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AMANITA EBURNEA—A NEW SPECIES FROM CENTRAL AMERICA

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Summary

Amanita eburnea is described as new from pine forests of Honduras and Belize.

Amanita eburnea Tulloss sp. nov. Holotypus: HONDURAS, Siguatepeque, vi.1976 M. H. Ivory s/63 (K).[1]

Etymology: *eburneus*, ivory white.

Pileus albus vel fumeus, 70 - 90 mm in mensura diametrica, primo globosus, sed deinde qui fit late campanulatus vel plano-convexus, margine nonstriata, nonappendiculata; materies volvica absentes. Lamellae condensae, albae, liberae. Stipes 45 - 110 × 10 - 18 *mm, albus. Volva membranacea, alba vel subflavida, vel* 45 *mm alta. Basidiae tetrasterigmaticae; fibulae absentes. Sporae* (7.0-) 8.0 - 11.0 (-12.0) × (4.8-) 5.2 - 6.5 (-7.5) µm, *ellipsoidae vel elongatae, amyloideae.*

Amanita eburnea (Fig. 1) is a member of section *Phalloideae.* It is a medium-sized white to smoke gray mushroom with a smooth dry pileus surface and an annulate stipe. It has a membranous universal veil forming several limbs on the rounded to slightly pointed stipe base; the limbs are free for about one third of their height. Microscopic characters serving to distinguish the species are ellipsoid to elongate spores with an overall average Q of 1.58; a ramose subhymenium containing many uninflated, short hyphal elements; and rather plentiful inflated cells in the partial veil. This entity is known from pine forests in Belize and Honduras.

PILEUS: 70 - 90 mm diam, white to smoke gray, dry, globose at first, then broadly campanulate, then planoconvex to planar; margin nonappendiculate, nonstriate;

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1. DTJ - private herbarium of Dr. D. T. Jenkins, University of Alabama, Birmingham, U.S.A.
IVORY - private herbarium of Dr. M. H. Ivory, Oxford Forestry Institute, University of Oxford, U.K.
K - Herbarium, Kew Botanic Gardens, U.K.

Fig. 1 *Amanita eburnea*. M. H. Ivory s/63 [×1.2].

pileipellis "papery" (Ivory); context white, firm to spongy. LAMELLAE: white, free, drying brown (6D6 or 7.5YR 5/8)[2], up to 6 mm broad, margin at times buff[3] and remaining pallid in exsiccatae. STIPE: 45 - 110 × 10 - 18 mm, white, cylindric, decorated with longitudinal striations; context white, firm to spongy, stuffed to solid; annulus white to buff, superior to subapical, membranous to submembranous, persistent or left in patches over gills or collapsing and sliding down stipe; base swollen to slender bulb, rounded or pointed below, 25± mm long; universal veil membranous, white to buff, appressed to base, limbate in several free lobes reaching to 45± mm from base of bulb, with limb free for up to 15± mm. One specimen (s/111) which was old enough to have lost its annulus, had a "moderately unpleasant smell" (Ivory).

PILEIPELLIS: filamentous undifferentiated branching hyphae 1.5 - 19.0 µm diam, gelatinizing just at surface, interwoven, not radially arranged; oleiferous hyphae 3.0 - 13.3 µm diam, with walls of the broadest thickened slightly up to 0.7 µm. PILEUS CONTEXT: tangled undifferentiated filamentous branching hyphae 1.8 - 15.0 µm diam; inflated cells ovoid, subpyriform, subglobose, plentiful, to 107 × 57 µm; branching oleiferous hyphae 1.0 - 7.5 µm diam. LAMELLA TRAMA: bilateral; frequently branching tangled filamentous undifferentiated hyphae 2.4 - 7.2 µm diam, dominating, occasionally with slightly inflated terminal segment (e.g. 56 × 10 µm) at 30°-40° to central stratum; inflated cells to 32 × 17 µm, thin-walled, intercalary, clavate to ovoid to ellipsoid; branching oleiferous hyphae present, relatively common, 2.2 - 7.0 µm diam. SUBHYMENIUM: branching chains of uninflated narrow short hyphal segments to slightly inflated short hyphal segments and ovoid to subglobose inflated cells (to 18 × 12.5 µm); basidia arising from cells of all types. BASIDIA: 37.5 - 46 × 9.7 - 11.0 µm, 4-spored, thin-walled; clamps not seen. UNIVERSAL VEIL: On the stipe base at the exterior surface: a thin layer of loosely interwoven undifferentiated filamentous branching hyphae largely without sublongitudinal orientation, 1.8 - 5.8 µm diam, at times in fascicles; some inflated terminal cells subclavate to clavate to ventricose, to 166 × 34 µm, all below the surface. On the stipe base below the surface: filamentous undifferentiated branching hyphae plentiful, 1.8 - 11.5 µm; inflated cells plentiful, ellipsoid to ovoid to subglobose (to 85 × 61 µm) and clavate (to 95 × 31 µm), occasionally with walls thickened up to 1.0 µm; oleiferous hyphae present 4.5 - 4.8 µm. On the stipe base, inner surface: extensively gelatinized, all structures similar to interior; in some regions there is a thin layer of sublongitudinally oriented hyphae. STIPE CONTEXT: acrophysalidic; branching filamentous undifferentiated hyphae 4.2 - 10.5 µm diam; oleiferous hyphae 2.8 - 7.7 µm diam; acrophysalides dominant to 315 × 64.5 µm; clamps not seen. PARTIAL VEIL: dominated by terminal inflated cells, difficult to reinflate, subglobose to ovoid (to 45 × 43 µm), subclavate to pyriform (to 82.5 × 25 µm, most less than 50 µm long) with walls infrequently thickened to 1.0 µm;

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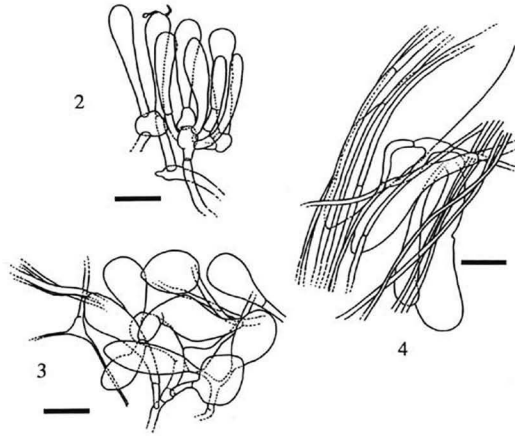
2. Color codes of the form "6D6" are from (Kornerup & Wanscher, 1978). Color codes of the form "7.5YR 5/8" are from (Munsell Color, 1975).

3. The color "buff" is mentioned for several parts of the fruiting body in the collector's notes. Dr. Ivory informs me (pers. corresp.) that this name was taken from Rayner (1970) in which it is noted as equivalent to the Munsell notation 1.2Y/8.1/4.1 and the ISCC-NBS name "pale yellow".

filamentous undifferentiated branching hyphae 1.5 - 7.8 µm diam, mostly quite narrow, collapsing; oleiferous hyphae present, 4.0 - 10.0 µm diam. All tissues pale yellow in 2% and 5% KOH and 10% NH_4OH.

BASIDIOSPORES: [108 from 5 specimens] (7.0-) 8.0 - 11.0 (-12.0) × (4.8-) 5.2 - 6.5 (-7.5) µm, (average length (per specimen) = 9.0 - 9.8 µm; overall average length = 9.3 µm; average width (per specimen) = 5.7 - 6.0 µm; overall average width = 5.9 µm; Q = (1.29-) 1.34 - 1.84 (-2.08); average Q (per specimen) = 1.50 - 1.66; overall average Q = 1.58), amyloid, thin-walled, hyaline, ellipsoid to elongate, rarely broadly ellipsoid or cylindrical, sometimes swollen at one end, often slightly adaxially flattened; contents guttulate; apiculus sublateral, cylindrical; white in deposit.

Distribution and habitat: Solitary to gregarious in troops under *Pinus oocarpa* Schiede & Deppe in grass on well-drained loamy soil and *P. caribaea* Mor. in bushy forest over poorly drained sandy soil, May to October, at elevations between 500 and 1100 m, in Honduras and Belize.



Figs. 2-4 *Amanita eburnea*. 2. Elements of hymenium and subhymenium (M. H. Ivory s/63). 3. Elements of partial veil (M. H. Ivory s/46b). 4. Elements of universal veil from free portion of limb near exterior surface (M. H. Ivory s/63). The bars in Figs. 2-4 represent 20 µm.

Collections examined: **BELIZE**: Augustine Forest Station, vi.1976 M. H. Ivory s/85 (K), vi.1976 M. H. Ivory s/93 (K, portion in IVORY), vi.1976 M. H. Ivory s/111 (K, portion in IVORY). **HONDURAS**: Siguatepeque, v.1976 M. H. Ivory s/46b (K, portion in IVORY), vi.1976 M. H. Ivory s/63 (holotype K, isotype with faded color photograph in IVORY).

DISCUSSION

The amyloid spores, nonappendiculate pileus margin and membranous volva on an expanded stipe base require *A. eburnea* to be placed in section *Phalloideae* (Bas, 1969). Five white taxa of section *Phalloideae* described from North America have spores of approximately the size or shape of those from *A. eburnea* (Jenkins, 1986). The group with similar values of overall average Q include the following (values in parentheses obtained from (Jenkins, 1986)):

— *Amanita parviformis* (Murrill) Murrill (1.40) - a small mushroom with pileus about 25 mm diam described from Florida (U.S.A.), differing from *A. eburnea* by having few inflated cells in its universal veil tissue, having an annulus composed exclusively of hyphae, and having spores 7.8 - 9.4 × 5.5 - 6.3 µm (based on study of the type by Jenkins (1979));

— *Amanita gwyniana* Coker (1.45) - a small mushroom with pileus up to 40 mm diam described from North Carolina (U.S.A.). differing from *A. eburnea* by having markedly rounded ends to the lamellae at the pileus margin, having a distinct odor of chloride of lime, and having spores 9.2 - 11.0 × 6.5 - 7.4 µm (based on the protologue (Coker, 1927));

— *Amanita hygroscopica* Coker (1.47) - described from North Carolina (U.S.A.), differing from *A. eburnea* by having lamellae that become pinkish with maturity, having a subhygrophanous pileus, having an annulus that is subsuperior to submedian (Coker, 1917: pl. 17 and 18), and having spores 9.0 - 12.2 × 6.2 - 8.1 µm (Jenkins (1986) states that the characters of lamellae and pileus here cited for *A. hygroscopica* are quite distinctive in *Amanita*);

— *Amanita magnivelaris* Peck (1.47) - described from Long Island, New York (U.S.A.), differing from *A. eburnea* by having no inflated cells in its partial veil, having few inflated cells in the universal veil at the stipe base, and having spores 8.6 - 10.9 × 5.5 - 7.8 µm (based on study of the type by Jenkins (1978));

— *Amanita elliptosperma* Atkinson (1.49) - described from North Carolina (U.S.A.), differing from *A. eburnea* by its having few inflated cells in its partial veil, having sparse inflated cells in its universal veil, and having spores 9.37 - 10.15 × 6.25 - 8.59 µm (based on study of the type by Jenkins (1982)).

Amanita magnivelaris has been reported from Mexico by Singer (1958). White species of section *Phalloideae* having globose to broadly ellipsoid spores which are also broader than those of *A. eburnea* (*A. bisporigera* Atkinson, *A. verna* (Bull. : Fr.) Roques, and *A. virosa* ss. auct. amer.) have also been reported from that country by several authors including Pérez-Silva *et al.* (1970). Pegler (1983) reports no records of any species in section *Phalloideae* in the Lesser Antilles. There is no white species of section *Phalloideae* reported for any locality in South America (Garrido & Bresinsky,

1985 and Raithelhuber, 1986).

The whitish pileus, habit, microscopic structure of the annulus and the overall average Q of the spores of *A. eburnea* are suggestive of the Mediterranean taxa *A. ovoidea* (Bull. : Fr.) Link, *A. ovoidea* var. *proxima* (Dumée) Bon & Courtecin, and *A. aminoalifatica* Filippi.[4] These entities are considered to form a closely related group in section *Amidella* (Gilbert, 1940 & 1941; Bas, 1969; Kühner & Romagnesi, 1974; Filippi, 1985). A distinguishing character of section *Amidella* is an appendiculate pileus margin which is absent in *A. eburnea*.

The collection M. H. Ivory s/244 (HONDURAS, Siguatepeque, x.1976) is believed by Dr. Ivory (pers. corresp.) to be conspecific with the collections of *A. eburnea* herein cited. Portions of Ivory s/244 were deposited in both K and DTJ. Neither portion can be located at present despite repeated searches.

ACKNOWLEDGMENTS

I am very grateful to all of the following: Dr. C. Bas, Rijksherbarium, Leiden, The Netherlands, for his comments on this article and for providing working space in his laboratory for the study of collections of *A. ovoidea*; Sig. Ilario Filippi, Florence, Italy, who provided the loan of co-type and paratype material of *A. aminoalifatica* and allowed me to retain some parts of these collections as well as a collection of *A. ovoidea* and several color transparencies; Dr. M. H. Ivory, Oxford Forestry Institute, University of Oxford, U. K., for his informative correspondence, for a gift of photographs, for providing me with a copy of his field notes and a loan of specimens, and for reviewing drafts of this article; Dr. David T. Jenkins, Department of Biology, University of Alabama in Birmingham, for his reviewing this article; Ms. Mary A. King, Roosevelt, New Jersey, for assistance in final preparation of the manuscript for publication; Mr. Neal Macdonald, Princeton, New Jersey, for preparing the illustrations; Dr. David N. Pegler, Herbarium, Royal Botanic Gardens, Kew, U. K., who originally put me in contact with Dr. Ivory, hosted me at K, located a number of collections, and provided work space and facilities for examination of material at K.

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4. An additional taxon possibly belonging to this group is known to me only from its protologue: *Amanita ovoidea* var. *amnophila* Beeli (1930: 129). The spore dimensions given by Beeli suggest a much thinner spore than is found in *A. ovoidea*: 7 - 10 × 4 - 5 µm. It appears average Q = 1.9±. No type is designated; but a collection made in September in Genck, Belgium is cited.

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LECANORA SECT. PETRASTERION (LICHENIZED ASCOMYCOTINA) IN NORTH AMERICA: LECANORA WEBERI RYAN, SP. NOV. (SUBSECT. PSEUDOCORTICATAE), FROM COLORADO

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ABSTRACT

A new crustose-squamulose lichen, Lecanora weberi **Ryan, sp. nov.**, is described from rocks in the vicinity of Boulder, Colorado. It is treated here under sect. Petrasterion subsect. Pseudocorticatae Poelt, and is probably closely related to L. novomexicana Magnusson, but is distinguished especially by its short, rather flattened and squamule-like lobes, which are grayish tinged and have a very thin upper cortex. It is also similar to forms of Rhizoplaca melanophthalma (DC.) Leuck. & Poelt sensu lato, but lacks a distinct umbilicus and lower cortex, and has adnate apothecia with orangish to yellowish brown discs and scarcely prominent margins.

This paper describes a new crustose-squamulose species of Lecanora, from Colorado. Although the new species resembles forms of both Lecanora novomexicana Magnusson sensu lato and Rhizoplaca melanophthalma (DC.) Leuck. & Poelt sensu lato, it does not fit well within either, and retains its identity when growing side by side with them. The type material has already been distributed (as "Lecanora sp. nov. Ryan") in the exsiccati series from the University of Colorado (COLO), and a diagnosis and description of the new species are given now. Until the relationship between the genera Lecanora Ach. emend. Massal. and Rhizoplaca Zopf can be clarified by further investigations, the supraspecific classification of the several apparently intermediate taxa (including L. nigromarginata Magnusson and L. opiniconensis Brodo, as well as the new species described below) is uncertain. For the present, taxa such as these, which lack a distinctly umbilicate-foliose thallus and well-developed lower cortex, appear to be best placed within Lecanora subg. Placodium (Pers. emend. Poelt) Poelt. The new species described here is treated under sect. Petrasterion subsect. Pseudocorticatae Poelt.

Unless noted otherwise, the methods and terminology used in this paper are as described by Ryan (1989a,b).

Colors (followed by numbers in parentheses), as viewed through a dissecting scope with fiber optic lighting, refer to the system of Kelly (1965). Chemical analyses were made by the standard thin-layer chromatographic (TLC) method of Culberson (1972), modified as described by Ryan (1989a).

