Phellopilus gen. nov. and its affinities within Phellinus s. lato and Inonotus s. lato (Basidiomycetes)

Tuomo Niemelä¹, Tobias Wagner², Michael Fischer² & Yu-Cheng Dai¹

¹) Finnish Museum of Natural History, Botanical Museum, P.O. Box 47, FIN-00014 University of Helsinki, Finland
²) Institut für Botanik, Universität Regensburg, D-93040 Regensburg, Germany

Received 9 August 2000, accepted 7 December 2000


Molecular analysis of a portion of the large ribosomal subunit was performed with Phellinus nigrolimitatus (Romell) Bourd. & Galzin and ten other species in Phellinus Quél. s. lato and Inonotus P. Karst. s. lato in order to establish their generic relationships. The microscopy of P. nigrolimitatus was revised. The species turned out to differ enough to be separated into a new genus, Phellopilus Niemelä, Wagner & Fischer. At the very onset of sporulation the spores are much longer and narrower (proterospores) than later on; this phenomenon is discussed. Morphological and anatomical characters were studied for a somewhat similar species from the Americas, Fomitiporia punctatiformis Murrill (Phellinus punctatiformis (Murrill) Ryvarden); it seems to fit best in the genus Fuscoporia. A new species, Fuscoporia montana Y.C. Dai & Niemelä, is described from Yunnan, China.

Key words: Molecular analysis, Phellinus nigrolimitatus, Phellopilus, proterospore

Introduction

While preparing the revision of the European Hymenochaetales, Fiasson and Niemelä (1984) had long discussions and some disagreement on the generic position of Phellinus nigrolimitatus (Romell) Bourd. & Galzin. Certain characters such as narrow, thin-walled spores and hyphal encrustations linked it with Fuscoporia viticola (Schwein.: Fr.) Murrill and other species of that genus. However, an overall analysis pointed more towards Phellinus s. stricto (‘Ochroporus’
at that time), i.e. the Phellinus igniarius complex, but none of the solutions was fully satisfactory. The problem reemerged and became more evident when molecular studies and phylogenetic analyses were carried out within Phellinus s. lato (Fischer 1996) and within the European poroid Hymenochaetales (Wagner & Fischer 2001): In both studies, P. nigrolimitatus had an isolated position outside the genera acknowledged so far.

On the basis of selected taxa of Phellinus s. lato and Inonotus s. lato the taxonomic position should be determined for P. nigrolimitatus. This should be achieved by microscopical studies and a sequence analysis of a 900 b fragment of the nuclear encoded large ribosomal subunit. Another problem addressed in this study refers to three unidentified strains from China. So far no sequence data are available for these specimens and so they were included in the molecular analysis as well.

Spore shape of Phellinus punctatiformis (Murrill) Ryvarden, P. cinchonensis (Murr.) Ryvarden and the Chinese material referred to above show some resemblance with P. nigrolimitatus, and they are discussed as well.

Materials and methods

Microscopy and herbarium material

In species descriptions the following abbreviations are used. Cotton Blue (CB), Melzer’s reagent (IKI) and 5% potassium hydroxide (KOH) are the media for making microscopic mounts. Their exact compositions are defined in Niemelä (1985). In presenting the variation of spore (setal) size, 5% of the measurements out of each end of the range are given in parentheses. \( L = \) mean length (arithmetical mean of all spores/setae), \( W = \) mean width, \( Q = \) length/width ratio, and \( n = \) the number of spores (or other structures) measured from given number of specimens.

Spores and other microscopic structures were measured in CB; setae were measured in even (length) or half (width) \( \mu \)ms only, from the apex to approximate base. Spores and other structures were measured with the accuracy of 0.1 \( \mu \)m, by using phase contrast illumination. The viscosity of CB in lactic acid arrest the spores, and measurements can easily be made. In IKI and especially KOH spores are moving all the time, making measuring troublesome.

