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# The simple-septate basidiomycetes: a synopsis

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**Abstract** The simple-septate basidiomycetes comprise more than 8,000 species that show a high morphological and ecological heterogeneity. To gain insight in the phylogenetic relationships within this group, we compared several ultrastructural features such as septal pore apparatus, form and behavior of the spindle pole bodies, types of host–parasite interaction, presence or absence of colacosomes, symplechosomes, atractosomes and cystosomes as well as nuclear rDNA sequences coding for small- and large-subunit rRNA. Based on our integrated analysis, we propose a new classification system for the simple-septate basidiomycetes with the subphylum Pucciniomycotina and the classes Agaricostilbomycetes, Atractiellomycetes, Classiculomycetes, Cryptomycocolacomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes and Pucciniomycetes. We also propose the pucciniomycotinous taxa Cystobasidiales, Erythrobasidiales, Helicobasidiales, Mixiales, Naohideales, Pachnocybales, Spiculogloeales and Kondoaceae and the new subphyla Agaricomycotina (equivalent to the current Hymenomycetes) and Ustilaginomycotina (equivalent to the current Ustilaginomycetes).

**Taxonomical Novelties** Agaricomycotina · Agaricostilbomycetes · Atractiellomycetes · Classiculomycetes · Cryptomycocolacomycetes · Cystobasidiales · Cystobasidiomycetes · Erythrobasidiales · Helicobasidiales · Kondoaceae · Microbotryomycetes · Mixiales · Mixiomycetes · Naohideales · Pachnocybales · Pucciniomycetes · Pucciniomycotina · Spiculogloeales · Ustilaginomycotina

**Keywords** Basidiomycetes · Fungal systematics · Molecular phylogenetics · Ultrastructure · Taxonomy

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## Introduction

Based on estimates by Kirk et al. (2001), simple-septate basidiomycetes (Urediniomycetes *sensu* Swann and Taylor 1995) consist of approximately 8,000 described species. The Pucciniales (Uredinales) with ca. 7,000 species is the largest order in this group, followed by the Septobasidiales and Microbotryales. Most simple-septate basidiomycetes have phragmobasidia. Holobasidia are extremely rare and occur only in *Pachnocybe*, *Chionosphaera* and *Curvibasidium* and possibly also in *Colacosiphon* (Kirschner et al. 2001; Sampaio et al. 2004; Oberwinkler and Bandoni 1982; Oberwinkler and Bauer 1989). Morphologically and ecologically, the simple-septate basidiomycetes exhibit a high degree of divergence. They comprise plant parasites as well as mycoparasites, saprobes and some human pathogens, but mutualistic symbionts are not known in this group so far (Weiß et al. 2004). Sporulation ranges from scattered basidia (as in *Kriegeria*) to resupinate fructifications (as in *Saccoblastia*) to complex stilboid or pycnidioid basidiomes (as in *Pachnocybe* and *Heterogastridium*, respectively). This tremendous morphological and ecological diversity is reflected by a great genetic heterogeneity (Weiß et al. 2004). We assume that the simple-septate basidiomycetes represent old basidiomycetous lineages, and that the current number of species may only represent the tip of the iceberg.

Although attempts have been made (e.g., Swann et al. 2001; Weiß et al. 2004), a supraordinal classification of the simple-septate basidiomycetes is far from settled. Thus, only one higher subgroup, the Microbotryomycetidae, has been described (Swann et al. 1999). This paper therefore focuses on the evolution and supraordinal classification of this heterogeneous group of basidiomycetes. To gain insight into the phylogenetic relationships within this group, we evaluated ultrastructural characters such as septal pore apparatus, form and behavior of the spindle pole bodies (SPBs), host–parasite interactions, colacosomes, symplechosomes, atractosomes, and cystosomes as well as nuclear genes coding for rRNA of the small and large ribosomal subunits (SSU and LSU, respectively).

This paper is influenced to a great extent by the so far more than 200 contributions of the series "Studies in heterobasidiomycetes" published by the Lehrstuhl Spezielle Botanik und Mykologie, Universität Tübingen, during the past 30 years.

## Materials and methods

### Transmission electron microscopy

For conventional chemical fixation, samples were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature overnight. Following six transfers in 0.1 M sodium cacodylate buffer, samples were postfixed in 1% osmium tetroxide in the same buffer for 1 h in the dark, washed in distilled water and stained in 1% aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, samples were dehydrated in acetone, using 10-min changes at 25, 50, 70, 95% and three times in 100% acetone. Samples were embedded in Spurr's plastic and sectioned with a diamond knife. Ultrathin serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min, and washed with distilled water. They were examined using a Zeiss transmission electron microscope operating at 80 kV.

For high-pressure freezing and freeze substitution, samples were removed with a 2-mm cork borer. To remove air from intercellular spaces, samples were infiltrated with distilled water containing 6% (v/v) (2.5 M) methanol for approximately 5 min at room temperature. Single samples were placed in an aluminum holder (one half with a hollow of 0.3 mm depth for the sample and the other with a flat top) and frozen immediately in the high-pressure freezer HPM 010 (Balzers Union, Lichtenstein) as described in detail by Mendgen et al. (1991). Substitution medium (1.5 ml per specimen) consisted of 2% osmium tetroxide in acetone, which was dried over calcium chloride. Freeze substitution was performed at −90, −60 and −30°C (8 h for each step) using a Balzers freeze substitution apparatus (FSU 010). The temperature was then raised to approximately 0°C over a 30-min period, and samples were washed in dry acetone for another 30 min. Infiltration with an Epon/Araldite mixture (Welter et al. 1988) was performed stepwise: 30% resin in acetone at 4°C for 7 h, 70 and 100% resin at 8 C for 20 h each and 100% resin at 18°C for approximately 12 h. Samples were then transferred to fresh medium and polymerized at 60°C for 10 h. Finally, samples were processed as described above for chemically fixed samples, except that the sections were additionally stained with 1% aqueous uranyl acetate for 1 h.

Specimens used in the transmission electron microscopy are specified in the figure legends using the following acronyms: ATCC, American Type Culture Collection; BM, private herbarium Bernhard Müller; CCM, Czech Collection of Microorganisms; F, culture collection of the Botanical Institute, University of Tübingen; FO, private herbarium F. Oberwinkler; GD, private herbarium G. Deml; GEL, private herbarium G. and E. Langer; HUV, Herbarium Ustilaginales Vánky; RB, private herbarium R. Bauer; RJB, private herbarium R.-J. Bandoni; and TUB, Herbarium of the Botanical Institute, University of Tübingen.

### Molecular phylogenetic analyses

DNA was isolated from cultures or herbarium specimens using a sodium dodecyl sulfate (SDS) method as described previously (Begerow et al. 1997). The D1/D2 region of the nuclear gene coding for the LSU rRNA was amplified using polymerase chain reaction and the primers NL1/NL4 (O'Donnell 1993). The nuclear gene coding for the SSU rRNA was amplified using several primer combinations to cover the whole gene (Gargas and Taylor 1992). The PCR products were purified using the QIAquick protocol (Qiagen). The dsDNA was directly sequenced using an ABI PRISM Dye-Termination Cycle Sequencing Kit (Applied Biosystems) on automated sequencers (ABI 373A and ABI 3100, Applied Biosystems). The sequences were manually edited and assembled with Sequencher 4.1 (Gene Codes Corporation).

The first data set consisted of LSU sequences of 87 species of simple-septate basidiomycetes and three ascomycetes as outgroup. The second set combined partial LSU and nearly complete SSU sequences of 61 basidiomycetes and three ascomycetes as outgroup. For both data sets, we followed a multiple-analysis strategy (Lee 2001) and aligned the sequences with four different alignment methods and programs, namely, DIALIGN 2.2.1, using the −n option (Morgenstern 1999); MAFFT, using the FFT-NS-i option (Katoh et al. 2002); MUSCLE 3.2 (Edgar 2004); and POA (Lee et al. 2002). For the combined analysis of LSU and SSU sequences, each region was aligned separately. The different alignments of the first data set were then analyzed according to different methods of phylogenetic reconstruction. Positions where most sequences contained leading or trailing gaps were excluded before inferring phylogenetic trees. For neighbor-joining analysis (Saitou and Nei 1987), the data were first analyzed with Modeltest version 3.04 (Posada and Crandall 1998) using the Akaike information criterion (AIC) to find the most appropriate model of DNA substitution. A general time-reversible

model of DNA substitution additionally assuming a percentage of invariable sites and Γ-distributed substitution rates at the remaining sites (GTR + I + G) was selected for all alignments, with model parameters optimized for the individual alignments using maximum likelihood. Pairwise distances estimated under the respective model were then used to calculate a neighbor-joining tree with PAUP* (Swofford 2002) using the BIONJ variant (Gascuel 1997). Support for internal nodes was inferred by bootstrap analysis (Felsenstein 1985) from 1,000 resampling replicates. PAUP* was also used for maximum parsimony analyses, which were performed as previously described (Begerow et al. 2002). Bayesian Markov chain Monte Carlo (MC) analyses were done with MrBayes 3.1, involving two independent MC processes, each over five Mio generations with a cold and three incrementally heated chains starting from random trees, using the GTR + I + G model of DNA substitution (with model parameters sampled during MC), sampling every 100th tree and discarding a burn-in of 5,000 trees from each process (Huelsenbeck and Ronquist 2001). Finally, we analyzed the alignments with the fast maximum likelihood method as implemented in PHYML (Guindon and Gascuel 2003), again using the GTR + I + G model of DNA substitution. Maximum likelihood bootstrap support was based on 100 replicates. For the phylogenetic analysis of the combined LSU/SSU data set, we used the same strategies as described above for the LSU data set but calculated only neighbor-joining and maximum parsimony trees. The alignments are available from the second author upon request.

The following species and specimens were analyzed [the first number refers to the GenBank (http://www.ncbi.nlm.nih.gov) accession number of the LSU sequence and, if present, the second number refers to the GenBank accession number of the SSU sequence]: Agaricostilbomycete ZP 344 (AY512890), *Agaricostilbum pulcherrimum* (Berk. & Broome) B.L. Brady, B. Sutton & Samson (L20277; U40809), *Anthracoidea sclerotiformis* (Cooke & Massee) Kukkonen (DQ363331; DQ363315), *Aspergillus fumigatus* Fresen. (AY660917; M55626), *Aspergillus sparsus* Raper & Thom (U17910), *Athelia bombacina* (Link) Pers. (AF279377; M55638), *Atractiella solani* (Cohn & J. Schröt.) Oberw. & Bandoni (AY512831; DQ198797), Atractiellomycete AH 33906 (DQ363323; DQ198796), Atractiellomycete FO 44664 (DQ363322; DQ198795), *Aurantiosporium subnitens* (J. Schröt. & Henn.) M. Piepenbr., Vánky & Oberw. (AF009846), *Auricularia polytricha* (Mont.) Sacc. (AF261554; L22255), *Bauerago vuyckii* (Oudem. & Beij.) Vánky (DQ363321), *Bensingtonia ciliata* Ingold (AY512832), *Bensingtonia musae* M. Takash., S.O. Suh & Nakase (AY512833), *Bensingtonia phyllada* (Van der Walt & Y. Yamada) Van der Walt, Nakagawa & Y. Yamada ex Boekhout (AY512836), *Bensingtonia yamatoana* (Nakase, M. Suzuki & Itoh) Nakase & Boekhout (AY512834; D38239), *Bensingtonia yuccicola* (Nakase & M. Suzuki) Nakase & Boekhout (AY512835), *Boletus satanas* Lenz (AF336242; M94337), *Calocera cornea* (Batsch) Fr. (AY293170; L22256), *Camptobasidium hydrophilum* Marvanová & Suberkr. (AY512837; DQ198783), *Chionosphaera apobasidialis* D.E. Cox (AF393470; U77662), *Cintractia amazonica* Syd. & P. Syd. (AJ236142; DQ363302), *Classicula fluitans* R. Bauer, Begerow, Oberw. & Marvanová (AY512838), *Colacogloea peniophorae* (Bourdot & Galzin) Oberw. & Bandoni (AY512839; DQ363320), *Colacosiphon filiformis* R. Kirschner, R. Bauer & Oberw. (DQ363324), *Coleosporium senecionis* (Pers.) Fr. (AY512840), *Conidiosporomyces ayresii* (Berk.) Vánky & R. Bauer (AJ235308; DQ363303), *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo (AF041494; M92991), *Cryptomycocolax abnormis* Oberw. & R. Bauer (AY512841), *Curvibasidium cygneicollum* J.P. Samp. (AY512880), *Cyrenella elegans* Goch. (AY512842), *Cystobasidium fimetarium* (Schumach.) P. Roberts (AY512843; AY124479), *Cystofilobasidium capitatum* (Fell, I.L. Hunter & Tallman) Oberw. & Bandoni (DQ363333; D12801), *Dacrymyces stillatus* Nees (AJ406405; L22258), *Dicellomyces scirpi* Raitv. (AF487385; DQ363304), *Doassansia hygrophilae* Thirum. (AF007524; DQ198788), *Entyloma ficariae* A. A. Fisch. Waldh. (AJ235295; DQ198790), *Entyloma gaillardianum* Vánky (AF007530; DQ363305), *Eocronartium muscicola* (Pers.) Fitzp. (AY512844), *Erratomyces patelii* (Pavgi & Thirum.) M. Piepenbr. & R. Bauer (AF009855; DQ363309), *Erythrobasidium hasegawianum* Hamam., Sugiy. & Komag. (AF131058: D12803), *Exobasidium vaccinii* (Fuckel) Woronin (AF009858; DQ198792), *Filobasidiella neoformans* Kwon-Chung (AJ551290; L05428), *Fulvisporium restifaciens* (D.E. Shaw) Vánky (AF009860), *Georgefischeria riveae* Thirum. & Naras. (AF009861; DQ363312), *Graphiola phoenicis* (Moug.) Poit. (AF009862; DQ363306), *Gymnosporangium sabinae* (Dicks.) G. Winter (AY512845), *Helicobasidium mompa* Tanaka (L20281), *Helicobasidium purpureum* (Tul.) Pat. (AY512846), *Helicogloea lagerheimii* Pat. (AY512849; DQ198794), *Helicogloea* sp. CCJ 1178 (AY512848), *Helicogloea* sp. FO 42773 (AY512847; DQ198793), *Helicogloea variabilis* K. Wells (L20282), *Herpobasidium filicinum* (Rostr.) Lind (AY512850), *Heterogastridium pycnidioideum* Oberw. & R. Bauer (AY512851; DQ198785), *Hyalopsora polypodii* (Pers.) Magnus (AY512852; AB011015), *Insolibasidium deformans* (C.J. Gould) Oberw. & Bandoni (AY646099), *Jaculispora submersa* H.J. Huds. & Ingold (AY512853; AY124477), *Jamesdicksonia dactylidis* (Pass.) R. Bauer, Begerow, A. Nagler & Oberw. (AF009853; DQ363310), *Kondoa malvinella* (Fell & I.L. Hunter) Y. Yamada, Nakagawa & I. Banno (AF131059), *Kondoa* sp. ZP 352 (AY12854), *Kriegeria eriophori* Bres. (L20288), *Kurtzmanomyces tardus* Gim.-Jurado & Uden (DQ363325), *Leucosporidiella fragaria* (J.A. Barnett & Buhagiar) J.P. Samp. (AY512879), *Leucosporidium antarcticum* Fell, Statzell, I.L. Hunter & Phaff (AY512855), *Leucosporidium fellii* Gim.-Jurado & Uden (AY512856), *Leucosporidium scottii* Fell, Statzell, I.L. Hunter & Phaff (AY512857), *Liroa emodensis* (Berk.) Cif. (AY512858), *Mastigobasidium intermedium* Golubev (AY512859; D38235), *Melampsora lini* (Ehrenb.) Lév. (L20283; AY125396), *Melampsora* sp.

