

# Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, *Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics

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**Abstract:** The *Cladosporium herbarum* complex comprises five species for which *Davidiella* teleomorphs are known. *Cladosporium herbarum* s. str. (*D. tassiana*), *C. macrocarpum* (*D. macrocarpa*) and *C. bruhnei* (*D. alicina*) are distinguishable by having conidia of different width, and by teleomorph characters. *Davidiella variabile* is introduced as teleomorph of *C. variabile*, a homothallic species occurring on *Spinacia*, and *D. macrospora* is known to be the teleomorph of *C. iridis* on *Iris* spp. The *C. herbarum* complex combines low molecular distance with a high degree of clonal or inbreeding diversity. Entities differ from each other by multilocus sequence data and by phenetic differences, and thus can be interpreted to represent individual taxa. Isolates of the *C. herbarum* complex that were formerly associated with opportunistic human infections, cluster with *C. bruhnei*. Several species are newly described from hypersaline water, namely *C. ramotenellum*, *C. tenellum*, *C. subinflatum*, and *C. herbaroides*. *Cladosporium pseudiridis* collected from *Iris* sp. in New Zealand, is also a member of this species complex and shown to be distinct from *C. iridis* that occurs on this host elsewhere in the world. A further new species from New Zealand is *C. sinuosum* on *Fuchsia excorticata*. *Cladosporium antarcticum* is newly described from a lichen, *Caloplaca regalis*, collected in Antarctica, and *C. subtilissimum* from grape berries in the U.S.A., while the new combination *C. ossifragi*, the oldest valid name of the *Cladosporium* known from *Narthecium* in Europe, is proposed. Standard protocols and media are herewith proposed to facilitate future morphological examination of *Cladosporium* spp. in culture, and neotypes or epitypes are proposed for all species treated.

**Taxonomic novelties:** *Cladosporium antarcticum* K. Schub., Crous & U. Braun, sp. nov., *C. herbaroides* K. Schub., Zalar, Crous & U. Braun, sp. nov., *C. ossifragi* (Rostr.) U. Braun & K. Schub., comb. nov., *C. pseudiridis* K. Schub., C.F. Hill, Crous & U. Braun, sp. nov., *C. ramotenellum* K. Schub., Zalar, Crous & U. Braun, sp. nov., *C. sinuosum* K. Schub., C.F. Hill, Crous & U. Braun, sp. nov., *C. subinflatum* K. Schub., Zalar, Crous & U. Braun, sp. nov., *C. subtilissimum* K. Schub., Dugan, Crous & U. Braun, sp. nov., *C. tenellum* K. Schub., Zalar, Crous & U. Braun sp. nov., *Davidiella macrocarpa* Crous, K. Schub. & U. Braun, sp. nov., *D. variabile* Crous, K. Schub. & U. Braun, sp. nov.

**Key words:** Clonality, *Davidiella*, homothallism, new species, phylogeny, recombination, taxonomy.

## INTRODUCTION

*Cladosporium herbarum* (Pers.: Fr.) Link, type species of the genus *Cladosporium* Link, is one of the most common environmental fungi to be isolated worldwide. It abundantly occurs on fading or dead leaves of herbaceous and woody plants, as secondary invader on necrotic leaf spots, and has frequently been isolated from air (Samson *et al.* 2000), soil (Domsch *et al.* 1980), foodstuffs, paints, textiles, humans (de Hoog *et al.* 2000) and numerous other substrates. It is also known to occur on old carpophores of mushrooms and other fungi (Heuchert *et al.* 2005) and to be a common endophyte (Riesen & Sieber 1985, Brown *et al.* 1998, El-Morsy 2000), especially in temperate regions. Under favourable climatic conditions *C. herbarum* also germinates and grows as an epiphyte on the surface of green, healthy leaves (Schubert 2005).

Persoon (1794) introduced *C. herbarum* as *Dematium herbarum* Pers., which was later reclassified by Link (1809) as *Acladium herbarum* (Pers.) Link. In 1816, Link included *C. herbarum* together with three additional species in his newly described genus *Cladosporium*. Clements & Shear (1931) proposed *C. herbarum* as lectotype species of the latter genus, a decision followed by de Vries (1952) and Hughes (1958). Several authors provided detailed treatments of *C. herbarum* (de Vries 1952, Ellis 1971, Domsch *et al.* 1980, Prasil & de Hoog 1988), and there are literally thousands of records of this species in the literature. McKemy & Morgan-Jones (1991) and Ho *et al.* (1999) examined *C. herbarum* in culture and published detailed descriptions of its features *in vitro*.

*Cladosporium macrocarpum* Preuss, a second component within the herbarum complex, has hitherto been known and treated as an allied, but morphologically distinct species on the basis of its

wider and somewhat larger, frequently 2–3-septate, more regularly verrucose conidia, shorter conidial chains and more pronounced prolongations of the conidiophores. Dugan & Roberts (1994) carried out examinations of morphological and reproductive aspects of both species, and in so doing demonstrated a morphological continuum between *C. macrocarpum* and *C. herbarum*, concluding that the name *herbarum* should have preference. Therefore, Ho *et al.* (1999) introduced the new combination *C. herbarum* var. *macrocarpum* (Preuss) M.H.M. Ho & Dugan. Although transitional forms have been discussed to occur between the two species, several authors still prefer to retain *C. macrocarpum* as a separate species.

In an attempt to elucidate the species within the *C. herbarum* complex, therefore, a multilocus DNA sequence typing approach was used, employing five genes, namely the internal transcribed spacers of the rDNA genes (ITS), actin, calmodulin, translation elongation factor 1- $\alpha$ , and histone H3. These data were supplemented with morphological examinations under standardised conditions, using light and scanning electron microscopy, as well as cultural characteristics and growth studies.

## MATERIAL AND METHODS

### Isolates

Isolates included in this study were obtained from the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands, or were freshly isolated from a range of different substrates. Single-conidial and ascospore isolates were obtained using the techniques as explained in Crous (1998) for species of *Mycosphaerella* Johanson and its anamorphs. Isolates

were inoculated onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), 2 % malt extract agar (MEA) and oatmeal agar (OA) (Gams *et al.* 2007), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. All cultures obtained in this study are maintained in the culture collection of the CBS (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank ([www.MycoBank.org](http://www.MycoBank.org)).

### DNA isolation, amplification and sequence 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), calmodulin (CAL), translation elongation factor 1- $\alpha$  (EF), histone H3 (HIS) and part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer, the 5.8S rRNA gene, the second internal transcribed spacer and the 5' end of the 28S rRNA gene (LSU). The nucleotide sequences were generated using both PCR primers to ensure good quality sequences over the entire length of the amplicon. 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](http://www.treebase.org)).

### Data analysis

The number of entities in the dataset of 79 strains was inferred with STRUCTURE v. 2.2 software (Pritchard *et al.* 2000, Falush *et al.* 2003) using an UPGMA tree of data of the ACT gene compared with CAL, EF and HIS with the exclusion of the nearly invariant ITS region. For this analysis group indications were derived from a tree produced with MrAIC (Nylander 2004). The length of the burn-in period was set to 1 000 000, number of MCMC repeats after burn-in 10 000, with admixture ancestry and allele frequencies correlated models, assuming that all groups diverged from a recent ancestral population and that allele frequencies are due to drift. Uniform prior for ALPHA was set to 1.0 (default) and allele frequencies with  $\lambda$  set to 1.0 (default). The numbers of MCMC repetitions after burn-in were set as 10 000 and 100 000. The number of clusters (K) in STRUCTURE was assumed from 5 to 7. Population differentiation  $F_{ST}$  (index:  $\theta$ ) was calculated with 1–6 runs using the same software. The null hypothesis for this analysis is no population differentiation. When observed theta ( $\theta$ ) is significantly different from those of random data sets ( $p < 0.05$ ), population differentiation is considered.

Association of multilocus genotypes was screened with the multilocus option in BioNUMERICS v. 4.5. To test for reproductive mode in each population, the standardised index of association ( $I^S_A$ ; Haubold *et al.* 1998) was calculated with START2 software (Jolley *et al.* 2001). The null hypothesis for this analysis is complete panmixia. The values of  $I^S_A$  were compared between observed and randomised datasets. The hypothesis would be rejected when  $p < 0.05$ . Mean genetic diversity (H) and diversities of individual loci were calculated with LIAN v. 3.5 (Haubold & Hudson 2000). Degrees of recombination or horizontal gene transfer were also visualised using SPLITSTREE v. 4.8 software (Huson & Bryant 2006). Split decomposition was carried out with default settings, i.e., character transformation using uncorrected (observed, "P") distances, splits transformation using "equal angle", and optimise boxes iteration set to 2.

### Morphology

As the present study represents the first in a series dealing with *Cladosporium* spp. and their *Davidiella* Crous & U. Braun teleomorphs in culture, a specific, standardised protocol was established by which all species complexes will be treated in future.

**Morphology of the anamorph:** Microscopic observations were made from colonies cultivated for 7 d under continuous near-ultraviolet light at 25 °C on SNA. Preparations were mounted in Shear's solution (Gams *et al.* 2007). 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 coverslip. Different types of conidia are formed by *Cladosporium* species for which different terms need to be adopted. **Ramoconidia** are conidia with usually more than one (mostly 2 or 3) conidial hilum, which typically accumulate at the tip of these conidia. Conidiogenous cells with more than one conidiogenous locus are first formed as apical parts of conidiophores. Such apical

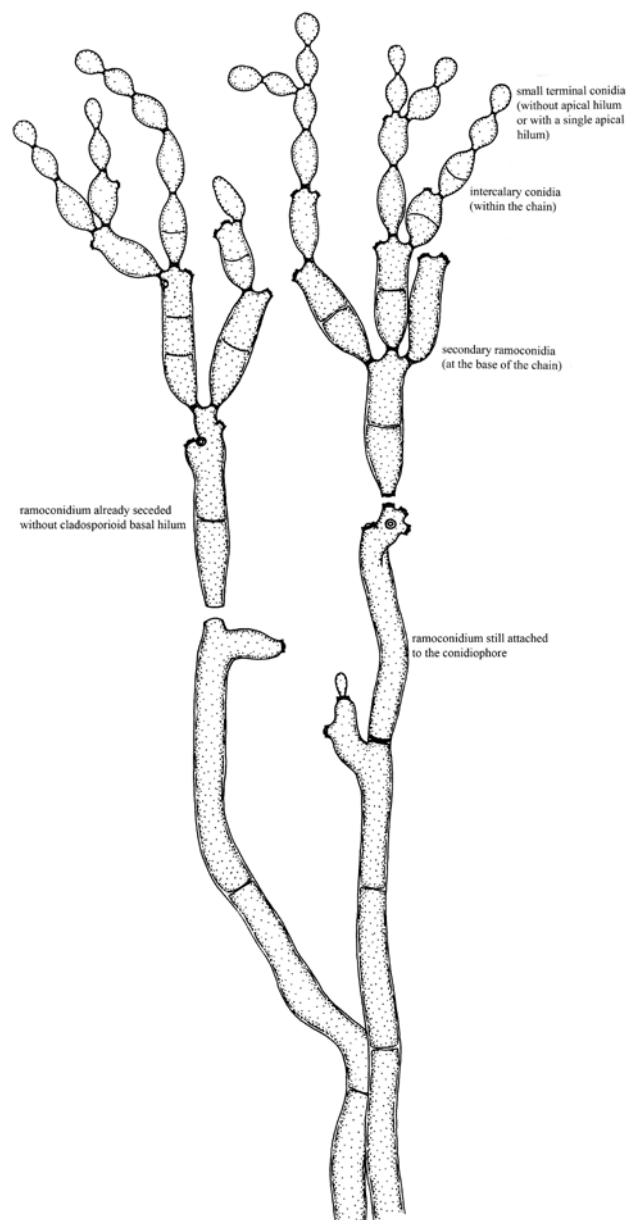


Fig. 1. *Cladosporium* conidiophore with ramoconidia, secondary ramoconidia, intercalary conidia, and small, terminal conidia. Scale bar = 10  $\mu$ m. K. Schubert del.

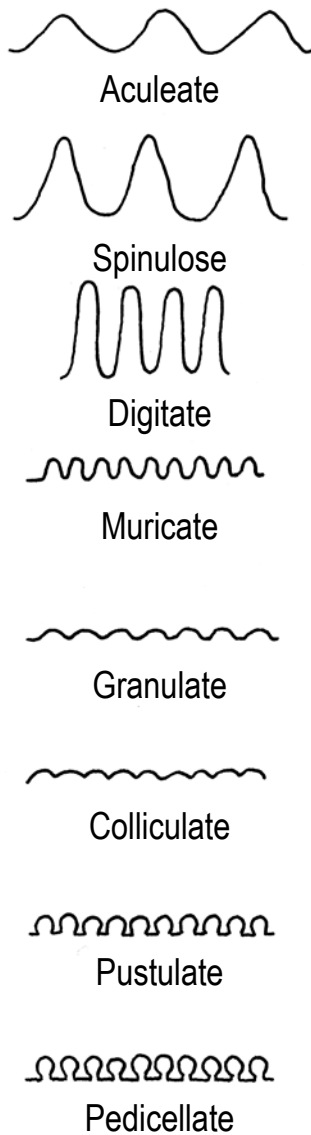


Fig. 2. Terms used to describe conidium wall ornamentation under the cryo-electron microscope. Adapted from David (1997).

parts of conidiophores are called **ramoconidia** if they secede at a septum from the conidiophore (Kirk *et al.* 2001). The septum at which the ramoconidium secedes often appears to be somewhat refractive or darkened. Ramoconidia are characterised by having a truncate, undifferentiated base (thus they lack a differentiated, coronate basal hilum formed in the context of conidiogenesis) and they can be very long, aseptate to sometimes multi-septate. Although they were formed initially as part of the conidiophore, they function as propagules. Only few of the species known until now have the ability to form true ramoconidia. **Secondary ramoconidia** also have more than one distal conidial hilum but they always derive from a conidiogenous locus of an earlier formed cell, which can be either a conidiogenous cell or a ramoconidium. Secondary ramoconidia are often shorter but somewhat wider than ramoconidia; they are often septate, and typically have a narrowed base with a coronate hilum (Fig. 1). Conidia in *Cladosporium* are cells with a coronate basal hilum, which is formed in the context of conidiogenesis and with either a single (when formed as intercalary units in unbranched parts of chains) or without any distal conidial hilum (when formed at the tip of conidial chains). For the first, the term “**intercalary conidium**” and for the latter, “**small terminal conidium**” is used. Intercalary conidia typically are larger and more pigmented and have a more differentiated surface ornamentation

than the small terminal conidia. In older literature true ramoconidia were often cited as “ramoconidia s. str.”, whereas secondary ramoconidia have been referred to as “ramoconidia s. lat.”

**Morphology of the teleomorph:** Teleomorphs were induced by inoculating plates of 2 % tap water agar onto which autoclaved stem pieces of *Urtica dioica* (European stinging nettle) were placed. Inoculated plates were incubated on the laboratory bench for 7 d, after that period they were further incubated at 10 °C in the dark for 1–2 mo to stimulate teleomorph development. Wherever possible, 30 measurements ( $\times 1\,000$  magnification) were made of conidia and ascospores, with the extremes of spore measurements given in parentheses. Cultural characteristics: Colonies were cultivated on PDA, MEA and OA plates 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, PDA and OA plates by inoculating three plates per isolate for each medium, and incubating them for 14 d at 25 °C, after that period colony diameters were determined.

### Low-temperature scanning electron microscopy

Isolates of *Cladosporium* spp. were grown on SNA with 30 g agar/L for 3–4 d at room temperature under black light. Relevant parts of the small colonies with conidiophores and conidia were selected under a binocular, excised with a surgical blade as small agar (3  $\times$  3 mm) blocks, and transferred to a copper cup for snap-freezing in nitrogen slush. Agar blocks were glued to the copper surface with frozen tissue medium (KP-Cryoblock, Klinipath, Duiven, Netherlands) mixed with 1 part colloidal graphite (Agar Scientific, Stansted, U.K.). Samples were examined in a JEOL 5600LV scanning electron microscope (JEOL, Tokyo, Japan) equipped with an Oxford CT1500 Cryostation for cryo-electron microscopy (cryoSEM). Electron micrographs were acquired from uncoated frozen samples, or after sputter-coating by means of a gold/palladium target for 3 times during 30 s (Fig. 2). Micrographs of uncoated samples were taken at an acceleration voltage of 3 kV, and consisted out of 30 averaged fast scans (SCAN 2 mode), and at 5 kV in case of the coated samples (PHOTO mode).

## RESULTS

### Phylogeny and differentiation

The manually adjusted alignment contained 80 sequences (including the outgroup sequence) and the five loci were represented by a total of 1 516 characters including alignment gaps which were used in the analysis. Of the 1 516 characters, 369 were parsimony-informative, 259 were variable and parsimony-uninformative, and 888 were constant.

Forty equally most parsimonious trees (TL = 1 933 steps; CI = 0.569; RI = 0.786; RC = 0.447), one of which is shown in Fig. 3, were obtained from the parsimony analysis of the combined genes. Neighbour-joining analysis using three substitution models (uncorrected “p”, Kimura 2-parameter and HKY85) on the sequence data yielded trees with identical topologies. These differed from the tree presented in Fig. 3 with regard to the placement of *C. macrocarpum* strain CPC 12054 which was placed as a sister branch to the *C. bruhnei* Linder clade in the distance analyses (results not shown) because it shares an identical CAL sequence. All cryptic species consisting of multiple strains are clustering in well-supported clades with bootstrap support values ranging from 71 % (*C. herbarum*) to 100 % [e.g. *C. ramotenellum* K. Schub.,

Table 1. Isolates subjected to DNA sequence analyses and morphological examinations.

Anamorph	Teleomorph	Accession number <sup>1</sup>	Host	Country	Collector	GenBank numbers <sup>2</sup> (ITS, EF, ACT, CAL, HIS)
<i>Cladosporium antarcticum</i>	—	CBS 690.92* (ex-type)	<i>Caloplaca regalis</i>	Antarctica	C. Möller	EF679334, EF679405, EF679484, EF679560, EF679636
<i>Cladosporium bruhnei</i>	<i>Davidiella allicina</i>	CBS 134.31 = ATCC 11283	—	Germany	—	EF679335, EF679406, EF679485, EF679561, EF679637
		CBS 157.82	<i>Quercus robur</i>	Belgium	—	EF679336, EF679407, EF679486, EF679562, EF679638
		CBS 159.54 = ATCC 36948	Man, skin	The Netherlands	—	EF679337, EF679408, EF679487, EF679563, EF679639
		CBS 161.55	Man, sputum	The Netherlands	—	EF679338, EF679409, EF679488, EF679564, EF679640
		CBS 177.71	<i>Thuja tinctoria</i>	The Netherlands	—	EF679339, EF679410, EF679489, EF679565, EF679641
		CBS 188.54 = ATCC 11290 = IMI 049638 = CPC 3686	—	—	—	AY251077, EF679411, EF679490, EF679566, EF679642
		CBS 366.80	Man, skin	The Netherlands	—	EF679340, EF679412, EF679491, EF679567, EF679643
		CBS 399.80	Man, skin	The Netherlands	—	AJ244227, EF679413, EF679492, EF679568, EF679644
		CBS 521.68	Air	The Netherlands	—	EF679341, EF679414, EF679493, EF679569, EF679645
		CBS 572.78	<i>Polyporus radiatus</i>	Russia	V.K. Melnik	DQ289799, EF679415, DQ289866, DQ289831, EF679646
		CBS 813.71	<i>Polygonatum odoratum</i>	Czech Republic	—	EF679342, EF679416, EF679494, EF679570, EF679647
		CBS 110024	Industrial water	Germany, Nordrhein-Westfalen	—	EF679343, EF679417, EF679495, EF679571, EF679648
		CBS 115683 = ATCC 66670 = CPC 5101	CCA-treated Douglas-fire pole	U.S.A., New York	C.J. Wang	AY361959, EF679418, AY752193, AY752224, AY752255
		CBS 121624* = CPC 12211 (neotype)	<i>Hordeum vulgare</i>	Belgium	J.Z. Groenewald	EF679350, EF679425, EF679502, EF679578, EF679655
		CPC 11386	<i>Tilia cordata</i>	Germany, Sachsen-Anhalt	K. Schubert	EF679344, EF679419, EF679496, EF679572, EF679649
		CPC 11840	<i>Ouirisia macrophylla</i>	New Zealand	A. Blouin	EF679345, EF679420, EF679497, EF679573, EF679650
		CPC 12042 = EXF-389	Hypersaline water from saltens	Slovenia	P. Zalar	EF679346, EF679421, EF679498, EF679574, EF679651
		CPC 12045 = EXF-594	Hypersaline water from saltens	Spain	P. Zalar	EF679347, EF679422, EF679499, EF679575, EF679652
		CPC 12046 = EXF-680	Air conditioning system	Slovenia	P. Zalar	EF679348, EF679423, EF679500, EF679576, EF679653
		CPC 12139	<i>Hordeum vulgare</i>	The Netherlands	—	EF679349, EF679424, EF679501, EF679577, EF679654
		CPC 12212	<i>Hordeum vulgare</i>	Belgium	J.Z. Groenewald	EF679351, EF679426, EF679503, EF679579, EF679656
		CPC 12921	<i>Eucalyptus</i> sp.	Australia	—	EF679352, EF679427, EF679504, EF679580, EF679657
<i>Cladosporium cladosporioides</i> complex	—	CBS 673.69	Air	The Netherlands	—	EF679353, EF679428, EF679505, EF679581, EF679658
	<i>Davidiella</i> sp.	CBS 109082	<i>Silene maritima</i>	United Kingdom	A. Aptroot	EF679354, EF679429, EF679506, EF679582, EF679659
	—	CPC 11606	<i>Musa</i> sp.	India	M. Arzanlou	EF679355, EF679430, EF679507, EF679583, EF679660
	—	CPC 11609	<i>Musa</i> sp.	India	M. Arzanlou	EF679356, EF679431, EF679508, EF679584, EF679661
<i>Cladosporium herbaroides</i>	—	CBS 121626* = CPC 12052 = EXF-1733 (ex-type)	Hypersaline water from saltens	Israel	P. Zalar	EF679357, EF679432, EF679509, EF679585, EF679662
<i>Cladosporium herbarum</i>	<i>Davidiella tassiana</i>	CBS 111.82	<i>Arctostaphylos uva-ursi</i>	Switzerland	E. Müller	AJ238469, EF679433, EF679510, EF679586, EF679663
		CBS 300.49	<i>Biscutella laevigata</i>	Switzerland	J.A. von Arx	EF679358, EF679434, EF679511, EF679587, EF679664
		CBS 121621* = CPC 12177 (epitype)	<i>Hordeum vulgare</i>	The Netherlands	—	EF679363, EF679440, EF679516, EF679592, EF679670
		CPC 11600	<i>Delphinium barbeyi</i>	U.S.A., Colorado	A. Ramalay	DQ289800, EF679435, DQ289867, DQ289832, EF679665
		CPC 11601	<i>Delphinium barbeyi</i>	U.S.A., Colorado	A. Ramalay	EF679359, EF679436, EF679512, EF679588, EF679666
		CPC 11602	<i>Delphinium barbeyi</i>	U.S.A., Colorado	A. Ramalay	EF679360, EF679437, EF679513, EF679589, EF679667
		CPC 11603	<i>Delphinium barbeyi</i>	U.S.A., Colorado	A. Ramalay	EF679361, EF679438, EF679514, EF679590, EF679668

				<i>Delphinium barbeyi</i>	U.S.A., Colorado	A. Ramalay	EF679362, EF679439, EF679515, EF679591, EF679689
				<i>Hordeum vulgare</i>	The Netherlands	—	EF679364, EF679441, EF679517, EF679593, EF679671
				<i>Hordeum vulgare</i>	The Netherlands	—	EF679365, EF679442, EF679518, EF679594, EF679672
				<i>Hordeum vulgare</i>	The Netherlands	—	EF679366, EF679443, EF679519, EF679595, EF679673
				<i>Hordeum vulgare</i>	The Netherlands	—	EF679367, EF679444, EF679520, EF679596, EF679674
				<i>Hordeum vulgare</i>	The Netherlands	—	EF679368, EF679445, EF679521, EF679597, EF679675
				<i>Iris</i> sp.	—	—	EF679369, EF679446, EF679522, EF679598, EF679676
				<i>Iris</i> sp.	The Netherlands	—	EF679370, EF679447, EF679523, EF679599, EF679677
				Water	Romania	—	EF679371, EF679448, EF679524, EF679600, EF679678
				<i>Mycosphaerella tulasnei</i>	—	—	AF222830, EF679449, EF679525, EF679601, EF679679
				<i>Triticum aestivum</i>	Turkey	—	EF679372, EF679450, EF679526, EF679602, EF679680
				<i>Spinacia oleracea</i>	U.S.A.	—	EF679376, EF679454, EF679530, EF679606, EF679684
				<i>Corylus</i> sp.	U.S.A.	—	EF679373, EF679451, EF679527, EF679603, EF679681
				Hypersaline water from saltens	Slovenia	P. Zalar	EF679374, EF679452, EF679528, EF679604, EF679682
				<i>Spinacia oleracea</i>	U.S.A.	—	EF679375, EF679453, EF679529, EF679605, EF679683
				<i>Spinacia oleracea</i>	U.S.A.	—	EF679377, EF679455, EF679531, EF679607, EF679685
				<i>Spinacia oleracea</i>	U.S.A.	—	EF679378, EF679456, EF679532, EF679608, EF679686
				<i>Spinacia oleracea</i>	U.S.A.	—	EF679379, EF679457, EF679533, EF679609, EF679687
				<i>Spinacia oleracea</i>	U.S.A.	—	EF679380, EF679458, EF679534, EF679610, EF679688
				<i>Narthecium ossifragum</i>	Norway	M. di Menna	EF679381, EF679459, EF679535, EF679611, EF679689
				<i>Narthecium ossifragum</i>	Norway	M. di Menna	EF679382, EF679460, EF679536, EF679612, EF679690
				<i>Iris</i> sp.	New Zealand	C.F. Hill	EF679383, EF679461, EF679537, EF679613, EF679691
				Hypersaline water from saltens	Slovenia	P. Zalar	EF679384, EF679462, EF679538, EF679614, EF679692
				Air conditioning system	Slovenia	P. Zalar	EF679385, EF679463, EF679539, EF679615, EF679693
				<i>Fuchsia excorticata</i>	New Zealand	A. Blouin	EF679386, EF679464, EF679540, EF679616, EF679694
				Hypersaline water from saltens	Slovenia	S. Soujak	EF679387, EF679465, EF679541, EF679617, EF679695
				Hypersaline water from saltens	Slovenia	P. Zalar	EF679388, EF679466, EF679542, EF679618, EF679696
				Hypersaline water from saltens	Slovenia	P. Zalar	EF679389, EF679467, EF679543, EF679619, EF679697
				<i>Carya illinoensis</i>	U.S.A.	—	EF679390, EF679468, EF679544, EF679620, EF679698
				Grape berry	U.S.A.	—	EF679391, EF679469, EF679545, EF679621, EF679699
				Grape berry	U.S.A.	—	EF679392, EF679470, EF679546, EF679622, EF679700
				Grape bud	U.S.A.	—	EF679393, EF679471, EF679547, EF679623, EF679701
				<i>Pinus ponderosa</i>	Argentina	A. Creslebin	EF679394, EF679472, EF679548, EF679624, EF679702
				<i>Pinus ponderosa</i>	Argentina	A. Creslebin	EF679395, EF679473, EF679549, EF679625, EF679703
				Bing cherry fruits	U.S.A.	—	EF679396, EF679474, EF679550, EF679626, EF679704
				Grape berry	U.S.A.	—	EF679397, EF679475, EF679551, EF679627, EF679705
				Hypersaline water from saltens	Slovenia	P. Zalar	EF679398, EF679476, EF679552, EF679628, EF679706
				Hypersaline water from saltens	Israel	P. Zalar	EF679401, EF679479, EF679555, EF679631, EF679709
CPC 11604							
CPC 12178							
CPC 12179							
CPC 12180							
CPC 12181							
CPC 12183							
CBS 107.20							
CBS 138.40* (epitype)							
CBS 175.82							
CBS 223.31 = ATCC 11287							
CBS 299.67							
CBS 121811* = CPC 12755 (neotype)							
CPC 11817							
CPC 12054 = EXF-2287							
CBS H-19855 = CPC 12752 = CBS 121623							
CPC 12756							
CPC 12757							
CPC 12758							
CPC 12759							
CBS 842.91* (epitype)							
CBS 843.91							
CBS 116463* = LYN 1065 = ICMP 15579 (ex-type)							
CBS 121628* = CPC 12043 = EXF-454 (ex-type)							
CPC 12047 = EXF-967							
CBS 121629* = CPC 11839 = ICMP 15819 (ex-type)							
CBS 102044							
CBS 119907* = CPC 12040 = EXF-334 (ex-type)							
CBS 121630* = CPC 12041 = EXF-343 (ex-type)							
CBS 172.52 = ATCC 11320							
CBS 113741							
CBS 113742							
CBS 113744							
CPC 12484							
CPC 12485							
CBS 113753							
CBS 113754*							
CPC 12044 = EXF-462							
CBS 121634* = CPC 12053 = EXF-1735 (ex-type)							
	<i>Cladosporium iridis</i>	<i>Davidiella macrospora</i>					
	<i>Cladosporium macrocarpum</i>	<i>Davidiella macrocarpa</i>					
	<i>Cladosporium ossifragi</i>	—					
	<i>Cladosporium pseudiridis</i>	—					
	<i>Cladosporium ramotenellum</i>	—					
	<i>Cladosporium sinuosum</i>	—					
	<i>Cladosporium spinulosum</i>	—					
	<i>Cladosporium subinflatum</i>	—					
	<i>Cladosporium sp.</i>	—					
	<i>Cladosporium subtilissimum</i>	—					
	<i>Cladosporium tenellum</i>	—					

Table 1. (Continued).

Anamorph	Teleomorph	Accession number <sup>1</sup>	Host	Country	Collector	GenBank numbers <sup>2</sup> (ITS, EF, ACT, CAL, HIS)
		CPC 11813	<i>Phyllactinia</i> sp. on <i>Corylus</i> sp.	U.S.A.	D. Glawe	EF679399, EF679477, EF679553, EF679629, EF679707
		CPC 12051 = EXF-1083	Hypersaline water from saltens	Israel	P. Zalar	EF679400, EF679478, EF679554, EF679630, EF679708
<i>Cladosporium variabile</i>	<i>Davidiella variabile</i>	CBS 121636* = CPC 12751 (epitype)	<i>Spinacia oleracea</i>	U.S.A.	—	EF679402, EF679480, EF679556, EF679632, EF679710
—	<i>Davidiella</i> sp.	CPC 12753	<i>Spinacia oleracea</i>	U.S.A.	—	EF679403, EF679481, EF679557, EF679633, EF679711
		CBS 289.49	<i>Allium schoenoprasum</i>	Switzerland	E. Müller	AY152552, EF679482, EF679558, EF679634, EF679712
		CBS 290.49	<i>Trisetum distichophyllum</i>	Switzerland	E. Müller	EF679404, EF679483, EF679559, EF679635, EF679713

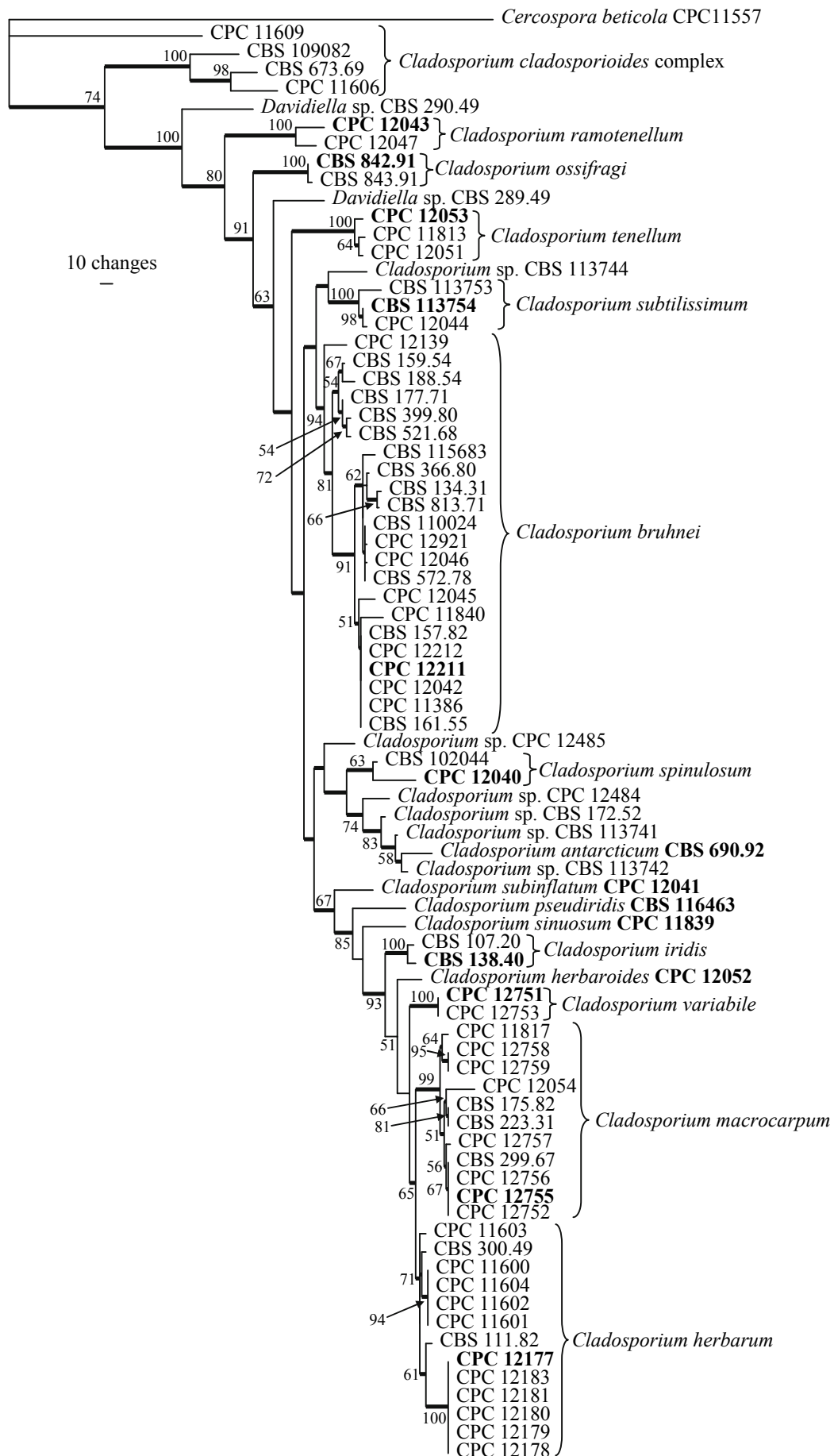
<sup>1</sup>ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; EXF: Extremophilic Fungi Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IMI: International Mycological Institute, CAB International, Egham, Basingstoke Lane, U.K.

<sup>2</sup>ACT: partial actin gene, CAL: partial calmodulin gene, EF: partial elongation factor 1-alpha gene, HIS: partial histone H3 gene, ITS: internal transcribed spacer region.

<sup>3</sup>Ex-type cultures.

Zalar, Crous & U. Braun and *C. ossifragi* (Rostr.) U. Braun & K. Schub.]. The intraspecific variation in the *C. bruhnei* clade is due to genetic variation present in the sequence data of all loci except for ITS, those in the *C. macrocarpum* clade in all loci except for ITS and ACT, and those in the *C. herbarum* clade in all loci except for ITS and CAL (data not shown). However, none of the variation for these species could be linked to host specificity or morphological differences. In general, ITS data did not provide any resolution within the *C. herbarum* complex, whereas EF data provided species clades with very little intraspecific variation and ACT, CAL and HIS revealed increasing intraspecific variation (ACT the least and HIS the most).

The mean genetic diversity (H) of the entire data set excluding the nearly invariant ITS region was 0.9307, with little difference between genes (ACT = 0.9257, CAL = 0.9289, EF = 0.9322, HIS = 0.9361). The loci showed different numbers of alleles (ACT: 22, CAL: 16, EF: 21, HIS: 20, ITS: 6). Differentiation of entities when calculated with STRUCTURE software using the admixture/correlated model showed highest value with K = 6. At this value  $F_{ST}$  varied between 0.1362 and 0.3381. Linkage disequilibrium calculated using the standardised index of association ( $I_{SA}^S$ ) for the entire dataset (observed variance  $V_o = 0.5602$ , expected variance  $V_e = 0.2576$ ) was 0.3914 ( $P = 0.0001$ ), consistent with a small amount of recombination that did not destroy the linkage between alleles. Only few groups appeared to be separated for all alleles; degrees of gene flow are indicated in Fig. 4. SPLITSTREE software produced unresolved star-shaped structures for all genes, without any sign of reticulation (Fig. 5).



**Fig. 3.** One of 40 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment (ITS, ACT, CAL, EF, HIS). The scale bar shows ten changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and strain numbers in bold represent ex-type sequences. The tree was rooted to sequences of *Cercospora beticola* strain CPC 11557 (GenBank accession numbers AY840527, AY840458, AY840425, AY840494, AY840392, respectively).





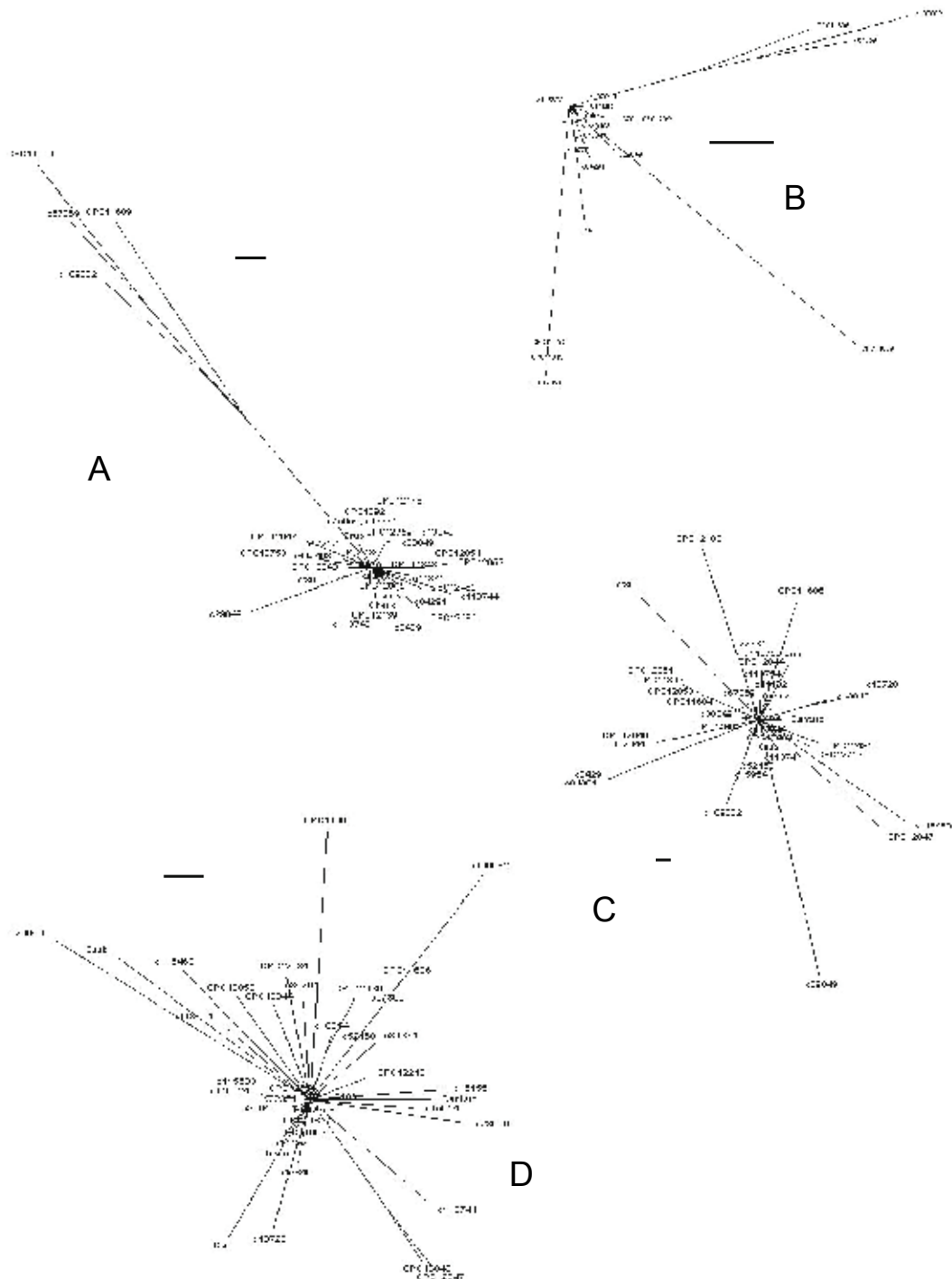


Fig. 5. Split decomposition of the *Cladosporium herbarum* complex using SPLITSTREE of 16–22 unique alleles obtained from 79 *Cladosporium* isolates for four loci. The star-like structures suggest clonal development. A = ACT, B = CAL, C = HIS, D = EF. Scale bars = 0.01 nucleotide substitutions per site.

