MYCOLOGICAL RESEARCH 111 (2007) 1366–1376

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/mycres

# The *Peniophorella praetermissa* species complex (*Basidiomycota*)

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ARTICLE INFO

*Article history:*
Received 13 October 2006
Received in revised form
22 July 2007
Accepted 1 October 2007
Published online 7 October 2007
*Corresponding Editor:*
Karl-Henrik Larsson

*Keywords:*
Cryptic species
*Hyphoderma*
Phylogeny
Phylogeography
Species concepts

ABSTRACT

The corticioid basidiomycete *Peniophorella praetermissa* has long been regarded as a morphologically variable species complex. An ITS-based phylogenetic study based on a worldwide sampling was carried out using parsimony and Bayesian inference. The resulting trees feature three major clades, further divided into well-supported subclades. These could be considered as distinct species, a contention that is further supported by crossing test data. Only two out of the eight phylogenetic lineages identified can be distinctly morphologically characterized: *P. odontiaeformis* and *P. subpraetermissa*. *P. odontiaeformis* is an odontioid species with a paleotropical distribution whereas the taxa in the remaining subclades have smooth basidiomata and are distributed in temperate areas. *P. subpraetermissa* is known only from the type collection and is distinguished microscopically by its reddish brown apically encrustated cystidia. Taxa in the remaining subclades are impossible to distinguish from each other morphologically, and therefore, are viewed as a species complex, *P. praetermissa s. lat.* One of the subclades, which is widely distributed but restricted to the Northern hemisphere, is proposed to represent *P. praetermissa s. str.* An epitype is selected from the same area as the holotype, among the specimens studied here. However, the geographically most widespread clade with many representatives from both hemispheres is here referred to as *P. pertenuis*, a taxon that was previously considered a synonym to *P. praetermissa*.



## Introduction

*Peniophorella praetermissa* (syn. *Hyphoderma praetermissum*) is a frequently collected wood-inhabiting, resupinate basidiomycete reported from all forested continents of the world. Its macroscopic appearance with a smooth, thin, whitish to cream-coloured basidiome makes the species inconspicuous and anonymous at a cursory glance (lat. *praetermissa* = overlooked), but its micro-structure contains several characteristic features helpful to species determination. The key diagnostics for *P. praetermissa* are broadly ellipsoid spores, lepto- and gloeocystidia, and so-called stephanocysts, which

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0953-7562/$ – see front matter 
doi:10.1016/j.mycres.2007.10.001

are globose cells involved in nematode trapping. An excellent description and illustrations of basidiome morphology of *P. praetermissa* are given in Eriksson & Ryvarden (1975: fig. 2). The species is known to show certain morphological variability and has been suspected to represent a species complex rather than a single species. This was first substantiated by Boidin (1950), who used crossing tests to demonstrate the presence of several distinct biological species within *P. praetermissa* s. lat. Similarly, Hallenberg *et al.* (1994) used protein electrophoresis, crossing tests data, and culture studies to distinguish and characterize groups within *P. praetermissa*, but no revisionary changes were undertaken.

Two species that have been proposed as closely related to *P. praetermissa* are *P. odontiaeformis* and *P. subpraetermissa* (Hjortstam & Larsson 1995; Larsson 2007). The former is similar to *P. praetermissa* in its micromorphology but has an odontioid hymenium; furthermore, it seems to be restricted to tropical and subtropical areas in the Old World. *P. subpraetermissa* differs by having cystidia with a reddish brown, amorphous, apical encrustation that does not dissolve in potassium hydroxide and that is visible as reddish dots under the lens. Stephanocysts are found more or less regularly in all three species, more easily so in cultured mycelia than in basidiomata (Hallenberg 1990). The genus *Peniophorella* was earlier considered a synonym to *Hyphoderma* but was recently reinstated to encompass some species formerly assigned to *Hyphoderma* (Larsson 2007). *Peniophorella* belongs to the hymenochaetoid clade, whereas *Hyphoderma* with its type species *H. setigerum* belongs to the phlebioid clade (Larsson *et al.* 2004).

Offering unparalleled resolution, DNA sequence data have become a popular information source for investigating speciation and biogeography in fungi (Petersen & Hughes 1999, 2007). Although many initiatives focus on agarics, an increasing number of studies on other fungi, including corticioids, are being published (e.g. Nilsson *et al.* 2003; Paulus *et al.* 2007). In general, these studies indicate that many species are widely distributed and that surprisingly few species are geographically restricted. Intra-continental dispersal within a suitable climatic range seems common, while the major oceans and climate zones may be effective barriers to dispersal. The extent to which the same species occur in the temperate areas of both northern and southern hemispheres is still largely unknown but probably low. A possible interpretation is that species with narrow geographical ranges have evolved more recently than those with intercontinental distributions. The ability to mate *in vitro* despite substantial geographical separation has been regarded as plesiomorphic, and such taxa are thought once to have had a broader continuous distribution. Such an area could have been split up by continental drift or more recent vegetation changes as a result of climate shifts (Hallenberg 1991; Hibbett *et al.* 1998; Petersen & Hughes 1999).

This study aims to further the understanding of the *P. praetermissa* species complex through phylogenetic analysis of 64 isolates of *P. praetermissa*, *P. odontiaeformis*, and *P. subpraetermissa*. Species limits – and their taxonomic implications – are given special attention; crossing tests and morphological comparisons are used as additional tools in these pursuits. The worldwide sampling of the specimens allows for questions on historical biogeography to be addressed.

## Materials and methods

### *Sampling*

The specimens were selected from the FCUG culture collection (http://www.systbot.gu.se/database/FCUG/FCUG.html) (Sweden), the National Museum of Natural Science of the Republic of China (Taiwan), and the Tottori Mycological Institute (Japan). Two additional cultures were provided by Gitta Langer and Mycotheque de l'Université catholique de Louvain (MUCL), one sequence was obtained from David Hibbett's laboratory, and two sequences were retrieved from GenBank. The cultures from 64 ingroup and seven outgroup specimens were used for DNA sequencing and, where applicable, crossing tests; their associated vouchers were used for morphological comparison. For the convenience of the reader, specimens are grouped in accordance with the results of the phylogenetic analyses (Fig 1, Table 1). The herbarium material used in the study is permanently preserved in the public herbaria GB and TMI and the cultures are stored in the culture collection FCUG.

