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

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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) µms only, from the apex to approximate base. Spores and other structures were measured with the accuracy of 0.1 µm, 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 µl 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 µl 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 µl 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.

Inocutis rheades (Pers.) Fiasson & Niemelä	Germany. Bavaria: 1995, *TW 385* (REG).
Inonotus hispidus (Bull.: Fr.) P. Karst.	Germany. Bavaria: 1992, *MF 92-829* (REG).
Phylloporia ribis (Schum.: Fr.) Ryvarden	Germany. Bavaria: 1982, *MF 82-828* (REG).
Porodaedalea pini (Brot.: Fr.) Murrill	Germany. Bavaria: 1984, *MF 84-71* (REG).
Fomitiporia punctata (P. Karst.) Murrill	Germany. Bavaria: 1987, *MF 87-511* (REG).
Phellinus igniarius (L.: Fr.) Quél.	Germany. Bavaria: 1983, *MF 83-1110a* (REG).
Phellinidium ferrugineofuscum (P. Karst.) Fiasson & Niemelä	Finland. Kemin Lappi: 1997, *TN 6121* (REG).
Phellopilus nigrolimitatus (Romell) Niemelä, Wagner & Fischer	Germany. Bavaria: 1985, *MF 85-823* (REG). Poland. Małopolska prov.: 1973, *TN 499a* (H). China. Changbai For. Res.: 1997, *YCD 2464* (H, REG).
Fuscoporia ferruginosa (Schrad.: Fr.) Murrill	Germany. Bavaria: 1982, *MF 82-930* (REG).
Fuscoporia contigua (Pers.: Fr.) Cunningham	Germany. Bavaria: 1995, *TW 699* (REG).
Fuscoporia montana Y.C. Dai & Niemelä	China. Yunnan: 1999, *Gao 25, Zhang 34*, n.d. *Yang 1378* (H).
Tremella foliacea Pers.: Fr.	Germany. Bavaria: 1997, *MF 97-1117* (REG).

ple-septate and branched generative hyphae, and brown skeletals 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–.

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

Polyporus nigrolimitatus Romell, Arkiv för Botanik 11(3): 18. 1911.
Phellinus nigrolimitatus (Romell) Bourd. & Galzin, Bull. Soc. Mycol. France 41: 193. 1925.

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 curry 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 curry 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 marmorated 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 basicly 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 skeletals 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 skeletals) at dissepiment edge hyaline, wavy, occasional generative hyphae with a capitate 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