## Taxonomy

### Key to the *Cladosporium* species treated

Morphological features used in the key to distinguish the species treated in this study were determined after 7 d growth at 25 °C on SNA using light microscopy, and cultural characteristics after 14 d incubation on PDA.

1. Conidia usually smooth, rarely minutely verruculose ..... ***C. cladosporioides*** (species complex)
1. Conidia with different surface ornamentation, minutely to distinctly verruculose, verruculose to echinulate or spiny ..... 2
2. Conidiophores uniform, macronematous; conidia solitary, sometimes formed in short unbranched chains ..... 3
2. Conidiophores both macronematous and micronematous; conidia always catenate, usually formed in branched chains ..... 5

3. Conidiophores due to geniculations often growing zigzag-like, (4–)5–7  $\mu\text{m}$  wide; conidia 9–21  $\times$  (5–)6–8  $\mu\text{m}$ , 0–1-septate; conidiogenous loci and conidial hila 1.2–2(–2.2)  $\mu\text{m}$  diam ..... **C. sinuosum**
3. Conidiophores not growing zigzag-like, wider, 6–11  $\mu\text{m}$ ; conidia very large and wide, 15–75(–87)  $\times$  (7–)10–19(–21)  $\mu\text{m}$ , often with more septa; conidiogenous loci and hila wider, (2–)2.5–4  $\mu\text{m}$  diam ..... 4
4. Conidia (18–)30–75(–87)  $\times$  (7–)10–16(–18)  $\mu\text{m}$ , (0–)2–6(–7)-septate, walls thickened, especially in older conidia, up to 1  $\mu\text{m}$  thick ..... **C. iridis**
4. Conidia shorter and wider, 15–55  $\times$  (9–)11–19(–21)  $\mu\text{m}$ , 0–3-septate, walls distinctly thickened, up to 2  $\mu\text{m}$ , usually appearing zonate ..... **C. pseudiridis**
- 5(2) Macronematous conidiophores nodulose or nodose with conidiogenous loci usually confined to swellings ..... 6
5. Macronematous conidiophores non-nodulose or only occasionally subnodulose due to geniculate proliferation, but conidiogenous loci not confined to swellings ..... 11
6. Macronematous conidiophores 3–6  $\mu\text{m}$  wide, swellings 5–11  $\mu\text{m}$  wide ..... 7
6. Macronematous conidiophores somewhat narrower, (1.5–)2.5–5  $\mu\text{m}$  wide, swellings 3–8  $\mu\text{m}$  wide ..... 8
7. Aerial mycelium twisted; conidial septa often distinctly darkened, becoming sinuous with age, apex and base of the conidia often appear to be distinctly darkened; slower growing in culture (29 mm after 14 d on PDA) ..... **C. variabile**
7. Aerial mycelium not twisted; conidial septa as well as apex and base not distinctly darkened, septa not sinuous with age; faster growing in culture (on average 38 mm after 14 d on PDA) ..... **C. macrocarpum**
8. Macronematous conidiophores (1.5–)2.5–4.5(–5.5)  $\mu\text{m}$  wide, swellings 3–6.5  $\mu\text{m}$  wide; conidia 4–17(–22)  $\mu\text{m}$  long, ornamentation variable, but usually densely echinulate, spines up to 0.8  $\mu\text{m}$  long ..... **C. subinflatum**
8. Macronematous conidiophores slightly wider, 3–5  $\mu\text{m}$ , swellings (4–)5–8(–9)  $\mu\text{m}$  wide; conidia longer, up to 25(–35)  $\mu\text{m}$ , ornamentation minutely verruculose to verrucose, but not echinulate or spiny ..... 9
9. Conidia formed by macronematous conidiophores 3–33  $\times$  (2–)3–6(–7)  $\mu\text{m}$ , with age becoming wider, (3.5–)5–9(–11)  $\mu\text{m}$ , darker and more thick-walled ..... **C. herbaroides**
9. Conidia formed by macronematous conidiophores not becoming wider and darker with age, usually up to 7  $\mu\text{m}$  wide ..... 10
10. Conidiophores usually with small head-like swellings, sometimes also with a second intercalary nodule; small terminal conidia 4–9  $\times$  2.5–3.5  $\mu\text{m}$ , secondary ramoconidia and occasionally formed ramoconidia 10–24(–31)  $\times$  3–5(–7)  $\mu\text{m}$  ..... **C. bruhnei**
10. Conidiophores with a single or often numerous swellings in short succession giving the stalk a knotty/gnarled appearance; conidia wider, small terminal conidia 4–10  $\times$  3–5(–6)  $\mu\text{m}$ , intercalary conidia 6–16  $\times$  4–6  $\mu\text{m}$ , secondary ramoconidia 12–25(–35)  $\times$  (3–)5–7(–9)  $\mu\text{m}$  ..... **C. herbarum**
- 11(5) Small terminal and intercalary conidia 4–15  $\times$  3–5  $\mu\text{m}$ , secondary ramoconidia 16–36(–40)  $\times$  (4–)5–8  $\mu\text{m}$ , 0–3(–4)-septate, ramoconidia absent ..... **C. ossifragi**
11. Small terminal conidia, ramoconidia and secondary ramoconidia distinctly narrower, 2–5(–6)  $\mu\text{m}$  wide, 0–2(–3)-septate ..... 12
12. Mycelium dimorphic, narrow hyphae 1–3  $\mu\text{m}$  wide, hyaline to subhyaline, thin-walled, hyphae of the second type wider, 3.5–8(–9)  $\mu\text{m}$ , pale to dark greyish olivaceous or olivaceous-brown, thick-walled, sometimes even two-layered, 1(–1.5)  $\mu\text{m}$  thick, hyphae appearing consistently enveloped in polysaccharide-like material or covered by a slime coat; conidiophores usually several times slightly to distinctly geniculate towards the apex, with numerous conidiogenous loci crowded towards the apex, up to 14 per conidiogenous cell ..... **C. antarcticum**
12. Mycelium not dimorphic, neither enveloped in polysaccharide-like material nor covered by a slime coat; conidiophores usually not geniculate, occasionally only slightly so ..... 13
13. Conidial ornamentation distinctly echinulate, spiny (baculate, digitate or capitate under SEM), spines 0.5–1.3  $\mu\text{m}$  long, loose to moderately dense, conidial hila usually situated on small peg-like prolongations or denticles ..... **C. spinulosum**
13. Conidial ornamentation different, minutely verruculose to verruculose, conidial hila not situated on peg-like prolongations ..... 14
14. Small terminal conidia narrowly obovoid, limoniform or fusiform, but neither globose nor subglobose; conidiogenous loci and conidial hila 0.5–2(–2.5)  $\mu\text{m}$  diam ..... **C. subtilissimum** (species complex)
14. Numerous small globose or subglobose terminal conidia formed, also ovoid or limoniform; conidiogenous loci and conidial hila somewhat smaller, 0.5–1.5(–2)  $\mu\text{m}$  diam ..... 15

15. Conidiophores usually with numerous conidiogenous loci forming sympodial clusters of pronounced scars at the apex, sometimes up to 10 or even more denticulate loci; conidia 3–20(–28) × 2.5–5(–6) µm, 0–1(–2)-septate, often with several apically crowded hila, up to 7(–9) ..... **C. tenellum**
15. Conidiophores usually only with few conidiogenous loci, mostly 1–3; conidia longer and narrower, 2.5–35 × 2–4(–5) µm, 0–3-septate, usually with up to three distal conidial hila ..... **C. ramotenellum**

### Key to the *Davidiella* species treated

1. Ascospores frequently wider than 7 µm when mounted in Shear's solution or lactic acid, apical cell obtusely rounded ..... 2
1. Ascospores not wider than 7 µm when mounted in Shear's solution or lactic acid, apical cell acutely rounded, ascospores (20–)25–27(–30) × (5.5–)6–7 µm ..... **D. allicina**
2. Pseudoparaphyses prominent; asci frequently >95 µm; ascospores (22–)23–26(–28) × (6–)6.5–7(–8) µm ..... **D. macrocarpa**
2. Pseudoparaphyses mostly absent in older ascomata; asci <95 µm ..... 3
3. Ascospores (22–)26–30(–35) × (7–)7.5–8(–9) µm; asci wider than 18 µm ..... **D. variabile**
3. Ascospores (17–)20–23(–25) × (6–)7(–8) µm; asci not wider than 18 µm ..... **D. tassiana**

### Generic concept of the teleomorph

The introduction of the teleomorph genus *Davidiella* was mainly based on phylogenetic studies within the *Mycosphaerellaceae* (Braun *et al.* 2003), where it could be demonstrated that "*Mycosphaerella*" species with *Cladosporium* anamorphs formed a sister clade to *Mycosphaerella* (Crous *et al.* 2000, 2001). Braun *et al.* (2003) transferred five species to *Davidiella* based on prior established anamorph-teleomorph connections, though no details were provided pertaining to morphological differences between *Davidiella* and *Mycosphaerella*. Aptroot (2006) transferred several additional species to *Davidiella*, and distinguished them from true *Mycosphaerella* species by the presence of distinct, irregular cellular inclusions (lumina) in their ascospores. Furthermore, Schoch *et al.* (2006) placed *Davidiella* in a separate family (*Davidiellaceae*) in the *Capnodiales*. During the course of the present study, several fresh specimens of *Davidiella* spp. were collected or induced in culture, making it possible to circumscribe the genus as follows:

***Davidiella*** Crous & U. Braun, Mycol. Progr. 2: 8. 2003, **emend.**

*Ascomata* pseudothecial, black to red-brown, globose, inconspicuous and immersed beneath stomata to superficial, situated on a reduced stroma, with 1(–3) short, periphysate ostiolar necks; periphysoids frequently growing down into cavity; wall consisting of 3–6 layers of *textura angularis*. *Asci* fasciculate, short-stalked or not, bitunicate, sessile, obovoid to broadly ellipsoid or subcylindrical, straight to slightly curved, 8-spored. *Pseudoparaphyses* frequently present in mature ascomata, hyaline, septate, subcylindrical. *Ascospores* bi- to multiseriate, hyaline, obovoid to ellipsoid-fusiform, with irregular lumina inclusions, mostly thick-walled, straight to slightly curved; frequently becoming brown and verruculose in asci; at times covered in mucoid sheath. *Cladosporium* anamorph usually produced in culture, but not in all taxa.

*Type species: Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 2: 8. 2003.

### Description of *Cladosporium* species

Based on morphological examinations (David 1997) and phylogenetic studies employing DNA sequence data (Crous *et al.* 2000, 2001,

2007 – this volume, Braun *et al.* 2003), the generic concept of the genus *Cladosporium* has been stabilised. *Cladosporium* is confined to *Davidiella* (*Davidiellaceae*, *Capnodiales*) anamorphs with coronate conidiogenous loci and conidial hila consisting of a central convex dome and a raised periclinal rim.

***Cladosporium antarcticum*** K. Schub., Crous & U. Braun, **sp. nov.** MycoBank MB504573. Figs 6–8.

*Etymology:* Refers to Antarctica, where the fungus was collected.

Differt a *Cladosporio* licheniphilo conidiophoris saepe non-ramosis, frequentibus geniculatis, angustioribus, (2–)3–4.5 µm, conidiis longioribus et angustioribus, 4–30 × 2.5–5 µm, 0–3-septatis, verruculosis vel verrucosis.

*Mycelium* immersed and superficial, dimorphic, branched, often with short lateral outgrowths, narrow hyphae 1–3 µm wide, hyaline to subhyaline, thin-walled, hyphae of the second type wider, 3.5–8(–9) µm, pluriseptate, often somewhat constricted at the septa, sometimes swollen, pale to dark greyish olivaceous or olivaceous-brown, smooth or verruculose, thick-walled, sometimes even two-layered (two distinct wall layers visible), 1(–1.5) µm thick, hyphae appearing consistently enveloped in polysaccharide-like material or covered by a slime coat. *Conidiophores* micronematous and macronematous, solitary or in loose groups, arising from plagiotropous or ascending hyphae, terminally or usually laterally. *Macronematous conidiophores* erect to somewhat decumbent, straight to somewhat flexuous or bent, cylindrical, once or several times slightly to distinctly geniculate towards the apex due to sympodial proliferation, unbranched or once branched, up to 120 µm long, 3–4.5 µm wide, sometimes slightly attenuated towards the apex, pluriseptate, up to eight septa, occasionally slightly constricted at the septa, pale to medium or even dark olivaceous-brown or greyish brown, paler towards apices, smooth to somewhat rough-walled, walls thickened but thinner-walled towards apices, sometimes slightly swollen at the base, up to 6 µm wide. *Conidiogenous cells* integrated, terminal and intercalary, once or several times slightly to distinctly geniculate, 10–33 µm long, proliferation sympodial, with several or numerous conidiogenous loci, at first terminal, later turning to one side of the stalk and situated on small lateral shoulders, up to 14 per cell, protuberant, denticulate, 1–1.5(–2) µm diam, thickened and darkened-refractive. *Micronematous conidiophores* as short lateral, peg-like outgrowths with a single apical scar or somewhat longer, occasionally once

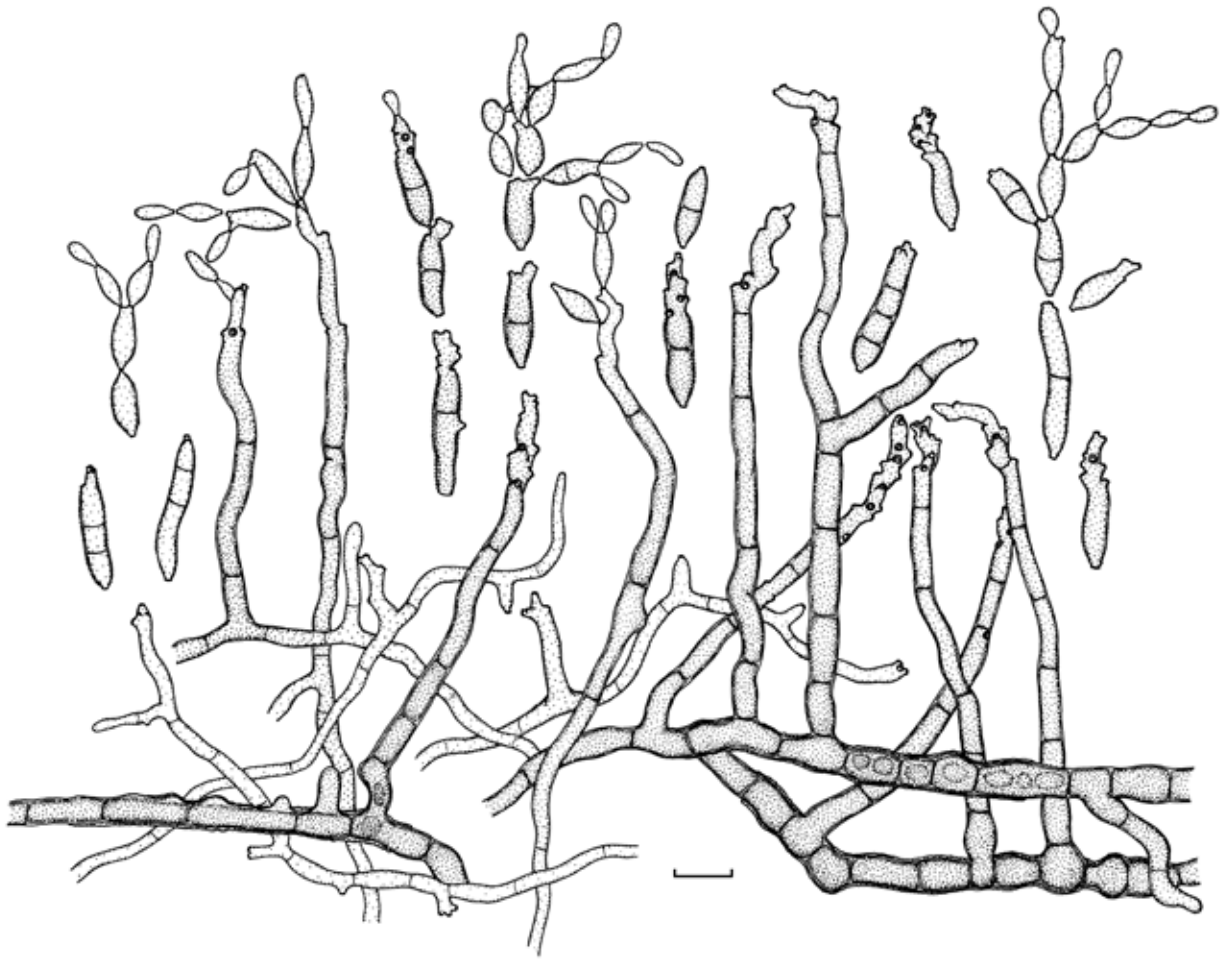


Fig. 6. *Cladosporium antarcticum* (CBS 690.92). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu$ m. K. Schubert del.

geniculate with several conidiogenous loci at the apex, 2–22  $\times$  2–3  $\mu$ m, pale greyish olivaceous, loci denticulate. *Ramoconidia* occasionally occurring, cylindrical, up to 30  $\mu$ m long, 4–5  $\mu$ m wide, 0–1-septate, concolorous with the tips of conidiophores, with a broadly truncate, unthickened and not darkened base, without dome and rim, 2.5  $\mu$ m wide. *Conidia* catenate, in branched chains, straight, small terminal conidia obovoid, limoniform or narrowly ellipsoid, 4–14  $\times$  2.5–4  $\mu$ m [av.  $\pm$  SD, 8.5 ( $\pm$  3.3)  $\times$  3.5 ( $\pm$  0.6)], 0(–1)-septate, secondary ramoconidia ellipsoid to cylindrical, often with several or numerous conidial hila crowded at the distal end, up to 12, 13–30  $\times$  4–5  $\mu$ m [av.  $\pm$  SD, 20.1 ( $\pm$  5.8)  $\times$  4.3 ( $\pm$  0.5)  $\mu$ m], 0–3-septate, sometimes slightly constricted at the median septum, pale olivaceous-brown or greyish brown, minutely verruculose to verrucose (granulate under SEM), walls more or less thickened, rounded or slightly attenuated towards apex and base, hila protuberant, denticulate, 0.8–1.5(–2)  $\mu$ m diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring.

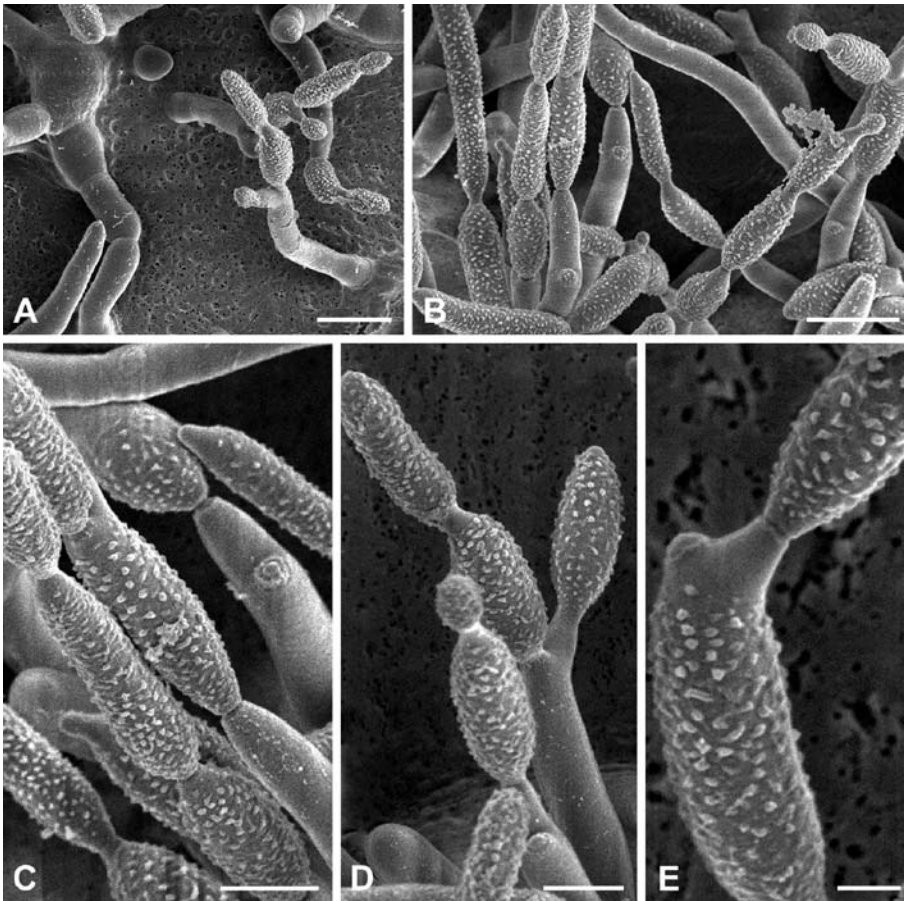
**Cultural characteristics:** Colonies on PDA attaining 9 mm diam after 14 d at 25  $^{\circ}$ C, greenish olivaceous to grey-olivaceous, at the margin becoming dull green, reverse with a pale olivaceous-grey centre and a broad olivaceous-black margin, margin narrow, regular, entire edge, white, feathery, aerial mycelium sparse but colonies appearing felty, growth flat with somewhat elevated colony centre, prominent exudates not formed, sporulation dense, covering almost the whole colony. Colonies on MEA attaining 12 mm diam after 14 d at 25  $^{\circ}$ C, olivaceous-grey to iron-grey, iron-grey reverse, velvety to powdery, aerial mycelium sparse, sporulation profuse. Colonies

on OA attaining 4 mm after 14 d at 25  $^{\circ}$ C, olivaceous-grey, aerial mycelium sparse, diffuse, growth flat, without prominent exudates, sporulating.

**Specimen examined:** **Antarctica**, King George, Arctowski, isolated from the lichen *Caloplaca regalis* (Teloschistaceae), C. Möller, No. 32/12, 1991, CBS-H 19857, **holotype**, isotype HAL 2024 F, culture ex-type CBS 690.92.

**Substrate and distribution:** On the lichen *Caloplaca regalis*; Antarctica.

**Notes:** This is the second genuine lichenicolous species of the genus *Cladosporium*. *Cladosporium licheniphilum* Heuchert & U. Braun, occurring on apothecia of *Pertusaria alpina* in Russia, is quite distinct from *C. antarcticum* by having subcylindrical or only slightly geniculate-sinuous, wider conidiophores, 5–8  $\mu$ m, with numerous characteristic terminal branches and much shorter, 0–1-septate, smooth conidia, 3.5–13  $\times$  3–7  $\mu$ m (Heuchert & Braun 2006). *Cladosporium lichenicola* Linds. was invalidly published and *C. arthoniae* M.S. Christ. & D. Hawksw. as well as *C. lichenum* Keissl. are to be excluded from the genus *Cladosporium* since they do not possess the typical cladosporioid scar structure but inconspicuous, unthickened conidiogenous loci and conidial hila (Hawksworth 1979, Heuchert et al. 2005). The fungicolous species *C. uredinicola* Speg. and the follicolous species *C. alneum* Pass. ex K. Schub. and *C. psoraleae* M.B. Ellis are morphologically superficially similar. However, *C. uredinicola*, a widespread fungus on rust fungi, downy mildews and powdery mildew fungi, differs in having somewhat longer and wider, smooth conidia, 3–39  $\times$  2–6.5(–8)  $\mu$ m, and wider conidiogenous loci and conidial hila, 0.5–3



**Fig. 7.** *Cladosporium antarcticum* (CBS 690.92). A. Overview of the growth pattern on SNA. Note the very large bulbous cells formed at the base of different conidiophores. Other conidiophores sprout from the agar surface. B. Overview of conidiophores and conidia. Note the large distance of the scars on the conidiophore and the different stages of conidial formation on the tips of other conidia. The long secondary ramoconidia are also visible, and sparse aerial hyphae. C. Detail of B with details of the ornamentation and scars. The absence of ornamentation at the apical (spore-forming) end of the secondary ramoconidium is clearly visible. D–E. Tubular structures on conidiophore (D) and secondary ramoconidium (E). Scale bars: A–B = 10  $\mu\text{m}$ , C–D = 5  $\mu\text{m}$ , E = 2  $\mu\text{m}$ .



**Fig. 8.** *Cladosporium antarcticum* (CBS 690.92). A–B. Macronematous conidiophores. C, G. Mycelium enveloped by a polysaccharide-like layer. D, F. Conidia. E. Micronematous conidiophore. H. Ramoconidium with numerous distal scars. Scale bars = 10  $\mu\text{m}$ .

$\mu\text{m}$  (Heuchert *et al.* 2005); *C. alneum*, which causes leaf spots on *Alnus glutinosa*, possesses longer and wider conidiophores, 25–260  $\times$  (2–)3–7(–8.5)  $\mu\text{m}$ , and somewhat shorter, smooth conidia (Schubert 2005, Schubert *et al.* 2006); and *C. psoraleae*, known

from Myanmar on *Psoralea corylifolia*, can easily be distinguished from *C. antarcticum* by its smooth and wider conidia, 3.5–7  $\mu\text{m}$ , and wider conidiogenous loci and conidial hila, 1–3  $\mu\text{m}$  diam (Ellis 1972, Schubert 2005).

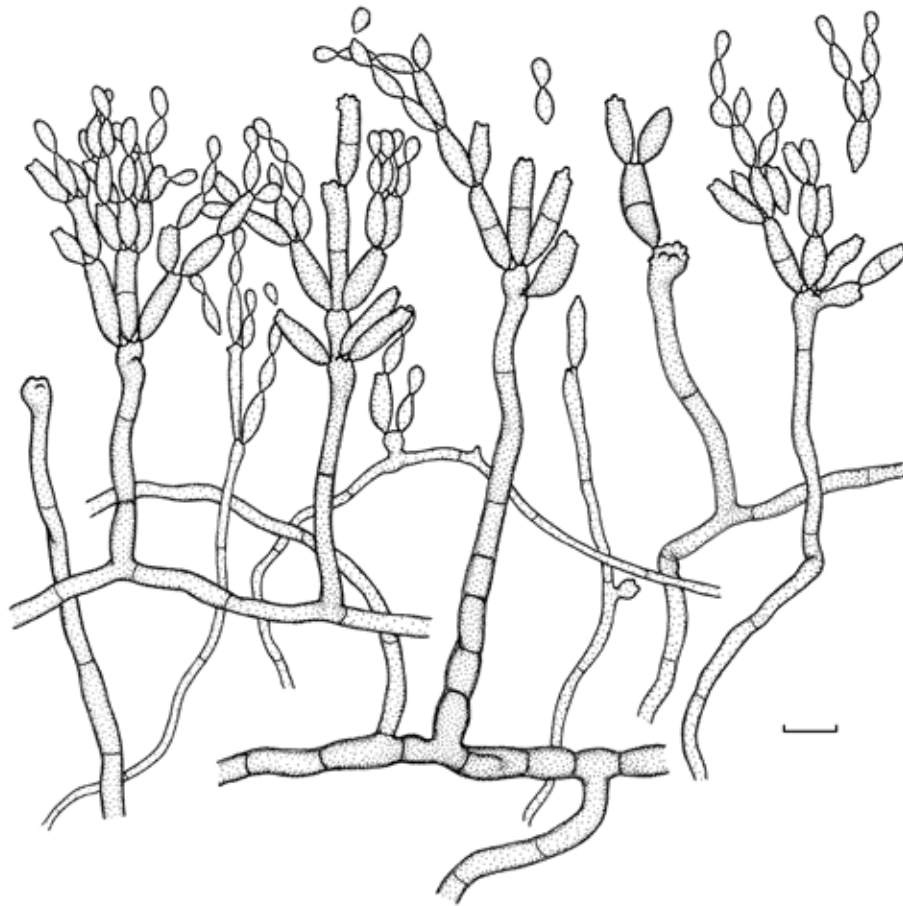


Fig. 9. *Cladosporium bruhnei* (CPC 12211). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu$ m. K. Schubert del.

***Cladosporium bruhnei*** Linder, Bull. Natl. Mus. Canada 97: 259. 1947. Figs 9–12.

≡ *Homodendrum hordei* Bruhne, in W. Zopf, Beitr. Physiol. Morph. nied. Org. 4: 1. 1894, non *C. hordei* Pass., 1887.

≡ *Cladosporium herbarum* (Pers.: Fr.) Link var. ( $\delta$ ) *cerealium* Sacc. f. *hordei* (Bruhne) Ferraris, Flora Ital. Crypt., Pars I, Fungi, Fasc.13: 882. 1914.

≡ *Cladosporium hordei* (Bruhne) Pidopl., Gribnaja Flora Grubych Kormov: 268. 1953, *nom. illeg.*, homonym, non *C. hordei* Pass., 1887.

**Teleomorph: *Davidiella allicina*** (Fr. : Fr.) Crous & Aptroot, in Aptroot, *Mycosphaerella* and its anamorphs: 2. *Conspectus of Mycosphaerella*. CBS Biodiversity Ser. 5: 30. 2006.

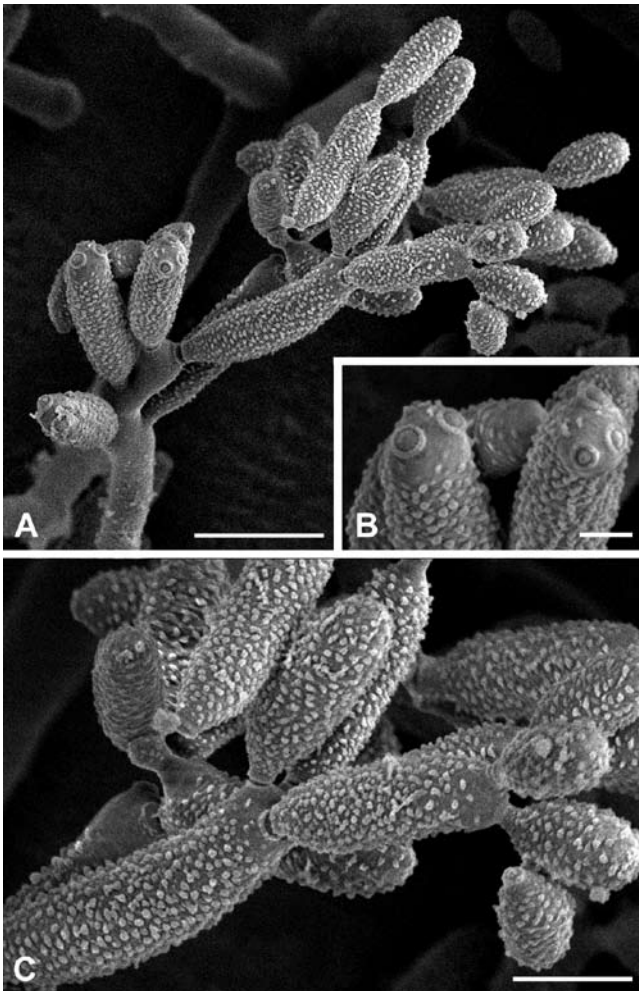
**Basionym: *Sphaeria allicina*** Fr., Kongl. Vetensk. Acad. Handl. 38: 247. 1817, sactioned by Fr., *Syst. Mycol.* 2: 437. 1823.

≡ *Sphaerella allicina* (Fr. : Fr.) Auersw., in Gonn. & Rabenh., *Mycol. Europaea* 5–6: 19. 1869.

**Ascomata** pseudothecial, black, superficial, situated on a small stroma, globose, up to 250  $\mu$ m diam; ostioles periphysate, with apical periphysoids present; wall consisting of 3–6 layers of reddish brown *textura angularis*. **Asci** fasciculate, bitunicate, sessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 65–90  $\times$  16–25  $\mu$ m; with pseudoparenchymatal cells of the hamathecium persistent. **Ascospores** tri- to multiseriate, overlapping, hyaline, with irregular lumina, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse basal end, and acutely rounded apical end, widest near the middle of the apical cell, medianly 1-septate, not to slightly constricted at the septum, (20–)25–27(–30)  $\times$  (5.5–)6–7  $\mu$ m.

**Mycelium** superficial, hyphae branched, 1.5–8  $\mu$ m wide, pluriseptate, broader hyphae usually slightly constricted at the septa and somewhat swollen, hyaline to subhyaline, almost smooth

to somewhat verruculose or irregularly rough-walled, sometimes appearing to have a slime coat, walls unthickened. **Conidiophores** macronematous, sometimes also micronematous, arising as lateral or terminal branches from plagiotropous or ascending hyphae, erect, straight to more or less flexuous, sometimes geniculate, nodulose, usually with small head-like swellings, sometimes also with intercalary nodules, sometimes swellings protruding and elongated to one side, unbranched, occasionally branched, (7–)20–330  $\mu$ m, sometimes even longer, (2–)3–5  $\mu$ m wide, swellings (4–)5–8  $\mu$ m wide, pluriseptate, not constricted at the septa, septa sometimes not very conspicuous, subhyaline to pale brown or pale olivaceous, smooth or somewhat verruculose, walls unthickened or almost so, more thickened with age. **Conidiogenous cells** integrated, usually terminal, cylindrical with a terminal head-like swelling, sometimes with a second swelling, 15–40  $\mu$ m long, proliferation sympodial, with few conidiogenous loci confined to swellings, up to five per swelling, loci protuberant, conspicuous, 1–2  $\mu$ m diam, thickened and darkened-refractive. **Conidia** catenate, formed in branched chains, straight to slightly curved, small terminal conidia subglobose, ovoid to obovoid or somewhat limoniform, 4–9  $\times$  2.5–3.5  $\mu$ m [av.  $\pm$  SD, 6.5 ( $\pm$  1.5)  $\times$  3.1 ( $\pm$  0.5)  $\mu$ m], aseptate; secondary ramoconidia and occasionally formed ramoconidia ellipsoid to subcylindrical or cylindrical, 10–24(–31)  $\times$  3–5(–7)  $\mu$ m [av.  $\pm$  SD, 16.1 ( $\pm$  4.1)  $\times$  4.1 ( $\pm$  0.8)  $\mu$ m], rarely up to 40  $\mu$ m long, 0–1(–3)-septate, very rarely 5-septate, subhyaline to pale brown or pale olivaceous, minutely verruculose to verruculose (mostly granulate with some muricate projections under SEM), walls unthickened or almost so, apex rounded or slightly attenuated towards apex and base, hila protuberant, conspicuous, 1–2  $\mu$ m wide, up to 1  $\mu$ m high, thickened and darkened-refractive; microcyclic conidiogenesis occurring.



**Fig. 10.** *Cladosporium bruhnei* (CPC 12211). A. Conidiophore with characteristic long secondary ramoconidium and complex conidiophore. B. Detail of hilum on secondary ramoconidia. C. Details of prominent ornamentation on conidia. Scale bars: A = 10  $\mu$ m, B = 2  $\mu$ m, C = 5  $\mu$ m.

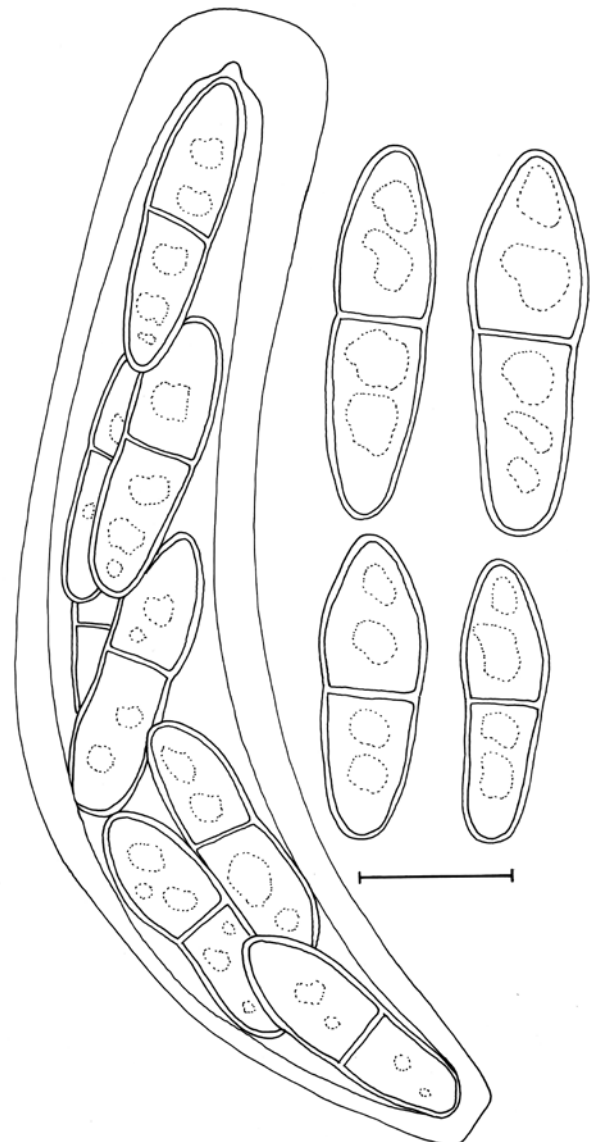
**Cultural characteristics:** Colonies on PDA reaching 22–32 mm diam after 14 d at 25 °C, olivaceous-grey to iron-grey, sometimes whitish, smoke-grey to pale olivaceous due to abundant aerial mycelium covering almost the whole colony, with age collapsing becoming olivaceous-grey, occasionally zonate, velvety to floccose, margin narrow, entire edge, white, glabrous to somewhat feathery, aerial mycelium sparse to abundant, white, fluffy, growth regular, flat to low convex, sometimes forming few exudates in the colony centre, sporulating. Colonies on MEA reaching 21–32 mm diam after 14 d at 25 °C, grey-olivaceous, olivaceous-grey to dull green or iron-grey, sometimes whitish to pale smoke-grey due to abundant aerial mycelium, olivaceous-grey to iron-grey reverse, velvety, margin narrow, entire edge to slightly undulate, white, radially furrowed, glabrous to slightly feathery, aerial mycelium sparse to abundant, mainly in the centre, white, fluffy, growth convex to raised, radially furrowed, distinctly wrinkled in the colony centre, without prominent exudates, sporulating. Colonies on OA reaching 20–32 mm diam after 14 d at 25 °C, smoke-grey, grey-olivaceous to olivaceous-grey, greenish black or iron-grey reverse, margin narrow, entire edge, colourless to white, glabrous, aerial mycelium sparse to abundant, dark smoke-grey, diffuse, high, later collapsed, felty, growth flat, without prominent exudates, sporulation profuse.

**Specimens examined:** *Sine loco et dato*, CBS 188.54 = ATCC 11290 = IMI 049638. **Australia**, N.S.W., Barrington Tops National Park, isolated from leaves of *Eucalyptus stellulata* (Myrtaceae), 3 Jan. 2006, B. Summerell, CPC 12921.

