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Discovery of a functional *Mycosphaerella* teleomorph in the presumed asexual barley pathogen *Septoria passerinii*

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Abstract

We studied the possibility of a teleomorph associated with the genotypically diverse septoria speckled leaf blotch (SSLB) pathogen of barley, *Septoria passerinii*. A teleomorph in the genus *Mycosphaerella* had been predicted previously based on phylogenetic analyses. This prediction was tested with experiments in the Netherlands and the United States by co-inoculating isolates with opposite mating types onto susceptible barley cultivars and monitoring leaves for sexual structures and for the discharge of ascospores. Characterization of putative hybrid progeny by both molecular (AFLP, RAPD, mating type, and ITS sequencing) and phenotypic analyses confirmed that a *Mycosphaerella* teleomorph of *S. passerinii* has been discovered approximately 125 years after the description of the anamorph. Progeny had recombinant genotypes of the molecular alleles present in the parents, and the identities of representative progeny isolates as *S. passerinii* were confirmed by ITS sequencing. A previously unknown sexual cycle explains the high degree of genetic variation among isolates found in nature. The experimental identification of a predicted teleomorph for *S. passerinii* indicates that cryptic sexual cycles may be common for many other "asexual" fungi with high levels of genotypic diversity.

Keywords: *Septoria passerinii*; Septoria speckled leaf blotch (SSLB); *Hordeum vulgare*; Barley; Teleomorph; Sexual cycle; *Mycosphaerella*; Crossing protocol

1. Introduction

Septoria passerinii Sacc. causes septoria speckled leaf blotch (SSLB) on barley (*Hordeum vulgare*) and was first discovered in Italy in 1879 (Passerini). Since then, SSLB has been reported around the globe in such areas as the Upper Midwest region of the United States, the Prairie Provinces of Canada, Northern Europe, Northern Africa, Western Asia, and Australia (Cunfer and Ueng, 1999; Mathre, 1997). Over the past decade, SSLB epidemics have increased in frequency, and SSLB has become one of the most important, albeit sporadic, foliar diseases of barley in the United States and in Canada (Mathre, 1997; Steffenson, 2003; Toubia-Rahme et al., 2003). Yield losses of up to 38% have been reported in Minnesota and North Dakota, with similar reports of losses up to 20% in Canada (Green and Bendelow, 1961; Toubia-Rahme and Steffenson, 1999). In addition to reductions in yield, SSLB can render the remaining barley grain unacceptable for malting due to reductions in both kernel size and amount of malt extract (Green and Bendelow, 1961).

Many barley cultivars are resistant to *S. passerinii* (Banttari et al., 1975; Buchannon, 1961; Green and Dickson, 1957; Koble et al., 1959; Rasmusson and Rogers,

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1963; Toubia-Rahme and Steffenson, 2004). Green and Dickson (1957) reported that 50 of 126 *H. vulgare* cultivars tested were resistant to this pathogen, but these resistant cultivars were of low malting quality. Extensive breeding programs exist for barley, but there has been little attempt to incorporate resistance to *S. passerinii* into new cultivars (Toubia-Rahme and Steffenson, 2004). This is because breeding programs mainly focus on developing cultivars with high yields and high malting qualities and thus have used parents with little or no resistance to SSLB. Consequently, all of the commercially important cultivars for malt and feed in the Upper Midwest region of the United States grown over the past 25 years have been and still are highly susceptible to this pathogen, even though the major cultivars have changed throughout the years (Helm et al., 2001; Toubia-Rahme and Steffenson, 1999, 2004). Toubia-Rahme and Steffenson (2004) argued that because of the increasing importance of SSLB, there should be more invested in the development of cultivars that incorporate resistance with high yield and malting quality characteristics. They reported that resistance could be found in cultivars from diverse geographical origins, such as North America, South America, Europe, North Africa, and East Asia.

Presently there is evidence of up to six genes controlling resistance to SSLB in barley (Buchannon, 1961; Metcalfe et al., 1970; Rasmusson and Rogers, 1963). These specific resistance genes in the host suggest the presence of avirulence genes in the pathogen. However, such avirulence genes have not yet been identified in *S. passerinii*. Furthermore, formal genetic analysis of the pathogen is not possible due to the fact that only the imperfect stage has been reported (Cunfer and Ueng, 1999). Our previous work, however, provided lines of evidence suggesting the possibility of sexual recombination in this fungus. Despite the fact that *S. passerinii* was generally considered to be an asexual fungus (Cunfer and Ueng, 1999), we used heterologous mating-type probes from the wheat pathogen *Mycosphaerella graminicola* (Waalwijk et al., 2002) to clone the mating-type genes of *S. passerinii* (Goodwin et al., 2003), based on a previously identified close phylogenetic relationship between these two species (Goodwin et al., 2001; Goodwin and Zismann, 2001). In addition, it was shown that both mating-type idiomorphs of *S. passerinii* were found commonly in natural populations on the same leaf among 22 isolates tested, suggesting that sexual recombination under field conditions was possible. This was further substantiated by combined isozyme and RAPD genotyping of these 22 isolates, which yielded 22 unique haplotypes, as expected for sexual, but not asexual, populations (Goodwin et al., 2003).

The purpose of this paper was to test the hypothesis that *S. passerinii* has a cryptic teleomorph in the genus *Mycosphaerella*. The relative ease of generating the predicted teleomorph of *S. passerinii*, which has not been noticed in nature over the past 120-plus years, has broad implications for mycology and indicates that many other fungi may be incorrectly classified as asexual.

2. Materials and methods

2.1. Isolates, crossing, and phenotyping procedures

Twelve isolates of *S. passerinii* and two isolates of *M. graminicola* were used in this study (Table 1). Crosses were made both in Wageningen, The Netherlands, and in West Lafayette, IN, USA. Inoculum preparation, inoculations, and crossing procedures were as described previously for *M. graminicola* by Kema et al. (1996c), except that spore suspensions were sprayed onto seedlings instead of being applied with cotton. Environmental conditions for growing seedlings both before and after inoculation were as described previously (Kema et al., 1996a). Isolate combinations for crosses are listed in Table 2. *S. passerinii* crosses were made on 10-day-old seedlings of the barley cvs. Topper 33 and/or Kindred. A cross between *S. passerinii* isolates with the same mating type was included as a negative control to differentiate ascospores generated from environmental contaminants on barley from those generated by *S. passerinii*. *M. graminicola* test crosses were made on the wheat cv. Taichung 29 and served as a positive control for the crossing procedure, as a negative control to differentiate ascospores generated from environmental contaminants on wheat, and as a reference for diagnostic comparison of *M. graminicola* ascospores with those potentially produced by the *S. passerinii* teleomorph, since we speculated earlier that ascospores from these species were likely to be similar morphologically (Goodwin et al.,

Table 1
Summary information about the isolates of *Septoria passerinii* and *Mycosphaerella graminicola* used in this study

Species	Isolate	Collection location	Mating type
S. passerinii[a]	P62	North Dakota, USA	*mat 1-1*
	P63	North Dakota, USA	*mat 1-1*
	P64	North Dakota, USA	*mat 1-1*
	P65	North Dakota, USA	*mat 1-1*
	P66	North Dakota, USA	*mat 1-2*
	P67	North Dakota, USA	*mat 1-2*
	P68	North Dakota, USA	*mat 1-1*
	P71[b]	North Dakota, USA	*mat 1-1*
	P75	North Dakota, USA	*mat 1-1*
	P78	Minnesota, USA	*mat 1-2*
	P81	Minnesota, USA	*mat 1-2*
	P83[b]	North Dakota, USA	*mat 1-2*
M. graminicola	IPO323	The Netherlands	*mat 1-1*
	IPO94269	The Netherlands	*mat 1-2*

[a] The isolates of *S. passerinii* were as reported previously by Goodwin et al. (2003).

[b] Cultures of these isolates have been deposited into the collection of the Fungal Biodiversity Center (Centraalbureau voor Schimmelcultures) in Utrecht, the Netherlands, under accession numbers: P71 = CBS 120383 and P83 = CBS 120382. Progeny isolates P71 × P83 A = CBS 120384 and P71 × P83 B = CBS 120385 also were deposited.

Table 2
In planta crosses between and among isolates of *Septoria passerinii* and *Mycosphaerella graminicola*

Isolates	Cultivars for crossing	Locations for crossing
P71 × P83	Topper 33 and Kindred	Wageningen and West Lafayette
P78 × P83	Topper 33	Wageningen
P62 × P81	Kindred	West Lafayette
P62 × P83	Kindred	West Lafayette
P62 × P81	Kindred	West Lafayette
P63 × P78	Kindred	West Lafayette
P64 × P81	Kindred	West Lafayette
P65 × P66	Kindred	West Lafayette
P68 × P67	Kindred	West Lafayette
P71 × P81	Kindred	West Lafayette
P71 × P83	Kindred	West Lafayette
P75 × P78	Kindred	West Lafayette
P63 × P67	Topper 33 and Kindred	Wageningen and West Lafayette
IPO323 × IPO94269	Taichung 29	Wageningen
P71 × IPO94269	Topper 33 and Taichung 29	Wageningen
IPO323 × P83	Topper 33 and Taichung 29	Wageningen

2003). Finally, we also performed interspecific crosses between *S. passerinii* and *M. graminicola* because of the suggested close phylogenetic relationship between these species (Goodwin and Zismann, 2001). Plants were placed on a rotating table in an inoculation cabinet, and spore suspensions (at concentrations of 10^7 per ml in a total of 30, 15 ml per parental isolate) were sprayed until run-off. Incubations in Wageningen and West Lafayette were conducted as described by Kema et al. (1996c) and Adhikari et al. (2003), respectively. After symptoms developed during incubation in the greenhouse (22 °C, >85% RH), seedlings were placed outside in large plastic pots covered with 1.5-mm-mesh plastic screens. Crosses were attempted seven times between September 2002 and May 2005. Leaf samples were collected once per week from 7 to 12 weeks after inoculation in the Netherlands and from 4 to 10 weeks in the U.S. for discharge of ascospores onto 2% water agar and for microscopical identification of the sexual structure. Proposed parental isolates and the resulting progeny were inoculated onto the susceptible barley cv. Topper 33 for phenotypic comparisons.

2.2. Comparative taxonomical analyses of discharged ascospores and of sexual structures

Because plants were outside for up to 10 weeks, they were exposed to sexual and asexual spores of naturally occurring contaminant fungal species. In addition, it was impossible to know for certain what type of ascospores to expect for *S. passerinii* because they had not been described previously. Therefore, all discharged ascospores were meticulously categorized for size, shape, number of cells, pigmentation, and germination pattern on 2% water agar. All non-*M. graminicola* ascospore types discharged from leaves inoculated with *M. graminicola*, as well as all ascospore types discharged from leaves that were co-inoculated with isolates of *S. passerinii* with the same mating type, were considered to be environmental contaminants. Examples of the different types of discharged ascospores were transferred as single spores to yeast-glucose broth (YGB) and then onto potato dextrose agar (PDA) for comparisons of growth with that of *S. passerinii*. Infected leaf samples that were co-inoculated with isolates of *S. passerinii* with opposite mating types were also screened microscopically to find the associated sexual structure.

2.3. DNA extraction and analyses

In preparation for DNA extraction, isolates were grown in YGB for 10 days, at which time spores were pelleted and subsequently lyophilized. Total genomic DNA was extracted from 10 mg of lyophilized spores using the Puregene DNA isolation kit (Gentra System Inc., Minneapolis, MN, USA) and eluted with 50 µl of TE buffer (pH 8.0). All PCRs were performed in either an MJ PTC-200 Peltier (MJ Research, Watertown, MA, USA) or a Perkin-Elmer 9600 (Perkin-Elmer, Foster City, CA, USA) thermal cycler. Primers and adapters used in this study are listed in Table 3.

To confirm ascospores as progeny from *S. passerinii* crosses and to determine allelic segregation ratios, parental isolates and presumed progeny were screened using mating-type PCR, Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Internal Transcribed Spacer (ITS) analysis. For the mating-type analysis, primers developed by Goodwin et al. (2003) were used. PCRs were done in 25-µl reactions, each containing 2.5 µl of 10 mM dNTPs, 2.5 µl of 10× PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 0.1 µl of 5 U/µl AmpliTaq DNA polymerase (Applied Biosystems), 2.5 µl of 0.01% G-2500 Gelatin (Sigma), 1.33 µl each of 4 µM

Table 3
Primers and adapters for *Septoria passerinii* used in this study

Name	Sequence (5′ to 3′)	Purpose
MT-F	CTTCTTGCCTGCGCCACAGG	*mat 1-1* and *mat 1-2* PCR
Alpha(1594)R	CGGTATGTGGATGGAAGAAAGG	*mat 1-1* PCR
HMG(849)R	TAGTCGGGACCTGAAGGAGTG	*mat 1-2* PCR
OPA-9	GGGTAACGCC	RAPD
*Eco*RI adapter	CTCGTAGACTGCGTACC AATTGGTACGCAGTC	AFLP
*Mse*I adapter	GACGATGAGTCCTGAG TACTCAGGACTCAT	AFLP
E00	GACTGCGTACCAATTC	AFLP
M00	GATGAGTCCTGAGTAA	AFLP
E19	GACTGCGTACCAATTCGA	AFLP
M16	GATGAGTCCTGAGTAACC	AFLP
ITS4	TCCTCCGCTTATTGATATGC	ITS sequencing
ITS5	GGAAGTAAAAGTCGTAACAAGG	ITS sequencing

MT-F, Alpha(1594)R, and HMG(849)R primers, 3 μl of 1 ng/μl target DNA, and 8.9 μl of sterile double-distilled (sdd) water. Thermal cycler conditions were as described previously (Goodwin et al., 2003), and the annealing temperature was 55 °C. For the RAPD analysis, PCRs were done in 25-μl reactions, each containing 2.5 μl of 2 mM dNTPs, 2.5 μl of 10× PCR + $MgCl_2$ buffer, 0.25 μl of 50 mM $MgCl_2$, 0.06 μl of 5 U/μl Taq DNA polymerase (Roche), 2.5 μl of 10 ng/μl OPA9 primer (Operon Technologies), 1.5 μl of 0.5 ng/μl DNA, and 15.69 μl of sdd water. Cycling parameters were as described previously by Kema et al. (1996c). Amplicons from both RAPD and mating-type PCRs were run on 1.2% agarose gels for visualization. Fluorescent AFLP analysis was done according to the protocol described previously by Flier et al. (2003). DNA was digested using enzymes *Eco*RI and *Mse*I with primers E00 and M00 and then ligated with *Eco*RI and *Mse*I adapters. Primary amplification was with primers E00 and M00, while secondary amplification was with primers E19 (fluorescent, Cy5-labeled) and M16, each with two selective nucleotides. Amplified bands were viewed using ALFwin Evaluation software (Amersham Pharmacia Biotech, Roosendaal, The Netherlands). For ITS sequencing, PCRs were done in 25-μl reactions, each containing 2.5 μl of 10 mM dNTPs, 2.5 μl of 10× Mango PCR buffer, 1.5 μl of 25 mM $MgCl_2$, 0.5 μl of 1U/μl Mango Taq DNA polymerase (Bioline), 2.5 μl each of 2 μM primers ITS4 and ITS5, 1 μl of 10 ng/μl target DNA, and 12 μl of sdd water. Cycling parameters were as described previously by Goodwin and Zismann (2001). Sequencing was done with the ThermoSequenase fluorescence-labeled primer cycle sequencing kit on an ALFexpress automated DNA sequencer (Amersham Pharmacia Biotech, Piscataway, NJ, USA) as described previously (Goodwin and Zismann, 2001). Digestions of ITS regions were done with the enzyme *Sau*3AI as described previously (Goodwin and Zismann, 2001).

3. Results

3.1. Comparative taxonomical analyses of discharged ascospores and of sexual structures

Routine test crosses between *M. graminicola* isolates IPO323 and IPO94269 that were used as positive controls for the crossing procedure discharged ascospores from eight through 12 weeks after inoculation. During weeks 11 and 12 (November 2002), we also identified a substantial number of two-celled ascospores (~80) from plants that were co-inoculated with *S. passerinii* isolates P71 and P83 that closely resembled those from *M. graminicola* in morphology and early growth development. Ascospores of the two species were similar in their germination patterns. Initially, two germ tubes arose from the polar ends and grew parallel to the long axis of the spore. Additional secondary germ tubes (1–2) arose at the ascospore septum and grew perpendicular to the long axis of the ascospore. Ascospores remained hyaline and did not develop additional septa during the initial phase of germination. We were able to isolate 17 of those as single-ascospore cultures for further analyses. Repeated attempts to cross *S. passerinii* resulted in a positive discharge of eight ascospores of the same type as mentioned above during May 2005 in West Lafayette, this time from cv. Kindred that was co-inoculated with *S. passerinii* isolates P63 and P67. One of these was isolated as a single-ascospore culture. The colonies developing from all 18 proposed progeny on PDA plates, as well as their morphology and growth rate in YGB cultures (not shown), were identical to those of the parental isolates.

In addition, numerous different types of ascospores were discharged from barley leaves that were co-inoculated with two *S. passerinii* isolates, including the control crosses between isolates of the same mating type, during this same time period. We monitored thousands of ascospore contaminants on barley, some of which could be identified. One species of *Didymella* with an *Ascochyta* anamorph, one species of *Leptosphaerulina*, and four species of *Paraphaeosphaeria* (including *P. michotii*) were isolated commonly. In addition, two-celled ascospores of *Davidiella tassiana*, the teleomorph of *Cladosporium herbarum*, also were encountered regularly on older leaf material. Single-spore isolates from a sampling of these contaminants did not show any similarity to *S. passerinii* in *in vitro* growth tests on PDA or in YGB (not shown).

The interspecies crosses between *S. passerinii* and *M. graminicola* resulted in numerous ascospores (two to four celled), but their growth on PDA and in YGB did not resemble that of either *S. passerinii* or *M. graminicola*. Subsequent RAPD characterization (data not shown) excluded them as interspecies hybrids, so they were considered to be contaminants.

Infected leaf samples inoculated with isolates of *S. passerinii* with opposite mating types from which *Mycosphaerella* ascospores were successfully harvested were examined microscopically to locate ripe ascomata. Despite numerous attempts over several years, only a single, partly decayed ascoma was found. Ascospores were observed to be hyaline, thin-walled, obovoid, and 10–15 × 3–4 μm. Due to the poor state of the material, the sexual stage could not be officially named, although it clearly resembled *M. graminicola* in general morphology. We therefore propose that the *S. passerinii* teleomorph belongs to the genus *Mycosphaerella*, as is indicated by its DNA phylogeny (Goodwin et al., 2001).

3.2. Genotyping

The 17 proposed progeny from the cross between *S. passerinii* isolates P71 and P83 were genotyped based on mating-type PCR, RAPD and AFLP markers, and by ITS analyses. The mating-type PCRs were positive and matched the expected 1:1 segregation ratio (*mat1-1*:*mat1-2* = 10:7; $\chi^2 = 0.53$; $P = 0.05$) typical for an organism with a heterothallic, bipolar mating system (data not shown).

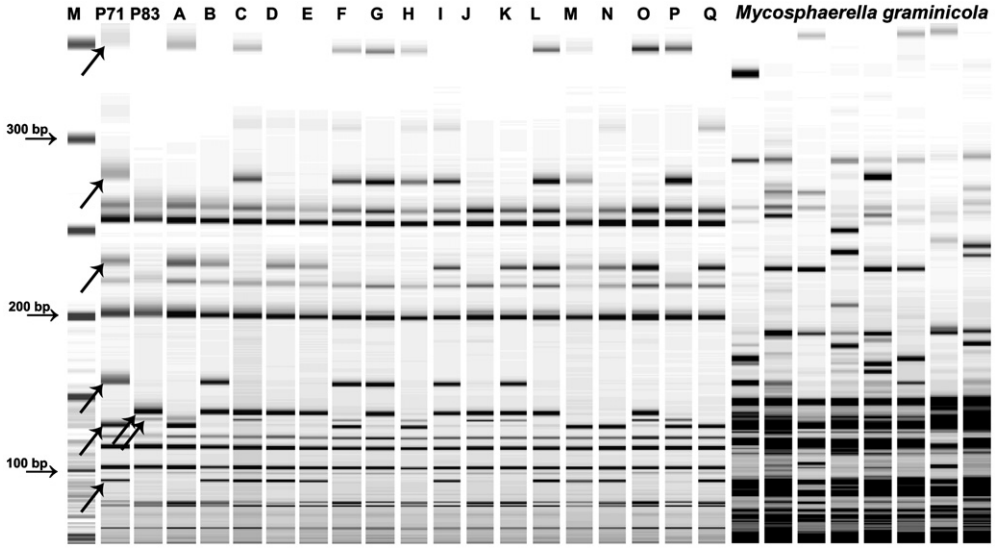

Fig. 1. Genotypes of the parental *Septoria passerinii* isolates P71 and P83 compared to 17 ascospore progeny (isolates A through Q) and seven single-ascospore isolates of *Mycosphaerella graminicola* using AFLP markers. Primers *Eco*RI-GA and *Mse*I-CC were used for all isolates. Diagonal arrows indicate polymorphic bands between the *S. passerinii* parental isolates (six from P71 and two from P83). Horizontal arrows indicate reference size markers.

Furthermore, the RAPD analysis showed that the majority of these progeny had recombinant genotypes based on just three markers (not shown), indicating that these *S. passerinii* isolates were the parents of the collected offspring. Genotyping of the ascospore set using AFLP confirmed this conclusion (Fig. 1). Results of the AFLP analysis with the primers *Eco*RI-GA and *Mse*I-CC showed that the parental isolates P71 and P83 had six and two unique bands, respectively, and had an additional 10 bands in common. All putative progeny isolates possessed these 10 shared bands and additionally displayed at least two of the eight unique bands observed for the parental isolates P71 and/or P83. All 17 proposed progeny had recombinant genotypes except for one that had the same genotype as P83, but this one had a recombinant genotype in the RAPD analysis. None of the progeny had bands that were not present in the parents. For comparison, seven isolates of *M. graminicola* were included on the same polyacrylamide gel using the same AFLP enzymes and primers. There was at least one (at ~230 bp) and possibly more shared bands between *M. graminicola* and *S. passerinii*, which can be expected since these species are closely related, but bands having the same size do not necessarily have the same sequences. However, the vast majority of bands were not shared between the two species, and the AFLP patterns clearly distinguish *S. passerinii* from *M. graminicola*.