Only specimens listed after species’ discussions were used for microscopic and/or morphologic analysis. Other materials in the herbarium of the Botanical Museum, University of Helsinki (H) were consulted, too. Types and other important collections were studied from the herbaria BPI, FLAS, PRM, S, SWFC (for herbarium abbreviations, see Holmgren et al. 1990). Colour terms follow Anonymous (1969) and Rayner (1970).

Molecular studies

DNA was isolated from cultured mycelium and/or herbarium specimens. Cultures were grown on 2% malt extract medium (ME; 2% malt extract, 2% agar, 0.05% yeast extract in distilled water) at 23 °C under permanent dark conditions. Total DNA was essentially isolated as described by Lee and Taylor (1990). The air-dried DNA pellets were resuspended in 100 \( \mu \)l TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). Quality and quantity of DNA was examined on 1% agarose gels.

Isolated DNA was diluted 1:500 or 1:1000 in distilled water. The polymerase chain reaction (PCR) was used to amplify a portion of the large subunit of the nuclear encoded ribosomal DNA unit defined by the primers prLR0R and prLR7 (for primer sequences, see Vilgalys & Hester 1990; additional information was kindly supplied by Rytas Vilgalys).

The PCR reactions were set up in 100 \( \mu \)l volumes. Thirty-seven cycles were performed on a TRIO-Thermoblock (Biometra, Germany), using the following parameters: 94 °C denaturation step (1 min), 47 °C annealing step (45 sec), and 72 °C primer extension (2 min). A final incubation step at 72 °C (7 min) was added after the final cycle.

The amplified products were purified with the QIAquick PCR Purification Kit (Qiagen) following the manufacturer’s instructions. DNA was suspended in 50 \( \mu \)l Tris-HCl buffer (10 mM,
pH 8.0).

Cycle sequencing was carried out with the primers prLR0R and prLR5 using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Sequences were generated in two directions, and thirty-five amplification cycles were carried out, using the following parameters: 96 °C denaturation step (30 sec), 48 °C annealing step prLR0R (15 sec) and 49 °C annealing step prLR5 (15 sec), and 60 °C primer extension (4 min).

DNA was precipitated by addition of 2 µl NaAc (3M, pH 4.8) and 55 µl of EtOH 100%, and was washed with 150 µl of EtOH 70%. The DNA pellet was resuspended in EDTA (50 mM, pH 8.0):formamide = 1:4.

The separation and detection of the cycle sequencing products were done on an ABI 377 automated sequencer (Perkin Elmer).

Approximately 900 bases of the sequences, starting from the primer prLR0R, were automatically aligned using the CLUSTAL X (version 1.64b) program (Thompson et al. 1997). A final alignment was performed by eye. Alignment gaps were treated as missing data.

The neighbour-joining tree was calculated with components of the PHYLIP 3.5c package (Felsenstein 1995). Bootstrap values for internal nodes were calculated by 1000 replications.

The field data of the fungal material used in the molecular studies are given in Table 1. The heterobasidiomycete Tremella foliacea Pers.: Fr. was chosen as an outgroup. Since no PCR product was obtained for P. punctatiformis, this species is not included in the sequence analysis.

### Taxonomic treatment

**Phellopilus** Niemelä, Wagner & Fischer, gen. nov.

*Fungi poroidei Phellino affines sed cum sporis obclavatis et contexto basidiocarpi bistrato.*

**Typus generis:** Polyporus nigrolimitatus Romell.

Basidiocarps perennial, poroid, brown; context made up of two layers (duplex) separated by thin, crustose demarcation. Hyphal system indistinctly dimitic or trimitic, with hyaline, sim-

---

**Table 1.** Species and specimens included in molecular analyses. YCD means Yu-Cheng Dai, TW Tobias Wagner, TN Tuomo Niemelä, MF Michael Fischer.

