Mycol. Res. **100** (2): 179–187 (1996) *Printed in Great Britain*

Phylogenetic studies in *Peniophora*

NILS HALLENBERG, ELLEN LARSSON AND MARGIT MAHLAPUU*

Department of Systematic Botany, Carl Skottsbergs gata 22, S-413 19 Göteborg, Sweden

* *Institute of Zoology and Botany, 21 Vanemuise St., EE-2400 Tartu, Estonia*

Phylogenetic relationships in *Peniophora* were investigated using ITS sequences from nuclear rDNA. The result was compared with parsimony analysis based on morphological characters, and a traditional classification based on basidiome morphology. Sixty-four specimens belonging to 24 species in *Peniophora* were sequenced. In particular, ITS2 sequences gave a good resolution in phylogenetic analyses and 3 distinct clades were distinguished. Intraspecific variation was constantly low in ITS2, and rarely exceeded 2%. In ITS1, the variation in certain species was considerable and hence most confidence was placed on the results from ITS2. Intercompatibility tests were done for the representatives of each taxon. In some species, intersterility had been found between representatives regarded as conspecific on morphological criteria. Such sibling species were not separated from each other in ITS2 sequences.

Peniophora Cooke is a well known genus of corticioid, wood-inhabiting basidiomycetes, comprising about 60 species. Most of them are found on branches and similar xerophytic habitats, and cause a white rot. Some of the species have been recorded worldwide. Like many other primary occupiers of wood, most *Peniophora* species show a strong selectivity for certain host species. The genus, identified by a number of morphological characters, has been regarded as natural. The culture characters have also been investigated and support a close relationship between the species (Stalpers, 1978; Boidin & Lanquetin, 1984, 1990; Nakasone, 1990). Several infrageneric subdivisions have been proposed (Parmasto, 1968; Boidin & Lanquetin, 1974; Eriksson, Hjortstam & Ryvarden, 1978; (Boidin, 1994). These have not, however, been focused on phylogenetic relationships explicitly but were aimed to be a practical classification system based on distinct characters (Table 1). For phylogenetic considerations, the value of certain prominent characters has probably been overestimated and possibilities for parallelisms neglected. One of the reasons for performing phylogenetic analyses based on molecular data, was to evaluate this situation more closely.

The biological species concept has been found to be particularly useful in *Peniophora*. This began with Eriksson (1950), who was the first to use crossing tests in taxonomy of Corticiaceae. *Peniophora* species are easily cultured and many studies have been carried out since. Moreover, in most cases there are distinct delimitations between different species in interspecific pairings. Some species in *Peniophora* seem to be

Table 1. Comparison between infrageneric arrangement proposed by Boidin (1994) and phylogenetic grouping found in this study. Only those species which have been treated in this study are mentioned. *P. manshurica* was not present in Boidin's treatment, but is morphologically very close to *P. quercina*

Subgenus	Group 1	Group 2	Group 3	Group 4
Peniophora	*P. quercina*	*P. cinerea*	—	*P. pini*
	P. simulans	*P. nuda*	—	*P. rufa*
	P. manshurica	*P. pseudonuda*	—	—
	P. piceae	*P. violaceolivida*	—	—
	P. rufomarginata	—	—	—
	P. limitata	—	—	—
	P. pilatiana	—	—	—
	P. pithya	—	—	—
Cristodendrella	—	*P. lycii*	—	*P. meridionalis*
Cryptochaete	—	—	—	*P. polygonia*
Duportella	—	—	—	*D. tristicula*
Gloeopeniophora	—	*P. laeta*	*P. aurantiaca*	*P. versicolor*
—	—	—	*P. erikssonii*	*P. proxima*
—	—	—	*P. laurentii*	—
—	—	—	*P. incarnata*	—

very closely related, in basidiome morphology only differing in a single character but still, they are separated by sterility barriers. In such cases, host preferences have been accepted as good additional characters in distinguishing species from each other. Sibling complexes have also been detected, with no morphological differences between the intersterile groups. Hypotheses have been proposed that sterility barriers cannot always be looked upon as species delimiting criteria (Hallenberg, 1991*a*, *b*; Hallenberg & Larsson, 1992; Hallenberg, Larsson & Larsson, 1994). Testing this hypothesis with molecular data was another object of this study.

The species and culture selection used in this study falls back on results from research carried out earlier at the institute in Göteborg, on speciation and species complexes in *Peniophora* (Hallenberg, 1984; Hallenberg, 1986; Hallenberg, 1988; Hallenberg & Larsson, 1991; Hallenberg & Larsson, 1992). Some cultures, regarded to be of importance for the study, have been obtained from the culture collections in Tartu and Villeurbanne. In this primary study it has not been the intention to cover the great diversity of *Peniophora* species which indeed are available as cultures elsewhere. Altogether, 64 specimens representing 24 species have been sequenced (ITS1 and ITS2 region, nuclear rDNA).

Table 3. Datamatrix based on 18 morphological characters for 23 species of *Peniophora*, 1 species of *Duportella*. Outgroup: *Amylostereum chailletii*

	Characters:																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P. quercina	0	1	0	0	1	1	0	1	0	1	1	1	1	0	1	0	1	1
P. simulans	0	1	0	0	1	1	0	1	0	1	1	1	1	0	1	0	1	1
P. manshurica	0	1	0	0	1	1	0	1	0	1	1	1	1	0	1	0	1	1
P. piceae	0	1	0	0	1	1	0	1	0	1	1	1	1	0	1	0	1	1
P. rufomarginata	0	1	0	0	1	1	0	1	0	1	1	1	1	0	1	0	1	1
P. limitata	0	1	0	0	1	1	0	1	0	1	0	1	1	0	1	0	1	1
P. pilatiana	0	1	0	0	1	1	0	1	0	1	0	1	1	0	1	0	1	1
P. pithya	0	1	0	0	1	1	0	1	0	1	0	0	1	0	1	0	1	1
P. pseudonuda	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	1	1
P. laeta	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	1
P. cinerea	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	0	1	1
P. nuda	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	0	1	1
P. lycii	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	1	1	1
P. violaceolivida	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	0	1	1
P. aurantiaca	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
P. erikssonii	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
P. laurentii	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1
P. incarnata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
P. pini	1	0	0	0	1	1	0	0	1	1	1	0	1	1	0	0	1	1
P. polygonia	1	0	0	0	1	1	0	0	1	0	1	0	1	0	0	1	1	1
P. rufa	1	0	0	0	1	1	0	0	1	1	1	0	1	1	0	0	1	1
P. meridionalis	0	0	0	0	0	1	1	0	0	1	0	1	1	0	1	1	1	1
P. versicolor	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	1
P. proxima	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1
Dup. tristicula	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	0	1	1
Amyl. chailletii	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	1	1

MATERIALS AND METHODS

Mycelia arising for single spores (SS) and polyspores were isolated after spore dispersal on malt extract agar (MEA). Crossing tests were performed between specimens of the same species, as determined by morphological criteria, to identify sibling species. For details of procedure, see Hallenberg (1988).