To further distinguish the *S. passerinii* progeny from *M. graminicola*, the ITS region was digested with the enzyme *Sau*3AI. All *S. passerinii* progeny showed the same pattern as both of the parental isolates, P71 and P83 (not shown). This pattern was different from the pattern of *M. graminicola* isolates IPO323, IPO94269, and T48. In addition, the ITS regions of parental isolates P71 and P83 and progeny A, E, K, and M were cloned and sequenced. The ITS sequences of all isolates were identical to one another and to archived sequences of several isolates of *S. passerinii* in a blastn search of GenBank. Isolates P71, P83, A, and B have been deposited into the culture collection of the Fungal Biodiversity Center (Centraalbureau voor Schimmelcultures) in Utrecht, the Netherlands. The one proposed progeny isolate from the cross between *S. passerinii* isolates P63 and P67 was characterized as *mat1-1*, and its ITS sequence also was identical to that of *S. passerinii*. This isolate must have been a progeny derived from isolates P63 and P67, because barley is not grown in central Indiana and *S. passerinii* has not been found on wild barley, so no source of natural inoculum exists.

3.3. Phenotyping

Plant inoculations confirmed the ability of the progeny isolates to infect barley. Inoculation of barley seedlings with spores from offspring from the cross between P71 and P83 caused the typical SSLB symptoms on barley (Fig. 2) that began as small chlorotic flecks at 10 days after inoculation. These slowly developed into larger chlorotic blotches that eventually turned necrotic at ~17 days after inoculation. These lesions contained numerous pycnidia, the asexual fructifications that produce the slender multicelled pycnidiospores typical for *S. passerinii*. In contrast, inoculations using *M. graminicola* on the barley cv. Topper 33 or *S. passerinii* on the wheat cv. Taichung 29 did not develop symptoms, even after extended incubation periods (data not shown).

4. Discussion

High genotypic diversity in natural populations, the identification of apparently intact mating-type genes, and the occurrence of both mating types within single leaves

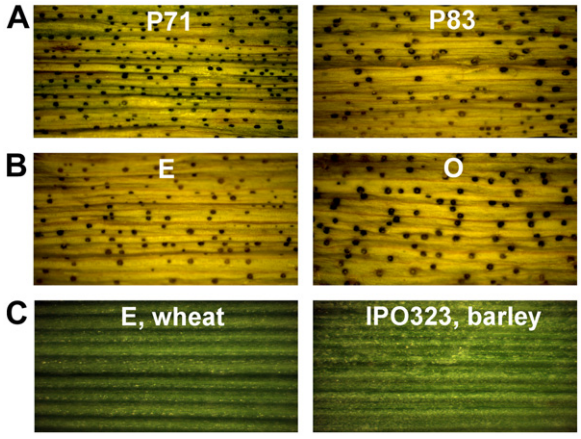

Fig. 2. Symptom development of isolates of *Septoria passerinii* and *Mycosphaerella graminicola* on the barley cultivar Topper 33 or the wheat cultivar Obelisk at 21 days post inoculation. (A) *S. passerinii* parental isolates P71 and P83 on cv. Topper 33. (B) *S. passerinii* progeny isolates E and O on cv. Topper 33. (C) Negative controls. (Left) *S. passerinii* progeny isolate E on cv. Obelisk. (Right) *M. graminicola* isolate IPO323 on cv. Topper 33.

all led to the speculation that *S. passerinii* had the capacity for sexual recombination (Goodwin et al., 2003). However, there was no concrete proof of a functional teleomorph for this fungus that was hitherto considered to be asexual (Cunfer and Ueng, 1999). Therefore, we proceeded to test the hypothesis of a functional teleomorph by crossing isolates of *S. passerinii* with opposite mating types using the *in planta* protocol developed for the closely related sexual species *M. graminicola* (Kema et al., 1996c). This led to the generation of the teleomorph for *S. passerinii* both in Europe and in the United States.

Even though we have generated and characterized sexual progeny from two crosses of *S. passerinii* isolates, we cannot formally describe the sexual stage as required by the International Code of Botanical Nomenclature due to the lack of well-preserved teleomorph material. The identification of the sexual structure has been hampered by the necessity to place inoculated plants outside for approximately two months. Due in part to this, the vast majority of the ascospores discharged from the inoculated barley leaves did not originate from crosses of *S. passerinii* isolates but instead were contaminants from fungi in the environment. Likewise, the vast majority of sexual structures observed on leaves were not produced by crosses of *S. passerinii* isolates but rather by naturally occurring contaminant species. This complicated the localization of the very few ascomata generated by the teleomorph of *S. passerinii*. Furthermore, our observations suggest that the inconspicuous, thin-walled, medium-brown ascomata degenerate quickly once ascospores are discharged, which could explain our difficulty in locating ripe ascomata on leaf sections known to harbor the teleomorph. Three species of *Mycosphaerella* have been described on *Hordeum* (barley), two on *Secale* (rye), and three on *Triticum* (wheat) (Corlett, 1991), but the dimensions of their ascospores as well as their associated anamorphs indicate that they are distinct from the *Mycosphaerella* teleomorph of *S. passerinii*.

It is noteworthy that the success rate of crosses and the number of ascospores obtained from successful crosses are much lower for *S. passerinii* than for *M. graminicola*. Two explanations for the observed sporadic recombination are that either the sexual cycle is much less active in *S. passerinii* on barley than in *M. graminicola* on wheat, or that conditions of the crossing procedure for *M. graminicola* on wheat need to be adapted to meet the environmental requirements for formation of the teleomorph of *S. passerinii* on barley. Thus far, we do not have an indication of what these environmental requirements are, especially since ascospores were harvested from the two successful crosses during cold and wet conditions in Europe (November 2002) and during warm and dry conditions in the United States (May 2005). Other crossing procedures have been attempted for both *S. passerinii* and *M. graminicola*, including leaving the inoculated plants in the greenhouse instead of placing them outside, following the *in vitro* crossing method used for *Mycosphaerella citri* (Mondal et al., 2004), and others. However, only the protocol developed by Kema et al. (1996c) resulted in ascospore production in both species.

The need to place inoculated plants outside to complete the sexual cycle makes them vulnerable to infection by environmental inoculum of *S. passerinii* and other fungi. Contamination by unrelated fungi can be identified and eliminated easily, as described above. However, we also must be certain that environmental inoculum of *S. passerinii* can be identified and excluded. The possibility of contamination by environmental inoculum in Indiana is essentially zero. Barley is not grown commercially in Indiana so there is no nearby source of inoculum. The only wild barley that occurs commonly is *Hordeum jubatum*, and speckled leaf blotch has never been reported on this host in Indiana. Furthermore, an isolate from *H. jubatum* in Minnesota had a different-sized amplicon with the mating-type PCR assay and a different ITS sequence compared to typical *S. passerinii*, so was considered to represent a new, unnamed species of *Septoria* (Goodwin and Zismann, 2001). Thus, there is essentially a zero probability that the progeny isolate in Indiana could have arisen from contamination by environmental inoculum of *S. passerinii*.

It also is extremely unlikely, if not impossible, that we have isolated ascospores from environmental inoculum of *S. passerinii* in the Netherlands. Despite the fact that *S. passerinii* is endemic in the Netherlands, it is not a major pathogen of barley. Moreover, the size of the barley crop in the Netherlands is very small (~50,000 ha) and concentrated at least 150 km from the experimental site. This reduces the chance for splash-borne inoculum to zero, as conidia (pycnidiospores) of the closely related (Goodwin and Zismann, 2001) *S. tritici* are dispersed only over very short distances (on the order of meters) (Bannon and Cooke, 1998; Shaw, 1999) with half distances of about 10 cm (Shaw, 1999). Dispersal ranges of conidia of

S. passerinii have not been estimated but presumably will be similar to those for *S. tritici*. Furthermore, none of the negative controls (those inoculated with isolates of the same mating type) discharged ascospores that could be tied to *S. passerinii*, and all of the segregating markers from the AFLP and RAPD analyses came from the two inoculated isolates with no evidence of migrant alleles. An abundance of genetic data in *M. graminicola* using the same mating protocol also showed no evidence of migrant alleles (Kema et al., 1996c, 2000). We therefore conclude that there is essentially no chance that any of the progeny isolates in Indiana or the Netherlands arose from environmental inoculum of *S. passerinii*.

Recently, many presumably asexual fungi have been found to be sexual, such as: *Colletotrichum acutatum* (teleomorph *Glomerella acutata*), a pathogen of flowering plants (Guerber and Correll, 2001); *Phaeoacremonium aleophilum* (teleomorph *Togninia minima*), associated with Petri disease in grapevines (Mostert et al., 2006); and *Beauveria bassiana* (teleomorph *Cordyceps bassiana*), a widely used biological control agent against insects (Huang et al., 2002). Similarly, the identification of mating-type genes in *S. passerinii* has led to the current discovery of a cryptically active sexual cycle. However, mating-type genes have been identified in many other fungal species in which a sexual cycle has not yet been confirmed. One such example is the barley pathogen *Rhynchosporium secalis*. After a phylogenetic analysis showed that this pathogen probably has a teleomorph in the genus *Tapesia* (Goodwin, 2002), two groups cloned its mating-type genes using degenerate primers designed from sequences of *T. yallundae* and *Pyrenopeziza brassicae* (Foster and Fitt, 2003; Linde et al., 2003). Screening of natural populations of *R. secalis* revealed high genetic diversity and a 1:1 ratio for *mat1-1*: *mat1-2* in most populations sampled (Linde et al., 2003). Another example is *Fusarium oxysporum*, a well-studied plant pathogen with a wide host range (Armstrong and Armstrong, 1981). Mating-type genes from *F. oxysporum* have been cloned by Arie et al. (2000). However, attempts to cross isolates of *F. oxysporum* with opposite mating types have not yielded sexual spores (S. Ware, unpublished), nor have these spores been found in nature, although high genotypic diversity in natural populations of *F. oxysporum* also suggests the possibility of a sexual cycle (Baayen et al., 2000; Bao et al., 2002). More recently, Paoletti et al. (2005) found evidence for sexuality in the opportunistic human pathogen *Aspergillus fumigatus*.

Almost certainly, many presumably asexual fungi are sexually recombining (see review by Taylor et al., 1999, for a parallel opinion with expanded arguments). In addition to the examples given already, a brief review of findings for the human pathogen *Cryptococcus neoformans* represents an excellent example of why the reproductive capabilities of fungi should not be underestimated. The anamorph *C. neoformans* was first described by Busse (1894) and was presumed to be asexual until the discovery of a bipolar heterothallic mating system in 1976, which led to the naming of the teleomorph *Filobasidiella neoformans* (Kwon-Chung, 1976). Twenty years later, monokaryotic fruiting between isolates with the same mating type was reported in *C. neoformans*, but this type of reproduction was considered to be strictly mitotic and asexual based on descriptions in other fungi (Wickes et al., 1996). However, in 2005 this monokaryotic fruiting was proven to be a second sexual form of mating for this pathogen (Lin et al., 2005). Thus, major ideas on mating for *C. neoformans* have changed three times since the description of the anamorph, and even a completely new type of sexual reproduction in fungi has been discovered. Therefore, the possibility and even probability of sexual recombination for presumably asexual fungi cannot be excluded, as has been demonstrated in our study.

The discovery of a functional sexual cycle for *S. passerinii* has potentially important consequences for future study of this pathogen as well as for resistance breeding efforts in the host. In a comparison between *S. passerinii* and *M. graminicola*, the time lapse between the description of the anamorph and the discovery of the corresponding teleomorph is similar (123 and 130 years, respectively). *S. tritici*, the anamorph of *M. graminicola*, was first reported in 1842. The teleomorph was discovered in 1894, but it was not linked to *S. tritici* until 1972 (Sanderson, 1972). Once this link was made, the emphasis of research efforts extended from epidemiological studies (Royle and Shaw, 1986; Shaw and Royle, 1993) to studies on population genetics (McDonald et al., 1995, 1999) and host-pathogen interactions (Kema et al., 1996a,b, 2000). The development of fungal genetics in *M. graminicola* (Kema et al., 1996c) had an important impact on the identification of resistance genes in wheat (Brading et al., 2002). To date, at least 12 resistance genes have been identified that are currently being used in practical breeding programs (Chartrain et al., 2005). In this study, we have identified the existence of the sexual stage of *S. passerinii* and report a crossing protocol that potentially can, with some adaptation, be used to generate a mapping population of *S. passerinii* progeny to study the genetics of avirulence on barley. We hypothesize that this will substantially benefit resistance breeding in barley to this economically important pathogen.

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Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments

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Abstract: Saprobic *Cladosporium* isolates morphologically similar to *C. sphaerospermum* are phylogenetically analysed on the basis of DNA sequences of the ribosomal RNA gene cluster, including the internal transcribed spacer regions ITS1 and ITS2, the 5.8S rDNA (ITS) and the small subunit (SSU) rDNA as well as β-tubulin and actin gene introns and exons. Most of the *C. sphaerospermum*-like species show halotolerance as a recurrent feature. *Cladosporium sphaerospermum*, which is characterised by almost globose conidia, is redefined on the basis of its ex-neotype culture. *Cladosporium dominicanum*, *C. psychrotolerans*, *C. velox*, *C. spinulosum* and *C. halotolerans*, all with globoid conidia, are newly described on the basis of phylogenetic analyses and cryptic morphological and physiological characters. *Cladosporium halotolerans* was isolated from hypersaline water and bathrooms and detected once on dolphin skin. *Cladosporium dominicanum* and *C. velox* were isolated from plant material and hypersaline water. *Cladosporium psychrotolerans*, which grows well at 4 °C but not at 30 °C, and *C. spinulosum*, having conspicuously ornamented conidia with long digitate projections, are currently only known from hypersaline water. We also newly describe *C. salinae* from hypersaline water and *C. fusiforme* from hypersaline water and animal feed. Both species have ovoid to ellipsoid conidia and are therefore reminiscent of *C. herbarum*. *Cladosporium langeronii* (= *Hormodendrum langeronii*) previously described as a pathogen on human skin, is halotolerant but has not yet been recorded from hypersaline environments.

Taxonomic novelties: *Cladosporium dominicanum* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. fusiforme* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. halotolerans* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. psychrotolerans* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. salinae* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. spinulosum* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. velox* Zalar, de Hoog & Gunde-Cimerman, sp. nov.
Key words: Actin, β-tubulin, halotolerance, ITS rDNA, phylogeny, SSU rDNA, taxonomy.

INTRODUCTION

The halophilic and halotolerant mycobiota from hypersaline aqueous habitats worldwide frequently contain *Cladosporium* Link isolates (Gunde-Cimerman *et al*. 2000, Butinar *et al.* 2005). Initially, they were considered as airborne contaminants, but surprisingly, many of these *Cladosporium* isolates were identified as *C. sphaerospermum* Penz. because they formed globoid conidia (data unpublished). *Cladosporium sphaerospermum*, known as one of the most common air-borne, cosmopolitan *Cladosporium* species, was frequently isolated from indoor and outdoor air (Park *et al.* 2004), dwellings (Aihara *et al.* 2001), and occasionally from humans (Badillet *et al.* 1982) and plants (Pereira *et al.* 2002). Strains morphologically identified as *C. sphaerospermum* were able to grow at a very low water activity (a_w 0.816), while other cladosporia clearly preferred a higher, less extreme water activity (Hocking *et al*. 1994). This pronounced osmotolerance suggests a predilection for osmotically stressed environments although *C. sphaerospermum* is reported from a wide range of habitats including osmotically non-stressed niches.

We therefore hypothesised that *C. sphaerospermum* represents a complex of species having either narrow or wide ecological amplitudes. The molecular diversity of strains identified as *C. sphaerospermum* has not yet been determined and isolates from humans have not yet been critically compared with those from environmental samples. Therefore, a taxonomic study was initiated with the aim to define phylogenetically and morphologically distinct entities and to describe their *in vitro* osmotolerance and their natural ecological preferences.

MATERIALS AND METHODS

Sampling

Samples of hypersaline water were collected from salterns located at different sites of the Mediterranean basin (Slovenia, Bosnia and Herzegovina, Spain), different coastal areas along the Atlantic Ocean (Monte Cristy, Dominican Republic; Swakopmund, Namibia), the Red Sea (Eilat, Israel), the Dead Sea (Ein Gedi, Israel), and the salt Lake Enriquillio (Dominican Republic). Samples from the Sečovlje salterns (Slovenia) were collected once per month in 1999. Samples from the Santa Pola salterns and Ebre delta river saltern (Spain) were taken twice (July and November) in 2000. A saltern in Namibia and one in the Dominican Republic were sampled twice (August and October) in 2002. Various salinities, ranging from 15 to 32 % NaCl were encountered in these ponds.

Isolation and maintenance of fungi

Strains were isolated from salterns using filtration of hypersaline water through membrane filters (pore diam 0.45 µm), followed by incubation of the membrane filters on different culture media with lowered water activity (Gunde-Cimerman *et al*. 2000). Only colonies of different morphology on one particular selective medium per sample were analysed further. Strains were carefully selected from different evaporation ponds, collected at different times, in order to avoid sampling of identical clones. Subcultures were maintained at the Culture Collection of Extremophilic Fungi (EXF, Biotechnical Faculty, Ljubljana, Slovenia), while a selection was deposited at the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands) and the Culture Collection of the National Institute of Chemistry (MZKI, Ljubljana, Slovenia). Reference strains were obtained from CBS, and were selected either on the basis of the strain history, name, or on the basis of their ITS rDNA sequence. Strains were maintained on oatmeal agar (OA; diluted OA, Difco: 15 g of Difco 255210 OA medium, 12 g of agar, dissolved in 1 L of distilled water) with or without 5 % additional NaCl. They were preserved in liquid nitrogen or by lyophilisation. Strains studied are listed in Table 1.

Table 1. List of *Cladosporium* strains, with their current and original names, geography, GenBank accession numbers and references to earlier published sequences.

Strain Nr.[a]	Source	Geography	GenBank accession Nr.[b]		
			ITS rDNA / 18S rDNA	actin	β-tubulin
Cladosporium bruhnei					
CBS 177.71	Thuja tincture	The Netherlands, Amsterdam	DQ780399 / DQ780938	EF101354	EF101451
CBS 812.71	*Polygonatum odoratum*, leaf	Czech Republic, Lisen	DQ780401 / –	–	–
Cladosporium cladosporioides					
CBS 170.54 **NT**	*Arundo*, leaf	U.K., England, Kew	AY213640 / DQ780940	EF101352	EF101453
EXF-321	Hypersaline water	Slovenia, Sečovlje saltern	DQ780408 / –	–	–
EXF-780			DQ780409 / –	–	–
EXF-946	Hypersaline water	Bosnia and Herzegovina, Ston saltern	DQ780410 / –	–	–
Cladosporium dominicanum					
CPC 11683	*Citrus* fruit (orange)	Iran	DQ780357 / –	EF101369	EF101419
EXF-696	Hypersaline water	Dominican Republic, saltern	DQ780358 / –	EF101367	EF101420
EXF-718	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780356 / –	EF101370	EF101418
EXF-720	Hypersaline water	Dominican Republic, saltern	DQ780355 / –	–	EF101417
EXF-727	Hypersaline water	Dominican Republic, saltern	DQ780354 / –	–	EF101416
EXF-732 **T**; CBS 119415	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780353 / –	EF101368	EF101415
Cladosporium fusiforme					
CBS 452.71	Chicken food	Canada	DQ780390 / –	EF101371	EF101447
EXF-397	Hypersaline water	Slovenia, Sečovlje saltern	DQ780389 / –	EF101373	EF101445
EXF-449 **T**; CBS 119414	Hypersaline water	Slovenia, Sečovlje saltern	DQ780388 / DQ780935	EF101372	EF101446
Cladosporium herbarum					
ATCC 66670, as *Davidiella tassiana*	CCA-treated Douglas-fir pole	U.S.A., New York, Geneva	AY361959[2] & DQ780400 / DQ780939	AY752193[11]	EF101452
Cladosporium halotolerans					
ATCC 26362	Liver and intestine of diseased frog	U.S.A., New Jersey	AY361982[2] / –	–	–
ATCC 64726	Peanut cell suspension tissue culture	U.S.A., Georgia	AY361968[2] / –	–	–
CBS 280.49	Stem of *Hypericum perforatum* identified as *Mycosphaerella hyperici*	Switzerland, Glarus, Mühlehorn	DQ780369 / –	EF101402	EF101432
CBS 191.54	Laboratory air	Great Britain	– / –	–	–
CBS 573.78	*Aureobasidium caulivorum*	Russia, Moscow region	– / –	–	–
CBS 626.82	–	Sweden, Stockholm	– / –	–	–
dH 12862; EXF-2533	Culture contaminant	Brazil	DQ780371 / –	EF101400	EF101422
dH 12941; EXF-2534	Culture contaminant	Turkey	– / –		EF101421
dH 12991; EXF-2535	Brain	Turkey	DQ780372 / –		EF101423
dH 13911; EXF-2422	Ice	Arctics	DQ780370 / –	EF101401	EF101430
EXF-228; MZKI B-840	Hypersaline water	Slovenia, Sečovlje saltern	DQ780365 / DQ780930	EF101393	EF101425
EXF-380	Hypersaline water	Slovenia, Sečovlje saltern	DQ780368 / –	EF101394	EF101427
EXF-564	Hypersaline water	Namibia, saltern	DQ780363 / –	EF101395	EF101433
EXF-565	Hypersaline water	Namibia, saltern	– / –	–	–
EXF-567	Hypersaline water	Namibia, saltern	– / –	–	–
EXF-571	Hypersaline water	Namibia, saltern	– / –	–	–
EXF-572 **T**; CBS 119416	Hypersaline water	Namibia, saltern	DQ780364 / –	EF101397	EF101424
EXF-646	Hypersaline water	Spain, Santa Pola saltern	DQ780366 / –	EF101398	EF101428
EXF-698	Hypersaline water	Dominican Republic, saltern	– / –	–	–
EXF-703	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780367 / –	EF101392	EF101426
EXF-944	Hypersaline water	Bosnia and Herzegovina, Ston saltern	– / –	–	–
EXF-972	Bathroom	Slovenia	– / –	–	–
EXF-977	Bathroom	Slovenia	DQ780362 / –	EF101396	EF101431

