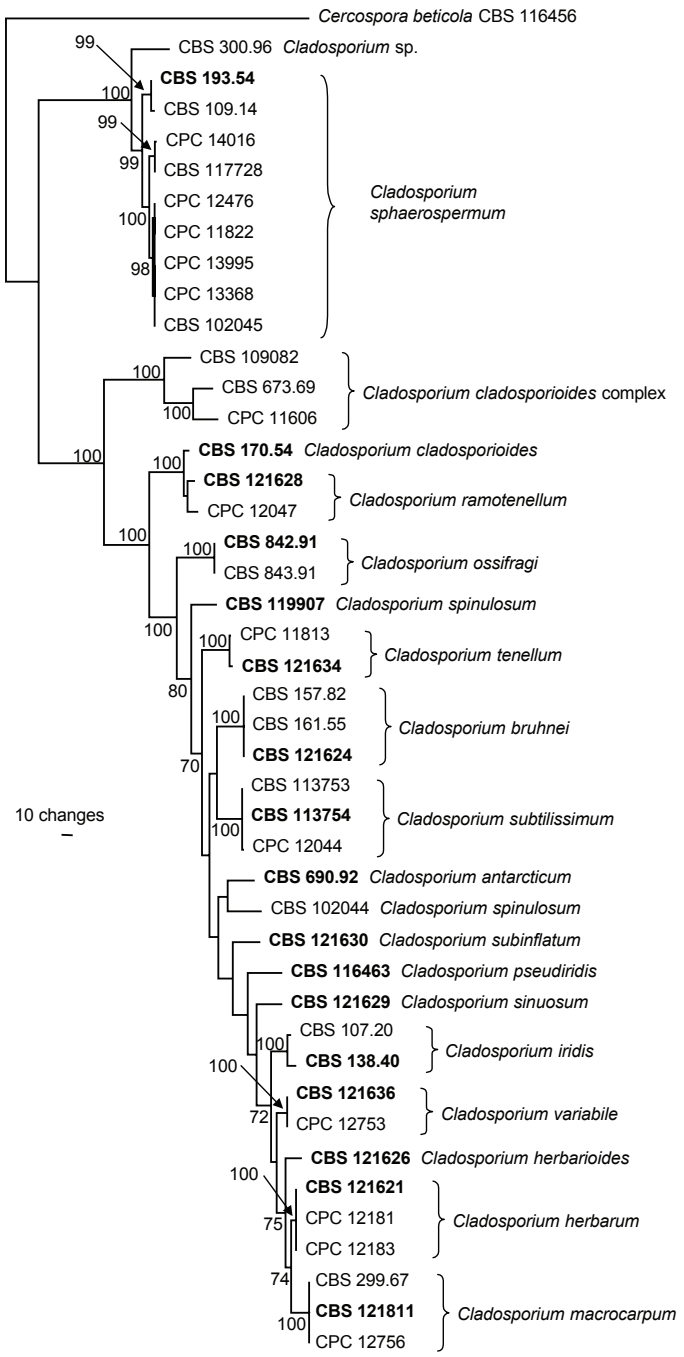
Morphology

Strain NRRL 8131 (= CBS 117728) was grown on 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), and 2 % malt extract agar (MEA) (Gams et al. 2007), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation or in the dark for assessing colony characters (Schubert et al. 2007b, Zalar et al. 2007), and suspensions of conidia were preserved in glycerol for long term storage at -80 °C and in liquid nitrogen. Subcultures on MEA plates were used for scanning electron microscopy, and SNA and MEA slide cultures or plates for light microscopy. Microscopic observations were made from colonies cultivated for 7 d under continuous near-ultraviolet light at 25 °C on SNA and MEA. Preparations were mounted in Shear's solution (Gams et al. 2007) or 85 % lactic acid. To study conidial development and branching patterns, squares of transparent adhesive tape (Titan Ultra Clear Tape, Conglom Inc., Toronto, Canada) were placed on conidiophores growing in the zone between the colony margin and 2 cm inwards, and mounted between two drops of Shear's solution under a glass cover slip. Conidial terminology follows that of Schubert et al. (2007b). Colonies were cultivated on PDA, SNA and MEA for 14 d at 25 °C in the dark, after which the surface and reverse colours were rated using the charts of Rayner (1970). Linear growth was determined on MEA and SNA plates by inoculating three plates of each medium and incubating them for 14 d at 25 °C.

