Ultrastructure in basidiomycetes – requirement for function*

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Three years ago, Robert Bauer, a very experienced and well known electron microscopist, agreed to contribute with an article on basidiomycetous ultrastructure for the symposium in memory of Josef Poelt. However, unexpectedly and tragically, Robert died September 7, 2014. Because of my 30 years excellent collaboration with him, I took over his duty for that purpose, and he is co-author for that reason, though there was no manuscript left by him.

Abstract: Most basidiomycetes are multicellular organisms, composed of hyphae with cell walls and apical growth. A normal set of heterotrophic eukaryotic cell organelles is present, and in addition, chitosomes and Spitzenkörper at hyphal tips. Specific for certain relationships are atractosomes and symplechosomes, and colacosomes occur in some mycoparasites. Spindle pole bodies organize the microtubules and are essential for nuclear division. Their changes in structure and position during nuclear divison have evolutionary backgrounds. Living cells are connected via septal pores of different ultrastructural complexities, and reliable phylogenetic markers. Sexual reproduction occurs in meiosporangia with nuclear changes from dikaryotic to diploid, and following meiosis in the same cell or in an outgrowth, the metabasidium, of the previous probasidium, generally structurally differentiated as a teliospore. Ballistospore development has prerequisites for forcible spore ejection. Ultrastructural differentiation of basidiospore walls is another evolutionary process, driven by ecological adaptations. In various groups of Pucciniomycotina and most Ustilaginomycotina, dimorphic life cycles are established and characterised by haploid yeast phases. While budding, basidiomycetous yeasts have characteristic cell wall differentiations, and those are also necessary when compatible yeast cells conjugate. Yeast budding is one of basidiomycetous asexual propagation strategies. In most rust fungi, different "spore stages" with various ultrastructural differentiations are involved in dispersal. Efficient substrate interactions are required for optimal nutrion uptake, realized by cellular and subcellular functional structures, e.g. haustoria, and exo- and endosomes. Myco- and animal parasitism is disjunct in lower basidiomycetes and structurally remarkably diverse. Ecologically and phylogenetically, symbioses of basidiomycetes with land plants play a most important role. This goes hand in hand with structurally highly adapted characters. Finally, ultrastructural features of basidiolichens will be discussed.

1. Introduction

Traditional light microscopy in the 19th and 20th century reached its resolution limits around 0.5 μm. However, experienced microscopists guessed the hidden subcellular diversity below that limitation, and routined physicists were eager to surmount the barriers by using electron beams. Few remarks on the historical development and final application of electron microscopes

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in biology can be found in Oberwinkler (2018), "How to understand cryptogams? The development of research methods and their impact on the knowledge of cryptogams", in this volume.

When I started to learn transmission electron microscopic techniques in 1965, including preparation procedures of that time for fungi, there was in general a clear distinction between electron microscopists and the "rest of biologists", as I was informed, unmistakably, by the person in charge of the single electron microscope for botany at that place. Fortunately, in a nearby university, the Institute of Physics was a leading one in electron microscopy, and its hospitable atmosphere was very helpful to get over problems in examining fungal materials. Some ten years later, ultrastructural research was integrated in biology as a discipline with sophisticated techniques.

It seems trivial to point to what is intended to be studied in the electron microscope. In reality, a very careful light microscopic pre-examination is indispensable, and in fact, the first challenge for the beginner in electron microscopy. For that purpose, training programmes in light microscopy are absolutely essential. This demand was strongly supported when fluorescent and confocal light microscopy advanced and nanoscopy led to diffraction-unlimited optical resolution.

Except of habit features, nearly all other structural characters in basidiomycetous fungi are cellular and subcellular differentiations, prerequisites for various kinds of functions.

The following treatise is not a historical review, instead it intends to bridge structural and functional features in basidiomycetes, complemented by few examples from other fungi.

2. Hyphae and their organelles

The apical growth of hyphae was already studied light microscopically by Reinhardt (1892) and Brunswik (1924) who detected the Spitzenkörper, verified by Girbardt (1957) using phase contrast for living hyphae of *Trametes versicolor* (*Polystictus v.*). One of the pioneering works in transmission electron microscopy of basidiomycetes was the three dimensional reconstruction of the growing hyphal apex, including the Spitzenkörper (Girbardt 1969, Fig. 1).

Various subcellular structures were detected in hyphae which are strongly polarized cells, and a formidable challenge for intensive further ultrastructural research was the consequence. The heterogeneity of vesicle contents at the hyphal apex of *Asergillus nidulans*, not seen by previously used conventional TEM, was revealed by electron tomography as applied by Hohmann-Marriott et al. (2006). Using fluorescent microscopy, Riquelme et al. (2007) studied

the Spitzenkörper localization and intracellular traffic of green fluorescent protein-labeled chitin synthases in living hyphae of *Neurospora crassa*. Macrovesicles surround a core of microvesicles, the chitosomes, ribosomes and F-actin. Chitosomes contain membrane proteins that are delivered to the hyphal growth region (Bracker et al. 1976, Bartnicki-Garcia 2006), and made available by fusion of the vesicles with the plasma membrane (Bartnicki-Garcia et al. 1989). – Myosin-5, Kinesin-1 and kinesin-3 are considered to deliver growth supplies to the hyphal tip. Microtubules, MTs, are the tracks for delivering vesicles to the actin microfilaments and motors in membrane traffic during hyphal growth of *Ustilago maydis* (Steinberg 2007).

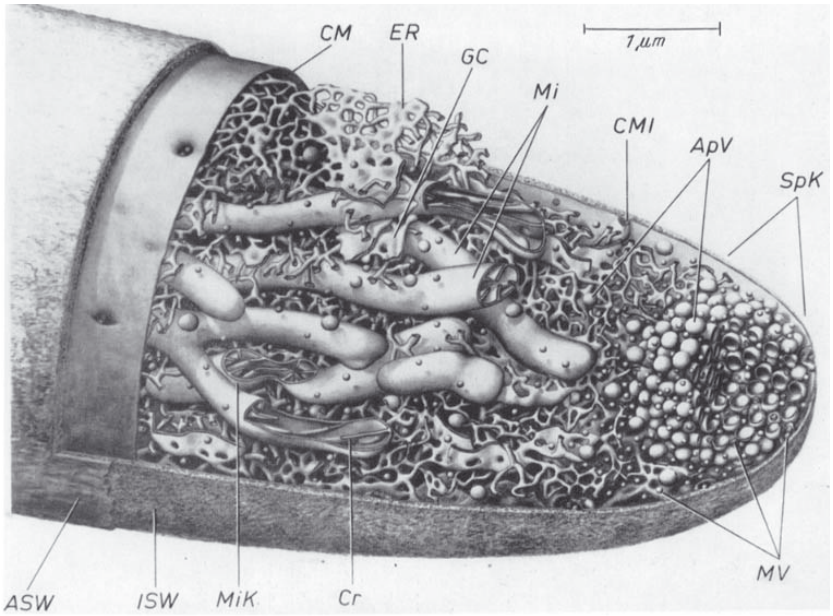


Fig. 1: Three dimensional reconstruction of the hyphal tip of *Trametes versicolor* with Spitzenkörper. ApV = apical macrovesicles, ASW = outer cell wall, CM = plasma membrane, CMI = plasma membrane invagination, Cr = mitochondrial crista, ER = endoplasmatic reticulum, GC = Golgi cisterna, ISW = inner fibrillar wall layer, Mi = mitochondrion, MiK = mitochondrial bend, MV = microvesicles, SpK = Spitzenkörper. From Girbardt (1969).

Fluorescent marker proteins and labelled subcellular structures have been used by Steinberg & Schuster (2011) for live cell imaging with laser-based epi-fluorescence microscopy of subcellular structures in the hyphal tip of *U. maydis* (Fig. 2) and for documenting cell organelle dynamics by movies. Kinesin-3 transports organelles along microtubules (Steinberg 2015), protein fibres, consisting of tubulin and recruiting part of the cytoskeleton. Filamentous actin (F-actin) is involved in this process (Xiang & Fischer 2004, Fischer et al. 2008). It constitutes the second component of the cytoskeleton and supports endocytosis in peripheral patches. Secretory vesicles containing proteins move from the endoplasmatic reticulum to the Golgi apparatus in which pro-

cessing and sorting of proteins occurs that finally are excreted by vesicle fusion with the plasma membrane. Golgi compartments appear to be spatially segregated within the hyphal tip. According to Harris (2013), the loss of normal Golgi organization stops polarized hyphal extension and triggers de-polarization of the hyphal tip. – The spheroidal, but often polymorphic chitosomes, mostly 40–70 nm in diameter, from *Mucor rouxii* and chitin microfibrils were studied transmission electron microscopically by Bracker et al. (1976). Chitosomes are chitin synthase containing vesicles (Sietsma et al. 1996, Riquelme et al. 2007), and immunocytochemical studies allowed to localize chitin synthase proteins in vesicle-like particles of *Ustilago maydis* yeast cells (Ruiz-Herrera et al. 2006). The nuclear envelope is connected with the endoplasmic reticulum, containing proteins, delivered to the nucleus (Steinberg & Schuster 2011). These authors also reported that early endosomes "have a dual role in sorting to the vacuole and recycling back to plasma membrane". In addition, they are continuously moving (Higuchi & Steinberg 2015), whereas mitochondria and peroxisomes appear mainly immobile. Molecular processes are driven by the energy, provided through mitochondria, and biosynthetic pathways of primary and secondary metabolites are localized in peroxisomes (Kiel et al. 2000, Schrader & Fahimi 2008, Sprote et al. 2009). Acetyl-CoA oxidases and -dehydrogenases are considered ancient features of peroxisomes (Camões et al. 2015).

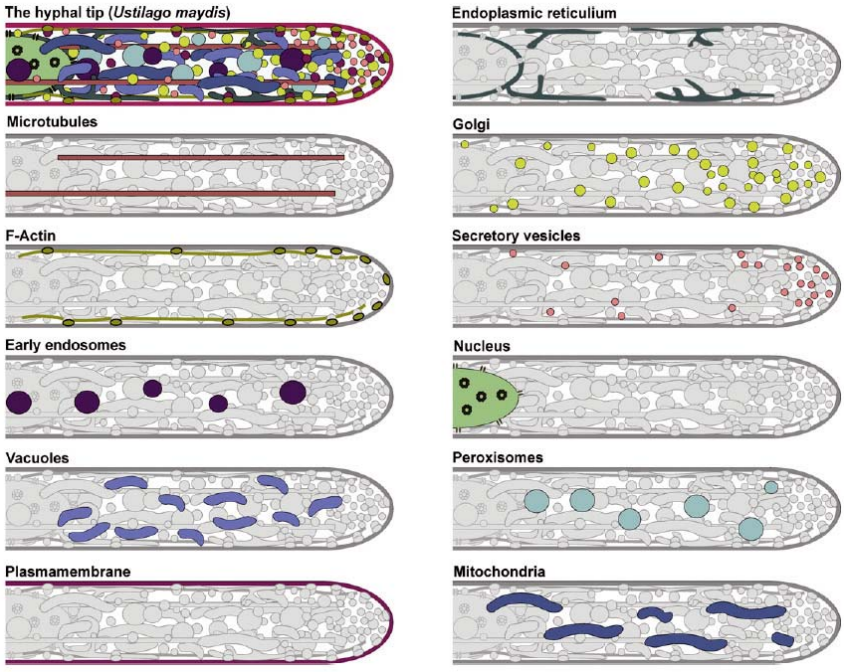


Fig. 2: The distribution of sub-cellular structures in the hyphal tip of *Ustilago maydis*. From Steinberg & Schuster (2011). For comments compare text.

Besides a general set of cell organelles of heterotrophic eukaryotes, and several special ones, as chitosomes, exosomes, and vesicles of the Spitzenkörper, as outlined above, unique sub-cellular structures were detected transmission electron microscopically, the symplechosome (Fig. 4), in *Atractiella solani, Phleogena faginea* and *Saccoblastia farinacea* (Oberwinkler & Bauer 1989, Bauer & Oberwinkler 1991a, Bauer et al. 2006, Oberwinkler 2012a). Also the pycnidial fungi *Basidiopycnis hyalina* and *Proceropycnis pinicola* contain symplechosomes (Oberwinkler et al. 2006). Symplechosomes consist of stacks of plate-like cisternae that are connected by hexagonally arranged bars. Such bars often link them with mitochondria (M). The occurrence of symplechosomes seems to be restricted to the Atractiellales (Oberwinkler & Bandoni 1982, Oberwinkler & Bauer 1989, Oberwinkler 2012b) of the Pucciniomycotina (Fig. 3). So far, nothing is known about their function. Until now, the report of symplechosomes in an atractiellaceous fungus appearing as an orchid mycorrhiza in Ecuador (Kottke et al. 2010) remains enigmatic.