### *DNA extraction, amplification, and sequencing*

As a source of DNA, single-spore mycelia were isolated, cultivated on malt agar plates (1.25 % malt extract), and subsequently placed in malt liquid solution (malt extract as above) for three weeks; polyspore mycelia were used in the absence of single-spore cultures. Mycelia were harvested and dried between sheets of sterile filter paper; approximately 2 mg D.W. of mycelium were used per specimen. DNA extraction was accomplished using the DNeasy® Plant Mini Kit (QIAGEN®, Hilden); during this and the following steps of the DNA preparation, purification, and sequencing, the recommendations of the respective manufacturer were followed.

The PCRs were carried out using Ready-To-Go® PCR Beads kits (Amersham Pharmacia Biotech, Uppsala), a Biometra TRIO-Thermoblock (Biometra, Göttingen), the PCR primers ITS1F and ITS4B, and the PCR set-up of Gardes & Bruns (1993). The PCR product was purified using the QIAquick® Spin procedure (QIAGEN®, Hilden) and the sequence reactions were conducted using 100 ng of template DNA and the CEQ 2000 Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter, Fullerton). Sequences were obtained using the CEQ 2000XL DNA Analysis System (Beckman Coulter, Fullerton) and edited in Sequencher© 4 (GeneCodes, Ann Arbor).

### *Alignment and phylogenetic analysis*

The sequences were aligned in MAFFT 5.731 (Katoh *et al.* 2005) and adjusted manually in Seaview (Galtier *et al.* 1996). Homology was deemed to be satisfactorily assessable for all characters. All analyses employed outgroup rooting: five specimens of *Peniophorella pubera* and two of *P. guttulifera* were employed as outgroup in light of their basal position relative to *P. praetermissa* (cf. Larsson *et al.* 2004).

Two separate phylogenetic analyses were performed. A heuristic parsimony search was set up in PAUP 4.0b10 (Swofford 2003) with 10K random addition sequence

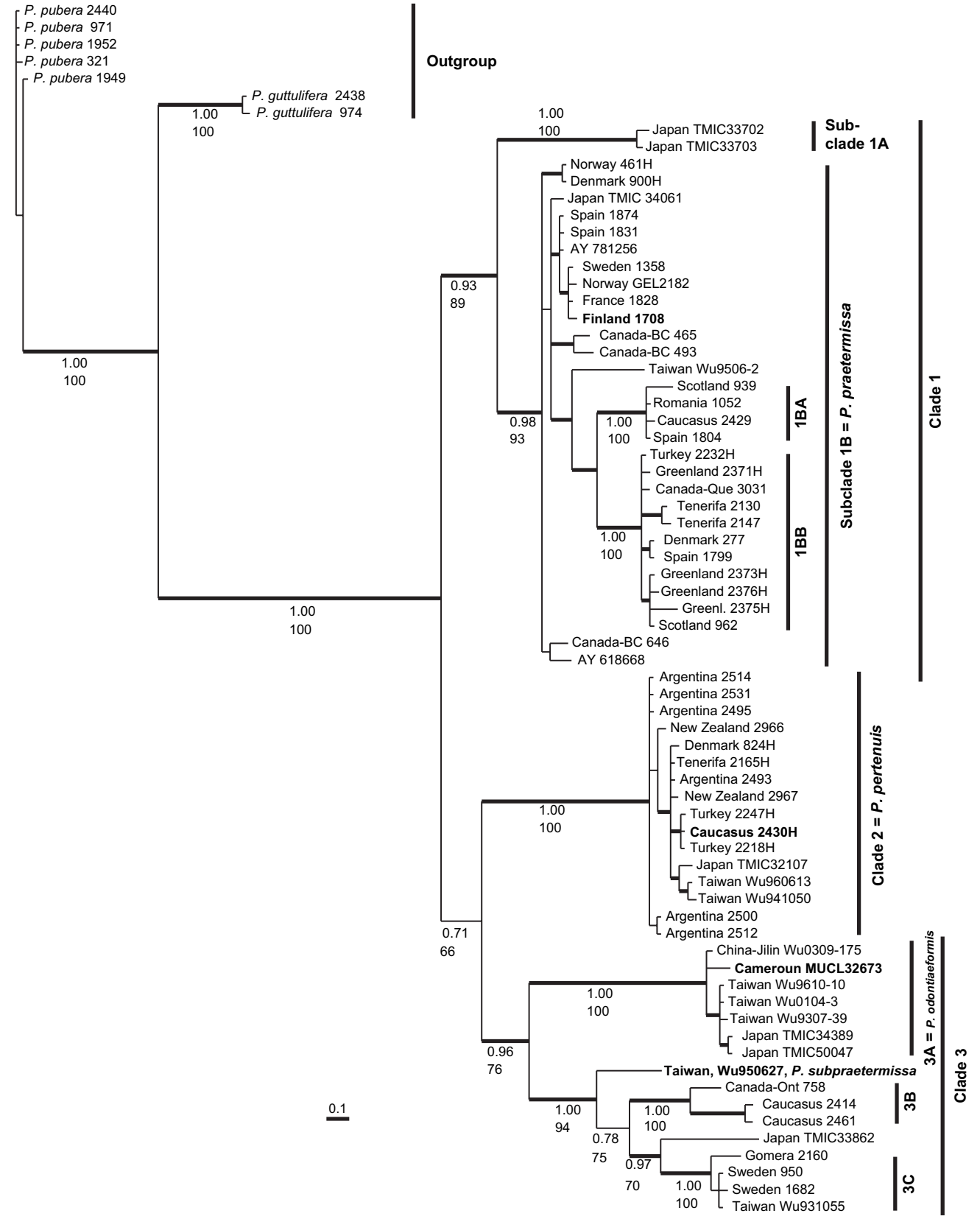


**Fig 1 – Phylogenetic relationships of representatives in the *Peniophorella praetermissa* complex from Bayesian analysis of 71 ITS sequences. PPs of higher value than 0.7 are given with corresponding jackknife values from the parsimony analysis below for major clades and subclades. All branches in bold indicate clades present in the strict concensus tree in the parsimony analysis. Taxa in bold represent holotypes or epitypes. *P. guttulifera* and *P. pubera* are used as outgroup for the parsimony analysis, *P. pubera* 2440 for the Bayesian analysis.**

replicates, each holding two trees per step. TBR branch swapping was employed, saving at most two trees per random sequence addition replicate. A strict consensus tree was retrieved from the resulting trees. Clade support was estimated in PAUP through a jackknife analysis, performing 25K replicates with 37 % deletion (Farris *et al.* 1996), each employing 50 rounds of random addition sequence with two trees held per step and TBR swapping with at most two trees saved per replicate.