Strain Nr.[a]	Source	Geography	GenBank accession Nr.[b]		
			ITS rDNA / 18S rDNA	actin	β-tubulin
EXF-1072	Hypersaline water	Israel, Dead Sea	DQ780373 / –	EF101399	EF101428
EXF-2372	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
UAMH 7686	Indoor air ex RCS strip, from *Apis mellifera* overwintering facility	U.S.A., Alta, Clyde Corner	AY625063[5] / –	–	–
–	rDNA from bottlenose dolphin skin infected with *Loboa loboi*	U.S.A., Texas	AF035674[6] / –	–	–
–	Microcolony, on rock	Turkey, Antalya	AJ971409[7] / –	–	–
–	Microcolony, on rock	Turkey, Antalya	AJ971408[7] / –	–	–
–	Tomato leaves	–	L25433[8] / –	–	–
Cladosporium langeronii					
CBS 189.54 **NT**	Mycosis	Brazil	DQ780379 / DQ780932	EF101357	EF101435
CBS 601.84	*Picea abies*, wood	Germany, Göttingen	DQ780382 / –	EF101360	EF101438
CBS 101880	Moist aluminium school window frame	Belgium, Lichtervoorde	DQ780380 / –	EF101359	EF101440
CBS 109868	Mortar of Muro Farnesiano	Italy, Parma	DQ780377 / –	EF101362	EF101434
dH 11736	Biomat in a lake	Antarctics	DQ780381 / –	EF101363	EF101436
dH 12459	Orig. face lesion	Brazil	DQ780378 / –	EF101358	EF101439
dH 13833	Ice	Arctics	DQ780383 / –	EF101361	EF101437
–	Nasal mucus	–	AF455525[4] / –	–	–
–	Nasal mucus	–	AY345352[4] / –	–	–
–	Mycorrhizal roots	–	DQ068982[9] / –	–	–
Cladosporium oxysporum					
ATCC 66669	Creosote-treated southern pine pole	U.S.A., New York, Binghamton	AF393689[10] / DQ780395	AY752192[11]	EF101454
ATCC 76499	Decayed leaf, *Lespedeza bicolor*	–	AF393720	–	–
CBS 125.80	*Cirsium vulgare*, seedcoat	The Netherlands	AJ300332[12] / DQ780941	EF101351	EF101455
EXF-697	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780392 / –	–	–
EXF-699	Hypersaline water	Dominican Republic, saltern	DQ780394 / –	–	–
EXF-710	Hypersaline water	Dominican Republic, saltern	DQ780393 / –	–	–
EXF-711	Hypersaline water	Dominican Republic, saltern	DQ780391 / –	–	–
Cladosporium psychrotolerans					
EXF-326	Hypersaline water	Slovenia, Sečovlje saltern	DQ780387 / DQ780934	–	EF101444
EXF-332	Hypersaline water	Slovenia, Sečovlje saltern	DQ780385 / DQ780933	EF101364	EF101441
EXF-391 **T**; CBS 119412	Hypersaline water	Slovenia, Sečovlje saltern	DQ780386 / –	EF101365	EF101442
EXF-714	Hypersaline water	Dominican Republic	DQ780384 / –	EF101366	EF101443
Cladosporium ramotenellum					
EXF-454 **T**; CPC 12043	Hypersaline water	Slovenia, Sečovlje saltern	DQ780403 / –	–	–
Cladosporium salinae					
EXF-322	Hypersaline water	Slovenia, Sečovlje	DQ780375 / –	EF101391	EF101403
EXF-335 **T**; CBS 119413	Hypersaline water	Slovenia, Sečovlje	DQ780374 / DQ780931	EF101390	EF101405
EXF-604	Hypersaline water	Spain, Santa Pola	DQ780376 / –	EF101389	EF101404
***Cladosporium* sp.**					
CBS 300.96	Soil along coral reef coast	Papua New Guinea, Madang, Jais Aben	DQ780352 / –	EF101385	–
EXF-595	Hypersaline water	Spain, Santa Pola saltern	DQ780402 / –	–	–
Cladosporium sphaerospermum					
ATCC 12092	Soil	Canada	AY361988[2] / –	–	–
ATCC 200384	Compost biofilter	The Netherlands	AY361991[2] / –	–	–
CBS 109.14; ATCC 36950	*Carya illinoensis* leaf scale	U.S.A.	DQ780350 / –	EF101384	EF101410
CBS 122.47; IFO 6377; IMI 49640; VKM F-772; ATCC 11292	Decaying stem of *Begonia* sp., with *Thielaviopsis basicola*	The Netherlands, Aalsmeer	AJ244228[1] / –	–	–
CBS 188.54; ATCC 11290; IMI 049638	de Vries (Engelhardt strain)	–	AY361990[2] & AY251077[3] / –	–	–
CBS 190.54; ATCC 11293; IFO 6380; IMI 49641	–	–	AY361992[2] / –	–	–
CBS 192.54; ATCC 11288; IMI 49636	Nail of man	–	AY361989[2] / –	–	–

Table 1. (Continued).

Strain Nr.[a]	Source	Geography	GenBank accession Nr.[b]		
			ITS rDNA / 18S rDNA	actin	β-tubulin
CBS 193.54 **NT**; ATCC 11289; IMI 49637	Human nails	–	DQ780343 & AY361958[2] / DQ780925	EF101380	EF101406
CBS 122.63	Plywood of *Betula* sp.	Finland, Helsinki	– / –	–	–
CBS 102045; EXF-2524; MZKI B-1066	Hypersaline water	Spain, Barcelona, Salines de la Trinitat	DQ780351 / –	EF101378	EF101411
CBS 114065	Outdoor air	Germany, Stuttgart	– / –	–	–
CPC 10944	Gardening peat substrate	Russia, Kaliningrad	DQ780350 / –	–	–
EXF-131; MZKI B-1005	Hypersaline water	Slovenia, Sečovlje saltern	AJ238670[1] / –	–	–
EXF-328	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-385	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-446	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-455	Hypersaline water	Slovenia, Sečovlje saltern	DQ780349 / –	EF101375	EF101412
EXF-458	Hypersaline water	Slovenia, Sečovlje saltern	DQ780345 / –	EF101374	EF101409
EXF-461	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-464	Hypersaline water	Slovenia, Sečovlje saltern	– / DQ780927	–	–
EXF-465	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-598	Hypersaline water	Spain, Santa Pola	– / –	EF101377	–
EXF-644	Hypersaline water	Spain, Santa Pola	– / –	–	–
EXF-645	Hypersaline water	Spain, Santa Pola	– / –	–	–
EXF-649	Hypersaline water	Spain, Santa Pola	– / –	–	–
EXF-715	Hypersaline water	Dominican Republic, saltern	– / –	–	–
EXF-738	Bathroom	Slovenia	DQ780348 / –	EF101383	EF101414
EXF-739	Bathroom	Slovenia	DQ780344 / –	EF101381	EF101407
EXF-781; MZKI B-899	Hypersaline water	Slovenia, Sečovlje	– / –	–	–
EXF-788	Hypersaline water	Slovenia, Sečovlje	– / –	–	–
EXF-962	Bathroom	Slovenia	DQ780347 / –	EF101382	EF101413
EXF-965	Bathroom	Slovenia	– / –	–	–
EXF-1069	Hypersaline water	Israel, Eilat saltern	– / –	EF101376	–
EXF-1061	Hypersaline water	Israel, Dead Sea	DQ780346 / –	EF101379	EF101408
EXF-1726	Hypersaline water	Israel, Dead Sea	– / –	–	–
EXF-1732	Hypersaline water	Israel, Eilat saltern	– / DQ780928	–	–
–	*Bryozoa* sp.	–	AJ557744 / –	–	–
–	Nasal mucus	–	AF455481[4] / –	–	–
Cladosporium spinulosum					
EXF-333	Hypersaline water	Slovenia, Sečovlje saltern	DQ780404 / –	–	–
EXF-334 **T**	Hypersaline water	Slovenia, Sečovlje saltern	DQ780406 / –	EF101355	EF101450
EXF-382	Hypersaline water	Slovenia, Sečovlje saltern	DQ780407 / DQ780936	EF101356	EF101449
Cladosporium subinflatum					
EXF-343 **T**; CPC 12041	Hypersaline water	Slovenia, Sečovlje saltern	DQ780405 / –	EF101353	EF101448
Cladosporium tenuissimum					
ATCC 38027	Soil	New Caledonia	AF393724 / –	–	–
EXF-324	Hypersaline water	Slovenia, Sečovlje saltern	– / DQ780926	–	–
EXF-371	Hypersaline water	Slovenia, Sečovlje saltern	DQ780396 / –	–	–
EXF-452	Hypersaline water	Slovenia, Sečovlje saltern	DQ780397 / –	–	–
EXF-563	Hypersaline water	Namibia, saltern	DQ780398 / –	–	–
Cladosporium velox					
CBS 119417 **T**; CPC 11224	*Bamboo* sp.	India, Charidij	DQ780361 / DQ780937	EF101388	EF101456
EXF-466	Hypersaline water	Slovenia, Sečovlje saltern	DQ780359 / –	EF101386	–
EXF-471	Hypersaline water	Slovenia, Sečovlje saltern	DQ780360 / –	EF101387	–

Cultivation and microscopy

For growth rate determination and phenetic description of colonies, strains were point inoculated on potato-dextrose agar (PDA, Difco), OA and Blakeslee malt extract agar (MEA, Samson *et al*. 2002) and incubated at 25 °C for 14 d in darkness. Surface colours were rated using the colour charts of Kornerup & Wanscher (1967). For studies of microscopic morphology, strains were grown on synthetic nutrient agar (SNA, Gams *et al*. 2007) in slide cultures. SNA blocks of approximately 1 × 1 cm were cut out aseptically, placed upon sterile microscope slides, and inoculated at the upper four edges by means of a conidial suspension (Pitt 1979). Inoculated agar blocks were covered with sterile cover slips and incubated in moist chambers for 7 d at 25 °C in darkness. The structure and branching pattern of conidiophores were observed at magnifications × 100, × 200 and × 400 in intact slide cultures under the microscope without removing the cover slips from the agar blocks. For higher magnifications (× 400, × 1 000) cover slips were carefully removed and mounted in lactic acid with aniline blue.

Morphological parameters

Morphological terms follow David (1997), Kirk *et al.* (2001) and Schubert *et al.* (2007 – this volume). Conidiophores in *Cladosporium* are usually ascending and sometimes poorly differentiated. Though the initiation point of conidiophore stipes could sometimes be determined only approximately, their lengths were in some cases useful for distinguishing morphologically similar species when observed in slide cultures. The branching patterns can be rotationally symmetric or unilateral. Characters of conidial scars were studied by light and scanning electron microscopy (SEM). Conidial chains show different branching patterns, determined by the numbers of conidia in unbranched parts, the nature of ramoconidia as well as their distribution in conidial chains. Measurements are given as (i) n_1–n_2 or (ii) (n_1–)n_3–n_4(–n_2), with n_1 = minimum value observed; n_2 = maximum value observed; n_3/n_4 = first/third quartile. For conidia and ramoconidia also average values and standard deviations are listed. The values provided are based on at least 25 measurements for the conidiophores of each strain, and at least 50 measurements for conidia.

Ecophysiology

To determine the degree of halotolerance, strains were point-inoculated on MEA without and with additional NaCl at concentrations of 5, 10, 17 and 20 % NaCl (w/v) and incubated at 25 °C for 14 d. To determine cardinal temperature requirements for growth, plates were incubated at 4, 10, 25, 30 and 37 °C, and colony diameters measured after 14 d of incubation.

DNA extraction, sequencing and analysis

For DNA isolation strains were grown on MEA for 7 d. DNA was extracted according to Gerrits van den Ende & de Hoog (1999) by mechanical lysis of approx. 1 cm^2 of mycelium. A fragment of the rDNA including the Internal Transcribed Spacer region 1, 5.8S rDNA and the ITS 2 (ITS) was amplified using the primers V9G (de Hoog & Gerrits van den Ende 1998) and LS266 (Masclaux *et al.* 1995). Sequence reactions were done using primers ITS1 and ITS4 (White *et al*. 1990). For amplification and sequencing of the partial actin gene, primers ACT-512F and ACT-783R were applied according to Carbone & Kohn (1999). For amplification and sequencing of the β-tubulin gene primers T1 and T22 were used according to O'Donnell & Cigelnik (1997). A BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) was used in sequence reactions. Sequences were obtained with an ABI Prism 3700 DNA Analyzer (Applied Biosystems). They were assembled and edited using SeqMan v. 3.61 (DNAStar, Inc., Madison, U.S.A.). Sequences downloaded from GenBank are indicated in the trees by their GenBank accession numbers; newly generated sequences are indicated by strain numbers (see also Table 1). Sequences were automatically aligned using ClustalX v. 1.81 (Jeanmougin *et al*. 1998). The alignments were adjusted manually using MEGA3 (Kumar *et al*. 2004). Phylogenetic relationships of the taxa were estimated from aligned sequences by the maximum parsimony criterion as implemented in PAUP v. 4.0b10 (Swofford 2003). Data sets of the SSU rDNA, ITS rDNA and the β-tubulin and actin genes are analysed separately. Species of *Cladosporium s. str.* were compared with various taxa of the *Mycosphaerellaceae* using SSU rDNA sequences and *Fusicladium effusum* G. Winter (*Venturiaceae*) as outgroup. The other data sets focus on *Cladosporium s. str.*, using *Cladosporium salinae* Zalar, de Hoog & Gunde-Cimerman as an outgroup, because this species was most deviant within *Cladosporium* in the SSU rDNA analysis (see below). Heuristic searches were performed on all characters, which were unordered and equally weighted. Gaps were treated as missing characters. Starting tree(s) were obtained via stepwise, random, 100 times repeated sequence addition. Other parameters included a "MaxTrees" setting to 9 000, the tree-bisection-reconnection as branch-swapping algorithm, and the "MulTrees" option set to active. Branch robustness was tested in the parsimony analysis by 10 000 search replications, each on bootstrapped data sets using a fast step-wise addition bootstrap analysis. Bootstrap values larger than 60 are noted near their respective branches. Newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov); their accession numbers are listed in Table 1. Alignments and trees were deposited in TreeBASE (www.treebase.org).

Table 1. (Page 158–160).

[a] Abbreviations used: 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, Utrecht, The Netherlands; dH = de Hoog Culture Collection, housed at CBS, Utrecht, The Netherlands; EXF = Culture Collection of Extremophilic Fungi, Ljubljana, Slovenia; IFO = Institute for Fermentation, Culture Collection of Microorganizms, Osaka, Japan; IMI = The International Mycological Institute, Egham, Surrey, U.K.; MZKI = Microbiological Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia; UAMH = University of Alberta Microfungus Collection, Alberta, Canada; VKM = All-Russian Collection of Microorganisms, Russian Academy of Sciences, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia; NT = ex-neotype strain; T = ex-type strain.

[b] Reference: [1]de Hoog *et al.* 1999; [2]Park *et al.* 2004; [3]Braun *et al.* 2003; [4]Buzina *et al.* 2003; [5]Meklin *et al.* 2004; [6]Haubold *et al.* 1998; [7]Sert & Sterflinger, unpubl.; [8]Curtis *et al.* 1994; [9]Menkis *et al.* 2005; [10]Managbanag *et al.* unpubl.; [11]Crous *et al.* 2004; [12]Wirsel *et al.* 2002. All others are newly reported here.

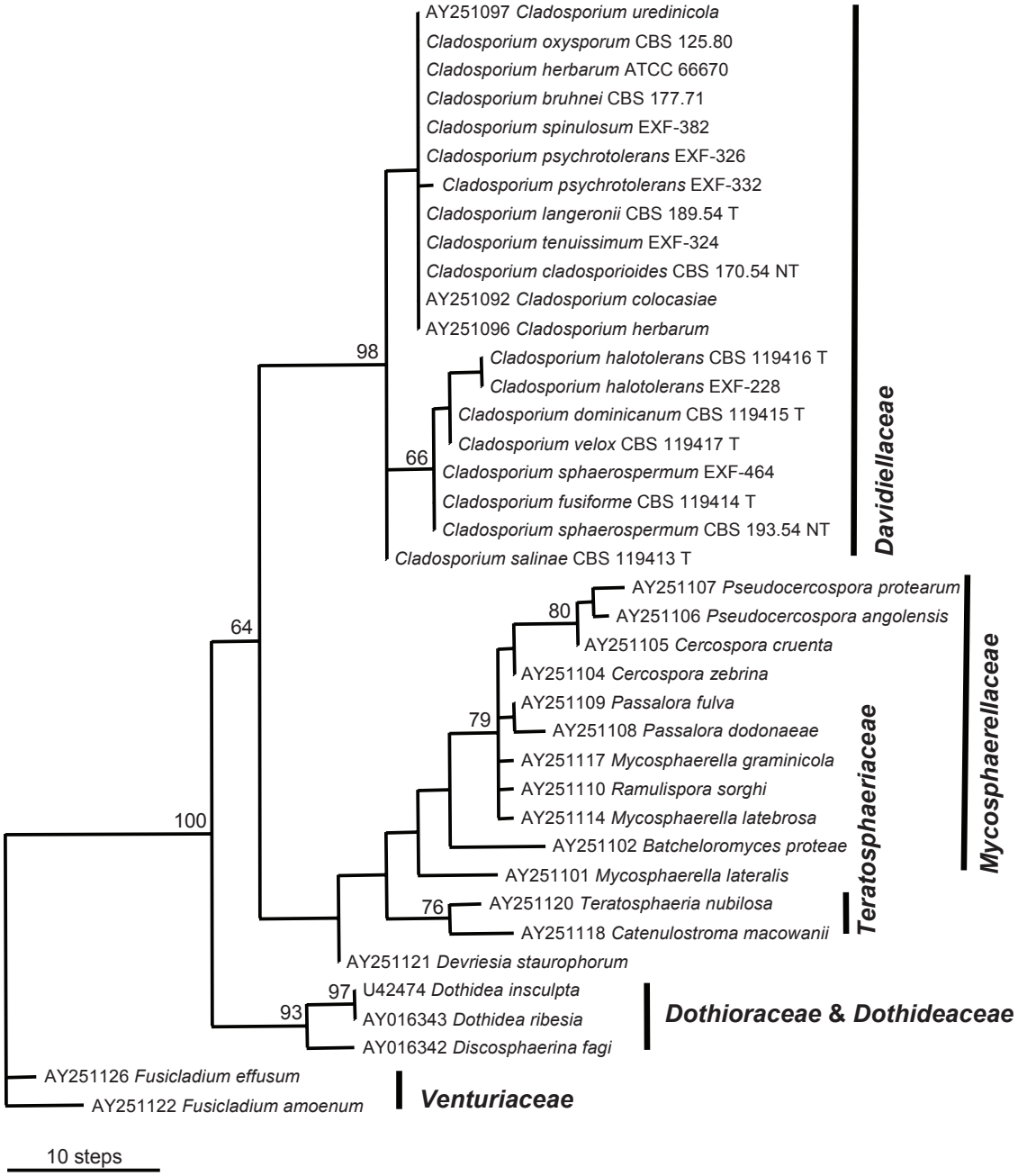

Fig. 1. One of 30 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned small subunit ribosomal DNA sequences. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Species of *Cladosporium s. str.*, including the seven newly described species, form a strongly supported monophyletic group among other taxa of the *Mycosphaerellaceae* (*Dothideomycetes*) (CI = 0.631, RI = 0.895, PIC = 50).

Table 2. Statistical parameters describing phylogenetic analyses performed on sequence alignments of four different loci.

Parameter	SSU rDNA	ITS rDNA[1]	β-tubulin[2]	Actin[3]
Number of alignment positions	1031	498	654	210
Number of parsimony informative characters (PIC)	50	68	220	103
Length of tree / number of steps	103	102	714	338
Consistency Index (CI)	0.631	0.804	0.538	0.586
Retention Index (RI)	0.895	0.975	0.883	0.885
Rescaled Consistency Index (RC)	0.565	0.784	0.475	0.518
Homoplasy index (HI)	0.369	0.196	0.462	0.414
Number of equally parsimonious trees retained	30	600	90	32

[1]Including the internal transcribed spacer region 1 and 2 and the 5.8S rDNA.

[2]Including partial sequences of 4 exons and complete sequences of 3 introns.

[3]Including partial sequences of 3 exons and 2 introns.

