Phylogenetic relationships in *Auriculariales* and related groups – hypotheses derived from nuclear ribosomal DNA sequences[1]

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In order to estimate phylogenetic relationships in the *Auriculariales sensu* Bandoni (1984) and allied groups we have analysed a representative sample of species by comparison of nuclear coded ribosomal DNA sequences, applying models of neighbour joining, maximum parsimony, conditional clustering, and maximum likelihood.

Analyses of the 5′ terminal domain of the gene coding for the 28 S ribosomal large subunit supported the monophyly of the *Dacrymycetales* and *Tremellales*, while the monophyly of the *Auriculariales* was not supported. The *Sebacinaceae*, including the genera *Sebacina*, *Efibulobasidium*, *Tremelloscypha*, and *Craterocolla*, was confirmed as a monophyletic group, which appeared distant from other taxa ascribed to the *Auriculariales*. Within the latter the following subgroups were significantly supported: (1) a group of closely related species containing members of the genera *Auricularia*, *Exidia*, *Exidiopsis*, *Heterochaete*, and *Eichleriella*; (2) a group comprising species of *Bourdotia* and *Ductifera*; (3) a group of globose-spored species of the genus *Basidiodendron*; (4) a group that includes the members of the genus *Myxarium* and *Hyaloria pilacre*; (5) a group consisting of species of the genera *Protomerulius*, *Tremellodendropsis*, *Heterochaetella*, and *Protodontia*. Additional analyses of the internal transcribed spacer (ITS) region of the species contained in group (1) resulted in a separation of these fungi due to their basidial types.

INTRODUCTION

While today it is obvious that the utilization of microscopic data was the decisive step towards a phylogenetic classification of the basidiomycetes, it was the type of basidium in *Auricularia* which for a long time veiled the phylogenetic position of this genus.

Since Tulasne (1853), variations of the basidium have more and more been regarded as important taxonomical markers, particularly to define higher taxonomic groups: Patouillard (1887) and Brefeld (1888) independently used basidial septation to divide the basidiomycetes into two major groups, 'homo-/hétérobasidiés' (Patouillard 1887) respectively 'Auto-/Protobasidiomyceten' (Brefeld 1888), a principle widely used to date. Since the establishment of the *Auriculariales* (Schroeter 1889), this taxon or its family pendant designated basidiomycetes – mostly exclusive of smuts and rusts – possessing transversely three-septate (auricularioid) basidia, despite the heterogeneity of this group with regard to other characters. Likewise the *Tremellales* (or *Tremellaceae*) included those basidiomycetes with longitudinally cruciate-septate basisia.

When ultrastructural data, particularly concerning the septal pore and the spindle pole body, became available and were found to be useful in basidiomycetes systematics, several revisions were proposed in order to describe more natural groups within the auricularioid fungi (Oberwinkler & Bandoni 1982). Bandoni (1984) integrated micromorphological, ultrastructural, ecological, and developmental data to propose an alternative classification for the *Auriculariales* and *Tremellales*. He excluded from the *Auriculariales* species with simple septal pores and emended this taxon to contain all dolipored heterobasidiomycetes with transversely or longitudinally septate basidia, hyphal haploid stages, and continuous parenthesomes. The *Tremellales* were restricted by him to taxa with the *Tremella* type septal pore complex and unicellular haploid stages. Thus, with the exception of the genus *Auricularia*, the *Auriculariales sensu* Bandoni now included only taxa previously assigned to the *Tremellales* by Martin (1945).

Within the *Auriculariales*, Bandoni (1984) recognized the following families, at this taxonomical level according to basidial types:

(1) *Auriculariaceae*: species of *Auricularia* (Fig. 1).
(2) *Exidiaceae*: species with longitudinally septate basidia and clamp connections (Fig. 2).
(3) *Sebacinaceae*: species with exidioid basidia and lacking clamp connections (Fig. 3).
(4) *Aporpiaceae*: species with myxarioid basidia where the basidial compartments giving rise to basidiospores do not completely fill the meiosporangium (Fig. 4).
(5) *Hyaloriaceae* to include only *Hyaloria* with myxarioid basidia of the gastroid type where basidiospores are not forcibly discharged (Fig. 5).

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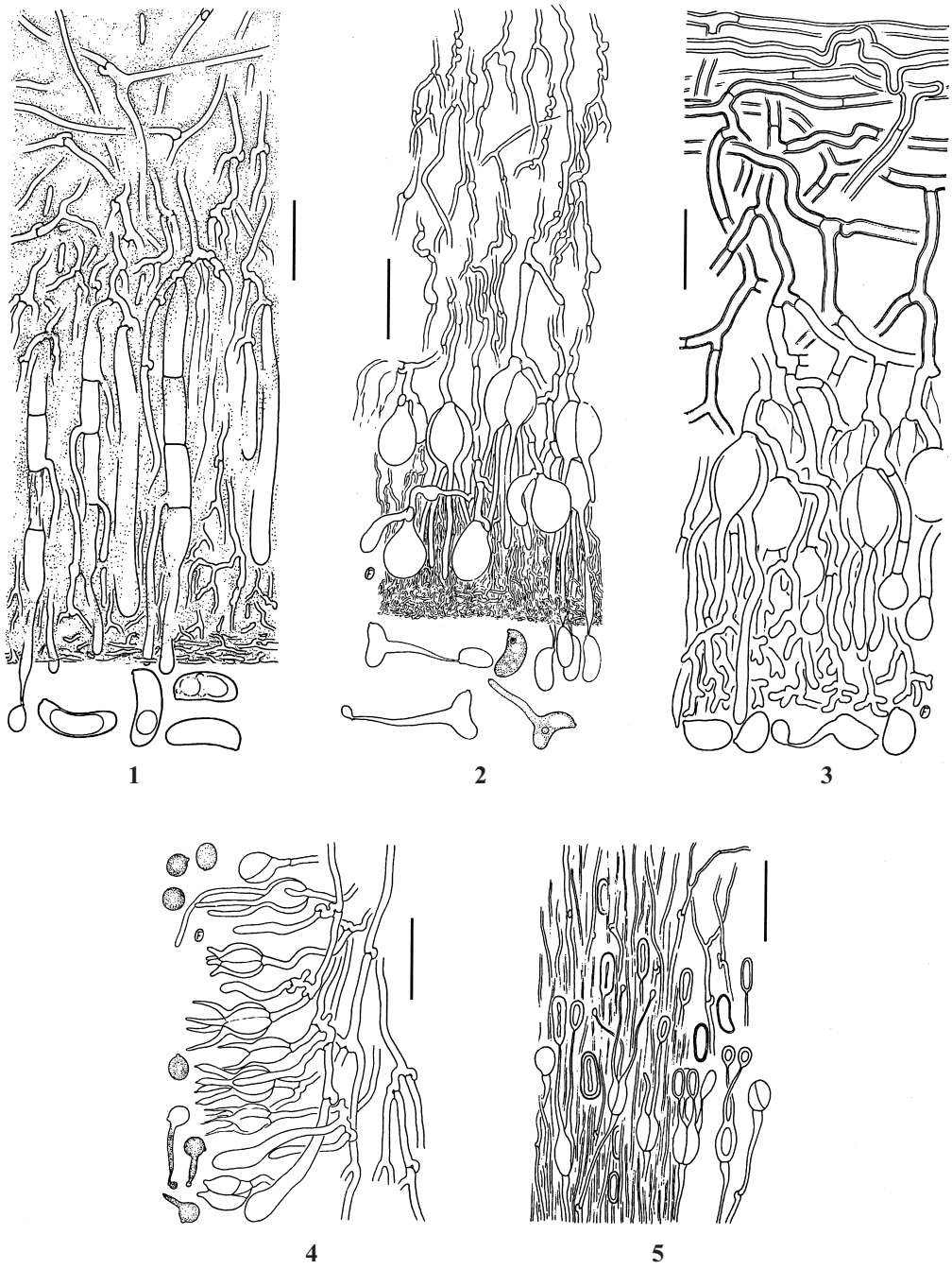

Figs 1–5. Basidial types in *Auriculariales*: sections through the hymenial region.
Fig. 1. *Auricularia auricula-judae*. **Fig. 2.** *Exidiopsis effusa*. **Fig. 3.** *Sebacina incrustans*. **Fig. 4.** *Pseudohydnum gelatinosum*. **Fig. 5.** *Hyaloria pilacre*. Bars = 20 µm.

