"Litmus cystidia": on the strange chemical behaviour of cystidial contents in some *Suillus* species

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Abstract: The cystidia (cheilo-, pleuro-, and caulocystidia) of *Suillus placidus*, *S. subalpinus*, and of a *Suillus* species from Spain, which has not been identified so far, stain deeply blue in alcaline solutions and red in acidic media, when the tissue samples are pretreated with concentrated ammonia. Whereas many other *Suillus* species react negatively, some hyphae in the gill and stipe trama of *Gomphidius glutinosus* and *G. gracilis* stain in the same way. A description of the applied methods is followed by a discussion of the taxonomic impact of this new microchemical reaction.

Zusammenfassung: Nach Behandlung mit konzentriertem Ammoniak färben sich die Zystiden (Cheilo-, Pleuro- und Caulozystiden) von *Suillus placidus*, *S. subalpinus* und einer bisher nicht identifizierten *Suillus*-Art aus Spanien in alkalischer Lösung tief blau, in sauren Medien mehr oder weniger rot. Hyphen der Lamellen- und Stieltrama von *Gomphidius glutinosus* und *G. gracilis* zeigen dasselbe Färbeverhalten, während die Zystiden oder die Tramahyphen zahlreicher weiterer *Suillus*- und *Gomphidius*-Arten sich nicht verfärben. Das Testverfahren wird beschrieben und dessen Ergebnisse illustriert. Die taxonomische Bedeutung dieser neuen mikrochemischen Farbreaktion wird diskutiert.

In autumn 1997, at the last evening of the XI Jornadas Micológicas in Esplugues, Barcelona, Spain, R. PÖDER was attracted by a collection of three boletes which were exhibited under the name *Xerocomus rubellus* QUÉLET. The general appearence of these specimens allowed such an identification, at least in a broad sense: medium-sized basidiomata with red caps, more or less dry; tubes, pores, and stipe yellow; slightly blueing here and there. Since *X. rubellus* is still a critical taxon, R. PÖDER removed an adequately sized part from one cap for microscopical examination of its pileipellis. Back in Austria, the microscopical features of the sample turned out to be those of a *Suillus* species (small spores, fasciculate cheilo- and pleurocystidia, pileipellis with ixotrichodermal hyphae). It was even more surprising that the cystidia stained deeply blue when observed in 3% potassium hydroxide. After a few simple experiments we could show that this phenomenon was caused by the ammonia component of the "soaking medium" (see CLÉMENÇON 1986) with which we had pretreated the dried material to achieve better sections. Since new characters are still welcome in taxonomy, we started more thorough studies on the specifity and reliability of this staining reaction.

Material and methods

Test methods: Hymenophoral or tramal tissue samples (thicker sections or small pieces) from dried basidiomata are soaked in 25% ammonia solution (about pH 12.5) for one hour. After evaporation of most of the ammonia solution (on filter paper), tissue samples are microscopically examined in strongly alcaline (pH 13-14) or strongly acidic (pH 1-1.5) solutions like 3% KOH, 5% NaOH, 50% or more concentrated H_2SO_4, and 20-30% HCl (aquous solutions). In positive cases, cystidia or hyphae stain distinctly bluish in the alcaline medium and reddish in acids. No staining reactions occur when untreated samples are examined in the bases or acids, or when samples are examined directly in 25% ammonia after soaking.

Positive results could not be obtained by pretreating the samples with 25% (w/v) aqueous ammonium chloride solution (NH_4Cl) or by exposing rehydrated material to a pure nitrogen atmosphere.

Micrographs (Nomarski interference contrast; oil immersion x 100) were taken using a Leitz Diaplan microscope with an automatic camera system Leitz Vario Orthomat 2; film: Kodak Ektachrome 64T). Colour notations in quotation marks follow KORNERUP & WANSCHER (1978).