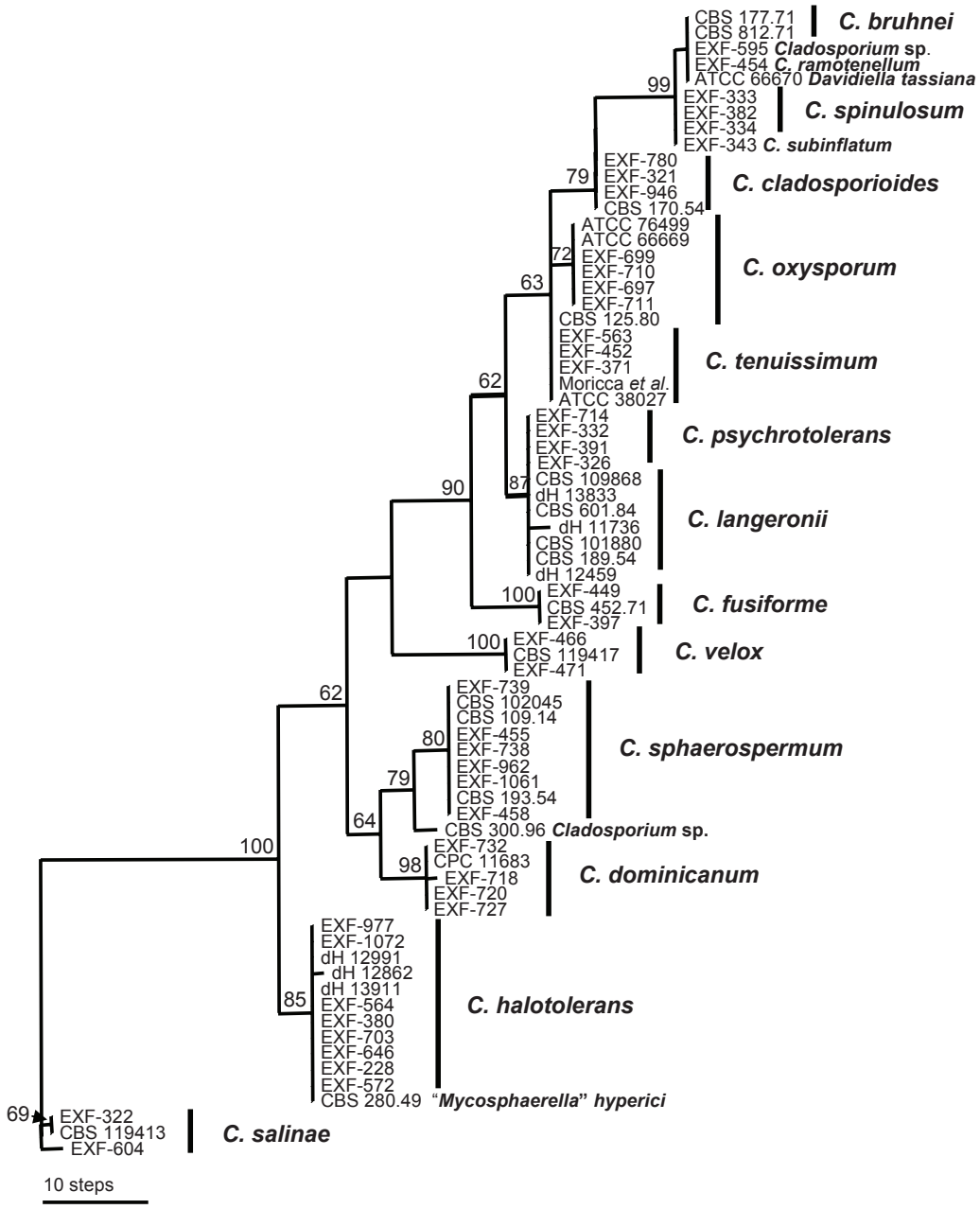

Fig. 2. One of 600 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned sequences of the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Trees were rooted with the strains of *Cladosporium salinae*. Most monophyletic species clades received high, but some deeper branches moderate, bootstrap support (CI = 0.804, RI = 0.975, PIC = 68).

RESULTS

Descriptive statistical parameters of phylogenetic analyses and calculated tree scores for each analysed sequence locus are summarised in Table 2. Mainly reference material such as ex-type or ex-neotype strains was analysed on the level of SSU rDNA sequences. Downloaded and newly generated SSU rDNA sequences of members of *Cladosporium s. str.* were compared with related taxa of the *Mycosphaerellaceae*, *Dothioraceae* and *Dothideaceae*. The somewhat more distantly related *Fusicladium effusum* (*Venturiaceae*) (Braun *et al.* 2003: Fig. 2) was selected as outgroup. *Anungitopsis amoena* R.F. Castañeda & Dugan (now placed in *Fusicladium* Bonord., see Crous *et al.* 2007b), also a member of the *Venturiaceae*, was included in the analyses. All taxa included in the SSU rDNA analysis belong to the *Dothideomycetes* (Schoch *et al.* 2006), within which the ingroup is represented by the orders *Capnodiales* (*Davidiellaceae*, *Mycosphaerellaceae*, *Teratosphaeriaceae*) and *Dothideales* (*Dothioraceae*, *Dothideaceae*) (see also Schoch *et al.* 2006). The genus *Cladosporium*, of which some species are linked to *Davidiella* Crous & U. Braun teleomorphs (Braun *et al.* 2003), forms a statistically strongly supported monophyletic group (*Davidiellaceae*). It also accommodates species newly described in this paper, namely, *C. halotolerans* Zalar, de Hoog & Gunde-Cimerman, *C. fusiforme* Zalar, de Hoog & Gunde-Cimerman, *C. dominicanum* Zalar, de Hoog & Gunde-Cimerman, *C. salinae*, *C. psychrotolerans* Zalar, de Hoog & Gunde-Cimerman, *C. velox* Zalar, de Hoog & Gunde-Cimerman and *C. spinulosum* Zalar, de Hoog & Gunde-Cimerman (Fig. 1). A sister group relationship of *Cladosporium s. str.* with a clade of taxa characterised, among others, by *Mycosphaerella* Johanson teleomorphs, containing various anamorphic genera such as *Septoria* Sacc.,

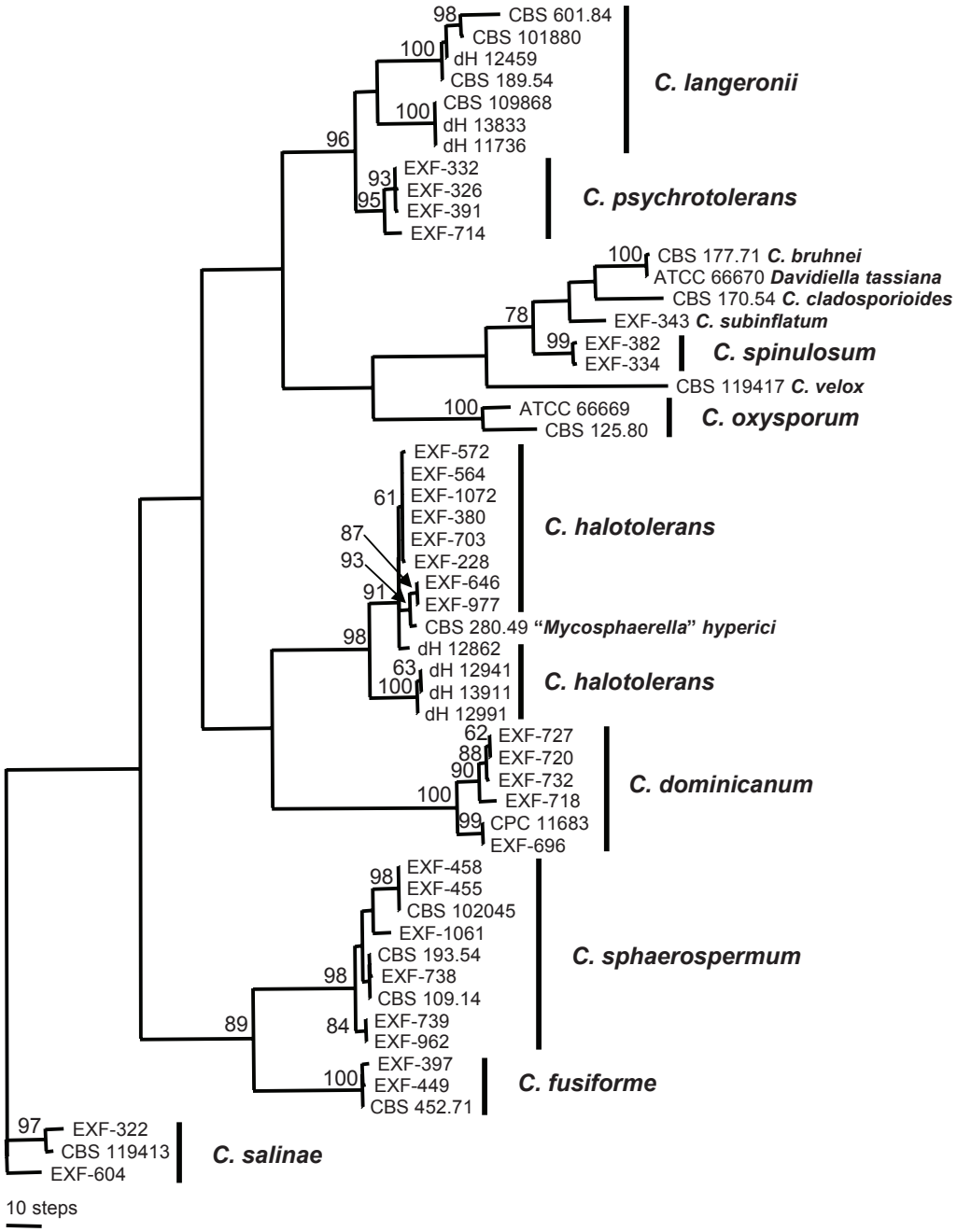

Fig. 3. One of 90 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned exons and introns of a part of the β-tubulin gene. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Trees were rooted with the strains of *Cladosporium salinae*. Most monophyletic species clades received high, but deeper branches weak or no, bootstrap support (CI = 0.538, RI = 0.883, PIC = 220).

Ramularia Unger, *Cercospora* Fresen., *Pseudocercospora* Speg., "*Trimmatostroma*" Corda (now *Catenulostroma* Crous & U. Braun) (see Crous *et al*. 2004, 2007a – this volume) and the somewhat cladosporium-like genus *Devriesia* Seifert & N.L. Nick. (Seifert *et al.* 2004), was statistically only moderately supported (Fig. 1), whereas in an analogous analysis by Braun *et al.* (2003: Fig. 2) it was highly supported. These data also support the conclusion by Braun *et al.* (2003) and Crous *et al.* (2006) that *Cladosporium* is not a member of the distantly related *Herpotrichiellaceae* (*Chaetothyriomycetes*), which is also rich in cladosporium-like taxa (Crous *et al.* 2006). None of the fungi isolated from hypersaline environments belonged to the *Herpotrichiellaceae*. The SSU rDNA sequences do not resolve a phylogenetic structure within *Cladosporium s. str*. Only a moderately supported clade comprising *C. halotolerans*, *C. dominicanum*, *C. velox*, *C. sphaerospermum* and *C. fusiforme* is somewhat distinguished from a statistically unsupported clade with *C. herbarum* (Pers. : Fr.) Link, *C. cladosporioides* (Fresen.) G.A. de Vries, *C. oxysporum* Berk. & Broome, *C. spinulosum*, and *C. psychrotolerans*, etc. Because *C. salinae* appeared most distinct within the genus *Cladosporium* in analyses of the SSU rDNA (Fig. 1), it was used as outgroup in analyses of the ITS rDNA and the β-tubulin and actin genes.

Analyses of the more variable ITS rDNA and partial β-tubulin and actin gene introns and exons supported the species clades of *C. halotolerans*, *C. dominicanum*, *C. sphaerospermum*, *C. fusiforme* and *C. velox* (Figs 2–4), of which *C. velox* was distinguished in the β-tubulin tree by a particular long terminal branch of the only sequenced strain (Fig. 3). *Cladosporium salinae* also clustered as a well-supported species clade in preliminary analyses using various *Mycosphaerella* species as outgroup (not shown). All strains of *C. langeronii* (Fonseca, Leão & Nogueira) Vuill. are particularly well distinguishable from other *Cladosporium* species by strikingly slow-

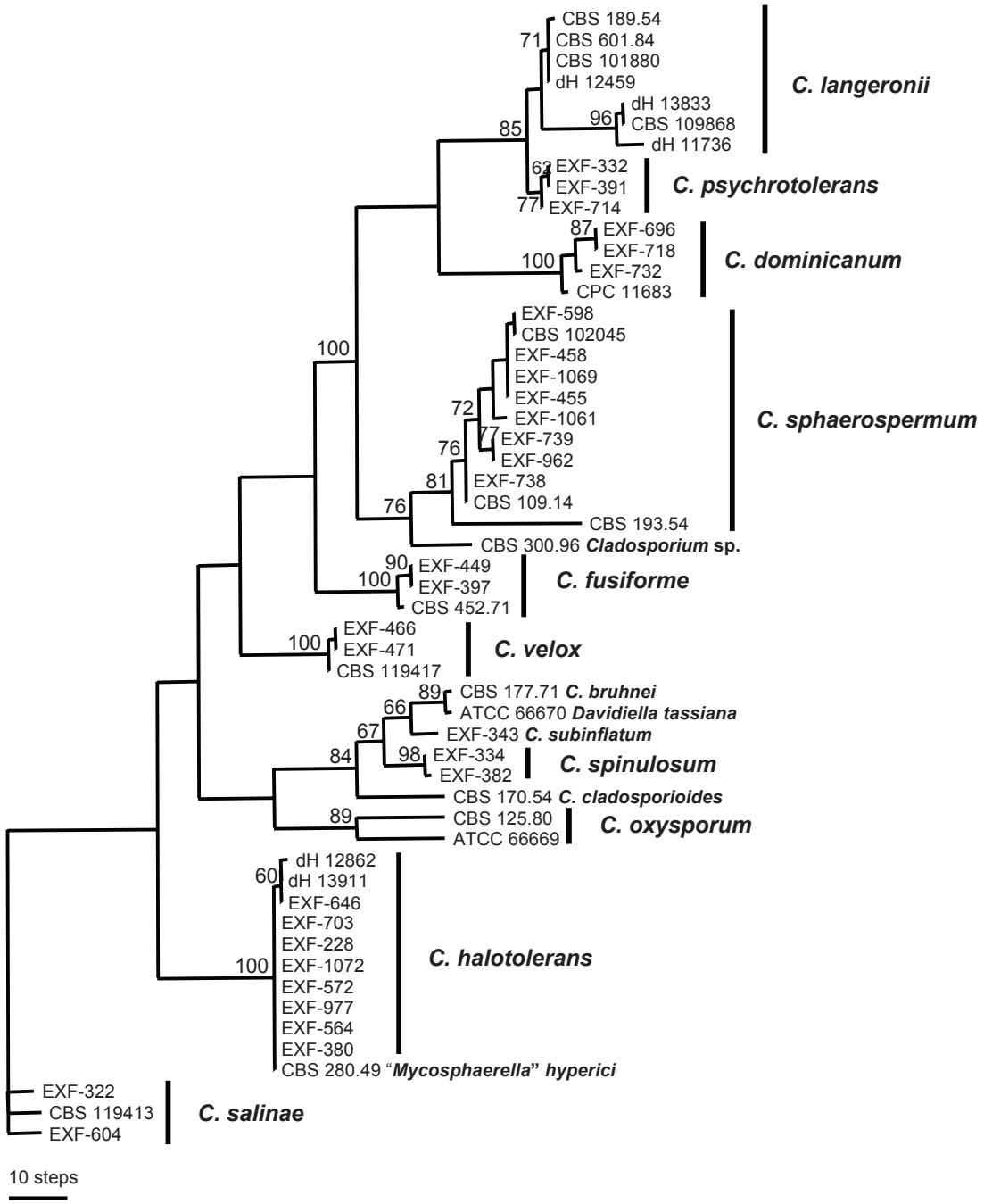

Fig. 4. One of 32 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned exons and introns of the partial actin gene. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Trees were rooted with the strains of *Cladosporium salinae*. Most monophyletic species clades received high, but deeper branches weak or no, bootstrap support (CI = 0.586, RI = 0.885, PIC = 103).

growing colonies at all tested temperatures and relatively large, oblong conidia. However, phylogenetic analyses of the β-tubulin and actin gene indicate that *C. langeronii* presents two cryptic species (Figs 3–4). The species clade of *C. psychrotolerans* is moderately supported in analyses of the actin gene but highly by means of the β-tubulin gene. It is evident from all three analyses (Figs 2–4) that *C. langeronii* and *C. psychrotolerans* are closely related species. The species node of *Cladosporium spinulosum*, which is morphologically clearly distinguished from all other species by its conspicuous ornamentation consisting of digitate projections (Fig. 5), is supported by β-tubulin (Fig. 3) and actin (Fig. 4) sequence data but not by those of the ITS rDNA (Fig. 2). Analyses of all loci, however, indicate that it is a member of the *C. herbarum* complex.

The analyses of sequences of the ITS and the β-tubulin and actin gene introns and exons (Figs 2–4) do not allow the full elucidation of phylogenetic relationships among these *Cladosporium* species. Statistical support of the interior tree branches resulting from analyses of the β-tubulin and actin genes is low (bootstrap values mostly < 50 %). While the sister group relationship of *C. sphaerospermum* and *C. fusiforme* is highly supported in the analysis based on the β-tubulin gene, analysis of the ITS rDNA indicate that these two species are unrelated, and that *C. sphaerospermum* is closely related to *C. dominicanum*. It is clear from the data that the species morphologically resembling *C. sphaerospermum* are not phylogenetically closely related and that the data we present here do not allow their classification in natural subgroups of the genus *Cladosporium*. Only *C. spinulosum* was placed in all analyses among species of the *C. herbarum* complex

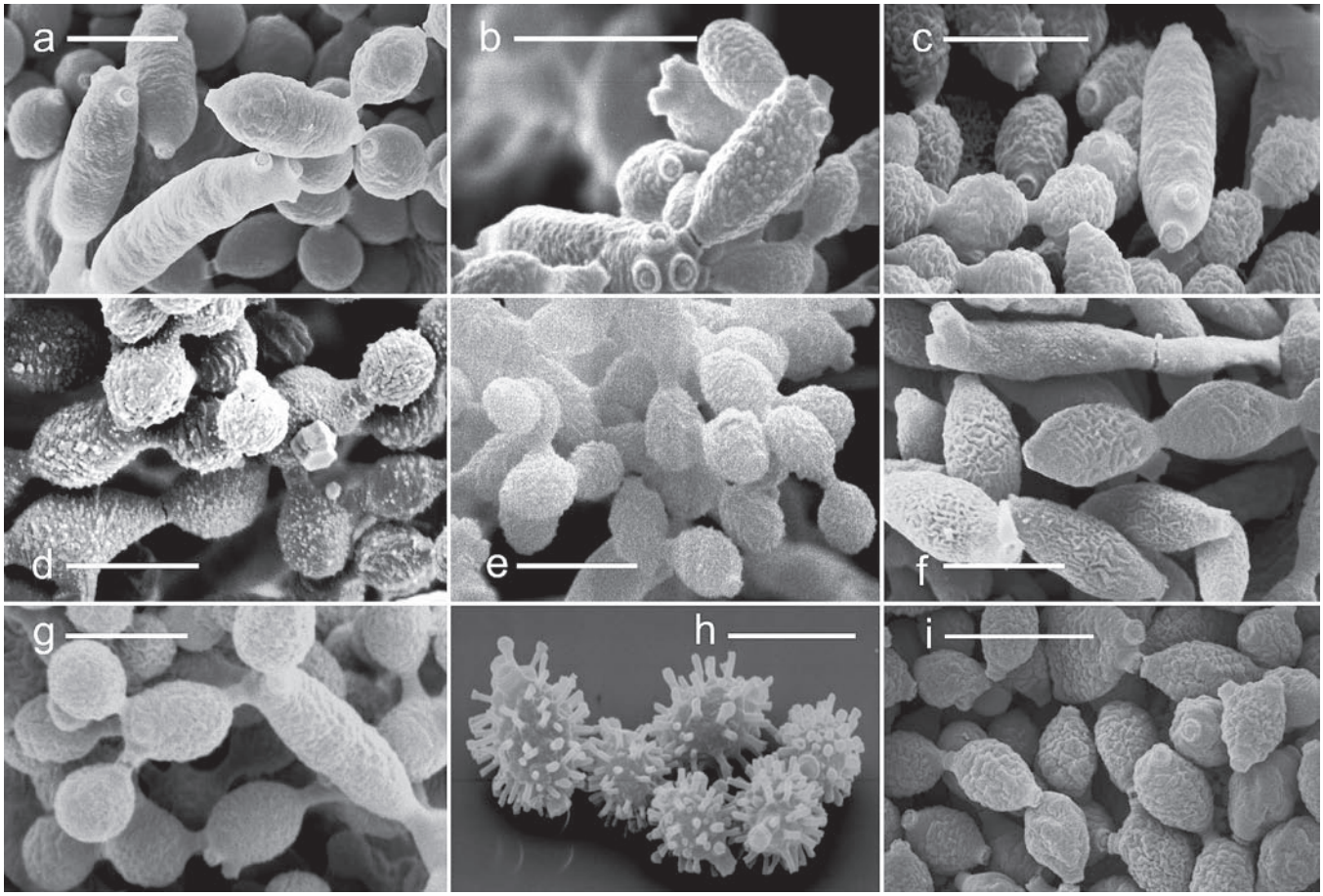

Fig. 5. Conidial scars and surface ornamentation of ramoconidia and conidia (SEM). A. *C. dominicanum* (strain EXF-732). B. *C. fusiforme* (strain EXF-449). C. *C. halotolerans* (strain EXF-572). D. *C. langeronii* (strain CBS 189.54). E. *C. psychrotolerans* (strain EXF-391). F. *C. salinae* (strain EXF-335 = CBS 119413). G. *C. sphaerospermum* (strain CBS 193.54). H. *C. spinulosum* (strain EXF-334). I. *C. velox* (strain CBS 119417). Scale bars = 5 µm. (Photos: K. Drašlar).

and all analyses supported close relatedness of *C. langeronii* and *C. psychrotolerans*.

The majority of species described here have slightly ornamented conidia ranging from minutely verruculose (*C. fusiforme*, *C. langeronii, C. psychrotolerans*, *C. sphaerospermum*, *C. velox*) to verrucose (*C. halotolerans*) (Fig. 5). The verrucose conidia of *C. halotolerans* can be recognised also under the light microscope and used as a distinguishing character. Almost smooth to minutely verruculose conidia are encountered in *C. dominicanum* and *C. salinae* (Fig. 5). *Cladosporium spinulosum*, a member of the *C. herbarum* species complex, has conidia with a digitate ornamentation that can appear spinulose under the light microscope; however, when using the SEM it became clear that its projections have parallel sides and a blunt end (Fig. 5).

DISCUSSION

The genus *Cladosporium* was established by Link (1816) who originally included four species, of which *C. herbarum* is the type species of the genus (Clements & Shear 1931). In 1950, von Arx reported a teleomorph connection for this species with *Mycosphaerella tassiana* (De Not.) Johanson. Based on SSU rDNA data the majority of *Mycosphaerella* species, including the type species of the genus, *M. punctiformis* (Pers.) Starbäck, clustered within the *Mycosphaerellaceae*, a family separated from *M. tassiana* (Braun *et al*. 2003). Therefore, *Mycosphaerella tassiana* was reclassified as *Davidiella tassiana* (De Not.) Crous & U. Braun, the type of the new genus *Davidiella.* All anamorphs with a cladosporium- and heterosporium-like appearance and with a supposed *Dothideomycetes* relationship were maintained under the anamorph name *Cladosporium*, morphologically characterised by scars with a protuberant hilum consisting of a central dome surrounded by a raised rim (David 1997).

