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Phellinus species on Betula. Mating tests, RFLP analysis of enzymatically amplified rDNA, and relations to Phellinus alni

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Mainly based on collections from Fennoscandia, Estonia, and Central Europe, four species of the genus *Phellinus* Quél. (Hymenochaetaceae), all belonging to the *P. igniarius* (L.:Fr.) Quél. group, are shown to occur on *Betula* L. These species are *P. nigricans* (Fr.) P.Karsten, *P. cinereus* (Niemelä) Fischer, *P. laevigatus* (Fr. ex P.Karsten) Bourd. & Galz., and *P. lundellii* Niemelä. The occurrence of *P. igniarius* and *P. alni* (Bond.) Parm. on *Betula* remains in doubt. *P. alni*, *P. nigricans*, *P. cinereus*, *P. laevigatus*, and *P. lundellii* were characterized by pairing tests of single spore mycelia and RFLP (restriction fragment length polymorphism) data of enzymatically amplified ribosomal DNA. A unique RFLP phenotype was assignable to each species except *P. nigricans*, which was identical with *P. alni*. Distribution of the taxa is throughout the area under study; however, *P. nigricans* seems to be limited to Fennoscandia. Two stocks from North America proved to represent *P. cinereus*. Pairing relationships between *P. nigricans*, *P. cinereus*, and *P. alni* were examined in detail and were found to differ according to the geographic origin of the stocks. *P. nigricans* was positive in numerous pairings with *P. alni* from Fennoscandia, Estonia, and Central Europe. In addition, it is positive with *P. cinereus* from Fennoscandia, but negative with *P. cinereus* from Central Europe, Estonia, and the United States. *P. laevigatus* and *P. lundellii* were intersterile with all other taxa.

Key words: *Betula*, pairing tests, PCR, *Phellinus*, RFLP, speciation

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Introduction

According to the literature, in Europe a considerable number of taxa of the genus *Phellinus* Quél. are found on *Betula* L. All these taxa are members of the *P. igniarius* (L.:Fr.) Quél. group. Species concepts within this group are primarily based on macroscopical and microscopical features, culture characteristics and host plants (Bondartsev 1953; Kreisel 1961, 1987; Jahn 1962, 1963, 1967, 1977, 1979; Donk 1971, 1974; Niemelä 1972, 1974, 1975, 1977; Domanski et al. 1973; Parmasto 1976, 1985, 1993; Fischer 1987, 1995).

Substrate specificity is pronounced for some taxa of the group. *P. tuberculosus* (Baumg.) Niemelä is almost exclusively found on species of *Prunus* L. (Bondartsev 1953; Kreisel 1961; Jahn 1963; Niemelä 1977; Breitenbach & Kränzlin 1986). *P. tremulae* (Bond.) Bond. & Borisov (Bondartsev 1953; Jahn 1962; Domanski et al. 1973; Niemelä 1974) and *P. populicola* Niemelä (Niemelä 1975; Jahn 1979; Kreisel 1987) are limited to *Populus* L. In Central Europe, the type species of the group, *P. igniarius*, so far has been demonstrated only for species of *Salix* L. (Fischer 1995). Possibly it also grows on *Populus* (Eriksson

1958; Kreisel 1961; Jahn 1963; Domanski et al. 1973; Niemelä 1975; Benkert 1977; Niemelä & Kotiranta 1982), but at least some of these findings represent *P. populicola* instead.

For some of the *Phellinus* taxa occurring on Betula this tree represents the main host; for others it is a host of secondary importance.

Phellinus igniarius (L.: Fr.) Quél.

The host preference of this taxon evidently varies with the geographic region. As mentioned above, in Central Europe it seems limited to species of *Salix*, though this is in contrast to reports by Kreisel (1961, 1987), Jahn (1963, 1979), Plank (1978), Kotlaba (1984) and Breitenbach and Kränzlin (1986), who suggest that it occurs on a wide variety of deciduous trees including *Betula*. Most likely, the majority of these reports properly refer to the closely related species *P. alni* (Bond.) Parm. In Fennoscandia, *Betula* is cited as the main host of *P. igniarius* (Eriksson 1958; Niemelä 1975; Niemelä & Kotiranta 1982; Erkkilä & Niemelä 1986; Renvall et al. 1991); besides, the taxon is found on *Salix* and numerous other trees. As well, in the eastern part of Europe and the territory of the former USSR *Betula* is reported as common host (Bondartsev 1953; Domanski et al. 1973). As pointed out by Niemelä (pers. comm.), however, in Finland most collections of *P. igniarius* from *Betula* in fact represent *P. cinereus* (Niemelä) Fischer (see below). *Betula* is not included as host of *P. igniarius* in the "Distribution maps of Estonian fungi" (Parmasto 1993). Nothing definite can be said about the collections of Eastern Europe, since for this part of its range the species concept of *P. igniarius* has not yet been fully clarified.

Phellinus alni (Bond.) Parm.

As with *P. igniarius*, occurrence of this taxon on *Betula* is questionable. Little is said in the literature about the host preferences of *P. alni*. *Alnus* Mill. is cited as substrate by Bondartsev (1912, 1953), who was the first to mention the taxon (first as *Fomes igniarius* f. *alni* Bond. and later as *P. igniarius* f. *alni* Bond.). *P. igniarius* var. *alni* was proposed as one of four varieties of *P. igniarius* by Niemelä (1975); occurrence is typically given as on Alnus glutinosa, but also on most of the other tree hosts of *P. igniarius*, possibly including *Betula*. *P. alni* was raised to specific rank in Parmasto's study on Yakutian fungi (Parmasto 1976), where it is reported as occurring on *Betula*. *Betula* is not mentioned as host of *P. alni* in Estonia (Parmasto 1993); however, numerous collections from *Betula* morphologically are very close to *P. alni* (Parmasto, pers. comm.).

Phellinus nigricans (Fr.) P.Karsten

Although the concept of this species is still unsettled (Donk 1971, 1974; Fischer 1995), it is usually recognized in the literature. The designations are variable, i.e. *P. igniarius* f. *nigricans* Bondartsev (Bondartsev 1953), *P. trivialis* f. *betularum* Jahn (Jahn 1963), and *P. nigricans* (Domanski et al. 1973; Niemelä 1975; Niemelä & Kotiranta 1982; Jülich 1984; Breitenbach & Kränzlin 1986; Parmasto 1993). In all cases *Betula* is given as the main host, preferentially dead or severely wounded stems. This is in contrast to *P. igniarius* and *P. alni*, both of which occur as parasites. The distribution of *P. nigricans* is boreal (Niemelä 1975; Jahn 1979), and it also is reported from Estonia (Parmasto 1993, pers. comm.). As noted by Niemelä and Kotiranta (1982), reports of *P. nigricans* from Central Europe and further south are due to a misunderstanding of the species concept. Since *P. trivialis* (Bres.) Kreisel is included in the species concept of *P. nigricans* by Domanski et al. (1973), the same may be true for the Polish collections of *P. nigricans*. The species is considered to exist in Switzerland; findings, mostly on *Betula*, are rare however (Breitenbach & Kränzlin 1986).

Phellinus cinereus (Niemelä) Fischer

Mainly on the basis of specimens from Central Europe, this taxon was just recently raised to specific level (Fischer 1995). Originally designated as *P. igniarius* var. *cinereus* Niemelä (Niemelä 1975), it has also been mentioned by Jahn and Jahn (1986), Parmasto (1993), and Luschka (1994). *P. cinereus* seems to be closely associated with *Betula*, occurrence on dead *Alnus incana* is reported by Niemelä (1975). Possibly it also grows on *Acer* L., *Fraxinus* L., and *Ulmus* L. (Parmasto, 1993). Ecologically *P. cinereus* is

very similar to *P. nigricans*, but differs in that it sometimes also occurs on standing, living trees. In Germany, one of us (M.F.) found *P. cinereus* exclusively in moist sites of dense forests, saprophytically on dead, standing birch.

P. laevigatus (Fr. ex P.Karsten) Bourd. & Galz.

This is another species mostly growing on *Betula*. Bourdot & Galzin (1927) and Bondartsev (1953) cite *Rhamnus* L. as host, but probably all their findings refer to *P. rhamni* (Bond.) Jahn (Jahn 1967). Distribution of *P. laevigatus* is scattered, but in suitable sites the species seems to occur in most parts of Europe (Bondartsev 1953; Eriksson 1958; Kreisel 1961, 1987; Jahn 1967; Domanski 1972; Niemelä 1972; Plank 1978; Niemelä & Kotiranta 1982; Kotlaba 1984; Breitenbach & Kränzlin 1986; Parmasto 1993; Luschka 1994). It prefers dead, fallen stems of birch in dense forests, where large, resupinate fruiting bodies often develop on the underside of the fallen tree. Occasionally it is found on *Alnus* (Niemelä 1972; Niemelä & Kotiranta 1982; Kotlaba 1984; Breitenbach & Kränzlin 1986). On birch, it often grows side by side with *Piptoporus betulinus* (Bull.:Fr.) P.Karsten and *Fomes fomentarius* (L.) Fr. (Jahn 1967).

