Sporendocladia bactrospora associated with wounds on native broadleaved trees in Norway and Sweden

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Running title: *Sporendocladia bactrospora* in Norway

Summary

A survey to identify Ophiostomatoid fungi that infect wounds on native Norwegian and Swedish broad leaved trees was undertaken during summer 2004. A fungus resembling a species of *Sporendocladia* was commonly isolated from the exposed cambium and inner bark of wounds. Morphological examination and comparisons of DNA sequence data for the ITS and 5.8S regions of the rRNA gene region led to its identification as *Sporendocladia bactrospora*. Pathogenicity trials on young *Populus tremula* and *Betula pubescens* trees showed that *S. bactrospora* is capable of causing lesions on these trees. There have been few previous reports of *S. bactrospora* and in most cases these have been as saprophytes on wood. In contrast, results of this study show that it is a common

inhabitant of freshly made wounds on native broad leaved trees in Scandinavia and it appears to contribute to staining of wood.

Key words: *Phialocephala*, *Populus*, *Betula*, wood stain, disease, Microascales

1. Introduction

Sporendocladia bactrospora (W.B. Kendr.) M.J. Wingf. is a wood inhabiting ascomycete, previously known as *Phialocephala bactrospora* W.B. Kendr. and originally treated in the *Leptographium* complex of asexual fungi (Kendrick 1961, 1963; Wingfield et al. 1987). It is thus part of a group of fungi generically known as the Ophiostomatoid fungi (Wingfield et al. 1993; Seifert et al., 2013). Distinctive morphological differences between *S. bactrospora* and other species of *Phialocephala* and *Leptographium* led to its placement in the genus *Sporendocladia* as *S. bactrospora*.

Sporendocladia bactrospora is characterized by bacilliform conidia produced in chains from phialides with cylindrical collarettes (Kendrick 1963; Wingfield 1985; Wingfield et al. 1987). Furthermore, the conidia are produced through a process of ring wall building (Kendrick 1963; Wingfield 1985; Wingfield et al. 1987) similar to that found in species of *Chalara* and *Thielaviopsis* (Nag Raj and Kendrick 1975; Paulin-Mahady et al. 2002). This is in contrast to *Leptographium* spp. which produce conidia through percurrent proliferation of the conidiogenous cells (Wingfield et al. 1987).

There are few reports of *Sporendocladia* spp., with only seven species listed in literature (Sutton 1993; Wingfield et al. 1987) and in Index Fungorum (www.indexfungorum.org). These reports list *Sporendocladia* spp. from chestnut cupules, leaf litter and wood (Arnaud 1954; Kendrick 1961; Luque et al. 2000; Onofri and Zucconi 1984; Sutton 1975, 1993) as well as a report from an unknown host in Japan (Jacobs et al. 2003). Although it is generally suggested that *Sporendocladia* spp. are saprophytes associated with dead plant material, *S. bactrospora* has been reported from the trunks of living *Quercus suber* L. trees in Spain (Luque et al. 2000), *Tilia* veneers in England (Kendrick 1961) and from the wood of *Populus trichocarpa* Torr. & A. Gray and *P. tremuloides* Michx. in Canada (Grünig et al., 2002; Kendrick 1961).

Species of *Ophiostoma* and *Ceratocystis* are often referred to as the Ophiostomatoid fungi, due to the previous confusion in their taxonomy and their similar biologies (Wingfield et al. 1993; Seifert et al. 2013). These fungi include some of the most important and devastating tree pathogens known, including the Dutch Elm disease pathogens, *Ophiostoma ulmi* (Buisman) Melin & Nannf. and *O. novo-ulmi* Brasier (Brasier 2000; Hubbes 1999), *Ceratocystis fagacearum* (Bretz) J. Hunt. the cause of oak wilt (Henry 1944; Sinclair et al. 1987) and *C. platani* (Walter) Engelbrecht & Harrington, the cause of canker and death of plane trees (Anonymous 1986; Walter 1946; Panconesi 1981). Despite the importance of the Ophiostomatoid fungi, relatively little is known about their diversity or geographic distribution, especially on broad leaved tree species. Reports from Scandinavian countries include mainly Ophiostomatoid fungi on coniferous hosts.

A study was, therefore, undertaken to identify the Ophiostomatoid fungi present on native broad leaved tree species in Norway and to gain knowledge of their relative importance. These investigations let to the collection of several Ophiostomatoid fungi (Roux et al. 2005; Fouche et al. 2007; Kamgan Nkuekam et al. 2010), including a fungus resembling a *Sporendocladia* sp. The aim of the present study was to identify the fungus, to consider its possible pathogenicity and to assess its potential role in disease of native broad leaved trees based on its association with wounds on these trees in Norway.