The concept of distinguishing ramoconidia from secondary ramoconidia has been adopted from Schubert *et al.* (2007). In the species described here, ramoconidia have been observed often in *C. sphaerospermum*, sometimes in *C. psychrotolerans*, *C. langeronii* and *C. spinulosum*, and only sporadically in all other species. Therefore, ramoconidia can be seen as important for distinguishing species although sometimes, they can be observed only with difficulty. When using ramoconidia as a diagnostic criterion, colonies only from SNA and not older than 7 d should be taken into account.

Cladosporium sphaerospermum was described by Penzig (1882) from decaying *Citrus* leaves and branches in Italy. He described *C. sphaerospermum* as a species with (i) branched, septate and dark conidiophores having a length of 150–300 µm and a width of the main conidiophore stipe of 3.5–4 µm, (ii) spherical to ellipsoid, acrogenously formed conidia of 3.4–4 µm diam, and (iii) ramoconidia of 6–14 × 3.5–4 µm. Penzig's original material is not known to be preserved. Later, a culture derived from CBS 193.54, originating from a human nail, was accepted as typical of *C. sphaerospermum.* However, de Vries (1952), incorrectly cited it as "lectotype", and thus the same specimen is designated as neotype in this study (see below), with the derived culture (CBS 193.54)

used as ex-neotype strain. Numerous strains with identical or very similar ITS rDNA sequences as CBS 193.54 were isolated from hypersaline water or organic substrata including plants or walls of bathrooms. It is not clear yet whether surfaces in bathrooms and of plants, colonised by *C. sphaerospermum*, can have a similar low water activity as salterns. In our experiments, the strains of this species, however, grew under *in vitro* conditions at a water activity of up to 0.860, while Hocking *et al.* (1994) and Aihara *et al.* (2002) reported that it can grow even at 0.815. Therefore, we consider *C. sphaerospermum* as halo- or osmotolerant. Hardly any reports are available unambiguously proving that *C. sphaerospermum* is a human pathogen. It is therefore possible that CBS 193.54 was not involved in any disease process but rather occurred as a contaminant on dry nail material. *Cladosporium sphaerospermum* is a phylogenetically well-delineated species (Figs 2–4).

Strains of *C. halotolerans* were isolated sporadically from substrata such as peanut cell suspension, tissue culture, bathroom walls and as culture contaminants. This surprising heterogeneity of substrata suggests that *C. halotolerans* is distributed by air and that it can colonise whatever substrata available, although it may have its natural niche elsewhere. We have recurrently isolated it from hypersaline water of salterns and other saline environments and it was also detected with molecular methods (but not isolated) from skin of a salt water dolphin. There are only few reports of this species from plants (Table 1). It is therefore possible that *C. halotolerans* is a species closely linked to salty or hypersaline environments although additional sampling is necessary to prove that. *Cladosporium halotolerans* is morphologically recognisable by relatively oblong to spherical, coarsely rough-walled conidia. The ITS rDNA sequence of a fungus in the skin of a bottlenose dolphin, suffering from lobomycosis, is identical to the sequences of *C. halotolerans*. This sequence was deposited as *Lacazia loboi* Taborda, V.A. Taborda & McGinnis (GenBank AF035674) by Haubold *et al*. (1998), who apparently concluded wrongly that a fungus with a cladosporium-like ITS rDNA sequence similar to that of *C. halotolerans* can be the agent of lobomycosis. Later, Herr *et al*. (2001) showed that *Lacazia loboi* phylogenetically belongs to the *Onygenales* on the basis of amplified SSU rDNA and chitin synthase-2 gene sequences generated from tissue lesions. By this, they confirmed an earlier supposition by Lacaz (1996) who reclassified the organism as *Paracoccidioides loboi* O.M. Fonseca & Silva Lacaz (*Onygenales*). It is therefore possible that *C. halotolerans* was not the main etiologic agent for the lobomycosis and it was colonising the affected dolphin skin secondarily while inhabiting other seawater habitats.

Cladosporium langeronii and *C. psychrotolerans* are closely related but *C. langeronii* is particularly well distinguishable from all other *Cladosporium* species by its slow growing colonies (1–7 mm diam / 14 d) and relatively large conidia (4–5.5 × 3–4 µm). *Cladosporium psychrotolerans* has smaller conidia (3–4 × 2.5–3 µm) but a similar length : width ratio and faster expanding colonies (8–18 mm diam / 14 d). *Cladosporium langeronii* is most likely a complex of at least two species. Strains isolated from the Arctic and the Antarctic may need to be distinguished from *C. langeronii s. str.* on species level. This inference is particularly supported by analyses of the β-tubulin and actin genes (Figs 3–4). *Cladosporium langeronii s. str.*, represented by an authentic strain of *Hormodendrum langeronii* Fonseca, Leão & Nogueira, CBS 189.54 (Trejos 1954), has been isolated from a variety of substrata but is tolerating only up to 10 % NaCl. It was originally described by da Fonseca *et al*. (1927a, b) and subsequently reclassified as *Cladosporium langeronii* by Vuillemin (1931). The authentic strain derived from an ulcerating nodular lesion on the arm of a human patient. Because other strains of this species are ubiquitous saprobes originating from various substrata, we suspect that *C. langeronii* is not an important human pathogen. *Cladosporium psychrotolerans* has been isolated from hypersaline environments only, and tolerates up to 20 % NaCl in culture media.

In general, the human- or animal-pathogenic role of the *C. sphaerospermum*-like species described here seems to be limited. It is possible that pathogenic species of *Cladophialophora* Sacc. have been misidentified as *C. sphaerospermum* or as other species of *Cladosporium* (de Hoog *et al*. 2000). Alternatively, true *Cladosporium* species isolated as clinical strains could have been secondary colonisers since they are able to dwell on surfaces poor in nutrients, possibly in an inconspicuous dormant phase and may then be practically invisible. More likely, they could be air-borne contaminations of lesions, affected nails etc. (Summerbell *et al*. 2005) or are perhaps disseminated by insufficiently sterilised medical devices, as melanised fungi can be quite resistant to disinfectants (Phillips *et al.* 1992). They can easily be isolated and rapidly become preponderant at isolation and thus difficult to exclude as etiologic agents of a disease. For example, in 2002, a case report on an intrabronchial lesion by *C. sphaerospermum* in a healthy, non-asthmatic woman was described (Yano *et al*. 2002), but we judge the identification of the causal agent to remain uncertain, as it was based on morphology alone and no culture is available. The present authors have the opinion that all clinical cases ascribed to *Cladosporium* species need careful re-examination.

General characteristics and description of *Cladosporium sphaerospermum*-like species

The present paper focuses on *Cladosporium* strains isolated from hypersaline environments. Comparison of data from deliberate sampling and analysis of reference strains from culture collections inevitably leads to statistical bias, and therefore a balanced interpretation of ecological preferences of the species presented is impossible. Nevertheless, some species appeared to be consistent in their choice of habitat, and for this reason we summarise isolation data for all species described. Strains belonging to a single molecular clade proved to have similar cultural characteristics and microscopic morphology. Although within most of the species there was some molecular variation noted (particularly when intron-rich genes were analysed), some consistent phenetic trends could be observed.

Conidiophores of all *C. sphaerospermum*-like species lack nodose inflations (McKemy & Morgan-Jones 1991). They are usually ascending and can sometimes be poorly differentiated from their supporting hyphae. Though the initiation point of conidiophore stipes could sometimes be determined only approximately, their lengths were in some cases useful for distinguishing morphologically similar species when observed in slide cultures. Generally, the branched part of a conidiophore forms a complex tree-like structure. The number and orientation of early formed secondary ramoconidia, however, determines whether it is rotationally symmetric or unilateral.

The variability in ITS rDNA sequences observed in all *C. sphaerospermum*-like species (about 10 %) spans the variation observed in all members of the genus *Cladosporium* sequenced to date. Thus, the *C. sphaerospermum*-like species described here may not present a single monophyletic group but may belong to various species complexes within *Cladosporium*. Verifying existing literature with sequence data of these species (Wirsel *et al.* 2002, Park *et al*. 2004), we noticed that names of the common saprobes seem to be distributed nearly at random over phylogenetic trees.

For most commonly used names, no type material is available for sequencing. Also verification of published reports is difficult without available voucher strains.

Cladosporium cladosporioides was incorrectly lectotypified based on CBS 170.54 (de Vries 1952), which Bisby considered a standard culture of *C. herbarum*. The *C. cladosporioides* species complex requires revision, and will form the basis of a future study. *Cladosporium herbarum* is maintained as a dried specimen in the Leiden herbarium; Prasil & de Hoog (1988) selected CBS 177.71 as a representative living strain. Strains, earlier accepted as living representatives of *C. herbarum*, CBS 177.71 and CBS 812.71 (Prasil & de Hoog 1988, Wirsel *et al.* 2002) and ATCC 66670 (Braun *et al.* 2003, as *Davidiella tassiana*) have been re-identified as *C. bruhnei* Linder by Schubert *et al.* (2007 – this volume). Ho *et al*. (1999) used strain ATCC 38027 as a representative of *C. tenuissimum* Cooke and this strain has identical ITS sequences as the non-deposited *C. tenuissimum* material used by Moricca *et al.* (1999). We tentatively accept this concept although we could not include ATCC 38027 in our analyses. The ITS sequence of strain CBS 125.80, identified by Wirsel *et al.* (2002) as *C. oxysporum*, is identical to the sequence of ATCC 38027. Strain ATCC 76499, published by Ho *et al.* (1999) as *C. oxysporum*, appears to be identical to a number of currently unidentified *Cladosporium* strains from Slovenian salterns that compose a cluster separate from all remaining species. Strains of this cluster, represented in Fig. 2 by strain ATCC 76499, morphologically resemble *C. oxysporum.*

Strain CBS 300.96 has not been identified to species level in the present study. It clusters outside the species clade of *C. sphaerospermum*, with the latter being its nearest relative. CBS 300.96 differs from *C. sphaerospermum* by having smaller structures: conidiophore stipes [(5–)20–80(–150) × (2–)2.5–3(–4) µm], 0–1 septate ramoconidia [(13–)19–27(–32) × 2–2.5 µm], conidia [(2.5–)3–3.5(–4) × (2–)2–2.5(–3) µm] and secondary ramoconidia [(5–)9–18(–30) × (2–)2.5–2.5(–3) µm]. However, based on a single isolate, we currently refrain from describing it as a new species.

Key to species treated in this study

Macro-morphological characters used in the key are from colonies grown on PDA and MEA 14 d at 25 °C, if not stated otherwise; microscopical characters are from SNA slide cultures grown for 7 d at 25 °C.

1. Conidial ornamentation conspicuously echinulate / digitate because of up to 1.3 µm long projections that have more or less parallel sides ..... ***C. spinulosum***
1. Conidial ornamentation verruculose to verrucose or smooth, not conspicuously echinulate or digitate 2

2. Conidiophores micronematous, poorly differentiated, once or several times geniculate-sinuous, short, up to 60 µm long; terminal conidia obovoid ..... ***C. salinae***
2. Conidiophores micro- or macronematous, not geniculate or only slightly so, usually up to 100 µm or 220 µm long or even longer; terminal conidia globose, subglobose to ovoid or fusiform 3

3. Secondary ramoconidia 0–3(–4)-septate; septa of conidiophores and conidia darkened and thickened 4
3. Secondary ramoconidia 0–1(–2)-septate; septa neither darkened nor thickened 5

4. Conidiophores (5–)10–50(–300) × (2–)2.5–3(–5.5) µm; terminal conidia (2–)3–4(–6) × (2–)2.5–3(–5) µm; secondary ramoconidia (5–)7–12(–37.5) × (2–)2.5–3(–6.5) µm; ramoconidia sporadically formed ..... ***C. halotolerans***
4. Conidiophores mostly longer and somewhat wider, (10–)45–130(–300) × (2.5–)3–4(–6) µm; terminal conidia mostly wider, (2.5–)3–4(–7) × (2–)3–3.5(–4.5) µm; secondary ramoconidia (4–)8.5–16(–37.5) × (2–)3–3.5(–5) µm; ramoconidia often formed, up to 40 µm long, with up to 5 septa ..... ***C. sphaerospermum***

5. Terminal conidia usually fusiform ..... ***C. fusiforme***
5. Terminal conidia globose, subglobose or ovoid 6

6. Conidia and secondary ramoconidia irregularly verruculose to sometimes loosely verrucose; radial growth on PDA at 25 °C after 14 d typically less than 5 mm ..... ***C. langeronii***
6. Conidia and secondary ramoconidia smooth to minutely verruculose; radial growth on PDA at 25 °C after 14 d typically more than 10 mm 7

7. Conidiophores (3–)3.5–4(–7.5) µm wide, thick-walled; conidiogenous loci and conidial hila 0.5–2 µm diam; ramoconidia sometimes formed with a broadly truncate, up to 2 µm wide non-cladosporioid base; no growth observed after 14 d at 30 °C on MEA ..... ***C. psychrotolerans***
7. Conidiophores mostly narrower, 2–4 µm wide, only with slightly thickened walls; conidiogenous loci and conidial hila narrower, 0.5–1.5 µm diam; ramoconidia rarely formed; colony showing at least weak growth after 14 d at 30 °C on MEA 8

8. Secondary ramoconidia (4–)6.5–13(–24.5) µm long; no visible colony growth after 14 d at 10 °C on MEA..... ***C. dominicanum***
8. Secondary ramoconidia mostly longer, (3.5–)5.5–19(–42) µm; radial growth of colonies after 14 d at 10 °C on MEA more than 5 mm ..... ***C. velox***

Description of *Cladosporium* species

Cladosporium dominicanum Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB510995. Fig. 6.

Etymology: Refers to the the Dominican Republic, where most strains were encountered.

Conidiophora lateralia vel terminalia ex hyphis rectis oriunda; stipes longitudine variabili, (5–)10–100(–200) × (1.5–)2–2.5(–3.5) µm, olivaceo-brunneus, levis vel leniter verruculosus, tenuitunicatus, plerumque unicellularis, simplex vel ramosus. Conidiorum catenae undique divergentes, ad 8 conidia in parte continua continentes. Cellulae conidiogenae indistinctae. Conidia levia vel leniter verruculosa, dilute brunnea, unicellularia, plerumque breviter ovoidea, utrinque angustata, (2.5–)3–3.5(–5.5) × (2–)2–2.5(–2.5) µm, long.: lat. 1.4–1.6; ramoconidia secundaria cylindrica vel quasi globosa, 0–1-septata, (4–)6.5–13(–24.5) × (2–)2.5–3(–4.5) µm, ad 4 cicatrices terminales ferentia; cicatrices inspissatae, protuberantes, 0.5–1.2 µm diam. Hyphae vagina polysaccharidica carentes.

Mycelium without extracellular polysaccharide-like material. *Conidiophores* arising laterally and terminally on erect hyphae, micronematous and semimacronematous, stipes of variable length, (5–)10–100(–200) × (1.5–)2–2.5(–3.5) µm, olivaceous-brown, smooth to minutely verruculose, thin-walled, almost non-septate, unbranched or branched. *Conidial chains* branching in all directions, up to eight conidia in the unbranched parts. *Conidiogenous cells* undifferentiated. *Ramoconidia* rarely formed. *Conidia* smooth to minutely verruculose, subhyaline to light brown, non-septate, usually short-ovoid, narrower at both ends, length : width ratio = 1.4–1.6; (2.5–)3–3.5(–5.5) × (2–)2–2.5(–2.5) µm [av. (± SD) 3.4 (± 0.6) × 2.2 (± 0.2)]; *secondary ramoconidia* cylindrical to almost spherical, 0–1-septate, (4–)6.5–13(–24.5) × (2–)2.5–3(–4.5) µm [av. (± SD) 10.3 (± 5.2) × 2.7 (± 0.6)], with up to four distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.5–1.2 µm diam.

Cultural characteristics: Colonies on PDA reaching 18–36 mm diam, olive-yellow (2D6), hairy granular, flat or slightly furrowed, with flat margin. Droplets of light reseda-green (2E6) exudate sometimes present. Reverse dark green to black. Colonies on OA reaching 19–34 mm diam, olive (2F5), loosely powdery with raised central part due to fasciculate bundles of conidiophores. Reverse dark green. Colonies on MEA reaching 30–32 mm diam, reseda green (2E6), velvety, furrowed, with undulate margin. Reverse dark green-brown. Colonies on MEA + 5 % NaCl reaching 37–41 mm diam, reseda-green (2E6), radially furrowed, velvety, sporulating in the central part or all over the colony, margin white and regular. Reverse brownish green.

Maximum tolerated salt concentration: 75 % of tested strains develop colonies at 20 % NaCl after 7 d, while after 14 d all strains grow and sporulate.

Cardinal temperatures: No growth at 4 and 10 °C, optimum 25 °C (30–32 mm diam), maximum 30 °C (2–15 mm diam), no growth at 37 °C.

Specimen examined: **Dominican Republic**, from hypersaline water of salt lake Enriquillo, coll. Nina Gunde-Cimerman, Jan. 2001, isol. P. Zalar 25 Feb. 2001, CBS H-19733, **holotype**, culture ex-type EXF-732 = CBS 119415.

Habitats and distribution: Fruit surfaces; hypersaline waters in (sub)tropical climates.

Differential parameters: No growth at 10 °C, ovoid conidia, large amounts of sterile mycelium.

Strains examined: CPC 11683, EXF-696, EXF-718, EXF-720, EXF-727, EXF-732 (= CBS 119415; ex-type strain).

Note: Cultures of *C. dominicanum* sporulate less abundantly than *C. sphaerospermum* and *C. halotolerans* and tend to lose their ability to sporulate with subculturing.

Cladosporium fusiforme Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB510997. Fig. 7.

Etymology: Refers to its usually fusiform conidia.

Conidiophora erecta, lateralia vel terminalia ex hyphis rectis oriunda; stipes longitudine variabili, (10–)25–50(–100) × (2–)2–3.5(–4) µm, olivaceo-brunneus, levis, crassitunicatus, compluries septatus (cellulis 9–23 µm longis), plerumque simplex. Conidiorum catenae undique divergentes, in parte continua ad 5 conidia continentes. Cellulae conidiogenae indistinctae. Conidia leniter verruculosus, dilute brunnea, unicellularia, plerumque fusiformia, utrinque angustata, (2.5–)3.5–5(–6.5) × (2–)2–2.5(–3) µm, long. : lat. 1.8–2.0; ramoconidia secundaria cylindrica, 0(–1)-septata, (5–)6–11(–22) × (2.5–)2.5–3(–3) µm, ad 4 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, 0.7–1.0 µm diam. Hyphae vagina polysaccharidica carentes.

Mycelium without extracellular polysaccharide-like material. *Conidiophores* erect, arising laterally and terminally from straight hyphae, stipes of variable length, (10–)25–50(–100) × (2–)2–3.5(–4) µm, olivaceous-brown, smooth- and thick-walled, regularly-septate (cell length 9–23 µm), mostly unbranched. *Conidial chains* branching in all directions, up to 5 conidia in the unbranched parts. *Conidiogenous cells* undifferentiated. *Ramoconidia* rarely formed. *Conidia* minutely verruculose, light brown, aseptate, usually fusiform and narrower at both ends, length : width ratio = 1.8–2.0; (2.5–)3.5–5(–6.5) × (2–)2–2.5(–3) µm [av. (± SD) 4.4 (± 0.8) × 2.2 (± 0.2)]; *secondary ramoconidia* cylindrical, 0(–1)-septate, (5–)6–11(–22) × (2.5–)2.5–3(–3) µm [av. (± SD) 9.0 (± 4.7) × 2.6 (± 0.3)], with up to 4 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.7–1.0 µm diam.

Cultural characteristics: Colonies on PDA reaching 20–26 mm diam, dull green (30E3), granular due to profuse sporulation, flat, with flat margin. Sterile mycelium absent. Reverse blackish green. Colonies on OA reaching 24–28 mm diam, olive (3F3), granular in concentric circles, consisting of two kinds of conidiophores (low and high), flat, with flat margin. Reverse black. Colonies on MEA reaching 23–28 mm diam, olive (3E5), deeply furrowed, velvety (sporulating all over) with undulate, white margin. Reverse brownish green. Colonies on MEA + 5 % NaCl reaching 28–43 mm diam, olive (3E6), granular due to profuse sporulation, slightly furrowed with flat, olive-grey (3F2) margin. Reverse dark green.

Maximum tolerated salt concentration: Only one of three strains tested (CBS 452.71) developed colonies at 17 % NaCl after 14 d, the other two strains grew until 10 % NaCl.

Cardinal temperatures: For one of three strains (CBS 452.71) the minimum temperature of growth was 4 °C (6 mm diam), for the other two 10 °C (8–9 mm diam); optimum 25 °C (23–28 mm diam), maximum 30 °C (only strain CBS 452.71 grew 5 mm diam), no growth at 37 °C.

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. L. Butinar, Dec. 1999, CBS H-19732, **holotype**, culture ex-type EXF-449 = CBS 119414.

Habitats and distribution: Osmotic environments worldwide.

Differential parameters: Oblong conidia, relatively low degree of halotolerance.

Strains examined: CBS 452.71, EXF-397, EXF-449 (= CBS 119414; ex-type strain).