Specimens examined: *Suillus aeruginascens* (OPAT.) SNELL: **Austria**, Tirol, Achenkirch, under *Larix decidua*, 30. 9. 1991, U. PEINTNER (IB 91/770); - Tirol, Tulfes, above Windegg, under *Larix decidua* in coniferous forest, 26. 7. 1979, M. MOSER (IB 79/224); - Tirol, Hötting/Innsbruck, below Stangensteig, under *Larix decidua*, 17. 7. 1996, M. MOSER (IB 96/13). *Suillus amabilis* (PECK) SING.: **USA**, Wyoming, Fourmile Meadow, Turpin Road, Teton National Forest, under *Pseudotsuga menziesii*, 7. 8. 1989, M. MOSER (IB 89/180); - Wyoming, trail from Togwotee Lodge to South Buffalo River, Teton National Forest, under *Pseudotsuga menziesii*, 26. 7. 1997, M. MOSER (IB 97/43). *Suillus americanus* (PECK) SNELL: **USA**, Michigan, Ann Arbor, Saginaw Forestry Farm, 23. 8. 1937, A. H. SMITH (IB 37/12). *Suillus bellini* (INZENGA) WATL.: **France**, Île de Porquerolle, under *Pinus halepensis* and *Pinus pinaster*, 3. 11. 1977, M. MOSER (IB 77/281). *Suillus boudieri* (QUÉL.) WATL.: **France**, Var, Nôtre Dame de Jerusalem, Tour de la Mar, Massif de l'Esterel, under *Pinus halepensis*, 4. 11. 1980, M. MOSER (IB 80/513). *Suillus brevipes* (PECK) O. KUNTZE: **USA**, Wyoming, Teton National Park, Flaggstaff Road, under *Pinus contorta*, *Picea* sp., 9. 8. 1987, M. MOSER (IB 87/187); - Wyoming, Yellowstone National Park, Lewis Lake, eastern side , under *Pinus contorta*, *Picea* sp., 10. 8. 1983, M. MOSER (IB 83/243). *Suillus brunnescens* SMITH & THIERS: **Italy**, Parma, Bedonia, Val di Taro, Park of Seminario Vescovile, under *Pinus nigra*, 4. 11. 1994, M. MOSER (IB 94/395). *Suillus clintonianus* PECK: **Russia**, Western Siberia, Jamal district, Labytnangi, under *Picea* sp., *Betula* sp., *Alnus* sp., *Larix sibirica*, 14. 8. 1996, U. PEINTNER (IB 96/762). *Suillus caerulescens* SMITH & THIERS: **USA**, California, Mendocino Co., Little Lake Road, three miles east of Mendocino, under *Pseudotsuga* sp., 23. 11. 1991, M. MOSER (IB 91/536). *Suillus collinitus* (FR.) O. KUNTZE: **Switzerland**, Jura, north of Aargau, at the edge of forest below *Pinus silvestris*, 10. 10. 1964, M. MOSER (IB 64/199); **Austria**, Tirol, Achenkirch, under *Pinus* sp., 28. 8. 1992, U. PEINTNER (IB 92/722); **Malta**, Buskett, south of Rabatt, under *Pinus halepensis*, 3. 12. 1990, M. MOSER (IB 90/211). *Suillus flavidus* (FR.) PRESL.: **USA**, California, Mendocino Co., Little Lake Road, 1.5 miles east of Mendocino, 5. 12. 1991, M. MOSER (IB 91/672); - Wyoming, Yellowstone National Park, Lily Pad Lake, with *Pinus contorta*, M. MOSER (IB 97/109). *Suillus flavoluteus* (SNELL) SINGER: **USA**, Wyoming, Mt. Washburn, Yellowstone National Park, near timberline, under *Pinus albicaulis*, 27. 8. 1983, M. MOSER (IB 83/379). *Suillus fuscotomentosus* THIERS & SMITH: **USA**, California, Yuba Co., Bullard's Bar Recreation Area, Schoolhous Camp Garden, under *Pinus ponderosa*, 9. 12. 1991, M. MOSER (IB 91/714). *Suillus granulatus* (L.: FR.) O. KUNTZE: **Austria**, Tirol, Achenkirch, under *Pinus* sp., 27. 8. 1992, U. PEINTNER (IB 92/578); - Tirol, Achenkirch, under *Pinus* sp., 23. 8. 1991, U. PEINTNER (IB 92/659); **Malta**, Ghargur Hill near Madliena, under *Pinus halepensis*, 6. 12. 1990, M. MOSER (IB 90/231). *Suillus grevillei* (KLOTZSCH: FR.) SINGER: **Austria**, Tirol, Piller, Matzlerwald, under *Larix decidua*, 16. 9. 1988, M. MOSER (IB 88/185); - Tirol, Sellrain, Hochlagenaufforstung Haggen, under *Larix decidua*, 20. 7. 1997, H. LADURNER (IB 97/822). *Suillus grisellus* PECK: **USA**, Michigan, Proud Lake, Recreation Area Milford, under *Larix* sp., 12. 10. 1991, M. MOSER (IB 91/464). *Suillus lakei* (MURR.) SMITH & THIERS: **USA**, Washington, Lake Crescent, Olympic National Park, under spruce and fir, 4. 10. 1935, A. H. SMITH & W. H. SNELL (IB 35/06); - California, near Ponit Reyes, along road side, under *Pseudotsuga menziesii*, 21. 11. 1991, M. MOSER (IB 91/494). *Suillus leptopus* (PERS.) MARCHAND: **France**, Île de Porquerolle, west of Por-

