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Neotypification of *Dothistroma septosporum* and epitypification of *D. pini*, causal agents of Dothistroma needle blight of pine

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Summary

Dothistroma needle blight (DNB) is one of the most devastating needle diseases on *Pinus* spp. worldwide. Ever since the description of the causal agent of the disease in Europe in 1911 as *Cytosporina septospora*, and independently in the USA in 1941 as *Dothistroma pini*, there has been considerable taxonomic discordance regarding the name of the pathogen used in literature. This was compounded both by the proposal of different varieties of the pathogen based on differences in spore size and the application of dual nomenclature where three names, *Scirrhia pini*, *Eruptio pini* and *Mycosphaerella pini*, were used to describe the sexual morph of the fungus. More recent studies using sequence-based methods revealed that DNB can be caused by either one of two distinct species, that is *D. septosporum* and *D. pini*. These important species have not been adequately typified, and this perpetuates lack of stability for their names. In this study, these names are fixed to reference sequences linked to living cultures representing type specimens. To achieve this goal, we designate an epitype for *D. pini* and a neotype for *D. septosporum*. The known polymorphism in the ITS region, the barcoding gene for these fungi, is characterized and a complete taxonomic history is provided for the genus *Dothistroma*.

1 | INTRODUCTION

Dothistroma needle blight (DNB), also commonly known as "red-band disease," "red spot" or "red-band needle blight," is one of the most important foliage diseases of *Pinus* spp. worldwide (Bradshaw, 2004; Drenkhan et al., 2016; Gibson, 1972). Symptoms of the disease include reddish spots or bands surrounding black erumpent conidiomata (acervuli) on necrotic needles. Recent reviews of the disease distribution have shown that it occurs in 76 countries spanning a wide array of geographic and climatic conditions (Drenkhan et al., 2016; Woods et al., 2016). The disease occurs in almost all areas where susceptible pines are found and has been documented on 95 *Pinus* species or their subspecies. Rare and sporadic occurrences of the disease have also been recorded on five non-*Pinus* genera of the Pinaceae including *Abies*, *Cedrus*, *Larix*, *Picea* and *Pseudotsuga*. But in all these cases, heavily diseased *Pinus* spp. have been in close proximity to those conifers (Drenkhan et al., 2016).

DNB can be caused by either one of two different fungal species, that is *D. septosporum* (Dorogin) M. Morelet and *D. pini* Hulbary (Barnes, Crous, Wingfield, & Wingfield, 2004). These two species can be clearly distinguished based on DNA sequence data (Barnes et al., 2004; Ioos et al., 2010). However, before 2004, they were considered as one species and the names were commonly used interchangeably. This confusion in their taxonomy stems from two independent roots of the species name, one in Europe and the other in the USA (see Table 1). In the USA, the asexual state of the pathogen was described by Robert L. Hulbary in 1941 as *Dothistroma pini* (Hulbary, 1941). The pathogen had also previously been described as *Actinothyrium marginatum* (Saccardo, 1920), *Cryptosporium acicola* (Dearness, 1928), *Septoria acicola* (Hedgcock, 1929) and it was confused with *Lecanosticta acicola* (Sydow & Petrak, 1924), a closely related, but distinctly different pathogen that causes brown-spot needle blight (Evans, 1984).

In Europe, the pathogen causing DNB was first described as *Cytosporina septospora* (Doroguine, 1911). As was true in the USA,

TABLE 1 Taxonomic history of *Dothistroma* species

Date	Species epithet	Reference	Area	Host (*Pinus*)	Date material was collected	Herbarium collection details/number	Description	Confusion caused/importance/significance
1896	*Hypostomum flichianum* Vuill.	Vuillemin (1896)	Theil-sur-Vanne, close to Sens (Yonne), France	*P. austriaca* and *P. mugo* Turra subsp. *P. mugo* (syn. *P. montana* Mill.)	April–Oct 1860	No material deposited	This is probably the first published description of Dothistroma needle blight. The work remained in obscurity until Morelet redescribed it as *Dothistroma flichianum* in 1980.	It is highly likely that this description is of either *D. septosporum* or *D. pini* in France. However, this cannot be validated as no herbarium material exists.
1911	*Cytosporina septospora* Dorogin	Doroguine (1911)	In the park of the Forestry Institute in Lesnoj, near to St. Petersburg, Russia	*P. mugo* Turra subsp. *P. mugo* (syn. *P. montana* Mill.)	Summer 1910	Holotype material lost	First recognized description of the Dothistroma pine needle pathogen.	Doroguine described the symptoms as "brownish–yellowish spots" or "darker brown spots" (Doroguine, 1912) causing this description to be overlooked in the literature. Later he transferred the name to *Brunchorstia pinea* – see Doroguine, 1926.
1920	*Actinothyrium marginatum* Sacc.	Saccardo (1920)	Orofino, Idaho, USA	*P. ponderosa* Laws	9 June 1917	IMI 91341; Shattuck Col. Weir No./Univ. Padova No. 10330	Name given to fungus causing red banding patterns on *P. ponderosa*.	Erroneously described two fungi - see Sydow & Petrak (1924).
1924	*Actinothyrium marginatum* = rejected as a *nomen confusum*	Sydow & Petrak (1924)	Orofino, Idaho, USA	*P. ponderosa* Laws	9 June 1917	IMI 91341; Shattuck Col. Weir No./Univ. Padova No. 10330	Realized that Saccardo had described the fruiting body of *Leptostroma decipiens* Petrak and the conidia of *Lecanosticta pini* Thüm. From red-band symptoms. Suggested that *A. marginatum* therefore does not exist and proposed that *L. pini* be synonymized with *L. decipiens* with the new name combination of *Lecanosticta acicola* (Thüm). Syd.	Called the fungus causing red band and brown spot the same thing, i.e. confused *Lecanosticta acicola* and *Dothistroma* spp. This confusion was perpetuated by Petrak (1961), Dearness (1928) as *Cryptosporium acicolum* Thüm., and Hedgcock (1929) as *Septoria acicola* (Thüm.) Sacc. See Siggers (1944).
1926	*Brunchorstia pinea* (P. Karst.) Höhn	Doroguine (1926)	As in 1911 and various others	As in 1911 and various others	As in 1911 and various others	No material deposited	Doroguine mistakenly decided that the fungus he described in 1911 and 1912 as *Cytosporina septospora* was actually *Brunchorstia pinea* (another pathogen altogether).	Called the red-band fungus *Brunchorstia pinea* and this name was incorrectly used to describe DNB in Eastern European literature – see Gremmen (1965) and Gremmen (1968).
1931	*Septoriella septospora* (Dorogin) Sacc. apud Trotter	Trotter (1931)					Saccardo transferred *Cytosporina septospora* to the genus *Septoriella* Oudem as *S. septospora* (Dorogin) Sacc.	Name change was never used in any further literature until 1968.

(Continues)

TABLE 1 (Continued)

Date	Species epithet	Reference	Area	Host (*Pinus*)	Date material was collected	Herbarium collection details/number	Description	Confusion caused/importance/ significance
1941	*Dothistroma pini* Hulbary	Hulbary (1941)	De Kalb County, Illinois, USA	*P. nigra* Arn. var. *austriaca*	29 Nov 1938	IILLS 27093; MBT128093; herb CBS H-12211; IMI 178710	Described the red-band fungus. A number of specimens from Ohio and Iowa considered conspecific with it.	New genus = *Dothistroma*.
1944	*Actinothyrium marginatum* Sacc. = *D. pini* Hulbary	Siggers (1944)	Various states of the USA	Various	Various	Weir no. 10330; 19906; 19930 (as *A. marginatum*); F.P. 20548; F.P.41675 (as *Cryptosporium acicolum*); F.P.18284 (as *Lecanosticta acicola*); F.P.54210; F.P.18237; F.P.46791 (as *Septoria acicola*)	Examined a range of material from the USA and determined that none of the various samples labelled as *Actinothyrium marginatum*, *Cryptosporium acicolum*, *Lecanosticta acicola* and *Septoria acicola* were *Lecanosticta acicola* but were probably conspecific with *D. pini* as described by Hulbary. Included in this was the original material used to describe *Actinothyrium marginatum* Sacc. (Weir no 10330).	Highlights the confusion often made in the literature between the brown-spot fungus and the red-band fungus. Clearly stated that *Lecanosticta acicola* and *Dothistroma pini* were different fungi. This work was later supported by Murray & Batko (1962) when they proposed the synonymy of *Actinothyrium marginatum* and *Dothistroma pini* and suggested retaining *D. pini*.
1957 (1880)	*Mycosphaerella pini* Rostr. apud Munk	Munk (1957)	Tvorup, Jutland, Denmark	*P. sylvestris* (possibly *P. maritima* or *P. nigra* var. *austriaca*)	28 Oct 1880	IMI 287842 (slides)	Sexual state described from material collected by Emil Rostrup in 1880.	Described the sexual stage of the pathogen but no link is made to any asexual stage.
1964	*Dothistroma pini* var. *pini* Hulbary	Thyr & Shaw (1964)	De Kalb County, Illinois, USA	*P. nigra* Arn. var. *austriaca*	29 Nov 1938	IILLS 27093; MBT128093; herb. CBS H-12211; IMI 178710	Variety *pini* described based on differences in the average conidial length.	Concluded that *Actinothyrium marginatum* and *Dothistroma* sp. were the same fungus. Described new variety of *Dothistroma pini*.
1964	*Dothistroma pini* var. *linearis* Thyr & C. G. Shaw	Thyr & Shaw (1964)	Meadow Creek, Clearwater Co. Idaho	*P. ponderosa*	June 1959	WSP 48361	Variety *linearis* described based on differences in the average conidial length.	Concluded that *Actinothyrium marginatum* and *Dothistroma* sp. were the same fungus. Described new variety of *Dothistroma pini*.
1966	*Scirrhia pini* A. Funk & A. K. Parker	Funk & Parker (1966)	Sooke, British Columbia, Canada	*P. contorta*	19 July 1965	DAVFP 16700; IMI 120997	The sexual state of *Dothistroma pini* is described as *Scirrhia pini* and is clearly differentiated from *Scirrhia acicola* (causal agent of brown-spot needle blight of pine). The authors suggest that the varieties of the asexual stage proposed by Thyr & Shaw (1964) might be premature.	*Scirrhia pini* described as the sexual stage of *Dothistroma pini*. No connection was made with *Mycosphaerella pini*, already described by Munk (1957).

