Mycol Progress (2010) 9:69–78
DOI 10.1007/s11557-009-0620-6

ORIGINAL ARTICLE

Two new species of *Leptographium* from *Dryocetes authographus* and *Hylastes cunicularius* in Norway

Karin Jacobs · Paal Krokene · Halvor Solheim · Michael J. Wingfield

Received: 28 April 2009 / Revised: 21 August 2009 / Accepted: 24 August 2009 / Published online: 13 September 2009

Abstract The anamorph genus *Leptographium* Lagerberg and Melin includes species that are typically bark beetle-associated fungi, with teleomorphs in *Grosmannia*. During a survey of ophiostomatoid fungi in Norway, two unusual species, that fit the broader morphological description of *Leptographium*, were isolated directly from the root-feeding beetles, *Dryocetes authographus* and *Hylastes cunicularius*, as well as from roots infested by these insects. The first of these could be distinguished from other described species based on a sparse sporulation, black spore drops and chlamydospores in older cultures. This species also produces a *Hyalorhinocladiella* synanamorph. The second species was characterised by distinctly curved conidia. Based on these unusual morphological characteristics and distinct DNA sequences, these fungi were recognised as new taxa for which the names *Leptographium chlamydatum* sp. nov. and *L. curvisporum* sp. nov. are provided.

Keywords Bark beetle-associated fungi · *Dryocetes authographus* · *Hylastes cunicularius* · *Leptographium chlamydatum* · *Leptographium curvisporum*

K. Jacobs (✉)
Department of Microbiology, University of Stellenbosch,
Stellenbosch 7600, South Africa
e-mail: kj@sun.ac.za

P. Krokene · H. Solheim
Norwegian Forest and Landscape Institute,
P.O. Box 115, 1431 Ås, Norway

M. J. Wingfield
Forestry and Agricultural Biotechnology Institute,
University of Pretoria,
Pretoria 0002, South Africa

Introduction

The genus *Leptographium* was described in 1927 for fungi causing blue-stain on timber in Europe (Lagerberg et al. 1927). The taxonomic history of the genus was confused for many years due to segregate genera being established for groups with differing modes of conidium development (Kendrick 1961, 1962; Wingfield 1985). However, studies including those based on DNA sequence comparisons during the past two decades have led to a group that is phylogenetically and ecologically clearly defined (Jacobs et al. 2001, 2004, 2005, 2006; Kim et al. 2004, 2005a; Lee et al. 2005). Thus, *Leptographium* species are generally characterised by dematiaceous, erect conidiophores terminating in penicillate branches giving rise to conidiogenous cells bearing single-celled conidia that accumulate in mucilaginous masses (Kendrick 1962; Jacobs and Wingfield 2001). These fungi are anamorphs of *Grosmannia*, a segregate of *Ophiostoma sensu lato* (Zipfel et al. 2006). Species of *Grosmannia* are typified by ascomata with globose bases, necks of variable length and cucullate ascospores produced in slimy masses (Jacobs and Wingfield 2001; Zipfel et al. 2006).

Leptographium species in Europe are mainly known from conifers where they exist in close association with insects, particularly bark beetles (Coleoptera: Curculionidae: Scolytinae), which may act as their primary vectors (Solheim and Långström 1991; Jacobs and Wingfield 2001). These include species such as *L. wingfieldii* M. Morlet, *L. lundbergii* Lagerb. & Melin and *L. penicillatum* Grosmann (Lagerberg et al. 1927; Grosmann 1931; Morelet 1988; Jacobs et al. 2005). *L. francke-grosmanniae* K. Jacobs & M.J. Wingf. is the only species known to occur specifically on hard woods in Europe (Davidson 1971).

A survey of ophiostomatoid fungi associated with the bark beetles *Dryocetes autographus* (Ratz.) and *Hylastes*

cunicularius E. in Norway led to the isolation of two unusual *Leptographium* species. The objective of this study was to compare the strains from Norway with described species of *Leptographium*. This was achieved using DNA sequence and morphological comparisons.

Materials and methods

Isolations and isolates

Strains treated in this study were isolated from Norway spruce (*Picea abies*) roots infested by intermingling *Dryocetes autographus* and *Hylastes cunicularius* beetles, as well as directly from the beetles. Roots of Norway spruce were examined for stained areas. Small pieces of the stained tissue were removed with a sterile scalpel and surfaces disinfected in commercial bleach, EtOH and dH_2O (10:10:80 v/v). The surface-disinfected tissue pieces were then cut in half and placed on 2% Malt Extract Agar (MEA) (20 g Biolab malt extract, 20 g agar, 1,000 ml distilled water), containing cycloheximide and streptomycin sulfate, incubated at 22°C and later examined for fungal growth. Isolations directly from bark beetles were done by rinsing each beetle (53 *H. cunicularius* and 20 *D. autographus*) in sterilised water and crushing them onto the surface of the same medium as above. *Leptographium* strains were identified under a dissection microscope and spore drops were transferred with a sterile needle to 2% MEA and incubated at 22°C.

Strains used in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (Table 1). Duplicates are also housed in the collection of the Norwegian Forest and Landscape Institute (NFLI), and representative isolates of taxonomically important strains have been deposited with the Centraalbureau voor Schimmelcultures (CBS), Netherlands and the herbarium of the ARC Plant Protection Research Institute (PREM) in Pretoria, South Africa.

Phylogenetic analyses

For phylogenetic comparisons, eight strains representing two *Leptographium* spp. that appeared different to the known *Leptographium* species, were grown on commercially available potato dextrose agar (PDA; Biolab, Johannesburg, South Africa) for 10 days at 25°C. Mycelium was scraped from the colonies of pure cultures and DNA was extracted using the method of Möller et al. (1992) modified by Jacobs et al. (2004). The presence of DNA was confirmed under UV-light on a 1% agarose gel stained with ethidium bromide.

