

CZECH MYCOLOGY

Publication of the Czech Scientific Society for Mycology

Volume 47

May 1994

Number 3

The ultrastructure of the spore wall and ornamentation in the *Xerocomus* group of *Boletus*

JAN HOLEC

Department of Botany, Charles University, Benátská 2, 128 01 Praha 2, Czech Republic

Holec J. (1994): The ultrastructure of the spore wall and ornamentation in the *Xerocomus* group of *Boletus*. – *Czech Mycol.* 47: 173–184

The spore wall of five selected species of the *Xerocomus* group of *Boletus* was studied with a transmission electron microscope (TEM). The wall is composed of five layers in all the species studied: a very thin electron-dense outer layer 1 (ectosporium), a moderately electron-dense layer 2 (perisporium), a thick and amorphous electron-dense middle layer 3a (exosporium) that passes gradually into a thinner, granular or granular-fibrillar and moderately electron-dense layer 3b (epispodium), and an almost electron-transparent layer 4 (endosporium). A smooth spore surface was found in *Boletus pulverulentus* and *B. chrysenteron*. A striate exosporium covered by the ectosporium and the perisporium was found in *B. pruinatus*, rough warts originating from a disrupted perisporium and ectosporium in *B. parasiticus*, and very fine warts composed of outgrowths of the ectosporium and part of the perisporium in *B. subtomentosus*. A species of another group of the *Boletales* with conspicuous ornamentation (*Strobilomyces strobilaceus*) was examined as comparative material. The results of this TEM study are compared with SEM photographs of the spores of *Xerocomus* published by other authors, and methodological problems with the examination of spore wall ornamentation are discussed. The data revealed confirm the high value of spore wall architecture and ornamentation in the taxonomy of this genus. The separation of *B. parasiticus* in a new genus *Pseudoboletus* Šutara is supported by its spore wall ornamentation that is unique in the *Boletaceae*.

Key words: *Xerocomus*, *Boletus*, *Strobilomyces*, ultrastructure, transmission electron microscope, spore wall, ornamentation, taxonomy

Holec J. (1994): Ultrastruktura stěny a ornamentiky spor u hřibů ze skupiny suchohřibů. – *Czech Mycol.* 47: 173–184

Pomocí transmisního elektronového mikroskopu byla studována stěna spor u pěti druhů hřibů ze skupiny suchohřibů. Stěna je tvořena pěti vrstvami: vnější, velmi tenkou elektrondenzní vrstvou 1 (ectosporium), pod ní je středně elektrondenzní vrstva 2 (perisporium), následuje tlustá a amorfni elektrondenzní vrstva 3a (exosporium), která postupně přechází v tenčí, granulární či granulárně-fibrilární a středně elektrondenzní vrstvu 3b (epispodium), pod kterou leží vnitřní, téměř elektrontransparentní vrstva 4 (endosporium). Spory druhů *Boletus pulverulentus* a *B. chrysenteron* mají hladký povrch. U druhu *B. pruinatus* byl zjištěn podélně rýhovaný povrch exosporia, pokrytý ectosporiem a perisporiem, u druhu *B. parasiticus* jsou na povrchu spor hrubé bradavky vzniklé roztrháním perisporia a ectosporia a u druhu *B. subtomentosus* jemné bradavky tvořené výrůstky ectosporia a části perisporia. Pro porovnání byl studován druh *Strobilomyces strobilaceus* jako zástupce jiné skupiny řádu *Boletales* s výraznou ornamentikou spor. Výsledky celého studia jsou porovnány s fotografiemi spor z řádkovacího elektronového

mikroskopu, publikovanými v literatuře. Jsou diskutovány metodologické problémy spojené se studiem ornamentiky spor. Výsledky potvrzují vysokou taxonomickou hodnotu stavby stěny a ornamentiky spor u této skupiny hřibů. Oddělení druhu *B. parasiticus* do samostatného rodu *Pseudoboletus* Šutara je podpořeno nálezem ornamentiky, která je v čeledi *Boletaceae* unikátní.

1. INTRODUCTION

There is a relatively high number of studies on the spore wall of the *Xerocomus* group of *Boletus*. Perreau-Bertrand (1961, 1965) made extensive light microscopic examination of the ornamentation, structure and composition of the spore wall with the use of various chemical agents. Later she studied the ultrastructure of the spore wall in *Xerocomus* and other genera of the *Boletales* with a TEM (Perreau-Bertrand 1967). She distinguished five layers in the spore wall of *Xerocomus* using permanganate fixation. All species studied had a smooth spore surface, except for one case. The ornamentation was of exosporial or exceptionally perisporial origin in other genera. The SEM investigation of the spore morphology in the *Boletales* (Pegler et Young 1981) showed that the spore surface is ornamented in several species of *Xerocomus*. The authors reported a finely rugulose to verrucose ornamentation seemingly of myxosporial origin. Recently, Heinemann et al. (1988) and Oolbekkink (1991) published SEM photographs of the spores in *Xerocomus*. Oolbekkink studied 17 species of the *Xerocomus* group of *Boletus* and distinguished four types of spore surface: smooth, fibrillose or floccose, pitted and striate. He supposes the ornamentation to be of either exosporial or of perisporial origin. With the same method Klofac et Krisai-Greilhuber (1992) found a smooth spore surface in several species of *Xerocomus* and finely or roughly striate ornamentation in some species of *Boletellus*.

Although the knowledge of spore wall architecture and ornamentation in *Xerocomus* seems to be sufficient, there are many unresolved questions. Perreau-Bertrand (1967) reports no ornamentation in *Xerocomus* (with only one exception). However, the presence of warts, striae, and other surface structures is distinct according to the SEM studies mentioned. The statements concerning the origin of the spore ornamentation published in these papers are only assumptions that are not confirmed by recent TEM investigation. Besides, there are several taxonomical problems in *Xerocomus* that relate to the spore ornamentation and its origin, e. g. the position of *Boletus pruinatus* or the taxonomic value of the conspicuously pitted spore surface in *Boletus parasiticus* Bull.: Fr.