Wells (1994) modified this system by merging *Aporpiaceae* and *Hyaloriaceae* into his concept of *Hyaloriaceae* and adding *Patouillardinaceae* for species with obliquely septate basidia and *Tremellodendropsidaceae* for species of *Tremellodendropsis* with sometimes partially septate basidia.

The separation of simple-pored species with auricularioid basidia from *Auricularia* has been supported by analyses of ribosomal RNA and DNA sequences (e.g. Gottschalk & Blanz 1985, Swann & Taylor 1993, Berres, Szabo & McLaughlin 1995, Begerow, Bauer & Oberwinkler 1997), but sequences of species of *Auriculariales sensu* Bandoni have been published only sporadically so far. We have analysed a representative sample of species of *Auriculariales* and related groups by means of DNA sequencing and sequence comparison in order to examine whether this group of heterobasidiomycetes is monophyletic, to derive its phylogenetic position in the *Hymenomycetes* and to estimate phylogenetic relationships among its members.

MATERIALS AND METHODS

For the present study we determined nuclear DNA sequences of the ribosomal genome (rDNA) from 93 species of *Auriculariales* and related groups of basidiomycetes. From

these organisms (Table 1), we sequenced the 5′ terminal domain of the nuclear gene coding for the 28 S ribosomal large subunit (LSU) and additionally the internal transcribed spacer (ITS) region including the gene coding for the 5.8 S ribosomal subunit from a subset of 18 species. We also determined ITS sequences of some species with myxarioid basidia, which we finally did not include in our ITS analysis because of aligning uncertainties. In our analyses of the LSU sequences we added eleven sequences determined by other authors (Table 1).

Extraction of genomic DNA

In most cases we extracted genomic DNA from herbarium specimens or from pure cultures (see Table 1), using a DNA isolation protocol which was modified from Edwards, Johnstone & Thompson (1991) and Henrion, Le Tacon & Martin (1992), as already described in detail by Weiß, Yang & Oberwinkler (1998): Ground samples are suspended in a lysis buffer, cell detritus is removed by centrifugation; after an RNase digestion the DNA is precipitated, washed, and eluated. In cases where this procedure did not yield enough DNA of sufficient high quality to be used as amplification template, we alternatively used the Qiagen DNeasy Plant Isolation Kit™ (QIAGEN, Hilden, Germany) according to the manufacturer's prescriptions. The ground and suspended samples are loaded to 2 ml spin columns in which DNA is precipitated and binds to a specific membrane while other soluable components are removed by centrifugation steps before the DNA is eluated from the membrane.

PCR and DNA sequencing

To amplify LSU sequences of about 600 base pairs, we performed the polymerase chain reaction (PCR; Mullis & Faloona 1987) using the primer combination NL1/NL4 (O'Donnell 1993) and a touch-down thermo profile; parameters are given in Weiß *et al*. (1998). ITS sequences were PCR amplified with the primer combination ITS1/ITS4 (White *et al*. 1990); in some cases we alternatively used the primer ITS1F (Gardes & Bruns 1993) instead of ITS1. Concentrations of PCR components for ITS amplification were the same as used in the LSU PCR (Weiß *et al*. 1998). We ran an annealing temperature of 52 °C in all of 35 amplification cycles.

Success and yield of the PCR reations was checked by agarose gel electrophoresis. The PCR products were purified with the QIAquick™ kit (QIAGEN, Hilden, Germany), which works according to the same principles as described above for this manufacturer's DNA isolation kit. We sequenced the double stranded PCR products in both directions using cycle sequencing with ABI PRISM™ dye terminator cycle sequencing kit and the automated sequencer ABI 373A (Applied Biosystems Perkin-Elmer) based on the dideoxynucleotide chain termination method (Sanger, Nicklen & Coulson 1977).

Sequence Comparison Analyses

To estimate phylogenetic relationships of the included species, we analysed the DNA sequences obtained using a variety of methods that follow different mathematical models. Alignments were made using the MegAlign module of the Lasergene software system (DNASTAR, Inc.) implementing the ClustalW algorithm (Thompson, Higgins & Gibson 1994), with some manual adjustions. The length of the LSU alignment is 575 nucleotide sites, the ITS alignment is 531 sites long, consisting of the ITS 1 region, the 5.8 S rDNA, and the ITS 2 region. Since the ITS sequences of myxarioid species were only dubiously alignable to those of the members of the *Exidia/Auricularia* group, we only used the latter to construct an ITS alignment.

The LSU alignment was analysed using the distance methods of neighbour joining (Saitou & Nei 1987) and conditional clustering (Lefkovitch 1993). The latter method gives an estimate of sets of species ('musters') that are supported by the distance matrix; see Weiß *et al*. (1998) for a brief survey of the method. Neighbour joining was combined with a bootstrap analysis (Felsenstein 1985) involving 1000 replication rounds. As genetic distances for neighbour joining as well as for conditional clustering we chose Kimura 2-parameter distances (Kimura 1980) as modified by Felsenstein (1995) with a transition/transversion ratio of 2.0. Additionally, we applied a heuristic maximum parsimony analysis (e.g. Fitch 1971) on the LSU alignment, treating gaps as uncertainties (500 replicates of heuristic search with random addition, tree-bisection-reconnection as branch-swapping algorithm, steepest descent option not in effect, MULPARS option in effect, MaxTrees setting 10000, auto-increased by 100).

In neighbour-joining and in maximum parsimony analysis we included an LSU sequence of *Ustilago cynodontis* as an outgroup organism to root the unrooted dendrograms that were obtained with these methods. The *Ustilago cynodontis* LSU sequence was not included in conditional clustering analysis. Genetic distances for neighbour joining and conditional clustering were computed with the Dnadist module of the PHYLIP software package, version 3.572 (Felsenstein 1995); the modules Neighbor, Seqboot, and Consensus of the same package were used to perform neighbour-joining and bootstrap analysis. For conditional clustering we used the computer program CONCLUS (Lefkovitch 1996); maximum parsimony analysis was carried out with test version 4d64 of the PAUP* computer program, written by D. L. Swofford in 1998. All programs employed, with the exception of CONCLUS, which is DOS executable, were used in Macintosh versions and run on Power Macintosh machines or a UMAX Power Macintosh clone.