Secondary mycelia, obtained from polyspore cultures, were grown at room temperature for 2 wk in 50 ml MYG liquid media (1% malt-extract, 0·4% yeast-extract, 1% glucose) for DNA isolation. After harvesting, mycelia were easily dried between filter paper, put into eppendorff-tubes and directly

Table 2. Characters used for parsimony analysis based on basidiome morphology, of 24 species of *Peniophora*, using *Amylostereum chailletii* as outgroup

Characters	Character states
1. Fruitbody:	0 = effused, 1 = wart-like
2. Attachment:	0 = adnate, 1 = broadly attached but, loosening at the margin
3. Hyphal system:	0 = monomitic, 1 = dimitic
4. Clamps:	0 = present, 1 = absent
5. Basal layer:	0 = thin, 1 = thick, conspicuous
Basal hyphae (6–9):	
6.	0 = only undifferentiated hyphae present, 1 = differentiated hyphae present
7.	0 = densely packed, pseudoparenchymatous hyphae absent, 1 = such hyphae present
8.	0 = thick-walled hyphae forming a distinct layer of parallel hyphae absent, 1 = such hyphae present
9.	0 = irregular hyphae with walls swelling in KOH absent, 1 = such hyphae present
10. Pigmentation:	0 = subhyaline, 1 = brown
11. Interhyphal gelatinous matrix in basal layer:	0 = absent, 1 = present
12. Sulphocystidia	0 = present, 1 = absent
13. Encrusted cystidia rooted, > 8 µm wide:	0 = present, 1 = absent
14. Encrusted cystidia, < 8 µm wide:	0 = absent, 1 = present
15. Metuloids:	0 = absent, 1 = present
16. Dendrohyphidia:	0 = absent, 1 = present
17. Basidia:	0 = wider than 10 µm, 1 = more narrow
18. Spores:	0 = > 8 µm wide, 1 = < 8 µm wide

Table 4. List of isolates used.

Group 1

Peniphora quercina (Pers.: Fr.) Cooke ssp. *quercina*: 1472/deciduous wood/Romania, Iasi. 2554/*Quercus robur*/Estonia, Tartu. 2548/*Quercus robur*/ Checheno-Ingushetia (N. Caucasus).
P. quercina (Pers.: Fr.) Cooke ssp. *caucasica* Parmasto & I. Parmasto: 2388/*Quercus*/Russia, Krasnodar. 2546/*Quercus longipes*/Aserbaijan. 2547/*Quercus longipes*/Aserbaijan. 2550/*Quercus longipes*/Aserbaijan.
P. manshurica Parmasto: 2541/*Quercus mongolica*/Russia, Khabarovsk. 2452/*Quercus mongolica*/Russia, Primorsk.
P. simulans D. A. Reid: LY 5152/*Fagus*/France, Corsica.
P. rufomarginata (Pers.) Litsch.: 2543/*Tilia*/Moldova, Kodry Nature Reserve. 1181/*Tilia*/Norway, Rogaland. 1473/*Tilia*/Romania, Iasi.
P. rufomarginata deviating (Hallenberg, 1991): 2110/deciduous wood/Spain, Tenerifa (Canary Islands).
P. piceae (Pers.) J. Erikss.: 1239/*Picea*/Norway, Oppland. 2306/*Picea*/Norway, Akerhus. 1822/*Abies*/Spain, Lerida.
P. limitata (Fr.) Cooke: 1167/*Fraxinus*/Sweden, Bohuslän. 1481/*Fraxinus*/Romania, Iasi. 2553/*Fraxinus excelsior*/Checheno-Ingushetia (N. Caucasus).
P. pilatiana Pouzar & Svrček: LY 9737/*Pistacia lentiscus*/France, Corsica.
P. pithya (Pers.) J. Erikss.: 2226/*Picea*/Turkey, Trabzon.

Group 2

P. lycii (Pers.) Höhn. & Litsch.: 1807/deciduous wood/Spain, Lerida. 2387/*Cornus*/Russia, Krasnodar.
P. violaceolivida (Sommerf.) Massee: 1808/deciduous wood/France, Pyrénées Orientale. 2316/*Salix*/Russia, S. Ural.
P. cinerea (Pers.: Fr.) Cooke ssp. *cinerea*: 1173/*Picea*/Sweden, Bohuslän. 1810/*Betula*/France, Pyrénées Orientale. 2026/*Quercus*/Denmark, Jutland. 2182/deciduous wood/Taiwan. 2330/*Tilia*/Canada, Ontario. 2331/*Prunus*/Canada, Ontario.
P. cinerea (Pers.: Fr.) Cooke ssp. *fagicola* Hallenb. & E. Larsson: 1788/*Fagus*/Spain, Huesca. 1910/*Fagus*/Denmark, Jutland.
P. nuda (Fr.) Bres.: 1809/*Betula*/France, Pyrenees Orientale. 1967/*Fagus*/Sweden, Halland.
P. nuda, deviating (Hallenberg, 1991): 2193/deciduous wood/Turkey, Trabzon.
P. pseudonuda Hallenb.: 2384/deciduous wood/Russia, Krasnodar. 2390/*Carpinus*/Russia, Krasnodar.
P. laeta (Fr.) Donk: 1475/*Carpinus*/Romania, Cluj. 1905/*Carpinus*/Sweden, Öland.