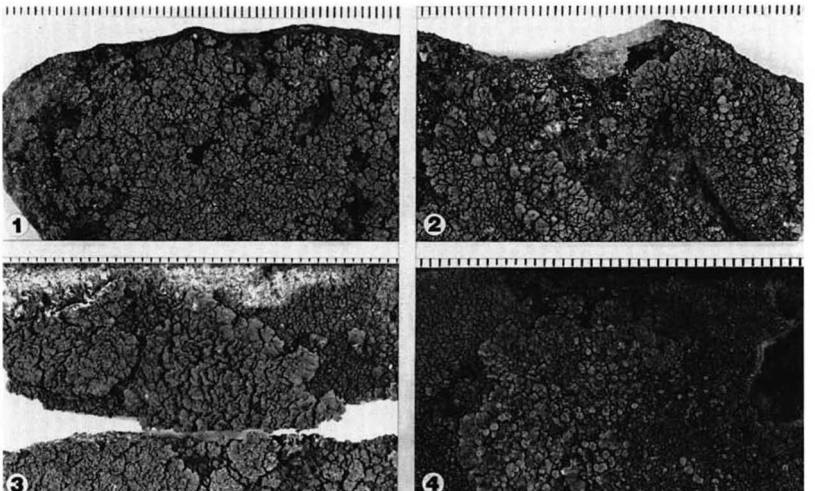
Lecanora weberi Ryan, sp. nov. (Fig. 1, 2)

In rubipus siliceis crescens, Colorado. Thallus areolatus ad squamosus, squamulis marginalibus eis centri subsimilibus vel nonnihil elongatis et lobiformibus, olivaceo-griseis, marginibus pallide flavovirescentibus instructis, non vel leviter pruinosis. Apothecia adnata ad sessilia, discis aurantiacis ad flavovirescentibus pruina flavoalbida tectis, margines leviter prominentes, continui ad crenati. Cortex superior valde tenuis, cellulas algarum emortuas includens; fasciculi hypharum subcorticales desunt vel non evoluti; cortex granulis flavescentibus inspersus; cortex inferior deest vel indistincte evolutus. Paraphyses subconglutinatae, apicibus clavatis; sporeae octonae, ellipsoidae, ca. 8-11 x 4-6 µm. Spermatia filiformia, ± curvata. Continet acidum usnicum in cortice, acida pingua (acida allo-pertusaricum, et al.) in medulla.

TYPE: U. S. A.: COLORADO: Boulder Co.: Boulder Mountain Parks, just S of city of Boulder, 1850 m, on scattered low fine-grained sandstone or quartzite boulders at base of Flatiron Mountains, in open stands of Pinus ponderosa, 10 Aug. 1986, W. Weber, COLO Exs. 685, **Holotype** (COLO!), **Isotype** (ASU!).

THALLUS areolate-squamulose, indistinctly radiating, closely appressed, not easily removed intact; areoles scattered to contiguous or weakly imbricated (fertile thalli) or becoming densely imbricated (sterile thalli); **lobes** similar to central areoles or somewhat larger and more lobe-like or squamule-like, isodiametric to moderately elongated, 1-2 mm long, 0.5-1.5 mm wide, simple or slightly branched, often strongly crenate, partly overlapping each other, flat to concave or undulate, 0.2-0.3 mm thick, appressed to slightly ascending, broadly adnate at least on side toward thallus center, often becoming free on side toward thallus margin; **thallus center** areolate-verrucose or partly areolate-squamulose in fertile thalli; areoles flat to slightly convex or undulating, with more-or-less

Figures 1-4. Lecanora weberi Ryan and similar taxa. Scale = mm. -1. Part of the holotype of L. weberi (COLO Exs. 685, COLO). -2. Part of a collection of L. weberi (Shushan and Weber S-3218, M), showing fertile thalli with verrucose areoles (upper right) and sterile thalli with imbricate squamules (below). -3. A specimen of Lecanora novomexicana Magnusson from the type locality of L. weberi (Weber, s.n., COLO). -4. Subcrustose form of Rhizoplaca melanophthalma (DC.) Leuck. & Poelt sensu lato from the type locality of L. weberi (Weber, s.n., COLO).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downward-turning margins, irregular in outline, to 1(-2) mm wide, sometimes rimosely divided into secondary areoles, 0.2-0.5(1.5) mm thick, thinner toward edge, becoming crenate, usually narrowed below, broadly attached to substrate centrally or more towards one side, by about one fourth to one half the lower side, the margins often becoming free from substrate (especially in sterile thalli); **color above** in fertile thalli when fresh usually with distinctly grayish green tone, light grayish olive (109) to light olive gray (112), paler and more grayish towards thallus margin, with the squamules edges mostly pale yellowish green (121) to pale greenish yellow (104), in older herbarium specimens fertile thalli grayish yellowish green (122) to grayish greenish (105), partly with orangish yellow tinges, and sterile thalli grayish yellow (90) to moderate yellow (87); **upper surface** mostly epruinose, more-or-less matt; squamule edges often somewhat roughened, with patches of whitish pruina; **color below** pale, more-or-less moderate yellowish brown (77), darker brown near attachment area; **upper cortex** with few to many dead algal cells, very thin, 5-10(-15)µm, even, without conelike hyphal bundles, mostly inspersed with fine yellowish granules (soluble in K); **algae** trebouxioid; algal layer 30-50(-75) µm thick, continuous to interrupted; **medulla** moderately loose, partly filled with clumps of gray granules; hyphae 3-5 µm diam.; **lower cortex** absent, or (on ascending squamules) 12-15 µm thick, often poorly developed, broken and penetrated by medullary hyphae; hyphae ca. 5 µm diam., lumina 2 µm.

APOTHECIA usually numerous and becoming crowded (but sometimes absent or rare), to three per areole, 1-1.3 mm diam., borne submarginally to laminally on the squamules, adnate to broadly sessile; **disc** often slightly concave when young, becoming plane to undulate when old, usually densely whitish-yellowish pruinose, but often epruinose when young, grayish yellow (90) to yellowish gray (93) or sometimes almost pale greenish yellow (104) with pruina, light to deep orange (51-52) to moderate yellowish brown (77) under pruina, often darker and more olivaceous when young; **margin** about 0.1-0.2 mm thick, soon irregularly with irregularly foveolate surface, entire or somewhat crenate, becoming flexuous or distorted in age, sometimes distinct and more-or-less raised from the start, persistent, often somewhat paler than thallus and weakly pruinose; **hymenium** hyaline, 50-65 µm high; epihymenium brownish, weakly inspersed with fine granules (partly soluble in K) and covered by 15-20 µm thick superficial layer of coarse granules (soluble in K); **paraphyses** distinct and more-or-less free in water, about 1.5-2 µm thick below, the tips 2-2.5 µm thick, colorless; **subhymenium** grayish, with oil droplets; **excipulum** hyphae gelatinized but somewhat distinct in water, densely packed, irregularly oriented, ca. 3-5(-7) µm diam., the lumina more-or-less short, 1 µm wide; **parathecium** hyphae parallel; **hypothecium** distinct from subhymenium, hyaline to slightly yellowish-brownish, to 300 µm thick in center; **amphithecium** similar in structure to thallus but **cortex** to 30-50 µm

thick in lower part; algal layer about 50 µm thick below hypothecium, continuous to interrupted; algae filling most of the margin; asci 35-40 x 10-12 µm, Lecanora-type; spores ellipsoid to oblong-ellipsoid (L:W = 1.8-2.4:1), about 8-11(-13) x (3-)4-6(-7) µm, with one or two oil droplets, 8 per ascus, the wall about 0.5 µm thick.

SPERMOGONIA immersed, the ostiole pale, the cavity ca. 100-150 um diam.; **spermatia** curved, (15-)20-25 µm long.

SPOT TESTS AND CHEMISTRY: Thallus and apothecia C-, Pd-; **cortex** K- or + yellowish; KC+ yellowish; with usnic acid (often only traces); **medulla** K-, KC-, I_2KI-; with fatty acids of the protolichesterinic group (allo-pertusaric and dihydropertusaric, constipatic, protoconstipatic and dehydroconstipatic acids), and sometimes traces of unknowns; **discs** K-, KC-. A specimen was analyzed by C. Leuckert (Shushan & Weber S-3218, COLO).

DISTRIBUTION AND ECOLOGY: Montane areas of western U. S. A. (Colorado, to Idaho and S. Dakota), on siliceous rocks (sandstone, conglomerate, quartzite, arkosite), usually in moderately shaded areas, on horizontal to sloping faces near tops of boulders or outcrops, in open forest, sometimes in canyons, 1050-1875 m; often very abundant, covering whole level surfaces of boulders; **associated organisms** include the vascular plants Pinus ponderosa or P. edulis, or Cercocarpus montanus, and the lichens Lecanora muralis, L. novomexicana, L. sp. (L. polytropa group?), Rhizoplaca melanophthalma sensu lato R. chrysoleuca sensu lato ("R. subdiscrepans" morphotype), Acarospora fuscata, Aspicilia caesiocinerea, Candelariella spp., Dimelaena oreina, Lecidea atrobrunnea, Melanelia spp., and Xanthoparmelia spp.

OTHER REPRESENTATIVE SPECIMENS EXAMINED: U. S. A. COLORADO: Boulder Co.: near Boulder, Shushan & Weber S-3218 (COLO, M, WIS); near Lyons, Weber L-72985 (COLO); **Garfield Co.:** 7 km N of Rifle, Ryan 20678 (ASU); **Larimer Co.:** km SW of Ft. Collins, Anderson S-20272 (COLO); **Moffat Co.:** 25.5 km N of Axial, Ryan 20637 (ASU). **IDAHO: Bonneville Co.:** Palisades Reservoir, Ryan 19590 (ASU). **SOUTH DAKOTA: Pennington Co.:** 6.5 km NW of Hermosa, Wetmore 10285 (MIN, UPS).

DISCUSSION: The new species is named after the collector of the type material, William Weber of the University of Colorado at Boulder, in honor of his work on lobate species of Lecanora in North America.

The two taxa with which L. weberi is most likely to be confused are L. novomexicana sensu lato and Rhizoplaca melanophthalma sensu lato (Figs. 3 and 4, respectively). All three taxa have pruinose apothecia and an upper cortex containing dead algal cells, and contain usnic acid and fatty acids of the pertusaric/constipatic group. At the type locality of L. weberi and other locations, the three taxa grow side by side, yet each remains distinct, with a different overall appearance produced by a combination of features. Lecanora weberi differs from the other two

species especially by its very thin cortex and lack of psoromic acid, and by its more grayish, pale-edged, frequently imbricated squamule-like lobes. Its marginal lobes are shorter, flatter and more loosely attached than those of L. novomexicana. Lecanora weberi differs from R. melanophthalma in that the new species lacks a distinct umbilicus and lower cortex and has apothecia that are adnate, with orangish to yellowish brown discs and scarcely prominent margins. Many of the characteristics of L. novomexicana and R. melanophthalma (e.g., chemistry, growth form, and colors of the thallus and apothecia) that are useful for distinguishing those species from L. weberi at the type locality of the latter do not apply at other localities. Especially in the case of R. melanophthalma, the material from the type locality of L. weberi is not very typical. In fact, both of these other species (as presently delimited by most authors) are extremely variable and plastic, can also be very difficult to distinguish from each other, and may well consist of several taxa.

The morphology and color of the thallus of L. weberi itself are also somewhat variable. Especially interesting is the difference between the more common, predominantly fertile forms (areolate-verrucose thallus center, squamulose-lobed margin) and the occasional, mostly sterile forms (more-or-less imbricate squamulose throughout) that occur on or next to the parent thalli. The origin of such dimorphism, which also occurs in various other lobate taxa, is unknown.

ACKNOWLEDGEMENTS

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TWO NEW SPECIES OF THE GENUS ASCOCHYTA LIB.

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ABSTRACT. Two new species of genus Ascochyta Lib. - A.urticicola Vanev et Bakalova, sp.nov. and A.digitalina Vanev et Bakalova, sp.nov., from Bulgaria, are described and illustrated.

During inventory investigations of genus Ascochyta Lib. (order Sphaeropsidales, class Deuteromycetes) in Bulgaria, two new species belonging to this genus were established.

ASCOCHYTA URTICICOLA Vanev et Bakalova sp.nov.

Maculae 0,1-0,8 cm in diam., orbiculares vel angulatae, solitariae interdum comfluentes, atro-brunneae vel nigrae, saepe concentric zonatae. Pycnides epiphyllae, sparsae, solitariae, globosae vel depresso-globosae, pallide brunneae, 110-180 µm in diam., poro orbiculari 25-35 µm in diam., cellulis parvis obscurioribus cincto. Conidia cylindrica vel elliptica utrinque rotundata, uni-vel biseptata, saepe ad septum leniter constricta, hyalina, (10) 13,5-17,5(21) x 4-5(6) µm (Fig. 1A).

In foliis Urticae dioicae L., Bulgaria, mons Pirin, Predela, 26.09.1975, S.G.Vanev, G.G.Bakalova, SOM 18962 M, holotypus.

Leaf spots 0,1-0,8 cm in diam., numerous, rounded or angular, single or often confluent, dark brown or black, more pale in the centre, occasionally concentric. Pycnidia spheric or flattened, single, scattered, pale-brown, 110-180 µm with circular ostiol, 25-35 µm in diam.,

surrounded by zone of darker cells. Conidia cylindric with rounded both ends or ellipsoidal, straight or slightly curved, hyaline, 1-2(rarely 3)septate, occasionaly constricted at the septa, (10)13,5-17 x 4-5(6) μm .

Specimen examined: on living leaves of Urtica dioica L., Bulgaria, Pirin mount., Predela, 26.09.1975, S.G. Vanev and G.G.Bakalova, SOM 18962 M (holotype).

There is only one Ascochyta species registered on plants belonging to genus Urtica- A.urticae A.L.Sm.et Ramsb.

Melnik(1977) delimited 2 subgenera of Ascochyta on the basis of the number and disposition of conidial septa. According to this author A.urticae belongs to subgenus Libertia Melnik since the conidia have only 1 central septum dividing the conidium into 2 nearly equal cells.

Ascochyta urticicola clearly differs from A.urticae in the size of conidia and the number of the conidial septa, 1 or 2, rarely 3 (Table 1).

Table 1.
Comparative morphologic data of Ascochyta urticicola and A.urticae

Subgenus	Species	Dimensions of conidia in μm		Number of septa in conidia
		lenght	width	
Ascochyta	A.urticicola	(10)13,5-17,5(21)	4-5(6)	1-2(3)
Libertia	A.urticae (by Melnik,1977)	7-12	2-4	1

For this reason it is necessary to place A.urticicola in subgenus Ascochyta Melnik.

There are 2 other Ascochyta species parasitic on plants of the same family (Urticaceae)- A.parietariae

Roum.et Fautr.(on Parietaria officinalis L.) and A.boehmeriae Woronichin (on Boehmeria ssp.) but they differ from A.urticicola not only in the host-plants but morphologically also.

ASCOCHYTA DIGITALINA Vanev et Bakalova, sp.nov.

Maculae 0,3-1,5 cm in diam., orbiculares, solitariae, globosae vel confluentes, ochraceae vel pallide brunneae, indistincte atropurpureo-marginatae. Pycnides epiphyllae sparsae, solitariae, globosae vel depresso-globosae, pallide brunneae immersae, 110-175 µm in diam. poro orbiculari 20-35 µm in diam., cellulis atrofuscis cincto. Conidia cylindrica utrinque rotundata, interdum oblongo-elliptica vel clavata, recta vel leniter curvata, distincte uni-vel biseptata, ad septum haud vel vix constricta interdum inaequaliter bicellulata, hyalina, (12,5)15-17,5 x (4)4,5-5(6) µm (Fig. 1B).

In foliis vivis Digitalidis viridiflorae Lindr., Bulgaria, mons Rodopi, Beglica, 2.08.1981, S.G.Vanev, SOM 18964 M, holotypus.

Leaf spots 0,3-1,5 cm in diam. rounded, single or confluent, ochraceous or palebrown with dark- brown or dark-violaceous wide border. Pycnidia more or less spheric, separate, scattered thin-walled, pale-brown, 110-175 µm in diam., with a single, circular ostiol 20-35 µm in diam., surrounded by darker cells. Conidia cylindric with rounded both ends, or ellipsoidal, rarely clavate, straight or slightly curved, hyaline, 1-2-septate, occasionally constricted at the septa (sometimes with 2 unequal cells), (12,5 15-17,5 x (4)4,5-5(6) µm .

Specimen examined: on living leaves of Digitalis viridiflora Lindr., Bulgaria, Rodopi mount., Beglica, 2.08.1981, S.G.Vanev, SOM 18964 M, (holotype).

Two different Ascochyta species parasitic on plants of the genus Digitalis are described in the literature- A.digitalis Fuckel and A.moelleriana Wint. Melnik (1977) has studied the type specimens of both species and has rejected A.digitalis as a misdetermined species. Acc-

ording to this author A.moelleriana is a later synonym of A.euphrasiae Oud. He has placed A.euphrasiae in subgenus Libertia because of the 1-septate conidia.

The new species A.digitalina belongs to subgenus Ascochyta and clearly differs from A.euphrasiae in size of conidia and the number and disposition of the septa (Table 2).

Table 2.
Comparative morphologic data of Ascochyta digitalina and A.euphrasiae

Subgenus	Species	Dimensions of conidia in μm		Number of septa in conidia
		lenght	width	
Ascochyta	A.digitalina	(12,5)15-17,5(20)	(4)4,5-5(6)	1-2
Libertia	A.euphrasiae (Melnik,1977)	7-12	3-4	0-1

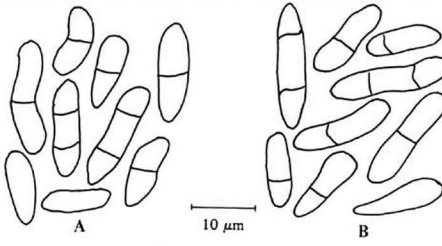

Fig.1 Conidia of: A. Ascochyta urticicola
B. A. digitalina

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TYPE STUDIES IN MARASMIOID AND COLLYBIOID FUNGI (TRICHOLOMATACEAE) II. AGARICUS GRAMINUM

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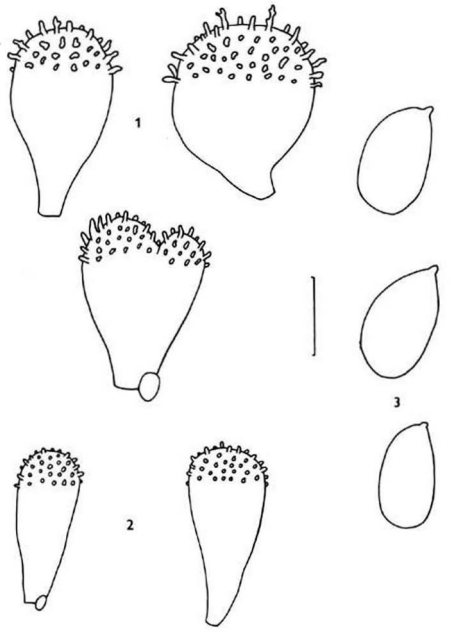
ABSTRACT

The results of the revision of the type specimens of Agaricus graminum Libert /= Marasmius graminum (Libert)Berk. et Br./ are published. Agaricus graminum in the original sense of Libert differs from Marasmius graminum in sense of contemporary authors. It is proposed to use the name Marasmius curreyi Berk. et Br. for the latter.

