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ORIGINAL ARTICLE

Sphaeropsis sapinea and fungal endophyte diversity in twigs of Scots pine (*Pinus sylvestris)* in Germany

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Abstract

Sphaeropsis sapinea is the causal fungal agent of Diplodia tip blight disease of Scots pine (*Pinus sylvestris*) and other coniferous trees of relevance to forestry in Germany. In this study, the distribution and occurrence of *S. sapinea* and accompanying endophytic fungi in twigs of healthy and diseased Scots pine was investigated on a spatial and temporal scale. Sampling of 26,000 twig segments from trees in 105 temperate coniferous forest stands in Germany resulted in isolation of 33,000 endophytic fungi consisting of 103 species identified based on morphological and ITS-DNA sequence analyses. Approximately 98% of the sample was represented by fungi in the Ascomycota, with only two species (*Peniophora pini* and *Coprinellus* sp.) belonging to the Basidiomycota. Four species were detected in a frequency greater than 10% (*Sphaeropsis sapinea*, *Sydowia polyspora*, *Microsphaeropsis olivacea,* and *Truncatella conorum-piceae*) from the collective sample. A typical inhabitant of Scots pine twigs *Desmazierella acicola* was isolated and additionally typical hardwood colonizers like *Biscogniauxia* spp. were detected. *S. sapinea*, an endophytic plant pathogen with saprobic capabilities, was isolated from more than 80% of the studied pine trees, but the majority of trees sampled showed no symptoms of Diplodia tip blight. No invasive, pathogenic quarantine fungi for Germany were isolated from healthy or diseased Scots pines. Advantages and disadvantages of isolation-based endophyte studies over studies using direct DNA-isolation are discussed. Knowledge of the fungal endophyte communities in twigs of Scots pine allowed for identification *S. sapinea* and other potential pathogens of pines and other forest trees that may possibly contribute to increased disease under repeated periods of drought and heat stress in the future.

Keywords Endophytic fungi · *Pinus sylvestris* · *Sphaeropsis sapinea* · Diplodia tip blight

Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most important conifer tree species in Northern and Central Europe. In Germany, this tree species is a very important factor in forest and timber management and economics. Natural Scots pine forests are rather rare in Germany, due primarily to its low competitiveness compared with other, more shade tolerant tree species such as European beech (*Fagus sylvatica* L.). Therefore, native pine forests are restricted to azonal vegetation types, for example, sand dunes or the edges of moors or moorlands. Most of the natural forests with Scots pine as the main tree species are found in Northeastern Germany. *P. sylvestris* has a wide ecological amplitude (Ellenberg and Leuschner 2010), it is considered to be a stress tolerant, easy to regenerate, versatile, and light demanding pioneer species. Typical for Northern parts of Germany are secondary or man-made pine stands. With ca. 2.4 million hectares, *P. sylvestris* has a 22.9% share of the German forested area (Thünen-Institut 2014); however, areas forested with pine are decreasing. This decline has several causes, but risk factors, such as global climate change and new or emerging fungal diseases, play an important role (Mason and Alía 2000; Drenkhan et al. 2016). Scots pine forests covers 28 million hectares in Europe and thus make up 20% of the commercial forest area of the European Union (Mason and Alía 2000).

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Sphaeropsis sapinea (Fr.) Dyko & B. Sutton (syn. *Diplodia pinea* (Desm.) J. Kickx f.) is recognized as the most widespread necrotrophic ascomycete pathogen responsible for dramatic losses of pine trees across the continents (Fabre et al. 2011; Phillips et al. 2013). It spreads from needles via stomata or injured tissue into the host and results in disease symptoms that include tip blight, stem canker, dieback of current year shoots, and blue staining of sapwood (Brookhouser and Peterson 1971; Munck et al. 2009). It is also known to be present and persistent as a symptomless endophyte in pine twigs (Langer et al. 2011; Fabre et al. 2011; Luchi et al. 2014). Pathogenic occurrence of *S. sapinea* can be triggered by stress, which is particularly important in the context of climate change with precipitation deficits and increased temperatures that weaken pine (Fabre et al. 2011; Bosso et al. 2017). This species of the Botryosphaeriaceae family has appeared as a pathogen in Central Europe since the 1980s (Swart and Wingfield 1991). In Germany, infections of Scots pines and Austrian pines (*Pinus nigra* J.F. Arnold) by *S. sapinea* causing serious forest health problems were observed in the middle of the 1990s by Heydeck and Dahms (2012) and later by Langer et al. (2011). The geographical origin of the native distribution of *S. sapinea* is not known (CABI 2014) and it is questionable whether the species is native to Europe (Desprez-Loustau et al. 2009). Knowledge of the latent distribution of *S. sapinea* in Germany and occurrence of other potential pine pathogens or quarantine pests is of great interest. The aim of this study is to determine the composition of cultivable fungal endophytes (in the sense of Petrini (1991)) of Scots pine in Germany at the species level and to assess the taxa regarding their significance for forestry.

The distribution of *S. sapinea* in Germany, and environmental factors triggering the outbreak of disease symptoms after a period of symptomless existence of *S. sapinea* in apparently healthy twigs of pines, were studied by Bußkamp (2018) in a doctoral thesis. Results of that research are presented as part of this study. The fungal endophytes of pine branches were previously studied by Kowalski and Kehr (1992). Various other authors have studied diverse tissues of Scots pine with regard to colonization by mycobiota (Carroll et al. 1977; Petrini and Fisher 1988; Fisher et al. 1991; Kowalski and Kehr 1992; Kowalski 1993; Pirttilä et al. 2003; Lygis et al. 2004; Pinto et al. 2006; Menkis et al. 2006; Kwaśna 2008; Giordano et al. 2009; Menkis and Vasaitis 2010; Peršoh et al. 2010; Terhonen et al. 2011; Martínez-Álvarez et al. 2012; Romeralo et al. 2012; Sanz-Ros et al. 2015; Millberg et al. 2015). In contrast to the aforementioned studies, in this work the incidence of disease and endophytic occurrence of *S. sapinea* was investigated on a German spatial base with a large and comprehensive spatial and temporal sample. Additionally, an assessment of the endophytes concerning their lifestyles, trophic status, and relevance to forest health is presented.