querolle, under *Pinus halepensis*, 4. 11. 1977, M. MOSER (IB 77/289). *Suillus luteus* (L.: FR.) S. F. GRAY: **Sweden**, Småland, Bastesjö, Femsjö, under *Pinus silvestris*, 19. 8. 1976, M. MOSER (IB 76/117). **Norway**, Tromsö, Skibotndalen, west of Haskievala, east of field station, under *Pinus silvestris*, 6. 8. 1995, M. MOSER (IB 95/60). *Suillus nueschii* SING.: **Austria**, Tirol, Pitztal, above Klausboden, ca. 1500 m s. m., under *Larix* sp., 3. 8. 1966, M. MOSER (IB 66/135); - Tirol, Pitztal, Hütte im Tiefental, on meadow under larch, 24. 8. 1966, M. MOSER (IB 66/95). *Suillus pictus* (PECK) SMITH & THIERS: **USA**, Maine, Old Town, Country Road, under *Pseudotsuga*, 12. 8. 1983, M. MOSER (IB 83/251). *Suillus placidus* (BON.) SING.: **Austria**, Tirol, Sellrain, Hochlagenaufforstung Haggen, under *Pinus cembra*, 23. 8. 1997, H. LADURNER (IB 97/821). *Suillus plorans* (ROLL.) SING.: **Austria**, Tirol, Ötztal, at timberline near Poschach, in *Pinus cembra* stand, 12. 8. 1958, M. MOSER (IB 58/28). *Suillus ponderosus* SMITH & THIERS: **USA**, Oregon, Clear Creek Camp Ground, Mt. Hood Area, under *Picea engelmannii*, *Pinus contorta*, *Abies* sp., *Tsuga* sp., 15. 10. 1995, M. MOSER & J. AMMIRATI (IB 95/359). *Suillus pseudobrevipes* SMITH & THIERS: **USA**, Wyoming, Teton National Park, Snake River, SE of Signal Mountain, under *Pinus contorta*, 6. 8. 1983, M. MOSER (IB 83/207). *Suillus punctatipes* (SNELL & DICK) SMITH & THIERS: **USA**, Washington, Washington Pass, Chelan City, under *Abies lasiocarpa*, 29. 8. 1989, M. MOSER (IB 89/357). *Suillus sibiricus* SING.: **Austria**, Vorarlberg, uppermost Lechtal, SW of Zuger Älpele, ca. 1850 m s. m., 5. 10. 1990, N. GERHOLD (IB 90/186); - Tirol, Sellrain, Hochlagenaufforstung Haggen, under *Pinus cembra*, 10. 9. 1996, H. LADURNER (IB 96/889); **Switzerland**, Kanton Waadt, Vallon den Nant, Bex, under *Pinus cembra*, 29. 8. 1986, M. MOSER (IB 86/112); **USA**, Wyoming, lake in the east of Two Ocean Mountain, Shoshone National Forest, above timberline with small *Pinus albicaulis*, 3150 m s. m., 28. 8. 1997, M. MOSER (IB 97/276). *Suillus* sp.: **Spain**, Catalonia, Barcelona, exhibited at the XIX Exposicio de Bolets in Esplugues de Llobregat, finding data unknown, 19. 10. 