RB 3003 (AY512866), *Melanopsichium pennsylvanicum* Hirschh. (AF009863; DQ363314), *Melanotaenium euphorbiae* (L.W. Lenz) Whitehead & Thirum. (AF009865; DQ198789), *Microbotryum intermedium* (J. Schröt.) Vánky (AY512858), *Microbotryum reticulatum* (Liro) R. Bauer & Oberw. (AY512861), *Microbotryum scorzonerae* (Alb. & Schwein.) G. Deml & Prillinger (AY512862), *Microbotryum stygium* (Liro) Vánky (AY512863), *Microbotryum violaceum* (Pers.) G. Deml & Oberw. FO 38227 (AF009866; DQ198782), *Microbotryum violaceum* (Pers.) G. Deml & Oberw. GD 1400 (AY512864), *Microstroma juglandis* (Berenger) Sacc. (AF009867; DQ363313), *Mixia osmundae* (Nishida) C.L. Kramer (AY512867; D14163), *Moesziomyces bullatus* (J. Schröt.) Vánky (AF009868; DQ363307), *Mrakia frigida* (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komag. (AJ866978; D12802), *Mycogloea* sp. FO 40962 (AY512868; DQ198791), *Naohidea sebacea* (Berk. & Broome) Oberw. (AF131061), *Occultifur externus* J.P. Samp., R. Bauer & Oberw. (AF131062), *Pachnocybe ferruginea* (Sow.: Fr.) Berk. (L20284), *Phleogena faginea* (Fr.) Link (AY512869; DQ198798), *Platygloea disciformis* (Fr.) Neuhoff (AY512870), *Platygloea vestita* Bourdot & Galzin (AY512872; AY124480), *Pseudohydnum gelatinosum* (Scop.) P. Karst. (DQ363332; L22260), *Puccinia obscura* J. Schröt. (AY512873), *Puccinia recondita* Dietel & Holw. (L08729), *Rhamphospora nymphaeae* D.D. Cunn. (AF007526; DQ363311), *Rhodosporidium sphaerocarpum* S.Y. Newell & Fell (AY512875), *Rhodotorula armeniaca* R.G. Shivas & Rodr. Mir. (DQ363326), *Rhodotorula aurantiaca* (Saito) Lodder (AY512877), *Rhodotorula bogoriensis* (Deinema) Arx & Weijman (AY512876), *Rhodotorula diffluens* (Ruinen) Arx & Weijman (AY512878), *Rhodotorula colostri* (T. Castelli) Lodder (AY512874), *Rhodotorula hordea* Rodr. Mir. & Weijman (AY5128881), *Rhodotorula javanica* (Ruinen) Arx & Weijman (AY5128882), *Rhodotorula minuta* (Saito) F.C. Harrison (DQ363327), *Rhodotorula philyla* (Van der Walt, D.B. Scott & Klift) Rodr. Mir. & Weijman (AY512883), *Russula compacta* Frost (AF287888; U59093), *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (J01355; J01353), *Sakaguchia dacryoidea* (Fell, I.L. Hunter & Tallman) Y. Yamada, K. Maeda & Mikata (AF131065), *Schizonella melanogramma* (DC.) J. Schröt. (AF009870; DQ363308), *Schizophyllum commune* Fr. (DQ071725; X54865), *Septobasidium carestianum* Bres. (L20281; DQ198787), *Sphacelotheca polygoni-serrulati* Maire (AY512884), *Spiculogloea* sp. RB 1040 (AY512885; DQ198784), *Sporidiobolus johnsonii* Nyland (AY512886), *Sporidiobolus salmonicolor* Fell & Tallman (AY512887), *Sporobolomyces coprosmicola* Hamam. & Nakase (AY512888), *Sporobolomyces lactophilus* Nakase, Itoh, M. Suzuki & Bandoni (AY512889), *Sporobolomyces oryzicola* Nakase & M. Suzuki (DQ363328), *Sporobolomyces xanthus* (Nakase, G. Okada & Sugiy.) Boekhout (AY512891), *Taphrina amentorum* Sadeb. (DQ363330), *Taphrina deformans* (Berk.) Tul. (U94948; U00971), *Tilletia caries* (DC.) Tul. & C. Tul. (AJ235308; U00972), *Tranzschelia pruni-spinosae* (Pers.) Dietel (DQ363329), *Tremella globispora* D.A. Reid (AF042243; U00976), *Udeniomyces puniceus* (Komag. & Nakase) Nakase & Takem. (AF075519; D31658), *Ustilago hordei* (Pers.) Lagerh. (L20286; U00973), *Ustilago maydis* (DC) Corda (L20287; X62396), *Ustilentyloma fluitans* (Liro) Vánky (AF009882; AY124481) and *Xerocomus chrysenteron* (Bull.) Quél. (AF514809; M94340).

## The proposed system

The classification system proposed here is consistent with an elevation of the three currently accepted basidiomycetous classes, Urediniomycetes, Ustilaginomycetes and Hymenomycetes, to subphyla, as recently proposed by the AFTOL (Assembling the Fungal Tree of Life project; http://ocid.nacse.org/research/aftol) consortium. For supraordinal taxa that include the rust fungi, we decided to adopt neither names based on "Uredinio-" [derived from Latin *uredinium* (uredosorus)] nor names based on "Uredino-" (based on the anamorphic rust genus *Uredo*), but to switch to names based on *Puccinia*, the largest genus in the simple-septate basidiomycetes. We hope that this radical switch prevents future confusion arising from Uredin(i)omycetes being interpreted in two widely different phylogenetic ranges, equivalent to our Pucciniomycotina and Pucciniomycetes. To homogenize the nomenclature of the basidiomycote subphyla, we also formally describe the Agaricomyotina and the Ustilaginomycotina, which are equivalent to the current Hymenomycetes and Ustilaginomycetes, respectively.

In all descriptions of new higher taxa we have not explicitly cited types, since automatical typification is applicable here, by which all these taxa are typified by the type of the generic name from which they are derived (Articles 10.6 and 10.7 of the International Code of Botanical Nomenclature; Greuter et al. 2000).

The references given in the text are exemplary, and the reader should consult the bibliography listed in the primary references for more information. For the morphological characters of the respective groups, see Oberwinkler (1977, 1978, 1982, 1985, 1987, 1993); for molecular phylogenetic hypotheses and support of the various groups, see Tables 1 and 2, Figs. 1 and 2 of the present study, Swann and Taylor (1993, 1995), Weiß et al. (2004); and for the assignment of mitosporic yeast taxa to suprageneric groups, see Fell et al. (2001), Sampaio (2004) and Scorzetti et al. (2002).

**Agaricomycotina** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., subphyl. nov. (Hymenomycetes *sensu* Swann and Taylor 1995)

*Fungi Basidiomycotum structura secundaria acidi nucleici ribosomalis 5S RNA typo B; carbohydratorum compositione parietum cellularum glucoso dominanti, xyloso praesente.* (Members of the Basidiomycota having a type B secondary structure of the 5S RNA and a cell wall carbohydrate composition with dominance of glucose and presence of xylose.)

**Ustilaginomycotina** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., subphyl. nov. (Ustilaginomycetes R. Bauer, Oberw. & Vánky)

*Fungi Basidiomycotum structura secundaria acidi nucleici ribosomalis 5S RNA typo B; carbohydratorum compositione parietum cellularum glucoso dominanti, xyloso absente.* (Members of the Basidiomycota having a type B secondary structure of the 5S RNA and a cell wall carbohydrate composition with dominance of glucose and absence of xylose.)

**Pucciniomycotina** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., subphyl. nov. (Urediniomycetes *sensu* Swann and Taylor 1995) (Figs. 3a,b, 4a–f, 5a–d, 6a–f, 7a–f, 8a–f, 9a–d and 10a–f)

*Fungi Basidiomycotum structura secundaria acidi nucleici ribosomalis 5S RNA typo A.* (Members of the Basidiomycota having a type A secondary structure of the 5S RNA.)
*Main characteristics*: Mannose as major cell wall carbohydrate (Prillinger et al. 1993), type A secondary structure of the 5S rRNA (Gottschalk and Blanz 1985), simple septal pores without membrane caps (Figs. 3a,b, 4a, 5a,b, 6a,c, 7a,b, 8a,b, 10a; Bauer and Oberwinkler 1994), SPBs discoidal (Figs. 4b, 5c, 6b,d, 7c, 8d, 9d, 10b; Bauer and Oberwinkler 1994)

**Agaricostilbomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Agaricostilbomycetidae sensu Swann et al. 2001; Fig. 4a–f)

*Fungi Puccioniomycotinum dimorphi, non-phytoparasitici; parietibus cellularum fucosum continentibus; septis hypharum sine corpusculis poris associatis; basidiosporis statu germinandi aseptatis; absentibus colacosomatibus, teliosporis, holobasidiis curvatis conidiisque radiatis.* (Dimorphic, nonphytoparasitic members of the Pucciniomycotina having fucose as cell wall carbohydrate component, septal pores without associated microbodies, aseptate basidiospores during germination and no colacosomes, teliospores, curved holobasidia and radiate conidia.)
*Main characteristics*: Septal pores without microbodies (Fig. 4a; Oberwinkler and Bauer 1989), nucleoplasmic SPB separation, metaphasic SPBs intranuclear (Fig. 4b; Bauer et al. 1992)

**Agaricostilbales** Oberw. & R. Bauer
Without tremelloid haustorial cells (Oberwinkler and Bandoni 1982)

**Agaricostilbaceae** Oberw. & R. Bauer
Basidiospores produced in a yeast-like manner (Bauer et al. 1992)

*Agaricostilbum* J.E. Wright emend. J.E. Wright, Bandoni & Oberw.
*Bengsingtonia* Ingold, emend. Nakase & Boekhout (*pro parte*, mitosporic)
*Sterigmatomyces* Fell, emend. Yamada & Banno (mitosporic)

**Chionosphaeraceae** Oberw. and Bandoni
Gasteroid basidia with simultaneous basidiospore production per basidium (Oberwinkler and Bandoni 1982)

*Chionosphaera* D.E. Cox
*Kurtzmanomyces* Yamada et al., emend. J. P. Samp. (mitosporic)
*Mycogloea* (*pro parte*)
*Stilbum* Tode ex Mérat

**Kondoaceae** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., fam. nov.

*Fungi Agaricostilbalium phragmobasidiis ballistosporas procreantibus*. (Members of the Agaricostilbales having ballistosporic phragmobasidia.)
*Main characteristics*: Ballistosporic phragmobasidia (Fonseca et al. 2000)

*Bensingtonia* Ingold, emend. Nakase & Boekhout (*pro parte*, mitosporic)
*Kondoa* Y. Yamada, Nakagawa & I. Banno emend. Á. Fonseca, J.P. Samp., Inácio & Fell

**Spiculogloeales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Agaricostilbomycetum cellulis haustorialibus tremelloideis*. (Members of the Agaricostilbomycetes having tremelloid haustorial cells.)
*Main characteristics*: Nanometer-fusion mycoparasitism (Fig. 4c–f; Bauer 2004) with tremelloid haustorial cells (Fig. 4e; Roberts 1996; Weiß et al. 2004)

*Mycogloea* L.S. Olive (*pro parte*)
*Spiculogloea* P. Roberts
*Sporobolomyces* Kluyver & C.B. Niel (*pro parte*, mitosporic)

**Atractiellomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Fig. 5a–d)

*Fungi Pucciniomycotinorum symplechosomatibus*. (Members of the Pucciniomycotina having symplechosomes.)
*Main characteristics*: Symplechosomes (Fig. 5d; Bauer and Oberwinkler 1991a; Oberwinkler and Bauer 1989), metaphasic SPBs in the nuclear envelope, SPB–ER caps (Fig. 5c)

Order **Atractiellales** Oberw. & Bandoni emend. Oberw. & R. Bauer
With characters of the class

**Phleogenaceae** Weese
Septal pores with atractosomes (Fig. 5b; Oberwinkler and Bauer 1989; Weiß et al. 2004)

*Atractiella* Sacc.
*Helicogloea* Pat. (*pro parte*)
*Phleogena* Link

**Saccoblastiaceae** Jülich
Septal pores with microbodies (Fig. 5a; Weiß et al. 2004)

*Helicogloea* Pat. (*pro parte*)
*Infundibura* Nag Raj & W.B. Kendrick (mitosporic)
*Saccoblastia* A. Møller s. str. (*S. farinacea* and related species)

**Taxa not ascribed to any family of the Atractiellales**

*Hobsonia* Berk. ex Massee (mitosporic)
*Leucogloea* R. Kirschner (mitosporic)

**Classiculomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Fig. 6a,b)

*Fungi Pucciniomycotinorum corpusculis poris septorum associatis cellulisque haustorialibus tremelloideis*. (Members of the Pucciniomycotina having septal pores associated with microbodies and tremelloid haustorial cells.)
*Main characteristics*: Septal pores with microbodies (Fig. 6a), metaphasic SPBs intranuclear (Fig. 6b), tremelloid haustorial cells (Bauer et al. 2003)

**Classiculales** R. Bauer, Begerow, Oberw. & Marvanová
With characters of the class

**Classiculaceae** R. Bauer, Begerow, Oberw. & Marvanová
With characters of the order

*Classicula* R. Bauer, Begerow, Oberw. & Marvanová
*Jaculispora* H.J. Huds. & Ingold (mitosporic)

**Cryptomycocolacomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Fig. 6c–f)

*Fungi Pucciniomycotinorum colacosomatibus corpusculisque poris septorum associatis*. (Members of the Pucciniomycotina having colacosomes and septal pores with microbodies.)
*Main characteristics*: Mycoparasitic, with colacosomes (Fig. 6e,f), septal pores with microbodies (Fig. 6c), metaphasic SPBs in the nuclear envelope (Fig. 6d), without teliospores (Kirschner et al. 2001; Oberwinkler and Bauer 1990)

**Cryptomycocolacales** Oberw. & R. Bauer
With characters of the class

**Cryptomycocolacaceae** Oberw. & R. Bauer
With characters of the order

*Colacosiphon* R. Kirschner, R. Bauer & Oberw.
*Cryptomycocolax* Oberw. & R. Bauer

**Cystobasidiomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Figs. 3a, 7a–f)

*Fungi Pucciniomycotinorum parietibus cellularum fucoso absente*. (Members of the Pucciniomycotina having a cell wall carbohydrate composition without fucose.)
*Main characteristics*: Cell wall carbohydrate composition without fucose (Takashima et al. 2000), cytoplasmic SPB separation, metaphasic SPBs intranuclear (Fig. 7c; Weiß et al. 2004)

**Cystobasidiales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Cystobasidiomycetum cellulis haustorialibus tremelloideis porisque septorum cystosomatibus*. (Members of the Cystobasidiomycetes having tremelloid haustorial cells and septal pores with cystosomes.)
*Main characteristics*: Nanometer-fusion mycoparasitism with tremelloid haustorial cells, septal pores with cystosomes (Fig. 7a,d–f; Bauer 2004; Oberwinkler 1990; Sampaio et al. 1999; Weiß et al. 2004)

**Cystobasidiaceae** Gäum.
With characters of the order

*Cystobasidium* (Lagerh.) Neuhoff
*Occultifur* Oberw.
*Rhodotorula* F.C. Harrison (*pro parte*, mitosporic)

**Erythrobasidiales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Cystobasidiomycetum systemate coenzymatis Q10 hydrogenato*. (Members of the Cystobasidiomycetes having a hydrogenated Coenzyme Q10 system.)
*Main characteristics*: Without tremelloid haustorial cells, septal pores without cystosomes (compare Fig. 7b), hydrogenated coenzyme Q10 system [CoQ10 (H2)] (Bauer 2004; Hamamoto et al. 1988, 2002; Suh et al. 1993)

*Bannoa* Hamam. (mitosporic)
*Erythrobasidium* Hamam., Sugiy. & Komag. (mitosporic)
*Rhodotorula* F.C. Harrison (*pro parte*, mitosporic)
*Sporobolomyces* Kluyver & C.B. Niel (*pro parte*, mitosporic)

**Naohideales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Cystobasidiomycetum haustoriis intracellularibus*. (Members of the Cystobasidiomycetes forming intracellular haustoria.)
*Main characteristics*: Nanometer-fusion mycoparasitism with intracellular haustoria, septal pores without cystosomes (Fig. 7b,f; Bauer 2004; Oberwinkler 1990; Weiß et al. 2004)

*Naohidea* Oberw.