2. Materials and Methods

2.1 Fungal Isolates

During the summer of 2004, three sites in Norway and one in Sweden were surveyed for the presence of Ophiostomatoid fungi on native broad leaf trees. Sampling was conducted over a period of two weeks in June. The sites sampled included a sawmill near the town of Ås, a sawmill near Svenneby, standing trees adjacent to Årungen Lake near the town of Ås (all in Norway) and a loading bay near Filipstad in Sweden. Ophiostomatoid fungi were collected from recently harvested logs at the sawmills and loading bays as well as from artificially induced wounds made six weeks prior to the surveys, on the stems of standing trees of *Populus tremula* L., *Betula* sp., *Quercus* sp., *Salix* sp. and *Sorbus aucuparia* L. The tree species targeted for artificial wounding were chosen because they were the most commonly occurring broad leaved trees in the Årungen Lake area near the town of Ås in Norway. Logs surveyed in sawmills

and loading bays were from *P. tremula* and *Betula pubescens* Ehrh., since these were the only species harvested at the time. Artificially induced wounds on standing trees were made using an axe to remove a strip of bark to expose the cambium (Barnes et al. 2003). A slit (1-5 mm thick) was also made into the xylem at the centre of the patches of exposed cambium where the bark had been removed. The wounds were approximately 5-10 cm^2, depending on the size of the tree wounded. One wound was made per tree at breast height (DBH). Trees ranged in diameter from 10-20 cm. Depending on the availability of suitably sized trees, one to ten trees for each of the above mentioned five species at Årungen Lake were wounded. All wounded trees were investigated for the presence of possible Ophiostomatoid fungi, six weeks after the wounds were made.

Isolations were made from all fruiting structures that resembled those produced by Ophiostomatoid fungi and which were growing on the bark and cambium of wounded standing trees and harvested logs. Spore drops or mycelial strands from fruiting structures were plated onto 2% Malt Extract Agar containing 100 mg/L Streptomycin (MEAS) (Sigma, Steinheim, Germany) and incubated at room temperature (~ 20 °C). Isolates were purified by transferring single spore drops to fresh unamended MEA. All isolates are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, and the culture collection of the Norwegian Forest and Landscape Institute (NFLI) with a sub-set deposited with the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

2.2 Fungal Identification

Isolates of a fungus resembling a *Sporendocladia* sp. were examined using a Carl Zeiss microscope with a Zeiss Axio vision camera system (Oberkochen, Germany) to confirm their identity. Fungal material was mounted in lactic acid (85%) for the light microscope studies. Descriptions of *Sporendocladia* (Wingfield et al. 1987) and morphologically similar genera such as *Leptographium* (Jacobs and Wingfield 2001) and *Phialocephala* (Kendrick 1961; Wingfield et al. 1987) were used to characterize isolates.

Comparisons of DNA sequence data were made to confirm the identity of the isolates resembling *Sporendocladia*. Two isolates of the *Sporendocladia*-like fungus from broad leaved trees sampled in Norway were grown on MEA plates for 14 days. Mycelium was scraped from the agar surfaces using a scalpel and ground to a fine powder using liquid nitrogen and sterile mortars and pestles. DNA was extracted from the ground mycelium using a modification of the method described by Möller et al. (1992) and as published by Roux et al. (2006).

The internal transcribed spacer (ITS) region and the 5.8S gene of the ribosomal DNA (rDNA) operon were amplified using the primers ITS1 and ITS4 (White et al. 1990). In each microcentrifuge tube, 1 µL of $MgCl_2$, 2 µL DNTP's, 2.5 µL Buffer with $MgCl_2$, 0.5 µL Primer, 17 µL water, 2 µL DNA and 0.5 µL Taq was included. The PCR programme was the same as that described by Roux et al. (2006). All PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide and visualized under UV illumination. Sizes of amplicons were estimated using a 100 bp molecular weight marker (XIV) (Roche

Diagnostics, Johannesburg, South Africa). Prior to sequencing, the PCR products were cleaned using the High Pure PCR product purification kit (Roche Diagnostics, Johannesburg, South Africa).

Purified PCR products were sequenced following the methods of Nakabonge et al. (2006) using the Big Dye Terminator Cycle sequencing reaction kit (Perkin Elmer Applied Biosystems, Foster City, CA) following the manufacturer's protocols. Sequence runs were done on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Primers ITS1 and ITS4 were used to sequence both strands of the amplicons.