Gene regions used for the comparisons included the internal transcribed spacer (ITS2), part of the large subunit (28S) of the rDNA operon, β-tubulin and elongation factor 1-α (EF 1-α) genes as previously described by Jacobs et al. (2004). PCR reactions were performed in 25 µl volumes (2.5 mM $MgCl_2$, 1× PCR buffer, 0.2 mM dNTP, 0.2 mM of each primer and 2.5 U *Taq*-polymerase enzyme). Primers used in the amplification reactions and for cycle sequencing were ITS3 and LR3 (White et al. 1990) for the ITS2 and 28S region, Bt2a and Bt2b (Glass and Donaldson 1995) for the β-tubulin gene and EF1F and EF2R for the elongation factor 1-α gene (Jacobs et al. 2004). PCR products were purified using the Qiaquick PCR purification kit (Qiagen) and sequenced using the Big Dye terminator cycle sequencing premix kit (Applied Biosystems) on an ABI PRISM 3100 automatic sequencer (Perkin Elmer Applied Biosystems). Sequence contigs were assembled using Sequence Navigator (Applied Biosystems), aligned in ClustalX (Thompson et al. 1997) and manually adjusted in Se-Al (Rambaut 2007).

Phylogenetic relationships for the taxa were inferred using distance analysis in PAUP* v.4.0b10 (Swofford 2001). Characters were treated as unweighted in the analysis and gaps were treated as missing data. A single tree for each dataset was obtained using neighbour-joining analysis with an uncorrected P-distance and rooted to midpoint. A bootstrap analysis (1,000 replicates using the neighbour-joining option) was performed to determine the confidence levels of the nodes. For all the datasets, ambiguously aligned regions were replaced by codes. Step matrices to assign different weights to these codes were computed using INAASE 2.3b (Lutzoni et al. 2000). Both a partition homogeneity test (Farris et al. 1995) and a Templeton Nonparametric Wilcoxon Signed Ranked Test (Kellogg et al. 1996) was performed to determine whether the three datasets could be combined.

Morphological comparisons

All measurements and microscopic observations were made from 14-day-old fungal structures grown on 2% MEA and oatmeal agar (OA) (30 g Jungle Oats; Tiger Consumer Brands, South Africa) (Gams et al. 1998) and incubated at 25°C. Fungal structures were mounted on slides in 85% lactic acid and examined using phase or differential interference contrast microscopy. Fifty measurements were made for each morphological character and averages and standard deviations were computed.

Results

Isolates and phylogenetic analyses

Eight isolates representing two distinct morphological groups were collected from roots infested by *Dryocetes*

Table 1 Strains used in DNA-based comparisons and GenBank accession nos

Species	Strain no.	ITS	β-tubulin	Elongation 1-α
L. truncatum	CMW 30	DQ062054	DQ061988	DQ062021
	CMW 28	DQ062052[a]	DQ061986[b]	DQ062019[c]
L. lundbergii	CMW 217	DQ062065[a]	DQ061999[b]	DQ062032[c]
	CMW 17264	DQ062068[a]	DQ062002[b]	DQ062035[c]
L. douglasii	CMW725	AY553380[a]	AY534928[b]	AY536174[c]
	CMW2078	AY553381[a]	AY534929[b]	AY536175[c]
L. neomexicanum	CMW2079	AY553382[a]	AY534930[b]	AY536176[c]
L. reconditum	CMW15	AY553383[a]	AY534931[b]	AY536177[c]
L. serpens	CMW193	AY553387[a]	AY534935[b]	AY536181[c]
	CMW60	AY553388[a]	AY534936[b]	AY536182[c]
L. aenigmaticum	CMW2199	AY553389[a]	AY534937[b]	AY536183[c]
	CMW2310	AY553390[a]	AY534938[b]	AY536184[c]
O. robustum	CMW2805	AY553396[a]	AY534944[b]	AY536190[c]
	CMW668	AY553397[a]	AY534945[b]	AY536191[c]
L. aureum	CMW709	AY553413[a]	AY534961[b]	AY536207[c]
	CMW714	DQ062071[a]	DQ062005 [b]	DQ062038[c]
L. pyrinum	CMW509	AY553414[a]	AY534962[b]	AY536208[c]
	CMW169	DQ062072[a]	DQ062006[b]	DQ062039[c]
L. yunnanensis	CMW5304	AY553415[a]	AY534963[b]	AY536209[c]
	CMW5152	DQ062073[a]	DQ062007[b]	DQ062040[c]
L. wingfieldii	CMW2095	AY553400[a]	AY534948[b]	AY536194[c]
	CMW 2096	AY553398[a]	AY534946[b]	AY536192[c]
	CMW2019	AY553399[a]	AY534947[b]	AY536193[c]
L. pineti	CMW3831	DQ062076[a]	DQ062010[b]	DQ062043[c]
	CMW3837	DQ062077[a]	DQ062011[b]	DQ062044[c]
L. americanum	CMW495	DQ062079[a]	DQ062013[b]	DQ062046[c]
	CMW2929	DQ062078[a]	DQ062012[b]	DQ062045[c]
L. abietinum	CMW2817	DQ062080[a]	DQ062014[b]	DQ062047[c]
	CMW3083	DQ062081[a]	DQ062015[b]	DQ062048[c]
L chlamydatum	CMW11592	EU979333[a]	EU979341[b]	EU979349[c]
	CMW11597	EU979334[a]	EU979342[b]	EU979350[c]
	CMW11623	EU979335[a]	EU979343[b]	EU979351[c]
L. curvisporum	CMW11608	EU979332[a]	EU979340[b]	EU979348[c]
	NFRI 95-593/1	EU979328[a]	EU979336[b]	EU979344[c]
	NFRI 95-593/2	EU979330[a]	EU979338[b]	EU979346[c]
	NFRI 95-593/3	EU979329[a]	EU979337[b]	EU979345[c]
	NFRI 95-593/4	EU979331[a]	EU979339[b]	EU979347[c]
L. laricis	CMW1980	DQ062074[a]	DQ062008[b]	DQ062041[c]
	CMW2014	DQ062075[a]	DQ062009[b]	DQ062042[c]

autographus and *Hylastes cunicularius,* or from the insects themselves although at low frequencies (≤5%). These isolates appeared to be morphologically distinct from all other species of *Leptographium*. Amplification of the ITS2 and 28S region, partial β-tubulin and partial EF 1-α genes resulted in fragments of approximately 700, 500 and 900 base pairs (bp), respectively. Results of both the partition homogeneity test (Farris et al. 1995) as well as a Templeton Nonparametric Wilcoxon Signed Ranked Test (Kellogg et al. 1996) showed that the three datasets could be combined.