Materials and methods

Sample collection

From 2014 to 2016, in different seasons, twigs were sampled from Scots pine (*Pinus sylvestris* L.) from 105 forest sites in Germany by climbing or felling trees or by archery (Fig. 1 and supplementary Table). A systematic sampling approach was used to collect *P. sylvestris* twigs along a transect from the Northeast to the Southwest of Germany in climate sensitive regions (91 sites, Fig. 1). Transect sampling was conducted at grid points of the Forest Condition Survey grid (Thünen-Institut 2020); and 14 additional pine stands were also examined with at least 6 trees per stand being sampled (Fig. 1). Trees sampled from the latter stands were diseased by Diplodia tip blight and were also infested with phyllophagous insects (e.g., *Dendrolimus pini* L., *Thecodiplosis brachyntera* Schwägrichen) or mistletoe (*Viscum album subsp. austriacum* (Wiesb.) Vollm.) at the time of sampling. At the localities Pfungstadt, Bad Freienwalde, and Lüderitz, pairwise comparisons of stands with healthy and diseased trees were performed. Monthly sampling, over a period of 13 months (September 2015–October 2016), of twigs collected from a single tree was carried out in a forest stand close to the city of Dransfeld in the South of Lower Saxony (Fig. 1). Sampled trees were older than 50 years and located 20 to 600 m above sea level in stands that were dominated by Scots pine. Most stands were secondary man-made forests, partially outside the natural range of Scots pine (EUFORGEN 2008). Three shoot tips per tree were arbitrarily selected, placed in a sterile plastic bag and transported in a cool box (8 °C) to the laboratory. Twig samples were divided into two categories based on occurrence of Diplodia tip blight symptoms (healthy twigs from trees with symptoms ($n = \sim 2800$ samples/segments) and twigs from trees without symptoms ($n = \sim 23{,}000$ samples/segments)). Ocular visible symptoms of Diplodia dieback of shoots and browning of the crown were also recorded. Symptomatic branches were examined in the laboratory for the presence of *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton.

Isolation and determination of fungi

Three randomly selected tips per tree from 3 to 4-year-old branches ($n = 190$ *P. sylvestris* trees) were defoliated, washed, and surface disinfested by treating for 1 min in 70% EtOH, 5 min in a 3% NaOCl, and 1 min in 70% EtOH (Bußkamp 2018). Pre-treatments (washing and brushing), different disinfestation methods (duration of soaking, concentration of NaOCl), and post-treatments (washing in water or EtOH, drying method) were tested in preliminary experiments (data not shown). The method selected for this study was verified by additional testing (imprinting, rolling in sporulating cultures of previously disinfested twigs and plating of rinse water,

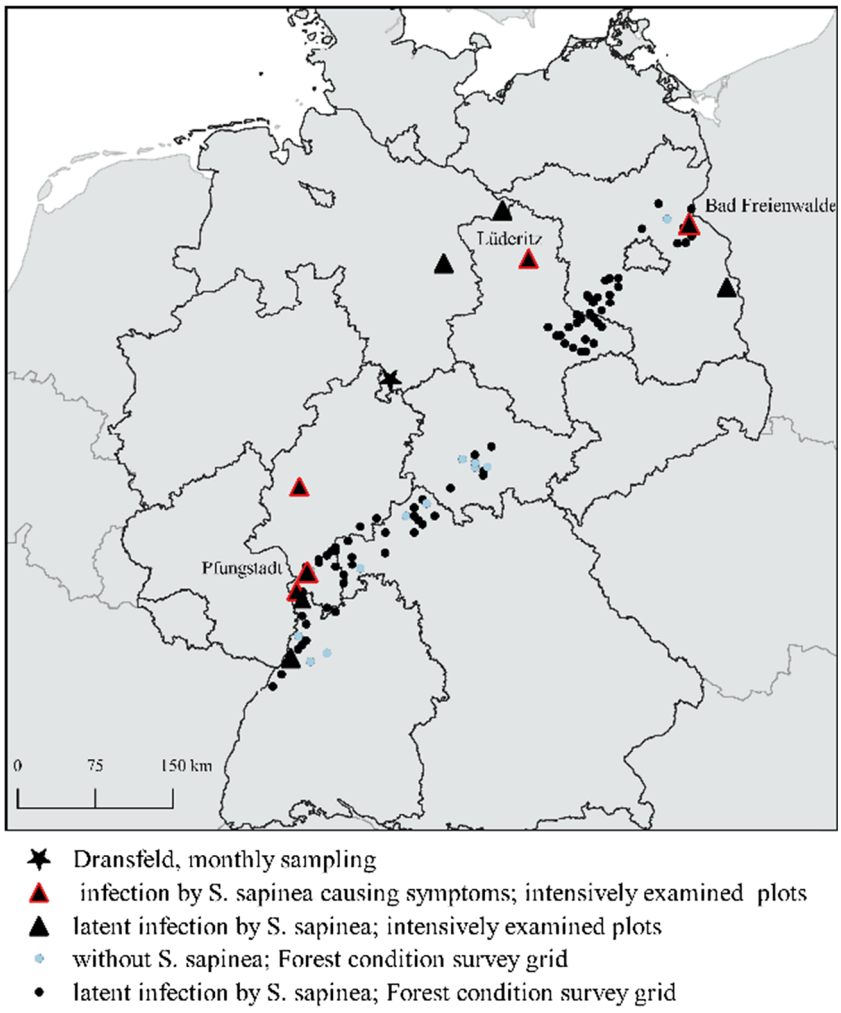

Fig. 1 Sampling sites in Germany 2014–2016; © GeoBasis-DE / BKG 2014 and © EuroGeographics

followed by monitoring of fungal growth). Thereafter, twigs were cut into 5-mm length pieces (hereinafter referred to as segments), plated on malt yeast peptone agar (MYP) modified according to Langer (1994) containing 0.7% malt extract (Merck 1.05391.0500, Darmstadt, Germany), 0.05% yeast extract (Fluka 70,161-100G, Seelze, Germany), 0.1% peptone (Merck 1.07272.0500), and 1.5% agar (Fluka 05040-1KG). Usually, 3 twig segments were placed on MYP medium in a 90-mm-diameter plastic Petri dish and incubated for up to 3 weeks at room temperature (ca. 22 °C) and ambient daylight. Twig segments were visually checked, weekly, for developing colonies. Emerging mycelia were sub-cultured separately on MYP medium. Isolated strains were initially assigned to mycelial morphotypes (MTs) further characterized based on micro-morphological characters and DNA sequence analysis. Representative fungal strains were stored on MYP slants at 4 °C.

From each morphotype, 1–2 mg of culture tissue was suspended in 100 μl TE buffer in a 1.5-ml tube. A microwave (600 W) was used twice for 1 min each time, with a pause of 30 s, to break up cells. Tubes were cooled to − 20 °C for 20 min and centrifuged at 10000 rpm for 5 min. A 100 times diluted portion of the supernatant was used for DNA with the polymerase chain reaction (PCR). Primer pairs for amplification of the ITS1, 5.8S, and ITS2 regions were ITS1F/ITS4 or ITS1/ITS4 (White et al. 1990; Gardes and Bruns 1993). PCR was performed with 45-μl Master mix from QIAGEN, Hilden, Germany, where 5 μl of extracted DNA was added. PCR was carried out using the primer pairs, with initial denaturation at 94 °C for 3 min, followed by 29 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 60 s; final elongation was performed at 72 °C for 7 min. PCR products were separated on 1% agarose gel stained with GelRed fluorescence dye (Biotium, Hayward, CA, USA), followed by a cleaning with QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Sanger sequencing of purified products (Sanger et al. 1977) was commissioned at GATC Biotech (Cologne, Germany). Editing and alignment of DNA sequences were performed with MEGA6 (Tamura et al. 2013) followed by submission to GenBank (Table 1).