For a Bayesian inference of phylogeny, MrModeltest 2.2 (Nylander 2005) was used to estimate separate best-fit models of evolution for the three markers ITS1, 5.8S, and ITS2. A heterogeneous Bayesian inference was set up in MrBayes 3.0B4 (Ronquist & Huelsenbeck 2003), with model parameters estimated separately for each data partition. Eight Metropolis-coupled MCMC (MCMCMC) chains with a temperature of 0.2 were initiated; these were run for 5 M generations with tree and parameter sampling every 4K generations (1250 trees). The initial burn-in was set to 20 % (250 trees). After establishing the fact that the analysis converged prior to the burn-in, a 50 % majority-rule consensus cladogram was computed from the remaining 1K trees; the proportions of this tree correspond to Bayesian PPs (BPP).

### *Crossing tests*

The crossing tests were restricted to specimens for which non-clamped, single-spore isolates were available. Several of the specimens used in this study were homothallic or available only as polyspore isolates and consequently left out of the crossing tests. Single-spore mycelia from different specimens were placed in pairs, 10–15 mm apart, on malt-extract agar (1.25 % malt extract) and left at room temperature for three weeks. From each specimen, two to four single-spore mycelia were used. Paired cultures were checked for clamp formation in three different regions: at the immediate contact zone, and on opposite sides of the inocula, some 20 mm from the respective inoculum. Plates with negative results were rechecked after three weeks. Di-mon tests were done to confirm intragroup compatibility when no single-spore cultures were available (see Table 2).

### *Morphological studies*

Studies of macro- and micro-morphology were carried out under dissection microscope (×12) and light microscope (×1250). Spore measurements were undertaken from spore prints when available, and from basidiomata in the remaining cases. For each specimen, 30 spores were measured for length and width.

## Results

### *Alignment and phylogenetic analyses*

After manual adjustment, the 71-taxon alignment consisted of 633 nucleotide characters, 421 of which were constant, and 30 of which were parsimony uninformative, leaving 182 (29 %) parsimony informative characters. The parsimony analysis returned 18,092 most parsimonious trees of 447 steps (CI = 0.6264, HI = 0.3736).

For the three separate ITS regions, MrModelTest (AIC) suggested HKY + G (ITS1 and ITS2) and SYM + I (5.8 S) as best-fit models; this information was transferred to MrBayes. Plotting the cold-chain likelihood values against the generation number revealed that stationarity was reached after approximately 80K generations. The chain mixing was found to be satisfactory. Fig 1 shows the 50 % majority-rule consensus phylogram from the Bayesian analysis, overlaid with support information from the parsimony analysis as applicable. The ingroup was found to be monophyletic with respect to the outgroup. The ingroup specimens fall into three major, geographically widespread clades. These clades are further divided into several well-supported subclades.

All files pertaining to the parsimony and Bayesian analyses are available for download at http://andromeda.botany.gu.se/praetermissum.zip (2 Mb).

### *Morphology*

According to the literature, *Peniophorella praetermissa* has 0.06 to 0.35 mm thick, whitish to cream-coloured basidiomata with a smooth hymenium; smooth, thin-walled spores that are narrowly ellipsoid to suballantoid with numerous oil-drops or grainy contents, and of 8–11 × 4–5 μm; and thin-walled cystidia of two types: (1) leptocystidia, capitate to cylindrical, 20–90 × 6–10 μm, apically widened, and frequently encrusted with crystals or amorphous material, sometimes projecting; and (2) gloeocystidia that are enclosed, usually fusiform and always tapering to the apex, with yellowish, homogeneous contents, 30–120 × 8–18 μm. In addition, another type of sterile element, stephanocysts, is usually found (10–20 × 10–13 μm). The basidia are subclavate and of 20–30 × 6–9 μm in size with four sterigmata and a basal clamp (Fig 2) (Eriksson & Ryvarden 1975; Lin & Chen 1990; Maekawa 1994).

*P. odontiaeformis* has resupinate basidiomata with a distinctly odontioid hymenium, 0.25–1 mm thick, aculei up to 0.5 mm, cream-coloured. Microscopically, it differs from *P. praetermissa* mainly by having only one kind of cystidia, which are widened at the base and have a long cylindrical neck or are apically tapering. Stephanocysts are abundant in basidiome tissue (Berthet & Biodin 1966; Lin & Chen 1990; Maekawa 1994). *P. odontiaeformis* was described from material from Cameroun, whereas the proposed synonym *H. mucronatum* is based on material from Japan. Material from both areas is included in this study and their synonymy is supported by ITS sequence data.

*P. subpraetermissa* is only known from the type specimen in Taiwan. It is easily distinguished from *P. praetermissa* by the reddish brown encrustation of amorphous material on apices of leptocystidia, and by somewhat smaller spores (Wu 1997).