DNA Sequence data were aligned online using MAFFT (Multiple alignment program for amino acid or nucleotide sequences) ver.5.667 (Katoh et al. 2005) (http://timpani.genome.ad.jp/~mafft/server/) and thereafter checked manually. Sequence analyses were done using MEGA version 5 (Tamura et al. 2011) and PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2002). The Heuristic search option in PAUP was used to generate phylogenetic trees. Sequences were analysed using parsimony, with the most parsimonious trees generated by heuristic searches, simple addition and Tree Bisection Reconstruction (TBR) branch swapping, with MULPAR effective. All missing, constant and uninformative data were excluded from the analysis. A bootstrap analysis using a 1000 replicates was performed (Felsenstein 1993). For the phylogenetic analysis, *Hypocrea americana* (Canham) Overton (Hypocreales) was used as the outgroup taxon.

2.3 Pathogenicity studies

To investigate the possible pathogenicity of the *Sporendocladia*-like fungus, *B. pubescens* and *P. tremula* saplings were inoculated under field conditions in Norway with four selected isolates of the fungus (CMW17908/NFLI2004-466/0141, CMW17910/NFLI2004-466/0283, CMW17912/NFLI2004-466/0201, CMW17913/NFLI2004-466/0461). Ten *B. pubescens* saplings, five at each of two sites near the town of Ås, were inoculated for each fungal isolate, while eight saplings (four at each site) served as controls. Ten *P. tremula* saplings for each isolate at a single site were also inoculated with the fungus, with four saplings serving as controls. All trees were 4-5 years old, with diameters at the point of inoculation at ~1-2 cm and heights of between two and three meters. Inoculations were done using ten-day-old fungal cultures. Discs of agar (3 mm diameter), overgrown with the test isolates were placed into wounds of equal size created with a metal cork borer. Plugs were placed into the wounds in such a manner that the mycelium faced the cambium. One inoculation wound was made per tree. Wounds were closed with Parafilm to prevent desiccation of the wounds and agar plugs. Control treatments were inoculated with plugs of sterile agar.

The development of lesions was evaluated and lesions measured after six weeks at which time re-isolations were also made from the resultant lesions. The lengths of the lesions produced in both the bark and cambium were measured. Results were analyzed statistically using the GLM Procedure in SAS (SAS Institute Inc. 1999). All data were tested for their agreement with the normal distribution and analysed according to the General Linear Model (GLM).

Adjustments for multiple comparisons were made using the Bonferroni procedure and variation between isolates tested at 95% Confidence limits.

3. Results

3.1 Fungal Isolates

Grey to blue mycelial mats were observed on the cambium surrounding wounds made on the stems of living trees, as well as on the cut ends of sampled logs where the bark had been damaged. Isolations made from these mats yielded several fungal genera including a fungus resembling a *Sporendocladia* sp., as well as species of *Ceratocystis*, *Ophiostoma* and *Thielaviopsis* (Roux et al. 2005; Fouche et al. 2007; Kamgan Nkuekam et al. 2010). A total of 18 isolates of the

Table 1. *Sporendocladia* isolates collected during a study of wounds (on standing trees and logs) on native broad leaved trees in Norway and Sweden.

Host	Area	Tissue type	Number of isolates[a]
Betula sp.	Årungen	Living, standing trees	2/9 (22)
	Filipstad, Sweden	Logs in loading bay	1/20 (5)
Populus tremula	Ås	Logs at sawmill	3/7 (43)
P. tremula	Årungen	Living, standing trees	1/1 (100)
P. tremula	Svenneby	Logs at sawmill	10/23 (43)
Quercus sp.	Årungen	Living, standing trees	1/4 (25)

[a]First number reflects number of trees from which *Sporendocladia* isolates were obtained and second number the trees of each species from which Ophiostomatoid fungi were isolated. % success is presented in brackets.

Sporendocladia sp. were collected from *Betula* spp., *P. tremula*, and *Quercus* sp. trees sampled from locations in Norway and South Western Sweden (Table 1). Four of the *Sporendocladia* isolates obtained from *Quercus* sp. and *P. tremula* at Årungen were obtained from standing trees, while the other 14 isolates were collected from logs at sawmills and in loading bays.

Table 2. Isolates used in phylogenetic study of ITS sequence data obtained from GenBank.