During preparation of a monograph of Central-European species of the genus Marasmius and type studies in these fungi, I was very surprised to find that the type specimen of Agaricus graminum Libert distributed in the collection of exsiccata "Plantae Cryptogamae Arduennae" differs from Marasmius graminum in sense of contemporary authors. I have found the same fungus in the Favre's herbarium in Geneve, which was published by Favre (1960) as a bisporic form of M. graminum. Marasmius graminum in the sense of contemporary authors is a cosmopolite species and all modern mycologists have this taxon in the same conception. Therefore, this fact mentioned above is very interesting. In the present paper, I give the descriptions of both species and also the reasons for a proposal to select as correct name Marasmius curreyi Berk. et Br. for the distinguished second species.

Marasmius graminum (Libert) Berkeley et Broome
Agaricus graminum Libert, Pl. Crypt. Arduennae, Fasc. II, No. 119, 1832.
Marasmius graminum (Libert) Berk. et Br., Outl. Brit. Fung., London, p. 222, 1860.

Macrofeatures (partly according to Favre's notes and partly from the type-specimens): PILEUS up to 4.5 mm broad but usually smaller, convex, opaque, with or without papilla, plicate, red-brown with the darker centre. LAMELLAE distant, L = (4-)6-8, l = 0, collariate, collarium very fine but prominent, not pure white but cream-whitish. STEM up to 25 mm long, filiform, smooth, lustrous, whitish above, blackbrown to almost black below.



Figs. 1-3. Marasmius graminum (type-specimen): 1. Pileipellis. 2. Cheilocystidia. 3. Basidiospores. Scale bar = 10 µm.

Microfeatures (figs. 1-3): BASIDIOSPORES ellipsoid, broadly ellipsoid to slightly amygdaliform, indextrinoid, hyaline, thin-walled, smooth, of two sizes: 7-12 x 2.5-4 µm and (9-)11-16(-18) x (5-)6.2-8(-10) µm; the second one is more common. BASIDIA clavate, clamped, 2- and 4-spored, e.g. 24 x 8 µm. BASIDIOLES clavate, broadly clavate, fusiform, sometimes with a broad and obtuse top, thin-walled, clamped, 13-29(-32) x (4.5-)6-10(-11) µm. CHEILOCYSTIDIA of the similar form as the broom cells of the pileus surface, clamped, thin-walled, 16-27 x (5-)6-10 µm, projections up to 2-4(-5) µm long. PLEUROCYSTIDIA none. SUBHYMENIUM of thin-walled, branched, cylindric, slightly dextrinoid hyphae, 2-4 µm broad. TRAMA of the lamellae of cylindric, thin-walled, dextrinoid, less branched, clamped, 3-8 µm broad hyphae; flesh of the pileus of interwoven, thin-walled, clamped, more or less cylindric, branched, slightly dextrinoid hyphae, sometimes finely incrusted, 2.2-9 µm broad; flesh of the stem in cortex of parallel, slightly dextrinoid, cylindric, clamped, slightly thick-walled (up to 0.8-1.2 µm), 3-4 µm broad hyphae, on the surface smooth, in medulla of parallel, dextrinoid, cylindric, thin-walled (up to 0.5 µm), clamped, 2-8 µm broad hyphae. PILEIPELLIS hymeniform, of cylindric-clavate, clavate, broadly clavate to almost globose cells, clamped, thin-walled, 13-28 x 8-24 µm, with irregular, simple to corraloid branched projections above, 1-3(-6) µm long, these broom cells mostly of the Rotalis-type but sometimes of a type transient to Siccus-type, the upper part of broom cells and the projections brown pigmented; between these cells are sometimes almost smooth to smooth cells and rare thin-walled hyphae with 4-6 µm long projections.

Material revised:
BELGIUM: Pl. Crypt. Arduennae, Fasc. II., No. 119, Libert, 1832, PRM 707065 (Lectotype), BPI, BP 18185, BR, K (ut Agaricus graminum).
SUISSE: Entre le God Trid et le God Purcher, vers 1900 m, val Trupschun pr. S-chauf, Hm Engadine, dans une aunaie sur graminées pourissantes, 9.IX.1955, J.Favre, G 14465 (ut Marasmius graminum forme bisporique).

This species is characterized macroscopically especially by the low number of lamellae and microscopically by the size of spores, character of broom cells of the pileus epicutis and especially of their projections.

The type-specimens from Libert's collection "Plantae Cryptogamae Arduennae" are preserved in many herbaria. The specimen from BPI was designated as an "isotype" (Gilliam, 1976). However, all the specimens of these exsiccata must be considered syntypes. The "isotype" (BPI) consists of two parts of the stem, with the absence of pileus, and thus this specimen has not any significance as a type. I revised these type-specimens from 5 important herbaria (BP, BPI, BR, K, PRM); the best specimen of them was that from the herbarium of the National Museum in Prague. Therefore, I propose it (PRM 707065) as a lectotype. However, the best revised ma-

terial of this species was the Favre´s collection from Suisse (G 14465)

The oldest synonym for Marasmius graminum treated in various papers and monographs of marasmioid fungi is Marasmius pruinatus Berk. et Curt. 1859 (not M. pruinatus Rea 1916). In monographs of Singer (1958, 1965, 1976), this name is mentioned as a synonym while Gilliam (1976) after the type study of the holotype-specimen (K) and authentic material of Berkeley et Curtis (FH) considers Marasmius pruinatus an independent species. According to this author, this species differs from M. graminum especially by the finely divided projections on the cuticular cells of M. pruinatus which contrast with the broader, discrete projections of M. graminum.

I have revised the specimen of M. pruinatus from Farlow Herbarium and my results agree with Gilliam´s solution. The microfeatures were the following (figs. 4-5): BASIDIOSPORES clavate to drop-shaped, indextrinoid, hyaline, thin-walled, smooth, 10.1-14.2 x 3.2-4.2 µm. BASIDIA clavate, 4-spored, clamped, 21-24 x 6.2-7.7 µm. BASIDIOLES clavate, fusiform, clamped, often with prominent obtuse top, thin-walled, 14-26 x 4.7-8 µm. CHEILOCYSTIDIA of the similar form as the broom cells of the pileus epicutis, clavate, with more or less nodulose projections, thin-walled, e.g. 16 x 7.5-8 µm. PLEUROCYSTIDIA none. SUBHYMENIUM of dextrinoid, thin-walled, clamped, 2-3.5 µm broad, hyaline hyphae. TRAMA of the lamellae of dextrinoid, clamped, thin-walled, sometimes branched, 2.5-7 µm broad hyphae; flesh of the pileus of dextrinoid, thin-walled, branched, clamped, hyaline,3-6 µm broad hyphae; flesh of the stipe of parallel, clamped, dextrinoid, 3-7 µm broad, thin-walled (in medulla) and thick-walled (up to 1-1.5 µm in cortex) hyphae; surface of the stipe smooth. PILEIPELLIS hymeniform, of broom cells of the Siccus-type, cells clavate, sometimes clamped, thin-walled to thick-walled in the upper part, with up to 5.5 µm long, more or less nodulose, obtuse projections, 9-14 x 5.2-8 µm; some cells coralloid branched with some thick-walled projections.

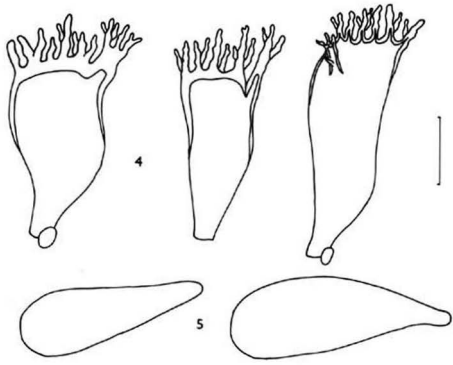
These microfeatures show unambiguously that this species belongs to the section Sicci. These results agree with conclusions of D.E.Desjardin on the revision label dated 3.II. 1988 and 10.III.1989.

Therefore, I have studied the type-specimen of the second older known synonym - Marasmius curreyi Berk. et Br. 1879. In my opinion, features of this specimen as well as Cooke´s table 1130B (Cooke, 1890), agree well with descriptions of the common "M. graminum" in the sense of contemporary authors and both taxa are conspecific. According to Dennis (1951) Marasmius exustus Berk. et Curt. 1879 could be identical with "M. graminum", too. The type-specimen is preserved in FH (not in K). However, according to Singer (1976) who revised this type, M. exustus represents a species from the section Hygrometrici. Therefore, I propose to use the name Marasmius curreyi as the correct one for the species called "M. graminum" now. A full description of this species follows now.

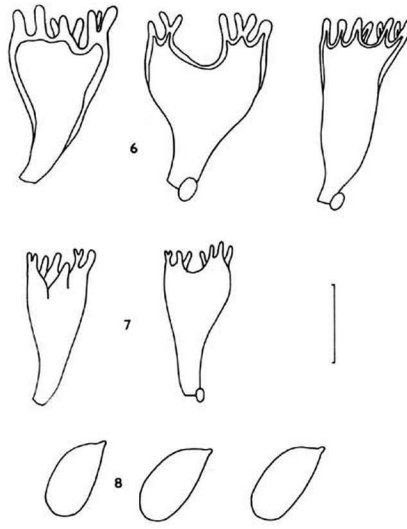
Marasmius curreyi Berkeley et Broome, Ann. Mag. Nat. Hist. V, 3:209, 1879.

Marasmius graminum (Libert) Berk. et Br. ss. auct., e.g. Gilliam (1976), Singer (1976).
Marasmius tritici Young, Phytopathology, 25:116, 1925.

Macrofeatures: PILEUS 4-6 mm broad, conical-hemispherical, then convex to expanded, sometimes with slightly revolute margin, with a small papilla, especially when young, then often with small umbilicus, smooth, under lens slightly tomentose, slightly plicate, young red-brown to rusty-brown to pinkish-brown. LAMELLAE distant, L = 9-13, l = 0, collariate, very rarely without distinct collarium, with concolorous edge, white. STEM 18-26 mm long, filiform, lustrous, smooth, whitish to white above, through brown to dark red-brown to black-brown toward the base.



Figs. 4-5. Marasmius pruinatus (authentic material): 4. Pileipellis. 5. Basidiospores. Scale bar = 5 µm.



Figs. 6-8. Marasmius curreyi: 6. Pileipellis. 7. Cheilocystidia. 8. Basidiospores. Scale bar = 10 µm.

Microfeatures (figs. 6-8): BASIDIOSPORES ellipsoid, cylindric-ellipsoid to slightly amygdaliform, smooth, thin-walled, hyaline, (7.7-)8-11(-13) x 4-5.7(-6.5) µm. BASIDIA clavate, clamped, 4- rarely 2-spored, 15-30 x 4-7 µm. BASIDIOLES clavate, cylindric-clavate, slightly fusiform, clamped, 18-28 x 4-8 µm. CHEILOCYSTIDIA of the similar form as the broom cells of the pileipellis, more or less clavate, hyaline,

10-16(-20) x 6-12 um, with 1-7(-8) um long, hyaline to slightly yellowish projections above. PLEUROCYSTIDIA none. SUBHYMENIUM of thin-walled, cylindric, clamped, branched, hyaline, 2-4 um broad hyphae. TRAMA of the lamellae of dextrinoid, thin-walled, clamped, hyaline, 3-8 um broad hyphae; flesh of the pileus of dextrinoid, branched, interwoven, thin-walled, clamped, hyaline, 3-7(-10) um broad hyphae; flesh of the stem in cortex of parallel, clamped, slightly thick-walled (up to 1 um), yellow-brown to brown pigmented, in medulla dextrinoid, parallel, thin-walled, clamped, hyaline, 2.2-8 um broad hyphae. PILEIPELLIS hymeniform, of broom cells of the Siccus-type, clavate or short cylindric-clavate, some of them thin-walled and hyaline, other (usually a great part of them) slightly thick-walled above, 9-22 x (6-) 8-14(-18) um, with 2-5(-7) um long, obtuse projections, cells hyaline below, yellow-brown obove and in the projections.

Habitat: on remnants of Poaceae, Juncaceae, Cyperaceae and rarely of some other plants.

Material revised:
BELGIUM: Westerloo (prov. Antwerpen), 1941, Tuymans (BR). - Bruxelles (Brabant), 1891, Delogne (BR).
CZECHOSLOVAKIA: Dolany near Unhošť, 1939, Herink (PRM 139045) - Řepy near Praha, 1952, Pouzar (PRM 707068). - Praha-Žižkov, 1968, Svrček (PRM 671895). - Praha, Kinského sady, 1963, Wichanský (PRM 600982 and 624000). - Praha, Stromovka, 1952, Pouzar (PRM 707057); 1948, Vacek (PRM 707075). - Mnichovice, 1918, Velenovský (PRC and PRM 707061). - Mnichovice, Myšlín, 1939, Velenovský (PRM 153986). - Mnichovice, Hubačov, 1939, Velenovský (PRM 154305). - Libochovičky, 1916, Velenovský (PRC and PRM 707062). - Kročehlavy near Kladno, 1941, Herink (PRM 707064). - Černolice near Dobřichovice, 1950, Pouzar (PRM 707069). - Mořinka near Dobřichovice, 1947, Svrček (PRM 707080). - Pyskočely near Stříbrná Skalice, 1951, Pouzar (PRM 707059, 707067, 707070, 707071). - Ruda near Nové Strašecí, 1939, Herink (PRM 139124). - Poříčko, 1950, Pouzar (PRM 707072). - Kosoř, 1950, Vacek (PRM 707073). - Libřice, 1941, Herink (PRM 707060). - Karlštejn, 1946, Vacek (PRM 707081); Svrček (PRM 707078). - Drahelčice, 1947, Vacek (PRM 514995). - Horní Slavěnice near Lomnice n. Luž., 1962, Svrček (PRM 567936). - Smržov near Lomnice n. Luž., 1961, Kubička (PRM 616353); 1962, Svrček (PRM 567935). - Hamr-Kosky, 1980, Kubička (CB 2344). - Záboří near Blatná, Skalický (PRC). - Lutová near Třeboň, 1945, Svrček (PRM 707126). - Třeboň, 1979, Kubičková et Kubička (CB 2161). - Vodňany, 1937, 1938 and 1943, Herink (PRM 490571, 490566, 499642 and 707077). - Čimelice near Písek, 1961, Svrček (PRM 616352). - Laziště near Písek, 1963, Svrček (PRM 612757). - Vrábsko near Čimelice, 1966, Svrček (PRM 626072 and 626073); 1963, Pouzar (PRM 612756). - Zvíkovské Povltaví, 1954, Pouzar (PRM 617489). - Golčův Jeníkov, 1940, Herink (PRM 707063 and 707066). - Nemyšl near Tábor, 1943, Svrček (PRM 707058). - Vidnava (Weidenau), 1912 and 1919, Hruby (BRNM 07311/39 and 07310/39). - Bojkovice-Bzová, 1985, Antonín (BRNM). - Kuřim, 1942, Šmarda (BRNM 313942). - Zdravá Voda near Žarošice, 1947, Vacek

(PRM 707076). - Prenčov, 1898 and 1901, Kmeť (BRA). - Turčianský Martin, 1951, Šmarda (BRNM 313941). - Rača, 1951, Krippelová et Šmarda (BRA). - Zemianské Podhradie (Ns.Podhragy), 1884, Bäumler (BP 19704).
ENGLAND: on Rye, Cooke (K). - Kew, Royal Bot. Gardens, 1966 (K). - Coughton (Warwicks), Clark (K). - Canseway, Slapton (Dorset), 1981, Clark (K). - Arington Court (N.Devon), 1978, Clark (K). - West Nolesey (Surrey), 1979, Spooner (K). - Hale Barnes Playing Fields (Cheshire), 1980, Newton (K). - Kew, 1912 (K). - Arbrook Common, Esher (Surrey), 1981, Spooner (K). - Holm Fen (Huntongdonshire), 1965, Houtton (K). - Cranbourne Park, Windsor Park (Berkshire), 1967, Dennis and Reid (K). - Lickey Hills (Worcestershire), 1968, Price (K). - Maidenhead (Berkshire), 1970, Verdcourt (K).
FIJI: Viti Levu, Savura Creek, 1976, Maddison and Kirby (K).
FRANCE: Santes, Courtecuisse (Herb. Courtecuisse).
GERMAN DEMOCRATIC REPUBLIC: Dienstädt (Ostthüringen), 1975, Hirsch (JE B 591/50). - Halle/Saale, Dölauer Heide (Sachsen-Anhalt), 1974, Hirsch and Braun (JE B 475/42). - Halle-Neustadt (Sachsen-Anhalt, 1971, Hirsch (JE B 48/4). - Bräunsdorf (Sachsen), 1970, Zschieschang and Ebert (JE). - Dübener Heide (Sachsen-Anhalt), 1977, Dörfelt (HAL); 1970, Hirsch (JE B 74/4). - Eisleben (Sachsen-Anhalt), 1972, Hirsch (JE B 201/15).
HUNGARY: Tiszacsege, Hortobagy (Hajdu-Bihar), 1974, Babos (BP 51592). - Tahi, Mts. Pilis, 1955, Bohus and Babos (BP 30833). - Csúcshégy, Mts. Budai, 1957, Bohus and Babos (BP 39251). - Tökhegy, Mts. Budai, 1954, Bohus and Babos (BP 39250).
THE NETHERLANDS: Osgstgeest (Zuid-Holland), 1955, Bas (BRNM 96794 and K).
POLAND: Wroclaw (Breslau), Schroeter (BRA).
ROMANIA: Cluj, 1959,Silaghi (PRM 533819).
SWEDEN: Göteborg, Stora "Änggården" (Västergötland), 1937, Nathorst-Windahl (PRM 103546 and K).
WEST PAKISTAN: Lahore and Sialkot, 1959, Sultan Ahmad (K).