The aligned combined dataset consisted of 1,992 characters. Twelve ambiguously aligned regions were identified (726 bp in total) and excluded from the analysis. These regions were replaced with weighted codes as calculated using INAASE 2.3b (Lutzoni et al. 2000). The tree topologies resulting from analysis of the three separate datasets as well as those of the combined dataset were similar (data not shown).

In all cases, strains from Norway spruce grouped in two clades, distinct from each other and from other species of

Leptographium. This was consistent with the fact that they are morphologically distinct from each other and apparently from other described species of *Leptographium*. The tree resulting from analysis of a combined dataset for all three gene regions showed that the strains represent two distinct taxa that are closely related to *L. abietinum* (Peck) M.J. Wingf. and *L. americanum* K. Jacobs & M.J. Wingf. (Fig. 1).

Morphology

The two unknown *Leptographium* spp. reflected by two distinct phylogenetic clades were morphologically distinct from each other as well as other *Leptographium* spp. (Table 2). Isolates from one of these clades could be distinguished from previously described species based on very sparse sporulation, black spore drops observed in older

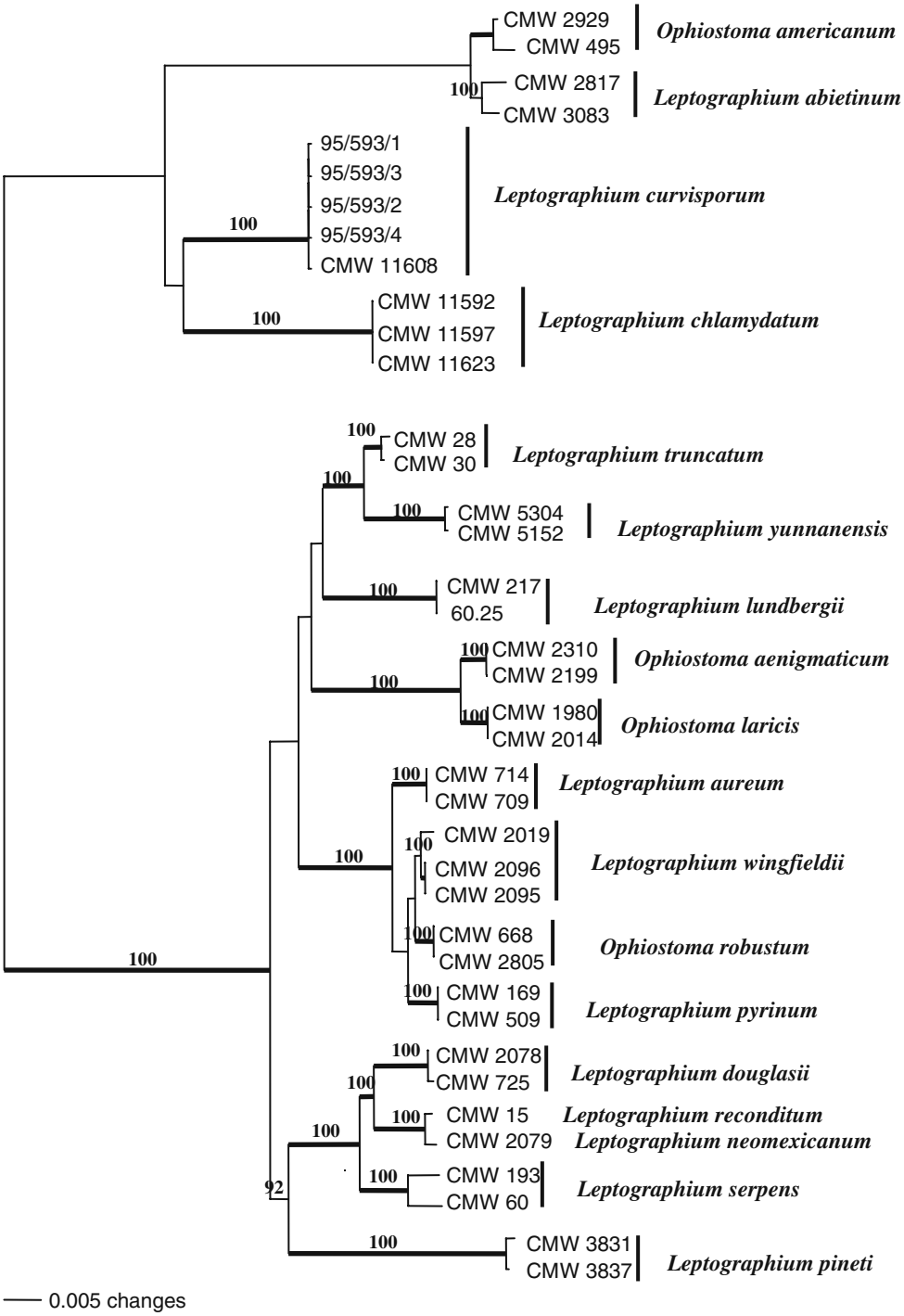

Fig. 1 Neighbour-joining tree derived from analysis of the combined dataset. Branches in *bold* have bootstrap values above 85

Table 2 Comparison of *L. chlamydatum* and *L. curvisporum* with closely related species based on morphology, ecology and geographic distribution