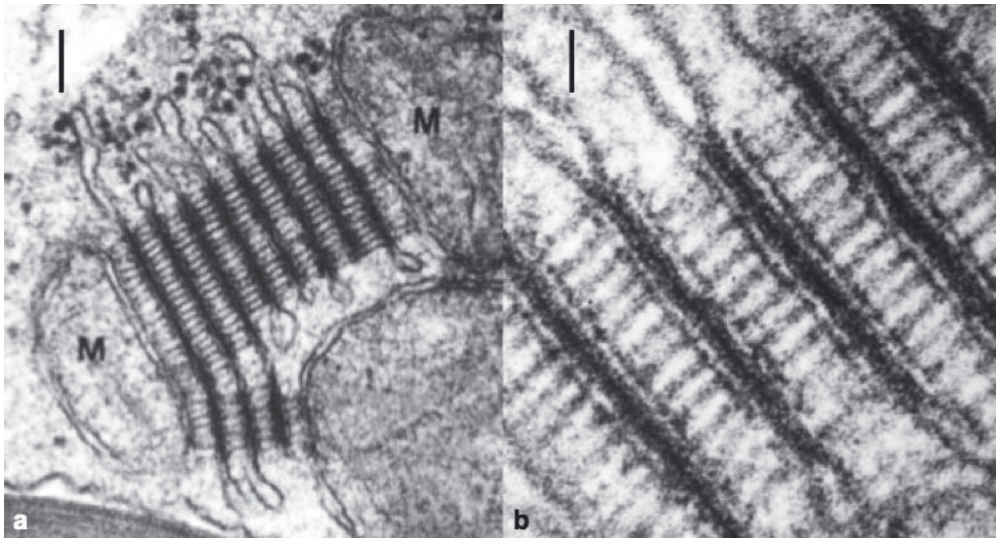


Fig. 3: Symplechosomes interconnect membrane staples which are marginally inflated. In addition, also bar connections to mitochondria (M) occur. a: bar = 0.5 μm; b: bar = 20 nm. From Bauer & Oberwinkler (2012a).

Ultrastructural features of organisms are categorized as general biology. However, the previous example, and following odd structures, occurring in fungal cells of scattered groups, may provide difficulties for proper assignment. To facilitate better understanding, we provide a phylogeny of Pucciniomycotina with the taxa considered, supplemented by the occurrence of unusual cell organelles, spindle pole body (SPB) and selected septal pore characteristics (Fig. 4). Comments on submicroscopic characteristics of the

basidiomycetous cell wall are provided in chapter 3 (septal pores) and chapter 6 (basidiomycetous yeasts).

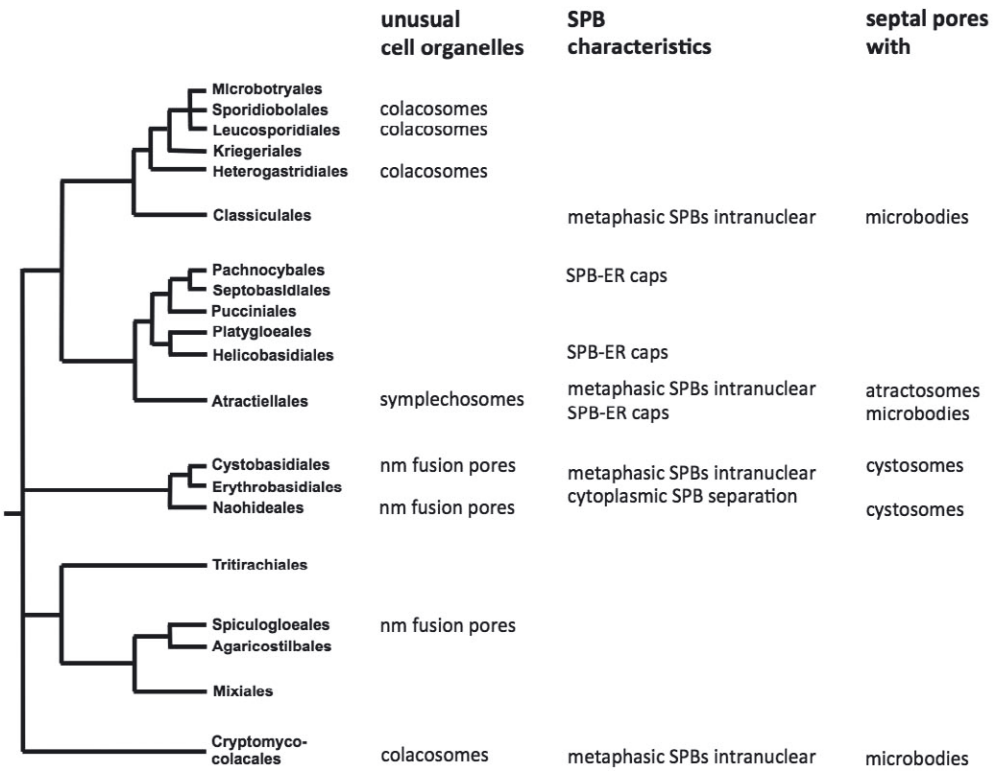


Fig. 4: Various specific subcellular structures in Pucciniomycotina and their phylogenetic distribution. The quoted characters will be discussed in the appertaining chapters 3 (septal pores), 4 (spindle pole bodies, SPBs) and 7 (mycoparasites). The phylogenetic tree is modified after Bauer et al. (2006) and used in following figures. Orig.

Basidiomycetous subcellular structures:

- Spitzenkörper
- Chitosomes, exo- and endosomes
- Symplechosomes
- General set of organelles of eukaryotic heterotrophs

Further data needed about:

- Functions of symplechosomes

3. Septal pore evolution

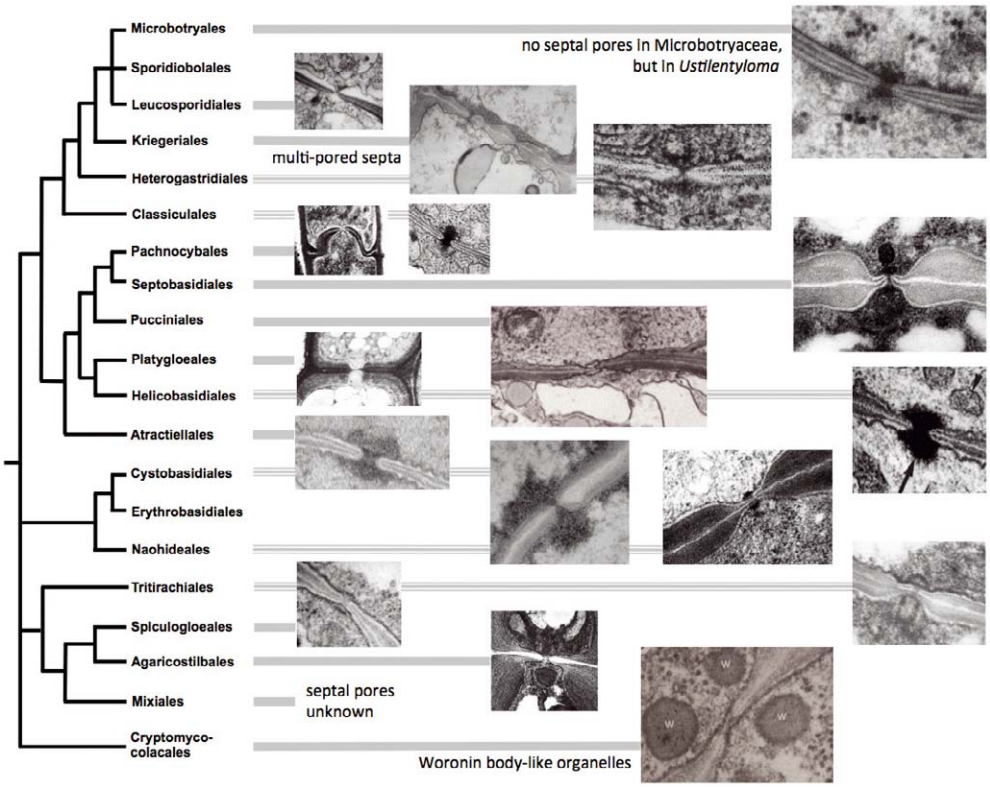


Fig. 5: Septal pores in Pucciniomycotina. The ordinal phylogeny serves as a guideline for the selected illustrations. Single pictures not to scale. Explanation in the text. Orig.

Using phase contrast microscopy, Girbardt (1958) found central swellings in septa of living cells of *Trametes versicolor* (*Polystictus v.*). Serial sections for transmission electron microscopy revealed channel-like structures (Girbardt l.c.) for which the terms dolipore and parenthesome were later introduced (Moore & McAlear 1962). In contrast, simple septal pores were found in the rust fungi *Puccinia podophyllii* (Moore 1963), *P. graminis* and *P. recondita* (Ehrlich et al. 1968, Ehrlich & Ehrlich 1969).

We arrange pore types according to higher relationships in Basidiomycota. Exclusively simple septal pores without membrane caps occur in Pucciniomycotina (Fig. 5). Pores of *Cryptomycocolax* are associated with Woronin-like bodies (W). Septal pores are not associated with microbodies in Agaricostilbales and Spiculogloeales, however they are present in *Helicogloea, Infundibura* and *Saccoblastia* of the Atractiellales, and atractosomes (Fig. 6a) were found in *Atractiella, Helicogloea, Phleogena* and in the pycnidial *Basidiopycnis* and *Proceropycnis* (Oberwinkler et al. 2006) of the same order. Also, species of

the Classiculales and Pucciniomycetes (Helicobasidiales, Platygloeales, Pucciniales, Septobasidiales and Pachnocybales) have microbody associated septal pores. Cystosomes (Fig. 6b) are characteristic for pores of Cystobasidiales species. In the Pucciniomycotina, multi-pored septa were only found in *Kriegeria* of the Kriegeriales. Curiously, also in *Bartheletia* of the Agaricomycotina, multiporate septa occur. Septal pores could not be found in Mixiales and Microbotryaceae.

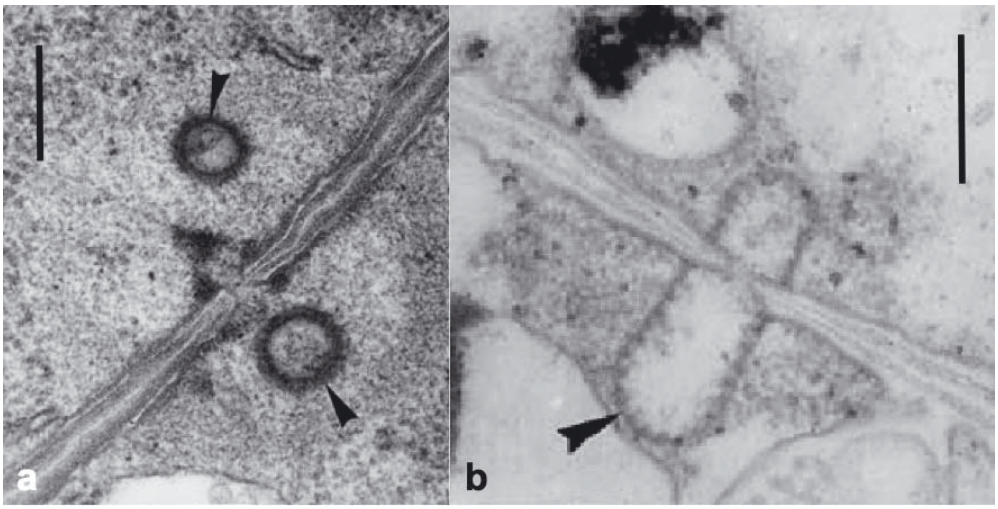


Fig. 6: **a** Atractosomes (arrowheads) of *Atractiella* sp. **b** Cystosome (arrowhead) of *Cystobasidium fimetarium*. Bars 20 = nm. From Bauer et al. (2006).

Ultrastructural studies in smut fungi were carried out in our group over more than ten years and then summarized in a cladistic approach to propose a new phylogeny for Ustilaginomycetes by Bauer et al. (1997). In the following years, these results could be tested indepently by molecularly based phylogenies of various smut taxa.