**Taxa not ascribed to any order of the Cystobasidiomycetes**

*Cyrenella* Goch. (mitosporic)
*Sakaguchia* Y. Yamada, K. Maeda & Mikata

**Microbotryomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Microbotryomycetidae Swann) (Fig. 8a–f)

*Fungi Pucciniomycotinorum colacosomatibus corpusculisque poris septorum associatis nullis et affines*. [Members of the Pucciniomycotina having colacosomes and septal pores without microbodies, and related taxa (see Figs. 1 and 2).]
*Main characteristics*: Taxa with colacosomes (Fig. 8e; Bauer 2004; Bauer and Oberwinkler 1991b; Sampaio et al. 2003) and taxa derived from colacosome fungi (Bauer et al. 1997), metaphasic SPBs intranuclear (Fig. 8d; Bauer et al. 1991; McCully and Robinow 1972a,b)

**Heterogastridiales** Oberw. & R. Bauer
Mycoparasitic, with colacosomes, without teliospores (Fig. 8e; Bauer 2004; Kirschner et al. 1999; Oberwinkler et al. 1990a,b)

**Heterogastridiaceae** Oberw. & R. Bauer
With characters of the order

*Atractocolax* R. Kirschner, R. Bauer & Oberw.
*Colacogloea* Oberw. & R. Bauer
*Heterogastridium* Oberw. & R. Bauer
*Krieglsteinera* Pouzar

**Leucosporidiales** J.P. Samp., M. Weiß & R. Bauer
Colacosomes (compare Fig. 8e), teliospores, white to cream-colored cultures (Sampaio et al. 2003)

**Leucosporidiaceae** J.P. Samp., M. Weiß & R. Bauer
With characters of the order

*Leucosporidiella* J.P. Samp. (mitosporic)
*Leucosporidium* Fell, Statzell, I.L. Hunter & Phaff (*pro parte*)
*Mastigobasidium* Golubev

**Sporidiobolales** J.P. Samp., M. Weiß and R. Bauer
Colacosomes (compare Fig. 8e), with teliospores, pink-colored cultures (Sampaio et al. 2003)

**Sporidiobolaceae** R.T. Moore emend. J.P. Samp., M. Weiß & R. Bauer
With characters of the order

*Rhodosporidium* I. Banno
*Rhodotorula* F.C. Harrison (*pro parte*, mitosporic)
*Sporidiobolus* Nyland
*Sporobolomyces* Kluyver & C.B. Niel (*pro parte*, mitosporic)

**Microbotryales** R. Bauer & Oberw.
Phytoparasitic (Fig. 8f), without colacosomes, teliospores (Bauer et al. 1997)

**Microbotryaceae** R.T. Moore
Without septal pores (Fig. 8c; Bauer et al. 1997)

*Bauerago* Vánky
*Liroa* Cif.
*Microbotryum* Lév.
*Sphacelotheca* de Bary
*Zundeliomyces* Vánky

**Ustilentylomataceae** R. Bauer & Oberw.
With septal pores (Bauer et al. 1997)

*Aurantiosporium* M. Piepenbr., Vánky & Oberw.
*Fulvisporium* Vánky
*Ustilentyloma* Savile

**Taxa not ascribed to any order of the Microbotryomycetes**

*Camptobasidium* Marvanová & Suberkr.
*Curvibasidium* J.P. Samp. & Golubev
*Kriegeria* Bres.
*Leucosporidium antarcticum* Fell, Statzell, Hunter & Phaff
*Leucosporidium fasciculatum* Babeva & Lisichk.

**Mixiomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Fig. 9a–d)

*Descriptio analoga ordinis Mixialium*.
*Main characteristics*: Multinucleate hyphae, multiple spores produced simultaneously on sporogenous cells (Fig. 9c; Nishida et al. 1995)

**Mixiales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Pucciniomycotinorum hyphis multinucleatis cellulisque sporogenis sporas multiplices simul procreantibus*. (Members of the Pucciniomycotina having multinucleate hyphae. Multiple spores produced simultaneously on sporogenous cells.)
*Main characteristics*: Multinucleate hyphae, multiple spores produced simultaneously on sporogenous cells (Nishida et al. 1995)

**Mixiaceae** C.L. Kramer
With characters of the order

*Mixia* C.L. Kramer

**Pucciniomycetes** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., class. nov. (Urediniomycetidae sensu Swann et al. 2001) (Fig. 10a–f)

*Fungi Pucciniomycotinorum duplicatione corporis nucleo associati intermeiotico metaphasico et affines*. [Members of the Pucciniomycotina having a metaphasic intermeiotic SPB duplication and related members (see Figs. 1 and 2)].
*Main characteristics*: Pores mainly with microbodies (Fig. 10a; Bauer and Oberwinkler 1994), metaphasic SPBs in the nuclear envelope (Fig. 10b; Bauer and Oberwinkler 1990a; O'Donnell and McLaughlin 1981a–c), mainly metaphasic intermeiotic SPB duplication (Fig. 10b,c; Bauer 1987; Bauer and Oberwinkler 1990a; O'Donnell and McLaughlin 1981b), clampless hyphae (Oberwinkler and Bandoni 1984)

**Septobasidiales** Couch ex Donk
Parasites on scale insects (Couch 1938).

**Septobasidiaceae** Maire
With characters of the order

*Auriculoscypha* Reid & Manimohan
*Coccidiodictyon* Oberw.
*Harpographium* Sacc. (*pro parte*, mitosporic)
*Johncouchia* S. Hughes & Cavalc. (mitosporic)
*Ordonia* Racib.
*Septobasidium* Pat.
*Uredinella* Couch

**Platygloeales** R.T. Moore
Phytoparasites mainly on stems and leaves (Oberwinkler and Bandoni 1984, Oberwinkler et al. 1990a), SPB–ER caps (Bauer and Oberwinkler 1994)

**Platygloeaceae** Racib.
On angiosperms (Oberwinkler and Bandoni 1984; Oberwinkler et al. 1990a)

*Platygloea* J. Schröt. s. str. (*P. disciformis* (Fr.) Neuhoff and related species)
*Insolibasidium* Oberw. & Bandoni

**Eocronartiaceae** Jülich
On mosses and ferns (Oberwinkler and Bandoni 1984)

*Eocronartium* Atkinson
*Herpobasidium* Lind emend. Oberw. & Bandoni
*Jola* A. Møller
*Platycarpa* Couch emend. Oberw. & Bandoni
*Ptechetelium* Oberw. & Bandoni

**Pucciniales** Clem. & Shear (Uredinales Arthur)
Phytoparasites mainly on stems and leaves (Fig. 10f; Gäumann 1959), without SPB–ER caps (Fig. 10b; Bauer 1987; O'Donnell and McLaughlin 1981a–c), complex life cycle with spermogonia etc. (Gäumann 1959)

**13 families** (see Cummins and Hiratsuka 2003) ca. **115 genera** (see Cummins and Hiratsuka 2003)

**Helicobasidiales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Pucciniomycetum in statu uninucleato in fungis Puccinialium parasitici*. (Members of the Pucciniomycetes having a rust-parasitic haplophase.)
*Main characteristics*: Parasites on roots of vascicular plants, rust-parasitic haplophase (Fig. 10e; Bauer et al. 2004; Lutz et al. 2004a–c), SPB–ER caps (Bourett and McLaughlin 1986)

*Helicobasidium* Pat.
*Tuberculina* Sacc. (mitosporic)

**Pachnocybales** R. Bauer, Begerow, J.P. Samp., M. Weiß & Oberw., ord. nov.

*Fungi Pucciniomycetum holobasidiati*. (Members of the Pucciniomycetes having holobasidia.)
*Main characteristics*: Saprobic, holobasidia, stilboid fruitbodies (Oberwinkler and Bandoni 1982; Oberwinkler and Bauer 1989), SPB–ER caps (Bauer and Oberwinkler 1990a)

**Pachnocybaceae** Oberw. & R. Bauer
With characters of the order

*Pachnocybe* Berk.

**Taxa not ascribed to any class of the Pucciniomycotina**

*Atractogloea* Oberw. & Bandoni
*Kryptastrina* Oberw.
*Paraphelaria* Corner
*Zygogloea* P. Roberts

**Table 1** Support values of Pucciniomycotina and their classes derived from different alignment algorithms and methods of phylogenetic inference applied to nuclear LSU rDNA sequences

| | MAFFT (679)[a] | | | | DIALIGN (772)[a] | | | | POA (674)[a] | | | | MUSCLE (648)[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NJ | MC | MP | ML | NJ | MC | MP | ML | NJ | MC | MP | ML | NJ | MC | MP | ML |
| Pucciniomycotina | 98 | 100 | 96 | 99 | 100 | 100 | 98 | 100 | 96 | 100 | 83 | 97 | 100 | 94 | 95 | 100 |
| Agaricostilbomycetes | <50 | 71 | <50 | 54 | <50 | 54 | – | <50 | <50 | – | – | <50 | 54 | 79 | <50 | 55 |
| Atractiellomycetes | <50 | – | – | <50 | <50 | – | – | <50 | <50 | – | – | <50 | – | – | – | – |
| Classiculomycetes | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 98 | 100 | 100 | 100 |
| Cryptomycocolacomycetes | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99 | 100 | 100 | 100 | 100 | 100 |
| Cystobasidiomycetes | 90 | 100 | 86 | 99 | 90 | 100 | 82 | 98 | 85 | 100 | 84 | 98 | 77 | 94 | 62 | 91 |
| Microbotryomycetes | 91 | 100 | 74 | 99 | 85 | 100 | 74 | 97 | 81 | 100 | 70 | 96 | 60 | 94 | 66 | 93 |
| Pucciniomycetes | 100 | 100 | 92 | 100 | 99 | 100 | 81 | 99 | 99 | 100 | 82 | 99 | 100 | 100 | 85 | 100 |

Bootstrap values are based on 1,000 replicates for NJ and MP; 100 replicates were used for ML. Posterior probabilities inferred from Markov chain MC are based of 5,000,000 generations

*NJ* Neighbor-joining, *MC* Monte Carlo analysis, *MP* maximum parsimony, *ML* maximum likelihood

[a]The numbers in parentheses refer to the respective alignment length. Values smaller than 50% indicate that the group is present in the tree obtained from the original alignment

## Discussion of the proposed taxa

### Pucciniomycotina

Within the Basidiomycota, the Pucciniomycotina are well characterized by a type A 5S rRNA secondary structure (Gottschalk and Blanz 1985) and mannose as the major cell wall carbohydrate component (Prillinger et al. 1993). However, our analyses reveal a high genetic heterogeneity for the species ascribed to the Pucciniomycotina, which is reflected by high branch lengths in our molecular phylogenetic analyses involving nuclear SSU and LSU rDNA sequences (Figs. 1 and 2), particularly concerning *Colacosiphon*, *Cryptomycocolax*, *Mixia*, and the taxa of the Agaricostilbomycetes (see synopsis above). The phylogenetic relationships between the several classes of Pucciniomycotina are not well resolved in the molecular phylogenetic analyses and might be affected by artifacts such as long-branch attraction. However, the monophyly of the Pucciniomycotina is well supported in the analysis of the second data set, including two genes and all major groups of Basidiomycota (Fig. 2; Table 2). The Agaricomycotina does not appear as a monophyletic group in some analyses (see Table 2), which is consistent with the very short branch that supports this group if it is present in the phylogenetic tree (Fig. 2). However, in those trees where the three subphyla were resolved, the Pucciniomycotina appears often basal to Ustilaginomycotina and Agaricomycotina (Fig. 2). A basal position within the Basidiomycota is consistent with the huge morphological and genetic diversity of the Pucciniomycotina and also with ultrastructural characteristics. The SPBs of the Pucciniomycotina, like those of ascomycetes (e.g., Nagler et al. 1989, Wells 1970), are discoidal in shape (Figs. 4b, 5c, 6b,d, 7c, 8d, 9d, 10b,c; Bauer et al. 1991; Bauer and Oberwinkler 1990a, 1994). As in the ascomycetes, the septal pores of the Pucciniomycotina

**Table 2** Support values of Agaricomycotina, Ustilaginomycotina, Pucciniomycotina and some classes derived from different alignment methods and methods of phylogenetic inference applied to nuclear SSU and LSU rDNA sequences

| | MAFFT (2,393)[a] | | DIALIGN (2,468)[a] | | | | POA (2,566)[a] | | MUSCLE (2,360)[a] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NJ | MP | NJ | MC | MP | ML | NJ | MP | NJ | MP |
| Agaricomycotina | – | <50 | <50 | – | <50 | – | <50 | – | 52 | <50 |
| Pucciniomycotina | 100 | 96 | 100 | 100 | 96 | 100 | 100 | 96 | 100 | 98 |
| Ustilaginomycotina | 100 | 70 | 100 | 100 | 81 | 100 | 100 | 74 | 100 | 76 |
| Agaricostilbomycetes | 59 | – | 89 | – | – | 78 | – | – | 57 | – |
| Atractiellomycetes | 51 | – | 81 | 100 | <50 | 86 | 90 | <50 | 67 | – |
| Cystobasidiomycetes | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Microbotryomycetes | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Pucciniomycetes | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Bootstrap values are based on 1,000 replicates for NJ and MP; 100 replicates were used for ML. Posterior probabilities inferred from Markov chain MC are based on 5,000,000 generations

*NJ* Neighbor-joining, *MP* maximum parsimony, *MC* Monte Carlo analysis, *ML* maximum likelihood

[a]The numbers in parentheses refer to the respective alignment length. Values smaller than 50% indicate that the group is present in the tree obtained from the original alignment