(Continues)

TABLE 1 (Continued)

Date	Species epithet	Reference	Area	Host (*Pinus*)	Date material was collected	Herbarium collection details/number	Description	Confusion caused/importance/ significance
1967	*Dothistroma acicola* (Thüm.) Schischkina & Tsanava; *Systremma acicola* (Dearn.) F. A. Wolf & Barbour	Shishkina & Tsanava (1967)	Georgia, USSR	Mostly *P. pithyusa*, but also various others	1962–1964	None recorded	Linked *Dothistroma pini* with a sexual stage of the fungus found in Georgia (USSR). They proposed the new combination *Dothistroma acicola* (Thüm.) Schischkina & Tsanava for the asexual stage (replacing *D. pini*) and *Systremma acicola* (Dearn.) F. A. Wolf & Barbour for the sexual stage.	Unfortunately, they considered the fungus causing red-band and brown-spot to be the same thing and complicated the taxonomy further with the proposal of the new asexual name of *D. acicola* and use of the old name of the sexual stage of the brown-spot fungus, *Systremma*. However, this classification seems not to have been adopted and has not been found in any subsequent literature.
1967	*Dothistroma pini* var. *keniensis* M. H. Ivory	Ivory (1967)	Muguga, Nairobi, Kenya	*P. radiata*	10 Jan 1966	IMI 116919	Variety described based on differences in the average conidial length.	Described new variety of *Dothistroma pini*.
1968	*Dothistroma septospora* (Dorogin) M. Morelet	Morelet (1968b)					Morelet (1968b and 1969) noticed the similarity between *Dothistroma pini* Hulbary and *Cytosporina septospora* Dorogin and made the new combination *Dothistroma septospora*.	Made the new combination of *D. septospora* by retaining the oldest epithet for the species name "*septospora*" from *C. septosporum* Dorogin and combining it with the genus name *Dothistroma* Hulbary.
1968	*Scirrhia pini* var. *galliensis* M. Morelet	Morelet (1968a)					Variety described based on differences in the average asci and ascospore size.	Linked *S. pini* var. *galliensis* with *D. septospora* var. *pini*.
1968	*Scirrhia pini* var. *pini* A. Funk & A. K. Parker	Morelet (1968a)					Variety described based on differences in the average asci and ascospore size.	Linked *S. pini* var. *pini* with *D. septospora* var. *lineare*.
1968	*Dothistroma pini* Hulbary	Gremmen (1968)	Snagov, Romania	*P. ponderosa*, *P. nigra* var. *austriaca*	None available		Gremmen (1968) noticed similarity between *Dothistroma pini* Hulbary and *Cytosporina septospora* Dorogin. He also (Gremmen, 1965, 1968) drew attention to the fact that it had been misidentified as *Brunchorstia pinea* in Romania (by Georgescu & Petrescu, 1952 and Săvulescu, 1948) and in Spain (by Martínez, 1933; later perpetuated by Martínez & Torres Juan, 1965).	Noticed similarity between *Dothistroma pini* Hulbary and *Cytosporina septospora* Dorogin between Russian and American collections of the fungus as well as the confusion by some authors of the fungi *Dothistroma pini* and *Brunchorstia pinea*.

(Continues)

TABLE 1 (Continued)

Date	Species epithet	Reference	Area	Host (*Pinus*)	Date material was collected	Herbarium collection details/number	Description	Confusion caused/importance/ significance
1980	*Dothistroma septospora* var. *septospora* (Dorogin) M. Morelet	Sutton (1980)	Worldwide	Various *Pinus* sp.		None specified	Reclassification of asexual variety from *Dothistroma pini* Hulbary var. *pini* to *Dothistroma septospora* var. *Septospora*.	New combination for asexual varieties.
1980	*Dothistroma septospora* var. *lineare* (Thyr & C. G. Shaw) B. Sutton	Sutton (1980)	Various countries	Various *Pinus* sp.		Various - see within text (Sutton, 1980)	Reclassification of asexual variety *Dothistroma pini* Hulbary var. *linearis* to *Dothistroma septospora* var. *lineare*.	New combination for asexual varieties.
1980	*Dothistroma septospora* var. *keniense* (M. H. Ivory) B. Sutton	Sutton (1980)	Kenya	*P. radiata*		IMI116919	Reclassification of asexual variety *Dothistroma pini* Hulbary var. *keniensis* to *Dothistroma septospora* var. *keniense*.	New combination for asexual varieties.
1980	*Dothistroma flichiana* (Vuill.) M. Morelet	Morelet (1980)	Theil-sur-Vanne, close to Sens (Yonne), France	*P. austriaca* and *P. mugo* Turra subsp. *P. mugo* (syn. *P. montana* Mill.)	April–Oct 1860	None available	*Hypostomum flichianum* = *Dothistroma flichiana* (Vuill.) M. Morelet	There is no specimen available for *D. flichiana* and the species can, therefore, never be validated.
1983	*Septoria septospora* (Dorogin) Arx; *Mycosphaerella pini* (A. Funk & A. K. Parker) Arx	Arx (1983)					Arx proposed the new combination *Septoria septospora* (Dorogin) Arx for the asexual stage and *Mycosphaerella pini* (A. Funk & A. K. Parker) Arx for the sexual stage.	The naming of *M. pini* here is invalid as it is a homonym of *M. pini* Rostrup apud Munk (see Munk, 1957) which remains the holotype of the sexual stage. The new combination, *Septoria septospora*, was rejected by Evans (1984), when he pointed out the genus *Septoria* is reserved for coelomycetes with pycnidia.
1984	Existence of varieties rejected	Evans (1984)	Various	Various	Various	Various	Rejected splitting the fungus into different varieties based on morphology of spore size.	Varietal names of *Dothistroma* no longer accepted. Agreed with the placement of the sexual stage in *Mycosphaerella* as opposed to *Scirrhia* (Funk & Parker, 1966).
1996	*Eruptio pini* (Rostr. apud Munk) M. E. Barr	Barr (1996)					Barr proposed the new combination *Eruptio pini* (Rostr. apud Munk) M. E. Barr for the sexual stage.	Renamed the sexual stage of the fungus from *Mycosphaerella pini* to *Eruptio pini*. This name was rejected by Crous, J-Kang, and Braun (2001) who showed that based on phylogeny, *Eruptio* is a synonym of *Mycosphaerella*. The name *M. pini* was therefore retained.

(Continues)

TABLE 1 (Continued)

Date	Species epithet	Reference	Area	Host (*Pinus*)	Date material was collected	Herbarium collection details/number	Description	Confusion caused/importance/ significance
2000	*Dothistroma rhabdoclinis* Butin	Butin et al. (2000)	Wolfenbüttel, Germany	*Pseudotsuga menziesii*	24 May 1998	CBS 102195; MB804420	New species of *Dothistroma* described associated with *Rhabdocline pseudotsugae* on *Pseudotsuga menziesii* (Douglas fir).	Description of a new *Dothistroma* species.
2004	*Dothistroma pini* Hulbary	Barnes et al. (2004)	USA				Distinct species based on multigene phylogenies.	DNB is caused by two different pathogens. Showed that morphological varieties not supported by molecular data.
2004	*D. septosporum* (Dorogin) M. Morelet	Barnes et al. (2004)	Europe				Distinct species based on multigene phylogenies.	DNB is caused by two different pathogens. Showed that morphological varieties not supported by molecular data.
2011	End of dual nomenclature	Hawksworth et al. (2011)					Dual nomenclature for pleomorphic fungi discontinued. ''One Fungus = One Name" (1F1N) implemented.	Use of all previous names linked to the teleomorphic stage of *Dothistroma* (e.g. *Mycosphaerella pini*) is discontinued. The genus should be only known as *Dothistroma*.
2013	*D. rhabdoclinis* = *Sphaerulina rhabdoclinis* (Butin) Quaedvl., Verkley & Crous	Quaedvlieg et al. (2013)	Wolfenbüttel, Germany	*Pseudotsuga menziesii*	24 May 1998	CBS 102195; MB804420	*Dothistroma rhabdoclinis* transferred to *Sphaerulina rhabdoclinis* based on multigene phylogenies.	Now only two *Dothistroma* species known and both cause Dothistroma needle blight of pine: *Dothistroma pini* and *Dothistroma septosporum*.
2016	*D. pini* Hulbary	This study	USA, Michigan, Montcalm County	*P. nigra*	2001	CMW 10951; CBS 116487; CBS H-12211; MBT62987	Epitypification of *D. pini*.	Living cultures available linked to type material.
2016	*D. septosporum* (Dorogin) M. Morelet	This study	Russia, St. Petersburg, Park Sosnovka	*P. sylvestris*	14 Nov 2013	CMW 44656; CBS 140339; CBS H-22299; MBT202423; TAAM 168554A	Neotypification of *D. septosporum*.	Living cultures available linked to type material.

various names were incorrectly applied to the pathogen including *Brunchorstia pinea* (Doroguine, 1926) and *Septoriella septosporum* (Trotter, 1931). A complete account of the taxonomic history of these fungi is provided in Table 1.

In the late 1960s, Michel Morelet reduced to synonymy all the names applied in the USA and Europe to the causal agent of DNB and referred to the asexual morph of the pathogen as *Dothistroma septosporum* (as "*septospora*") (Morelet, 1968a, 1969). Yet, for more than four decades, both the names *D. septosporum* and *D. pini* were interchangeably used with *D. pini* being preferentially applied in the USA, New Zealand and Africa and *D. septosporum* typically used in Europe (Bradshaw, 2004). It was not until Barnes et al. (2004), who applied DNA sequence data to a global collection of isolates, showed that DNB is caused by two distinct species and both names, *D. septosporum* and *D. pini*, were retained. An ongoing initiative, strongly promoted by the objectives of the DIAROD EU COST Action FP1102 (Determining Invasiveness And Risk Of Dothistroma, http://www.cost.eu/COST_Actions/fps/FP1102?), is now in place to continually use molecular methods to correctly identify the species of *Dothistroma* reported in old literature and to establish the current global distribution of both pathogens (Drenkhan et al., 2016).

Recent advances in fungal taxonomy have led to the abandonment of the dual nomenclature system for pleomorphic fungi (Hawksworth, 2015; Hawksworth et al., 2011). This has led to a situation where the application of names for both sexual and asexual morphs is no longer appropriate. As a consequence, the "One Fungus = One Name" (1F1N) concept is in the process of being implemented (Taylor, 2011; Wingfield et al., 2012) and where entire genera are being reclassified with single names being fixed to type species (Crous et al., 2014; Rossman et al., 2015; Wijayawardene et al., 2014). The names for the sexual morphs of the DNB pathogens, described as *Scirrhia pini* (Funk & Parker, 1966), *Mycosphaerella pini* (Munk, 1957) and *Eruptio pini* (Barr, 1996), are no longer appropriate and the asexual genus name *Dothistroma* has been retained for both the DNB pathogens (Quaedvlieg, Groenewald, De Jesús Yáñez-Morales, & Crous, 2012).

DNA barcoding has been established to advance and streamline the molecular identification of fungal species and the discovery of potentially new species (Quaedvlieg et al., 2012; Schoch et al., 2012; Stielow et al., 2015). The barcoding region considered most appropriate for fungi is the Internal Transcribed Spacer (ITS) region due to its robust amplification success and the extensive databases that are currently available for this region (Schoch et al., 2012, 2014; Stielow et al., 2015).

The two pathogens causing DNB have very similar morphology, and they give rise to the same disease symptoms (Barnes, Kirisits, Wingfield, & Wingfield, 2011). Consequently, the ITS region is being used to effectively distinguish between them (Piškur, Hauptman, & Jurc, 2013; Queloz, Wey, & Holdenrieder, 2014; Tsopelas, Barnes, Soulioti, & Wingfield, 2013). Not surprisingly, diagnostic methods using ITS-RFLP have also been developed to rapidly distinguish between *D. pini* and *D. septosporum* (Barnes et al., 2004; Pehl, Burgermeister, & Wulf, 2004). However, point mutations in the ITS region of both *D. septosporum* (Mullett & Fraser, 2015) and *D. pini* have recently been reported (Barnes, Walla, Bergdahl, & Wingfield, 2014). It is consequently not yet known whether the restriction sites used in diagnostic protocols have been affected in these new haplotypes.

Descriptions of the majority of new fungal species being described are supported with DNA sequence data, commonly for more than one gene region. It has consequently become imperative to have DNA sequence data linked to type specimens in order to validate already described species. In this regard, an important challenge is that cultures linked to appropriate type material are often not available, or DNA cannot be extracted from inordinately old fungarium material. This problem can be circumvented by neotypification or, in the case of living cultures not being available, epitypification (Ariyawansa et al., 2014).