Marasmius curreyi differs from the above mentioned M. graminum ss.str. especially by the broom cells of the pileipellis solely of the Siccus-type and the smaller, rather ellipsoid to cylindric-ellipsoid spores. The number of the lamellae is usually lower in the first species.

According to these results, seven species of Marasmius section Marasmius occur in Europe - Marasmius alniphilus Favre, M. bulliardii Quél., M. curreyi Berk. et Br., M. graminum (Libert) Berk. et Br., M. limosus Quél., M. rotula (Scop.: Fr.) Fr. and M. wettsteinii Sacc. et Syd.

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VACCINIUM FUNGI: HELICOMA VACCINII SP. NOV.

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An apparently undescribed species of *Helicoma* Corda was found on *Vaccinium elliotii* Chapman stems during a survey of fungi on *Vaccinium* spp. in the eastern U.S. The fungus is described as a new species and illustrated from culture and host tissue. It represents the first report of a *Helicoma* from *Vaccinium*.

The *Helicoma* sp. was colonizing a vertical scar on a stem section of *V. elliotii* collected near the Satilla River, Ware Co., Georgia. Conidia were streaked onto Difco Bacto-Agar and single germinating conidia transferred to the following types of media: Difco malt extract agar (MEA), Difco corn meal agar (CMA), full and half-strength Difco potato dextrose agar (PDA) and V8 agar (Stevens, 1974). Cultures were maintained under laboratory bench conditions of fluctuating light and temperature (18-20 C).

Helicoma vaccinii L. M. Carris sp. nov. Figs. 1-13.

Coloniae in agaro cum Zeae farina composito cultae aetate quinque hebdomadum diametrum 0.6-1.0 cm attingentes; mycelio atro-brunneo, lanato vel caespitoso. Coloniae in caulibus effusae, hirsutae, atro-brunneae. Mycelium immersum vel superficiale, ex hyphis septatis, brunneis, 1.7-3.4 μm crassis. Conidiophora plerumque simplicia vel raro ramosa, flexuosa, atro-brunnea, cellulis apicalibus pallide brunneis, interdum rugosa, 4-10 septata, 64-145 μm long., 4.2-5.0 μm lat. ad basim, 2.5-3.3 μm lat. ad apicem, proliferationibus percurrentibus pluribus (1-3). Cellulae conidiogenae mono- vel polyblasticae, denticulatae; denticula 1.3-2.7 μm long., 1.0-1.3 μm lat. Filamenta conidica in 1.5-1.75 spiris convoluta, 4-8 septata, apice rotundato, basi truncata, 2.0-4.0 μm crassa. Conidia hyalina vel pallide brunnea, laevia, 8.0-13.0 μm lata.

Habitat in caulibus *Vaccinii elliotii*, Satilla River, Ware Co., Georgia. N. Vorsa, 9.vii.1988 (herb. BPI) HOLOTYPUS.

Five-wk-old colonies on CMA 0.6-1.0 cm diam, dark brown, woolly to floccose, sporulation abundant, border even, slightly crenate, reverse dark brown. Colonies on stem effuse, hairy, dark brown. Mycelium immersed and superficial, hyphae septate, brown, 1.7-3.4 μm diam. Conidiophores (Figs. 1B, 1E, 3, 9) simple or rarely branched, cylindrical, flexuous, dark brown with pale apical cells, occasionally roughened, 4-10 septate, 64-145 μm long, 4.2-5.0 μm wide at base, 2.5-3.3 μm wide at apex, usually with 1-2 percurrent proliferations (Fig. 6). Conidiogenous cells (Figs. 4, 5) mono- or polyblastic, denticulate, denticles 1.3-2.7 μm in length, 1.0-1.3 μm wide. Conidial filament coiled 1.5-1.75 times, 4-8 septate, rounded at apex and tapering to a truncate base, 2.0-4.0 μm diam. Conidia (Figs. 1E, 1D, 7, 8, 10, 11) hyaline to pale brown, smooth, 8.0-13.0 μm diam.

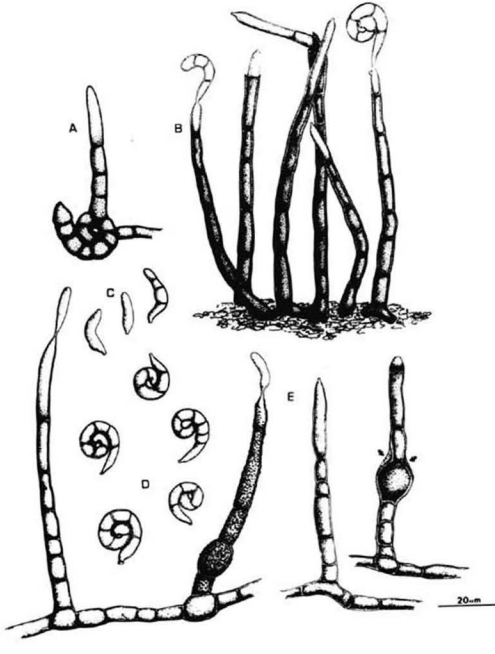
HABITAT: On stem scars of *Vaccinium elliotii*.
DISTRIBUTION: Ware Co., Georgia, U. S. A.

MATERIAL EXAMINED: LMC 0043, HOLOTYPE, deposited as stem section, dried culture, and prepared slides in BPI. ISOTYPE specimen deposited at WSP, living culture deposited as ATCC 66068.

OTHER PERTINENT MATERIAL EXAMINED: *Helicoma muelleri* Corda, IMI 78575, FH-Linder No. 1341; *Tubeufia pezizula* (Berk. & Curt.) Barr (*H. muelleri* anamorph), IMI 73306; *H. ambiens* Morgan, FH-co-type.

Only minor differences were noted between colonies on host tissue (Fig. 2) and in culture. Conidiophores formed in culture frequently had roughened walls and enlarged cells with a dark, vesicle-like outer wall layer (Fig. 1E) that were not evident in colonies on host material.

Fig. 1. *Helicoma vaccinii*. A. Conidiophore developing from cell of conidium in culture on CMA. B. Conidiophores on *Vaccinium elliotii*. Note proliferations. C. Immature conidia. D. Mature conidia. E. Conidiophores formed in culture on CMA. Arrows indicate vesicle-like outer wall.


A
B
C
D
E
20 µm

Colonies were restricted on all media used, and did not exceed 6-7 mm diameter after two months' growth.

Goos (1986) monographed the genus *Helicoma*, recognizing 32 species and four sections. The sections are delimited primarily on mode of conidial development and conidium attachment to the conidiogenous cell. *Helicoma vaccinii* fits well in Sect. *Helicoma*, a group comprised of species with conidia borne acrogenously on distinct denticles. Goos (1986) noted the morphological similarities between the ten species in Sect. *Helicoma*. There is considerable overlap in characters, including: conidiophore length; number of septa; and number of coils in the conidium. The diagnostic characters most useful in differentiating species include diameter of conidia and width of conidial filaments. Nine of the species in Sect. *Helicoma* produce conidia >13 µm in diameter. Only *H. taiwanensis* Matsushima has relatively small conidia (7-15 µm in diameter) in the size range of the conidia of *H. vaccinii*. The conidia of *H. taiwanensis* are otherwise different from the conidia of *H. vaccinii* in having wider conidial filaments (4.0-6.0 µm versus 2.5-4.5 µm for *H. vaccinii*) and blunt basal cells. In addition, *H. taiwanensis* produces a second, phragmosporous type of conidium and conidiophores with multiple, inflated conidiogenous nodes (Matsushima, 1983).

Goos (1986), Linder (1931), and Pirozynski (1972) noted the shape of the conidium basal cell as a useful character for differentiating *H. muelleri* and *H. ambiens*, two morphologically similar species in Sect. *Helicoma*. Likewise, the long, tapering basal cells of *H. vaccinii* (5.4-14.7 µm in length) distinguish this fungus from other species in Sect. *Helicoma*. For example, the basal cells of *H. muelleri* conidia (IMI 78575) are 4.7-6.7 µm long.

The conidiogenous cells of *H. vaccinii* are predominately monoblastic, both on host tissue and in culture (Figs. 1B, 1E, 4-6, 9). Occasionally, conidiogenous cells with 2-3 denticles are found. More frequently, the

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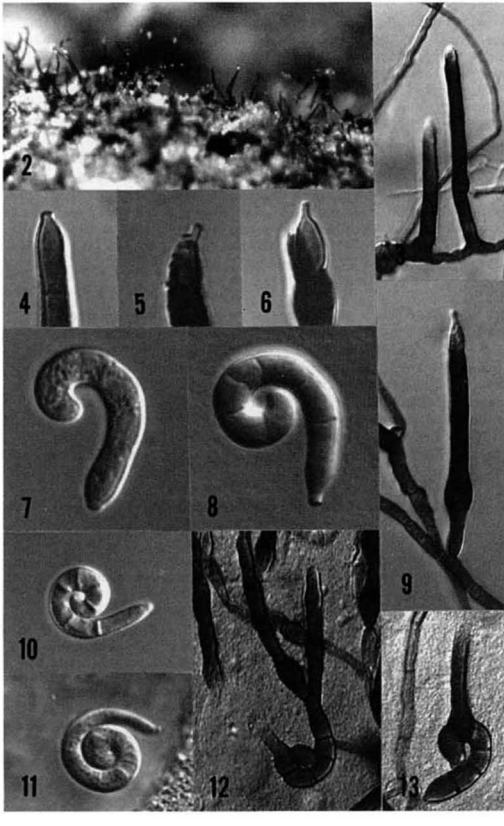
Figs. 2-13. *Helicoma vaccinii*. 2. Conidiophores on *Vaccinium elliotii*. X 90. 3. Conidiophores formed in culture on CMA. X 600. 4, 5. Conidiophore apices. Note denticles. X 1400. 6. Proliferation of conidiophore apex with ruptured outer wall. X 1400. 7, 8. Immature conidia. X 2000. 9. Conidiophore formed on CMA. X 800. 10, 11. Mature conidia. X 1000. 12, 13. Conidiophores developing from cells of conidia. X 600.


2
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conidiogenous cells proliferate percurrently, with a new conidiogenous cell emerging through the ruptured darkened outer wall of the conidiophore apex (Figs. 1B, 6). This type of proliferation was illustrated by Pirozynski (1972) for the *H. muelleri* anamorph of *Tubeufia pezizula* [cited as *Thaxteriella pezizula* (Berk. & Curt.) Petrak]. An illustration of a conidiophore apex of *H. ambiens* (Goos, 1980; Fig. 29) also resembles the conidiogenous cell proliferation in *H. vaccinii* shown in Fig. 6.

Germination of *H. vaccinii* conidia frequently occurs from the basal cell, in a way similar to *H. muelleri* as reported by Linder (1929), but germ tubes may develop from any cell in the conidium of the former. In colonies on CMA and MEA, conidia often become swollen and melanized, giving rise directly to conidiophores (Figs. 1A, 12, 13).

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LONG-CHAIN FATTY ACID COMPOSITION AS A TOOL FOR DIFFERENTIATING SPOILAGE WINE YEASTS

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SUMMARY

The use of conventional methods for yeast identification is not pratical enough for industrial application due to the complexity and morosity of the techniques. Furthermore, misinterpretation of the results is frequent. The use of chemotaxonomic methods for pratical uses enables a faster and easier identification. In this work the analysis of long-chain fatty acids was used to differentiate several yeast strains belonging to the genera *Saccharomyces*, *Zygosaccharomyces*, *Torulaspora*, *Candida* and *Pichia*.

INTRODUCTION

The identification of yeasts according to morphological, sexual, physiological and biochemical characteristics has been traditionally used by Lodder (1970), Barnett *et al.* (1983) and Kreger-van Rij (1984). The work of Lodder (1970) and Kreger-van Rij (1984) results from a revision of the book edited formerly by both authors (Lodder and Kreger-van Rij, 1952). Unfortunately for those who deal with practical aspects of yeasts the frequent changes of nomenclature make the identification more difficult, due to an increase in the synonymy among the species classified by these authors in their different editions, which led van der Walt (1987) to propose that the fermentation industry should develop a specific taxonomy.

Nowadays the more significant developments on taxonomy are due to molecular methods (for a review see Kurtzman and Phaff, 1987). The chemical constituition of each microorganism is regarded as a result of natural evolution and so, the species with similar composition are seen as being closely related. The fact that the above mentioned methodologies are time consuming and/or of difficult industrial use, lead to the development of more rapid techniques, such as analysis of compounds and metabolites. These, by revealing also a phylogenetic relatedness, enable an easier practical delimitation of species.

Among other compounds analysed are the long-chain fatty acids, which have been used with several microorganisms since the work of Abel *et al.* (1963) using bacteria. In our work we tried to differentiate several spoilage wine yeasts based on the cellular long-chain fatty acid composition following, primarily, the methodology proposed by Lategan and his co-workers (Kock *et al.* 1985, 1986; Cottrell *et al.* 1985, 1986; Viljoen *et al.* 1986, 1987, 1988; Tredoux *et al.* 1987a, 1987b). The spoilage wine yeasts can be divided into three main groups: the film-forming (e. g. *Candida vini, Pichia membranaefaciens* and *Brettanomyces* spp.), the fermenting (*Saccharomyces cerevisiae*), and those of more difficult control, the "sensu stricto" spoilage yeasts (e.g. *Zygosaccharomyces bailii*). Our aim was to obtain a test which could rapidly determine whether or not the isolated yeasts belonged to the species more dangerous to wine stability. *Z. bailii* is difficult to differentiate from *Torulaspora delbrueckii* when the sexual conjugation is not evident. Therefore several strains of these species, isolated from other origins, were also analysed.

MATERIAL AND METHODS

The yeast strains were either obtained from the yeast culture collection of the Instituto Gulbenkian de Ciência (IGC), Oeiras, Portugal, or isolated from Portuguese bottled dry white wines with "sandy" sediments (table 1). Stock cultures of all strains were maintained on Wickerham agar slopes and the isolation was made by plating, using the same medium. All isolated strains were identified according to conventional methods (Barnett *et al.* 1983, and Kreger-van Rij, 1984) in the IGC laboratory.

Growth on liquid medium followed the methodology proposed by Kock *et al.* (1985). Cells were grown in 250 mL Erlenmeyer flasks with 80 mL of medium composed by 80 $g.L^{-1}$ glucose (Merck) and 6.7$g.L^{-1}$ Yeast Nitrogen Base (YNB) (Difco) at 30±0.1 C for 16 h, in a water bath with magnetic stirring. Then, 10 mL of the suspension were transferred to 1 L Erlenmeyer flasks with 400 mL of the previous medium, in the same environmental conditions, for 48 h. The cells were harvested during the stationary phase by centrifugation and washed twice with cold demineralized water. The biomass obtained was either freeze dried or used immediately. For growth on solid medium the cells, from stock cultures, were suspended in Ringer solution and a drop was placed in plates of Wickerham agar. Incubation was carried at 25±0.1 C for 48 h, after which the biomass was taken from the agar and used immediately.