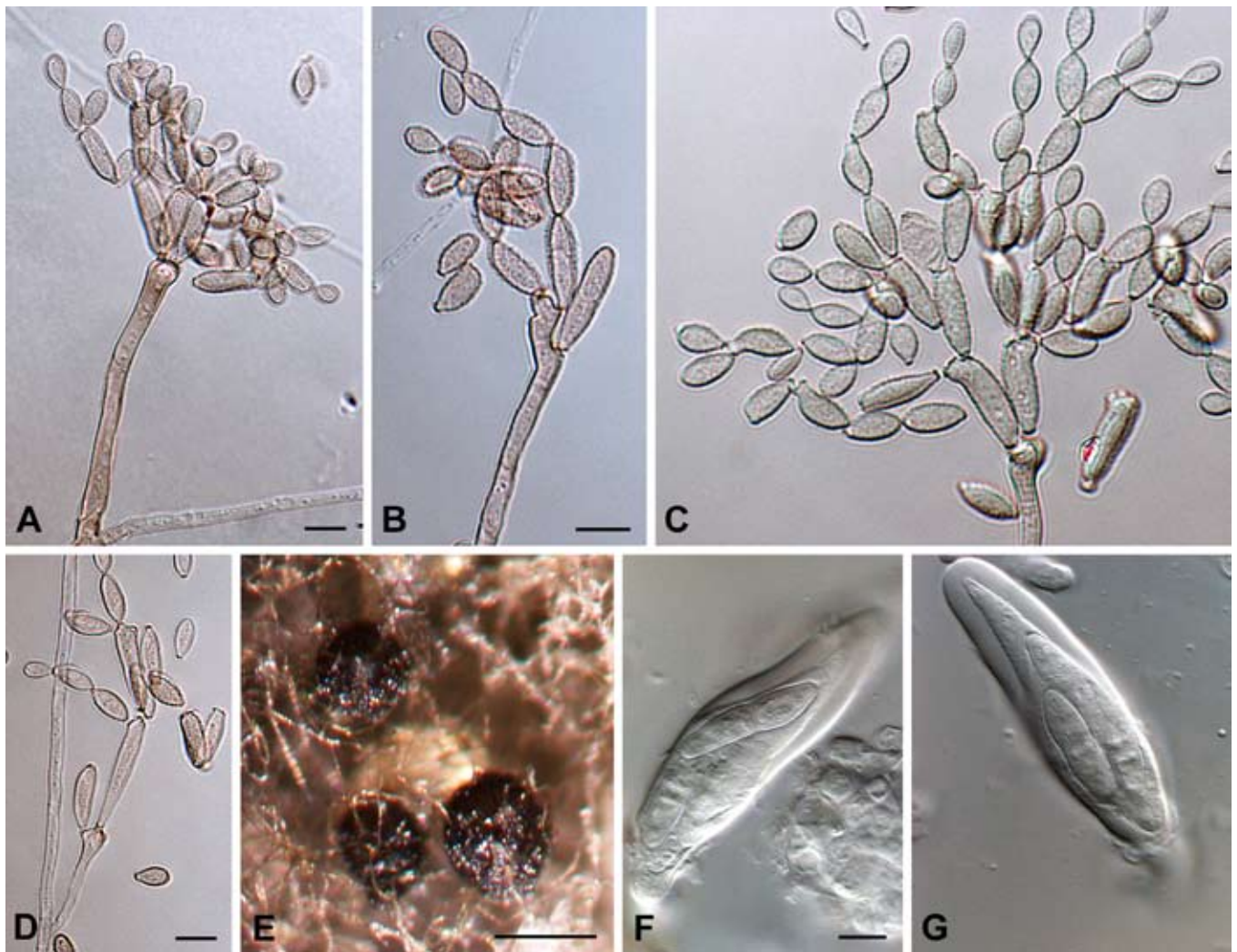
**Belgium**, isolated from *Quercus robur* (Fagaceae), CBS 157.82; Kampenhout, isolated from *Hordeum vulgare* (Poaceae), 26 June 2005, J.Z. Groenewald, CBS-H 19856, **neotype designated here** of *C. bruhnei*, isoneotype HAL 2023 F, cultures ex-type CBS 121624 = CPC 12211, CPC 12212. **Czech Republic**, Lisen, isolated from *Polygonatum odoratum* (Liliaceae), CBS 813.71, albino mutant of CBS 812.71. **Germany**, CBS 134.31 = ATCC 11283 = IMI 049632; Nordrhein-Westfalen, Mülheim an der Ruhr, isolated from industrial water, IWW 727, CBS 110024; Sachsen-Anhalt, Halle (Saale), Robert-Franz-Ring, isolated from leaves of *Tilia cordata* (Tiliaceae), 2004, K. Schubert, CPC 11386. **Netherlands**, isolated from air, CBS 521.68; isolated from *Hordeum vulgare*, 1 Jan. 2005, P.W. Crous, CPC 12139; isolated from man, skin, CBS 159.54 = ATCC 36948; Amsterdam, isolated from Thuja tincture, CBS 177.71; Geleen, St. Barbara Ziekenhuis, isolated from man, skin, CBS 366.80, CBS 399.80; isolated from man, sputum, Aug. 1955, CBS 161.55. **New Zealand**, Otago, Lake Harris, isolated from *Ourisia macrophylla* (Scrophulariaceae), 30 Jan. 2005, A. Blouin, Hill 1135, CPC 11840. **Russia**, Moscow region, isolated from *Polyporus radiatus* (Polyporaceae), Oct. 1978, CBS 572.78 = VKM F-405. **Slovenia**, Ljubljana, isolated from an air conditioning system, 2004, M. Butala, EXF-680 = CPC 12046; Sečovlje, isolated from hypersaline water from salterns (reserve pond), 2005, P. Zalar, EXF-389 = CPC 12042. **Spain**, Ebro Delta, isolated from hypersaline water from salterns (crystallisation pond), 2004, P. Zalar, EXF-594 = CPC 12045. **Sweden**, Skåne, on tip blight of living leaves of *Allium* sp. (Alliaceae), Fr. no. F-09810, UPS-FRIES, **holotype** of *Davidiella allicina*. **U.S.A.**, New York, Geneva, isolated from CCA-treated Douglas-fir pole, CBS 115683 = ATCC 66670 = CPC 5101.

**Substrate and distribution:** Living and decaying plant material, man, air, hypersaline and industrial water; widespread.

**Literature:** Saccardo (1899: 1076), Linder (1947: 289).



**Fig. 11.** *Davidiella allicina* (F-09810, UPS-FRIES, holotype). Ascus and ascospores. Scale bar = 10  $\mu$ m. P.W. Crous del.



**Fig. 12.** *Cladosporium bruhnei* (CPC 12211) and its teleomorph *Davidiella allicina*. A–B. Macronematous conidiophores. C. Conidial chains. D. Micronematous conidiophore. E. Ascomata of the teleomorph formed on the host. F–G. Asci. Scale bars: A–B, D, F = 10  $\mu$ m, E = 200  $\mu$ m.

**Notes:** *Cladosporium bruhnei* proved to be an additional component of the herbarum complex. The species resembles *C. herbarum* s. str. as already stated by Linder (1947), but possesses consistently narrower conidia, usually 2.5–5  $\mu$ m wide, and the conidiophores often form only a single apical swelling. The species was described by Bruhne (*l.c.*) as *Hormodendrum hordei* from Germany but type material could not be located. Linder (1947) examined No. 1481a-5 (Canada, N. Quebec, Sugluk, on *Elymus arenarius* var. *villosus*, 31 Jul. 1936, E. Meyer), presumably in the National Museum, and stated that this specimen agreed well with the description and illustration given by Bruhne (*l.c.*). Although the species occurs on numerous substrates and is widely distributed, it has not yet been recognised as a distinct species since it has probably been interpreted as a narrow variant of *C. herbarum*.

Based on morphology and DNA sequence data, the CBS strain CBS 177.71 chosen by Prasil & de Hoog (1988) as representative living strain of *C. herbarum*, rather clusters together with isolates of *C. bruhnei*. The strain CBS 813.71 is an albino mutant of the latter species as it does not appear to contain colour pigment. Furthermore, all isolates from humans treated until now as *C. herbarum* proved to be conspecific with the narrow-spored *C. bruhnei*.

Although *Davidiella tassiana* (ascospores 17–25  $\times$  6–8.5  $\mu$ m, RO) was treated as synonymous to *D. allicina* (ascospores 20–27  $\times$  6–7  $\mu$ m, UPS) in Aptroot (2006), they differ in apical ascospore

taper, with ascospores of *D. allicina* being acutely rounded, while those of *D. tassiana* are obtusely rounded. The same ascospore taper was also observed in the teleomorph of *C. bruhnei*, and thus the name *D. allicina* is herewith linked to *C. bruhnei*, which is distinct from *C. herbarum*, having *D. tassiana* as teleomorph.

***Cladosporium herbaroides*** K. Schub., Zalar, Crous & U. Braun, sp. nov. MycoBank MB504574. Figs 13–15.

**Etymology:** Refers to its morphological similarity to *Cladosporium herbarum*.

Differt a *Cladosporio herbaro* conidiis polymorphis, 3–33  $\times$  (2–)3–6(–7)  $\mu$ m, postremo latioribus, (3.5–)5–9(–11)  $\mu$ m, fuscis et crassitunicatis; et a *Cladosporio macrocarpo* conidiophoris leniter angustioribus, 3–5  $\mu$ m latis, nodulis angustioribus, 5–8  $\mu$ m latis.

**Mycelium** branched, (1–)2–8  $\mu$ m wide, septate, often with small swellings and constrictions, subhyaline to pale brown or pale olivaceous-brown, smooth or almost so to somewhat verruculose, walls unthickened or almost so. **Conidiophores** macronematous and micronematous, arising lateral from plagiotropous hyphae or terminally from ascending hyphae. **Macronematous conidiophores** erect, straight to slightly flexuous, often geniculate, nodulose, with unilateral or multilateral swellings, often numerous swellings in short succession giving them a gnarled appearance, often forming somewhat protruding or prolonged lateral swellings or a branch-



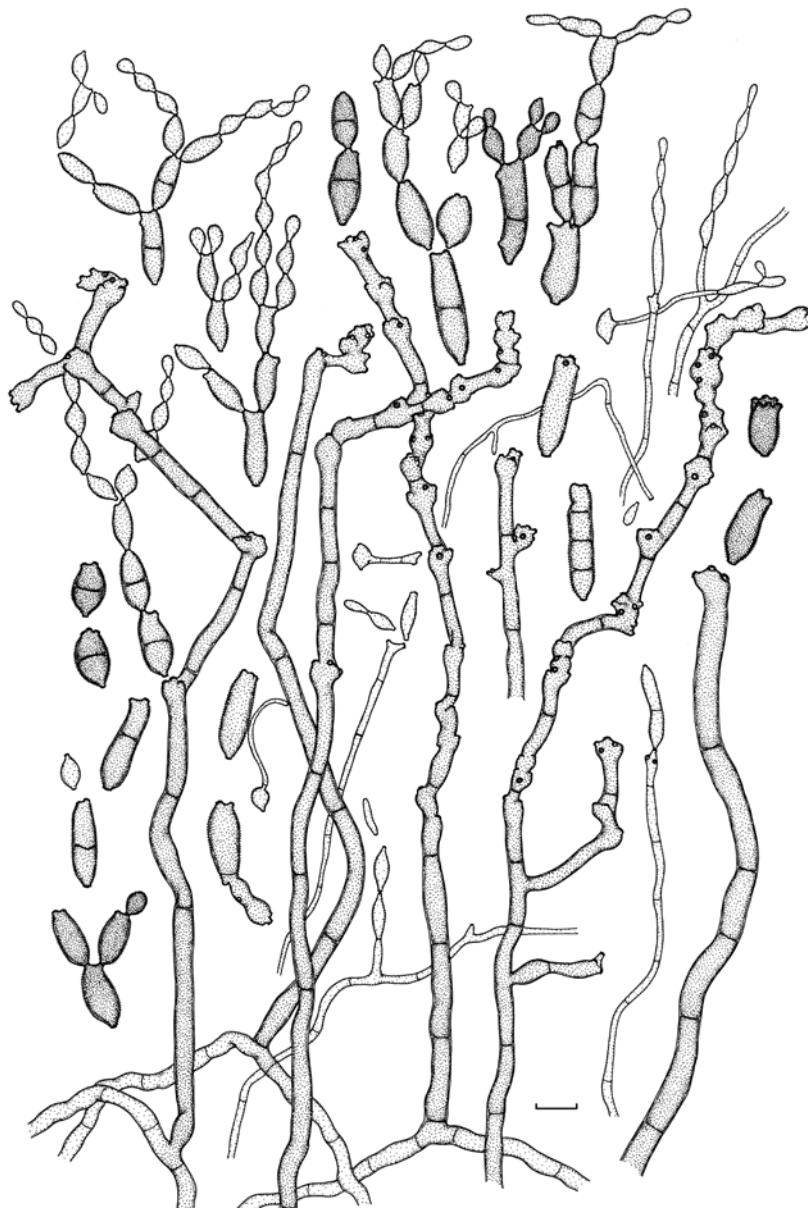
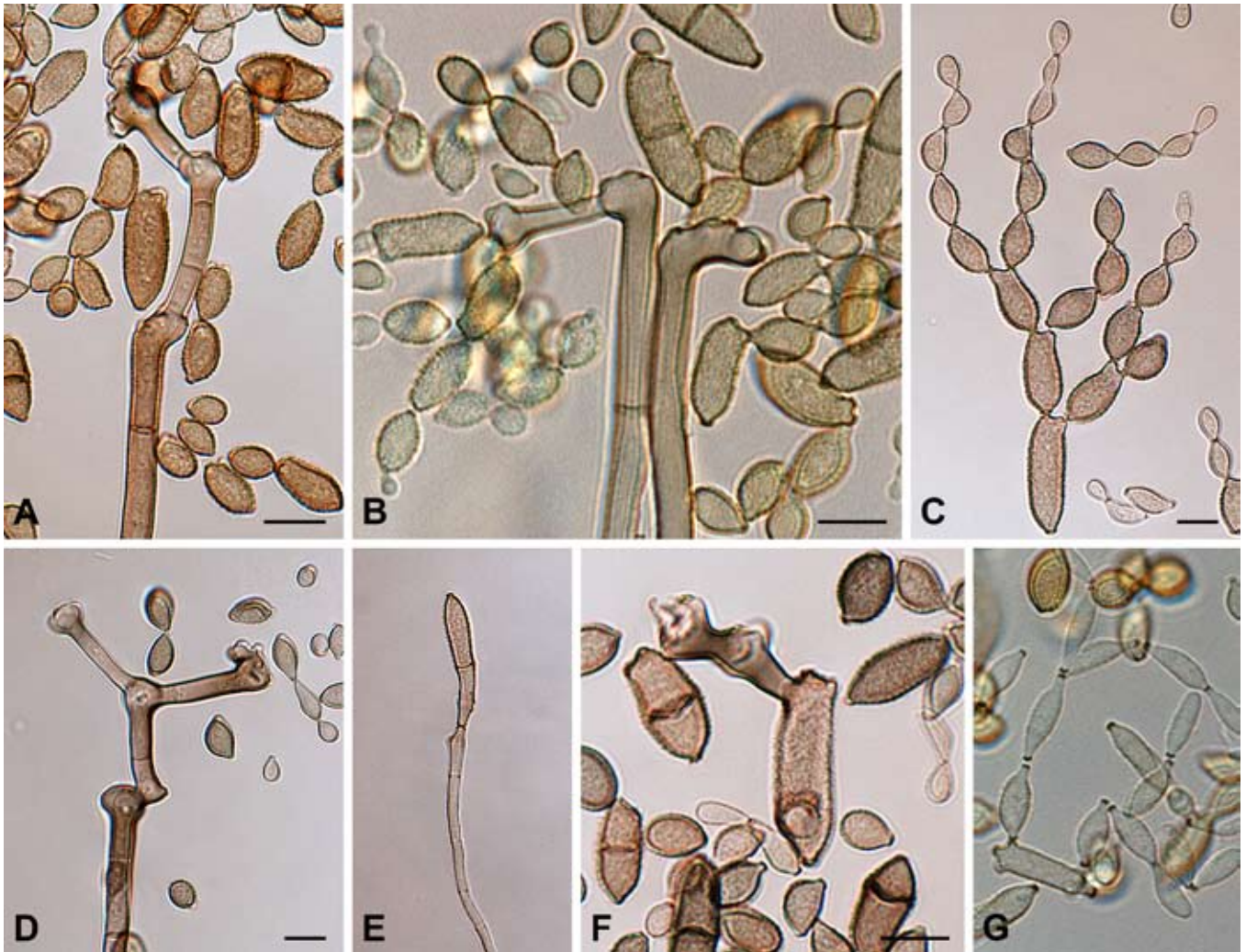


Fig. 13. *Cladosporium herbaroides* (CPC 12052). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

like prolongation below the terminal swelling (due to sympodial proliferation), unbranched or sometimes branched, 30–230  $\mu\text{m}$  long or even longer, 3–5  $\mu\text{m}$  wide, swellings 5–8  $\mu\text{m}$  wide, septate, not constricted at septa, pale to medium olivaceous-brown, smooth or almost so, walls slightly thickened. *Conidiogenous cells* integrated, terminal or intercalary, cylindrical, usually nodulose to nodose forming distinct swellings, sometimes geniculate, 15–55  $\mu\text{m}$  long, with numerous conidiogenous loci usually confined to swellings or situated on small lateral shoulders, sometimes on the top of short peg-like prolongations or denticles, loci protuberant, 1–2  $\mu\text{m}$  diam, thickened and darkened-refractive. *Micronematous conidiophores* much shorter, narrower, paler, neither nodulose nor geniculate, arising laterally from plagiotropous hyphae, often only as short lateral denticles or branchlets of hyphae, erect, straight, conical to cylindrical, unbranched, 3–65  $\times$  2–3  $\mu\text{m}$ , mostly aseptate, sometimes up to five septa, subhyaline, smooth, walls unthickened. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, conidiogenous loci solitary or sometimes as sympodial clusters of pronounced denticles, protuberant, 1–1.5  $\mu\text{m}$  diam, thickened and somewhat darkened-refractive. *Conidia* polymorphous, two main morphological types recognisable, formed

by the two different types of conidiophores, conidia formed by macronematous conidiophores catenate, in branched chains, straight to slightly curved, subglobose, obovoid, limoniform, ellipsoid to cylindrical, 3–33  $\times$  (2–)3–6(–7)  $\mu\text{m}$  [av.  $\pm$  SD, 14.5 ( $\pm$  7.9)  $\times$  5.2 ( $\pm$  1.2)  $\mu\text{m}$ ], 0–2(–3)-septate, sometimes slightly constricted at septa, septa median or somewhat in the lower half, pale to medium olivaceous-brown, verruculose to verrucose (granulate under SEM), walls slightly thickened, with up to three rarely four distal scars, with age becoming medium or even dark brown (chocolate brown), wider and more thick-walled, 5.5–33  $\times$  (3.5–)5–9(–11)  $\mu\text{m}$  [av.  $\pm$  SD, 14.4 ( $\pm$  6.9)  $\times$  7.2 ( $\pm$  1.9)  $\mu\text{m}$ ], walls up to 1  $\mu\text{m}$  thick, hila protuberant, 0.8–2(–2.5)  $\mu\text{m}$  diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring. *Conidia* formed by micronematous conidiophores paler and narrower, mostly formed in unbranched chains, sometimes in branched chains with up to three distal hila, straight to slightly curved, limoniform, narrowly fusiform, almost filiform to subcylindrical, 10–26(–35)  $\times$  2–3.5  $\mu\text{m}$  [av.  $\pm$  SD, 15.6 ( $\pm$  6.2)  $\times$  2.9 ( $\pm$  0.5)  $\mu\text{m}$ ], 0–1(–3)-septate, subhyaline to pale brown, almost smooth to minutely verruculose, walls unthickened, hila protuberant, 1–1.5  $\mu\text{m}$  diam, thickened and somewhat darkened-refractive.



**Fig. 14.** *Cladosporium herbaroides* (CPC 12052). A–B, D. Macronematous conidiophores. C. Conidial chain. E. Microneumatous conidiophore. F. Microcyclic conidiogenesis. G. Conidia formed by microneumatous conidiophores. Scale bars = 10  $\mu\text{m}$ .

**Cultural characteristics:** Colonies on PDA attaining 23 mm diam after 14 d at 25 °C, grey-olivaceous to olivaceous, olivaceous-grey reverse, velvety, margin regular, entire edge, narrow, feathery, aerial mycelium abundantly formed, loose, with age covering large parts of the colony, woolly, growth flat with somewhat elevated colony centre, folded, regular, deep into the agar, with few prominent exudates, sporulation profuse. Colonies on MEA attaining 24 mm diam after 14 d at 25 °C, grey- to greenish olivaceous, olivaceous-grey or iron-grey reverse, velvety to powdery, margin narrow, colourless, entire edge, somewhat feathery, aerial mycelium pale olivaceous-grey, sparse, growth convex, radially furrowed, folded in the colony centre, without prominent exudates, sporulating. Colonies on OA attaining 23 mm diam after 14 d at 25 °C, grey-olivaceous, margin more or less regular, entire edge, colourless, somewhat feathery, aerial mycelium whitish to smoke grey, at first sparse, later more abundantly formed, growth flat, without exudates, sporulation profuse.

**Specimen examined:** Israel, from hypersaline water of Eilat salterns, 2004, coll. N. Gunde-Cimerman, isol. M. Ota, CBS-H 19858, **holotype**, isotype HAL 2025 F, culture ex-type CBS 121626 = EXF-1733 = CPC 12052.

**Substrate and distribution:** Hypersaline water; Israel.

**Notes:** *Cladosporium herbaroides* is morphologically similar to *C. herbarum* but differs in having somewhat longer conidia becoming wider, darker and even more thick-walled with age [at first conidia 3–33  $\times$  (2–)3–6(–7)  $\mu\text{m}$ , with age (3.5–)5–9(–11)  $\mu\text{m}$  wide]. Besides

that, the species often produces a second conidial type formed on microneumatous conidiophores, giving rise to unbranched conidial chains which are almost filiform, limoniform, narrowly fusiform to subcylindrical, much narrower and paler than the ones formed by macroneumatous conidiophores, 10–26(–35)  $\times$  2–3.5  $\mu\text{m}$ . In *C. herbarum*, conidia formed by microneumatous conidiophores do not occur as frequently as in *C. herbaroides*, and differ in being often clavate and somewhat wider, up to 4(–5)  $\mu\text{m}$  wide. *Cladosporium macrocarpum* is easily distinguishable by having somewhat wider conidiophores (3–)4–6  $\mu\text{m}$ , with distinctly wider swellings, 5–10  $\mu\text{m}$  wide, and the conidia are usually (3–)5–9(–10)  $\mu\text{m}$  wide.

***Cladosporium herbarum*** (Pers. : Fr.) Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37. 1816: Fr., Syst. mycol. 3(2): 370. 1832. Figs 16–19.

**Basionym:** *Dematium herbarum* Pers., Ann. Bot. (Usteri) 11: 32. 1794: Fr., Syst. mycol. 3(2): 370. 1832.

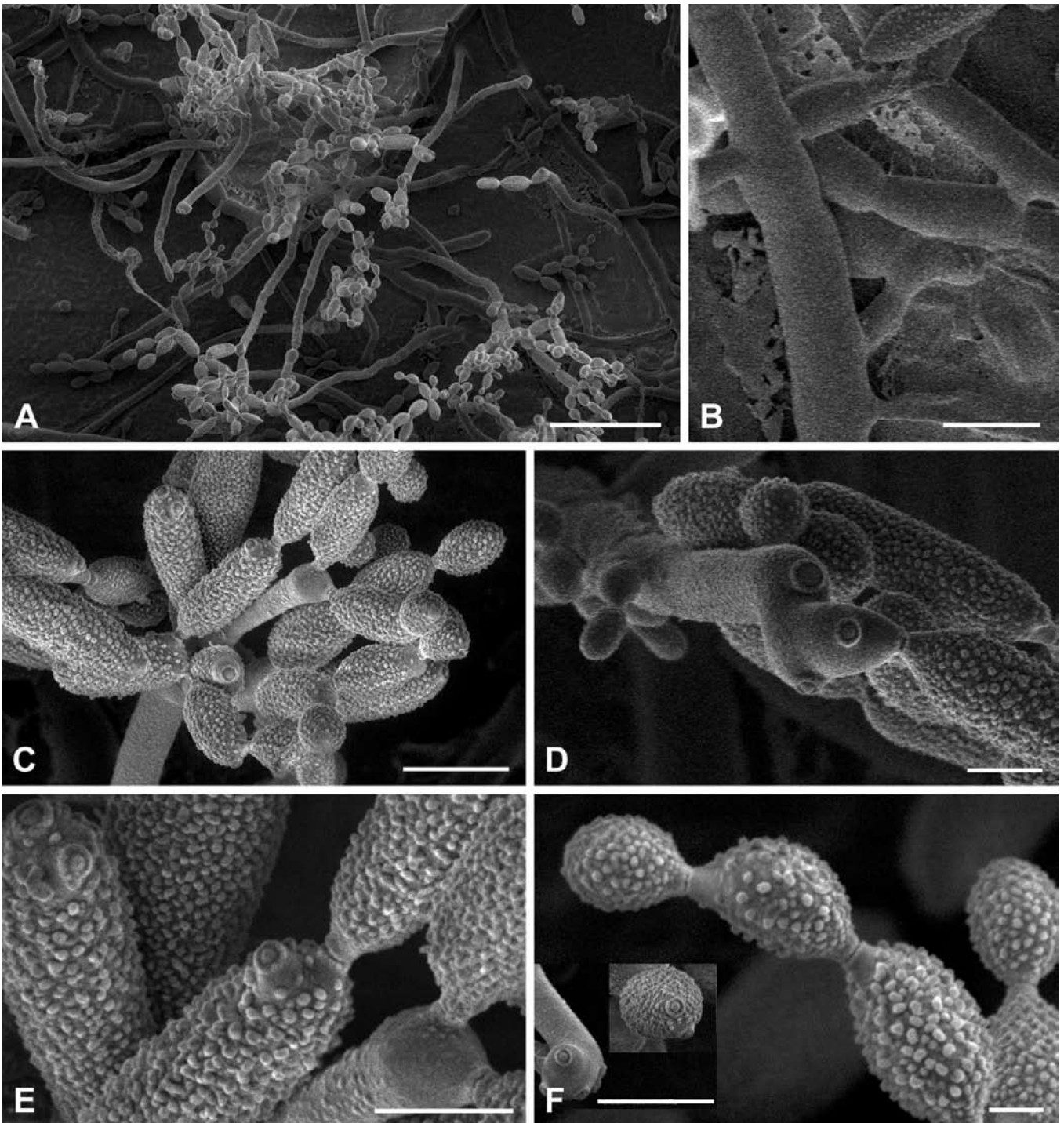
= *Dematium epiphyllum* var. ( $\beta$ ) *chionanthi* Pers., Mycol. eur. 1: 16. 1822, **syn. nov.**

For additional synonyms see Dugan *et al.* (2004), Schubert (2005).

**Teleomorph:** *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 2: 8. 2003.

**Basionym:** *Sphaerella tassiana* De Not., Sferiacei Italici 1: 87. 1863.

= *Mycosphaerella tassiana* (De Not.) Johanson, Öfers. Förh. Kongl. Svenska Vetensk.-Akad. 41: 167. 1884.



**Fig. 15.** *Cladosporium herbaroides* (CPC 12052). A. Overview of the growth characteristics of this fungus. Broad hyphae run over the surface of the agar, and possibly give rise to conidiophore branches. The conidiophores of this fungus can be rather long, resembling aerial hyphae. Clusters of conidia are clearly visible in this micrograph. B. The very wide surface hyphae can anastomose. C. Conidiophore with secondary ramoconidia and conidia. Note the variation in scar size. D. A very elaborate, complex conidiophore with different scars of variable size, one being more than 2  $\mu\text{m}$  wide! E. Details of secondary ramoconidia and hila. Note the rather strong ornamentation in which smaller "particles" are between larger ones. F. Three conidia in a row. Note the scar formation in the chain and the reduction of the size of the cells throughout the spore-chain. The inset shows the resemblance of the scars on a conidiophore and on a secondary ramoconidium. Scale bars: A = 50  $\mu\text{m}$ , B–C, F (inset) = 10  $\mu\text{m}$ , D–E = 5  $\mu\text{m}$ , F = 2  $\mu\text{m}$ .

*Ascomata* pseudothecial, black, globose, erumpent to superficial, up to 200  $\mu\text{m}$  diam, with 1(–3) short, periphysate ostiolar necks; wall consisting of 3–6 layers of medium red-brown *textura angularis*. *Asci* fasciculate, bitunicate, sessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 65–85  $\times$  13–17  $\mu\text{m}$ . *Pseudoparaphyses* absent in host material, but remnants observed when studied in culture, hyaline, septate, subcylindrical, anastomosing, 3–4  $\mu\text{m}$  wide. *Ascospores* tri- to multiseriate, overlapping, hyaline, with irregular luminal inclusions, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse

ends, widest near middle of apical cell, medianly 1-septate, not to slightly constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (17–)20–23(–25)  $\times$  (6–)7(–8)  $\mu\text{m}$ ; becoming brown and verruculose in asci. *Ascospores* germinating after 24 h on MEA from both ends, with spore body becoming prominently constricted at the septum, but not distorting, up to 7  $\mu\text{m}$  wide, hyaline to pale brown and appearing somewhat verruculose, enclosed in a mucoid sheath, with germ tubes being irregular, somewhat nodular.

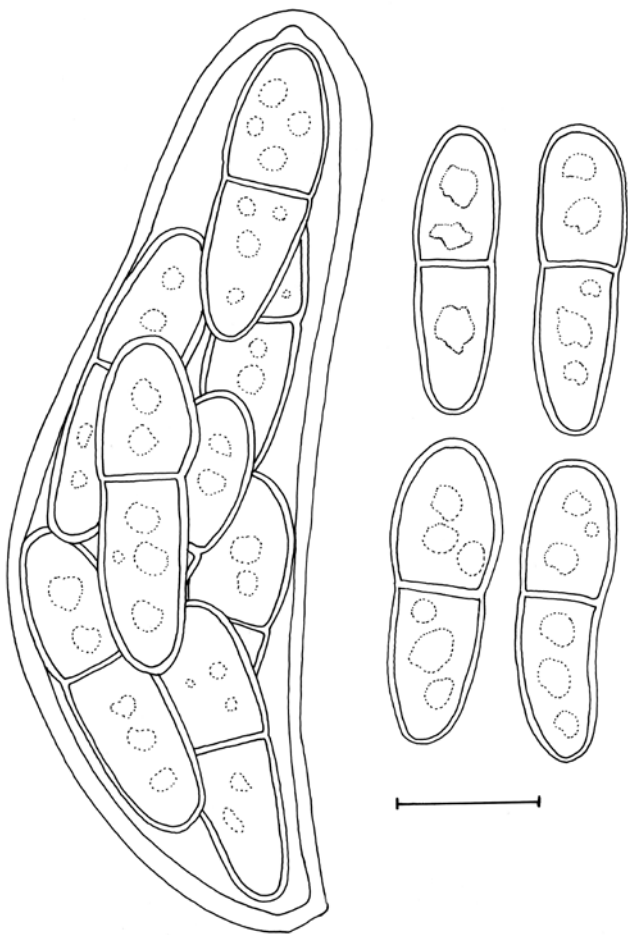


Fig. 16. *Davidiella tassiana* (RO, holotype). Ascus and ascospores. Scale bar = 10  $\mu\text{m}$ . P.W. Crous del.

*Mycelium* superficial, loosely branched, (0.5–)1–5  $\mu\text{m}$  wide, septate, sometimes constricted at septa, hyaline, subhyaline to pale brown, smooth or almost so to verruculose or irregularly rough-walled, sometimes appearing irregular in outline due to small swellings and constrictions, walls unthickened to somewhat thickened, cell lumen appearing to be granular. *Conidiophores* both macro- and micronematous, arising laterally from plagiotropous hyphae or terminally from ascending hyphae. *Macronematous conidiophores* erect, straight to flexuous, somewhat geniculate-sinuous, nodulose to nodose with unilateral or multilateral swellings, with a single to numerous swellings in short succession giving the stalk a knotty/gnarled appearance, unbranched or occasionally branched, up to three times, sometimes with a lateral branch-like proliferation below or at the apex, 10–320  $\times$  3.5–5  $\mu\text{m}$ , swellings 5–8(–9)  $\mu\text{m}$  wide, pluriseptate, septa sometimes constricted when formed after a node, pale to medium brown, older ones almost dark brown, paler towards the apex, smooth or minutely verruculose, walls thickened, sometimes even two-layered. *Conidiogenous cells* integrated, terminal or intercalary, nodulose to nodose, with a single or up to five swellings per cell, 10–24  $\mu\text{m}$  long, proliferation sympodial, with several conidiogenous loci confined to swellings, mostly situated on small lateral shoulders, more or less protuberant, broadly truncate to slightly convex, 1.5–2.5  $\mu\text{m}$  diam, thickened and somewhat darkened-refractive. *Micronematous conidiophores* hardly distinguishable from hyphae, sometimes only as short lateral outgrowth with a single apical scar, short, conical to almost filiform or narrowly cylindrical, non-nodulose, not geniculate, unbranched, 5–120  $\times$  1.5–3(–4)  $\mu\text{m}$ , pluriseptate, not constricted at septa, cells usually very short, 5–15  $\mu\text{m}$  long, subhyaline to pale brown,

almost smooth to minutely verruculose or irregularly rough-walled, sometimes forming clavate conidia, up to 33  $\mu\text{m}$  long, 0–2-septate. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, narrowly cylindrical or filiform, with a single or two loci. *Conidia* catenate, in unbranched or loosely branched chains with branching mostly occurring in the lower part of the chain, straight to slightly curved, small terminal conidia without distal hilum obovoid, 4–10  $\times$  3–5(–6)  $\mu\text{m}$  [av.  $\pm$  SD, 7.8 ( $\pm$  1.9)  $\times$  4.7 ( $\pm$  0.9)  $\mu\text{m}$ ], aseptate, intercalary conidia with a single or sometimes up to three distal hila limoniform, ellipsoid to subcylindrical, 6–16  $\times$  4–6  $\mu\text{m}$  [av.  $\pm$  SD, 12.4 ( $\pm$  1.6)  $\times$  5.3 ( $\pm$  0.6)  $\mu\text{m}$ ], 0–1-septate, *secondary ramoconidia* with up to four distal hila, ellipsoid to cylindrical-oblong, 12–25(–35)  $\times$  (3–)5–7(–9)  $\mu\text{m}$  [av.  $\pm$  SD, 18.8 ( $\pm$  4.5)  $\times$  6.2 ( $\pm$  0.9)  $\mu\text{m}$ ], 0–1(–2)-septate, rarely with up to three septa, sometimes distinctly constricted at the septum, septum median or somewhat in the upper or lower half, pale greyish brown or brown to medium brown or greyish brown, minutely verruculose to verruculose, walls slightly to distinctly thickened, guttulate to somewhat granular, usually only slightly attenuated towards apex and base, apex obtuse or slightly truncate, towards the base sometimes distinctly attenuated with hila situated on short stalk-like prolongations, hila slightly to distinctly protuberant, truncate to slightly convex, (0.8–) 1–2.5(–3)  $\mu\text{m}$  wide, 0.5–1  $\mu\text{m}$  high, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occurring, conidia forming micro- and macronematous secondary conidiophores.

*Cultural characteristics*: Colonies on PDA reaching 19–37 mm diam after 14 d at 25  $^{\circ}\text{C}$ , grey-olivaceous to olivaceous-grey, whitish to smoke-grey or pale olivaceous-grey due to abundant aerial mycelium, velvety, reverse olivaceous-grey or iron-grey, margin almost colourless, regular, entire edge, glabrous to feathery, aerial mycelium abundant mainly in the colony centre, dense, felty, woolly, sometimes becoming somewhat reddish brown, fawn coloured, growth regular, flat to low convex with an elevated colony centre, sometimes forming few large prominent exudates, sporulation profuse. Colonies on MEA reaching 17–37 mm diam after 14 d at 25  $^{\circ}\text{C}$ , smoke-grey to pale olivaceous-grey towards margin, olivaceous-grey to iron-grey reverse, velvety, margin white, entire edge to slightly undulate, aerial mycelium abundant, dense, fluffy to felty, growth low convex or raised, radially furrowed, folded and wrinkled in the colony centre, without prominent exudates but sporulating. Colonies on OA reaching 12–28 mm diam after 14 d at 25  $^{\circ}\text{C}$ , olivaceous-grey to iron-grey, due to abundant aerial mycelium pale olivaceous-grey, olivaceous-grey reverse, margin narrow, more or less undulate, white, aerial mycelium white, loose to dense, high, fluffy to felty, covering large parts of the colony, growth flat to low convex, without prominent exudates, sporulating.

*Specimens examined*: *Sine loco, sine dato*, L 910.225-733, **lectotype** of *C. herbarum*, selected by Prasil & de Hoog, 1988. *Sine loco*, on leaves of *Chionanthus* sp. (Oleaceae), L 910.255-872 = L-0115833, **holotype** of *Dematium epiphyllum* var. ( $\beta$ ) *chionanthi*. **Netherlands**, Wageningen, isolated from *Hordeum vulgare* (Poaceae), 2005, P.W. Crous, CBS-H 19853, **epitype designated here** of *C. herbarum* and *D. tassiana*, isoeotype HAL 2022 F, ex-type cultures, CPC 12177 = CBS 121621, CPC 12178–12179, 12181, 12183. **Italy**, on upper and lower surface of dead leaves of *Carex nigra* ["*fusca*"] (Cyperaceae), Tassi no. 862, RO, **holotype** of *Davidiella tassiana*. **U.S.A.**, Colorado, San Juan Co., above Little Molas Lake, isolated from stems of *Delphinium barbeyi* (Ranunculaceae), 12 Sep. 2004, A. Ramaley, CBS-H 19868 (teleomorph), single ascospore isolates, CBS 121622 = CPC 11600, CPC 11601–11604.

*Substrate and distribution*: On fading and decaying plant material, on living leaves (phylloplane fungus), as secondary invader, as an endophyte, isolated from air, soil, foodstuffs, paints, textiles and numerous other materials; cosmopolitan.

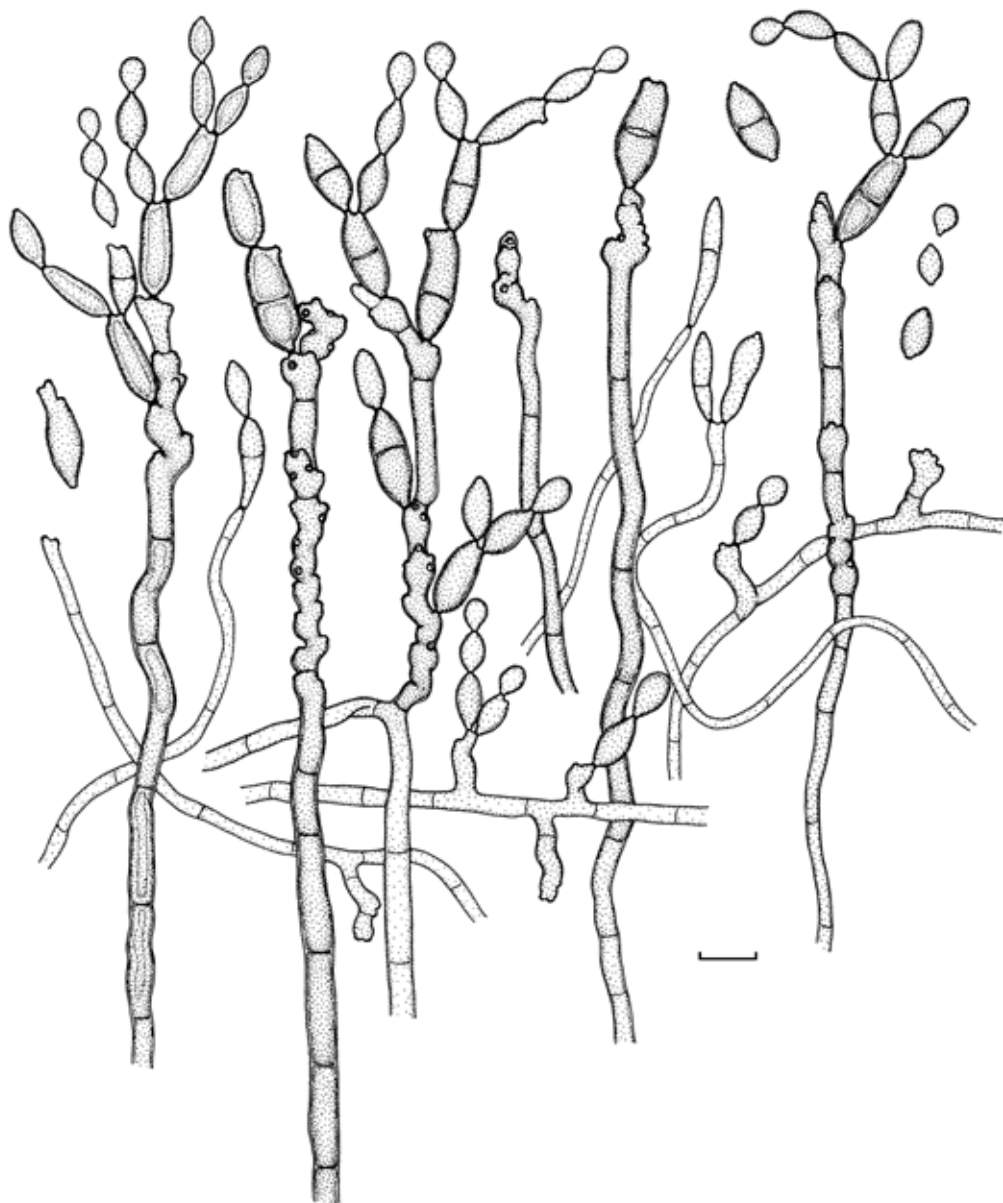


Fig. 17. *Cladosporium herbarum* (CPC 11600). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

*Literature:* de Vries (1952: 71), Hughes (1958: 750), Ellis (1971: 313), Domsch *et al.* (1980: 204), Sivanesan (1984: 225), Ellis & Ellis (1985: 290, 468, 1988: 168), Prasil & de Hoog (1988), Wang & Zabel (1990: 202), McKemy & Morgan-Jones (1991), Dugan & Roberts (1994), David (1997: 59), Ho *et al.* (1999: 129), de Hoog *et al.* (2000: 587), Samson *et al.* (2000: 110), Samson *et al.* (2001).