Group 3

P. aurantiaca (Bres.) Höhn. & Litsch.: 0682/*Alnus*/Canada, B.C. 1991/*Alnus*/Denmark, Greenland.
P. erikssonii Boidin: 1690/*Alnus*/Finland, North Häme.
P. laurentii (S. Lundell) Nann.: 2321/*Betula*/Russia, S. Ural.
P. incarnata (Pers.: Fr.) P. Karst.: 1689/*Populus*/Finland, North Häme. 1909/*Corylus*/Denmark, Jutland. 2349/*Salix*/Denmark, Greenland. 2494/*Nothofagus*/Argentina, Tierra del Fuego. 2498/*Nothofagus*/Argentina, Tierra del Fuego. 2519/*Nothofagus*/Argentina, Chubut.

Group 4

Duportella tristicula (Berk. & Broome) Reinking: LY 12609/Reunion. LY 12653/Reunion.
P. proxima Bres.: 1795/*Buxus*/Spain, Lerida.
P. meridionalis Boidin: 2116/deciduous wood/Spain, Gomera (Canary Islands). 2336/*Quercus*/France. 2441/dedicuous wood/Spain, Salamanca.
P. versicolor (Bres.) Sacc. & Syd.: 1156/*Pinus*/Spain, Gran Canaria (Canary Islands). 2108/*Persea*/Spain, Tenerifa (Canary Islands). 2115/*Erica*/Spain, Gomera (Canary Islands).
P. pini (Schleich.: Fr.) Boidin: 2399/*Pinus*/Russia, Krasnodar.
P. polygonia (Pers.: Fr.) Bourd. & Galz.: 1903/*Populus*/Sweden, Västergötland. 2315/*Salix*/Russia, S. Ural.
P. rufa (Pers.: Fr.) Boidin: 2402/*Populus*/Russia, Krasnodar.

Outgroup

Amylostereum chailletii (Pers.: Fr.) Boidin: 2025/*Abies*/Denmark, Jutland.

mixed with lysis buffer (Lee & Taylor, 1990). The samples were homogenized with a pellet mixer. Chloroform extract was done three times, and DNA precipitated with 0·5 vol. ammonium acetate and 0·6 vol. isopropanol. A portion of nuclear rDNA (*ca* 800 bp) was amplified, using the primers ITS1F and ITS4B (Gardes & Bruns, 1993). This portion includes the internal transcribed spacer regions, ITS1 and ITS2. PCR conditions and temperature cycling were according to Gardes & Bruns (1993). Amplified fragments were cleaned with Magic PCR Preps (Promega), according to the manufacturer's instructions.

DNA sequencing was performed using a cycle sequencing reagent kit (fmol DNA Sequencing System, Promega), with α-^{35} dATP and α-^{35}S dTTP. Sequence primers were ITS1F and ITS3 (White *et al.*, 1990).

The DNA was separated in polyacrylamide gels (6% acrylamide; 8 M urea) *ca* 3 h at 2100 V. Sequences were aligned using CLUSTALV (Higgins, Bleasby & Fuchs, 1992) but could as well be done by eye with the same result. The aligned sequences were entered into PAUP, version 3.1.1 (Swofford, 1993). ITS1 and ITS2 sequences were analysed separately, omitting the flanking regions. Because no large insertions were present, gaps were treated as a fifth character. Parsimony analyses were performed using outgroup rooting and the heuristic search option. Support for phylogenetic groups was assessed by bootstrap analyses using 100 data sets. All sequences are available upon request from the senior author.

Characters for parsimony analysis based on basidiome morphology were obtained from detailed study of herbarium specimens (see 'list of species', below) and from Eriksson *et al.* (1978). Character list and data matrix are given in Tables 2 and 3. The heuristic search was used here. No constraints were introduced, and all characters were treated as unordered.

List of species

The specimens and isolates of *Peniophora* species shown in Table 4 were used for molecular analyses, intercompatibility tests, and morphological studies. Information concerning the studied specimens is given in the following order: species/culture number (FCUG if not stated otherwise)/ substrate/locality. Species are arranged into phylogenetic groups found in the present analysis.

RESULTS

Nucleotide sequences for the internal transcribed spacer regions ITS1 and ITS2 (rDNA) were obtained for 56 and 64 specimens of *Peniophora*, respectively. A high variation in ITS1 sequences were found for some species, resulting in a tree where such species where distinctly separated from others with a supposed close relationship, to judge from morphological data (see 'ITS1 sequencing', below). Consequently, additional cultures acquired during the progress of the study were only sequenced in the ITS2 region. Almost all parsimony analyses presented here were carried out on ITS2 data only. Intraspecific pairing tests were performed in advance to evaluate the biological species concept as compared with sequence data.

Sequencing was done for one strand only but in some cases the opposite strand was sequenced as a control. No ambiguities were found. The ITS1 region started 47 positions from the 5′ end of primer ITS1F and was found to be 191–199 bp long. The ITS2 region followed after 111 bp from 5′ end of primer ITS3 and varied between 191–196 bp. For ITS1, the number of informative characters (sites) was 51, while 74 were variable. For ITS2, 70 informative characters and 80 variable were found.

The choice of outgroup became problematic for ITS2 analyses, because only alignment of closely related species seemed to be possible. *Duportella tristicula* was selected first, as it is well separated from *Peniophora* in basidiome morphology, but still close enough. Among more distantly related taxa *Amylostereum chailletii* was found to have an ITS2 sequence which also could be aligned. It deviated by being only 170 bp long, due to a few deletions (up to 5 bp). Using *A. chailletii* as outgroup resulted in a consensus tree which had a topology identical to the tree with *D. tristicula* as outgroup, except for the internal arrangement within group 4. When *A. chailletii* and *D. tristicula* together were used as outgroup, the resulting consensus tree was identical to that with *A. chailletii* alone.

Gaps were treated as 'fifth base' to increase the number of informative sites. Except for *A. chailletii*, gaps were only single or double.

Results from crossing tests show that sibling complexes are present in a number of species. Such sibling complexes are particularly indicated in Figs 2–6. In all other cases where a species is represented by more than one specimen, intraspecific compatibility was recorded.