P. lundellii Niemelä

This taxon is readily found in suitable places in Northern Europe (Niemelä 1975; Niemelä & Kotiranta 1982). It is rare in Estonia (Parmasto 1993) and there is only one report from Poland (Domanski et al. 1973). Occurrence in Germany was reported only recently (Jahn 1977). Yet, also in Central Europe the species seems to occur regularly in low mountains and bogs or marshes (Kotlaba 1984; Breitenbach & Kränzlin 1986; Luschka 1994). The ecology of *P. lundellii* is very similar to that of *P. laevigatus* in that fruiting bodies mostly are developed on dead trunks of birch. Sometimes *P. lundellii* also occurs on *Alnus* and other deciduous trees. Compared with *P. laevigatus*, *P. lundellii* more likely is to be found in remote areas, and its fruiting bodies usually appear at a later stage. (Niemelä 1972; Niemelä & Kotiranta 1982).

Betula is the main substrate for four of the species listed above: *P. nigricans*, *P. cinereus*, *P. laevigatus* and *P. lundellii*. Because no unequivocal data exist for *P. igniarius* and *P. alni*, occurrence of these taxa on *Betula* in Europe remains doubtful.

Macroscopical and microscopical characters will usually allow a reliable identification of specimens in *P. laevigatus* and *P. lundellii*, but identification is much more difficult in *P. igniarius*, *P. alni*, *P. nigricans*, and *P. cinereus*. A species concept that is applicable in one region may fail in another. Characters that are less dependent on environmental factors — restriction data of enzymatically amplified ribosomal DNA as well as pairing tests of single spore mycelia and identification of the pattern of sexuality — were introduced recently as an additional aid in identifying specimens (Fischer 1995). Mainly on the basis of material from Central Europe, results were as follows: *P. igniarius*, *P. alni*, *P. nigricans* and *P. cinereus* are unifactorial. Pairing tests demonstrated a full intersterility between *P. igniarius*, *P. alni* and *P. cinereus*. *P. nigricans* (two stocks from Fennoscandia) was positive in numerous pairings with *P. alni* (mostly from Central Europe), and in one case also positive with *P. cinereus* (from Central Europe). Because of limited material, a full evaluation of the interrelations between these taxa was not possible.

It was the main purpose of this study to include additional stocks from outside of Central Europe. Specimens from *Betula* designated as *P. igniarius* s. str. were not available and therefore this species was not included. Special attention was paid to the relationships between *P. alni*, *P. nigricans*, and *P. cinereus*. No unambiguous material of *P. alni* on *Betula* was available, and stocks from different substrata were included instead. Numerous collections from Fennoscandia were provided by Dr. T. Niemelä, and stock cultures from Estonia and Russia were provided by Dr. E. Parmasto. For the first time the mode of reproduction as well as RFLP data are presented for *P. laevigatus* and *P. lundellii*. As was shown in the *P. pini* (Brot.:Fr.) Ames group, North American taxa cannot be related to known European species (Fischer 1994). For this reason, two North American collections from *Betula* were additionally tested in this study.

Material and methods

The collections used are listed in Table 1.

Table 1. *Phellinus* on *Betula*: Field data of collections studied

Stock number	Testers	Date collected	Location	Host
Phellinus sp.:				
76-18[a]		25-VI-1976	Estonia	*Betula pendula* Roth
P. alni:				
TN 3214a[b]	2,6,7,8	17-VI-1985	Finland	*Alnus incana* (L.) Moench
85–87[a]	1,4,5,7	7-VIII-1985	Estonia	*Alnus incana*
87–1028	1,2,3,5	28-X-1987	Germany	*Malus domestica* Borkh.
90–822	1,2,3,8	22-VIII-1990	Germany	*Sorbus aucuparia* L.
TN 5750[b]	1,2,3,4	24-IV-1994	Finland	*Alnus glutinosa* (L.)Gaertn.
71–9[a]		10-V-1971	Estonia	*Alnus incana*
P. nigricans:				
TN 3301[b]	1,2,8,11	5-X-1985	Finland	*Betula*
TN 4405[b]	1,2,3,5	24-VI-1987	Finland	*Betula pubescens* Ehrh.
P. cinereus:				
85–917a	1,3,4,5	17-IX-1985	Germany	*Betula pubescens*
85–917b	1,2,3,4	17-IX-1985	Germany	*Betula pubescens*
85–917c	1,2,5,8	17-IX-1985	Germany	*Betula pubescens*
TN 3308[b]	1,2,3,5	5-X-1985	Finland	*Betula*
86–9a	1,2,4,6	IX-1986	Germany	*Betula pubescens*
86–9b	1,4,5,6	IX-1986	Germany	*Betula pubescens*
86–9c	1,3,5,6	IX-1986	Germany	*Betula pubescens*
89–822a[c]	1,2,3,4	22-VIII-1989	Estonia	*Betula*
89–826[c]	2,3,4,5	26-VIII-1989	Estonia	*Tilia cordata* L.
89–828b[c]	1,3,4,5	28-VIII-1989	Estonia	*Betula*
90–826a	1,2,3,4	26-VIII-1990	Germany	*Betula pubescens*
90–826b	1,2,5,6	26-VIII-1990	Germany	*Betula pubescens*
92–926b	1,3,4,5	26-IX-1992	USA/Michigan	*Betula papyrifera* Marsh.
92–927	1,2,3,4	27-IX-1992	USA/Michigan	*Betula papyrifera*
TN 5745[b]	1,4,6,7	24-IV-1994	Finland	*Betula pubescens* Ehrh.
TN 5747[b]	1,2,3,4	24-IV-1994	Finland	*Betula*
TN 5748[b]	1,4,5,6	24-IV-1994	Finland	*Betula*
TN 5756[b]		31-V-1994	Finland	*Betula pubescens*
TN 5759[b]	2,3,4,5	8-VI-1994	Finland	*Betula pubescens*
70–4[a]		19-XI-1970	Estonia	*Betula pendula*
71–15[a]		17-V-1971	Estonia	*Betula pendula*
71–22[a]		20-VIII-1971	Russia Vassiliev	*Betula paraermanii*
P. laevigatus:				
83–912	1,2,3	12-IX-1983	Germany	*Betula pendula*
TN 3260[b]	1,2,3,5	3-IX-1985	Finland	*Betula pubescens*
TN 5769[b]	2,3,4,5	5-VII-1994	Finland	*Betula*
P. lundellii:				
TN 3259[b]		3-IX-1985	Finland	*Betula pubescens*
86–1125[d]	1,6,7,8	25-XI-1986	Germany	*Betula pubescens*
89–119[d]	1,3,4,5	9-XI-1989	Germany	*Betula*
TN 5760[b]		8-VI-1994	Finland	*Alnus incana*

[a] provided by Erast Parmasto (TAA)
[b] provided by Tuomo Niemel (H)
[c] provided by Andreas Bresinsky (REG)
[d] provided by Norbert Luschka (REG)

Terms

Stock refers to a fruiting body with specific mating type factors. *Collection* is synonymous with stock. A collection may consist of one to several fruiting bodies. *Line of demarcation* refers to a dark pigmented line in the medium between two mycelia that exhibit antagonistic behaviour (Adams & Roth, 1967). *Single spore mycelium* is synonymous with isolate and refers to a mycelium developing from a single basidiospore. *Secondary mycelium* is a mycelium developing when two compatible single spore mycelia are paired. Such a mycelium can be subcultured as a stable heterokaryon.

Cultural conditions

All cultures were grown on malt agar (2% agar, 2% malt extract, 0.05% yeast extract) at 23°C and 65% humidity in the dark. The pH was usually adjusted to about 5.0–5.5 with sodium citrate buffer (0.05 M).

Isolation of single spores

A section of the hymenium was attached to the inside of a petri dish lid. Discharged spores were dispersed with Ringer's solution (NaCl: 0.225%, KCl: 0.01%, CaCl: 0.0045%, $NaHCO_3$: 0.005%) and aseptically isolated after germination.

Pairing tests

Intrastock pairings were made using 8–12 single spore mycelia, which were paired in all possible combinations. Inocula from each culture were placed 1 cm apart in petri dishes. Usually two pairings were made in an 8.5 cm petri dish. Paired mycelia were incubated for 4–5 wks before examination. For interstock pairings, two different isolates per mating type were selected from each stock as testers; these were paired in all possible combinations. Additional testers were included in some interstock pairings. For interpreting the pairing tests the terminology of Chase and Ullrich (1985) is used, i.e., incompatibility (= homogenic incompatibility) and compatibility denote pairing responses controlled by mating type factors. Intersterility (heterogenic incompatibility) and interfertility imply pairing reactions regulated by factors other than mating type.

DNA isolation

DNA isolation from lyophilized mycelium (homokaryotic and heterokaryotic) was performed as described by Lee and Taylor (1990) with only slight modifications. DNA pellets were air dried and were resuspended in 100 fl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Concentration of DNA was examined on 1% agarose gels (Boehringer, Mannheim). DNA was also isolated from fresh mycelium; 35–40 mg of fresh mycelium was scraped from a petri dish and ground in liquid nitrogen. Further treatment was as described above.

Generally the DNA isolated from lyophilized mycelium was more abundant and better in quality than the DNA from fresh mycelium. The latter often was very viscous and difficult to resuspend in TE buffer, perhaps due to the presence of polysaccharides and/or carbohydrates (Bruns et al. 1990; Kim et al. 1990).