The cellular long-chain fatty acids were extracted and methylated according to Moss *et al.* (1974), using a solution of 5% (w/v) NaOH in 50% (v/v) aqueous methanol and borontrifluoride methanol (Merck). The final methyl ester mixture was evaporated under a nitrogen stream and redissolved in hexane. The samples were analysed in a gas chromatograph (Perkin Elmer 8410) with a FID detector, using a stainless steel column (1/8 in x 3.5m) packed with 8% Carbowax 20M W-AW (100-120 mesh). The column temperature was 230 C at an outlet N_2 flow of 40 $mL.min^{-1}$. The methyl ester identification was done by retention times of myristic (C14:0), myristoleic (C14:1), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid esters, relative to the palmitic acid ester. The peak areas were determined by automatic integration and the results, for each ester,

Table 1. List of analysed yeast strains

Species	Designation	Origin[a)]
Saccharomyces cerevisiae Meyen ex Hansen	A000 and A063	Commercial wine starters
S. cerevisiae (syn.[b)] *S. bayanus* Saccardo)	A065	Commercial wine starter
S. cerevisiae (syn. *S. bayanus* Saccardo)	A028, A026 and A029	Isolated from bottled wines
Zygosaccharomyces bailii (Lindner) Guilliermond	A006	Type strain, IGC 2470, CBS 680
Z. bailii (Lindner) Guilliermond	G227	IGC 4227, NRRL S9 144
Z. bailii (Lindner) Guilliermond	A022, A023, A024, A025, A027 and A031	Isolated from bottled wines
Z. bailii (Lindner) Guilliermond (syn. *Saccharomyces elegans* Lodder & Kreger-van Rij)	G899	IGC 2899
Pichia membranaefaciens (Hansen) Hansen	A030	Isolated from bottled wines
Candida vini (Desmazières) van Uden & Buckley	A007	IGC 2597, CBS 639
Torulaspora delbrueckii (Lindner) Lindner	G166	Type strain, IGC 4166, CBS 1146, NRRL Y-886
T. delbrueckii (Lindner) Lindner (syn. *Saccharomyces delbrueckii* Lindner)	G477	IGC 2477
	G500	IGC 2500, kefyr grains
	G501	IGC 2501, kefyr grains
	G502	IGC 2502, bottled kefyr
T. delbrueckii (Lindner) Lindner (syn. *Saccharomyces rosei* (Guilliermond)Lodder & Kreger-van Rij)	G209	IGC 3209, CBS 865, potato starch
	G713	IGC 2713, CBS 817
T. delbrueckii (Lindner) Lindner (syn. *S. rosei* and *Schwanniomyces hominis* Batista *et al.*)	G844	IGC 2844, human skin
T. delbrueckii (Lindner) Lindner (syn. *S. rosei* and *Torulopsis stellata* (Kroemer & Krumbholz) Lodder var. *cambresieri* Lodder & Kreger-van Rij)	G907	IGC 2907
	G911	IGC 2911, CBS 158, sugar
	G998	IGC 2998, man
	G999	IGC 2999, seawater
T. delbrueckii (Lindner) Lindner (syn. *Torulopsis colliculosa* (Hartmann) Saccardo)	G916	IGC 2916, CBS 133, javanensi ragi
Unindentified strains	A032, A033, A067, A068, A069, A070, A071, A072, A074, A075, A076, A077, A078, A079, A080, A081 and A082	Isolated from bottled wines

a) Abbreviations: IGC, Instituto Gulbenkian de Ciência, Oeiras, Portugal; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands and NRRL, Northern Regional Research Center, US Department of Agriculture, Peoria, Illinois, USA.

b) Synonyms of the species described by Barnett et al. (1983) and Kreger-van Rij (1984).

expressed as a percentage by weight of the total area.

Principal Component Analysis (PCA) enables us to analyse observed values of a set of continous variables (in our case, percentages of fatty acids) for a set of experimental units (in our case, yeast strains), in order to build new variables, called principal components, which represent the directions of the axes of greatest variability. By projecting the initial variables on the planes defined by the first few principal components, we obtain graphical representations whose appropriate analysis enables the identification of the principal contrasts among the initial continuous variables. The coordinates of the strains in the first four axes were used to perform an ascending hierarchical classification according to the reciprocal neighbours method. Next, we apply a cut-off in the dendogram obtained from the hierarchical classification, this obtaining three classes which were characterized by the initial variables. All these analyses were carried out by the SPAD (Système Portable pour l'Analyse des Données) software (Lebart *et al.* 1985).

RESULTS AND DISCUSSION

The results from growth in liquid medium (Fig. 1) showed a clear distinction between the yeast groups previously established, enabling their separation in agreement with their practical spoilage importance. Other authors (Tredoux *et al.* 1987a, 1987b) have characterized a larger number of yeasts but the groups proposed have not a consistent enological meaning.

The methods in wine microbiological control should give rapid results and be simple to operate, therefore, in order to reduce time of analysis and to simplify the procedures, the yeasts were grown on solid medium. The fatty acid profiles (Fig. 2) were similar to those obtained on liquid medium with the same basic differences among the three groups. The major difference is on the percentage of C18:2 in *Z. bailii* strains, but this does not affect the distinction among the three groups. The influence of growth conditions on the fatty acid composition is largely referred in literature (for a review see Ingram and Buttke, 1984, and van Uden, 1985). This similarity of the profiles in different conditions was, therefore, somewhat unexpected although, probably, the media composition and incubation conditions are not distinct enough to produce large differences. For example, Hunter and Rose (1972) had already noticed that there was little change in the fatty acid composition of *S. cerevisiae* cells grown at 15 C and 30 C. These results are of particular importance to industry because the procedures are simplified and, principally, because it is possible to make the analysis directly from microbiological control plates, if the biomass grown is enough for the determination.

The statistical treatment by PCA establishes a correlation matrix, comprising the long-chain fatty acid compositions for each strain. This allows distribution of the strains in a projection plan according to their fatty acid similarities, confirming the differences between the three spoilage yeast groups (Fig. 3). The unindentifyed strains A068, A070, A074, A076, A077, A079, A081 and A082 were clustered on the *S. cerevisiae* group, strains A032, A067, A075, A078, and A080 were in the *Z. bailii* group, and strains A033, A069, A071 and A072 were in the *P. membranaefaciens* group (Fig. 3). Further tests, on a larger number of strains and species, are being made in order to improve the reliability of this analysis, for that it will be necessary to ensure the stability of the defined clusters. If this analysis proves suitable it will be possible to create a data bank allowing a direct yeast identification according to its phylogenetic proximity.

The PCA of the thirteen studied strains of *T. delbrueckii* showed that their fatty acid profiles are clearly different from those of the *Z. bailii* strains (Fig. 4). In fact, whereas all strains of *Z. bailii* appear in one single cluster, except for one strain, G899 formerly classified as *Saccharomyces elegans*, strains of *T. delbrueckii* are

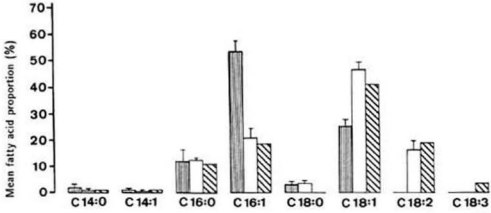

Fig. 1 - Long-chain fatty acids profiles from strains grown on Wickerham liquid medium, at 30 C for 48h. ▥-Fermenting yeasts, *S. cerevisiae* A000, A063, A065, A028 and A029. □-"Sensu stricto" spoilage yeasts, *Z. bailii* A006, A024, A027 and A031. ▨-Film-forming yeast, *P. membranaefaciens* A030. Results are the mean of two or three replicates. Standard deviations indicated by small vertical bars.

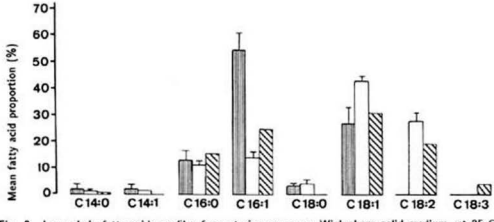

Fig. 2 - Long-chain fatty acids profiles from strains grown on Wickerham solid medium, at 25 C for 48h. ▥-Fermenting yeasts, *S. cerevisiae* A000, A063, A065, A028, A026 and A029. □-"Sensu stricto" spoilage yeasts, *Z. bailii* A006, G227, A022, A023, A024, A025 and A031. ▨-Film-forming yeast, *P. membranaefaciens* A030. Standard deviations indicated by small vertical bars.

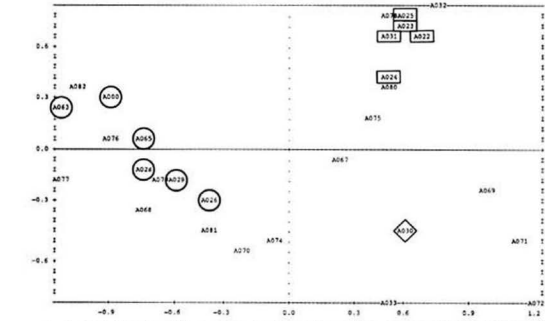

Fig. 3 - Projection plan of the yeasts, isolated from wines and from commercial wine starters, on the axes 1 (horizontal) and 2 (vertical). *S. cerevisiae* ○ ; *Z. bailii* □ ; and *P. membranaefaciens* ◇ . Unidentified strains do not have symbols.

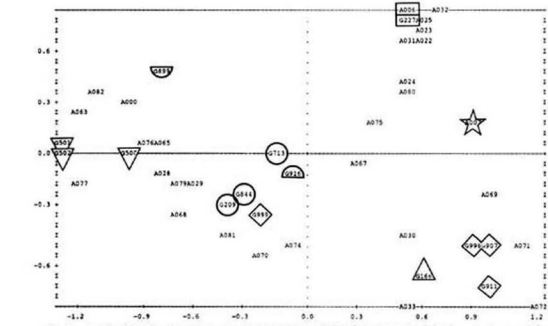

Fig. 4 - Projection plan of the yeasts, isolated from wines, from commercial wine starters and from other origins, on the axes 1 (horizontal) and 2 (vertical). *S. rosei* and *S. hominis* ○; *S. delbrueckii* ▽; *T. stellata* var. *cambresieri* ◇ ; *T. colliculosa* ⌓ ; *T. delbrueckii* (type strain) △ ; *S. elegans* ⌓; *Z. bailii* □ ; and *C. vini* ☆. Strains isolated from wines and from commercial wine starters do not have symbols. the yeasts isolated from other origins are considered illustratives, not influencing the definition of the axes. Strains A026, A078 and G477 are covered by strains G209, G227 and G501, respectively.

scattered on the projection plan. Nevertheless it was possible to distinguish several subgroups among strains of the species now considered as *T. delbrueckii* (Barnett *et al.* 1983, and Yarrow, 1984), approximately related to the former classification for the species *S. delbrueckii, S. rosei* and *T. stellata* var. *cambresieri*.

From all this a question arises: are the former classifications more consistent, in phylogenetic terms, than the present ones (Barnett *et al.* 1983, and Yarrow, 1984)?

Although the number of studied strains is too small to enable a definite conclusion, our results showed that the long chain fatty-acid composition might be of great convenience in the distinction between *Z. bailii* and *T. delbrueckii*.

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MYCOTAXON

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DESCRIPTION OF THE ANAMORPH OF VALSEUTYPELLA MULTICOLLIS IN CULTURE

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INTRODUCTION

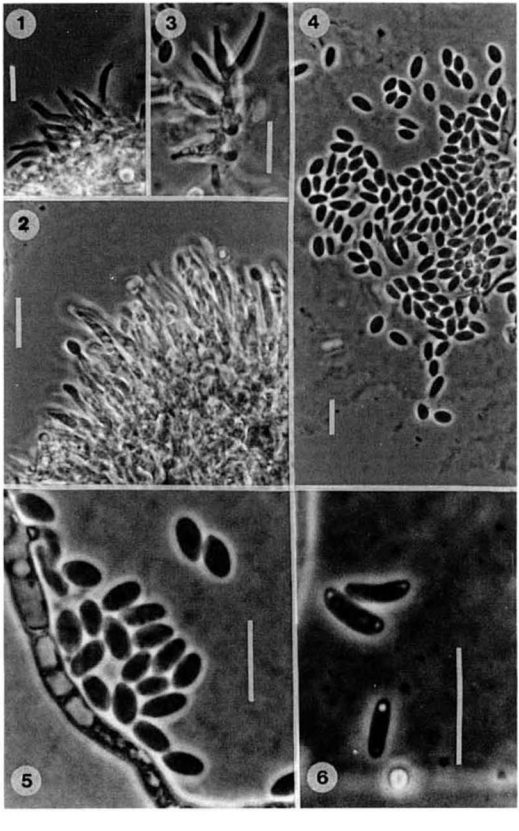
The genus *Valseutypella* Höhnel is characterized by its receptacle-shaped stroma formed by pseudoparenchymatous and sclerotial cells.

This genus had remained monospecific until the description of *V. multicollis* Checa, Moreno & Barr (Checa et al., 1986), which differs from the type species *V. tristicha* (de Not.) Höhnel, by its larger stromata containing numerous perithecia, as well as by the size of its ascospores and its habitat. *Valseutypella tristicha* is host specific on *Rosa* spp., whereas the Spanish species occurs on *Quercus ilex* ssp. *rotundifolia*.

The anamorph of *V. tristicha* belongs to the genus *Cytospora* (Hubbes, 1960) and has not been described in detail. The anamorph of *V. multicollis* also belongs to *Cytospora*.

METHODS

With ascospores of *V. multicollis*, obtained from stromata grown on wood of *Quercus ilex* ssp. *rotundifolia*, we started cultures in PCA (potato carrot agar) with antibiotic. The fungus is conserved at the fungal culture collection of the CIB (IJFM) as IJFM A-522. After several months of incubation at room temperature, we observed the production of pycnidia, more quickly and abundantly on MEA (malt agar).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DESCRIPTION

Colonies on MEA growing quickly with scarce aerial mycelium. Producing a diffusible dark brown pigment and forming abundant pycnidia in concentric zones. Pycnidia black, variable in size (300-700 µm),stromatic, globose to pyriform, with one or several ostioles, which disperse the conidia by means of drops of exudate. Phialides cylindrical (Figs. 1-2) sometimes narrowed at the base, 8-18 x 1,5-2 µm, in verticils of 3-5, on branched hyphae which grow filling up the greater part of the pycnidial cavity (Fig.3). Conidia hyaline, cylindrical-oval in front view (Figs.4-5), allantoid in side view (Fig.6) and becoming ellipsoidal (4-6 x 2,5 µm) and apiculated on one end. None of the cultures developed perithecia.

The comparison with the anamorph of *V. tristicha* (CBS 465.59) was not possible because this strain did not produce pycnidia in culture.

DISCUSSION

This pycnidial form must be placed in the genus *Cytospora* because of its stromatic pycnidia with dark walls, cylindrical and verticillated phialides not restricted to the base of the pycnidial cavity, and particularly because of the presence of allantoid conidia. However it includes ellipsoidal forms too, thus being different from the greater part of the species of *Cytospora*.

The definitive clarification of the systematic position of the anamorphs of *Valseutypella* continues the revision of the genus *Cytospora*, which includes anamorphs of *Valsa* (Spielman, 1985) and *Leucostoma*, as well as numerous anamorphic species whose variability in culture has to be determinated (Sutton, 1980).

We thank Dr. M.E. Barr of the University of Massachusetts, for reviewing the manuscrip and Mr. M. Heykoop for the English corrections.

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Figs.1-6: *Cytospora* sp. 1-2.- Cylindrical phialides, verticils, on branched conidiophores. 3.- Branched conidiogenous hyphae in the pycnidial cavity. 4-5.- Oval to ellipsoidal conidia. 6.- Allantoid conidia. The bars indicate 10 µm .

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STUDIES ON J. B. CLELAND'S FUNGAL HERBARIUM - 2: CORTINARIUS SUBGENUS MYXACIUM (CORTINARIALES)

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Abstract

Collections of Cortinarius, subgenus Myxacium in the J.B. Cleland herbarium have been re-examined. Of the seven species described and the one recorded from South Australia, five are accepted, two are treated as synonyms, and one new species is described: Cortinarius bundarus.

Introduction

Cortinarius subgenus Myxacium is distinguished from other subgenera by the gelatinizing universal veil. This subgenus contains small to large fleshy species with brown, yellow, blue or vinaceous-red pigmentation. Pigment is epimembranal intercellular or plasmatic. Hyphae of the cuticle are 7-20 µm wide, the spores are verrucose, subglobose to amygdaliform or lemon-shaped. Cheilocystidia are present or absent. (Singer, 1986:635).

J.B. Cleland described or recorded eight species within this subgenus. Fresh material could not be obtained to supplement the macroscopic descriptions provided by Cleland or to amplify distributional data. The macroscopic descriptions in this paper are adapted from Cleland's handbook (1934-1935), his papers (1928 & 1933), and the collection notes accompanying each collection. Cleland recorded colours using the colour handbook of Ridgway (1912). In this study, microscopic data were recorded from fragments of basidiome stained with ammoniacal Congo Red and then mounted in a 3% aqueous solution of potassium hydroxide.

KEY TO THE INCLUDED SPECIES OF SUBGENUS MYXACIUM

1. Basidiome red.....C. erythraeus 1.
1*. Basidiome differently coloured.....2.
2. Basidiome yellowish brown.....3.
2*. Basidiome with lilac or violet tints.....4.
3. Spores approaching subfusiform, 12.2-18.0 x 7.0 -9.9 µm.....C. subarvinaceus 2.
3*. Spores ellipsoid, 6.8-9.4 x 4.6-5.7 µm.....C. sinapicolor 3.
4. Pileus whitish to light buff; lamellae ochraceous buff; stipe with a trace of violet; odour of fenugreek when dry.....C. austroalbidus 4.
4*. Pileus violet-brown or violet becoming brown; lamellae violet-tinted; stipe pallid to pale violet or violet.....5.
5. Stipe with a distinct annulus, deep dull bluish violet below, pallid above; spores large, amygdaliform, 11.4-15.8 [-17.6] x 6.4-8.6 [-9.4] µm.....C. archeri 5.
5*. Stipe with a cortina, paler than above; spores smaller, short ellipsoid to elongate ellipsoid, up to 11.6 µm long.....6.
6. Spores short ellipsoid to ellipsoid, 6.4-8.6 x 4.8-8.3 µm..... C.microarcheri 6.
6*. Spores ellipsoid to elongate ellipsoid or approaching cylindric, longer than 8.6 µm....7.
7. Spores elongate ellipsoid, sometimes approaching cylindric, 8.6-11.6 x 4.8-6.1 µm.....C. subarcheri 7.
7*. Spores ellipsoid, 9.4-10.8 [-11.6] x 6.0-7.3 µm .....C. bundarus 8.