The original fungarium material of *Cytosporina septospora* collected by Georges Doroguine in 1910 from *P. mugo* Turra subsp. *P. mugo* (syn. *P. montana* Mill.) in Saint Petersburg, Russia (Doroguine, 1911), has been lost. According to the curators of these herbaria, it is neither maintained at the Komarov Botanical Institute of the Russian Academy of Sciences, Saint Petersburg (formerly, Leningrad, LE), nor at the All-Russian Research Institute of Plant Protection (LEP). This implies that the name-bearing type material for *D. septosporum* is no longer available. Although the type material of *D. pini* collected by James C. Carter in Illinois in 1938 is available, repeated attempts to amplify the ITS region from conidiomata on this specimen have not been successful (Barnes et al., 2004). A serious situation thus exists where there is no appropriate type material or cultures to allow for robust DNA-based classification of *Dothistroma* species, or indeed other members of this genus.

The purpose of this study was to provide a neotype for *D. septosporum* and to designate an epitype for *D. pini* for which cultures and sequence data are available. These strains can then be used as the authentic material for all future morphological and DNA-based comparative studies on *Dothistroma*. As the ITS region is used as the barcoding gene for the genus, our aim was to characterize the different haplotypes found in the ITS region of both species and provide an ITS map for easy annotation. Lastly, we have provided a complete taxonomic history for *Dothistroma* and the DNB pathogens.

2 | MATERIAL AND METHODS

2.1 | Isolates

In Russia, four sampling areas were chosen for the collection of possible neotype material for *D. septosporum*. All needle samples were collected by Rein Drenkhan and Dmitry L. Musolin in November 2013. The first site (59.991°N, 30.344°E) was the park of Saint Petersburg Forestry Institute, in Lesnoj (now St. Petersburg State Forest Technical University), where Georges Doroguine collected symptomatic needles in 1910 and then described *Cytosporina septospora* (Doroguine, 1911, 1912; Fig. 1). Although typical DNB symptoms were not found, several needles were collected from various conifer species and the species-specific conventional PCR (Ioos et al., 2010) was used to directly screen the plant material for the presence of the pathogens.

FIGURE 1 The Russian description of *Dothistroma septosporum* (Doroguine, 1912). A cover of the Lesnoy Zhurnal (Forest Journal), issue 10 of 1912 from the collection of The Fundamental Library of Saint Petersburg State Forest Technical University, St. Petersburg, Russia (top), and figure with its legend: *Cytosporina septospora* nov. spec. Right: a diseased needle; centre: cross section through a fungus fruit conceptacle and a diseased needle (× 450); left: one chamber of the conceptacle with spores (× 600)

A second sampling site in St. Petersburg was ca. 3 km from the first site, in Park Sosnovka (60.02278°N, 30.35167°E). This 360 ha area was a natural pine forest used as a recreational area before it was established as a city park in 1960. Needle samples were collected from nine symptomatic local, but planted, *P. sylvestris* and two *P. mugo* trees, ca. 10–15 years old.

The third and fourth sampling sites were from two natural pine stands 30 km from St. Petersburg. At the third location (60.28500°N, 29.81200°E), samples were collected from 19 symptomatic *P. sylvestris* trees 15–20 years old. At the fourth sampling location (60.20060°N, 29.96010°E), 13 symptomatic *P. sylvestris* trees 10–15 years old were sampled. At all sampling sites, 2- to 3-year-old needles were collected from the lower parts of the tree canopies and needle samples from different trees were placed in separate sterile plastic bags.

In seeking an epitype for *D. pini*, it was not possible for the authors to sample in De Kalb County, northern Illinois, where James C. Carter collected the material on *P. nigra* subsp. (var.) *austriaca* in 1938 and from which Robert L. Hulbary made his original descriptions of the genus *Dothistroma* and the species, *D. pini* (Hulbary, 1941). However, needle samples collected in 2001 by Gerry Adams from *P. nigra* in Stanton, Montcalm County, Michigan, and cultures generated from this material (Barnes et al., 2004) were used for this purpose.

Most of the remaining isolates used in this study were made from symptomatic needles of different pine species collected from various countries during the course of the last few years (Table 2). Single conidial isolations from mature conidiomata were made from all needle collections following the methods described by Mullett & Barnes (2012), on 2% Dothistroma sporulating media (DSM: 20 g malt extract, 5 g yeast extract and 15 g agar) supplemented with 100 mg/L streptomycin (Sigma-Aldrich, St. Louis, USA). Additional cultures were also obtained from international culture collections (Table 2). All cultures

TABLE 2 Detailed information of the isolates used in this study

Species	Locality	Sampling date	Collected/isolated and identified by	Host species	CMW number[a]	Other collection numbers[b]	ITS GenBank Accession No.[c]	ITS haplotype
Dothistroma septosporum	Colombia, Armenia, Quindio	March 2011	Rodas C; Barnes I	*Pinus elliotii* x *taeda*	37193	–	**KU948387**	Ds_HAP.1
D. septosporum	Denmark, Copenhagen, Arboretum in Hørsholm	June 2013	Thomsen IM; Barnes I	*P. aristata*	40004	–	**KU948388**	Ds_HAP.1
D. septosporum	England, East Anglia	2006	Mullett MS	*P. nigra* subsp. *laricio*	47224	D291	**KU948389**	Ds_HAP.1
D. septosporum	England, New Forest	21 July 2005	Mullett MS	*P. nigra* subsp. *laricio*	47220	D152	**KU948390**	Ds_HAP.1
D. septosporum	England, North York Moors	10 July 2006	Mullett MS	*P. nigra* subsp. *laricio*	47221	D245	**KU948391**	Ds_HAP.1
D. septosporum	England, Sherwood & Lincs	22 Aug 2007	Mullett MS	*P. nigra* subsp. *laricio*	47226	D429	**KU948392**	Ds_HAP.1
D. septosporum	Finland, Suonenjoki district	Aug 2011	Barnes I	*P. sylvestris*	37537	–	**KU948393**	Ds_HAP.1
D. septosporum	France, Villefranche-sur-Cher	13 Aug 2012	Mullett MS; Barnes I	*P. nigra* subsp. *laricio*	41502	–	**KU948394**	Ds_HAP.1
D. septosporum	Greece, Lagadas, Thessaloniki Prefecture	Dec 2011	Tsopelas P; Barnes I	*P. brutia*	37965	–	**KU948395**	Ds_HAP.1
D. septosporum	Guatemala, Jalapa, Finca Forestal Soledad	Oct 2010	Barnes I	*P. oocarpa*	36892	–	**KU948396**	Ds_HAP.1
D. septosporum	Guatemala, Tactic, Alta Verapaz	July 2011	Barnes I	*P. maximinoi*	38528	–	**KU948397**	Ds_HAP.1
D. septosporum	Guatemala, Salamá, Sierra de Chuacús	28 April 1983	Evans HC	*P. tecunumanii*	42207	IMI 281626	**KU948398**	Ds_HAP.1
D. septosporum	Netherlands, Lunteren	June 2009	Quaedvlieg W	*P. mugo*	45414	CBS 128782	**KU948399**	Ds_HAP.1
D. septosporum	New Zealand, South Island	2005	Doherty B; Dick M	*P. radiata*	–	CBS 128990	BioProject PRJNA74753	Ds_HAP.1
***D. septosporum* (neotype)**	**Russia, St. Petersburg, Park Sosnovka**	**14 Nov 2013**	**Drenkhan R; Musolin D; Adamson K**	***P. sylvestris***	**44656**	**CBS 140339; CBS H-22299; MBT202423; TAAM 168554A**	**KU948400**	Ds_HAP.1
D. septosporum	Russia, St. Petersburg, Park Sosnovka	14 Nov 2013	Drenkhan R; Musolin D; Adamson K	*P. sylvestris*	44657	CBS 141531; CBS H-22300; TAAM 168554	**KU948401**	Ds_HAP.1
D. septosporum	Russia, near St. Petersburg, natural pine stand	13 Nov 2013	Drenkhan R; Musolin D; Adamson K	*P. sylvestris*	44658	CBS 140340; CBS H-22301; TAAM 168555	**KU948402**	Ds_HAP.1
D. septosporum	Russia, near St. Petersburg, natural pine stand	13 Nov 2013	Drenkhan R; Musolin D; Adamson K	*P. sylvestris*	44659	CBS 140684; CBS H-22302; TAAM 168552	**KU948403**	Ds_HAP.1
D. septosporum	Russia, Vladivostok	27 Aug 2014	Drenkhan R; Solheim H; Adamson K	*P. sylvestris*	–	TAAM 168553	**KU948404**	Ds_HAP.1
D. septosporum	Scotland, Aberdeen, Cruikshank Botanic Garden	July 2013	Mullett MS; Fraser S	*Cedrus atlantica* subsp. *glauca*	47231	D1200.1; IMI 504778	KP317915	Ds_HAP.2
D. septosporum	Scotland, Cowal and Trossachs	11 Aug 2010	Mullett MS	*P. sylvestris*	47229	D524	**KU948405**	Ds_HAP.1
D. septosporum	Scotland, Moray	5 July 2006	Mullett MS	*P. nigra* subsp. *laricio*	47222	D258	**KU948406**	Ds_HAP.1

(Continues)

TABLE 2 (Continued)

Species	Locality	Sampling date	Collected/isolated and identified by	Host species	CMW number[a]	Other collection numbers[b]	ITS GenBank Accession No.[c]	ITS haplotype
D. septosporum	Scotland, North Highland	19 July 2010	Mullett MS	*P. sylvestris*	47230	D539	**KU948407**	Ds_HAP.1
D. septosporum	Scotland, Tay	15 Aug 2007	Mullett MS	*P. nigra* subsp. *laricio*	47225	D386	**KU948408**	Ds_HAP.1
D. septosporum	Spain, Girona Province, Sant Hilari Sacalm, La Selva	May 2013	Soler CC; Barnes I	*P. sylvestris*	39978	–	**KU948409**	Ds_HAP.1
D. septosporum	USA, Alaska, Haines	26 Aug 2015	Mulvey R; van der Nest A; Barnes I	*P. contorta* subsp. *contorta*	47456	–	**KU948410**	Ds_HAP.1
D. septosporum	USA, Alaska, Pt. Bridget State Park, Juneau	23 May 2013	Mulvey R; van der Nest A; Barnes I	*P. contorta* subsp. *contorta*	47460	–	**KU948411**	Ds_HAP.3
D. septosporum	USA, Idaho, Lochsa Historical Ranger Station	22 June 2004	Carris LM; Barnes I	*P. ponderosa*	15077	CBS H-12204; MBT62980	AY808299	Ds_HAP.1
D. septosporum	USA, Oregon, Bandon County	Jan 1983	–	*P. ponderosa*	14822	ATCC MYA-610	AY808300	Ds_HAP.1
D. septosporum	USA, Oregon, Lincoln, near Seal Rocks	23 April 2014	Shaw D; Barnes I	*P. contorta* subsp. *contorta*	44519	–	**KU948412**	Ds_HAP.4
D. septosporum	USA, Oregon, Lincoln, near Seal Rocks	23 April 2014	Shaw D; Barnes I	*P. contorta* subsp. *contorta*	44520	–	**KU948413**	Ds_HAP.4
D. septosporum	USA, Montana, Missoula Lola National Forest	May 2006	Six D; Barnes I	*P. contorta* subsp. *latifolia*	23780	–	**KU948414**	Ds_HAP.1
Dothistroma pini	Czech Republic, Chodská Lhota	Sep 2013	Bergová E; Kryštofová A	*P. jeffreyi*	43394	–	**KU948415**	Dp_HAP.1
D. pini	France, Selles-Saint-Denis	13 Aug 2012	Mullett MS; Barnes I	*P. nigra* subsp. *laricio*	43903	–	**KU948416**	Dp_HAP.2
D. pini	France, Villefranche-sur-Cher	13 Aug 2012	Mullett MS; Barnes I	*P. nigra* subsp. *laricio*	41496	–	**KU948417**	Dp_HAP.4
D. pini	France, Villefranche-sur-Cher	13 Aug 2012	Mullett MS; Barnes I	*P. nigra* subsp. *laricio*	41477	–	**KU948418**	Dp_HAP.1
D. pini	Hungary, Csabrendek	May 2007	Kirisits K; Barnes I	*P. nigra*	29371	CBS 127874	**KU948419**	Dp_HAP.1
D. pini	Romania, Voluntari	30 May 2015	Costache C	*P. nigra*	46789	–	**KU948420**	Dp_HAP.2
D. pini	Russia, Krasnosulinsky	12 May 2007	Bulgakov TS; Barnes I	*P. nigra*	29368	CBS 127871	**KU948421**	Dp_HAP.2
D. pini	Russia, Tarasovsky	7 May 2007	Bulgakov TS; Barnes I	*P. nigra* subsp. *pallasiana*	29366	–	**KU948422**	Dp_HAP.2
D. pini	Slovenia, Pivka	June 2012	Jurc D, Piškur B	*P. nigra* subsp. *nigra*	43409	CBS 134689	KC149562	Dp_HAP.1
D. pini	Ukraine, Tsjurupinsk area, Kherson region	9 Sep 2013	Davydenko K; Siziba V	*P. nigra* subsp. *pallasiana*	42947	–	**KU948423**	Dp_HAP.2
D. pini	USA, Indiana, Shelby County	20 May 2011	Walla J; Barnes I	*P. nigra*	37786	–	**KU948424**	Dp_HAP.1
***D. pini* (epitype)**	**USA, Michigan, Montcalm County**	**2001**	**Adams G; Barnes I**	***P. nigra***	**10951**	**CBS 116487; CBS H-12211; MBT62987**	AY808302	Dp_HAP.1
D. pini	USA, Minnesota	1970	–	*P. nigra*	14820	ATCC MYA-609	**KU948425**	Dp_HAP.1
D. pini	USA, Nebraska, Lancaster County	14 July 2011	Walla J; Barnes I	*P. nigra*	37623	–	**KU948426**	Dp_HAP.1