PCR amplification

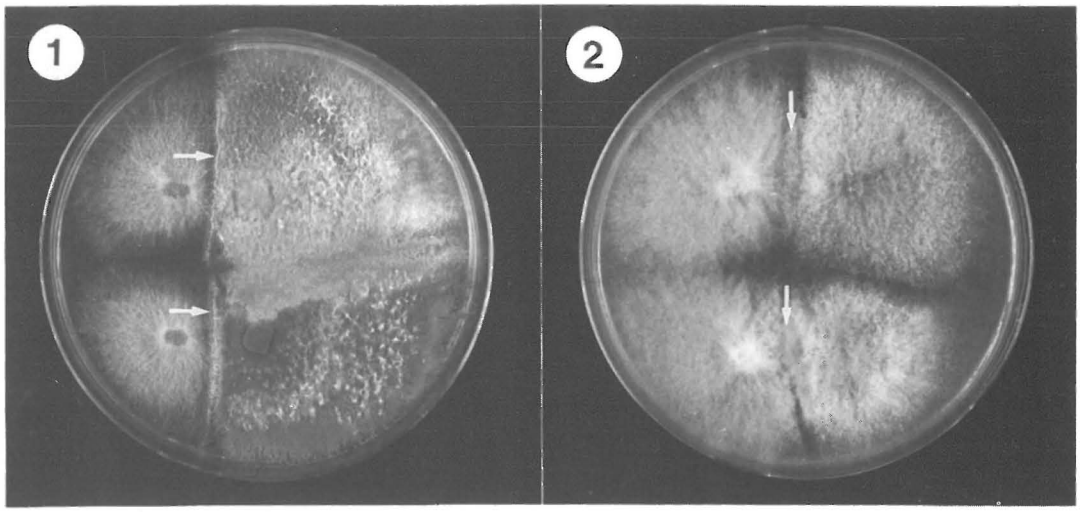
DNA samples were diluted in distilled water. The PCR was used to amplify a fragment of about 1.85 kb homologous to a region in *Saccharomyces cerevisiae* rDNA from base position 34 in the 5.8S RNA to base position 1448 in 25S RNA (Hibbett & Vilgalys 1991; Vilgalys & Hester 1990). Primer sequences (LR7, 5.8S-R) are given by Vilgalys and Hester (1990). The PCR reactions were set up in 100 fl volumes and were overlayed with two drops of mineral oil. Hot start PCR was applied throughout (d'Aquila et al. 1991).

Thirty cycles were performed (Biometra TRIO-Thermoblock), using the following parameters: 94°C denaturation step (1'), 50°C annealing step (45"), 72°C primer extension (2'45"). A final incubation step at 72°C (7') was added after the final cycle. Usually 3 or 5 fl of each PCR reaction was electrophoresed on 1% agarose gels. DNA molecular weight marker VI (Boehringer, Mannheim) was used as standard.

Restriction analysis of PCR products

The amplified products were extracted with one volume of phenol:chloroform (1:1) and centrifuged at 10 000 rpm for 15 min (Eppendorf Centrifuge 5415 C). After this, 80 fl of the aqueous portion was removed, and DNA was precipitated by addition of 8 fl of NaAc (pH 8.0) and 190 fl of 100% EtOH (> 1 h, –20°C). Precipitates were collected by centrifugation (11 000 rpm, 15 min), washed with 750 fl of 70% EtOH, air dried and resuspended in 60 fl TE buffer.

For restriction analysis, usually 10 fl of each PCR product was digested in 20 fl volumes. The four-base restriction enzymes Cfo I, Hae III, Hpa II, Msp I (isoschizomere of Hpa II), Nla III, and Taq I were used according to the manufacturer's instructions (New England Biolabs; Boehringer, Mannheim; Pharmacia). The restriction products were separated on 2% agarose gels. Size of the fragments was estimated by comparison with molecular weight marker VI (Boehringer, Mannheim). Results were recorded by photographing gels over a UV transilluminator.



Figs. 1–2. *Phellinus lundellii*, stock 86–1125. — 1: Incompatible pairings of single spore isolates with a line of demarcation (arrow). — 2: Compatible pairings with a secondary mycelium forming in the contact zone (arrow).

Results

Intrastock pairings (sexuality)

All species of *Phellinus* are without clamp connections; moreover, homokaryons and heterokaryons cannot be distinguished by the number of nuclei per hyphal segment and thus different criteria have to be used for the interpretation of pairing reactions (Fischer & Bresinsky 1992; Fischer 1994).

As has been shown for all other members of the *P. igniarius* group examined to date, the sexuality of *P. laevigatus* and *P. lundellii* is controlled by a unifactorial mating type system (Tables 2, 3). The reactions observed in intrastock pairings were as follows: i) Selfing always resulted in intermingling of isolates. ii) Pairing of incompatible isolates (A=) resulted in the formation of a line of demarcation in the contact zone (Fig. 1). iii) Pairing of compatible isolates (A=) resulted in the formation of a secondary mycelium in the contact zone (Fig. 2). Generally, formation of secondary mycelium was more distinct in *P. lundellii* than in *P. laevigatus*. A unifactorial pattern of sexuality was also observed

Table 2. *Phellinus laevigatus*: Results of pairing eight single spore isolates in stock TN 3260

single spore isolate	1	2	3	4	5	6	7	8
1 (A1)a	/b	+	+	+	–	–	+	–
2 (A2		/	–	–	+	+	–	+
3 (A_2)			/	–	+	+	–	+
4 (A_2)				/	+	+	–	+
5 (A_1)					/	–	+	–
6 (A_1)						/	+	–
7 (A_2)							/	+
8 (A_1)								/

a mating type factor;

b intermingling of single spore isolates = /; formation of secondary mycelium = +; formation of a line of demarcation = –

in the newly introduced stocks from the other species.

Interstock pairings within taxa (multiple allelism)

Interstock pairings within *P. alni* (based on numerous stocks) and *P. nigricans* have been presented previously. Compatibility within the taxa was more or less complete, suggesting multiple alleles in the mating type locus (Fischer 1995).

Our three stocks of *P. laevigatus*, all from *Betula* but from different geographic regions, were fully compatible with each other. Six different mating type factors were assigned (A_1–A_6).

Single spore isolates of only two stocks were available for *P. lundellii*; these were fully compatible, resulting in four different mating type factors (A_1–A_4)

Eighteen stocks proved to be *P. cinereus*. Except for stock 89–826, from *Tilia*, all were from *Betula*. The geographic origin was variable, including Finland, Estonia, Germany, and the United States. The pairing results between the stocks are summarized in Table 4. Compatibility between stocks was not complete, especially in pairings with 89–826 (*Tilia*, Estonia), TN 5745 (*Betula*, Finland), and 92–926b and 92–927 (*Betula*, USA). Here there were several pairings that were not assignable to any of the reaction types described above (Fig. 3). Leaving aside 89–826, TN 5745, 92–926b, and 92–927, altogether 25 different mating type factors were recognized (A_1–A_{25}). Common mating type factors were found in 85–917a × 85–917b, 85–917b × 85–917c, and 90–826a × 90–826b.

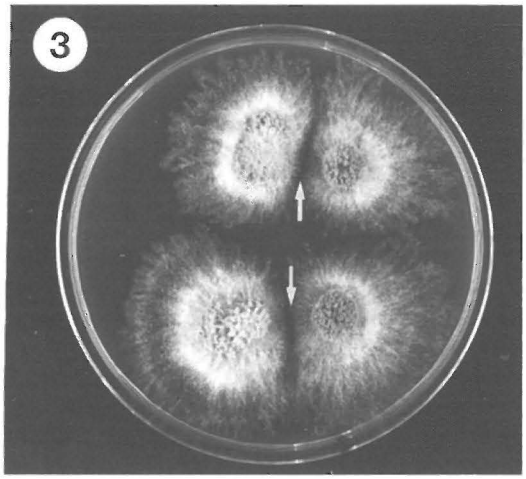


Fig. 3. *Phellinus cinereus*, stocks 89–826 × 92–927. Questionable pairings of single spore isolates; a zone free of mycelium has developed between paired isolates (arrow).