The present study was conducted to determine the structure of the spore wall and to reveal the origin of the spore wall ornamentation of selected "key" species in the *Xerocomus* group of *Boletus*. The results could contribute to a species delimitation in *Xerocomus* and a generic delimitation in *Boletaceae*.

2. MATERIAL AND METHODS

The collections used for this study are from the herbarium of the National Museum in Prague, Czechoslovakia (PRM): *Boletus chrysenteron* Bull., Bohemia, Roztocký háj, 9 Sept. 1978, leg. and det. Z. Pouzar, PRM 814499; *Boletus pruvinatus* Fr. et Hök, Bohemia, Dlouhopolsko, 4 Oct. 1972, leg. and det. Z. Pouzar (as *B. fragilipes*), PRM 814351; *Boletus parasiticus* Bull.: Fr., Bohemia, Soběslavská blata, 7 Sept. 1980, leg. and det. F. Kotlaba, PRM 830591; *Boletus pulverulentus* Opat. in Wiegmann. Slovakia, Mláčik at Železná Breznica, leg. and det. Z. Pouzar, 30 Aug. 1986, PRM 842268; *Boletus subtomentosus* L.: Fr., Bohemia, Průhonice Park, leg. and det. F. Kotlaba, 6 Jul. 1983, PRM 831196; *Strobilomyces strobilaceus* (Scop.: Fr.) Berk., Bohemia, Kulivá hora, leg. and det. E. Wichanský, 4 Sept. 1964, PRM 603912.

For transmission electron microscopy (TEM), the fragments of the tubes were rehydrated in distilled water for 30 min. and then fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 during several days. Following transfers in 0.1 M cacodylate buffer with 10 min. intervals, the material was post-fixed in 1 % osmium tetroxide in 0.1 M cacodylate buffer for 1 h in the dark, washed six times in distilled water and stained in 1 % uranyl acetate for 1 h in the dark. After 3 washes in distilled water, the material was dehydrated with 10 min. intervals at 10 %, 25 %, 50 %, 70 %, 90 % and 3 x 100 % acetone. The material was embedded in Spurr's resin (Spurr 1969). The sections were cut on a Reichert ultramicrotome using a diamond knife and mounted on Formvar-coated single slot copper grids. They were stained with lead citrate (Reynolds 1963) at room temperature for 5 min. and washed four times with distilled water. The ultrathin sections were observed with a Zeiss EM 109 transmission electron microscope.

Abbreviations used: B. - *Boletus*, S. - *Strobilomyces*. Wherever the name *Xerocomus* is mentioned, "the *Xerocomus* group" of *Boletus* is meant.

3. RESULTS

3.1 Structure of the spore wall

The structure of the spore wall is very similar in the observed species of *Xerocomus* (Figs. 1, 3, 5, 8, 15). The spore wall is composed of the following layers: a very thin electron-dense outer layer 1, a moderately electron-dense layer 2 (25 - 95 nm), a thick middle layer 3 composed of two sublayers: an almost opaque outer layer 3a (120 - 220 nm) and a less electron-dense inner layer 3b (35 - 120 nm), and an electron transparent innermost layer 4 (45 - 80 nm). The thickness of the whole spore wall varies from 350 to 500 nm in the species studied.

Layer 1 is a pellicle covering the spore surface (e. g. Figs. 1, 3, 4, 7, 14, 16). It is often disrupted and only remains on some parts of the spore wall (Figs. 6, 7, 10, 11, 17). The appearance of layer 1 varies from a distinct and thin "line" (Figs. 4, 9) to an unequally thick and slightly floccose layer (e. g. Figs. 2, 3, 15, 16). These differences are probably caused by the use of herbarium material for the examination, by uneven staining of various parts of layer 1 or by the ultrathin sections not being exactly transversal.

The appearance and thickness of layer 2 is very similar in almost all the species studied (Figs. 2, 3, 5, 15; 25 – 94 nm). Only in *Boletus parasiticus* (Figs. 7, 8, 9, 11) layer 2 is almost twice as thick (about 150 nm) and discontinuous. In other species, this layer is smooth and covers the whole spore. It is closely attached to layer 3a and consists of a moderately electron-dense and finely granular substance.

