Classification and phylogenetic relationships of *Hymenochaete* and allied genera of the Hymenochaetales, inferred from rDNA sequence data and nuclear behaviour of vegetative mycelium

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Based on nuclear rDNA sequences of 18 species of the corticioid genus *Hymenochaete*, one species of the stipitate *Stipitochaete,* two species of the hydnoid *Hydnochaete,* one species of the hydnoid *Asterodon,* two species of the cyclolamellate to poroid *Cyclomyces,* two species of the cyclolamellate to poroid *Coltricia* and 11 more species of the poroid Hymenochaetales, we examined the position of *Hymenochaete* within the Hymenochaetales, the infrageneric structure of *Hymenochaete*, and the relationships between *Hymenochaete, Stipitochaete, Hydnochaete, Cyclomyces* and *Coltricia*. In addition, the nuclear behaviour of the vegetative mycelium was studied for 10 selected taxa of *Hymenochaete* and one taxon of *Hydnochaete*. *Hymenochaete* appears to be polyphyletic, and a new genus, *Pseudochaete*, is proposed to accommodate the holocoenocytic *H. tabacina*, which is more closely related to the poroid Hymenochaetales, *Onnia* and *Porodaedalea*. The splitting of *Hymenochaete* into four anatomically distinct sections is not supported by our data. *Stipitochaete* is not a separate genus. *Hydnochaete duportii* and *H. japonica*, as well as *Cyclomyces fuscus* and *C. tabacinus* are not distinctly separated and are suggested to be transferred to *Hymenochaete*.

Taxonomical novelties: *Pseudochaete* T. Wagner & M. Fischer, *Pseudochaete tabacina* (Sow.: Fr.) T. Wagner & M. Fischer, *Hymenochaete duportii* (Pat.) T. Wagner & M. Fischer, *Hymenochaete hydnoides* T. Wagner & M. Fischer, *Hymenochaete cyclolamellata* T. Wagner & M. Fischer *Hymenochaete porioides* T. Wagner & M. Fischer.

The corticioid *Hymenochaete* Lév., with *H. rubiginosa* (Dicks.: Fr.) Lév. (Fig.1) as type, was described by LÉVEILLÉ (1846) in the Thelephoraceae Chev. Mainly based on the occurrence of setae in the hymenium, Léveillé combined 19 species from *Corticium* Pers., *Stereum* Pers.: S.F. Gray and *Thelephora* Fr. in his new genus, which he conceived as a natural group. PATOUILLARD (1900) included *Hymenochaete* besides hydnoid and poroid genera in his „Série des Igniaires". DONK (1948) described the Hymeno-chaetaceae Donk, which OBERWINKLER (1977) rised to the level of an order, the Hymenochaetales. The Hymenochaetales defined by Oberwinkler include taxa exhibiting distinct types of hymenial structure (corticioid, hydnoid or poroid) and basidiocarps (resupinate, pileate or stipitate); the main characters are the xanthochroic reaction, the lack of clamps, the frequent occurrence of setae and the cause of a white rot. Recently, phylogenetic analysis of sequence data of nSSU and mtSSU rDNA (HIBBETT & DONOGHUE 1995, LANGER 1998, HIBBETT & THORN 2001), as well as nLSU rDNA (unpublished data), demonstrated several aphyllophoralean taxa, such as *Hyphodontia* J. Erikss., *Schizopora* Velen., *Oxyporus* (Bourd. & Galz.) Donk, *Basidioradulum* Nobles and *Trichaptum* Murrill as closely related to the Hymenochaetales sensu Oberwinkler. The available data on the ultrastructure of the septal pores showed nonperforated parenthosomes to be a common characteristic for hymenochaetoid taxa (MOORE 1980, KELLER 1997, MÜLLER et al. 2000).

Taxa of *Hymenochaete* exhibit all the typical characters as listed above. The hyphal system is monomitic and basidiospores are mostly cylindrical or ellipsoid. Although the high variability in macroscopic and microscopic features may indicate a polyphyletic origin of *Hymenochaete*, until now only the two genera *Hydnochaete* Bres. (comprising 8 species), with a hydnoid hymenophore, and *Stipitochaete* Ryvarden (comprising 2 species; Fig. 2), with stipitate basidiocarps, were separated from *Hymenochaete* (RYVARDEN 1982, 1985). In this way, *Hymenochaete* is restricted to corticioid taxa. However, transitions of corticioid, hydnoid, cyclolamellate or poroid basidiocarps are common within the hymenochaetoid taxa, on generic level for instance within *Cyclomyces* Fr., *Hyphodontia*, *Schizopora* and on species level within *Coltricia montagnei* (Fr.) Murrill (RYVARDEN 1985, GILBERTSON & RYVARDEN 1986, CORNER 1991, LANGER 1994, 1998, DAI 1999).

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Fig. 1. Effused-reflexed fruit body of *Hymenochaete rubiginosa*.

According to FIASSON & NIEMELÄ (1984) and JÜLICH (1984), *Hymenochaete* together with *Hydnochaete* and *Asterodon* Pat. forms the family Hymenochaetaceae. At present *Hymenochaete* comprises more than 100 species, mostly tropical or subtropical. An extensive and detailed survey of the genus is offered by the monographs of JOB (1990) and LÉGER (1998). Concerning the pattern of reproduction, the few data available show the genus to be homothallic (LÉGER 1998). Cytological data are available for 28 species, showing binucleate mycelial segments for all species except for *H. tabacina*, which is multi-nucleate (holocoenocytic sensu BOIDIN 1971), and *H. pseudoadusta* Léger & Lanquetin and *H. acanthophysata* Léger, which are oligonucleate (BOIDIN 1971, BOIDIN & LANQUETIN 1984, LÉGER 1998).