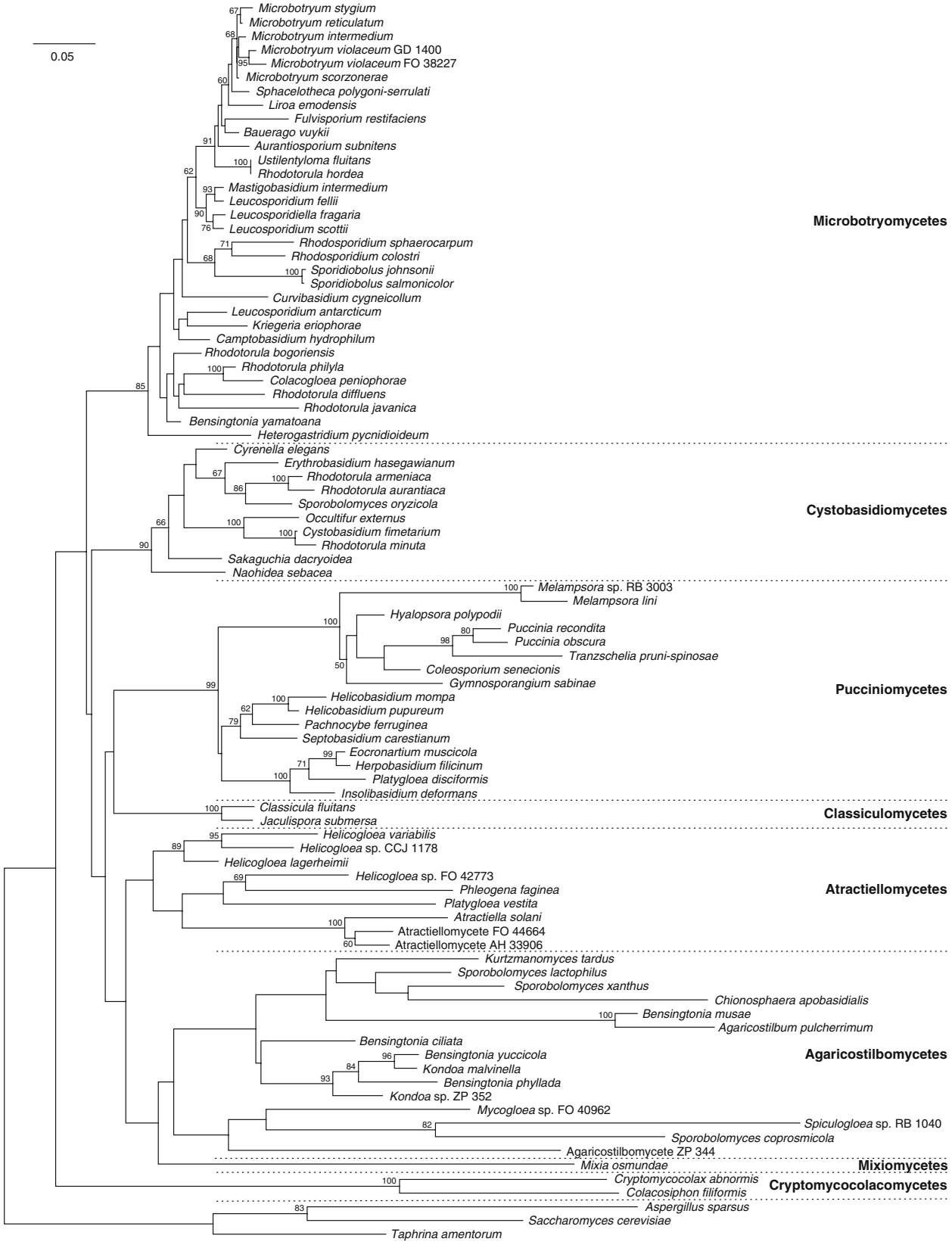


**Fig. 1** Neighbor-joining analysis of nuclear LSU rDNA sequences of 87 members of Pucciniomycotina. The shown tree was inferred from a DIALIGN alignment with PAUP* using the BIONJ variant of neighbor-joining under a GTR + I + G model of nucleotide substitution and rooted with three ascomycetes. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. Bootstrap values are given for 1,000 replicates; values below 50% are omitted

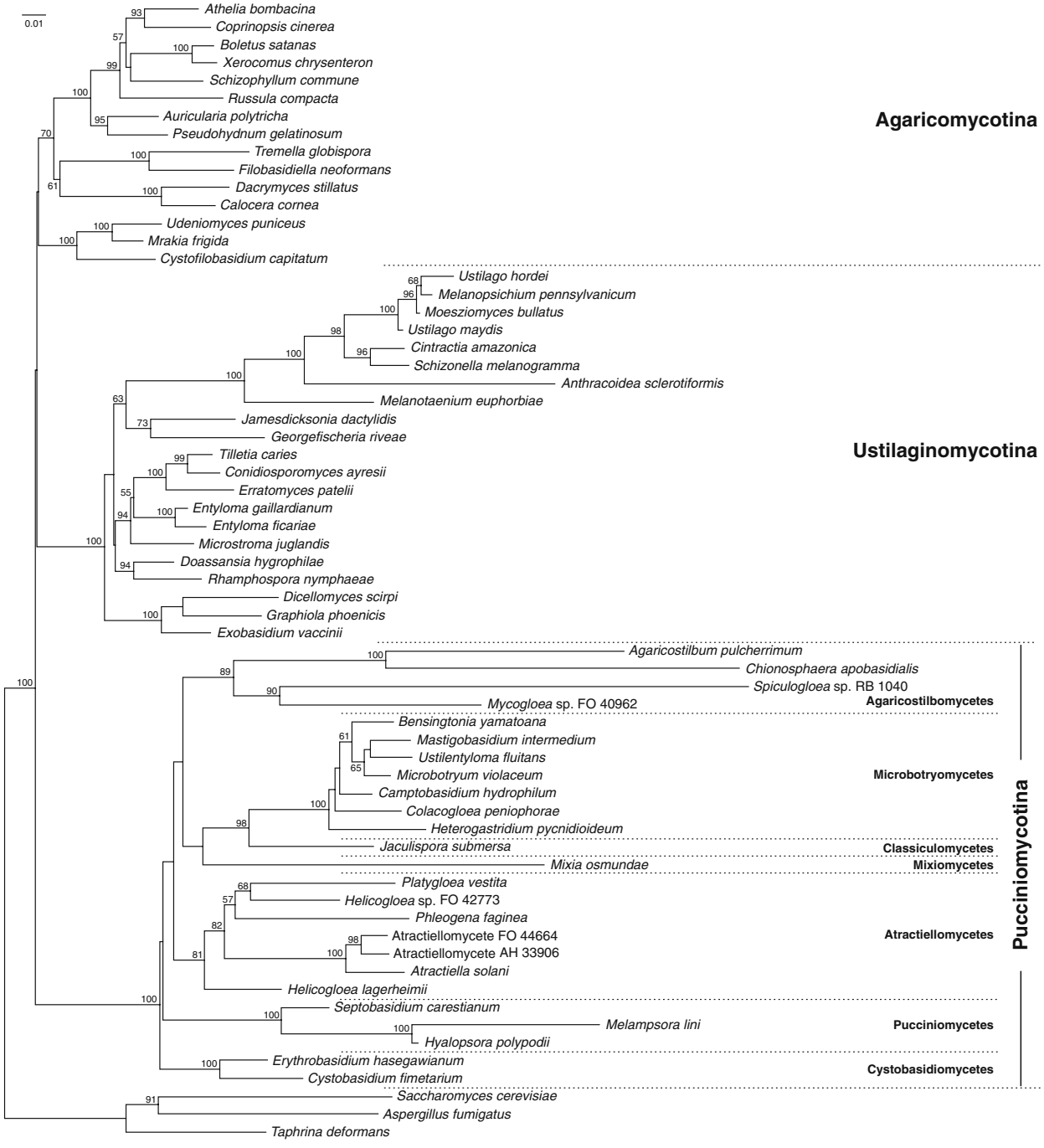


**Fig. 2** Neighbor-joining analysis of nuclear SSU and LSU rDNA sequences of 61 basidiomycetes, including most classes of Pucciniomycotina. The shown tree was inferred from a DIALIGN alignment of 2,468 bp. The phylogram was calculated with PAUP* using the BIONJ variant of neighbor-joining under a GTR + I + G model of nucleotide substitution and rooted with three ascomycetes. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. Bootstrap values are given for 1,000 replicates; values below 50% are omitted

are simple (Figs. 3a,b, 4a, 5a,b, 6a,c, 7a,b, 8a,b, 10a; see also Bauer and Oberwinkler 1994 and the references therein) and lack membrane-bounded caps in contrast to those simple pores occurring in the Ustilaginomycotina (Ustilaginomycetes) (Fig. 3c,d; Bauer et al. 1995, 1997). The vast majority of Agaricomycotina (Hymenomycetes) possess dolipores (Fig. 3e,f; Oberwinkler 1985).

## Agaricostilbomycetes, Spiculogloeales and Kondoaceae

Because of the morphological and ecological divergence, the monophyly of the Agaricostilbomycetes is difficult to discern. In our molecular analyses this diversity is reflected in the long terminal branches within that group (Fig. 1).

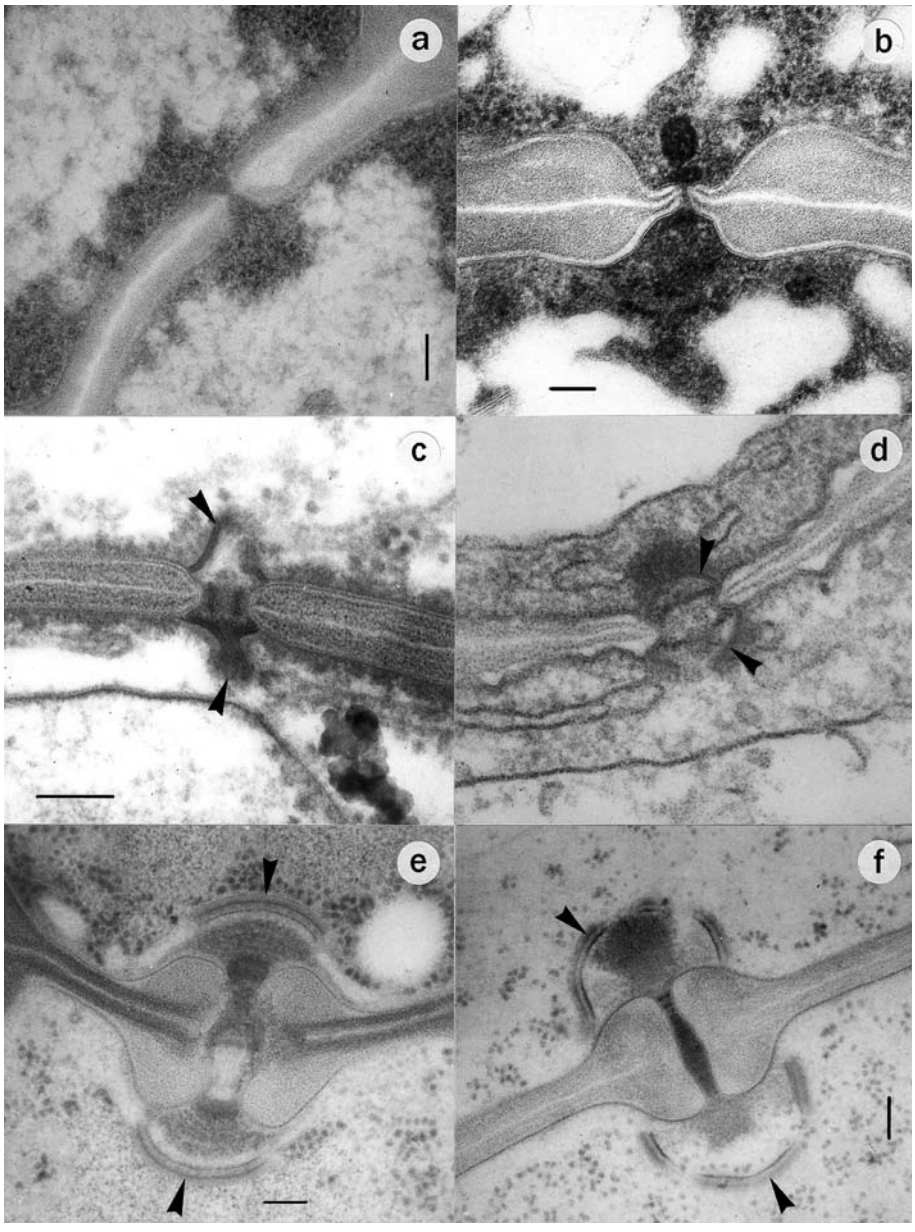


**Fig. 3** Basidiomycetous septal pore apparatus. Bars = 0.1 µm. Material illustrated in c, e, f was prepared using high-pressure freezing and freeze substitution **a**, **b** Simple pores of *Cyrenella elegans* Goch. F 189 (a) and *Septobasidium carestianum* Bres. RB 928 (b) with lack of membrane caps and parenthesomes, representative for the Pucciniomycotina **c**, **d** Simple pores with membrane caps (arrowheads) of *Exobasidium pachysporum* Nannf. RB 947 (c) and *Urocystis ranunculi* (Lib.) Moesz RB 609 (d, magnification as in c), representative for the Ustilaginomycotina (Ustilaginomycetes) **e**, **f** Dolipore with imperforate parenthesomes (arrowheads) of *Dacrymyces stillatus* Nees RB 3102 (e) and dolipore with perforate parenthesomes (arrowheads) of *Schizophyllum commune* Fr. FO 41666 (f), representative for the Agaricomycotina (Hymenomycetes)

The monophyly of the Agaricostilbomycetes is only weakly supported by LSU data (Table 1), but there is a higher support for the group in the combined analysis of SSU and LSU sequences, at least in the neighbor-joining tree (Fig. 2). It is possible that the Agaricostilbomycetes have to be separated into two classes in the future, but at the moment, a separation appears to be premature. Maximum parsimony analyses of the LSU/SSU data set often group *Mixia* within the Agaricostilbomycetes, with a bootstrap support of up to 70% (data not shown). The unstable position of *Mixia* in the various analyses might be a result of a long-branch attraction artifact.

The teleomorphic genera of the Agaricostilbomycetes are *Agaricostilbum*, *Chionosphaera*, *Stilbum*, *Kondoa Mycogloea* and *Spiculogloea*. All are dimorphic. Except for *Chionosphaera* (Oberwinkler and Bandoni 1982), all sexual species form phragmobasidia. Within the Spiculogloeales at least *Spiculogloea*, and in the Agaricostilbales only *Kondoa* are ballistosporic (Fonseca et al. 2000). In our LSU tree (Fig. 1) *Kondoa* forms a well-supported subgroup

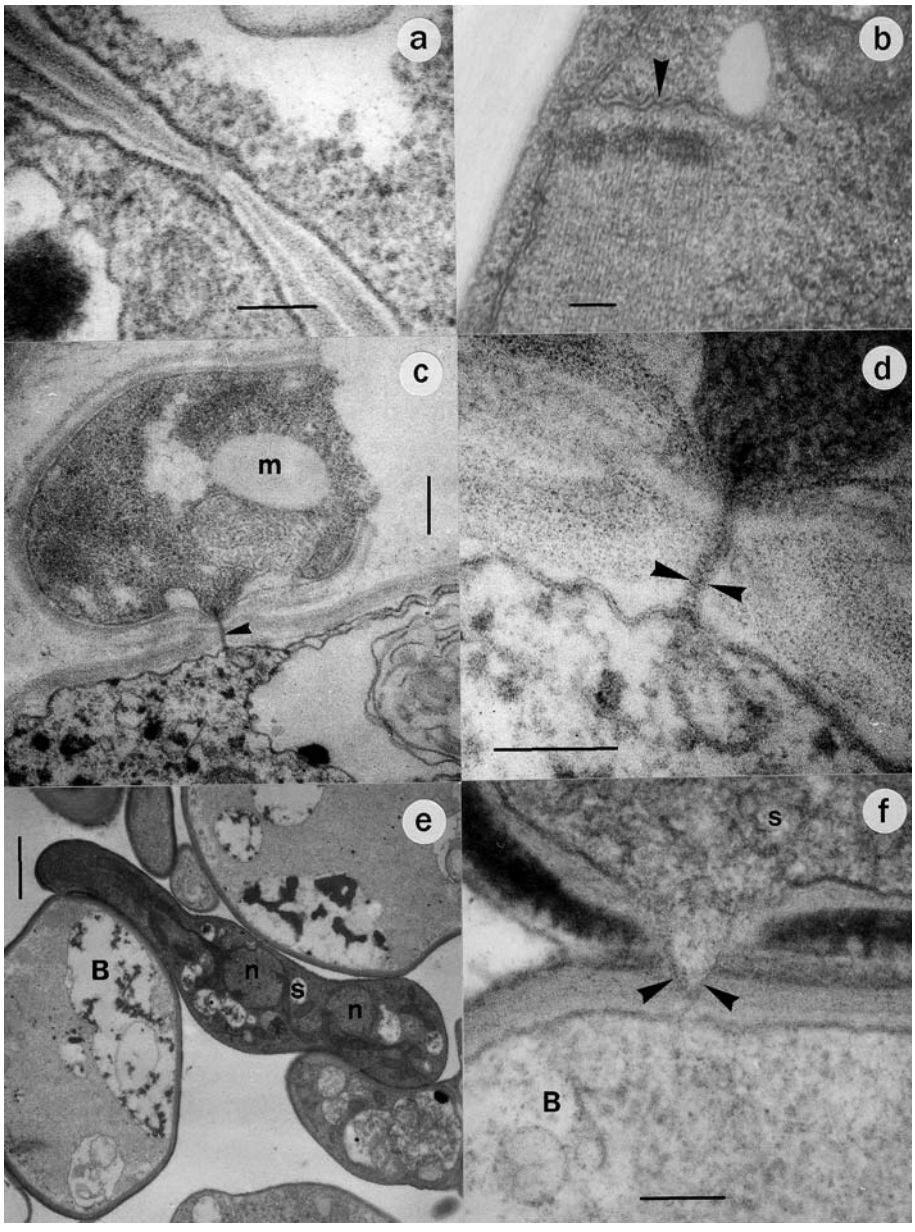


**Fig. 4** Ultrastructural characteristics of the Agaricostilbomycetes. Bars = 0.1 µm in a, b, d, f, 0.2 µm in c, and 1 µm in e **a** Simple septal pore of *Spiculogloea occulta* P. Roberts GEL 607 **b** Intranuclear metaphase I SPB of *Agaricostilbum pulcherrimum* (Berk. & Broome) B.L. Brady, B. Sutton & Samson F 129. Nuclear envelope is visible at arrowhead **c** Tremelloid haustorial cell of *Mycogloea* sp. FO 40962 (m) in contact with a host hypha demonstrating the fusion pore (arrowhead) **d** Detail of e. Note that the pore membrane (arrowheads) is continuous with the plasma membranes of both cells **e** Tremelloid haustorial cell of *Spiculogloea minuta* P. Roberts FO 38413 (s) in contact with a host hypha of *Botryobasidium subcoronatum* (v. Höhn. & Litsch.) Donk (B). Note that two nuclei (n) within the tremelloid haustorial cell are sectioned **f** Fusion pore between *Spiculogloea minuta* P. Roberts FO 38413 (s) and *Botryobasidium subcoronatum* (v. Höhn. & Litsch.) Donk (B) in detail. Note that the pore membrane (arrowheads) is continuous with the plasma membranes of both cells

with some *Bensingtonia* species. We propose the family Kondoaceae for this subgroup.

