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Epitypification of *Ophiostoma galeiformе* and phylogeny of species in the *O. galeiformе* complex

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Abstract: *Ophiostoma galeiformе* was described first in 1951 from *Larix kaempferi* in Scotland, where it was found to be associated with the bark beetles *Hylurgops palliatus, Dryocoetes autographus,* and the ambrosia beetle *Trypodendron lineatum.* The taxonomy of this fungus has been uncertain because of a lack of sexual structures on the type specimen and contamination of a preserved ex-type culture. The aim of this study was to clarify application of the species name, *O. galeiformе,* by designating an epitype and to consider phylogenetic relationships of the species. Nineteen isolates resembling *O. galeiformе* from different parts of the world were used, including collections from *Pinus sylvestris* infested with *Tomicus piniperda* in Scotland and the contaminated ex-type culture. Morphological characteristics of isolates from Sweden, South Africa, Scotland, Chile and Austria corresponded well with those originally described for *O. galeiformе,* and an isolate from Scotland is designated as the epitype. A detailed description is provided. Results of interfertility tests showed that *O. galeiformе* is heterothallic. Analysis of ITS rDNA sequences showed that the isolates representing *O. galeiformе* were distinct from three morphologically similar isolates from the USA and Mexico, which probably represent an undescribed taxon.

Key words: bark beetles, bluestain fungi, ITS, sapstain, *Tomicus piniperda*

INTRODUCTION

Bark beetles (Coleoptera: Scolytidae) commonly occur in most coniferous forest ecosystems, and several species are regarded as important forest pests (Wood and Bright 1992). Most coniferous bark beetle species act as vectors of fungi, specifically *Ophiostoma* species (Beaver 1989, Paine et al 1997, Whitney 1982, Wingfield et al 1993). The genus *Ophiostoma* includes a few primary tree pathogens as well as many agents of sapstain (Brasier 1979, 1991; Brasier and Mehrotra 1995; Harrington 1993; Lagerberg et al 1927; Seifert 1993).

The sapstain fungus *Ophiostoma galeiformе* (Bakshi) Mathiesen-Käärik originally was described from Scotland as *Ceratocystis galeiformе* Bakshi (Bakshi 1951, Mathiesen-Käärik 1953). This fungus was isolated from the bark of *Larix kaempferi* infested with the bark beetles *Hylurgops palliatus* (Gyllenhal), *Dryocoetes autographus* (Ratzeburg), and the ambrosia beetle *Trypodendron lineatum* (Olivier) (Bakshi 1951). Later the fungus was found on *Picea abies* infested with *Hylastes cunicularius* (Errichson) in Sweden (Mathiesen-Käärik 1953, 1960). *Ophiostoma galeiformе* also is associated with exotic pine-infesting bark beetles in Chile and South Africa (Zhou et al 2001, 2004a). In addition, an *O. galeiformе*-like isolate recently has been isolated from *Dendroctonus mexicanus* (Hopkins) infesting *Pinus pseudostrobus* in Mexico (Zhou et al 2004b).

The taxonomic status of *O. galeiformе* is uncertain because the type specimen lacks perithecia (Hunt 1956, Upadhyay 1981). Although Hunt (1956) accepted the species in his study of the genus *Ceratocystis,* Upadhyay (1981) and Seifert et al (1993) considered it a species of uncertain status. Article 9.7 of the International Code of Botanical Nomenclature

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(Greuter et al 2000) allows the designation of a specimen and/or a culture as an epitype where the holotype does not show the necessary distinguishing characters. The aim of this study was to reconsider the taxonomic status of *O. galeiforme* and designate an epitype for the species. Light microscopy was employed to examine isolates, and sequences of the ITS (internal transcribed spacer) region of the ribosomal RNA operon were used to assess phylogenetic relationships.