The shape of basidiocarps is variable in *Hymenochaete* (Figs. 1, 3), and even varies within single species. For instance, *H. rubiginosa* and *H. tabacina* (Sow.: Fr.) Lév. can range from resupinate to flabellate. BURT (1918) was the first to distinguish three different layers within the basidiocarps of *Hymenochaete*: a setigerous layer (containing the setae), a context (hyphal layer without setae) and a cortex (agglutinated hyphae, tomentum). Based on the specific occurrence of these layers within taxa he divided *Hymenochaete* into three groups, which ESCOBAR (1978) recognized as sections *Hymenochaete*, *Gymnochaete* and *Fultochaete*. These sections were accepted by JOB (1990) and LÉGER (1998), who added a fourth section, *Paragymnochaete*. The sections are defined as follows:

- Sect. *Hymenochaete* – Cortex, context and setigerous layer all present. Type: *H. rubiginosa* (Dicks.: Fr.) Lév.
- Sect. *Fultochaete* Escobar: Léger – Context and setigerous layer present, cortex absent. Type: *H. damicornis* (Link: Fr.) Lév.
- Sect. *Gymnochaete* Escobar: Léger – Only setigerous layer present; context and cortex absent. Type: *H. corrugata* (Fr.: Fr.) Lév.
- Sect. *Paragymnochaete* Léger – Setigerous layer and cortex present, context absent. Type: *H. sphaerospora* Léger & Lanquetin.

PARMASTO (1995) examined the relationships between these infrageneric groups by a phylogenetic analysis inferred from 36 different morphological and anatomical features. His analysis included 86 species from *Hymenochaete*, representing all four sections, 7 species from *Hydnochaete* and two species from *Stipitochaete*. The results suggested that *Hymenochaete*, *Stipitochaete* and *Hydnochaete* are paraphyletic, and that the sections of *Hymenochaete* are polyphyletic.

The goals of our study were to verify, a) whether *Hymenochaete* is monophyletic or whether the corticioid type of hymenophore occurs in different lineages within the Hymenochaetales, b) whether the infrageneric structure of the genus, as inferred from the anatomy of basidiocarps, is reliable, and c) the relationships of *Hymenochaete* within the Hymenochaetales.

To address these questions, we analyzed nuclear large subunit rDNA sequences. We also studied cytological data which

Fig. 2. Stipitate fruit body of *Stipitochaete damicornis*.

have been shown to provide important insights in the relationships between members of the Hymenochaetales (FISCHER 1996, WAGNER & FISCHER 2001).

Material and Methods

Cultures were grown on 2 % malt extract agar, (2 % malt extract, 2 % agar, 0.05 % yeast extract in distilled water) at 23°C and 65 % humidity in the dark.

Slide cultures were used for staining of vegetative cultures (VAN UDEN 1951). Nuclei were stained with Giemsa for light microscopy (KORHONEN & HINTIKKA 1974, FISCHER 1987).

DNA was isolated from cultured mycelia or herbarium specimens as described by LEE & TAYLOR (1990). DNA pellets were air-dried and resuspended in 100 μL TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0).

DNA usually was diluted 1:1000 in distilled water. Approximately 1400 bases (b) of the nLSU rDNA were amplified using Taq polymerase (Eurogentec) and primers LR0R (ACC CGC TGA ACT TAA GC) and LR7 (TAC TAC CAC CAA GAT CT) on a Biometra TRIO-Thermoblock, using the following parameters: 94° C denaturation step (1 min.), 47° C annealing step (45 sec.), and 72° C primer extension (2 min.). The cycle was repeated 37 times. A final incubation step at 72° C (7 min.) was added after the final cycle. PCR products were purified with the QIAquick PCR Purification Kit (Qiagen). Cycle sequencing was performed with primers LR0R and LR5 (TCC TGA GGG AAA CTT CG) using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA). Parameters for the reactions were: 96° C denaturation step (30 sec.), 48° C (LR0R) and 49° C (LR5) annealing step (15 sec.), and 72° C primer extension (4 min.). The cycle was repeated 35 times. Cycle sequencing products were run on an ABI 377 automated DNA sequencer (Applied Biosystems).

We performed a phylogenetic analysis of nuclear large subunit ribosomal DNA (nLSU rDNA) sequencing data for a selec-ted range of taxa (40 species, see Tab. 1; sequences partially previously published in WAGNER 2001, WAGNER & FISCHER 2001). Taxon sampling included 18 species of *Hymenochaete* (including the type species and two isolates of *Hymenochaete tabacina* (Sow.: Fr.) Lév.), representing all four sections, the type species from the stipitate *Stipitochaete* (Fig. 2), two species from the hydnoid *Hydnochaete*, the type species from the hydnoid *Asterodon*, two species from the cyclolamellate to poroid *Cyclomyces*, including the type species, two species from the cyclolamellate to poroid *Coltricia* S.F. Gray, including the type species and a cyclolamellate specimen of *C. montagnei* (Fr. in Mont.) Murrill, and 11 more species of the poroid Hymenochaetales, representing the genera *Phellinus* Quél. (type species), *Inonotus* P. Karst. (type species), *Porodaedalea* Murrill (type species), *Fomitiporia*