The species of *Agaricostilbum Chionosphaera* and *Stilbum* develop stilboid basidiocarps (Oberwinkler and Bandoni 1982). *Agaricostilbum* species have frequently been found on dead material of palms, whereas *Stilbum vulgare* Tode has been collected from heterogeneous substrates. *Chionosphaera* species have sometimes been suspected to be mycoparasitic due to their frequent association with other fungi (e.g., Kirschner et al. 2001). The basidiocarps of *Mycogloea* are minute and are normally found in association with sporocarps of ascomycetes (Bandoni 1995). A distinctive feature of *Mycogloea* are the deciduous basidia that easily detach from the probasidia. The auricularioid basidial stage of *Kondoa* is known only from cultures (Fonseca et al. 2000). Species of

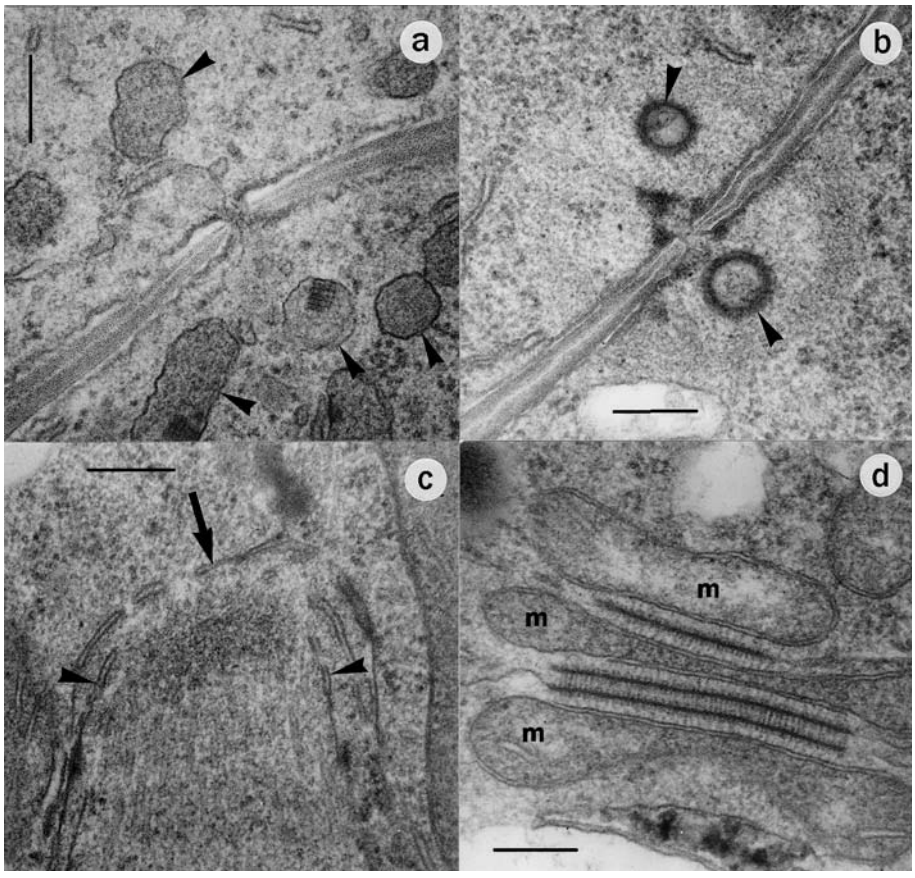


**Fig. 5** Ultrastructural characteristics of the Atractiellomycetes. Bars = 0.2 µm **a** Septal pore apparatus of *Saccoblastia farinacea* (Höhn.) Donk FO 39218 with nonswollen pore margin and associated microbodies (arrowheads) in a more or less circular arrangement **b** Septal pore apparatus of *Atractiella* sp. RB 3100 with an organelle-free zone and atractosomes (arrowheads) **c** Metaphase I SPB of *Atractiella* sp. RB 3100 inserted into the nuclear envelope (arrowheads). Note an ER-cap (arrow) surrounding the SPB **d** Symplechosomes of *Atractiella* sp. RB 3100 connected with mitochondria (m)

*Spiculogloea* are intrahymenial mycoparasites with auricularioid basidia that are ornamented with fine spicules (Roberts 1996, 1997).

In our phylogenetic analyses of the joint SSU/LSU data set, *Spiculogloea* sp. RB 1040 and *Mycogloea* sp. FO 40962 represent a well-supported clade (Fig. 2). The members of this clade are mycoparasites having tremelloid haustorial cells (Figs. 4c–f; Bauer 2004; Roberts 1996; Weiß et al. 2004). A clamp is subtended to each tremelloid haustorial cell, which consists of a subglobose basal part with one or more thread-like filaments. These specific cells were first described and designated as "haustoria" by Olive (1947). Such cells are typical for the mycoparasitic Tremellales of the Agaricomycotina (Hymenomycetes) (Bandoni 1984; Oberwinkler et al. 1984), but surprisingly, they also occur in mycoparasitic members of the Pucciniomycotina. Thus, tremelloid haustorial cells are known from species of the pucciniomycotinous genera *Classicula*, *Cystobasidium*, *Jaculispora*, *Mycogloea*, *Occultifur*, *Spiculogloea* and *Zygogloea* (Bauer 2004; Roberts 1994, 1996, 1997; Sampaio et al. 1999; Oberwinkler 1990; see also below). It is unclear whether the tremelloid haustorial cells occurring in the Tremellales and those of members of the Pucciniomycotina are homologous. However, in *Classicula* (Marvanová and Bandoni 1987), *Spiculogloea* (Fig. 4e) and *Occultifur* (Fig. 7d) the tremelloid haustorial cells are binucleate, whereas available data indicate that those occurring in the Tremellales are mononucleate (Oberwinkler et al. 1984). Nevertheless, as in the Tremellales (Bauer and Oberwinkler 1990b,c; Zugmaier et al. 1994), the tremelloid haustorial cells in *Spiculogloea* and *Mycogloea* sp. FO 40962 are capable of fusing with host cells via a small pore of approximately 14–19 nm width (nanometer-fusion interaction, see Figs. 4c–f and Bauer 2004).

Meiosis has been studied in detail in *Agaricostilbum pulcherrimum* (Berk. & Broome) B.L. Brady, Sutton & Samson. In metaphase, the SPBs are intranuclear (Fig. 4b; Bauer et al. 1992). We have also found intranuclear SPBs in *Stilbum* and *Spiculogloea*. *Agaricostilbum pulcherrimum* possesses a unique trait among the Pucciniomycotina investigated. In prophase I the SPBs have a nucleoplasmic separation; the SPB with elongating middle piece enters the nucleoplasm through a disruptered nuclear envelope. All other Pucciniomycotina studied have a cytoplasmic SPB separation (see Bauer et al. 1991 and the references

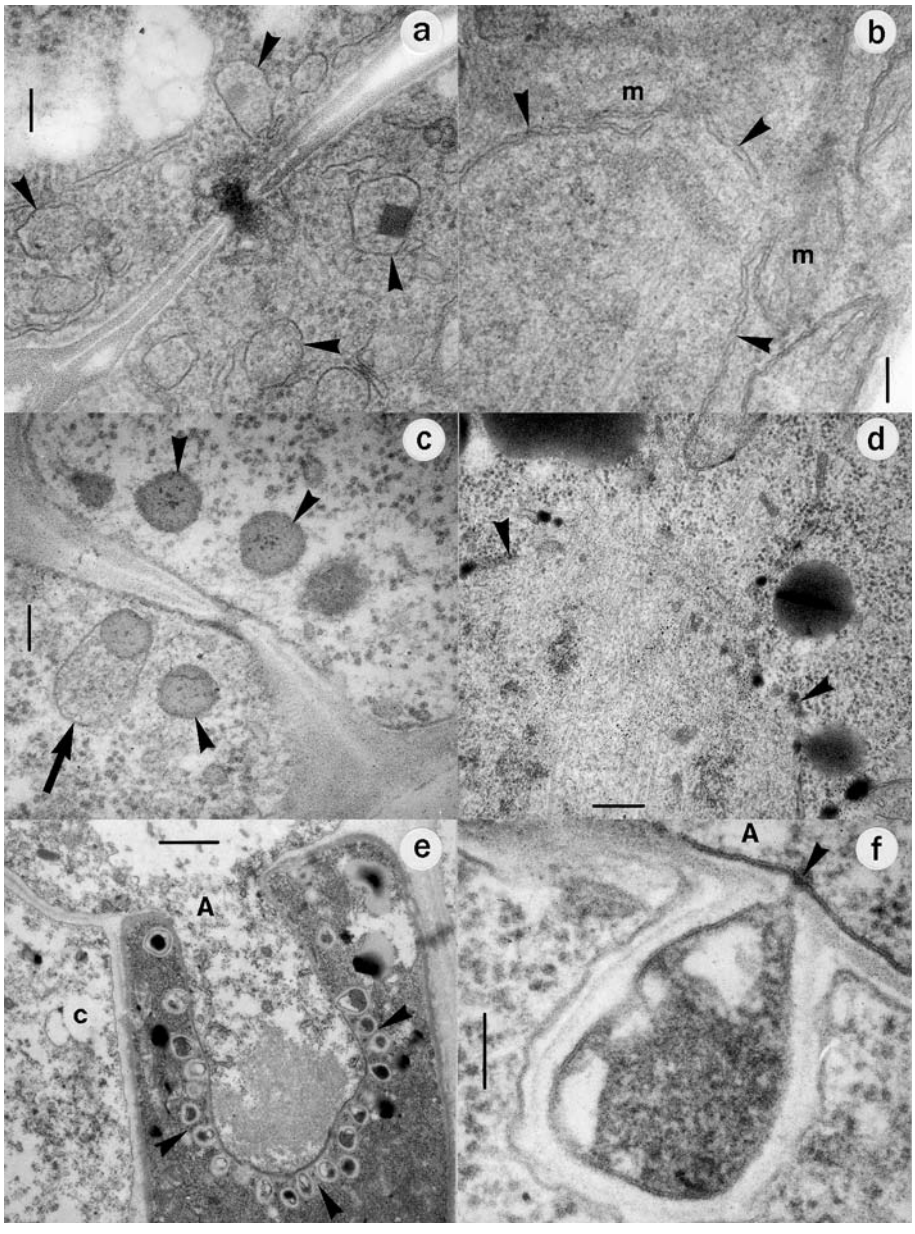


**Fig. 6** Ultrastructural characteristics of the Classiculomycetes (a, b) and Cryptomycocolacomycetes (c-f). Bars = 0.1 µm in a-c, f, 0.2 µm in d, and 1 µm in e **a** Septal pore apparatus of *Classicula fluitans* R. Bauer, Begerow, Oberw. & Marvanová ATCC 64713 with nonswollen pore margin and associated microbodies (arrowheads) in a more or less circular arrangement. Note that the organelle-free zone is of poor contrast **b** Intranuclear mitotic metaphase SPB of *Jaculispora submersa* H.J. Huds. & Ingold CCM 8127; nuclear envelope (arrowheads), mitochondria (m) **c** Simple septal pore of *Cryptomycocolax abnormis* Oberw. & R. Bauer FO 40023 associated with microbodies (arrow) and bodies resembling Woronin bodies (arrowheads) **d** Metaphase I SPB of *Cryptomycocolax abnormis* Oberw. & R. Bauer FO 40023 inserted into the nuclear envelope (arrowheads) **e** Host cell (A) intruding into a hyphal cell of *Cryptomycocolax abnormis* Oberw. & R. Bauer FO 40023 (c). Colacosomes are visible at arrowheads **f** Colacosome of *Cryptomycocolax abnormis* Oberw. & R. Bauer FO 40023 in contact with host cytoplasm (A) showing the fusion pore (arrowhead). Note that the pore membrane is continuous with both the host plasma membrane and the membrane surrounding the core of the colacosome

therein). It should be noted, however, that *Agaricostilbum pulcherrimum* is the only member of the Agaricostilbomycetes that has been investigated so far in this respect.