	L. abietinum	*L. americanum*	*L. chlamydatum*	*L. curvisporum*
Conidiophore length	74–535(–570) µm	(150–)210–455(–730) µm	(123–)191–303(–359) µm	(139–)179–349(–645) µm
Conidium shape	Distinctly curved at the base	Oblong to obovoid	Oblong to obovoid	Distinctly curved at the base
Conidium size	(3–)4–5(–7)1–2 µm	3–22×1–3 µm	2–5(–7)×1–2 µm	3–4(–5)×1–2 µm
Rhizoids	Absent	Absent	Absent	Occasionally present
Chlamydospores	Absent	Absent	Present	Absent
Teleomorph	Absent	Ophiostoma	Absent	Absent
Synanamorph	Absent	Absent	Hyalorhinocladiella	Absent
Host	*Picea mariana* *P. engelmannii* *Pseudotsuga menziesii* *Pinus contorta* *P. sylvestris* *P. ponderosa* *P. aristata* *P. mugo* *P. monticola*	*Larix decidua*	*Picea abies*	*Picea abies*
Insect associations	*Dendroctonus pseudotsugae* *D. rufipennis* *Hylastes longicollis* *Hylurgops porosus* *Hylurgops planirostris*	*Dendroctonus simplex*	*Hylastes cunicularius* *Dryocetes autographus*	*Hylastes cunicularius* *Dryocetes autographus*
Geographic distribution	Northwestern United States	Northeastern United States	Norway	Norway

cultures as well as the presence of chlamydospores. This species is also unusual in that it produces a distinct *Hyalorhinocladiella* synanamorph, a character usually observed in anamorphs of *Ophiostoma*. The other group of isolates had distinctly curved conidia. In this respect, it resembled *L. abietinum,* which is a common inhabitant of various conifers infested by bark beetles in North America (Jacobs and Wingfield 2001). It could, however, be distinguished from this species based both on morphological differences and DNA sequence comparisons.

Based primarily on phylogenetic differences but also morphological features and a distinct ecology, the two *Leptographium* spp. from Norway are described as follows.

Leptographium chlamydatum K. Jacobs, M.J. Wingf. & H. Solheim sp. nov. (Figs. 2 and 3)

MycoBank: 515125

Etymology The specific epithet refers to the chlamydospores in older cultures of this species.

Description Conidiophora singula e mycelio proxime orientia, macronemata, mononemata, (144–) 195–396 (–580) µm longa, sine structuris rhizoidiformibus. Apparatus conidiogenus (25–) 37–60 (–72) µm longus, massa conidiorum exclusa, ramis cylindricis multiseriatis. Conidia hyalina non septata, oblonga vel obovoidea basibus truncatis apicibus rotundatis 3–5 (–6) × 2–3 µm. Synanamorpha in firma Hyalorhinocladiellae (7–) 8–72 (–137) µm longa, 2–3 µm lata. Chlamydosporae in culturis vetustioribus videntur (5–) 9–16 (–20) µm diametro.

Conidiophores occurring singly arising directly from the mycelium, macronematous, mononematous, (144–) 195–396 (–580) µm in length, rhizoid-like structures absent (Figs. 2a, 3a). *Stipes* light olivaceous, cylindrical, simple, 6–24-septate, (119–) 150–344 (–529) µm long, apical cell not swollen, basal cell occasionally slightly swollen. *Conidiogenous apparatus* (25–) 37–60 (–72) µm long, excluding the conidial mass, with multiple series of cylindrical branches. *Primary branches*, 2–4, light olivaceous, smooth, cylindrical, aseptate, (9–) 10–15 (–17) µm long and 4–5 (–6) µm wide, arrangement of the primary branches on the stipe-type B (more than two branches) (Fig. 2b). *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (7–) 9–13 (–18) µm long and 1–3 µm wide. Conidium development according to van Wyk et al. (1988) (Fig. 2c). *Conidia*

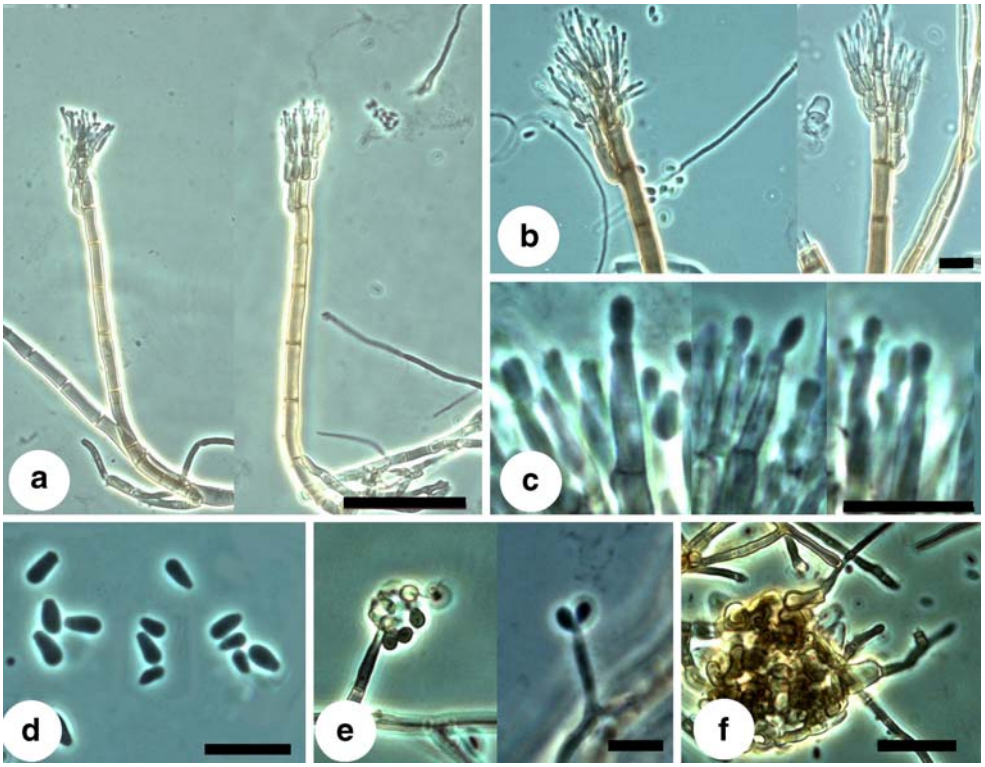

Fig. 2 Light micrographs of the morphological characters of *Leptographium chlamydatum* (CMW 11592). **a** Conidiophore morphology, *bar* 20 µm. **b** Conidiogenous apparatus with two primary branches. **c** Conidiogenous cells showing annelidic conidium development and delayed secession of conidia, *bar* 10 µm. **d** Obovoid conidia with almost tapered ends, *bar* 10 µm. **e** *Hyalorhinocladiella* synanamorph *bar* 10 µm. **f** Chlamydospore-like structures, *bar* 20 µm