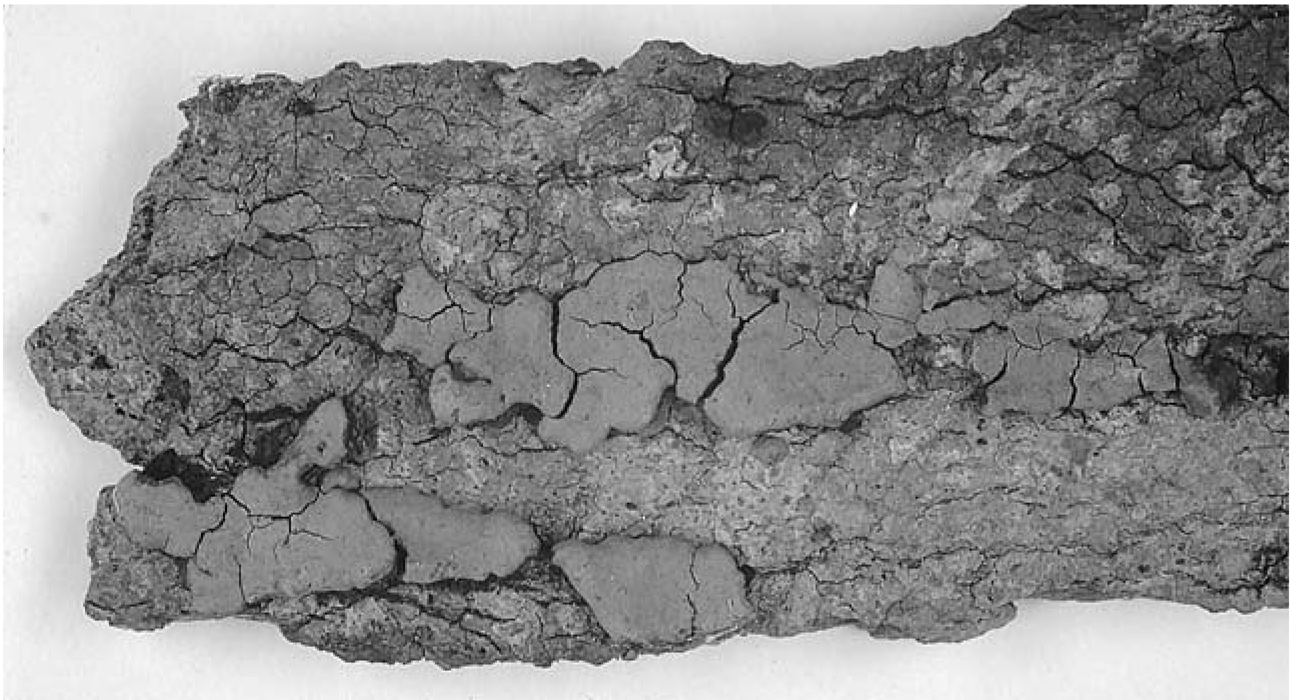

Fig. 3. Resupinate fruit body of *Hymenochaete carpatica*.

Murrill (type species), *Fuscoporia* Murrill (type species), *Phellinidium* (Kotl.) Fiasson & Niemelä (type species), *Phellopilus* Niemelä, Wagner & Fischer (type species), *Inocutis* Fiasson & Niemelä (type species), *Inonotopsis* Parmasto (type species), *Onnia* P. Karst. (type species), and *Phylloporia* Murrill (among others discussed by MURRILL 1904, 1905, 1907, DONK 1969, FIASSON & NIEMELÄ 1984, DAI 1995, 1999, HIBBETT & DONOGHUE 1995, LANGER 1998, HIBBETT & THORN 2001, NIEMELÄ et al. 2001, WAGNER 2001, WAGNER & FISCHER 2001).

Previous DNA data suggested russuloid taxa as closely related to the hymenochaetoid taxa (HIBBETT & DONOGHUE 1995, HIBBETT & THORN 2001). Therefore, three species from the russuloid clade, *Russula violacea* Quél., *Bondarzewia montana* (Quél.) Sing. and *Stereum hirsutum* (Wild.: Fr.) S.F. Gray, were chosen as outgroups.

Approximately 900 b of the sequences, starting from primer LR0R, were automatically aligned using ClustalX (THOMPSON et al. 1997). A final alignment was performed by eye. Gaps in the alignment were treated as missing data. All positions were included in the final alignment. The sequences obtained have been deposited in GenBank (for accession nos. see Tab.1). Sequence alignments have been deposited in Tree BASE as submission no. SN 825.

Maximum parsimony (MP) analysis was performed with PAUP 4.0b2 (SWOFFORD 1999). The characters were unordered with equal weighting. One hundred heuristic searches were performed, with tree-bisection-reconnection (TBR) branch-swapping algorithm, random taxon addition sequences, MulTrees option in effect, and zero length branches collapsed. No more than 100 trees were saved in each replicate. 10 trees were held at each step during stepwise addition. Maxtrees option was set to autoincrease. A bootstrap parsimony analysis (FELSENSTEIN 1985) was performed with 1000 heuristic replicates.

The distance analysis was calculated with components of the PHYLIP 3.5c packet (FELSENSTEIN 1995) integrated in ClustalX. The distance matrix was generated using Kimura 2-parameter distances, weighting transition:transversion ratio 2:1. Neighbour-joining (NJ, standard parameters) was used to calculate the tree. Bootstrap analysis was run with 1000 replicates.

Both the NJ tree and the MP tree were rooted with the outgroups.

To rate the results of the parsimony analysis, constrained trees were created in MacClade version 3.04 (MADDISON & MADDISON 1992). In the heuristic search TBR branch swapping algorithm and MulTrees option were in effect. Collapse zero length-branches option was on. Maxtrees option was set to 100. Unconstrained and constrained trees were compared using the Kishino-Hasegawa test (KISHINO & HASEGAWA 1989) and the Wilcoxon signed-ranks test (TEMPLETON 1983).

Results

Sequence data

Within the Hymenochaetales included in this study the length of the sequenced nLSU rDNA region ranged from 866 bases (*Fuscoporia ferruginosa* (Schrad.: Fr.) Murrill) to 878 bases (*Phylloporia ribis* (Schum.: Fr.) Ryvarden). A noticeable exception was *Coltricia perennis* (L.: Fr.) Murrill with 852 bases. Within the outgroups the fragment size ranged from 863 bases (*Stereum hirsutum*) to 870 bases (*Russula violacea*). The sequences of the two isolates of *Hymenochaete tabacina* were identical, and only one sequence was included in the final analysis. The alignment of the 40 sequences resulted in 905 sites with 339 variable characters, 219 of which were parsimony informative.

The MP analysis produced 98 most parsimonious trees on one island (MADDISON 1991), each with 1032 steps, consistency index (CI) 0.47, retention index (RI) 0.60 and rescaled consistency index (RC) 0.28. The strict consensus of the 98 most parsimonious trees (Fig. 4) showed the Hymenochaetales as well separated from the outgroups (bootstrap support: 100 %). Except for *Hymenochaete tabacina* all species of *Hymenochaete* were grouped together in one highly supported clade (100 %, 'Hymenochaete' clade) which also contains *Stipitochaete damicornis* (Link) Ryvarden, the two species of *Hydnochaete,* and the two species of *Cyclomyces*. The taxa belonging to the four different sections of *Hymenochaete* formed no section specific clades, but were intermingled and spread over the whole 'Hymenochaete' clade.

