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

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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.

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$_2$O (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).

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]

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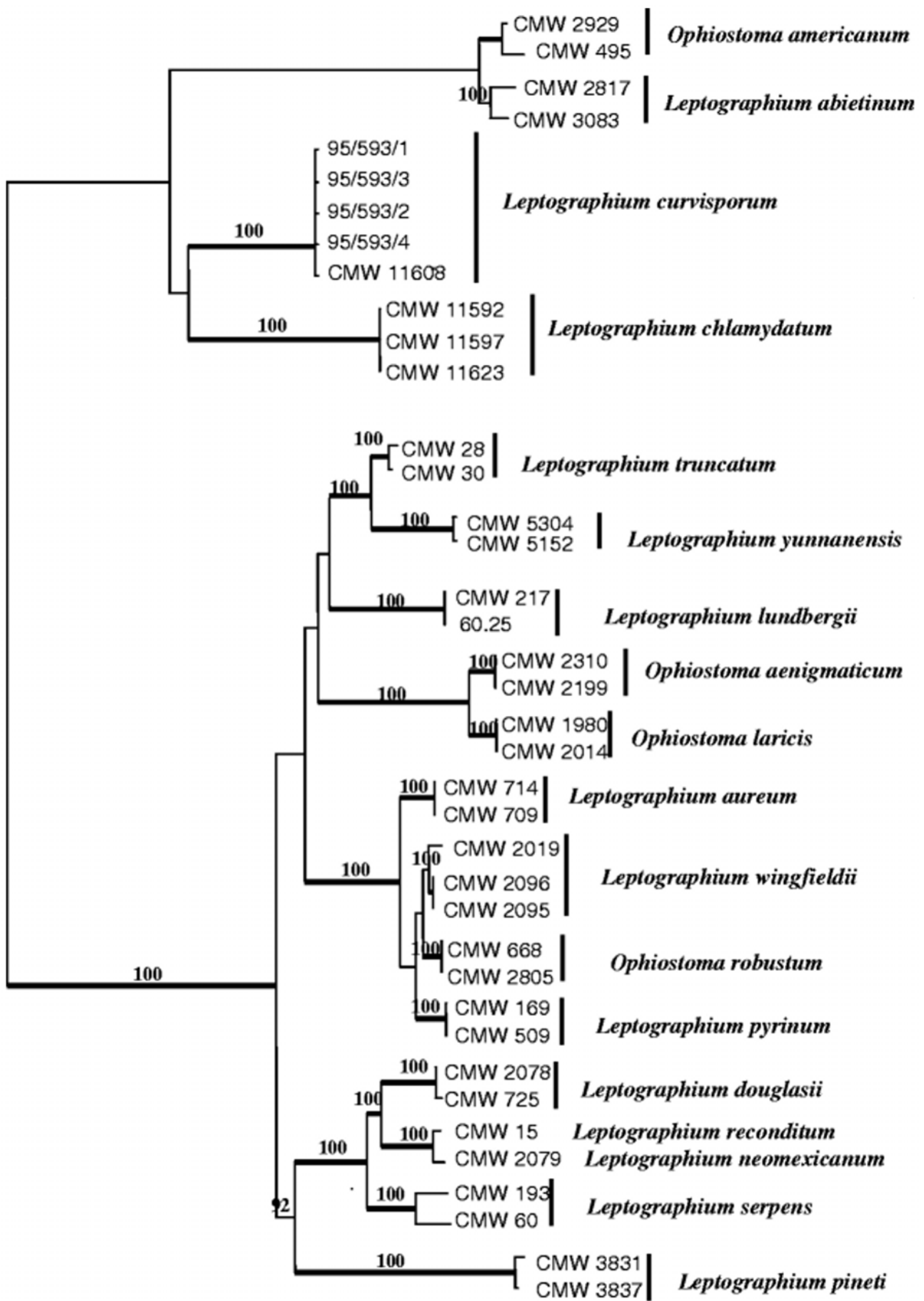
Fig. 1. Neighbour-joining tree derived from analysis of the combined dataset. Branches in bold have bootstrap values above 85.

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 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.