Pairing tests between taxa

Pairing results between the taxa are presented in Tables 4 and 5. *P. laevigatus* and *P. lundellii* were fully intersterile with all other taxa. Estimation of the interrelations between *P. alni* (Finland, Estonia, Germany), *P. nigricans* (Finland), and *P. cinereus* (Finland, Estonia, Germany, USA) is difficult. Intersterility

Table 3. *Phellinus lundellii*: Results of pairing eight single spore isolates in stock 86–1125

single spore isolate	1	2	3	4	5	6	7	8
1 (A_1)[a]	/[b]	–	+	–	+	–	+	+
2 (A_1)		/	+	–	+	–	+	+
3 (A_2)			/	+	–	+	–	–
4 (A_1)				/	+	–	+	+
5 (A_2)					/	+	–	–
6 (A_1)						/	+	+
7 (A_2)							/	–
8 (A_2)								/

[a] mating type factor;
[b] intermingling of single spore isolates = /; formation of secondary mycelium = +; formation of a line of demarcation = –

Table 4. *Phellinus cinereus* and *P. nigricans*: Interstock pairings of European and North American collections

P. cinereus P. nigricans Stocks 4405	85–917a	85–917b	85–917c	86–9a	86–9b	86–9c	TN 3308	89–822a	89–826	89–828b	90–826a	90–826b	TN 5745	TN 5747	TN 5748	TN 5759	92–926b	92–927	TN 3301	TN
P. cinereus:																				
85–917a	50[a]	75	+	+	+	+	+	+	(+)	+	+	+	+	+	+	+	+	+	–	–
85-917b		50	75	+	+	+	+	+	(+)	+	+	+	(+)	(+)	+	(+)	(+)	(+)	–	–
85–917c			50	+	+	+	+	+	(+)	+	+	+	+	+	+	+	(+)	(+)	–	(–)
86–9a				50	+	+	(+)	+	(+)	+	+	+	(+)	+	+	+	+	+	–	–
86–9b					50	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
86–9c						50	+	+	(+)	+	+	+	+	+	+	+	(+)	(+)	–	–
TN 3308							50	+	(+)	+	+	+	+	+	(+)	+	(+)	(+)	+	(+)
89–822a								50	(+)	+	+	+	+	+	+	+	(+)	+	–	–
89–826									50	+	(+)	(+)	(+)	+	(+)	(+)	(+)	(+)	–	–
89–828b										50	+	+	+	+	+	+	+	(+)	–	–
90–826a											50	75	(+)	+	+	+	+	+	–	–
90–826b												50	+	+	+	+	+	(+)	–	–
TN 5745													50	+	+	(+)	(+)	+	+	+
TN5747														50	+	+	(+)	(+)	+	+
TN 5748															50	+	(+)	(+)	+	+
TN 5759																50	+	(+)	+	+
92–926b																	50	+	–	–
92–927																		50	–	–
P. nigricans:																				
TN 3301																			50	+
TN 4405																				50

—[a] unifactorial pattern = 50; compatibility complete = +; compatibility incomplete = (+); one mating type factor in common = 75; intersterility complete = –; intersterility incomplete = (–).

between *P. cinereus* and *P. alni* was complete (Table 5). As was partly shown before (Fischer 1995), there are numerous positive results between *P. alni* (Finland, Estonia, Germany) and *P. nigricans* (Finland). The pairing results between *P. cinereus* and *P. nigricans* varied according to the geographic origin of the stocks (Fig. 4, Table 4): the German, Estonian, and North American stocks of *P. cinereus* were fully intersterile with *P. nigricans*, whereas the Finnish stocks of *P. cinereus* were fully compatible with *P. nigricans* except for TN 3308 (*P. cinereus*), which exhibited a reduced compatibility with TN 4405 (*P. nigricans*).

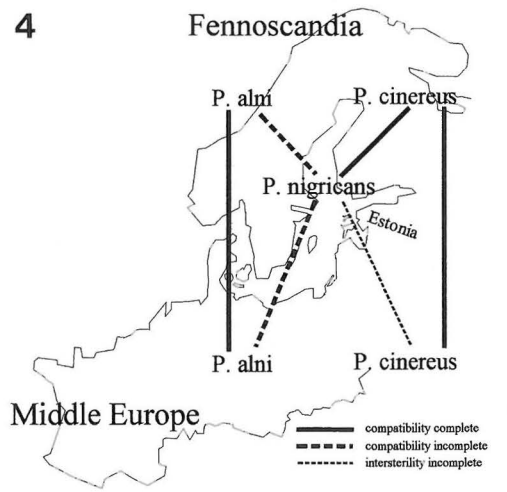


Fig. 4. Pairing relationships between *Phellinus alni, P. nigricans,* and *P. cinereus* from Central Europe, Estonia, and Fennoscandia.

PCR amplification

PCR amplification was performed for all stocks and several single spore isolates of *P. cinereus*, *P. alni*, *P. nigricans*, and *P. lundellii* as well as for two stocks of *P. laevigatus* and the unidentified stock 76–18. Amplification products were always obtained with DNA from lyophilized mycelium; for this a DNA dilution of 1:500 in distilled water usually was successful. Amplification products were sometimes weak or absent with DNA from fresh mycelium, and in these cases an additional phenolization step was applied and/or DNA was diluted up to 1:2000. In Fig. 5 gel electrophoresis of PCR products is shown for *P. alni*, 76–18, P. nigricans, *P. laevigatus*, *P. lundellii*, and some stocks of *P. cinereus*. No length heterogeneity of the PCR product was observed and a uniform band of approximately 1.85 kb was consistently revealed. This was also true for the stocks of *P. cinereus* not included in Fig. 5.

Probably due to insufficient specificity of the PCR amplification, additional bands were observed in some stocks. In 76–18 several bands appeared between approximately 350 bp and 600 bp, and in TN 5759 (*P. cinereus*) one additional band was detected at approximately 510 bp (Fig. 5). The latter also appeared in some other stocks of P. cinereus. Usually a reduction of the primer concentration improved the specificity of the reaction. No PCR products were obtained in the controls.

Table 5. *Phellinus* on *Betula* and *P. alni*: Pairings of European and North American collections

	I	II	III	IV	V	VI	VII
P. alni (I)	+[a]	(+)	–	–	–	–	–
P. nigricans (II)		+	(–)	–	–	–	–
P. cinereus (III) (Central Europe, Estonia)			(+)	(+)	(+)	–	–
P. cinereus (IV) (Fennoscandia)				+	(+)	–	–
P. cinereus (V) (USA, Michigan)					+	–	–
P. laevigatus (VI)						+	–
P. lundellii (VII)							+

[a] compatibility complete = +; compatibility incomplete = (+); intersterility complete = –; intersterility incomplete = (–)

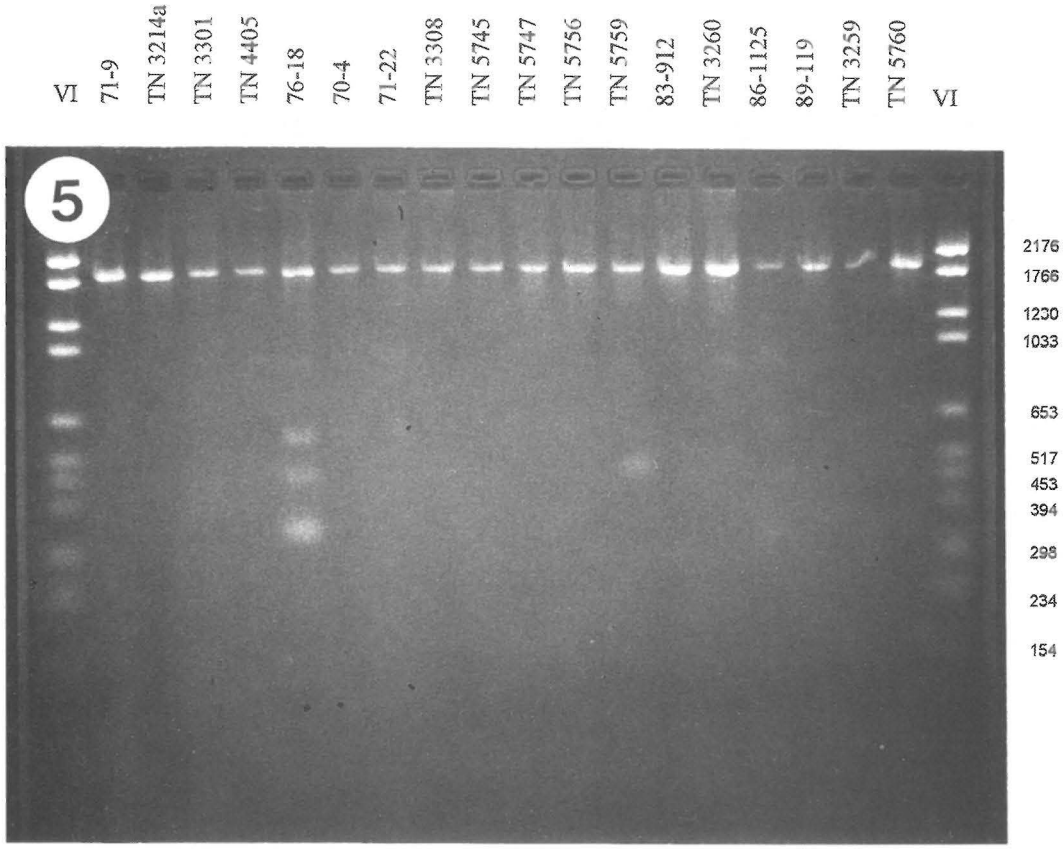


Fig. 5. Gel electrophoresis of PCR products of rDNA on 1% agarose. Estimated size of fragment is 1.85 kb. VI = molecular weight standard; fragment sizes in base pairs. 71–9, TN 3214a (*Phellinus alni*); TN 3301, TN 4405 (*P. nigricans*); 76–18 (unidentified); 70-4, 71-22, TN 3308, TN 5745, TN 5747, TN 5756, TN 5759 (*P. cinereus*); 83-912, TN 3260 (*P. laevigatus*); 86–1125, 89–119, TN 3259, TN 5760 (*P. lundellii*).