MATERIALS AND METHODS

Fungal isolates and morphological investigation.—The available morphological structures on the holotype of *O. galeiforme* (IMI20168) were examined. A culture from the type (CBS137.51) was found to be contaminated with a *Cadophora* sp., and repeated attempts to purify it failed. Eighteen other isolates, morphologically and ecologically similar to *O. galeiforme,* were included in the study (TABLE I). Single-conidial cultures were prepared for all these isolates and were grown on 2% MEA (20 g Biolab malt extract, 20 g Biolab agar and 1000 mL de-ionized water). One isolate from Scotland (CMW5290), originating from ascospore masses taken from a perithecium occurring in the galleries of the bark beetle *Tomicus piniperda* on *Pinus sylvestris* produced perithecia in culture. This isolate was grown on malt agar (2% malt, 1.6% agar) supplemented with pieces of Norway spruce sapwood and on oatmeal agar (Gams et al 1998) at room temperature and diffuse daylight. After onset of sporulation, fungal structures were mounted on slides in lactophenol. Forty perithecia, ascospores, conidiophores and conidia were measured using a light microscope. Ranges and averages were computed for all fungal cultures, which are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa, and/or the culture collection of TC Harrington (C), Department of Plant Pathology, Iowa State University, Iowa.

Mating experiments.—Four isolates (CMW578 and CMW580 from South Africa, CMW4441 and CMW4443 from Scotland) (TABLE I) resembling *O. galeiforme* were chosen to determine thallism and to obtain perithecia. Two crosses, one (DM270) between the two South African isolates and a second (DM269) between the two isolates from Scotland, produced perithecia; 20 single ascospore cultures each (DM270-1 to DM270-20, and DM269-1 to DM269-20) were prepared from perithecia obtained from each of the two crosses, and eight progeny from each set were selected and paired in every possible combination. Four tester strains each (two of each mating type) of each progeny set were paired in every possible combination and with a culture derived from the epitype (CMW5290). All pairings were conducted on MEA with pine twigs as described by Harrington et al (2001) and monitored for the appearance of perithecia.

DNA sequencing and phylogenetic analysis.—Hyphal-tip cultures of selected isolates were prepared for DNA extraction and sequencing (TABLE I). DNA was extracted using a modified version of the extraction method developed by Raeder and Broda (1985). The ITS1 and ITS2 (internal transcribed spacer) regions, including the 5.8S gene of the ribosomal RNA operon, were amplified using primers ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al 1990). PCR products were sequenced with the same primers used for PCR, as well as two additional internal primers, CS2 (Wingfield et al 1996) and ITS3 (White et al 1990). Conditions for PCR amplification and sequencing reactions were as described by Zhou et al (2004a). A search of similar ITS sequences using BLAST (National Center for Biotechnology Information, USA), indicated that the sequence of *Leptographium guttulatum* MJ Wingfield & K Jacobs was closest to *O. galeiforme*. Two isolates (CMW1310 and CMW742) (TABLE I) of *L. guttulatum* then were sequenced and included in this study because only part of the ITS sequence of the species is available from GenBank. The resulting sequences first were aligned using Clustal X (1.81) and further manually aligned using Sequence Navigator version 1.01 (ABI PRISM, PerkinElmer). Phylogenetic relationships among the isolates were determined using distance analyses in PAUP* version 4.0b10 (Phylogenetic Analysis Using Parsimony) (Swofford 1998). Trees were constructed using the neighbor-joining tree-building algorithm (Saitou and Nei 1987). An isolate of *O. cucullatum* Solheim was used as outgroup taxon. Bootstrap analysis (1000 replicates) was run to determine confidence of the branching points (Felsenstein 1985).

RESULTS

Morphology.—All *O. galeiforme* isolates produced conidiophores in culture. A continuum of conidiophores existed from mononematous to synnematous, although the synnemata dominated in all cultures. Morphological comparisons showed no differences among the conidiophores and conidia of the isolates from Sweden, South Africa, Scotland, Chile and Austria and those present on the holotype specimen (IMI20168). Only one of the isolates (CMW5290) derived from an ascospore mass, produced perithecia in culture, and it probably consisted of both mating types or it was a heterokaryon. Measurements of the teleomorph and anamorph corresponded well with those described previously (TABLE II). Three *O. galeiforme*-like isolates from Mexico (CMW9490), Georgia, USA (C527), and California, USA (C1293), morphologically were similar to the *O. galeiforme* isolates from Sweden, South Africa, Scotland, Chile and Austria, but colony color of the isolates from Mexico and the USA was distinctly lighter than that of other isolates.