ITS1 sequencing

To begin with, it was not possible to align *A. chailletii* at all, and *P. pini* was chosen as outgroup. Phylogenetic analysis of the whole dataset gave 64 most parsimonious trees with length = 189 and consistency index = 0·450. Specimens of group 1 and parts of group 2 were resolved in a rather similar way as for ITS2 sequences, while the other representatives were unresolved. Intraspecific variation was considerable for some species, as was the separation of a few taxa which are closely related according to their morphology. *P. laurentii* was linked to *P. pseudonuda* and *P. proxima*, *P. meridionalis* and *P. polygonia* were included in group 1, in *P. cinerea* ssp. *fagicola* FCUG 1788 was clearly separated from FCUG 1910, the two *P. manschurica* representatives were widely separated, and *P. pilatiana* was distinctly separated from *P. limitata*. All these results are unlikely when compared with morphological data. The ambiguities found in the ITS1 analysis are obviously related to extensive divergence in certain species and is not further commented upon here. For group 3 species, ITS1 data are referred to below (Fig. 5).

ITS2 sequencing, phylogenetic analyses of complete set of taxa

Intraspecific variation in ITS2 sequences was constantly low, rarely exceeding 4 bp (2%). A greater variation could be related to allopatric sampling or sterility barriers. These cases are mentioned under each species group (below). Using *A. chailletii* as outgroup gave a good resolution in phylogenetic analysis. Analysis of the whole dataset (65 specimens) resulted in 504 most parsimonious trees, tree length = 292, consistency index = 0·606 (Fig. 1). Because of the large size of the data matrix it was not possible to do a bootstrap analysis. Three phylogenetic groups were identified among the present selection of *Peniophora* species, specified in the list of species above. Remaining species were not joined into a distinct group but for practical reasons of the analysis referred to a fourth group. Also in the morphological analysis this fourth group was heterogeneous. Group four species were, however, easily aligned with the other groups in both ITS1 and ITS2 sequences, which supports a view that all *Peniophora* species treated here could be regarded as congeneric. The consensus tree obtained from the complete dataset formed a basis for subsequent analysis of the four species groups, which in general were even better resolved, with higher consistency indices (Figs 2–6). For these subsets proper bootstrap analyses could be performed.

ITS2 sequencing, phylogenetic analyses of subgroups

Group 1 (Fig. 2). In *P. piceae*, 1822 (Spain) was found to be incompatible with 1239 and 2306 (both Norway). In the sequence data, 1822 is separated from the other two specimens in 2 sites (ITS2) or 8 sites (ITS1). Corresponding figures for differences between 1239 and 2306 were 0 and 1, respectively. In *P. manshurica*, the two available specimens were incompatible and differed from each other in 2 sites (ITS2), but in ITS1 an extensive divergence was found (20 sites). In *P. rufomarginata*, one specimen is separated in the consensus tree (2110). This specimen is only partially compatible with the other ones, but here sequence differences are restricted to one site (ITS2) or 2 sites (ITS1).