hyaline, aseptate, oblong to obovoid with truncate bases and rounded apices, 3–5 (–6) × 2–3 µm (Figs. 2d and 3c). *Hyphae* submerged in agar with abundant aerial mycelium, smooth, serpentine, occasionally constricted at the septa, (2–) 3–5 (–8) µm wide. *Hyalorhinocladiella*-type synanamorph, (7–) 8–72 (–137) µm long, 2–3 µm wide (Figs. 2e and 3c). *Chlamydospores* present in older cultures, 2–4 weeks, (5–) 9–16 (–20) µm in diameter (Fig. 2f and 3d).

Specimens examined Norway, Ås, Akershus, all collected by H. Solheim and M.J. Wingfield, Aug. 1995; isolated from *Dryocetes autographus* PREM 60044 (CMW 11592; NFLI 95-593/82) (holotype); *additional specimens:* isolated from *Hylastes cunicularius* CMW 11623 (NFLI 95-593/131; CBS 123915); CMW 11597 (NFLI 95-593/132).

Leptographium curvisporum K. Jacobs, M.J. Wingf. & H. Solheim sp. nov. (Figs. 4 and 5)

MycoBank: 515126

Etymology The specific epithet refers to the distinctly curved conidia of this species.

Description Conidiophora singula e mycelio proxime orientia, macronemata, mononemata, (139–) 179–349 (–645) µm longa, aliquando cum structuris rhizoidiformibus. Apparatus conidiogenus (36–) 52–82 (–125) µm longus, massa conidiorum exclusa, ramis cylindricis multiseriatis. Conidia hyalina non septata, oblonga vel obovoidea basibus truncatis subcurvatis, apicibus rotundatis 3–4 (–5) × 1–2 µm.

Conidiophores occurring singly arising directly from the mycelium, macronematous, mononematous, (139–) 179–349 (–645) µm in length, rhizoid-like structures occasionally present. Stipes light olivaceous, cylindrical, simple, 4–8-septate, (76–) 118–278 (–545) µm long, apical cell not swollen, basal cell not swollen (Figs. 4a and 5a). *Conidiogenous apparatus* (36–) 52–82 (–125) µm long, excluding the conidial mass, with multiple series of cylindrical branches. *Primary branches*, 2–3, light olivaceous, smooth, cylindrical, aseptate, (9–) 11–16 (–21) µm long and (2–) 3–5 (–6) µm wide, arrangement of the primary branches on the stipe-type B (more than two branches) (Fig. 4b). *Conidiogenous cells* discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (10–) 11–19 (–25) µm long and 1–2 µm wide. Conidium development as described by van Wyk et al. (1988) (Fig. 4c). *Conidia* hyaline, aseptate, oblong to obovoid with truncate bases that are slightly curved and rounded apices, 3–4 (–5) × 1–2 µm (Figs. 4d and 5c).

Specimens examined Norway, Ås, Akershus, *Picea abies* infested with *Dryocetes autographus* and *Hylastes cunnicularius*, collected by H. Solheim and M.J. Wingfield, Aug.

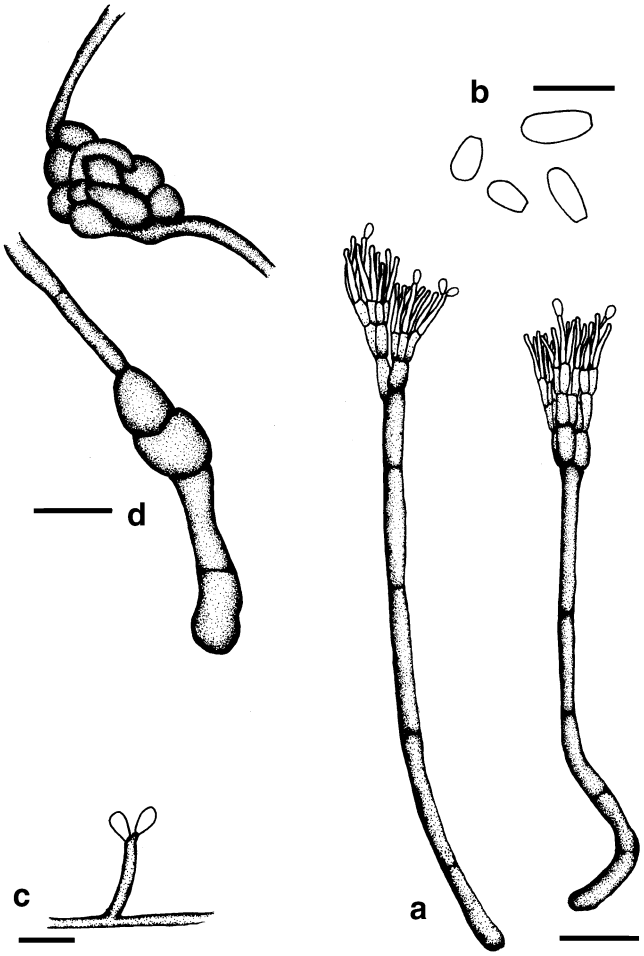

Fig. 3 Line drawings of the morphological characters of *Leptographium chlamydatum* (CMW 11592). **a** Conidiophore morphology, *bar* 10 μm. **b** Obovoid conidia with almost tapered ends, *bar* 10 μm. **c** *Hyalorhinocladiella* synanamorph of *L. chlamydatum*, *bar* 20 μm. **d** Chlamydospore-like structures, *bar*10 μm

1995, PREM 60035 (CMW 17260; NFLI95-593/1; CBS123914) (holotype). *Additional specimens:* Norway, Ås, Akershus, *Picea abies* infested with *Dryocetes autographus* and *Hylastes cunicularius*, collected by H. Solheim and M.J. Wingfield, Aug. 1995, CMW 17261 (NFLI 95-593/2); CMW 17262 (NFLI 95-593/3; CBS 124006); CMW 11608 (NFLI 95-593/4); CMW 17263 (PREM 60036; NFLI 95-593/5).