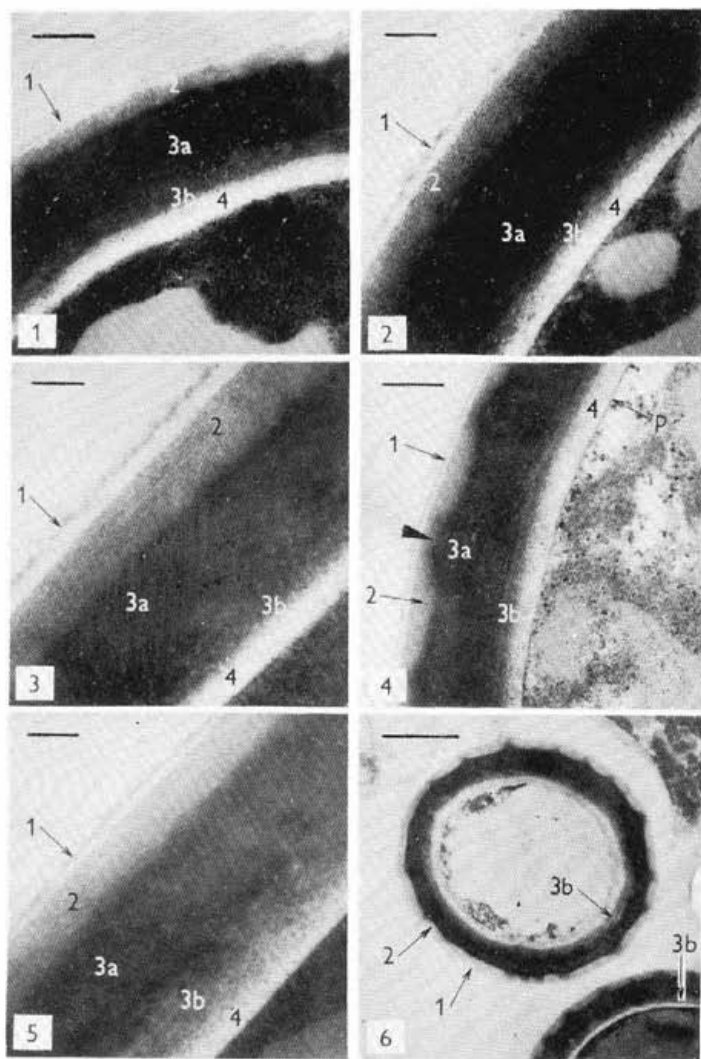
Layer 3 constitutes the thickest part of the spore wall (200 – 300 nm). On the basis of different electron density and structure, it can be divided into two sublayers (Figs. 1, 3, 5, 6, 8, 13, 15). The outer layer 3a appears very homogenous and contains an amorphous electron-dense substance. On the other hand, the inner layer 3b is approximately twice as thin and characterised by a moderately electron-dense and granular or granular-fibrillar substance (this is particularly obvious in the Figs. 1, 3, 5, 6, and 15). The transition of these two sublayers is mostly gradual, in some cases almost indistinguishable (Figs. 3, 4, 8), but in other cases also very distinct (Figs. 1, 5, 6, 15). When all spores in one ultrathin section were carefully observed, these two layers were always distinguishable in the species studied.

Layer 4 forms the innermost part of the spore wall and is adjacent to the plasma membrane. It is amorphous, electron-transparent and in some cases filled with scattered granules (Figs. 5, 8, 9, 14, 15). It is present in both not quite mature (Fig. 4) and mature spores.

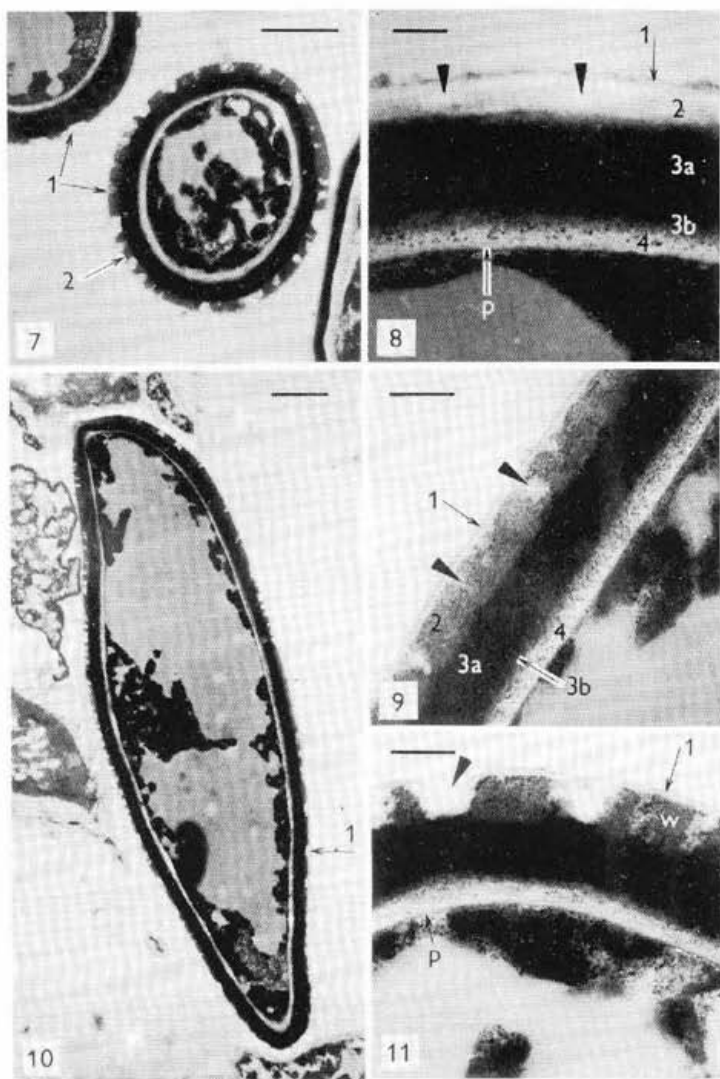
This description shows the general arrangement of the spore wall layers in *Xerocomus*. However, there are some specific features in the individual species and above all remarkable differences in the appearance and origin of the ornamentation between the species studied.