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*	*Larix decidua*	*Picea abies*	*Picea abies*
	P. engelmannii			
	Pseudotsuga menziesii			
	Pinus contorta			
	P. sylvestris			
	P. ponderosa			
	P. aristata			
	P. mugo			
	P. monticola			
Insect associations	*Dendroctonus pseudotsugae*	*Dendroctonus simplex*	*Hylastes cunicularius*	*Hylastes cunicularius*
	D. rufipennis		*Dryocetes autographus*	*Dryocetes autographus*
	Hylastes longicollis			
	Hylurgops porosus			
	Hylurgops planirostris			
Geographic distribution	Northwestern United States	Northeastern United States	Norway	Norway

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.
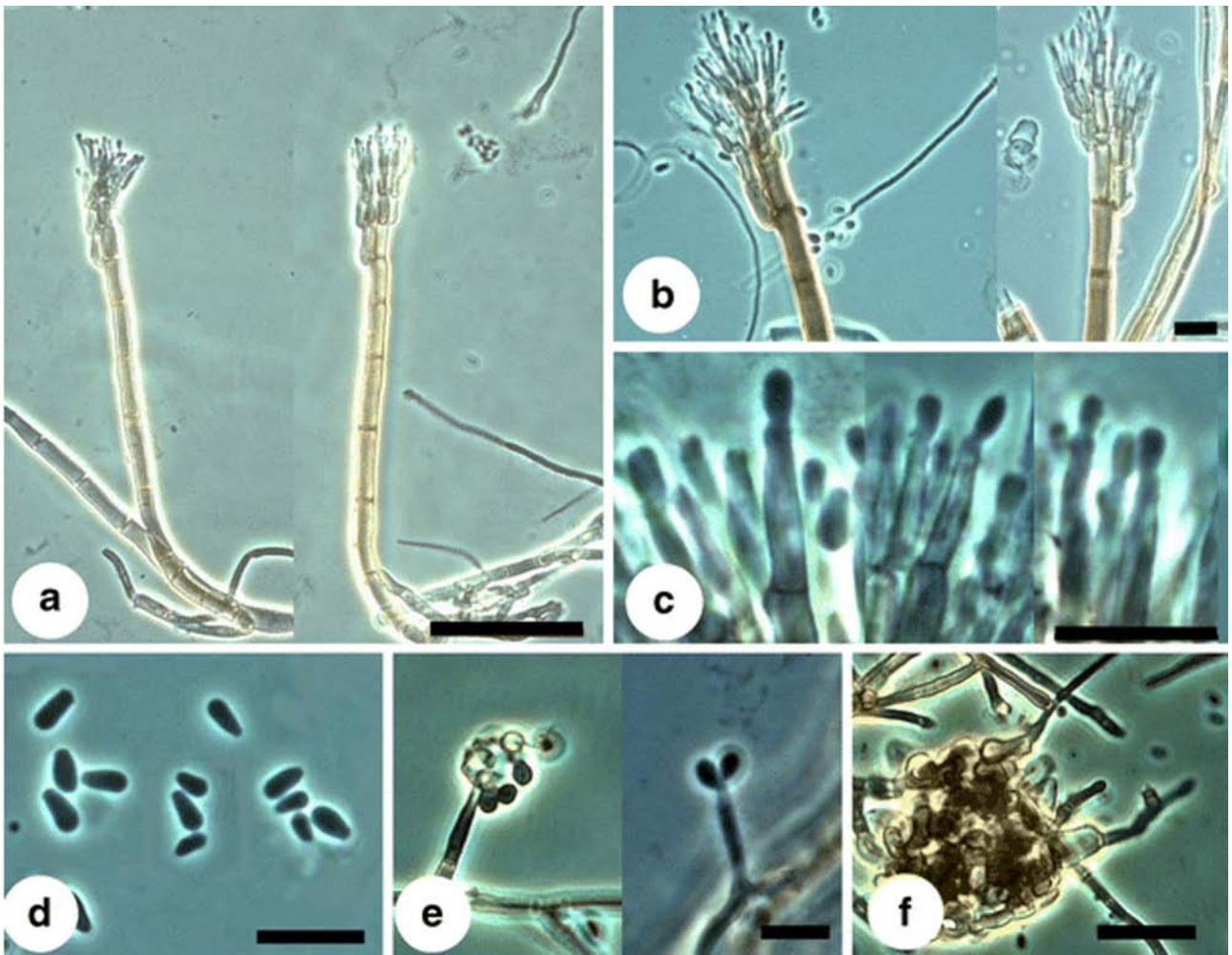
Fig. 2. Light micrographs of the morphological characters of *Leptographium chlamydatum* (CMW 11592). **a** Conidiophore morphology, bar 20 µm. **b** Conidiogenous apparatus with two primary branches. **c** Conidiogenous cells showing annelidic conidium development and delayed secession of conidia, bar 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.