Mating experiments.—Perithecia with fertile ascospores were formed in pairings between two South African isolates (CMW578 and CMW580) and between two Scottish isolates (CMW4441 and

TABLE I. Fungal isolates included in this study

Species	Isolate No.	Other No.	Collector or supplier
Ophiostoma galeiforme	CMW4426[a]		MJ Wingfield, T. Kirisits
	CMW4441	C1239[b]	MJ Wingfield, T Kirisits
	CMW4443	C1238	MJ Wingfield, T Kirisits
	CMW4447		MJ Wingfield, T Kirisits
	CMW5290	IFFF Scotland/5[c]; CBS115711	T Kirisits, MJ Wingfield
	CMW9478		MJ Wingfield, XD Zhou
	CMW9479		MJ Wingfield, XD Zhou
	CMW9482		MJ Wingfield, XD Zhou
	CMW9483		MJ Wingfield, XD Zhou
	CMW9494		XD Zhou
	CMW9495		XD Zhou
	CMW578	C1235	L Strauss
	CMW580	C1236	L Strauss
	CMW9988	CBS150.54[d]	A Mathiesen-Käärik
	CMW12686	CTK115[c]	T Kirisits
O. galeiforme-like		C527	M Baldwin
		C1293	D Hofstra
	CMW9490		MJ Wingfield, XD Zhou
Leptographium guttulatum	CMW1310	PREM56310[e]	JN Gibbs
	CMW742	PREM56307	M Morelet
O. cucullatum		C1216 (NFRI 81-83/2[f])	H Solheim

[a] CMW = Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

[b] C = Culture collection of TC Harrington, Department of Plant Pathology, Iowa State University, Iowa, USA.

[c] IFFF, CTK = Culture collection of the Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU—University of Natural Resources and Applied Life Sciences, Vienna, Austria.

[d] CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.

[e] PREM = National Collection of Fungi, Pretoria, South Africa.

[f] NFRI = Culture Collection of the Norwegian Forest Research Institute, Ås, Norway.

CMW4443). None of the 20 single-ascospore cultures derived from either of these crosses gave rise to perithecia, but pairings among the single ascospore progeny indicated that the fungus is heterothallic and has two mating types. Pairings between the tester strains selected from the above crosses showed that the South African isolates were sexually compatible with the Scottish isolates (TABLE III). A self-sterile culture, CMW5290, which was used to generate the epitype specimen, also was compatible with some tester strains (TABLE III). Compatible crosses resulted in perithecia with viable ascospores 4–8 wk after being crossed.

DNA Sequence analysis.—Fragments approximately 510 bp in size were amplified from the DNA of all isolates (TABLE I), except from two isolates of *L. guttulatum* (580 bp) and the culture (CBS137.51) labeled as ex-type (463 bp). This latter fragment was sequenced, and a BLAST search revealed that it was similar to a species of *Cadophora* and, thus, was a contaminant. Manual alignment of the remaining sequences resulted in a total of 592 characters that were used in a distance analyses. Three main clusters were evident in the phylogram (FIG. 1) obtained with the neighbor-joining, tree-building algorithm. The first cluster, including three similar ITS sequence, represented the *O. galeiforme* group and had a bootstrap support of 98%. Isolates from Chile, South Africa and Sweden shared one ITS sequence, five isolates from Scotland had a second sequence and the Austrian isolate had a third sequence. The second cluster, with a bootstrap support of 59%, included the three isolates from Mexico and the USA. The third cluster consisted of two isolates of *L. guttulatum,* with 100% support.

TAXONOMY

The five *O. galeiforme* isolates from Scotland morphologically are indistinguishable, and the anamorph characteristics also are indistinguishable from those on the holotype specimen originating from Scotland. These isolates had identical ITS sequences, differed only by one base pair from the Austrian isolate and differed by two base pairs from the isolates from Chile, South Africa and Sweden. Furthermore, prog-

TABLE I. Extended

Host	Insect	Origin	Genbank No.
Pinus sylvestris	*Tomicus piniperda*	Elgin, Scotland	AY649774
P. sylvestris	*T. piniperda*	Elgin, Scotland	AY649775
P. sylvestris	*T. piniperda*	Elgin, Scotland	AY649776
P. sylvestris	*T. piniperda*	Elgin, Scotland	AY649777
P. sylvestris	*T. piniperda*	Elgin, Scotland	AY649778
P. radiata	*Hylastes ater*	Valdivia, Chile	AY649769
P. radiata	*H. ater*	Valdivia, Chile	AY649770
P. radiata	*Hylurgus ligniperda*	Valdivia, Chile	AY649771
P. radiata	*H. ligniperda*	Valdivia, Chile	AY649772
P. elliiottii	*H. ligniperda*	KwaZulu-Natal, South Africa	AY649764
P. elliiottii	*H. ligniperda*	KwaZulu-Natal, South Africa	AY649765
P. pinaster	—	Grabouw, South Africa	AY649766
P. pinaster	—	Grabouw, South Africa	AY649767
Picea abies	*Hylastes cunicularius*	Västerbotten, Sweden	AY649768
P. abies	*H. cunicularius*	Stinatz, Burgenland, Austria	AY649773
Pinus taeda	—	Georgia, USA	AY649779
P. radiata	—	California, USA	AY649780
P. pseudostrobus	*Dendroctonus mexicanus*	Chiapas, Mexico	AY649781
P. sylvestris	*T. piniperda*	Hampshire, England	AY649782
P. sylvestris	*T. piniperda*	Orleans, France	AY649783
Picea abies	*Ips typographus*	Norway	AY649784