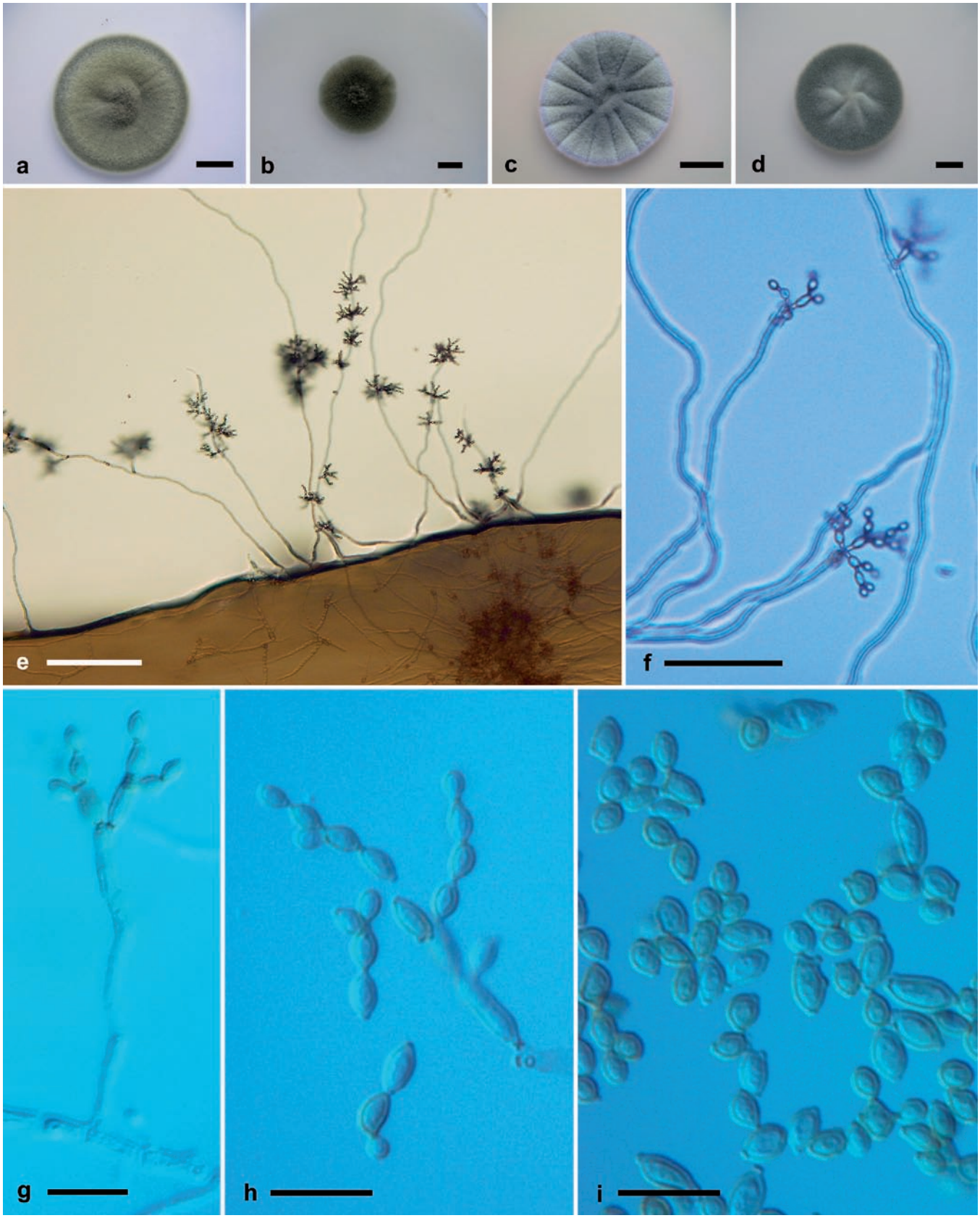

Fig. 6. *Cladosporium dominicanum*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G. Conidiophore. H–I. Secondary ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A, D, F–H, from EXF-2519; B, C, E from EXF-727; I, EXF-732 (ex-type strain). Scale bars A–D = 10 mm, E = 100 µm, F = 30 µm, G–I = 10 µm.

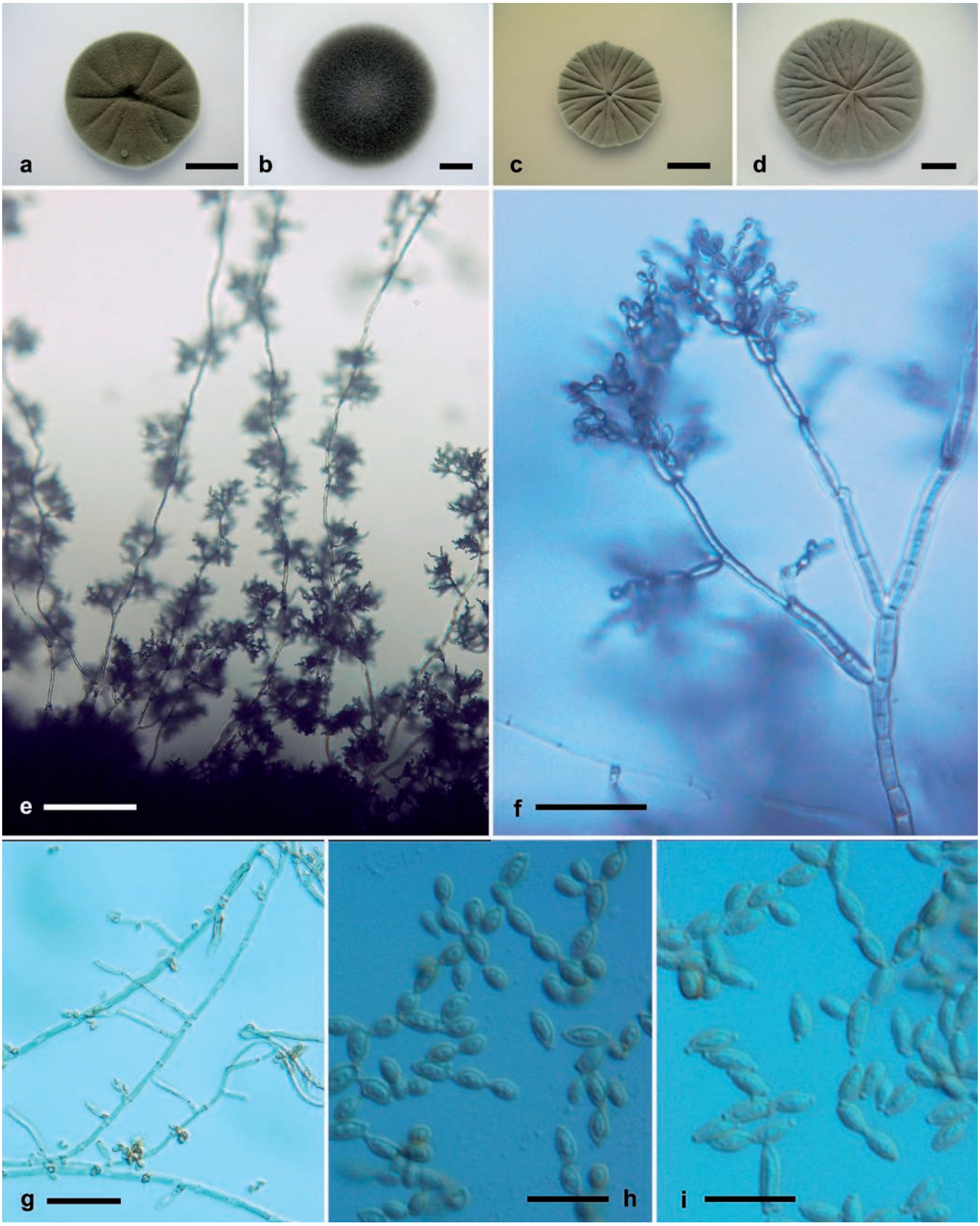

Fig. 7. *Cladosporium fusiforme*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–G. Habit of conidiophores. H–I. Ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A–H, from EXF-449 (ex-type strain); I, from CBS 452.71. Scale bars A–D = 10 mm, E = 100 µm, F–G = 30 µm, H–I = 10 µm.

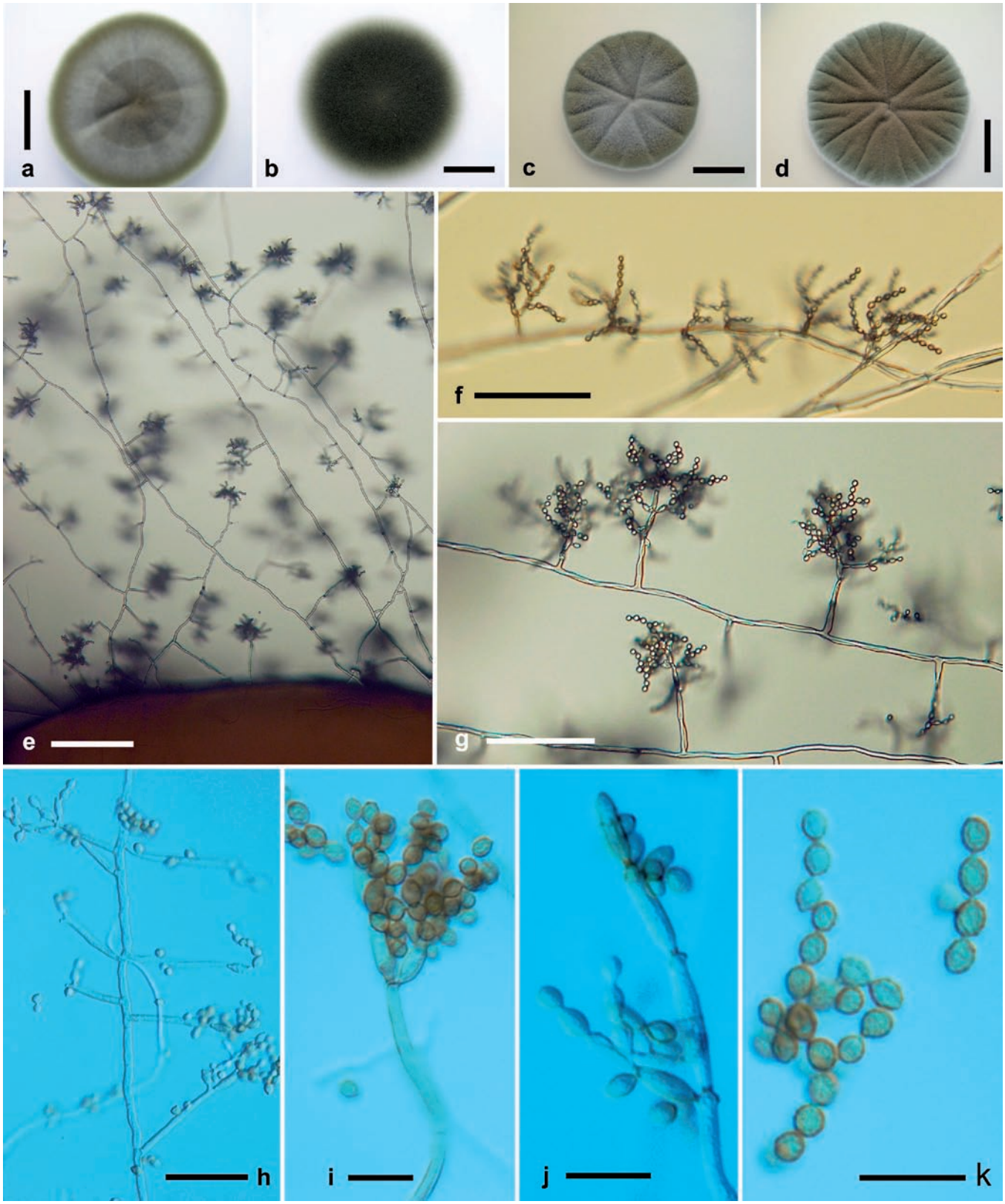

Fig. 8. *Cladosporium halotolerans.* Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–H. Habit of conidiophores. I. Conidiophore. J. Succession of secondary ramoconidia. K. Conidia. E–K. All from 7-d-old SNA slide cultures. A–B, from EXF-572 (ex-type strain); C–D, from EXF-977; E, G, from EXF-972; F, from EXF-564; H, I, K, from EXF-1072; J, from dH 12862. Scale bars A–D = 10 mm, E = 100 µm, F–G = 50 µm, H = 30 µm, I–K = 10 µm.

Cladosporium halotolerans Zalar, de Hoog & Gunde-Cimerman **sp. nov.** MycoBank MB492439. Fig. 8.

Etymology: Refers to its halotolerant habit.

Conidiophora erecta, lateralia vel terminalia ex hyphis rectis oriunda; stipes longitudine variabili, (5–)10–50(–300) × (2–)2.5–3(–5.5) µm, pallide olivaceo-brunneus, levis vel leniter verruculosus, tenuitunicatus, 0–3-septatus, interdum pluriseptatus, simplex, denticulatus. Conidiorum catenae undique divergentes, terminales ad 9 conidia continentes. Cellulae conidiogenae indistinctae. Conidia verrucosa, brunnea vel fusca, unicellularia, plerumque subglobosa vel globosa, raro breviter ovoidea, utrinque angustata, (2–)3–4(–6) × (2–)2.5–3(–5) µm, long. : lat. 1.2–1.5; ramoconidia secundaria cylindrica vel quasi globosa, 0(–1)-septata, (5–)7–12(–37.5) × (2–)2.5–3(–6.5) µm , ad 4 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, protuberantes, 0.7–1.0(–1.5) µm diam. Hyphae vagina polysaccharidica carentes.

Mycelium partly submerged, partly superficial; hyphae without extracellular polysaccharide-like material. *Conidiophores* erect, arising laterally and terminally from straight hyphae, stipes of variable length, (5–)10–50(–300) × (2–)2.5–3(–5.5) µm, pale olivaceous-brown, smooth to minutely verruculose, thin-walled, 0–3-septate, unbranched, with pronounced denticles. *Conidial chains* branching in all directions, terminal chains with up to 9 conidia. *Conidiogenous cells* undifferentiated. *Ramoconidia* rarely formed. *Conidia* verrucose, brown to dark brown, non-septate, usually subglobose to globose, less often short-ovoid, narrower at both ends, length : width ratio = 1.2–1.5; (2–)3–4(–6) × (2–)2.5–3(–5) µm [av. (± SD) 3.5 (± 0.7) × 2.7 (± 0.5)]; *secondary ramoconidia* cylindrical to almost spherical, 0–1-septate, (5–)7–12(–37.5) × (2–)2.5–3(–6.5) µm [av. (± SD) 10.3 (± 4.8) × 2.9 (± 0.6)], with up to 4 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.7–1.0(–1.5) µm diam.

Cultural characteristics: Colonies on PDA reaching 27–43 mm diam, olive (2F5), slightly furrowed, often covered with grey secondary mycelium, except at the marginal area where only sporulating structures can be observed. Margin white and regular, with submerged hyphae. Reverse pale green to black. Colonies on OA reaching 29–40 mm diam, olive (2F6), flat, uniform, granular due to profuse sporulation and fasciculate bundles of conidiophores, without sterile mycelium. Reverse dark green to black. Colonies on MEA reaching 18–44 mm diam, highly variable in colour, but mainly olive (2E5), and from flat with regular margin to deeply furrowed with undulate margin. Colony centre wrinkled with crater-shaped appearance. Reverse pale to dark green. Colonies on MEA + 5 % NaCl reaching 24–48 mm diam, olive (3E8), furrowed, velvety, with more pale, undulate margins. Reverse dark green to black.

Maximum tolerated salt concentration: Only 15 % of tested strains develop colonies at 20 % NaCl after 7 d, whereas after 14 d all cultures grow and sporulate.

Cardinal temperatures: No growth at 4 °C, optimum 25 °C (18–44 mm diam), maximum 30 °C (6–23 mm diam). No growth at 37 °C.

Specimen examined: **Namibia**, from hypersaline water of salterns, coll. Nina Gunde-Cimerman, 1 Sep. 2000, isol. P. Zalar, 1 Oct. 2000, CBS H-19734, **holotype**, culture ex-type EXF-572 = CBS 119416.

Habitats and distribution: Hypersaline water in subtropical climates; indoor environments; Arctic ice; contaminant in lesions of humans and animals; plant phyllosphere; rock.

Literature: Haubold *et al.* (1998), Meklin *et al.* (2004).

Differential parameters: Verrucose conidia, short unbranched and non-septate conidiophores which arise laterally alongside erect hyphae.

Strains examined: CBS 191.54, CBS 573.78, CBS 626.82, dH 12862, dH 12991, dH 13911, EXF-228, EXF-380, EXF-565, EXF-567, EXF-571, EXF-572 (= CBS 119416; ex-type strain), EXF-646, EXF-698, EXF-703, EXF-944, EXF-972, EXF-977, EXF-1072, EXF-2372.

Notes: *Cladosporium halotolerans* strongly resembles *C. sphaerospermum*. Several strains of this species such as dH 12862, dH 12941, CBS 191.54 and UAMH 7686 have been isolated sporadically from various indoor habitats in Europe, Brazil and the U.S.A. and repeatedly from bathrooms in Slovenia (Table 1). Probably sometimes as uncertain culture contaminations, it has been isolated from plants (GenBank accession no. L25433), inner organs of a diseased frog (AY361982) and human brain (Kantarcioglu *et al.* 2002). The presence of *C. halotolerans* species in gypsum sediments entrapped in Arctic ice, the fact that it was repeatedly isolated from hypersaline water and possibly its presence in dolphin skin (see Discussion) suggest that it has a clear preference for (hyper)osmotic habitats. This is supported by its ability to grow at 20 % NaCl.

The teleomorph of *C. halotolerans* is predicted to be a *Davidiella* species. Strain CBS 280.49 was isolated by J.A. von Arx from teleomorphic material of a fungus labelled as *Mycosphaerella hyperici* (Auersw.) Starbäck on *Hypericum perforatum* in Switzerland. According to Aptroot (2006) this species may belong in *Davidiella* and produces a *Septoria* anamorph. In the original herbarium specimen, CBS H-4867, a *Mycosphaerella* teleomorph was present, but no sign of a *Cladosporium* anamorph. We assume that CBS 280.49 was a culture contaminant.

Cladosporium langeronii (Fonseca, Leão & Nogueira) Vuill., Champ. Paras.: 78. 1931. Fig. 9.

Basionym: *Hormodendrum langeronii* Fonseca, Leão & Nogueira, Sci. Med. 5: 563. 1927.

≡ *Cladosporium langeronii* (Fonseca, Leão & Nogueira) Cif., Manuale di Micologia Medica, ed. 2: 488 (1960), comb. superfl.

Mycelium partly submerged, partly superficial; hyphae sometimes enveloped in polysaccharide-like material. *Conidiophores* erect or ascending, micronematous and macronematous, stipes of variable length, (20–)50–130(–200) × (3–)3.5–4.5(–6.5) µm, dark brown, rough- and thick-walled, regularly septate (cell length 9–22 µm), arising laterally and terminally from submerged or aerial hyphae, branched. *Conidial chains* dichotomously branched, up to 6 conidia in the unbranched parts. *Conidiogenous cells* undifferentiated, sometimes seceding and forming ramoconidia. *Ramoconidia* cylindrical, 0–1 septate, (10–)11–22(–42) × (3–)3.5–4.5(–5) µm, base broadly truncate, 2–3.5 µm wide, slightly thickened and somewhat darkened. *Conidia* irregularly verruculose to sometimes loosely verrucose, dark brown, non-septate, usually ovoid, length : width ratio = 1.3–1.5; conidial size (3–)4–5.5(–8) × (2–)3–4(–5) µm [av. (± SD) 4.8 (± 1.0) × 3.5 (± 0.6)]; *secondary ramoconidia* cylindrical to almost spherical, mostly 0–1(–2)-septate, (5.5–)7.5–12.5(–35.5) × (2.5–)3–4.5(–5.5) µm [av. (± SD) 10.7 (± 4.7) × 3.6 (± 0.8)], with 2, rarely 3 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.9–1.5(–2.3) µm diam.

Cultural characteristics: Colonies on PDA, OA and MEA with restricted growth, attaining 2.5–4.5, 1.5–7.0 and 1.0–5.5 mm diam, respectively. Colonies flat or heaped (up to 3 mm), dark green (30F4), with black reverse and slightly undulate margin with immersed mycelium. Sporulating on all media. On MEA + 5 % NaCl growth is faster, colonies attaining 8.5–12.0 mm diam, sporulating and growing deeply into the agar.

Maximum tolerated salt concentration: All strains develop colonies at 17 % NaCl after 14 d.

Cardinal temperatures: No growth at 4 °C, optimum / maximum 25 °C (1.0–5.5 mm diam), no growth at 30 °C.

Specimen examined: **Brazil**, from man ulcero-nodular mycosis of hand and arm, 1927, coll. and isol. da Fonseca, CBS H-19737, **holotype**, culture ex-type CBS 189.54.