On the ITS alignment we applied neighbour joining with bootstrap, conditional clustering and heuristic maximum parsimony analyses with the same parameters and computer programs as specified above. Only heuristic search in parsimony analysis was performed with 10000 rounds of random addition (and is thus very likely to have in fact found the most parsimonious trees), using the 1999 test version 4d65 of the PAUP* computer program. Additionally, a maximum likelihood analysis (Felsenstein 1981) was performed on the ITS alignment, implemented as a heuristic algorithm in the Dnaml module of the PHYLIP package (transition/transversion ratio 2.0, empirical base frequencies, one category of substitution rates, global rearrangements option in effect,

Table 1. List of specimens used in molecular phylogenetic analyses.

Species	Collection no.*	Material†	Provenance	GenBank accession no. (LSU/ITS)‡	
Specimens sequenced for this study					
Agaricus augustus	FO 46713	H	Germany	AF291286	
Agrocybe praecox	FO 46614	H	Germany	AF291287	
Amphinema byssoides	MW 379	H	Germany	AF291288	
Auricularia auricula-judae	MW 446	H	Germany	AF291289	AF291268
A. delicata	USJ 54470	K	Costa Rica	AF291290	AF291269
A. fuscosuccinea	MW 530	K	Costa Rica	AF291291	AF291270
Auricularia mesenterica	FO 25132	H	Germany	AF291292	AF291271
Basidiodendron caesiocinereum var. *caesiocinereum*	MW 320	H	Germany	AF291293	
B. caesiocinereum var. *trachysporum*	MW 393	K	Germany	AF291294	
B. cinereum	MW 377	H	Germany	AF291295	
B. eyrei	MW 529	H	Germany	AF291296	
B. grandinioides	MW 528	H	Germany	AF291297	
B. rimosum	USJ 53885	H	Costa Rica	AF291298	
Basidioradulum radula	FO 6790	H	Germany	AF291299	
Boletus edulis	FO 46874	H	Germany	AF291300	
Bourdotia galzinii	FO 2278	H	Germany	AF291301	
Calocera cornea	MW 55	H	Germany	AF291302	
Ceratobasidium pseudocornigerum	RoKi 197	K	Germany	AF291303	
Ceratosebacina calospora	FO 30317	K	Canada	AF291304	
Collybia dryophila	FO 21603	H	Germany	AF291305	
Coprinus plicatilis	FO 46718	H	Germany	AF291306	
Cortinarius percomis	FO 46876	H	Germany	AF291307	
Craterocolla cerasi	FO 36456	H	Germany	AF291308	
Dacrymyces stillatus	FO 28136	K	Germany	AF291309	
D. variisporus	FO 24617	K	Austria	AF291310	
Dacryomitra pusilla	FO 38346	K	Germany	AF291311	
Dacryopinax spathularia	FO 42687	K	Taiwan	AF291312	
Daedalea quercina	MW 437	H	Germany	AF291313	
Ditiola haasii	RoKi 100	K	Germany	AF291314	
Ductifera pululahuana	KW 1733	H	USA: Illinois	AF291315	
D. sucina	KW 2155	H	USA: California	AF291316	
Efibulobasidium rolleyi	RJB 6889	H	Canada	AF291317	
Eichleriella deglubens	FO 12006	H	Germany	AF291318	AF291272
Exidia glandulosa	MW 355	K	Germany	AF291319	AF291273
E. japonica	TAA 42689	H	Russia (Far East)	AF291320	AF291274
E. pithya	MW 313	K	Germany	AF291321	AF291275
E. recisa	MW 315	H	Germany	AF291322	AF291276
E. saccharina	RoKi 88	F	Germany	AF291323	AF291277
E. thuretiana	MW 373	K	Germany	AF291324	AF291278
E. truncata	MW 365	K	Germany	AF291325	AF291279
Exidiopsis calcea	MW 331	K	Germany	AF291326	AF291280
E. gloeophora	FO 46549	S	Germany	AF291327	
E. grisea	RoKi 162	K	Germany	AF291328	AF291281
E. sp.	FO 46291	K	Germany	AF291329	AF291282
Femsjonia pezizaeformis	FO 25100	K	Germany	AF291330	
Fomes fomentarius	FO 46637	H	Germany	AF291331	
Guepiniopsis buccina	FO 31571	K	Germany	AF291332	
Hapalopilus nidulans	FO 29328	H	Germany	AF291333	
Heterochaete hirneoloides	USJ 55480	H	Costa Rica	AF291334	AF291283
H. shearii	USJ 54609	H	Costa Rica	AF291335	AF291284
H. sp.	USJ 55639	H	Costa Rica	AF291336	AF291285
Heterochaetella dubia	RoKi 96	H	Germany	AF291337	
Hyaloria pilacre	TI 2768	H	Venezuela	AF291338	
Hymenochaete rubiginosa	FO 46767	H	Germany	AF291339	
Hypholoma fasciculare	FO 46696	H	Germany	AF291340	
Inonotus nodulosus	FO 46797	H	Germany	AF291341	
Kuehneromyces mutabilis	FO 46698	H	Germany	AF291342	
Lactarius torminosus	MW 25	H	Germany	AF291343	
Lepiota oreadiformis	FO 46679	H	Germany	AF291344	
Marasmius graminum	FO 46723	H	Germany	AF291345	
Merulius tremellosus	MW 332	K	Germany	AF291346	
Mycena pura	FO 46623	H	Germany	AF291347	
Myxarium granulum	USJ 54532	H	Costa Rica	AF291348	
M. grilletii	RoKi 218	H	Germany	AF291349	

M. mesonucleatum	USJ 55354	H	Costa Rica	AF291350
M. nucleatum	ZP TRE2M	K	Portugal	AF291351
M. subhyalinum	MW 527	H	Germany	AF291352
M. sp.	FO 35744	H	Norway	AF291353
Oudemansiella mucida	FO 46729	H	Germany	AF291354
Pluteus pouzarianus	HKAS 31456	H	Germany	AF291355
Polyporus varius	FO 46745	H	Germany	AF291356
Protodontia subgelatinosa	USJ 54661	H	Costa Rica	AF291357
Protomerulius africanus	Ryv. 9800 (O)	H	Kenya	AF291358
P. brasiliensis	Ryv. 19735 (O)	H	Argentina	AF291359
Pseudohydnum gelatinosum	MW 298	H	Germany	AF291360
Russula cyanoxantha	FO 46621	H	Germany	AF291361
Sarcodon imbricatus	FO 46793	H	Germany	AF291362
Sebacina aff. *epigaea*	MW 526	H	Germany	AF291363
S. dimitica	MW 525	H	Germany	AF291364
S. incrustans	MW 524	H	Germany	AF291365
S. vermifera (*sensu* Warcup & Talbot 1967)	NIAES 5830	K	Australia	AF291366
S. sp.	RoKi 179	K	Germany	AF291367
Stropharia albonitens	FO 46892	H	Germany	AF291368
Stypella vermiformis	MW 417	H	Germany	AF291369
Suillus grevillei	FO 46699	H	Germany	AF291370
Trametes gibbosa	FO 46707	H	Germany	AF291371
Tremella encephala	MW 338	K	Germany	AF291372
T. foliacea	MW 299	K	Germany	AF291373
T. moriformis	MW 335	K	Germany	AF291374
Tremellodendropsis sp.	USJ 54427	H	Costa Rica	AF291375
Tremelloscypha gelatinosa	GG 23605 (KW)	H	Mexico	AF291376
Tremiscus helvelloides	MW 337	H	Germany	AF291377
Tricholoma vaccinum	FO 46876	H	Germany	AF291378