In summary, presently, the Agaricostilbomycetes are characterized by negative characters (for the individual characters, see the discussion of the respective classes below). In contrast with the Atractiellomycetes, they are dimorphic; in contrast with the Classiculomycetes, they have septal pores without microbodies; in contrast with the Cryptomycocolacomycetes, they lack colacosomes; in contrast with the Cystobasidiomycetes, they have fucose as a cell wall carbohydrate component; in contrast with the Microbotryomycetes, they are neither phytoparasitic (Microbotryales, *Kriegeria*) nor do they have colacosomes (Heterogastridiales, Leucosporidiales, Sporidiobolales), teliospores (Leucosporidiales, Micro-

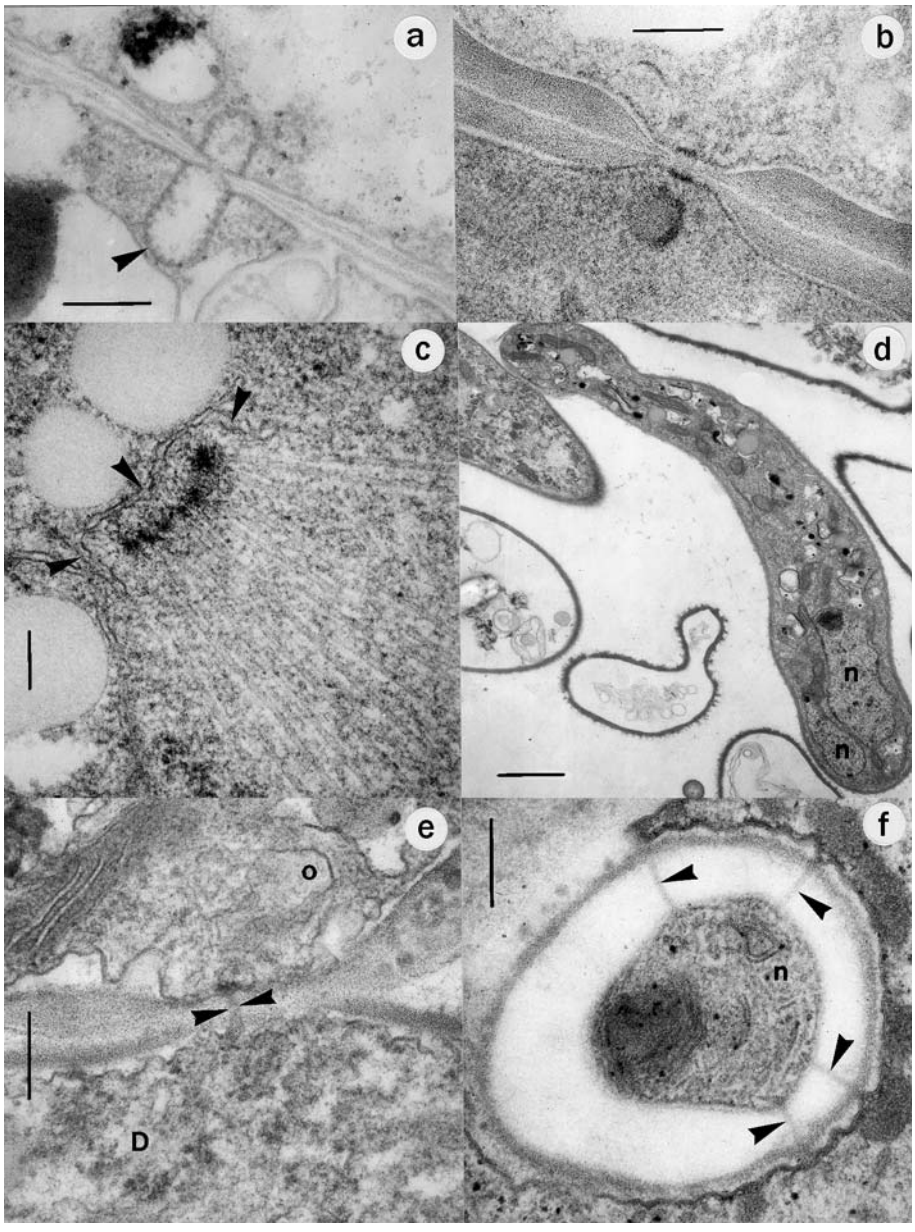


**Fig. 7** Ultrastructural characteristics of the Cystobasidiomycetes. Bars = 0.2 µm in a, b, e, f, 0.12 µm in c, and 1 µm in d **a** Septal pore of *Cystobasidium fimetarium* (Schum.) P. Roberts RB 3103 (obtained from K.-H. Rexer) occluded by a cystosome (arrowhead) **b** Simple septal pore of *Naohidea sebacea* (Berk. & Broome) Oberw. RJB 7257. Note that the pore is not occluded by a cystosome **c** Intranuclear metaphase II SPB of *Occultifur internus* (L.S. Olive) Oberw. FO 31775. Nuclear envelope is visible at arrowheads **d** Tremelloid haustorial cell of *Occultifur internus* (L.S. Olive) Oberw. FO 31775 in contact with a host hypha. Note that two nuclei (n) are visible in the haustorial cell **e** Detail of d showing the fusion pore between *Occultifur internus* (L.S. Olive) Oberw. FO 31775 (o) and its dacrymycetous host (D). Note that the pore membrane (arrowheads) is continuous with the plasma membranes of both cells **f** Haustorial apex (n) of *Naohidea sebacea* (Berk. & Broome) Oberw. RJB 7257 showing four sectioned fusion bridges (arrowheads)

botryales, Sporidiobolales, *Leucosporidium antarcticum*, *L. fasciculatum*), curved holobasidia (*Curvibasidium*; Sampaio et al. 2004) or radiate conidia (*Camptobasidium*; Marvanová and Suberkropp 1990); in contrast with the Mixiomycetes, they are not phytoparasitic; and in contrast with the Pucciniomycetes, they are either dimorphic or the basidiospores remain aseptate during germination (Septobasidiales). However, the present knowledge of the group is certainly restricted to a high degree by the low number of species that have so far been described in the Agaricostilbomycetes.

## Atractiellomycetes

The Atractiellomycetes are well characterized by the formation of symplechosomes (Fig. 5d; Bauer and Oberwinkler 1991a; Oberwinkler and Bauer 1989). Each

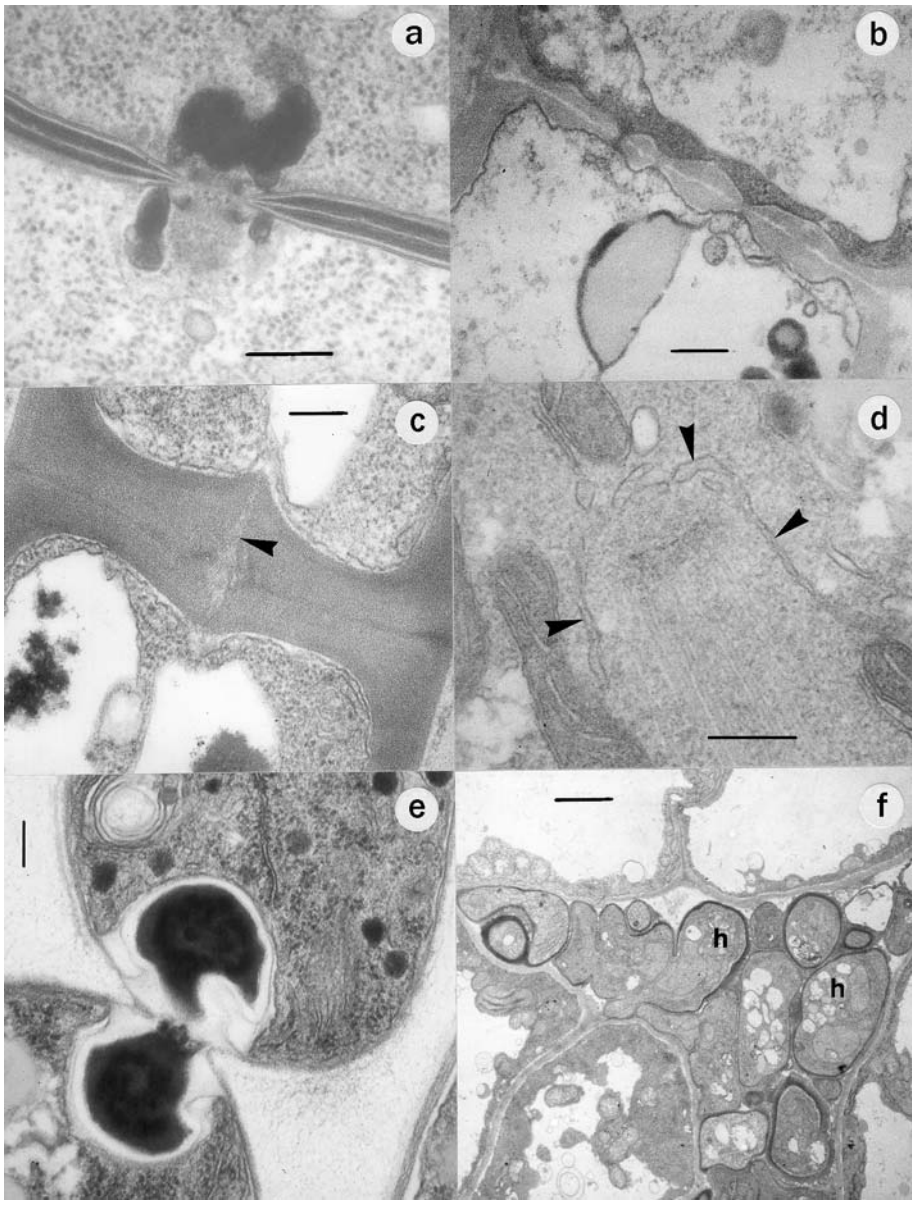


**Fig. 8** Ultrastructural characteristics of the Microbotryomycetes. Bars = 0.2 in a-e, and 2 µm in f. Material illustrated in a was prepared using high-pressure freezing and freeze substitution **a** Simple septal pore of *Heterogastridium pycnidioideum* Oberw. & R. Bauer FO 40023a associated with electron-opaque material **b** Multiperforate septum of *Kriegeria eriophori* Bres. RB 1030 **c** Pore equivalent in *Sphacelotheca hydropiperis* (Schum.) de Bary HUV 13667 with a median swelling traversed by a non-membrane-bounded line (arrowhead) **d** Intranuclear metaphase II SPB of *Heterogastridium pycnidioideum* Oberw. & R. Bauer ATCC 48561. Nuclear envelope is visible at arrowheads **e** Self-parasitism by colacosomes of two neighboring hyphae of *Colacogloea peniophorae* (Bourdot & Galzin) Oberw. & Bandoni FO 36346 **f** Hyphae of *Microbotryum scabiosae* (Sow.) G. Deml & Prillinger FO 29227 in the intercellular space of *Knautia arvensis* (L.) Coult.

symplechosome consists of stacked cisternae of the endoplasmic reticulum (ER), which are interconnected by hexagonally arranged filaments. Mature symplechosomes are usually interconnected at both sides with mitochondria by the same filament system. LSU data support the monophyly of this group only weakly (Fig. 1 and Table 1). However, the combined analysis of SSU and LSU data resulted in a good support (up to 100%) for some alignments (Table 2). Thus, the applied algorithms might have been affected differently from the diversity of the sequences of the members of this class.

The Atractiellomycetes are saprotrophic and not dimorphic: basidiospore germination with hyphae usually results in the formation of a saprobic haplophase with the formation of haploid conidia (Bauer and Oberwinkler 1986, Ingold 1992, Oberwinkler and Bandoni 1982). The fructifications of the Atractiellomycetes are highly diverse. Thus, *Helicogloea* and *Saccoblastia* have resupinate fructifications (Baker 1936), whereas *Atractiella* and *Phleogena* form stilboid basidiocarps (Oberwinkler and Bandoni 1982). In addition, we have recently detected two new members of the Atractiellomycetes (designated as

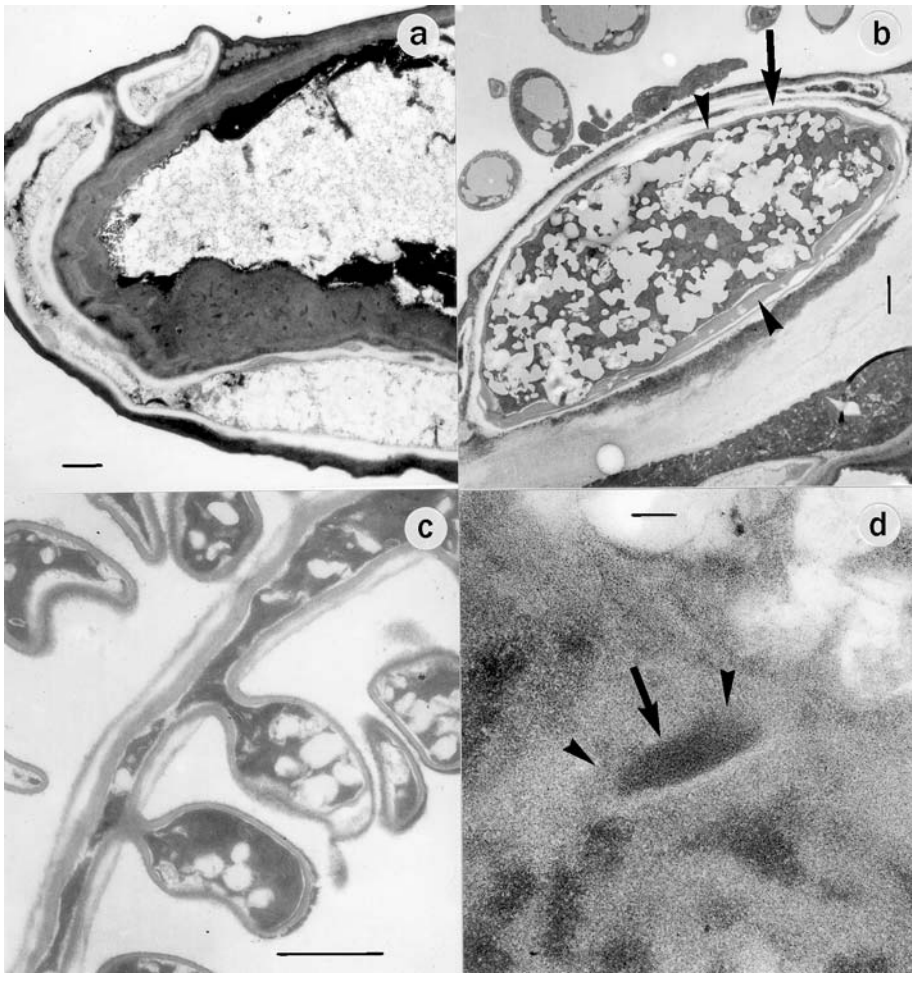


**Fig. 9** Ultrastructural characteristics of the Mixiomycetes. Bars = 1 µm in a-c, and 0.1 µm in d. Material illustrated is from a herbarium specimen obtained from J. Sugiyama, Japan **a** Hypha of *Mixia osmundae* (Nishida) C.L. Kramer RB 3101 in contact with a host cell of *Osmunda regalis* L. Note the electron-transparent appearance of the hyphal cell wall; the host cell wall is thickened at the contact area **b** Sac-like swelling of *Mixia osmundae* (Nishida) C.L. Kramer RB 3101 under the epidermal cuticle. Note that the wall of the swelling is roughly two-layered: the outer layer is electron-transparent (arrow), whereas the inner, secondary layer is electron-opaque (arrowheads) **c** Sporogenous cell of *Mixia osmundae* (Nishida) C.L. Kramer RB 3101 with two enteroblastically developing spores. Note that the walls are electron-opaque **d** Longitudinally sectioned interphase SPB of *Mixia osmundae* (Nishida) C. L. Kramer RB 3101 showing two SPB elements (arrowheads) connected by a middle piece (arrow)

Atractiellomycete AH 33906 and Atractiellomycete FO 44664 in Figs. 1 and 2) that have pycnidioid basidiocarps. The species of *Helicogloea* and *Saccoblastia* are ballistosporic, whereas the others have gastroid sporulation (Oberwinkler and Bandoni 1982). Several anamorphic hyphomycetes such as *Hobsonia*, *Infundibura* and *Leucogloea* have recently been ascribed to the Atractiellales (Kirschner 2004).

As in most Pucciniomycetes, the septal pore apparatus in the Atractiellomycetes possess an organelle-free zone. At least in the type of *Saccoblastia*, *S. farinacea* (v. Höhn.) Donk, the septal pores are associated with microbodies (Fig. 5a), whereas in the type of *Helicogloea*, *H. lagerheimii* Pat. and in *Atractiella*, *Phleogena* and the two new species (Atractiellomycetes AH 33906 and FO 44664 mentioned above), the microbodies are substituted by atractosomes (Fig. 5b; Weiß et al. 2004). Atractosomes originate from ER cisternae: an ER cisterna curves at the margin to form a globular compartment, the atractosome. *Helicogloea* and *Saccoblastia* are characterized by basidia with a lateral probasidial sac in which karyogamy occurs (Baker 1936). The delimitation between the two genera is insufficiently based only on the texture of the resupinate basidiocarps. It was therefore surprising to find different septal pore apparatus at least in the types of the two genera. The puccinialean septal pore architecture in *Saccoblastia* (Fig. 5a) suggests that this genus obtains a basal position within the Atractiellomycetes.