Remaining subgroups are impossible to distinguish from each other morphologically, and the view of them as a species complex – *P. praetermissa s. lat.* – is best retained. Most morphological characters, such as spore size (Fig 3), were found to be variable with substantial overlap between clades, and no consistent characters of efficacious diagnostic value could be discerned. Other characters used in the diagnosis of

**Table 1 – Collection data and GenBank accession numbers of the specimens studied**

| Taxon Culture no.[a] | Locality | Substrate[b] | Voucher | GenBank accession no. |
|---|---|---|---|---|
| Clade 1A | | | | |
| *Peniophorella praetermissa s. lat.* | | | | |
| TMIC 33702 | Japan, Tokyo | Ang. branch | TMI 20528 | DQ647439 |
| TMIC 33703 | Japan, Tokyo | Ang. branch | TMI 20529 | DQ647440 |
| Clade 1B except clade 1BA, 1BB | | | | |
| *P. praetermissa s. str., pro parte* | | | | |
| FCUG 461H | Norway, S. Tröndelag | *Picea* | KHL 2835B | DQ647450 |
| FCUG 900H | Denmark, Jylland | *Picea* | NH 7717 | DQ647451 |
| FCUG **465** | Canada, B.C. | *Picea* | NH 6608 | DQ647448 |
| FCUG **493** | Canada, B.C. | *Picea* | NH 6719 | DQ647449 |
| FCUG 646 | Canada, B.C. | *Thuja* | NH 6851 | DQ647447 |
| | Canada, B.C. | Wood | | AY618668 |
| FCUG **1358** | Sweden, Dalsland | *Fagus* | NH 5170 | DQ647441 |
| | Sweden | Wood | | AY781256 |
| GEL 2182 | Norway, Akershus | *Betula* | | DQ647442 |
| FCUG **1708**, epitype | Finland, Pyhä Häki | *Betula* | NH 9536 | DQ647444 |
| FCUG **1828** | France, Pyrennées Orient. | *Pinus* | NH 10164 | DQ647443 |
| FCUG **1831** | Spain, Lerida | *Abies* | NH 9943 | DQ647446 |
| FCUG **1874** | Spain, Lerida | *Abies* | NH 9996 | DQ647445 |
| TMIC 34061 | Japan, Akita | Ang. wood | TMI 20953 | DQ647452 |
| Wu 9506-2 | Taiwan, altitude 2000 m | Ang. branch | | DQ647468 |
| Clade 1BA | | | | |
| *P. praetermissa s. str., pro parte* | | | | |
| FCUG **939** | Scotland, Perthshire | *Betula* | NH 7807 | DQ647464 |
| FCUG **1052** | Romania, Iasi | Ang. wood | NH 7987 | DQ647465 |
| FCUG **1804** | Spain, Huesca | *Fagus* | NH 9811 | DQ647467 |
| FCUG **2429** | Russia, the Caucasus | *Quercus* | NH 12268 | DQ647466 |
| Clade 1BB | | | | |
| *P. praetermissa s. str., pro parte* | | | | |
| FCUG **277** | Denmark, Jylland | Ang. wood. | NH 3494 | DQ647453 |
| FCUG **962** | Scotland, Perthshire | *Pinus* | NH 7827 | DQ647460 |
| FCUG **1799** | Spain, Huesca | *Fagus* | NH 9815 | DQ647454 |
| FCUG **2130** | Spain, Tenerifa | *Pinus* | NH 11192 | DQ647461 |
| FCUG **2147** | Spain, Tenerifa | *Pinus* | NH 10986 | DQ647462 |
| FCUG 2232H | Turkey, Trabzon | *Alnus* | NH 11451 | DQ647455 |
| FCUG 2371H | Greenland, Narssarssuaq | *Betula* | NH 11803 | DQ647456 |
| FCUG 2373H | Greenland, Narssarssuaq | *Betula* | NH 11941 | DQ647457 |
| FCUG 2375H | Greenland, Quinqua v. | *Betula* | NH 11919 | DQ647459 |
| FCUG 2376H | Greenland, Narssarssuaq | *Betula* | NH 11948 | DQ647458 |
| FCUG 3031H | Canada, Quebec | Ang. wood | AN 3031 | DQ647463 |
| Clade 2 | | | | |
| *P. pertenuis* | | | | |
| FCUG 824H | Denmark, Sjaelland | Ang. wood | NH 3868 | DQ647479 |
| FCUG 2165H | Spain, Tenerifa | *Erica* | NH 11063 | DQ647485 |
| FCUG 2218H | Turkey, Trabzon | *Corylus* | NH 11553 | DQ647483 |
| FCUG 2247H | Turkey, Trabzon | *Picea* | NH 11357 | DQ647481 |
| FCUG 2430H, epitype | Russia, the Caucasus | *Abies* | NH 12146 | DQ647482 |
| FCUG 2493 | Argentina, T. del Fuego | *Nothofagus* | NH 12429 | DQ647486 |
| FCUG 2495 | Argentina, T. del Fuego | *Nothofagus* | NH 12436 | DQ647492 |
| FCUG 2500 | Argentina, T. del Fuego | *Nothofagus* | NH 12490 | DQ647488 |
| FCUG 2512 | Argentina, T. del Fuego | *Nothofagus* | NH 12587 | DQ647489 |
| FCUG 2514 | Argentina, T. del Fuego | *Nothofagus* | NH 12591 | DQ647490 |
| FCUG **2531** | Argentina, Chubut | *Austrocedrus* | NH 12756 | DQ647491 |
| TMIC 32107 | Japan, Oita | *Elaeagnus* | TMI 16118 | DQ647484 |
| Wu 9606-13 | Taiwan, altitude 2650 m | Ang. branch | | DQ647477 |
| Wu 9410-50 | Taiwan, altitude 2100 m | Ang. branch | | DQ647478 |
| FCUG 2966 | New Zealand, Franz Josef | Podocarp | NH 15101 | DQ647480 |
| FCUG 2967 | New Zealand, Franz Josef | Podocarp | NH 15115 | DQ647487 |