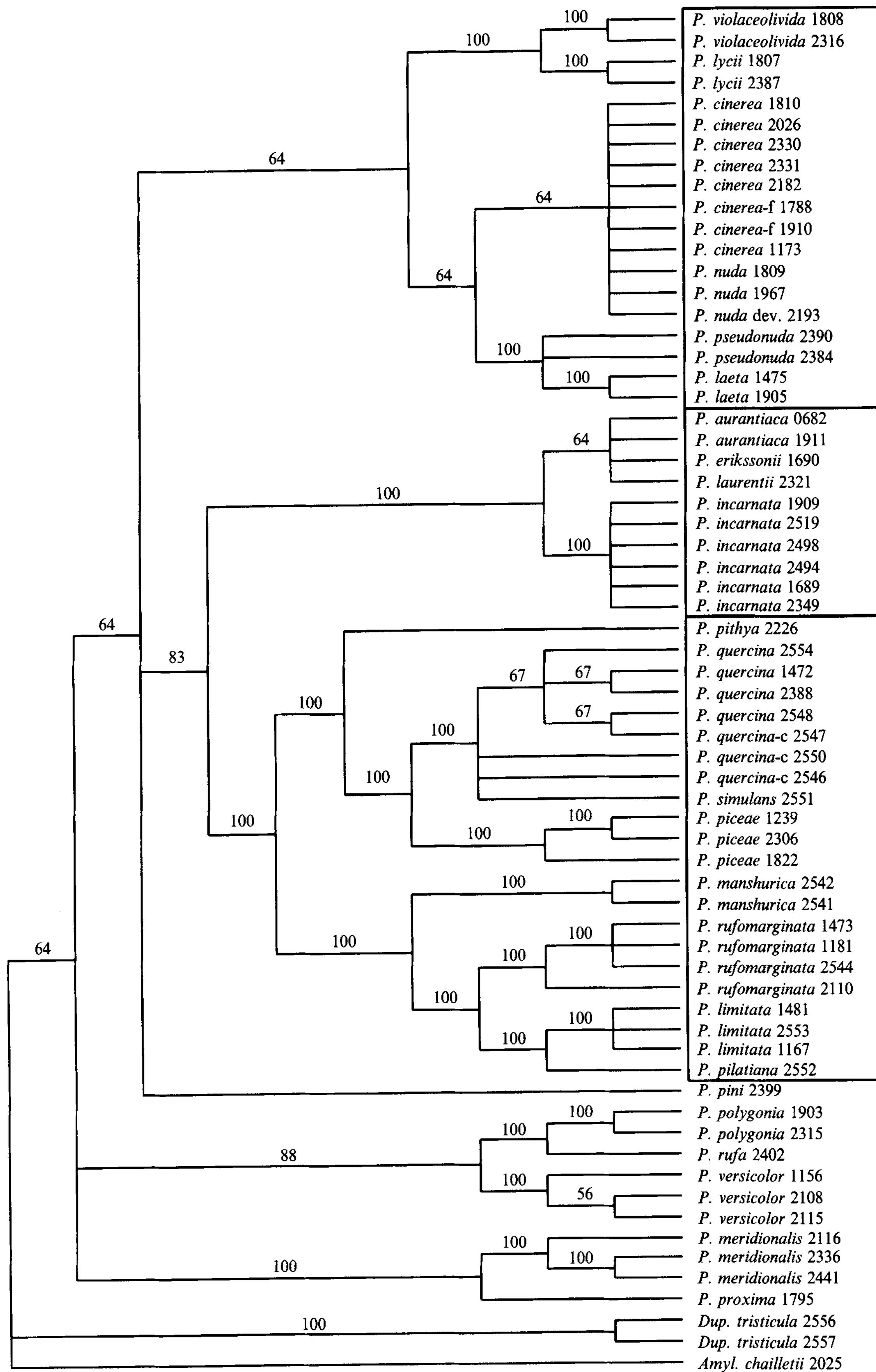

Fig. 1. Investigated specimens of the genus *Peniophora*. Fifty percent majority-rule consensus tree (of 504 most parsimonous trees), based on outgroup-rooted analyses of rDNA sequence characters (ITS2). Figures on branches give the percentage of trees in which the respective clade is represented. Monophyletic groups in boxes. Tree length = 292 steps, consistency index = 0·606. Outgroup: *Amylostereum chailletii*.

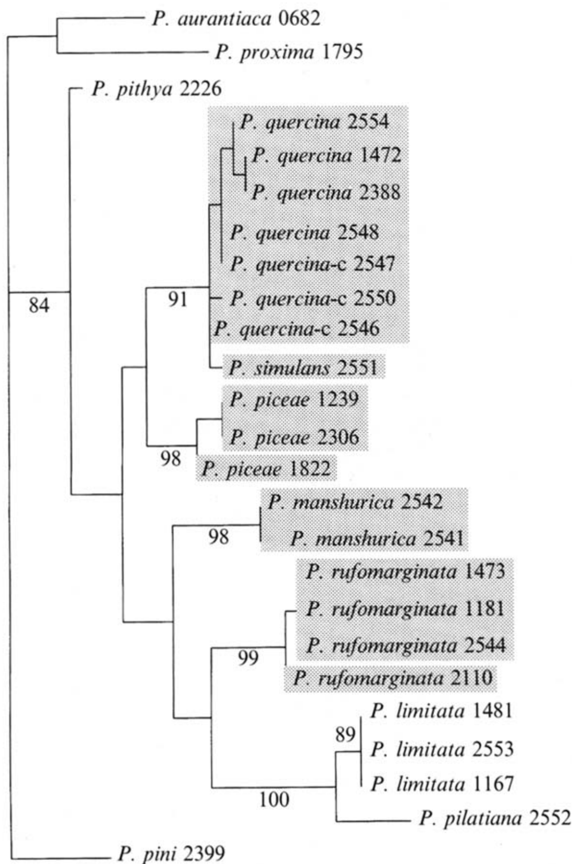

Fig. 2. Phylogram for group 1 specimens. One of 72 most parsimonious trees, based on outgroup-rooted analyses of rDNA sequence characters. This tree has a similar topology as the fifty percent majority-rule consensus tree. Bootstrap values given in bold. Tree length = 100 steps, consistency index = 0·750. Number of informative characters = 36, variable = 43. Outgroup: *P. aurantiaca* (0682), *P. proxima* (1795), and *P. pini* (2399). Intraspecific intersterility groups obtained from crossing tests are shadowed (*P. simulans* regarded as synonymous to *P. quercina*).

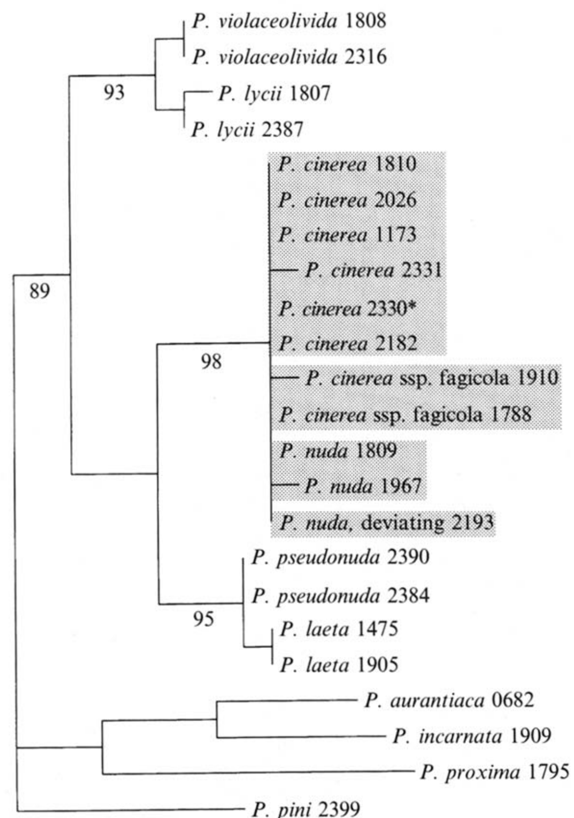

Fig. 3. Phylogram for group 2 specimens. Consensus tree (one most parsimonious tree), based on outgroup-rooted analyses of rDNA sequence characters. Bootstrap values given in bold. Number of informative characters = 16, variable = 20. Tree length = 59 steps, consistency index = 0·797. Outgroup: *P. aurantiaca* (0·682), *P. incarnata* (1909), *P. proxima* (1795), and *P. pini* (2399). Intraspecific intersterility groups obtained from crossing tests are shadowed. The indicated (*) specimen of *P. cinerea* (2330) has been found to be interincompatible with almost all other representatives of the species, except for those from NE North America.

Group 2 (Fig. 3). In this species group the total variation in number of sites is particularly small. *P. cinerea* cannot be separated from *P. nuda*. Total variation in this subclade is 1 (ITS2) or 4 (ITS1). With well supported bootstrap values (> 90%), the total variation between subclades does not exceed 7 steps (ITS2).

Group 3 (Figs 4, 5). In *P. aurantiaca*, 0682 (Canada, B.C.) differed from 1911 (Greenland) in 6 sites (ITS2) or 1 site (ITS1). The very closely related *P. erikssonii* differed from *P. aurantiaca* (0682) in 5 or 2 sites respectively. Despite the world wide sampling, there were no differences in *P. incarnata* sequences (ITS2) while in ITS1, 1909 was separated from the other representatives in 6 sites. In addition to the specimens of *P. incarnata* analysed above, a specimen from Finland (1689) was sequenced (ITS2 only). This was also identical to the other specimens.