Within the 'Hymenochaete' clade three subgroups were evident: one subgroup was formed by the two species of *Hydnochaete* (100 %), with *Hymenochaete acanthophysata* Léger as a sister taxon (90 %); *H. cervinoidea* Léger & Lanquetin was next related to these species (55 %). A second subgroup comprised the sister taxa *H. cruenta* (Pers.: Fr.) Donk and *H. denticulata* Léger & Lanquetin (90 %) together with *H. pinnatifada* Burt (76 %). The remaining taxa of *Hymenochaete* were grouped together in a bigger subgroup (81 %), where sister taxa were formed by *H. rubiginosa* and *H. ochromarginata* Talbot (98 %) as well as *H. pseudoadusta* Léger & Lanquetin, *H. adusta* Har. & Pat., *H. berteroi* Pat., *Stipitochaete damicornis*, *Cyclomyces fuscus* Fr. and *C. tabacinus* (Mont.) Pat. (59 %). In the bootstrap consensus tree (not shown), the remaining taxa in this subgroup showed a polytomy of unresolved lineages.

Next to the 'Hymenochaete' clade were several unresolved lineages, leading to *Inonotus* and *Inocutis* (sistergroups, 62 %), *Phellinus* and *Phylloporia*. Only very low support (50 %) was evident for *Hymenochaete tabacina* next to *Porodaedalea* and *Onnia* (sistergroups, 66 %), which were positioned next to a larger clade (80 %) consisting of *Coltricia*, *Fuscoporia*, *Inonotopsis*, *Phellinidium*, *Phellopilus*, and *Asterodon*.

Bootstrap values were mostly higher in the NJ analysis; otherwise the topology of the NJ tree (Fig. 5) was mostly congruent with the MP tree (Fig. 4). A divergence was noteworthy within the 'Hymenochaete' clade, where *H. rubiginosa* and *H. ochromarginata* are grouped together with *H. fuliginosa* and *H. cinnamomea* (71 %, Fig. 5). The relationship between *Porodaedalea* and *Onnia* was supported by a higher bootstrap value (98 %). The NJ analysis showed these taxa as more closely related to *Fomitiporia, Phellinus, Inonotus, Inocutis* and *Phylloporia*. As in the MP tree, *H. tabacina* appeared as related to *Porodaedalea* and *Onnia* (60 %). *Asterodon*, *Phellinidium*, *Phellopilus*, *Fuscoporia*, and *Inonotopsis* formed a separate clade (74 %). Next to this clade were *Coltricia perennis* and *C. montagnei*, which were sistergroups (100 %). The arrangement of the latter taxa was slightly different in the two trees, with *Coltricia* and *Fuscoporia* as basal groups and next related to *Phellopilus* and *Asterodon* and the group of *Phellinidium* and *Inonotopsis* in the NJ tree (62 %, Fig. 5).

To evaluate the monophyly of *Hymenochaete*, a constrained analysis was performed, that forced monophyly of the 'Hymenochaete' clade, including *H. tabacina.* The constrained analysis produced 100 trees that were 7 steps longer than the unconstrained trees. The monophyly was not rejected by the Kishino Hasegawa test (0.3273–0.4253) and the Wilcoxon signed ranks-test (0.2819–0.3900).

According to the results of the NJ analysis, we also performed a constrained analysis that forced the monophyly of a clade comprising *Porodaedalea*, *Onnia* and *Hymenochaete tabacina*. The constrained analysis produced 100 trees that were only 1 step longer than the unconstrained trees. The monophyly was not rejected by the Kishino Hasegawa test (0.8085–0.8695) and the Wilcoxon signed ranks-test (0.8084–0.8728).

Nuclear data

The results of the karyological studies in selected species of *Hymenochaete* and *Hydnochaete* were as follows: Binucleate hyphal segments were observed in *Hymenochaete adusta* Har. & Pat., *H. carpatica* Pilàt, *H. cervinoidea* Léger & Lanquetin, *H. cinnamomea* (Pers.: Fr.) Bres., *H. nanospora* Léger, *H. ochromarginata*, *H. pinnatifada*, *H. separabilis* Léger, *H. separata* Cunn., and *Hydnochaete japonica* Lloyd. Besides the dominating binucleate hyphal segments, also some trinucleate and tetranucleate segments were observed, mostly so in the hyphal tips. In accordance with BOIDIN (1971), a holocoenocytic nuclear behaviour was observed in *H. tabacina*. While most of the segments contained 4 to 10 nuclei in this species, the number of nuclei increased up to 20 and more in the hyphal tips.

Discussion

Monophyly of *Hymenochaete*

Hymenochaete was shown as a polyphyletic genus in this study, and *H. tabacina* was distinctly separated from the other taxa of *Hymenochaete* (Figs. 4, 5). The two trees presented in the study of PARMASTO (1995) revealed no separate position for *H. tabacina*. However, the affinity of this species was inconclusive, and it clustered together with different taxa of *Hymenochaete* and *Hydnochaete*. *H. tabacina* is a cosmopolitan species, developing resupinate, semipileate or pileate basi-

