

## A new hyphal type found in *Xerocomus pruinatus*

HEIDI LADURNER

REINHOLD PÖDER  
Institute of Microbiology  
Leopold-Franzens-University Innsbruck  
Technikerstrasse 25  
A-6020 Innsbruck, Austria

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**Key words:** *Boletales*, *Xerocomaceae*, *Xerocomus pruinatus*. – Hyphal anatomy, taxonomy.

**Abstract:** A special type of context hyphae was found in basidiomata of *Xerocomus pruinatus*. These hyphae are characterised by a particularly structured thickening of their inner wall surface that stains blue with Melzer's solution. This hyphal type could not be found in other European *Xerocomus* or *Boletellus* species so far. The topology, morphology, and the staining behaviour of these hyphae are described and illustrated, their taxonomical impact is shortly discussed.

**Zusammenfassung:** In Fruchtkörpern von *Xerocomus pruinatus* konnten auffallend differenzierte Hyphen beobachtet werden: sie sind durch speziell strukturierte Verdickungen ihrer inneren Wandoberfläche charakterisiert, die sich mit Melzers Reagenz blau färbt. Dieser Hyphentyp konnte bisher nicht in anderen europäischen *Xerocomus*- bzw. *Boletellus*-Arten gefunden werden. Topologie, Morphologie und Färbeverhalten dieser Hyphen werden beschrieben und illustriert, deren taxonomische Bedeutung wird kurz diskutiert.

The taxonomy of the "*Boletus-Boletellus-Xerocomus*" complex still raises questions about the delimitation of genera and species. In recent years this task has grown even more complex: several new species, e.g., *Xerocomus persicolor* ENGEL & al. (1996), *Boletellus ripariellus* REDEUILH (1997), *B. catalaunicus* PÖDER & al. (1997), and *B. fennicus* HARMAJA (1999), have been described that are not easily separable without careful microscopical examination. Concerning the traditional *Xerocomus chrysenteron* (BULL.) QUÉL., for example, SMITH & THIERS (1971) meant: "It is doubtful if any species of bolete has been more frequently misidentified than this one." It seems that very little progress has been made to date with these problems. Therefore, we carried out comprehensive morphological-anatomical studies on this group of fungi in the past few years. One of our findings, a special type of context hyphae in *X. pruinatus* facilitates the delimitation of this species from *X. chrysenteron* and from other similar taxa.

### Material and methods

**Test methods:** Stipe tissue samples (thicker vertical hand-sections or small pieces taken near the stipe base) from fresh or dried basidiomata are soaked in Melzer's solution for five min. After repeated careful washing in chloral hydrate solution, tissue samples are microscopically examined in chloral hydrate solution. The described cell type stains from slightly greyish blue to deep violet-blue (= "amy-lon positiv" following IMLER 1950).

Micrographs were taken using a Leitz Diaplan microscope (Nomarski interference contrast) with an automatic camera system Leitz Vario Orthomath 2; film: Kodak Ektachrome 64T.

**Collections examined:** *Xerocomus pruinatus*: **Austria:** Niederösterreich, Purkersdorf, Tulbinger Kogel, under *Fagus*, *Quercus*, 12. 11. 1989, leg. W. KLOFAC, det. W. KLOFAC & I. KRISAI-GREILHUBER (WU 8523). Steiermark, Grebenzen, under *Fagus* and *Picea*, 8. 10. 1995, leg. & det. M. KIRCHMAIR & R. PÖDER (IB 1995/970); - - under *Fagus* and *Picea*, 7. 10. 1995, leg. & det. U. PEINTNER & R. PÖDER (IB 1995/971). Tirol, Sistrans, Speckweg, under *Picea*, 15. 9. 1996, leg. U. PEINTNER, det. H. LADURNER (IB 1996/1056). Kärnten, near Knappenberg, under *Picea*, 8. 10. 1998, leg. & det. R. PÖDER (IB 1998/366).