LSU sequences from other studies

	GenBank accession no.	Sequence author
Amanita muscaria	AF024465	Weiß 1998
Calocera viscosa	AF011569	Weiß 1997
Filobasidiella neoformans	L14068	Fan et al. 1994
Protodontia piceicola	AF291266	Begerow 2000
Sebacina epigaea	AF291267	Begerow 2000
Sirobasidium magnum	AF042242	Chen 1998
Thelephora palmata	AF291265	Eberhardt 2000
Tremella fuciformis	AF042254	Chen 1998
Tremella mesenterica	AF011570	Weiß 1997
T. nivalis	AF042237	Chen 1998
Ustilago cynodontis	AF009881	Begerow 1997

* Acronyms and herbaria: FO, F. Oberwinkler (private collection); GG, G. Guzmán; HKAS, Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica, People's Republic of China; KW, K. Wells (private collection); MW, M. Weiss (private collection); NIAES, National Institute of Agro-Environmental Sciences, Japan; O, Herbarium of the University of Oslo; RJB, R. J. Bandoni (private collection); RoKi, R. Kirschner (private collection); Ryv, L. Ryvarden; TAA, Herbarium of the Institute of Zoology and Botany of the Estonian Academy of Sciences, Tartu; TI, T. Iturriaga, Plant Pathology Herbarium, Cornell University; USJ, Herbarium of the University of Costa Rica, San José; ZP, J. P. Sampaio (private collection).

† F, fresh collection; H, herbarium material; K, pure culture; S, spore print.

‡ ITS sequences (ITS 1, 5.8 S, ITS 2) were not determined for all specimens.

performing 10 rounds of random addition). Since aligning outgroup sequences to the alignment of ITS sequences as marked in Table 1 was not possible without conspicuous uncertainties, we did not include an outgroup species in our ITS analysis.

RESULTS

Phylogenetic hypotheses derived from the LSU alignment

The results obtained with neighbour-joining analysis from the LSU alignment can be inferred from the dendrogram shown in Fig. 6. Among well supported groups of higher taxonomic levels in the heterobasidiomycetes are clusters representing *Tremellales*, *Dacrymycetales*, and the family of *Sebacinaceae*, which was split away from all the remaining genera of *Auriculariales sensu* Bandoni (1984) that were present in our study. *Ceratobasidium pseudocornigerum* was placed as a neighbour to the *Dacrymycetales* cluster; the species pair of *Exidiopsis gloeophora* and *Ceratosebacina calospora* appeared adjacent to the main group of *Auriculariales*. In the homobasidiomycetes, we found well supported clusters representing *Agaricales*, *Boletales*, *Russulales s. str.* (the latter two appeared as sister taxa), and *Hymenochaetales*.

Bootstrap values exceeding 95% suggest that the following clusters of species assigned to the *Auriculariales* represent natural groups:

(1) a cluster containing the genera *Auricularia*, *Exidia*, *Exidiopsis*, and *Heterochaete*, which we will refer to as the '*Exidia*/*Auricularia* group'.

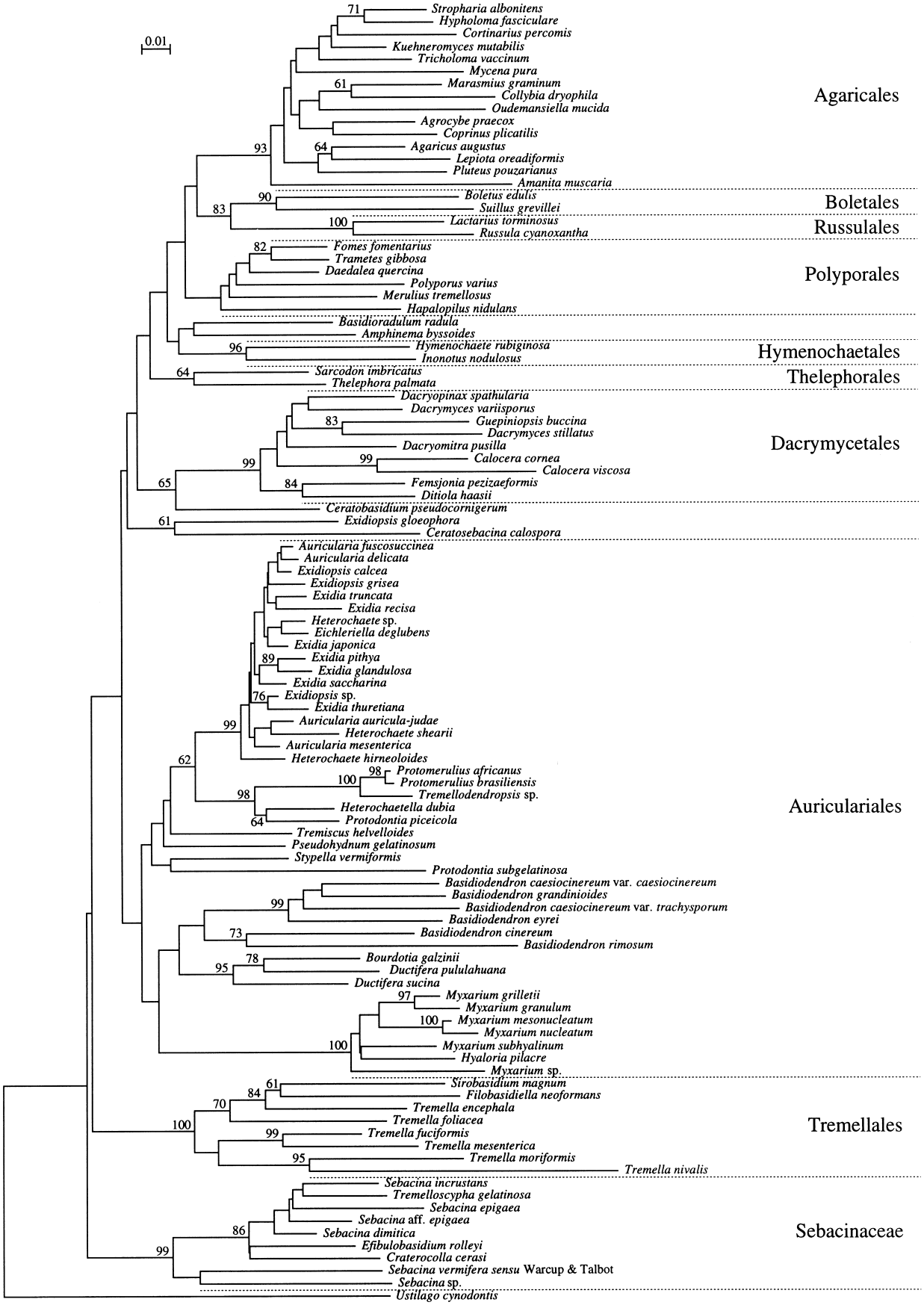

Fig. 6. Neighbour-joining analysis of an alignment of nuclear DNA coding for the 5′ terminal domain of the 28 S ribosomal large subunit. Genetic distances were computed according to the Kimura two-parameter model. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. The topology was rooted with *Ustilago cynodontis*. Numbers on branches are bootstrap values (1000 replicates, numbers rounded to next integers, values smaller than 60% not shown).