Discussion

Two new species of *Leptographium* emerged from this study. These fungi are potential associates of the root-feeding bark beetles *Dryocetes autographus* and *Hylastes cunicularius*. One of the fungi, *L. chlamydatum*, was isolated from the bark beetles, while *L. curvisporum* was isolated from diseased *P. abies* tissue infested by these beetles. These results are not unusual as many *Leptographium* spp. are associated with root-feeding bark beetles. For example, *L. wageneri* var. *pseudotsugae* T.C. Harr. & F.W. Cobb is commonly associated with *Hylastes nigrinus* Mann. in western North America (Witcosky et al. 1986; Harrington 1993), and *L. truncatum* (M.J. Wingf. & Marasas) M.J. Wingf. and *L. serpens* (Goid.) M.J. Wingf. are closely associated with *Hylastes angustatus* (Herbst) in South Africa, where the insect is an introduced exotic (Wingfield and Marasas 1980, 1981). Likewise, *L. huntii* M.J. Wingf. is commonly associated with the introduced *H. ater* (F.) in both Chile (Zhou et al. 2004) and New Zealand (Kim et al. 2005b).

Leptographium chlamydatum can easily be distinguished from other *Leptographium* spp. by the presence of clumps of thick-walled cells in older cultures, usually older than 2 weeks. These resemble the chlamydospores occasionally observed in *Phoma* spp. (Camyon and Gerhardson 1997). Chlamydospores are commonly found in a number of other fungal genera where they are believed to facilitate survival. Although various species of *Ceratocystis* produce well-developed and very obvious chlamydospores (Upadhyay 1981; Barnes et al. 2003; Johnson et al. 2005), this character has been observed only in one other species of *Leptographium, L. piriforme* Greif, Gibas & Currah (Greif et al. 2006).

Leptographium clamydatum also produces a very distinct *Hyalorhinocladiella* synanamorph in culture and these structures can aid in identifying the fungus. Production of synanamorphs in *Leptographium* spp. is not common but is also not without precedent. For example, *L. elegans* M.J. Wingf., Crous & Tzean and L. bistatum J.-J. Kim & G.-H. Kim produce very obvious *Sporothrix* states (Wingfield et al. 1994; Kim et al. 2004). As in the case of the chlamydospores, *Hyalorhinocladiella*-type synanamorphs have been described only in *Leptographium piriforme*, although they are common in species of *Ophiostoma* (Hunt 1956; Upadhyay 1981; Kirschner and Oberwinkler 1999; Jacobs and Kirisits 2003; Jacobs et al. 2003). *Hyalorhinocladiella* anamorphs might be considered reduced forms of the conidiogenous cells of *Leptographium* or *Pesotum*, but those formed in *L. chlamydatum* appear to represent a distinctly functional anamorph state.

Leptographium curvisporum is phylogenetically closely related to *L. chlamydatum*. Both these species group together with *L. abietinum* and *L. americanum* in a clade distinct from other *Leptographium* spp. Morphologically, *L. curvisporum* most closely resembles *L. abietinum*. The latter species is primarily known from the northwestern USA and Western Canada, where it occurs on various conifers, is carried by bark beetles and is considered to be

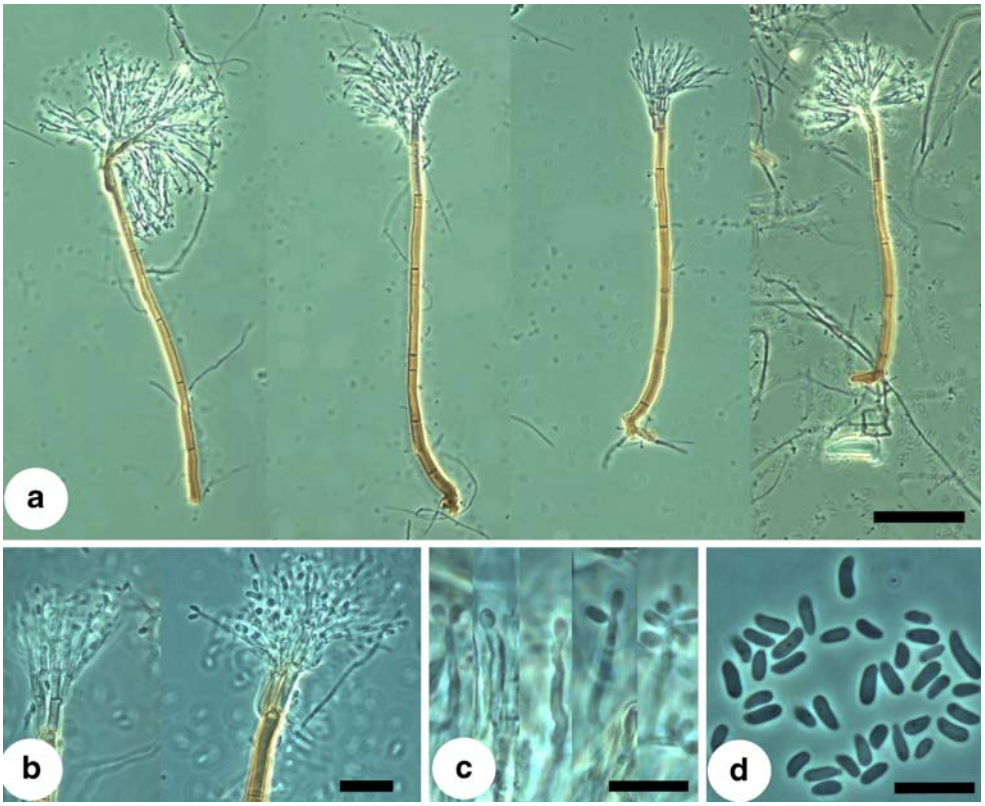

Fig. 4 Light micrographs of the morphological characters of *Leptographium curvisporum* (CMW 11608). **a** Variation in conidiophore morphology, *bar* 20 µm. **b** Conidiogenous apparatus with two or three primary branches. **c** Conidiogenous cells showing annelidic conidium development and delayed secession of conidia, *bar* 10 µm. **d** Curved conidia with tapered ends, *bar* 10 µm