(Continues)

TABLE 2 (Continued)

Species	Locality	Sampling date	Collected/isolated and identified by	Host species	CMW number[a]	Other collection numbers[b]	ITS GenBank Accession No.[c]	ITS haplotype
D. pini	USA, South Dakota, Brookings County	15 July 2011	Walla J; Barnes I	*P. ponderosa*	38037	–	**KU948427**	Dp_HAP.1
D. pini	USA, North Dakota, Cass County	29 June 2011	Walla J; Barnes I	*P. ponderosa*	37633	NDSU 75299	KJ933441	Dp_HAP.3
D. pini	USA, North Dakota, Cass County	06 July 2011	Walla J; Barnes I	*P. cembra*	37634	NDSU 7724	**KU948428**	Dp_HAP.1
D. pini	USA, North Dakota, Pembina County	17 June 2010	Walla J; Barnes I	*P. ponderosa*	41115	–	**KU948429**	Dp_HAP.3
D. pini	USA, North Dakota, Pembina County	30 July 2011	Walla J; Barnes I	*P. ponderosa*	37618	–	**KU948430**	Dp_HAP.5
Amycosphaerella africana	South Africa, Western Cape Province, Pampoenvlei	7 Nov 1994	Crous PW	*Eucalyptus cladocalyx*	45395	CBS 110843	AY725545	–
Lecanosticta acicola	USA, New Hampshire, Blackwater	15 Jun 2011	Ostrofsky B; Crous PW	*P. strobus*	45427	CBS 133791	KC012999	–
Lecanosticta brevispora	Mexico	24 Oct 2009	de Jesús Yáñes-Morales M; Mendez-Inocencio C; Crous PW	*Pinus* sp.	45424	CBS 133601	JX901763	–
Lecanosticta gloeospora	Mexico, Nuevo León	16 May 1983	Evans HC	*P. pseudostrobus*	42645	IMI 283812	**KU948431**	–
Lecanosticta guatemalensis	Guatemala	28 April 1983	Evans HC	*P. oocarpa*	42206	IMI 281598	JX901764	–
Lecanosticta longispora	Mexico, Nuevo León, Galeana, Cerro del Potosi	24 Oct 2009	de Jesús Yáñes-Morales M; Mendez-Inocencio C; Crous PW	*Pinus* sp.	45429	CBS 133602	JX901766	–
Stromatoseptoria castaneicola	Netherlands	29 Aug 1999	Verkley GJM	*Castanea sativa*	–	CBS 102322	KF251271	–
Sphaerulina rhabdoclinis	Germany, Wolfenbüttel	24 May 1998	Butin H	*Pseudotsuga menziesii*	12519	CBS 102195	AY808308	–

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

[b]D = Culture collection at Forest Research, Alice Holt Lodge, Surrey, England; IMI = International Mycological Institute; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; MBT = Mycobank Typification number (www.mycobank.org); TAAM = Mycological herbaria in Estonia (http://natarc.ut.ee/seenekogud.php); ATCC = American Type Culture Collection, Virginia, U.S.A; NDSU = accession number for the herbarium collection at the North Dakota State University Dale E Herman Research Arboretum.

[c]ITS sequence deposited in GenBank in this study are indicated in BOLD.

for DNA isolation were grown on DSM at 21°C at natural day/night light intervals for 3–4 weeks.

2.2 | DNA isolation, amplification and analyses

For DNA extractions, mycelium obtained from cultures growing on DSM plates was freeze-dried overnight and ground into a powder using the Retsch GmbH MM301 homogenizer (Haan, Germany). Total DNA was extracted from (30 mg) ground mycelia using the Zymo Research Fungal DNA MiniPrep kit (Irvine California, USA) and eluted in a volume of 50 µl. DNA concentrations were measured using a Thermo Scientific NanoDrop® ND-1000 spectrophotometer (Wilmington, DE, USA) and diluted to a working stock of 20 ng μl^{-1}.

The ITS barcoding region was amplified using the primers ITS1 and ITS4 (White et al., 1990). Each PCR mix included 2.5 µl of 10 × FastStart PCR buffer, 2.5 µl $MgCl_2$ (25 mM), 0.2 µl of FastStart Taq polymerase (5 U μl^{-1}) (Roche Diagnostics, Indianapolis, USA), 2 µl dNTP mix (10 mM), 0.5 µl of each forward and reverse primer (10 mM), 1 µl of DNA and dsH_2O to a total volume of 25 µl. PCRs were run on a Bio-Rad iCycler thermocycler (BIO-RAD, Hercules, CA, USA) with the following thermal cycling conditions: 1 cycle at 95°C for 4 min, 10 cycles of 95°C for 20 s, 56°C for 45 s, 72°C for 45 s, 25 cycles of 95°C for 20 s, 56°C for 45 s (with an increase of 5 s after each cycle), 72°C for 45 s, followed by a final extension cycle at 72°C for 10 min. For each sample, 5 µl of PCR amplicon was electrophoresed on 2% agarose gel (Merck, Darmstadt, Germany) with 2 µl GelRed™ (Biotium, California) and visualized under UV light using the GelDoc™ EZ Imager (BioRad, Johannesburg, South Africa). Amplicons were purified using G-50 sephadex (SIGMA-Aldrich, Steinheim, Germany) in Centri-sep Spin Columns (Princeton separations Inc., Adelphia, USA).

ITS PCR amplicons were sequenced in both directions using the ABI PRISM™ Big Dye ready reaction kit (Applied BioSystems, Foster City, CA, USA). Sequencing reactions consisted of 0.5 µl Big Dye reaction mix, 2.1 µl 5 × Big Dye sequencing buffer, 0.5 µl primer, 60–100 ng amplified PCR product and dsH_2O to a total volume of 12 µl. Cycling conditions included 1 cycle of 96°C for 10 s and 35 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Sequencing reactions were run on an ABI PRISM™ 3500xl capillary autosequencer (Applied BioSystems).

Forward and reverse sequences were assembled in CLC Main Workbench V. 6.6.2 (CLC Bio, www.clcbio.com). To validate the correct orientation and annotation of the ITS sequences for this, and future studies (Nilsson et al., 2014), and to identify the different haplotypes, an ITS map for *Dothistroma* was constructed in CLC Main Workbench V. 6.6.2. The full length of the 18S, ITS1, 5.8, ITS2 and 28S sequence of the *D. septosporum* strain NE1 was obtained from Scaffold 18, downloaded from the Joint Genome Institute (JGI) website (De Wit et al., 2012; http://genome.jgi.doe.gov/Dotse1/Dotse1.home.html), and used as the reference strain. The different ITS haplotypes of *D. pini* and *D. septosporum* were identified and annotated using CLC Main Workbench V. 6.6.2.

For the phylogenetic analyses, all sequences were aligned using the online version of Mafft Version 7 (Katoh & Standley, 2013; http://mafft.cbrc.jp/alignment/server/) with default settings. Alignments were manually checked and adjusted in Mega (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Sequences for the outgroup taxa were downloaded from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) (Table 2). *Stromatoseptoria castaneicola* and *Amycosphaerella africana* (syn.: *Mycosphaerella ellipsoidea* and *M. africana*, respectively) were included in the alignments as they are phylogenetically the most closely related taxa to *Dothistroma* (Quaedvlieg et al., 2012, 2013). *Sphaerulina rhabdoclinis* was included as it was previously considered a member of *Dothistroma* as *D. rhabdoclinis* (Butin, Kehr, & Pehl, 2000; Quaedvlieg et al., 2013). All known *Lecanosticta* species were also included in the analyses as a consequence of their commonly being confused with *Dothistroma* spp. due to similar disease symptoms and morphological characters (Table 1).

Phylogenetic analyses included maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). The MP analysis was conducted using the software package Paup* Version 4.0b10 (Swofford, 2003). Gaps were treated as a fifth character state, and 1000 random stepwise addition heuristic searches were performed with tree bisection reconnection (TBR) selected as the branch-swapping algorithm. The consistency index (CI), homoplasy index (HI), rescaled consistency index (RC), retention index (RI) and tree length (TL) were recorded for the resulting trees. The branch node confidence levels were estimated by performing 1000 bootstrap replicates.

For both likelihood methods (ML and BI), the best-fit substitution models for the data set were determined using jModelTest Version 0.1.1 (Posada, 2008). Maximum likelihood analysis was performed with the program PhyML Version 3.0 (Guindon & Gascuel, 2003), and the confidence levels for nodes were estimated with 1000 bootstrap replicates.

The BI analysis was conducted in MrBayes Version 3.1.2 (Ronquist et al., 2012) by applying the Markov chain Monte Carlo (MCMC) method. Four independent MCMC chains were randomly initiated and run for six million generations, applying the best substitution model determined with jModelTest Version 0.1.1. Trees were sampled every 100 generations. Tracer Version 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) was used to determine burn-in values by comparing the log likelihoods, and trees sampled in the burn-in phase (10%) were discarded. The remaining trees were used to construct majority rule consensus trees and to determine posterior probabilities for the tree topology.

2.3 | Morphology

For morphological observations, cultures were subcultured onto 2% DSM and Spezieller Nahrstoffarmer agar (SNA), and incubated at 18°C for two weeks under ultraviolet light to induce sporulation. Slide preparations were made by mounting fungal material in clear, 80% lactic acid and morphological structures were observed using a Zeiss

Axioskop microscope (Carl Zeiss, Germany). Images were captured electronically using a Zeiss Axio Vision (Carl Zeiss) camera system and software. Type specimens were deposited in MycoBank (www.MycoBank.org).

3 | RESULTS

3.1 | Isolates

All attempts to isolate *Dothistroma* from the pine needles collected in the Saint Petersburg State Forest Technical University Park, where the original description of *Dothistroma* was made, were unsuccessful. However, screening conifer needles from 12 different species with species-specific primers as described in Ioos et al. (2010), confirmed the presence of *D. septosporum* in *Pinus sibirica*, *P. ponderosa* and *Pseudotsuga menziesii*.