(2) The genus *Myxarium* extended by *Hyaloria pilacre*.
(3) A group which comprises members of the genera *Bourdotia* and *Ductifera*.
(4) A group containing *Protomerulius brasiliensis*, *Protomerulius africanus*, a species of *Tremellodendropsis*, *Heterochaetella dubia*, and *Protodontia piceicola*.
(5) A group comprising the species of genus *Basidiodendron* with globose basidiospores.

Heuristic search for the most parsimonious tree topology derivable from the LSU alignment yielded 96 equally parsimonious trees, from which we computed a strict consensus tree (not shown). Compared to the results of the neighbour-joining analysis (Fig. 6) as described above, the maximum parsimony consensus tree gives essentially the same phylogenetic hypotheses with the following exceptions. A cluster containing both the *Myxarium* group and the species pair of *Basidiodendron cinereum* and *B. rimosum* was placed outside the *Auriculariales* main group; the species pair of *Exidiopsis gloeophora* and *Ceratosebacina calospora* was placed amongst homobasidiomycetous taxa next to *Boletales* and *Russulales*; the *Bourdotia-Ductifera* group detected significantly in neighbour-joining analysis remained unresolved, and *Pseudohydnum gelatinosum* was placed in one cluster with *Stypella vermiformis* and *Protodontia subgelatinosa*.

Conditional clustering analysis of the LSU alignment detected musters that are for the most part compatible to the species groups obtained by neighbour-joining and maximum parsimony analysis. In addition to these compatible musters, conditional clustering also revealed a muster comprising *Pseudohydnum gelatinosum* and *Protodontia subgelatinosa*, and a singleton muster containing *Stypella vermiformis*.

Phylogenetic hypotheses derived from the ITS alignment

The dendrogram obtained by neighbour-joining analysis of the ITS alignment is shown in Fig. 7. High bootstrap support was computed for the cluster of *Auricularia delicata* and *Auricularia fuscosuccinea*. Also high bootstrap values were achieved for the pair of *Exidia glandulosa* and *Exidia pithya* and for the pair of *Eichleriella deglubens* and the unassigned species of genus *Heterochaete*. Other phylogenetic relationships could not be significantly resolved.

The same groups as significantly validated in neighbour-joining bootstrap analysis are also present in the tree with the highest likelihood that was found in heuristic maximum likelihood analysis of the ITS alignment (not shown). With the exception of the species pair of *Auricularia delicata* and *Auricularia fuscosuccinea*, these groups are also present in the strict consensus (topology not shown) of 10 equally parsimonious trees which we obtained by heuristic maximum parsimony analysis of the ITS alignment.

As a result of conditional clustering of the ITS data set the following musters were found: the cluster of the *Auricularia* species; the species pair of *Eichleriella deglubens* and the unassigned *Heterochaete* species; and a cluster comprising *Exidiopsis grisea*, the unassigned species of *Exidiopsis* and all of the *Exidia* species that were present in this study.

DISCUSSION

Relationships between groups at higher taxonomic levels

It is characteristic for the present study as well as it is often the case in molecular phylogenetic studies (e.g. Swann & Taylor 1993, Berres *et al*. 1995, Hibbett *et al*. 1997) that topologies remain poorly resolved at higher hierarchical levels. However, the phylogenetic reconstruction estimated by neighbour joining from our LSU data (Fig. 6) contains arrangements that, although without significant bootstrap support, may reflect aspects of a natural classification of the *Hymenomycetes*.

Thus, the consecutive placement of the clusters of *Auriculariales*, *Ceratosebacina*, *Ceratobasidium*, and *Dacrymycetales* is in agreement with close relationships between these phragmobasidious and holobasidious taxa as proposed by Martin (1945), who stressed the resemblance of the 'tenuous and more arid Sebacinas' and *Ceratobasidium* and also proposed a linkage of the latter genus to the *Dacrymycetales via Cerinomyces crustulinus*. Both *Ceratobasidiaceae* and *Dacrymycetaceae*, together with the *Tulasnellaceae*, were included in Lowy's *Metabasidiomycetidae* (Lowy 1968), which he regarded as intermediate between his concepts of *Heterobasidiomycetidae* and *Homobasidiomycetidae*. A similar dolipore ultrastructure (dolipore with continuous parenthesomes) unites the groups of the *Auriculariales sensu* Bandoni (1984), *Tulasnellales*, and *Dacrymycetales* (Wells 1994). The same type of dolipore can be found in the *Hymenochaetales* (Oberwinkler 1985).

LSU sequences of species of *Tulasnella* could not be unambigously aligned to the other sequences used in this study and, therefore, were excluded from the phylogenetic analyses. Difficulty in treating *Tulasnella* in comparative molecular analyses has been reported by Gottschalk & Blanz (1985) in their study of 5 S rRNA of various basidiomycetes. These problems are possibly due to a higher substitution rate in some parts of the ribosomal genome in this group. On the other hand, molecular analyses using the complete 18 S rDNA gene indicated a close relationship of a *Tulasnella* species to the Auriculariales (M. Weiß, unpubl.), which is correlated with ultrastructural data.

A taxonomic problem is the relationship among the species of the genus *Ceratobasidium*. While *C. calosporum*, the type species of the genus, has dolipores with continuous parenthesomes and apically partially septate basidia (F. Oberwinkler, unpubl.), the parenthesomes of other species that have been ascribed to this genus are perforated with large pores (Wells 1994). The character of discontinuous parenthesomes was used by Roberts (1999) as a constitutive part of his definition of the order *Ceratobasidiales*. More species of *Ceratobasidium* need to be studied ultrastructurally and by molecular analyses to elucidate phylogenetic relationships in these holobasidiate heterobasidiomycetes.

Our LSU analyses provide a conceivable hypothesis on the evolutionary root of the gilled basidiomycetes. A bootstrap value of 83% suggests that the *Boletales* are the sister group to the *Russulales* (at least *sensu stricto*, comprising *Russula* and *Lactarius*), and the neighbour-joining topology (Fig. 6) points to a common evolutionary origin of *Agaricales*, *Boletales*, and

Russulales and hence for the vast majority of gilled basidiomycetes. It is, of course, probable that some gilled representatives (e.g. *Lentinus*, *Panus*) are members of other lines of descent (Hibbett *et al*. 1997).

Dacrymycetales

This is so far the largest number of species of the *Dacrymycetales* which have been included in a molecular phylogenetic study, but much more species would have to be added to derive meaningful hypotheses about phylogenetic relationships in this well-defined group of heterobasidiomycetes the internal classification of which is still unclear (Reid 1974, Oberwinkler 1993).

In the neighbour-joining LSU analysis (Fig. 6) the cyphelloid species of *Ditiola haasii*, a nearly non-pigmented dacrymycetous species (Oberwinkler 1989), and *Femsjonia pezizaeformis* are clustered with a bootstrap support of 84%. Members of *Femsjonia* and *Ditiola* are similar in their heterogenous anatomical structure with thick-walled hyphae in the stipe and thin-walled hyphae in the hymenium, which led Reid (1974) to synonymize *Femsjonia* with *Ditiola*.

Calocera cornea and *C. viscosa* were significantly joined, contradictory to Oberwinkler's (1993) reintroduction of the genus *Corynoides* to accomodate *Calocera cornea* and *Dacryomitra pusilla*, clavarioid species that lack conspicuous rooting bases and the three-zonal hyphal system characteristic of *Calocera viscosa*. The position of *D. pusilla* adjacent to the species of *Calocera* reflects a close relationship of these clavarioid taxa which have been united in *Calocera* by several authors (e.g. McNabb 1965, Reid 1974).