<table>
<thead>
<tr>
<th>Species and specimens included in molecular analyses</th>
<th>Germany. Bavaria: 1995, TW 385 (REG).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylloporia ribis (Schum.: Fr.) Ryvarden</td>
<td>Germany. Bavaria: 1984, MF 84-71 (REG).</td>
</tr>
<tr>
<td>Porodaealea pini (Brot.: Fr.) Murrill</td>
<td>Germany. Bavaria: 1987, MF 87-511 (REG).</td>
</tr>
</tbody>
</table>
ple-septate and branched generative hyphae, and brown skeletal which also bear scattered simple septa and apical ramifications. Hymenial setae present, other types of setae absent. Hyphae at the dissepiment edges sometimes with an apical encrustation. Spores smooth- and thin-walled, narrowly obclavate (almost cylindric), faintly cyanophilous, IKI–.

**Phellolopilus nigrolimitatus** (Romell) Niemelä, Wagner & Fischer, *comb. nova* (Figs. 1–3)


**LECTOTYPUS:** Sweden, Södermanland prov., Bedarö, *Picea abies*, 16.IV.1905 Romell (S 12499, studied).

Basidiocarp perennial, pileate or effused-reflexed, rarely resupinate, pilei up to 5 cm thick at base, projecting up to 7 cm, but usually much smaller, resupinate areas up to several tens of cm across, 5–20 mm thick. Upper surface of the pileus of two kinds: (a) regular type smooth or rough, at first matted and curly yellow or cinnamon, older parts pellicular and cigar brown, finally hard, vinaceous brown or almost black; (b) spongy type swollen, tomentose, with uneven surface full of round cavities of various sizes, at first curly yellow, finally cinnamon, orange-brown or fawn. Edge of the pileus rounded in actively growing and spongy basidiocarps, very sharp in old regular ones. Underside fawn, greyish brown or finally fuscous, poroid area receding in old specimens (both pileate and resupinate), tube mouths even, pores round or angular, (5–)6–8–(10) per mm (*n* = 70/6).

Section: Context corky, cinnamon, in the regular type duplex with variably thick upper context covered with thin pellicle, and paler lower context a few mm thick; the layers separated by thin, wavy, black, crustose zone. In the spongy type context marmoreal with dark brown basic mycelium, irregularly rounded spaces of paler mycelium, and round empty cavities; different areas separated by hair-thin crustose zones. Tube layer 2–10 mm thick, pale greyish brown, annual layers indistinct.

Hyphal system dimitic/trimitic, all septa without clamp connections, hyaline hyphae faintly cyanophilous (CB+), all IKI–, brown hyphae darkening in KOH but otherwise unchanged.

**Upper context** composed of one type of hyphae (distinction between generative and skeletal hyphae cannot be made), which are mostly thick-walled, dull brown, less often fairly thin-walled and paler, (1.5–)4–5(–6) µm (*n* = 30/1), often flattened, sparingly septate, occasionally branched, intermixed with upward-radial orientation, hyphal apices at surface hyaline, thin-walled and merging into amorphous pellis. Crustose separating zone 30–50 µm thick, made up of densely packed, tortuose, reddish brown, thick-walled hyphae in a resinous matrix. **Lower context** dimitic/trimitic, generative hyphae hyaline, thin-walled, readily branched and septate, numerous; skeletal hyphae (1.8–)2.9–5(–5.5) µm (*n* = 90/3), thick- to very thick-walled, yellow brown or ochraceous, interwoven with radial orientation, mostly straight, sparingly septate and seldom branched; among them (especially close to upper limit) thin, branched, brown, thick-walled hyphae or hyphal tips; all transitions present. Spongy context composed basically of one type of hyphae, which are (1.8–)2–4.6(–5.1) µm (*n* = 70/2), deep dull brown, thick-walled, occasionally septate and branched, loosely interwoven without orientation, but close to surface vertically aligned, pale yellow and at surface hyaline, making no pellis (Fig. 1e); paler areas in spongy context having more yellowish hyphae and common hyaline generative hyphae.