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 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).
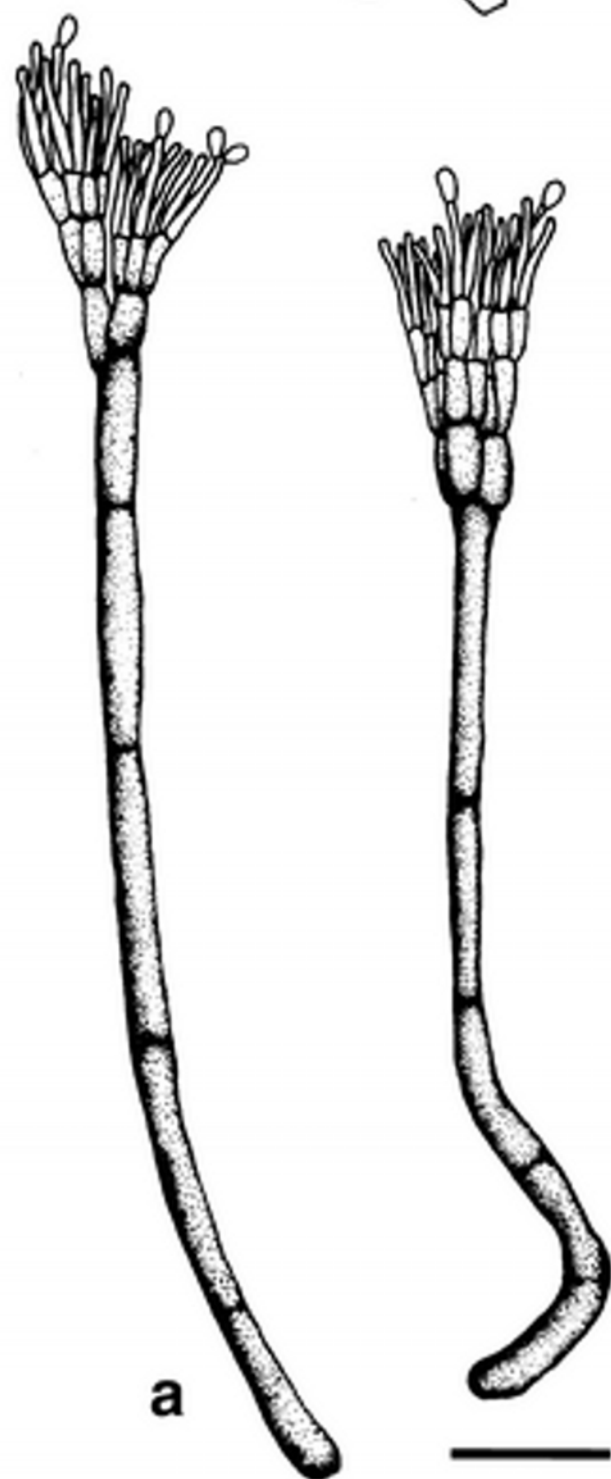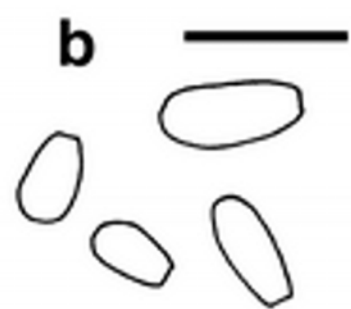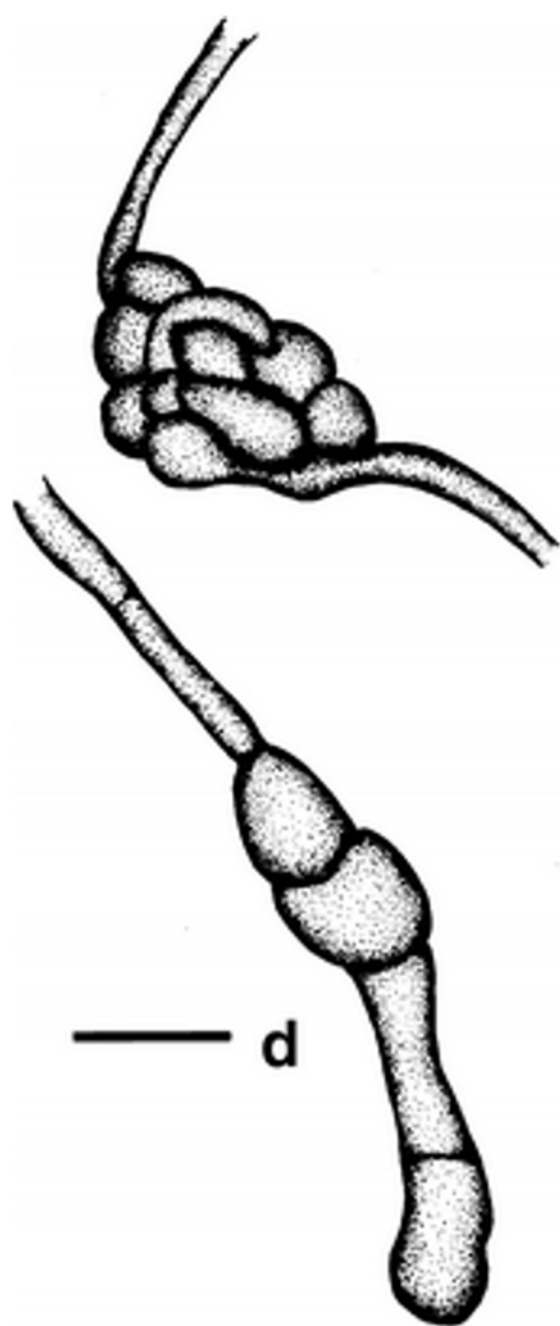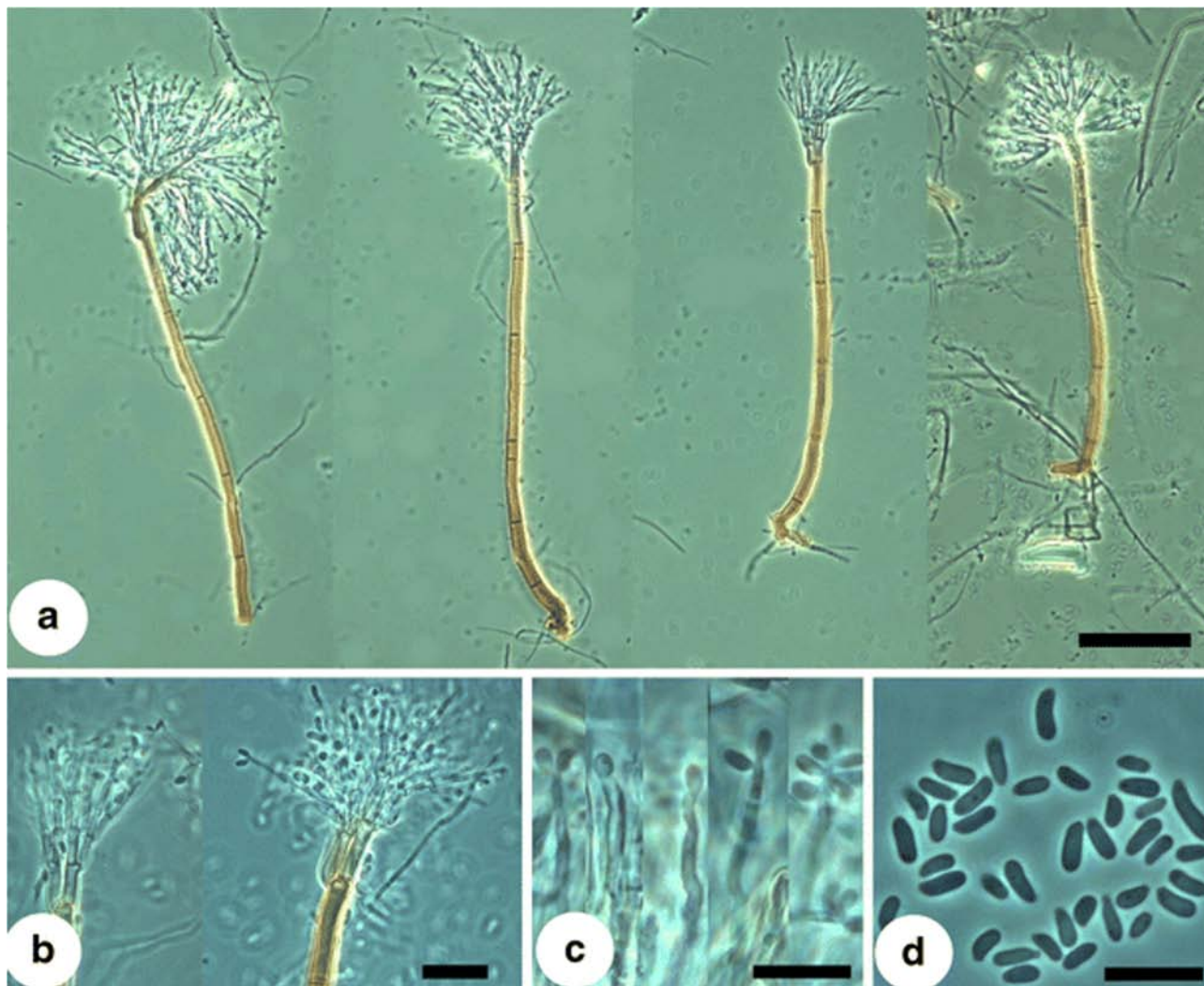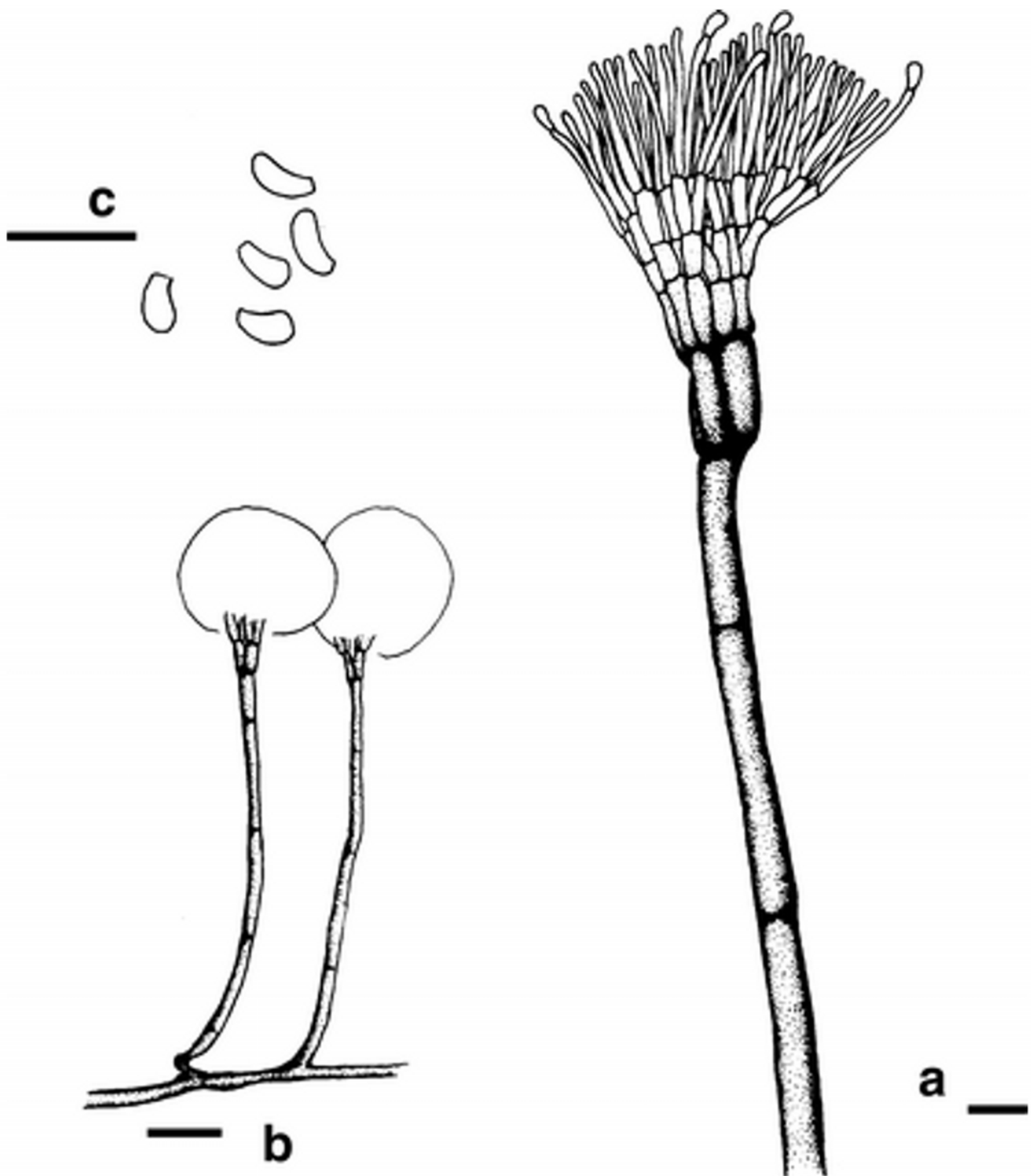
Fig. 3. Line drawings of the morphological characters of *Leptographium chlamydatum* (CMW 11592). **a** Conidiophore morphology, bar 10 μm. **b** Obovoid conidia with almost tapered ends, bar 10 μm. **c** *Hyalorhinocladiella* synanamorph of *L. chlamydatum*, bar 20 μm. **d** Chlamydospore-like structures, bar10 μm

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)

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

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. 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 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.

Acknowledgments

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