Habitats and distribution: Polar ice and biomats; conifer wood and window frame in Europe; humans; strains originating from nasal mucus (Buzina *et al*. 2003) have 100 % sequence homology with

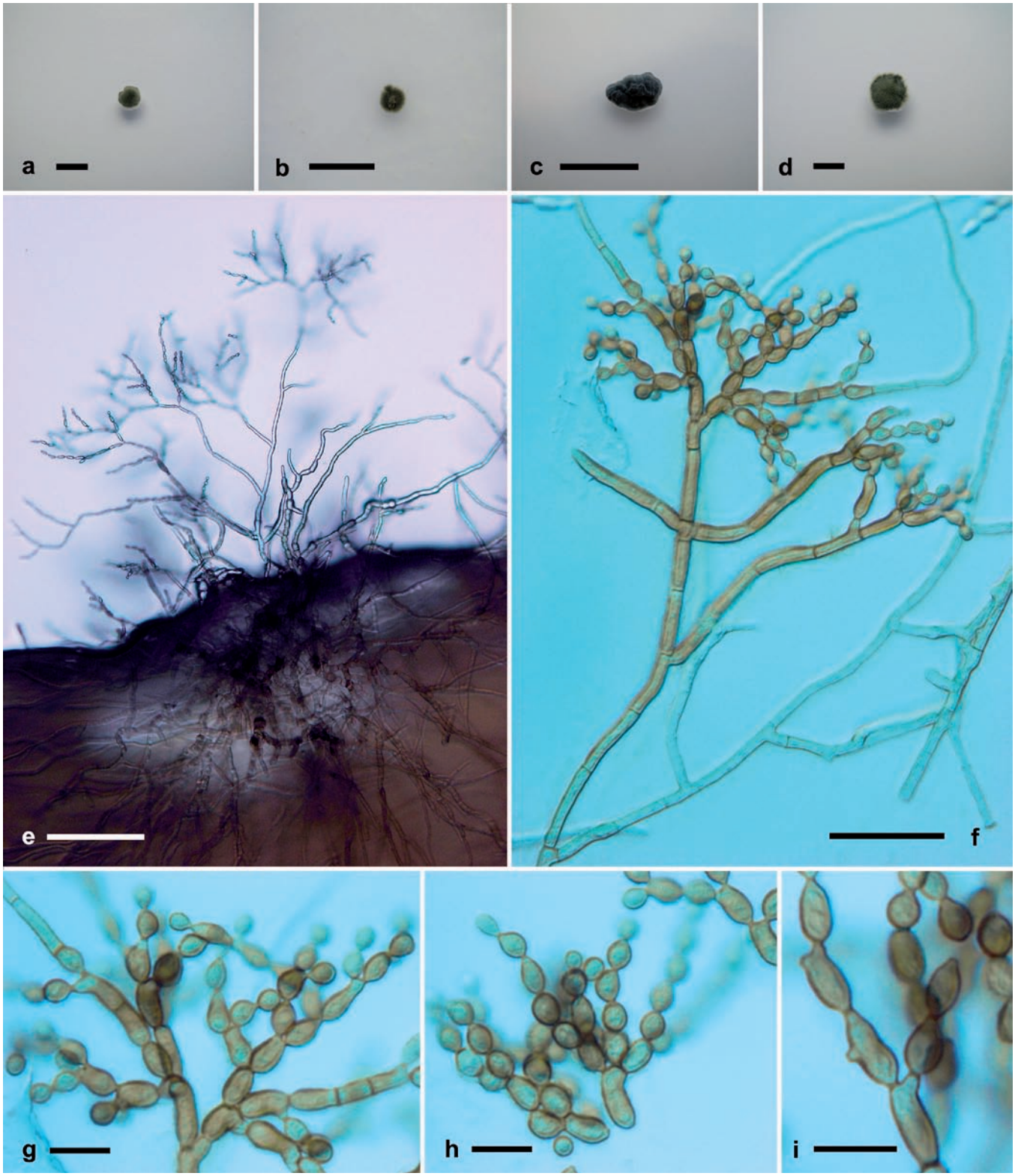

Fig. 9. *Cladosporium langeronii*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G–I. Ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A–D, from CBS 189.54 (ex-type strain); E, from CBS 109868; F–I, from EXF-999. Scale bars A, C–D = 10 mm, B = 5 mm, E = 100 µm, F = 30 µm, G–I = 10 µm.

the strains studied, as well as with a clone from mycorrhizal roots (Menkis *et al*. 2005). The species is distributed worldwide, without any apparent predilection for a particular habitat. The strains from clinical cases probably were culture contaminants.

Literature: da Fonseca *et al.* (1927a, b).

Differential parameters: Restricted growth; lowest salt halotolerance taxon of all *C. sphaerospermum*-like species.

Strains examined: CBS 189.54 (ex-type strain), CBS 601.84, CBS 101880, CBS 109868, dH 11736, dH 12459 = EXF-999, dH 13833 = EXF-1933.

Notes: De Vries (1952) synonymised the isolate identified as *Hormodendrum langeronii* with *C. sphaerospermum*. Strains of this species have often been identified as *C. cladosporioides* (Buzina *et al.* 2003, Menkis *et al.* 2005) although it has slightly longer conidia.

Cladosporium psychrotolerans Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB492428. Fig. 10.

Etymology: Refers to its ability to grow at low temperatures.

Mycelium partim submersum; hyphae vagina polysaccharidica carentes. Conidiophora erecta vel adscendentia; stipes (10–)50–100(–150) × (3–)3.5–4(–7.5) µm, olivaceo-brunneus, levis, crassitunicatus, compluries regulariter septatus (cellulis 10–40 µm longis), identidem dichotome ramosus. Conidiorum catenae undique divergentes, terminales partes simplices ad 4 conidia continentes. Cellulae conidiogenae indistinctae. Ramoconidia primaria cylindrica, (18–)19–22(–43) × (2.5)3–3.5(–4.5) µm, 0(–1)-septata. Conidia leves vel leniter verruculosa, dilute brunnea, unicellularia, globosa vel ovoidea, (2.5–)3–4(–4.5) × (2–)2.5–3(–3) µm, long.: lat. 1.3–1.4; ramoconidia secundaria cylindrica, 0–1(–2)-septata, (5–)8–16(–36) × (2–)2.5–3(–5) µm, ad 4 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, 0.5–2 µm diam.

Mycelium partly superficial partly submerged; hyphae without extracellular polysaccharide-like material. *Conidiophores* erect or ascending, macronematous, stipes (10–)50–100(–150) × (3–)3.5–4(–7.5) µm, olivaceous-brown, smooth or almost so, thick-walled, regularly septate (cell length 10–40 µm), arising laterally from aerial hyphae, repeatedly dichotomously branched. *Conidial chains* branching in all directions, up to 4 conidia in the unbranched parts. *Ramoconidia* sometimes formed, cylindrical, (18–)19–22(–43) × (2.5)3–3.5(–4.5) µm, aseptate, rarely 1-septate, with a broadly truncate base, up to 2 µm wide, unthickened or slightly thickened, somewhat darkened-refractive. *Conidia* smooth to minutely verruculose, light brown, non-septate, spherical to ovoid, length : width ratio = 1.3–1.4; conidial size (2.5–)3–4(–4.5) × (2–)2.5–3(–3) µm [av. (± SD) 3.4 (± 0.5) × 2.5 (± 0.2)]; *secondary ramoconidia* cylindrical, 0–1(–2)-septate, (5–)8–16(–36) × (2–)2.5–3(–5) µm [av. (± SD) 12.7 (± 6.5) × 3.0 (± 0.5)], with up to 4 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.5–2 µm diam.

Cultural characteristics: Colonies on PDA reaching 13–18 mm diam, velvety, olive (3F4) due to profuse sporulation, flat with straight margin. Reverse dark green. Colonies on OA reaching 13–15 mm diam, olive (2F8), of granular appearance due to profuse sporulation; aerial mycelium sparse. Margin regular. Reverse black. Colonies on MEA reaching 8–15 mm diam, olive (2F4), velvety, radially furrowed with undulate white margin. Colonies on MEA with 5 % NaCl growing faster than on other media, reaching 25–27 mm diam, olive (3E6) and granular due to profuse sporulation, either slightly furrowed or heavily wrinkled with regular or undulate margin. Reverse dark green.

Maximum tolerated salt concentration: 17 % NaCl after 14 d.

Cardinal temperatures: Minimum at 4 °C (5 mm diam), optimum and maximum at 25 °C (8–15 mm diam).

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. S. Sonjak, May 1999, CBS H-19730, **holotype**, culture ex-type EXF-391 = CBS 119412.

Habitats and distribution: Hypersaline water in the Mediterranean basin.

Differential parameters: Growth at 4 °C; maximal NaCl concentration 17 % NaCl, which differentiates it from other species with similar conidia, like *C. sphaerospermum*, *C. halotolerans* and *C. dominicanum*.

Strains examined: EXF-326, EXF-332, EXF-391 (= CBS 119412; ex-type strain), EXF-714.

Cladosporium salinae Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB492438. Fig. 11.

Etymology: Refers to salterns (= Latin *salinae*) as the habitat of this species.

Mycelium partim submersum; hyphae multa rostra lateralia ferentes, hyphae vagina polysaccharidica involutae. Conidiophora vix distincta, lateralia vel terminalia ex hyphis aeriis oriunda; stipes longitudine variabili, (5–)25–50(–60) × (2–)2.5–3(–4) µm, olivaceo-brunneus, levis vel leniter verruculosus, crassitunicatus, irregulariter dense septatus (cellulis 6–29 µm longis), simplex, interdum ramosus. Conidiorum catenae undique divergentes, terminales ad 6 conidia continentes. Cellulae conidiogenae nonnumquam integratae, in summo sequentiam sympodialem denticulorum formantes. Conidia levia, interdum leniter verruculosa, dilute brunnea, unicellularia, plerumque fusiformia, (4.5–)5.5–7.5(–10) × (2–)2.5–3(–3.5) µm, long. : lat. 1.9–2.4; ramoconidia secundaria cylindrica, 0–1(–2)-septata, (7.5–)9.5–13.5(–19) × (2.5–)2.5–3.5(–4.5) µm, ad 5 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, protuberantes, 0.7–1.8 µm diam.

Mycelium partly superficial partly submerged, with numerous lateral pegs, consistently enveloped in polysaccharide-like material. *Conidiophores* poorly differentiated, micronematous, stipes (5–)25–50(–60) × (2–)2.5–3(–4) µm, olivaceous-brown, smooth to often minutely verruculose or irregularly rough-walled, thick-walled, irregularly densely septate (length of cells 6–29 µm), arising laterally and terminally from aerial hyphae, unbranched, occasionally branched. *Conidial chains* branching in all directions, terminal chains with up to 6 conidia. *Conidiogenous cells* sometimes integrated, producing sympodial clusters of pronounced denticles at their distal ends. *Conidia* usually smooth, occasionally minutely verruculose, light brown, aseptate, usually oblong ellipsoidal to fusiform, length : width ratio = 1.9–2.4; (4.5–)5.5–7.5(–10) × (2–)2.5–3(–3.5) µm [av. (± SD) 6.7 (± 1.3) × 2.9 (± 0.4)]; *secondary ramoconidia* cylindrical, 0–1(–2)-septate, (7.5–)9.5–13.5(–19) × (2.5–)2.5–3.5(–4.5) µm [av. (± SD) 12.1 (± 3.3) × 3.2 (± 0.6)], with up to 5 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.7–1.8 µm diam.

Cultural characteristics: Colonies on PDA reaching 10–27 mm diam, granular, olive (2E4) due to profuse sporulation, with white undulate margin. Aerial mycelium absent. Colonies either heaped or radially furrowed, in the marginal area growing deeply into the agar. Reverse dark brown to dark green. Colonies on OA reaching 7–20 mm diam, olive (3E6), of granular appearance due to profuse sporulation, aerial mycelium present. Margin either undulate or arachnoid, deeply furrowed. Reverse pale brown to dark green. Colonies on MEA reaching 8–19 mm diam, velvety, reseda-green (2E6), heaped. Margin furrowed, growing deeply into the agar. Colonies on MEA with 5 % NaCl growing much faster than on other media, reaching 25–38 mm diam, of different colours, mostly reseda-green (2E6) and granulate due to profuse sporulation, margin olive-yellow (2D6). Reverse yellow to dark green.

Maximum tolerated salt concentration: MEA + 17 % NaCl after 14 d.

Cardinal temperatures: No growth at 4 °C, optimum and maximum temperature at 25 °C (8–19 mm diam), no growth at 30 °C.

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. S. Sonjak, Feb. 1999, CBS H-19731, **holotype**, culture ex-type EXF-335 = CBS 119413.

Habitats and distribution: Hypersaline water in the Mediterranean basin.

Differential parameters: Sympodial conidiogenous cells with pronounced denticles, narrow temperature amplitude.

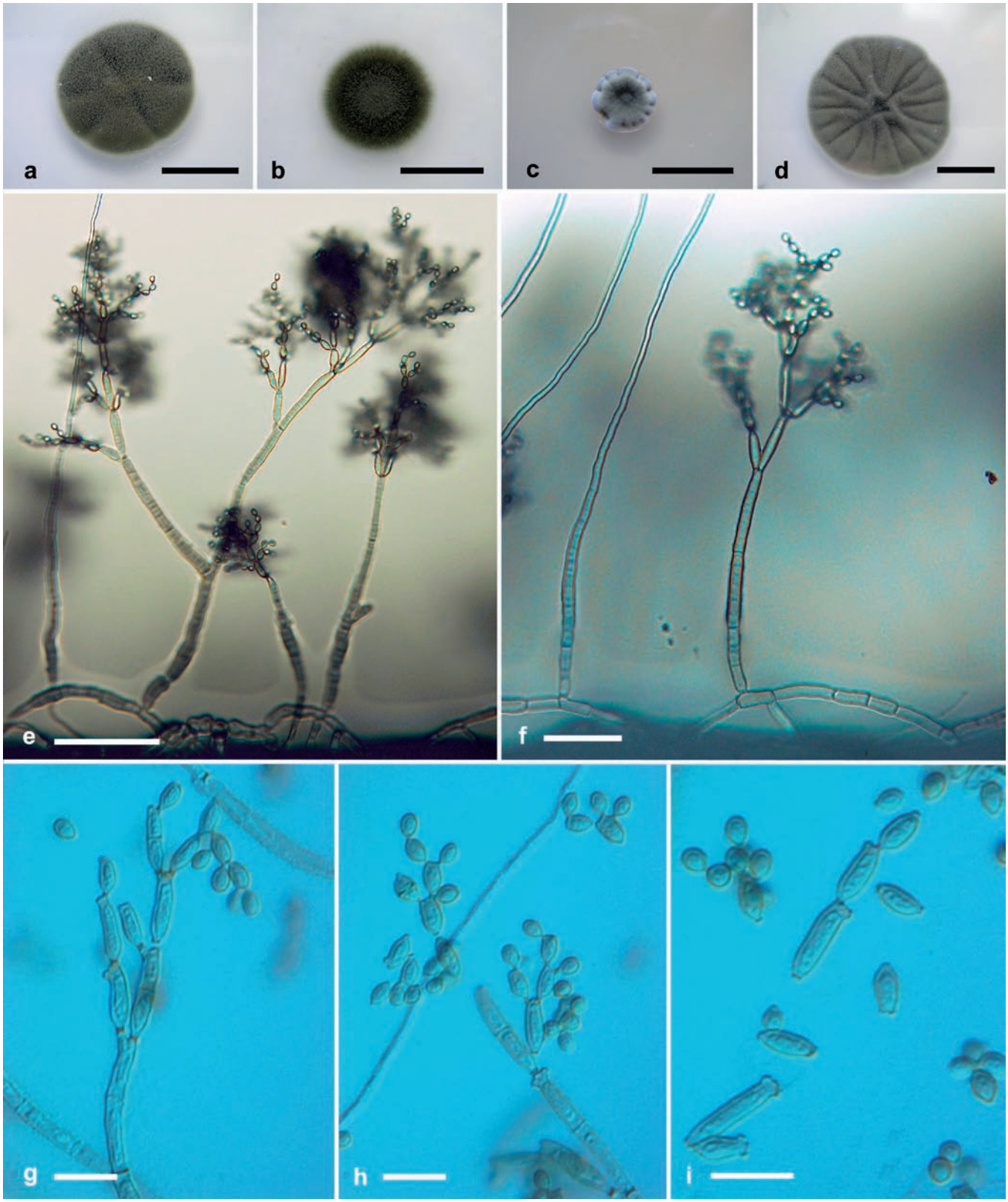

Fig. 10. *Cladosporium psychrotolerans*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Conidiophores. G. Apical part of a conidiophore. H–I. Secondary ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. All but C, from EXF-391 (ex-type strain); C, from EXF-714. Scale bars A–D = 10 mm, E = 100 µm, F = 50 µm, G–I = 10 µm.

Strains examined: EXF-322, EXF-335 (= CBS 119413; ex-type strain), EXF-604.

Notes: *Cladosporium salinae* morphologically resembles species of the genus *Fusicladium* because its conidia are oblong ellipsoidal to fusiform and conidiogenous loci of ramoconidia are placed closely together. As any other *Cladosporium* species, its conidia show typical cladosporioid scar structures, however. *Cladosporium salinae* seems to have a separate position within the genus *Cladosporium* since it seems to be distantly related to any other described *Cladosporium* species or currently known species complex within *Cladosporium*.

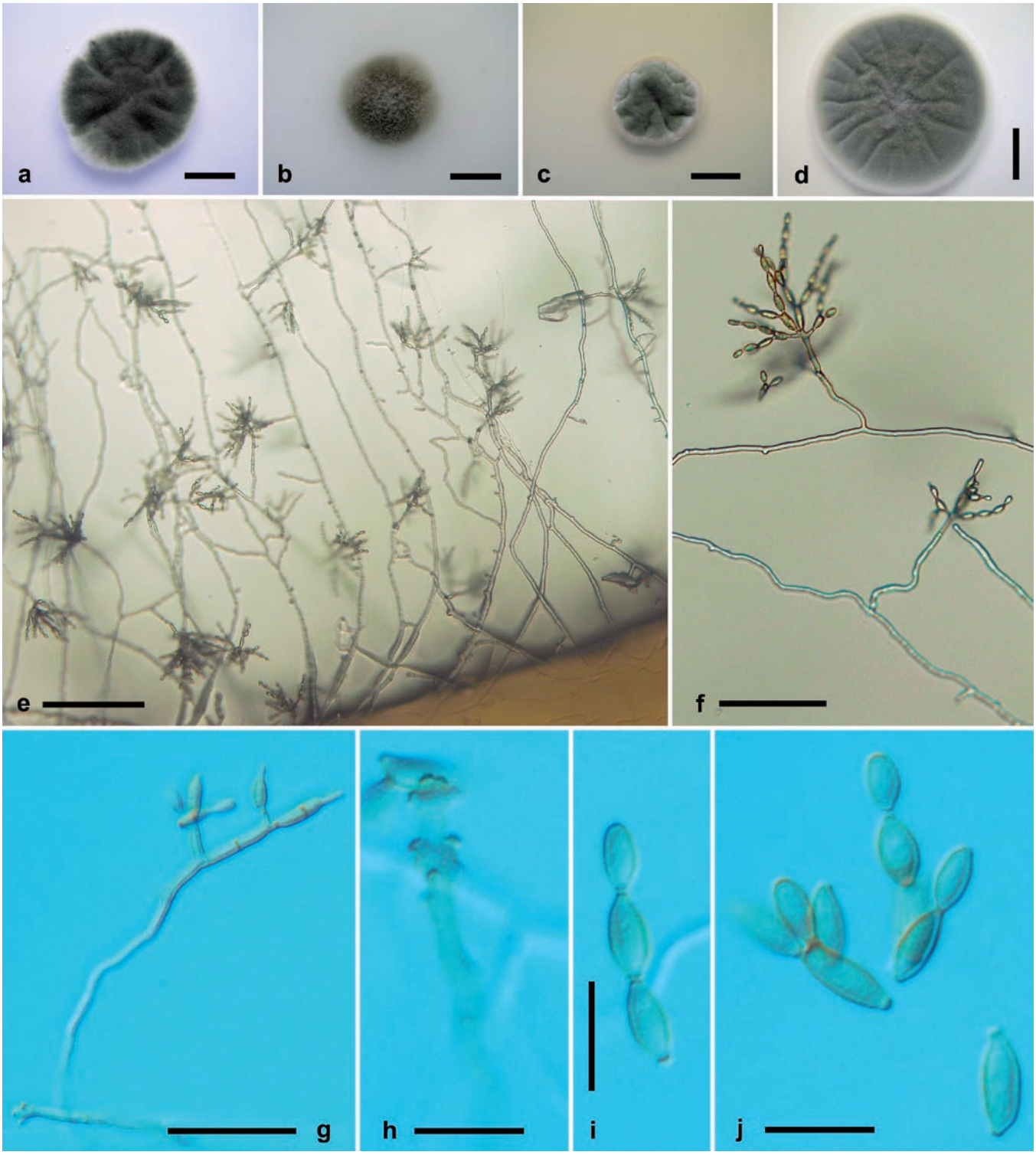

Fig. 11. *Cladosporium salinae*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G. Conidiophore. H. Detail of apical part of conidiophore. I. Conidia. J. Secondary ramoconidia and conidia. E–J. All from 7-d-old SNA slide cultures. A–D, from EXF-604; E–J, from EXF-335 (ex-type strain). Scale bars A–C = 5 mm, D = 10 mm, E = 100 µm, F = 50 µm, G = 30 µm, H–J = 10 µm.

Cladosporium sphaerospermum Penzig, Michelia 2(8): 473. 1882. Fig. 12.