**Italy:** Prov. Trento, Vezzano, on calcareous soil under *Picea abies* (L.) KARSTEN, 17. 9. 1983, leg. R. PÖDER & B. CETTO, det. H. LADURNER (IB 1983/701); - Vetriolo, 19. 9. 1993, leg. & det. A. ZUCCHERELLI (IB 1993/107); - Val Calamanto, under *Fagus*, *Abies*, *Picea*, 20. 9. 1993, leg. & det. M. MOSER (IB 1993/198). Prov. Cuneo, Boves, under conifers, 1. 10. 1993, leg. P. CAZZOLI, det. G. SIMONINI (GS 1013). Prov. Parma, Val di Taro, Vighini, under *Castanea sativa* MILL., 6. 10. 1996, leg. & det. R. PÖDER & H. LADURNER (IB 1996/1052); - Val di Taro, Stabielle, under *Quercus* and *Castanea*, 5. 10. 1996, leg. & det. R. PÖDER & H. LADURNER (IB 1996/1055); - Val di Taro, Baselica, Casella, 17. 10. 1998, leg. U. PEINTNER & M. MOSER, det. H. LADURNER (IB 1998/365). Prov. Emilia Romagna, Civago, fondo valle, under *Castanea*, *Fagus*, *Corylus*, 24. 9. 1998, leg. & det. G. REDEUILH, R. PÖDER & H. LADURNER (IB 1998/371).

**Spain:** Prov. Girona, Cantonigrós, under *Fagus*, 11. 10. 1998, leg. Anonymous, det. R. PÖDER & H. LADURNER (IB 1998/370).

**Sweden:** Småland, Femsjö, Hylteberg, 5. 9. 1957, leg. M. MOSER, det. H. LADURNER (IB 1957/10); - - spruce forest, 24. 6. 1979, leg. M. MOSER, det. H. LADURNER (IB 1979/508).

**Type material of other species of this complex:** *Boletellus catalaunicus* PÖDER, MORENO, TABARÉS & ROCABRUNA: **Spain:** Catalonia, Riells de Montseny, among litter on sandy soil under mixed hardwoods, 6. 10. 1994, leg. A. ROCABRUNA & M. TABARÉS (IB 1994/617, holotype). *Xerocomus erubescens* CANDIÑANOS & MUÑOZ: **Spain:** Burgos, Loc. Barrasa de Mena, under *Quercus faginea* LAM. and *Erica vagans* KOCH (on calcareous soil, alt. 450 m s. m.), 14. 10. 1992, leg. J. A. MUÑOZ & J. A. CANDIÑANOS, (BAR 1656-92, holotype). *Boletellus fennicus* HARMAJA: **Finland:** EH, Lammi, Pappila, Biological Station of the University of Helsinki, in park under *Betula*, 9. 9. 1968, leg. et det. H. HARMAJA (Museum Botanicum Universitatis, Helsinki). *Boletus fraternus* PECK: **USA:** Alabama, Ala, near sidewalk to Auburn, July 1896, leg. C. H. PECK, New York Botanical Garden Nr. 5878 (holotype). *Boletellus intermedius* SMITH & THIERS: **USA:** Michigan, Livingstone, E. S. George Reserve near Pinckney, 11. 9. 1965, leg. R. HOMOLA, The University of Michigan Herbarium, (A. H. Smith 12559). *Xerocomus persicolor* ENGEL, KLOFAC & GRÜNERT: **Italy:** Verona, Garda lake, S. Vigilio, ca. 100 m s. m., in mixed shrub forest with *Ostrya*, *Pinus*, *Quercus*, 25. 9. 1994, leg. R. GRÜNERT, Fungarium GRÜNERT Nr. 1391 (holotype). *Xerocomus ripariellus* REDEUILH: **France:** Étang d'Or, under *Quercus* and *Salix*, 28. 9. 1995, leg. G. REDEUILH, Herbarium REDEUILH Nr. 22541 (holotype).