**Table 1 – (*continued*)**

| Taxon Culture no.[a] | Locality | Substrate[b] | Voucher | GenBank accession no. |
|---|---|---|---|---|
| Clade 3A | | | | |
| *P. odontiaeformis* | | | | |
| TMIC 34389 | Japan, Haha-Jima Isl. | Ang. branch | TMI 21347 | DQ647496 |
| TMIC 50047 | Japan, Tottori | *Fagus* | TMI 6824 | DQ647500 |
| MUCL 32673 holotype | Cameroun | Branch | LY3562 | DQ647498 |
| Wu 0309-175 | China, Jilin | Ang. wood | | DQ647497 |
| Wu 9307-39 | Taiwan, altitude 700 m | Ang. branch | | DQ647499 |
| Wu 0104-3 | Taiwan, altitude 400 m | Ang. branch | | DQ647495 |
| Wu 9610-10 | Taiwan, altitude 700 m | Ang. branch | | DQ647494 |
| *P. subpraetermissa* holotype | | | | |
| Wu 950627 | Taiwan, altitude 2250 m | Ang. branch | Holotype | DQ647493 |
| Clade 3B | | | | |
| *P. praetermissa s. lat.* | | | | |
| FCUG 758 | Canada, Ontario | *Tsuga* | NH 7440 | DQ647471 |
| FCUG **2414** | Russia, the Caucasus | *Abies* | NH 12066 | DQ647469 |
| FCUG **2461** | Russia, the Caucasus | *Abies* | NH 12198 | DQ647470 |
| Clade 3C | | | | |
| *P. praetermissa s. lat.* | | | | |
| FCUG **950** | Sweden, Västergötland | *Ulmus* | KHL 4363 | DQ647472 |
| FCUG **1682** | Sweden, Västergötland | Ang. wood | NH 6283 | DQ647473 |
| FCUG **2160** | Spain, Gomera | Ang. wood | NH 11131 | DQ647474 |
| Wu 9310-55 | Taiwan, altitude 1950 m | Ang. branch | | DQ647475 |
| TMIC 33862 | Japan, Ogasawara Islands | *Calophyllum* | TMI 20754 | DQ647476 |
| **Outgroup taxa** | | | | |
| *P. guttulifera* | | | | |
| FCUG 974 | Scotland, Perthshire | *Betula* | NH 7813 | DQ647502 |
| FCUG 2438 | Russia, Krasnodar | *Pinus* | NH 12012 | DQ647501 |
| *P. pubera* | | | | |
| FCUG 321 | Sweden, Västmanland | Ang. wood | NH 3950 | DQ647506 |
| FCUG 971 | Sweden, Öland | Ang. wood | EL 4439 | DQ647503 |
| FCUG 1949 | Denmark, Jylland | *Picea* | NH 10380 | DQ647504 |
| FCUG 1952 | Denmark, Jylland | *Betula* | NH 10512 | DQ647505 |
| FCUG 2440 | Russia, Krasnodar | *Abies* | NH 12069 | DQ647507 |

Clades of the ingroup are listed and numbered in accordance with Fig 1.
a Culture numbers in bold were used for crossing tests. An ''H'' after the culture number indicates a homothallic strain.
b The substrate is specified to the extent known. The abbreviation ''Ang.'' refers to angiosperm.

*P. praetermissa s. lat.* are capitate leptocystidia and ventricose gloeocystidia with light-refracting contents. Their size variations are shown in Table 3. They are clearly of little value in delimitation of individual clades because of the large overlap. Usually, one of these two types of cystidia was found to be more abundant than the other. A formalized substructure of *P. praetermissa* complex is proposed here, mainly based on ITS phylogeny and crossing tests.

### *Species delimitation and taxonomy of the* Peniophorella praetermissa *species complex*

Three main clades are identified in the phylogenetic analysis (Fig 1) according to the following:

*Clade 1, subclade 1A*. This subclade is well-supported, but there are just two specimens originating from the same locality in Japan. At present, this clade is not recognized as a distinguishable distinct species, but is here included within the concept of *Peniophorella praetermissa s. lat.*

*Clade 1, subclade 1B*. *Peniophorella praetermissa sensu stricto*. This taxon is well-supported and widely distributed over the northern hemisphere. The holotype of *P. praetermissa* is in good condition, but the material is scanty and can not be used for sequencing. Therefore, an epitype originating from the same country as the holotype (Finland) is selected among the material sequenced for this study.

*Typi*: **Finland**: *Tavastia australis*: Tammela, Mustiala, *in ligno Alni incanae*, 15 Nov 1888, *PA Karsten* (Herb. Karsten 1247—holotype, H). **Finland**: Western Finland: Pyhä-Häkki National Park, on *Betula*, 21 Aug. 1986, *N. Hallenberg* (NH 9536—***epitypus hic designatus***; GB; FCUG 1708—cultura viva).

Even though the species is monophyletic in this ITS phylogeny it includes two well-supported internal clades (Fig 1). The representatives of these two groups are compatible within groups to a high extent. Between them incompatibility is likewise recorded to a high extent, although not completely (Table 2). Representatives from these two clades (1BA, 1BB)

**Table 2 – Summary of the crossing tests that were performed**

| Clade | Origin | FCUG culture | Clade 1B – (1BA + 1BB) – 465 | Clade 1B – (BA + 1BB) – 493 | Clade 1B – (1BA + 1BB) – 1358 | Clade 1B – (1BA + 1BB) – 1708 | Clade 1B – (1BA + 1BB) – 1828 | Clade 1B – (1BA + 1BB) – 1831 | Clade 1B – (1BA + 1BB) – 1874 | Clade 1BA – 939 |
|---|---|---|---|---|---|---|---|---|---|---|
| Clade 1B – (1BA + 1BB) | Canada-BC | 465-1,5 | | | | | | | | |
| Clade 1B – (1BA + 1BB) | Canada-BC | 493-1,2 | $+^{4}$ | | | | | | | |
| Clade 1B – (1BA + 1BB) | Sweden | 1358-1,2 | $+^{4}$ | | | | | | | |
| Clade 1B – (1BA + 1BB) | Finland | 1708-1,2 | | | | | | | | |
| Clade 1B – (1BA + 1BB) | France | 1828-1,2 | $+^{4}$ | $+^{4}$ | $+^{4}$ | $+^{4}$ | | | | |
| Clade 1B – (1BA + 1BB) | Spain | 1831-1,2,5 | $+^{4}$ | | | $+^{4}$ | $+^{4}$ | | | |
| Clade 1B – (1BA + 1BB) | Spain | 1874-1,2 | $+^{4}$ | | | | $+^{4}$ | $+^{4}$ | | |
| Clade 1BA | Scotland | 939-1,2 | $-^{4}$ | | | | $-^{4}$ | | | |
| Clade 1BA | Romania | 1052-1,2 | $-^{4}$ | | | $-^{4}$ | $-^{2}$ | $-^{4}$ | | $+^{8}$ |
| Clade 1BA | Spain | 1804-4,5,7 | $-^{2}$ | | | | $-^{4}$ | | | $+^{8}$ |
| Clade 1BA | Caucasus | 2429-1,2,5,6 | | | $-^{4}$ | | | $-^{4}$ | | $+^{8}$ |
| Clade 1BB | Denmark | 277-PS | | | | | | | | |
| Clade 1BB | Scotland | 962-PS | | | | | | | | |
| Clade 1BB | Spain | 1799-1,2,3,4 | | $-^{4}$ | $-^{4}$ | | $-^{4}$ | $-^{4}$ | $-^{4}$ | $+^{4}$ |
| Clade 1BB | Tenerifa | 2130-1,3 | $-^{4}$ | | | $-^{4}$ | | $-^{4}$ | | |
| Clade 1BB | Tenerifa | 2147-1,3,4 | | $-^{4}$ | $-^{4}$ | | $-^{4}$ | $-^{4}$ | $-^{4}$ | $-^{4}$ |
| Clade 2 | Argentina | 2531-2,4,5 | | | | $-^{4}$ | $-^{4}$ | | | |
| Clade 3B | Caucasus | 2414-1,5 | | | $-^{4}$ | $-^{2}$ | | | | |
| Clade 3B | Caucasus | 2461-1,2,3,4 | | | $-^{6}$ | $-^{2}$ | | | | |
| Clade 3C | Sweden | 950-1,2 | $-^{4}$ | | | | $-^{4}$ | $-^{2}$ | | |
| Clade 3C | Sweden | 1682-2,3 | $-^{4}$ | | | | $-^{4}$ | $-^{4}$ | | |
| Clade 3C | Gomera | 2160-2,3,4,5 | $-^{6}$ | $-^{4}$ | $-^{4}$ | $-^{6}$ | $-^{2}$ | $-^{6}$ | $-^{4}$ | $-^{4}$ |