Species	**GenBank Accession Number**
Ceratocystis fimbriata	AY157956
C. moniliformis	AY529000
C. polonica	AY233925
Corollospora fusca	JN943385
C. maritima	JN943387
Custingophora olivacea	AM267269
Doratomyces nanus	JN104558
Graphium basitruncatum	EF165016
G. penicillioides	AB038432
Gondwanamyces capensis	EU660447
G. scolytodis	AM267268
Hypocrea americana	DQ491488
Microascus manginii	JN942891
M. trigonosporus	AM774156
Petriella setifera	AJ784398
P. sordida	GU586850
Pseudallescheria boydii	AY877359
Scopulariopsis brevicaulis	JN942890
Scedosporium aurantiacum	AB461361
S. dehoogii	JQ302817
Sporendocladia bactrospora	AF486123
S. bactrospora	AF486130

3.2 Fungal identification

While having the peripheral appearance of a *Leptographium* sp. with dark mononematous conidiophores, the *Sporendocladia*-like fungus isolated from broad leaved trees in Norway and Sweden had very distinct, tubular collarettes at

the apices of the conidiogenous cells. Conidia accumulated in slimy masses at the apices of these conidiophores. Closer examination revealed cylindrical conidia with truncate ends produced in chains. Based on morphology, this fungus was identified as *Sporendocladia bactrospora*.

Phylogenetic analyses comparing sequences for the isolates from Norway with those of published, related species obtained from GenBank (Table 2), confirmed the identity of the isolated fungus as a *S. bactrospora* (Fig. 1). Isolates from Norway showed a 100% sequence homology for the ITS region to that available in GenBank. One tree was obtained from the heuristic searches, using 443 parsimony informative characters. The Consistency Index (CI), Retention Index (RI) and Rescaled Consistency Index (RC) values for the consensus trees were 0.551, 0.613 and 0.338, respectively. Our results also confirmed previous reports (Jacobs et al. 2003) that *S. bactrospora* resides in the Microascales. Of the genera included in the analyses, *S. bactrospora* isolates grouped most closely to fungi in the Ceratocystidaceae and Gondwanamycetaceae (Seifert et al, 2013), as well as *Custingophora olivacea* Stolk, Hennebert & Klopotek (Fig. 1).