The analyses did not unite the species of *Dacrymyces* present in this study, indicative of the unsatisfactory limitations of this genus and particularly its vague generic delimitation to *Guepiniopsis* (Reid 1974). This is reflected by a bootstrap value of 83% for the species pair of *G. buccina*, the type species of *Guepiniopsis*, and *Dacrymyces stillatus*, the type species of *Dacrymyces*.

Tremellales

Optimal bootstrap support was obtained for the cluster containing the species ascribed by Bandoni (1984) to his emended concept of *Tremellales* which were present in our study. The separation of at least two evolutionary lines with members possessing longitudinally cruciate-septate basidia, the discovery of which started when Brefeld (1888) distinguished *Exidia* from *Tremella* on the basis of different germination types, can meanwhile be held as proven.

Chen's (1998) results in his morphological and molecular phylogenetic studies of *Tremella* are, in general, in agreement with our results. The studies differ in that Chen found *Sirobasidium* as a sister taxon to the genus *Tremella*, whereas in our analyses *Sirobasidium* appears to have evolved within the *Tremella* group. If this holds true, then it supports Olive's theory that the *Tremella* basidial type precedes the catenulate basidial type of *Sirobasidium* (Olive 1958), in contrast to what is believed to be the course of evolution of this character by other authors (e.g. Möller 1895, Bandoni 1957). However, recent molecular studies (Fell *et al*. 2000) propose a more complex phylogeny for *Tremellales* and allied genera, suggesting that both *Tremella* and *Sirobasidium* might be polyphyletic.

The resupinate species of Auriculariales

Since the Tulasne brothers (Tulasne & Tulasne 1871) erected the genus *Sebacina* to split resupinate basidiomycetes with longitudinally septate basidia from *Thelephora*, generic concepts in this group of heterobasidiomycetes have often been a subject of controversial discussion. Other taxonomic units at generic or infrageneric level were introduced to make possible refined classifications of species assignable to this complex. *Exidiopsis* was established to accommodate species strongly resembling species of *Exidia* in their micromorphology (Johan-Olsen, *in* Brefeld 1888). Habit of growth and basidiocarp morphology were the segregating characters of *Heterochaete* (Patouillard, *in* Patouillard & Lagerheim 1892), *Stypella* (Möller 1895), *Eichleriella* (Bresadola 1903), and *Serendipita* (Roberts 1993). Thick-walled cystidia distinguished *Heterochaetella* (Bourdot 1920), gloeocystidia defined *Bourdotia* (Bresadola 1908) and *Endoperplexa* (Roberts 1993), and basidial morphology was the basis for *Basidiodendron* (Rick 1938), *Ceratosebacina*, and *Microsebacina* (Roberts 1993).

Authors have proposed a variety of systems (e.g. Bourdot & Galzin 1928, McGuire 1941, Ervin 1957, Wells 1961, 1994, Luck-Allen 1963, Oberwinkler 1964, Wells & Oberwinkler 1982, Roberts 1993), but there is no unanimously accepted taxonomic concept. McGuire's (1941) statement that 'it is quite probable that our inability to recognize the true relationships in the group is due simply to lack of sufficient information concerning these inconspicuous forms' is still true. Nevertheless, our DNA analyses provide some indications of a more natural grouping of these fungi parts of which have already been proposed.

Sebacinaceae

Supported by a bootstrap value of 99% in the LSU neighbour-joining analysis (Fig. 6), we found a cluster well corresponding to the family *Sebacinaceae*, which was erected for species with exidioid basidia without clamp connections throughout the fructifications and thickened walls of tramal hyphae (Wells & Oberwinkler 1982). It had been suspected that the absence of clamp connections and the consistency of the fruit bodies were characters to separate the *Sebacina incrustans* group from other resupinate forms, particularly to those of *Exidiopsis*, and to connect this group with *Tremellodendron*, which contains clavarioid species (McGuire 1941, Ervin 1957, Wells 1961).

Wells & Oberwinkler (1982) included in their concept of *Sebacinaceae* the genera *Sebacina s. str.*, *Tremellodendron*, *Tremelloscypha*, and, tentatively, *Efibulobasidium*, which was later transferred to the *Exidiaceae* by Wells (1994). Species of *Efibulobasidium* can be found as pustules on corticate twigs or leaves (Wells 1975), while members of the other genera included in the *Sebacinaceae* comprise terricolous species, which is quite an unusual ecological feature in the jelly fungi.

Our analysis supports the inclusion of *Efibulobasidium* in the *Sebacinaceae* (Fig. 6) and expands the morphological and

ecological range of this family by the addition of *Craterocolla cerasi*, a pulvinate wood-decayer with a macroscopically different anamorphic stage (Brefeld 1888), and *Sebacina vermifera sensu* Warcup & Talbot (1967). Both species fit in the *Sebacinaceae* concept in so far as they lack clamp connections. The undetermined species of *Sebacina* also belonging in this group (Fig. 6) is a thin, hyaline, resupinate form also lacking clamp connections (R. Kirschner, pers. comm.).

There is some taxonomic confusion regarding *Sebacina vermifera*. As originally described by Oberwinkler (1964) from Bavaria, this species possesses subbasidial clamp connections; the type grew intrahymenial in a fructification of *Uthatobasidium fusisporum* on rotten wood. Later, similar organisms were isolated from orchid roots in Australia (Warcup & Talbot 1967), and one of such isolates is included in this study, from within vesicular-arbuscular mycorrhizas of *Phyllanthus calycinus*, and from soil (Warcup 1988). Additional collections were reported from Denmark (Hauerslev 1976), Great Britain, and the Balearic Islands (Roberts 1993, 1996). There is a conspicuous deviance in the measurements of the extremely long, vermiform basidiospores (Roberts 1993). In contrast to the type material, these fungi were always characterized as lacking clamp connections.

Since the isolate obtained from orchid roots which we analysed in this study is clearly to be included in the *Sebacinaceae* cluster (Fig. 6) and all of the species of this cluster lack clamp connections, there is evidence that

(1) *Sebacina vermifera* in its original sense is not a member of the *Sebacinaceae*;
(2) *Sebacina vermifera sensu* Warcup & Talbot (1967) possibly represents a new species or an assemblage of closely related new species, as was also considered by Warcup (1988), of the *Sebacinaceae*. The name *Serendipita vermifera* is not applicable to these, since it is based on the clamped *Sebacina vermifera*.

Regarding the spectrum of habitats that now can be related to species of *Sebacina* – Warcup (1988) even observed the forming of ectomycorrhizas with *Melaleuca uncinata* – mycorrhizal associations may be considered also for other (at least for the terrestrial) species in the *Sebacinaceae*.

Ceratosebacina

Roberts (1993) erected the genus *Ceratosebacina* for inconspicuous, resupinate species with longitudinally septate basidia that resemble in shape the holobasidia in species of *Ceratobasidium*. In cultures of the type species, *Ceratosebacina longispora*, Ingold (1992) observed globose microconidia, a unique character in the *Auriculariales*.