3.2 Ornamentation of the spore wall

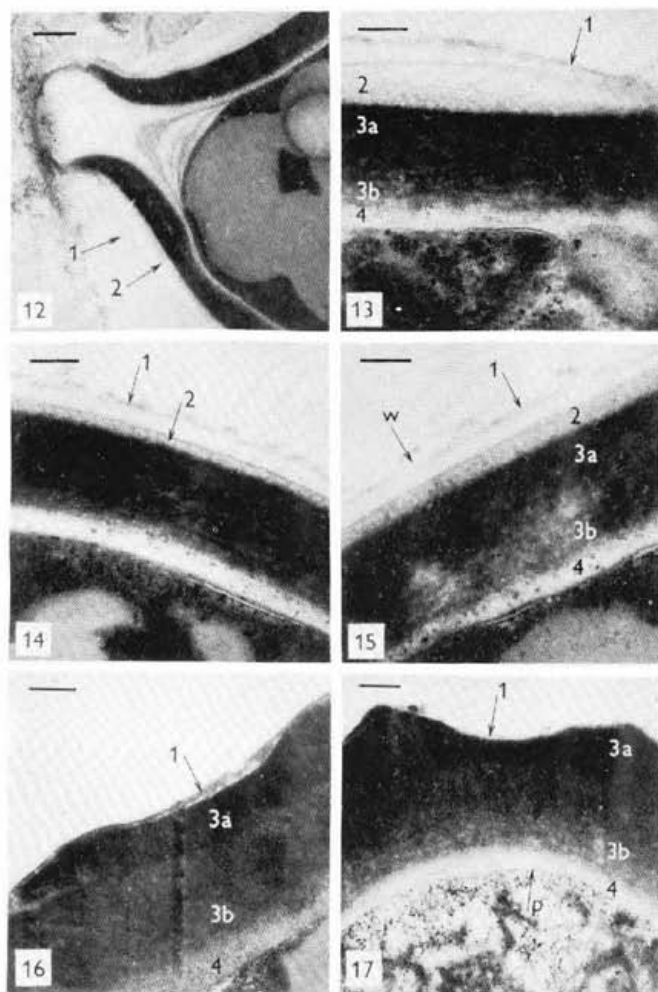
A smooth spore surface was only observed in *Boletus pulverulentus* (Figs. 1, 2) and *Boletus chrysenteron* (Fig. 3). *B. chrysenteron* is reported to have longitudinally striate spores according to Heinemann et al. (1988) and Oolbekkink (1991), but such an ornamentation was not found in any of the spores observed. Low prominences (about 50 nm) changing with shallow depressions were found in layer 3a in cross sections of the spores in *Boletus pruinatus* (Figs. 4, 6). Longitudinal sections (Fig. 5) show no prominences in layer 3a. This means that layer 3a forms longitudinal ridges (striae) that are covered by layers 2 and 1. The distance between individual



Figs. 1 - 6. Spore wall of *Boletus pulverulentus*, *B. chrysenteron*, and *B. pruinatus*. - 1. *B. pulverulentus*, cross section. Mature spore with smooth spore surface and relatively distinct layer 3b. (x 50 000, Bar = 0.2 μ m). - 2. *B. pulverulentus*, cross section of mature spore. Layer 3a passes gradually into layer 3b that is less electron dense and slightly granular (x 85 000, Bar = 0.1 μ m). - 3. *B. chrysenteron*, cross section. Mature spore with smooth spore surface and slightly floccose layer 1. Layer 3a passes gradually into moderately electron-dense and granular layer 3b (x 85 000, Bar = 0.1 μ m). - 4. *B. pruinatus*, cross section of not quite mature spore. Layer 3a forms low prominences (arrow-head) that are covered by layers 1 and 2. Layer 3b has a granular-fibrillar structure (x 50 000, Bar = 0.2 μ m). - 5. *B. pruinatus*, longitudinal section of mature spore. No prominences of layer 3a are present (x 85 000, Bar = 0.1 μ m). - 6. *B. pruinatus*, cross section of mature spore (lower right corner) and not quite mature spore (in the center) that is covered by rests of layer 1. The moderately electron-dense layer 3b is very distinct on both spores. The prominences forming longitudinal ridges on the surface of layer 3a are more pronounced on the mature spore (x 12 000, Bar = 1 μ m). Abbreviation: p = plasma membrane.



Figs. 7 - 11. Spore wall of *Boletus parasiticus*. - 7. Cross section of mature spores. Layer 2 is separated into fragments that are partly covered by layer 1 (x 12 000, Bar = 1 μ m). - 8. The initial stage of spore ornamentation development, longitudinal section. Layer 2 begins to break up (arrow-heads), seemingly by the growth of the spore. Layer 3b differs from layer 3a on the basis of its electron density and finely granular structure. (x 85 000, Bar = 0.1 μ m). - 9. Later stage of spore ornamentation development, longitudinal section. Layer 2 begins to separate into fragments (x 50 000, Bar = 0.2 μ m). - 10. Mature spore, longitudinal section. The verrucose spore ornamentation is formed by fragments (warts) of layer 2 that are partly covered by layer 1 (x 4 400, Bar = 2 μ m). - 11. Final stage of spore ornamentation development, cross section. Layer 2 is divided into fragments that form warts on the spore surface (see also Fig. 10). The disruption of layer 1 (arrow-head) indicates that the warts developed during the enlargement of the spore volume and resulting disintegration of layer 2 (x 50 000, Bar = 0.2 μ m). Abbreviations: p = plasma membrane, w = wart.



Figs. 12 - 17. Spore wall of *Boletus subtomentosus* and *Strobilomyces strobilaceus*. - 12. *B. subtomentosus*, longitudinal section of mature spore. The distance between the layers 1 and 2 is relatively great ($\times 30\ 000$, Bar = $0.2\ \mu\text{m}$). - 13. *B. subtomentosus*, longitudinal section of not quite mature spore near the apiculus. Layer 1 is smooth and separated from layer 2 by an electron-transparent substance ($\times 85\ 000$, Bar = $0.1\ \mu\text{m}$). - 14. *B. subtomentosus*, longitudinal section of mature spore. Layer 1 forms fine warts on the spore surface ($\times 85\ 000$, Bar = $0.1\ \mu\text{m}$). - 15. *B. subtomentosus*, explanations see Fig. 14. Layer 3b is well discernable on the basis of its finely granular-fibrillar structure (see also Fig. 14) and moderate electron density. - 16. *Strobilomyces strobilaceus*, longitudinal section of not quite mature spore near the apiculus. The electron-dense layer 3a is covered directly by a thin layer that is very similar to layer 1 in the *Xerocomus* species examined. No layer resembling the moderately electron-dense and finely granular layer 2 of *Xerocomus* species is present ($\times 30\ 000$, Bar = $0.25\ \mu\text{m}$). - 17. *S. strobilaceus*, cross section of young spore. The transition of the layers 3a and 3b is gradual. However, layer 3b is discernable on the basis of its moderate electron-density and granular structure. The prominences of layer 3a are still relatively low. Layer 1 forms only small rests between these prominences ($\times 30\ 000$, Bar = $0.25\ \mu\text{m}$). For abbreviations see Figs. 7 - 11.

ridges differs in various parts of the spore wall (Fig. 6). The ridges are considerably higher and more distinct in mature spores (Fig. 6, in the lower right corner) than in spores of earlier stages of development. (Fig. 6, in the centre).