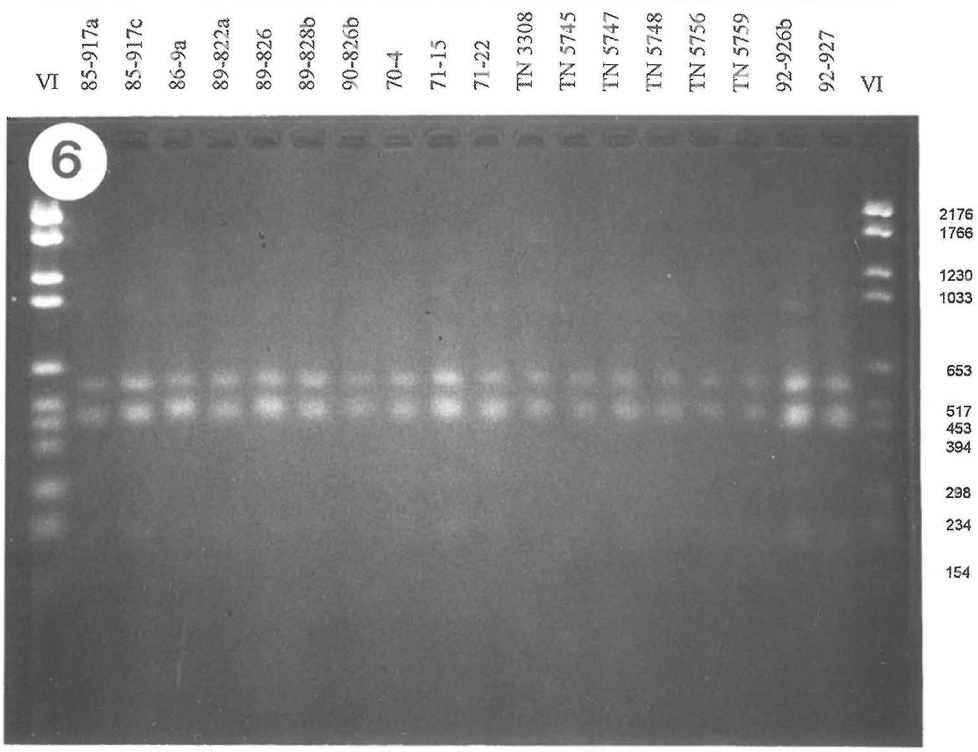
RFLP analysis of PCR products

Within stocks no divergence was observed between homokaryons and heterokaryons (data not shown).

The possible existence of intraspecific variation was examined in more detail in *P. cinereus*. The 18 stocks available were digested with Cfo I, Hae III, Hpa II (Msp I), Nla III, and Taq I. The RFLP phenotypes for Nla III and Taq I are presented in Figs. 6 and 7, respectively, and were identical for all stocks. This was also true for the other enzymes (data not shown). The sum of fragment sizes always corresponded well with the size of the undigested PCR product.

The following stocks were used for interspecific comparison: 71–9, TN 3214a (*P. alni*); 76–18 (unidentified); TN 3301, TN 4405 (*P. nigricans*); 70–4, 71–22, TN 3308, TN 5745, TN 5747, TN 5756, TN 5759 (*P. cinereus*); 83–912, TN 3260 (*P. laevigatus*); 86–1125, 89–119, TN 3259, TN 5760 (*P. lundellii*). The enzymes used were as given above. The RFLP phenotypes for Cfo I, Hpa II, and

Figs. 6–7. Restriction fragments. VI = molecular weight standard; fragment sizes in base pairs. 71–9, TN 3214a (*Phellinus alni*); 76–18 (unidentified); TN 3301, TN 4405 (*P. nigricans*); 70–4, 71–22, TN 3308, TN 5745, TN 5747, TN 5756, TN 5759 (*P. cinereus*); 83–912, TN 3260 (*P. laevigatus*); 86- 1125, 89–119, TN 3259, TN 5760 (*P. lundellii*). — 6: Nla III restriction fragments electrophoresed on 2% agarose. — 7: Taq I restriction fragments electrophoresed on 2% agarose.


VI
85-917a
85-917c
86-9a
89-822a
89-826
89-828b
90-826b
70-4
71-15
71-22
TN 3308
TN 5745
TN 5747
TN 5748
TN 5756
TN 5759
92-926b
92-927
VI
6
2176
1766
1230
1033
653
517
453
394
298
234
154

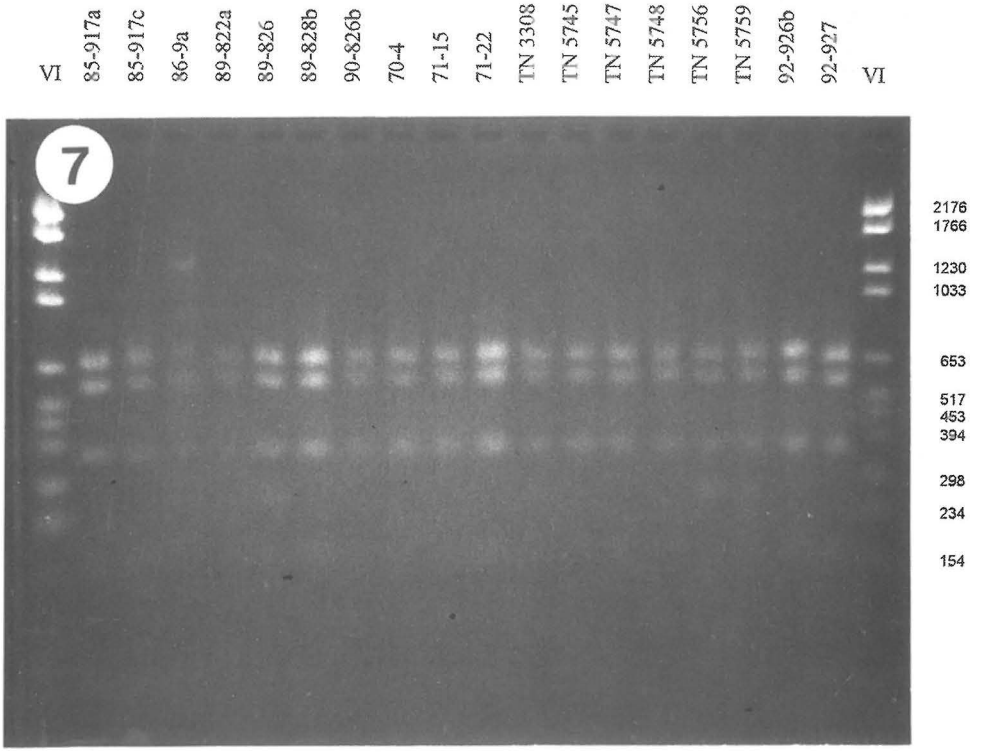

VI
85-917a
85-917c
86-9a
89-822a
89-826
89-828b
90-826b
70-4
71-15
71-22
TN 3308
TN 5745
TN 5747
TN 5748
TN 5756
TN 5759
92-926b
92-927
VI
7
2176
1766
1230
1033
653
517
453
394
298
234
154

Table 6. Distribution of restriction gragments.

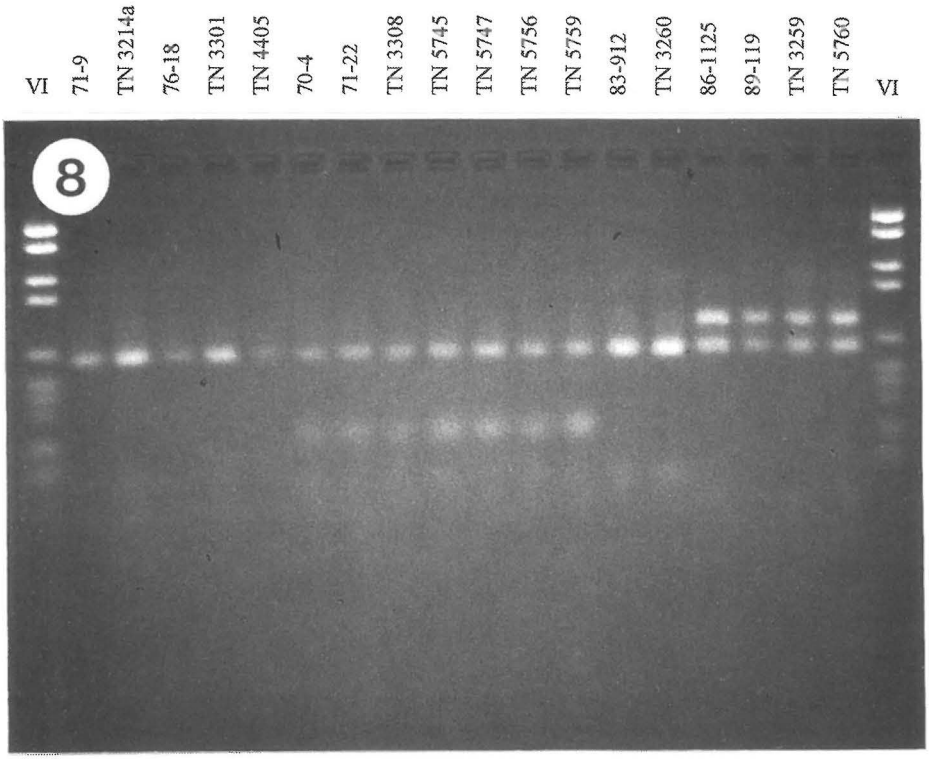
Enzyme	No.	Size [a]	Stocks [b] 71-9	TN 3214a	76-18	TN 3301	TN 4405	70-4	71-22	TN 3308	TN 5745	TN 5747	TN 5756	TN 5759	83-912	TN 3260	86-1125	89-119	TN 3259	TN 5760
Cfo I	1	760	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	2	630	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	3	620	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0
	4	320	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0
	5	320	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0
	6	210	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	7	170	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
	8	140	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hae III	1	850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	2	650	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
	3	630	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	4	440	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	5	260	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0*	0*	0*	0*
	6	260	1	1	1	1	1	1	1	1	1	1	1	1	1*	1*	1	1	1	1
	7	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
	9	150	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
	10	130	1	1	1	1	1	1	1	1	1	1	1	1	1*	1*	1*	1*	1*	1*
Hpa II	1	900	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1
	2	880	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	3	700	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	430	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	280	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6	280	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7	180	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8	180	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Nla III	1	610	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	500	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
	3	470	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
	4	460	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	5	450	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	6	370	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
	7	220	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	8	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
Taq I	1	650	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	2	570	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1
	3	530	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	4	360	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	170	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	6	130	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