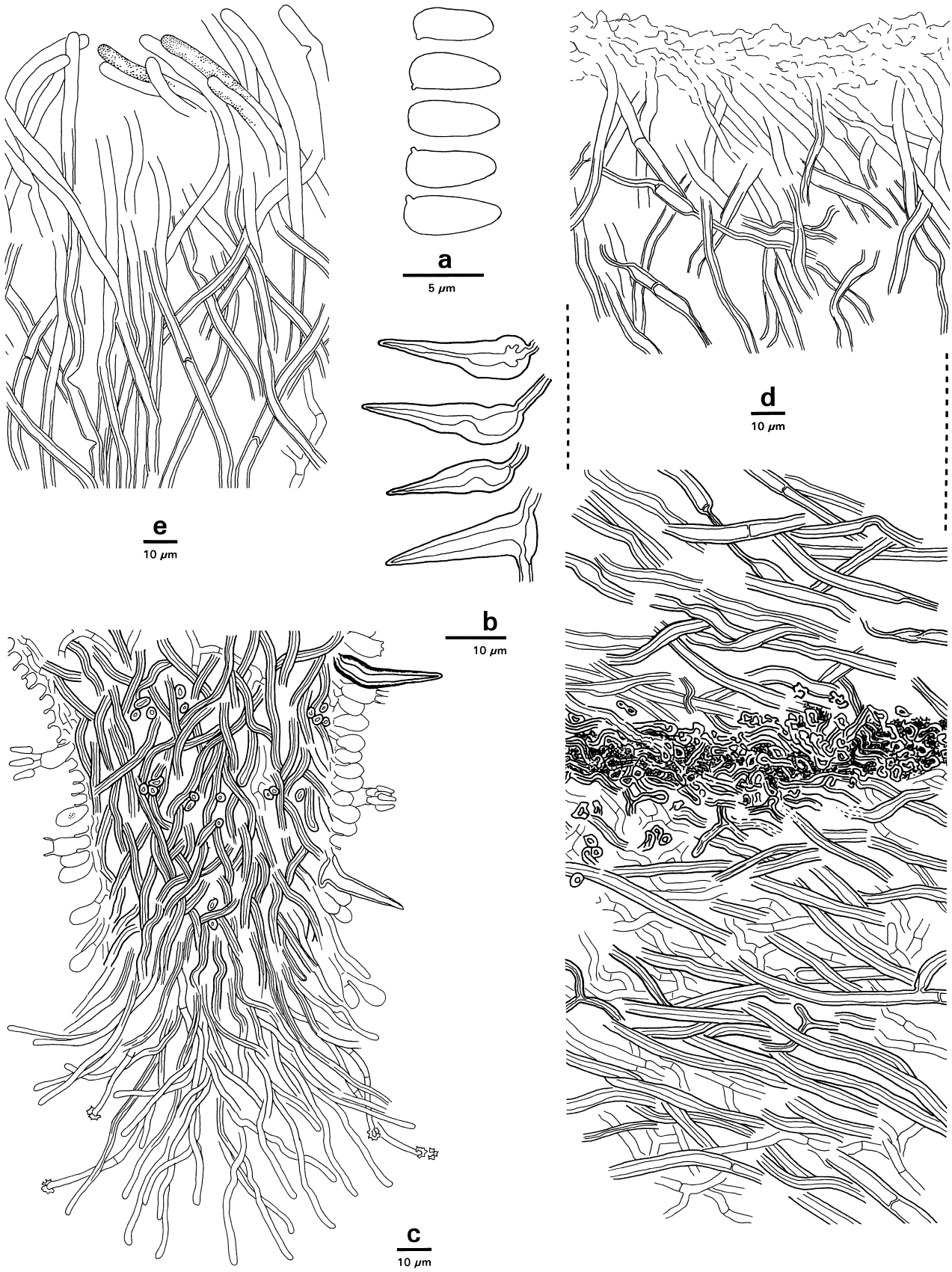

Fig. 1. *Phellopilus nigrolimitatus* Niemelä, Wagner & Fischer, specimens *Niemelä 6533 & Dai* (**a**–**d**) and *Renvall 376a* (**e**), drawn in Cotton Blue. — **a**: Spores; — **b**: Hymenial setae; — **c**: Dissepiment edge; — **d**: Vertical section through context up to the upper pellicle; — **e**: Surface of the basidiocarp of the spongy type.

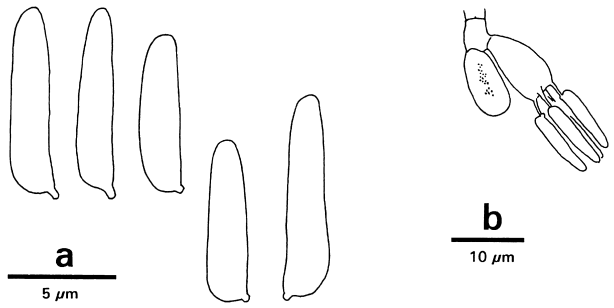

Fig. 2. *Phellopilus nigrolimitatus* Niemelä, Wagner & Fischer, specimen *Niemelä 499a*. — **a**: Exceptionally narrow and long spores, proterospores; — **b**: Basidium with attached spores.

(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, sometimes with a heel, usually terminal and regular, but a few pleural or double-pointed or otherwise irregular. Other kinds of setae absent; no cystidia; no cystidioles, but basidioles give soon rise to secondary hyphae which finally fill the old tubes. Collapsed old hymenium 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.

Notes on *Phellopilus 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 skeleto-binding hyphae, referring to the similar but hyaline elements of the genus *Polyporus*; the terminology of hyphal systems fits poorly to the Hymenochaetales. The septa in the skeletal hyphae are very distinct. The curious habit to produce wildly growing spongy context may also be characteristic of the new genus.