In *B. parasiticus*, the ornamentation originates from layer 2. This layer is continuous and smooth in immature spores (Fig. 8) but then begins to break up (Fig. 8, 9), seemingly by the growth of the spore. Finally, layer 2 consists of more or less separated fragments with an approximately square or rectangular cross section (Fig. 11). These fragments are covered with the continuous layer 1 (Fig. 9) which later becomes disrupted (Fig. 11). The presence of fragments both in longitudinal (Fig. 10) and cross sections of the spore wall (Fig. 7) shows that layer 2 forms verrucose or irregularly reticulate spore surface. The ornamentation is comparatively high as layer 2 is nearly as thick (about 170 nm) as the whole layer 3 (about 200 nm).

Layer 1 forms small outgrowths on the spore surface of *B. subtomentosus*. These structures are very fine in not quite mature spores and near the apiculus (Fig. 13) but very distinct in mature spores (Fig. 14, 15). They are approximately 25 – 35 nm high and irregularly arranged. The distance between layer 1 and 2 is much greater than in spores of other species studied. The data obtained show that the spore surface is covered by very faint and irregularly distributed warts of ectosporial origin.

From these data follows that the spore ornamentation of the observed species of *Xerocomus* is formed by: a) a ridged surface of layer 3a (*B. pruinatus*), b) disintegration of layer 2 and 1 (*B. parasiticus*) or c) fine warts formed by layer 1 together with part of layer 2 (*B. subtomentosus*). These types of ornamentation are of a different origin with respect to the layer of the spore wall and way of development.

To compare the results obtained in *Xerocomus* with another group of the order *Boletales* with ornamented spores, the related species (after Pegler et Young 1981) *Strobilomyces strobilaceus* was also studied. This species has a distinctly reticulate spore ornamentation (Perreau-Bertrand 1961, Pegler et Young 1981). The layers of the spore wall are arranged in other way than in *Xerocomus* (Figs. 16 – 19). The innermost layer (about 100 nm) seems to be identical with layer 4 in *Xerocomus*. The adjacent layer consists of granular and moderately electron-dense material that resembles the substance of layer 3b in *Xerocomus*. However, in contrast to *Xerocomus* species this layer is very thick (400 – 430 nm) and very distinct. Besides, narrow "columns" protrude from this layer into the outer layer (Figs. 18, 19). Around these "columns" an electron-dense substance is accumulated. This substance is strongly reminiscent of the material of layer 3a in *Xerocomus* and forms the reticulate ornamentation together with "columns" mentioned (200 – 220 nm without the ornamentation, about 480 nm including the ornamentation). In immature spores, there is an electron-dense pellicle present (Fig. 16) that covers

all outgrowths of the spore wall and resembles layer 1 in *Xerocomus*. The pellicle breaks up by the growth of the spore (Fig. 17) and it is not present (Figs. 18, 19) on mature spores.

The comparison shows that the spore wall in *S. strobilaceus* is much thicker than in *Xerocomus* species (about 1100 nm). Layer 2, which was observed in all spores of *Xerocomus*, is absent in *S. strobilaceus*. The next layers seem to be homologous with layers described for *Xerocomus* species but their arrangement and thickness is different. The ornamentation of mature spores is not covered by layer 1.

4. DISCUSSION

4.1 The spore wall

Using a TEM, Perreau-Bertrand (1967) distinguished five layers in the spore wall of several *Xerocomus* species. She used the following terms for their description: ectosporium, perisporium, exosporium, episporium and endosporium (originally ectospor, perispor, etc.; this form is not correct according to Singer, 1986). The ultrastructure of layers revealed by the present author (1, 2, 3a, 3b and 4 respectively) correlate to Perreau-Bertrand's description of the layers mentioned above. There are, however, several differences – after Perreau-Bertrand, the exosporium is clearly delimited from the episporium (the transition of these two layers is gradual in my case), the perisporium on her photographs is much thinner, etc. Nevertheless, the spore wall architecture is similar in both cases. Consequently, the spore wall layers are named in the following text as: 1 – ectosporium, 2 – perisporium, 3a – exosporium, 3b – episporium, 4 – endosporium. Although these terms are usually used for the description of the spore wall in light microscopy (Pegler et Young 1971, Singer 1986), they seem to be fully acceptable for description at the ultrastructural level in this case. They express the relative position of the individual layers of the spore wall as well as the differences in their structure in accordance with the terminology of the authors mentioned above.

The fact that layers with different appearance in the ultrathin sections are of different chemical composition was demonstrated by Rast et Hollenstein (1977). They revealed a mixture of granules and amorphous material together with some fibrils in the electron-dense outer layer of *Agaricus bisporus* spore wall, chitin fibrils embedded in a β – glucan-protein matrix in a moderately electron-dense middle layer and an almost electron-transparent mucous covering adjacent to the plasma membrane. The authors showed that the ultrastructure of the individual layers of the spore wall is in close relation to their chemical composition. Based on the great similarity of the ultrastructure of some layers in *A. bisporus* and selected species of *Xerocomus*, the electron-transparent endosporium in *Xerocomus* consists probably of a mucous substance. Further more, although the episporium (layer 3 b)