Successful isolations were made from symptomatic pine material collected at Park Sosnovka and from the two locations near St. Petersburg, but these were only from *P. sylvestris* trees. Pine needles from a single *P. sylvestris* tree, collected at Park Sosnovka, and the associated culture obtained from these needles provided the material to neotypify *D. septosporum* (see Taxonomy section below, Table 2).

All cultures generated in this study are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. Representative reference strains and pine needle specimens were deposited in international culture collections and fungaria including the CBS-KNAW Fungal Biodiversity Centre in Utrecht, The Netherlands, and the Mycological Herbarium of the Estonian University of Life Sciences (TAAM; http://natarc.ut.ee/seenekogud.php) (Table 2).

3.2 | DNA isolation, amplification and analyses

Amplification of the ITS region (part of the nuclear rDNA region) in *Dothistroma* generated PCR products in the range of 535–536 bp (Fig. 2). The complete sequenced fragments included 30 bp of the 3′ end of the 18S nrRNA gene (SSU), 146–147 bp of the internal transcribed spacer 1 (ITS1), 158 bp of the 5.8S nrRNA gene, 144 bp of the internal transcribed spacer 2 (ITS2) and 58 bp of the 5′ end of the 28S nrRNA gene (LSU) (Fig. 2).

Four different ITS haplotypes were identified in *D. septosporum* (Ds_HAP.1, Ds_HAP.2, Ds_HAP.3 and Ds_HAP.4) and five in *D. pini* (Dp_HAP.1, Dp_HAP.2, Dp_HAP.3, Dp_HAP.4 and Dp_HAP.5) based on either point mutations or single nucleotide insertions (Table 2; Fig. 3). To construct the ITS map for *Dothistroma*, the sequence of *D. pini* haplotype 4 (Dp_HAP.4) was used (Table 2). This isolate from France contained an extra A in position 75 thus making it the longest ITS fragment. Polymorphisms were observed at 11 sites in the ITS fragment (Fig. 3), eight of which were found in the ITS1 region and three in the ITS2 region. Of these polymorphisms, four were fixed and distinct between *D. septosporum* and *D. pini* (see sites at bp 99, 146, 349, and 472) and were used to define the species. The polymorphism at site 349, a G in *D. septosporum* and an A in *D. pini*, gave rise to the *Alu*I restriction site in *D. pini*, which can also be used to distinguish between the two species using an ITS-RFLP method (Barnes et al., 2004). This restriction site was maintained regardless of the mutations observed in the different haplotypes within the two species.

For the phylogenetic analyses, the final data set consisted of 59 taxa with 547 aligned nucleotides, including gaps. In the MP analysis, 367 characters were constant, 72 characters were parsimony

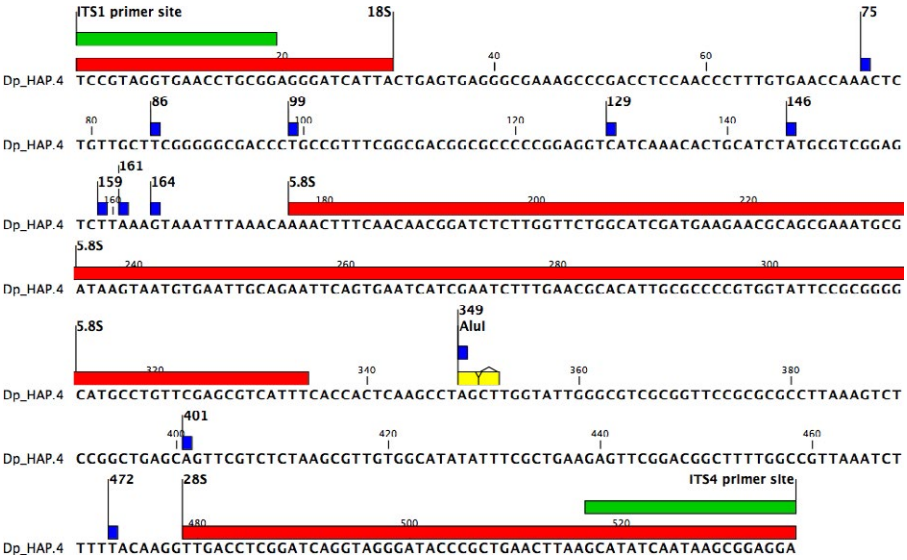

FIGURE 2 The complete 537-bp ITS barcoding map of *Dothistroma pini* (haplotype Dp_HAP.4), amplified by primers ITS1/ITS4 (White et al. 1990). Full primer binding sites are indicated by green boxes. The partial 18S, complete 5.8S, and partial 28S genes are indicated by red boxes. Blue boxes indicate sites where polymorphisms are observed in the ITS regions, creating the different ITS haplotypes in *Dothistroma* (see Fig. 3). The 4-bp blunt-end *Alu*I restriction site (AGCT), used for *D. pini* identification, is indicated in yellow. A polymorphism at site 349 from an A to G in all *D. septosporum* isolates removes this restriction site. This sequence is represented by GenBank accession number KU948417

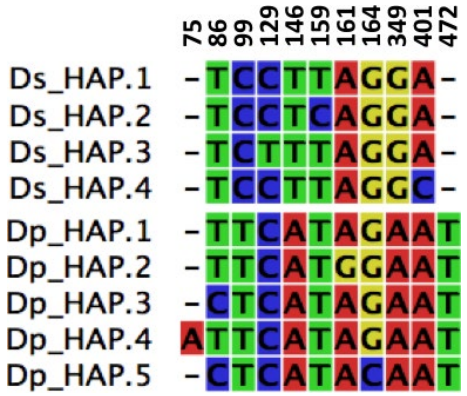

FIGURE 3 The different ITS haplotypes found in *Dothistroma* species. Four haplotypes are found in *Dothistroma septosporum* (Ds) while five are found in *Dothistroma pini* (Dp). Numbers at the top indicate the site where the polymorphisms are positioned in the ITS fragment amplified with primers ITS1/ITS4 (see Fig. 2)

uninformative and 108 characters were parsimony informative. The CI, HI, RC, RI and TL were 0.936, 0.064, 0.906, 0.968 and 233, respectively. After the heuristic search, two trees were retained of which one was chosen for presentation (Fig. 4). The best-fit substitution model for ML and BI was selected by Akaike information criterion (AIC) and was TIM2 (Posada, 2008) with rate variations among sites (+G). Because the MP, ML and BI analyses all resulted in similar tree topologies, significant bootstrap support (for MP and ML) and posterior probabilities (for BI) are all indicated on the branches of the MP tree (Fig. 4).

Ds_HAP.1 was the most frequent haplotype (88%) found in *D. septosporum* (Fig. 4). The other three haplotypes were represented by only 1–2 individuals each. Ds_HAP.2 is represented by an isolate of *D. septosporum* that was obtained from *Cedrus atlantica* subsp. *glauca* and has a 1-bp difference from Ds_HAP.1 at site 159 (Fig. 3). Isolates from the USA had the most variable number of *D. septosporum* haplotypes (three of the four). This was also true for the *D. pini* haplotypes where three of the five haplotypes of *D. pini* occurred in the USA isolates. The sequences of haplotypes represented by only one individual were double-checked to ensure they had not been generated as a result of a sequencing error. All ITS sequences generated in this study were deposited in GenBank (Table 2).

3.3 | Taxonomy

The morphological and culture characteristics of the isolates from Russia, St. Petersburg, Park Sosnovka (CMW 44656 and CMW 44657; Fig. 6), were the same as those described for *D. septosporum* in Barnes et al. (2004). In addition, the regions sequenced confirmed the identity of these isolates as *D. septosporum* (Fig. 4). The morphological characteristics and DNA sequence data of *D. pini* isolate CMW 10951 were previously presented in Barnes et al. (2004) and were available for this study (Fig. 5). These results allowed us to designate a neotype for *D. septosporum* and an epitype for *D. pini*. These typifications are described below.

Classification: *Dothistroma*, Mycosphaerellaceae, Capnodiales, Dothideomycetidae, Dothideomycetes, Pezizomycotina, Ascomycota, Fungi.

Dothistroma pini Hulbary, Bull. Ill. St. Nat. Hist. Surv. 21: 235. 1941. Figs 3–5 (type of the genus).

See Barnes et al. (2004) for a full description of *D. pini* based on isolate CBS 116487.

Holotype: USA, northern Illinois, De Kalb County from *P. nigra* subsp. (var.) *austriaca*, 29 November 1938, J. Cedric Carter, MBT128093, herb. ILLS 27093, herb. CBS H-12211 (= isotype).

Epitype designated: USA, Michigan, Montcalm County, Stanton, Evergreen Township, from *Pinus nigra*, 2001, G. Adams, MBT62987, herb. CBS H-12211, culture ex-epitype CMW 10951 = CBS 116487.

***Notes*:** No ex-type cultures are available from the holotype material and DNA could not be recovered from the herbarium material. The epitype designated here represents *Dothistroma pini* ITS haplotype 1 (DP_HAP.1) (Figs 3,4, Table 2). Sequences available on GenBank: Genome (PRJNA212510), ITS (AY808302), BT1 (AY808197), BT2 (AY808232) and TEF1α (AY808267).

Dothistroma septosporum (Dorogin) M. Morelet (as "*septospora*"), Bull. Soc. Sci. Nat. Archéol. Toulon Var. 177: 9. 1968. Figs 3,4,6.

Basionym. *Cytosporina septospora* Dorogin, Bull. Trimestriel Soc. Mycol. France 27: 106. 1911.

- *Septoriella septospora* (Dorogin) Sacc. apud Trotter, Syll. Fung. 25: 480. 1931.
- *Septoria septospora* (Dorogin) Arx, Proc. Kon. Ned. Akad. Wetensch. C 86, 1: 33. 1983.
- *Actinothyrium marginatum* Sacc., Nuovo Giorn. Bot. Ital. 27: 83. 1920.
- *Mycosphaerella pini* Rostr., in Munk, Dansk Bot. Ark. 17(1): 312. 1957.
- *Eruptio pini* (Rostr.) M. E. Barr, Mycotaxon 60: 438. 1996.
- *Dothistroma pini* var. *lineare* Thyr & C. G. Shaw (as "*linearis*"), Mycologia 56: 107. 1964.
- *Dothistroma septosporum* (as *"septospora"*) var. *lineare* (Thyr & C. G. Shaw) B. Sutton, The Coelomycetes. Fungi imperfecti with pycnidia acervuli and stromata (Kew): 173. 1980.
- *Scirrhia pini* A. Funk & A. K. Parker, Canad. J. Bot. 44: 1171. 1966.
- *Mycosphaerella pini* (A. Funk & A. K. Parker) Arx, Proc. Kon. Ned. Akad. Wetensch., Ser. C 86(1): 33 (1983) (homonym, nom. illegit., Art. 53).
- *Dothistroma pini* var. *keniense* M. H. Ivory (as "*keniensis*"), Trans. Brit. Mycol. Soc. 50: 294. 1967.
- *Dothistroma septosporum* (as *"septospora"*) var. *keniense* (M. H. Ivory) B. Sutton, in Sutton, The Coelomycetes. Fungi imperfecti with pycnidia acervuli and stromata (Kew): 174. 1980.

See Barnes et al. (2004) for a full description of *D. septosporum* based on isolate CBS 116488.

Holotype: *Cytosporina septospora* Dorogin, Lesnoj, near St. Petersburg, *Pinus montana* Mill., summer 1910, G. Doroguine.