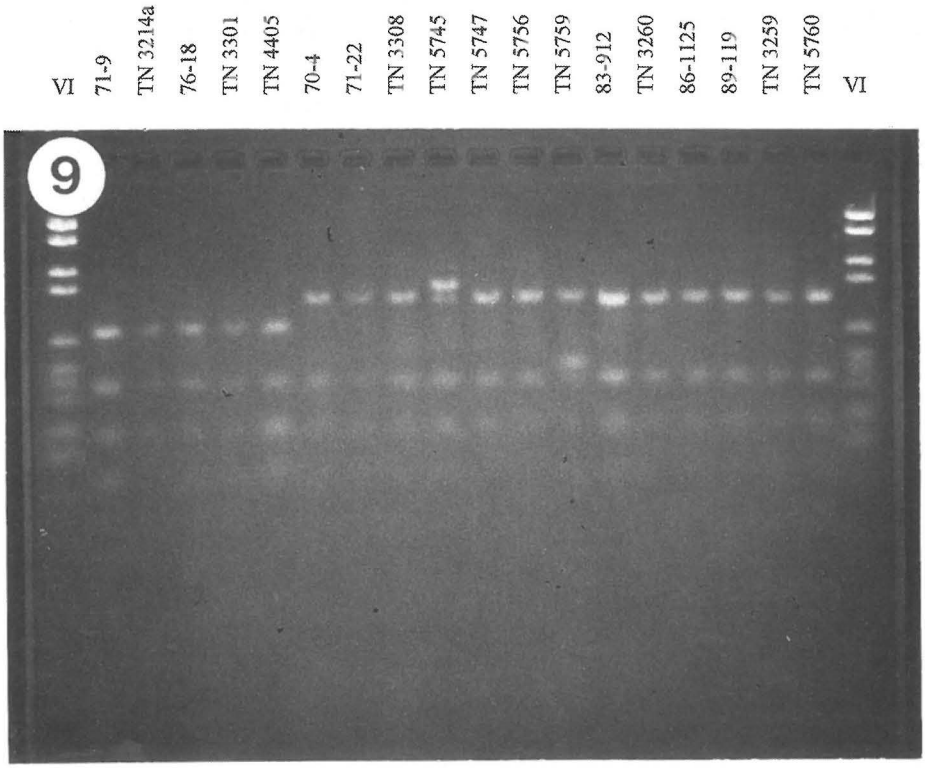
[a] Fragment sizes estimated by comparison with molecular weight standard;

[b] stocks tested: 71-9, TN 3214a (Phellinus alni); 76-18 (unidentified); TN 3301, TN 4405 (P. nigricans); 70-4, 71-22, TN 3308, TN 5745, TN 5747, TN 5756, TN 5759 (P. cinereus); 83-912, TN 3260 (P. laevigatus); 86-1125, 89-119, TN 3259, TN 5760 (P. lundellii);

* fragments questionable.

Figs. 8–9. Restriction fragments. VI = molecular weight standard; fragment sizes in base pairs. 71–9, TN 3214a (*Phellinus alni*); 76–18 (unidentified); TN 3301, TN 4405 (*P. nigricans*); 70–4, 71–22, TN 3308, TN 5745, TN 5747, TN 5756, TN 5759 (*P. cinereus*); 83–912, TN 3260 (*P. laevigatus*); 86 — 1125, 89–119, TN 3259, TN 5760 (*P. lundellii*). — 8: Cfo I restriction fragments electrophoresed on 2% agarose. — 9: Hpa II restriction fragments electrophoresed on 2% agarose.


VI
71-9
TN 3214a
76-18
TN 3301
TN 4405
70-4
71-22
TN 3308
TN 5745
TN 5747
TN 5756
TN 5759
83-912
TN 3260
86-1125
89-119
TN 3259
TN 5760
VI
8
2176
1766
1230
1033
653
517
453
394
298
234
154


VI
71-9
TN 3214a
76-18
TN 3301
TN 4405
70-4
71-22
TN 3308
TN 5745
TN 5747
TN 5756
TN 5759
83-912
TN 3260
86-1125
89-119
TN 3259
TN 5760
VI
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2176
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394
298
234
154

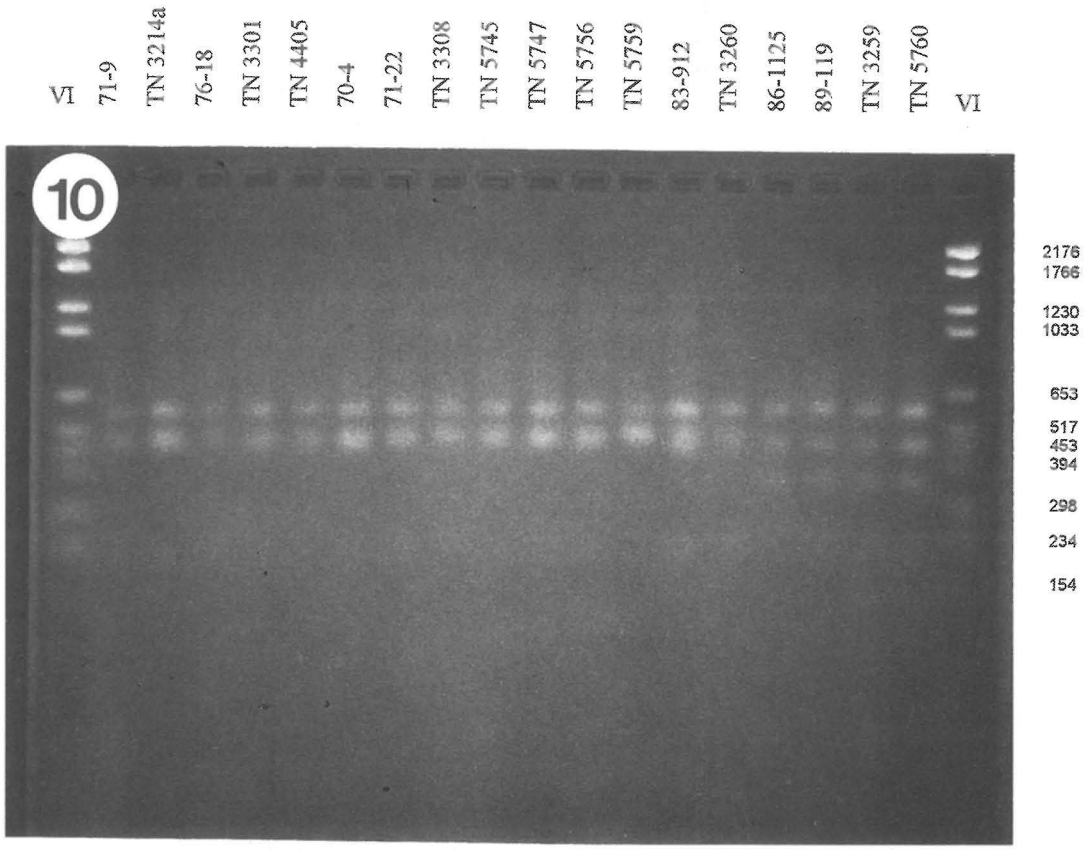

Fig. 10. Nla III restriction fragments electrophoresed on 2% agarose. VI = molecular weight standard; fragment sizes in base pairs. 71–9, TN 3214a (*Phellinus alni*); 76–18 (unidentified); TN 3301, TN 4405 (*P. nigricans*); 70–4, 71–22, TN 3308, TN 5745, TN 5747, TN 5756, TN 5759 (*P. cinereus*); 83–912, TN 3260 (*P. laevigatus*); 86–1125, 89–119, TN 3259, TN 5760 (*P. lundellii*).