eny from crossings of Scottish and South African isolates were fully interfertile. These isolates, therefore, are considered to represent a single species whose morphological characteristics agree well with the original description of *O. galeiforme* (Bakshi 1951). A dried culture (2% malt and 1.6% agar with pieces of Norway spruce sapwood), bearing perithecia and mononematous and synnematous anamorphs of a Scottish isolate (CMW5290), is designated as the epitype of *O. galeiforme* because it originated from the same geographical region as the holotype specimen. The epitype specimen has been deposited in the National Collection of Fungal Specimens of South Africa (PREM57491), and the culture is maintained in the collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa (CMW5290), as well as with the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (CBS115711). The species description provided below is based on the epitype specimen and culture of *O. galeiforme*.

Ophiostoma galeiforme (Bakshi) Mathiesen-Käärik, Meddel. Statens Skogs-Forskningsinst. 43:47. 1953 ≡ *Ceratocystis galeiforme* Bakshi, Mycol. Pap. 35:13. 1951.

Colonies reaching 30 mm diam in 10 d on 2% MEA at 25 C. Colonies light grey (19″d, Rayner 1970) to dark brown (13′′′′′k) with age, appressed with yeasty appearance. *Perithecia* rarely and sparsely produced in culture but readily formed in pairings between sexually compatible strains after 4–8 wk incubation. Perithecial bases globose, dark brown to black, 60–240(–550) μm diam (FIG. 2C), with few ornamental hyphae. Perithecial necks dark brown to black, 260–585(–840) μm long, 20–52(–93) μm wide at the base, 8–25(–54) μm wide at the apex. *Ostiolar hyphae* absent (FIG. 2B). *Asci* not observed. *Ascospores* hyaline, aseptate, with brim, bean shaped in side view, 2.0–3.5(–5.0) × 1.0–1.5(–3.0) μm (FIG. 2A).

Synnematous anamorph predominant in culture. *Conidiophores* with apex hyaline to light grey, stalk brown, 50–260(–740) μm long, 7–29(–97) μm wide at base, 10–48(–190) μm wide at head (FIG. 2F). *Conidiogenous cells,* 8–12(–15) × 1.0–1.5(–2.0) μm (FIG. 2E). *Conidia* hyaline, cylindrical, 2.0–4.0(–6.0) × 1.0–2.0(–3.0) μm (FIG. 2D).

Mononematous anamorph rarely produced in culture. *Conidiophores* with up to 10 septa, 60–92(–130) μm long (FIG. 2I). *Primary branches,* cylindrical, 10–20(–25) μm long. *Secondary branches,* 8–15(–20) μm long. *Conidiogenous cells,* cylindrical, 5.5–10(–16) × 1.0–1.5(–2.0) μm (FIG. 2H). *Conidia* hyaline, cylindrical to ellipsoid, with a truncate base, 2.0–3.5(–4.5) × 1.0–1.5(–2.0) μm (FIG. 2G).