For identifying MTs, a ZEISS Axiostar plus microscope was used and the standard procedures for fungi described in Lee and Langer (2012) were followed. In addition to standard literature recommended by Oertel (2003) for determination of fungi and forest diseases, the following literature was used, e.g., Guba 1961; Booth 1971; Domsch et al. 1993; Arx 1981; Gerlach and Nirenberg 1982; Breitenbach and Kränzlin 1984; Ju et al. 1998; Verkley 1999; Samson et al. 2010; and Butin 2011. Names of fungal species follow Index Fungorum (www.indexfungorum.org). At least one representative strain of each morphotype was used for DNA sequence analysis. Sequences were submitted to GenBank and the BLAST algorithm (http://www.ncbi.nlm.nih.gov/genbank, Altschul et al. 1997) was used for fungal taxon confirmation. Intraspecific ITS sequence similarity of 98–100% was used to determine species identity. Query coverage of all taxa was between 90 and 100%. Except for *Truncatella conorum-piceae* (54%) where there was only a single reference for comparison, the arithmetic mean of overlap was 97.7%. The frequency of each fungal species sampled from twigs was specified as the percentage of this particular fungus in all outgrowing fungi.

The map (Fig. 1) was made using QGIS (www.qgis.org). Evaluation of the endophyte data was analyzed using ordination (Detrended Correspondence Analysis and Canonical Correspondence Analysis, data not shown); test of significance was performed using R (R Core Team 2019, www.r-project.org).

Results

From 190 analyzed trees at 105 sites, 103 species of endophytic fungi were sampled (Table 1). With 101 species belonging to the Ascomycota and two species to the Basidiomycota (*Peniophora pini* (Schleich. ex DC.) Boidin and *Coprinellus* sp.). No fungi were recovered from approximately 5% of the sampled twig segments.

Fungi belonging to the Pleosporales represented the most frequent sample group of fungi (27% of the isolated strains) and showed a high species-richness (19 species: *Alternaria alternata* (Fr.) Keissl.; *A. infectoria* E.G. Simmons; *Alternaria* sp., *Drechslera* sp., *Epicoccum nigrum* Link; *Microsphaeropsis olivacea* (Bonord.) Höhn.; *Paraphaeosphaeria neglecta* Verkley, Riccioni, & Stielow; *Pa. verruculosa* Verkley, Göker, & Stielow; *Periconia* sp., *Phoma eupyrena* Sacc., 4 unidentified *Phoma* species, 2 unidentified *Preussia* species, and 3 unidentified Pleosporaceae), whereas, e.g., the Dothideales grew out frequently (22% of the species belong to Dothideales), but only one species was detected in this order (*Sydowia polyspora* (Bref. & Tavel) E. Müll.). Ca. 13% of the isolated strains were assigned to Botryosphaeriales (2 species: *Camarosporium brabeji* Marinc., M.J. Wingf. & Crous and *Sphaeropsis sapinea*), and respectively 10% Amphisphaeriales (3 species: *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, *Truncatella conorum-piceae* (Tubeuf) Steyaert, and *Truncatella* sp. 2), 9% Xylariales (19 species: *Arthrinium kogelbergense* Crous, *Biscogniauxia mediterranea* (De Not.) Kuntze, *B. nummularia* (Bull.) Kuntze, *Daldinia childiae* J.D. Rogers & Y.M. Ju, *D. concentrica* (Bolton) Ces. & De Not., *Daldinia* sp., *Hypoxylon fragiforme* (Pers.) J. Kickx f., *H. rubiginosum* (Pers.) Fr., *Nemania diffusa* (Sowerby) Gray, *N. serpens* (Pers.) Gray, two unidentified *Pestalotiopsis* species, two unidentified *Rosellinia* species, *Xylaria longipes* Nitschke, *X. polymorpha* (Pers.) Grev., and three unidentified *Xylaria* species), 5% Sordariales (10 species: *Chaetomium globosum* Kunze, two unidentified *Chaetomium* species, *Jugulospora rotula* (Cooke) N. Lundq., *Podospora curvicolla* (G. Winter) Niessl, *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not., two unidentified *Sordaria* species, *Trichocladium* sp., and an unidentified Sordariales species), 5% Diaporthales (10 species: three not further identified *Apiognomonia* species, *Cytospora* sp., four unidentified *Diaporthe* species, *Plagiostoma* sp., and an unidentified Gnomoniaceae), 4% Helotiales (8 species: *Botrytis cinerea* Pers., *Lambertella* sp., *Pezicula cinnamomea* (DC.) Sacc., *P. eucrita* (P. Karst.) P. Karst., *P. neosporulosa* Z.L. Yuan & Verkley, two unidentified *Pezicula* species, and *Phacidium lacerum* Fr.), and 2% Pezizales (5 species: *Chromelosporium carneum* (*Pers.*) Hennebert, *Desmazierella acicola* Lib., *Peziza varia* (Hedw.) Alb. & Schwein., *Pyronema domesticum* (Sowerby) Sacc., and an unidentified Pezizales species). The rest of the isolated species occurred with a lower abundance in the study: 6 species of the Hypocreales (*Trichoderma* sp., *Lecanicillium psalliotae* (Treschew) Zare & W. Gams, *Fusarium* sp., *Fusarium* sp. 1, *Fusarium solani* complex, and *Beauveria bassiana* (Bals.-Criv.) Vuill.), *Nigrospora oryzae* (Berk. & Broome) Petch, *Nigrospora* sp., two Capnoidales species (two *Cladosporium* sp.), three Coniochaetales (two *Lecythophora* species and *Coniochaeta ligniaria* (Grev.) Cooke, a single Eurotiales (*Penicillium* sp.), a single Umbelopsidales (*Umbelopsis isabellina* (Oudem.) W. Gams), and few other Ascomycota not determined to the order level (Fig. 2 and Table 1).