differ from each other slightly in their mean spore sizes, whereas the spore sizes of the remaining parts of the species encompass the ranges of both (Fig 3).

*Clade 2*. This clade is well-supported and seems to represent a species of its own. For the abovementioned reasons a potential name for this taxon could be searched for among the taxonomic synonyms to *Peniophorella praetermissa*. The oldest available synonym seems to be *Corticium pertenue* and the new combination is made below.

There are only minor morphological differences in spore width between *Peniophorella praetermissa* and *P. pertenuis*: *P. pertenuis*: 4–4.5 μm, *P. praetermissa:* 4–5.5 μm. Still, this taxon deserves to be a species of its own because the studied representatives (1) belong to a strongly supported monophyletic clade, clearly distinguished from *P. praetermissa* in the ITS phylogeny, (2) feature ITS sequences of very low variability, and (3) are geographically widely distributed with specimens both from the northern and southern hemispheres.

*Clade 3*. This clade is divided into three well-supported subclades and two single specimens on long branches. Subclade 3A is *P. odontiaeformis*, which is morphologically easily distinguished from *P. praetermissa* by the odontioid hymenium and occurrence in subtropical–tropical areas (Cameroun, Japan, Taiwan). Subclades 3B and 3C are small well-supported clades, apparently widely distributed. One of the single specimens on a long branch is *P. subpraetermissa*, only known from the type collection. The species is easily recognized by its reddish brown encrustation of amorphous material on apices of leptocystidia, readily observed under the lens (×10 magnification). The subclades 3B, 3C, and the remaining single specimen on a long branch (TMIC 33862), are left without specific taxon names, but are referred to as *P. praetermissa s. lat.*

### *Crossing tests*

The species delimitation outlined above is supported by crossing test data (Table 2). However, within clade 2 in *Peniophorella praetermisssa*, one of the specimens (FCUG 1799) seems to lack incompatibility barriers towards representatives of clade 1. A similar situation with 'illegal' matings across the borders of biological species has earlier been reported in *Peniophora cinerea* and may indicate incomplete speciation (Hallenberg & Larsson 1992). Within subclade 1BA there are also several intragroup matings resulting in incompatibility. This could be a further indication of an incomplete lineage sorting between two closely related sister taxa.

## Taxonomy

**Peniophorella pertenuis** (P. Karst.) Hallenb. & R.H. Nilsson, **comb. nov**.

| Clade 1BA – 1052 | Clade 1BA – 1804 | Clade 1BA – 2429 | Clade 1BB – 277 | Clade 1BB – 962 | Clade 1BB – 1799 | Clade 1BB – 2130 | Clade 1BB – 2147 | Clade 2 – 2531 | Clade 3B – 2414 | Clade 3B – 2461 | Clade 3C – 950 | Clade 3C – 1682 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| +/− | | | | | | | | | | | | |
| $+^{4}$ | +/− | | | | | | | | | | | |
| $+^{8}$ | +/− | $+^{4}$ | | | | | | | | | | |
| | $-^{4}$ | $-^{4}$ | $+^{2}$ | $+^{2}$ | | | | | | | | |
| $-^{4}$ | $-^{4}$ | | | | | $+^{4}$ | | | | | | |
| | | $-^{12}$ | | | $-^{12}$ | $-^{4}$ | | | | | | |
| | $-^{2}$ | $-^{2}$ | | | $-^{4}$ | $-^{4}$ | | | | | | |
| | $-^{2}$ | $-^{4}$ | | | $-^{6}$ | $-^{6}$ | | $-^{12}$ | $+^{4}$ | | | |
| | $-^{4}$ | | | | $-^{4}$ | | $-^{4}$ | | | | | |
| | | $-^{4}$ | | | $-^{4}$ | | $-^{4}$ | | $-^{6}$ | $-^{6}$ | | |
| $-^{2}$ | $-^{4}$ | $-^{4}$ | | | $-^{2}$ | $-^{2}$ | $-^{4}$ | $-^{12}$ | $-^{4}$ | $-^{6}$ | $+^{4}$ | $+^{6}$ |

The number of tests are indicated for each combination (superscript). Under FCUG culture, the specific numbers for single spore cultures are given after the (''−'').

MycoBank no.: MB 510167
Basionym: *Corticium pertenue* P. Karst., *Hedwigia* **29**: 270 (1890).