In our molecular analyses *Ceratosebacina calospora* was placed outside the main group of *Auriculariales* and clustered with *Exidiopsis gloeophora* (Fig. 6), another species with quite inconspicuous fructifications. A distinctive character of the latter species is the hyphae which become up to 5 µm wide adjacent to the basidial bases and often contain yellowish cytoplasm (Oberwinkler 1964). Since *Exidiopsis gloeophora* does not fit into the concept of *Ceratosebacina* and according to our analyses it is very distant from other members of *Exidiopsis*, this species might be placed in a new genus. However, regarding the lack of significant morphological characters for these fungi, there is a need to include more species in molecular phylogenetic analyses before new taxa are introduced.

'Calospored' species exist in *Ceratosebacina*, *Ceratobasidium*, and *Tulasnella*, e.g. *Tulasnella deliquescens*. It may well be that this is indicative of a close phylogenetic relationship between these groups, all the more so as *Ceratobasidium calosporum* possesses imperforate parenthesomes and partially septate basidia (see above; F. Oberwinkler, unpubl.). Possibly also the *Sebacinaceae* might be connected to this complex: Warcup & Talbot (1967) noted that there are similar 'moniliform blastospores' in cultures of a *Tulasnella* species (*Tulasnella deliquescens*, *fide* Roberts 1999) and cultures of the *Sebacina* species which they ascribed to *Sebacina vermifera* (see above). Ingold (1992) reported monilioid hyphae in cultures of *Ceratosebacina longispora*. Another connection may be that members of *Ceratosebacina*, *Ceratobasidium*, *Tulasnellales*, and *Sebacinaceae* are mycobionts in orchid mycorrhizas (see above and the references compiled by Roberts (1999)).

Gloeocystidiate species

Species in the resupinate *Auriculariales* possessing gloeocystidia have originally all been ascribed to one genus, *Bourdotia*, which had been introduced by Bresadola (1908) as a subgenus of *Sebacina* to accommodate *Sebacina galzinii* As noted by several authors, e.g. Rogers (1933), McGuire (1941), Ervin (1957), and Wells (1959), most of the species then included in *Bourdotia* showed the unusual character that in older fructifications collapsed basidia form an involucre-like sheath around fertile hyphae, a feature missing in *Bourdotia galzinii*. Luck-Allen (1963) used this character to separate all species but the type from *Bourdotia* and to transfer them to *Basidiodendron*. This was widely accepted and is used in current systematical treatments of these species (Wells & Raitviir 1975, Wells 1994).

Our analyses partly support this concept. In neighbour-joining analysis the species of *Basidiodendron* present in our study form a cluster, though not significantly supported, which is separated from *Bourdotia galzinii* (Fig. 6). The round-spored species of *Basidiodendron* constitute a well-supported subgroup, to which *Basidiodendron cinereum* and *Basidiodendron rimosum* are connected. However, the latter species appear adjacent to the genus *Myxarium* in heuristic maximum parsimony analysis. The two subgroups of *Basidiodendron* found do not reflect the two subgenera *Basidiodendron* and *Asarcogloea* proposed by Luck-Allen (1963): she placed *Basidiodendron rimosum* and *B. cinereum*, which were clustered together in our dendrograms, in different subgenera.

The cluster of the round-spored species of *Basidiodendron* is morphologically homogeneous. Because the genetic distance between *B. caesiocinereum* var. *caesiocinereum* and *B. caesiocinereum* var. *trachysporum* inferred from the LSU 5′ terminal domain is high, suggesting that they represent distinct species, we have sequenced an additional collection of each variety. Intra-variety distances below 0.4% and inter-variety distances exceeding 7.6% support the recognition of the varieties as distinct species. However, more specimens should

be examined morphologically and by molecular analysis before this should formally be proposed.

In our phylogenetic estimates (Fig. 6) *Bourdotia galzinii*, the type species of the genus *Bourdotia*, is included in a well-supported group together with *Ductifera pululahuana*, the type species of *Ductifera* (*fide* Wells 1958), and *D. sucina*, two gloeocystidiate species of the *Auriculariales* which develop cerebriform fructifications. Interestingly, Bourdot & Galzin (1928) had included the species now known as *Ductifera pululahuana* in their concept of the genus *Bourdotia*, treating *Bourdotia galzinii* as a subspecies of *Bourdotia pululahuana*. Species of *Ductifera* have exidioid basidia (Wells 1958) as present in species of *Basidiodendron*, while basidia of *Bourdotia galzinii* are myxarioid (Wells 1959). Wells & Raitviir (1975) distinguish between the 'petiolate' type of basidium of *Bourdotia galzinii* and the 'sphaeropedunculate' type present in species of *Myxarium* and consider these two types as convergently evolved. Our results provide further evidence for this hypothesis and suggest that *Bourdotia* and *Ductifera* should be united into one genus.

The Exidia/Auricularia *group*

We detected a high similarity in the studied part of the LSU rDNA gene for the species of *Exidia*, *Exidiopsis*, *Heterochaete*, *Eichleriella*, and *Auricularia*, which were present in our analyses (Fig. 6). This DNA region is evidently not appropriate to reconstruct phylogenetic relationships among species of these genera; however, our results strongly support their monophyletic origin. Particularly, the species of *Sebacina s. str.* were separated from the genus *Exidiopsis* with high significance (see also above).

The *Exidia/Auricularia* group unites species with different basidial types, the transversely septate basidium of *Auricularia* (Fig. 1) and the longitudinally cruciate-septate type present in *Exidia* and the remaining genera included in this group (Fig. 2). This is in agreement with Bandoni's hypothesis (1984) 'that the basidium of *Auricularia* species is directly derived from that of the exidioid fungi.' For more than hundred years, starting with the introduction of microscopic characters as a base for taxonomy in mycology, species with the auricularioid type were separated from species with the exidioid type, though there exist intermediate forms between the two basidial types, e.g. *Patouillardina cinerea* with obliquely septate basidia (Oberwinkler 1982, Bandoni 1984, Wells 1994).

Species of *Auricularia* and *Exidia* are similar with respect to morphology and anatomy of fruitbodies, pigmentation, and also in their anamorphic stages. As has already been noted and regarded as a primary taxonomic character by Brefeld (1888), species of *Auricularia*, *Exidia*, and *Exidiopsis* are capable of forming C-shaped ('häkchenförmige') microconidia upon basidiospore germination. Microconidia of this sort have also been reported from *Heterochaete* (Kisimova-Horovitz, Oberwinkler & Gómez 1997). Yet the formation of C-shaped microconidia is not exclusively found in members of the *Exidia/Auricularia* group and has been observed in *Myxarium nucleatum* (Ingold 1984), *Ductifera sucina* (Möller 1895; as *Exidia sucina*), *Basidiodendron* (F. Oberwinkler, unpubl.), and even in *Dicellomyces scirpi* (Ingold 1985) and *Platygloea effusa* (Ingold 1988). Moreover, there are microconidia with a distinct morphology in *E. saccharina* (Brefeld 1888; as *Ulocolla saccharina*), and in *E. recisa* (Ingold 1995). The occurrence of C-shaped microconidia is thus of only limited significance as a phylogenetic marker.

We have tried to gain a higher resolution of phylogenetic relationships in the *Exidia/Auricularia* group by applying sequences of the more variable ITS region (Fig. 7). In doing so we found too large a variation outside the 5.8 S part to align without too much ambiguities sequences of members of the *Exidia/Auricularia* group to sequences from other species of the *Auriculariales* and thus decided not to include an outgroup sequence in our ITS analyses.

Neighbour-joining, maximum likelihood, and also maximum parsimony analyses of our ITS data set yielded consistent results (Fig. 7). The unrooted dendrograms allow for a separation of the auricularioid from the exidioid species. The exidioid species, which are very similar in their micromorphology, did not cluster according to the genera in which they are currently placed.