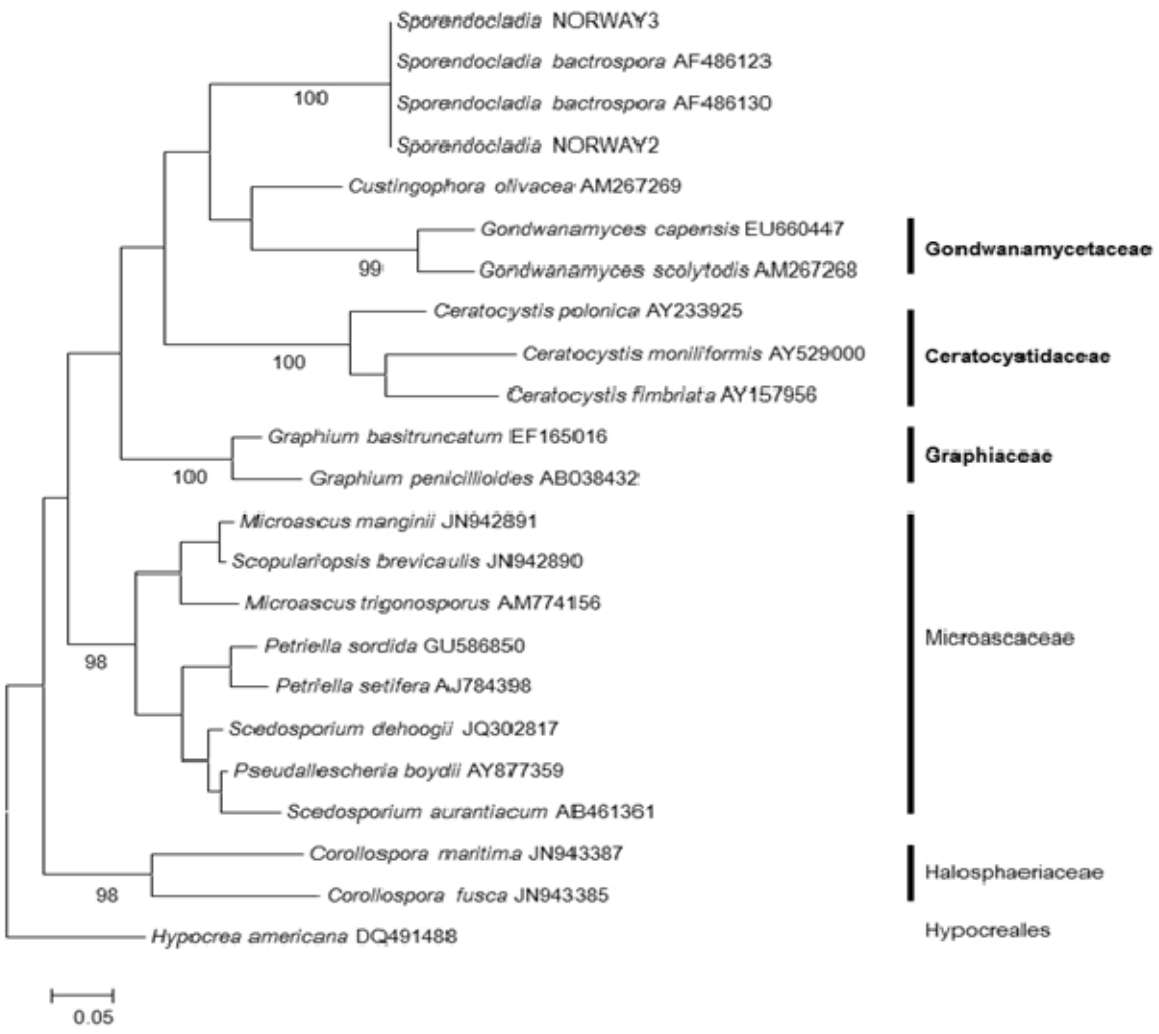

Figure 1 Maximum likelihood tree produced using Mega and showing the identity of *S. bactrospora* isolates from Norway collected in this study. *Hypocrea americana* was used as the outgroup taxon. Bootstrap values above 80% are indicated below the branches and the respective families of the Microascales are indicated on the right. The *S. bactrospora* isolates are more similar to Ophiostomatoid fungi in the Microascales, than to the other families in the order.

3.3 Pathogenicity studies

Distinct lesions developed on both *B. pubescens* and *P. tremula* saplings after inoculation with *S. bactrospora* (95% Confidence Limits). Lesions in the xylem (Fig. 2), in the form of light to dark brown streaks, were much longer than those