*Notes:* De Vries (1952) incorrectly selected a specimen of Link's herbarium at herb. B as lectotype. Prasil & de Hoog (1988) discussed this typification and designated one of Persoon's original specimens as lectotype in which *C. herbarum* could be recognised. The latter material, which is in poor condition, could be re-examined within the course of these investigations and showed conidia agreeing with the current species concept of *C. herbarum* being (6–)9.5–14.5(–21)  $\times$  (5–)6–7(–8)  $\mu\text{m}$ . Since the identity of the strain CBS 177.71 chosen by Prasil & de Hoog (1988) as representative living strain of *C. herbarum* could not be corroborated, an epitype with a living ex-epitype culture is designated. The holotype specimen of *D. tassiana* (RO) is morphologically similar to that observed on the epitype of *C. herbarum*, having ascospores which are (17–)21–

23(–25)  $\times$  (6–)7–8(–8.5)  $\mu\text{m}$ , turning brown and verruculose in asci with age. However, no hamathecial remnants were observed in ascomata *in vivo*.

The connection to the teleomorph *D. tassiana* could be confirmed, which is in agreement with the findings of von Arx (1950) and Barr (1958). Ascospore isolates formed the typical *C. herbarum* anamorph in culture, and these anamorph cultures developed some immature fruiting bodies within the agar. When inoculated onto water agar plates with nettle stems, numerous ascomata with viable ascospores were formed in culture.

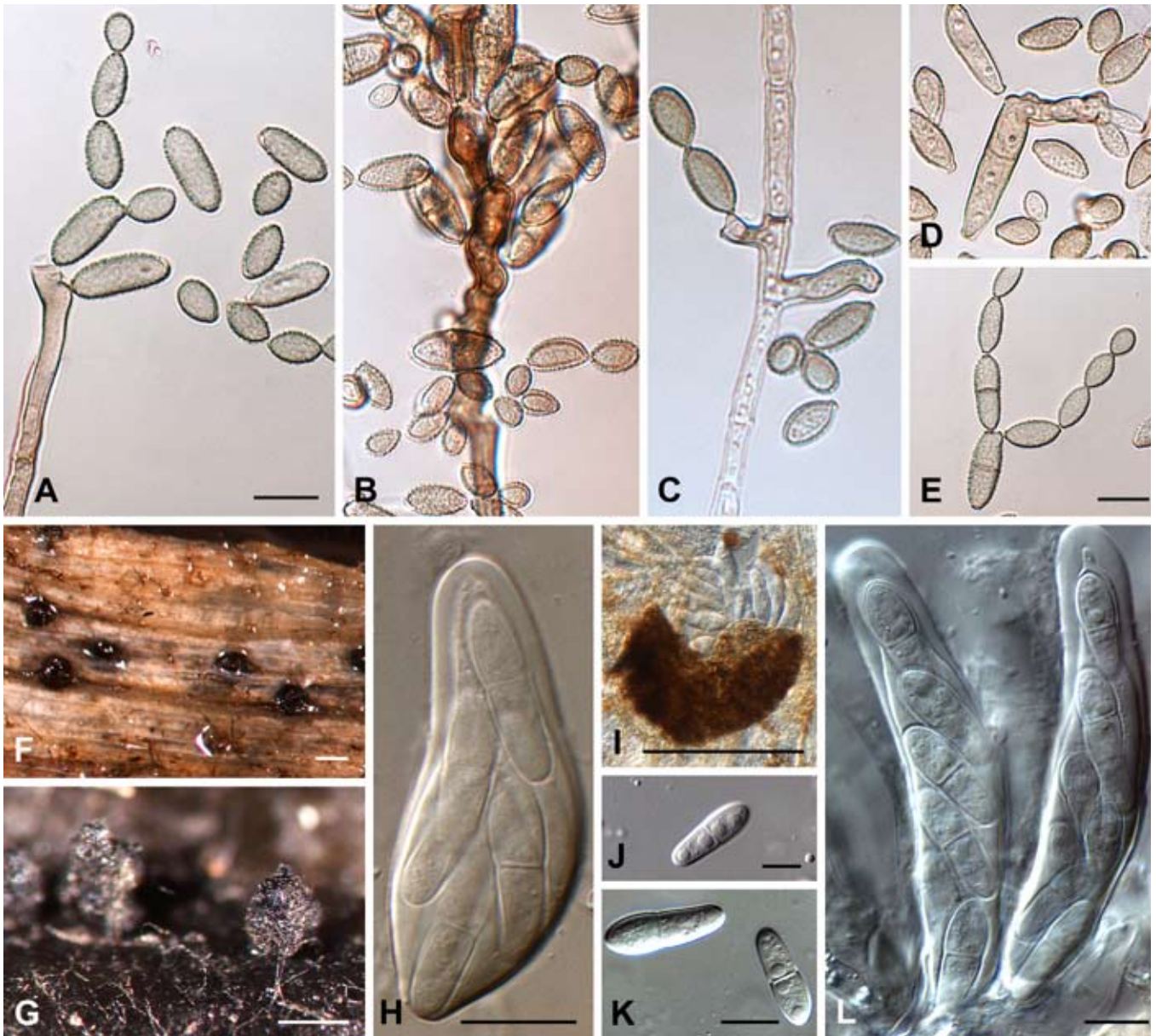
***Cladosporium iridis*** (Fautrey & Roum.) G.A. de Vries, *Contr. Knowl. Genus Cladosporium*: 49. 1952. Figs 20–21.

*Basionym:* *Scolicotrichum iridis* Fautrey & Roum., *Rev. Mycol. (Toulouse)* 13: 82. 1891.

$\equiv$  *Heterosporium iridis* (Fautrey & Roum.) J.E. Jacques, *Contr. Inst. Bot. Univ. Montréal* 39: 18. 1941.

For additional synonyms see Dugan *et al.* (2004).

*Teleomorph:* ***Davidiella macrospora*** (Kleb.) Crous & U. Braun, *Mycol. Progr.* 2: 10. 2003.



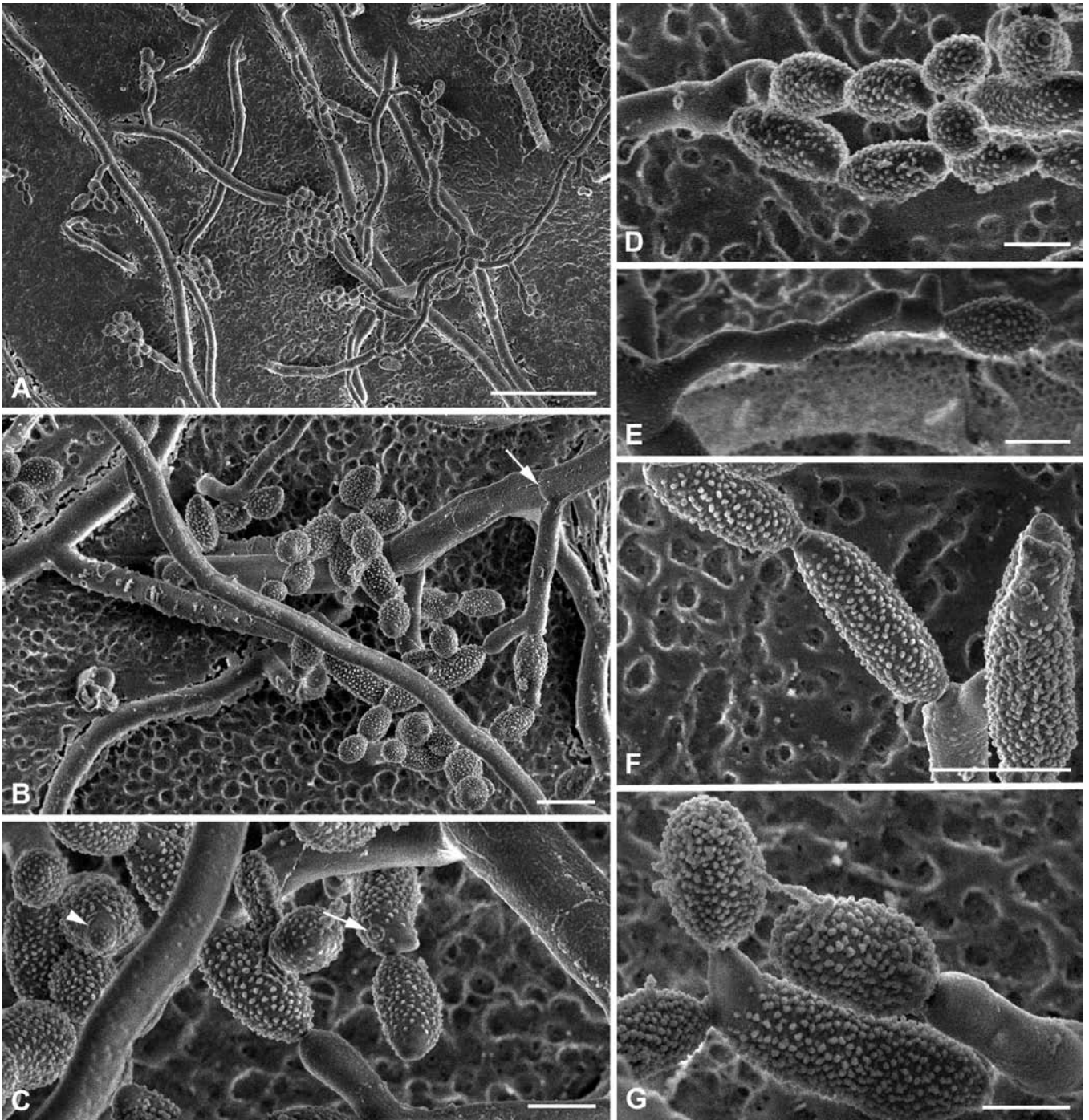
**Fig. 18.** *Cladosporium herbarum* (CPC 11600) and its teleomorph *Davidiella tassiana* (from the host and CPC 12181). A–B. Macronematous conidiophores. C. Micronematous conidiophore. D. Microcyclic conidiogenesis. E. Conidial chain. F. Ascomata on the leaf. G. Ascomata formed in culture on nettle stems. H–I. Asci on the host. J–K. Ascospores in culture. L. Asci in culture. Scale bars: A, E, H, J–L = 10  $\mu$ m, F–G, I = 200  $\mu$ m.

**Basionym:** *Didymellina macrospora* Kleb., Ber. Deutsch. Bot. Ges. 42: 60, 1924. 1925.

$\equiv$  *Mycosphaerella macrospora* (Kleb.) Jørst., Meld. Stat. Plantepatol. Inst. 1: 20. 1945.

**Mycelium** branched, 2–8  $\mu$ m wide, septate, not constricted at the septa, hyaline to pale brown, smooth, walls slightly thickened, sometimes guttulate. **Conidiophores** very long, usually terminally arising from ascending hyphae, erect to subdecumbent, slightly to distinctly flexuous, geniculate-sinuous, usually several times, subnodulose due to geniculate, sympodial proliferation forming swollen lateral shoulders, unbranched, rarely branched, up to 720  $\mu$ m long, 6–11  $\mu$ m wide, swellings 8–11(–14)  $\mu$ m wide, pluriseptate, often very regularly septate, not constricted at the septa, pale to medium olivaceous-brown, somewhat paler towards the apex, smooth to minutely verruculose, walls only slightly thickened. **Conidiogenous cells** integrated, terminal as well as intercalary, cylindrical-oblong, 15–55  $\mu$ m long, proliferation percurrent to sympodial, usually with a single geniculation forming laterally swollen shoulders often below a septum, conidiogenous loci confined to swellings, usually one locus per swelling, rarely

two, protuberant, (2–)2.5–4  $\mu$ m diam, somewhat thickened and darkened-refractive. **Conidia** solitary, sometimes in short, unbranched chains, straight to curved, young conidia pyriform to subcylindrical, connection between conidiophore and conidium being rather broad, subhyaline to pale olivaceous-brown, walls slightly thickened, then enlarging and becoming more thick-walled, cylindrical-oblong, soleiform with age, both ends rounded, usually with a slightly to distinctly bulbous base, visible from a very early stage, but broadest part often towards the apex not at the base, (18–)30–75(–87)  $\times$  (7–)10–16(–18)  $\mu$ m [av.  $\pm$  SD, 53.3 ( $\pm$  17.8)  $\times$  12.6 ( $\pm$  2.2)  $\mu$ m], (0–)2–6(–7)-septate, usually not constricted at the septa, rarely slightly constricted, septa often becoming sinuous with age, pale to medium olivaceous-brown, sometimes darker, verrucose to echinulate, walls thickened, especially in older conidia, up to 1  $\mu$ m thick, hila protuberant, often stalk-like or conically prolonged, up to 2  $\mu$ m long, (2–)2.5–3.5(–4)  $\mu$ m diam, with age becoming more sessile, sometimes just visible as a thickened plate just below the outer wall layer, especially in distal scars of branched conidia, periclinal rim often distinctly visible, hila somewhat thickened and darkened-refractive; microcyclic conidiogenesis not observed.



**Fig. 19.** *Cladosporium herbarum* (CPC 11600). A. Overview of hyphal growth and conidiophore formation of a colony on SNA. Conidiophores are often formed on very wide (approx. 10 µm), septate hyphae that often grow near the agar surface. B. A more detailed view on colony organisation reveals the ornamented conidia. Note the septum near the conidiophore (arrow). C. Detail of spore ornamentation and hila on a secondary ramoconidium (arrow). Ornamentation is visible during early stages of spore formation (arrow). D. Structure of the conidiophore, illustrating the complex morphology of the spore-forming apparatus. In addition, secondary ramoconidia, conidia, and a hilum on the conidium are visible. E. Complex structure of the spore-forming apparatus. F. Details of secondary ramoconidia with complex scar-pattern on the right cell. G. Details of a secondary ramoconidium giving rise to conidia. Note the lack of ornamentation at the location of spore formation. Scale bars: A = 50 µm, B, F = 10 µm, C–E, G = 5 µm.

**Cultural characteristics:** Colonies on PDA reaching 19–23 mm diam after 14 d at 25 °C, pale greenish olivaceous, smoke-grey to olivaceous-grey due to abundant aerial mycelium, greenish olivaceous to olivaceous reverse, margin broad, regular, entire edge to slightly undulate, feathery, aerial mycelium abundantly formed, felty, fluffy, covering large parts of the colony, mainly in the central parts, high, growth low convex with a somewhat raised colony centre. Colonies on MEA reaching 9–23 mm diam after 14 d at 25 °C, pale olivaceous-grey to olivaceous-grey, olivaceous-grey reverse, felty, margin slightly undulate, white, somewhat raised, aerial mycelium abundant, loose, diffuse, high, growth low convex,

radially furrowed, slightly folded. Colonies on OA reaching 10–19 mm diam after 14 d at 25 °C, olivaceous, margin broad, undulate, white, aerial mycelium white, very high, loose, diffuse, hairy, growth flat, due to the mycelium low convex, without prominent exudates and sporulating on all media.

**Specimens examined:** Isolated from *Iris* sp. (*Iridaceae*), CBS 107.20. **France**, Cote d'Or, Jardin de Noidan, on leaves of *Iris germanica*, Jul. 1880, F. Fautrey, Roumeguère, Fungi Sel. Gall. Exs. No. 5689, PC, **lectotype** of *C. iridis*, selected by David, 1997; K, isolectotype. **Netherlands**, Boterenbrood, isolated from leaves of *Iris* sp., Aug. 1940, CBS-H 19859, **epitype designated here** of *C. iridis*, culture ex-epitype CBS 138.40.

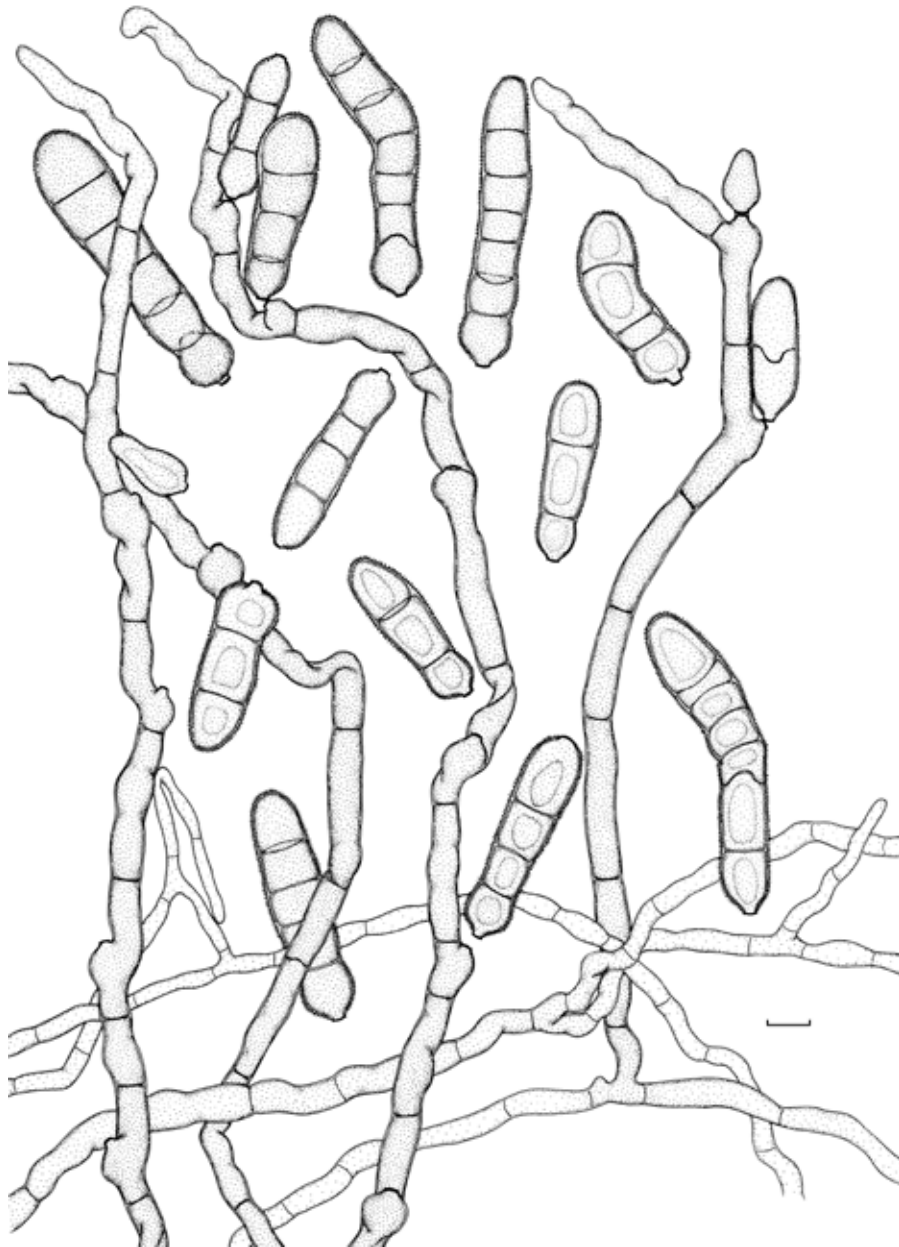


Fig. 20. *Cladosporium iridis* (CBS 138.40). Conidiophores and conidia. Scale bar = 10  $\mu$ m. K. Schubert del.

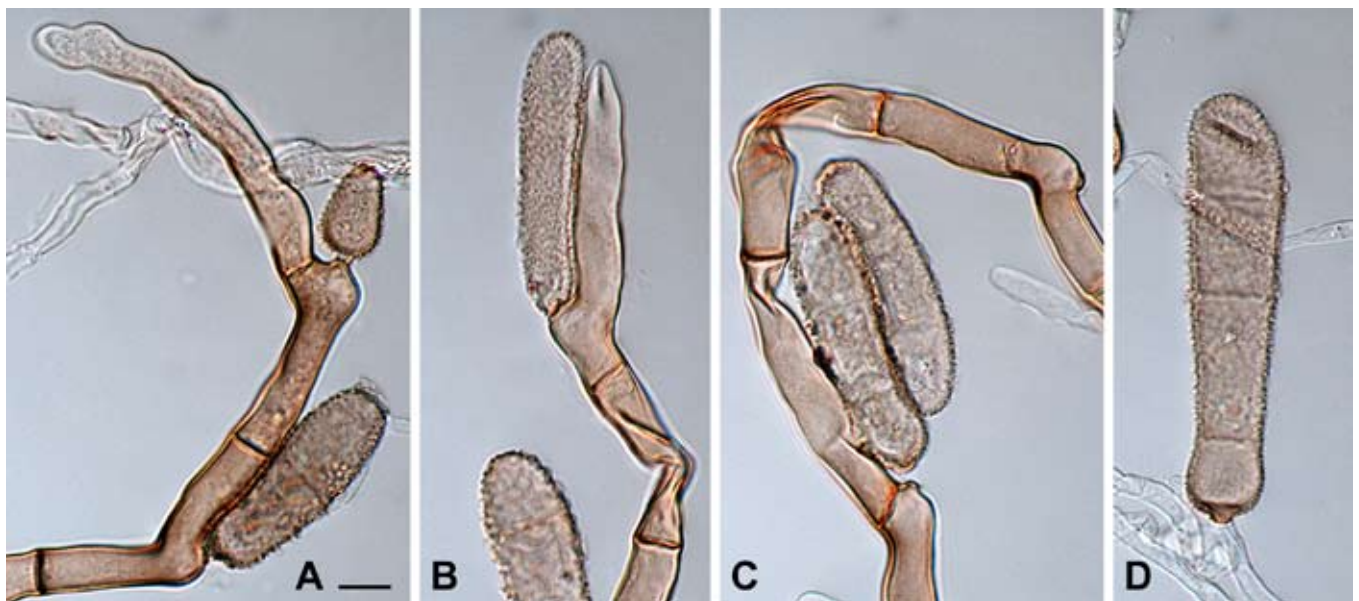


Fig. 21. *Cladosporium iridis* (teleomorph *Davidiella macrospora*) (CBS 138.40). A–C. Conidiophores with conidia. D. Conidium. Scale bar = 10  $\mu$ m.



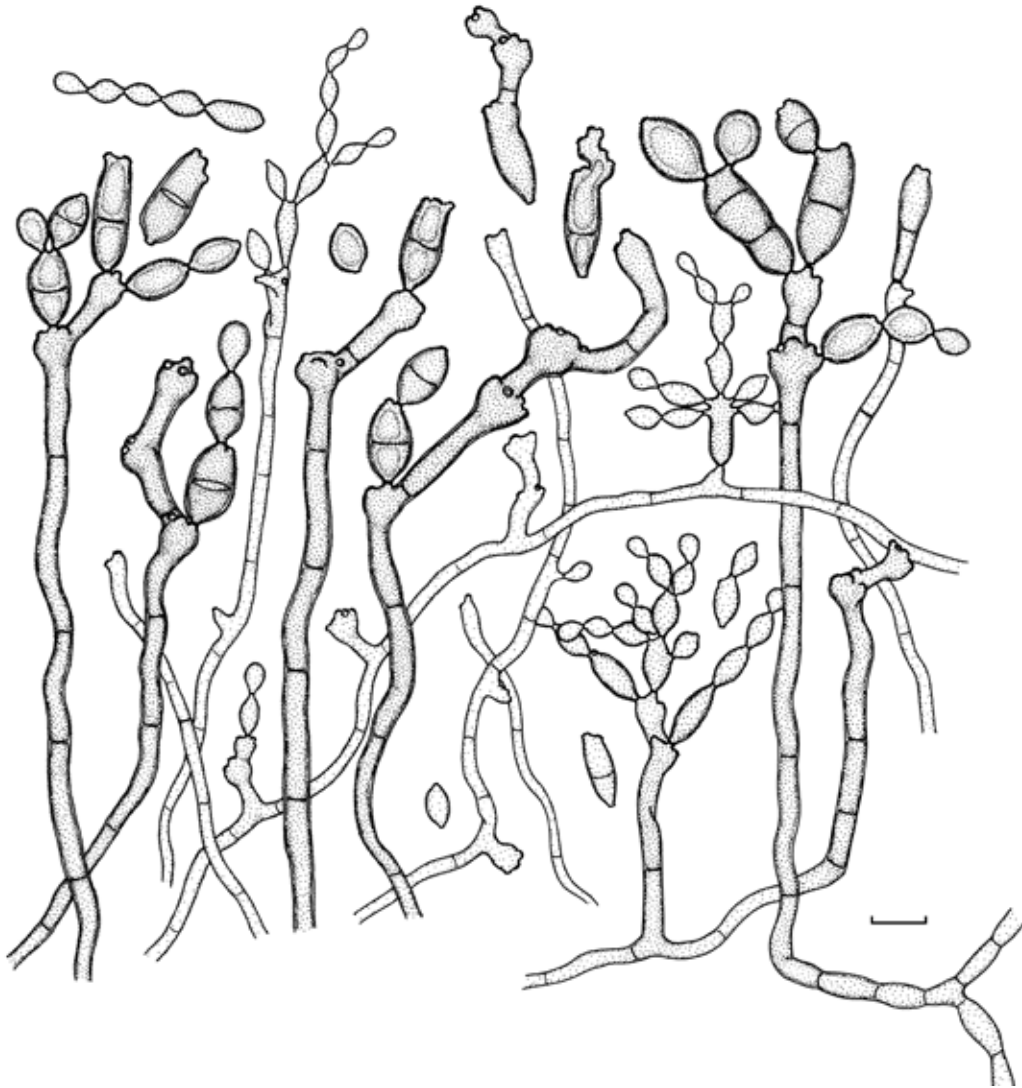


Fig. 22. *Cladosporium macrocarpum* (CBS 299.67). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

**Substrates and distribution:** Leaf spot and blotch of *Iris* spp. including *I. crocea*, *I. florentina*, *I. foetidissima*, *I. germanica*, *I. gueldenstaediana*, *I. kamaonensis*, *I. pallida*, *I. plicata* (= *I. swertii* Hort.), *I. pseudacorus*, *I. pumila*, *I. spuria* ssp. *halophila*, and other species, also on *Belacamanda chinensis* (= *Gemmingia chinensis*), *Hemerocallis fulva*, *Gladiolus gandavensis*; Africa (Algeria, Morocco, South Africa, Zambia, Zimbabwe), Asia (Armenia, Azerbaijan, China, Georgia, India, Iran, Israel, Japan, Kazakhstan, Kirgizstan, Korea, Russia, Turkey, Turkmenistan, Uzbekistan), Australasia (Australia, New Zealand), Europe (Austria, Belgium, Belorussia, Cyprus, Czech Republic, Denmark, Estonia, France, Germany, Great Britain, Greece, Italy, Latvia, Lithuania, Malta, Moldavia, Montenegro, Netherlands, Norway, Poland, Romania, Russia, Serbia, Spain, Sweden, Ukraine), North America (Canada, U.S.A.), Central & South America (Argentina, Chile, Jamaica, Panama, Uruguay).

**Literature:** Ellis (1971: 312), Ellis & Waller (1974), Sivanesan (1984: 222), McKemy & Morgan-Jones (1990), David (1997: 43), Shin *et al.* (1999).

**Notes:** The description of the morphological parameters in culture is based on the isolate sporulating on PDA, since sporulation on SNA was not observed. The conidiophores and conidia *in vivo* are usually wider than in culture [conidiophores (6–)9–15(–17)  $\mu\text{m}$  wide, conidia (11–)15–23(–28)  $\mu\text{m}$ ].

***Cladosporium macrocarpum*** Preuss, in Sturm, Deutsch. Fl. 3(26): 27. 1848. Figs 22–25.

= *Cladosporium herbarum* var. *macrocarpum* (Preuss) M.H.M. Ho & Dugan, in Ho *et al.*, Mycotaxon 72: 131. 1999.

= *Dematium herbarum* var. ( $\beta$ ) *brassicae* Pers., Syn. meth. fung. 2: 699. 1801, **syn. nov.**

= *Dematium graminum* Pers., Mycol. eur. 1: 16. 1822, **syn. nov.**

= *Dematium vulgare* var. ( $\delta$ ) *typharum* Pers., Mycol. eur. 1: 14. 1822, **syn. nov.**

= *Dematium vulgare* var. ( $\beta$ ) *foliorum* Pers., Mycol. eur. 1: 14. 1822, **syn. nov.**

For additional synonyms see Dugan *et al.* (2004), Schubert (2005).

**Teleomorph: *Davidiella macrocarpa*** Crous, K. Schub. & U. Braun, **sp. nov.** MycoBank MB504582.

*Davidiellae tassianae* similis, sed pseudoparaphysisbus prominentibus et ascosporis maioribus, (22–)23–26(–28)  $\times$  (6–)6.5–7(–8)  $\mu\text{m}$ .

**Ascomata** superficial on a small stroma, black, up to 200  $\mu\text{m}$  diam, globose, separate, but developing with 1–3 necks with age; ostioles consisting of pale brown to subhyaline cells, periphysate, with periphysoids growing into the cavity; wall consisting of 3–6 layers of medium brown *textura angularis*. *Pseudoparaphyses* present, hyaline, subcylindrical, septate, anastomosing, 3–4  $\mu\text{m}$  diam; hamathelial cells persistent in cavity. *Asci* fasciculate, bitunicate, subsessile, broadly ellipsoid with a long tapered stalk, straight to curved, 8-spored, 70–110  $\times$  15–20  $\mu\text{m}$ . *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, irregular lumina

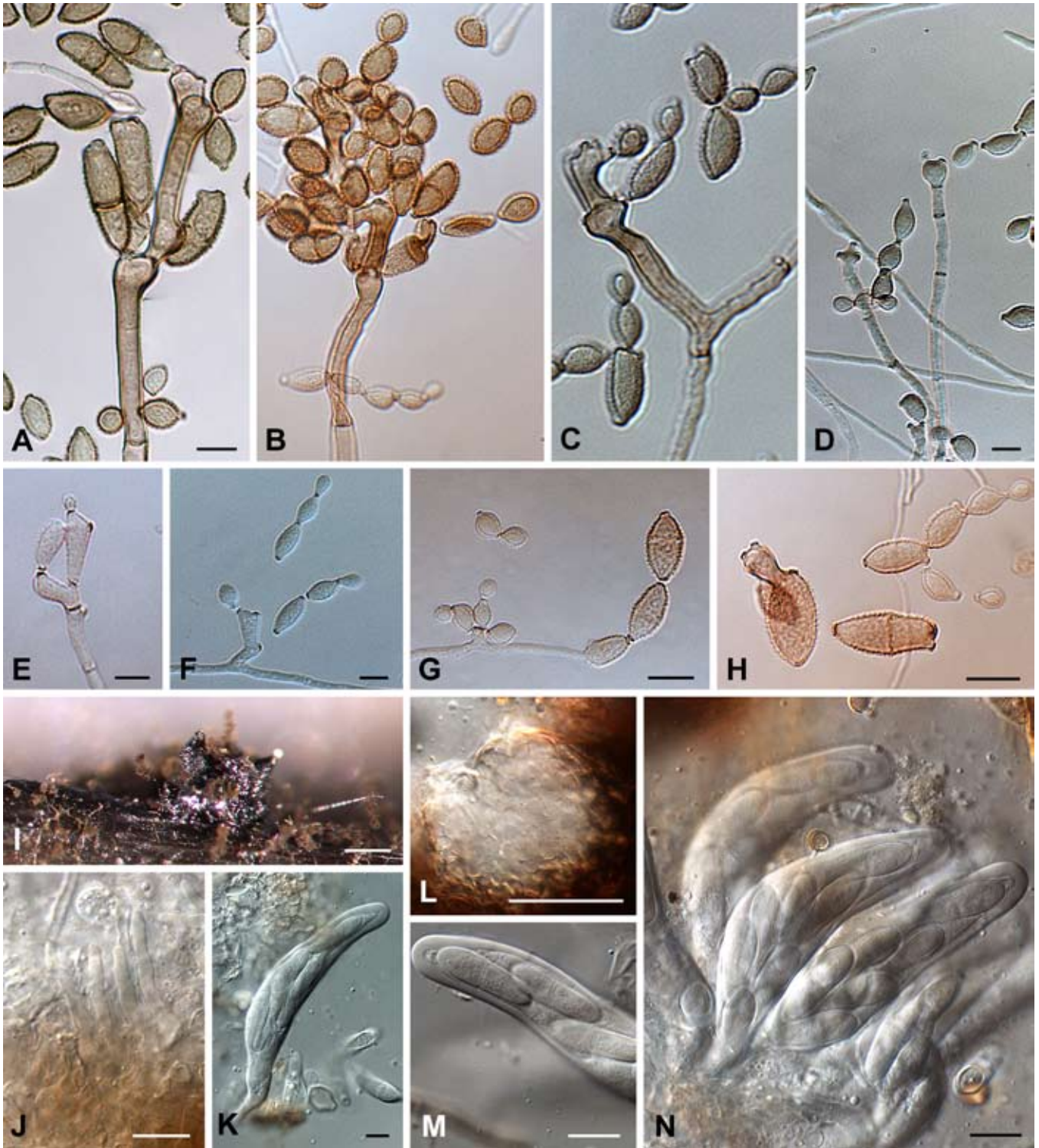
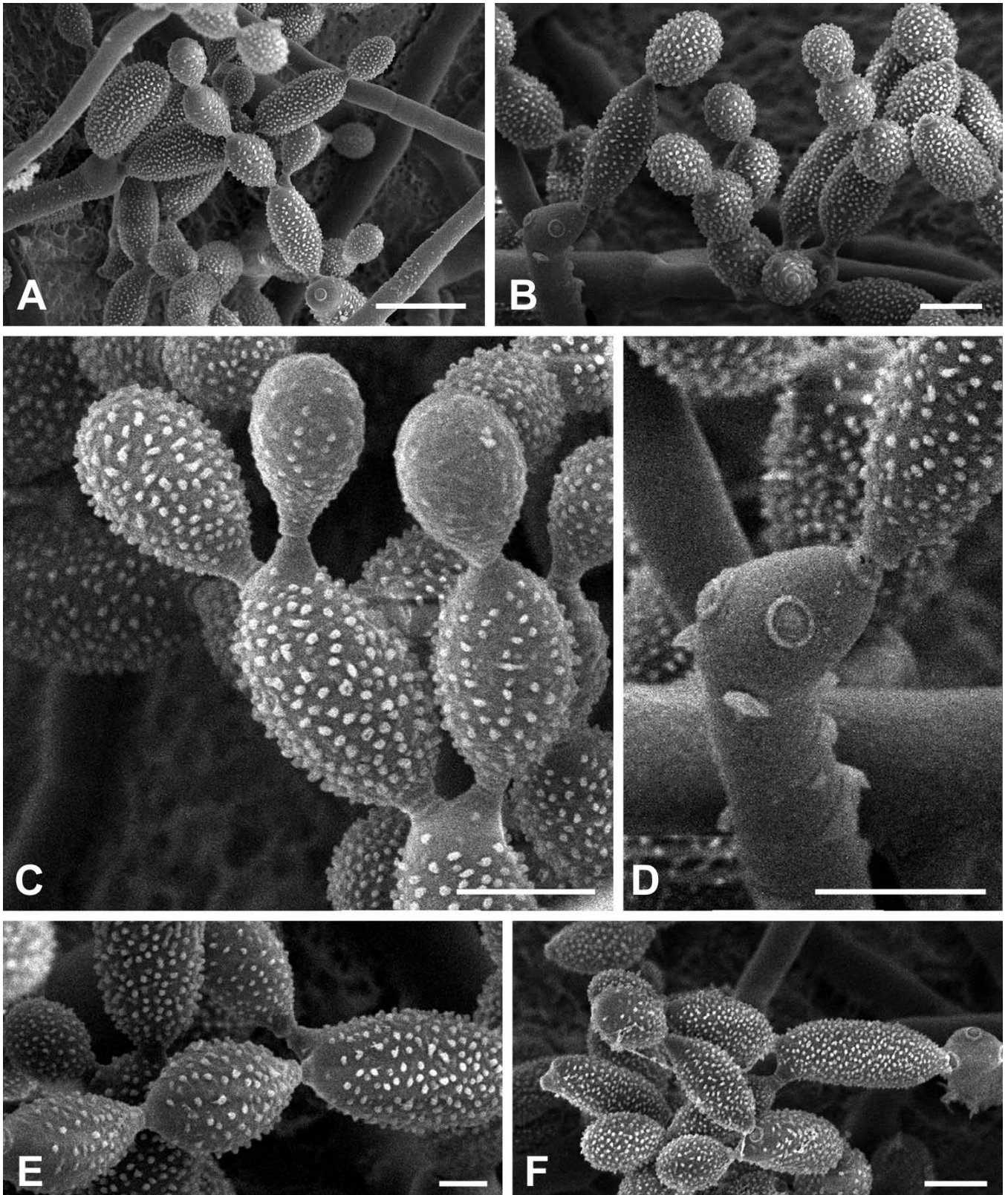


Fig. 23. *Cladosporium macrocarpum* (CBS 299.67) and its teleomorph *Davidiella macrocarpa* (CPC 12755). A–C. Macronematous conidiophores and conidia. D–G. Micronematous conidiophores. H. Microcyclic conidiogenesis. I. Ascomata formed on nettle stems in culture. J.Periphyses. K, M–N. Asci. L. Ostiole. Scale bars: A, D–H, J–N = 10  $\mu$ m, I = 200  $\mu$ m.

rarely observed, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest in the middle of the apical cell, medianly 1-septate, not to slightly constricted at the septum, tapering towards both ends, but more prominently towards lower end, (22–)23–26(–28)  $\times$  (6–)6.5–7(–8)  $\mu$ m; mucoid sheath rarely observed, mostly absent.