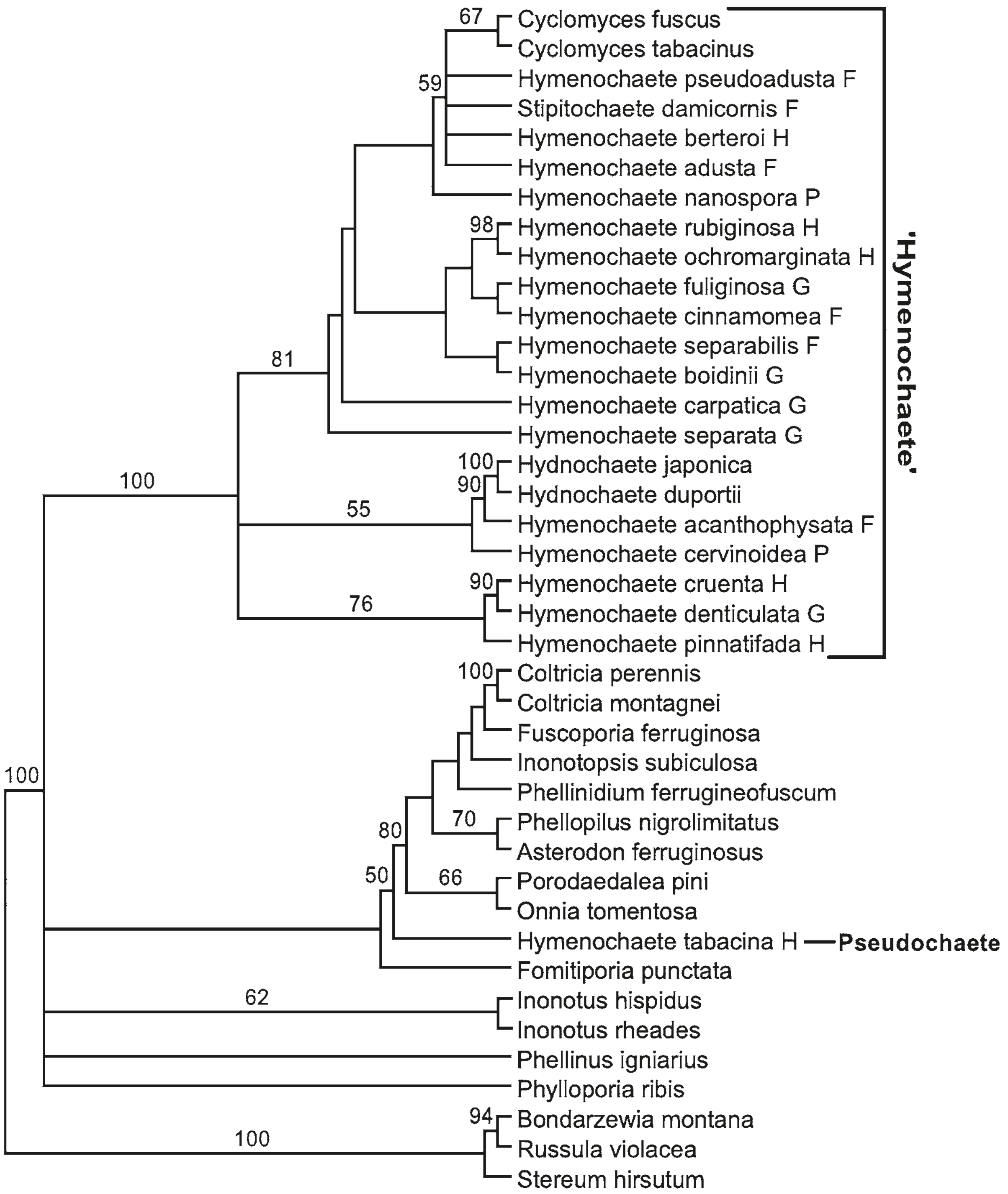

Fig. 4. Strict consensus tree (1032 steps) of 98 best trees obtained from parsimony analysis of nLSU rDNA sequences of 37 taxa of the Hymenochaetales. The poroid *Bondarzewia montana*, the agaricoid *Russula violacea* and the stereoid *Stereum hirsutum* were used as outgroups. The tree was rooted with the outgroups. Bootstrap analysis was performed with 1000 replicates; only bootstrap values higher than 50 % are indicated above the branches. Large caps after names indicate sections within *Hymenochaete* (H = Sect. Hymenochaete; F = Sect. Fultochaete; G = Sect. Gymnochaete; P = Sect. Paragymnochaete).