1997, R. PÖDER (IB 97/820). *Suillus spectabilis* (PECK) O. KUNTZE: **USA**, Michigan, Pickerol Lake, Pinekney Recreation Area, Washtenaw Co., under *Larix laricina* on swampy ground, 21. 10. 1991, M. MOSER (IB 91/476). *Suillus subalpinus* MOS.: **USA**, Wyoming, just below Amphitheatre Lake, Teton National Park, under *Pinus albicaulis*, 20. 8. 1989, M. MOSER (IB 89/293); - Wyoming, lake in the east of Two Ocean Mountain, Shoshone National Forest, under *Pinus albicaulis*, *Picea* sp., 7. 8. 1997, M. MOSER (IB 97/130). *Suillus tomentosus* (KAUFFM.) SING., SNELL & DICK: **USA**, Michigan, Marquette County, Michigan State Forest, Jack Pine Flats, 19. 8. 1972, S. J. MAZZER (IB 72/450). *Suillus tridentinus* (BRES.) SING.: **Austria**, Tirol, Achenkirch, near *Larix decidua*, 27. 8. 1992, U. PEINTNER (IB 92/565). *Suillus variegans* (SWARTZ: FR.) O. KUNTZE: **Austria**, Kärnten, Sattnitz, Göltschach, under *Pinus silvestris*, 23. 9. 1986, M. MOSER (IB 86/271); - Tirol, Gnadenwald, under *Pinus silvestris*, 12. 9. 1988, M. MOSER (IB 88/168); - Tirol, Achenkirch, 29. 7. 1992, U. PEINTNER (IB 92/729). *Gomphidius glutinosus* (SCHFF.) FR.: **Austria**, Tirol, Achenkirch, Christlum, coniferous forest, 28. 8. 1992, U. PEINTNER (IB 92/632). *Gomphidius gracilis* BERK.: **Italy**, Südtirol, Gadertal, Störes-Wald S of St. Kassian, under *Larix decidua*, *Picea abies*, on dolomitic soil, 6. 9. 1996, M. MOSER (IB 96/84); **Austria**, Tirol, Imst, Linserhof, under *Larix decidua*, 20. 7. 1986, J. THIEN & M. MOSER (IB 86/53); - Tirol, Matrei, under *Larix* sp., 18. 10. 1990, M. MOSER (IB 90/196); - Tirol, Nauders, 1500 m s. m., borderline Kastel-Wald and Martanneswiesen, 31. 8. 1988, N. GERHOLD (IB 88/500). *Gomphidius maculatus* (SCOP.) FR.: **Austria**, Tirol, between Steinach and Trins at south side of valley, under *Larix decidua*, 14. 7. 1981, M. MOSER (IB 81/91). *Gomphidius roseus* (FR.) FR.: **Sweden**, Femsjö, wood near Flahult, under *Pinus silvestris* together with *Suillus bovinus*, 13. 9. 1994, M. MOSER (IB 94/249); **Italy**, Südtirol, Antholzer Biotop, under *Pinus* sp., 7. 9. 1996, U. PEINTNER (IB 96/689). *Gomphidius subroseus* KAUFFM.: **USA**, Washington, Callam Co., Olympic Hot Springs, Olympic National Park, under *Pseudotsuga*, 3. 10. 1995, M. MOSER (IB 95/226); - California, near Point Reyes, along road side, under *Pseudotsuga*, 21. 11. 1991, M. MOSER (IB 91/496).