*Mycelium* unbranched or loosely branched, 1–4.5(–5)  $\mu$ m wide, septate, sometimes slightly constricted at septa, hyaline to pale brown, smooth to minutely verruculose, walls unthickened or slightly thickened. *Conidiophores* micronematous and macronematous,

solitary, arising terminally from plagiotropous hyphae or terminally from ascending hyphae. *Macronematous conidiophores* erect, straight to somewhat flexuous, cylindrical-oblong, nodulose to nodose, with a single apical or usually several swellings either somewhat distinct from each other or often in short succession giving conidiophores a knotty appearance, swellings sometimes laterally elongated or formed at the top of a branch-like outgrowth below the apical swelling, sometimes distinctly geniculate, unbranched, sometimes branched, 12–260  $\times$  (3–)4–6  $\mu$ m, swellings 5–10  $\mu$ m wide, pluriseptate, sometimes slightly constricted at septa, pale to medium brown or olivaceous-brown, somewhat paler at apices,



**Fig. 24.** *Cladosporium macrocarpum* (CBS 299.67). A. Survey of a conidiophore that forms several secondary ramoconidia and conidia. Several aerial hyphae are also visible in this picture. B. Conidiophore with broadly ellipsoid secondary ramoconidia and obovoid conidia. Note the different scars on the conidiophore at the lower left. C. Ellipsoid or obovoid conidia with notable areas of scar formation. The ornamentation is relatively widely distributed over the body of the cell and similar to *C. variabile*. D. Detail of a conidiophore (see B) with scars. Note the relatively shallow rings of the scars. E. Details of conidia and a secondary ramoconidium. F. Conidiophore with a secondary ramoconidium and conidia. Note the hila on several spores and the lack of ornamentation at the site where spores are formed. Scale bars: A–C, = 10 µm, D, F = 5 µm, E = 2 µm.

smooth to minutely verruculose or verruculose, walls somewhat thickened, sometimes even two-layered. *Conidiogenous cells* integrated, terminal or intercalary, cylindrical, nodulose with lateral shoulders or nodose with swellings round about the stalk, with conidiogenous loci confined to swellings, 12–37 µm long, with up to

12 loci per cell, usually with up to six, loci conspicuous, protuberant, (1–)1.5–2 µm diam, somewhat thickened and darkened-refractive. *Micronematous conidiophores* almost indistinguishable from hyphae, straight, narrowly filiform, non-nodulose or with a single or few swellings, mostly with small head-like swollen apices, usually

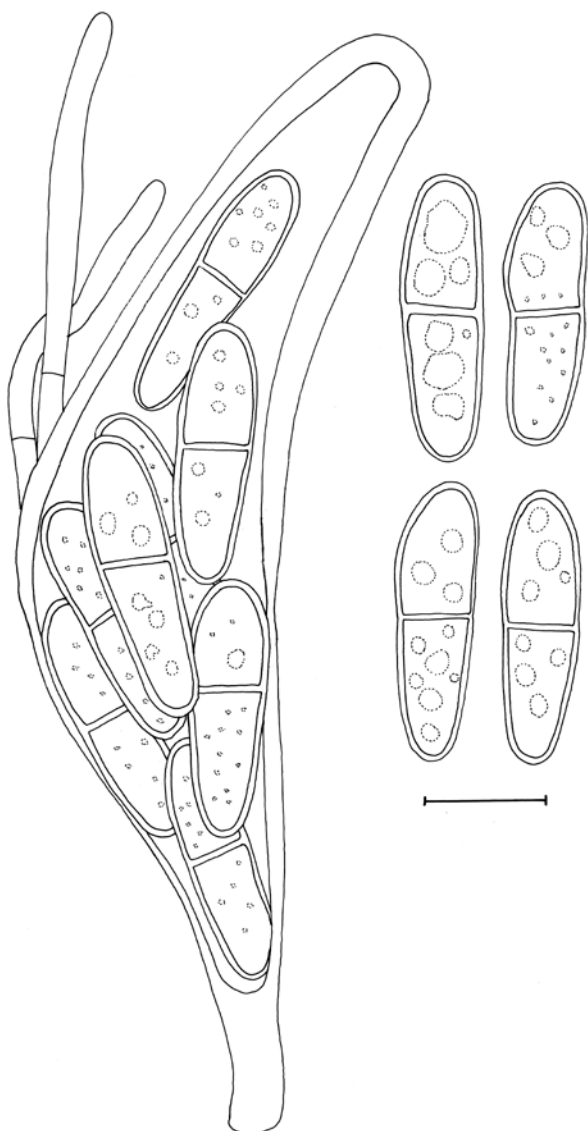


Fig. 25. *Davidiella macrocarpa* (CPC 12755). Ascus and ascospores. Scale bar = 10  $\mu\text{m}$ . P.W. Crous del.

only few micrometer long, 1.5–3  $\mu\text{m}$  wide, aseptate or with only few septa, subhyaline, smooth or almost so, walls unthickened, with a single or only few conidiogenous loci, narrow, 0.8–1.2  $\mu\text{m}$  diam, thickened and somewhat darkened-refractive. *Conidia* catenate, in branched chains, small terminal conidia subglobose, obovoid, oval, limoniform, 4–11  $\times$  (3–)4–6  $\mu\text{m}$  [av.  $\pm$  SD, 7.6 ( $\pm$  1.9)  $\times$  5.0 ( $\pm$  0.8)  $\mu\text{m}$ ], aseptate, intercalary conidia broadly ovoid-ellipsoid, 10–17  $\times$  (4.5–)5–9  $\mu\text{m}$  [av.  $\pm$  SD, 12.7 ( $\pm$  2.1)  $\times$  6.8 ( $\pm$  0.8)  $\mu\text{m}$ ], 0–1-septate; secondary ramoconidia broadly ellipsoid to subcylindrical, 14–25(–30)  $\times$  (5–)6–9(–10)  $\mu\text{m}$  [av.  $\pm$  SD, 19.4 ( $\pm$  3.5)  $\times$  7.6 ( $\pm$  1.0)  $\mu\text{m}$ ], 0–2(–3)-septate, sometimes slightly constricted at the septa, septa somewhat sinuous with age, pale brown to medium olivaceous-brown or brown, sometimes even dark brown, verruculose to echinulate (muricate under SEM), walls thickened, up to 1  $\mu\text{m}$  thick, mostly broadly rounded at apex and base, sometimes attenuated, sometimes guttulate by oil drops, with up to three apical hila, mostly 1–2, hila sessile (apparently somewhat immersed) to somewhat protuberant, 1–2(–2.5)  $\mu\text{m}$  diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring with conidia forming secondary micro- and macronematous conidiophores, conidia often germinating with long hyphae. *Conidia* formed by micronematous conidiophores usually smaller, narrower and paler, catenate, in short unbranched

or branched chains, subglobose, obovoid to limoniform, ellipsoid or fusiform, 2.5–16  $\times$  1.5–5  $\mu\text{m}$ , 0(–1)-septate, few longer conidia subcylindrical to clavate, up to 37(–43)  $\mu\text{m}$  long, 0–2(–3)-septate, occasionally with up to four septa, sometimes slightly constricted at the septa, subhyaline to pale brown, almost smooth to minutely verruculose, walls unthickened, hila 0.8–1.2  $\mu\text{m}$  diam, thickened and darkened-refractive.

**Cultural characteristics:** Colonies on PDA reaching 30–43 mm in diam after 14 d at 25  $^{\circ}\text{C}$ , dark dull green to olivaceous-grey, olivaceous-grey, dark olivaceous- to iron-grey reverse, pulvinate, velvety, sometimes somewhat zonate, paler zones towards the margin, margin regular, entire edge, almost colourless to white, glabrous to feathery, aerial mycelium sparse to more abundant in the colony centre or covering large areas of the colony, hairy, fluffy or felty, whitish to smoke-grey, sometimes becoming reddish, livid red to vinaceous, growth flat, regular, sometimes forming few prominent exudates, exudates sometimes slightly reddish, sporulation profuse with two kinds of conidiophores, low and high. Colonies on MEA reaching 31–50 mm in diam after 14 d at 25  $^{\circ}\text{C}$ , grey-olivaceous to olivaceous-grey or iron-grey, sometimes pale olivaceous-grey to whitish due to abundant aerial mycelium, olivaceous-grey or iron-grey reverse, velvety or powdery, margin narrow, entire edge, colourless to white, glabrous, aerial mycelium sparse to abundant, hairy or felty, growth regular, flat to low convex, radially furrowed, without prominent exudates, sporulation profuse. Colonies on OA reaching 29–40 mm in diam after 14 d at 25  $^{\circ}\text{C}$ , grey-olivaceous, olivaceous-grey to dark smoke-grey, olivaceous-black or iron grey reverse, margin entire edge, narrow, colourless or white, glabrous, aerial mycelium sparse, mainly in the colony centre, felty, white to smoke-grey or grey-olivaceous, felty, growth flat, regular, without exudates, sporulating.

**Specimens examined:** *Sine loco et dato*, L 910.255-723 = L-0115836, **lectotype designated here** of *Dematium graminum*. *Sine loco*, on dead stems of *Brassica* sp. (*Brassicaceae*), No. 601, L 910.255-716 = L-0115849, **holotype** of *D. herbarum* var. ( $\beta$ ) *brassicae*. *Sine loco*, on leaves of *Iris* (*Iridaceae*), *Quercus* (*Fagaceae*), *Brassica* etc., L 910.255-736 = L-0115871, **holotype** of *D. vulgare* var. ( $\beta$ ) *foliorum*, isotype L 910.255-718 = L-0115872. *Sine loco et dato*, L 910.255-698 = L-0115852, **lectotype designated here** for *D. vulgare* var. ( $\delta$ ) *typharum*. Isolated from “*Mycosphaerella tulasnei*”, CBS 223.32 = ATCC 11287 = IMI 049635. **Romania**, isolated from water, CBS 175.82. **Slovenia**, Sečovelje, isolated from hypersaline water from saltens (precristallisation pond), 2004, P. Zalar, EXF-2287 = CPC 12054. **Turkey**, Ankara, Tekeli, isolated from *Triticum aestivum* (*Poaceae*), isol. S. Tahsin, ident. A.C. Stolk, CBS 299.67. **U.S.A.**, Seattle, University of Washington Campus, 47.6263530, -122.3331440, isolated from cleistothecia of *Phyllactinia guttata* (*Erysiphaceae*) on leaves of *Corylus* sp. (*Corylaceae*), 16 Sep. 2004, D. Glawe, CPC 11817; Washington, isolated from *Spinacia oleracea* (*Chenopodiaceae*), 1 Jan. 2003, L. DuToit, CBS-H 19855, **neotype designated here** for *C. macrocarpum*, and **holotype** of *D. macrocarpa*, isoneotype HAL 2020 F, isotype HAL 2021 F, culture ex-type CPC 12752, 12756–12759, CPC 12755 = CBS 121623.

**Substrate and distribution:** Decaying plant material, human, hypersaline water, water; widespread.

**Literature:** de Vries (1952: 76), Ellis (1971: 315), Domsch *et al.* (1980: 208), Ellis & Ellis (1985: 290, 468), Matsushima (1985: 5), McKemy & Morgan-Jones (1991), Dugan & Roberts (1994), David (1997: 71), Samson *et al.* (2000: 112).

**Notes:** In the absence of Preuss’s type material (not preserved) de Vries (1952) “lectotypified” *C. macrocarpum* by a specimen in Saccardo’s herbarum (Herb. Myc. P.A. Saccardo no. 419, PAD). This material, subsequently distributed in Mycotheca Italica no. 1396, should correctly be regarded as neotype (David 1997). A single collection of Saccardo’s Mycotheca Italica no. 1396 from herb. HBG, which can be considered as isoneotype material,

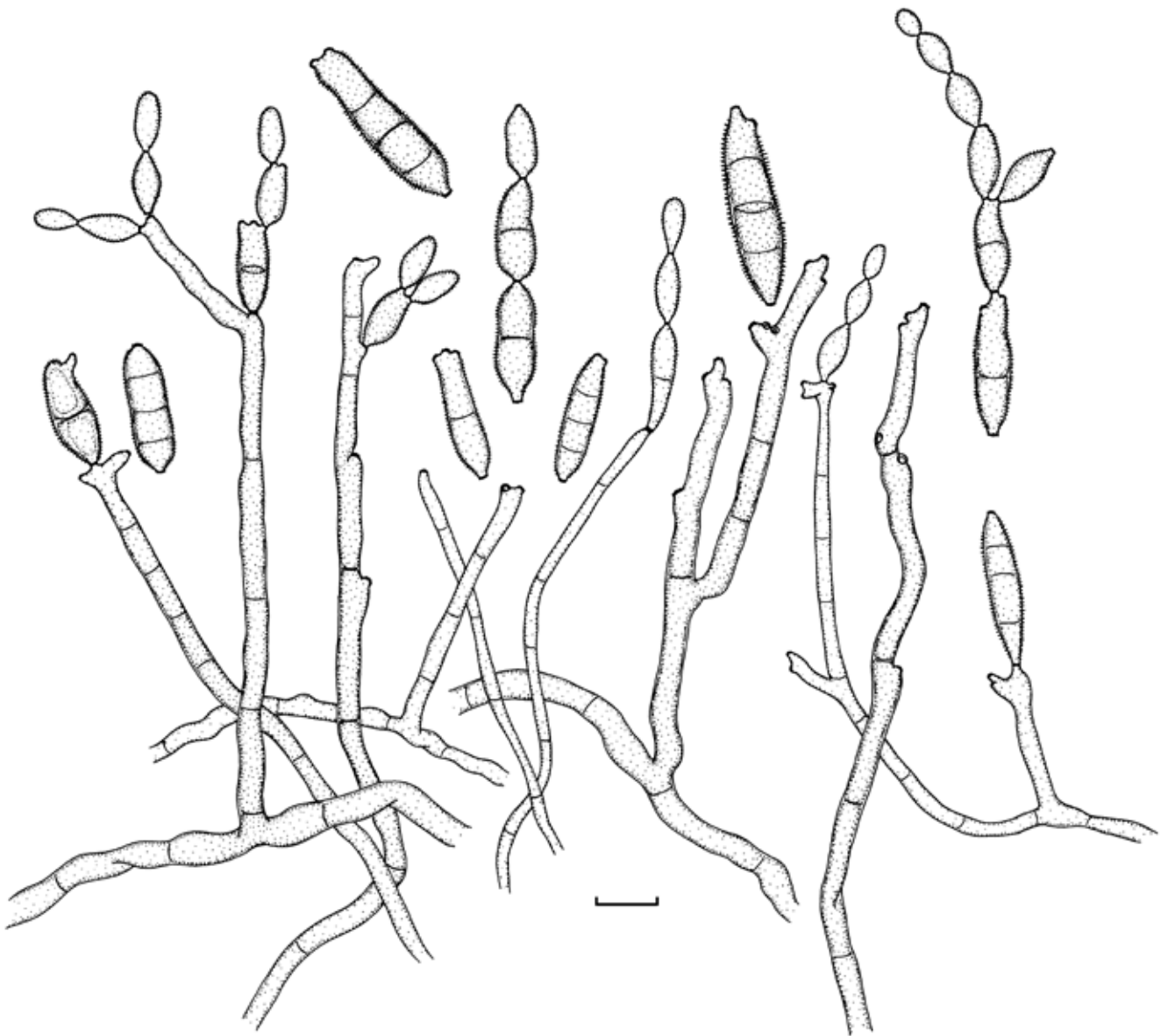


Fig. 26. *Cladosporium ossifragi* (CBS 842.91). Conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

was re-examined and proved to rather agree with the species concept of *C. herbarum* s. str. The conidia were formed in simple, rarely branched chains,  $6\text{--}26 \times (4\text{--})5.5\text{--}8\text{--}(9) \mu\text{m}$ , 0–3-septate, almost smooth or minutely to densely verruculose or verrucose (Schubert 2005). However, since de Vries' "lectotypification" was incorrect according to the code (ICBN, Art. 9.2, 9.17), a neotype is designated.

The delimitation of *C. macrocarpum* as a morphologically distinct species from *C. herbarum* has been controversially discussed by several authors (McKemy & Morgan-Jones 1991, Dugan & Robert 1994, Ho *et al.* 1999). Based on molecular as well morphological studies, it can be shown that *C. macrocarpum* is a well-defined species distinguishable from *C. herbarum* s. str. by forming conidiophores with wider nodes,  $5\text{--}10 \mu\text{m}$ , wider and more frequently septate conidia [small terminal conidia  $4\text{--}11 \times (3\text{--})4\text{--}6 \mu\text{m}$  versus  $4\text{--}10 \times 3\text{--}5\text{--}(6) \mu\text{m}$  in *C. herbarum*, intercalary conidia  $10\text{--}17 \times (4.5\text{--})5\text{--}9 \mu\text{m}$  versus  $6\text{--}16 \times 4\text{--}6 \mu\text{m}$  in *C. herbarum*, secondary ramoconidia  $14\text{--}25\text{--}(30) \times (5\text{--})6\text{--}9\text{--}(10) \mu\text{m}$  versus  $12\text{--}25\text{--}(35) \times (3\text{--})5\text{--}7\text{--}(9) \mu\text{m}$  in *C. herbarum*] and by being connected to *Davidiella macrocarpa*. On natural substrates the conidiophores are usually somewhat wider than in culture,  $4\text{--}8\text{--}(10) \mu\text{m}$  wide, and also the conidia can be somewhat wider, sometimes up to  $13\text{--}(15) \mu\text{m}$ .

*Cladosporium graminum*, described by Persoon (1822), as well as *C. brunneum* and *C. gracile*, introduced by Corda (1837), are older synonyms of *C. macrocarpum* and, according to the code, would have priority. However, since *C. macrocarpum* is a well established, currently used name with numerous records in literature, a proposal to conserve the name against these older names is in preparation for formal publication in *Taxon*.

A characteristic difference between ascomata of *C. macrocarpum* in comparison to those of *C. herbarum*, are the smaller, globose pseudothecia, asci with longer stalks, prominence of pseudoparaphyses, and rather inconspicuous luminal ascospore inclusions.

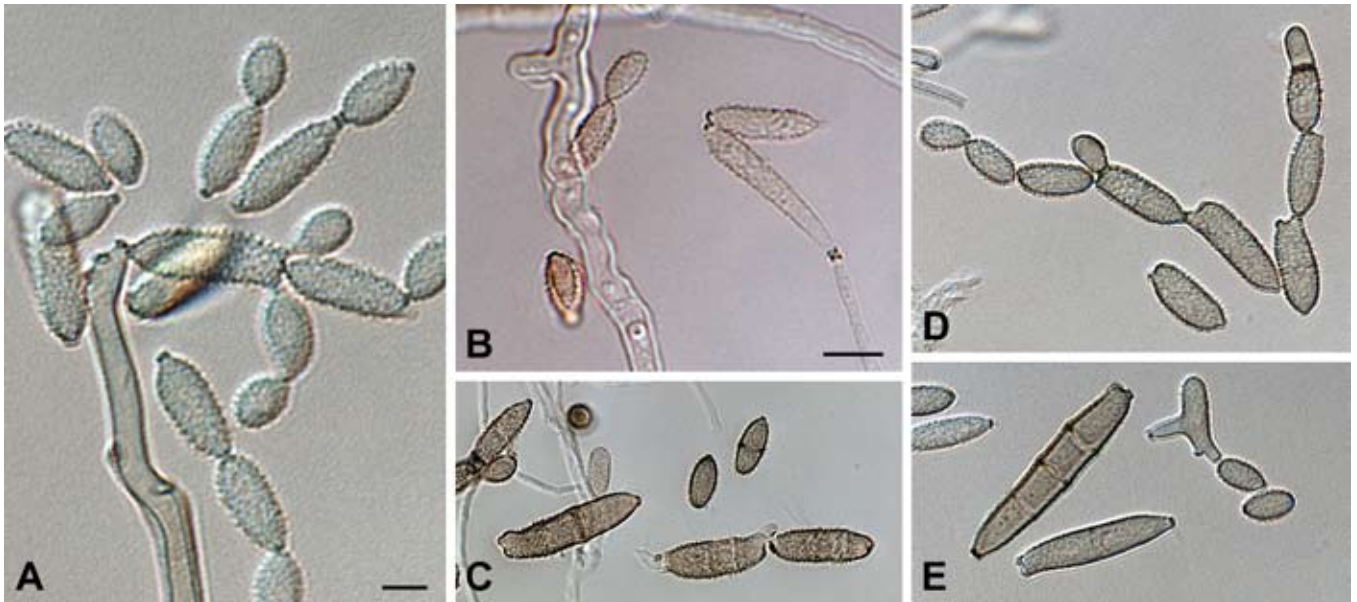
***Cladosporium ossifragi* (Rostr.) U. Braun & K. Schub., comb. nov.** MycoBank MB504575. Figs 26–28.

*Basionym:* *Napicladium ossifragi* Rostr., Bot. Færøes 1: 316. 1901.

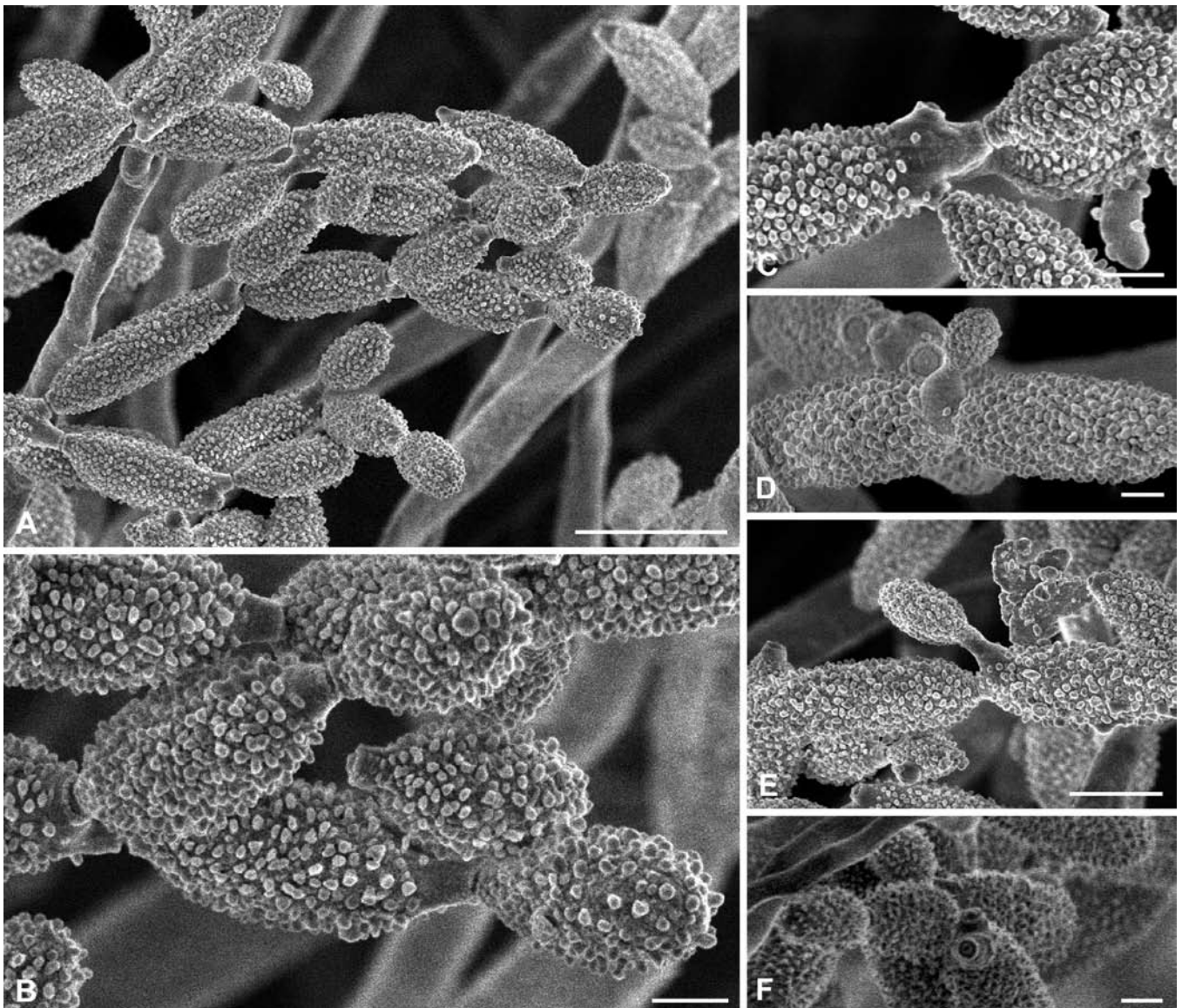
≡ *Heterosporium ossifragi* (Rostr.) Lind, Dan. fung.: 531. 1913.

= *Heterosporium magnusianum* Jaap, Schriften Naturwiss. Vereins Schleswig-Holstein 12: 346. 1902.

≡ *Cladosporium magnusianum* (Jaap) M.B. Ellis in Ellis, More Dematiaceous Hyphomycetes: 337. 1976.



**Fig. 27.** *Cladosporium ossifragi* (CBS 842.91). A. Macronematous conidiophore. B. Micronematous conidiophore. C–D. Conidia. E. Conidia and microcyclic conidiogenesis. Scale bars = 10  $\mu$ m.



**Fig. 28.** *Cladosporium ossifragi* (CBS 842.91). A. Survey on different secondary ramoconidia and conidia. B. Details of conidia and hila. Note the very pronounced ornamentation and the absence of ornamentation near the site of spore formation. C. Detail of the end of a secondary ramoconidium with pronounced hila. D. Formation of a new conidium. Note the broad scar behind it (> 1  $\mu$ m). E. Formation of a new conidium from a smooth-walled stalk. F. Hila on a secondary ramoconidium. This micrograph is from the sample before coating with gold-palladium and shows similar features as the sample after sputter coating. Scale bars: A = 10  $\mu$ m, B–D, F = 2  $\mu$ m, E = 5  $\mu$ m.

*Mycelium* abundantly formed, twisted, often somewhat aggregated, forming ropes, branched, 1–5 µm wide, septate, often irregularly swollen and constricted, hyaline or subhyaline to pale brown, smooth, walls unthickened or only slightly thickened. *Conidiophores* macronematous and micronematous, arising from plagiotropous hyphae, terminally or laterally, erect to subdecumbent, more or less straight to flexuous, cylindrical, sometimes geniculate, subnodulose with loci often situated on small lateral shoulders, unbranched, sometimes branched, often very long, up to 350 µm long, 3–4.5(–5) µm wide, pluriseptate, shorter ones aseptate, not constricted at septa, pale to pale medium brown, paler towards apices, sometimes subhyaline, smooth to minutely verruculose, especially towards apices, walls somewhat thickened, up to 0.5 µm, sometimes appearing two-layered. *Conidiogenous cells* integrated, terminal as well as intercalary, cylindrical, sometimes geniculate, subnodulose, 5–31 µm long, proliferation sympodial, with few loci (1–3) per cell, loci usually confined to small lateral shoulders, protuberant, conspicuous, short cylindrical, 1–2 µm wide, up to 1 µm high, somewhat thickened, darkened-refractive. *Conidia* catenate, in short, unbranched or branched chains, straight, small terminal and intercalary conidia subglobose, obovoid to ellipsoid, 4–15 × 3–5 µm [av. ± SD, 9.3 (± 3.7) × 4.0 (± 0.7) µm], 0–1-septate, not constricted at the septa, pale brown, hila 0.8–1 µm diam, secondary ramoconidia cylindrical, sometimes ellipsoid or subfusiform, 16–36(–40) × (4–)5–8 µm [av. ± SD, 26.6 (± 7.4) × 6.0 (± 1.2) µm], (0–)1–3(–4)-septate [*in vivo* wider, (6–)7–9(–11) µm, and with up to five, rarely seven septa], not constricted at the septa, septa sometimes slightly sinuous, pale brown to pale medium brown, densely verruculose, verrucose to echinulate (densely muricate under SEM), walls unthickened to somewhat thickened, rounded or somewhat attenuated at apex and base, hila protuberant, conspicuous, sometimes situated on short, small prolongations, 1–2.5 µm diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occasionally occurring.

**Cultural characteristics:** Colonies on PDA reaching 53 mm diam after 14 d at 25 °C, greenish olivaceous, grey-olivaceous to olivaceous-grey or iron-grey, appearing somewhat zonate, dull green to olivaceous-black reverse, margin colourless, regular, entire edge, aerial mycelium abundantly formed, covering at first the colony centre later most of the surface, dense, high, growth flat with elevated colony centre, somewhat folded. Colonies on MEA reaching 54 mm diam after 14 d at 25 °C, pale olivaceous-grey to olivaceous-grey in the centre, iron-grey reverse, velvety, margin colourless to white, entire edge, radially furrowed, aerial mycelium abundantly formed, fluffy to felty, growth flat with somewhat raised, folded colony centre. Colonies on OA attaining 52 mm diam after 14 d at 25 °C, olivaceous-grey to iron-grey, iron-grey to greenish black reverse, margin white, entire edge, aerial mycelium diffuse, loose, growth flat, prominent exudates absent, sporulation profuse on all media.

**Specimens examined:** **Denmark**, Undallslund, on leaves of *Nartheceum ossifragum* (*Melanthiaceae*), 13 Sep. 1885, E. Rostrup, CP, **neotype designated here** of *C. ossifragi*; Tønder, Rómó near Twismark, 19 Aug. 1911, H. Sydow, Sydow, Mycoth. Germ. 1047, M. **Germany**, Hamburg, Eppendorfer Moor, on leaves of *Nartheceum ossifragum*, 12 Sep. 1897, O. Jaap, HBG, **lectotype selected here** of *C. magnusianum*; 4 Sep. 1903, O. Jaap, Jaap, Fungi Sel. Exs. 49, M; Wernerwald near Cuxhaven, Aug. 1927, A. Ludwig, Petrak, Mycoth. Gen. 146, M. **Norway**, Bjerkreim County, isolated from leaves of *Nartheceum ossifragum*, M. di Menna, CBS-H 19860, **epitype designated here** of *C. ossifragi*, culture ex-epitype CBS 842.91 = ATCC 200946; Møre og Romsdal County, isolated from leaves of *Nartheceum ossifragum*, M. di Menna, CBS 843.91.

**Substrate and distribution:** Causing leaf spots on *Nartheceum ossifragum*; Europe (Austria, Denmark, Germany, Great Britain, Ireland, Norway).

**Literature:** Ellis & Ellis (1985: 390), David (1995a; 1997: 85–86, 88), Ho *et al.* (1999: 132).

**Notes:** Type material of *Napicladium ossifragi* is not preserved in Rostrup's herbarium (on *Nartheceum ossifragum*, Faeroe Islands, Viderö, Viderejde and Österö, Svinaa, *sine dato*, leg. Ostenfeld & Harz). However, other authentic collections seen and examined by Rostrup are deposited at CP. Lind (1913) re-examined these samples, synonymised *N. ossifragi* with *H. magnusianum* and correctly introduced the combination *H. ossifragi*. Nevertheless, the correct oldest name for this fungus has been ignored by most authors. David (1997), who clearly stated that *N. ossifragi* is the earliest name for this species, preferred to use the name *C. magnusianum* because the typification of Rostrup's name was still uncertain. Despite the lacking type material, there is no doubt about the correct identity of *N. ossifragi* since authentic material of this species, examined by and deposited in Rostrup's herbarium (CP), is preserved. Therefore, there is no reason to reject the oldest valid name for this species. The original collection of *C. magnusianum* cited by Jaap (1902) (on leaves of *Nartheceum ossifragum*, Denmark, Tønder, Rómó, peatbog by Twismark, Jul.–Aug. 1901, Jaap), but not designated as type, is not preserved (David 1997). It is neither deposited at B, HBG nor S. However, in the protologue Jaap (1902) also referred to material of this species found near Hamburg, which is, hence, syntype material available for lectotypification.

***Cladosporium pseudiridis*** K. Schub., C.F. Hill, Crous & U. Braun, **sp. nov.** MycoBank MB504576. Figs 29–30.

**Etymology:** Epithet derived from its similar morphology to *Cladosporium iridis*.

Differt a *Cladosporio iridis* conidiis 0–3-septatis, brevioribus et latoribus, 15–55 × (9–)11–19(–21) µm.

*Mycelium* sparingly branched, 2–7 µm wide, septate, not constricted at the septa, subhyaline to pale brown, smooth or almost so, walls somewhat thickened, guttulate or protoplasm appearing granular, sometimes enveloped by a slime coat. *Conidiophores* arising mostly terminally from ascending hyphae, sometimes also laterally from plagiotropous hyphae, erect, more or less straight, broadly cylindrical-oblong, once or several times slightly to distinctly geniculate-sinuous, forming more or less pronounced lateral shoulders, nodulose, unbranched, 100–320(–500) × 7–11 µm, swellings 10–14 µm wide, becoming narrower and paler towards the apex, septate, not constricted at the septa, septa mainly basal, apical cell often very long, pale to medium olivaceous-brown, subhyaline at the apex, smooth or almost so, sometimes minutely verruculose, walls usually distinctly thickened, sometimes even two-layered, up to 1(–2) µm thick, protoplasm granular, often clearly contrasting from the outer wall. *Conidiogenous cells* integrated, terminal and intercalary, cylindrical-oblong, slightly to distinctly geniculate-sinuous, nodulose with conidiogenous loci confined to swellings or lateral shoulders, 30–110 µm long, proliferation percurrent to sympodial, with a single or three, sometimes up to five geniculations per cell, usually only a single locus per swelling, protuberant, very prominent, short cylindrical, peg-like, clearly composed of a dome and surrounding rim, dome often higher than the periclinal rim, broad, somewhat paler than rim, conically narrowed, (2–)2.5–4 µm wide, up to 2 µm high, thickened and darkened-refractive.

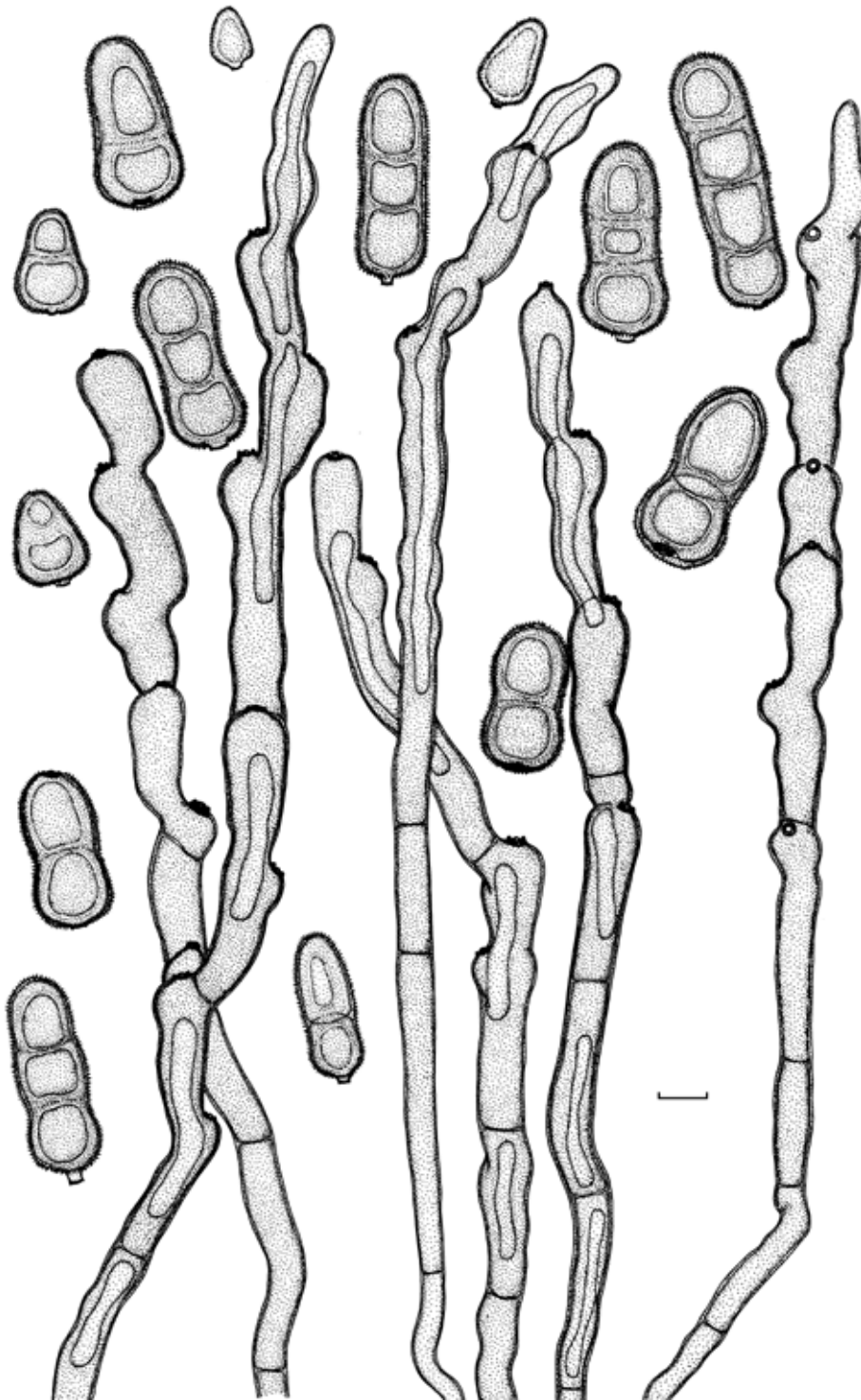


Fig. 29. *Cladosporium pseudiridis* (CBS 116463). Conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

*Conidia* solitary, sometimes in short unbranched chains of two or three, straight to slightly curved, young conidia small, 0–1-septate, broadly ovoid to pyriform, 15–26  $\times$  (9–)11–16(–18)  $\mu\text{m}$  [av.  $\pm$  SD, 19.2 ( $\pm$  4.3)  $\times$  14.2 ( $\pm$  3)  $\mu\text{m}$ ], first septum somewhat in the upper half, the upper cell is much smaller but gradually extending as the conidium matures, mature conidia 1–3-septate, broadly pyriform, cylindrical-oblong or soleiform, usually with a distinctly bulbous base, 30–55  $\times$  12–19(–21)  $\mu\text{m}$  [av.  $\pm$  SD, 41.5 ( $\pm$  6.8)  $\times$  17.1 ( $\pm$  2.1)  $\mu\text{m}$ ], broadest part of conidia usually at the bulbous base, mostly attenuated towards the basal septum, septa becoming sinuous with age, pale to medium olivaceous-brown or brown, usually echinulate, sometimes coarsely verrucose, walls distinctly thickened, up to 2

$\mu\text{m}$  thick, often appearing layered with a large lumen in the centre of the cell, broadly rounded to flattened at apex and base, hila often very prominent, often peg-like elongated, up to 3  $\mu\text{m}$  long, with age becoming less prominent, visible as a thickened flat plate just below the outer echinulate wall layer, slightly raised towards the middle, 2–3.5  $\mu\text{m}$  diam, thickened and darkened-refractive; microcyclic conidiogenesis not observed.

*Cultural characteristics:* Colonies on PDA attaining 6 mm diam after 14 d at 25  $^{\circ}\text{C}$ , whitish, smoke-grey to pale olivaceous-grey due to abundant aerial mycelium, olivaceous-black reverse, margin narrow, white, more or less crenate, aerial mycelium zonate, fluffy, covering most of the colony, mainly in the colony centre, growth



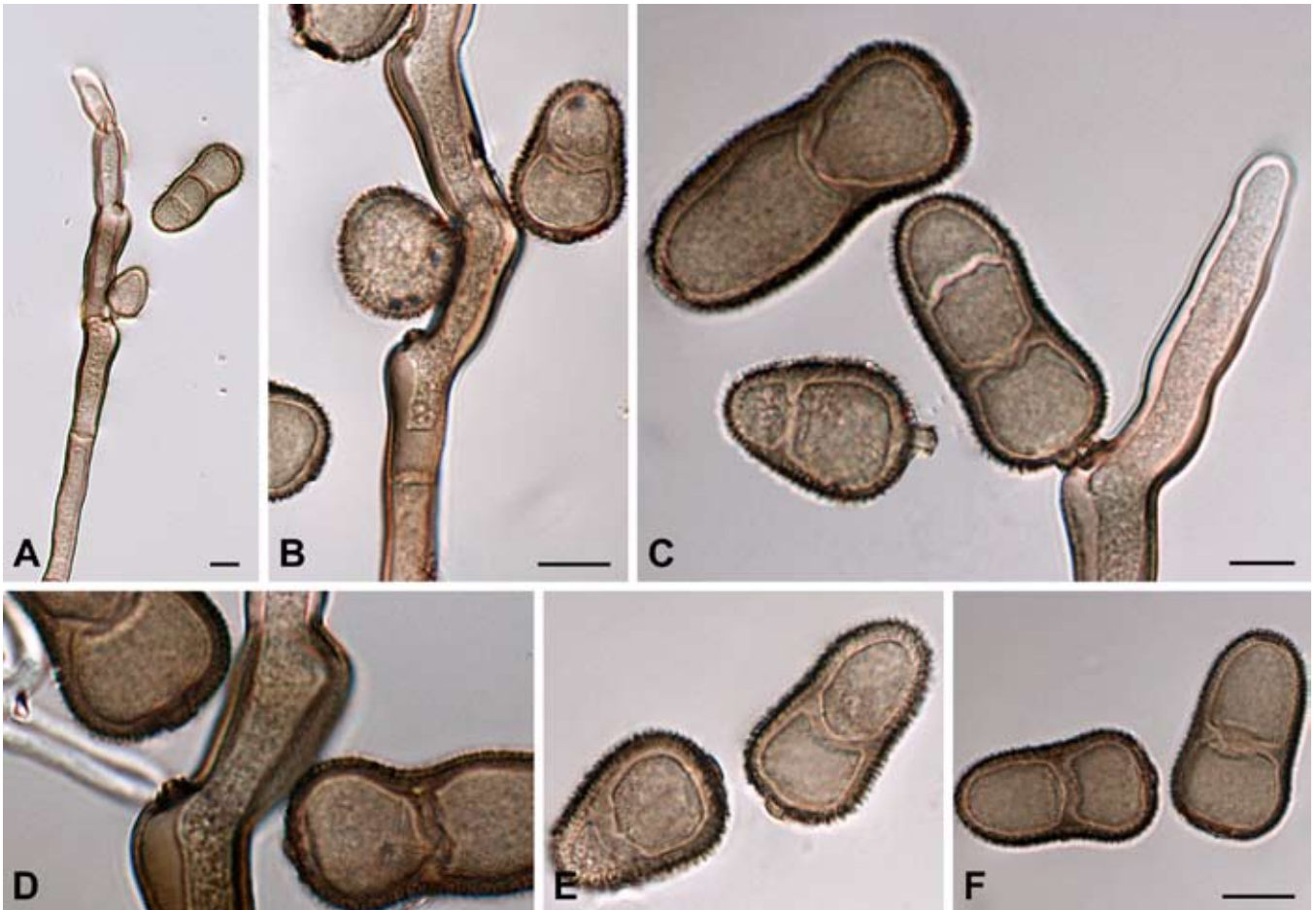


Fig. 30. *Cladosporium pseudiridis* (CBS 116463). A–C. Conidiophores and conidia. D. Part of a conidiogenous cell showing a protuberant cladosporioid conidiogenous locus. E–F. Conidia. Scale bars = 10 µm.

convex to raised, deep into the agar, with age few large prominent exudates formed, sparingly sporulating. Colonies on MEA attaining 7 mm diam after 14 d at 25 °C, olivaceous-grey, pale olivaceous-grey to pale rosy-buff due to abundant aerial mycelium covering almost the whole colony, iron-grey reverse, margin colourless or white, broad, regular, more or less glabrous, aerial mycelium fluffy, dense, high, growth convex to umbonate, sometimes with elevated colony centre, prominent exudates lacking, sporulation sparse. Colonies on OA attaining 8 mm diam after 14 d at 25 °C, white, pale buff to pale olivaceous-grey in the centre, margin grey-olivaceous, olivaceous- to iron-grey reverse, margin entire edge or somewhat undulate, somewhat feathery, growth raised with a somewhat depressed centre forming an elevated outer rim, without prominent exudates, sporulation more abundant.