## Results

The particular hyphal compartments ("cells") were mainly found in the lower half of the stipe tissue, only exceptionally they could be observed in the context of the upper part of the stipe or in the cap. They originate at the cross-walls of inconspicuous, thin-walled, and moderately broad hyphae from where they extend more or less abruptly to strikingly thick-walled, up to 30 µm broad, irregularly-shaped cells of variable length (Fig. 1). The wall thickening is formed by material deposits on the inner wall surface of the hyphae, which, depending on their individual developmental stage, show a weak to strong amyloid reaction. In its most marked form the inner wall surface is ornamented by small, densely packed, crater-like structures (Colour fig. I). Fusions (anasto-

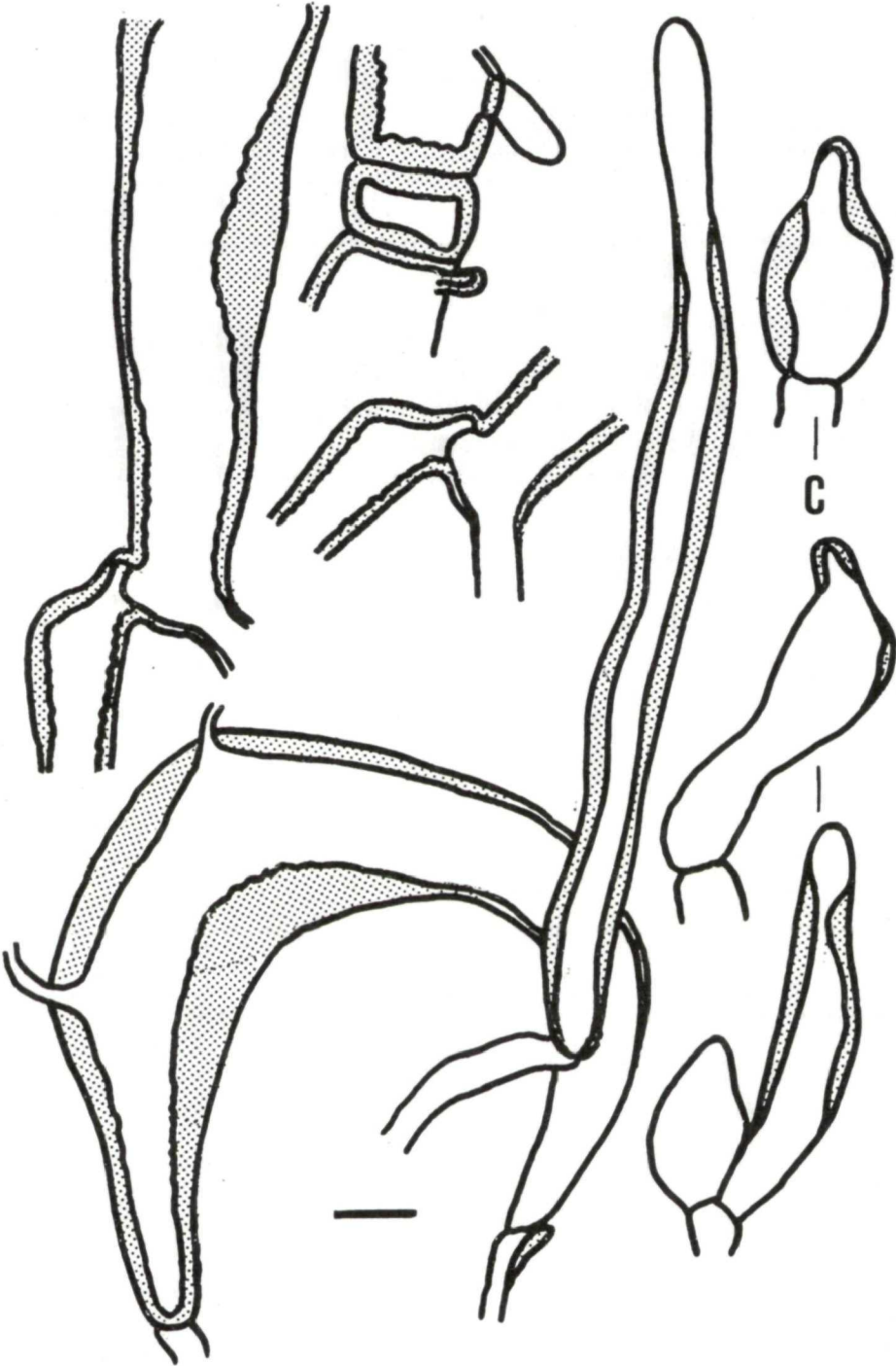


Fig. 1. Morphological variation of thick-walled, amyloid hyphal compartments in the stipe tissue of *Xerocomus pruinatus*. C Short, cystidia-like terminal elements on the lower stipe surface. Bar: 5  $\mu$ m.

moses) with ordinary, thin-walled hyphae as well as strange ramifications resembling skeleto-ligative hyphae as outlined by PEGLER (1996) were frequently observed.

The frequency of these hyphae differs in each of the 16 examined *X. pruinatus* collections which came from all over Europe but they could not be found in the following 18 European or extra-European taxa (the number of examined collections is given in parenthesis): *Xerocomus armeniacus* (QUÉL.) QUÉL. (14), *X. badius* (FR.: FR.) GILB. (1), *X. chrysenteron* (BULL.) QUÉL. (19), *X. dryophilus* (THIERS) SING. (6), *X. erubescens* CANDIÑANOS & MUÑOZ (2), *X. fennicus* (10), *X. ichnusanus* ALESSIO, GALLI & LITTINI (1), *X. moravicus* (VACEK) HERINK (4), *X. persicolor* (4), *X. porosporus* IMLER (2), *X. ripariellus* (31), *X. rubellus* QUÉL. ss. auct. mult. (39), *Boletus fraternus* PECK (1), *B. roseoalbidus* (ALESSIO & LITTINI) MORENO & al. (3), *X. subtommentosus* (L.: FR.) QUÉL. (20), *Boletellus catalaunicus* (2), *B. intermedius* SMITH & THIERS (3), and *B. zelleri* (MURR.) SING., SNELL & DICK (3). Furthermore, in the area of the stipe base terminal elements of these hyphae form noticeable cystidoid cells with amyloid, apical wall thickenings (Fig. 1).

## Discussion

Regarding the difficult species delimitation within the genus *Xerocomus* s. l. and the long-lasting discussions about the systematical position of *X. pruinatus*, the new hyphal type provides a reliable distinguishing feature: in addition to the weakly striated spores of *X. pruinatus* these hyphae constantly occur in all of its colour variants, but have not been found in any other related taxon.

In this context it should also be mentioned that our findings contradict SINGER (1965) who excluded any amyloid reaction of basidiomatal tissues within the genus *Xerocomus*.

Furthermore, none of the hyphal types described so far (for an overview see PEGLER 1996) matches our observations. From a morphological point of view the storage hyphae in sclerotia of some *Poriales*, e.g., in *Wolfiporia* and *Lentinus* spp. (CLÉMENÇON 1998), seem to be similar to some extent. Thus, a possible nutritional function of these hyphae might explain the different individual frequency of their occurrence, variations in "wall" thickness, and their varying staining behaviour (from slightly greyish blue to deeply violet-blue). Future investigations might show whether a correlation exists between different developmental stages of the basidiomata and the changing properties of these hyphae.