Table 2 Statistical parameters describing the sequence alignments of three different loci and the combined alignment.

Parameter	ITS[1]	ACT[1]	EF[1]	Combined
Number of alignment positions	495	219	380	1094
Number of parsimony informative characters	37	99	177	313
Number of variable and parsimony-uninformative characters	100	30	54	184
Number of constant characters	358	90	149	597

[1] ACT: partial actin gene; EF: partial elongation factor 1-α gene; ITS: internal transcribed spacer region.



SEM was conducted by mounting small (2–3 mm^3) sections of MEA agar with fungal growth onto SEM stubs, subjecting the mounts to osmium tetroxide vapours for 2 h, and gold-coating the specimens with a Technics Hummer V sputter coater. Specimens were observed and photographed with a Hitachi S-570 scanning electron microscope.

RESULTS

DNA phylogeny

The manually adjusted concatenated alignment contained 44 sequences (including the outgroup sequence) and the three loci were represented by a total of 1094 characters including alignment gaps which were used in the analysis (Table 2). The result of the partition homogeneity test (P = 0.685) indicated that the loci were congruent and the sequence data could therefore be analysed as a concatenated alignment. A single most parsimonious tree (TL = 1253 steps; CI = 0.663; RI = 0.871; RC = 0.578), which is shown in Fig. 1, was obtained from the parsimony analysis of the combined genes. Phylogenetic analyses using the concatenated alignment as well as the individual loci (data not shown) all conclusively demonstrated that CBS 117728 clustered with *C. sphaerospermum* in accordance with combined ITS, ACT and EF sequences, and that it was the sister taxon of MRC 10263 (= CPC 14016) (Fig. 1). Neighbour-joining analysis using three substitution models (uncorrected 'p', Kimura 2-parameter and HKY85) on the sequence data yielded trees with identical topologies (data not shown).

Taxonomy

In PRM 657823, only a trimmatostroma-like hyphomycete was found. The three other specimens, including the type material (PRM 155424 holotype, PRM 657824, and PRM 657821) exclusively contained *C. herbarum*, well-characterised by having nodulose conidiophores and verrucose conidia (Schubert et al. 2007b). The type of *C. lignicola* was also examined by Hughes (1958), who reduced it to synonymy with *C. herbarum*, a treatment that was confirmed during the course of the present study. The examination of NRRL 8131 '*C. lignicola*' clearly showed that this fungus is quite distinct from

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Fig. 1 The single most parsimonious tree obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment (ITS, ACT, and EF). The scale bar shows ten changes, and bootstrap support values from 1 000 replicates are shown at the nodes. The tree was rooted to sequences of *Cercospora beticola* strain CBS 116456 (GenBank accession numbers AY840527, AY840458, AY840494, respectively).

the true *C. lignicola* (= *C. herbarum*) by having non-nodulose conidiophores and almost smooth to verruculose, often short rostrate conidia (Fig. 2, 3). The general habit of this fungus is obviously cladosporioid, including conspicuous, somewhat protuberant conidiogenous loci. The periclinal rim is evident, but the central dome is rather low and not very conspicuous when viewed by light microscopy (Fig. 2). SEM studies confirmed that scar structure conforms to the coronate *Cladosporium* type (Fig. 3).