Here, we use a molecular phylogeny and insert the major types of smut septal pore types, complemented by illustrations of teleomorph stages (Fig. 7). Membranous pore caps are an ultrastructural synapomorphy in true smut fungi. Urocystales were characterized by non membranous pore bands, and Ustilaginales and Georgefischeriales by the loss of septal pores. In contrast, Tilletiales and *Entorrhiza* have dolipores, but parenthesomes are lacking. The latter genus was included in our studies from the very beginning, and placed in a separate position in our phylogenetic scheme. Recently, an own division was proposed for this genus (Bauer et al. 2015).

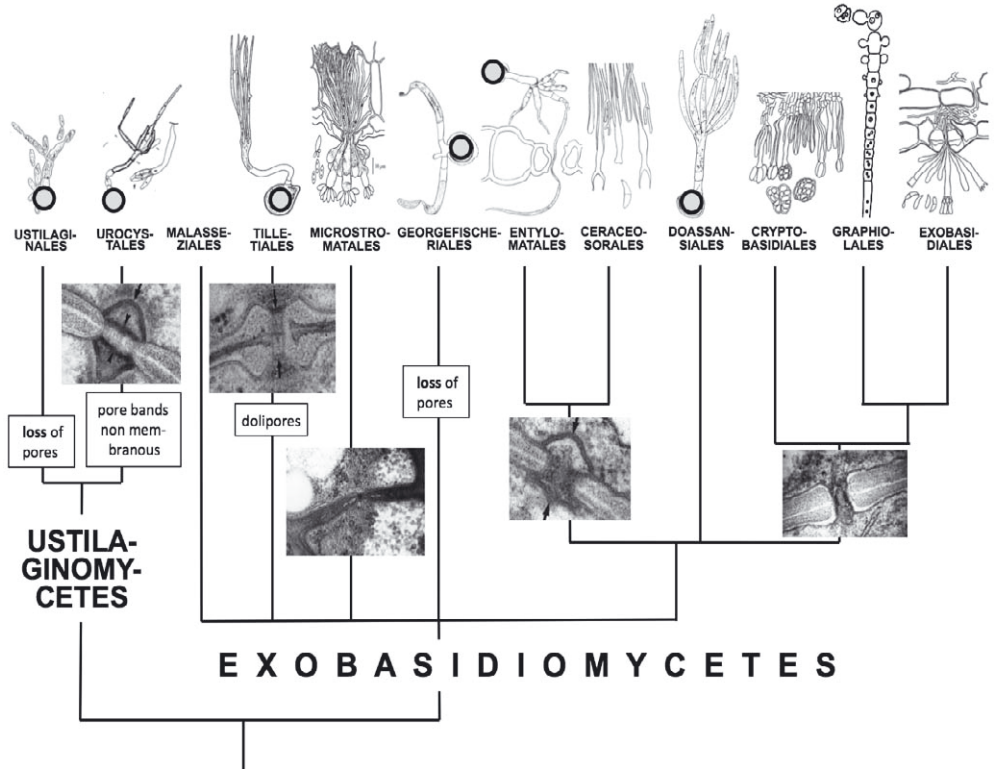


Fig. 7: Septal pores in Ustilaginomycotina. The molecularly based phylogeny is complemented with septal pore types and teleomorph stages in line drawings. Single pictures are not to scale. Explanation in the text. Modified after Oberwinkler (2012a).

In general, species of the Agaricomycotina have dolipores with parenthesomes (Fig. 8). As mentioned above, Girbardt (1957) detected this septal pore type in *Trametes versicolor*. The many reports on dolipores in higher basidiomycetes cannot be cited here. Instead, we show a small, but representative selection of different types in an evolutionary sequence, based on a molecular hypothesis (Fig. 8). The tubular parenthesome parts of the *Tremella* dolipore are at large characteristic for Tremellomycetes. The so-called "continuous parenthesome" has a tiny central micropore, only visible by chance or in one of a number of serial sections. Such pores are typical for Dacrymycetales, Sebacinales, Auriculariales, some Cantharellales, Geastrales and Hymenochaetales. Differences in ultrastructural features of these septal pores are either lacking or hardly to discriminate. The distribution in the orders has a remarkable phylogenetic bearing. Therefore, exceptions of the unique feature cannot be interpreted, as in Cantharellales and Hymenochaetales, in which also species with perforate parenthesomes occur (van Driel et al. 2009). Such cases definitely require careful restudies, not only electron microscopically but also molecularly. A curious and unverified finding of Robert Bauer (in Hibbett et al.

2014) was that *Sphaerobolus stellatus* should have dolipores with continuous and perforate parenthesomes. For more evolved basidiomycetes, apparently exclusively dolipores with perforate parenthesomes have been reported.

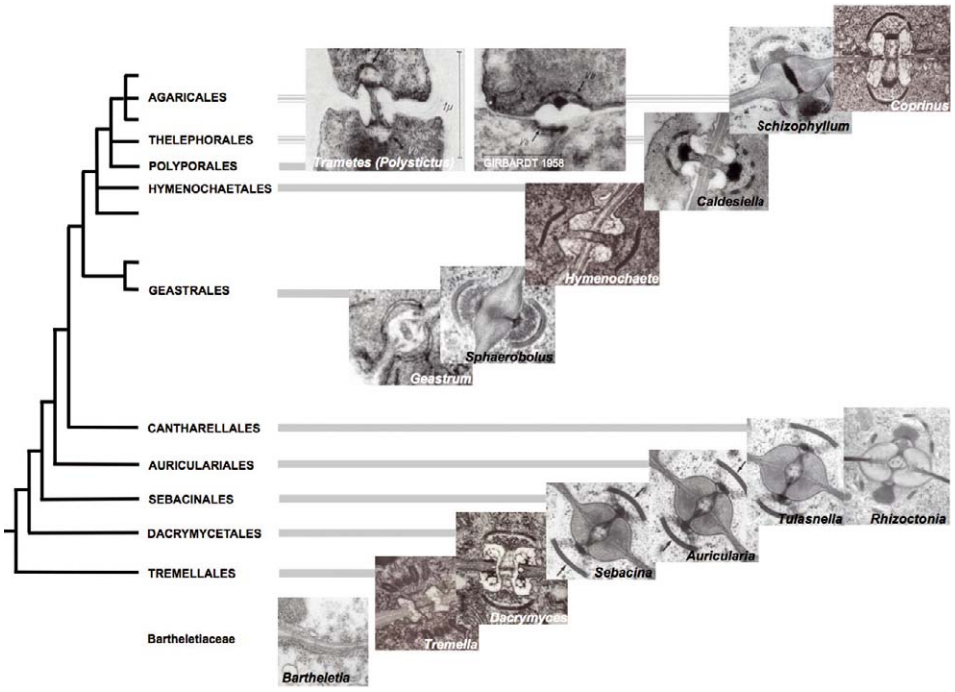


Fig. 8: Septal pores of selected representatives of Agaricomycotina in a molecularly based phylogenetic arrangement. Single pictures are not to scale. *Trametes* after Girbardt (1958). Phylogenetic tree after Hibbett et al. (2014), strongly modified. Orig. comments in the text.

Wallemia species are anamorphic, xerophilic basidiomycetes, tolerating high osmotic pressure, e.g. hypersaline environments (Cantrell et al. 2011, Zajc et al. 2014). *Wallemia sebi* is reported to be very common in house dust (Kirchmair & Neuhauser 2012, Desroches et al. 2014) and has mycotoxigenic and pathogenic potentials (Jančič et al. 2014). *Wallemia sebi* produces spores in chains (Fig. 9b). These have been interpreted as conidia (Padamse et al. 2012) or as possible basidiospores (Okada & Takashima 2002). The septal pore ultrastructure of *Wallemia* has been studied several times (Moore 1986, van Driel et al. 2009, Padamse et al. 2012, Weiss et al. 2014, own studies, unpubl. Fig. 9c–e). The dolipore has tremelloid parenthesome sacs, indicating a relationship with the Tremellomycetes. – In a SSU rDNA phylogeny with a sampling of Ustilaginomycota, Tremellomycetes, *Dacrymyces*, and the major outgroup Taphrinomycetes, Zalar et al. (2005) found the three *Wallemia* species in a basal position of the Basidiomycota. Matheny et al. (2006) analyzed the phylogenetic position using 3451 nucleotide characters of the 18S, 25S

and 5.8S rRNA genes and 1282 amino acid positions of rpb1, rpb2 and tef1 nuclear protein-coding genes across 91 taxa. Their results were ambiguous since Agaricomycotina or Ustilaginomycotina became sister groups. In a combined gene tree even Pucciniomycotina were sister clade to basally placed Wallemiomycetes. The phylogenetic position of Wallemiomycetes was also dicussed by Padamse et al. (2012) who sequenced the genome of *W. sebi.* Analyses of 71 proteins grouped *Wallemia* as the earliest diverging lineage in Agaricomycotina with a 96 % bootstrap support. – The reported differing phylogenetic positions of *Wallemia* cannot be explained at present. Even more enigmatic is that the genome based phylogenies do not resolve a well supported clade.

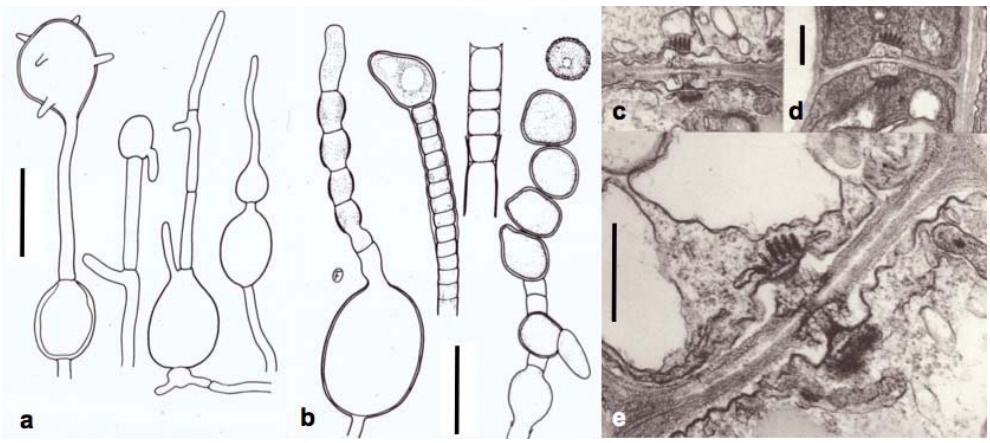


Fig. 9: *Wallemia sebi*. **a** Different ontogenetic stages of resting cells; bar = 20 µm. **b** Spore development in chains, finally disintegrating; bar = 10 µm. **c**–**e** Hyphal septa and septal dolipores with tremelloid parenthesomes; bars = 1 µm. Orig.

As mentioned already for *Kriegeria*, also *Bartheletia paradoxa* (Fig. 8) has simple multi-pored septa (Scheuer et al. 2008). Though basidial morphology and molecular data are in favour of a relationship with Tremellales, the correct phylogenetic position of this species remains unsolved.

Ontogenetic stages of septal pores clearly indicate, that transport mechanisms must occur between living cells. After that phase, septal pores are becoming plugged.

Unfortunately, no experimental studies are available until now for elucidating functional issues.

Evolutionary trends in basidiomycetous septal pores:

- Septal pore simple → dolipore, lacking parenthesomes → dolipore with parenthesomes
- Simple septal pores with Woronin-like bodies → with associated microbodies → without microbodies
- Parenthesomes lacking → tubulate
- Parenthesomes continuous → perforate

Further data needed:

- Experimental approaches to learn about septal pore functional aspects

4. Nuclear divisions

Nuclear divisions are essential developmental events in eukaryotes. Mitosis and meiosis can be observed with the light microscope remarkably well in living cells, and after specific staining, in contrasted preparations. However, details of the processes remained unclear until sophisticated techniques in transmission electron microscopy were available. As an example, we have chosen meiosis in the fern parasite *Herpobasidium filicinum* (Bauer & Oberwinkler 1994) and illustrate major phases in their ontogenetic sequence (Fig. 10). During division, the nucleus is completely wrapped by endoplasmatic reticulum and the spindle pole body (SPB) is extended into the cytoplasm. These features are distinctive from comparable meiotic phases in rust fungi.