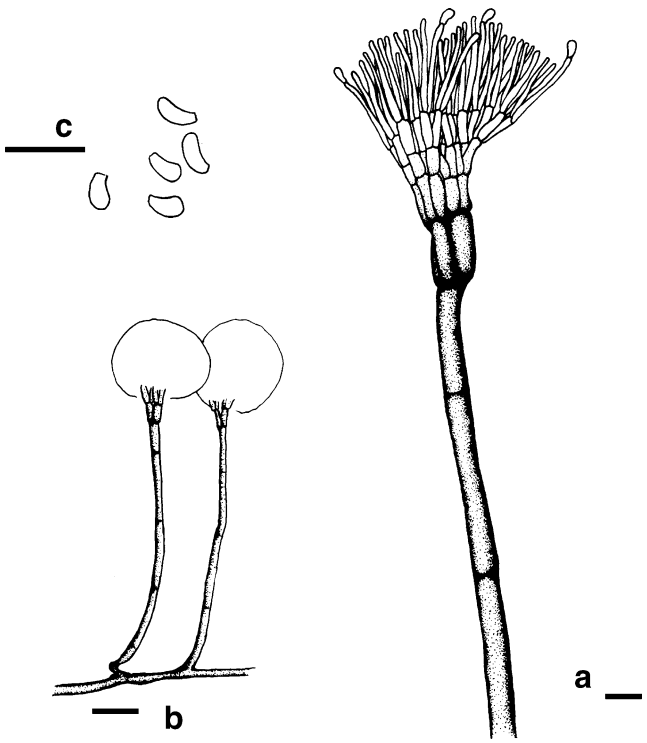

Fig. 5 Line drawings of the morphological characters of *Leptographium curvisporum* (CMW 11608). **a** Conidiogenous apparatus with three primary branches, *bar* 10 µm. **b** Habit sketch showing single arrangement of condiophores, *bar* 20 µm. **c** Curved conidia with tapered ends, *bar* 10 µm

weakly pathogenic (Kendrick 1962; Reynolds 1992; Ross and Solheim 1997; Solheim and Safranyik 1997; Solheim and Krokene 1998). Other than its distinct ecological niche, *L. curvisporum* can be distinguished from *L. abietinum* based on the presence of knob-like rhizoids, which are absent in *L. abietinum*. The conidia of *L. curvisporum* are also distinctly smaller than those of *L. abietinum*.

Ophiostomatoid fungi have been relatively well studied in Europe and the discovery of two new species associated with relatively well-known insects was surprising. This might be explained by the fact that the insects have a root-feeding habit and this niche has not been afforded much attention in the past (Mathiesen-Käärik 1953). Clearly, the inventory of *Leptographium* species, even in countries where these fungi are well known, is incomplete. More intensive surveys and especially those focussed on root-feeding insects are likely to yield substantial numbers of new *Leptographium* spp., which will expand our understanding of these interesting and often important fungi.

Acknowledgements We acknowledge the National Research Foundation (NRF), Tree Pathology Cooperative Programme (TPCP), University of Pretoria, South Africa; Norwegian Research Council (NFR) and Norwegian Forest and Landscape Institute (Skog og landskap) for financial support. We are also indebted to Dr. Hugh Glen for providing the Latin diagnoses.

References

Barnes I, Roux J, Wingfield BD, Dudzinski MJ, Old KM, Wingfield MJ (2003) *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. Mycologia 95:865–871

Camyon S, Gerhardson B (1997) Formation of pseudosclerotia and bacteria-induced agents *of* chlamydospores in *Phoma foveata*. Eur J Pl Pathol 103:467–470

Davidson RW (1971) New species of *Ceratocystis*. Mycologia 63:5–15

Farris JS, Källersjö M, Kluge AG, Bult C (1995) Testing significance of incongruence. Cladistics 10:315–319

Gams W, Hoekstra ES, Aproot A (1998) CBS course of mycology, 4th edn. Centraalbureau voor Schimmelcultures, Baarn

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microb 61:1323–1329

Greif MD, Gibas CFC, Currah RS (2006) *Leptographium piriforme* sp. nov. from a taxonomically diverse collection of anthropods collected in an aspen-dominated forest in western Canada. Mycologia 98:771–780

Grosmann H (1931) Contributions to the knowledge concerning the life partnership between bark beetles and fungi. Z ParasitKde 3:56–102

Harrington TC (1993) Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*. In: Wingfield MJ, Seifert KA, Webber JF (eds) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. APS Press, St. Paul, Minnesota, USA, pp 161–172

Hunt J (1956) Taxonomy of the genus *Ceratocystis*. Lloydia 19:1–58

Jacobs K, Kirisits T (2003) *Ophiostoma kryptum* sp. nov. from *Larix decidua* and *Picea abies* in Europe, similar to *O. minus*. Mycol Res 107:1231–1242

Jacobs K, Wingfield MJ (2001) *Leptographium* species: tree pathogens, insect associates and agents of blue-stain. APS Press, St. Paul, Minnesota, USA

Jacobs K, Wingfield MJ, Wingfield BD (2001) Phylogenetic relationships in *Leptographium* based on morphological and molecular characters. Can J Bot 79:719–732