is not distinctly delimited from the exosporium (layer 3 a) in *Xerocomus* species, it seems to be of a different chemical composition than the exosporium. Its granular to slightly fibrillar structure resembles the appearance of the middle layer of *A. bisporus* spore wall. The presence of some fibrillar material (perhaps chitin) in this layer is possible. The exosporium in *Xerocomus* species is very similar to the outer layer in *A. bisporus* and seems to consist of an amorphous and probably pigmented substance. Cléménçon (1970, 1977, 1986) writes that layers of the eusporium (= endosporium + episporium) are produced by different degree of dispersion of an electron-opaque "tunica" substance in a transparent "corium" matrix. His assumption seems to be incorrect with respect to the findings of Rast et Hollenstein (1977). This is another reason to use the terms endosporium and episporium for the layers 4 and 3b and not the terms corium and coriotunica. Besides, a hypothesis concerning the spore wall ontogeny should be supported by thorough developmental studies. The spore development in one species of the *Boletales* - *Boletus rubinellus* - was investigated by Yoon et McLaughlin (1984, 1986). They described six phases of the spore development based on nuclear behaviour and changes in wall layers and cytoplasm. The authors observed considerable changes of the spore wall ultrastructure during the development but from stage 2 the wall was composed of four layers: pellicle, perisporium, episporium and endosporium. These layers seem to correspond with the layers 1, 2, 3a and 4 in *Xerocomus*. The granular and moderately electron-dense layer (3b in *Xerocomus*) was not observed in mature spores of *Boletus rubinellus*. However, Yoon et McLaughlin (1986) observed that the electron-dense layer forming the thickest part of the spore wall in *B. rubinellus* became multi-layered during stage 6. This phenomenon was not observed in fully mature spores of *Xerocomus* species. The presence of the not distinctly delimited layers 3a and 3b could indicate immaturity of the spores with respect to the possibility of removing the fully mature spores during preparation of the material for TEM study. However, the layers 3a and 3b were discernible in all observed spores of all the species studied and parts of the tubes used for preparation were taken from quite mature fruitbodies. Besides, the maturity of spores in the fruitbody was controlled beforehand under the light microscope. Therefore, in accordance with Perreau-Bertrand (1967) the spore wall in the *Xerocomus* group of *Boletus* seems to be really five-layered. The trilamellate structure of the outermost layer (ectosporium) reported by Yoon et McLaughlin (1986), was not observed, probably because of the use of herbarium material.

It is necessary to discuss here the availability of herbarium material for a study. The use of herbarium specimens was an attempt to find out the degree of conservation of the ultrastructure of the spore wall after considerably long storing in a herbarium (the specimens used for this study were collected in the years 1964, 1972, 1978, 1980, 1983, and 1986). The comparison with the ultrastructure of spores from fresh material in *Boletus rubinellus* (Yoon et McLaughlin 1984, 1986) is very

interesting. The changes in the cytoplasm of the spores from herbarium material are considerable and the artefacts are clearly visible in many photographs, e. g. the absence or bad preservation of membranes (Figs. 4, 13, 14, 15) or disintegrated vesicles and the presence of particles without any structure (Fig. 17). Consequently, the interpretation of the structure of cytoplasm and membranes is problematical. The aggregations of lipids in a dense cytoplasm are discernible in some spores (e. g. Figs. 1, 7, 10, 12, 19) which is a typical feature of mature spore. On the other hand, the ultrastructure of the spore wall layers is quite comparable with the ultrastructure of the spores from living fruitbodies. The spore wall as a structure saving the cytoplasm between spore release and germination seems to be very resistant as to preservation of the structure in dry state. This conclusion is supported by the good preservation of ectosporium and perisporium on my spores, at least in the form of small remnants.

Pegler et Young (1981) reported that the epitunica (= exosporium) forms a thin but distinct layer in *Xerocomus*. This finding was not confirmed, besides, the term epitunica was delimited for the description of the spore wall layer in *Cortinarius* (Cléménçon 1970, 1973) only.

4.2 Spore ornamentation

The spore ornamentation revealed by the use of a TEM is compared with SEM photographs of the spores in the *Xerocomus* group of *Boletus* made by other authors.

The finding of a smooth spore surface in *B. pulverulentus* (Fig. 1, 2) is in accordance with data by Oolbekkink (1991). However, Oolbekkink (1991) and Heinemann et al. (1988) report faint to distinct longitudinal striae on the spore surface of *B. chrysenteron* (the other species with smooth spores according to this paper, Fig. 3). Recently, Klofac et Krisai-Greilhuber (1992) confirmed that the spore surface is smooth in *B. chrysenteron* and the spores photographed by Oolbekkink (1991) and Heinemann et al. (1988) were taken from another species, probably *B. pruinatus*. Pegler et Young (1981) also found smooth spores in *B. chrysenteron*.

B. pruinatus shows distinct longitudinal ridges (striae) of exosporial origin. It is interesting that the ridges covered by perisporium and ectosporium (Fig. 4, 6) are visible on SEM photographs of the spore surface (Heinemann et al. 1988, Oolbekkink 1991, Klofac et Krisai-Greilhuber 1992). This phenomenon may be caused by the fact that the spores prepared by these authors were dry, whereas spores prepared by the present author were rehydrated in water for 30 minutes. The mucous perisporium seems to be closely attached to the exosporium in dry spores and does not fill the depressions between the striae. Rehydration of the perisporium may be the precondition of natural appearance of this layer. The same situation was observed by Farr (1983) in *Pholiota terrestris* and *P. highlandensis*