*Specimen examined:* **New Zealand**, Auckland, Mt. Albert, Carrington Road, Unitec Campus, isolated from large leaf lesions on *Iris* sp. (*Iridaceae*), 15 Aug. 2004, C.F. Hill, CBS-H 19861, **holotype**, culture ex-type CBS 116463 = LYN 1065 = ICMP 15579.

*Substrate and distribution:* On living leaves of *Iris* sp.; New Zealand.

*Notes:* *Cladosporium pseudiridis* closely resembles *C. iridis*, a common and widespread species causing leaf spots on numerous *Iris* spp. and a few additional hosts of the host family *Iridaceae*, but the latter species is easily distinguishable by having longer and narrower, more frequently septate conidia, (18–)30–75(–87) × (7–)10–16(–18) µm, (0–)2–6(–7)-septate.

It is unlikely that *C. pseudiridis* is of New Zealand origin since the genus *Iris* is not indigenous to New Zealand. All *Iris* species that are found in this country have been introduced, mainly for horticultural purposes. The species is, therefore, probably more common than indicated above. However, within the course of the recent monographic studies in the genus *Cladosporium* numerous herbarium specimens, mainly of European origin, have been examined and proved to be correctly identified agreeing with the species concept of *C. iridis*. Additional collections and cultures are necessary to determine its distribution.

***Cladosporium ramotenellum*** K. Schub., Zalar, Crous & U. Braun, **sp. nov.** MycoBank MB504577. Figs 31–33.

*Etymology:* Refers to the morphological similarity with *Cladosporium tenellum*.

Differt a *Cladosporio* cladosporioide conidiophoris et conidiis leniter angustioribus, 2–4(–5) µm latis, conidiis 0–2(–3)-septatis, semper verruculosus; et a *Cladosporio* tenello locis conidiogenis non numerosis et non aggregatos ad apicem, conidiis longioribus et angustioribus, 2.5–35 × 2–4(–5) µm, 0–3-septatis.

*Mycelium* unbranched or only sparingly branched, 1.5–4 µm wide, septate, without swellings and constrictions, hyaline or subhyaline, smooth, sometimes irregularly rough-walled, walls unthickened. *Conidiophores* solitary, macronematous and micronematous, arising as lateral branches of plagiotropous hyphae or terminally from ascending hyphae, erect, straight or slightly flexuous, cylindrical, neither geniculate nor nodulose, without head-like swollen apices or intercalary swellings, unbranched, sometimes

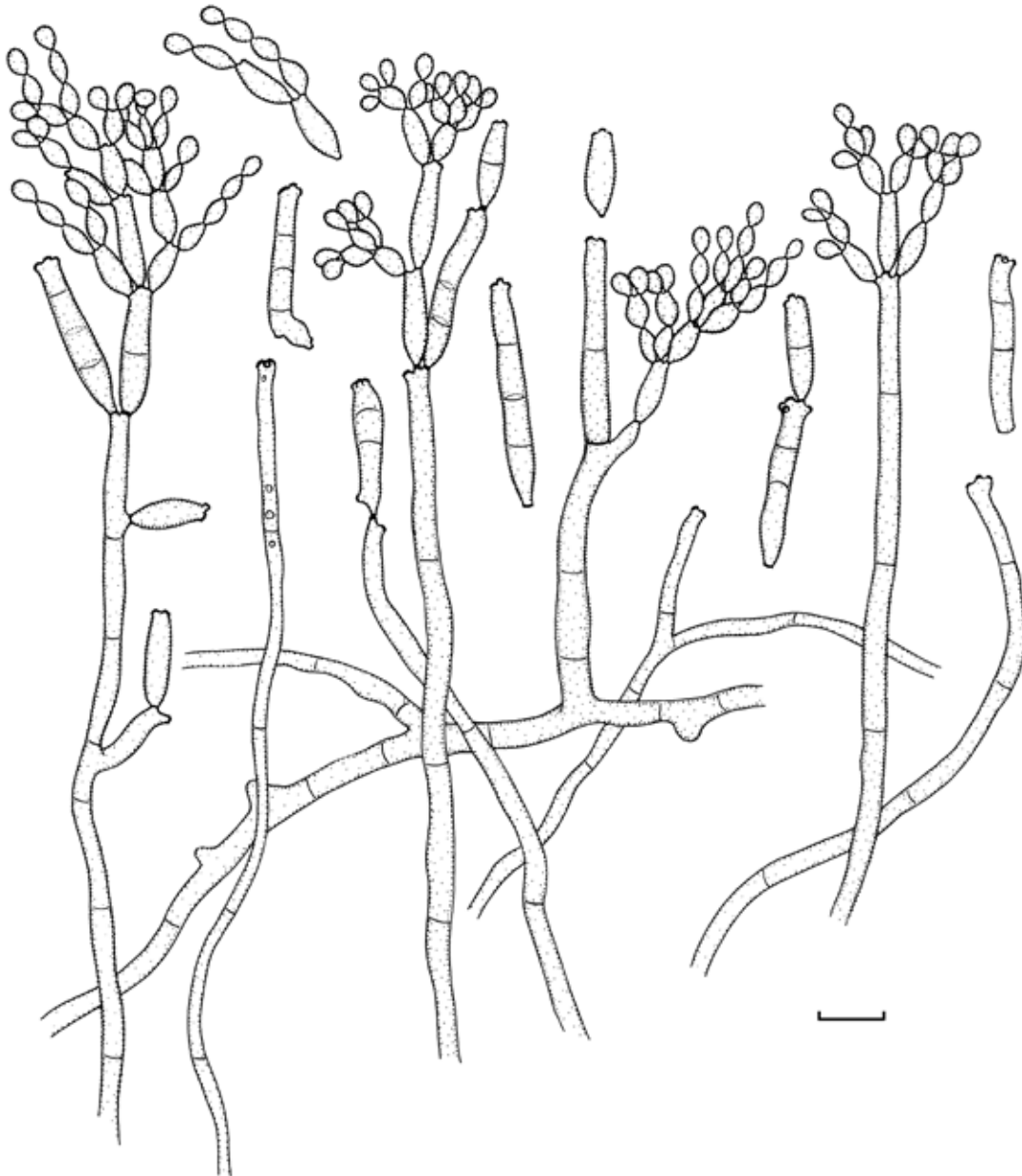
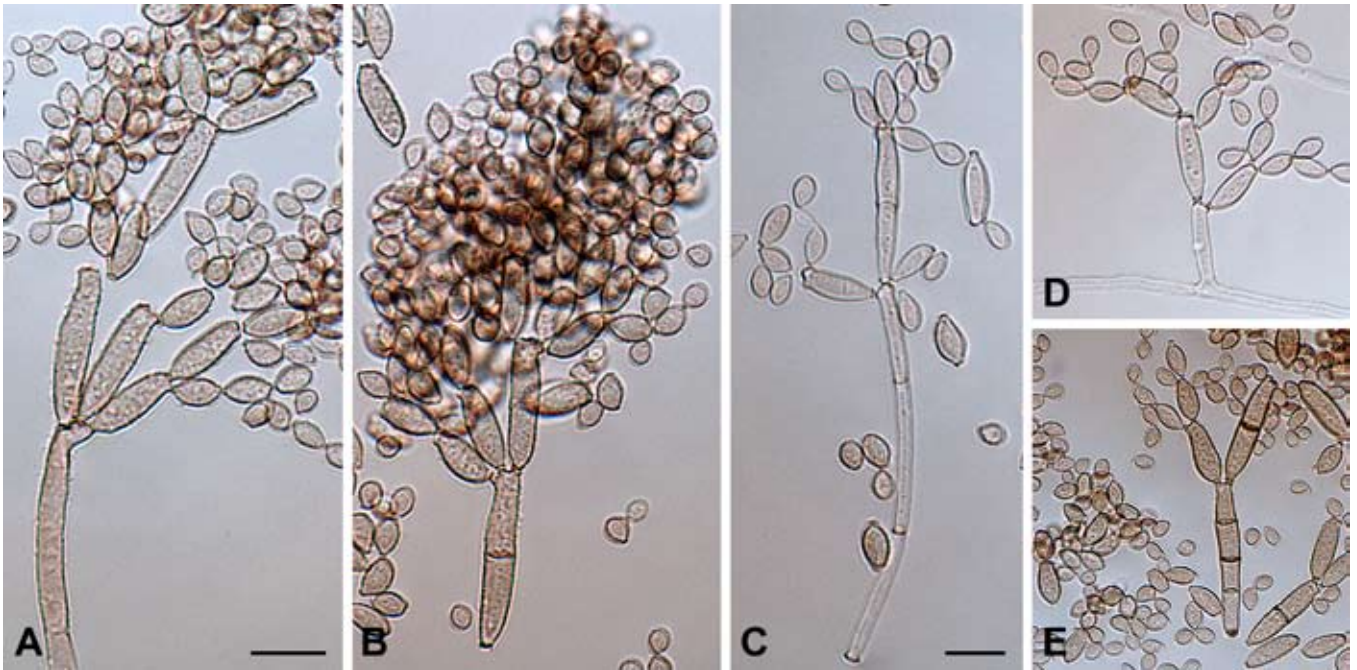


Fig. 31. *Cladosporium ramotenellum* (CPC 12043). Conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

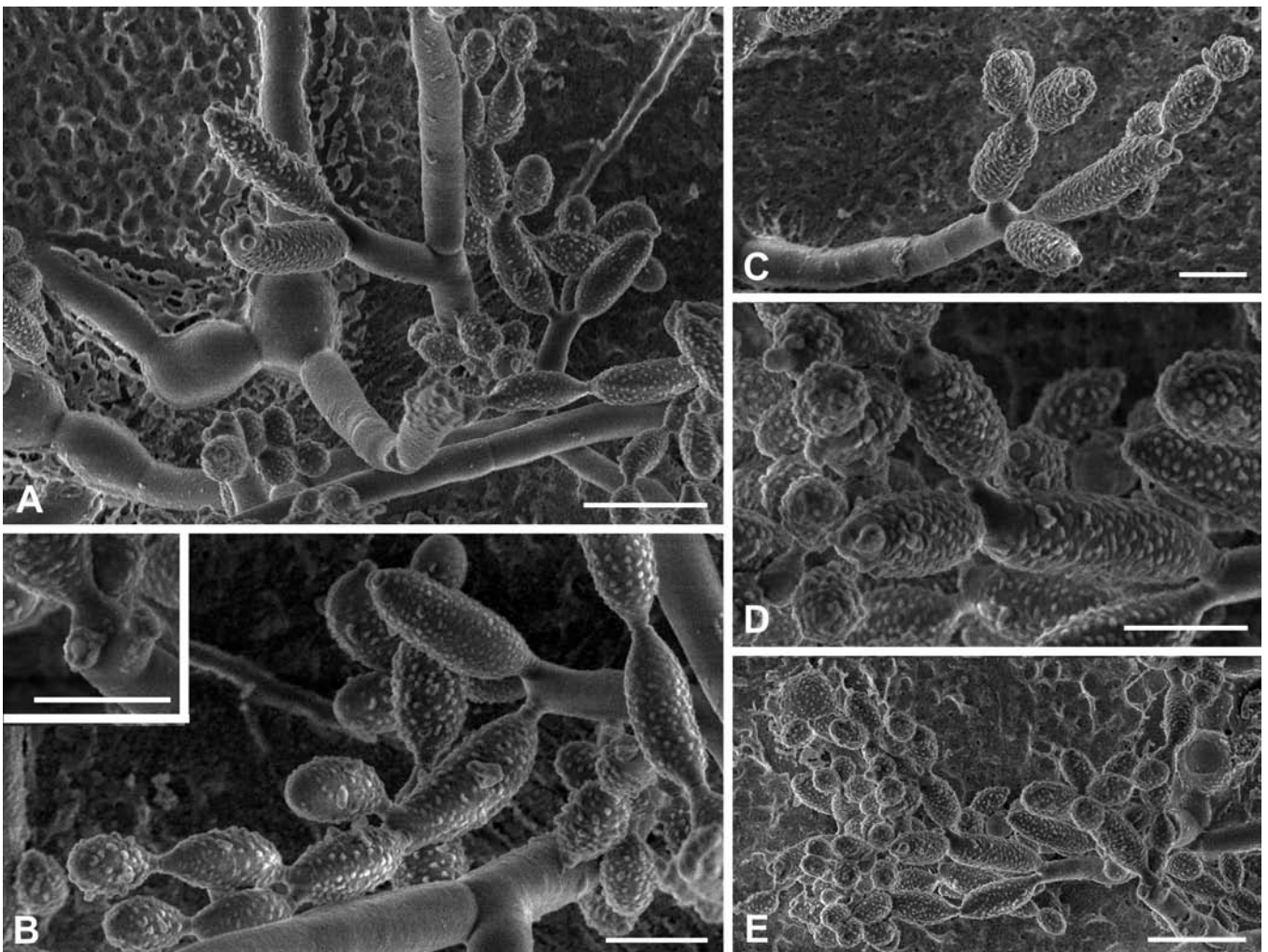
branched, branches often only as short lateral prolongations, mainly formed below a septum, 14–110  $\times$  2–4  $\mu\text{m}$ , septate, not constricted at the septa, subhyaline to pale olivaceous or brown, smooth to minutely verruculose, walls unthickened, sometimes guttulate. *Conidiogenous cells* integrated, terminal, sometimes also intercalary, cylindrical, not geniculate, non-nodulose, 10–28(–50)  $\mu\text{m}$  long, proliferation sympodial, with few conidiogenous loci, mostly 1–3, loci sometimes situated on small lateral prolongations, protuberant, 0.5–1.5(–2)  $\mu\text{m}$  diam, thickened and somewhat darkened-refractive. *Ramoconidia* formed, cylindrical-oblong, up to 47  $\mu\text{m}$  long, 2–4  $\mu\text{m}$  wide, 0–1-septate, rarely up to 4-septate, subhyaline to very pale olivaceous, smooth or almost so, with a broadly truncate base, without any dome and raised rim, 2–3  $\mu\text{m}$  wide, not thickened but somewhat refractive. *Conidia* numerous, polymorphous, catenate, in branched chains, straight, sometimes slightly curved, small terminal conidia numerous, globose, subglobose or ovoid, obovoid or limoniform, 2.5–7  $\times$  2–4(–4.5)  $\mu\text{m}$  [av.  $\pm$  SD, 5.1 ( $\pm$  1.3)  $\times$  3.1 ( $\pm$  0.6)  $\mu\text{m}$ ], aseptate, without distal hilum or with a single apical scar, intercalary conidia ellipsoid to

subcylindrical, 8–15  $\times$  3–4(–4.5)  $\mu\text{m}$  [av.  $\pm$  SD, 11.5 ( $\pm$  2.4)  $\times$  3.6 ( $\pm$  0.5)  $\mu\text{m}$ ], 0–1-septate; secondary ramoconidia subcylindrical to cylindrical-oblong, 17–35  $\times$  3–4(–5)  $\mu\text{m}$  [av.  $\pm$  SD, 22.5 ( $\pm$  5.6)  $\times$  3.7 ( $\pm$  0.5)  $\mu\text{m}$ ], 0–3-septate, not constricted at the septa, subhyaline to very pale olivaceous, minutely verruculose (granulate under SEM), walls unthickened or almost so, apex broadly rounded or slightly attenuated towards apex and base, sometimes guttulate, hila protuberant, conspicuous, 0.8–1.5(–2)  $\mu\text{m}$  diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occurring.

*Cultural characteristics*: Colonies on PDA reaching 46–49 mm diam after 14 d at 25  $^{\circ}\text{C}$ , olivaceous to grey-olivaceous due to abundant sporulation, appearing zonate in forming concentric zones, margin entire edge to slightly undulate, white, glabrous, aerial mycelium absent or sparse, growth flat with a somewhat folded and wrinkled colony centre, without prominent exudates, sporulation profuse. Colonies on MEA reaching 48–49 mm diam after 14 d at 25  $^{\circ}\text{C}$ , grey-olivaceous to olivaceous-grey, velvety, olivaceous-grey to



**Fig. 32.** *Cladosporium ramotenellum* (CPC 12043). A, C. Macronematous conidiophore. B. Conidial chain. D. Micronematous conidiophore. E. Ramoconidia and conidia. Scale bars = 10 µm.



**Fig. 33.** *Cladosporium ramotenellum* (CPC 12043). A. Survey of colony development showing a large bulbous "foot cell" that gives rise to conidiophores, which can be branched. B. Details of conidiophores showing secondary ramoconidia and conidia. The inset shows scar formation on a conidiophore. C. Conidiophore and several conidia. D. Details of ornamentation on conidia. Note the wide, but relatively low ornamentation units. E. A micrograph illustrating the organisation within a conidiophore. Scale bars A–D = 5 µm, E = 10 µm.

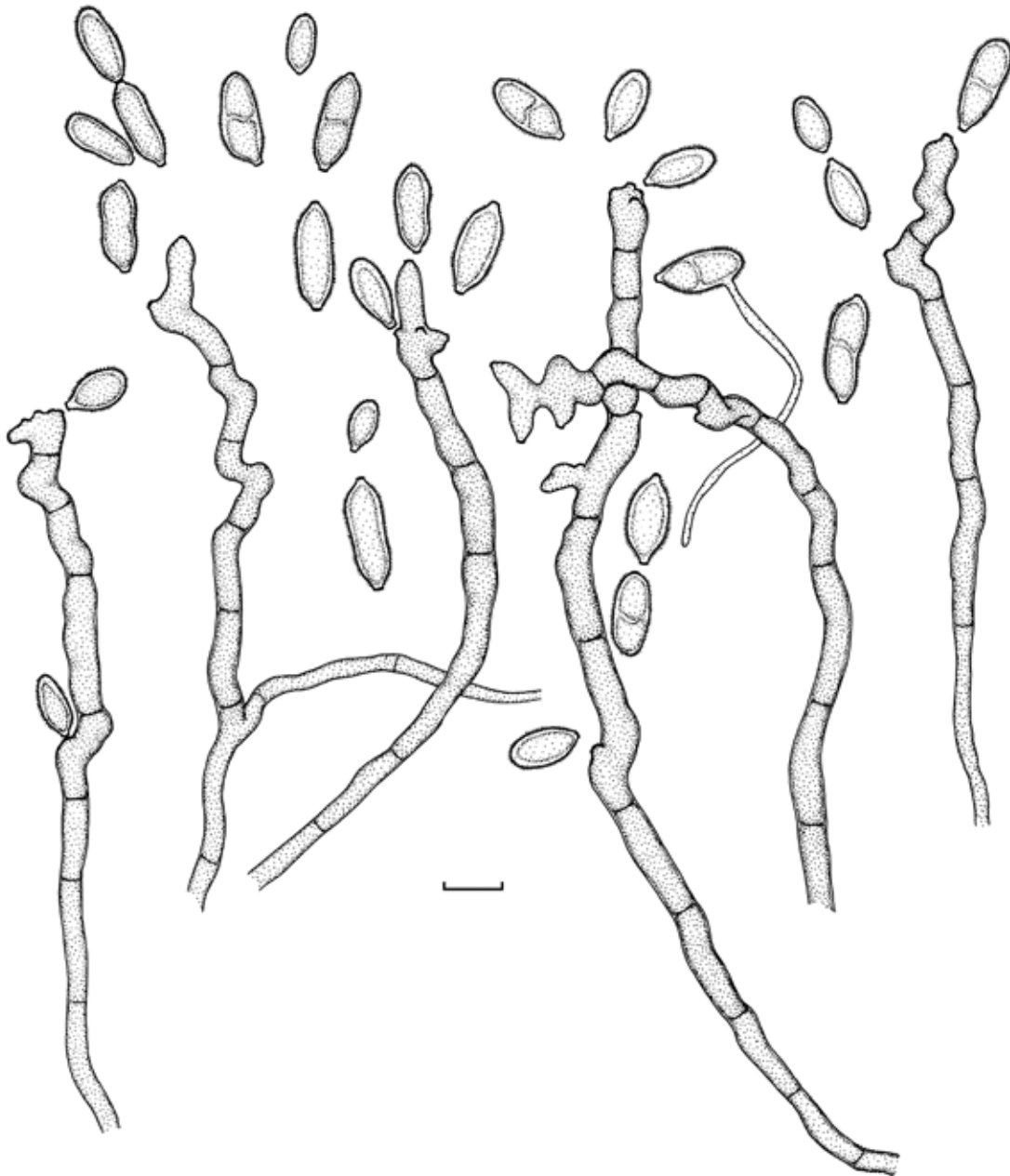


Fig. 34. *Cladosporium sinuosum* (CPC 11839). Conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

iron-grey reverse, margin entire edge to undulate, radially furrowed, colourless, glabrous to feathery, aerial mycelium sparse, diffuse, growth flat with slightly elevated colony centre, distinctly wrinkled, prominent exudates not formed, abundantly sporulating. Colonies on OA attaining 40 mm diam after 14 d at 25 °C, grey-olivaceous, margin entire edge, colourless or white, glabrous, aerial mycelium absent or sparse, growth flat, without exudates, sporulation profuse.

*Specimens examined:* **Slovenia**, Ljubljana, isolated from an air conditioning system (bathroom), 2004, M. Butala, CBS 121627 = CPC 12047 = EXF-967; Sečovlje, isolated from hypersaline water from reverse ponds, salterns, 2005, P. Zalar, CBS-H 19862, **holotype**, isotype HAL 2026 F, culture ex-type CBS 121628 = CPC 12043 = EXF-454.

*Substrate and distribution:* Hypersaline water, air; Slovenia.

*Notes:* *Cladosporium ramotenellum*, which appears to be a saprobe in air and hypersaline water, morphologically resembles *C. cladosporioides* and *C. tenellum* K. Schub., Zalar, Crous & U. Braun, but is quite distinct from *C. cladosporioides* by having somewhat narrower conidiophores and conidia, 2–4(–5)  $\mu\text{m}$

wide, and 0–3-septate, always minutely verruculose conidia. *Cladosporium tenellum*, a newly introduced species (see below) isolated from hypersaline water and plant material, possesses conidiophores with numerous conidiogenous loci, usually crowded towards the apex forming sympodial clusters of pronounced scars, and shorter and somewhat wider, 0–1(–2)-septate conidia, 3–20(–28)  $\times$  (2.5–)3–5(–6)  $\mu\text{m}$ . Besides these morphological differences, *C. ramotenellum* is faster growing in culture than *C. tenellum*.

*Cladosporium arthrinioides* Thüm. & Beltr. and *C. hypophyllum* Fuckel are also close to *C. ramotenellum*, but *C. arthrinioides*, known from Italy on leaves of *Bougainvillea spectabilis*, deviates in having shorter and wider, 0–1(–2)-septate, mostly smooth conidia (2–18  $\times$  2–6.5  $\mu\text{m}$ ) which become larger and more frequently septate with age (up to 32  $\mu\text{m}$  long and with up to four septa); and *C. hypophyllum* occurring in Europe on leaves of *Ulmus minor* differs in having often mildly to distinctly geniculate-sinuous, sometimes subnodulose conidiophores and shorter and somewhat wider, 0–1(–3)-septate conidia, 4–17(–19)  $\times$  2–5  $\mu\text{m}$ , becoming distinctly swollen, darker, longer and wider with age, 5–7  $\mu\text{m}$ , with the septa often being constricted (Schubert 2005).

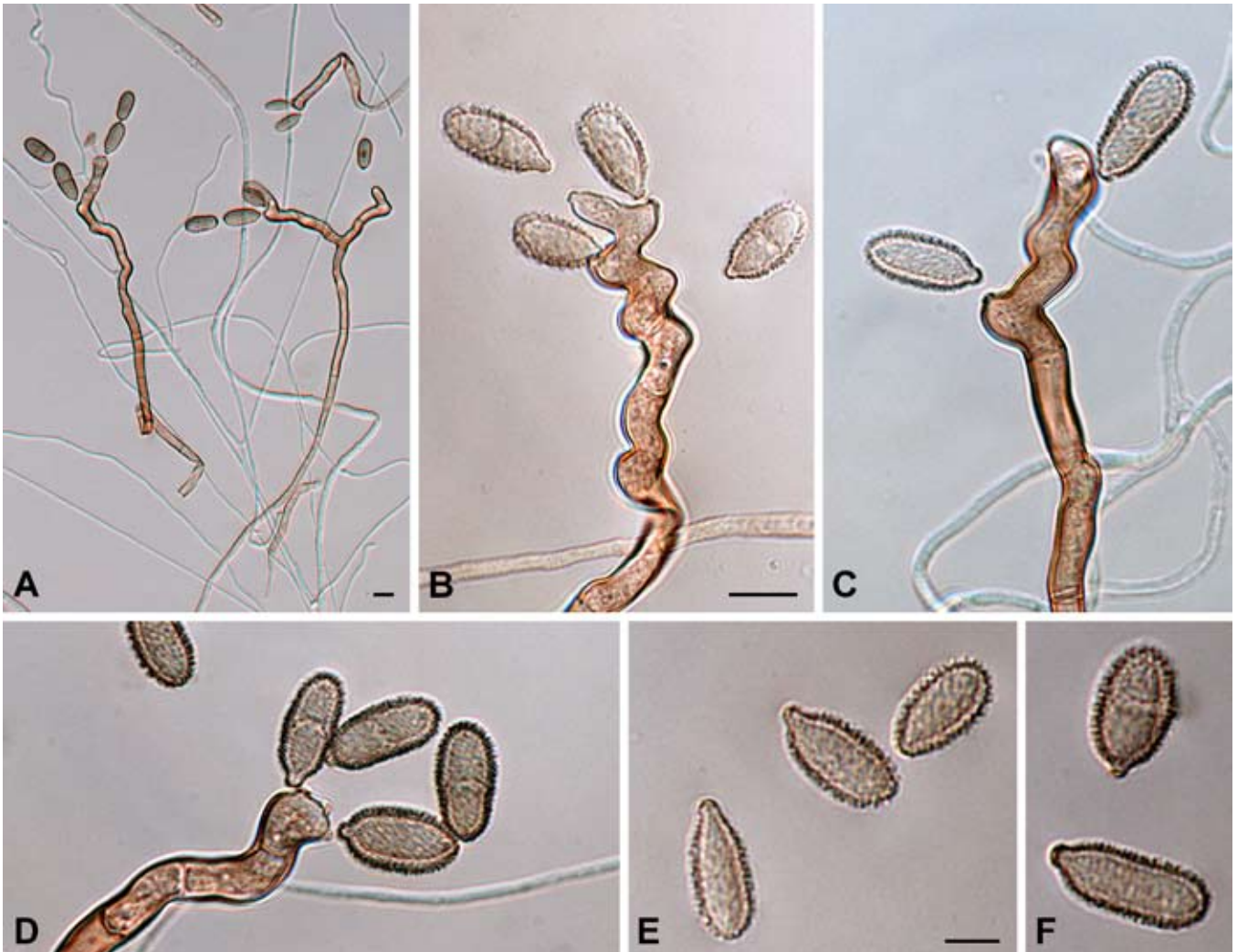


Fig. 35. *Cladosporium sinuosum* (CPC 11839). A–D. Conidiophores. E–F. Conidia. Scale bars = 10  $\mu$ m.

***Cladosporium sinuosum*** K. Schub., C.F. Hill, Crous & U. Braun, sp. nov. MycoBank MB504578. Figs 34–35.

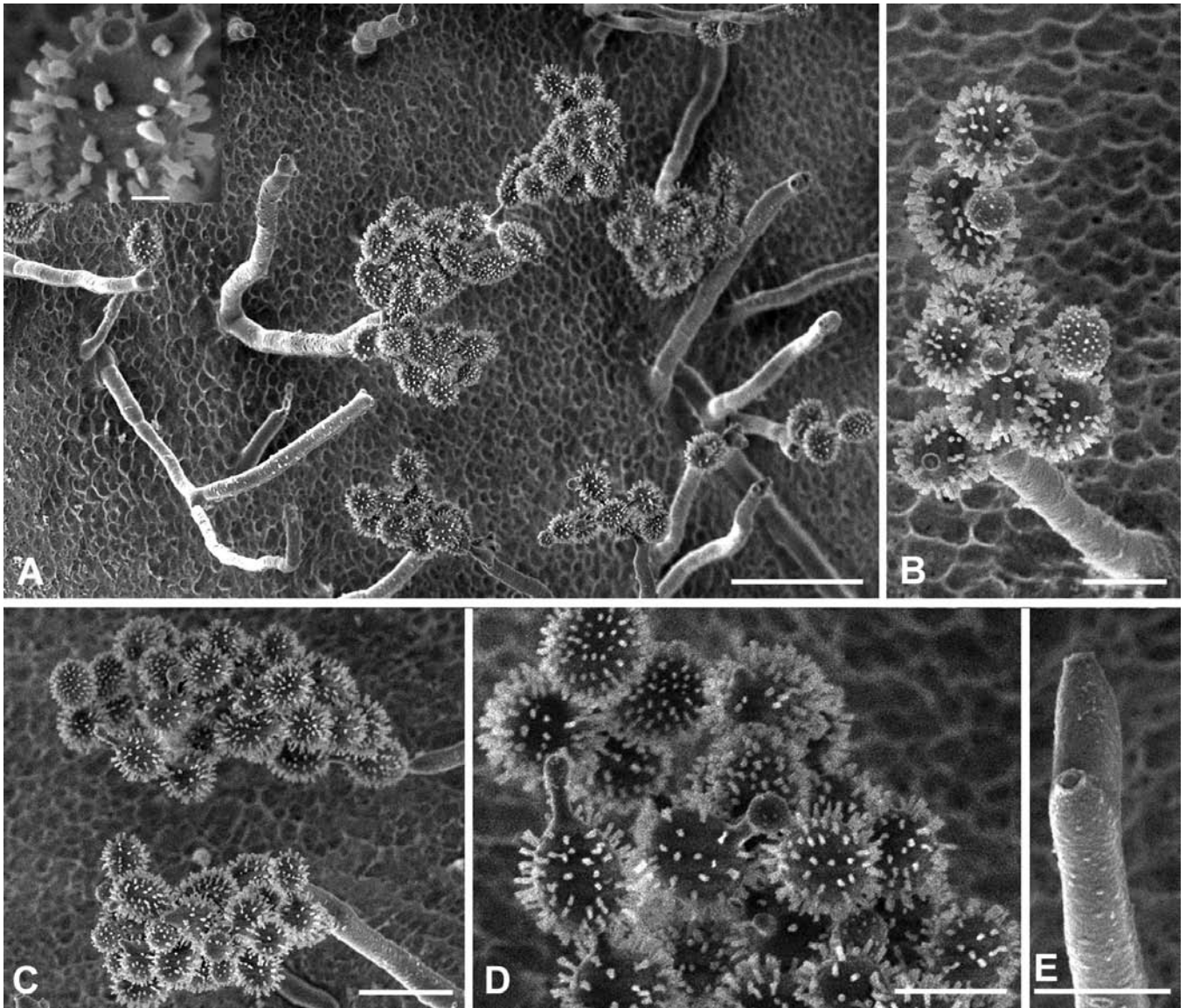
**Etymology:** Refers to the usually distinctly sinuous conidiophores.

Differt a *Cladosporio* herbaro conidiophoris distincte sinuosis, conidiis solitariis vel breve catenatis, catenis non ramosis, echinulatis.

**Mycelium** sparingly branched, 1–7  $\mu$ m wide, septate, not constricted at the septa, subhyaline to pale brown, smooth to minutely verruculose, walls unthickened or slightly thickened, sometimes with small swellings. **Conidiophores** arising laterally from plagiotropous hyphae or terminally from ascending hyphae, erect, more or less straight to flexuous, often once or several times slightly to distinctly geniculate-sinuuous, sometimes even zigzag-like, nodulose with small to large lateral shoulders, shoulders somewhat distant from each other or in close succession giving them a knotty/gnarled appearance, unbranched or once branched, 25–260  $\times$  5–7  $\mu$ m, shoulders up to 10  $\mu$ m wide, pluriseptate, septa sometimes in short succession, not constricted at the septa, pale brown to medium brown, smooth to minutely verruculose, walls thickened, often distinctly two-layered, up to 1  $\mu$ m thick. **Conidiogenous cells** integrated, terminal or intercalary, often slightly to distinctly geniculate-sinuuous, nodulose with small to large laterally swollen shoulders, 8–30  $\mu$ m long, proliferation sympodial, with a single or up to three conidiogenous loci, usually confined to lateral

shoulders, protuberant, often denticle-like or on the top of short cylindrical stalk-like prolongations, 1.2–2(–2.2)  $\mu$ m diam, mainly 2  $\mu$ m, somewhat thickened and darkened-refractive, dome often slightly higher than the surrounding rim. **Conidia** solitary or in short unbranched chains with up to three conidia, straight, obovoid, oval, broadly ellipsoid to subcylindrical or sometimes clavate (broader at the apex), 9–21  $\times$  (5–)6–8  $\mu$ m [av.  $\pm$  SD, 14.5 ( $\pm$  2.5)  $\times$  6.6 ( $\pm$  0.7)  $\mu$ m], 0–1-septate, not constricted at the septa, septum more or less median, pale greyish brown, densely echinulate, spines up to 1  $\mu$ m long, walls thickened, apex mostly broadly rounded or sometimes attenuated, towards the base mostly distinctly attenuated forming a peg-like prolongation, up to 2  $\mu$ m long, hila protuberant, 1.2–2  $\mu$ m diam, mainly 2  $\mu$ m, somewhat thickened and darkened-refractive; microcyclic conidiogenesis not observed.

**Cultural characteristics:** Colonies on PDA attaining 20 mm diam after 14 d at 25  $^{\circ}$ C, pale olivaceous-grey due to abundant aerial mycelium, olivaceous-grey towards margins, iron-grey to olivaceous-black reverse, margin regular, entire edge, aerial mycelium abundant, cottony, dense, high, growth regular, low convex, radially furrowed in the centre, growing deep into the agar, with age numerous small to large prominent exudates, sporulation sparse. Colonies on MEA attaining 16 mm diam after 14 d at 25  $^{\circ}$ C, white to pale smoke-grey, fawn reverse, velvety, margin undulate, glabrous, aerial mycelium abundant, dense, high, fluffy, growth raised with elevated colony centre, laterally furrowed, without



**Fig. 36.** *Cladosporium spinulosum* (CPC 12040). A. Overview on agar surface with conidiophores arising from the surface. The spore clusters on the conidiophore are very compact. Note several simple, tubular conidiophore ends. The inset shows details of a conidium showing two pronounced hila and a unique, very distinct ornamentation on the cell wall. B. Conidiophore with globose or subsphaerical secondary ramoconidia and conidia. Note the newly forming cells and hila. C. Two conidiophores. D. Details of spores and spore formation. E. The end of a conidiophore and two scars. Scale bars: A = 20  $\mu\text{m}$ , A (inset) = 1  $\mu\text{m}$ , B, D–E = 5  $\mu\text{m}$ , C = 10  $\mu\text{m}$ .

prominent exudates. Colonies on OA attaining 18 mm diam after 14 d at 25 °C, olivaceous, white to pale olivaceous-grey in the centre due to abundant aerial mycelium, olivaceous-grey reverse, margin white, entire edge, glabrous, aerial mycelium loose to dense, high, fluffy to felty, growth flat to low convex, regular, without prominent exudates, sporulating.

*Specimen examined:* **New Zealand**, Te Anau, isolated from leaves of *Fuchsia excorticata* (Onagraceae), 31 Jan. 2005, A. Blouin, Hill 1134A, CBS-H 19863, **holotype**, culture ex-type CBS 121629 = CPC 11839 = ICMP 15819.

*Substrate and distribution:* On living leaves of *Fuchsia excorticata*; New Zealand.

*Notes:* This new species is well characterised by its slightly distinctly geniculate-sinuous, often zigzag-like conidiophores and its conidia formed solitary or rarely in short unbranched chains and is therefore morphologically not comparable with any of the species described until now. Most *Cladosporium* species with conidia usually formed solitary or in short unbranched chains have previously been treated as species of the genus *Heterosporium* Klotzsch ex

Cooke, now considered to be synonymous with *Cladosporium*. All of them, including the newly introduced *C. arthropodii* K. Schub. & C.F. Hill from New Zealand, which also belongs to this species complex (Braun *et al.* 2006), possess very large and wide, often pluriseptate conidia quite distinct from those of *C. sinuosum* (David 1997). *Cladosporium alopecuri* (Ellis & Everh.) U. Braun, known from the U.S.A. on *Alopecurus geniculatus* is also quite different by having larger and wider conidia, 20–40  $\times$  7–13(–15)  $\mu\text{m}$ , and wider conidiogenous loci and conidial hila, 3.5–5  $\mu\text{m}$  diam (Braun 2000).

*Cladosporium herbarum* is superficially similar but the conidiophores of the latter species are sometimes only slightly geniculate-sinuous but never zigzag-like and the verruculose to verrucose conidia are frequently formed in unbranched or branched chains.

***Cladosporium spinulosum*** Zalar, de Hoog & Gunde-Cimerman, *Studies in Mycology* 58: 180. 2007 – this volume. Fig. 36.

*Note:* This new species is described and illustrated in Zalar *et al.* (2007 – this volume).

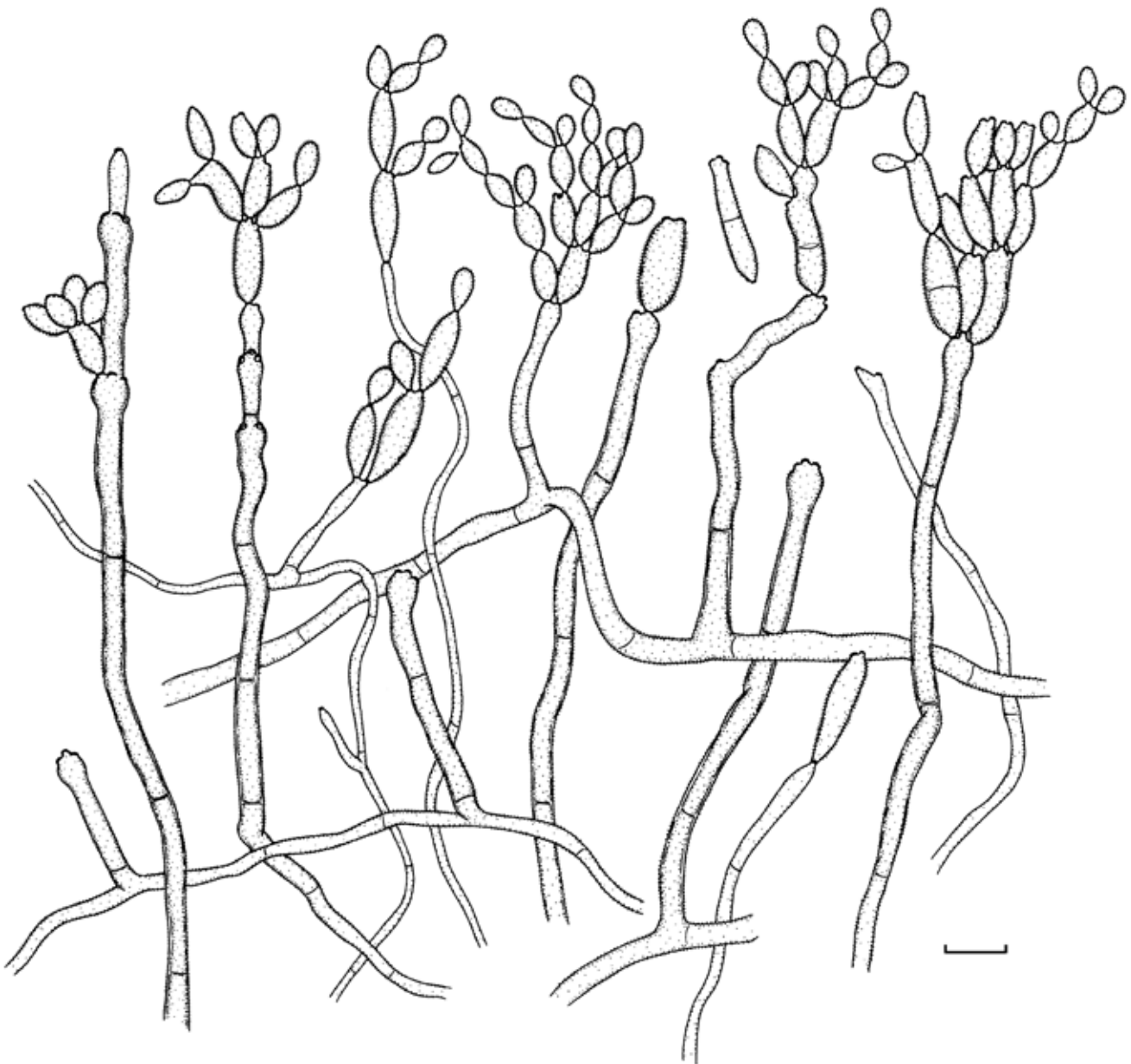


Fig. 37. *Cladosporium subinflatum* (CPC 12041). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

***Cladosporium subinflatum*** K. Schub., Zalar, Crous & U. Braun, sp. nov. MycoBank MB504579. Figs 37–39.

*Etymology*: Refers to its nodulose conidiophores.