Trama dimitic, with common, hyaline generative hyphae, and regular, unbranched skeletal which are reddish brown, thick-walled, interwoven, (1.3–)1.9–2.5(–2.9) µm (*n* = 60/2), only seldom septate. Hyphal tips (mostly skeletal) at dissepiment edge hyaline, wavy, occasional generative hyphae with a capitulate rosette of crystals. Subhymenium thin and indistinct, made up of parallel, glued-together generative hyphae.

**Hymenium:** Basidia ca. 8.4–9.8–(10.3) × 4.5–5.2 µm, thick club-shaped or ellipsoid. Basidioles (5.9–)8–9.2–(9.9) × (3.3–)4–5 µm, subglobose or thick club-shaped. Hymenial setae...
Fig. 1. *Phellopilus nigrolimitatus* Niemelä, Wagner & Fischer, specimens Niemelä 6533 & Dai (a–d) and Renvall 376a (e), drawn in Cotton Blue. — a: Spores; — b: Hymenial setae; — c: Dissepiment edge; — d: Vertical section through context up to the upper pellicle; — e: Surface of the basidiocarp of the spongy type.
As to its microscopy, Phellolopilus remains somewhere between the genera Phellinus s. stric-
to (the P. igniarus complex), Phellinidium (the
Phellinus ferrugineofuscus complex) and Fuscopa-
ria (the Phellinus contiguus complex), and
perhaps also Porodaedalea (the Phellinus pini
complex). Senescent hymenial cells collapse,
but remain visible because of their glued-togeth-
er bases in the so-called honeycomb structure,
so characteristic of Phellinus igniarus and relat-
d species, but also Phellinidium. Setae are
fairly similar to those of Phellinus s. stricto,
although being more robust in Phellolopilus. The
pellicular cover of Phellolopilus differs from the
well-developed crust of Phellinus igniarus (see
Niemeiä 1975: fig. 20a) and its relatives. Por-
adaedalea is characterized by a trichoderm. Hy-
phal encrustations link the new genus with Fusco-
poria and Porodaedalea, but in Phellolopilus there
is only a single rosette of crystals attached to
hyphal tip at the dissepiment edge, while in the
other genera numerous crystals cover apical
segments of generative hyphae (‘Kristallifere
Hyphen’ of Jahn 1967). In young basidiocarps
of P. nigrolimitatus no crystals were seen, and
usually they are scanty.

The present description of Phellolopilus ni-
grolimitatus is based on the type and other speci-
mens from North and Central Europe. The lecto-
type is fertile and agrees with the other specimens
mentioned, although spores are slightly narrower
than usually. It was selected as type by J.L. Lowe,
and illustrated in the original description (Romell
1911: fig. 3). In a thorough revision of Phellinus
in East Asia, Dai (1999) reported the spores to be
somewhat smaller, in particular narrower (L =
5.17 µm, W = 1.88 µm, Q = 2.75), but the
dimensions are well overlapping.

Spore characters of one collection from Po-
land (Małopolska prov., Babia Góra, Picea abies,
15.VIII.1973 Niemeiä 499a, H) were so deviat-
ing that the specimen was thought to represent
another species (Fig. 2), even though its other
microscopy, macroscopy, and even the charac-
teristic pocket rot agreed with Phellolopilus ni-
grolimitatus. Our sequence data, however,
confirmed its inclusion in that species. Most of
the spores were extremely long, and narrower
than usual: (5.3–)5.6–9.3–(9.8) × (1.7–)1.8–2.2
µm, L = 7.08 µm, W = 1.97 µm, Q = 3.59 (n =

Notes on Phellolopilus nigrolimitatus

Characteristic features of the species (and hence
the new genus) are the stratified structure of the
context, and the special shape of the thin-walled
spores. The brown, thick-walled hyphae of the
context are occasionally branched, unlike in the
other genera of Phellinus s. lato. Dai (1999)
called such ramified hyphae as skeleton-binding
hyphae, referring to the similar but hyaline
elements of the genus Polyporus; the terminolo-
gy of hyphal systems fits poorly to the Hymeno-
chaetales. The septa in the skeletal hyphae are
very distinct. The curious habit to produce wild-
ly growing spongy context may also be charac-
teristic of the new genus.