## Classiculomycetes

This class contains only two species, the teleomorphic *Classicula fluitans* R. Bauer, Begerow, Oberw. & Marvanová and the anamorphic *Jaculispora submersa* Huds. & Ingold (Bauer et al. 2003), which may be representatives of a pucciniomycotinous group of aquatic mycoparasites. Among the Pucciniomycotina, *Classicula*

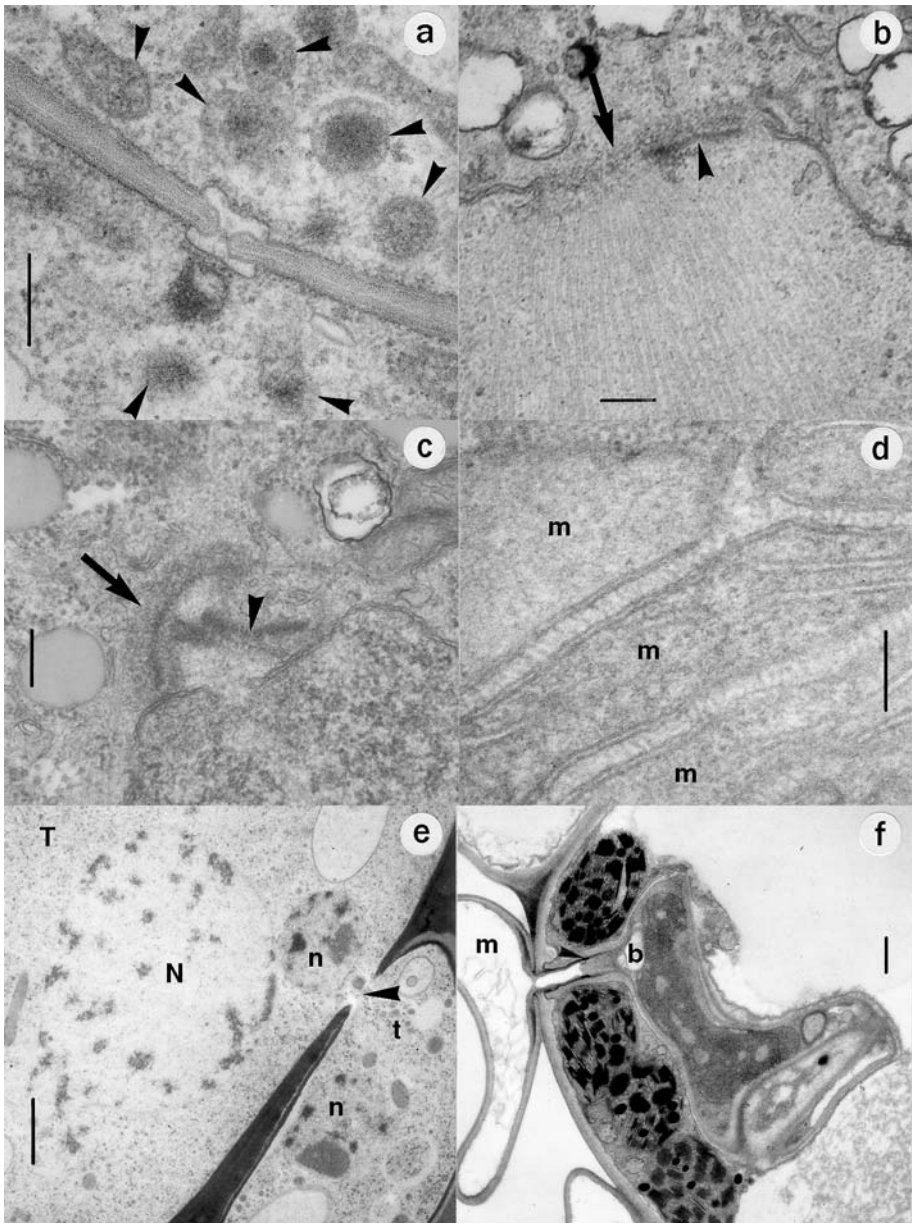


**Fig. 10** Ultrastructural characteristics of the Pucciniomycetes. Bars = 0.2 µm in a-c, 0.1 µm in d, and 1 µm in e, f. Material illustrated in e was prepared using high-pressure freezing and freeze substitution **a** Simple septal pore of *Puccinia suaveolens* (Pers.) Rostr. BM 3663 surrounded by microbodies (arrowheads) in a more or less circular arrangement **b** Metaphase I SPB of *Gymnosporangium clavariiforme* (Pers.) DC. GD 849 inserted in the nuclear envelope. A co-disc (arrowhead) has developed within the layers of the parent disc (arrow) **c** Late telophase I – interphase I in *Gymnosporangium clavariiforme* (Pers.) DC. GD 849. The co-disc (arrowhead) detaches from the parent disc (arrow) **d** Mitochondrial complex in *Septobasidium carestianum* Bres. RB 928. Mitochondria (m) are connected by fine filaments **e** Interaction stage between *Tuberculina persicina* (Ditmar) Sacc. (t) and *Tranzschelia pruni-spinosae* (Pers.) Dietel (T) TUB 011530 with the fusion pore (arrowhead), two sectioned nuclei of *Tuberculina* (n) and one sectioned nucleus of *Tranzschelia* (N). Note that one *Tuberculina* nucleus is located within the hyphal cell of *Tranzschelia* **f** Haustorial profile of *Coleosporium senecionis* (Pers.) Fr. FO 25095 with haustorial mother cell (m), haustorial neck with neckband (arrowhead) and haustorial body (b)

is unique in having subapically swollen sterigmata. Conidia of *Classicula* are similar to those of *Jaculispora* in size and in form: in both species, the conidia are naviculate, with three to four distal setose branches. These conidia in *Classicula* and in *Jaculispora* with broadly diverging branches resemble those of aquatic hyphomycetes (Ingold 1979). In fact, as noted by Marvanová and Bandoni (1987), *Classicula* and *Jaculispora* were frequently found in freshwater habitats. *Classicula* shares with *Jaculispora* two other significant characteristics, the septal pore architecture and the formation of tremelloid haustorial cells.

The septal pore apparatus in both species is composed of a simple pore surrounded by microbodies in a circular arrangement (Fig. 6a; Bauer et al. 2003). In basidiomycetes, this type of septal pore apparatus occurs only in the

Pucciniomycotina. While it is common in Pucciniomycetes and Cryptomycocolacomycetes (Figs. 6c and 10a; Bauer and Oberwinkler 1994; Kirschner et al. 2001; Oberwinkler and Bauer 1990), it occurs sporadically also in some members of the Atractiellomycetes. Thus, *Saccoblastia* in the *Atractiellomycetes* possesses this septal pore type (Fig. 5a; Weiß et al. 2004). We hypothesize that this septal pore type, which occurs in these three distinct lineages and also in *Classicula* and *Jaculispora*, reflects a common ancestral type.

Both *Classicula* and *Jaculispora* form tremelloid haustorial cells. Formation of tremelloid haustorial cells suggest that *Classicula* and *Jaculispora* are mycoparasites or at least have the potential for mycoparasitism (see the discussion of the Agaricostilbomycetes and Cystobasidiomycetes). *Classicula*, as other mycoparasites, is also capable of self-parasitism (Fig. 8e; Jeffries and Cuthbert 1984; Kirschner et al. 1999).

Except for *Classicula* and *Jaculispora*, all pucciniomycotinous mycoparasites with tremelloid haustorial cells possess septal pore apparatus without associated microbodies (Figs. 4a, 7a,b; Oberwinkler 1990; Sampaio et al. 1999). In other words, within the Pucciniomycotina, pore-associated microbodies and tremelloid haustorial cells occur only in *Classicula* and *Jaculispora*. This is consistent with the molecular results. *Classicula* and *Jaculispora* form a well-supported clade, separated from the other mycoparasites with tremelloid haustorial cells (Fig. 1 and Table 1).

## Cryptomycocolacomycetes

In our molecular phylogenetic analyses, *Cryptomycocolax abnormis* Oberw. & R. Bauer and *Colacosiphon filiformis* R. Kirscher, R. Bauer & Oberw. form a well-supported clade (Fig. 1 and Table 1). This phylogenetic placement agrees well with morphological, ultrastructural, and ecological results. Both fungi have uniquely long basidia, and they are both mycoparasites, interacting with their ascomycetous hosts by colacosomes (Fig. 6e,f; Kirschner et al. 2001; Oberwinkler and Bauer 1990). In addition, the septal pore apparatus in *Cryptomycocolax* is essentially identical to that of *Colacosiphon*: in both species the septal pores are surrounded by microbodies and bodies resembling Woronin bodies (Fig. 6c). Like in ascomycetes, the SPB discs in *Cryptomycocolax* (*Colacosiphon* has not been investigated in this respect) are large in size, they are inserted into an otherwise intact nuclear envelope (Fig. 6d), and they duplicate by fission (Oberwinkler and Bauer 1990). Thus, SPB and septal pore features indicate a very basal phylogenetic position of the Cryptomycocolacomycetes within the basidiomycetes.

We are convinced that the colacosomes occurring in the Cryptomycocolacomycetes are homologous to those occurring in the Microbotryomycetes (compare Figs. 6e and 6f with Fig. 8e; see also the discussion of the Microbotryomycetes below). At first glance, it may therefore be surprising that in the molecular analyses the representatives of the Cryptomycocolacomycetes are separated from the Microbotryomycetes (Fig. 1). However, a closer inspection reveals high ultrastructural divergence between the Cryptomycocolacomycetes and Microbotryomycetes. In fact, the puccinialean septal pores of *Cryptomycocolax* and *Colacosiphon* (Fig. 6c) are similar to the septal pores of *Saccoblastia* of the Atractiellomycetes (Fig. 5a), to those of most Pucciniomycetes (Fig. 10a; see Bauer and Oberwinkler 1994 and the references therein) and the members of the Classiculomycetes (Fig. 6a; Bauer et al. 2003), but definitely not to those of the Microbotryomycetes (Fig. 8a–c; Oberwinkler and Bauer 1990; Kirschner et al. 1999; Oberwinkler et al. 1990a,b). Furthermore, *Cryptomycocolax* (*Colacosiphon* has not been investigated in this respect) the discoidal SPBs are inserted in a closely fitting pore of the nuclear envelope during nuclear division (Fig. 6d; Oberwinkler and Bauer 1990). *Cryptomycocolax* shares this feature with the Pucciniomycetes (Fig. 10b; Bauer 1987; Bauer and Oberwinkler 1990a, 1994; Boehm and McLaughlin 1989; Bourett and McLaughlin 1986; O'Donnell and McLaughlin 1981a–c) and with the Atractiellomycetes (Fig. 5c), but not with the Microbotryomycetes (Fig. 8d; Bauer et al. 1991; Berbee et al. 1991). In summary, there is a deep gap between the Cryptomycocolacomycetes and Microbotryomycetes with respect to ultrastructural characters.

In *Cryptomycocolax* (*Colacosiphon* has been unsufficiently investigated in this respect) a second type of colacosome was found along the cytoplasmic intrusions of the host formed into the hyphae of the parasite (Fig. 6e,f; Oberwinkler and Bauer 1990). Colacosomes of that type have a more electron-transparent core, and they are capable of fusing with host cells via a small pore. Fusion with the host cytoplasm may be the ancestral function of colacosomes, distinguishing the mycoparasitic behavior of *Cryptomycocolax* (Cryptomycocolacomycetes) from that of the Microbotryomycetes (compare Fig. 6e,f with Fig. 8e). Accordingly, the evolution of the Microbotryomycetes may have been accompanied by the loss of the colacosome-fusion interaction. In addition, the question arises whether or not the colacosome-fusion mycoparasitism of *Cryptomycocolax* is homologous to the nanometer-fusion interaction occurring in the Cystobasidiomycetes, Agaricostilbomycetes and possibly also in the Classiculomycetes (compare Fig. 6f with Fig. 4c–f and Fig. 7d–f; see also Bauer 2004 and the discussion of the Agaricostilbomycetes and Cystobasidiomycetes). It is plausible that the colacosome-fusion interaction occurring in *Cryptomycocolax* represents the ancestral type of the nanometer-fusion interaction. In a first evolutionary step, an apoplastic compartment, the colacosome, developed that was able to fuse with the host cytoplasm. In a second step, the colacosome was reduced, and the parasitic cytoplasm fused directly with the host cytoplasm. Unfortunately, we were not able to amplify SSU rDNA from *Cryptomycocolax* or *Colacosiphon* so far. Thus, we could not analyze this class in the combined data set, which might have shed light

on its relationship to the Microbotryomycetes. The basal position of the *Cryptomycocolacomycetes* in Fig. 1 has not been supported by bootstrap values higher than 50%, regardless which alignment algorithm or method of phylogenetic reconstruction was used.

## Cystobasidiomycetes, Cystobasidiales, Erythrobasidiales and Naohideales

Among the Pucciniomycotina, the lack of fucose as cell wall carbohydrate component characterizes the Cystobasidiomycetes (Takashima et al. 2000). The group is well supported in all molecular analyses (Figs. 1 and 2; Tables 1 and 2), and Cystobasidiales and Erythrobasidiales were supported in most analyses of LSU rDNA sequences with 70–100% bootstrap values.

The sexual genera of the Cystobasidiomycetes are *Cystobasidium*, *Occultifur*, *Sakaguchia* and *Naohidea*. All members are phragmobasidiate and dimorphic, producing a yeast phase in the haploid stage. *Naohidea* produces a yeast stage with white colonies, whereas all other Cystobasidiomycetes have a yeast stage with pink-orange colonies. *Bannoa* and *Erythrobasidium* are not truly teleomorphic genera because the proposed basidial stages are in fact conidial stages (Sampaio et al. 1999). Among the Cystobasidiomycetes, *Bannoa* and *Erythrobasidium* share a hydrogenated coenzyme Q10 system (CoQ10 (H2)) (Hamamoto et al. 1988, 2002). In molecular analyses *Bannoa* and *Erythrobasidium* form a well-supported subcluster of the Cystobasidiomycetes (Sampaio 2004). Surprisingly, the basidiomycetous anamorph *Cyrenella elegans* Goch. is also a member of the Cystobasidiomycetes. The conidia of *Cyrenella* with radiating arms resemble those of aquatic hyphomycetes and may have evolved as an adaptation to water dispersal (Ingold 1979). This fungus is dimorphic and produces an orange-pigmented yeast stage. Like *Cyrenella*, *Sakaguchia* has also been isolated from aquatic habitats. Moreover, both taxa produce teliospores, although teliospore germination in *Cyrenella* has never been observed. While *Cyrenella* was found in a freshwater-related environment, *Sakaguchia* was isolated from seawater.

*Naohidea* builds gelatinous, film-like basidiocarps on pyrenomycetous hosts. *Cystobasidium* and *Occultifur internus* (L.S. Olive) Oberw. are intrahymenial parasites lacking basidiocarps (Oberwinkler 1990, Roberts 1997), whereas *Occultifur externus* J.P. Samp., R. Bauer & Oberw. is known only from culture (Sampaio et al. 1999). *Cystobasidium*, *Naohidea* and *Occultifur* are nanometer-fusion mycoparasites (Fig. 7d–f; Bauer 2004; see also the discussion of the Agaricostilbomycetes above). *Cystobasidium* and *Occultifur* interact with their respective hosts with tremelloid haustorial cells that are capable of fusing with host cells via a small pore of approximately 14–19 nm (Fig. 7d,e; Weiß et al. 2004). Thus, a direct cytoplasm–cytoplasm connection between the parasites and their respective hosts is formed. Besides the formation of tremelloid haustorial cells, the members of *Cystobasidium* and *Occultifur* share a distinct septal pore apparatus: the simple pores are enclosed by a peculiar organelle with a reticulate surface, the cystosome (Fig. 7a; Sampaio et al. 1999; Weiß et al. 2004). We have found cystosomes only in members of these two genera; this organelle may therefore be an apomorphy for this subgroup (compare Fig. 7a with Fig. 7b). This phylogenetic indication agrees well with the molecular results. Thus, in our LSU analysis, the sister group relationship of *Cystobasidium* and *Occultifur* is well supported by a bootstrap value of 100% (Fig. 1).