Mycelium partly submerged, partly superficial; hyphae thick, darkly pigmented and densely septate in submerged mycelium, not enveloped in polysaccharide-like material. *Conidiophores* erect or ascending, micronematous and macronematous, stipes of variable length, (10–)45–130(–300) × (2.5–)3–4(–6) µm, olivaceous-brown, smooth to minutely verruculose, thick-walled, with relatively dense septation (cells mostly 4.5–23 long), septa darkened and somewhat thickened, arising laterally and terminally from immersed or aerial hyphae, either unbranched or branched. *Conidial chains* branching in all directions, up to 6 conidia in the unbranched parts. *Conidiogenous cells* not differentiated. *Ramoconidia* often formed, cylindrical, (11.5–)20.5–40(–48) × (2.5–)3(–3.5) µm, with up to 5 septa, base broadly truncate, 2 µm wide, slightly thickened and somewhat darkened-refractive. *Conidia* verruculose, brown to dark brown, non-septate, usually subspherical to spherical, less often short-ovoid, narrower at both ends, with length : width ratio = 1.1–1.5; conidial size (2.5–)3–4(–7) × (2–)3–3.5(–4.5) µm [av. (±

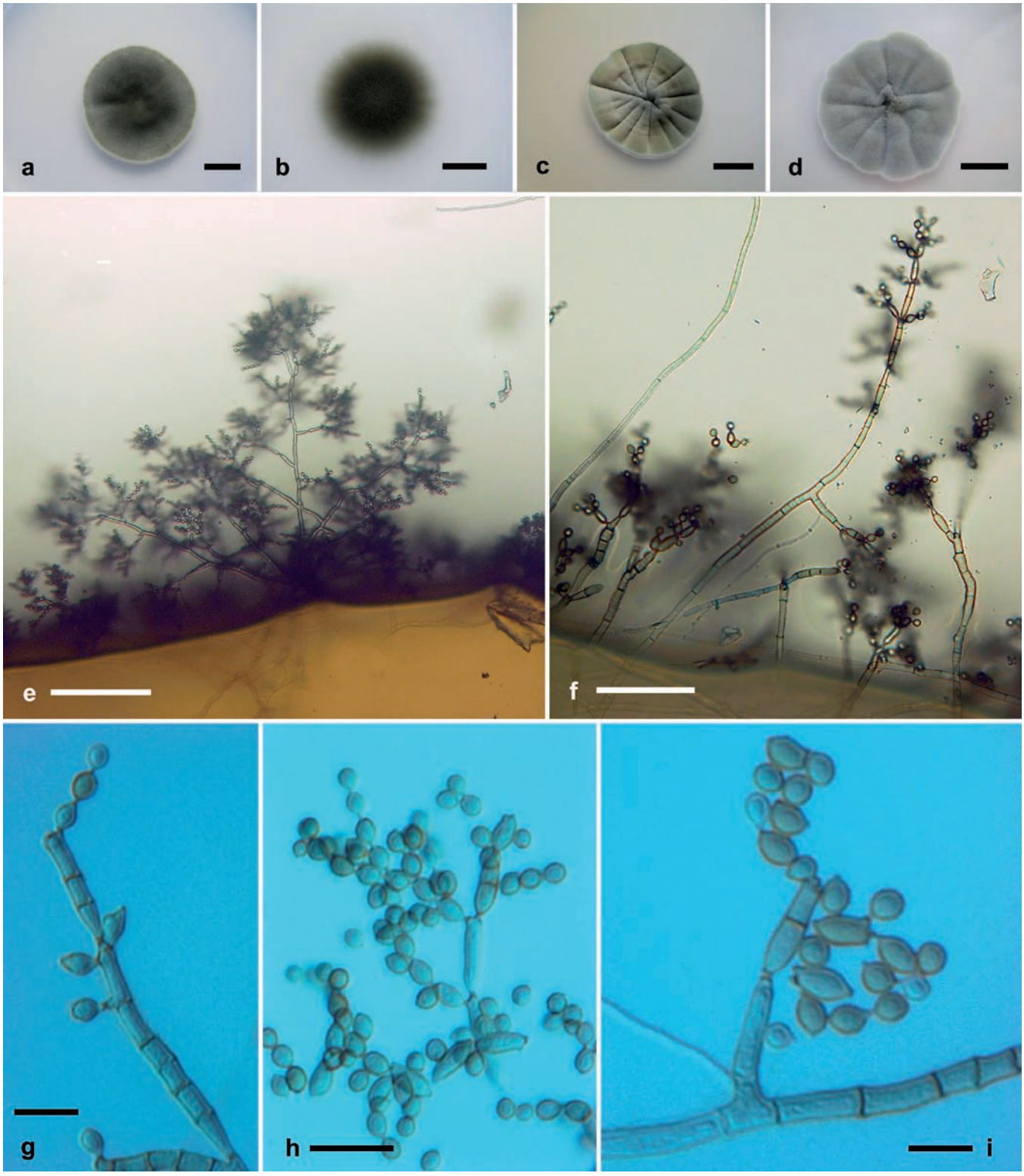

Fig. 12. *Cladosporium sphaerospermum*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G–I. Ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A, C–D, F–H, from CBS 193.54 (ex-neotype strain); B, from EXF-738; E, EXF-455; I, EXF-458. Scale bars A–D = 10 mm; E = 100 µm; F = 50 µm; G–I = 10 µm.

SD) 3.8 (± 0.8) × 3.1 (± 0.4)]; *secondary ramoconidia* cylindrical to almost spherical, 0–3(–4) septate, (4–)8.5–16(–37.5) × (2–)3–3.5(–5) µm [av. (± SD) 13.1 (± 6.3) × 3.2 (± 0.5)], with up to 4, rarely up to 6 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.9–1.1(–1.4) µm diam.

Cultural characteristics: Colonies on PDA reaching 21–44 mm diam, velvety, olive (2F5) due to profuse sporulation, either with white and regular, or exceptionally undulate margin. Aerial mycelium sparse. Colonies flat or rarely radially furrowed with elevated colony centre. Exudates not prominent, some strains release green soluble pigments into the agar. Reverse blackish blue to pale green. Growth deep into the agar. Colonies on OA reaching 21–38 mm diam, olive (2F8), of granular appearance due to profuse and uniform sporulation, almost no aerial mycelium. Margin either regular or arachnoid, deeply radially furrowed. Reverse black. Colonies on MEA reaching 15–35 mm diam, velvety, linden-green (2C5), radially furrowed. Colony centre wrinkled, forming a crater-like structure; margin furrowed, lighter in colour, consisting of

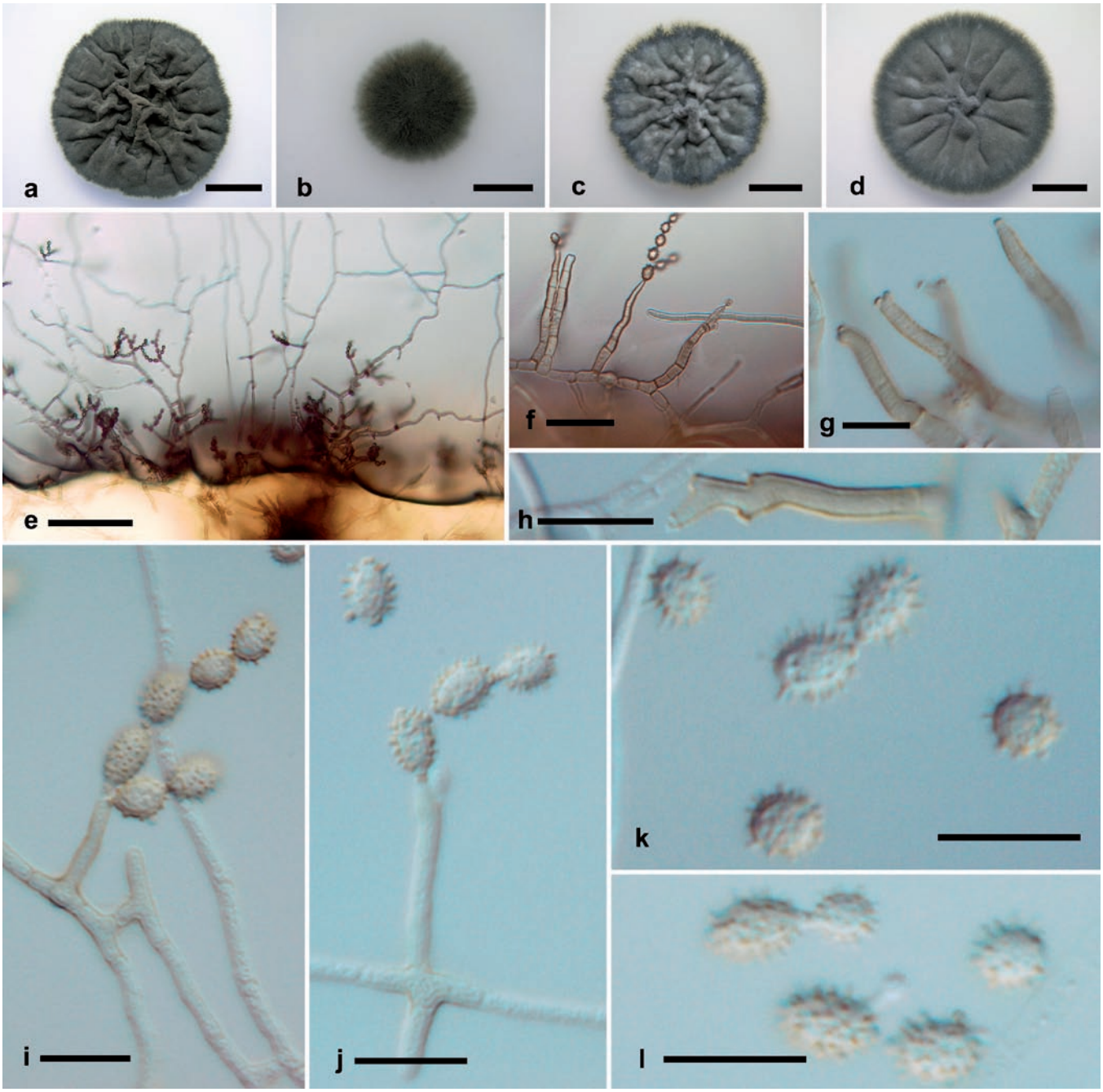

Fig. 13. *Cladosporium spinulosum*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E. Habit of conidiophores. F–J. Conidiophores. K–L. Conidia (also visible in I–J). E–L. All from 7-d-old SNA slide cultures. A–L, from EXF-334 (ex-type strain). Scale bars A–D = 10 mm, E = 100 µm, F = 30 µm, G–L = 10 µm.

submerged mycelium. Reverse pale to dark brown. Colonies on MEA with 5 % NaCl growing faster than on other media, reaching 31–60 mm diam, mainly olive (2D4), either being almost flat or radially furrowed, with margin of superficial mycelium; sporulation dense. Reverse ochraceous or dark green.

Maximum tolerated salt concentration: On MEA + 20 % NaCl 89 % of all strains tested develops colonies after 7 d, 96 % after 14 d.

Cardinal temperatures: No growth at 4 °C, optimum 25 °C (15–35 mm diam), maximum 30 °C (2–15 mm diam). No growth at 37 °C.

Specimen examined: **Netherlands**, from nail of man, 1949, coll. and isol. R.W. Zappey, CBS H-19738, **neotype designated here,** incorrectly selected by de Vries (1952) as "lectotype", culture ex-neotype CBS 193.54 = ATCC 11289 = IMI 049637.

Habitats and distribution: Hypersaline water in mediterranean and tropics; soil and plants in temperate climates; indoor wet cells; humans. The species does not seem to have any particular preference. Human isolates were probably culture contaminants.

Literature: Penzig (1882), de Vries (1952), Ellis (1971), de Hoog *et al.* (2000), Samson *et al.* (2002).

Diagnostic parameters: Thick-walled, melanised, densely septate mycelium, almost spherical, verruculose to verrucose terminal conidia, growth on 20 % NaCl after 7 d.

Strains examined: CBS 109.14, CBS 122.63, CBS 190.54, CBS 192.54, CBS 193.54 (ex-neotype strain), CBS 102045, CPC 10944, EXF-131, EXF-328, EXF-385, EXF-446, EXF-455, EXF-458, EXF-461, EXF-464, EXF-465, EXF-598, EXF-644, EXF-645, EXF-649, EXF-715, EXF-738, EXF-739, EXF-781, EXF-962, EXF-965, EXF-1061, EXF-1726, EXF-1732.

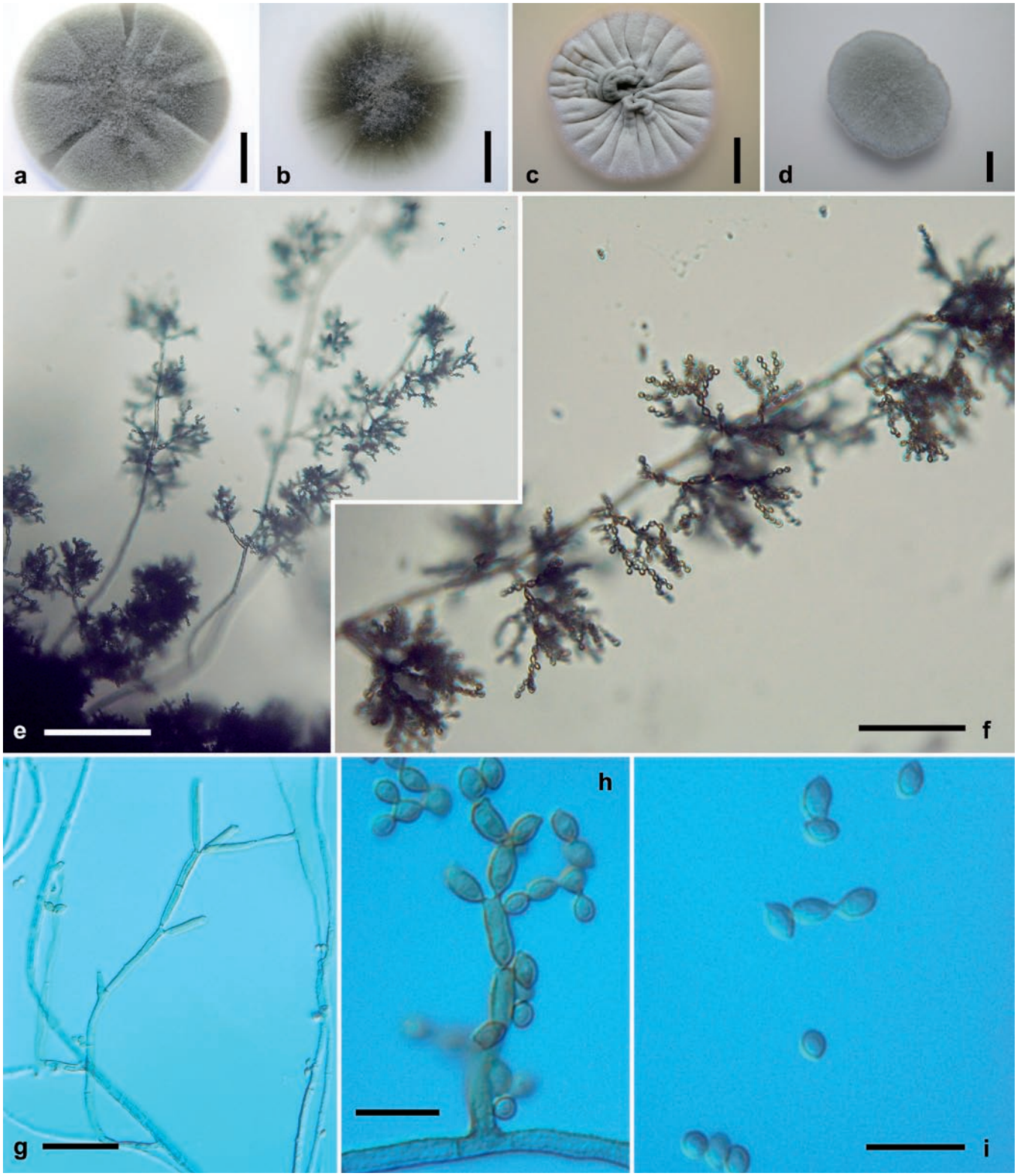

Fig. 14. *Cladosporium velox*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G. Conidiophore. H–I. Secondary ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A–D, G, from CBS 119417 (ex-type strain); E–F, H–I, from EXF-466. Scale bars A–D = 10 mm, E = 100 µm, F = 50 µm, G = 30 µm, H–I = 10 µm.

Cladosporium spinulosum Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB501099. Fig. 13.

Etymology: Refers to its conspicuously digitate conidia.

Conidiophora erecta vel adscendentia; stipites longitudine variabili, (15–)25–50(–155) × (2.5–)3–4(–5) µm, olivaceo-brunneus, levis, crassitunicatus, 0–6(–9)-septatus (cellulis 6–20 µm longis), ex hyphis submersis vel aeriis lateraliter vel terminaliter oriundus, simplex vel ramosus. Conidiorum catenae undique ramosae, ad 4 conidiis in partibus linearibus continuis cohaerentibus. Cellulae conidiogenae integratae vel discretae, acervos distales denticulorum conspicuorum sympodialium proferentes. Conidia echinulata vel digitata, brunnea vel fusca, continua, vulgo subglobosa vel globosa, (4.5–)5.5–7(–8) × (3–)4–4.5(–5) µm, long.: lat. = 1.1–1.6, digiti 0.6–1.3 µm longi; ramoconidia secundaria etiam digitata, cylindrica vel subglobosa, 0(–1)-septata, (6–)6.5–8(–18) × (4–)4.5–5(–5.5) µm, 1–3 cicatrices distales ferentia. Cicatrices inspissatae, conspicuae, protuberantes, 0.8–1.2 µm diam. Hyphae nonnumquam polysaccharido circumdatae.

Hyphae sometimes enveloped in polysaccharide-like material. *Conidiophores* erect or ascending, stipes of variable length, (15–)

25–50(–155) × (2.5–)3–4(–5) µm, olivaceous-brown, smooth, sometimes irregularly rough-walled to verrucose near the base, thick-walled, 0–6(–9)-septate (cells mostly 6–20 µm long), arising laterally and terminally from immersed or aerial hyphae, either unbranched or branched, somewhat tapering towards the apex. *Conidial chains* branching in all directions, up to 4 conidia in the unbranched parts. *Conidiogenous cells* sometimes integrated, producing sympodial clusters of pronounced denticles at their distal ends. *Ramoconidia* rarely formed. *Conidial wall ornamentation* conspicuously digitate, with up to 1.3 µm long projections having parallel sides and blunt ends. *Conidia* brown to dark brown, aseptate, usually subspherical to spherical, length : width ratio = 1.1–1.6; conidial size (4.5–)5.5–7(–8) × (3–)4–4.5(–5) µm [av. (± SD) 6.2 (± 1.0) × 4.2 (± 0.5)]; *secondary ramoconidia* ornamented as conidia, cylindrical to almost spherical, 0(–1)-septate, (6–)6.5–8(–18) × (4–)4.5–5(–5.5) µm [av. (± SD) 8.6 (± 4.0) × 4.8 (± 0.4)], with up to 3 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.8–1.2 µm diam.

Cultural characteristics: Colonies on PDA reaching 20–30 mm diam, velvety, dull green (29E4) to dark green (29F6) due to profuse sporulation, either with white and regular, or undulate margin. Aerial mycelium sparse. Colonies flat or radially furrowed with elevated colony centre. Growth deep into the agar. Exudates not prominent. Colonies on OA reaching 20–25 mm diam, dull green (29E4) to dark green (29F6), sometimes olive (3D4), of granular appearance due to profuse and uniform sporulation; almost without aerial mycelium. Margin arachnoid. Reverse pale brown to black. Colonies on MEA reaching 17–28 mm diam, velvety, dull green (29E4) to dark green (29F6), either flat or radially furrowed. Colony centre wrinkled, forming a crater-like structure; margin furrowed, paler in colour, consisting of submerged mycelium only. Reverse pale to dark green. Colonies on MEA with 5 % NaCl reaching 12–18 mm diam, of different colours, greenish grey (29D2), greyish green (29D5) to dark green (29F6); colony appearance variable, mostly either being almost flat with immersed colony centre or radially furrowed, with white to dark green margin consisting of superficial mycelium; sporulation dense. Reverse pale to dark green.

Maximum tolerated salt concentration: On MEA + 17 % NaCl, two of three strains tested developed colonies after 14 d.

Cardinal temperatures: Growth at 4 °C, optimum and maximum at 25 °C (17–28 mm). No growth at 30 °C.

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. S. Sonjak, Feb. 1999, CBS H-19796, **holotype**, culture ex-type EXF-334 = CBS 119907.

Habitats and distribution: Hypersaline water in temperate climate.

Diagnostic parameters: Conidia and ramoconidia with a digitate ornamentation.

Strains examined: EXF-334 (= CBS 119907; ex-type strain), EXF-382.

Notes: *Cladosporium spinulosum* is a member of the *C. herbarum* species complex (Figs 2–4) although its globoid conidia are reminiscent of *C. sphaerospermum*. Within *Cladosporium*, the species is unique in having conspicuously digitate conidia and ramoconidia. The two strains are differing in the size of conidia. The average size of conidia in EXF-334 is 6.2 (± 0.9) × 4.2 (± 0.5) µm, and in EXF-382 it is 3.9 (± 0.6) × 3.3 (± 0.4) µm.

Cladosporium velox Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB492435. Fig. 14.

Etymology: Refers to the quick growth of strains of this species.

Mycelium partim submersum; hyphae vagina polysaccharidica carentes. Conidiophora erecta, lateralia vel terminalia ex hyphis aeriis oriunda; stipes (10–)25–150(–250) × (2.5–)3–4(–4.5) µm, olivaceo-brunneus, levis, crassitunicatus, ad 7–septatus (cellulis 10–60 µm longis), identidem dichotome ramosus. Conidiorum catenae undique divergentes, terminales partes simplices ad 5 conidia continentes. Cellulae conidiogenae indistinctae. Conidia levia vel leniter verruculosa, dilute brunnea, unicellularia, ovoidea, (2–)3–4(–5.5) × (1.5–)2–2.5(–3) µm, long. : lat. 1.4–1.7; ramoconidia secundaria cylindrica, 0–1-septata, (3.5–)5.5–19(–42) × (2–)2.5–3(–4.5) µm, ad 4(–5) cicatrices terminales ferentia; cicatrices inspissatae, protuberantes, conspicuae, 0.5–1.5 µm diam.

Mycelium partly superficial partly submerged; hyphae without extracellular polysaccharide-like material. *Conidiophores* erect, stipes (10–)25–150(–250) × (2.5–)3–4(–4.5) µm, slightly attenuated towards the apex, olivaceous-brown, smooth- and thick-walled, arising terminally and laterally from aerial hyphae, dichotomously branched [up to 5(–7)-septate, cell length 10–60 µm]. *Ramoconidia* rarely formed. *Conidial chains* branching in all directions, terminal chains with up to 5 conidia. *Conidia* smooth to very finely verruculose, pale brown, non-septate, ovoid, length : width ratio = 1.4–1.7; (2–)3–4(–5.5) × (1.5–)2–2.5(–3) µm [av. (± SD) 3.6 (± 0.6) × 2.3 (± 0.2)]; *secondary ramoconidia* cylindrical, 0–1-septate, (3.5–)5.5–19(–42) × (2–)2.5–3(–4.5) µm [av. (± SD) 13.4 (± 10.2) × 2.8 (± 0.5)], with up to 4(–5) distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.5–1.5 µm diam.

Cultural characteristics: Colonies on PDA reaching 35–45 mm diam, velvety, dark green due to profuse sporulation, on some parts covered with white sterile mycelium, flat with straight white margin. Reverse dark green to black. Colonies on OA reaching 30–43 mm diam, dark green, mycelium submerged, aerial mycelium sparse. Margin regular. Reverse black. Colonies on MEA reaching 30–42 mm diam, pale green, radially furrowed, with raised, crater-shaped central part, with white, undulate, submerged margin. Sporulation poor. Colonies on MEA with 5 % NaCl reaching 35–45 mm diam, pale green, velvety, flat with regular margin. Reverse pale green. Sporulation poor.

Maximum tolerated salt concentration: 20 % NaCl after 14 d.

Cardinal temperatures: Minimum at 10 °C (9 mm diam), optimum at 25 °C (30–42 mm diam) and maximum at 30 °C (5–18 mm diam).

Specimen examined: **India**, Charidij, isolated from *Bambusa* sp., W. Gams, CBS H-19735, **holotype**, culture ex-type CBS 119417.

Habitats and distribution: Hypersaline water in Slovenia; bamboo, India.

Strains examined: CBS 119417 (ex-type strain), EXF-466, EXF-471.

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