(18–)20–32–(34) × (3–)4.5–7.5–(9) µm, L =
26.02 µm, W = 6.26 µm, Q = 3.91–4.29 (n =
150/5), red-brown, thick-walled, subulate, some-
times with a heel, usually terminal and regular,
but a few pleural or double-pointed or otherwise
irregular. Other kinds of setae absent; no cystid-
ia; no cystidioles, but basidioles give soon rise
to secondary hyphae which finally fill the old
tubes. Collapsed old hymenial making up a
strongly-developed honeycomb structure which
stains bright blue in CB.

Spores: Basidiospores (4.1–)4.7–6.3–(7.2) ×
(1.9–)2–2.5–(2.8) µm, L = 5.49 µm, W = 2.23 µm,
Q = 2.30–2.61 (n = 180/6), obclavate (narrow,
tapering to the distal end), less often cylindric
even then with slightly tapering apex), often in
tetrads; wall thin, smooth, faintly CB+, IKI–;
apiculus minute, laterally positioned.

Fig. 2. Phellolopilus nigrolimitatus Niemeiä, Wagner &
Fischer, specimen Niemeiä 499a. — a: Exceptional-
ly narrow and long spores, proterosporae; — b: Ba-
sidium with attached spores.
The range of variation is not clearly seen from these numbers, because often the longest spores were the narrowest (Fig. 2). Among the rod-shaped spores, also fairly or fully normal ones were seen; both types were also seen attached to the basidia. Evidently this is an example of the so-called proterospores, as described by Nuss (1982) in *Ganoderma*: the first spores during the onset of sporulation may deviate in shape and size from the typical ones which develop after the spore production has been established. The very young age of the basidiocarp, and thinness of the walls of the longest spores support that interpretation. Spore polymorphism was also discussed by Parmasto & Parmasto (1987) and Clémençon (1997). Spore characters of this specimen were not included in the description of *P. nigrolimitatus*.


**Fuscoporia montana** Y.C. Dai & Niemelä, sp. nova (Figs. 4–5)

*Carpophorum perenne, fuscum, cum poris 7–8 per mm; systema hypharum dimiticum, sporae 6.5–8.2 × 3.2–4.2 µm, inamyloideae, indextrinoidae, acyanophilae; setae hymeniales 24–37 × 5–7.5 µm.*

**Holotypus:** China. Yunnan prov., Hekou, III.1999 Gao 25 (H, isotypus SWFC).

Basidiocarp perennial, resupinate but sometimes making up a swollen edge, single basidiocarps 5–7 cm across. Surface of the swollen edge matted, uneven and with rounded annual zones, pale cigar brown or snuff brown, older
zones closer to the substrate date brown or umber; sterile margin bordering the whole basidiocarp more yellowish, fulvous. Poroid surface pale cigar brown or fawn, smooth, in old basidiocarps pulvinate, pores round and regular, (6–)7–8(–9) per mm (n = 30/3).

Section: Subiculum hard corky, 0.5–4 mm thick, cinnamon brown, homogeneous. Tube layer 5–10 mm thick, concolorous with lower surface, annual zones 2–4 mm, distinct, often separated by a thin layer of mycelium.

Hyphal system dimitic in all parts, all septa without clamp connections, all hyphae CB–, IKI–, brown hyphae darkening in KOH but otherwise unchanged.

Subiculum hyphae interwoven, generative hyphae very thin-walled, ca. 2–3.5 μm in diam., rather uncommon; skeletal hyphae (2.4–)2.6–4(–4.6) μm (n = 60/2), unbranched, thick-walled, yellow-brown, with few and inconspicuous septa.