Specimens examined. SCOTLAND: Perthshire, Blair Atholl. Bark of *Larix kaempferi* (Japanese larch), associated with bark beetles *Hylurgops palliatus* and *Dryocoetes autographus,* 1951, *BK Bakshi* (HOLOTYPE. IMI20168). SCOTLAND: Elgin. *Pinus sylvestris* (Scotch pine) infested with the bark beetle *Tomicus piniperda,* 29 Aug 1997, *T Kirisits and MJ Wingfield* (EPITYPE: PREM57491; CMW5290 = CBS115711). SCOTLAND: Elgin. *P. sylvestris* infested with

TABLE II. Comparison of the morphology of the epitype specimen and previous descriptions of *O. galeiforme* (all measurements in μm)

			Epitype (PREM57491)	Bakshi, 1951	Mathiesen-Käärik, 1953	Hunt, 1956
Teleomorph	Perithecial base	Color	Dark brown to black	Brown to black	Black	Same as Bakshi (1951)
		Diameter	(60–) 240 (–550)	(182–) 218 (–273)	(184–) 221 (–255)	
		Ornamentation	Very few hair-like hyphae	Present	Hairless or with few single hairs	
	Perithecial neck	Length	(260–) 585 (–840)	(539–) 640 (–700)	(620–) 760 (–930)	
		Base width	(20–) 52 (–93)	(39–) 49 (–60)	(45–) 51 (–65)	
		Apex width	(8–) 25 (–54)	(15–) 25 (–28)	(22–) 25 (–28)	
	Ostiolar hyphae		Absent	Absent	Absent	
	Ascospores	Color	Hyaline	Hyaline	Hyaline	
		Septation	Absent	Absent	Absent	
		Shape	Bean shaped, with brim	Bean shaped, with brim	Bean shaped, with appendages	
		Size	(2.0–) 3.5 (–5.0) × (1.0–) 1.5 (–3.0)	(4.0–) 4.6 (–5.3) × (2.1–) 2.5 (–3)	3.8–2.0, without sheath	
Anamorph	Synnematal anamorph		Synnemata 50–260(–740) μm long, 10–48(–190) μm wide at head, Conidia (2.0–) 4.0 (–6.0) × (1.0–) 2.0 (–3.0)	Conidia one-celled, elongate, (4.0–) 4.6 (–5.2) × (1.9–) 2.0 (–2.2)	Real synnemata: stipe 200–400 long, head up to 100–500, hyaline or light greenish	Stalks brown to black at base, hyaline in the upper part, up to 300 × 60, conidia same as those of *Leptographium*
	Mononematal anamorph		Rare in culture, conidiophores with up to 10 septa, 60–90(–130) μm long, Conidia cylindrical to ellipsoid, (2–) 3.5 (–4.5) × (1.0–) 1.5 (–2.0)	Conidia one-celled, elongate, (4.0–) 4.6 (–5.2) × (1.9–) 2.0 (–2.2)	Simple conidiophore: stipe 150–250 long, 4–8 septate, head up to 50–60, conidia egg-shaped, (4.0–) 4.8 (–5.5) × (2.0–) 2.2 (−2.4)	Stalks brown, thick-walled, up to 8 septate, up to 300 × 3–5, head up to 50, conidia hyaline, cylindrical to ellipsoid, 5–6 × 2.5–3
	'*Cephalosporium*' anamorph		Not observed	Conidia one-celled, oval, hyaline, (2.2–) 2.7 (–3.1) × (1.5–) 1.7 (–2.0)	*Cephalosporium*-like head: one celled, hyaline, oval, (2.2–) 2.8 (–3.6) × (1.2–) 1.9 (–2.3)	Hyaline (conidiophores and conidia), spores ellipsoid to oval, 3–3.5 × 2–2.5

TABLE III. Mating experiments of *O. galeiforme* tester strains and the epitype strain (CMW5290)

	Recipient strain								
Donor strain	DM269-1 (mat a)[c]	DM269-3 (mat a)	DM270-16 (mat a)	DM270-17 (mat a)	DM269-5 (mat b)[c]	DM269-7 (mat b)	DM270-10 (mat b)	DM270-12 (mat b)	CMW5290 (mat b)
DM269-1[a]	−[d]	−	−	−	+	+	+	−	+
DM269-3	−	−	−	−	+	+	−	−	−
DM270-16[b]	−	−	−	−	+	+	+	+	−
DM270-17	−	−	−	−	+	+	+	+	−
DM269-5	+[d]	+	+	−	−	−	−	−	−
DM269-7	+	+	+	−	−	−	−	−	−
DM270-10	+	+	+	+	−	−	−	−	−
DM270-12	+	+	+	+	−	−	−	−	−
CMW5290	+	+	+	−	−	−	−	−	−

[a] Tester strains DM269-1, DM269-3, DM269-5 and DM269-7 obtained from a cross between two Scottish isolates.
[b] Tester strains DM270-10, DM270-12, DM270-16 and DM270-17 obtained from a cross between two South African isolates.
[c] Mat a represents mating type a; mat b represents mating type.
[d] + indicates formation of perithecia with viable ascospores; − indicates no perithecia or ascospores produced.