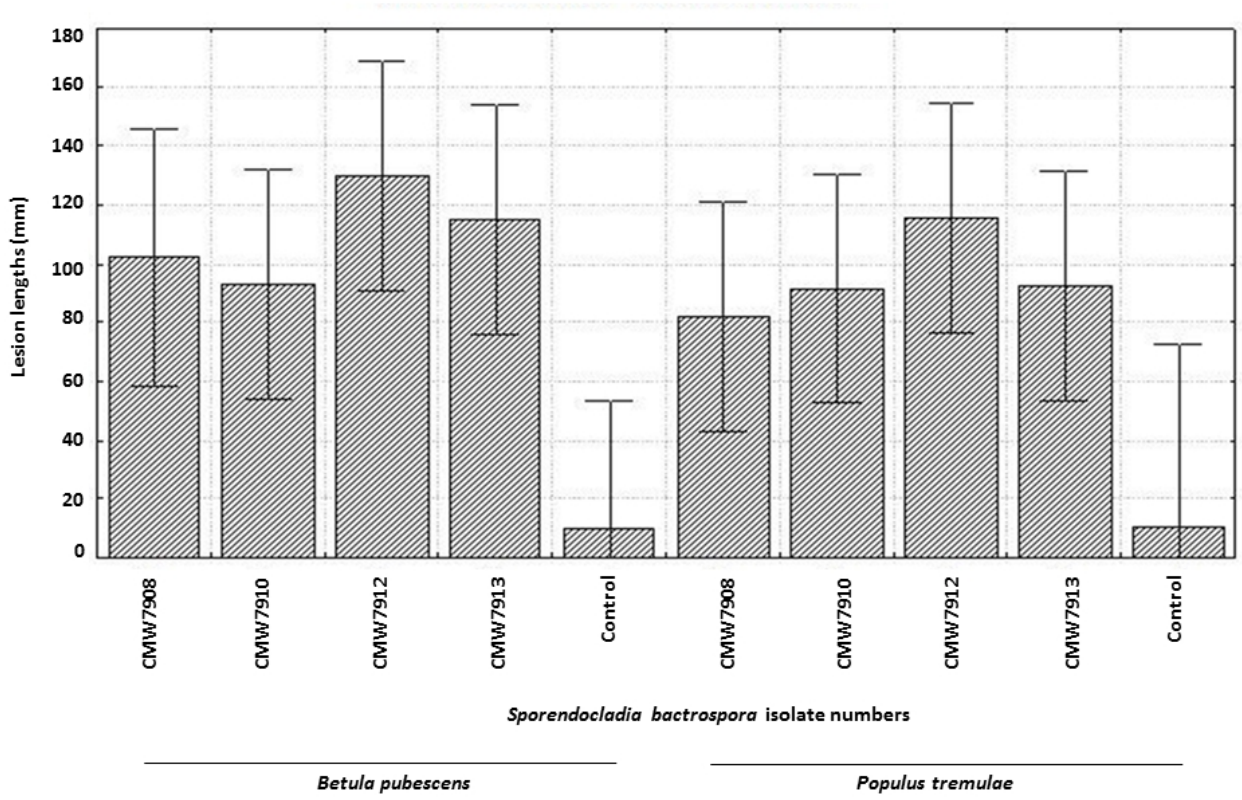

Figure 2 Xylem lesions (mm) produced by *S. bactrospora* on *Betula* sp. and *Populus* sp. after six weeks. (R-square = 0.24 and Coeff Var = 81.66 for *Betula* sp.; R-square = 0.31 and Coeff Var = 49.01 for *Populus* sp.). Bars indicate the 95% Confidence Limits for each isolate on each host.

produced in the bark (Fig. 3). Other than those produced in the xylem of *P. tremula*, lesions produced were significantly different from the controls. For the xylem lesions, there were no significant differences between the two hosts or between the isolates used (95% Confidence limits; Pr<0.0001). Lesions on the bark of *P. tremula* were generally longer than those on *B. pubescens,* however, this was not supported statistically for all isolates (95% Confidence Limits; Pr<0.0001). The *S. bactrospora* isolates that were used to inoculate the saplings were re-isolated from all of the *Betula* and *Populus* trees on which lesions had developed.

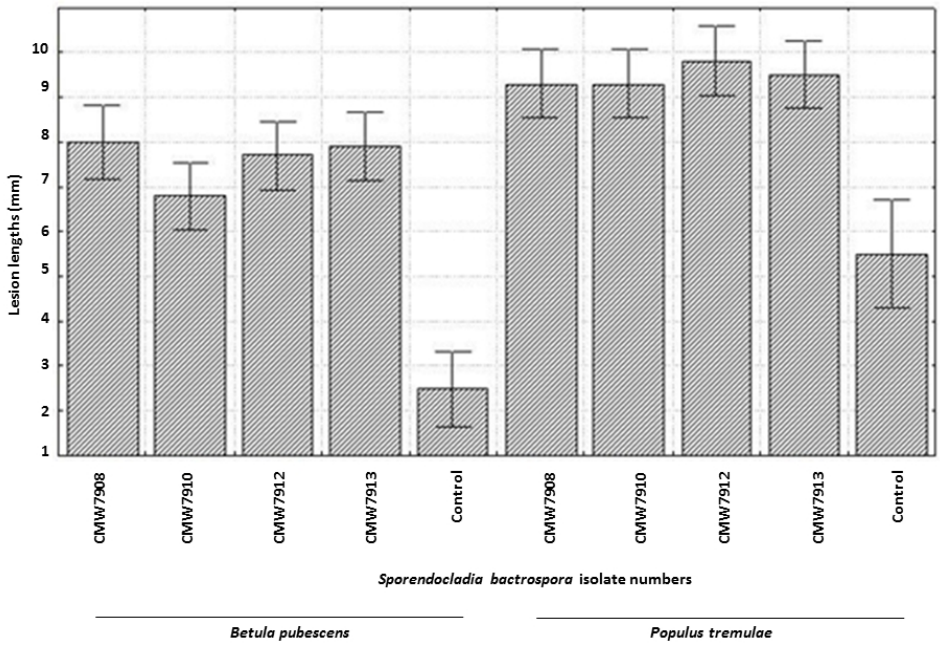

Figure 3 Bark lesions (mm) produced by *S. bactrospora* on *Betula* sp. and *Populus* sp. after six weeks. (R-square = 0.73 and Coeff Var = 19.03 for *Betula* sp.; R-square = 0.53 and Coeff Var = 12.58 for *Populus* sp.) (P<0.0001). Bars indicate the 95% Confidence Limits for each isolate on each host.

4. Discussion

This study reports on new hosts and a new niche for *S. bactrospora*. This is the first report of *S. bactrospora* from Norway and Sweden. Previous records of the fungus include those from Canada (Grünig et al., 2002; Kendrick 1961), England (Kendrick 1961), Japan (Jacobs et al. 2003) and Spain (Luque et al. 2000). *Sporendocladia bactrospora* appears to be a unique member of the genus as most other species are saprophytes isolated from rotting wood or rotting plant material. In this study, the fungus was isolated from fresh wounds on living trees, as well as from harvested logs.