Group 4 (Fig. 6). In *P. polygonia*, 2315 (Russia, Ural) differs from 1903 (Sweden) in 7 sites (ITS2) or 21 (ITS1). The Russian collection, however, deviated clearly in morphology and was also incompatible with 1903. It represents a taxon of its own (probably undescribed) even if *P. polygonia* is the closest relative.

Phylogenetic analysis based on morphological characters

In the first attempts to make a phylogenetic analysis with PAUP, the characters were taken from species descriptions and keys (Eriksson *et al.*, 1978). The result was an almost complete polytomy. After careful re-examination of the species concerned, additional characters or subdivision of characters were obtained (Table 2, datamatrix in Table 3). The resulting consensus tree (50% majority-rule) was in good accordance with analysis based on ITS2 sequences, except for group 4 species (Fig. 7). The heterogeneity of the latter group is discussed below.

DISCUSSION

The phylogenetic analyses based on ITS2 sequencing and morphological characters support a segregation into three different species groups. These groups partially coincide with already established subgenera in *Peniophora*.

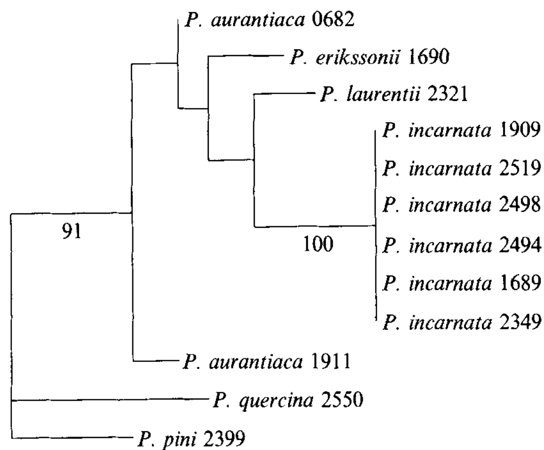

Fig. 4. Phylogram for group 3 specimens. One of three most parsimonious trees, based on outgroup-rooted analyses of rDNA sequence characters from ITS2. Bootstrap values given in bold. Number of informative characters = 12, variable = 23. Tree length = 57 steps, consistency index = 0·825. Outgroup: *P. quercina* (2550) and *P. pini* (2399).

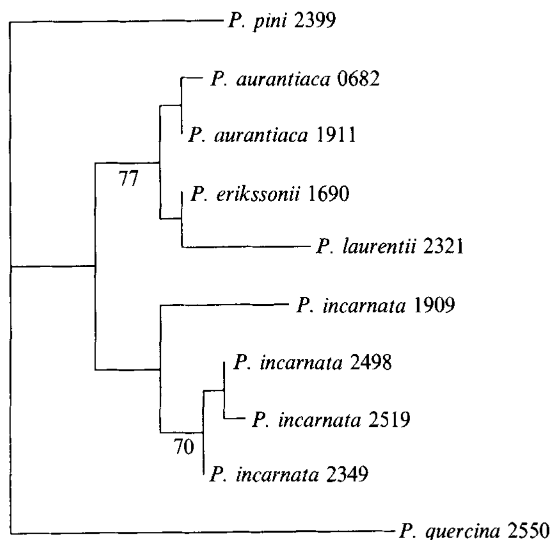

Fig. 5. Phylogram for group 3 specimens. One of three most most parsimonious trees, based on outgroup-rooted analyses of rDNA sequence characters from ITS1. Bootstrap values given in bold. Number of informative characters = 11, variable = 20. Tree length = 57 steps, consistency index = 0·825. Outgroup: *P. quercina* (2550) and *P. pini* (2399).

P. quercina *group*

Basidiomata of the species in this group are characterized by margins curling up upon drying. The micromorphological structures responsible for this xerophytic adaptation are found in a thick basal layer of brown, parallel hyphae. The encrusted cystidia are distinct and unrooted. The species in this group were all treated in subgenus *Peniophora* (Boidin, 1994).

P. cinerea *group*

Basidiomata are thin, dense and closely attached to the substrate. The basal layer is composed of brown-pigmented

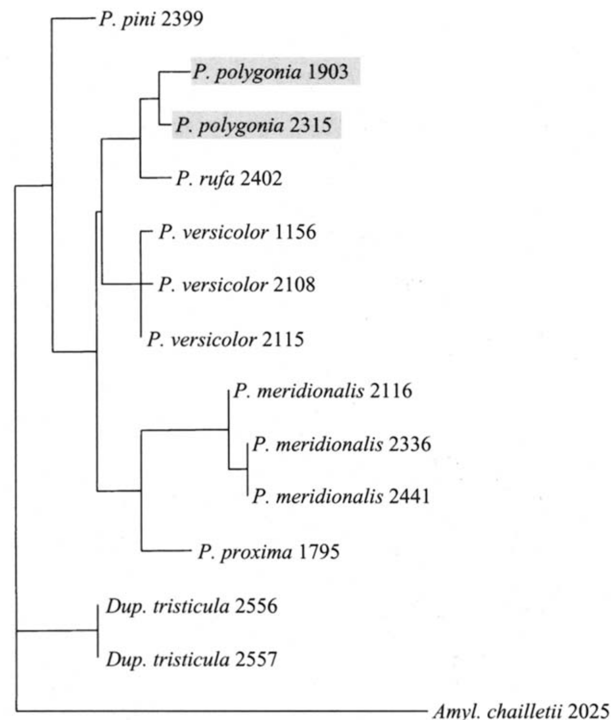

Fig. 6. Phylogram for group 4 specimens. One of three most parsimonious trees, based on outgroup-rooted analyses of rDNA sequence characters. High bootstrap values in this group were only obtained for representatives of the same species. Tree length = 127 steps, consistency index = 0·709. Number of informative characters = 43, variable = 54. Outgroup: *Amylostereum chailletii* (2025). Intraspecific intersterility groups obtained from crossing tests are shadowed.

hyphae, densely branched into a pseudoparenchymatous tissue. Encrusted cystidia are likewise unrooted, but less distinct than above. This species group was also treated in subgenus *Peniophora* (Boidin, 1994).