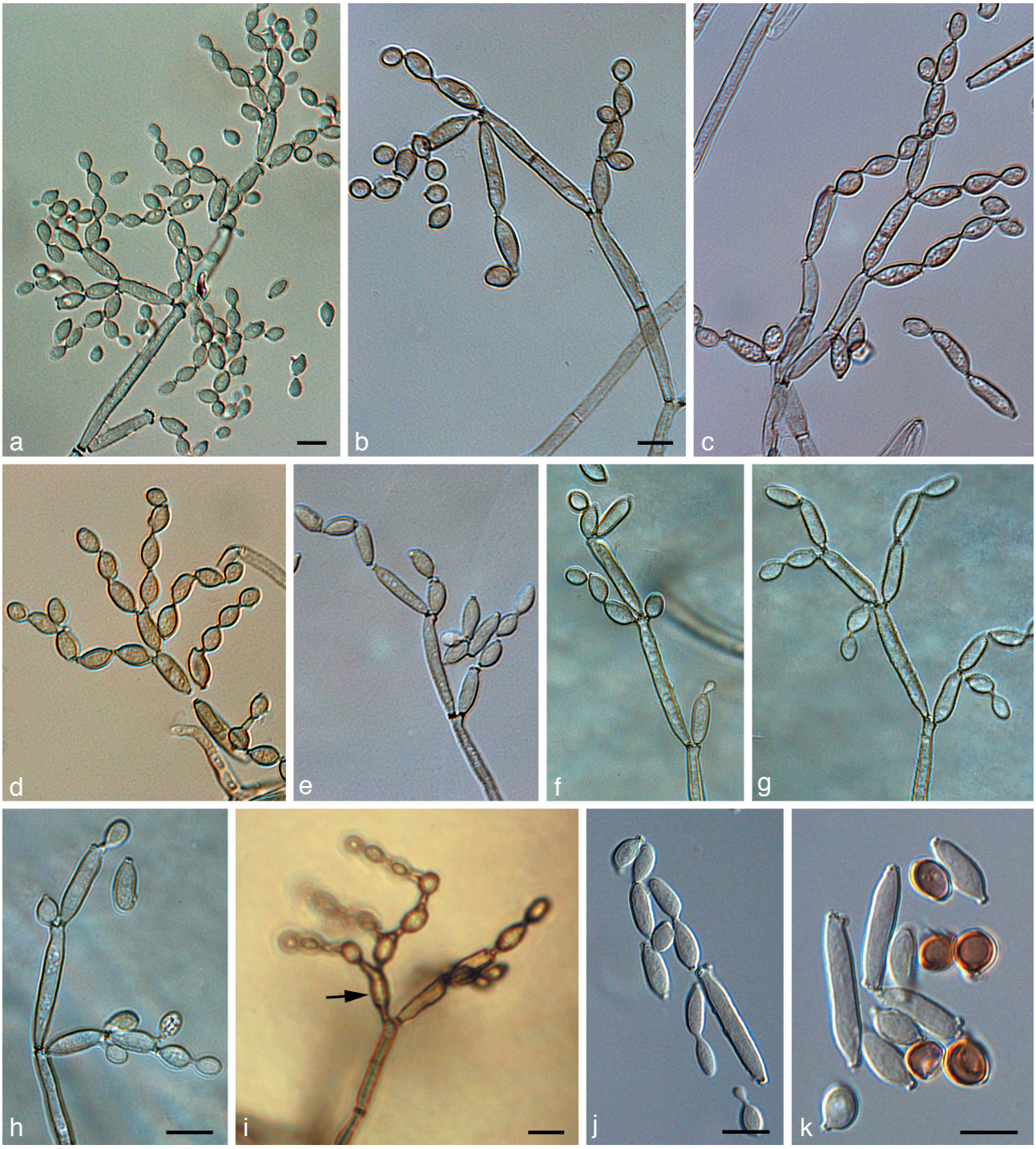

Fig. 2 Light micrographs of *Cladosporium sphaerospermum* NRRL 8131. a–h. Conidiophores at various stages of development, showing their characteristic branching patterns, ramoconidia, secondary ramoconidia, intercalary conidia, and small, terminal conidia (all on SNA); i. conidiophore with alternarioid secondary ramoconium (arrow), formed on MEA; j, k. secondary ramoconidia and intercalary conidia (note older intercalary conidia, which become dark brown and globose). — Scale bars = 10 µm.