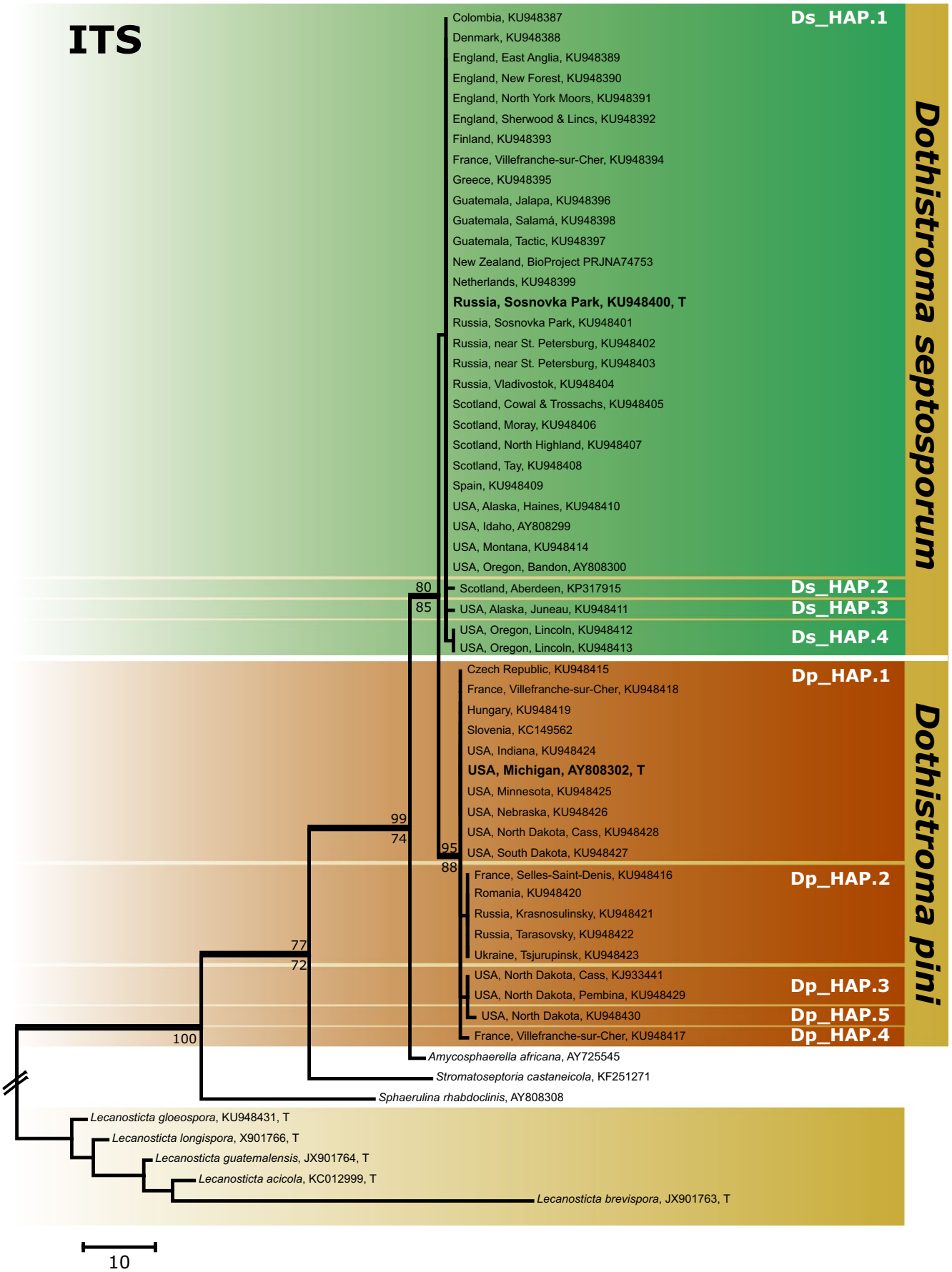

FIGURE 4 The most parsimonious tree representing the four different haplotypes of *Dothistroma septosporum* and five of *D. pini* generated from the ITS region. MP bootstrap support (>70%) are indicated above branches while ML, below branches. Bold branches indicate BI values > than 0.95. *Lecanosticta* species were used as the outgroup taxa. All represented type species are indicated with a T

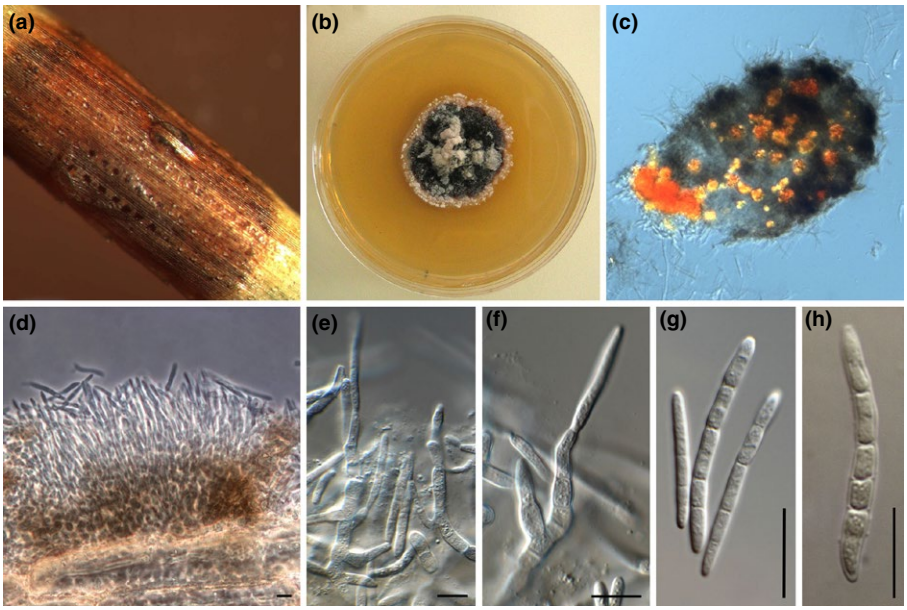

FIGURE 5 DNB symptoms, cultural morphology, and spore characteristics of the epitype material for *Dothistroma pini* from Michigan, USA (CBS 116487, MBT62987, CMW 10951); (a) typical red band surrounding black erumpent conidiomata on *P. nigra*, (b) culture morphology of CMW10951 on 2% MEA, (c) dothistromin (red discolouration) produced in culture as seen under a light microscope, (d) conidiomata, (e–f) conidiophores with conidia, (g–h) morphology of conidia showing multiple septa. Scale bars = 5 μm

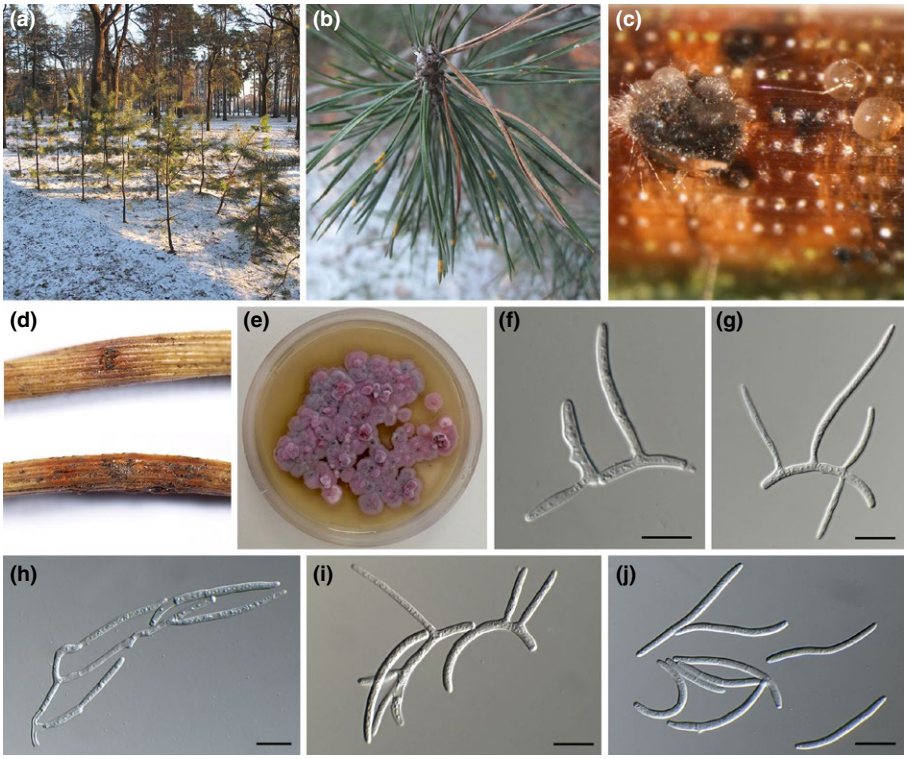

FIGURE 6 DNB symptoms, cultural morphology, and spore characteristics of the neotype material for *Dothistroma septosporum* from Russia (CBS 140339, MBT202423, CMW 44656); (a) planted local *Pinus sylvestris* in Park Sosnovka in Saint Petersburg, (b) characteristic primary DNB symptoms of yellowing on green needles and red banding on necrotic needles, (c) conidiomata with conidia aggregating in cream to brownish slimy masses, (d) typical red bands on necrotic needles surrounding erumpent black conidiomata, (e) culture morphology of CMW 44656 on 2% DSM, (f–i) conidiogenous cells and various stages of microcycle conidiation, (j) long, hyaline conidia. Scale bars = 5 μm

Neotype designated: Park Sosnovka, St. Petersburg, Russia, from planted but native *P. sylvestris*, 14 November 2013, R. Drenkhan and D. L. Musolin, MBT202423, herb. CBS H-22299, culture ex-neotype CMW 44656 = CBS 140339 = TAAM 168554A.

Notes: The herbarium material of the holotype has been lost from the Cryptogamic herbarium of the Komarov Botanical Institute, St. Petersburg, and no longer exists. It is also not preserved at LEP. A neotype is designated here and represents *Dothistroma septosporum* ITS haplotype 1 (DS_HAP.1) (Figs 3,4, Table 2). Ex-neotype sequences available on GenBank: ITS (KU948400), BT1 (KX364412), BT2 (KX364411), TEF1α (KX364410).

Other specimens examined: Russia, Park Sosnovka, St. Petersburg, from planted but native *P. sylvestris*, 14 November 2013, R. Drenkhan and D. L. Musolin, herb. CBS H-22300, culture CMW 44657 = CBS 141531 = TAAM 168554; Russia, near St. Petersburg, from natural pine stand of *P. sylvestris*, 13 November 2013, R. Drenkhan and D. L. Musolin, herb. CBS H-22301, culture CMW 44658 = CBS 140340 = TAAM 168555; Russia, near St. Petersburg, from natural pine stand of *P. sylvestris*, 13 November 2013, R. Drenkhan and D. L. Musolin, herb. CBS H-22302, culture CMW 44659 = CBS 140684 = TAAM 168552.

4 | DISCUSSION

The results of this study have made it possible to provide reliable specimens on which the names *D. septosporum* and *D. pini* can be stabilized in the future. The lack of such material arose for a number of reasons including the long-standing and confused taxonomy of these fungi. Both species were described before molecular genetic tools were available to provide insights into species boundaries and have consequently lacked DNA barcodes (Doroguine, 1911; Hulbary, 1941; Schoch et al., 2012). The need to neotypify *D. septosporum* has been recognized for many years and dates back to the time when Morelet (1968a) provided a new combination of *D. septosporum*. Because the holotype material has been lost, proposals to establish a neotype based on material linked to Doroguine's original collection were recommended (Morelet, 1968a). This material, labelled as *Cytosporina septospora* Dorogin, was collected in Ukraine, Kiev Guberniya, in the town of Smiela, from *P. sylvestris* on the 25 March, 1914, by L. Kaznowski (LE 116244, herb. CBS 11381). DNA could not be obtained from this specimen, and it was thus not considered as appropriate material for the present study. Similarly, DNA could not be extracted from the holotype material of *D. pini* (Barnes et al., 2004; Hulbary, 1941). In this study, we were able to fix the application of the names by generating DNA barcodes for the neotype designated here for *D. septosporum* and the epitype for *D. pini* after appropriate fresh specimens had been collected. Ex-neotype and ex-epitype cultures have also been secured, and these can be used in future comparative studies that should ensure taxonomic stability of two of the world's most important pine pathogens.