1. Cortinarius erythraeus Berk. Fig. 1 A-B.

Cortinarius erythraeus Berk. in Lond. J. Bot.4:48, 1845.
C. ruber Clel. in Trans. R. Soc. S. Australia 51: 303-304, 1927.

PILEUS up to 51 mm diam., convex, then irregularly wavy and convex, subgibbous, viscid, near Dragon's Blood Red (XIII) passing into Rufous (XIV); flesh white, moderately thick over the stipe, thinning outwards. LAMELLAE slightly sinuately adnexed, moderately close, a little ventricose, Tawny Olive (XXIX). STIPE up to 38 mm long, rather short and

stout, bulbous, to 19 mm diam. below and to 13 mm diam. above, viscid, solid or somewhat hollow, whitish and slightly striate above, concolorous with the pileus below the remains of the veil, with some yellowish mycelium at the base and whitish rooting mycelial strands below, flesh discoloured. CORTINA glutinous, yellowish red or red.

BASIDIOSPORES (55/6)[1], 8.0 - 11.0 ($\bar{x}$=9.8) x 5.9 - 8.3 ($\bar{x}$=6.9) µm, L/B=1.4[2], ellipsoid, finely warty rough, slightly thick-walled, pale yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (27/5), [23.2-] 32.0 - 51.2 ($\bar{x}$=36.9) x 8.6 - 13.0 ($\bar{x}$=10.6) µm, with sterigmata up to 4.8 µm long, four-spored, clavate. CYSTIDIA not observed. UNIVERSAL VEIL of filamentous, smooth, gelatinized hyphae, (38/2), 2.0 - 7.4 ($\bar{x}$=5.0) µm diam. CLAMP CONNECTIONS present.

HABIT, HABITAT AND PHENOLOGY - gregarious, occasionally solitary or subcaespitose on the ground under eucalypts. Specimens collected in June and July.

MATERIAL - SOUTH AUSTRALIA: Kinchina, 7.vii.1923, AD 4291, Miss J. Buxton, watercolour no. 12 (syntype of C. ruber). Belair Recreation Park, 21.vii.1923, AD 4290; 5.vii.1924, AD 4289 (syntype of C. ruber); 12.vi.1952, AD 4287. Morialta, 3.vi.1933, AD 4284; 3.vii.1937, AD 4288. Mt Bold, 9.vii.1939, AD 4286.

The orange to blood-red glutinous universal veil indicates that this species belongs in Section Pyromyxae (Moser & Horak, 1975: 227, 229, 574; Singer, 1986:636).

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1 (55/6), fifty-five measurements from six collections (see Bas, C. p. 290 (1969)).

2 L/B., length-breadth ratio

2. Cortinarius subarvinaceus Clel. Fig. 2 A-B.

Cortinarius subarvinaceus Clel. in Trans. R. Soc. S. Australia 51:304, 1927.

PILEUS 46 - 87 mm diam., convex sometimes repand, finally irregularly revolute, edge a little involute, sometimes substriate round the edge, very viscid, Mars Yellow (III), Ochraceous Tawny (XV), becoming much darker and shining in the centre; flesh slightly brownish, when old becoming semi-translucent, thick over the disc, thin outwards, cuticle thick and dark brown. LAMELLAE adnate or subsinuate, moderately close, slightly ventricose, pallid greyish cinnamon then Raw Sienna (III), Sayal Brown (XXIX) or Tawny Olive (XXIX) and darker. STIPE 37 - 75 mm long, stout, to 17 mm diam., cylindric or base sometimes slightly bulbous, mealy, fibrillose, base viscid, whitish becoming brownish. SPORE PRINT near Tawny Olive (XXIX).

BASIDIOSPORES (70/4), 12.2 - 18.0 ($\bar{x}$=14.6) x 7.0 - 9.9 ($\bar{x}$=8.4) µm, L/B=1.7, approaching subfusiform with the apices often drawn into obtuse mucrones, finely warty rough, slightly thick-walled, yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (27/3), 34.4 - 50.0 ($\bar{x}$=43.9) x 11.2 - 16.0 ($\bar{x}$=13.7) µm, with sterigmata up to 7.2 µm long, four-spored, clavate. CYSTIDIA absent. UNIVERSAL VEIL of repent, filamentous, loosely tangled hyphae, (40/1), 2.4 - 7.6 ($\bar{x}$=4.3) µm diam., gelatinized, hyaline, cuticle to 460 µm deep, subcuticular hyphae with pale reddish brown internal pigment, oleiferous hyphae scattered, cylindric, dark reddish brown. CLAMP CONNECTIONS not observed.

HABIT, HABITAT AND PHENOLOGY - gregarious, occasionally solitary or subcaespitose on the ground under eucalypts. Specimens collected from April to July.

MATERIAL - SOUTH AUSTRALIA: Mt Lofty, 25.iv.1921, AD 4323. Stirling West, 23.vii.1927, AD 4328 (holotype). Encounter Bay, 24.v.1928, AD 4322. Mt Robinson, via Upper Willow Creek, Encounter Bay, 30.v.1939, AD 4325. Echunga, 12.v.1939, AD 4326.

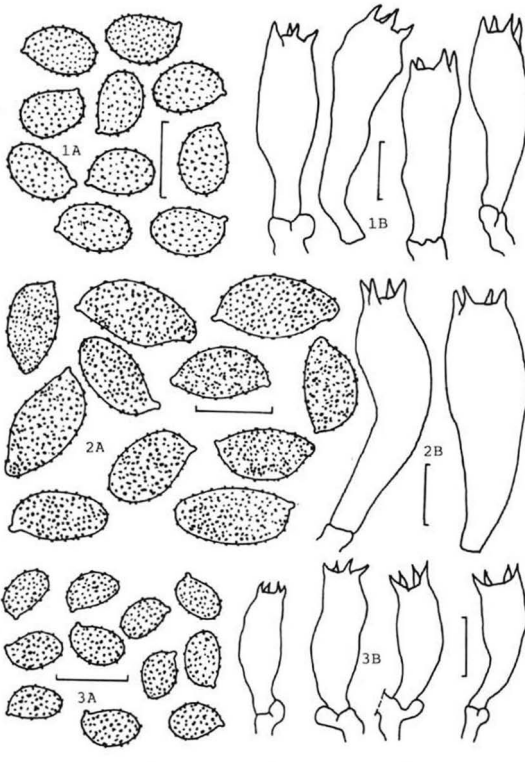

Fig. 1. Cortinarius erythraeus.- A. Basidiospores. B. Basidia. Fig. 2. C. subarvinaceus.- A. Basidiospores. B. Basidia. Fig. 3. C. sinapicolor - A. Basidiospores. B. Basidia. Bars = 10 µm.

The large subfusiform spores and absence of clamp connections indicate that this species belongs in Section Defibulati, as defined in Singer (1986:637).

3. Cortinarius sinapicolor Clel. - Fig. 3 A-B

Cortinarius sinapicolor Clel. in Trans. R. Soc. S. Australia 57:191, 1933.
C. ochraceus Clel. in Trans. R. Soc. S. Australia 51: 304, 1927 non Peck.

PILEUS up to 76 mm diam., slightly convex to convex, sometimes umbonate, then repand, finally irregularly upturned, edge sometimes undulating, very glutinous, Mustard Yellow (XVI), Yellow Ochre (XV) or Citrine Drab (XL) but with a brownish tinge around the edge, with the centre darker or passing into Amber Brown (III); flesh soapy-looking, thick over the disc, rapidly attenuated outwards. LAMELLAE adnate to sinuately adnexed, moderately close to close, to 8 mm deep, Buffy Brown (XL) when young, then near Sudan Brown (III) or Buckthorn Brown (XV). STIPE up to 76 mm long, moderately slender to moderately stout, to 13 mm diam., flexuous, base a little bulbous with white mycelial threads, then cylindric, striate or fibrillose above, very glutinous, pallid becoming yellowish brown or tinted yellow, flesh turning slightly watery yellowish. CORTINA pale yellowish.

BASIDIOSPORES (50/1), 6.8 - 9.4 ($\bar{x}$=8.0) x 4.6 - 5.7 ($\bar{x}$=5.0) µm, L/B=1.6, ellipsoid, finely warty rough, slightly thick-walled, yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (25/1), 22.4 - 32.8 ($\bar{x}$=26.5) x 6.8 - 10.0 ($\bar{x}$=8.6) µm, with sterigmata up to 5.6 µm long, four-spored rarely two-spored, clavate. CYSTIDIA not observed. UNIVERSAL VEIL of repent, filamentous hyphae, (31/1), 2.8 - 7.0 ($\bar{x}$=4.6) µm diam., radially arranged, loose, gelatinized, with abundant epimembranal pigment. CLAMP CONNECTIONS present.

HABIT, HABITAT AND PHENOLOGY - gregarious on the ground under eucalypts. Specimens collected in June and July.

MATERIAL - SOUTH AUSTRALIA: Mt Lofty, 9.vii.1927, AD 4246 (holotype of C. ochraceus). Belair Recreation Park, 20.vi.1931, AD 4654 (holotype of C. sinapicolor).

Moser and Horak (1975:574) recorded Cortinarius ochraceus Clel. non Peck as a variety (var. australiensis) of C.paraochraceus Moser. However, examination of the holotype of C. ochraceus Clel. non Peck has shown it to have smaller spores than those quoted for C. paraochraceus var. australiensis Moser: 6.8 - 9.4 ($\bar{x}$=8.6) x 4.9 - 5.6 ($\bar{x}$=5.2) µm as compared to 8.5 - 10.5 x 4.3 - 5.2 µm for the latter variety. Moser and Horak did not examine the holotype of C. ochraceus Clel. non Peck, but rather another collection with larger spores, belonging to a different species (Mt Lofty, 19.vi.1921, AD 4243).

The yellowish to ochraceous pileus, brownish lamellae, cylindric, non radicating stipe, and gregarious habit indicate that this species belongs in Section Pyromyxae, as defined in Singer (1986:636).

4. Cortinarius austroalbidus Fig. 4 A-B

Cortinarius austroalbidus Clel. & Harris in Rec. S. Aust. Mus. 9:54, 1948.
C. albidus Clel. in Trans. R. Soc. S. Australia 57: 191, 1933 non Fr.

PILEUS 37 - 62 mm diam., convex becoming plane, subumbonate, glutinous, smooth, edge subfibrillose when old, white with occasional tints of Light Buff (XV), when old near Light Buff (XV); flesh thin, attenuated outwards, with a very faint tint of violet. LAMELLAE sinuately adnexed, moderately close, ventricose, up to 9 mm deep, Light Ochraceous Buff (XV) to near Ochraceous Buff (XV). STIPE up to 62 mm long, moderately stout, to 13 mm diam., cylindric or slightly bulbous below,

sticky, fibrillose, white with a very faint tint of violet. VEIL brownish. ODOUR of fenugreek when dry.

BASIDIOSPORES (52/1), 9.3 - 11.6 [-12.8] ($\bar{x}$=10.4) x 5.8 - 7.4 ($\bar{x}$=6.5) µm, L/B=1.6, ellipsoid to elongate ellipsoid, finely warty rough, slightly thick-walled, pale yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (24/1), 28.2 - 40.0 ($\bar{x}$=33.6) x 8.2 - 11.7 ($\bar{x}$=9.4) µm, with sterigmata up to 5.2 µm long, four-spored, clavate. CYSTIDIA absent. UNIVERSAL VEIL of filamentous, gelatinized hyphae, (31/1), 2.4 - 6.0 ($\bar{x}$=3.8) µm diam., loosely repent, undulating, pigment not seen. CLAMP CONNECTIONS not seen.

HABIT, HABITAT AND PHENOLOGY - gregarious on the ground under eucalypts. Specimen collected in June.

MATERIAL - SOUTH AUSTRALIA: Belair Recreation Park, 29.vi.1932, AD 4113 (holotype).

The small to medium-sized basidiome, whitish pileus, pale lamellae, and clavate to somewhat bulbous stipe indicate that this species belongs in Section Malvacei, as defined in Singer (1986:638).

5. Cortinarius archeri Berk. - Fig. 5 A-B

Cortinarius archeri Berk. in Hook. f. Fl. Tasm. 2: 247, t.181/7, 1860.

PILEUS 63 - 89 mm diam., deeply convex then convex becoming plane, finally often with upturned edges and irregular, or with deep depressions and bosses, very viscid, deep violet becoming brown (Verona Brown (XXIX) to Bistre (XXIX) with a violet tint); flesh thin except over disc. LAMELLAE slightly sinuate to adnate, moderately close, to 10 mm deep, sometimes with reticulated ridges on the sides, Snuff Brown (XXIX) with a violet tint, especially on the edges. STIPE 63 - 89 mm long, stout, 18 - 25 mm diam. or more, at first bulbous below, usually slightly attenuated in the middle, sometimes flattened, striate above, hollow below, Deep Dull Bluish Violet (XXIV) below the glutinous subdistant ring, paler lilac above.

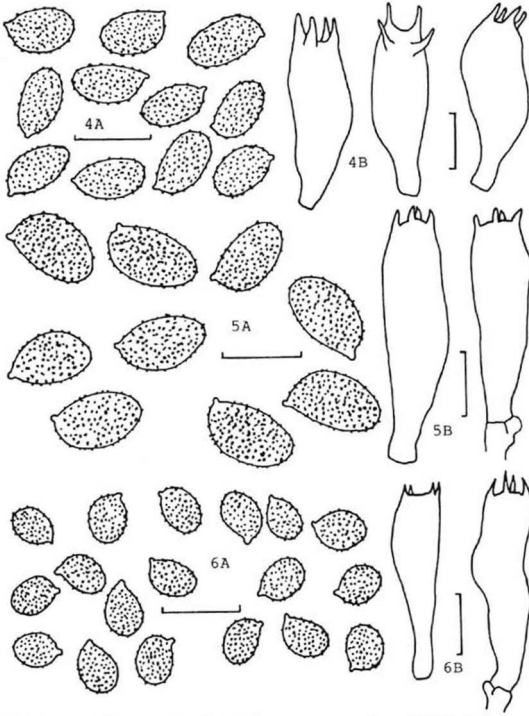

Fig. 4. Cortinarius austroalbidus.- A. Basidiospores. B. Basidia. Fig. 5. C. archeri.- A. Basidiospores. B. Basidia. Fig. 6. C. microarcheri - A. Basidiospores. B. Basidia. Bars = 10 µm.

BASIDIOSPORES (61/5), 11.4 - 15.8 [-17.6] ($\bar{x}$=13.4) x 6.4 - 8.6 [-9.4] ($\bar{x}$=7.6) µm, L/B=1.8, amygdaliform, finely warty rough, thick-walled, pale yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (20/2), 32.0 - 43.2 ($\bar{x}$=37.4) x 8.2 - 12.0 ($\bar{x}$=9.8) µm, with sterigmata up to 7.6 µm long, four-spored occasionally two-spored, clavate. CYSTIDIA not observed. UNIVERSAL VEIL glutinous, to 590 µm deep, of filamentous, loosely repent to ascending hyphae, (20/2), 2.0 - 4.2 ($\bar{x}$=3.5) µm diam. PILEAL CUTICLE a narrow band of repent, filamentous to cylindric hyphae. CLAMP CONNECTIONS present.

HABIT, HABITAT AND PHENOLOGY - solitary, gregarious or caespitose on the ground. Specimens collected in March, May and June.

MATERIAL - TASMANIA: Cheshunt, 16.iv.1856, K holotype, W. Archer. NEW SOUTH WALES: Mosman, Sydney, 20.v.1917, AD 4103. SOUTH AUSTRALIA: Mt Lofty, 25.v.1920, AD 4105; 29.iii.1924, AD 4099. Belair Recreation Park, 16.v.1931, AD 4101. Woodside, 19.vi.1946, AD 4102. Adelaide hills, v.1954, AD 4100, N. Atkinson.

This species can be distinguished from Cortinarius subarcheri Clel. and C. bundarus Grgurinovic by its large, amygdaliform spores. Cortinarius archeri constitutes the type of Section Archeriani.

6. Cortinarius microarcheri Clel. - Fig. 6 A-B

Cortinarius microarcheri Clel. in Trans. R. Soc. S. Australia 57:191, 1933.

PILEUS 18 - 62 mm diam., convex to nearly plane, edge sometimes striate, glutinous, deep violet or violet-brown, drying from the centre to earth-brown or light brown (Amber Brown, XL), flesh violet-tinted or whitish. LAMELLAE slightly sinuate to adnexed, moderately close, pallid violet to violet-brown, (Buffy Brown (XL), with violet tints), then earth-brown (Snuff Brown, XXIX). STIPE 31 - 50 mm long, rather slender, base a little thickened, fibrillose, slightly hollow or solid, pallid or pale violet.

BASIDIOSPORES (54/6), 6.4 - 8.6 ($\bar{x}$=7.1) x 4.8 - 8.3 ($\bar{x}$=5.3) µm, L/B=1.3, short ellipsoid to ellipsoid, warty rough, slightly thick-walled, yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (17/2), 27.2 - 41.2 ($\bar{x}$=33.7) x 6.4 - 9.6 ($\bar{x}$=8.4) µm, with sterigmata up to 6.4 µm long, four-spored, clavate. CYSTIDIA absent. UNIVERSAL VEIL to 250 µm deep, of filamentous hyphae, (11/1), 3.1 - 4.8 ($\bar{x}$=4.2) µm diam., gelatinized, lower portion of loosely ascending hyphae, upper portion of loosely repent hyphae. PILEAL CUTICLE to 96 µm deep, of repent, filamentous hyphae, (7/1), 3.6 - 5.5 ($\bar{x}$=4.8) µm diam., with plasmatic pigment. CLAMP CONNECTIONS present.