Jacobs K, Seifert KA, Harrison KJ, Kirisits T (2003) Identity and phylogenetic relationships of Ophiostomatoid fungi associated with invasive and native *Tetropium* spp. (Coleoptera: Cerambycidae) in Atlantic Canada. Can J Bot 81:316–329

Jacobs K, Bergdah DR, Wingfield MJ, Halik S, Seifert KA, Bright DE, Wingfield BD (2004) *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycol Res 108:411–418

Jacobs K, Solheim H, Wingfield BD, Wingfield MJ (2005) Taxonomic re-evaluation of *Leptographium lundbergii* based on DNA sequence comparisons and morphology. Mycol Res 109:1149–1161

Jacobs K, Eckhardt L, Wingfield MJ (2006) *Leptographium profanum* sp. nov. a new species of *Leptographium* from hardwood roots in North America. Can J Bot 84:759–766

Johnson JA, Harrington TC, Engelbrecht CJB (2005) Phylogeny and taxonomy of the North American clade of the *Ceratocystis fimbriata* complex. Mycologia 97:1067–1092

Kellogg EA, Appels R, Mason-Gamer RJ (1996) When genes tell different stories: the diploid genera of *Triticeae*. Syst Bot 21:321–347

Kendrick WB (1961) The *Leptographium* complex. *Phialocephala* gen.nov. Can J Bot 39:1080–1085

Kendrick WB (1962) The *Leptographium* complex. *Verticicladiella* S. Hughes. Can J Bot 40:771–797

Kim JJ, Lim YW, Wingfield MJ, Breuil C, Kim GH (2004) *Leptographium bistatum* sp. nov. a new species with a *Sporothrix* synanamorph from *Pinus radiata* in Korea. Mycol Res 108:699–706

Kim J-J, Lim YW, Breuil C, Wingfield MJ, Zhou XD, Kim G-H (2005a) A new *Leptographium* species associated with *Tomicus piniperda* infesting pine logs in Korea. Mycol Res 109:275–284

Kim GH, Kim JJ, Lim YW, Breuil C (2005b) Ophiostomatoid fungi isolated from *Pinus radiata* logs imported from New Zealand to Korea. Can J Bot 83:272–278

Kirschner R, Oberwinkler F (1999) A new *Ophiostoma* species associated with bark beetles infesting Norway spruce. Can J Bot 77:247–252

Lagerberg T, Lundberg G, Melin E (1927) Biological and practical researches into blueing in pine and spruce. Sver SkogsvFör Tidskr 25:145–272

Lee S, Kim JJ, Breuil C (2005) *Leptographium longiclavatum* sp. nov. a new species associated with the mountain pine beetle, *Dendroctonus ponderosae*. Mycol Res 109:1162–1170

Lutzoni F, Wagner P, Reeb V, Zoller S (2000) Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst Biol 49:628–651

Mathiesen-Käärik A (1953) Eine Übersicht über die gewöhnlichsten mit Borkenkärfern assiziierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze. Meddn St SkogforskInst 43:3–74

Möller EM, Bahnweg G, Sandermann H, Geiger HH (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucl Acids Res 20:6115–6116

Morelet M (1988) Observations sur trios Deutéromycètes inféodés aux pins. Ann Soc Sci Nat Archéol Toulon 40:41–43

Rambaut A (2007) Se-Al: sequence alignment editor. Available from http://evolve.zoo.ox.ac.uk/

Reynolds KM (1992) Relations between activity of *Dendroctonus rufipennis* Kirby on Lutz spruce and blue-stain associated with *Leptographium abietinum* (Peck) Wingfield. For Ecol Manage 47:71–86

Ross DW, Solheim H (1997) Pathogenicity to Douglas-fir of *Ophiostoma pseudotsugae* and *Leptographium abietinum*, fungi associated with the Douglas-fir beetle. Can J Bot 27:39–43

Solheim H, Krokene P (1998) Growth and virulence of *Ceratocystis rufipenni* and three blue-stain fungi isolated from the Douglas-fir beetle. Can J Bot 76:1763–1769

Solheim H, Långström B (1991) Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity. Ann Sci For 48:149–156

Solheim H, Safranyik L (1997) Pathogenicity to Sitka spruce of *Ceratocystis rufipenni* and *Leptographium abietinum*, blue-stain fungi associated with the spruce beetle. Can J Bot 27:1336–1341

Swofford DL (2001) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sinauer Associates, Sunderland, MA, USA

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 24:4876–4882

Upadhyay HP (1981) A monograph of *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press, Athens, GA

Van Wyk P, Wingfield MJ, Marasas WFO (1988) Differences in synchronisation of stages of conidial development in *Leptographium* species. Trans Br Mycol Soc 90:451–456

Wingfield MJ (1985) Reclassification of *Verticicladiella* based on conidial development. Trans Br Mycol Soc 85:81–93

Wingfield MJ, Marasas WFO (1980) *Verticicladiella alacris* sp. nov. associated with root disease of pines in South Africa. Trans Br Mycol Soc 75:21–28

Wingfield MJ, Marasas WFO (1981) *Verticicladiella alacris*, a synonym of *V. serpens*. Trans Br Mycol Soc 76:508–510

Wingfield MJ, Crous PW, Tzean SS (1994) *Leptographium elegans*: a new species from Taiwan. Mycol Res 98:781–785

Witcosky JJ, Schowalter TD, Hansen EM (1986) *Hylastes nigrinus* (Coleoptera: Scolytidae), *Pissodes fasciates,* and *Steremnius carinatus* (Coleoptera: Curculionidae) as vectors of black-stain root disease of Douglas-fir. Environ Entomol 15:1090–1095

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenitics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: A guide to methods and applications. Academic, San Diego, USA, pp 315–322

Zhou XD, de Beer ZW, Ahumada R, Wingfield BD, Wingfield MJ (2004) *Ophiostoma* and *Ceratocystis* spp associated with two pine infesting bark beetles in Chile. Fungal Divers 15:261–274

Zipfel RD, de Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ (2006) Multigene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Stud Mycol 55:75–97