Four fungal species were sampled with a frequency higher than 10% as follows: *M. olivacea* (23%), *Sy. polyspora* (22%), *S. sapinea* (12%), and *T. conorum-piceae* (10%). Fifteen species had a frequency exceeding 1%. All other species were isolated less often. *S. sapinea*, *M. olivacea*, *Sy. polyspora*, *T. conorum-piceae*, *So. fimicola*, and *A. alternata* were isolated from more than 50% of sampled trees. The most common species were isolated from more than 80% of the studied stands along the transect: *Sy. polyspora* (99%), *T. conorum-piceae* (98%), *M. olivacea* (97%), and *S. sapinea* (88%). Other fungi that were found in more than 50% of the study areas were as follows: *Diaporthe* sp. 2 and *A. alternata* as well as *Ne. serpens*. Neither fungal quarantine pests (according to EPPO A1 and A2 List (EPPO 2020a, b)) nor alien species (Desprez-Loustau 2009) for Germany were identified. The following 24 species

Table 1 Species list, frequency (rounded) of twig-inhabiting fungal endophytes of *Pinus sylvestris*, the GenBank accession numbers. Assessment of the significance for forestry, only made for fungi determined to species level, *first time described as an endophyte of *P. sylvestris* twigs, +isolated from diseased trees in this study

Taxon name	Frequency	% Sites	Systematics	GenBank accession	GenBank accession Blast	Assessment of the significance for forestry
Acremonium sp.	< 0.01	1.9	Hypocreales	MG098294	KJ194115	
Alternaria alternata+	1.5	60.0	Pleosporales	MG098257	KU179665	Generalist; Samson et al. (2010)
Alternaria infectoria+	0.3	30.5	Pleosporales	MG098290	JQ781841	Generalist, Pathogen; Samson et al. (2010)
Alternaria sp.+	0.1	3.8	Pleosporales	MG098289	KJ789851	
Apiognomonia sp. 3	< 0.1	1.0	Diaporthales	MK066898	KX776422	
Apiognomonia sp. 1	< 0.1	4.7	Diaporthales	MG098319	EU255016	
Apiognomonia sp. 2	< 0.1	< 1	Diaporthales	MG098323	AJ888475	
*Arthrinium kogelbergense**	< 0.01	< 1	Xylariales	MG098297	KF144895	Generalist, saprophyte; Crous and Groenewald (2013)
Ascomycete sp. 1 +	< 0.01	1.9		MG098282	EF42004	
Ascomycete sp. 2 +	< 0.1	2.9		MG098293	AJ698477	
Ascomycete sp. 3 +	<0.1	2.9		MG098310	AY561220	
Ascomycete sp. 4	< 0.01	1.0		MK066901	KY792589	
Beauveria bassiana+	0.1	1.9	Hypocreales	MG098278	KM114549	Entomopathogen; Brady (1979)
Biscogniauxia mediterranea+	1.8	48.6	Xylariales	MG098274	KT823762	Pathogen, endophyte, typical hard wood colonizer; Jong and Rogers (1972); Breitenbach and Kränzlin (1984); Henriques et al. (2014a, b)
*Biscogniauxia nummularia**+	0.7	39.0	Xylariales	MG098283	EF155488	Typical hard wood colonizer, endophyte, weakness pathogen, saprophyte; Fournier and Magni (2004a); Luchi et al. (2006)
*Botrytis cinerea**+	0.1	5.7	Helotiales	MG098288	CP009808	Pathogen; Domsch et al. (1993); Samson et al. (2010)
*Camarosporium brabeji**+	0.5	30.5	Botryosphaeriales	MG098280	EU552105	Pathogen and saprophyte; Botella and Diez (2011)
*Chaetomium globosum**+	0.1	10.5	Sordariales	MG098250	FN868476	Generalist; Minter (2006); Samson et al. (2010)
Chaetomium sp. 1+	0.3	12.4	Sordariales	MG098335	FJ666356	
Chaetomium sp. 2+	< 0.1	4.8	Sordariales	MG098270	KT371334	
*Chromelosporium carneum**+	< 0.01	1.9	Pezizales	MG098273	FJ872075	Saprophyte; Hennebert (1973)
Cladosporium sp. 1	< 0.01	< 1	Capnodiales	MG098291	KM520367	
Cladosporium sp. 2	< 0.01	1.0	Capnodiales	MG098295	HF952649	
*Coniochaeta ligniaria**+	< 0.1	8.6	Coniochaetales	MG098287	AY198390	Generalist; Weber (2002)
Cytospora sp.	< 0.01	1.0	Diaporthales	MK066904	KY051969	
*Daldinia childiae**+	0.4	6.7	Xylariales	MG098286	KJ957789	Typical hard wood colonizer, saprophyte; Stadler et al. (2014)
*Daldinia concentrica**+	0.2	18.1	Xylariales	MG098285	AM292046	Typical hard wood colonizer, saprophyte; Breitenbach and Kränzlin (1984); Stadler et al. (2014)
Daldinia sp.	< 0.01	< 1	Xylariales	MK066899	AM292038	
Desmazierella acicola+	2.1	43.8	Pezizales	MG098266	LN589957	

Table 1 (continued)

Taxon name	Frequency	% Sites	Systematics	GenBank accession	GenBank accession Blast	Assessment of the significance for forestry
						Typical saprophyte of *P. sylvestris* needles; Przybył et al. (2008); Martinović et al. (2016)
Diaporthe sp. 1^{+}	0.4	24.8	Diaporthales	MG098256	KC145855	
Diaporthe sp. 2^{+}	3.2	60.0	Diaporthales	MG098258	EF155490	
Diaporthe sp. 3	0,2	15.2	Diaporthales	MG098269	KJ609006	
Diaporthe sp. 4^{+}	0.6	2.8	Diaporthales	MG098318	KC343205	
Drechslera sp.	0.1	2.9	Pleosporales	MG098259	GU067763	
Epicoccum nigrum^{+}	1.3	47.6	Pleosporales	MG098251	GU566259	Generalist; Fávaro et al. (2011); Samson et al. (2010)
Fusarium solani complex	< 0.01	< 1	Hypocreales	MK066900	MF327377	Potential pathogen; Butin (2011)
Fusarium sp. 1^{+}	0.3	23.8	Hypocreales	MG098263	HQ630964	
Fusarium sp. 2	< 0.01	< 1	Hypocreales	MK066908	AF310980	
Gnomoniaceae sp.	0.5	39.0	Diaporthales	MG098311	DQ872667	
Hypoxylon fragiforme^{+}	1.0	28.6	Xylariales	MG098276	EF155528	Typical hard wood colonizer, saprophyte; Greenhalgh and Chesters (1968); Breitenbach and Kränzlin (1984); Rogers et al. (2002)
*Hypoxylon rubiginosum**	< 0.01	< 1	Xylariales	MG098300	KC968929	Typical hard wood colonizer, saprophyte; Breitenbach and Kränzlin (1984); Rogers et al. 2002)
*Jugulospora rotula**	< 0.01	< 1	Sordariales	MG098304	AY999136	Saprophyte; Lundqvist (1972)
Lambertella sp.	< 0.1	1.0	Helotiales	MG098309	KF499362	
*Lecanicillium psalliotae**$^{+}$	0.1	3.8	Hypocreales	MG098334	AY261180	Entomopathogen; generalist; Zare and Games (2003); Arevalo et al. (2009)
Lecythophora sp. 1^{+}	0.0	1.9	Coniochaetales	MG098271	KF367565	
Lecythophora sp. 2^{+}	0.1	10.5	Coniochaetales	MG098277	KT264687	
Leotiomycetes sp.	< 0.1	< 1		MG098324	KC595271	
*Microdochium nivale**	< 0.1	1.9	Amphisphaeriales	MG098306	AM502266	
Microsphaeropsis olivacea^{+}	23.0	97.1	Pleosporales	MG098249	JX681101	Generalist; Liu et al. (2015); Chen et al. (2015)
*Nemania diffusa**$^{+}$	0.7	35.2	Xylariales	MG098265	KT323181	Typical hard wood colonizer, saprophyte; Fournier and Magni (2004b); Balasuriya and Adikaram (2009)
Nemania serpens^{+}	1.5	50.5	Xylariales	MG098299	EF155504	Typical hard wood colonizer; Breitenbach and Kränzlin (1984)
Nigrospora oryzae^{+}	1.3	15.2	Incertae sedis	MG098254	EU436680	Generalist; Sivanesan and Holliday (1971)
Nigrospora sp.	< 0.1	1.9	Incertae sedis	MG098279	KC505176	
*Paraphaeosphaeria neglecta**	< 0.01	< 1	Pleosporales	MG098298	JX496092	Generalist; Verkley et al. (2014)
*Paraphaeosphaeria verruculosa**	< 0.1	2.9	Pleosporales	MG098314	JX496059	Generalist; Verkley et al. (2014)
Penicillium sp.	< 0.01	< 1	Eurotiales	MG098313	KF367536	
Periconia sp.	< 0.01	< 1	Pleosporales	MG098330	JX981482	