HABIT, HABITAT AND PHENOLOGY - solitary to gregarious on the ground. Specimens collected in June.

MATERIAL - SOUTH AUSTRALIA: Mt Lofty, 16.vi.1917, AD 4231 (lectotype, here designated); 19.vi.1921, AD 4219 (syntype); 23.vi.1928, AD 4224 (syntype); 8.vi.1931, AD 4222 (syntype); 8.vi.1931, AD 4223 (syntype). Eagle on the Hill, 5.vi.1932, AD 4230 (syntype). Kersbrook, 25.vi.1933, AD 4227, Dr Rogers.

The violet pileus, violet-brown lamellae, and ellipsoid spores indicate that this species belongs in Section Archeriani, Stirps Iodes (Moser & Horak, 1975:578; Singer, 1986:638).

7. Cortinarius subarcheri Clel. - Fig. 7

Cortinarius subarcheri Clel. in Trans. R. Soc. S. Australia 52:220, 1928 p.p.

PILEUS 25 - 60 mm diam., at first deeply convex then expanding to convex, occasionally with a depression in the centre, viscid, becoming shining, violet becoming brownish violet to violet (Brownish Vinaceous to Deep Brownish Vinaceous (XXXIX) with tints of Deep Purplish Vinaceous (XLIV) or Mars Brown (XV) and darker towards the edge). LAMELLAE adnate or sinuate, close, narrow, becoming slightly ventricose, violet becoming

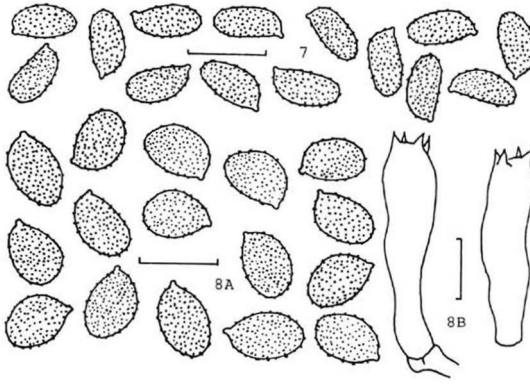

Fig. 7. Cortinarius subarcheri.- Basidiospores.
Fig. 8. Cortinarius bundarus.- A. Basidiospores. B. Basidia. Bars = 10 µm

Vinaceous Fawn (XL), Sorghum Brown (XXXIX), Prout's Brown (XV) or Apricot Buff (XIV). STIPE 35 - 51 mm long, stout, cylindric or base slightly bulbous, root rather conical, 15 - 25 mm diam., fibrillose, solid, sticky when moist, whitish to violet (Pale Lobelia Violet, XXXVII, Grayish Lavender to Deep Grayish Lavender, XLIII). FLESH white with a faint violet tint in stipe, or paler than Grayish Lavender. VEIL white.

BASIDIOSPORES (54/4), 8.6 - 11.6 ($\bar{x}$=9.5) x 4.8 - 6.1 ($\bar{x}$=5.3) µm, L/B=1.8, elongate ellipsoid, sometimes approaching cylindric, finely warty rough, slightly thick-walled, yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (3/1), 34.7 - 37.6 x 7.7 - 8.7 µm, with sterigmata up to 2.4 µm long, four-spored, clavate. CYSTIDIA not observed. UNIVERSAL VEIL to 354 µm deep, of loosely arranged, filamentous, gelatinized hyphae, (10/1), 2.4 - 4.3 ($\bar{x}$=3.5) µm diam. PILEAL CUTICLE

a narrow band of filamentous hyphae, with brownish pigment. CLAMP CONNECTIONS present.

HABIT, HABITAT AND PHENOLOGY - solitary, usually gregarious on the ground under Eucalyptus baxteri (Benth.) Maiden & Blakely, etc. Specimens collected in May and June.

MATERIAL - SOUTH AUSTRALIA: Mt Lofty, 16.vi.1917, AD 4220; 16.vi.1917, AD 4225; 19.vi.1920, AD 4226. Kinchina, 7.vii.1923, AD 4661. Mt Burr Forest Reserve, 30.v.1928, AD 4316 (lectotype, here designated). Morialta, 3.vi.1933, AD 4311. Willunga Hill, v.1932, AD 4313. Echunga, 12.vi.1939, AD 4662.

The violet-brown pileus and lamellae, and the elongate ellipsoid spores indicate that this species belongs in Section Archeriani, Stirps Iodes (Moser & Horak, 1975:578; Singer, 1986:638).

8. Cortinarius bundarus Grgurinovic, sp.nov. - Fig. 8 A-B

Cortinarius subarcheri Clel. in Trans. R. Soc. S. Australia 52:220, 1928 p.p.

Pileus usque ad 89 mm diametro, irregulariter convexus, violaceus deinde versus apicem pallide brunneolus. Lamellae sinuatae, moderate confertae, ex violaceo caryophyllaceo-cinnamomeae. Stipes usque ad 38 mm longus, crassus, fibrillosus, ex violaceo pallidus. Sporae 9.4 - 10.8 [-11.6] ($\bar{x}$=10.1) x 6.0 - 7.3 ($\bar{x}$=6.7) µm, ellipsoideae, verrucosae. Basidia 32.0 - 44.4 ($\bar{x}$=37.5) x 8.0 - 10.8 ($\bar{x}$=9.4) µm, clavata, 4-sporigera. Holotypus: South Australia, Bundaleer State Forest, vi.1928, AD 4329.

PILEUS up to 89 mm diam., irregularly convex, violet, becoming pale brownish in centre. LAMELLAE sinuate, slightly toothed, moderately close, Pinkish Cinnamon (XXIX) tinged with violet. STIPE up to 38 mm long, stout, to 38 mm diam., downy-fibrillose, violet-tinted. FLESH with violet or lilac tints.

BASIDIOSPORES (50/1), 9.4 - 10.8 [-11.6] ($\bar{x}$=10.1) x 6.0 - 7.3 ($\bar{x}$=6.7) µm, L/B=1.5, ellipsoid, coarsely warty rough, slightly thick-walled, yellowish brown in 3% aqueous solution of potassium hydroxide. BASIDIA (15/1), 32.0 - 44.4 ($\bar{x}$=37.5) x 8.0 - 10.8 ($\bar{x}$=9.4) µm, with sterigmata up to 4.0 µm long, four-spored, clavate. CYSTIDIA not observed. UNIVERSAL VEIL to 230 µm deep, of filamentous, gelatinized hyphae, (11/1), 1.9 - 4.3 ($\bar{x}$=3.1) µm diam., loosely repent to somewhat ascending. CLAMP CONNECTIONS present.

HABIT, HABITAT AND PHENOLOGY - gregarious on the ground. Specimen collected in June.

MATERIAL - SOUTH AUSTRALIA: Bundaleer State Forest, vi.1928, AD 4329 (holotype).

This species is distinguished from Cortinarius subarcheri by its ellipsoid and slightly larger spores. The violet pileus, violet tinged lamellae and ellipsoid spores indicate that this species belongs in Section Archeriani, Stirps Iodes, as defined in Singer (1986:638). The specific epithet is derived from the aboriginal word "bundara", meaning a "clump of trees".

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STUDIES ON *GALEROPSIS* AND *GASTROCYBE* (BOLBITIACEAE, AGARICALES)

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ABSTRACT

After we have studied the holotype of *Galeropsis desertorum* Velen. & Dvorak, type specimen of the genus *Galeropsis*, the presence of a hymeniform epicutis is proved contrary to the traditional interpretation as a cutis. The same happens with *G. bispora* Vasil'kov and *G. andina* Singer. The genus *Gastrocybe* Watling is considered as synonymous of *Galeropsis* Velen. We propose the following new combinations: *Galeropsis lateritia* (Watling) comb. nov., *G. deceptiva* (Baroni) comb. nov. and *G. desertorum* var. *bispora* (Vasil'kov) comb. et status nov.

INTRODUCTION

The genus *Galeropsis* Velen. was described by VELENOVSKY (1930) and in his original description the following microscopic characters were specified: "Basidiis subglobosis, quadristerigmatosis. Pulvere fusco. Sporis ovato-ellipticis, ochraceo-luteis, glabris. Cystidiis nullis.", without any reference to the structure of the epicutis.

Later on, SINGER (1963, 1986), WATLING (1968), SINGER & PONCE DE LEON (1982) and MORENO & al. (1987) considered the epicutis of *Galeropsis* as a cutis. On the other hand WATLING (1968) described the genus *Gastrocybe* with a hymeniform epicutis as the main differentiating character.

However, there are some references in the literature on the presence of a hymeniform epicutis in the genus *Galeropsis*, e.g. the comments given by Singer and compiled by HEIM (1950) in relation to *G. plantaginiformis* (Lebedeva) Singer which we reproduce he-

* This paper was presented at the "X Congress of European Mycologists" celebrated in USSR (20-25 August 1989), and at the "VIII Simposios de Ciencias Criptogámicas" celebrated in Melilla (23-26 September 1989).

re: "Le péridium est composé de deux couches, l'enveloppe extrême consiste en cellules en palissade, claviformes, brunes ou hyalines (10-11,5 µm de diam.), l'autre plus épaisse, devient vers l'intérieur une couche de jonction orientée transversalement, généralement plus dense vers l'intérieur".

WASSER (1979) described the following: "Epicutis of the cap consists of brownish and colourless cells (17-22 x 5-10 µm) and globate hyphae (10-12 µm diam.). Dermatocystidi absent".

The studies on the epicutis, very important in the differentiation of these genera, have not been mentioned neither in the studied species by HEIM (1950), nor in the following taxa described as new to science: *Galeropsis allospora* Singer, *G. angusticeps* (Peck) Singer, *G. liberata* (Kalchbr.) Heim, *G. madagascariensis* Singer and *G. mitraeformis* (Berk.) Heim.

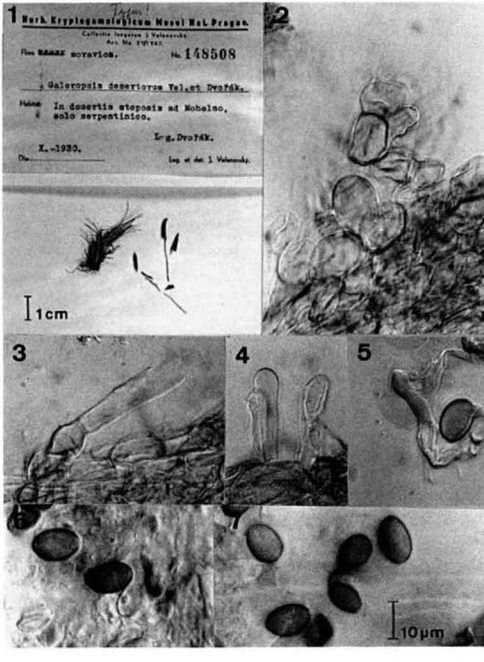
However SINGER (1963), when he described *Galeropsis andina* Singer, pointed out: "epicutis of the peridium not gelatinized, of elongated hyphae which forms a cutis", which makes us think on the possible existence of taxa without a hymeniform structure in their epicutis, conclusion which is wrong, as we will show further on (the revision of *G. andina* has proven that this species has a hymeniform epicutis).

The presence of pileocystidia has not been described for *Galeropsis desertorum*, *G. plantagiiniformis* and *G. bispora*. However, they have been confirmed in *Gastrocybe iberica* (MORENO & al., 1987).

Material examined: *Galeropsis desertorum* (holotypus) PR 148508; *Galeropsis bispora* (isotypus) PR 678864; *Galeropsis plantagiiniformis* (isotypus) PR 678867; *Galeropsis desertorum* var. *bispora* (as *G. desertorum*), on roots of *Poa bulbosa*, Iran (Kabus), leg. T.F. Hewes, MA-Fungi 16932. *Galeropsis andina* Singer (isotypus) BAFC 31514.

Conclusions: We think that *Galeropsis desertorum* Velen. & Dvorak (Figs. 1-7), type specimen of the genus *Galeropsis* Velen., is very variable in the size of its carpophores and the measurements of its spores; this species presents lageniform pileocystidia (sometimes difficult to observe if the material has been badly dried or colapssed), and a hymeniform epicutis formed by a single layer of cells instead of a cutis as traditionally was thought. The latter makes that we believe the genus *Galeropsis* must be emended and, besides, that the genus *Gastrocybe* is synonymous of *Galeropsis* and therefore its described species have to be transferred to the genus *Galeropsis*.

The bisporic species *Galeropsis bispora* Vasil'kov (Figs. 16-24) and *G. plantagiiniformis* (Lebedeva) Singer (Figs. 8-15) have also been revised, and it turns out that we consider *G. bispora* as a bisporic variety of *G.*



Figs. 1-7. *Galeropsis desertorum*. Basidiocarps, epicutis, pileocystidia and spores. (Holotypus, PR 148508)

desertorum, being the main character to differentiate it the constancy of its bisporic basidia, without having observed any tetrasporic. On the other hand, the fact it has spores with a bigger size than *G. desertorum* is logical in a bisporic variety. The epicutis and the presence of pileocystidia are similar to those of *G. desertorum*, and they were not described up to date.

The species *Gastrocybe iberica* Moreno, Illana & Heykoop (Figs. 25-29) is considered by us as synonymous of *Galeropsis bispora* (vide comparative table).

The typus of *Galeropsis plantaginiformis* presents tetrasporic basidia and very uncommonly some bisporic. The morphology of the spores (spore wall, germ pore) and the measurements are similar to those of *G. desertorum*, therefore both species are considered as synonymous by us as well as did PILAT (1948) and VASIL'KOV (1954), contrary to the opinion of SINGER (1936, 1951, 1956), HEIM (1950) and KOTLABA & POUZAR (1959).

After studying an isotypus of *Galeropsis andina* we have observed that it presents a hymeniform epicutis (Figs. 30-35), formed by a single layer of cells, and not a cutis as pointed out by SINGER (1963). The morphology of the spores and basidia agree with the description given by this author.

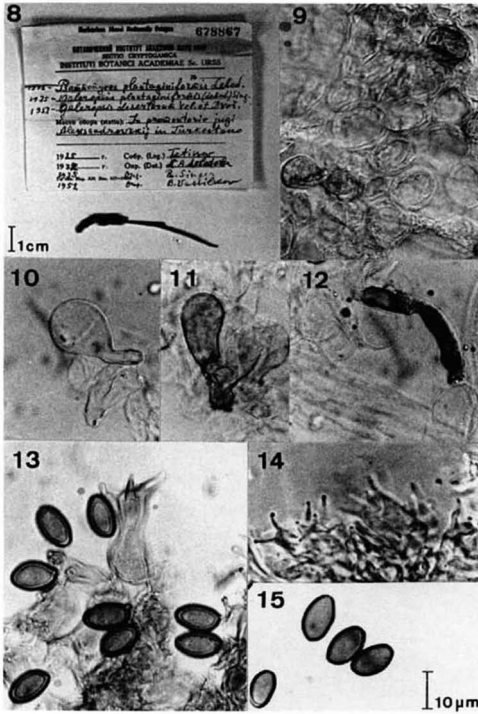
GALEROPSIS Velenovsky, Mykologia, Praha 7:105 (1930.
= ***GASTROCYBE*** Watling, The Michigan Botanist 7:20 (1968).
= ***PSAMMOMYCES*** Lebedeva, Tr. Zasc. Rast. 5(1):116 (1932).
= ***CYTTAROPHYLLUM*** (Heim) Singer, Beih. bot. Cbl. 56/B:147 (1936).

Galeropsis lateritia (Watling) Moreno, Heykoop & Illana comb. nov.
≡ ***Gastrocybe lateritia*** Watling, The Michigan Botanist 7:20 (1968).

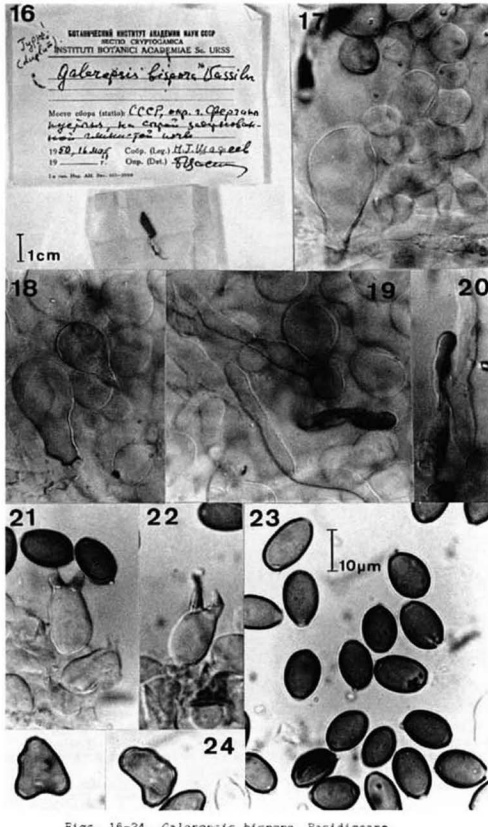
Galeropsis deceptiva (Baroni) Moreno, Heykoop & Illana comb. nov.
≡ ***Gastrocybe deceptiva*** Baroni, Mycologia 73:181-182 (1981).

Galeropsis desertorum Velen. & Dvorak var. bispora (Vasil'kov) Moreno, Heykoop & Illana comb. et status nov.
≡ ***Galeropsis bispora*** Vasil'kov, Proc. Bot. Inst. Acad. Sci. USSR, ser. 2. 9:463 (1954).
= ***Gastrocybe iberica*** Moreno, Illana & Heykoop, Cryptogamie, Mycol. 8:323-324 (1987).