Results of this study are consistent with those from a previous report showing that *S. bactrospora* may be a weak pathogen of trees (Luque et al. 2000). In a study of oak decline in Spain, *S. bactrospora* was one of several fungi isolated from diseased and dying *Q. suber* trees. Stem inoculations using *S. bactrospora* showed that it could produce lesions on these trees, although it did not result in seedling mortality. The study of Luque et al. (2000) also considered the impact of environmental stress, such as water stress, on potential disease development, showing that increased water stress resulted in decreased lesion sizes for *S. bactrospora*. Our inoculation studies showed that *S. bactrospora* has the potential to result in significant stain of the xylem of young *B. pubescens* and *P. tremula* trees. Further studies are, however, required to understand the role of this fungus on living trees and also how it moves from one tree to another. Given

the ecology of related fungi such as the anamorphs of *Ceratocystis* spp., it seems likely that sap feeding insects carry it from one tree to another.

Jacobs et al. (2003), showed, based on SSU & LSU DNA sequence data, that *S. bactrospora* resides in the Microsascales. Of the four genera included in their study, *S. bactrospora* grouped most closely with *Ceratocystis* spp. In the present study where we used sequence data only to confirm the identity of this fungus, we included twelve genera in the Microascales. Results confirmed that *Sporendocladia* is a member of the Microascales and has a closer relationship to species of *Ceratocystis*, *Gondwanamyces* and *Custingophora* than to *Graphium* and *Petriella* spp. No sexual state is, however, known for *S. bactrospora* and efforts to induce one in this study have failed.

This study confirms that *S. bactrospora*, initially considered a colonist of dead and rotting plant material, could play a role in tree disease and that it deserves further consideration. Its ecological role on trees should be investigated in greater detail, especially its potential to cause economic damage to timber. Results have considerably expanded available knowledge for a little known fungus, which is worthy of further study.

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REFERENCES

Anonymous 1986: *Ceratocystis fimbriata* (Ell. & Halsted) f.sp. *platani* (Walter). Bulletin OEPP/EPPO Bulletin **16**, 21-24.

Arnaud, G., 1954: Mycologie concrete: Genera. Bull. Trim. Soc. Mycol. Fr. **68**, 181-223.

Barnes, I.; Roux, J.; Wingfield, B.D.; Dudzinski, M.J.; Old, K.M.; Wingfield; M.J., 2003: *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. Mycologia **95**, 865-871.

Brasier, C.M., 2000: Intercontinental spread and continuing evolution of the Dutch elm disease pathogens, in: Dunn CP (eds), The elms: breeding, conservation and disease management. Kluwer Academic Publishers., Boston USA, Dordrecht The Netherlands, London UK, PP 61-72.

Felsenstein, J., 1993: DNABOOT-Bootstrap Confidence Interval on DNA parsimony 3.1. University of Washington, Seattle, USA.

Fouche, N.; Roux, J.; Solheim, H.; Heath, R.N.; Kamgan Nkuekam, G.; Chimwamurumbe, P.; Pegg, K.G.; Wingfield, M.J., 2007: Identification of *Ceratocystis* species isolated from hardwood trees in Namibia, Australia and Norway. 45th Congress of the Southern African Society for Plant Pathology. 21-24 January 2007, Kopanong Conference Centre, Benoni, South Africa.

Grünig, C.R.; Sieber, T.N.; Rogers, S.O.; Holdenrieder, O., 2002: Genetic variability among strains of *Phialocephala fortinii* and phylogenetic

analysis of the genus *Phialocephala* based on rDNA ITS sequence comparisons. Can. J. Bot. **80**, 1239-1249.

Henry, B.W., 1944: *Chalara quercina* n.sp., the cause of oak wilt. Phytopath **34**, 631-635.

Hubbes, T., 1999: The American Elm and Dutch Elm disease. For Cron **75**, 265-273.

Jacobs, K.; Wingfield, M.J., 2001: Leptographium species: tree pathogens, insect associated and agents of blue-stain. American Phytopathological Society Press, St. Paul, USA. ISBN 0-89-54-278-3.

Jacobs, A.; Coetzee, M.P.A.; Jacobs, K.; Wingfield M.J., 2003: Phylogenetic relationships among *Phialocephala* species and other ascomycetes. Mycologia **95**, 637-645.