A search on Index Fungorum (http://www.indexfungorum.org/Names/Names.asp) shows that in the 120 years during which the fungi causing the red-band needle disease have been studied, five different species have been linked to the name *Dothistroma*. The taxonomy of the pathogens causing the disease is beset with confusion that has been exacerbated by the lack of easily accessible or available literature, often compounded by problems such as language barriers. This is evident throughout the history of the pathogen as outlined in Table 1.

One of the most vivid examples illustrating the problems relating to the taxonomy of *Dothistroma* is found in the description of *Dothistroma flichianum*. In 1896, Jean P. Vuillemin produced a detailed description of a fungus that causes red-band symptoms on pine (Vuillemin, 1896). He established a new genus, *Hypostomum*, to accommodate this fungus and named the pathogen *Hypostomum flichianum* Vuill. The epithet "*flichianum*" honoured M. Fliche who collected the material during 1860 from infected *P. austriaca* and *P. mugo* subsp. P. *mugo* (syn. *P. montana*) in the Champfètu woods, Theil-sur-Vanne, close to Sens (Yonne), France. This literature remained in complete obscurity for nearly 70 years until Morelet (1980) established the new combination *Dothistroma flichianum* (Vuill.) M. Morelet (as "*flichiana*") (=*Hypostomum flichianum* Vuill.), but without any explanation (Morelet, 1980).

The genus *Hypostomum* is monotypic and older than *Dothistroma* (Hulbary, 1941; Vuillemin, 1896). Consistent with the rules of the International Code of Botanical Nomenclature (ICBN) at that time, *Dothistroma* should have been reduced to synonymy under *Hypostomum*. It is not known why Morelet retained the name *Dothistroma* instead. The only possible explanation is that there was no type material available for *Hypostomum* for comparison, and the taxonomy of this genus could thus not be confirmed. It does not serve any purpose at this point in time to revert the current name of *Dothistroma* back to *Hypostomum* because it would cause substantial confusion among plant pathologists. The decision made here is to retain the name *Dothistroma*, which is now a well-established genus name applied to globally important tree pathogens. Although it is highly likely that *D. flichianum* represents *D. septosporum* (or *D. pini*), it is not possible to validate this fact. In the absence of material linked to the name, the species *Dothistroma flichianum* will remain a taxonomic obscurity. However, in order to ensure long-term stability, a formal application to conserve *Dothistroma* and the species name "*septosporum*" linked to it is currently underway.

The names of the two other *Dothistroma* species listed in Index Fungorum (*Dothistroma acicola* and *Dothistroma rhabdoclinis*) are no longer accepted (Table 1). *Dothistroma acicola* (Thüm.) Schischkina & Tsanava was reduced to synonymy with the brown-spot fungus as *Lecanosticta acicola* (Thüm.) Syd (Quaedvlieg et al., 2012). *Dothistroma rhabdoclinis*, originally described as a hyperparasite of *Rhabdocline pseudotsugae* on Douglas fir (*Pseudotsuga menziesii*) (Butin et al., 2000), was transferred to *Sphaerulina* based on multigene DNA sequence data (Quaedvlieg et al., 2013). As a consequence of the dual nomenclature for fungi being abandoned (Hawksworth et al., 2011), all names associated with the teleomorph/sexual morph of *Dothistroma*, including *Mycosphaerella pini* (Munk, 1957), *Scirrhia pini* (Funk & Parker, 1966), *Scirrhia pini* var. *pini* (Morelet, 1968b), *Scirrhia pini* var. *galliensis* (Morelet, 1968b) and *Eruptio pini* (Barr, 1996) (Table 1) are redundant and, therefore, obsolete. The rejection of all varietal names based on

morphology by Evans (1984) and phylogenetic inference by Barnes et al. (2004), results in only two valid species in the genus *Dothistroma* with one name each: *D. pini* and *D. septosporum*.

The two pathogens causing DNB have very similar morphology, produce disease symptoms that are indistinguishable and in some cases, they have been found infecting the same needle (Barnes et al., 2008; Ioos et al., 2010). The only reliable means to differentiate between *D. septosporum* and *D. pini* is by applying DNA-based molecular methods (Barnes et al., 2004; Groenewald et al., 2007; Ioos et al., 2010). These methods include DNA sequence comparisons for the ITS, β-tubulin and Elongation factor (EF1-α) gene regions (Barnes et al., 2004, 2011), amplification using species-specific mating type markers (Groenewald et al., 2007), conventional and real-time PCR markers (Ioos et al., 2010), and ITS-RFLPs (Barnes et al., 2004; Pehl et al., 2004).

The ITS region is currently the most widely used gene region to distinguish between the DNB pathogens (Hanso & Drenkhan, 2008; Piškur et al., 2013; Queloz et al., 2014; Rodas, Wingfield, Granados, & Barnes, 2016; Tsopelas et al., 2013). In the present study, with the annotated ITS map generated, we have identified at least nine different ITS haplotypes in these pathogens. Our investigations of the positions of the point mutations have shown that the phylogenetic species concept remains sound for these species based on at least three fixed polymorphisms (Figs 3,4). Although variations exist in eight other positions, mainly in the ITS1, none of the known polymorphisms disrupt the *Alu*I restriction site and this can still be used with confidence for ITS-RFLP diagnostic purposes (Barnes et al., 2004).

Nothing is known regarding the variability in pathogenicity of the different *Dothistroma* ITS haplotypes; neither is anything known about the difference in pathogenicity between the two DNB pathogens. Preliminary studies have, however, shown that different strains of *D. septosporum* produce varied levels of dothistromin (Bradshaw, Ganley, Jones, & Dyer, 2000), a toxin that has been shown to be a virulence factor during infection (Kabir, Ganley, & Bradshaw, 2015). McTaggart et al. (2016) have recently alluded to the fact that name-based taxonomy fails to provide an adequate knowledge base for biosecurity. They emphasize the fact that there is a pressing need to reconsider how quarantine and biosecurity issues are considered and that genotypes rather than species names need to be considered more seriously. This is highlighted by the fact that a unique ITS haplotype of *D. septosporum* was isolated from a non-pine species, *Cedrus atlantica* var. *glauca* (Mullett & Fraser, 2015).

Currently, lists of species names are utilized by phytosanitary services to implement biosecurity measures and it is essential that these remain up-to-date with current taxonomic changes. *D. septosporum* has, for example, been on the EU Annex II/A2 list since 1992 and the IAPSC A2 list since 1989 (EPPO, 2016), but there is still no quarantine status for *D. pini*. This is despite the fact that this pathogen has been clearly defined since 2004 (Barnes et al., 2004). The results of this study show that plant material having haplotypes (genets), of either *D. septosporum* or *D. pini*, different to those present in a country should be actively excluded by quarantine services. In this case, quarantine measures should especially target countries where many different haplotypes of these pathogens occur. Accidental introductions of new haplotypes could pose a serious risk for local populations of *Pinus* spp. with potentially serious economical outcomes for commercial forestry (Wingfield, Brockerhoff, Wingfield, & Slippers, 2015).

Substantial efforts are currently underway to clarify the different geographic locations and host ranges of the two *Dothistroma* species responsible for DNB (Drenkhan et al., 2016; http://arcgis.mendelu.cz/monitoring/). This study contributes to this goal in providing DNA data confirming the presence of *D. septosporum* from 11 new geographic locations in six countries (Denmark, England, Russia, Scotland, Spain, and the USA) and *D. pini* in the Czech Republic and Romania. The DNA sequence data linked to type material of *D. septosporum* and *D. pini* emerging from the present study will also contribute substantially to future studies on *Dothistroma* and will provide a sound basis for molecular comparisons between species and genotypes. This is essential where phylogenetic analyses are conducted or where new species descriptions are being considered.

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REFERENCES

Ariyawansa, H. A., Hawksworth, D. L., Hyde, K. D., Jones, E. B. G., Maharachchikumbura, S. S. N., Manamgoda, D. S., … Daranagama, A. (2014). Epitypification and neotypification: Guidelines with appropriate and inappropriate examples. *Fungal Diversity*, *69*, 57–91.

Arx, J. A. V. (1983). *Mycosphaerella* and its anamorphs. *Proc. K. Ned. Akad. Wet., Ser. C.*, 15-54.

Barnes, I., Crous, P. W., Wingfield, B. D., & Wingfield, M. J. (2004). Multigene phylogenies reveal that red band needle blight of *Pinus* is caused by two distinct species of *Dothistroma*, *D. septosporum* and *D. pini*. *Studies in Mycology*, *50*, 551–565.

Barnes, I., Kirisits, T., Akulov, A., Chhetri, D. B., Wingfield, B. D., Bulgakov, T. S., & Wingfield, M. J. (2008). New host and country records of the Dothistroma needle blight pathogens from Europe and Asia. *Forest Pathology*, *38*, 178–195.

Barnes, I., Kirisits, T., Wingfield, M. J., & Wingfield, B. D. (2011). Needle blight of pine caused by two species of *Dothistroma* in Hungary. *Forest Pathology*, *41*, 361–369.

Barnes, I., Walla, J. A., Bergdahl, A., & Wingfield, M. J. (2014). Four new host and three new state records of Dothistroma needle blight caused by *Dothistroma pini* in the United States. *Plant Disease*, *98*, 1443.

Barr, M. E. (1996). Planistromellaceae, a new family in the Dothideales. *Mycotaxon*, *60*, 433–442.

Bradshaw, R. E. (2004). Dothistroma (red-band) needle blight of pines and the dothistromin toxin: A review. *Forest Pathology*, *34*, 163–185.

Bradshaw, R. E., Ganley, R. J., Jones, W. T., & Dyer, P. S. (2000). High levels of dothistromin toxin produced by the forest pathogen *Dothistroma pini*. *Mycological Research*, *104*, 325–332.

Butin, H., Kehr, R., & Pehl, L. (2000). *Dothistroma rhabdoclinis* sp. nov. associated with *Rhabdocline pseudotsugae* on Douglas fir. *Forest Pathology*, *30*, 195–203.

Crous, P. W., Giraldo, A., Hawksworth, D. L., Robert, V., Kirk, P. M., Guarro, J., … Trakunyingcharoen, T. (2014). The Genera of Fungi: Fixing the application of type species of generic names. *IMA Fungus*, *5*, 141–160.

Crous, P. W., J-Kang, C., & Braun, U. (2001). A phylogenetic redefinition of anamorph genera in *Mycosphaerella* based on ITS rDNA sequence and morphology. *Mycologia*, *93*, 1081–1101.

De Wit, P. J. G. M., Van Der Burgt, A., Okmen, B., Stergiopoulos, I., Abd-Elsalam, K. A., Aerts, A. L., … Bradshaw, R. E. (2012). The genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* reveal adaptation to different hosts and lifestyles but also signatures of common ancestry. *PLoS Genetics*, *8*, e1003088.

Dearness, J. (1928). New and noteworthy fungi V. *Mycologia*, *20*, 235–246.

Doroguine, G. (1911). Une maladie cryptogamique du Pin. *Bulletin Trimestriel de la Société Mycologique de France*, *27*, 105–106.

Doroguine, G. (1912). A fungal disease of mountain pine. Lesnoy Zhurnal, St Petersburg, Russia, *42*, 1292–1294. (in Russian).