Results

Best staining results are achieved when dried tissue samples are soaked in 25% ammonia solution for one hour, and 3% KOH or 50% H_2SO_4 is subsequently used for microscopical examination. In those *Suillus* species which react positively both the content and the secretion of cheilocystidia, pleurocystidia, and caulocystidia, some-

times also parts of their basal hyphae, stain "vivid blue", "ultramarine", "deep blue", "dark turquoise", or blackish olive when observed in 3% KOH. In sulphuric acid, the same structures turn red, as when stained with Congo red, or at least distinctly "brownish red" (Colour fig. III). Even in the latter case the colour clearly differs from that of untreated cells. The same chemical reaction can also be observed in a few basidia and basidiospores. Such basidia are characterized by brownish, intracellular pigments before staining; they are exclusively found as integrated elements of the cystidia fascicles. Very likely, the spores reacting positively originate from those basidia.

Pretreated cells of non-staining species as well as all untreated cells become darker in 3% KOH whereas intensity and shades of their final colouration vary depending on the quantity of pigments originally formed by the individual cells: their content is ochraceous, greyish brown, reddish brown, sometimes dark chocolate brown. However, bluish or greenish tints were never observed.

The same microchemical behavior was found in two of five *Gomphidius* species. In contrast to the three *Suillus* species, only the context hyphae (incl. hyphae of the gill trama) stain positively: in *Gomphidius gracilis* nearly all hyphae of the context stain blue whereas in *G. glutinosus* a greater number of hyphae remains unstained. *Gomphidius maculatus*, *G. roseus*, and *G. subroseus* do not show any reaction.

Discussion

The terms "litmus cystidia" used in the title should roughly paraphrase the observed colour changes which resemble those of the classic pH-indicator "litmus". Although we cannot explain the chemical mechanism of the described reaction, our experiments indicate that a pretreatement of fungal tissue with NH_3 is essential for positive staining results. In this context, we found a very interesting footnote in SINGER (1970) referring to *Suillus amabilis*, one of the species which react negatively in our tests: "In some of the numerous sections we have made, the sepia-brown pigment of the base of the cystidia fascicles dissolved after having been previously treated with NH_4OH 5%, dissolving to a deep blue pigment which dyed the entire hyaline contents of both spores and cystidia. Since this "reaction" cannot be repeated at will, it may be due to unknown factors or even substances foreign to the fungus itself." Moreover, according to BESL & BRESINSKY (1997) "...the characteristic encrustations on the fasciculated cystidia which are coloured by KOH..." are formed by substances called "lipophilic pigments" (prenylated phenols and quinones including their oxidation products; such pigments were found in 26 of 37 species studied). These "lipophilic pigments" are considered by the above mentioned authors "...to be indicator substances for the genus *Suillus* (and for the *Gomphidiaceae/Rhizopogonaceae*)." However, 19 *Suillus* species with "lipophilic pigments" tested by us did not show any positive reaction while *S. placidus* - it contains these compounds also - reacted clearly positive. Consequently, we have to assume that the staining reaction is based either on a special type of "lipophilic pigments" not produced by many *Suillus* species, or on other compounds. Nevertheless, the occurrence of the same staining phenomenon in *Suillus* and in *Gomphidius* provides further evidence for their close relationship. Addressing the latter issue, we have to point to the following taxa recently introduced (for contrasting views see ARPIN & KÜHNER 1977 and HØILAND 1987): *Suillaceae* (SINGER) BESL &

BRESINSKY including the genera *Boletinus*, *Suillus*, and *Gastrosuillus*; *Boletales* subordo *Suillineae* BESL & BRESINSKY including the families *Gomphidiaceae*, *Suillaceae*, and *Rhizopogonaceae*. This concept is mainly based on a careful revision of chemotaxonomical and molecular data (DNA analyses), taking into consideration different morphological levels of organization (types of hymenophores) as well (BESL & BRESINSKY 1997). The taxonomical view of BESL & BRESINSKY (1997) is well-founded, although we find it difficult to follow their argumentation regarding a "secondary regressive metamorphosis of tubulate to a lamellate hymenophore", which sometimes could have occurred within the *Boletales* (e.g. within the *Paxillaceae*), whereas the members of the genera *Gomphidius* and *Chroogomphus* could represent "primarily lamellate fungi". In our opinion, the current body of evidence suggests that reliable reconstructions of evolutionary pathways on the morphological level are still an open and promising field of research not only for mycological tasks.

With regard to delimitation problems on the species level, the occurrence of "litmus cystidia" (or "litmus hyphae" in the genus *Gomphidius*) might represent a relevant taxonomic character. While the European, ivory coloured *S. placidus* with its typical habitat under *Pinus cembra* barely can be mistaken, the identification of some North American *Suillus* species seems somewhat more difficult. *Suillus subalpinus*, for example, could be confused with older basidiomata of *S. brunnescens* or with *S. albidipes* (PECK) SINGER (MOSER 1997). The cystidia of a critical *S. brunnescens* collection from Italy discussed by MOSER (1997) reacted negatively in our test; *S. albidipes* has not been examined. Unfortunately, no name can be given to the positively reacting, "rubelloid" Spanish *Suillus* collection mentioned in the introduction. Very likely, it represents an undescribed species, not only because we have not been able to identify it, but also because our Spanish and Italian colleagues do not know a similar species.