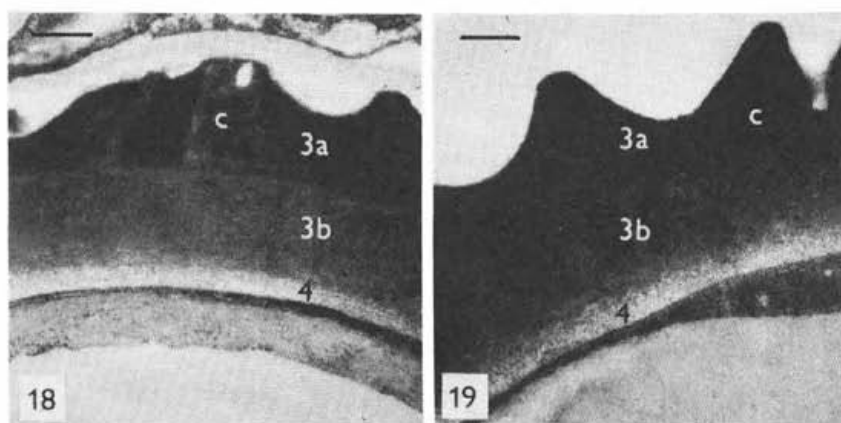
where the distinct perisporial ornamentation was discernable with a SEM on fresh spores fixed in glutaraldehyde or on dried spores rehydrated in ethanol and water. On the other hand, no perisporial ornamentation was revealed on the spores from herbarium material without prior rehydration.

In *B. parasiticus* perisporial ornamentation was found, composed by fragments of layer 2 (Figs 9, 10, 11). It was supposed to be verrucose to irregularly reticulate. The SEM photographs of Pegler et Young (1981) and Oolbekkink (1991) prove this assumption. Oolbekkink names this ornamentation "conspicuously pitted" and supposes that it is formed by disintegration of ecto- and perisporium. This assumption is now confirmed by the images demonstrating that the ornamentation develops really in this way.

In *B. subtomentosus* fine warts of ectosporial and partly also perisporial origin were found on the spore surface (Figs. 14, 15). The spore surface is finely floccose or verrucose on the SEM photographs of Pegler et Young (1981), Heinemann et al. (1988) and Oolbekkink (1991) which is in accordance with my findings. However, the ornamentation is not formed by disintegration of ectosporium and perisporium, as Oolbekkink reports, but by fine verrucose outgrowths of the ectosporium.

Generally, the exosporial or perisporial origin of spore ornamentation was revealed by many authors, e. g. Perreau-Bertrand (1967, 1973, 1976), Pegler et Young (1971) and Singer (1986). The existence of ornamentation formed above all by the ectosporium is relatively surprising because this layer is supposed to be very fine and often disappearing. Its preservation seems to depend on the way of preparation of the material. Although the perisporial ornamentation is very distinct with a TEM and SEM in *B. parasiticus*, it is not visible with a light microscope which is confirmed by e. g. Šutara (1991). Only the exosporial ornamentation in *B. pruinosus* (Pouzar 1981, Klofac et Krisai-Greilhuber 1992, own light-microscopic observations) is faintly visible with a light microscope. It is obvious that the use of a SEM is necessary to distinguish finer types of ornamentation in *Xerocomus* (perisporial, ectosporial-perisporial). The origin of the ornamentation can be revealed only on the basis of a TEM study.

The TEM photographs of the spore wall in *Strobilomyces strobilaceus* (Figs. 18, 19) show that the reticulate ornamentation depicted by Perreau-Bertrand (1961) and observed by Perreau et Heim (1969) and Pegler et Young (1981) with a SEM is of exosporial but partly also of episporial origin. The episporium forms "columns" around which the exosporium is located. This is the fourth type of ornamentation (as regards its origin) revealed in the species studied. At maturity the spore surface is almost not covered by an ectosporium. The perisporium reported by Perreau-Bertrand (1967) was not observed.



Figs. 18 - 19. Spore wall of *Strobilomyces strobilaceus*. - 18. Longitudinal section of mature spore near apiculus. Layer 3b is clearly different from layer 3a and forms "columns" projecting into layer 3a. Layer 1 is not present in the mature spore. - 19. Cross section of mature spore. Material of layer 3a is accumulated around narrow "columns" projecting from layer 3b, and forms "outgrowths" that compose reticulate ornamentation on the spore surface of this species (x 30 000, Bar = 0.25 μ m).

4.3 Taxonomic implications

The taxonomic value of the ornamentation of spores in the *Xerocomus* group of *Boletus* was discussed by Oolbekkink (1991). The results of this study and findings published by Klofac et Krisai-Greilhuber (1992) somewhat change his conclusions. Oolbekkink (1991) insists that "the taxa of *B. chrysenteron* complex can be distinguished from *B. subtomentosus* complex by their striate spores". However, the observations of Pouzar (1981), Pegler et Young (1981), Klofac et Krisai-Greilhuber (1992), and my own results show that the true *B. chrysenteron* has smooth spores. A similar species with striate spores is *B. fragilipes* C. Martin that was reinstated by Pouzar (1981) and considered to be a nomen dubium by Oolbekkink (1991). Klofac et Krisai-Greilhuber (1992) showed after a thorough discussion that the basionym of this species should be *Boletus pruinatus* Fr. et Hök. They proposed the new combination *Boletellus pruinatus* (Fr. et Hök) Klofac et Krisai-Greilhuber, above all on the basis of its striate spores. This is really a very important feature of the genus *Boletellus* but the second one is the boletoid trama (in comparison with a phylloporoid trama in *Xerocomus*). However, Klofac et Krisai-Greilhuber (1992) do not specify the trama type in their description of *Boletellus pruinatus*. They mention that the European species of the section *Chrysenteroidei* Sing. of *Boletellus* are expected to have a boletoid trama in young fruitbodies only and later the lateral stratum is almost parallel and resembles that in *Xerocomus badius*. Similarly, they mention the description of Watling (1968) where the trama is characterised as

almost phylloporoid. Thus, the position of *Boletus pruinus* is still unclear. The presence of spores with a striate exosporium is confirmed, but the problem of the trama type needs thorough study of the trama ontogeny.