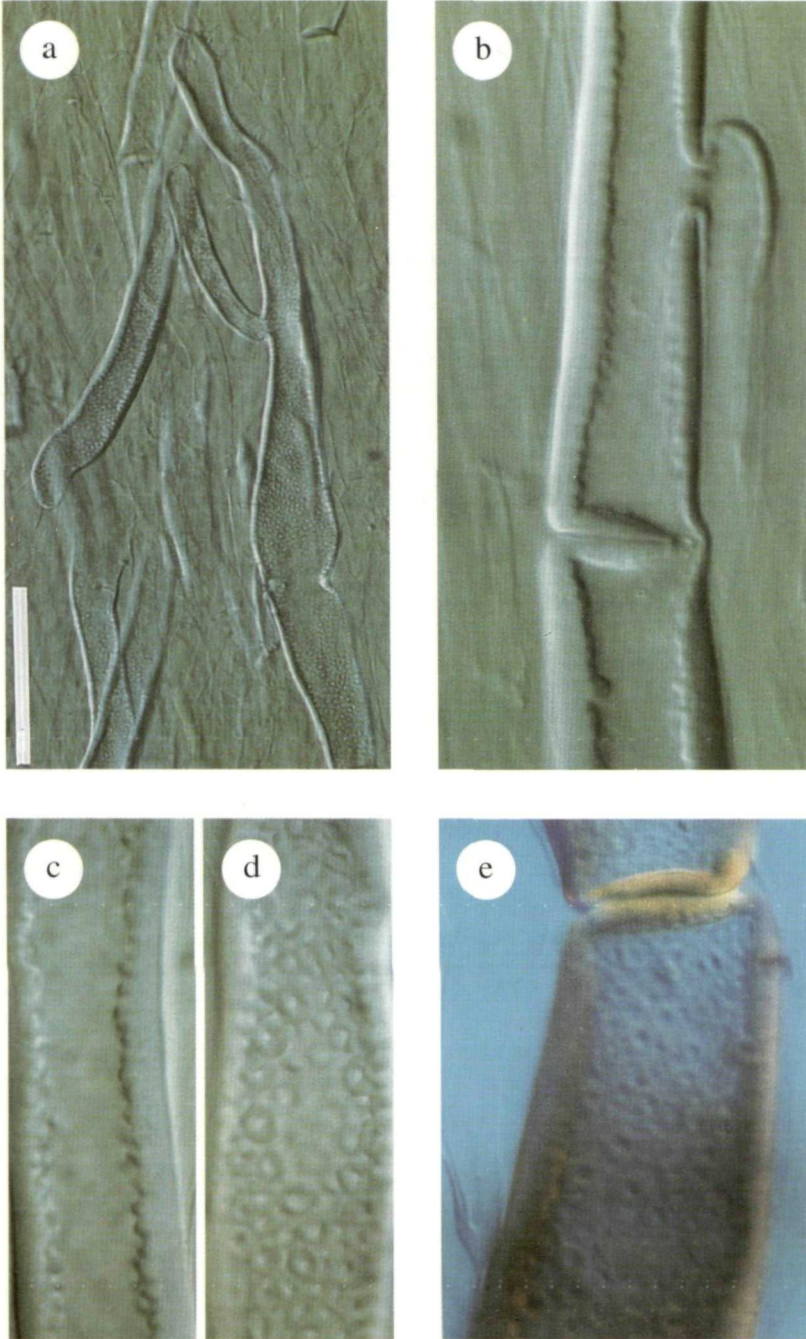
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Colour fig. 1: Micrographs of the new hyphal type in the stipe tissue of *Xerocomus pruinatus*. *a* Hyphal compartments in the stipe tissue (overview). *b* Hyphal sector with unilaterally developing double septum; the branching hypha shows a transitional state of wall thickness. *c, d* Detail of wall structure with crater-like ornamentation of the inner wall surface at different focal planes. *e* Two hyphal compartments separated by an intensively-stained double septum and well developed inner wall structure (staining: Congo red). Bar: *a* 50  $\mu\text{m}$ , *b-e* 10  $\mu\text{m}$ . – Phot. REINHOLD PÖDER.

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