We included in our data set *Exidia japonica*, the type species of *Tremellochaete* (Raitviir 1964). Since according to its ITS sequence it was positioned among other species of *Exidia*, even in close neighbourhood to *E. glandulosa*, the type species of *Exidia*, we think that *Tremellochaete* should be retained in *Exidia*. It may well be that current generic delimitations in the exidioid species of the *Exidia/Auricularia* group overemphasize fruitbody morphology.

The phylogenetic relevance of the myxarioid basidium

The occurrence of myxarioid basidia, i.e. longitudinally septate basidia in which the basidial compartments giving rise to basidiospores do not completely fill the basidium, a character that was often neglected in older taxonomic treatments, is considered an important marker in modern phylogenetic concepts of the *Auriculariales* (Bandoni 1984, Wells 1994). Roberts (1998) rejected this concept and, emphasizing fruitbody morphology, transferred the type species of *Myxarium*, *M. nucleatum*, to *Exidia*. Myxarioid basidia are a constant character in species of *Myxarium*, *Stypella*, *Pseudohydnum*, *Tremiscus* and others, but this type of basidium has also been noted in species of *Exidiopsis* (Wells & Raitviir 1977), *Eichleriella* (Wells & Raitviir 1980), and even for *Sebacina epigaea* (Roberts 1996). However, in the latter genera this feature seems to be noticeable only sporadically.

According to the LSU neighbour-joining analysis, the character of the constant occurrence of myxarioid basidia is not randomly distributed over the genera studied. With the exception of *Bourdotia galzinii*, the basidial type of which was regarded as a special, 'petiolate' type by Wells & Raitviir (1975), consistently myxarioid basidia are present only in several taxa which form consecutive branches basal to the *Exidia/Auricularia* group in the phylogenetic tree (Fig. 6), and in a well supported group perfectly matching the genus *Myxarium* extended by *Hyaloria pilacre*. This seems to support the hypothesis that the constant occurrence of myxarioid basidia is indeed a valuable character for a natural classification in the *Auriculariales*, which is emphasized by the significant

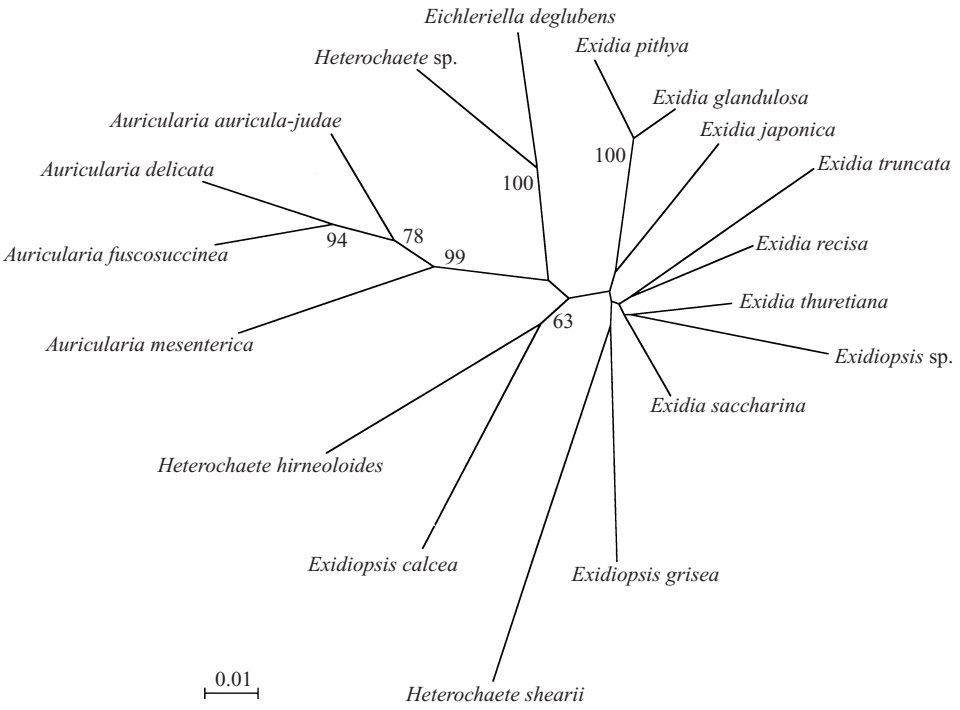

Fig. 7. Neighbour-joining analysis of an alignment of the nuclear internal transcribed spacer (ITS) region including the 5.8 S rDNA gene. Genetic distances were computed according to the Kimura two-parameter model. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. Numbers on branches are bootstrap values (1000 replicates, numbers rounded to next integers, values smaller than 60% not shown).

inclusion of *Hyaloria pilacre* in the *Myxarium* group: While the latter species does not forcibly discharge its basidiospores (Fig. 5) and is also distinguished from species of *Myxarium* by its stipitate fruitbodies, it clearly represents the myxarioid basidial type as do all species of *Myxarium* examined (Fig. 4).

The *Myxarium*/*Hyaloria* group is not only supported by a bootstrap value of 100%, but also by the large genetic distance separating this group from other taxa of the *Auriculariales* in the LSU neighbour-joining analysis (Fig. 6). Roberts's hypothesis (Roberts 1998) that the genus *Myxarium* is an artificial taxon is not supported; Bandoni's opinion (Bandoni 1984) that *Hyaloria* would possibly 'be better placed with other myxarioid fungi rather than in a separate family', as also suggested by Wells (1994), is confirmed.

Roberts's proposal (1998) to transfer *Protodontia subgelatinosa* to the genus *Stypella* is in agreement with our results. *P. subgelatinosa* appears joined to *S. vermiformis* and quite distant to *P. piceicola*, the other species of *Protodontia* present in this study.

In addition to the *Myxarium*/*Hyaloria* group, we have detected another strongly supported group of myxarioid species in our analyses, comprising *Heterochaetella dubia*, *Protodontia piceicola*, a species of *Tremellodendropsis*, *Protomerulius brasiliensis*, and *P. africanus*. Though exhibiting a wide range of forms of fruitbodies, i.e. resupinate, odontioid, clavarioid, and even poroid fructifications, all of these species possess a characteristic combination of two characters: all have myxarioid basidia and thick-walled hyphae (Bandoni, Oberwinkler & Wells 1982, Setliff & Ryvarden 1982). While thick-walled hyphae are known to occur also in a number of other species of the *Auriculariales*, e.g. in *Exidiopsis* (Wells & Raitviir 1977), *Eichleriella* (Wells & Raitviir 1980), and in members of the *Sebacinaceae* (Oberwinkler 1963, Wells & Oberwinkler 1982), this is a rather unusual feature of myxarioid species. However, the poroid species, such as those belonging to *Aporpium*, *Elmerina*, *Protodaedalea*, and *Protomerulius* form fruitbodies with thick-walled hyphae. More poroid species of the *Auriculariales* need to be examined in subsequent sequence analyses to test whether they could be placed near the species of *Protomerulius* included in this study. Careful morphological and microscopic reexaminations and molecular studies of the type species should also help to clarify the taxonomic confusion connected with the genera of the poroid, myxarioid *Auriculariales*, for which several constellations of synonymy have been proposed (Reid 1992, Ryvarden 1993, Núñez 1997).

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