The presence of distinct ornamentation in *Boletus parasiticus* is a very important feature. An ornamentation formed by disintegration of the perisporium and ectosporium is not known in *Xerocomus* and *Boletus*. This feature can serve as a further confirmation of the opinion published by Šutara (1991) on the isolated position of this species within *Xerocomus* and *Boletus*. *B. parasiticus* differs from other species of these genera by the parasitic way of life and above all by the sterile stipe surface. Consequently, Šutara (1991) described the new genus *Pseudoboletus* Šutara that includes *Pseudoboletus parasiticus* (Bull.: Fr.) Šutara. The unique spore ornamentation seems to support this classification.

A c k n o w l e d g e m e n t s

The TEM study was carried out in the Institut für Biologie, Lehrstuhl Spezielle Botanik/ Mykologie of the Eberhard-Karls-Universität in Tübingen, Germany. I would like to express my sincere thanks to the staff of the Institut, especially to Dr. I. Kottke for inviting me to Tübingen, Dr. R. Bauer for introducing me to electron microscopy, Prof. F. Oberwinkler for the possibility to work in the Institut as well as to Miss S. Süßbrich and O. Ebinger for technical assistance with the TEM. I also thank Dr. Z. Pouzar from the National Museum, Prague, for the theoretical and practical help in the selection of the herbarium specimens. The study was a part of my stay at the University of Tübingen that was supported by a grant from TEMPUS.

R E F E R E N C E S

- CLÉMENÇON H. (1970): Bau der Wände der Basidiosporen und ein Vorschlag zur Benennung ihrer Schichten. – *Z. Pilzk.* 36: 113–133.
- CLÉMENÇON H. (1973): Die Wandstrukturen der Basidiosporen III. *Cortinarius* und *Dermocybe*. – *Z. Pilzk.* 39: 121–144.
- CLÉMENÇON H. (1977): Die Strukturen der Basidiosporenwand und des Apikulus, und deren Beziehung zur Exogenization der Spore. – *Persoonia* 9: 363–380.
- CLÉMENÇON H. (1986): Electron microscopy and spore teguments. – In: SINGER R., *Agaricales in modern taxonomy*, Ed. 4: 83–87, Koenigstein.
- FARR D. F. (1983): Scanning electron microscopic observations of basidiospores in *Pholiota* and related genera. – *Can. J. Bot.* 63: 603–610.
- HEINEMANN P., RAMMELOO J. and RULLIER E. (1988): L'ornementation sporale des Xerocomaceae a spores dites lisses. – *Bull. Jard. bot. natn. Belg.* 58: 513–534.
- KLOFAC W. and KRISAI-GREILHUBER I. (1992): *Xerocomus chrysenteron* und ähnlich aussehende Röhrlinge. – *Öst. Z. Pilzk.* 1: 19–59.
- OOLBEKKINK G. T. (1991): The taxonomic value of the ornamentation of spores in "the *Xerocomus* group" of *Boletus*. – *Persoonia* 14: 245–273.
- PEGLER D.N. and YOUNG T. W. K. (1971): Basidiospore morphology in the Agaricales. – *Beih. Nova Hedwigia* 35., Vaduz.
- PEGLER D.N. and YOUNG T. W. K. (1981): A natural arrangement of the Boletales, with reference to spore morphology. – *Trans. Br. Myc. Soc.* 76: 103–146.

- PERREAU-BERTRAND J. (1961): Recherches sur les ornementsations sporales et la sporogénèse chez quelques espèces des genres *Boletellus* et *Strobilomyces* (Basidiomycetes). – *Annls. Sci. nat. (Bot.)* 2: 399–489.
- PERREAU-BERTRAND J. (1965): Structure membranaire et différenciations apicales chez les spores des genres *Xerocomus*, *Boletellus*, *Heimiella* et *Strobilomyces*. – *C. r. hebd. Seanc. Acad. Sci., Paris* 260: 4245–4248.
- PERREAU-BERTRAND J. (1967): Recherches sur la différenciation et la structure de la paroi sporale chez les Homobasidiomycetes a spores ornées. – *Annls. Sci. nat. (Bot.)* 8: 639–735.
- PERREAU J. (1973): Valeur de l'ornementation sporale dans la systématique des Basidiomycetes supérieurs. – In: SUBRAMANIAN C. V. (ed.) *Taxonomy of fungi*, pp 594–604, Madras.
- PERREAU J. (1976): Développement, morphologie et structure de la basidiospore. – *Inform. Scient.* 2: 55–75.
- PERREAU J. and HEIM R. (1969): L'ornementation des basidiospores au microscope électronique a balayage. – *Rev. Mycol.* 33: 329–340.
- POUZAR Z. (1981): Co jsou podzimní "babky"? (*Boletus fragilipes* u nás). – *Mykol. listy* 3: 8–14.
- RAST D. and HOLLENSTEIN G. O. (1977): Architecture of the *Agaricus bisporus* spore wall. – *Can. J. Bot.* 55: 2251–2262.
- REYNOLDS E. S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. – *J. Cell. Biol.* 17: 208–212.
- SINGER R. (1986): The Agaricales in modern taxonomy, Ed. 4. – Koenigstein, 981 p.
- SPURR A. R. (1969): A low-viscosity epoxy resin embedding medium for electron microscopy. – *J. Ultrastr. Res.* 26: 31–43.
- ŠUTARA J. (1991): *Pseudoboletus*, nový rod řádu Boletales. – *Čes. Mykol.* 45: 1–9.
- WATLING R. (1968): Records of Boleti and notes on their taxonomic position. – *Notes Roy. Bot. Gard. Edinburgh* 28: 301–315.
- YOON K.S. and McLAUGHLIN D. J. (1984): Basidiosporogenesis in *Boletus rubinellus*. I. Sterigmatal initiation and early spore development. – *Amer. J. Bot.* 7: 80–90.
- YOON K.S. and McLAUGHLIN D. J. (1986): Basidiosporogenesis in *Boletus rubinellus*. II. Late spore development. – *Mycologia* 78: 185–197.