Differt a *Cladosporio bruhnei* conidiophoris cum nodulis angustioribus, 3–6.5  $\mu\text{m}$  latis, conidiis brevioribus, 4–17(–22)  $\mu\text{m}$  longis, spinulosis, cum spinulis ad 0.8  $\mu\text{m}$  longis; et a *Cladosporio spinuloso* conidiophoris nodulosis, conidiis spinulosis, cum spinulis brevioribus, ad 0.8 longis, locis conidiogenis et hilis latioribus, (0.5–)1–2  $\mu\text{m}$  latis.

*Mycelium* unbranched or occasionally branched, 1.5–3  $\mu\text{m}$  wide, later more frequently branched and wider, up to 7  $\mu\text{m}$  wide, septate, not constricted at the septa, hyaline or subhyaline, almost smooth to somewhat verruculose or irregularly rough-walled, walls unthickened. *Conidiophores* mainly macronematous, sometimes also micronematous, arising terminally from ascending hyphae or laterally from plagiotropous hyphae, erect or subdecumbent, straight or flexuous, sometimes bent, cylindrical, nodulose, usually with small head-like swellings, sometimes swellings also on a lower level or intercalary, occasionally geniculate, unbranched, occasionally branched, (5–)10–270  $\times$  (1.5–)2.5–4.5(–5.5)  $\mu\text{m}$ ,

swellings 3–6.5  $\mu\text{m}$  wide, aseptate or with few septa, not constricted at the septa, pale brown, pale olivaceous-brown or somewhat reddish brown, smooth, usually verruculose or irregularly rough-walled and paler, subhyaline towards the base, walls thickened, sometimes appearing even two-layered, up to 1  $\mu\text{m}$  thick. *Conidiogenous cells* integrated, usually terminal or conidiophores reduced to conidiogenous cells, cylindrical, nodulose, usually with small head-like swellings with loci confined to swellings, sometimes geniculate, 5–42  $\mu\text{m}$  long, proliferation sympodial, with several loci, up to four situated at nodules or on lateral swellings, protuberant, conspicuous, denticulate, (0.8–)1–2  $\mu\text{m}$  diam, thickened and darkened-refractive. *Conidia* catenate, in branched chains, more or less straight, numerous globose and subglobose conidia, ovoid, obovoid, broadly ellipsoid to cylindrical, 4–17(–22)  $\times$  (2.5–)3.5–5.5(–7)  $\mu\text{m}$  [av.  $\pm$  SD, 11.7 ( $\pm$  4.6)  $\times$  4.5 ( $\pm$  0.8)  $\mu\text{m}$ ], 0–1(–2)-septate, not constricted at septa, pale brown or pale olivaceous-brown, ornamentation variable, mainly densely verruculose to echinulate (loosely muricate under SEM), spines up to 0.8  $\mu\text{m}$  high, sometimes irregularly verruculose with few scattered tubercles



Fig. 38. *Cladosporium subinflatum* (CPC 12041). A–C. Macronematous conidiophores. D–E. Conidia. Scale bar = 10 µm.

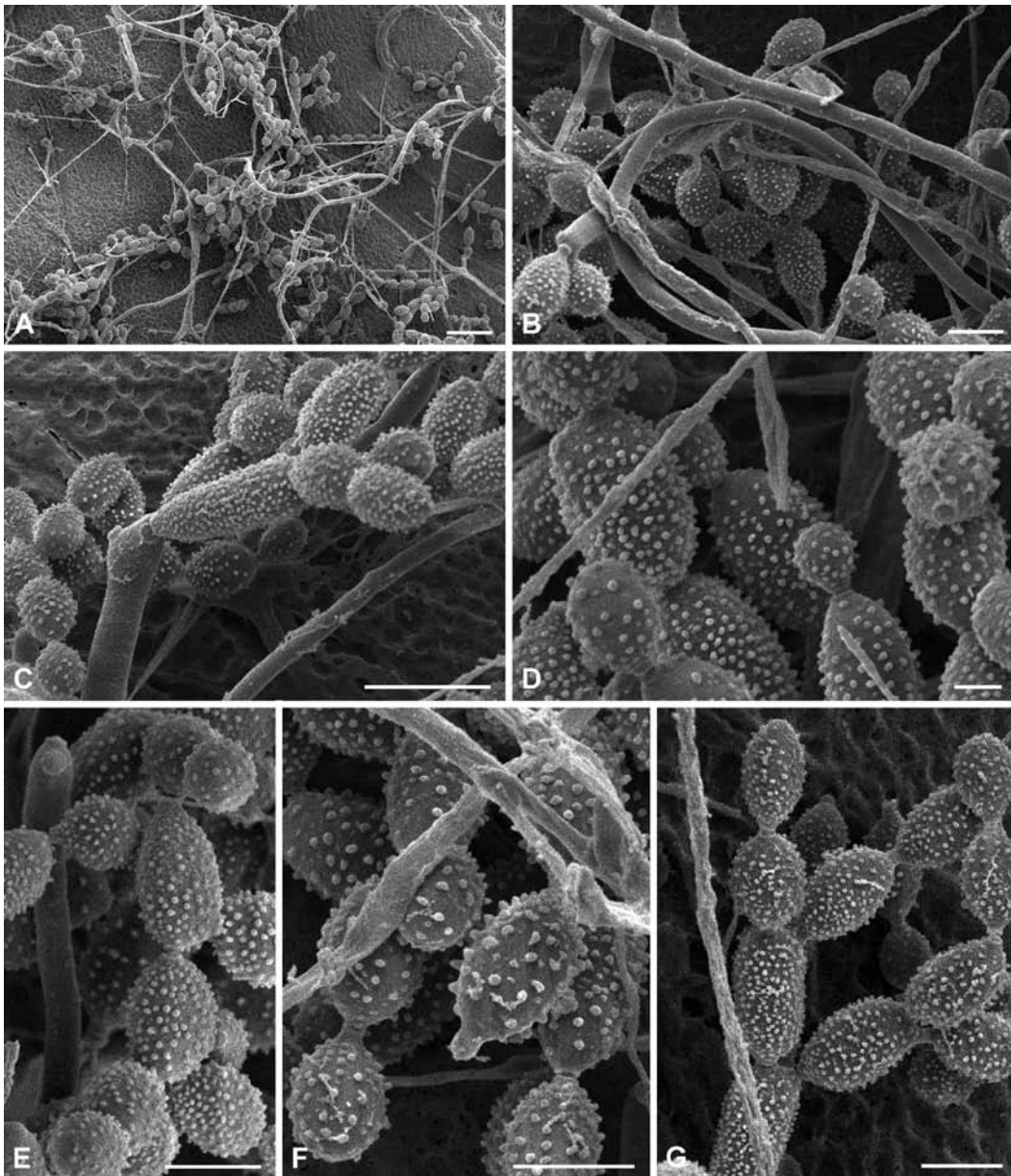


Fig. 39. *Cladosporium subinflatum* (CPC 12041). A–G. Images of an 11-d-old culture on SNA. A. Overview of colony with clusters of conidia and aerial hyphae. Many of the hyphae have a collapsed appearance. B. Detail of colony with conidiophores, conidia and aerial hyphae that are partly collapsed. C. Detail of a conidiophore end and a secondary ramoconidium. Note the scars at the end of the conidiophore. D. Details of conidia and ornamentation. The ornamentation consists out of markedly defined units, which have a relatively large distance from each other. Note the hilum on the right conidium. E. Conidiophore with large scars and conidia. F. Different blastoconidia with very early stages of new spore formation in the middle of the picture. G. Pattern of spore development. Scale bars: A = 20 µm, B, E–G = 5 µm, C = 10 µm, D = 2 µm.



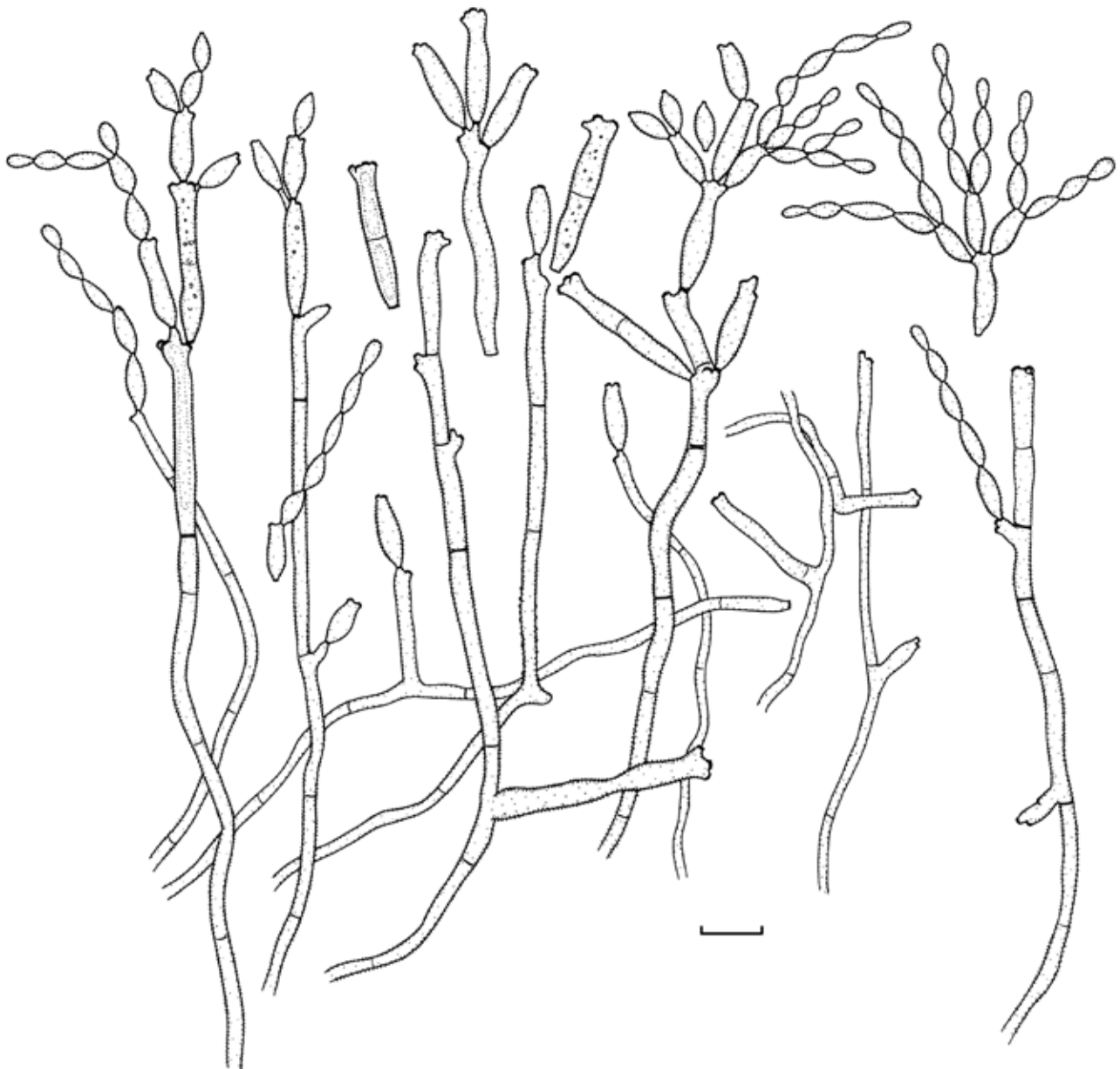
or irregularly echinulate, walls unthickened or slightly thickened, apex rounded or slightly attenuated towards apex and base, hila conspicuous, protuberant, denticulate, 0.5–2  $\mu\text{m}$  diam, thickened and darkened-refractive; microcyclic conidiogenesis observed.

**Cultural characteristics:** Colonies on PDA attaining 29 mm diam after 14 d at 25  $^{\circ}\text{C}$ , olivaceous-black to olivaceous-grey towards margin, margin regular, entire edge, narrow, colourless to white, glabrous to feathery, aerial mycelium formed, fluffy, mainly near margins, growth flat, somewhat folded in the colony centre, deep into the agar, few prominent exudates formed with age, sporulation profuse. Colonies on MEA attaining 25 mm diam after 14 d at 25  $^{\circ}\text{C}$ , olivaceous-grey to olivaceous due to abundant sporulation in the colony centre, pale greenish grey towards margin, iron-grey reverse, velvety to powdery, margin crenate, narrow, white, glabrous, radially furrowed, aerial mycelium diffuse, growth convex with papillate surface, wrinkled colony centre, without prominent exudates, sporulation profuse. Colonies on OA attaining 26 mm diam after 14 d at 25  $^{\circ}\text{C}$ , olivaceous, iron-grey to greenish black reverse, growth flat, deep into the agar, with a single exudate, abundantly sporulating.

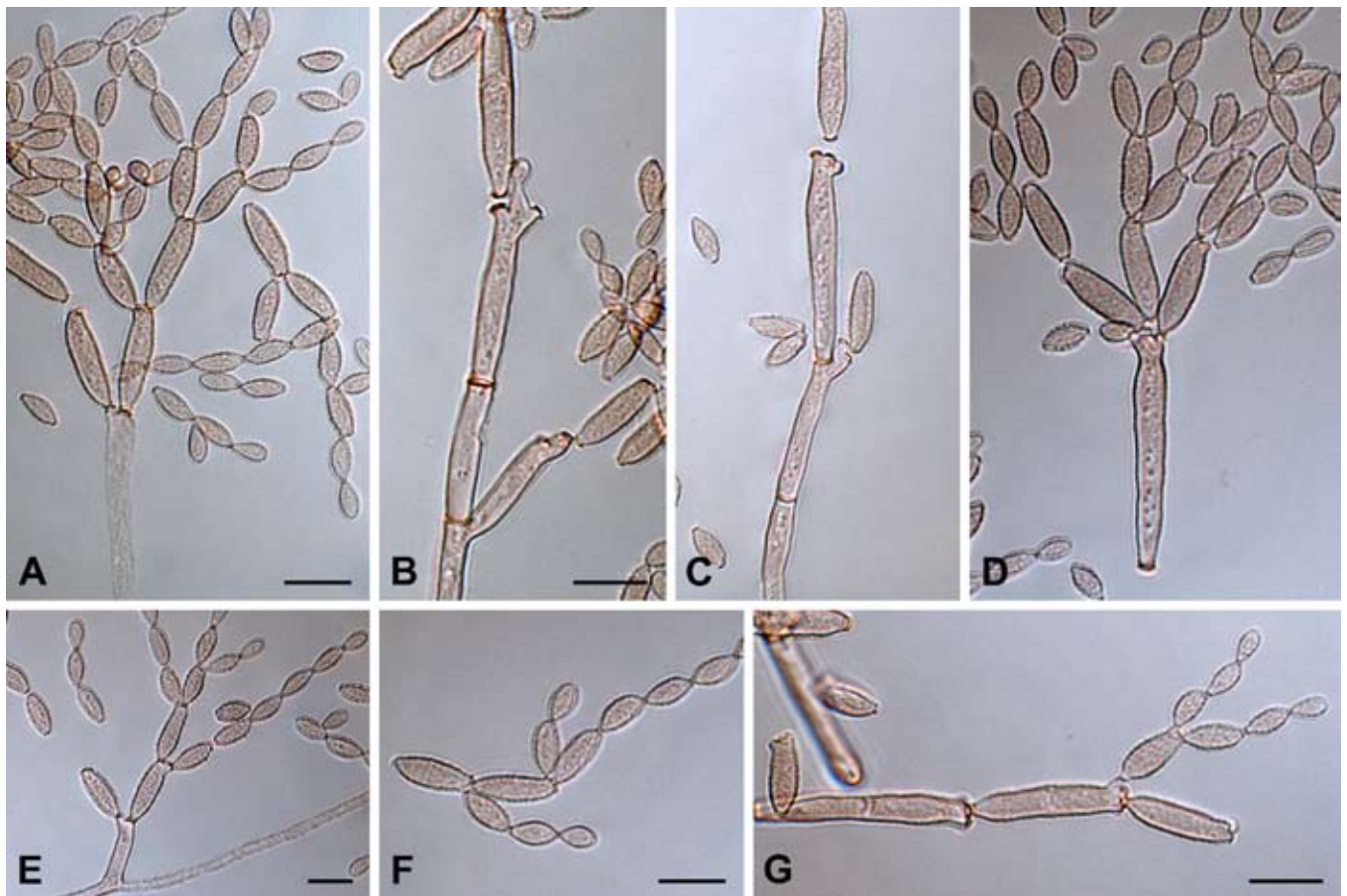
**Specimen examined:** Slovenia, Sečovlje, isolated from hypersaline water from crystallization ponds, salterns, 2005, S. Sonjak, CBS-H 19864, **holotype**, isotype HAL 2027 F, culture ex-type CBS 121630 = CPC 12041 = EXF-343.

**Substrate and distribution:** Hypersaline water; Slovenia.

**Notes:** *Cladosporium subinflatum*, an additional saprobic species isolated from hypersaline water, was at first identified as *C. spinulosum*, but proved to be both morphologically as well as phylogenetically distinct from the latter species in having somewhat wider [(1.5–)2.5–4.5(–5.5)  $\mu\text{m}$ ], nodulose macronematous conidiophores with conidiogenous loci confined to swellings, wider conidiogenous loci and hila, (0.8–)1–2  $\mu\text{m}$ , and spiny conidia with shorter spines than in *C. spinulosum* (up to 0.8  $\mu\text{m}$  versus 0.5–1.3  $\mu\text{m}$  long) (Zalar *et al.* 2007). With its narrow, nodulose macronematous conidiophores and catenate conidia, *C. bruhnei* is morphologically also similar but differs by having conidiophores with wider swellings, (4–)5–8  $\mu\text{m}$ , and longer conidia 4–24(–31)  $\mu\text{m}$ , rarely up to 40  $\mu\text{m}$  long which are minutely verruculose to verrucose but not spiny.



**Fig. 40.** *Cladosporium subtilissimum* (CBS 113754). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert *del.*



**Fig. 41.** *Cladosporium subtilissimum* (CBS 113754). A–C. Macronematous conidiophores. D. Conidial chain. E. Micronematous conidiophore. F–G. Conidia. Scale bars = 10  $\mu\text{m}$ .

***Cladosporium subtilissimum*** K. Schub., Dugan, Crous & U. Braun, **sp. nov.** MycoBank MB504580. Figs 40–42.

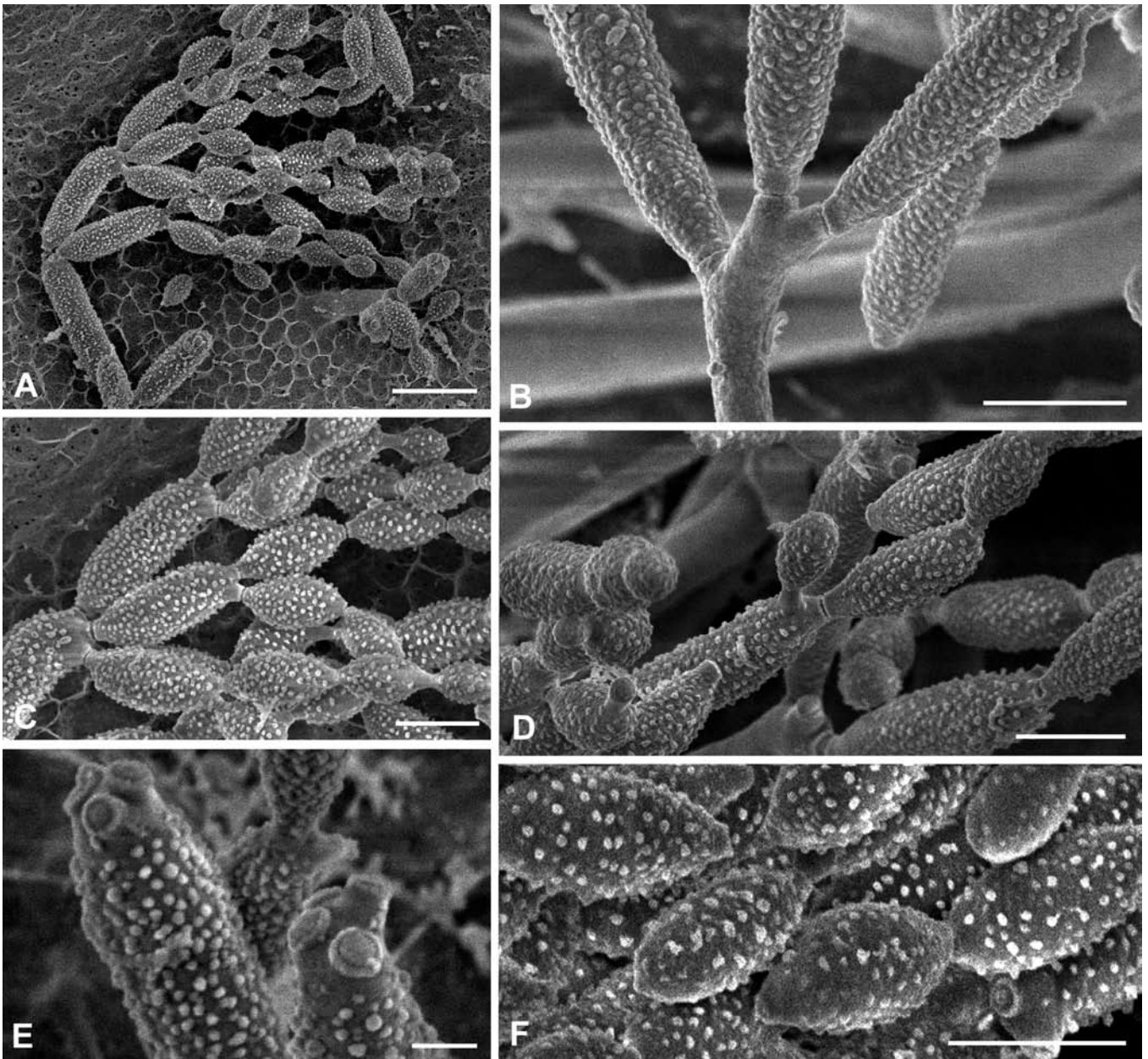
**Etymology:** Refers to its narrow conidiophores and conidia.

Differt a *Cladosporio cladosporioides* conidiophoris et conidiis semper asperulatis ad verruculositas, conidiis 0–1(–2)-septatis.

**Mycelium** unbranched or sparingly branched, 1–5  $\mu\text{m}$  wide, septate, without swellings and constrictions, hyaline to subhyaline or pale brown, smooth to minutely verruculose, walls unthickened or almost so, protoplasm somewhat guttulate or granular. **Conidiophores** macronematous and micronematous, arising laterally from plagiotropous hyphae or terminally from ascending hyphae, erect, straight to slightly flexuous, filiform to cylindrical-oblong, non-nodulose, sometimes geniculate towards the apex, unbranched or once branched, branches short to somewhat longer, usually formed below a septum, sometimes only short, denticle-like or conical, 25–140  $\times$  2–4  $\mu\text{m}$ , 0–4-septate, not constricted at the septa, subhyaline to pale brown, almost smooth, minutely verruculose to verruculose, sometimes irregularly rough-walled in the lower part, walls unthickened or slightly thickened, protoplasm guttulate or somewhat granular. **Conidiogenous cells** integrated, terminal or pleurogenous, sometimes also intercalary, filiform to narrowly cylindrical, non-nodulose, sometimes geniculate, 14–57  $\mu\text{m}$  long, with usually sympodial clusters of pronounced conidiogenous loci at the apex or on a lower level, denticle-like or situated on short lateral prolongations, up to five loci, intercalary conidiogenous cells usually with a short denticle-like lateral outgrowth below a septum, protuberant, denticulate, somewhat truncate, 1.2–2  $\mu\text{m}$  diam, thickened and darkened-refractive. **Ramoconidia** sometimes occurring, conidiogenous cells seceding at one of the upper septa

of the conidiophore and behaving like conidia, filiform or cylindrical, 20–40(–55)  $\mu\text{m}$  long, 1.5–4  $\mu\text{m}$  wide, 0–1-septate, concolorous with conidiophores, not attenuated towards apex and base, base broadly truncate, non-cladosporioid, without any dome and raised rim, 2–3.5  $\mu\text{m}$  wide, neither thickened nor darkened, sometimes slightly refractive. **Conidia** catenate, in branched chains, up to 12 or even more in a chain, straight, small terminal conidia numerous, subglobose, narrowly obovoid, limoniform or fusiform, 4–9  $\times$  2–3.5  $\mu\text{m}$  [av.  $\pm$  SD, 6.4 ( $\pm$  1.5)  $\times$  2.8 ( $\pm$  0.4)  $\mu\text{m}$ ], with up to three distal scars, aseptate, hila (0.5–)0.8–1  $\mu\text{m}$  diam, intercalary conidia narrowly ellipsoid, fusiform to subcylindrical, 9–18  $\times$  3–4(–6)  $\mu\text{m}$  [av.  $\pm$  SD, 13.0 ( $\pm$  2.5)  $\times$  3.8 ( $\pm$  0.3)  $\mu\text{m}$ ], 0(–1)-septate, hila 1–1.2(–1.8)  $\mu\text{m}$  diam, with up to four distal scars, secondary ramoconidia ellipsoid, fusiform or subcylindrical, (13–)17–32(–37)  $\times$  3–5(–6)  $\mu\text{m}$  [av.  $\pm$  SD, 21.4 ( $\pm$  4.4)  $\times$  4.1 ( $\pm$  0.5)  $\mu\text{m}$ ], 0–1(–2)-septate, septum median or somewhat in the lower half, usually not constricted at the septa, with up to six distal hila crowded at the apex, hila (1.2–)1.5–2(–2.5)  $\mu\text{m}$  diam, apex often somewhat laterally enlarged or prolonged with hila crowded there, very pale or pale brown or olivaceous-brown, minutely verruculose to verruculose (granulate under SEM), walls unthickened or only slightly thickened, often slightly attenuated towards apex and base, protoplasm often guttulate or granular, hila protuberant, denticulate, (0.5–)0.8–2(–2.2)  $\mu\text{m}$  diam, thickened and darkened-refractive; microcyclic conidiogenesis occasionally observed.

**Cultural characteristics:** Colonies on PDA attaining 24 mm diam after 14 d at 25  $^{\circ}\text{C}$ , grey-olivaceous to olivaceous, olivaceous-grey, iron-grey or olivaceous-black reverse, velvety, margin regular, entire edge, white or pale greenish olivaceous, glabrous to feathery, aerial mycelium sparse, only few areas with abundant



**Fig. 42.** *Cladosporium subtilissimum* (CBS 113754). A. Overview on the organisation of spore formation. The micrograph shows a large basal secondary ramoconidium which has chains of secondary ramoconidia, intercalary and small terminal conidia. The conidia are formed in rows of often three cells. Note the size difference in the different cells. B. Conidiophore showing very pronounced scars that almost appear as branches. C. Detail of (A), illustrating the scar formation between the cells. D. Conidia during different stages of formation. E. Details of pronounced hila, and prominent ornamentation on secondary ramoconidia with the central dome-formed area. F. Different conidia and hila. Scale bars: A = 10  $\mu\text{m}$ , B–D, F = 5  $\mu\text{m}$ , E = 2  $\mu\text{m}$ .

mycelium, diffuse, growth regular, flat or with a raised and wrinkled colony centre, radially furrowed, effuse, usually without prominent exudates, with age several exudates formed, sporulation profuse, colonies consisting of two kinds of conidiophores, short and a few longer ones. Colonies on MEA reaching 25 mm diam after 14 d at 25 °C, greenish olivaceous to grey-olivaceous in the centre, olivaceous-grey to iron-grey reverse, velvety, margin entire edge, crenate or umbonate, narrow, pale greenish olivaceous, sometimes radially furrowed, aerial mycelium absent or sparse, growth low convex with distinctly wrinkled colony centre, without prominent exudates, abundantly sporulating. Colonies on OA attaining 25 mm diam after 14 d at 25 °C, dark grey-olivaceous to olivaceous due to profuse sporulation, iron-grey reverse, sometimes releasing some olivaceous-buff pigments into the agar, velvety, margin regular, entire edge or crenate, narrow, colourless or white, glabrous or feathery, aerial mycelium sparse, growth flat with slightly raised colony centre, prominent exudates lacking, sporulation profuse.

*Specimens examined:* **Slovenia**, Sečovlje, isolated from hypersaline water from salterns (reserve pond), 2005, P. Zalar, CPC 12044 = EXF-462. **U.S.A.**, isolated from bing cherry fruits, F. Dugan, CBS 113753; isolated from a grape berry, F. Dugan, wf 99-2-9 sci 1, CBS-H 19865, **holotype**, isotype HAL 2028 F, culture ex-type CBS 113754.

*Excluded strains within the subtilissimum complex:* **Argentina**, isolated from *Pinus ponderosa* (*Pinaceae*), 2005, A. Greslebin, CPC 12484, CPC 12485. **U.S.A.**, isolated from grape berry, F. Dugan, CBS 113741, CBS 113742; isolated from grape bud, F. Dugan, CBS 113744.

*Substrate and distribution:* Plant material and hypersaline water; Slovenia, U.S.A.

*Notes:* *Cladosporium cladosporioides* is morphologically comparable with the new species but deviates in having usually smooth conidiophores and conidia, with the conidia being mainly aseptate. *C. subtilissimum* is represented by three isolates of different origins

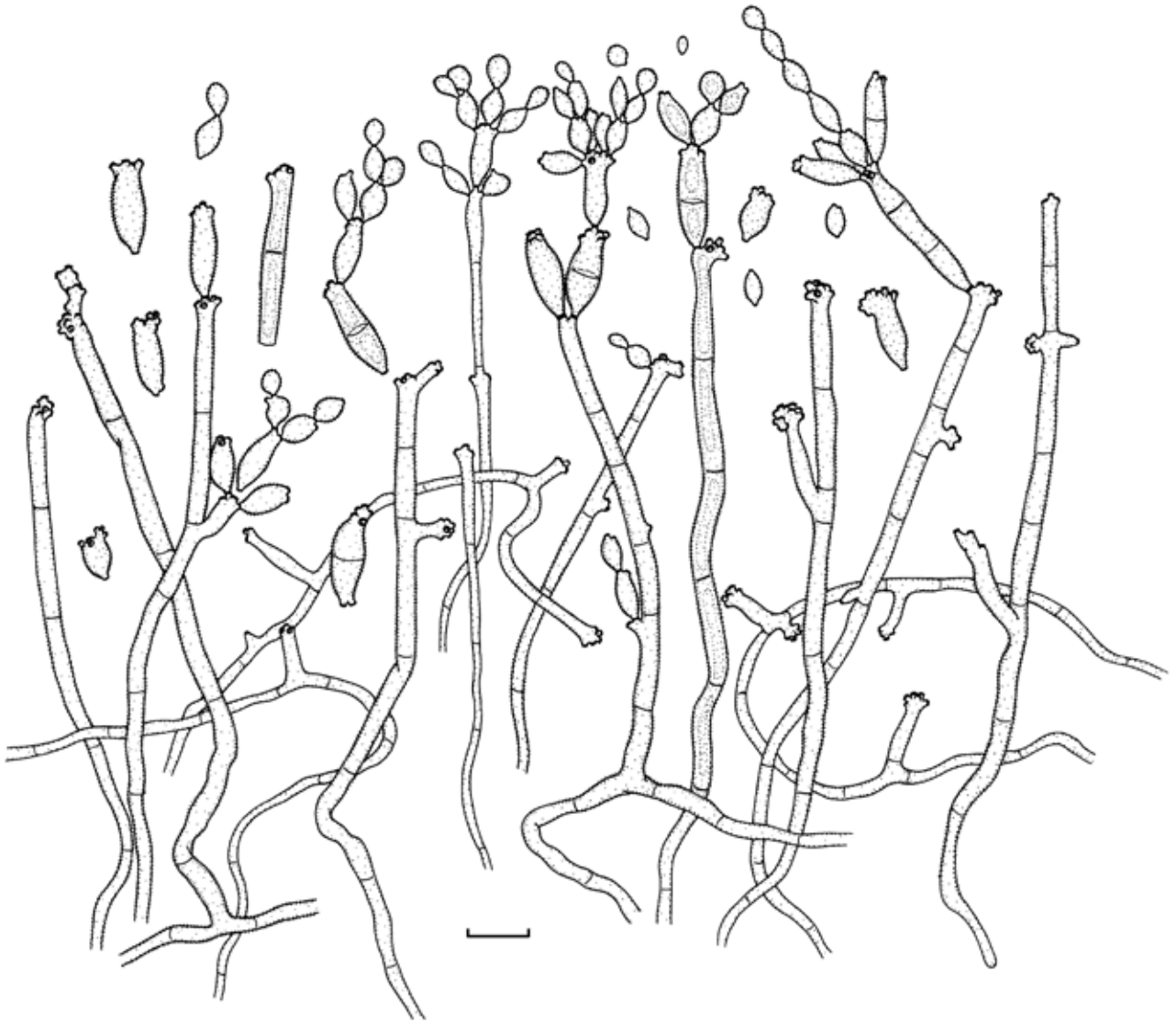


Fig. 43. *Cladosporium tenellum* (CPC 12053). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu$ m. K. Schubert del.

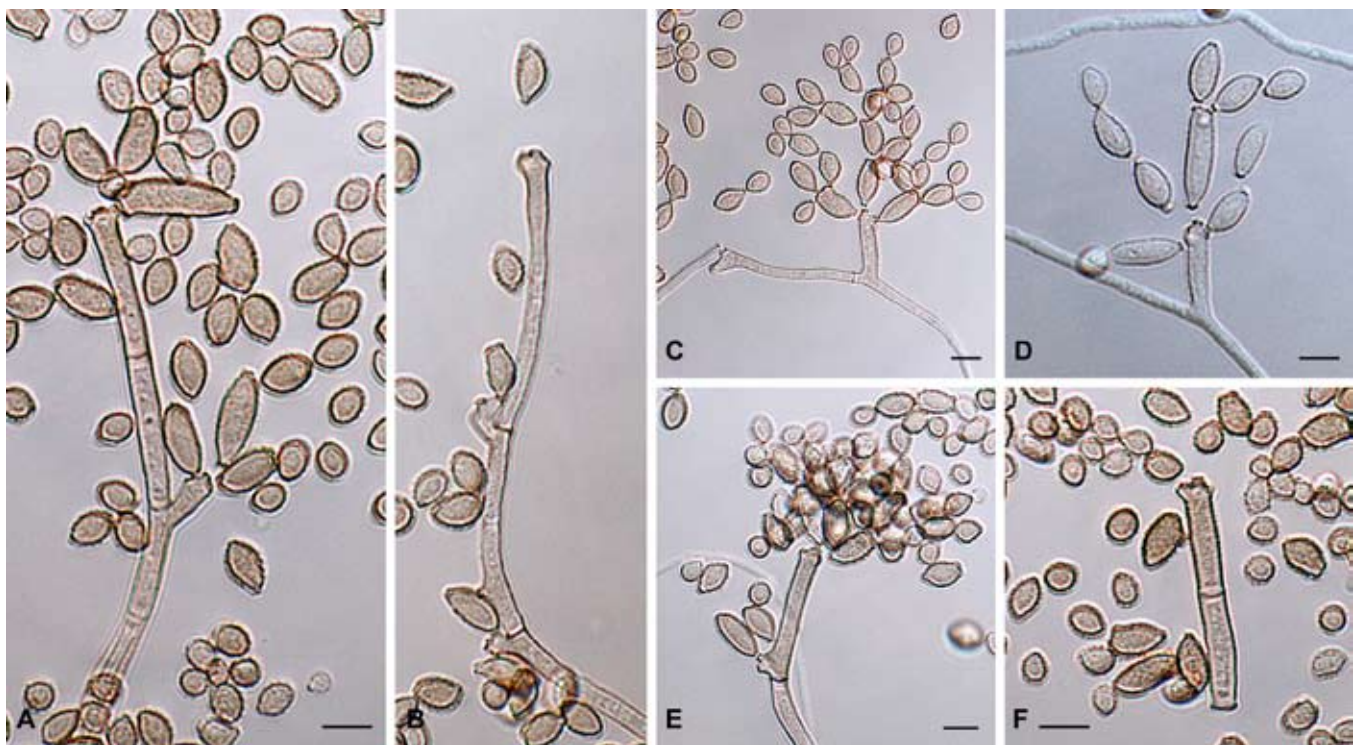
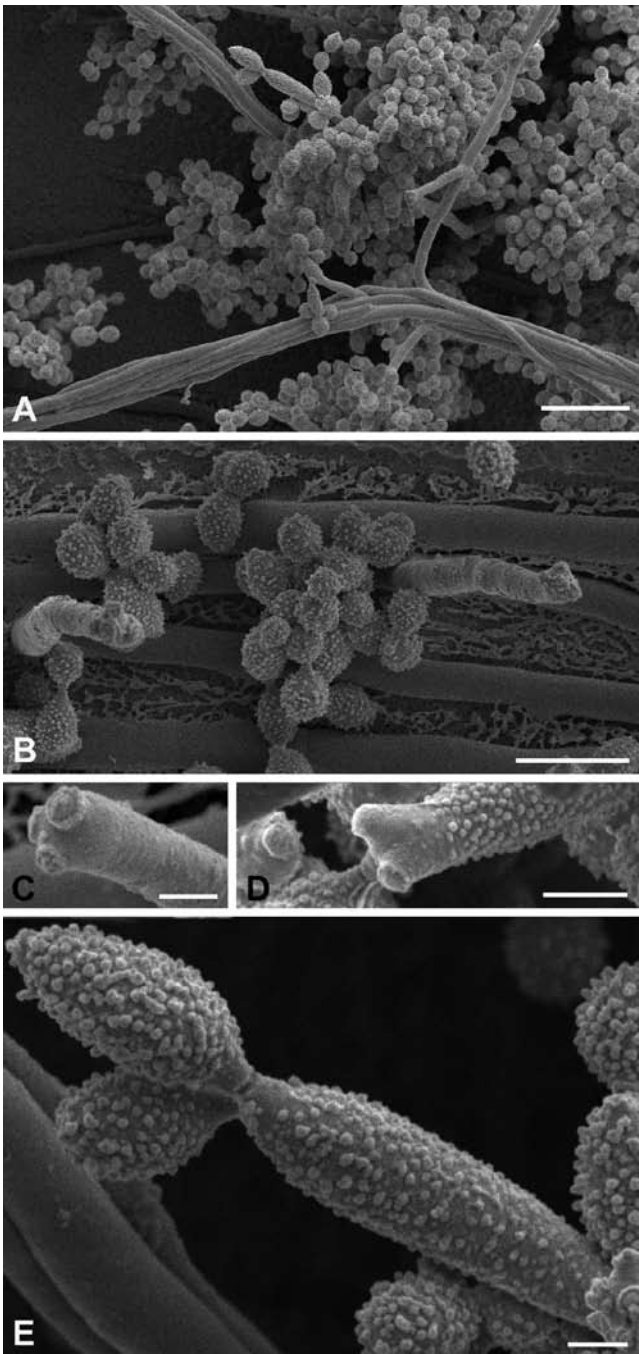


Fig. 44. *Cladosporium tenellum* (CPC 12053). A–C, E. Macro-nematous conidiophore. D. Micro-nematous conidiophore. F. Ramoconidium and conidia. Scale bars = 10  $\mu$ m.



**Fig. 45.** *Cladosporium tenellum* (CPC 12053). A. A bird's eye view of a colony of *C. tenellum* with its very characteristic bundles of aerial hyphae. Numerous conidia are visible, formed on simple conidiophores. B. Hyphae that run on the agar surface give rise to conidiophores and numerous conidia, that are relatively rounded. C. Conidiophore ends are rather simple and have large scars. D. Hila on a secondary ramoconidium with non-ornamented area. E. Detail of the prominent ornamentation on a secondary ramoconidium. Scale bars: A = 20  $\mu\text{m}$ , B = 10  $\mu\text{m}$ , C, E = 2  $\mu\text{m}$ , D = 5  $\mu\text{m}$ .

and substrates. Besides these strains, several additional isolates listed under excluded strains are morphologically indistinguishable from *C. subtilissimum* in culture, but genetically different, clustering in various subclades. They are indicated as *Cladosporium* sp. in the tree (Fig. 3).