P. incarnata *group*

The species are characterized by undifferentiated and unpigmented, basal hyphae. Encrusted cystidia are rooted, and both basidia and spores are big in comparison with other groups. This is particularly true for *P. aurantiaca* and *P. erikssonii*. The species in this group belong to the subgenus *Gloeopeniophora* (Höhn. & Litsch.) Boidin & Lanq.

The remaining species in this study are referred to the heterogeneous group 4. The molecular analysis reveals that these species are separated by a greater number of sites than in the other species groups. In the morphological analysis there are also weak connections among the species. *P. rufa* is close to *P. polygonia* in the molecular analysis, which is also obvious from morphological data, *P. pini* is separated from *P. rufa* in 18 sites (ITS2), although morphologically similar. The closest relative of *D. tristicula* is *P. proxima*, separated in 21 sites (ITS2) or 12 sites (ITS1).

From morphology alone it is obvious that *D. tristicula* represents a genus other than *Peniophora*. This species was included in the analysis because sequence data indicate that it

Fig. 7. Fifty percent majority-rule consensus tree (of one hundred and eleven most parsimonious trees), based on eighteen morphological characters. Figures on branches give the percentage of trees in which the respective clade is represented. Tree length = 34 steps, consistency index = 0·529. Outgroup: *Amylostereum chailletii*. Monophyletic species groups from the molecular analysis in boxes.

is a close relative to *Peniophora*. At the moment, it is impossible to make further conclusions about phylogenies within the group. This would require sequence data from additional species in both *Peniophora* and *Duportella*. The latter genus has also been treated as *Peniophora* subgen. *Duportella* (Pat.) Boidin & Lanq.

It is obvious that the infrageneric subdivision inferred from this analysis differs from earlier subdivisions based on morphological characters alone. After a re-evaluation of morphological characters, however, a phylogenetic estimate similar to that based on molecular data, was obtained. Additional characters were found in the segregation of different types of encrusted cystidia. Other characters were found in the microstructure of the basal layer. In both cases phylogenetically informative characters were found. Sulfocystidia, on the other hand, have a more unanticipated occurrence and seem to be a case of parallel evolution. Sulphocystidia appear to be absent in the *P. quercina* group with the exception of *P. pithya*, the most loosely attached species. Presence of dendrohyphidia is another distinct, morphological character which does not seem to be phylogenetically informative. Finding that *P. violaceolivida* (without dendrohyphidia) was the closest relative to *P. lycii* (with dendrohyphidia) was an unexpected result.

Although the new data presented here could be used to modify the infrageneric arrangement in the genus this is not done, mainly because of the bad resolution in group 4 and the need for incorporating more species in the analysis. No formal nomenclatural changes are proposed, but a comparison with an earlier arrangement is done (Table 1). For this purpose, the system proposed by Boidin (1994) was chosen, because of its completeness and coverage of all species treated in this study. Other published arrangements (Eriksson *et al.*, 1978; Parmasto, 1968) differ only slightly. It seems clear that *P. cinerea*, *P. nuda*, and *P. violaceolivida* will have to leave subg. *Peniophora* and join into a group of their own, together with *P. laeta*, earlier placed in subg. *Gloeopeniophora*. Also *P. pini* has to leave subg. *Peniophora*, while *P. versicolor* and *P. proxima* have to leave subg. *Gloeopeniophora*. *P. polygonia* was placed in subg. *Cryptochaete* (P. Karst.) Boidin & Lanq. but is referred to the heterogeneous group 4 here. In traditional arrangements *P. lycii* and *P. meridionalis* – both species with dendrohyphidia – have been regarded as closely related species and included in subgen. *Cristodendrella* Boidin & Lanq. This has not been justified in the present study.

In this study, several species are represented by a number of specimens. This is particularly interesting for genetic interpretation of sibling species complexes found in the genus. *P. simulans* is a species which obviously only exists in the type collection, originating from Corsica. It is intersterile with *P. quercina*, a species from which it is morphologically indistinguishable. Sequence data do not support a segregation from *P. quercina*. The two subspecies described in *P. quercina* were not separated by sequence data either. In *P. rufomarginata*, one specimen (2110) was found to deviate morphologically and by being only partially compatible with European representatives of the species (Hallenberg, 1991*b*). In the sequence data it was also distinguished. The two specimens of *P. manshurica* were both collected in far eastern Russia and were morphologically indistinguishable from each other. Still, they were found to be intersterile and ITS1 sequence data indicate an extensive genetic divergence (see above). A similar, but less striking case, was found in *P. piceae* where the morphologically indistinguishable 1822 from the Pyrénées was incompatible with two Norwegian specimens. The sequence differences were noticeable in ITS1 only.

In *P. cinerea*, no convincing differences could be found to separate the two subspecies described (Hallenberg & Larsson, 1992). Not even *P. nuda* could be separated from *P. cinerea*. The two species are well distinguished from each other in Europe but the difference is mainly in one character, the possession of sulfocystidia in *P. nuda*. Specimens from N. America were found to be compatible with European specimens of *P. cinerea* but incompatible with *P. nuda*, despite their possession of sulfocystidia. The existence of *P. nuda* in N. America has therefore been doubted. In fact, this is a good example of incongruence between a biological species concept and a morphological one, but no further comments on this matter are made here. From a morphological standpoint, *P. violaceolivida* has been treated as a species almost indistinguishable from *P. nuda*. In sequence data it is well separated, although close. Obviously, too much emphasis has been put on sulphocystidia *per se* as a distinguishing character. The shape

and frequency of sulfocystida are, however, quite different in *P. violaceolivida* when compared with *P. nuda*, which is why the two species can be kept separate using morphological criteria.

While good correspondance was found in ITS2 sequence data for all compatible specimens in the different species analysed, *P. aurantiaca* differed in this respect. The two allopatric representatives (W. British Columbia and S. Greenland) differed distinctly from each other. In *P. incarnata*, a specimen from Denmark was likewise distinctly separated from American representatives (Greenland and Argentina; ITS1 only). It must be noted, however, that although compatibility was recorded between specimens from Tierra del Fuego and Denmark, clamp connections were scarce in the matings, and restricted to the confrontation line or unidirectional. This must not be interpreted as an indication of restricted but possible genetic exchange between geographically separated areas. The only conclusion to be drawn is that (partial) intercompatibility is an evidence for a very close relationship.