T. piniperda, 29 Aug 1997, *MJ Wingfield and T Kirisits* PREM57492 (CMW4426). SCOTLAND: Elgin. *P. sylvestris* infested with *T. piniperda,* 29 Aug 1997, *MJ Wingfield and T Kirisits* PREM57493 (CMW4447).

DISCUSSION

We have confirmed previous reports that the holotype of *O. galeiforme* contains only the anamorph of the fungus and that the ex-type culture deposited at CBS is contaminated. An epitype thus has been designated based on a collection from the same geographical area where *O. galeiforme* was first collected. The results of this study have also confirmed that *O. galeiforme* occurs in Chile, South Africa and Sweden and provide the first report of the fungus from Austria and Central Europe. This is also the first report of the association between this fungus and *Tomicus piniperda*. Three isolates from the USA and Mexico thought to represent this species are different and probably represent an undescribed taxon.

Results of interfertility tests showed that *O. galeiforme* is heterothallic. Pairings between isolates of opposite mating types produced perithecia and those between isolates of the same mating type were negative. However, the field-collected epitype strain (CMW5290) used in this study produced perithecia and ascospores, which probably accounts for the fact that Bakshi (1951) reported that *O. galeiforme* is homothallic. We believe that field isolates with perithecia represent either heterokaryons or are mixtures of hyphae of opposite mating type.

In the descriptions of *O. galeiforme* by Bakshi (1951) and Hunt (1956), the conidial states were assigned to three genera: *Graphium, Leptographium* and *Cephalosporium*. Both Mathiesen-Käärik (1953) and Hunt (1956) mentioned that the fungus formed a continuum of conidiophore structures varying from single, simple conidiophores to true synnemata, typical of the genus *Graphium,* now referred to *Pesotum* (Harrington et al 2001). Wingfield (1993) stated that it was difficult to assign a generic name to the anamorph of *O. galeiforme* because the species has both synnematous and mononematous states. Scanning electron microscopy studies further showed a continuum in patterns of conidium development including those typical for *Sporothrix, Hyalorhinocladiella* and *Graphium* (now *Pesotum*) (Benade et al 1997). Harrington et al (2001) restricted *Pesotum* to those species forming both synnemata and *Sporothrix* conidiophores with prominent denticles on the conidiogenous cells, which are members of the *O. piceae* complex. They proposed that the synnemata of *O. galeiforme* are loosely to tightly fused *Leptographium*-like conidiophores and that the anamorph would be better placed in *Leptographium* than in *Pesotum*. The anamorph of the closely related *O. cucullatum* was transferred to *Phialographium* (as *P. erubescens* (Mathiesen-Käärik) Harrington et McNew) based on its phialidic conidial ontogeny (Harrington et al 2001). In this study, both the synnematal and mononematal forms of the anamorph of *O. galeiforme* were observed, although the synnematal form was predominant, and the conidiogenous cells are sympodial and phialidic. We do not believe that it is necessary to provide a formal name for the anamorph, but if necessary we preferentially would refer to it as *Leptographium*.

Analysis of sequence data for *O. galeiforme* isolates from Chile and South Africa presented in this study has shown that these isolates phylogenetically are re-