***Cladosporium tenellum*** K. Schub., Zalar, Crous & U. Braun, **sp. nov.** MycoBank MB504581. Figs 43–45.

**Etymology:** Refers to its narrow conidiophores and conidia.

Differt a *Cladosporio* cladosporioide conidiophoris et conidiis semper asperulatis, locis conidiogenis apicalibus, numerosis, hilis quoque numerosis, conidiophoris angustioribus, (1–)1.5–3.5(–4)  $\mu\text{m}$  latis; et a *Cladosporio* subtilissimo loci conidiogenis et hilis apicalibus, numerosis, angustioribus, saepe 1–1.5  $\mu\text{m}$  latis, conidiis minutis numerosis, saepe globosis.

**Mycelium** sparingly branched, 1–3  $\mu\text{m}$  wide, septate, septa often not very conspicuous, not constricted at the septa, sometimes slightly swollen, subhyaline, smooth, walls unthickened. **Conidiophores** macronematous and micronematous, solitary, arising terminally or laterally from plagiotropous or ascending hyphae, erect or subdecumbent, almost straight to more or less flexuous, cylindrical, sometimes geniculate towards the apex, but not nodulose, sometimes with short lateral prolongations at the apex, unbranched to once or twice branched (angle usually 30–45° degree, sometimes up to 90°), branches usually below a septum, 6–200  $\times$  (1–)2–4(–5)  $\mu\text{m}$ , septate, septa not very conspicuous, not constricted at the septa, subhyaline to pale brown, almost smooth to usually asperulate, walls unthickened or almost so. **Conidiogenous cells** integrated, terminal or intercalary, sometimes conidiophores reduced to conidiogenous cells, cylindrical, sometimes geniculate, non-nodulose, 6–40  $\mu\text{m}$  long, proliferation sympodial, with several conidiogenous loci often crowded at the apex and sometimes also at a lower level, situated on small lateral shoulders, unilateral swellings or prolongations, with up to 6(–10) denticulate loci, forming sympodial clusters of pronounced scars, intercalary conidiogenous cells with short or somewhat long lateral outgrowths, short denticle-like or long branches with several scars at the apex, usually below a septum, loci protuberant, 1–1.5(–2)  $\mu\text{m}$  diam, thickened and darkened-refractive. **Ramoconidia** sometimes occurring, cylindrical, up to 32  $\mu\text{m}$  long, 2.5–4  $\mu\text{m}$  wide, with a broadly truncate, unthickened base, about 2  $\mu\text{m}$  wide. **Conidia** catenate, formed in branched chains, straight, small terminal conidia globose, subglobose, ovoid, oval, 3–6  $\times$  2.5–3.5  $\mu\text{m}$  [av.  $\pm$  SD, 4.5 ( $\pm$  1.3)  $\times$  2.8 ( $\pm$  0.4)  $\mu\text{m}$ ], aseptate, asperulate, with 0–2 distal hila, intercalary conidia and secondary ramoconidia ellipsoid-ovoid, ellipsoid to subcylindrical, 3.5–20(–28)  $\times$  (2.5–)3–5(–6)  $\mu\text{m}$  [av.  $\pm$  SD, 12.4 ( $\pm$  5.4)  $\times$  4.1 ( $\pm$  0.7)  $\mu\text{m}$ ], 0–1-septate, rarely with up to three septa, sometimes slightly constricted at the septa, subhyaline, pale brown to medium olivaceous-brown, asperulate or verruculose (muricate, granulate or colliculate under SEM), walls unthickened or slightly thickened, apex rounded or slightly to distinctly attenuated towards apex and base, often forming several apical hila, up to 7(–9), crowded, situated on small lateral outgrowths giving them a somewhat irregular appearance, hila protuberant, 0.5–1.5  $\mu\text{m}$  diam, thickened and darkened-refractive; microcyclic conidiogenesis sometimes occurring.

**Cultural characteristics:** Colonies on PDA reaching 27–34 mm diam after 14 d at 25 °C, smoke-grey, grey-olivaceous to olivaceous-grey, olivaceous-grey to iron-grey reverse, velvety to powdery, margin regular, entire edge, narrow, colourless to white, aerial mycelium absent or sparingly formed, felty, whitish, growth regular, flat, radially furrowed, with folded and elevated colony centre, deep into the agar, with age forming few to numerous prominent exudates, sporulation profuse, few high conidiophores formed. Colonies on MEA reaching 25–44 mm diam after 14 d at 25 °C, olivaceous-grey to olivaceous- or iron-grey due to abundant sporulation in the colony centre, velvety, margin regular, entire edge, narrow, colourless, white to pale olivaceous-grey, aerial mycelium loose, diffuse, growth convex with papillate surface, radially furrowed, wrinkled, without prominent exudates, sporulating. Colonies on OA reaching 23–32 mm diam after 14 d at 25 °C, grey-olivaceous, olivaceous-

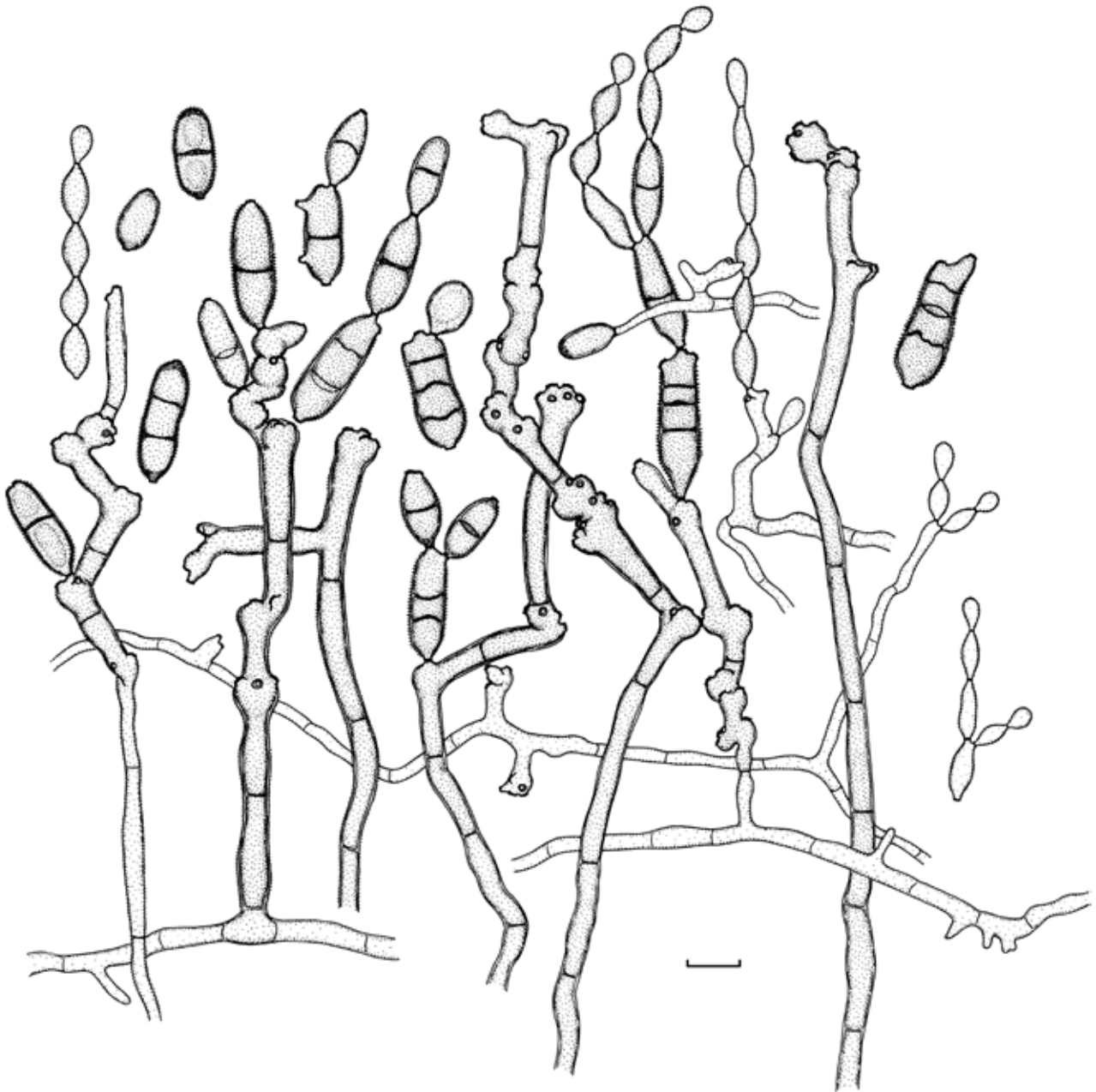


Fig. 46. *Cladosporium variabile* (CPC 12751). Macro- and micronematous conidiophores and conidia. Scale bar = 10  $\mu\text{m}$ . K. Schubert del.

grey to olivaceous due to abundant sporulation in the colony centre, olivaceous- or iron-grey reverse, velvety, margin regular, entire edge, narrow, colourless or white, aerial mycelium sparse, diffuse, floccose, growth flat to low convex, radially furrowed, wrinkled, without prominent exudates, sporulation profuse.

*Specimens examined:* Israel, Eilat, isolated from hypersaline water from salterns, 2004, N. Gunde-Cimerman, CBS 121633 = CPC 12051 = EXF-1083; Ein Bokek, isolated from hypersaline water of the Dead Sea, 2004, M. Ota, CBS-H 19866, **holotype**, isotype HAL 2029 F, culture ex-type CBS 121634 = CPC 12053 = EXF-1735. **U.S.A.**, Seattle, University of Washington campus, isolated from *Phyllactinia* sp. (*Erysiphaceae*) on leaves of *Corylus* sp. (*Corylaceae*), 16 Sep. 2004, D. Glawe, CPC 11813.

*Substrates and distribution:* Hypersaline water and plant material; Israel, U.S.A.

*Notes:* *Cladosporium subtilissimum* and *C. cladosporioides* are morphologically comparable with the new species *C. tenellum*, but *C. cladosporioides* deviates in having usually smooth conidiophores

and conidia with only few conidiogenous loci and conidial hila crowded at the apex and somewhat wider conidiophores, 3–5(–6)  $\mu\text{m}$ ; and in *C. subtilissimum* the small terminal conidia are not globose but rather narrowly obovoid to limoniform, the conidiogenous loci and conidial hila are somewhat wider, (0.5–)0.8–2(–2.2)  $\mu\text{m}$ , and at the apices of conidiophores and conidia only few scars are formed.

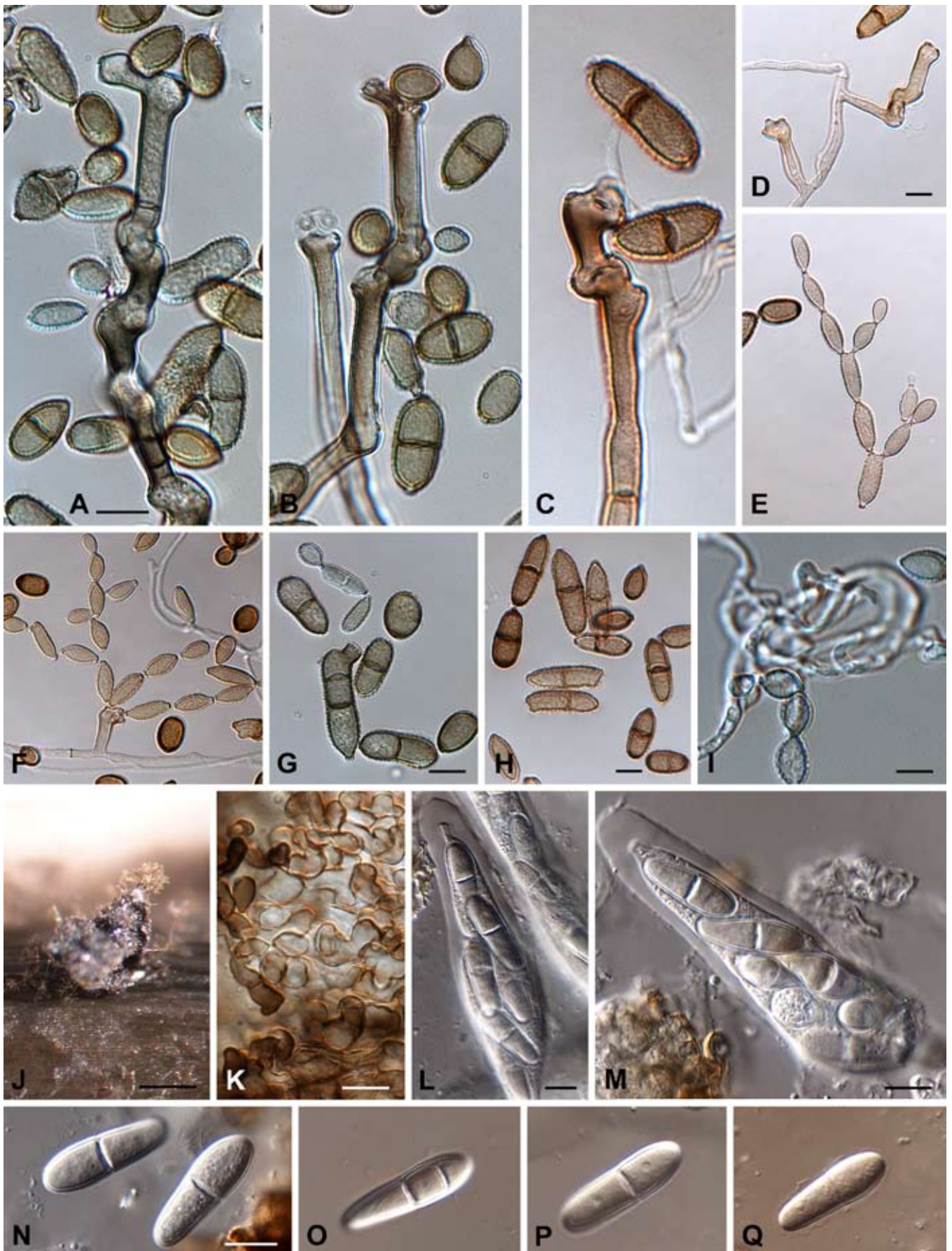
*Cladosporium ramotenellum*, which morphologically also resembles *C. tenellum*, possesses longer and narrower, 0–3-septate conidia, 2.5–35  $\times$  2–4(–5)  $\mu\text{m}$ , but forms only few conidiogenous loci and conidial hila at the apices of conidiophores and conidia.

***Cladosporium variabile*** (Cooke) G.A. de Vries, *Contr. Knowl. Genus Cladosporium*: 85. 1952. Figs 46–48.

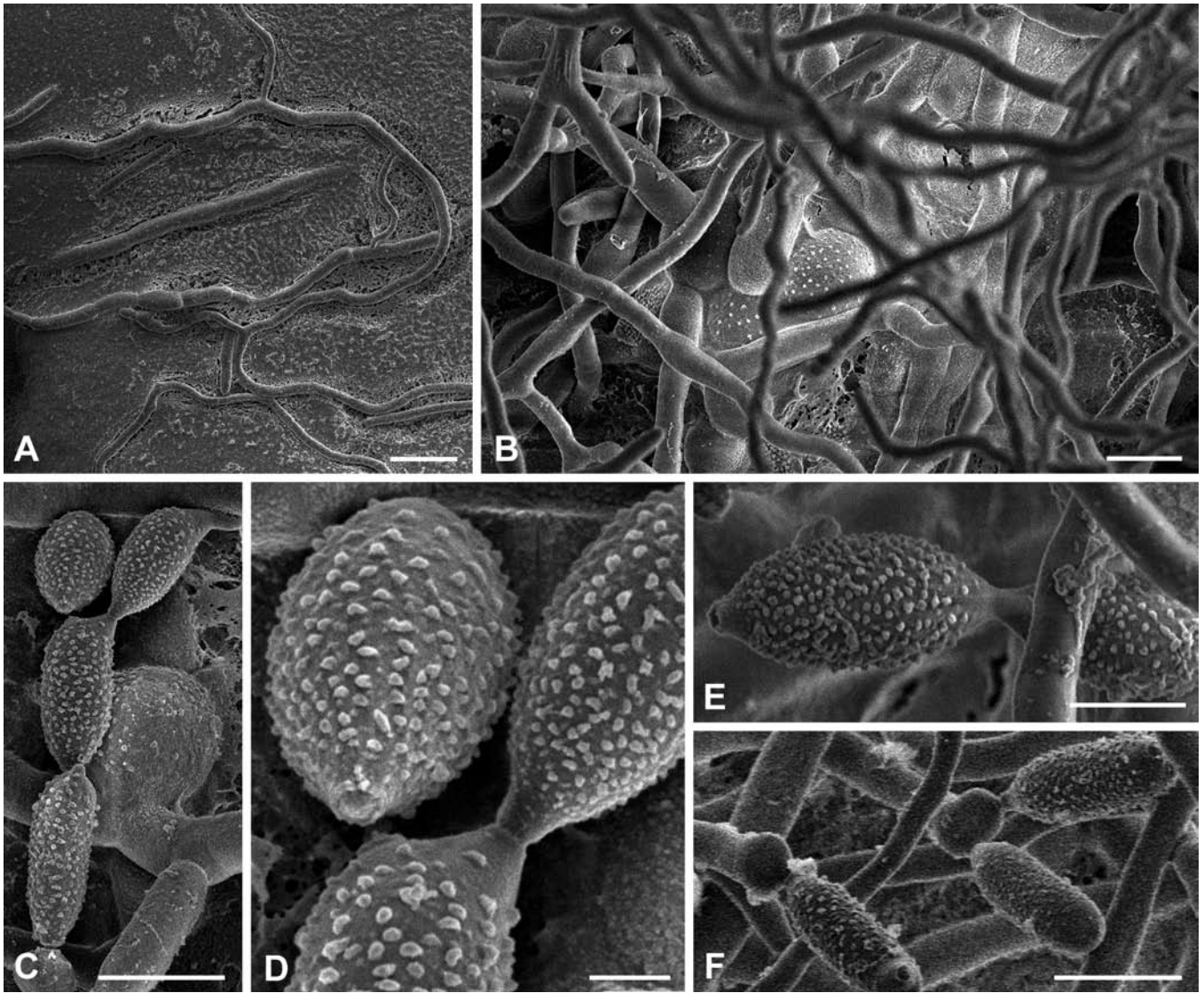
*Basionym:* *Heterosporium variabile* Cooke, *Grevillea* 5(35): 123. 1877.

= *Helminthosporium variabile* Cooke, *Fungi Brit. Exs. Ser. 1*, No. 360. 1870, *nom. inval.*

= *Cladosporium subnodosum* Cooke, *Grevillea* 17(83): 67. 1889.



**Fig. 47.** *Cladosporium variabile* and its teleomorph *Davidiella variabile* (CPC 12751). A–C. Macronematous conidiophores. D, F. Micronematous conidiophores. E, G–H. Conidia. I. Twisted aerial mycelium. J. Ascomata formed on nettle stem in culture. K. Surface view of ascomal wall of *textura epidermoidea*. L–M. Asci. N–P. Ascospores. Q. Ascus with a sheath. Scale bars A, D, G–J, K–N = 10  $\mu$ m, J = 250  $\mu$ m.



**Fig. 48.** *Cladosporium variabile* (CPC 12753). A. Survey of hyphae that grow on the agar surface. Some of the fungal cells have a swollen appearance and could develop into a "foot cell" that gives rise to a conidiophore. B. A number of aerial hyphae obstruct the swollen, large structures on the agar surface, which give rise to conidiophores. Some of them appear ornamented. C. A series of conidia formed on a conidiophore (bottom of the micrograph). D. Detail of the ornamented conidia. The ornamentations are isolated and dispersed. Note also the ornamentation-free scar zone and the hilum of the left cell. E. Two conidia behind an aerial hypha. F. Two conidiophores forming secondary ramoconidia. Note the bulbous shape of the spore-forming apparatus. This micrograph is from an uncoated sample. Scale bars: A–C, F = 10  $\mu\text{m}$ , D = 2  $\mu\text{m}$ , E = 5  $\mu\text{m}$ .

**Teleomorph: *Davidiella variabile*** Crous, K. Schub. & U. Braun, **sp. nov.** MycoBank MB504583.

*Davidiellae tassianae* similis, sed ascosporis maioribus, (22–)26–30(–35)  $\times$  (7–)7.5–8(–9)  $\mu\text{m}$ , et ascis latoribus, plus quam 18  $\mu\text{m}$ .

*Ascomata* pseudothecial, black, superficial, situated on a small stroma, globose, up to 250  $\mu\text{m}$  diam, with 1–3 ostiolate necks; ostioles periphysate, with apical periphysoids present; wall consisting of 3–6 layers of dark brown *textura angularis*, *textura epidermoidea* in surface view. *Asci* fasciculate, bitunicate, sessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 70–95  $\times$  18–28  $\mu\text{m}$ ; with pseudoparenchymatal cells of the hamathecium persistent. *Ascospores* tri- to multiseriate, overlapping, hyaline, with irregular lumina, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest near the middle of the apical cell, medianly 1-septate, not to slightly constricted at the septum, at times developing a second septum in each cell, several ascospores with persistent, irregular mucoid sheath, (22–)26–30(–35)  $\times$  (7–)7.5–8(–9)  $\mu\text{m}$ .

*Mycelium* immersed and superficial, irregularly branched, aerial mycelium twisted and spirally coiled, 1–3  $\mu\text{m}$  wide, septate, sometimes with swellings or small lateral outgrowths, hyaline to subhyaline, smooth, walls unthickened, hyphae which give rise to conidiophores somewhat wider, 3–4.5  $\mu\text{m}$ , subhyaline to pale brown, almost smooth to minutely verruculose, sometimes enveloped by a polysaccharide-like cover. *Conidiophores* usually macronematous, but also micronematous, arising terminally from ascending hyphae or laterally from plagiotropous hyphae. *Macronematous conidiophores* erect, more or less straight to flexuous, often distinctly geniculate-sinuuous forming lateral shoulders or unilateral swellings, sometimes zigzag-like or somewhat coralloid, nodulose, swellings at first terminal, then becoming lateral due to sympodial proliferation, often as distinct lateral shoulders, unbranched, sometimes once branched, 6–180  $\times$  (2.5–)3–6  $\mu\text{m}$ , swellings (3–)6–11  $\mu\text{m}$  wide, septate, not constricted at the septa, pale to medium olivaceous-brown or brown, usually verruculose, walls somewhat thickened, about 1  $\mu\text{m}$  thick, sometimes appearing to be two-layered. *Conidiogenous cells* integrated, terminal and intercalary, cylindrical, nodulose to nodose,



with a single or two swellings per cell, swellings apart from each other or formed in short succession, loci confined to swellings, up to six per node, protuberant, 1–2 µm diam, thickened and darkened-refractive. *Micronematous conidiophores* erect, straight to slightly flexuous, unbranched, usually without swellings, filiform to narrowly cylindrical, sometimes only as short lateral outgrowths of hyphae, often almost indistinguishable from hyphae, up to 50 µm long, 1.5–2.5(–3) µm wide, longer ones pluriseptate, septa appear to be somewhat more darkened, with very short cells, 4–12 µm long, subhyaline to pale brown, smooth, walls unthickened or almost so. *Conidiogenous cells* integrated, usually terminal, rarely intercalary, cylindrical, non-nodulose, with a single, two or few conidiogenous loci at the distal end, protuberant, up to 2 µm diam, thickened and darkened-refractive. *Conidia* catenate, in branched chains, straight, subglobose, obovoid, oval, broadly ellipsoid to cylindrical, sometimes clavate, 4–26(–30) × (3.5–)5–9(–10) µm [av. ± SD, 16.8 (± 6.9) × 6.5 (± 1.4) µm], 0–3-septate, usually not constricted at the septa, septa becoming sinuous with age, often appearing to be darkened, pale to medium or even dark brown or olivaceous-brown, verruculose to densely verrucose or echinulate (granulate under SEM), walls slightly to distinctly thickened in larger conidia, apex and base broadly rounded, sometimes broadly truncate or somewhat attenuated, apex and base often appear to be darkened or at least refractive, hila protuberant to somewhat sessile (within the outer wall ornamentation), (0.8–)1–2 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring.

**Cultural characteristics:** Colonies on PDA attaining 29 mm diam after 14 d at 25 °C, olivaceous to olivaceous-grey or iron-grey, iron-grey or olivaceous-grey reverse, velvety to powdery, margin regular, entire edge to fimbriate, almost colourless, aerial mycelium whitish turning olivaceous-grey, sometimes reddish, greyish rose, woolly-felty, growth flat with elevated colony centre, somewhat folded or radially furrowed, with age forming several very small but prominent exudates, sporulation profuse. Colonies on MEA attaining 27 mm diam after 14 d at 25 °C, olivaceous-grey to iron-grey, white to pale olivaceous-grey in the centre due to abundant aerial mycelium, velvety, margin very narrow, colourless, more or less entire edge, radially furrowed, aerial mycelium fluffy to floccose, dense, growth low convex with wrinkled and folded centre, without exudates, sporulation profuse. Colonies on OA attaining 25 mm diam after 14 d at 25 °C, iron-grey or olivaceous, margin regular, entire edge, narrow, white, glabrous, aerial mycelium whitish, at first mainly in the colony centre, high, dense, floccose, growth flat, abundantly sporulating, no exudates.

**Specimens examined:** Great Britain, Wales, Montgomeryshire, Welshpool, Forden Vicarage, on *Spinacia oleracea* (Chenopodiaceae), J.E. Vize, Cooke, Fungi Brit. Exs. Ser. I, No. 360, K, holotype. U.S.A., Washington, isolated from *Spinacia oleracea*, 1 Jan. 2003, L. DuToit, CBS-H 19867, epitype designated here of *C. variabile* and *D. variabile*, cultures ex-epitype CBS 121635 = CPC 12753, CPC 12751.

**Substrate and distribution:** Leaf-spotting fungus on *Spinacia oleracea*; Asia (China, India, Iraq, Pakistan), Europe (Austria, Belgium, Cyprus, Denmark, France, Germany, Great Britain, Hungary, Italy, Montenegro, Netherlands, Norway, Romania, Spain, Turkey), North America (U.S.A.).

**Literature:** de Vries (1952: 85–88), Ellis (1971: 315), Ellis & Ellis (1985: 429), David (1995b; 1997: 94, 96–98), Ho *et al.* (1999: 144).

**Notes:** *In vivo* the conidia are usually longer, somewhat wider and more frequently septate, (6.5–)10–45(–55) × (4.5–)6–14(–17) µm,

0–4(–5)-septate (Schubert 2005). In culture the dimensions tend to be smaller, which was already mentioned by de Vries (1952).

This leaf-spotting fungus superficially resembles *C. macrocarpum*, but besides its pathogenicity to *Spinacia*, *C. variabile* differs from the latter species in having distinctly larger and more frequently septate conidia on the natural host, forming twisted and spirally coiled aerial mycelium in culture and in having lower growth rates in culture (29 mm after 14 d on PDA versus 38 mm on average in *C. macrocarpum*). Furthermore, the conidial septa of *C. variabile* are often distinctly darkened, become sinuous with age and the apex and base of the conidia often appear to be distinctly darkened. A *Davidiella* teleomorph has not previously been reported for this species.

### The cladosporioides complex

This species complex will be treated in an additional paper in this series, dealing with the epitypification of this common and widespread species, and with numerous isolates identified and deposited as *C. cladosporioides*.

## DISCUSSION

In the present study, a multilocus genealogy supported by light and SEM microscopy, and cultural characteristics was used to redefine species borders within *Cladosporium*, especially within the *C. herbarum* complex. Most of the diagnostic features used for species delimitation on host material (Heuchert *et al.* 2005, Schubert 2005), proved to be applicable in culture. However, morphological features were often more pronounced *in vivo* than *in vitro*. For instance, conidiophore arrangement is not applicable to cultures, conidiophore and conidium widths were often narrower in culture than on the natural host, and macro- as well as microconidiophores were often observed in culture, but not on host material. All species belonging to the *C. herbarum* complex are characterised by possessing conidia which are ornamented, the ornamentation ranging from minutely verruculose to verrucose, echinulate or spiny whereas in the *C. sphaerospermum* complex species with both smooth-walled as well as ornamented conidia are included (Zalar *et al.* 2007). The surface ornamentation varies based on the length of surface protuberances and in the density of ornamentation. Furthermore, the conidia are mainly catenate, formed in unbranched or branched chains. However, species previously referred to the genus *Heterosporium*, which usually produce solitary conidia or unbranched chains of two or three conidia at the most on the natural host, also belong to this species complex (e.g., *C. iridis*). *In vitro* these chains can become longer and may even be branched. The conidiophores formed in culture are mostly macro- but may also be micronematous, sometimes forming different types of conidia that vary in shape and size from each other. Most of the species possess nodulose conidiophores with the conidiogenesis confined to the usually lateral swellings. However, this phenetic trend is not consistently expressed in all of the species belonging to the *C. herbarum* complex. The various *Cladosporium* species within the *C. herbarum* complex were observed to have subtle differences in their phenotype which were visible via cryo-electron microscopy (cryoSEM), and are discussed below.

**Fungal colonies:** CryoSEM provides the opportunity to study the organisation of the fungal colony at relatively low magnifications. *Cladosporium tenellum* proved to be the only fungus able to form

aerial hyphal strands under the conditions studied. *Cladosporium variabile* formed abundant aerial hyphae, but in *C. spinulosum* these were sparse, and only conidiophores were observed on the agar surface. Three-day-old colonies of *C. subinflatum* formed numerous, long aerial hyphae, and no conidiophores could be discerned under the binocular. After 11 d the aerial hyphae seemed to have disappeared, giving rise to conidiophores. *Cladosporium antarcticum*, *C. variabile* and *C. ramotenellum* showed very large, swollen (> 10 µm) cells which gave rise to conidiophores. With *C. variabile* possible earlier stages of these cells were visible (Fig. 48), which gave rise to conidiophores. More than one conidiophore could be formed on such a structure (*C. variabile* and *C. ramotenellum*). *Cladosporium herbarum* has very wide hyphae on the agar surface, which gave rise to conidiophores as lateral branches. These wide hyphae were observed to anastomose, which may provide a firm interconnected supporting mycelium for these conidiophores. In *C. herbaroides* these wide hyphae could also be discerned, but conidiophore formation was less obvious. Similarly, *C. tenellum* has wide, parallel hyphae that gave rise to conidiophores.

These observations reveal fungal structures in *Cladosporium* that have not previously been reported on, and that raise intriguing biological questions. For instance, why are hyphal strands observed in some species (*C. tenellum*), and not in others, and what happens to the aerial hyphae during incubation in some species such as *C. subinflatum*? Furthermore, these preliminary results suggest that CryoSEM provide additional features that can be used to distinguish the different species in the *C. herbarum* complex.

*Fine details of morphological structures:* CryoSEM provides the opportunity to study fine details of the conidiophore, (ramo)conidia and scars. Samples can be studied at magnification up to × 8 000, revealing details at a refinement far above what is possible under the light microscope (LM) (Fig. 2). However, the LM micrographs provide information about the different compartments of ramoconidia, as well as the thickness and pigmentation of the cell wall of different structures. With other words, the different techniques are complementary, and both reveal fungal details that build up the picture that defines a fungal species.

Conidiophores can vary with respect to their width and the length. *Cladosporium ramotenellum*, *C. antarcticum* and *C. variabile* have tapered conidiophores formed on large globoid “foot cells”. The conidiophore itself can be branched. *Cladosporium spinulosum* has conidiophores that rise from the agar surface, but can have a common point of origin. These conidiophores are not tapered, but parallel and slender. The conidiophores of *C. bruhnei* and *C. herbaroides* are rather long, and can appear as aerial hyphae.

An important feature of the conidiophore is the location where the conidia are formed. Conidiophore ends can be simple and tubular, or rounded to more complex, several times geniculate, with several scars. Conidiophore ends become more elaborate over time. *Cladosporium spinulosum* and *C. tenellum* have nearly tubular conidiophore ends, with often very closely aggregated scars. The conidiophore ends of *C. subinflatum* are also near tubular with a hint of bulbousness. *Cladosporium subtilissimum* is similar, but with somewhat more elevated scars that look denticulate. *Cladosporium variabile* has nodulose, somewhat swollen apices with often sessile, almost inconspicuous scars. In the case of *C. macrocarpum*, these structures are also nodulose to nodose and somewhat bent, with only slightly protuberant loci. *Cladosporium ramotenellum* has tubular conidiophore ends with pronounced scars. *Cladosporium antarcticum* has very characteristic, tapered ends, and widely dispersed (5 µm) scars. More complex conidiophore ends are more irregular in shape, and have scars dispersed over a longer

distance, such as observed in *C. bruhnei*, *C. herbaroides*, and *C. herbarum*.

Secondary ramoconidia are usually the first conidia formed on a conidiophore. They are often multicellular, and have one basal cladosporioid hilum, and more at the apex. Few *Cladosporium* species additionally form true ramoconidia representing apical parts of the conidiophore which secede at a septum resulting in an undifferentiated non-coronate base and function as conidia. Ramification of conidial chains is realised through these conidia. They can occur in up to three stages, which results in elaborated spore structures. The basal secondary ramoconidium is invariably the largest, and cell size decreases through a series of additional secondary ramoconidia, intercalary conidia, and small, terminal conidia. The elongation of secondary ramoconidia varies among the different species. *Cladosporium macrocarpum* has broadly ellipsoid to cylindrical secondary ramoconidia usually with broadly rounded ends, like *C. variabile*, while *C. spinulosum* has secondary ramoconidia that can often hardly be discerned from the conidia that are formed at later stages. The conidia of the other species roughly fall between these species. The most notable structures on these conidia are their ornamentation, scar pattern and morphology. *Cladosporium spinulosum* forms numerous globose to subsphaerical spores with digitate, non-tapered surface ornamentation, which is unique for all the species discussed here. In his study on *Cladosporium* wall ornamentation, David (1997) recognised three classes of echinulate surfaces (aculeate, spinulose, digitate), and five classes of verrucose surfaces (muricate, granulate, colliculate, pustulate and pedicellate) (Fig. 2). The ornamentation particles vary in shape, width, height and density. The most strongly ornamented conidia of the species examined by SEM are formed by *C. ossifragi*, with the ornamentation both large (up to 0.5 µm wide) and high, and can be regarded as densely muricately ornamented. Strong ornamentation is also seen in *C. herbaroides*, which is mostly granulate. *Cladosporium tenellum* (with muricate, granulate and colliculate tendencies) and *C. bruhnei* (mostly granulate with some muricate projections) have relatively large ornamentation structures with slightly more space between the units than the other two species. *Cladosporium antarcticum*, *C. ramotenellum*, *C. variabile* and *C. subtilissimum* exhibit rather large granulate ornamentations that have a more irregular and variable shape. *Cladosporium subinflatum* shows the widest dispersed structures of the series, being muricate. In contrast, *C. macrocarpum* has a very neat and regular pattern of muricate ornamentation. The area of formation of new spores on conidia is invariably not ornamented, and hila all have the typical *Cladosporium* morphology with a central dome and a ring-like structure around it.

*Branching patterns:* Spores usually show a “line of weakness” between them where the coronate scars form. It seems that scars at both sides of the line of weakness have the central dome structure, which appears to play a major role in the effective mechanism *Cladosporium* employs for spore dispersal, with the dome actively pushing the conidia apart. This mechanism is also illustrated in David (1997, fig. 2E). Indeed, conidia of *Cladosporium* are very easily dislodged; even snap freezing or the electrical forces inside the SEM often result in dislodgement of the spores in a powdery “wave”. It is no surprise, therefore, that *Cladosporium* conidia are to be found in most air samples. In *Cladosporium*, conidia are mostly formed in chains, with the size invariably decreasing from the base to the apex of the row. Upon formation each conidium is separated from the conidiophore, or previously formed conidium, and hence from its nutrients. The basal ramoconidium or secondary ramoconidia have the nutrients and metabolic power to produce

a number of additional secondary ramoconidia that in turn could produce a chain of intercalary conidia, and finally, some small, single-celled, terminal conidia. Further research is still necessary to determine if specific branching patterns can be linked to different species.

A surprising finding from the present study is the huge diversity in species and genotypes that exist in nature, be it in the indoor environment, on fruit surfaces, or in extreme ecological niches such as salterns, etc. It is clear that detailed studies would be required to find and characterise other species of *Cladosporium* and obtain a better understanding of their host ranges and ecology. A further surprise lay in the fact that several of these species are capable of sexual reproduction, and readily form *Davidiella* teleomorphs in culture. The *Davidiella* states induced here were all from homothallic species. Further attention now needs to be given to elucidating teleomorphs from other species which, as in *Mycosphaerella* (Groenewald *et al.* 2006, Ware *et al.* 2007) could be heterothallic, and experiencing clandestine sex.

Despite the occurrence of many different genotypes in variable genes, the degree of diversity in the entire data set was low. For the majority of the species ITS was almost invariant, with only six genotypes in the entire dataset. This suggests a very recent evolution. The standardised index of association ( $I_A^S$ ) was high (0.3914), indicating an overabundance of clonality and / or inbreeding, the latter possibly matching with observed homothallism of *Davidiella* teleomorphs. Clonality was visualised with SPLITSTREE software, where star-shaped representations without any sign of reticulation were obtained for all genes, though at different branch lengths (Fig. 5). With STRUCTURE software an optimal subdivision was achieved at six putative groups. Some of them were distinctly separated, yielding a theta ( $\theta$ ) around 0.14, but in most cases there was considerable overlap in representation of motifs, with  $\theta$  at significantly higher values. Results are difficult to interpret due to the small size of the data set compared to the number of predicted groups, and due to unknown but probably large sampling effects. With optimal subdivision of the 79 strains at a hypothesised value of  $K = 6$  (Fig. 4), still a large degree of inter-group similarity was noted, as was the case at any other level of  $K$ . This was particularly obvious when data from the most variable genes (EF and ACT) are superimposed (Fig. 4). The ACT groups are further subdivided by EF data, but in many cases the same EF motif (indicated with arrows) was encountered in different (multilocus) species, for example in *C. antarcticum*, *C. spinulosum*, *Davidiella* sp., and the various clusters comprising *Cladosporium* strains which are phenotypically almost indistinguishable but genetically distinct from *C. subtilissimum*. A similar situation was found with the distribution of EF genotypes (indicated with doughnuts) in *C. herbarum* and *C. macrocarpum*. Nevertheless, the data set showed significant structuring, partly correlating with geography, e.g. the EF-determined cluster of *C. bruhnei* that contained isolates from different sources in The Netherlands. Differences may be over-accentuated by known sampling effects, particularly in *C. herbarum* and *C. macrocarpum*, where single-spore isolates from a single collection are included.

Taken together the data suggest a recent, preponderantly clonal evolution, combined with limited natural selection at a low level of evolutionary pressure. As a result, many genotypes produced by hot spots in the genes analysed have survived, leading to nearly random variation in the data set. Many combinations of motifs that possibly could emerge have maintained in the course of time due to the absence of recombination. This indicates that the observed structure is that of populations within a single species, and consequently a distinction of clonal "species" could be redundant.

This conclusion is underlined by the fact that a single source in a single location can be colonised by various genotypes, such as grapes in the U.S.A. containing three different, closely related genotypes. However, the phenomenon of co-inhabitation by different *Mycosphaerella* species on the same lesion of *Eucalyptus* has been described before (Crous 1998, Crous *et al.* 2004) and it is therefore not surprising that different genotypes occurring close together are also observed for the related genus *Cladosporium*. There is no obvious ecological difference between genotypes, and hence isolates seem to have equal fitness.

However, in general we noticed a remarkable concordance of genetic and phenetic characters. The morphological study was done prior to sequencing, and nearly all morphotypes clustered in separate molecular entities. There are some exceptions, such as with *C. antarcticum* with striking morphology that was almost identical on the molecular level to *Cladosporium* spp. that resemble *C. subtilissimum* and would normally have been interpreted to be a mutant. Conversely, nearly all genetically distinguishable groups proved to be morphologically different, with the exception of members of the *C. subtilissimum* s. lat. complex (indicated as *Cladosporium* sp. in Fig. 3 and Table 1). The possibility remains that the found genetic parameters correlate with phenetic markers other than morphology, such as virulence, toxins or antifungal susceptibilities. For this reason we introduce the established entities here as formal species. They can be diagnosed by ACT sequencing or by phenetic characters provided in the key. For simple routine purposes, however, they can be seen and treated as the "*C. herbarum* complex", based on their close phylogenetic relationships.

## ACKNOWLEDGEMENTS

Several colleagues from different countries provided material and valuable cultures without which this work would not have been possible. In this regard, we thank particularly Alina G. Greslebin (CIEFAP, Esquel, Chubut, Argentina), Frank Dugan and Lindsey DuToit (Washington State Univ., U.S.A.), and Annette Ramaley (Colorado, U.S.A.). Konstanze Schubert was financially supported by a Synthesys grant (No. 2559) which is gratefully acknowledged. We thank Marjan Vermaas for preparing the photographic plates, Rianne van Dijken for the growth studies, and Arien van Iperen for maintaining the cultures studied. Bert Gerrits van den Ende is acknowledged for assisting with the molecular data analyses.

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