*Typi:* **Finland:** *Tavastia australis*: Tammela, Mustiala, *in ligno Pini*, 2 Nov. 1890, *PA Karsten* (Herb. Karsten 1384—lectotype, H). **Russia:** *Krasnodar:* Mostovskoj, Umpyr, on a fallen log of *Abies*, 10 Sep. 1991, *N. Hallenberg* (NH 12146—***epitypus hic designatus***; GB; FCUG 2430 —cultura viva).

## Discussion

As mentioned in the introduction, *Peniophorella praetermissa* has been looked upon as an unresolved species complex for decades. Consequently, several names have been introduced for this taxon. *Corticium tenue* was a frequently used name until 40 y ago, but apparently with no type specimen preserved. In fact, the original description and illustration indicates a species different from *P. praetermissa* (Patouillard 1885, 1886). Other purported synonyms include *Corticium pertenue*, *C. flavicans*, *Peniophora albugo*, and *P. taxodii*. None of these names have been in use for the last 50 y (http://andromeda.botany.gu.se/cortbase.html), but *C. pertenue* is the oldest synonym. *Peniophorella pertenuis* together with a redefined *P. praetermissa s. str.* now seem to make up most of the *P. praetermissa* complex.

The results of the phylogenetic analyses leave little doubt that our understanding of evolution and speciation in the *P. praetermissa* species complex is far from complete. There is no key presented to the recognized taxa because keys are only relevant when distinguishing morphological or ecological characters are available. Still it makes sense to delimit important phylogenetic taxa within this complex to facilitate future research on biodiversity, biogeography, and speciation.

Both *P. pertenuis* and *P. praetermissa* are widely distributed. *P. pertenuis* is the only taxon in the ingroup found in the southern hemisphere, in addition to East Asia, Europe, and areas adjacent to Europe (Turkey, Caucasus, and the Canary Islands). *P. praetermissa* is abundant among samples from Europe, but it has also been recorded from East Asia and North America. Clade 1BB has representatives from western Europe, Turkey, Canary Islands, Greenland, and north-eastern parts of North America. Clade 1BA has a somewhat similar distribution, being found in Europe, Turkey, and the Caucasus. Clades 3B and 3C appear to be widespread on the northern hemisphere, though the number of specimens in this study is low. Clade 1A is entirely Japanese. Surprisingly, samples of the tropical–subtropical *P. odontiaeformis* are joined in a well-supported

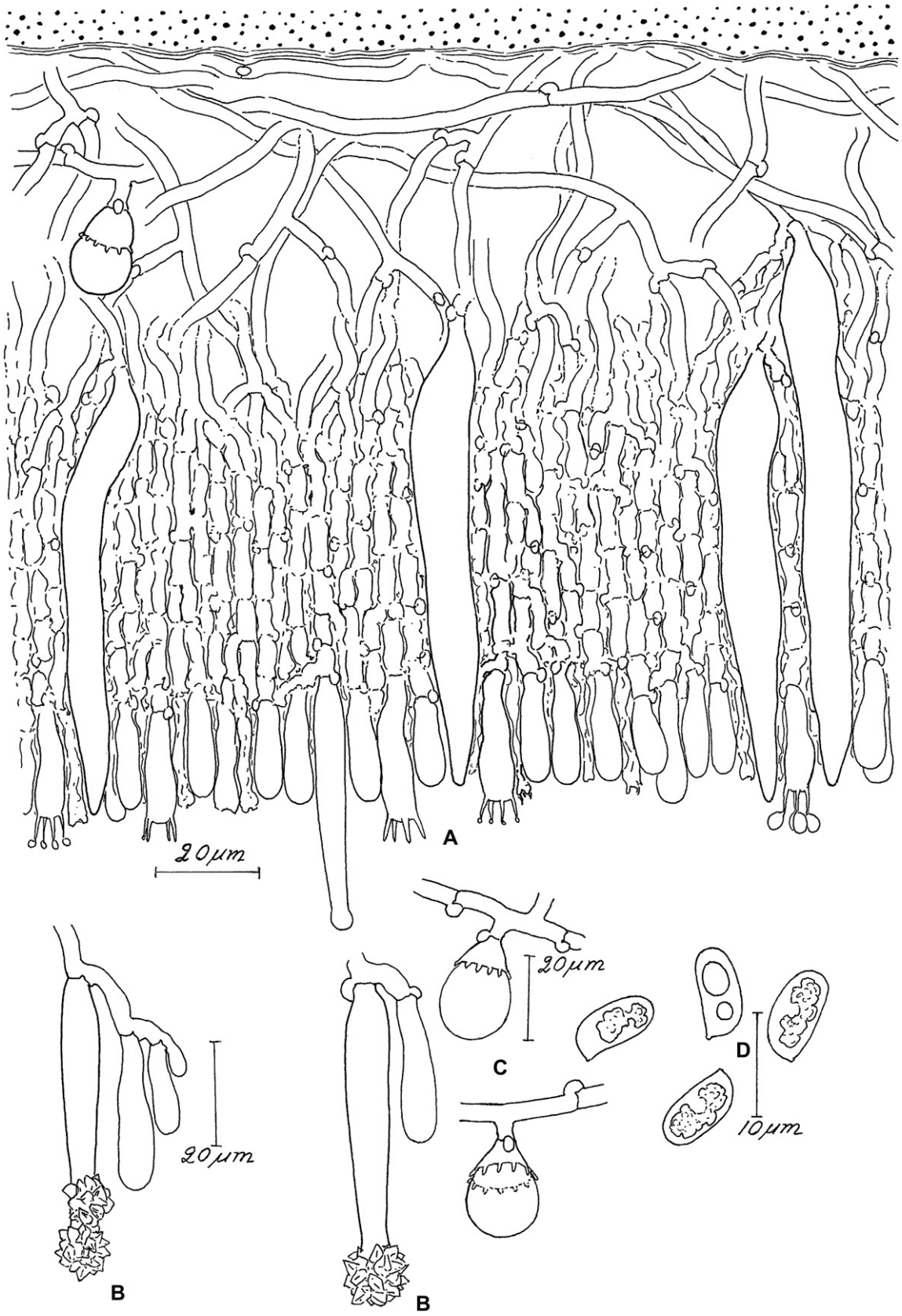


**Fig 2 – *Peniophorella praetermissa*. (A) Section through a fruit body; (B) capitate cystidia; (C) stephanocysts; (D) spores. – Coll. *Strid 7963* (from Eriksson & Ryvarden 1975, reproduced with permission from *Fungiflora*).**