Table 1 (continued)

Taxon name	Frequency	% Sites	Systematics	GenBank accession	GenBank accession Blast	Assessment of the significance for forestry
Pestalotiopsis sp. 1	< 0.1	< 1	Xylariales	MG098316	KM199328	
Pestalotiopsis sp. 2	< 0.01	< 1	Xylariales	MG098325	EU552147	
Pezicula cinnamomea	0.7	< 1	Helotiales	MG098321	KR859162	Typical hard wood colonizer, Pathogen; Verkley (1999)
Pezicula eucrita+	0.9	26.7	Helotiales	MG098307	KR859188	Saprophyte; Verkley (1999)
*Pezicula neosporulosa**	0.6	< 1	Helotiales	MG098320	KR859231	Pathogen; Yuan and Verkley (2015)
Pezicula sp. 1	1.4	< 1	Helotiales	MG098317	KR859220	
Pezicula sp. 2	0.3	< 1	Helotiales	MG098322	AF141166	
*Peziza varia**	< 0.01	< 1	Pezizales	MG098331	FN868472	Saprophyte; Breitenbach and Kränzlin (1984)
Pezizales sp.	< 0.01	< 1	Pezizales	MK066903	MG762599	
Pezizomycetes sp. 3	< 0.01	< 1		MK066897	JQ758844	
Pezizomycetes sp. 1+	0.2	16.2		MG098267	GQ153018	
Pezizomycetes sp. 2	< 0.01	< 1		MG098332	FN868473	
Phacidium lacerum	0.2	13.3	Helotiales	MG098312	KU942438	Typical saprophyte of *P. sylvestris* needles; Nawrot-Chorabik et al. (2013, 2016); Crous et al. (2014)
*Phoma eupyrena**+	< 0.1	5.7	Pleosporales	MG098275	AJ890436	Pathogen; Hampel (1970); Domsch et al. (1993); Hansen et al. (2013)
Phoma sp. 1	< 0.01	< 1	Pleosporales	MG098301	JX421725	
Phoma sp. 2	< 0.01	< 1	Pleosporales	MG098303	JQ318008	
Phoma sp. 3	< 0.01	< 1	Pleosporales	MG098328	EU167565	
Phoma sp.4	< 0.01	< 1	Pleosporales	MK066907	JX160059	
Plagiostoma sp.	< 0.01	< 1	Diaporthales	MK066906	MH864035	
Pleosporaceae sp.	< 0.1	1.0	Pleosporales	MG098327	KT268913	
Pleosporales sp. 1	< 0.01	1.0	Pleosporales	MK066896	JF288544	
Pleosporales sp. 2	< 0.01	< 1	Pleosporales	MK066905	KT268865	
*Podospora curvicolla**	< 0.1	1.0	Sordariales	MG098296	GQ922546	Saprophyte; Mirza and Cain (1969); Lundqvist (1972)
Preussia sp. 1	< 0.1	1.0	Pleosporales	MG098305	FJ210518	
Preussia sp. 2	0.1	< 1	Pleosporales	MG098329	JN225886	
*Pyronema domesticum**+	0.3	14.3	Pezizales	MG098284	HQ115722	Burning areas; Breitenbach and Kränzlin (1984)
Rosellinia sp. 1	< 0.01	1.0	Xylariales	MG098260	FN435734	
Rosellinia sp. 2+	1.1	38.1	Xylariales	MG098268	KC311485	
Sordaria fimicola+	4.8	22.9	Sordariales	MG098253	FN868475	Generalist; Lundqvist (1972)
Sordaria sp. 1+	0.1	8.6	Sordariales	MG098252	JN207268	
Sordaria sp. 2	< 0.01	< 1	Sordariales	MK066902	KY742564	
Sordariales sp.	< 0.01	1.9	Sordariales	MG098264	AY999126	
Sphaeropsis sapinea+	12.2	87.6	Botryosphaeriales	MG098333	KF766159	

Table 1 (continued)

Taxon name	Frequency	% Sites	Systematics	GenBank accession	GenBank accession Blast	Assessment of the significance for forestry
						Pathogen, saprophyte, typical endophyte of *P. sylvestris*; Swart and Wingfield (1991)
Sydowia polyspora[+]	22.1	99.0	Dothideales	MG098248	GQ412728	Typical Endophyte of *P. sylvestris* twigs, potential pathogen; Sutton and Waterston (1970); Brener et al. (1974); Heydeck (1991); Talgø et al. (2010); Heydeck and Dahms (2012)
Trichocladium sp.[+]	< 0.1	3.8	Sordariales	MG098272	KC311502	
Trichoderma sp.	0.1	6.7	Hypocreales			
*Truncatella conorum-piceae**[+]	10.4	98.1	Amphisphaeriales	MG098255	FN868480.	Weakness pathogen, saprophyte; Guba (1961); Maharachchikum-bura et al. (2011); Landeskompetenzzentrum Forst Eberswalde (2016)
Truncatella sp. 1	< 0.1	< 1	Amphisphaeriales	MG098315	GU566260	
*Umbelopsis isabellina**	< 0.1	1.9	Umbelopsidales	MG098308	AJ876493	Generalist; Meyer and Gams (2003)
*Xylaria longipes**[+]	0.8	42.9	Xylariales	MG098261	JX501293	Typical hard wood colonizer; Breitenbach and Kränzlin (1984)
*Xylaria polymorpha**	0.1	5.7	Xylariales	MG098262	GU322460	Typical hard wood colonizer, saprophyte; Breitenbach and Kränzlin (1984)
Xylaria sp. 1[+]	0.1	12.4	Xylariales	MG098281	AY315404	
Xylaria sp. 2	< 0.01	< 1	Xylariales	MG098292	JX515704	
Xylaria sp. 3	< 0.1	1.9	Xylariales	MG098302	HQ823756	
Coprinellus sp.	0.1	1.0	Agaricales	MG098326	KM010302	
*Peniophora pini**	< 0.01	< 1	Russulales	MG547963	EU118651	Typical saprophyte of *P. sylvestris*; Bernicchia and Gorjón (2010)