Doroguine, G. (1926). A note on *Cytosporina septospora*. *Bolezni Rastenij*, *15*, 48–50. (in Russian).

Drenkhan, R., Tomešová-Haataja, V., Fraser, S., Bradshaw, R. E., Vahalik, P., Mullett, M., … Barnes, I. (2016). Global geographic distribution and host range of *Dothistroma* species: A comprehensive review. *Forest Pathology*, doi: 10.1111/efp.12290

EPPO. (2016). PQR-EPPO database on quarantine pests (available online). http://www.eppo.int

Evans, H. C. (1984). *The genus Mycosphaerella and its anamorphs Cercoseptoria, Dothistroma and Lecanosticta on pines*. CMI Mycol. Surrey, UK: Commonwealth Agricultural Bureau, 1–102.

Funk, A., & Parker, A. K. (1966). *Scirrhia pini* n. sp., the perfect state of *Dothistroma pini* Hulbary. *Canadian Journal of Botany*, *44*, 1171–1176.

Georgescu, C., & Petrescu, M. (1952). Boala inrosirii acelor de pin provocata de *Brunchorstia destruens* Eriks. *Revista Padurilor*, *9*, 19–22.

Gibson, I. A. S. (1972). Dothistroma needle blight of *Pinus radiata*. *Annual Review of Phytopathology*, *10*, 51–72.

Gremmen, J. (1965). *Brunchorstia pinea* (Karst.) Hohn., een ernstige ziekte van de Oostenrijkse en Corsicaanse den. *Nederlands Bosbouw Tijdschrift*, *37*, 87–98.

Gremmen, J. (1968). The presence of *Scirrhia pini* Funk et Parker in Romania (Conidial stage: *Dothistroma pini* Hulb.). *Bulletin Trimestiel de la Société Mycologique de France*, *84*, 489–492.

Groenewald, M., Barnes, I., Bradshaw, R. E., Brown, A. V., Dale, A., Groenewald, J. Z., … Crous, P. W. (2007). Characterization and distribution of mating type genes in the dothistroma needle blight pathogens. *Phytopathology*, *97*, 825–834.

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*, 696–704.

Hanso, M., & Drenkhan, R. (2008). First observations of *Mycosphaerella pini* in Estonia. *Plant Pathology*, *57*, 1177.

Hawksworth, D. L. (2015). Proposals to clarify and enhance the naming of fungi under the International Code of Nomenclature for algae, fungi, and plants. *IMA Fungus*, *6*, 199.

Hawksworth, D. L., Crous, P. W., Redhead, S. A., Reynolds, D. R., Samson, R. A., Seifert, K. A., … Zhang, N. (2011). The Amsterdam declaration on fungal nomenclature. *IMA Fungus*, *2*, 105–112.

Hedgcock, G. G. (1929). *Septoria acicola* and the brown spot disease of pine needles. *Phytopathology*, *19*, 993–999.

Hulbary, R. L. (1941). A needle blight of Austrian pine. *Illinois Natural History Survey Bulletin*, *21*, 231–236.

Ioos, R., Fabre, B., Saurat, C., Fourrier, C., Frey, P., & Marcais, B. (2010). Development, comparison, and validation of real-time and conventional PCR tools for the detection of the fungal pathogens causing brown spot and red band needle blights of pine. *Phytopathology*, *100*, 105–114.

Ivory, M. H. (1967). A new variety of *Dothistroma pini* in Kenya. *Transactions of the British Mycological Society*, *50*, 289–297.

Kabir, M. S., Ganley, R. J., & Bradshaw, R. E. (2015). Dothistromin toxin is a virulence factor in dothistroma needle blight of pines. *Plant Pathology*, *64*, 225–234.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780.

Martínez, J. B. (1933). Una grave micosis del pino observada por primera vez en España. *Boletín de la Real Sociedad Española de Historia Natural. Sección Geológica*, *33*, 25–30.

Martínez, J. B., & Torres Juan, J. (1965). *Enfermedades de las coníferas españolas*. Madrid: Instituto Forestal de Investigaciones y Experiencias, *88*, 60.

McTaggart, A. R., Van Der Nest, M. A., Steenkamp, E. T., Roux, J., Slippers, B., Shuey, L. S., … Drenth, A. (2016). Fungal genomics challenges the dogma of name-based biosecurity. *PLoS Pathogens*, *12*, e1005475.

Morelet, M. (1968b). De aliquibus in mycologia novitatibus. *Bulletin de la Societe des Sciences Naturelles et D'Archeologie de Toulon et du Var*, *175*, 5–6.

Morelet, M. (1968a). De aliquibus in mycologia novitatibus (3e note). *Bulletin de la Societe des Sciences Naturelles et D'Archeologie de Toulon et du Var*, *177*, 9.

Morelet, M. (1969). *Scirrhia pini*: Note complémentaire. *Bulletin Mensuel de la Société Linnéenne de Lyon*, *38*, 268–270.

Morelet, M. (1980). Sur quatre Dothideales (14e note). *Bulletin de la Société des Sciences Naturelles et D'Archéologie de Toulon et du Var*, *227*, 14–15.

Mullett, M. S., & Barnes, I. (2012). *Dothistroma* isolation and molecular identification methods. Retrieve from Meetings of the DIAROD COST Action: http://www.forestry.gov.uk/fr/infd-8rvfdb.

Mullett, M. S., & Fraser, S. (2015). Infection of *Cedrus* species by *Dothistroma septosporum*. *Forest Pathology*, doi:10.1111/efp.12214

Munk, A. (1957). Danish Pyrenomycetes. A preliminary flora. *Dansk Botanisk Arkiv*, 17, 1–312. [In Danish].

Murray, J. S., & Batko, S. (1962). *Dothistroma pini* Hulbary: A new disease on pine in Britain. *Forestry*, *35*, 57–65.

Nilsson, R. H., Hyde, K. D., Pawłowska, J., Ryberg, M., Tedersoo, L., Aas, A. B., … Antonelli, A. (2014). Improving ITS sequence data for identification of plant pathogenic fungi. *Fungal Diversity*, *67*, 11–19.

Pehl, L., Burgermeister, W., & Wulf, A. (2004). *Mycosphaerella*-Nadelpilze der Kiefer-Identifikation durch ITS-RFLP-Muster. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes*, *56*, 239–244.

Petrak, F. (1961). Die *Lecanostica*-Krankheit der Föhren in Österreich. *Sydowia*, *15*, 252–256.

Piškur, B., Hauptman, T., & Jurc, D. (2013). Dothistroma needle blight in Slovenia is caused by two cryptic species: *Dothistroma pini* and *Dothistroma septosporum*. *Forest Pathology*, *43*, 518–521.

Posada, D. (2008). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, *25*, 1253–1256.

Quaedvlieg, W., Groenewald, J. Z., De Jesús Yáñez-Morales, M., & Crous, P. W. (2012). DNA barcoding of *Mycosphaerella* species of quarantine importance to Europe. *Persoonia_Molecular Phylogeny and Evolution of Fungi*, *29*, 101–115.

Quaedvlieg, W., Verkley, G. J. M., Shin, H. D., Barreto, R. W., Alfenas, A. C., Swart, W. J., … Crous, P. W. (2013). Sizing up *Septoria*. *Studies in Mycology*, *75*, 307–390.

Queloz, V., Wey, T., & Holdenrieder, O. (2014). First record of *Dothistroma pini* on *Pinus nigra* in Switzerland. *Plant Disease*, *98*, 1744.

Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). Tracer v1.6. Retrived from http://beast.bio.ed.ac.uk/Tracer

Rodas, C. A., Wingfield, M. J., Granados, G. M., & Barnes, I. (2016). Dothistroma needle blight: An emerging epidemic caused by *Dothistroma septosporum* in Colombia. *Plant Pathology*, *65*, 53–63.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542.

Rossman, A. Y., Crous, P. W., Hyde, K. D., Hawksworth, D. L., Aptroot, A., Bezerra, J. L., … Boonmee, S. (2015). Recommended names for pleomorphic genera in Dothideomycetes. *IMA Fungus*, *6*, 507.

Saccardo, P. A. (1920). Mycetes Boreali-Americani. Nuovo. *Giornale Botanico Italiano*, *27*, 13.

Săvulescu, T. (1948). Cinquième contribution à la connaissance des micromycètes de Roumanie. *Analele Academiei Romane Memoriile Sectiunii Stiintifice. ser. 3, XXIII*. 23–90.

Schoch, C. L., Robbertse, B., Robert, V., Vu, D., Cardinali, G., Irinyi, L., … Miller, A. N. (2014). Finding needles in haystacks: Linking scientific names, reference specimens and molecular data for fungi. *Database (Oxford)*, *2014*, doi:10.1093/database/bau1061

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., … Crous, P. W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences*, *109*, 6241–6246.

Shishkina, A. K., & Tsanava, N. I. (1967). *Systremma acicola* (Dearn.) Wolf & Barbour – the perfect stage of *Dothistroma acicola* (Thüm.) A. Schischk. et N. *Tzan. Novosti Sistematiki Nizshih Rasteniy*, *4*, 276–277.

Siggers, P. V. (1944). *The brown spot needle blight of pine seedlings*. U.S.D.A. Techincal Bulletin, *870*, 1–16.

Stielow, J. B., Lévesque, C. A., Seifert, K. A., Meyer, W., Irinyi, L., Smits, D., … Chaduli, D. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia_Molecular Phylogeny and Evolution of Fungi*, *35*, 242–263.

Sutton, B. C. (1980). The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute.

Swofford, D. L. (2003). PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b 10. Sinauer Associates.

Sydow, H., & Petrak, F. (1924). Zweiter Beitrag zur kenntnis der pilzflora nordamerikas, insoesondere der nordwestlichen staaten. *Annales Mycologici*, *22*, 387–409.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*, 2725–2729.

Taylor, J. W. (2011). One fungus = One name: DNA and fungal nomenclature twenty years after PCR. *IMA Fungus*, *2*, 113–120.

Thyr, B. D., & Shaw, C. G. (1964). Identity of the fungus causing red band disease on pines. *Mycologia*, *56*, 103–109.

Trotter, A. (1931). P. A. Saccardo's Supplementum Universale. *Sylloge Fungorum*, *25*, 480.

Tsopelas, P., Barnes, I., Soulioti, N., & Wingfield, M. J. (2013). *Dothistroma septosporum* identified in Greece on *Pinus brutia* and *Pinus nigra* plantations. *Plant Disease*, *97*, 1247–1248.

Vuillemin, M. P. (1896). Les Hypostomaceés, nouvelle famille de champignons parasites. *Bulletin de la Societe Des Sciences de Nancy*, *29*, 15–52.

Wijayawardene, N. N., Crous, P. W., Kirk, P. M., Hawksworth, D. L., Boonmee, S. Braun, U., … Hyde, K. D. (2014). Naming and outline of *Dothideomycetes*–2014 including proposals for the protection or suppression of generic names. *Fungal Diversity*, *69*, 1–55.

Wingfield, M. J., Brockerhoff, E. G., Wingfield, B. D., & Slippers, B. (2015). Planted forxest health: The need for a global strategy. *Science*, *349*, 832–836.

Wingfield, M. J., De Beer, Z. W., Slippers, B., Wingfield, B. D., Groenewald, J. Z., Lombard, L., & Crous, P. W. (2012). One fungus, one name promotes progressive plant pathology. *Molecular Plant Pathology*, *13*, 604–613.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (Eds.), *PCR protocols: a guide to methods and applications* (pp. 315–322). San Diego, CA: Academic Press.

Woods, A. J., Martín-García, J., Bulman, L., Vasconcelos, M. W., Boberg, J., La Porta, N., … Brown, A. (2016). Dothistroma needle blight, weather and possible climatic triggers for the disease's recent emergence. *Forest Pathology*, doi:10.1111/efp.12248