Kamgan Nkuekam, G.; Solheim, H.; De Beer, Z.W.; Grobbelaar, J.W.; Jacobs, K.; Wingfield, M.J.; Roux, J., 2010: *Ophiostoma* species, including *Ophiostoma borealis* sp. nov., infecting wounds of native broad-leaved trees in Norway. Crypt. Mycol. **31**, 285-303.

Katoh, K.; Kuma, K.; Toh, H.; Miyata, T., 2005: MAFFT version 5: Improvement in accuracy of multiple sequence alignment. Nucl. Acid Res. **33**, 511-518.

Kendrick, W.B., 1961: The *Leptographium* complex. *Phialocephala* gen. nov. Can. J. Bot. **39**, 1079-1085.

Kendrick, W.B., 1963: The *Leptographium* complex. Two new species of *Phialocephala*. Can. J. Bot. **41**, 1015-1023.

Luque, J.; Parladé, J.; Pera, J., 2000: Pathogenicity of fungi isolated from *Quercus suber* in Catalonia (NE Spain). For. Pathol. **30**, 247-263.

Möller, E.M.; Bahnweg, G.; Sandermann, H.; Geiger, H.H., 1992: A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucl. Acid Res. **20**, 6115-6116.

Nag Raj, T.R.; Kendrick, W.B., 1975: A monograph of *Chalara* and allied genera. Wilfrid Laurier University Press.

Nakabonge, G.; Gryzenhout, M.; Roux J.; Wingfield, M.J., 2006: *Celoporthe dispersa* gen. et sp. nov. from native Myrtales in South Africa. Stud. Mycol. **55**, 259-271.

Onofri, S.; Zucconi, L., 1984: Two new species of the genus *Phialocephala*. Mycotaxon **20**, 185-195.

Panconesi, A., 1981: Canker stain of plane trees: A serious danger to urban plantings in Europe. J Plant Path **81**, 3-15.

Paulin-Mahady, A.E.; Harrington, T.C.; McNew, D.L., 2002: Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis*, and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia **94**, 62-72.

Roux, J.; Solheim, H.; Wingfield, M.J., 2005: *Ceratocystis* and *Ophiostoma* species infecting native broad leaf trees in Norway. Proceedings of the 43rd Congress of the Southern African Society for Plant Pathology, Hartenbos Beach Resort, Mosselbaai, 23-26 January 2005.

Roux, J.; Mthalana, B.L.; De Beer, Z.W.; Eisenberg, B.; Wingfield, M.J., 2006: Quambalaria leaf and shoot blight on *Eucalyptus nitens* in South Africa. Austral. Plant Path. **35**, 427-433.

SAS Institute Inc., SAS/STAT Users Guide, Version 8, Cary NC: SAS Institute Inc., 1999. ISBN 1-58025-494-2

Seifert, K.A.; De Beer, Z.W.; Wingfield, M.J., 2013: The Ophiostomatoid Fungi: Expanding Frontiers. Biodiversity Series 12 CBS, Utrecht, the Netherlands.

Sinclair, W.A.; Johnson, W.T.; Lyon, H., 1987: Diseases of trees and shrubs. Cornell University Press, Ithaca, New York, USA, PP. 574.

Sutton, B.C., 1975: Hyphomycetes on cupules of *Castanea sativa*. Trans. Brit. Mycol. Soc. **64**, 405-426.

Sutton, B.C., 1993: Mitosporic fungi from Malawi. Mycological Papers **167**, 93 pp.

Swofford, D.L., 2002: PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K.; Peterson, D.; Peterson, N.; Stecher, G.; Nei, M.; Kumar, S., 2011: MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution **28**, 2731-2739.

Walter, J.M., 1946: Canker stain of plane trees. USDA Circular, No. 742

White, T.J.; Bruns, T.; Lee, S.; Taylor, J., 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis,

A., Gelfand D.H, Sninsky J.J. and White T.J. (Eds.), PCR Protocols: A sequencing guide to methods and applications. M. Academic Press, San Diego, pp. 315-322.

Wingfield, M.J., 1985: Reclassification of *Verticicladiella* based on conidial development. Trans. Brit. Mycol. Soc. **85**, 81-93.

Wingfield, M.J.; Seifert, K.A.; Webber, J.F., 1993: *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. American Phytopatholocal Society Press, St. Paul Minnesota, USA.

Wingfield, M.J.; Van Wyk, P.S.; Wingfield, B.D., 1987: Reclassification of *Phialocephala* based on conidial development. Trans. Brit. Mycol. Soc. **89**, 509-520.