Description — On MEA or SNA, *hyphae* 1–5 µm wide, sparingly to richly branched (angles between 45 and 90°), sometimes anastomosing, loosely to densely septate, thin-walled, almost smooth to distinctly rough-walled, subhyaline in narrow hyphae to medium dark olivaceous-brown, subcylindrical to irregular in outline by swellings and constrictions at septa. *Conidiophores* little differentiated, micronematous, barely discernable and distinguishable from ordinary hyphae, becoming macronematous on SNA after 14 d (15–)31–125(–250) × (2.5–)3–4.5(–5.5) µm, predominantly unbranched. *Conidiogenous cells* integrated, terminal and intercalary, 10–25 × (2.5–)3–4(–5) µm, with a single or up to three conidiogenous loci, 1–1.5 µm diam, coronate, but central dome low and not very conspicuous when viewed by light microscopy. *Conidia* catenate, in simple or usually branched chains, subglobose, ellipsoid-ovoid, obovoid, broadly fusiform, limoniform, straight to somewhat curved; terminal conidia with a single basal hilum and intercalary conidia with two hila on MEA (3–)4–10(–13) × (2–)3–4(–5) µm, 0(–1)-septate, length becoming shorter towards the apex; on SNA (2.5–)3.5–8(–10.5) × 2.5–4.5(–5) µm (mean 5.9 (std dev 1.4) × 3.4 (std dev 0.4), n = 50), length/width 1.1–3.8 (mean = 1.8); *secondary ramoconidia* on MEA with 3–5 hila, (6–)10–20(–25) × 3–5 µm; on SNA with 2–4 hila, 8.5–20 × 2.5–4.5 µm (mean 12.5 (std dev 2.7) × 2.9 (std dev 0.4), n = 25), 0–1-septate, sometimes alternarioid, obclavate, subrostrate (the alternarioid ones seldom observed when cultivated on SNA after 7 d, but readily observed on PDA and MEA); *ramoconidia* ('true ramoconidia' sensu Crous et al. 2007b) on SNA (11–)13–34(–43) × (2.5–)3–4 µm; *ramoconidia* and *small terminal conidia* in general subhyaline to pale olivaceous or olivaceous-brown, thin-walled, almost smooth to distinctly verruculose, hila conspicuous, 0.75–1.25 µm diam, coronate, often at the end of protuberant, short, terminal projections, 1–2 µm long or even longer in secondary ramoconidia with beak-like ends.