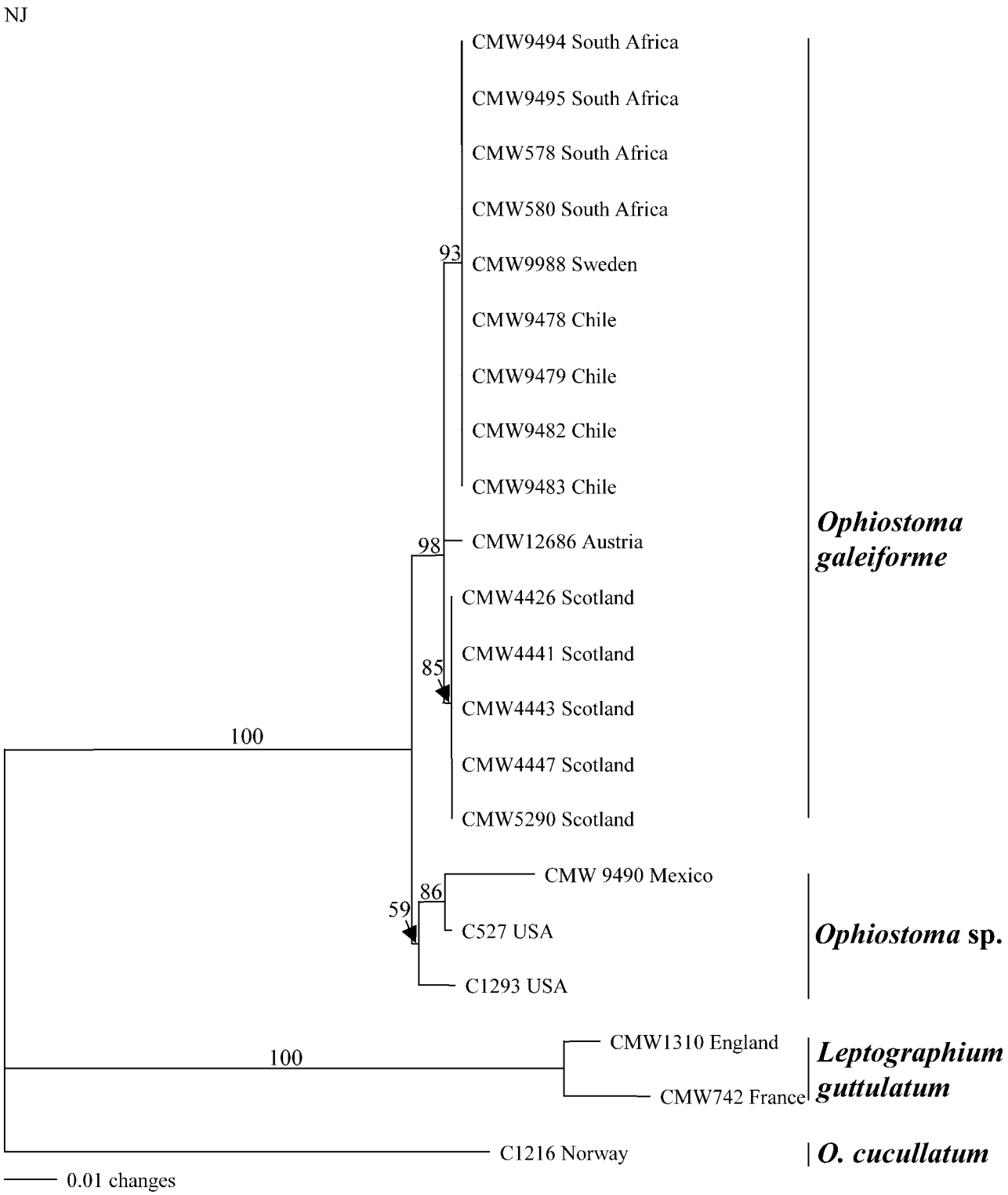

FIG. 1. Phylogram of the *Ophiostoma galeiforme* complex based on ITS sequences (ITS1 and ITS2 regions, as well as 5.8S rRNA gene) generated with the neighbor-joining, tree-building algorithm in PAUP*. Bar = total nucleotide differences between taxa. Bootstrap values (%) are indicated above the branches.