We found that *H. filicinum* shares more important nuclear traits with *Pachnocybe ferruginea, Eocronartium muscicola, Helicobasidium mompa* and *Helicobasidium brebissonii* than with the Uredinales or Cryptomycocolacales. No evidence for meiosis II was found in basidia of *H. filicinum*. Instead, the genesis of the interphase I SPB in *H. filicinum* is essentially identical to the basidiomycetous postmeiotic and intermitotic spindle pole body duplication. For comparison with *Cryptomycocolax abnormis*, we refer to the article of Oberwinkler "How to understand cryptogams?", Fig. 13, in this volume.

Microtubule-organizing centers, SPBs, are essential cell organelles, functionally involved in cell division. The SPB cycles were analysed when meioses of *Puccinia malvacearum* (O'Donnell & McLaughlin 1981a, 1981b, 1981c) and *Ustilago maydis* (O'Donnell & McLaughlin 1984a, 1984b) were studied electron microscopically (Fig. 11b). Similar studies were carried out for *Pachnocybe ferruginea* (Bauer & Oberwinkler 1990, Fig. 11a), *Cryptomycocolax abnormis* (Oberwinkler & Bauer 1990), *Microbotryum violaceum*

(Berbee et al. 1990), *Sphacelotheca polygoni-serrulati* (Bauer et al. 1991) and *Agaricostilbum pulcherrimum* (Bauer et al. 1992). In these studies it was found that SPBs in basal clades of the Pucciniomycotina are disk-like, as in Ascomycota. In contrast, in derived taxa of the Pucciniomycotina SPBs are subglobose, and globose ones are typical for Ustilaginomycotina and Agaricomycotina. SPB features of Agaricomycetes were compiled by Celio et al. (2006). – In summary, the data available on SPB ontogenetic cycles document considerable differences between Ascomycota and Basidiomycota.

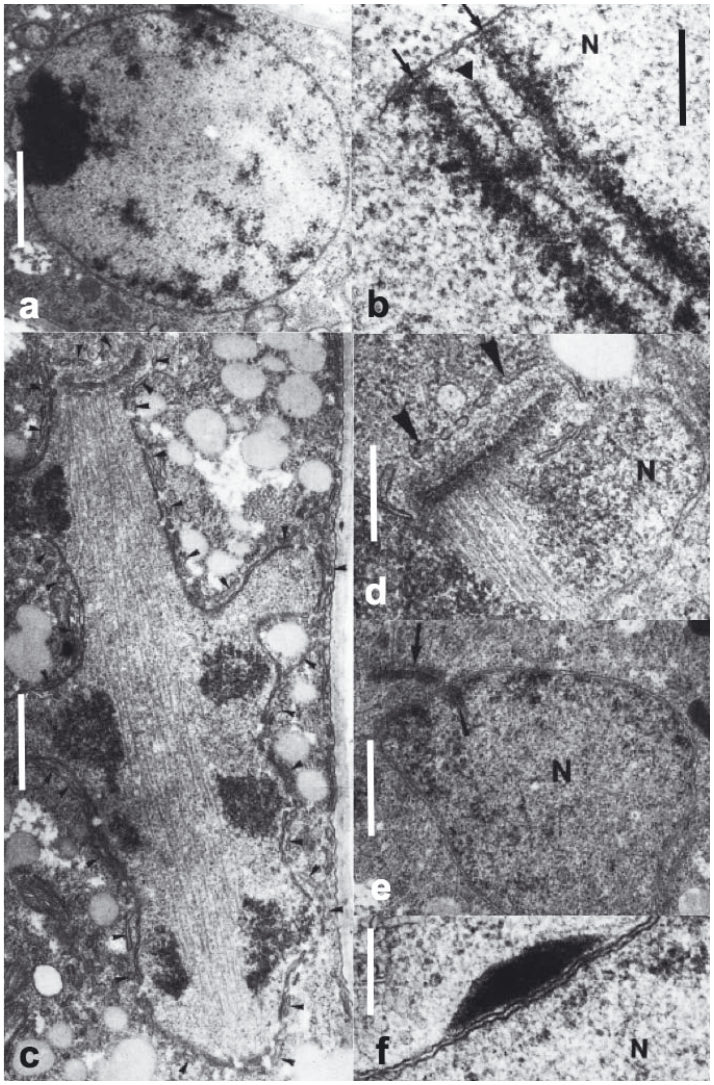


Fig. 10: Meiosis in *Herpobasidium filicinum*, N = nucleus. **a** Prophase I nucleus with dark contrasted, extranuclear SPB above and dark nucleolus to the left; bar = 1 µm. **b** Synaptonemal complex of prophase I. Only lateral elements (arrows) are attached to the nuclear envelope; bar = 0.2 µm. **c** Metaphase I nucleus with central spindle surrounded by chromatin. The nucleus is enclosed by fenestrated endoplasmic reticulum (arrowheads); bar = 0.5 µm. **d** Telophase **I** with central spindle and discoidal SPB overlaping the nuclear envelope; bar = 0.1 µm. **e** Interphase **I** with extranuclear SPB (arrow); bar = 1 µm. **f** Fully developed extranuclear interphase I SPB with two discs and prominent middle piece; bar = 0.2 µm. Modified after Bauer & Oberwinkler (1994).

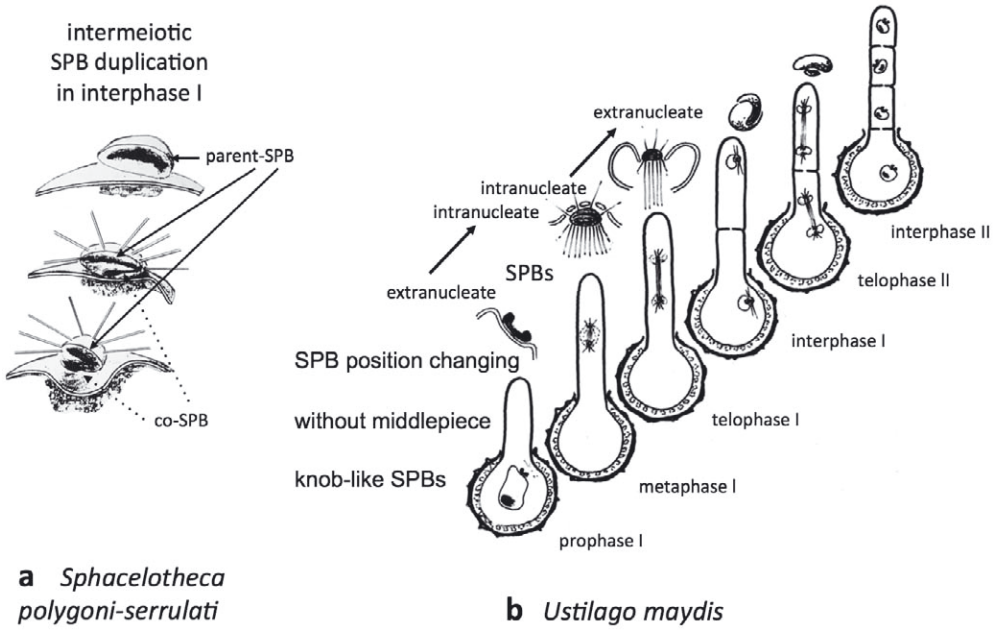


Fig. 11: **a** SPB duplication in *Sphacelotheca polygoni-serrulati*. **b** Meiosis and SPB cycle in Ustilago maydis. After karyogamy, a diploid nucleus is in the smut spore (prophase I) and associated with an extranuclear SPB. During metaphase I of the first meiotic nuclear division, the SPBs are intranuclear, and the meiosporangium is divided by a transverse septum into two cells. Between interphase I and II, a second nuclear division follows, and the final meiosporangium is four-celled, including the smut spore. a Orig. b from OBERWINKLER (2012a), strongly modified after O'DONNELL & MCLAUGHLIN (1984a, 1984b).

Differences of SPBs in Pucciniomycotina, Ustilaginomycotina and Agaricomycotina indicating evolutionary trends:

- SPB disk-like → knob-like
- SPB with middlepiece → without middlepiece
- SPB position during nuclear division extranucleate; intranucleate → extranucleate

Additional desirable investigations:

- More studies in nuclear division, especially in Agaricomycotina

5. Sporulation and meiospores

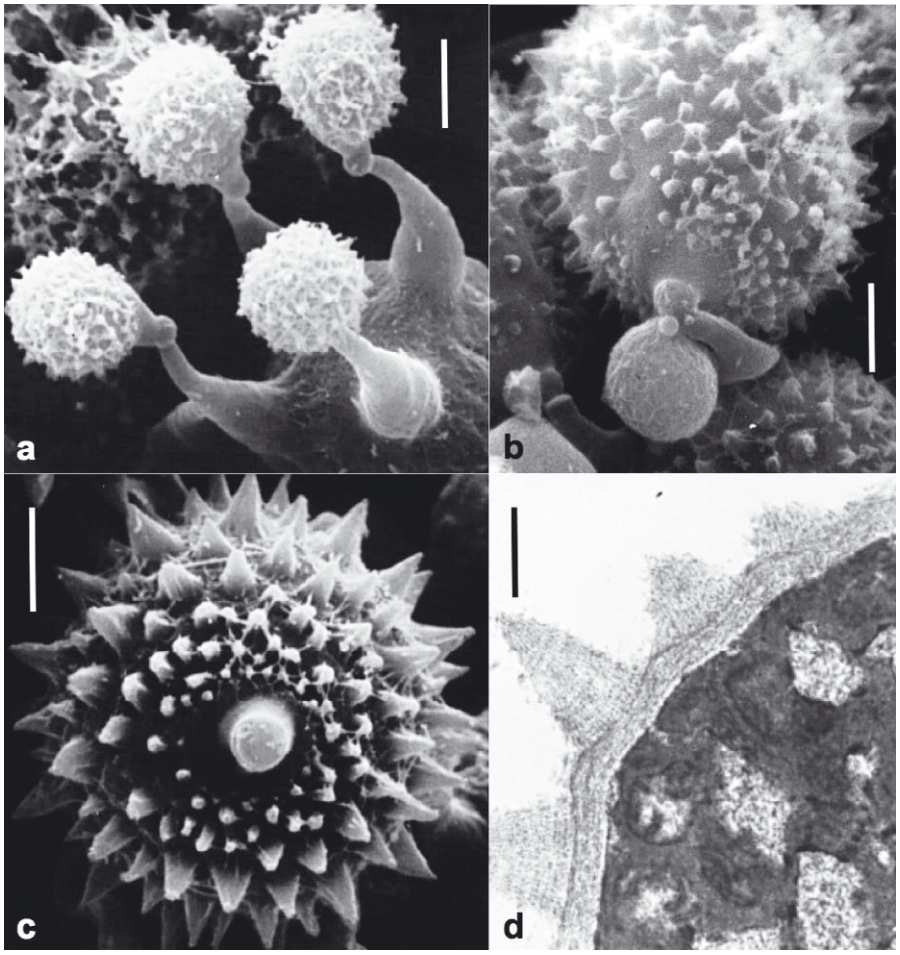


Fig. 12: Basidiospore development in *Laccaria laccata.* **a** Basidial apex with four sterigmata and young basidiospores; bar = 3 µm. **b** Basidiospore in the moment of discharge, ejection drop visible as small globule; bar = 2 µm. **c** Apiculus view of mature basidiospore; bar = 2 µm. **d** Ultrastructural details of spore wall and spore ornamentation; bar = 0.5 µm. Orig.

The most important propagules in Agaricomycotina are meiospores, and mostly they are ejected from sterigmata as ballistospores. This mechanism is a synapomorphy for Basidiomycota that requires bent sterigmata, asymmetrical outgrowth of basidiospores, and the formation of a drop prior to ejection and opposite to the apicular spore attachment (Figs. 12, 13). These details can be observed light microscopically, however, substrucural details are much better resolved by electron microscopy, both SEM and TEM (Fig. 13).

Typically, basidiospores are unicellular, hyaline, thin- and smooth-walled. However, in Agaricomycetes, diverse evolutionary developments in spore

wall architecture occurred. These can be considered as adaptations to terrestrial life conditions (Oberwinkler 1982, 1985). For Hymenochaetales, Thelephorales and Russulales, characteristic spore wall features were only slightly modified during evolution (Fig. 13). In contrast, Agaricales underwent strong ecological pressures to evolve highly adapted basidiospores for better survival and for facilitated spore germination (Garnica et al. 2007).