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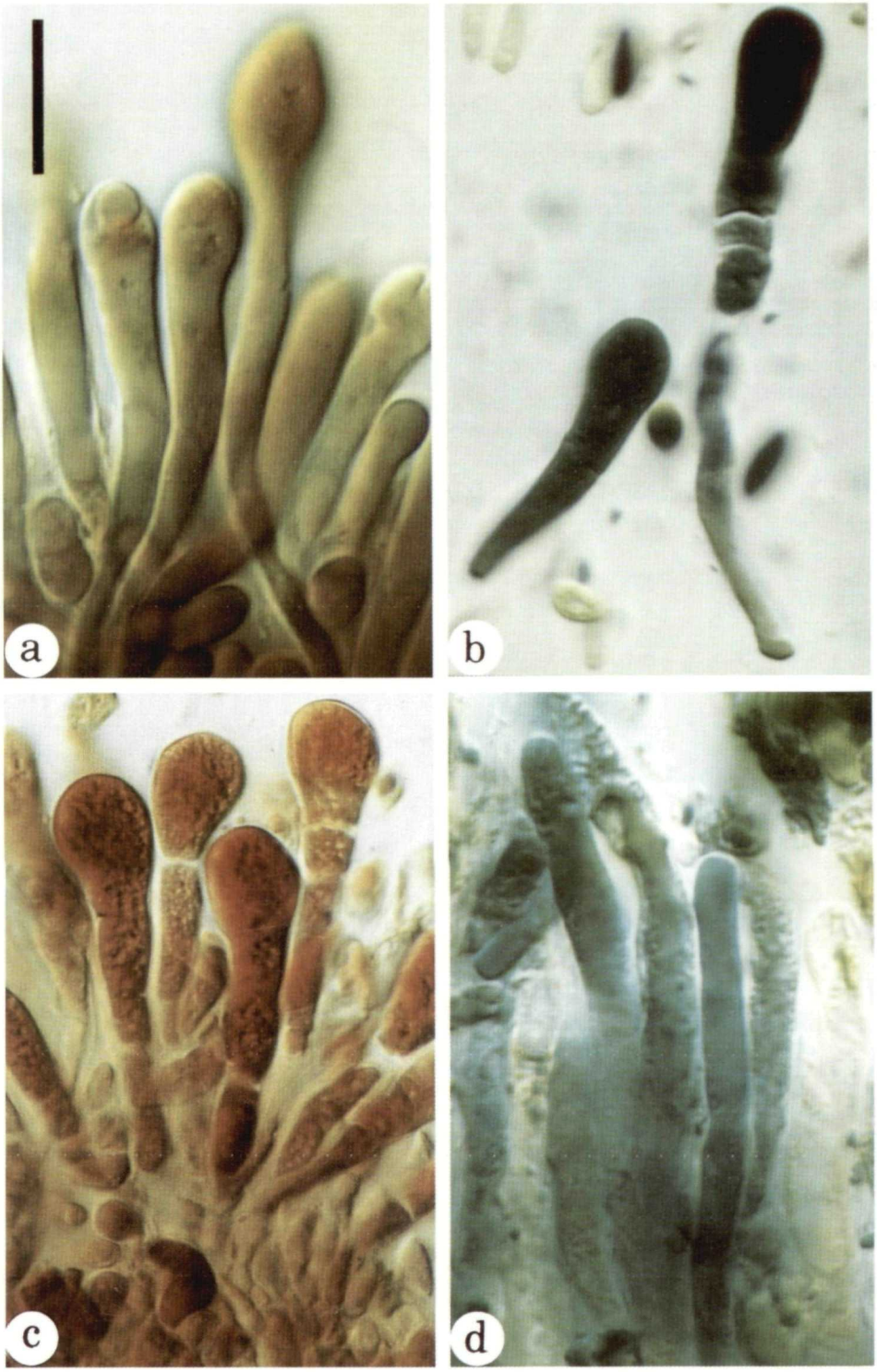

Colour fig. III. a-c Pleurocystidia of *Suillus placidus*; a in 3% KOH without pretreatment, b in 3% KOH after pretreatment with 25% ammonia, c pretreated sample in 25% HCl. d Pretreated pleurocystidia of an unidentified *Suillus* species from Spain in 3% KOH. Bar: 20 µm. - Phot. REINHOLD PÖDER.