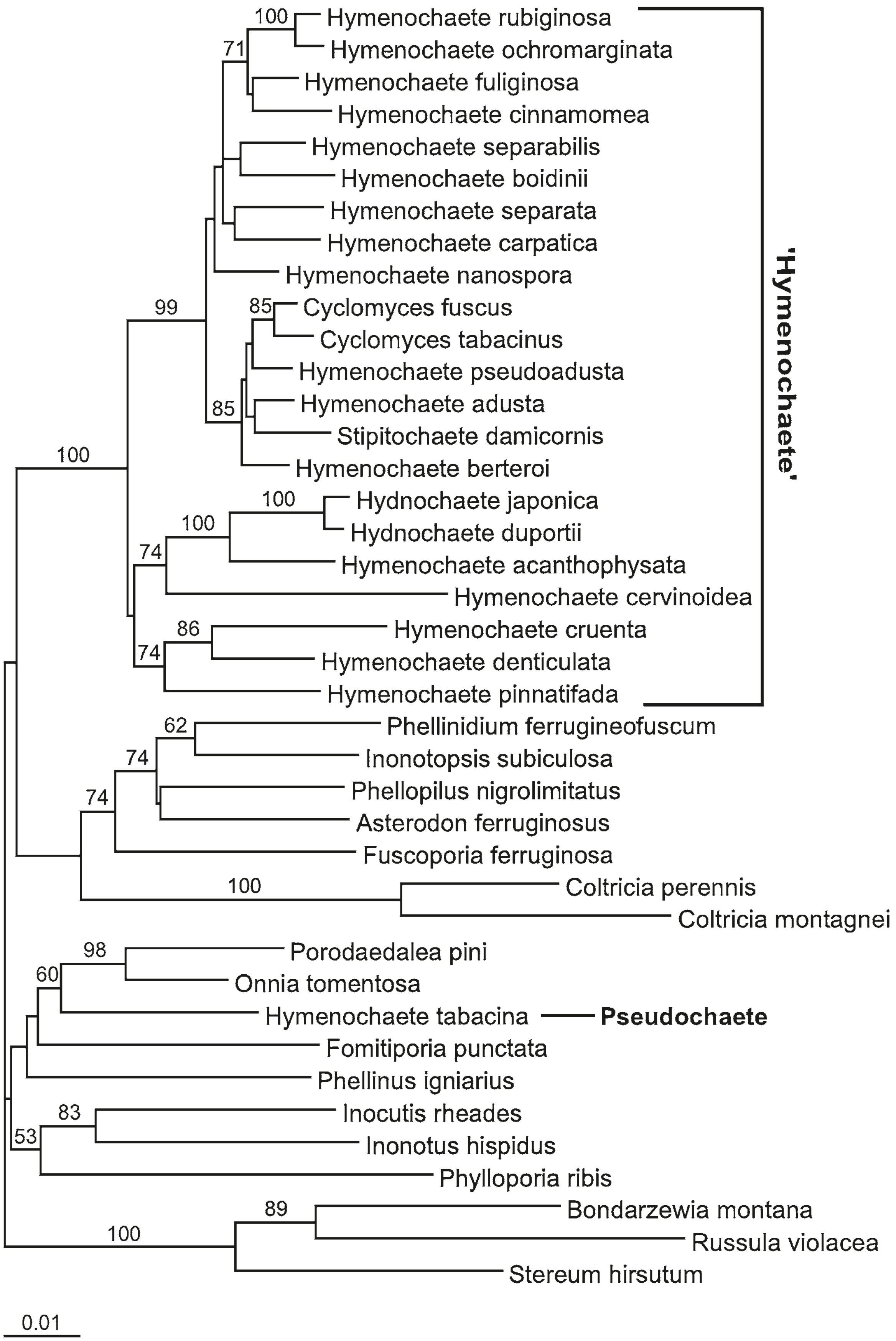

Fig. 5. Phylogram obtained by neighbour-joining analysis of nLSU rDNA sequences of 37 taxa of the Hymenochaetales. The poroid *Bondarzewia montana*, the agaricoid *Russula violacea* and the stereoid *Stereum hirsutum* were used as outgroups. The tree was rooted with the outgroups. Bootstrap analysis was performed with 1000 replicates. Bootstrap values higher than 50 % are indicated above the branches. Scale bar at the bottom indicates the percentage of difference.

diocarps. Important characters are the yellow orange margin of young basidiocarps, the radiating cracks in the hymenium, the horizontal or oblique setal hyphae which are ascending into the context, and the large and robust setae which are verrucous or incrusted at the tip. Among the species of *Hymenochaete* investigated in this study, incrusted setae are also present in *H. cervinoidea*, and setal hyphae are present in *H. cruenta* (Pers. Fr.) Donk and *H. berteroi* Pat. However, these taxa show no relationship to *H. tabacina* or to each other, and are positioned in different clades (Figs. 4, 5). *H. tabacina* is most divergent from the remaining species of *Hymenochaete* in its holocoenocytic nuclear behaviour. Another species, *H. cinnamomea*, was reported as holocoenocytic by JOB (1986). Our results, however, verify the binucleate behaviour stated by BOIDIN & LANQUETIN (1984).

The results of the constrained analysis were inconclusive, and neither the monophyly of *Hymenochaete*, including *H. tabacina*, nor the monophyly of a clade including *Porodaedalea*, *Onnia* and *H. tabacina* were rejected. However, the trees of the constrained analysis forcing *Porodaedalea*, *Onnia* and *H. tabacina* were only one step longer than the unconstrained trees and 6 steps shorter than those of the analysis forcing *Hymenochaete*, including *H. tabacina*. In addition the P-values of the the Kishino-Hasegawa test and the Wilcoxon signed-ranks test were much higher for the analysis forcing *Porodaedalea*, *Onnia* and *H. tabacina*. All in all, the results of the phylogenetic analysis of molecular data together with characters taken from morphology, anatomy and cytology delimit *H. tabacina* from *Hymenochaete*. Hence we propose to separate *H. tabacina* into a new genus:

Pseudochaete T. Wagner & M. Fischer *gen. nov.*

Typus: *Auricularia tabacina* Sow., Brit. Fung., pl. 25, 1797

Basidiomata annua, resupinata, semipileata vel pileata; hymenium leve vel sulcatum vel verrucosum; hyphae fibulis carentes; systema hypharum monomiticum, cellulae holocoenocyticae; setae copiosae, partim incrustatae; basidiosporae hyalinae, cylindricae vel allantoideae.

Basidiocarps annual, resupinate, semipileate or pileate; hymenium smooth, cracked or verrucous; hyphae without clamps; hyphal system monomitic; nuclear behaviour holocoenocytic; setae abundant, partially incrusted; basidiospores hyaline, cylindrical to allantoid.

Pseudochaete tabacina (Sow.: Fr.) T. Wagner & M. Fischer comb. nov.

Basionym: *Auricularia tabacina* Sow., Brit. Fung., pl. 25, 1797

Synonyms: *Thelephora tabacina* (Sow.: Fr.) Fr., Syst. Myc. 1: 437, 1821. – *Stereum tabacinum* (Sow.: Fr.) Fr., Epicr.: 550, 1838. – *Hymenochaete tabacina* (Sow.: Fr.) Lév., Ann. Soc. Nat., Bot III, 5: 152, 1846.

Possibly additional species have to be separated from *Hymenochaete*. Further studies should focus on taxa with different types of basidiospores. Within poroid taxa of the Hymenochaetales, shape of spores have been demonstrated as an important character for the delimitation of natural groups (WAGNER & FISCHER 2001). For instance, globose spores are diagnostic for the genera *Fomitiporia* and *Pseudoinonotus* T. Wagner & M. Fischer.

Infrageneric structure of *Hymenochaete*

Both trees do not support the infrageneric splitting of *Hymenochaete* into the four sections, *Hymenochaete*, *Fultochaete*, *Gymnochaete* and *Paragymnochaete* (Figs. 4, 5), suggesting that the anatomy of basidiocarps can not be used for the infrageneric classification of *Hymenochaete*. The taxa studied show no morphological or anatomical pattern that is in accordance with the phylogenetic analysis. Some characters appear as species specific or are only present within very few taxa. Such characters are for instance the small spores of *H. nanospora*, the acanthophysis within *H. acanthophysata*, the dendrophysis within *H. cruenta* and *H. pinnatifada* or the toothed setae of *H. denticulata* Léger & Lanquetin.

Relationships between *Hymenochaete*, *Stipitochaete*, *Hydnochaete* and *Cyclomyces*

Our sequence data do not separate *Stipitochaete damicornis*, *Hydnochaete duportii*, *H. japonica*, *Cyclomyce fuscus* and *C. tabacinus* from *Hymenochaete*. In accordance with previous results (PARMASTO 1995), taxa of *Hydnochaete* are placed within *Hymenochaete*, by which *Hymenochaete* becomes a paraphyletic taxon. Consequently PARMASTO (1995) suggests such taxa to be excluded from *Hydnochaete*. The sole feature delimiting *Hydnochaete* from *Hymenochaete* is the hymenophore type, which is hydnoid in *Hydnochaete* and smooth in *Hymenochaete*. However, hymenophore teeth are small in *H. duportii*, up to 700 μm, and *H. japonica*, 200–400 μm (RYVARDEN 1982), which may indicate a relationship to taxa of *Hymenochaete*. In order to preserve *Hymenochaete* as a monophyletic taxon, we suggest that *H. duportii* and *H. japonica* should be transferred to *Hymenochaete*.