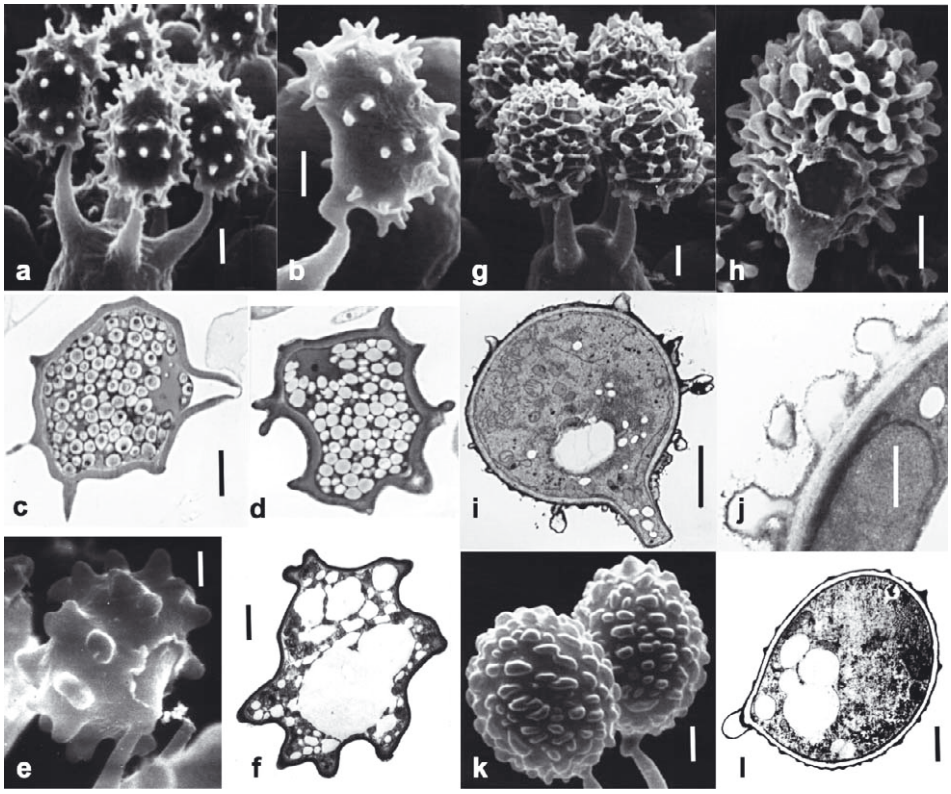


Fig. 13: Basidiospores of Thelephorales and Russulales. **a–d** *Thelephora terrestris*, SEM and TEM photos; bars = 3 µm. **e, f** *Sarcodon imbricatus*, SEM and TEM photo; bars = 1 µm. **g–l** Russulales, SEM and TEM photos: **g–i** *Russula mairei*; bar = 2 µm. **j** *Russula ochroleuca*, TEM photos with details of basidiospore wall and ornamentation; bar = 1 µm. **k** *Auriscalpium vulgare*, SEM photo; bar = 1 µm. **l** *Heterobasidion annosum*, median section of basidiospore, TEM photo; bar = 1 µm. a, b, c, d, i from Oberwinkler (1977), l from Honold (1982), others orig.

Since microscopists studied basidiospores of agarics comparatively, the high structural diversity of these propagules became obvious. The assumption then was that simple spore cell walls should represent older evolutionary stages than complex ones. However, a robust phylogenetic hypothesis for mushrooms became not available until recently (Garnica et al. 2007). Not unexpectedly for the experts, it turned out that basidiospore evolution could be traced along the premise "from simple to complex" (Fig. 14). Now, the former

believe that spores of agarics have an evolutionary bearing appears scientifically justified. Though there is no simple key, a general trend can be presumed as originating from thin-walled, smooth and unpigmented walls to thick-walled, ornamented and pigmented ones. Again, it is convincing to argue that the evolution of germ pores was a phylogenetic imperative for further facilitating spore germination. Consequently, mushroom spore wall features are also indicators for natural relationships in Agaricales. In the study of Garnica et al. (2007), molecular data supported the Agaricaeae, Bolbitiaceae and Psathyrellaceae as monophyletic groups. These authors also found that *Cortinarius* is phylogenetically close to *Phaeolepiota* (Fig. 14), *Cystoderma*, *Crucibulum* and *Cyathus*. In contrast, *Naucoria, Galerina, Gymnopilus* and *Hebeloma*, appear to be related to the Strophariaceae, and apparently the light-spored *Laccaria* (Fig. 12) evolved within the dark-spored agarics (Fig. 14).

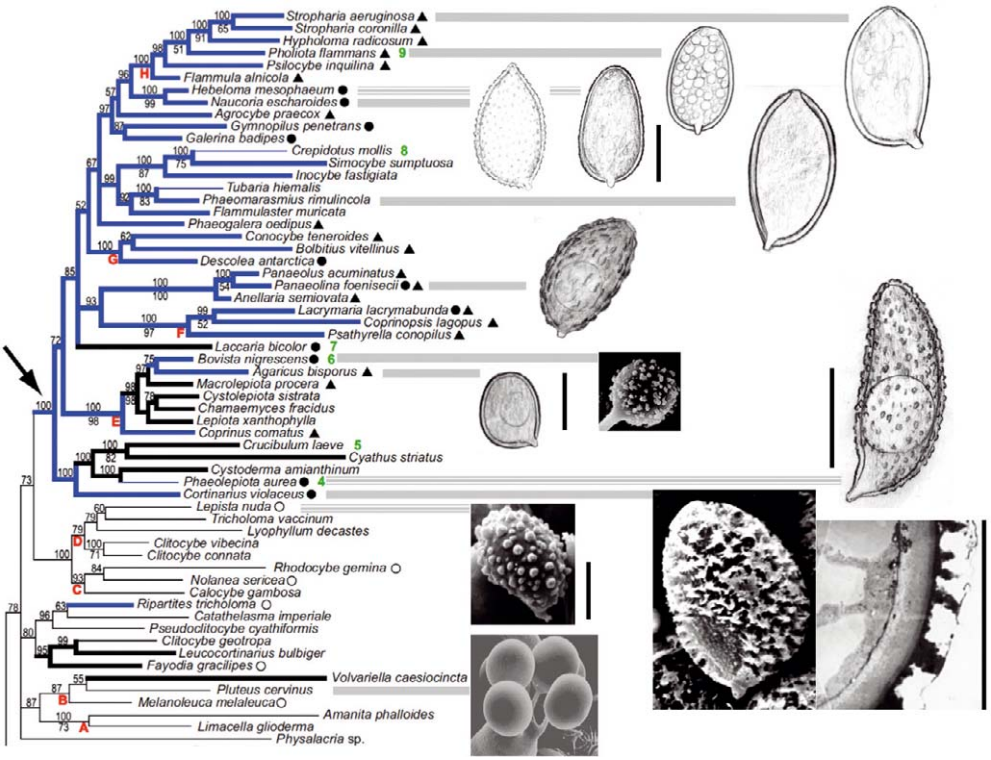


Fig. 14: Evolution of basidiospores in Agaricales. For illustrating basidiospores of representative species, SEM and TEM photos were used and connected to the appropriate taxon. Bars = 5 µm. In addition, also line drawings are included for which light microscopy yields sufficient resolution. Bold lines mark species with spore walls exceeding 200 nm in width, blue lines indicate those with dark coloured spore walls. A synapomorphy for complex spore walls is assumed for a phylogenetically derived relationship and marked by the arrow. The red letters are abbreviations for the following families: A = Amanitaceae; B = Pluteaceae; C = Entolomataceae; D = Tricholomataceae s.str.; E = Agaricaceae; F = Psathyrellaceae; G = Bolbitiaceae; H = Strophariaceae. Filled circles: spores with connected ornaments; empty circles: spores with isolated ornaments; triangles: spores with germ pore. Phylogenetic tree and corresponding legends from Garnica et al. (2007). Orig.

An additional article on Amanitaceae by Zhu-Liang YANG is available in this volume.

Quite different propagation strategies succeeded in rust fungi. Compare the contributions of Reinhard BERNDT (2018), Peter ZWETKO † and Paul BLANZ (2018) and Franz OBERWINKLER (2018) in this volume. Also smut fungi, producing teliospores, use these predominantly for dispersal and to overwinter. Further information will be found in the article of BEGEROW and KEMLER (2018) in this volume.

Basidiospore adaptations as propagules:

- Primary ballistospores → loss of active spore discharge
- Spore germination variable: yeast budding, secondary spores, microconidia, hyphae → exclusively hyphae
- Spore wall thin, smooth → spore wall ornamented → spore wall thick → spore wall thick and ornamented
- Spore not pigmented → spore pigmented
- Spore wall without a germination pore → spore wall with germination pore

6. Basidiomycetous yeasts and secondary spores

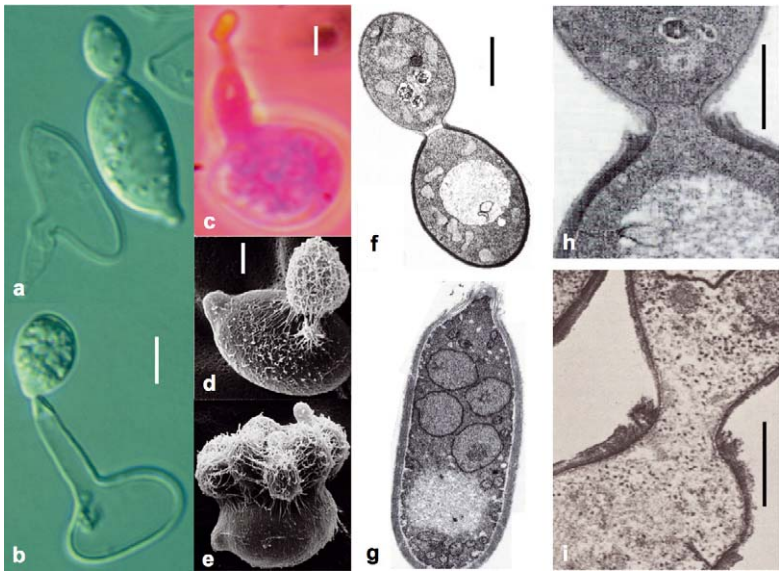


Fig. 15: Yeast budding and secondary spore formation. **a, b** Phase contrast microscopy of *Udeniomyces megalosporus*; **a** Young stage of yeast budding; **b** Note that the cytoplasm of the mother cell (below) is completely transferred into the secondary spore. **c** *Sebacina epigaea*, young stage of secondary spore formation; note the nucleus in the sterigma; preparation stained with phloxine. **d, e** *Tremella mesenterica* basidiospore budding with yeast cells; the extracellular slime is dried up to an irregular network by critical point fixation for SEM microscopy. **f–h** *Agaricostilbum pulcherrimum*, different stages of yeast budding photographed by TEM; **g** note four haploid nuclei in the yeast cell and budding scar on top; **h** detail of budding scar consisting of various cell wall layers. **i** *Graphiola phoenicis*, yeast budding stage comparable to figure h. Bars = 2 µm. a–e orig. f–h from OBERWINKLER & BANDONI (1982) and OBERWINKLER ET AL. (1982).

Often, basidiospore germination in Pucciniomycotina and Ustilaginomycotina as well as basal groups of the Agaricomycotina is unfixed, i.e. yeasts, secondary spores (Fig. 15), microconidia or hyphae may primarily develop. Only the huge bulk of higher Agaricomycotina species has basidiospores that germinate exclusively with hyphae.

Historically, yeast research and its technological applications represented an own discipline in mycology. Though early workers, like Brefeld (1838–1895), analysed and fully understood the dimorphic ontogenies of various basidiomycetes, it took a century until physiological (Prillinger et al. 1990–1993) and molecular studies (Swann & Taylor 1995) were carried out that allowed to dig out and verify the old knowledge. The three major clades, now called Pucciniomycotina, Ustilaginomycotina and Agaricomycotina (Bauer et al. 2006), were established at that time, and all of them include yeasts, however, the latter one only in Tremellomycetes.

Not surprisingly, the yeast of the basidiomycetous model organism *Ustilago maydis* was studied intensively for elucidating principal cell organelle differentiation processes during budding (Fig. 16).