In contrast with *Occultifur* and *Cystobasidium*, *Naohidea sebacea* (Berk. & Br.) Oberw. does not form tremelloid haustorial cells. However, *N. sebacea* interacts with its pyrenomycetous hosts by the formation of intracellular haustoria, in which a prominent electron-transparent space separates the haustorial cell from the host cytoplasm. Like in the other nanometer-fusion mycoparasites, plasmodesmata-like membrane-bounded fusion bridges traversing the electron-transparent space connect the cytoplasm of the haustorial filament with the host cytoplasm in the interaction between *N. sebacea* and its host (Fig. 7f; Bauer 2004). *Naohidea* appears in a basal position within the Cystobasidiomycetes in our LSU analysis (Fig. 1), which suggests that the Cystobasidiomycetes may have arisen from nanometer-fusion mycoparasites (Bauer 2004).

As discussed above, the Tremellales s. l. (including the Christianseniales and Filobasidiales) of the Agaricomycotina (Hymenomycetes) (Bandoni 1984, 1995; Wells and Bandoni 2001) are also typical nanometer-fusion mycoparasites (Bauer and Oberwinkler 1990b,c; Zugmaier et al. 1994). There are two possible explanations for the occurrence of nanometer-fusion mycoparasites in the Pucciniomycotina on the one hand and in the Tremellales on the other: (1) the nanometer-fusion interaction occurring in these two distinct groups is a result of convergent evolution, or, alternatively, (2) the common ancestor of both groups or of the Basidiomycota was a nanometer-fusion mycoparasite. The nanometer-fusion interaction occurring in some Agaricostilbomycetes, Cystobasidiomycetes and possibly also in the Classiculomycetes (see above) could be of a common phylogenetic origin, representing an apomorphy of these three lineages.

## Microbotryomycetes

With few exceptions, the Microbotryomycetes are dimorphic, producing a yeast phase in the haploid stage. Except for *Curvibasidium* (Sampaio et al. 2004), all teleomorphic members of the Microbotryomycetes are phragmobasidiate. The monophyly of the Microbotryomycetes is highly supported in all molecular analyses (Figs. 1 and 2; Tables 1 and 2). Morphologically and ecologically, however, the members of this class are diverse. Sporulation ranges from basidia produced from single teliospores as in *Leucosporidium* or *Microbotryum* to the formation of scattered basidia (e.g., *Kriegeria*) to complex pycnidioid basidiomes, like those of *Heterogastridium*. There are phytoparasitic taxa (e.g., Microbotryales or *Kriegeria*) and

mycoparasites (Heterogastridiales) (Weiß et al. 2004). A third group includes organisms usually regarded as saprobes, such as the Sporidiobolales, Leucosporidiales and *Camptobasidium*. However, since the life cycles of the species in the Leucosporidiales and Sporidiobolales have not been investigated under natural conditions, parasitism cannot be ruled out. In fact, except for the Microbotryales, *Kriegeria*, *Camptobasidium*, *Leucosporidium antarcticum*, *L. fasciculatum* and *Curvibasidium*, all teleomorphic members of the Microbotryomycetes possess colacosomes (Fig. 8e; Sampaio et al. 2003). Therefore, mycoparasitism may be more widespread among Microbotryomycetes than currently assumed.

Colacosomes were first described in detail from the interaction of the parasite *Colacogloea peniophorae* (Bourdot & Galzin) Oberw. & Bandoni and its host *Hyphoderma praetermissum* (Karst.) Erikss. & Strid. (Bauer and Oberwinkler 1991b, Oberwinkler et al. 1990a). During interaction, the membrane-bounded core of the colacosomes intrudes into the host cell wall. Thus, colacosomes combine the hyphal cells of the parasite with those of the host fungus, serving as connecting agents. The formation of colacosomes thus increases the host–parasite interface. Bauer (2004) summarized the colacosome interactions, demonstrating their morphological and functional variety. Thus, in *Colacogloea peniophorae*, colacosomes develop in a large number close to each other. It is evident that hyphae possessing colacosomes and their host hyphae are closely attached over a relatively long distance. *Colacogloea papilionacea* R. Kirschner & Oberw. forms hyphal coils possessing colacosomes around the hyphae of its ascomycetous host (Kirschner and Oberwinkler 2000), whereas in *Colacogloea bispora* (Hauerslev) Oberw. & Bauer (Oberwinkler et al. 1999), *Heterogastridium* (Bauer 2004), and *Krieglsteinera* (Bauer 2004), the appearance of colacosomes is associated with curious interaction structures: filamentous outgrowths of the host cells are intimately enclosed by galloid parasite cells. Numerous colacosomes are present along the contact area between the host intrusion and the parasite cell. These host intrusions always terminate in the parasite cell.

The Leucosporidiales and the Sporidiobolales are well supported in our molecular analyses (Fig. 1). However, our molecular analyses neither support nor reject the monophyly of the Heterogastridiales (see the synopsis above) significantly. In fact, the resolution of the phylogenetic backbone of the Microbotryomycetes is quite low in our trees (Fig. 1), and further studies are needed to clarify this question. However, our molecular analyses at least suggest that the Microbotryomycetes arose from colacosome-forming mycoparasites with subsequent transitions to plant parasitism (Fig. 8f). Thus, the representatives of the Heterogastridiales appear in a basal position within this group (Fig. 1). Transition from mycoparasitism to plant parasitism was apparently accompanied by the loss of colacosomes. In fact, the phytoparasitic Microbotryales and *Kriegeria* do not form colacosomes (Bauer et al. 1997). Members of *Sporidiobolus* and some species of *Rhodosporidium* have been frequently isolated from the phylloplane or other plant-related substrates (Sampaio et al. 2003). Since they have colacosomes, it is possible that they parasitize other fungi sharing the same habitat. Possibly, the ancestors of the Microbotryales originally grew on the phylloplane and then gradually changed from mycoparasitism to plant parasitism. Starting two decades ago, increasingly persuasive data indicating that the species of *Microbotryum* and their relatives are phylogenetically distant from other smut fungi (e.g., Bauer et al. 1991, 1997; Begerow et al. 1997; Berbee et al. 1991; Blanz and Gottschalk 1984; Deml 1987; Prillinger et al. 1993; Swann and Taylor 1993, 1995) resulted in the erection of the order Microbotryales. Today it is clear that the Microbotryales vs Ustilaginales *sensu* Bauer et al. (1997) represent one of the most fascinating examples of fungal convergence. Both groups are similar with respect to soral morphology, teliosporogenesis, life cycle, and basidial morphology (Bauer et al. 2001).

## Mixiomycetes and Mixiales

*Mixia osmundae* (Nishida) C.L. Kramer parasitizes members of *Osmunda* (Mix 1947). Hyphal cells are multinucleate (Kramer 1958). In the host tissue they grow strictly on the surface of the host cells (Fig. 9a). The host cell walls are thickened at the contact area with the hyphal cells (Fig. 9a). Like in ascomycetes (Nagler et al. 1989), the hyphal cell walls in *Mixia* are electron-transparent and nonfibrillate (Fig. 9a). Because we have not detected septa in our ultrastructural analysis using serial sections, we hypothesize that septation occurs very rarely. Haustoria or other intracellular structures are lacking. Under the cuticle of both the upper and lower epidermis, the hyphae form large sac-like swellings (Fig. 9b). Initially, the cell walls of the hyphal swellings are electron-transparent and nonfibrillate. Subsequently, an electron-opaque, fibrillate, basidiomycetous secondary cell wall develops (Fig. 9b). From these swellings, sporogenous cells arise (Kramer 1958). As typical for basidiomycetes, the cell walls of the sporogenous cells are electron-opaque and fibrillate (Fig. 9c; Nishida et al. 1995). Numerous spores are produced exogenously, enteroblastically and simultaneously from the sporogenous cells (Fig. 9c; Nishida et al. 1995). Like in other basidiomycetes (Bauer et al. 1991), the interphasic SPBs are double-structured in *Mixia*: two SPB elements are connected by a middle piece (Fig. 9d).

With respect to this combination of characters *Mixia* is unique among the Pucciniomycotina. This is consistent with the molecular results (Figs. 1 and 2). *Mixia* is separated from other taxa of the Pucciniomycotina by a long terminal branch, indicating a high genetic divergence. In our molecular phylogenetic analyses involving various alignment algorithms and methods of phylogenetic inference applied to the LSU and LSU/SSU data sets, the placement of *Mixia* was unstable: in LSU analyses always outside the other classes, and in LSU/SSU analyses

sometimes within the Agraricostilbomycetes, which might be caused by long-branch attraction (see discussion above). We therefore propose a separate new class for *Mixia*.

## Pucciniomycetes, Helicobasidiales, and Pachnocybales

In our molecular phylogenetic analyses this lineage was always well supported (Figs. 1 and 2; Table 1). Consistently, cladistic analysis of the 5S rRNA secondary structure has revealed an apomorphic GC in position 8:112 for this lineage (Müller 1989; Wolters 1987). Pucciniomycetes comprise the phytoparasitic Pucciniales, Platygloeales and Helicobasidiales, the Septobasidiales, which are associated with scale insects and, surprisingly, also the saprobic stilboid holobasidiomycete *Pachnocybe ferruginea* (Sow.: Fr.) Berk. (Oberwinkler and Bandoni 1982). The trophic mode of *Platygloea disciformis* (Fr.) Neuhoff may also be phytoparasitic. This fungus sporulates on dead twigs of *Tilia* species. A potential for mycoparasitism has sometimes been presumed (e.g., Oberwinkler et al. 1990a), but no solid evidence for this has been found. The rusts form the largest group of Pucciniomycetes, accounting for about 7,000 species (Kirk et al. 2001; Cummins and Hiratsuka 2003). They possess several apomorphies such as the formation of spermogonia and the subsequently formed aecia, a phytoparasitic haplophase and a life cycle with host alternation (Gäumann 1964). The dikaryotic interaction apparatus of the rusts with a specialized haustorial mother cell, a haustorial neck with neck band and a haustorial body, is unique in the basidiomycetes (Fig. 10f; Littlefield and Heath 1979), differing significantly from that of the Platygloeales (Boehm and McLaughlin 1988; Weiß et al. 2004). In the Pucciniomycetes only the Septobasidiales are dimorphic, forming a yeast phase in the haploid state. In vivo, under aqueous conditions and in vitro on agar discharged aseptate basidiospores of *Septobasidium* species become septate, and the compartments subsequently germinate by budding (Oberwinkler 1987). In vivo, the yeast cells are capable of infecting young scale insects, where dikaryotic coiled hyphae are formed (Couch 1938). The fungus eventually kills the infected scale insect and subsequently, dikaryotic hyphae with basidia arise from the insect body.

All Pucciniomycetes possess clampless hyphae and—perhaps except for the Septobasidiales (Fig. 3b) and *Platygloea disciformis*—the septal pores are of the typical puccinialean type (Littlefield and Heath 1979): an organelle-free zone, delimited by microbodies, surrounds each side of the pore (Fig. 10a; see also Bauer and Oberwinkler 1994 and the references therein). In the Septobasidiales and *P. disciformis*, microbodies surrounding the septal pores in a more or less circular arrangement appear to be lacking, but the pores are often associated with nonmembrane-bounded electron-opaque globules and bands (Fig. 3b; Oberwinkler et al. 1990a). In view of the known fixation problems with these species, these structures might in fact be microbodies that artificially lack the surrounding membrane. Outside the Pucciniomycetes, septal pores associated with microbodies occur also in the Saccoblastiaceae of the Atractiellomycetes (Fig. 5a; Weiß et al. 2004), in the Cryptomycocolacales (Fig. 6c; Oberwinkler and Bauer 1990) and in the Classiculomycetes (Fig. 6a; Bauer et al. 2003).

In all species of the Pucciniomycetes examined, the discoidal SPBs are inserted in a close-fitting pore of the nuclear envelope during nuclear division (Fig. 10b; Bauer 1987; Bauer and Oberwinkler 1994; Boehm and McLaughlin 1989; Bourett and McLaughlin 1986; O'Donnell and McLaughlin 1981a–c). This SPB–nuclear envelope relationship is shared only with the Crytomycocolacales (Fig. 6d; Oberwinkler and Bauer 1990) and the Atractiellales (Fig. 5c; McLaughlin 1987). In *Pachnocybe* (Bauer and Oberwinkler 1990a), *Herpobasidium* (Bauer and Oberwinkler 1994), *Helicobasidium* (Bourett and McLaughlin 1986) and *Eocronartium* (Boehm and McLaughlin 1989), but definitely not in the rusts (Fig. 10b; Bauer 1987; O'Donnell and McLaughlin 1981a–c), a characteristic cap of endoplasmic reticulum (ER cap) encloses the SPB discs during nuclear division. Owing to fixation problems with lack of contrast and loss of membranes, the SPB–ER relationship could not be studied in members of the Septobasidiales. Outside the Pucciniomycetes, ER caps enclosing the SPBs during nuclear division are also characteristic for the Atractiellomycetes (Fig. 5c; McLaughlin 1987). Therefore, this feature may be plesiomorphic for the Pucciniomycetes and apomorphic for the common ancestor of the Pucciniomycetes and Atractiellomycetes. Accordingly, the Atractiellomycetes may represent the sister lineage of the Pucciniomycetes, as already suggested by phylogenetic analysis of 5S rRNA (Wolters 1987). Unfortunately, the phylogenetic relationships between the different classes remained unresolved in our molecular analyses (Figs. 1 and 2).

A specific mode of intermeiotic SPB duplication is known from the rusts and from *P. ferruginea* (Fig. 10b,c; Bauer 1987; Bauer and Oberwinkler 1990a; O'Donnell and McLaughlin 1981b). The SPBs begin replication in metaphase I. A co-disc develops within the original disc and later, it detaches from the original disc. We have found this mode also in *Septobasidium* and in *Helicobasidium*. Probably, this specific mode of intermeiotic SBP duplication represents an apomorphy for the Pucciniomycetes.

Surprisingly, our LSU analysis suggests that *Helicobasidium* does not belong in the Platygloeales (Fig. 1). Like the Platygloeales, *Helicobasidium* is phytoparasitic. In contrast with the Platygloeales, *Helicobasidium* species parasitize roots of ferns and spermatophytes, causing the violet root rot *Thanatophytum* (*Rhizoctonia*) *crocorum*. However, the most significant characteristic of *Helicobasidium*, which distinguishes this genus from the Platygloeales, is its rust-parasitic haploid *Tuberculina* stage (Lutz et al. 2004a–c). *Helicobasidium* interacts with haploid rust stages via large fusion pores in the micrometer

size range through which *Helicobasidium* organelles are transferred to rust cells (Fig. 10e; Bauer et al. 2004). Regarding this curious situation, Bauer et al. (2004) hypothesized that the *Tuberculina* mycoparasitism evolved from the sexual interaction of the common ancestor of *Helicobasidium* and the rusts. Possibly, *Helicobasidium* is more closely related to the rusts than currently assumed.

Molecular data as well as septal pore and SPB characteristics indicate that *Pachnocybe ferruginea* is a member of the Pucciniomycetes (Bauer and Oberwinkler 1990a, Oberwinkler and Bauer 1989). Morphologically and ecologically, however, there is a deep gap between *Pachnocybe* and the other members of the Pucciniomycetes. In contrast with all other members of the Pucciniomycetes, *Pachnocybe ferruginea* is saprobic (Kropp and Corden 1986) and forms stilboid basidiocarps with holobasidia (Oberwinkler and Bandoni 1982). Thus, *P. ferruginea* is morphologically unique among the Pucciniomycetes.

In cells of *Pachnocybe*, mitochondria are often arranged in complexes. Each complex consists of two or more mitochondria that are directly interconnected by filaments (Kleven and McLaughlin 1989; Oberwinkler and Bauer 1989). The mitochondria-connecting filaments are identical in size and spacing to those of symplechosomes (Fig. 5d; Bauer and Oberwinkler 1991a; Oberwinkler and Bauer 1989), which are also connected to mitochondria at their edges. However, in contrast with the symplechosomes, the mitochondrial complexes of *Pachnocybe* are not composed of stacked ER cisternae. We have also found mitochondrial complexes of the *Pachnocybe* type in some *Septobasidium* species (Fig. 10d). The mitochondrial complexes might thus represent an apomorphy for *Pachnocybe* and the Septobasidiales.

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