As to its microscopy, *Phellopilus* remains somewhere between the genera *Phellinus s. stricto* (the *P. igniarius* complex), *Phellinidium* (the *Phellinus ferrugineofuscus* complex) and *Fuscoporia* (the *Phellinus contiguus* complex), and perhaps also *Porodaedalea* (the *Phellinus pini* complex). Senescent hymenial cells collapse, but remain visible because of their glued-together bases in the so-called honeycomb structure, so characteristic of *Phellinus igniarius* and related species, but also *Phellinidium*. Setae are fairly similar to those of *Phellinus s. stricto*, although being more robust in *Phellopilus*. The pellicular cover of *Phellopilus* differs from the well-developed crust of *Phellinus igniarius* (*see* Niemelä 1975: fig. 20a) and its relatives. *Porodaedalea* is characterized by a trichoderm. Hyphal encrustations link the new genus with *Fuscoporia* and *Porodaedalea*, but in *Phellopilus* 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 *Phellopilus nigrolimitatus* is based on the type and other specimens from North and Central Europe. The lectotype 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 Poland (Małopolska prov., Babia Góra, *Picea abies*, 15.VIII.1973 *Niemelä 499a*, H) were so deviating that the specimen was thought to represent another species (Fig. 2), even though its other microscopy, macroscopy, and even the characteristic pocket rot agreed with *Phellopilus nigrolimitatus*. 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* =

Fig. 3. *Phellopilus nigrolimitatus* Niemelä, Wagner & Fischer, basidiocarp photographed *in situ*, specimen *Niemelä 6533 & Dai*, × 1.

30/1). 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*.

Specimens examined: **Finland**. Satakunta: Viljakkala, 1982 *Niemelä 2501* (H). Koillismaa: Taivalkoski, 1976 *Ohenoja 15* (OULU). Kittilän Lappi: Kittilä, 1999 *Niemelä 6533 & Dai* (H). Sompion Lappi: Savukoski, 1988 *Renvall 376a* (H). **Norway**. Nord-Trøndelag: Lierne, 1986 *Kotiranta 6064* (H). **Poland**. Podlaskie prov.: Białowieża, 1996 *Niemelä 5995* (H). **Sweden**. Uppland: Lidingö, 1913 *Romell* (*S 12500*). Stockholm, 1900 *Romell* (*S 12497*). Södermanland: Bedarö, 1905 *Romell* (*S 12499*, type), 1910 *Romell* (*S 13544, 13545*). Lule Lappmark: Gällivare, 1910 *Romell* (*S 13542*).

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, indextrinoideae, 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

Fig. 4. *Fuscoporia montana* Y.C. Dai & Niemelä, herbarium specimens, holotype (up and left) and *Zhang 34* (right), × 1.

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 (skeletals) 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.