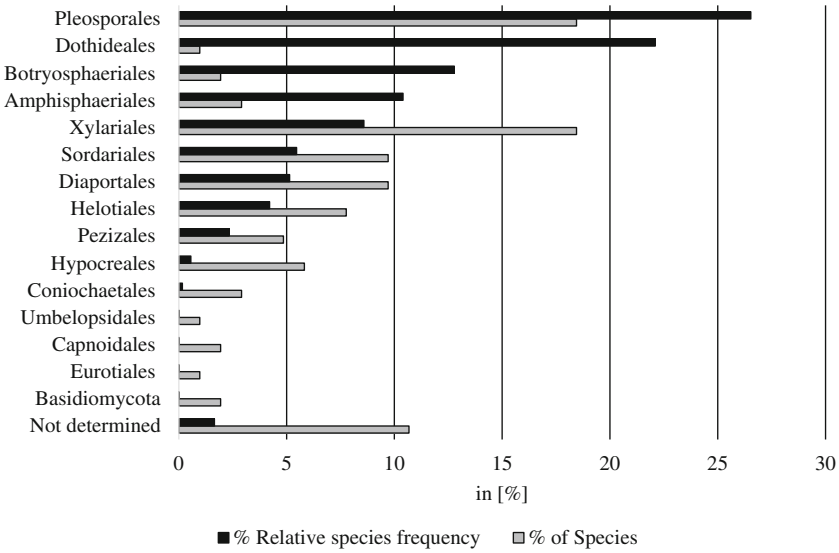

Fig. 2 Frequency of isolated fungi based on their respective taxonomic orders ($n = \sim 33{,}000$). Relative species frequency and portion of species numbers of fungal orders in relation to total number of proved species ($n = 103$), not determined represents species where no classification to order level was possible or Incertae sedis: *Nigrospora oryzae*, Pezizomycetes sp. 1, *Nigrospora* sp., Ascomycete spp., Leotiomycetes spp.)

were found as endophytes in pine twig for the first time: *Ar. kogelbergense*, *B. nummularia*, *Bo. cinerea*, Ca. *brabeji*, *Ch. globosum*, *Co. ligniaria*, *Chr. carneum*, *Da. childiae*, *Hy. rubiginosum*, *Ju. rotula*, *Le. psalliotae*, *Mi. nivale*, *Ne. diffusa*, *Pa. neglecta*, *Pa. verruculosa*, *Pen. pini*, *Pez. neosporulosa*, *Pez. varia*, *Ph. eupyrena*, *Py. domesticum*, *Po. curvicolla*, *T. conorum-piceae*, *U. isabellina*, and *X. polymorpha* (Table 1, species denoted with a plus * symbol).

Along the transect, 5–22 endophyte species per tree were detected, on average 13 species per plot. The number of identified species per plot increased with the number of investigated samples. Typically three pine twigs were sampled per tree (between 22 and 213 studied segments per tree). But a single tree located in the sampling area "Dransfeld" was sampled monthly over 13 months (~ 7500 studied segments) and 84 species were isolated. Seasonal differences in the frequency and number of species were apparent. For this tree, the colonization rate (number of species and frequency) was lower in December to March than from April to November. The occurrences of *Sy. polyspora* and *M. olivacea* contrasted, with *Sy. polyspora* dominating in the summer months (June–July) and *M. olivacea* in December–March.

At least one *S. sapinea*-strain was sampled from 88% of the 105 studied pine stands. In forest stands without symptoms of Diplodia tip blight, *S. sapinea* was detected with a relative frequency ranging from 0 to 68%. The analysis of the endophytic occurrence of *S. sapinea* along the studied transect across Germany exhibited that geographic longitude and altitude do not significantly influence the occurrence of the dieback fungus. However, *S. sapinea* tended to be isolated in a lower frequency at sites with higher altitudes (Pearson correlation coefficient = − 0.29).

From diseased tree twig samples, 45 fungal species were isolated (See Table 1, species denoted with a plus + symbol). All species of endophytic fungi found in diseased trees could also be isolated from trees without symptoms. A pairwise comparison of sites with and without symptoms of Diplodia tip blight revealed that the number of endophytic species isolated in trees with and without symptoms at sites in Pfungstadt and Bad Freienwalde were similar (Pfungstadt 34 and 33 species respectively, Bad Freienwalde in each case 23 species), with the occurrence of the different species also varying little between symptomatic and symptom-free trees in intensively examined stands. However, differences were found between healthy and diseased trees with regard to the infection rate with *S. sapinea*: 9% in healthy trees and 40% in diseased trees. In all three comparative pairs (Pfungstadt, Bad Freienwalde, and Lüderitz), the frequency of occurrence of *S. polyspora* and *Desmazierella acicola* were higher in healthy trees.

Twelve isolated species were identified as potential pathogens on woody plants as follows: *A. infectoria*, *B. mediterranea*, *B. nummularia*, *Bo. cinerea*, Ca. *brabeji*, *Fusarium solani* complex, *Pez. cinnamomea*, *Pez. neosporulosa*, *Ph. eupyrena*, *S. sapinea*, *Sy. polyspora*, *and T. conorum-piceae* (Table 1).

Discussion

The comparison of the endophyte fungal twig communities of diseased and non-diseased Scots pine trees revealed few differences. Except *S. sapinea*, there were no isolated species which occurred or were specific to diseased Scots pines. Botella et al. (2010) detected *Sy. polyspora* in twigs of diseased Aleppo pine. Additionally, several other potentially pathogenic fungi, e.g., *Gremmeniella abietina* (Lagerb.) M. Morelet, *Cytospora* sp., *Naemacyclus niveus* (Pers.) Fuckel ex Sacc., and *Pestalotia stevensonii* Peck were observed by Botella et al., but these pathogens were not found in the tested Scots pine twigs in the present

study. *Sy. polyspora* was isolated on nearly every studied site (99%) in this study. On the one hand, it was classified as a typical endophyte because no pathogenic occurrence (no typical disease symptoms) was observed in all tested trees. On the other hand, it could be assessed as a potential pathogen for (coniferous) trees based on various published studies (Table 1).