Tramal hyphae subparallel or interwoven with downward orientation, generative hyphae (2–)2.2–3.5(–3.7) μm (n = 30/2), very thin-walled, common especially in subhymenium and having a distinct dolipore septum there (seen in CB, phase contrast); skeletal hyphae (2.3–)2.7–3.8(–4.1) μm (n = 90/3), thick-walled, yellow-brown, non-septate. Hyphal tips at dissepiment edge light brown and stiff (skeletal) or very thin-walled and winding (generative), the latter ones very often bearing scattered rosettes of crystals.

Hymenium: Basidia (11–)12–17(–18) × (5.5–)6–8 μm (n = 40/2), thick club-shaped, pear-shaped or ellipsoid, sterigmata four. Basidioles very variable in size, (8.8–)9.5–15(–17.5) × (3.5–)4–7(–7.5) μm (n = 35/2), subulate, subglobose or broadly pear-shaped. Hymenial setae (17–)24–37(–43) × (4–)5–7.5(–8) μm, L = 30.57 μm, W = 6.02, Q = 4.47–5.67 (n = 90/3), red-brown, thick-walled, narrow subulate, rarely with a heel, usually terminal but rarely pleural; covered with aligned generative hyphidia. No other kinds of setae; no cystidia; sharp-pointed, hyphoid cystidioles (13–)16–30(–37) × (2.7–)3–4.3(–4.6) μm (n = 53/3), scattered in fertile hymenium but abundant in senescent one (specimen 1378). Hymenial cells with delicate walls, making up no sturdy honeycomb structure; generative hyphae in lower hymenium bearing scattered rosettes of crystals.

Spores: Basidiospores (6–)6.5–8.2(–9) × (2.9–)3.2–4.2(–4.4) μm, L = 7.22 μm, W = 3.76 μm, Q = 1.79–2.08 (n = 90/2), narrow ovoid (distal end tapering) or narrow ellipsoid, mature spores separate, guttulate; wall thin, smooth, CB–, IKI–; apiculus minute.
Notes on *Fuscoporia montana*

Spore shape and the presence of encrusted hyphae in lower hymenium and at dissepiment edges link *Fuscoporia montana* with *F. contigua* (Pers.: Fr.) Cunningham. Pores are much smaller in the new species, however, and setae are shorter; there are no mycelial setae in its marginal mycelium, occasionally found in *F. contigua*. The spores of *F. montana* are ovoid, i.e., narrower at their distal end, in particular in juvenile spores still attached to the basidia. Spore shape is much more rounded than in *P. nigrolimitatus*. Homogeneous structure of the subiculum, and the presence of richly encrusted hyphae at dissepiment edges link the new species in *Fuscoporia*, but not in *Phellopilus*; molecular results support that decision (see below). Only three collections are known by now, and in particular the morphology will be outlined more exactly when additional material becomes available.

*Fomitiporia punctatiformis* Murrill (*Phellinus punctatiformis* (Murrill) Ryvarden) is somewhat similar to the two species described above, and at first we considered its inclusion in the genus *Phellopilus*. Original collections were studied; the specimens are totally resupinate and round-pored [(7–)8–9(–10) pores per mm], spores are cylindric or slightly tapering, thin-walled,
CB−, (−5−)5.2−7.2(−8.4) × (2−)2.1−2.7(−3) µm, L = 6.16 µm, W = 2.46 µm, Q = 2.30−2.75 (n = 60/2), hymenial setae are subulate, (13−)16−31(−34) × (−4)−4.5−7(−8) µm, L = 22.88 µm, W = 5.75 µm, Q = 3.49−4.42 (n = 60/2). Hyphal structure is dimitic, skeletal (2.5−)2.7−3.7(−3.8) µm (n = 30/1), neither septate nor branched. Generative hyphae are richly covered with crystal rosettes at dissepiment edges. No strong honeycomb structure develops in senescent hymenium. These characters rule out the inclusion of this species in Phellolopus; an appropriate genus is Fuscoporia. We refrain from making the combination, since recent microscopical study of this species from the samples examined by Dai (1999) indicates the absence of true fertile material. Also Poraria cinnamomea Rick, Fomitiporella altocedronensis Murrill and Fomitiporia cinchonensis (Murrill et al., 1932) should be restudied before nomenclatural changes are made.