Nla III are shown in Figs. 8, 9, and 10. Altogether 40 unique bands were resolved among the 18 stocks in five taxa (Table 6). Three restriction phenotypes were detected for Cfo I, Hae III, Hpa II, and Nla III, and two for Taq I. No single enzyme allowed the identification of all taxa. When the results for all enzymes were pooled, except for *P. nigricans* all taxa could be unequivocally assigned to one unique rDNA type (Table 6). The RFLP data of *P. nigricans* and 76–18 were identical with *P. alni*. Different taxa partly exhibited common restriction phenotypes. For Cfo I, *P. alni* and *P. laevigatus* were identical; for Hpa II, *P. cinereus* and *P. lundellii* were identical; for Hae III and Nla III, *P. alni* and *P. cinereus* were identical; and for Taq I, *P. alni*, *P. cinereus*, and *P. lundellii* were identical. Only *P. cinereus* and *P.laevigatus*, and *P. laevigatus* and *P. lundellii* were different for each enzyme. As is shown in detail for *P. cinereus* (Figs. 6, 7), no intraspecific variation was observed for any of the taxa (Table 6).

There was good agreement between size estimates for the undigested PCR products and the sums of fragment sizes. Except for Nla III and Taq I the estimate of the fragment size sum was sometimes hindered by the presence of double bands. In addition, identification of small restriction fragments was questionable for Cfo I and Hae III. After application of running parameters especially suitable for resolution of double bands and/or small fragments, some fragments still remained questionable; these are indicated by an asterisk in Table 6.

Partial digestion was only rarely observed. The irregular band of about 510 bp, noted for Hpa II (and its isoschizomere, Msp I) in TN 5759 (Fig. 9), probably corresponds to the secondary band observed in the PCR amplification (Fig. 5). This band was not obtained in digestions with the other enzymes. For Hpa II (and Msp I), another product of partial digestion of approximately 1000 bp appeared in TN 5745. The origin of this fragment is unknown.

Discussion

Pairing tests

A unifactorial pattern of sexuality seems common for all members of the *P. igniarius* group; the mating type factor, A, throughout proves to be multiallelic (multiple allelism, A_1–A_n). The results of pairing tests can therefore be used to assign specimens to specific taxa. In this study four taxa were shown to occur on *Betula*: *P. nigricans, P. cinereus, P. laevigatus*, and *P. lundellii*. Except for *P. cinereus*, within these taxa all the interstock pairings were fully compatible (Tables 4, 5). Negative reactions in *P. cinereus* were partly due to homogenic incompatibility (common mating type factors, Fig. 1; see Raper 1966), and probably partly due to heterogenic incompatibility (functioning epistatically to the mating type compatibility system; see Chase & Ullrich 1985, 1990a,b; Wells & Wong 1989; Fischer & Bresinsky 1992). Common mating type factors were observed only in stocks of the same geographic origin, for example in 85–917a, 85–917b, and 85–917c, all collected in a bog within the Bayerischer Wald National Park, Germany.

The reasons for the questionable reactions (Fig. 3) in *P. cinereus* remain uncertain. Such reactions have been noted also in other species of *Phellinus* (Fischer 1994, 1995); assignment as positive or negative is not possible with the data at hand. Appearance of this reaction type is similar to the so-called barrage, indicating a common B-factor in the bifactorial genus Pleurotus (Fr.) Kumm. (Hilber 1982; Bresinsky et al. 1987). Questionable reactions were mainly observed in pairings with 89–826 (*Tilia*, Estonia), TN 5745, and TN 5759 (*Betula*, Finland) and 92–926b and 92–927 (*Betula*, USA). The differences in the collections from Estonia and North America may indicate an early stage of speciation, caused by different hosts or geographic isolation.

P. nigricans seems to serve as a linkage between *P. alni* and *P. cinereus* in Fennoscandia, where, to a different extent, it is positive with both taxa (Fig. 4). Otherwise, when paired with collections from Central and Eastern Europe, Fennoscandian *P. nigricans* is mostly compatible with *P. alni* (from different substrata, not including *Betula*), but with one exception intersterile with *P. cinereus* (Fischer, 1995). It is also intersterile with *P. cinereus* from the United States.

This shows that speciation between *P. alni*, *P. nigricans*, and *P. cinereus* is not yet complete in Fennoscandia, where via positive pairings of *P. nigricans* with both *P. cinereus* and *P. alni* a gene flow between these otherwise intersterile taxa is possible. Including Estonia and Central Europe, a process of even wider scale can be seen: via positive pairings between Central European *P. alni* and Fennoscandian *P. alni* and/or *P. nigricans*, genetic information can reach Fennoscandia, and hence be transferred to *P. cinereus*. Since no mating barriers exist between P. cinereus from Fennoscandia and Central Europe, genetic information from Central European *P. alni* may finally pass to Central European *P. cinereus*.

Nothing is known about the vitality of secondary mycelia established in vivo between different taxa — for example, *P. alni* × *P. nigricans*; it very well might be reduced as compared to intraspecific secondary mycelia. Distribution of species of *Phellinus* occurs by airborne spores; since the prevailing winds are to the west in Central Europe, the establishment of propagules between Central European and Fennoscandian stocks may in any case be exceptional.

Central European findings of *P. nigricans* would have been most helpful in addressing this set of problems, but they were not available to us. As shown for Fennoscandian *P. nigricans*, also in *Armillaria* (Fr.:Fr.) Staude one taxon is positive with two other, intersterile, taxa; however, the compatible taxa are geographically isolated in *Armillaria*, originating from Europe and North America (Anderson et al. 1980).

PCR and RFLP

The chosen rDNA region, containing both highly conserved and more variable sequences, has been repeatedly used as an aid in molecular taxonomy of closely related fungal species (Hibbett &

Vilgalys 1991; Chen & Hoy 1993; Fischer 1995). Gel electrophoresis of undigested PCR products consistently revealed a uniform band of approximately 1.85 kb in *P. alni, P. nigricans, P. cinereus, P. laevigatus,* and *P. lundellii.*

Four RFLP phenotypes were observed, one each for *P. cinereus, P. laevigatus*, and *P. lundellii* and one in common for *P. alni* and *P. nigricans*. There was no full agreement between pairing test data and RFLP data. *P. nigricans* and *P. cinereus* are compatible in Fennoscandia, but exhibit different RFLP phenotypes for Cfo I and Hpa II. Which type of fragment pattern is to be expected in secondary mycelia resulting from compatible pairings between isolates with different RFLP phenotypes? Apparently secondary mycelia are heterokaryotic, containing nuclei of both the paired homokaryons (Fischer 1987; Fischer & Bresinsky 1992). Thus, in positive pairings between *P. nigricans* and *P. cinereus*, nuclei representing both RFLP types should be recovered in the secondary mycelium. As a means of characterizing the particular rDNAs, isolation and subculturing of interspecific secondary mycelia is currently under way.

The RFLP phenotype of the unidentified stock 76–18 was identical with *P. alni* and *P. nigricans*; because of lack of single spore mycelia the final assignment of 76–18 to one of these taxa is not possible. Within the area under study, *P. alni* apparently does not occur on *Betula*; that 76–18 represents *P. nigricans* seems more likely therefore.

Conclusions

The occurrence of four and possibly even more species of *Phellinus* on a single host genus is unusual. *P. laevigatus* and *P. lundellii* are almost exclusively, and *P. nigricans* and *P. cinereus* mainly found on dead trees (Jahn 1963, 1967; Niemelä 1972, 1975, pers. comm.; Domanski 1972; Domanski et al. 1973; Plank 1978; Niemelä & Kotiranta 1982; Breitenbach & Kränzlin 1986; Parmasto 1993, pers. comm.). Speciation in parasitic species of *Phellinus* often is correlated with host preferences. This probably is due to varying rates of basidiospore germination on different substrata in vivo. Amazingly, at least in vitro, basidiospores of extremely specialized taxa are able to germinate on numerous substrata other than the actual host, as has been demonstrated for *Piptoporus betulinus* (Paine 1968; Paine & Merrill 1971) or *Fomitopsis cajanderi* (P.Karsten) Kotl. & Pouz. (Tsuneda & Kennedy 1980). Similar observations have been made for parasitic species of the *P. igniarius* group, although results are preliminary (Fischer 1987). In saprophytic taxa, as are those on *Betula*, fungus-host relationships seem less important. Even so, the taxa treated here only rarely share the same tree, possibly due to somewhat divergent habitat preferences, i.e., humidity or decay grade of the wood. Both *P. nigricans* and *P. cinereus* mostly occur on still standing but dead trees; however, *P. cinereus* also is found as a saproparasite on damaged, but still living trees. *P. laevigatus* and *P. lundellii* both grow on fallen, dead trunks; in rare cases, they are found side by side (Niemelä, pers. comm.).

P. nigricans apparently is a rare species. With the data at hand, it has been demonstrated only for Fennoscandia. An unequivocal identification of *P. nigricans* is not possible without pairing tests and RFLP data. To date no reliable species delimitation between *P. nigricans, P. alni*, and *P. cinereus* is possible with use of traditional characters. At least, the specific status of *P. nigricans* is somewhat less uncertain now than just recently (Fischer 1995), thanks to the unique pairing behaviour demonstrated for *P. alni* and *P. cinereus*.

P. cinereus has a wide distribution. It is the first species of the *P. igniarius* group shown by pairing tests and RFLP data to exist in North America. Most findings from *Betula* that are designated as *P. igniarius*, *P. alni*, or *P. nigricans* probably refer to *P. cinereus*. There is some evidence that the species also occurs in East Asia (preliminary results).

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References

Adams, D.H. & Roth, L.F. 1967: Demarcation lines in paired cultures of Fomes cajanderi as a basis for detecting genetically distinct mycelia. — Canad. J. Bot. 45:1583–1589.