It was apparent that pines with Diplodia tip blight studied in Germany exhibited a very high rate of infection with *S. sapinea* (~ 40%). Although high rates of infection were, rarely, also found in symptomless and vital pine stands (e.g., 68% *S. sapinea* at a sampling point in Hesse), the average rate of infection of symptomless pines was lower (~ 9%). The number of species isolated from diseased trees was generally less ($n = 45$, see Table 1) than those isolated out of symptomless pines ($n = 103$). This may be partially explained by the fast growth of *S. sapinea* in culture (Slippers and Wingfield 2007; Decourcelle et al. 2015), which possibly resulted in an underrepresentation of slow-growing fungal species. The sample numbers for the two categories (symptomless and Diplodia-diseased pines) in this study differed (2800 tested twig segments of pine twigs from trees with symptoms or 23,000 without symptoms of the Diplodia tip blight). This was probably the most significant influence on the detected number of species. In this study, *S. sapinea* was isolated from twigs with a relative frequency of 12% (mean value for all examined twig segments). From 88% of the 105 examination sites, it was possible to isolate at least one *S. sapinea* strain. The endophytic occurrence of *S. sapinea* in this study, measured in frequency of colonization, is higher than in other studies like by Bihon et al. (2011), Flowers et al. (2001, 2003), and Maresi et al. (2007). Bihon et al. (2011) assumed that the low isolation frequency of endophytic *S. sapinea* could be explained by the specific position of its propagules in symptomless pine tissue. The colonization with *S. sapinea* in buds and bark of black pine is not continuous, as presented in the study of Flowers et al. (2003). In their experiment, they bisected buds and bark and could not always isolate *S. sapinea* from both parts. Maresi et al. (2007) detected more endophytic growing *S. sapinea* strains with the PCR-method, compared with using isolation on nutrient media, from twigs of black pine. There are *S. sapinea* selective media, such as Swart's medium (Swart et al. 1987) or Blodgett's medium (Blodgett et al. 2003). Rigling et al. (1989) described the highest frequency of isolation on Bavendamm's medium. In our opinion, the use of a selective medium is not always necessary, since *S. sapinea* grows well, fast, and competitive on the used MYP medium.

On sites with a higher altitude, *S. sapinea* tended to be isolated with a lower frequency. This tendency was first described by Fabre et al. (2011) who also found a decrease in *S. sapinea* colonization of pine cones with increasing elevation. These researchers assume, therefore, that the milder temperature in winter at sites with lower elevation could be an explanation. In this study, the range in height of the examined sites was between ~ 20 and 600 m above sea level, while the researcher group around Fabre et al. (2011) examined sites up to ~ 1500 m above sea level.

The widespread latent/endophytic infection of pines with *S. sapinea* constitutes a danger to weakened pine stands. It is presumed that *S. sapinea* already present in a pine can easily change from endophytic to parasitic lifestyle in a weakened host. In greenhouse experiments, Stanosz et al. (1997) and Flowers et al. (2001) proved that *S. sapinea* strains obtained from healthy pine tissues show an equally high pathogenic potential as those strains obtained from diseased tissues. Usually numerous pycnidia of *S. sapinea* are present in forest stands occurring on twigs, cones, and needles of pine. They represent a constant risk for infection due to airborne dispersal of conidia. A high latent infection rate may pose a large risk when there are disease-triggering factors, e.g., hail or insect feeding or extreme weather conditions such as heat and drought, as in the years 2018 und 2019 in Germany.

The question of whether *S. sapinea* is native in Germany could not be answered with the presented results but the wide endophytic distribution suggests that this is possible.

Sydowia polyspora was the fungus with the highest abundance in this study. Twenty-two percent of all isolates were identified as *Sy. polyspora* and it was detected on nearly all sites (99%). Additionally, its infection rate in healthy trees was much higher than in the studied diseased pines. This is in agreement with the results of other studies, which demonstrate that *Sy. polyspora* occurs often as an epiphyte or endophyte of conifers and is widely distributed around the world (Muñoz-Adalia et al. 2017; Pan et al. 2018). It was found to be the second most common fungus in twig tissues of *P. sylvestris* (Sanz-Ros et al. 2015). *Sy. polyspora* lives predominantly saprophytically on a dead plant material and occurs on previously damaged needles and twigs as a weak pathogen (Heydeck 1991). Ascomata and pycnidia appear on dead pine branches and needles (Gremmen 1977). *Sy. polyspora* is also a wound pathogen and a blue stain fungus (Sutton and Waterston 1970). This is contrary to observations that this species causes damage in other conifers (Butin 1964), e.g., current season needle necrosis (CSNN) on true fir (Talgø et al. 2010), distinct chlorosis, or discoloration of needles and phloem lesions in *Pinus yunnanensis* Franchet (Pan et al. 2018). Therefore, it seems to be a potential pathogen for the studied Scots pine trees.

Microsphaeropsis olivacea (basionym: *Coniothyrium olivaceum* Bonord.) was isolated from nearly all studied stands (97%) and it was the third most common fungus in this study, with an abundance of 23%. Surprisingly, *M. olivacea* is only mentioned in three other studies as an endophyte of pine (Petrini and Fisher 1988; Kowalski and Kehr 1992; Kowalski 1993). It is also an endophyte in other tree species (e.g., Hormazabal et al. 2005). Anamorphic, *Coniothyrium*-like fungi are often colonizers of wood and leaves of woody plants (Damm et al. 2008). *M. olivacea* is known to be plurivorous and was found on twigs and branches of *Cytisus*, *Hedera*, *Laurus*, *Lycium*, and *Sambucus* (Ellis and Ellis 1985). Recently it was found to be the causal agent of brown spine

rot of Camelthorn (*Alhagi maurorum* Medik.) (Razaghi and Zafari 2016). Additionally, it was identified as an etiological agent of human skin infection (Guarro et al. 1999).

Truncatella conorum-piceae (≡ *Pestalotia conorum-piceae* Tubeuf) was also a frequently isolated endophyte in this study (frequency 10%, isolated on 98% of all sites). This was unexpected, because in other studies on endophytes in twigs of *P. sylvestris* no fungi allied to *Truncatella* s. l. were mentioned (Petrini and Fisher 1988; Kowalski and Kehr 1992; Peršoh et al. 2010; Martínez-Álvarez et al. 2012; Sanz-Ros et al. 2015). This fungus is mainly a saprobe and is only known as a subsequent decomposer of pre-damaged needles of pine (Landeskompetenzzentrum Forst Eberswalde (LFE) 2016). In other tissues of Scots pine *Truncatella angustata*, respectively, *Truncatella* spp. were rarely isolated (Menkis et al. 2006; Menkis and Vasaitis 2010; Terhonen et al. 2011).

Desmazierella acicola (≡ *Verticicladium trifidum* Preuss) is a common colonizer of pine needles (Martinović et al. 2016) but is also found as an endophyte in stems and xylem of pine (Petrini and Fisher 1988). The anamorphs fructify after needles fall through needle stromata (Maanen and Gourbière 1997) and apothecia occur on decaying needles. The increased occurrence of *D. acicola* in apparently healthy and symptomless trees in comparison with diseased pines could be explained by competition with other fungal strains colonizing the diseased twigs. The latter may inhibit the growth of *D. acicola* from its common niche, pine needles, into the shoots.