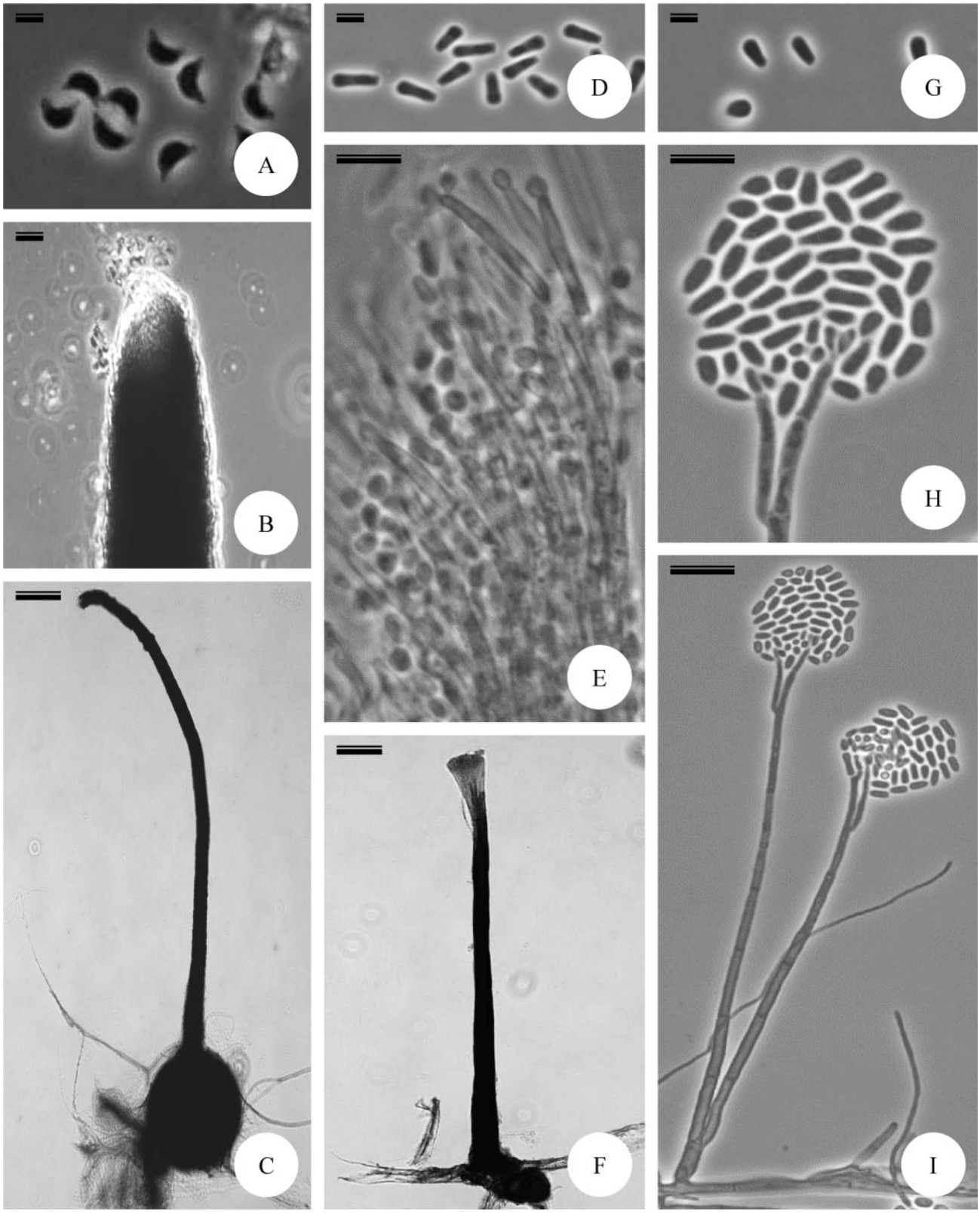

FIG. 2A–I. *Ophiostoma galeiforme* (CMW 5290) on malt agar with pieces of Norway spruce sapwood. A. Bean-shaped ascospores (Bar = 2.5 μm). B. Apex of the neck lacking ostiolar hyphae (Bar = 10 μm). C. Perithecium with long neck (Bar = 85 μm). D. Conidia of synnematous anamorph (Bar = 5 μm). E. Conidiogenous apparatus of synnematous anamorph (Bar = 8 μm). F. Synnematous anamorph (Bar = 80 μm). G. Conidia of mononematous anamorph (Bar = 5 μm). H. Conidiogenous apparatus of mononematous anamorph (Bar = 8 μm). I. Mononematous anamorph (Bar = 20 μm).

lated closely to those from Sweden, Scotland and Austria. Occurrence of mating between the isolates from South Africa and Scotland affirmed that these isolates represent a single biological species. Some of the *Ophiostoma* species on Pinaceae in countries such as South Africa and Chile are carried by bark beetles that accidentally were introduced into these countries from Europe (Ciesla 1988, Tribe 1992). *Ophiostoma galeiforme* apparently is associated commonly with a wide range of bark beetles in Europe, and it was likely introduced into South Africa and Chile with one or more of these European insects. In South Africa, *O. galeiforme* is associated with *Hylurgus ligniperda* (Fabricius) (Zhou et al 2001), and in Chile we commonly have isolated it from both *Hylastes ater* and *Hylurgus ligniperda* (Zhou et al 2004a). None of these Eurasian insects has been connected with *O. galeiforme* in their natural habitat, but this is probably due only to the lack of studies of fungi associated with these insects.

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