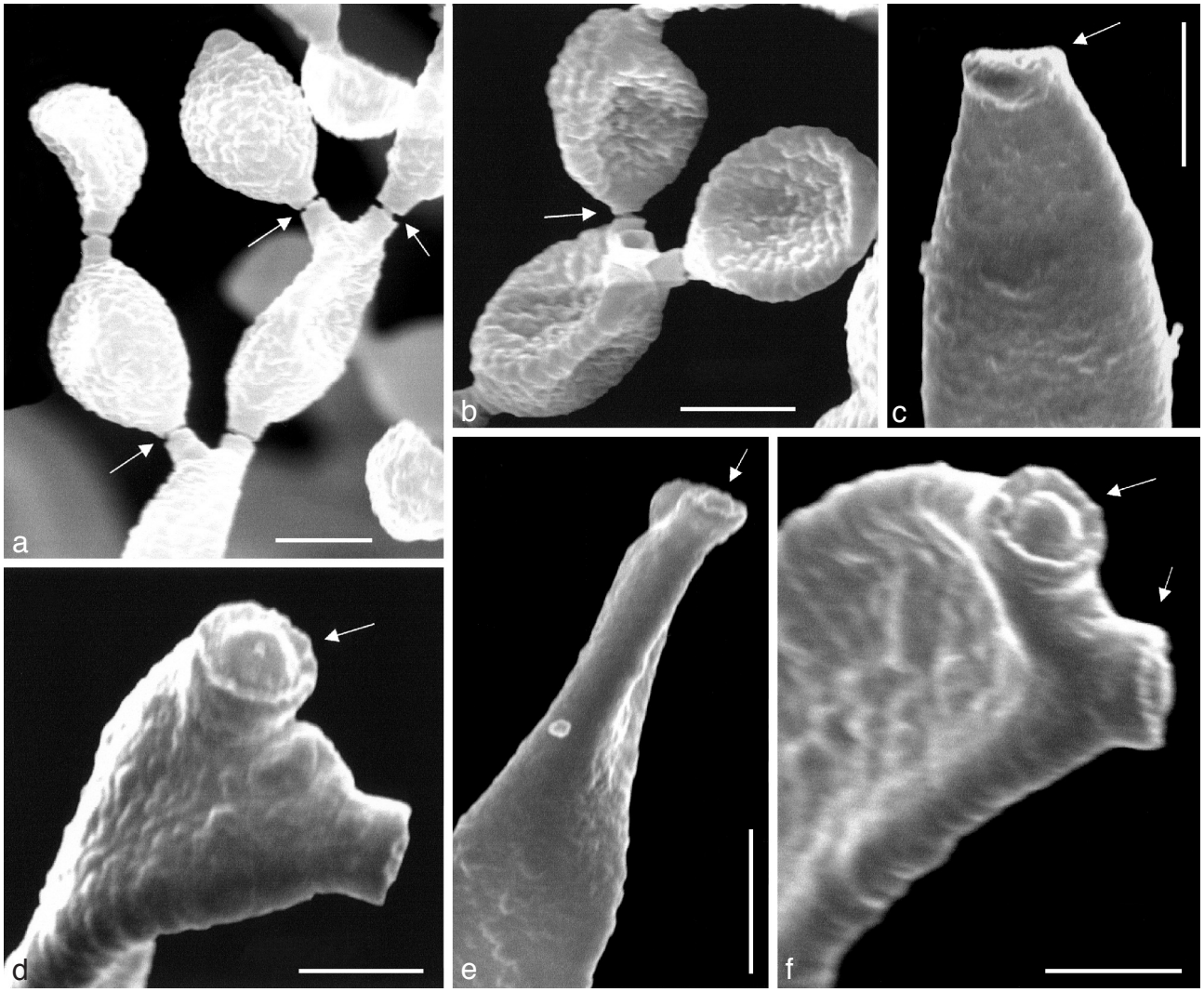

Fig. 3 Scanning electron micrographs of *Cladosporium sphaerospermum* NRRL 8131. a, b. Branching chains of conidia, showing conidiogenous loci with disjunctors (arrows); c. apex of conidiophore with conidiogenous scar in profile (arrow); d. two conidiogenous loci at apex of a secondary ramoconidium, the upper (arrow) clearly coronate; e. two conidiogenous loci at apex of a conidiophore, the one facing the viewer is clearly coronate (arrow); f. two conidiogenous loci (arrows) at apex of a secondary ramoconidium are coronate. — Scale bars: a–c = 2.5 µm, d = 1 µm, e = 5 µm, f = 1.25 µm.

Cultural characteristics — On MEA, colonies attaining 45 mm diam at 25 °C after 14 d; dark olivaceous, powdery, velvety, reverse dark olivaceous-grey. Colonies on PDA attaining 50 mm diam at 25 °C after 14 d, olivaceous-grey in centre, iron-grey in outer region, reverse iron-grey. On SNA, colonies attaining 30 mm diam at 25 °C after 14 d, semi-translucent and olivaceous-grey to iron-grey, with wide translucent margin.

Specimen examined. USA (no additional data known), isolated from wood, CBS-H 20086 (HAL 1846 F), dried culture ex ATCC 38493, cultures ATCC 38493 = CBS 117728 = CPC 12098 = NRRL 8131.

Notes — NRRL 8131 differs from previously known isolates of *C. sphaerospermum* in having mostly unbranched, micronematous conidiophores, only becoming macronematous with age, and frequently subrostrate, occasionally 'alternarioid' conidia. Also, conidia on MEA and SNA exceed in size conidia of other isolates. Conidial length/width (mean 1.8, n = 50) on SNA exceeds that from the standard description (range = 1.1–1.5, Zalar et al. 2007), with 32 % of conidia of NRRL 8131 falling within the range from Zalar et al. (2007).

DISCUSSION

Re-examination of type material of *C. lignicola* and a putatively North American strain NRRL 8131, originally referred to the latter species, demonstrated clearly that the two fungi are distinct. The type of *C. lignicola* was formerly examined by Hughes (1958), who reduced it to synonymy with *C. herbarum*, a treatment confirmed during the course of the present investigations.