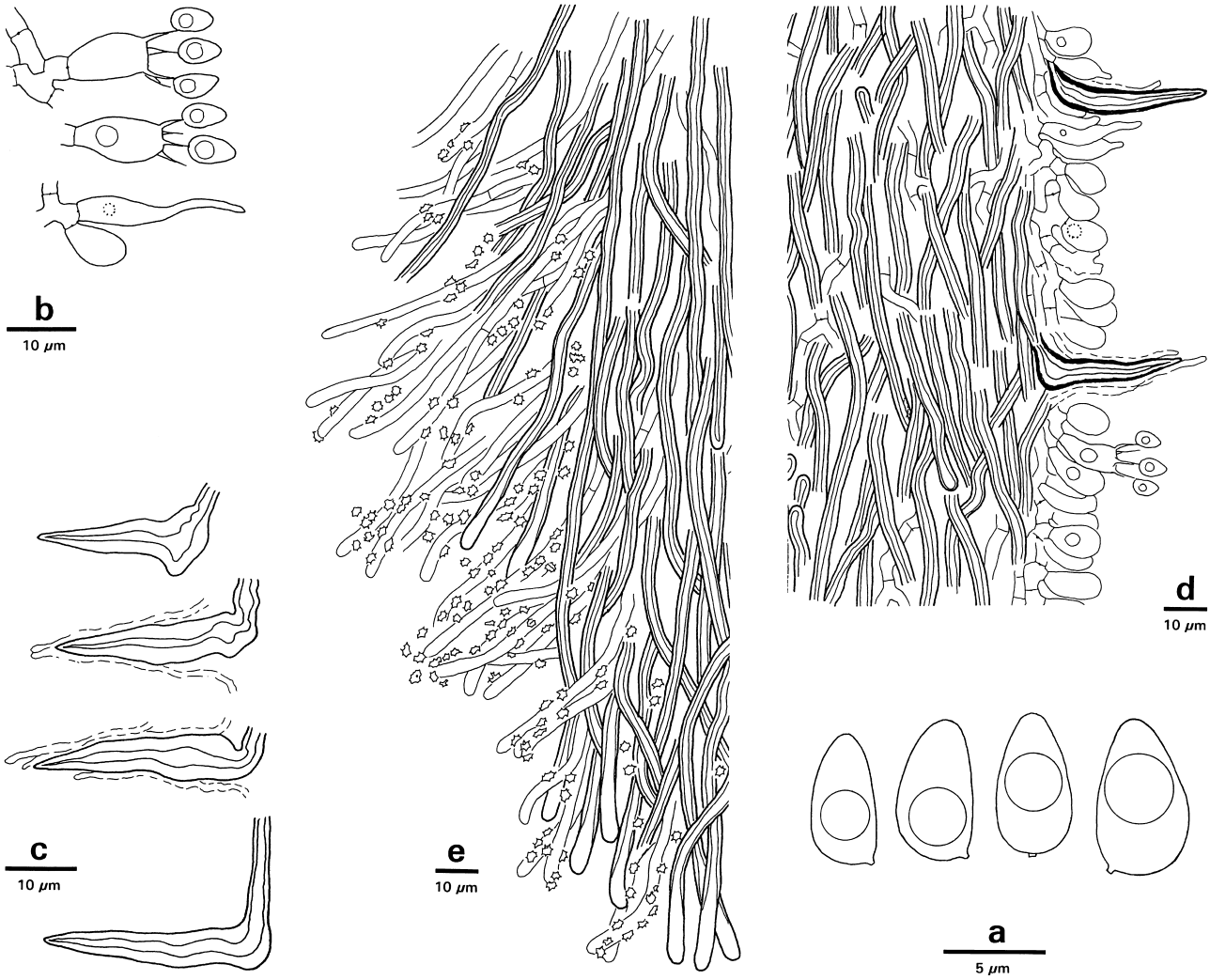

Fig. 5. *Fuscoporia montana* Y.C. Dai & Niemelä, drawn from the type in Cotton Blue. — **a**: Spores; — **b**: Basidia, a cystidiole and a basidiole; — **c**: Hymenial setae, some of them with accompanying hyphidia; — **d**: Vertical section through trama and hymenium; — **e**: Dissepiment edge with richly encrusted generative hyphae.

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, skeletals (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 *Phellopilus*; an appropriate genus is *Fuscoporia*. We refrain from making the combination, until more fertile material will be studied. Also *Poria cinnamomea* Rick, *Fomitiporella altocedronensis* Murrill and *Fomitiporia cinchonensis* Murrill (*Phellinus cinchonensis*) should be restudied before new nomenclatural changes are made.

Specimens examined: *Fuscoporia montana*: **China**. Yunnan: Hekou, III.1999 *Gao 25* (type); n.d. *Yang 1378*. Dali, Pianma, 10.III.1999 *Zhang 34*. All in H & SWFC.

Specimens examined: *Phellinus punctatiformis*: **U.S.A.** Florida: Cocoa, *Quercus*, 10.VI.1937 *Rhoads* (*BPI 0245344, 0241690*, isotypes), Highland, *Xanthocylum fagara*, 8.III.1937 *Shear 958* (*BPI 0241691*); same loc., 25.I.1944 *Shear* (*BPI 0241688*); Tallahassee, *Prunus caroliniana*, 29.VI.1952 *Rhoads* (*BPI 0241840*); Seminole, 25.X.1961 *Schallert F57* (*BPI 0241689*). **Brasil**. Rio Grande do Sul: Saõ Leopoldo, 1932 *Rick* (‘*Poria laevigata* Fr.’, *BPI 0241687*).

Molecular analysis

Beside *Phellopilus 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)
Fuscoporia ferruginosa and *F. contigua* (*Phellinus ferruginosus* group)
Phellinidium ferrugineofuscum (*Phellinus ferrugineofuscus* group)
Phylloporia 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 (*Phylloporia ribis*); the size of the fragment was 870 nucleotides for *Phellopilus 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) *Phellopilus nigrolimitatus* exhibits an isolated position. The molecular sequences of the three strains of *Phellopilus nigrolimitatus*, originating from Europe and East Asia, came out as nearly identical. *Phellinidium ferrugineofuscum* was positioned as next related to *Phellopilus nigrolimitatus*. The sister group to *Phellinidium* comprises the taxa *Fuscoporia 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 *Phylloporia ribis*.