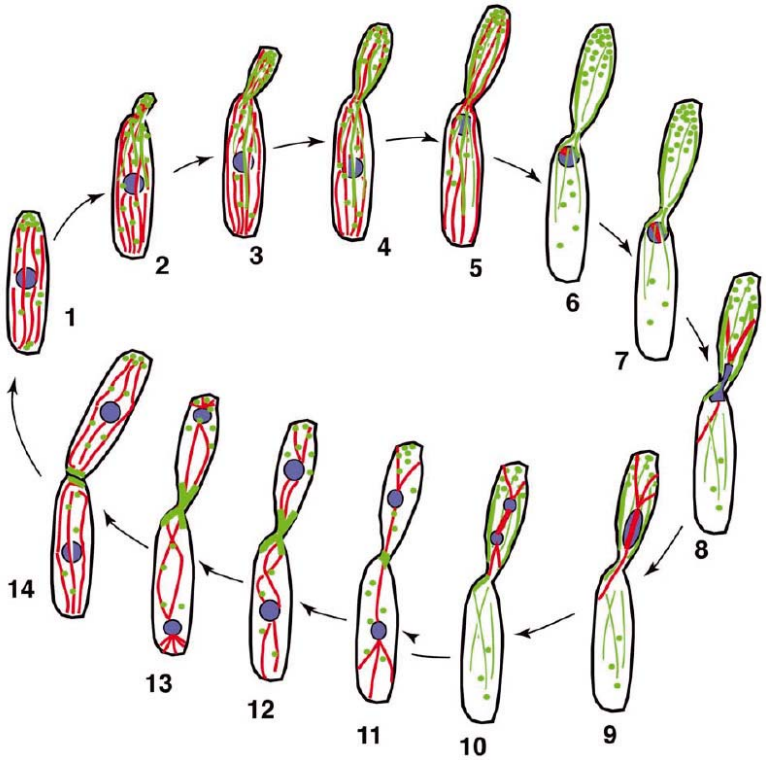


Fig. 16: Yeast budding in *Ustilago maydis*, showing sequential changes of essential cell organelles during this development. Nuclei in blue, actin in green, and tubulin in red. From Banuett & Herskowitz (2002).

Steinberg et al. (2001) found that microtubules in *Ustilago maydis* are highly dynamic and determine cell polarity, and Banuett & Herskowitz (2002) showed that bud elongation occurs through apical growth, reminding hyphal elongation (Fig. 16). The actin cytoskeleton polarizes to sites of bud emergence, including the neck region and the bud tip. Consequently, in a survey of the genome of *U. maydis*, genes coding for cell polarity, exocytosis, actin and microtubule organization, microtubule plus-end associated proteins, kinesins, and myosins were detected (Banuett et al. 2008).

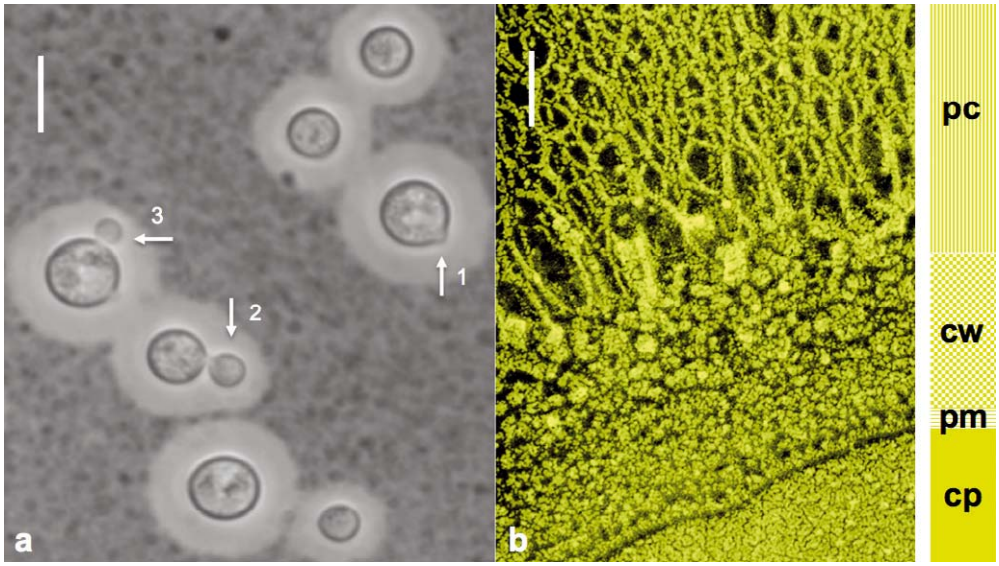


Fig. 17: ***Cryptococcus neoformans***. **a** Yeast grown in tissue culture medium and then stained with India ink; the capsules indirectly shown by exclusion of the ink; note arrows 1–3 with sequential stages of budding; bar = 5 µm. **b** Quick-freeze deep-etch electron micrograph of a cryptococcal cell, showing segments of cytoplasm (cp), plasma membrane (pm), cell wall (cw), and inner part of the polysaccharide capsule (pc), consisting of radiating fibers; bar = 0.1 µm. Illustrations from Kumar et al. (2011), rearranged and labelled.

Though subcellular structures preferably are observed in living cells with highly sophisticated light microscopy, still electron microscopic techniques are needed in ultrastructural investigations. In the case of another basidiomycetous model species, *Cryptococcus neoformans* the conspicuous polysaccharide capsule (Fig. 17) is structurally and functionally very important for its virulance as human pathogen (Kumar et al. 2011). The interaction of extracellular vesicles with the cell wall was studied with TEM and SEM by Wolf et al. (2014). The rather curious result was reported and illustrated by cryo-SEM micrographs that vesicles were released from the cell, crossed the cell wall, and are assumed to act as "virulence bags" with a high payload. – A methodically integrative study, including TEM, of *C. neoformans* by Kozubowsky et al. (2013) revealed a nonclustered state of centromeres and the

apparent localization near the nuclear envelope in nondividing cells, features reminiscent of some metazoans. Also combining fluorescent microscopy with TEM, Kopecká et al. (2013) found a new F-actin structure in *C. neoformans* that surrounds the cell nucleus.

Considering Basidiomycota as such, it is most likely that their origin goes back to yeasts with specific features, different from ascomycetous ones (Oberwinkler 2012).

Evolutionary trends in basidiomycetous yeasts:

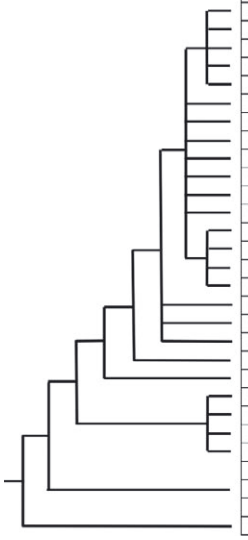
- Life cycle in the single cell stage → dimorphic ontogeny
- Dimorphic development → loss of yeast phase
- Hyphal stage parasitic → yeast stage saprobic
- Hyphal stage possibly mycoparasitic → yeast stage human and animal parasitic

7. Ultrastructural implications in basidiomycetous substrate interactions

As heterotrophic organisms, fungi depend on organic nutrients, generally classified as living or dead substrates, and utilized parasitically, symbiotically, or saprobically. Organisms serving as substrates play crucial roles. Therefore, the distinction of fungal, plant and animal parasites, and plant and algal symbionts is meaningful (Fig. 18). However, in many cases this schematical classification is not more than an approximation. Few examples will be used in the following to illustrate the impact of subcellular structures for better understanding basidiomycetous mycoparasitism, plant parasitism and mycorrhizae, as well as basidiolichens.

The overview of figure 18 aims to summarize quantitatively the distribution of nutritional modes in Basidiomycota. It appears that mycoparasites are concentrated in basal basidiomycetous lineages, Pucciniomycotina and Tremellales of the Agaricomycotina. Plant parasites dominate in Pucciniomycotina, Ustilaginomycotina, Hymenochaetales and Polyporales, and few in other clades. In wood decay, often parasitic stages intergrade into saprobic ones. Remarkably, saprobic substrate interactions are mostly difficult to characterize on a cellular level of host and fungus. Dacrymycetales, Phallales and most of the Auriculariales are saprobic. Widely and in many species distributed are saprobic Basidiomycota also in the Hymenochaetales, Polyporales, Russulales, Atheliales, Boletales and Agaricales. In contrast, all mycorrhizal associations of basidiomycetes are highly differentiated cellularly. By far on a global scale, most of ectomycorrhizae (ECM) have basidiomycetes as mycobionts.

Their structural characteristics are highly elaborate and uniform. Predominantly ectomycorrhizal partners constitute the Cantharellales, Thelephorales, Russulales, Boletales and Agaricales. The most effective mycorrhizal radiation obviously occurred in the Sebacinales, comprising the main types besides ECM. – In comparison to the very species-rich ascolichens, basidiolichens appear poor in species. However, there is a remarkable structural diversity in algal fungal associations on the cellular and subcellular level.



order	mycoparasites	plant parasites	saprobes	mycorrhizae	lichens
AGARICALES	few	few	many	many	many
AMYLOCORTICIALES			few		
BOLETALES	few			many	
JAAPIALES			few		
ATHELIALES	few	few	few	few	few
RUSSULALES		few	many	many	
GLOEOPHYLLALES			few		
CORTICIALES			few		
POLYPORALES		many	many		
THELEPHORALES				many	
HYMENOCHAETALES		many	many	few	
TRECHISPORALES			few		
PHALLALES			few		
HYSTERANGIALES			few	few	
GEASTRALES			many	few?	
GOMPHALES			few	many	
CANTHARELLALES			many	many	few
TULASNELLALES			few	few	
AURICULARIALES			many		
SEBACINALES				many	
DACRYMYCETALES			many		
TRICHOSPORONALES			few		
CYSTOFILOBASIDIALES			few		
FILOBASIDIALES	few		few		
TREMELLALES	many				
USTILAGINOMYCOTINA		many			
PUCCINIOMYCOTINA	many	many		few?	

Fig. 18: Evolution of Basidiomycota and simplified distribution patterns of main trophic stages. Orig.

The three major basidiomycetous mycoparasitic cellular and subcellular interactions (Bauer 2004), nanometer-fusion and micrometer-fusion pores and colacosomes, are distributed in the Pucciniomycotina (Fig. 19). The latter ones and the *Tuberculina* interaction with rust fungi are unique and only known from this basal subdivision. The nanometer-fusion type is reported from cystobasidial and tremelloid haustoria. Cell penetration occurs in the agaricoid mycoparasite *Nyctalis parasitica* and probably also in few other agaricoid and boletoid mycoparasites.

Because of their unique ultrastructure and their rare reports, colacosomes will be treated here at some length. They have been first detected in *Platygloea peniophorae*, a mycoparasitic species that was then transferred in an own genus, *Colacogloea*, by Oberwinkler et al. (1990, Bauer & Oberwinkler 1991b). At about the same time, two different types of colacosomes were found in *Cryptomycocolax abnormis* (Oberwinkler & Bauer 1990). The host of *Cryptomycocolax a.* is an ascomycete that is forced to invaginate cells of the para-

site (Figs. 20, 21). The colacosome with a central pore, surrounded by a membrane that finally fuses with the host plasmalemma, thus providing direct contact of host and parasite cytoplasm, was considered the ancestral one of the two types. An ontogenetic scheme (Fig. 20d) illustrates the main developmental stages of the parasitic interaction. The phylogenetic distance between *Cryptomycocolax* and the colacosome fungi of the Microbotryomycetes, according to hypotheses based on molecular data (Fig. 19), cannot be explained.

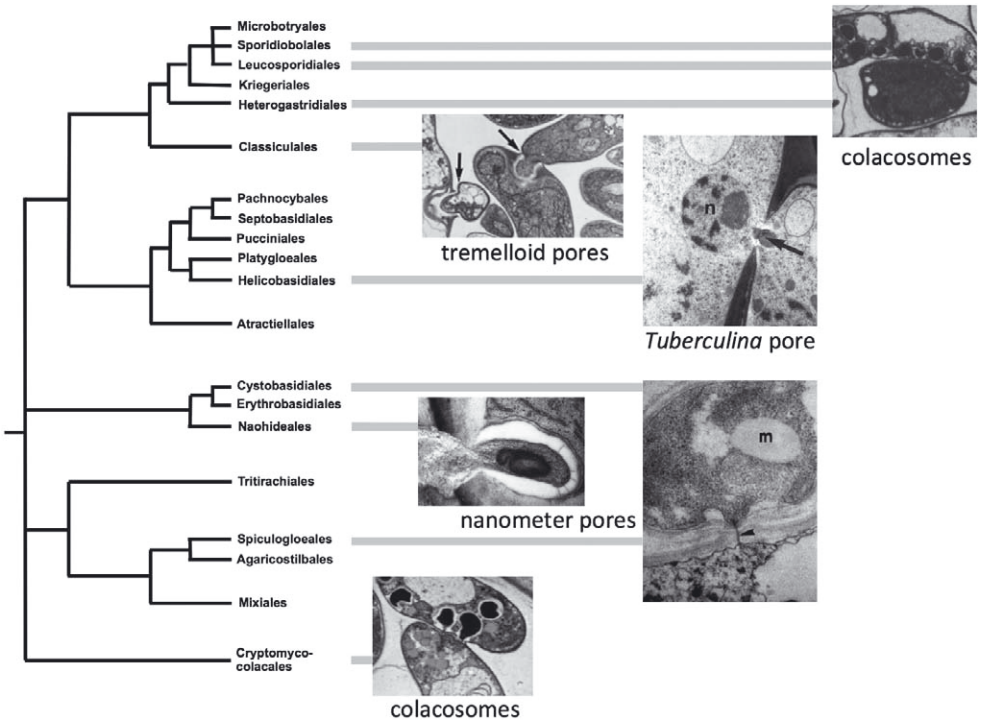


Fig. 19: Main types of mycoparasites with colacosomes, nanometer and micrometer pores, and their distribution in the Pucciniomycotina. Nanometer pores are present in the tremelloid haustoria. Micrometer pores occur in *Tuberculina*. Colacosomes and nanometer pores appear to be paraphyletic. Figures not to scale. Orig. Compare text.