With regard to forest protection, the frequency of Xylariales species (9%, 19 species) is important, especially of *Biscogniauxia mediterranea* (frequency 2%, found in 49% of the studied sites) and *Biscogniauxia nummularia* (frequency 0.7%, found in 39% of the studied sites). Both species are known endophytes (Nugent et al. 2005), colonizers of hardwood, and pathogens that take advantage of the weakness of their hosts. They prefer warmer temperatures and seem to benefit from climate warming. In this study, *B. mediterranea* had a higher frequency in pine stands with oak trees or with neighboring oaks in the warmer climate of south Germany than in northern parts. Because it also causes Charcoal canker on *Quercus suber* L. and other hardwoods in countries of the Mediterranean Basin (Ragazzi et al. 2011; Henriques et al. 2014a, 2014b, 2015), the occurrence of *B. mediterranea* as an endophyte in pine could, in view of climate warming, be evaluated as a potential risk for neighboring oaks in the same forest stand. *B. nummularia* is known to fructify (anamorphic and teleomorphic) only on beech species (*Fagus sylvatica* and *F. orientalis* Lipsky) in Eurasia (Læssoe et al. 1999). It is a common endophyte of European beech, although it can induce severe damage, e.g., strip-cankers and wood decay on trees stressed by drought (Greenhalgh and Chesters 1968; Luchi et al. 2015). The occurrence as an endophyte is not confined to beech, as it was also found symptomless in pine, fir, and Douglas fir (authors own, unpublished results) and in other tree species (Petrini 1985). In Northwestern Germany, beech is very often planted as advance regeneration under pine or is associated with pine. In view of global warming, this leads us to assign *B. nummularia* in pine as a potential risk for neighboring beech.

The diversity and number of fungal species detected from pine twigs in this study is higher compared with other studies on endophytes in twigs of *P. sylvestris*, where between 10 and 44 species were recorded (Petrini and Fisher 1988; Kowalski and Kehr 1992; Peršoh et al. 2010; Martínez-Álvarez et al. 2012; Sanz-Ros et al. 2015). One explanation for this difference seems to be the much higher number of twigs sampled in this study. Whether the isolation of endophytes on artificial culture medium fully reflects the natural colonization of pine twigs remains questionable. The reason for the low isolation rate of fungi in the Basidiomycota could not be clearly determined in the present study. Plausible explanations for these results in our endophyte isolation study may be that (1) few Basidiomycota fungi live in this ecological niche, (2) sampling and media culturing methods for isolation favored fungi in the Ascomycota, or (3) surface disinfestation of twigs was not effective in killing hyphae of fast-growing epiphytic fungi. Point three was evaluated in a study by Bußkamp (2018) and could be refuted.

In other comparable culture-based studies on endophytes in branches of Scots pine, no fungi in the Basidiomycota were detected (Petrini and Fisher 1988; Kowalski and Kehr 1992; Peršoh et al. 2010; Martínez-Álvarez et al. 2012; and Sanz-Ros et al. 2015). In an endophyte study of Scots pine and mistletoe by Peršoh et al. (2010), which used a culture-based isolation method, only a single basidiomycetous fungus was detected in the mistletoe. In a second study by Peršoh (2013), who worked with direct sequencing of plant tissue, the detection and identification of fungi in the Ascomycota and Basidiomycota were similar.

There is evidence that some endophytes cannot be cultured on nutrient medium (Allen et al. 2003; Arnold 2007; and Unterseher et al. 2007). Numerous studies have shown that Basidiomycetes, in particular, are undetectable on nutrient medium (Kowalski and Kehr 1992; Hoff et al. 2004; Lygis et al. 2005; Menkis et al. 2006; Zamora et al. 2008; Botella and Diez 2011; Sanz-Ros et al. 2015), which is why the detection of endophytes with the help of direct sequencing could be helpful. Ascomycetes that are not cultivable could thus also be detected (Arnold et al. 2007; Rajala et al. 2013, and Sanz-Ros et al. 2015). Extraction of total DNA of studied tissues could be examined to better determine endophyte communities (Rajala et al. 2013). But the fact that this method cannot distinguish between endo- and epiphytes can be problematic, since surface sterilization may not destroy the DNA of the epiphytes (Schulz and Boyle 2005). A direct comparison between culture-based and molecular methods using needles from *P. taeda* was carried out by Arnold et al. (2007). Their samples were cultured on malt extract agar medium and analyzed with ITS and LSUrDNA. Their results suggest that when isolated on nutrient medium, fungi in the

Basidiomycota were underrepresented, compared with results obtained by direct sequencing methods (Arnold et al. 2007). In contrast, Sordariomycetes were not adequately detected by direct sequencing of plant tissue (Arnold et al. 2007). Fungi in the taxonomic class Sordariomycetes are common endophytic fungi in plant tissue and comprised 31% of all isolations in the present study. Similar results were shown in studies by Sanz-Ros et al. (2015), in which Sordariomycetes accounted for 32% of all isolations from pine branches.

Gazis et al. (2011) argue that many endophyte studies deal only with a single DNA locus. The fungal barcode ITS region is generally used to identify species presence, as in the present work. Advantages of focusing on the ITS region are that this marker is simple to amplify and that, due to its frequent use, many sequences for comparison are available in public data repositories. However, the use of ITS sequences poses problems, as it sometimes shows high intraspecific variation (Lacap et al. 2003). The use of the ITS sequence as barcode region of the fungal genome is insufficient in very diverse genera or species complexes (Lacap et al. 2003; Hoffman and Arnold 2008). The limits of using the ITS region for species delimitation were apparent in the present study, e.g., for the species of the genus *Pestalotiopsis* Steyaert (Maharachchikumbura et al. 2011, 2014) or the genus *Diaporthe* (Gomes et al. 2013). Another problem resulting from the use of ITS sequences is the lack of high-quality reference sequences in the databases (Bridge et al. 2003; Nilsson et al. 2006; Arnold et al. 2007). For improving the number of reliable sequences, initiatives for barcoding fungi were launched (e.g., www.fungalbarcoding.org). Nevertheless, these databases contain at present less than 1% of the expected diversity of fungi (Hawksworth 2012). The seasonal differences in the isolation frequency of *Sy. polyspora* and *M. olivacea* correspond well with physiological studies of these fungi (Bußkamp 2018). *Sy. polyspora* dominated in the summer months and had a temperature optimum at approx. 22 °C in vitro, whereas *M. olivacea*, which dominated in the colder months of the year, had an optimum temperature at approx. 12 °C in vitro.

In summary, a foundational baseline to describe naturally occurring fungal endophyte communities of Scots pine twigs in Germany was established. In the future, it may be possible to identify potential endophytic fungi that suppress *S. sapinea*.

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Dedication The results are published in honor of Prof. Dr. Franz Oberwinkler, † 15 March 2018, Tübingen in memory of his 80th birthday, 22.05.2019.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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