Our sequence data show that *Phellopilus* 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, *Fuscoporia*. The putative relationship to *Fuscoporia* is confirmed by some microscopic features, which are presented in detail above.

As mentioned above, a possible differentiation within *Phellopilus 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

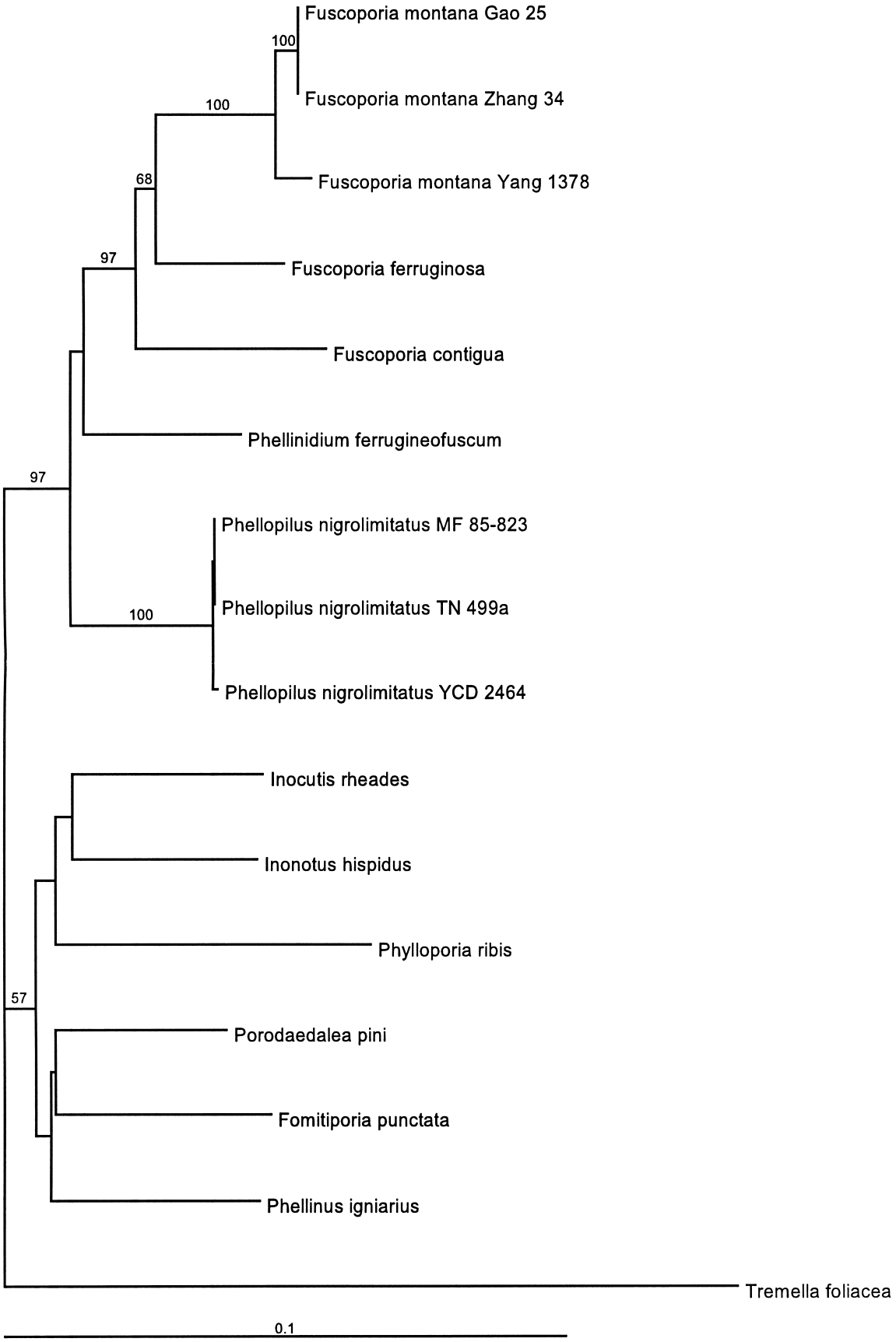

Fig. 6. Relationships between *Phellopilus nigrolimitatus* and related taxa inferred from 885 nucleotide sites of the large subunit of the nuclear ribosomal DNA using the neighbour-joining method. Support from 1000 bootstrap replications is indicated above the branches, values less than 50% are not noted.

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.

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