In *Colacogloea*, three more species were detected so far, *C. bispora* (Oberwinkler et al. 1999), with collections from Denmark and Taiwan, *C. papilionacea* with zygoconidia (Kirschner & Oberwinkler 2000) and *C. allantospora* from Canada (Bandoni et al. 2002).

A second genus in the Cryptomycocolacomycetes, *Colacosiphon*, isolated from fungi associated with bark beetles and cultured on natural media, is devoid of clamps and colacosomes type I. The elongated sporangium reminds very much to that of *Cryptomycocolax*, however, meiosis could not be observed, and transverse septation is lacking during its development (Kirschner et al. 2001).

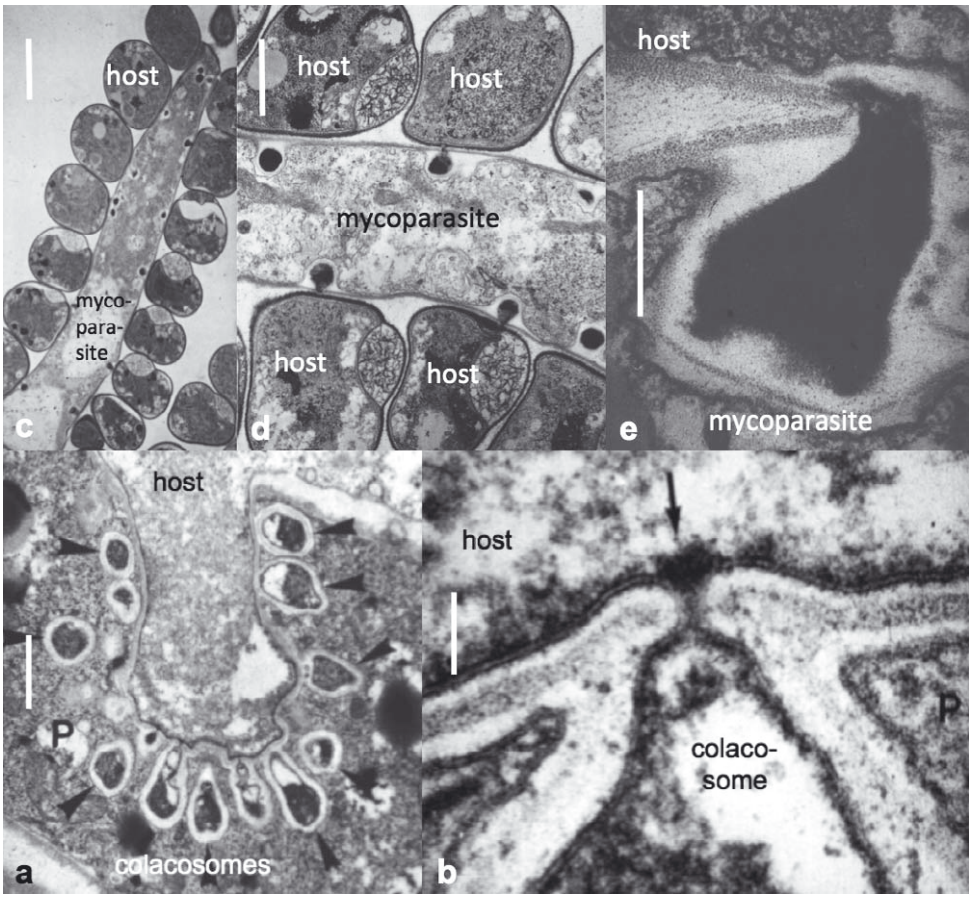


Fig. 20: Colacosomes. **a, b** *Cryptomycocolax abnormis* parasitizing an ascomycete. **a** Host intrusion in the parasite cell, apparently initiated by the colacosomes; arrowheads point to colacosomes in the cytoplasm of *Cryptomycocolax*; bar = 1 µm. **b** Nanometer pore (arrow) of approximately 20 nm between the colacosome and the host cytoplasm; bar = 0.1 µm. **c–e** *Colacogloea* sp. forcing the host to grow spirally around itself; bar = 5 µm. **d** Detail of c; bar = 5 µm. **e** Colacosome interacting with the host; note that a micropore connection could not be found in serial sctions; bar = 0.5 µm. Modified from Bauer (2004) and supplemented.

Atractocolax pulvinatus was collected as pustular basidiocarps from bark beetle galleries in *Picea abies* and *Pinus sylvestris* in Germany and Switzerland (Kirschner et al. 1999). Axenic cultures, derived from single beetles, produced fructifications in petri dishes, in which self-parasitism by colacosomes was observed.

Structures that show colacosomes, but not recognized as such, were already reported by Kreger-van Rij & Veenhuis (1971) from *Sporidiobolus*. Also *Leucosporidium, Mastigobasidium* and *Rhodosporidium* are colacosome fungi (Sampaio et al. 2003).

These examples, together with the one illustrated here (Fig. 21a–c), underline the assumption that colacosome mycoparasites may exist in many more species than hitherto detected. No means exist for a targeted search. In fact, the microsopic nature of these fungi and their submicroscopic colacosomes require extremely careful light microscopy. Understandably, there is no indication whatsoever about their ecological relevance.

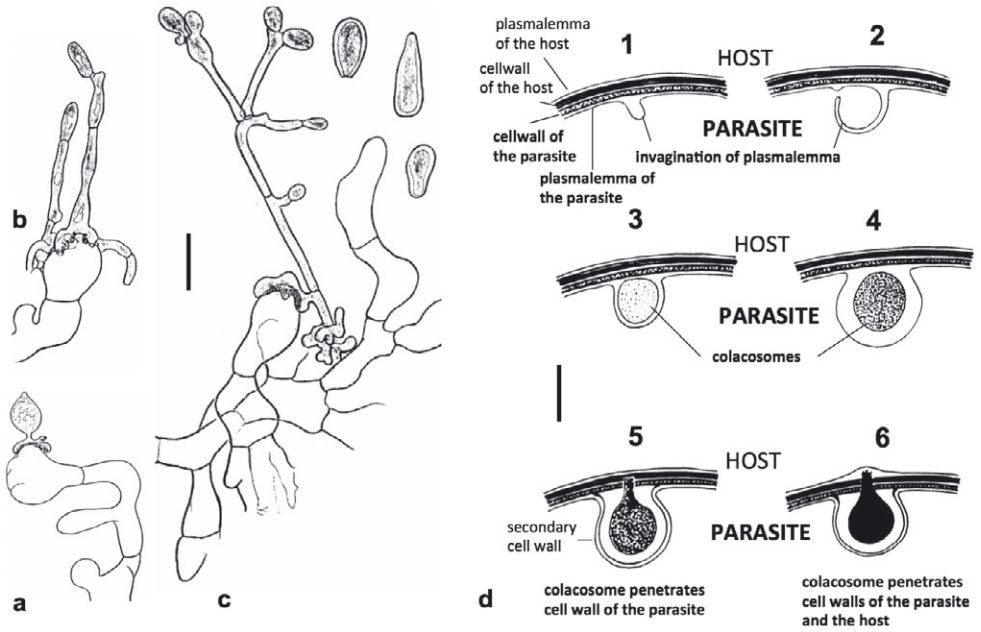


Fig. 21: Mycoparasitism by colacosomes. a–c First ontogenetic stages of *Colacogloea* sp. on an unidentified host; bar = 10 µm. a *Colacogloea* spore attached to a host cell through a germination hypha, already producing colacosomes. b Hyphal outgrowth of the parasite. c First conidial stages of the parasite. d Developmental stages of the colacosome in *Colacogloea peniophorae*, parasitizing *Hyphoderma praetermissum*; after Bauer & Oberwinkler (1991): (1) after a first step of invagination of the parasite plasmalemma, it recurves (2), is compartmented (3), its content becomes electron dense (4), the own cell wall is protruded (5), and in the final stage, the colacosome penetrates the host cell wall (6); bar = 1 µm. a–c Orig.

Most of adequately studied species in Tremellomycetes proved to be mycoparasites, equipped with the synapomorphic tremelloid haustorium, short hyphal branches, subtended by a clamp, basally swollen and apically tapering into a narrow filament, a peculiarity that might appear to be unique. However, a morhologically similar, mycoparasitically interactive structure also is found in some Pucciniomycotina, e. g. species of the genera *Classicula* of the Classiculales (Bauer et al. 2003), *Cystobasidium* (Sampaio & Oberwinkler 2011) and *Occultifur* (Oberwinkler 1990) of the Cystobasidiales, *Spiculogloea* (Langer & Oberwinkler 1998) of the Spiculogloeales, or *Zygogloea* (Bauer 2004). In addition, and surprisingly, in both cases, the parasite-host-interaction is realized by nanometer pores (Fig. 19).

The mycoparasitic *Platygloea sebacea* has been transferred into an own genus, *Naohidea*, by Oberwinkler (1990). Intracellular haustoria with nanometer-fusion pores were detected by Bauer (2004).

An unexpected discovery was that the rust mycoparasite *Tuberculina* is a developmental stage of the plant parasitic *Helicobasidium* (Lutz et al. 2004a, 2004b). The cellular interaction is performed by a micrometer-fusion channel (Fig. 19). Infection experiments showed a high diversity of *Tuberculina*, indicating pronounced host specificities (Lutz et al. 2004c).

To our knowledge, the ultrastructure of agaricoid and boletoid mycoparasites has not been studied so far. Macroscopically well known are *Nyctalis* species growing on *Lactarius* and *Russula* hosts. Light microscopically, intracellular hyphae were found but no haustorial structures could be detected, and no ultrastructural data are available. As summarized by Oberwinkler (2012a), also details for the mycoparsitic interactions in the host specific species of the genus *Squamanita* and of *Pseudoboletus parasiticus* are lacking.

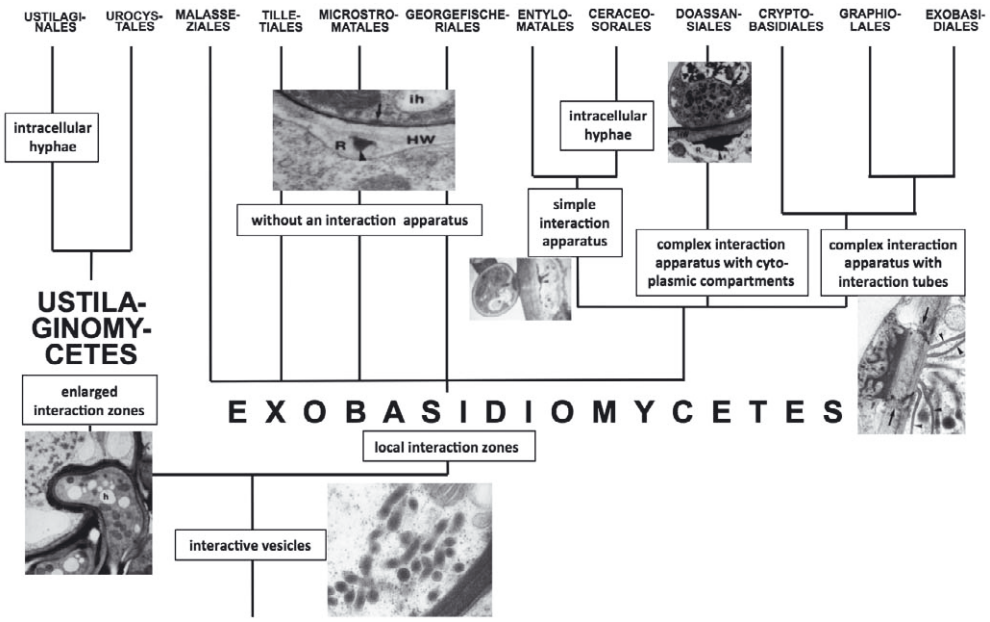


Fig. 22: Host parasite interactions in Ustilaginomycotina, based exclusively on structural features of the interaction sites. Figures not to scale. Compiled and modified after Bauer et al. (1997, 2001) and Begerow et al. (2014). Orig. Compare corresponding text.