Hymenochaete duportii (Pat.) T. Wagner & M. Fischer comb. nov.

Basionym: *Hydnochaete duportii* Pat., Bull. Soc. Mycol. Fr. 31: 76, 1915.

Hymenochaete hydnoides T. Wagner & M. Fischer nom. & comb. nov.

Basionym: *Hydnochaete japonica* Lloyd, Mycol. Writ. 4, 1916.

Non *Hymenochaete japonica* Yasuda in Lloyd, Myc. Writ. 5, letter 5: 8, note 542, 1917.

With the data at hand the status of other taxa of *Hydnochaete* remains questionable

Concerning the position of *Stipitochaete damicornis*, our results are congruent with those of PARMASTO (1995). Both analyses suggest that *S. damicornis* is closely related to *H. adusta* and *H. pseudoadusta*, and so both species of *Stipitochaete*, i.e. *S. damicornis* and the closely related *S. reniformis* (Fr.) Ryvarden should remain within *Hymenochaete,* as *H. damicornis* (Link: Fr.) Lév. and *H. reniformis* (Fr.) Lév. The occurrence of stipitated basidiocarps within the Hymenochaetales is often associated with a growth on the ground, as for instance within *Coltricia*, *Onnia* or *Aurificaria* Reid. Most of these species are suggested to be lignicolous, however, and only for *Coltricia perennis* a mycorrhizal association with jack pine and bearberry has been reported (DANIELSON 1984). For *Stipitochaete damicornis* a parasitical growing on roots is suggested by RYVARDEN (1985), while JOB (1990) describes the species as terrestrial; extensive studies on a possible mycorrhizal association are still missing.

Cyclomyces fuscus and *C. tabacinus* are morphologically separated from *Hymenochaete* by a poroid to concentric lamellate hymenophore and a thin black zone in the context. *Cyclomyces* is similar by the monomitic hyphal system and the shape of basidiocarps and basidiospores (RYVARDEN & JOHANSEN 1980, GILBERTSON & RYVARDEN 1986-1987, CORNER 1991, DAI 1999). The closely related *H. adusta* and *H. pseudoadusta* both exhibit a hymenophore with concentric wrinkles (LÉGER 1998), which supports a possible relation-ship with *Cyclomyces*. Therefore we suggest to transfer *C. fuscus* and *C. tabacinus* to *Hymenochaete*.

Hymenochaete cyclolamellata T. Wagner & M. Fischer nom. & comb. nov.

Basionym: *Cyclomyces fuscus* Fr., Linnaea 5: 512, 1830.

Non *Hymenochaete fusca* (P. Karst.) Saccardo, Syll. Fung. 14: 218, 1900 (= *Hymenochaetella fusca* P. Karst., Hedwigia 35: 174, 1896).

Hymenochaete porioides T. Wagner & M. Fischer nom. & comb. nov.

Basionym: *Polyporus tabacinus* Mont., Ann. Sci. Nat. (Ser. 3) 3: 349, 1835.

Synonyms: *Inonotus tabacinus* (Mont.) P. Karst., Rev. Mycol.3: 19, 1881. – *Cyclomyces tabacinus* (Mont.) Pat., Essai Tax. Hymen., 98, 1900.

Non *Hymenochaete tabacina* (Sow.: Fr.) Lév., Ann. Soc. Nat., Bot III, 5: 152, 1846 (= *Auricularia tabacina* Sow., Brit. Fung., pl. 25, 1797).

Cyclomyces, with *C. fuscus* as type species, represents a homogeneous genus, presently comprising 5 species (RYVARDEN & JOHANSEN 1980, GILBERTSON & RYVARDEN 1986, CORNER 1991, DAI 1999). Although *C. iodinus* (Mont.) Pat. and *C. setiporus* (Berk.) Pat. exhibit a poroid hymenophore, CORNER (1991) regarded them as varieties of *C. fuscus* only. In our opinion all accepted species of *Cyclomyces* should be transferred to *Hymenochaete*, and the name *Hymenochaete* should be conserved in preference to *Cyclomyces*.

Position of *Hymenochaete* within the Hymenochaetales

The separation of *Pseudochaete* from *Hymenochaete* suggests that within the Hymenochaetales the corticioid type of hymenophore occurs in different lineages. The same is true for the hynoid type, occurring within *Asterodon*, *Hydnochaete* and *Hymenochaete* (WAGNER 2001). Closer relationships between *Asterodon* and *Hymenochaete* are not supported by the phylogenetic analysis, and the affinities are still unresolved for most taxa of *Hydnochaete*. Furthermore, also the cyclolamellate type of hymenophore is evident in different lineages. The cyclolamellate sample of *Coltricia montagnei* included in this study is clearly allied with *Coltricia perennis* and shows no correlation to the cyclolamellate *Cyclomyces fuscus*. Anatomically *C. montagnei* is distinctly separated by coloured basidiospores and the lack of setae.

Within certain genera of the Hymenochaetales, such as *Phellinus*, *Inonotus*, *Fomitiporia*, *Fuscoporia* and *Porodaedalea*, different types of basidiocarps occur side by side. This is also evident for *Hymenochaete*. Morphologically, *Hymenochaete* represents a most heterogeneous genus, comprising different types of basidiocarps, from resupinate to stipitate and hymenophore, such as corticioid, hydnoid, concentric lamellate and poroid. This was also demonstrated for *Hyphodontia* and *Schizopora* (LANGER 1994, 1998), both taxa comprising species with corticioid, hydnoid and poroid hymenophore. Evolutionary tendencies from smooth to hydnoid or to poroid, or from resupinate to pileate or stipitate can not be inferred from our data. The type of hymenophore or the shape of the basidiocarp seem not suitable as characters indicative of evolutionary groups within the Hymenochaetales.