Molecular analysis

Beside Phellolopius nigrolimitatus, the following taxa representing important subgroups of the former genera Phellinus s. lato and Inonotus s. lato were included in the molecular studies (for field data, see Table 1):

Phellinus igniarius (Phellinus s. stricto)
Fomitiporia punctata (Phellinus robustus group)
Porodaedalea pini (Phellinus pini group)
Fuscosporia ferruginosa and F. contigua (Phellin
nus ferruginosus group)
Phellinidium ferrugineofuscum (Phellinus ferrugineofuscus group)
Phyllopora ribis (Phellinus ribis)
Inocutis rheades (Inonotus rheades group)
Inonotus hispidus (Inonotus s. stricto)

Except for the outgroup, Tremella foliacea, with 849 nucleotides, the length of the sequenced fragment ranged between 864 nucleotides (F. ferruginosa) and 879 nucleotides (Phyllopora ribis); the size of the fragment was 870 nucleotides for Phellolopus nigrolimitatus. The total length of the alignment was 885 nucleotides, 277 of which were variable and 145 phylogenetically informative. A phylogenetic tree was generated using the neighbour-joining method (Fig. 6). The enclosed taxa fall into two large clades, each subdivided into smaller subgroups.

Within the first large clade (above in Fig. 6) Phellolopus nigrolimitatus exhibits an isolated position. The molecular sequences of the three strains of Phellolopus nigrolimitatus, originating from Europe and East Asia, came out as nearly identical. Phellinidium ferrugineofuscum was positioned as next related to Phellolopus nigrolimitatus. The sister group to Phellinidium comprises the taxa Fuscosporia ferruginosa and F. contigua as well as the three strains identified as F. montana. The monophyletic character of this group is strongly supported by a bootstrap value of 97%. Even though they were all collected within a limited area of SW China, a considerable genetic variation is evident in the strains of F. montana.

For all internal nodes of the second large clade (below in Fig. 6) the bootstrap values were < 50%, which supports the idea of a generic state for these taxa. A somewhat closer relationship is evident between Phellinus igniarius, Fomitiporia punctata and Porodaedalea pini, and between Inocutis rheades, Inonotus hispidus and Phyllopora ribis.

Our sequence data show that Phellolopus is sharply delimited from any other group of Phellinus s. lato and Inonotus s. lato that was included in this study. The genus comes out as related to Phellinidium, and, less distinctly, Fuscosporia. The putative relationship to Fuscosporia is confirmed by some microscopic features, which are presented in detail above.

As mentioned above, a possible differentiation within Phellolopus nigrolimitatus is suggested by the dimensions of the basidiospores, which, when compared to material from East Asia, are slightly longer and broader in European specimens (Dai 1999). The length and shape
Fig. 6. Relationships between Phellopilus nigrolimitatus and related taxa inferred from 885 nucleotide sites of the large subunit of the nuclear ribosomal DNA using the neighbour-joining method. Support from 1000 bootstrap replications is indicated above the branches, values less than 50% are not noted.
of the spores also varies between the European collections, but this seems to be due to the stage of sporulation. The variability of spore dimensions is not reflected by the molecular sequence data (Fig. 6).

The overall topology of the presented tree is in good accordance with the one presented by Wagner and Fischer (2001), even though the latter was based on a differing set of taxa. Both studies show that the large genera *Phellinus* *s. lato* and *Inonotus* *s. lato* are not supported by molecular means.

**Acknowledgements**

Loans from herbaria BPI, FLAS, PRM and S are gratefully acknowledged. The Curator of herb. BPI is thanked for permission to take samples of *Phellinus punctatiformis* for molecular analyses. Prof. Teuvo Ahti (Helsinki) revised the Latin.

**References**


Wagner, T. & Fischer, M. 2001: Natural groups and a revised system for the European poroid Hymenomycetes (Basidiomycota) supported by molecular sequence data. — *Mycological Res.* 105. [In press].