In all major taxa of Basidiomycota, parasites of plants are present, with dominating numbers in Puccinomycotina, Ustilaginomycotina, Hymenochaetales and Polyporales (Fig. 18). Substrate interactions on the cellular and subcellular level have been studied extensively in many cases, especially in rusts and smuts. For few comments on plant parasitism of Pucciniales we refer to

the article of Franz Oberwinkler, dealing with cryptogams, chapter 4 and Fig. 10, and to Ustilaginomycotina by Begerow & Kemler, both in this volume. – Nonetheless, there are two main reasons why we do not exclude smut parasitic interactions from our considerations here. First, we had a many years ongoing research in smut ultrastructure in our former institute, and second, this example seems to us as one of the best, to objectively show the indispensability of subcellular structural features for a better understanding of organismic interactions. Principal interactive structures are included in a condensed scheme of a phylogenetic context (Fig. 22). Vesicle derived host-parasite interactions are most likely synapomorphies (Bauer et al. 1997). Continuous production of interactive vesicles results in enlarged interaction zones, independent of the hyphal location in the smut. Intracellular hyphae are characteristic for Ustilaginales. In contrast, local interaction zones are limited in both, time and space. Simple interactions are found in Entylomatales and Ceraceosorales, a complex interaction apparatus with cytoplasmic compartments occurs in Doassansiales, and a local apparatus with interaction tubes is present in Exobasidiales, Graphiolales and Cryptobasidiales. Finally, an interaction apparatus is lacking in Georgefischeriales, Microstromatales and Tilletiales.

A cladistic method, using these characters, led to the phylogenetic hypothesis applied here, that, fortunately, could be verified later on with molecular approaches.

Mycorrhizal systems are presented by Ingeborg Haug in this volume. Therefore, we refer only to one old investigation on the orchid mycorrhiza of *Neottia nidus-avis* (Magnus 1900), and to a transmission electron microscopic work and molecular analysis of a recntly detected ectomycorrhizal type in *Cavendishia nobilis* (Setaro et al. 2006).

It is impressive and admirable how light microscopic studies were carried out already in the second half of the 19th century. Definitely, the limitations of resolution were reached in Magnus' cellular studies of *N. nidus-avis* mycorrhization (Fig. 23a, b), and the borders of ultrastructure were touched herewith at very early times in such kind of research.

The molecular identification of the mycobiont in the *Cavendishia* ectendomycorrhiza as a *Sebacina* sp. could be firmly proven by TEM with a specific dolipore type (Fig. 23f). Such kind of an integrative study is considered paradigmatic and worth copying. More than 100 years after Magnus' study in *Neottia*, molecular results of the mycobiont of this heterotrophic orchid allow its putative identifiaction as a member of the Sebacinales which appear to be the dominant fungi in this host (McKendrick et al. 2002, Selosse et al. 2002, Oberwinkler et al. 2012).

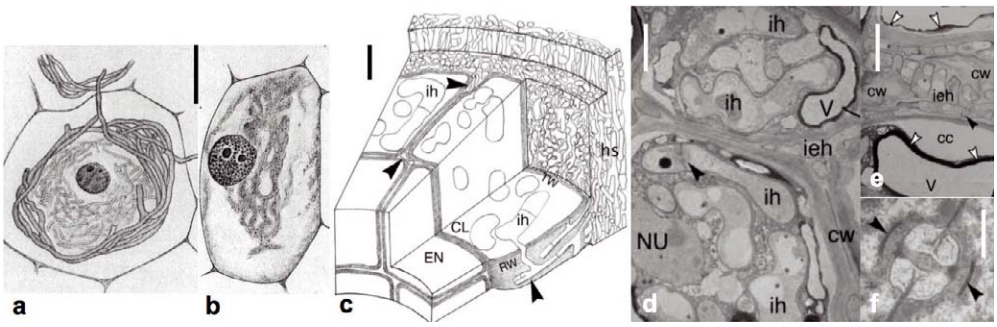


Fig. 23: **a, b** Light microscopy of orchid mycorrhiza (ORM) in *Neottia nidus-avis*, from MAGNUS (1900); bar = 10 µm. **a** Fungal hyphal coil in a living orchid cell surrounding the nucleus. **b** Later developmental stage with partly digested hyphae; the nucleus of the host is prominent. **c–e** Cavendishioid mycorrhiza, from SETARO et al. (2006). **c** Block diagram of the mycorrhiza of *Cavendishia nobilis*: (hs) hyphal sheath formed by thin septate hyphae; fine intercellular hyphae (arrowheads) and large intracellular hyphae (ih); (CL) cortical layer; (EN) endodermis; (RW) radial wall; (TW) tangential wall; bar = 5 µm. **d, e** Longitudinal sections through the cortical layer; (ih) large intracellular hyphae; (ieh) intercellular hyphae growing between the cell walls (CW); (CC) cortical cells; (NU) plant nucleus; (V) plant vacuole; intercellular hyphae with finger-like branches and a septum with a doliporus (black arrowhead); phenolics (white arrowheads) on the tonoplast; bar = 5 µm. **f** Doliporus with imperforate parenthesomes (arrowheads); bar = 0.5 µm.

The ultrastructure of fungus-alga-interactions in basidiolichens has been summarized by OBERWINKLER (2012a, 2012b, 2012c). This is another example where light and electron microscopy have been applied simultaneously and were used as mutual proofs (OBERWINKLER 1980, 1984).

Three main interaction types can be distinguished (Fig. 24):

(1) hyphal sheaths of *Dictyonema* (Fig. 24b, c) from which haustoria-like hyphae originate and penetrate *Rhizonema* (formerly assigned to *Scytonema*) trichomes (Fig. 24i, k). The central hypha can be seen in water preparations with 5 % KOH for light microscopy, especially well in phase contrast (Fig. 24g, h). Also SEM yields some information on the spatial arrangement of myco- and photobiont (Fig. 24d–f). However, interaction details of penetration into the cyanobacterial centroplasm (Fig. 24i and k: solid arrow) can only be demonstrated unambiguously by TEM, as reconstructed in the block diagram.

(2) A second and common interactive type in basidiolichens is a solid globular cluster, formed by densely arranged hyphae, surrounding green algae without haustoria, only precisely visible by TEM (Fig. 24a). Such structures are characteristic for what originally was named *Botrydina vulgaris*, but the construction of *Coriscium viride* is essentially composed of the same elements and integrated in a typical lichen thallus. Of course, this is also clear, when correct light microscopy is applied. But again, the ultrastructural details are in need of TEM investigations.

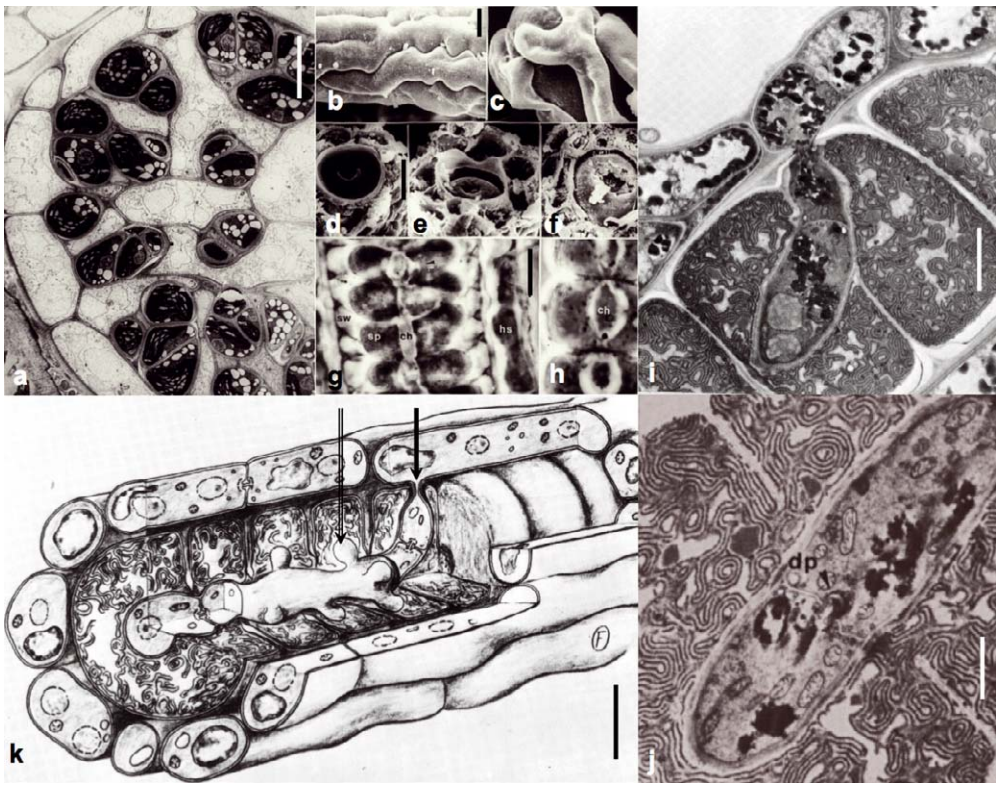


Fig. 24: Cellular and subcelluar interactions in basidiolichens. **a**, Transverse section of the globose thallus of *Lichenomphalia umbellifera*; bar = 5 µm. **b–k** *Dictyonema glabratum*, **b–f** SEM micrographs; bars 5 = µm. **g, h** Phase contrast photographs; bar = 5 µm. **i, j** Transmission electron micrographs of median sections of *Rhizonema* trichomes. **i** Haustorium of *Dictyonema*, penetrating the cyanobacterium; bar = 3 µm. **j** Hypha of *Dictyonema* growing in the centroplasm of the host; bar = 1 µm. **k** Three dimensional reconstruction of the *Dictyonema* interaction; solid arrow points to haustorial neck, double-lined arrow marks a protruberance of the central hypha; bar = 5 µm. From Oberwinkler (1980, 1984). See comments in the text.

(3) Hyphae close to algae in *Lepidostroma calocerum* become attached to them and develop into morphologically distinct appressoria. Unfortunately, our fixation of living thalli in situ was not effective enough to use the material for adequate TEM studies (Oberwinkler 1980, 1984). In illustrations of *Lepidostroma akaragae* (as *Multiclavula a.*) and *L. rugaramae* (as *Multiclavula r.*) by Fischer et al. (2007), such cellular details remain unresolved.

Ultrastructural features of substrate interactions in basidiomycetes:

- Cellular contacts of fungi and hosts involve subcellular interaction structures and reveal functional features
- The ultrastructure of mycoparasites is highly diverse

- Monokaryotic and dikaryotic rust haustoria are structurally different; the latter ones have gymno- and velopedunculate types
- Smut interaction systems are manifold and evolved coevolutionarly
- Basidiomycetous mycorrhizae comprise the commonly distinguished types which include different ultrastructural characteristics
- Basidiolichens have three main, ultrastructurally different, myco- photobiont types

8. Conclusions and outlook

Ultrastructural research in basidiomycetes started in the 1950s and culminated in the 1990s. Within this period of half a century, an enormous amount of subcellular structural differentiations were elucidated and used for better understanding of functional aspects. These comprise patterns of hyphal growth, mitotic and meiotic nuclear divions, and hyphal interactions with various kinds of substrates. Here, we summarized some of the most important mycoparasitic, plant parasitic and symbiontic assoziations on their cellular and subcellular levels. We put emphasis on explaining the intergrading methods of light and electron microscopy and the advantages for their simultaneous application.

The strongly increasing availability of molecular data from many, and sometimes perhaps all organisms, is one of the great stimuli for structurally, and especially ultrastructurally oriented biology. And vice versa, the mass of data on organisms themselves, in their individual interactions, and in the community dimensions, can only be understood when the structural prerequisites are known, most of them bound to cellular and subcellular features.

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