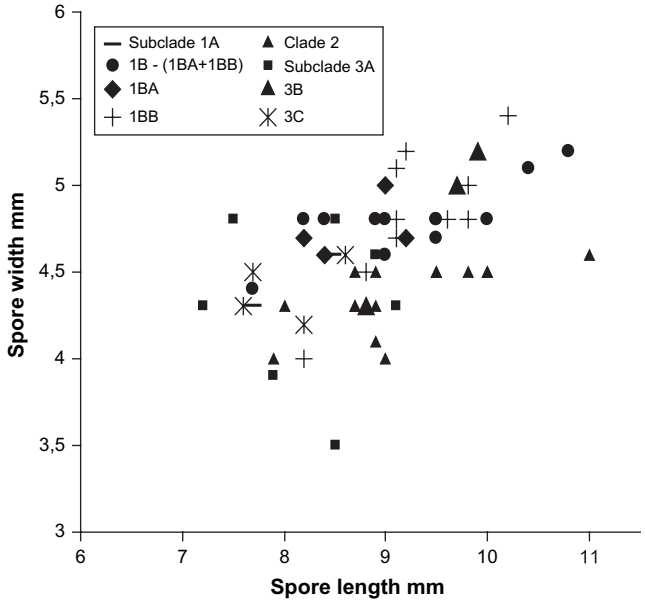


**Fig 3 – Spore sizes measured as average values for 30 spores per specimen. Symbols in the diagram refer to species or clades in the *Peniophorella praetermissa* complex.**

clade despite the great geographical distance between East Asia and the type locality of *Hyphoderma odontiaeforme* in Cameroun.

The wide geographical distribution of phylogenetically close specimens has interesting biogeographical implications. Biogeographical patterns for wood-inhabiting basidiomycetes have been commented upon in several studies. It has been shown that geographically delimited populations may often be distinguished from each other genetically and phylogenetically (Vilgalys & Sun 1994; Lickey *et al.* 2002; Nilsson *et al.* 2003). Contrastingly, disjunctions have been reported between East Asia and eastern North America in *Grifola frondosa* (Shen *et al.* 2002), between Europe–East Asia and eastern North America in *Pleurotus pulmonarius* (Vilgalys & Sun 1994), and between Europe, East Asia, and eastern North America in *Flammulina velutipes* (Methven *et al.* 2000). In *Panellus stypticus*, Jin *et al.* (2001) found close connections between Europe, East Asia, and western North America, thus indicating a coherent distribution from Europe to western North America, but with a clear distinction from eastern North American populations. Moreover, populations in Australia–New Zealand were clearly divergent from northern hemispherical populations. Minor but distinct differences in ITS sequences were found when comparing *Schizopora paradoxa* specimens from Europe, southern Argentina, and New Zealand–Australia (Paulus *et al.* 2000). Hibbett *et al.* (1998) showed that Australasian populations of *Lentinula* formed a distinct clade, but within it, several independent lineages could be delimited by geography.

An explanation to these apparently contrasting biogeographical patterns has been that geographically delimited groups represent ancient, endemic populations that have been separated for a considerable amount of time. According to Jin *et al.* (2001), extant fungal species may even have originated before tectonic separation of continents. Therefore, speciation by vicariance has been suggested in a few cases. *P. pertenuis* may well be of ancient origin because it is geographically widespread. However, even in that case, intermittent events with transoceanic dispersal could have taken place and dispersals between New Zealand and Australia are well documented (Paulus *et al.* 2000; Sanmartín & Ronquist 2004).

We conclude that *P. praetermissa* is indeed a species complex and should in its widest sense be taken to include numerous biological and phylogenetic species. Although it is easy to identify a specimen to *P. praetermissa s. lat.*, the lack of distinguishing morphological characters often precludes identification to species level without molecular methods. Although it is apparent that many more collections will be needed for a more detailed picture to emerge, the present study still shows that interesting biogeographical patterns may lay behind the phylogenetic hypothesis proposed. However, it is somewhat disappointing to note that over 70 specimens were not enough to fully resolve this species complex, and that many of its components will have to await more dense sampling, or perhaps additional molecular markers, before definite conclusions can be drawn. Contemporary mycology abounds with species complexes, and it seems clear that nothing but a worldwide sampling and collaboration will do to address them.

**Table 3 – Summary of size variation in characteristic microscopic details within the different clades of the *Peniophorella praetermissa* complex**

| Taxon\characters | Spore size (μm) | Capitate cystidia (μm) | Ventricose cystidia (μm) |
|---|---|---|---|
| Clade 1B – (1BA + 1BB) (12) | 7.5–11 × 4–5.5 | 38–72 × 3.5–12 | 36–105 × 6–13 |
| Clade 1BA (4) | 8–9.5 × 4.5–5 | 46–65 × 6–9 | 43–110 × 7–13 |
| Clade 1BB (10) | 8–10 × 4–5 | 29–120 × 5–14 | 29–130 × 6–13 |
| Clade 2 (15) | 8–11 × 4–5 | 35–95 × 6–13 | 38–90 × 6–12 |
| Clade 1A (2) | 7.5–9 × 4–5 | 29–55 × 4.5–5 | 43–72 × 7–11 |
| Clade 3B (3) | 8.5–10 × 4–5.5 | 34–58 × 6–9.5 | 36–130 × 5–12 |
| Clade 3C (4) | 7.5–9 × 4–5 | 38–96 × 5–11 | 36–84 × 7–12 |

For spore sizes, the mean value for each specimen is used for calculations. Number of specimens, upon which the measurements are based, is given in parenthesis.

## Acknowledgements

We are much indebted to Vivian Aldén for technical assistance. This study was financially supported through research grants to N.H. and R.H.N. from the Vidfelt foundation and to N.H. from the Royal Academy of Arts and Sciences in Göteborg, both of which are gratefully acknowledged.

## Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.mycres.2007.10.001.

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