The result from this study support the hypothesis proposed by Hallenberg & Larsson (1992), that sterility barriers are not always distinct boundaries between species, but can in some cases be regarded as the result of a propagation strategy within the limits of a species. This seems particularly to be the case when genetic differences between intersterile representatives are very small.

The results of this study also support the observation made by Vilgalys & Johnson (1987) and Vilgalys (1991), that representatives from different continents may differ noticeably genetically, while still retaining the ability to mate with each other (allopatric divergence). It is reasonable to accept that sterility barriers, as well as allopatric divergence, may be included in intraspecific variation, but can also be the causes of speciation. Any practical subdivision of taxa on species or subspecies levels must be an expert judgement, where all available data are considered. For the same practical reasons it is necessary to underline that sufficient morphological and ecological differences for recognition must be present in all subdivisions where nomenclature is involved.

The authors thank Erast Parmasto, Tartu, and Paule Lanquetin, Villeurbanne, for valuable discussions. Financial support was given by the Swedish Natural Science Research Council, the University of Gothenburg, and the foundation of Anna and Gunnar Vidfelt.

REFERENCES

Boidin, J. (1994). Les Peniophoraceae des parties tempérées et froides de l'hémisphère nord (Basidiomycotina). *Bulletin Mensuel de la Société Linnéenne de Lyon* **63**, 317–334.

Boidin, J. & Lanquetin, P. (1974). *Peniophora* (subg. *Duportella) kuehneri et halimi* novae sp.; réflexions sur les genre *Peniophora et Duportella. Bulletin Mensuel de la Société Linnéenne de Lyon* **43** (Numéro Spécial), 47–60.

Boidin, J. & Lanquetin, P. (1984). Répertoire des données utiles pour effectuer les tests d'intercompatibilité chez basidiomycètes. III. Aphyllophorales non porées. *Cryptogamie, Mycologie* **5**, 193–245.

Boidin, J. & Lanquetin, P. (1990). Répertoire des données utiles pour effectuer les tests d'intercompatibilité chez basidiomycètes. IV. Aphyllophorales non porées (Premier supplément). Non poroid Aphyllophorales. *Cryptogamie, Mycologie* **11**, 175–188.

Eriksson, J. (1950). *Peniophora* Cke Sect. *Coloratae* Bourd. & Galz. A taxonomical study with special reference to the Swedish species. *Symbolae Botanicae Upsalienses* **10(5)**, 1–76.

Eriksson, J., Hjortstam, K. & Ryvarden, L. (1978). *The Corticiaceae of North Europe*. **Vol. 5**. Fungiflora, Oslo, pp. 887–1047.

Gardes, M. & Bruns, T. D. (1993). ITS primers with enhanced specifity for basidiomycetes – application to the identification of mycorrhizae und rusts. *Molecular Ecology* **2**, 113–118.

Hallenberg, N. (1984). Compatibility between species of Corticiaceae s.l. (Basidiomycetes) from Europe and North America. *Mycotaxon* **21**, 335–388.

Hallenberg, N. (1986). On speciation and species delimitation in *Peniophora cinerea*-group (Corticiaceae, Basidiomycetes). *Windahlia* **16**, 73–80.

Hallenberg, N. (1988). Species delimitation in Corticiaceae (Basidiomycetes). *Mycotaxon* **31**, 445–466.

Hallenberg, N. (1991*a*). Speciation and distribution in Corticiaceae (Basidiomycetes). *Plant Systematics and Evolution* **177**, 93–110.

Hallenberg, N. (1991*b*). Pairing tests with species of Aphyllophorales (Basidiomycetes) from two phytogeographically isolated areas. *Mycotaxon* **42**, 355–386.

Hallenberg, N. & Larsson, E. (1991). Distinctions between culture characters and electrophoretic patterns of sibling species in four different species complexes (Corticiaceae, Basidiomycetes). *Mycologia* **83**, 131–141.

Hallenberg, N. & Larsson, E. (1992). Mating Biology in *Peniophora cinerea* (Basidiomycetes). *Canadian Journal of Botany* **70**, 1758–1764.

Hallenberg, N., Larsson, K.-H. & Larsson, E. (1994). On the *Hyphoderma praetermissum* complex (Corticiaceae, Basidiomycetes). *Mycological Research* **98**, 1012–1018.

Higgins, D. G., Bleasby, A. J. & Fuchs, R. (1992). CLUSTAL V: improved software for multiple sequence alignment. *Computer Applications in the Biological Sciences* **8**, 189–191.

Lee, S. B. & Taylor, J. W. (1990). Isolation of DNA from fungal mycelia and single spores. In: *PCR protocols. A guide to methods and applications* (ed. M. Innis, D. Gelfand, J. Sninsky & T. White), pp. 282–287. Academic Press: New York.

Nakasone, K. K. (1990). Cultural studies and identification of wood-inhabiting Corticiaceae and selected Hymenomycetes from North America. *Mycologia Memoir* No. **15**. J. Cramer, Berlin, Stuttgart.

Parmasto, E. (1968). *Conspectus Systematis Corticiacearum*. Tartu.

Stalpers, J. A. (1978). Identification of wood-inhabiting Aphyllophorales in pure culture. *Studies in Mycology* No. **16**, 1–248.

Swofford, D. L. (1993). *Phylogenetic analysis using parsimony (PAUP version 3.1.1)*. Illinois Natural History Survey: Champaign, Illinois.

Vilgalys, R. (1991). Speciation and species concepts in the *Collybia dryophila* complex. *Mycologia* **83**, 758–773.

Vilgalys, R. J. & Johnson, J. L. (1987). Extensive genetic divergence associated with speciation in filamentous fungi. *Proceedings of the National Academy of Science of the U.S.A.* **84**, 2355–2358.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols. A Guide to Methods and Applications* (ed. M. Innis, D. Gelfand, J. Sninsky & T. White), pp. 315–321. Academic Press: New York.

(Accepted 7 June 1995)