The North American strain (NRRL 8131) is *C. sphaerospermum*, but differs in morphology from previously known isolates of that species. It is easily distinguishable from *C. herbarum* (including *C. lignicola*) and all other know species of *Cladosporium* s.str., by having obclavate, short rostrate, sometimes 'alternarioid' conidia. Individual conidia often conformed to the spherical shape generally typical of isolates of *C. sphaerospermum*, but such conidia of NRRL 8131 could be somewhat larger than the upper limits of 4(–7) × 3.5(–4.5) µm given for *C. sphaerospermum* in Zalar et al. (2007). Furthermore, the conidiophores are at first consistently micronematous, much later they may become more macronematous, and they are usually unbranched. The conidiophores in other isolates of *C. sphaerospermum* are often branched in vivo as well as in vitro. However, not only did NRRL 8131 cluster with strains of *C. sphaerospermum* (Fig. 1), but the neotype of *C. sphaerospermum* (CBS 193.54) occasionally displayed subrostrate 'beaks' on ramoconidia (e.g., fig. 5G in Zalar et al. 2007). Because our sequence data conclusively place NRRL 8131 into *C. sphaerospermum* (Fig. 1) and because the subrostrate 'beaks' could also be located in the neotype, we have refrained from designating the morphologically distinct NRRL 8131 as a new variety.

In their treatment of *C. sphaerospermum*-like species (Zalar et al. 2007) some variation was observed in the ITS sequence data of all members studied, suggesting that they may not present a single monophyletic group, but could belong to a species complex within *Cladosporium*. *Cladosporium sphaerospermum* was described by Penzig (1882) from decaying *Citrus* leaves and branches in Italy. Isolate CBS 193.54, originating from a human nail, was accepted as neotype (de Vries 1952, Zalar et al. 2007). During the course of this study, attempts to generate the EF sequence data of isolate CBS 193.54 (ex-type of *C. sphaerospermum*) failed due to the presence of numerous double-peaks in the raw sequence, indicative of contaminated DNA or a possible hybrid. However, sequencing this gene for isolates cultured from single spores derived from isolate CBS 193.54 also generated the same results, and therefore led us to discard the idea of contaminated DNA. It was only possible to obtain the correct sequence (when compared to the EF sequences of other *C. sphaerospermum* isolates) upon cloning the obtained EF amplicon and sequencing the clones. The correct sequence was one of three sequence types obtained from the clones; blastn results of these three sequences all had distant affinity with *Cladosporium* EF sequences in GenBank. The origin of the problematic EF sequences is difficult to explain. As part of a larger project we have generated more than 400 EF sequences for *Cladosporium* spp., none of which gave a similar problem. Whether isolate CBS 193.54 contains pseudogene copies of the translation elongation factor 1-α gene, or whether it is a hybrid, remains to be investigated. Numerous strains with similar ITS rDNA sequences have in subsequent years been isolated from hypersaline water or organic substrata including plants and bathroom walls. Zalar et al. (2007) reported that strains of *C. sphaerospermum* could grow under in vitro conditions at a water activity of up to 0.860 (Hocking et al. 1994), or even 0.815 (Aihara et al. 2002). Therefore, Zalar et al. (2007) considered *C. sphaerospermum* as halo- or osmotolerant. Although *C. sphaerospermum* has commonly been isolated from osmotically stressed environments, it is also known from non-stressed niches, though the exact niche of NRRL 8131 (presumably from wood), remains unknown. *Cladosporium sphaerospermum* is a cosmopolitan species that has been studied from the perspectives of phylogeny, halo-tolerance and general ecology (summarised in Zalar et al. 2007), biodegradative capacities (e.g., Weber et al. 1995, Prenafeta-Boldú et al. 2001, Potin et al. 2004, Nieves-Rivera et al. 2006, Kim et al. 2007), and clinical aspects (summarised in de Hoog et al. 2000 and Zalar et al. 2007).

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REFERENCES

Aihara M, Tanaka T, Ohta T, Takatori K. 2002. Effect of temperature and water activity on the growth of Cladosporium sphaerospermum and Cladosporium cladosporioides. Biocontrol Science 7: 193–196.

Braun U, Crous PW, Dugan FM, Groenewald JZ, Hoog GS de. 2003. Phylogeny and taxonomy of cladosporium-like hyphomycetes, including Davidiella gen. nov., the teleomorph of Cladosporium s.str. Mycological Progress 2: 3–18.

Brown KB, Hyde KD, Guest DI. 1998. Preliminary studies on endophytic fungal communities of Musa acuminata species complex in Hong Kong and Australia. Fungal Diversity 1: 27–51.

Crous PW, Braun U, Groenewald JZ. 2007a. Mycosphaerella is polyphyletic. Studies in Mycology 58: 1–32.

Crous PW, Braun U, Schubert K, Groenewald JZ. 2007b. Delimiting Cladosporium from morphologically similar genera. Studies in Mycology 58: 33–56.

Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ. 2006. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology 55: 235–253.

David JC. 1997. A contribution to the systematics of Cladosporium. Revision of the fungi previously referred to Heterosporium. Mycological Papers 172: 1–157.

Dugan FM, Schubert K, Braun U. 2004. Check-list of Cladosporium names. Schlechtendalia 11: 1–103.

El-Morsy EM. 2000. Fungi isolated from the endorhizosphere of halophytic plants from the Red Sea Coast of Egypt. Fungal Diversity 5: 43–54.

Gams W, Verkley GJM, Crous PW. 2007. CBS Course in Mycology, 5th ed. CBS, Utrecht, Netherlands.

Ho MH-M, Castañeda RF, Dugan FM, Jong SC. 1999. Cladosporium and Cladophialophora in culture: descriptions and an expanded key. Mycotaxon 72: 115–157.

Hocking AD, Miscamble BF, Pitt J. 1994. Water relations of Alternaria alternata, Cladosporium cladosporioides, Cladosporium sphaerospermum, Curvularia lunata and Curvularia pallescens. Mycological Research 98: 91–94.

Hoog GS de, Guarro J, Gené J, Figueras MJ. 2000. Atlas of Clinical Fungi, 2nd ed. CBS, Utrecht, Netherlands, and Universitat Rovira i Virgili, Reus, Spain.

Hughes SJ. 1958. Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis. Canadian Journal of Botany 36: 727–836.

Kim JJ, Kang S-M, Choi Y-S, Kim G-H. 2007. Microfungi potentially disfiguring CCA-treated wood. International Biodeterioration & Biodegradation 60: 197–201.

Nieves-Rivera ÁM, Rodríguez NJ, Dugan FM, Zaidi BR, Williams Jr EH. 2006. Characterization of Cladosporium oxysporum and C. sphaerospermum using polyaromatic hydrocarbons (PAHs) as their sole carbon source in tropical coastal seawater. In: Mendez-Vilas A (ed.), Modern multidisciplinary applied microbiology: Exploiting microbes and their interactions: 483–487. Wiley-VCH, Weinheim, Germany.

Penzig AJO. 1882. Fungi Agromiculi. Michelia 2: 385–503.

Potin O, Veignie E, Rafin C. 2004. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by Cladosporium sphaerospermum isolated from an aged PAH contaminated soil. FEMS Microbiology Ecology 51: 71–78.

Prenafeta-Boldú FX, Kuhn A, Luykx DMAM, Anke H, Groenestijn JW van, Bont JAM de. 2001. Isolation and characterization of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycological Research 4: 477–484.

Rayner RW. 1970. A Mycological Colour Chart. Commonwealth Mycological Institute & British Mycological Society, Kew, Surrey, England.

Riesen T, Sieber T. 1985. Endophytic fungi in winter wheat (Triticum aestivum L.). Swiss Federal Institute of Technology, Zürich.

Schoch C, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98: 1041–1052.

Schubert K, Braun U, Groenewald JZ, Crous PW. 2007a. Cladosporium leaf-blotch and stem rot of Paeonia spp. caused by Dichocladosporium chlorocephalum gen. nov. Studies in Mycology 58: 95–104.

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink MS, Hill CF, Zalar P, Hoog GS de, Crous PW. 2007b. Biodiversity in the Cladosporium herbarum complex (Davidiellaceae, Capnodiales), with standardisation of methods for Cladosporium taxonomy and diagnostics. Studies in Mycology 58: 105–156.

Simmons EG. 2007. Alternaria: An identification manual. CBS Biodiversity Centre, Utrecht, Netherlands.

Vries GA de. 1952. Contribution to the knowledge of the genus Cladosporium Link ex Fr. Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

Weber FJ, Hage KC, Bont JA de. 1995.Growth of the fungus Cladosporium sphaerospermum with toluene as the sole carbon and energy source. Applied and Environmental Microbiology 61:3562–3566.

Zalar P, Hoog GS de, Schroers H-J, Crous PW, Groenewald JZ, Gunde-Cimerman N. 2007. Phylogeny and ecology of the ubiquitous saprobe Cladosporium sphaerospermum, and descriptions of seven new species from hypersaline environments. Studies in Mycology 58: 157–183.