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Table 1. List of taxa, included in the phylogenetic analysis. Taxa included in the cytological investigations are marked by asterisks. Source of fungal material; HB: Helmut Besl, YCD: Yu-Cheng Dai, DSH: David S. Hibbett, MF: Michael Fischer, LK: Lothar Krieglsteiner, TN: Tuomo Niemelä, TAA: Erast Parmasto, TW: Tobias Wagner, REG: Herbarium of the University of Regensburg, and GenBank accession number.

Taxa	**Origin of stocks (stock number)**	**Substrate**	**Accession number**
Hymenochaetales:			
Asterodon ferruginosus Pat.	Finland (YCD 3186) REG	on fallen trunk of *Pinus sylvestris*	AF323735
Coltricia S.F. Gray			
C. montagnei (Fr.) Murrill	USA (96/96) REG	on ground in mixed forest	AY039683
C. perennis (L.: Fr.) Murrill	Germany (MF 92-96) REG	on ground under *Pinus sylvestris*	AF311004
Cyclomyces Fr.:			
C. fuscus Fr.	CBS 100106	unknown	AF385163
C. tabacinus (Mont.) Pat.	India (CBS 311.39)	*Shorea robusta*	AF385164
Fomitiporia punctata (P. Karst.) Murrill [=*Phellinus punctatus* (Fr.) Pilát]	Germany (MF 85-74) REG	*Corylus avellana*	AF311007
Fuscoporia ferruginosa (Schrad.: Fr.) Murrill [=*Phellinus ferruginosus* (Schrad.: Fr.) Pat.]	Germany (MF 82-930) REG	*Fagus sylvatica*	AF311032
Hydnochaete Bres.:			
H. duportii Pat.	France; La Réunion (CBS 941.96)	*Casuarina equisetifolia*	AF323740
H. japonica Lloyd *	Japan (CBS 499.76)	unknown	AF385153
Hymenochaete Lév.:			
Sect. *Hymenochaete*:			
H. berteroi Pat.	France, La Réunion (CBS 733.86)	unknown	AF385160
H. cruenta (Pers.: Fr.) Donk	Germany (HB 149/80) REG	*Abies alba*	AF385152
H. ochromarginata Talbot *	Thailand (CBS 928.96)	unknown	AF385150
H. pinnatifada Burt. *	France, La Réunion (CBS 770.91)	Fabacae	AF385149
H. rubiginosa (Dicks.: Fries) Lév.	Germany (TW 22.9.97) REG	on dead *Quercus* spec.	AF323741
H. tabacina (Sow.: Fr.) Lév. *	Germany (LK 12.10.97) REG	*Salix cinerea*	AF385145
H. tabacina (Sow.: Fr.) Lév. *	France (TAA 95-49)	unknown	
Sect. *Fultochaete* Escobar: Léger:			
H. acanthophysata Léger	Ivory Coast (CBS 925.26)	unknown	AF385144
H. adusta Har. & Pat. *	Thailand (TAA 95-37)	unknown	AF385161
H. cinnamomea (Pers.: Fr.) Bres. *	Germany (LK 27.9.97) REG	deciduous wood	AF385156
H. pseudoadusta Léger & Lanquetin	Thailand (TAA 95-38)	unknown	AF385148
H. separabilis Léger *	France, La Réunion (CBS 738.86)	*Raphia ruffia*	AF385146
Sect. *Gymnochaete* Escobar: Léger:			
H. boidinii Léger & Lanquetin	France, La Réunion (CBS 762.91)	*Solanum auriculatum*	AF385159
H. carpatica Pilat *	Germany (TW 27.9.97) REG	*Acer pseudoplatanus*	AF385158
H. denticulata Léger & Lanquetin	France, La Réunion (CBS 780.91)	unknown	AF385155

Table 1. continued

H. fuliginosa (Pers.) Lév.	France (CBS 933.96)	*Abies* spec.	AF385154
H. separata Cunn. *	France, La Réunion (TAA 95-24)	unknown	AF385147
Sect. *Paragymnochaete* Léger:			
H. cervinoidea Léger & Lanquetin *	France, La Réunion (CBS 736.86)	unknown	AF385157
H. nanospora Léger *	Central African Republic (CBS 924.96)	unknown	AF385151
Inocutis rheades (Pers.) Fiasson & Niemelä [=*Inonotus rheades* (Pers.) Bond. & Sing.]	Germany (TW 385) REG	*Populus tremula*	AF311019
Inonotopsis subiculosa (Peck) Parm. [=*Inonotus subiculosus* (Peck) J. Erikss. & Strid]	China (YCD 2203) REG	*Abies alba*	AF311020
Inonotus hispidus (Bull.: Fr.) P. Karst.	Germany (MF 92-829) REG	*Fraxinus excelsior*	AF311014
Onnia tomentosa (Fr.) P. Karst. [=*Inonotus tomentosus* (Fr.) Teng]	Germany (TW 445) REG	rotten wood	AF311023
Phellinidium ferrugineofuscum (P. Karst.) Fiasson & Niemelä [=*Phellinus ferrugineofuscus* (P. Karst.) Bourd. & Galz.]	Finland (TN 6121) REG	*Picea abies*	AF311031
Phellinus igniarius (L.: Fr.) Quél.	Germany (MF 83-1110a) REG	*Salix fragilis*	AF311033
Phellopilus nigrolimitatus (Romell) Niemelä, T. Wagner & M. Fischer [=*Phellinus nigrolimitatus* (Romell) Bourd. & Galz.]	Germany (MF 85-823) REG	*Larix decidua*	AF311036
Phylloporia ribis (Schumach.: Fr.) Ryvarden [=*Phellinus ribis* (Schum.: Fr.) P. Karst.]	Germany (MF 82-828) REG	*Ribes uva-crispa*	AF311040
Porodaedalea pini (Brot.: Fr.) Murrill [=*Phellinus pini* (Brot.: Fr.) Ames]	France (TW 11.4.97) REG	dead wood of *Pinus* spec.	AF311037
Stipitochaete damicornis (Link) Ryvarden	Puerto Rico (DSH 98-006)	unknown	AF385162
Outgroups:			
Stereum hirsutum (Wild.: Fr.) S.F. Gray	Germany (TW 235) REG	*Fagus sylvatica*	AF385165
Sequences obtained from GenBank:			
Bondarzewia montana (Quél.) Sing.			AF042646
Russula violacea Quél.			AF218559