Anderson, J.B., Korhonen, K. & Ullrich, R.C. 1980: Relationships between European and North American biological species of Armillaria mellea. — Exp. Mycol. 4:87–95.

Benkert, D. 1977: Die Porlinge und Schichtpilze der Potsdamer Umgebung. — Gleditschia 5:165–202.

Bondartsev, A.S. 1912: Griby, sobrannye na stvolakh lesnykh porod v Bryanskom opytnom lesnichestve. — Trudy Lesn. Opytn. Delu Rossii 37:1–56.

Bondartsev, A.S. 1971: The Polyporaceae of the European USSR and Caucasia. — 896 pp. Israel Progr. Sci. Transl., Jerusalem.

Bourdot, H. & Galzin, A. 1927: Hymnomyctes de France. — 761 pp. Paul Lechevalier, Paris.

Breitenbach, J. & Kränzlin, F. 1986: Pilze der Schweiz 2. Nichtblтterpilze (Heterobasidiomycetes, Aphyllophorales, Gastromycetes). — 416 pp. Mykologia, Luzern.

Bresinsky, A., Fischer, M., Meixner, B. & Paulus, W. 1987: Speciation in Pleurotus. — Mycologia 79:234–245.

Bruns, T.D., Fogel, R. & Taylor, J.W. 1990: Amplification and sequencing of DNA from fungal herbarium specimens. — Mycologia 82:175–184.

Chase, T.E. & Ullrich, R.C. 1985: Genetics of intersterility in Heterobasidion annosum. — Mycol. Soc. Amer. Newslett. 36(1): 20.(Abstract)

Chase, T.E. & Ullrich, R.C. 1990a: Genetic basis of biological species in Heterobasidion annosum: Mendelian determinants. — Mycologia 82:67–72.

Chase, T.E. & Ullrich, R.C. 1990b: Five genes determining intersterility in Heterobasidion annosum. — Mycologia 82:73–81.

Chen, W. & Hoy, J.W. 1993: Molecular and morphological comparison of Pythium arrhenomanes and P. graminicola. — Mycol. Res. 97:1371–1378.

D'Aquila, R.T., Bechtel, L.J., Videler, J.A., Eron, J.E., Gorczyca, P. & Kaplan, J.C. 1991: Maximizing sensitivity and specificity of PCR by preamplification heating. — Nucleic Acids Res. 19:3749.

Domanski, S. 1972: Fungi. Polyporaceae I, Mucronoporaceae I. — 235 pp. Foreign Sci. Publ. Dept., Warsaw.

Domanski, S., Orlos, H. & Skirgiello, A. 1973: Fungi. Polyporaceae II (pileatae), Mucronoporaceae II (pileatae), Ganodermataceae, Bondarzewiaceae, Boletopsidaceae, Fistulinaceae. — 332 pp. Foreign Sci. Publ. Dept., Warsaw.

Donk, M.A. 1971: Notes on European polypores. IX. On some species of Hymenochaetaceae. — Proc. Koninkl. Nederlandse Akad. Wetensch. C. 74:405–421.

Donk, M.A. 1974: Check list of European polypores. — 469 pp. North-Holland Publ. Co., Amsterdam & London.

Eriksson, J. 1958: Studies in the Heterobasidiomycetes and Homobasidiomycetes — Aphyllophorales of Muddus National Park in North Sweden. — Symb. Bot. Upsalienses 16:1–172.

Erkkilä, R. & Niemelä, T. 1986: Polypores in the parks and forests of the City of Helsinki. — Karstenia 26:1–40.

Fischer, M. 1987: Biosystematische Untersuchungen an den Porlingsgattungen Phellinus Qul. und Inonotus Karst. — Biblioth. Mycol. 107:1–133.

Fischer, M. 1994: Pairing tests in the Phellinus pini group. — Mycologia 86:524–539.

Fischer, M. 1995: Phellinus igniarius and its closest relatives in Europe. — Mycol. Res. 99.

Fischer, M. & Bresinsky, A. 1992: Phellinus torulosus: sexuality and evidence of intersterility groups. — Mycologia 84:823–833.

Hibbett, D.S. & Vilgalys, R. 1991: Evolutionary relationships of Lentinus to the Polyporaceae: Evidence from restriction analysis of enzymatically amplified ribosomal DNA. — Mycologia 83:425–439.

Hilber, O. 1982: Die Gattung Pleurotus (Fr.) Kummer unter besonderer Bercksichtigung des Pleurotus eryngii-Formenkomplexes. — Biblioth.Mycol. 87:1–448.

Jahn, H. 1962: Der Espen-Feuerschwamm (Phellinus tremulae), ein gefrchteter Feind der Espe. — Westflische Pilzbriefe 3:94–102.

Jahn, H. 1963: Mitteleuropische Porlinge (Polyporaceae s. l.) und ihr Vorkommen in Westfalen (unter Ausschlua der resupinaten Arten). — Westflische Pilzbriefe 4:1–143.

Jahn, H. 1967: Die resupinaten Phellinus-Arten in Mitteleuropa. — Westflische Pilzbriefe 6:37–108.

Jahn, H. 1977: Phellinus lundellii und sein Vorkommen in Deutschland (BRD). — Westflische Pilzbriefe 11:59–66.

Jahn, H. 1979: Pilze die an Holz wachsen. — 268 pp. Busse, Herford.

Jahn, H. & Jahn, M.A. 1986: Konstanz und Fluktuation der Pilzvegetation in Norra Warleda (Uppland). Beobachtungen auf einem schwedischen Bauernhof. — Westflische Pilzbriefe 10–11:352–378.

Jülich, W. 1984: Die Nichtblтterpilze, Gallertpilze und Bauchpilze. — 626 pp. G. Fischer, Stuttgart & New York.

Kim, W.K., Mauthe, W., Hausner, G. & Klasen, G.R. 1990: Isolation of high molecular weight DNA and double-stranded RNAs from fungi. — Canad. J. Bot. 68:1898–1902.

Kotlaba, F. 1984: Zemepisn rozsireni a ekologie chorosu (Polyporales s. l.) v Ceskoslovensku. — 194 pp., 123 maps. Academia, Praha.

Kreisel, H. 1961: Die phytopathogenen Großpilze Deutschlands. — 284 pp. G. Fischer, Jena.

Kreisel, H. 1987: Pilzflora der Deutschen Demokratischen Republik. — 281 pp. G. Fischer, Jena.

Lee, S.B. & Taylor, J.W. 1990: Isolation of DNA from fungal mycelia and single cells. Pp. 282–287. — In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), PCR protocols, a guide to methods and applications. — Academic Press, San Diego.

Luschka, N. 1994: Die Pilze des Nationalparks Bayerischer Wald. — Hoppea 53:1–374.

Niemelä, T. 1972: On Fennoscandian polypores II. Phellinus laevigatus (Fr.) Bourd. & Galz. and P. lundellii Niemelä, n. sp. — Ann. Bot. Fennici 9:41–59.

Niemelä, T. 1974: On Fennoscandian polypores III. Phellinus tremulae (Bond.) Bond. et Borisov. — Ann. Bot. Fennici 11:202–215.

Niemelä, T. 1975: On Fennoscandian polypores IV.

Phellinus igniarius, P. nigricans and P. populicola, n. sp. — Ann. Bot. Fennici 12:93–122.

Niemelä, T. 1977: The effects of temperature on two culture types of Phellinus tremulae (Fungi, Hymenochaetaceae). — Ann. Bot. Fennici 14:21–24.

Niemelä, T. & Kotiranta, H. 1982: Polypore survey of Finland 2. The genus Phellinus. — Karstenia 22:27–42.

Paine, R.L. 1968: Germination of Polyporus betulinus basidiospores on non-host species. — Phytopathology 58:1062.

Paine, R.L. & Merrill, W. 1971: Host specificity of Polyporus betulinus. — Phytopathology 61:905.

Parmasto, E. 1976: Studies on Yakutian fungi II. — Eesti NSV Tead. Akad. Toimed. Biol. 25:316–321.

Parmasto, E. 1985: The species concept in Hymenochaetaceae (Fungi, Hymenomycetes). — Proc. Indian Acad. Sci. 94:369–380.

Parmasto, E. 1993: Distribution maps of Estonian fungi 1: 1–34. Inst. Zool. Bot., Tartu.

Plank, S. 1978: kologie und Verbreitung holzabbauender Pilze im Burgenland. — Wissensch. Arb. Burgenland 61:1–207.

Raper, J.R. 1966: Genetics of sexuality in higher fungi. — 283 pp. Ronald Press, New York.

Renvall, P., Renvall, T. & Niemelä, T. 1991: Basidiomycetes at the timberline in Lapland 2. An annotated checklist of the polypores of northeastern Finland. — Karstenia 31:13–28.

Tsuneda, J. & Kennedy, L.L. 1980: Basidiospore germination and substrate preference in Fomes fomentarius and Fomitopsis cajanderi. — Mycologia 72:204–208.

Vilgalys, R. & Hester, M. 1990: Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. — J. Bacteriol. 172:4238–4246.

Wells, K. & Wong, G. 1989: Partial intersterility and evidence of allopatric speciation in Exidiopsis plumbescens (Exidiaceae). — Mycologia 81:567–586.

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