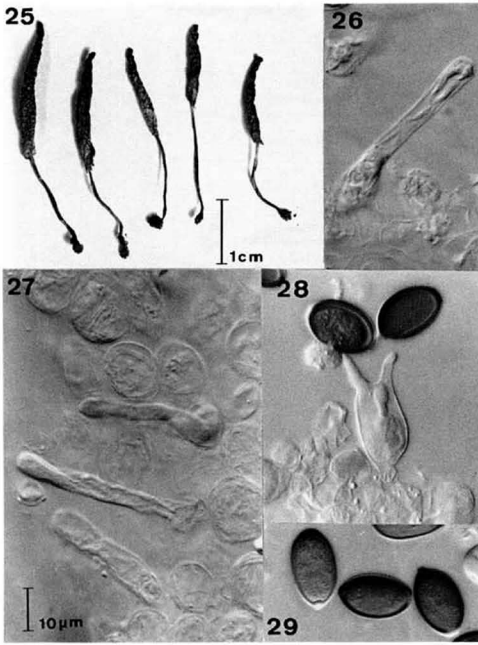
On the following table we compare the epicutis, basidia and basidiospores of *Galeropsis desertorum* Velen. & Dvorak, *G. plantaginiformis* (Lebedeva) Singer, *G. bispora* Vasil'kov and *Gastrocybe iberica* Moreno, Illana & Heykoop.



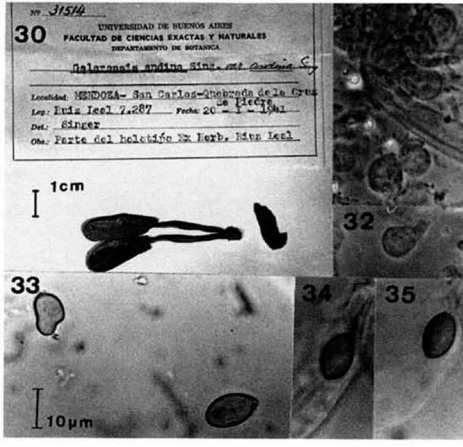
Figs. 8-15. *Galeropsis plantaginiformis*. Basidiocarp, epicutis, pileocystidium, basidia and spores. (Isotypus, PR 678867).



Figs. 16-24. *Galeropsis bispora*. Basidiocarp, epicutis, pileocystidia, basidia and spores. (Isotypus, PR 678864).



Figs. 25-29. *Gastrocybe iberica*. Basidiocarps, epicutis, pileocystidia, basidium and spores. (Holotypus, H.AH 9990).



Figs. 30-35. *Galeropsis andina*. Basidiocarps, epicutis and spores. (Isotypus, BAFC 31514).

Galeropsis desertorum

Epicutis
Hymeniform with lageniform pileocystidia

Basidia
Tetrasporic

Spores
11-14 x 7-8 µm (KOTLABA & POUZAR, 1959)

Galeropsis plantaginiformis

Epicutis
Hymeniform with lageniform pileocystidia

Basidia
Bisporic (SINGER, 1963) tetrasporic predominate (personal revision)

Spores
10-12,5 x 5,5-8,3 µm (HEIM, 1950)
9-12,5 x 5,5-7,2 µm (KOTLABA & POUZAR, 1959)
10,5-13 x 6-7,5 µm (personal revision)

Galeropsis bispora

Epicutis
Hymeniform with lageniform pileocystidia

Basidia
bisporic

Spores
14-16 x 10-11 µm (KOTLABA & POUZAR, 1959)
12,8-15(17,5) x 7,5-9,8 (10,5) µm (personal revision)

Gastrocybe iberica

Epicutis
Hymeniform with lageniform pileocystidia

Basidia
bisporic

Spores
15-20(23) X 9-13(16) µm MORENO & al., 1987)

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PHYTOPHTHORA ERYTHROSEPTICA

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ABSTRACT

Phytophthora erythroseptica was established by Pethybridge in 1913 as the fungus causing pink rot of potato tubers. It was characterized by formation of nonpapillate sporangia in water and the production in single cultures of abundant relatively large oogonia with amphigynous antheridia. Recently, several authors questioned the species concept of P. erythroseptica by including isolates that produced both amphigynous and paragynous antheridia. The present study was undertaken to compare 14 isolates of P. erythroseptica from various parts of the world in order to re-define the species based on morphological and cultural characteristics. It was concluded that isolates producing paragynous antheridia should not be assigned to P. erythroseptica.

INTRODUCTION

Phytophthora erythroseptica Pethyb. was named by Pethybridge (1913) as the incitant of pink rot of potato tubers. It was characterized by the formation in water of "conidia.....more or less ovate or inversely pear-shaped, with a rather blunt or even somewhat flattened apex" and the production in single culture of abundant sex organs so that "the oogonial incept enters the antheridium at or near its bases, grows up through it and out at the top, expanding there to form the oogonium proper in which the oospore develops". This unique method of sexual reproduction in P. erythroseptica was confirmed by Murphy (1918) who described the antheridium as "amphigynous" in contrast to the "paragy-nous antheridium which grew up to the side of the oogonium".

Since the erection of P. erythroseptica, three varieties have been proposed: var. atropa on Atropa

belladonna (Alcock, 1926), var. pisi on pea (Bywater & Hickman, 1959) and var. drechsleri as a new combination for P. drechsleri Tucker (Sarejanni, 1936). They were all rejected by Waterhouse (1963) who considered var. atropa not significantly different from var. erythroseptica and var. drechsleri synonymous with P. drechsleri which she retained and var. pisi, invalidly published for failing to cite the type specimen. But she retained var. pisi in her key and considered P. himalayensis (Dastur, 1948) identical to P. erythroseptica. Regardless of their taxonomic status, members of these taxa had one character in common: the production of entirely amphigynous antheridia.

However, Cooper (1928) studied the type culture of P. erythroseptica and found no amphigynous antheridia. Instead, he found paragynous antheridia closely appressed at the base of the oogonia. Converse and Schwartz (1968) identified the causal agent of root rot of red raspberry as P. erythroseptica sensu lato even though paragynous antheridia were produced in addition to the abundant amphigynous antheridia. Savage et al. (1968) also found that some isolates of P. erythroseptica produced paragynous antheridia and amphigynous antheridia in varying proportions. They pointed out the similarity between P. erythroseptica and P. megasperma Drechs. in producing nonpapillate sporangia and both antheridial types in single cultures as well as pathogenicity to potato tubers. Recently, Pratt (1981) identified some fast growing isolates from arrowleaf clover tentatively as P. erythroseptica although the antheridia were predominantly paragynous in agar but mostly amphigynous in broth cultures. He also questioned the distinction between P. erythroseptica and P. megasperma based on the antheridial type. Thus, Erwin (1983) listed P. erythroseptica as one of the few species of Phytophthora where particular nomenclature problems exist requiring critical re-evaluation.

The present study was undertaken to compare colony morphology and culture characteristics of a wide variety of isolates of P.erythroseptica under uniform conditions in order to define the species more precisely.

MATERIALS AND METHODS

Isolates and media: Specific information on the isolates of P. erythroseptica used is given in Table 1. All isolates were obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. Unless otherwise stated, clarified V-8 juice agar medium (Ribeiro, 1978) supplemented with sitosterol (30 mg/l) (CV8) was used for culture.

Morphology: Colony characteristics on CV8 were compared after incubating the cultures in darkness at 20C

Table 1. Sources of Phytophthora erythroseptica isolates

ATCC	Host	Origin	Source
10923(a)	Unknown	USA	Jeffers
10924	Potato	Unknown	Jeffers
16698	Unknown	USA	Gallegly N65
16699(T*)	Potato	India	Gallegly N117=CMI 17028
28766	Potato	Scotland	Pitt 38
36302	Potato	USA	Rowe 100
46725	Potato	Tasmania	Zentmyer P340
53014(T)	Potato	Ireland	Ho H14.1 = CMI 34684
64047	Wild rice	USA	Ho H14.12 Gunnell
64127	Unknown	Unknown	CBS 233.30
64128	Tulipa	Unknown	CBS 380.61
64155	Unknown	United Kingdom	CMI 146453
64156	Potato	United Kingdom	CMI 181716
64861	Potato	United Kingdom	UCR P3513

(a) Received as P. drechsleri
(T*) Type of P. himalayensis
(T) Type Culture

ATCC -- identified by American Type Culture Collection accession number
CMI -- Commonwealth Mycological Institute, Kew, Surrey, England
UCR -- University of California, Riverside

for 7 days. The colony diameters were measured at right angles through the inoculum, and the width of primary hyphae measured using a light microscope. The minimal and maximal temperatures for growth were tested by growing the isolates at 5 C and 35 C. Sporangia formation was induced by incubating small mycelial agar discs on CV8 in freshly collected stream water that had been filtered through 0.45 µm pore size membrane discs and incubating the produced discs under light at 20 C. Sex organs in single cultures were examined periodically by microscopy through the bottom of the petri dish. If the isolate failed to produce sex organs in single culture, it was paired with the appropriate mating types of P. nicotianae (ATCC 38606, A_2 and ATCC 38607, A_1). In case of a successful mating, the ability of the isolate to produce sex organs by selfing was confirmed by pairing it with the compatible

strain across a polycarbonate membrane to prevent physical contact between the cultures (Ko, 1978). They were examined for sex organs after 2-3 wk incubation in the dark at 20 C. If these attempts failed to induce formation of sex organs, the isolate was grown on tryptophan medium (Ribeiro, 1978) and sterilized oat grains (Van der Zwet & Forbes, 1961) and examined after 4-5 months.

RESULTS

Colony morphology

Isolates of P. erythroseptica grew well on CV8 (4-8 mm/day at 20 C) forming uniform, non-fluffy to moderately fluffy colonies with a diffuse and slightly irregular outline and no special growth pattern. ATCC 64047 colonies were distinctly fluffy with tall, erect aerial hyphae almost reaching the cover of the petri plate. ATCC 16699 colony radial growth was exceptionally slow (2mm/day), whereas ATCC 58104 grew faster than the others (11 mm/day). No isolates grew at 35 C, while some grew at 5 C (Table 2). The leading hyphae were uniform and fine, measuring 5-6 μm wide. In most isolates, small spherical to angular swellings in clusters or in chains were produced when mycelial agar discs were transferred to water although ATCC 46725 produced them also in old cultures.

Sporangia

All isolates produced sporangia readily in water within 24-48 hr, except ATCC 16698 and ATCC 64861 which formed them sparsely only in non-sterile stream water. The sporangia were non-deciduous, non-papillate and borne terminally on short sympodial branches of a slender sporangiophore. They were mostly 40-55 x 25-30 μm (Table 2), obpyriform to ovoid with rounded base although elongated ellipsoidal sporangia with tapering bases were also found, especially in ATCC 36302, ATCC 46725 and ATCC 58104. ATCC 64047 produced distinctly larger sporangia (68±11 x 35±7 μm). The apex of the sporangium in all isolates flattened easily when prepared for microscopy, and the empty sporangium partially collapsed after oospore release. The base of the sporangium was often plugged, and the ability of sporangia to proliferate internally varied with the isolate. Internal proliferation of sporangia was rare in ATCC 16699, ATCC 28766, ATCC 36302, ATCC 46725 and ATCC 58104 and occurred mostly in nonsterile stream water only, but it was commonly found in ATCC 16698 and especially ATCC 64047. Repeated emergence of zoospores was observed only in ATCC 46725.

Sex organs

Most isolates of P. erythroseptica produced abundant

Table 2. Growth and sporangial characteristics of Phytophthora erythroseptica

ATCC	Colony	Growth at 5 C	Growth at 35 C	Hyphal Swellings(a)	Sporangium Length(L) (µm)	Sporangium Breadth(B (µm)	Sporangium L/B	Sporangium Median Constriction	Sporangium Flat apex	Sporangium Internal proliferation
10923	MF	+/-	--	+	48+6(b)	31+4	1.6+0.2	+/-	+/-	+/-
10924	MF	--	--	+	47+8	26+4	1.8+0.3	+/-	+	+/-
16698	MF	--	--	+	47+5	28+3	1.7+0.1	--	--	+/-
16699	MF	--	--	+	38+5(c)	23+3	1.7+0.3	--	--	--
28766	SF	--	--	+/-	54+6	31+5	1.8+0.3	--	+/-	+/-
36302	SF	--	--	+	56+7	28+3	2.0+0.1	+/-	+/-	+/-
46725	SF	+	--	+	49+7	27+5	2.0+0.4	+	+	+/-
58104	SF	+	--	+	42+8	24+3	1.8+0.4	+	+	+/-
64047	F	--	--	+/-	68+11	35+7	2.0+0.5	+/-	+	+
64127	NF	+	--	-	48+6	30+3	1.6+0.2	--	+/-	--
64128	MF	+/-	--	-	51+6	30+4	1.7+0.2	--	+/-	--
64155	NF	+	--	-	41+5	26+3	1.6+0.1	+/-	+/-	+/-
64156	NF	+	--	-	47+3	31+2	1.5+0.1	+/-	+/-	+/-
64861	NF	-	--	+	39+7	24+3	1.6+0.2	+	+	--

(a) Small, angular, clustered or in chain

(b) Mean + standard error, based on 50 measurements

(c) Less than 50 measurements due to paucity of sporangia

sex organs readily either in water immediately following sporangial production or in single cultures on agar plates in 2-3 wk. The spherical oogonia were rather large, mostly 35-40 μm diam (Table 3), narrowing abruptly to a tubular stalk and were non-pigmented, although they eventually became faintly yellow or straw-colored with age. The oospores were aplerotic to markedly aplerotic with oospore wall 2-4 μm thick. The antheridium was unicellular, amphigynous and cylindrical. Occasionally, structures resembling paragynous antheridia, as described by Ho et al. (1983), were found in addition to the amphigynous antheridium. The dimensions of the sex organs are summarized in Table 3.

Table 3. Dimensions of sex organs of Phytophthora erythroseptica

ATCC	Oogonium diameter(μm)	Oospore diameter(μm)	Free oogonial space (a)	Antheridium		
				Type	Length(μm)	Width(μm)
10923	34±3 (b)	28±3	32%	A	14±2	13±2
10924	37±4	31±3	30%	A	11±2(b)	12±1
16698	40±3	31±5	40%	A	12±2	16±1
16699	No sex organs					
28766	38±2	32±2	29%	A	12±2	12±2
36302	38±3	32±3	29%	A	12±2	13±1
46725	38±3	31±3	33%	A	14±3	13±2
58104	No sex organs produced in single culture					
64047	41±1	31±4	43%	A	17±3	14±2
64127	37±3	31±3	30%	A	12±2	12±2
64128	37±2	30±2	34%	A	13±2	12±1
64155	34±4	27±3	37%	A	11±2	13±1
64156	37±2	31±2	30%	A	12±2	12±1
64861	No sex organs produced in single cell					

(a) Free oogonial space = $\frac{\text{Oogonium diam} - \text{oospore diam} \times 100}{\text{Oogonium diam}}$

(b) Mean ± standard error, based on 50 measurements
A = Amphigynous

ATCC 46725 produced sex organs only in old cultures or on oat grains, whereas ATCC 16699 never produced sex organs. Sex organs were produced by ATCC 10924 only once in water but were consistently induced by pairing it with A1 mating types of P. nicotianae and other heterothallic species of Phytophthora (Table 4). Similarly, ATCC 58104 never produced sex organs in single cultures but produced them readily when crossed with an A2 mating type of P. nicotianae and other heterothallic species. None of the other isolates of P. erythroseptica demonstrated heterothallic behavior. However, the oogonia of ATCC 10924 and ATCC 58104 formed heterothallically by selfing on membranes were smaller, mostly 29-33 μm diam and pigmented (brown), different from those normally formed in single cultures.

Table 4. Production of sex organs in crosses between ATCC 10924 and ATCC 58104 Phytophthora erythroseptica and other species of Phytophthora

Name	ATCC	Mating Type	P. erythroseptica ATCC 10924	P. erythroseptica ATCC 58104
P. cinnamomi	32992	A2	---	+++
	32993	A1	+++	---
P. cryptogea	46721	A1	+++	---
	52403	A2	---	+++
P. nicotianae	38606	A2	---	+++
	38607	A1	+++	---
P. palmivora	26200	A2	---	+++
	26201	A1	+++	---
P. erythroseptica	10924		---	+++
	58104		+++	---

Chlamydospores

No chlamydospores was produced by any isolate of P. erythroseptica under the stated cultural conditions.

DISCUSSION

Phytophthora erythroseptica is well known as the causal agent of pink rot of potato tubers throughout the world (Hickman, 1958; Stamps, 1978) although it has minor hosts in belladonna (Alcock, 1926), tulip (Buddin, 1938), calla (Tompkins & Tucker, 1947,1950), sugarcane (Steib & Chilton, 1950; Singh, 1955), onion (Hickman, 1958), pea (Bywater & Hickman, 1959), tomato (Walker & McLeod, 1970), cineraria (Lucas, 1977), and calceolaria (anonymous, 1980). The disease in potato is characterized by formation of a deep salmon-pink coloration when affected tissue is exposed to the air due to a tyrosinase reaction (White, 1946) and was initially considered as a diagnostic symptom. It was later found that similar or identical symptoms could be induced by other species of Phytophthora by artificial inoculation, and in nature P. megasperma, P. cryptogea and P. drechsleri were also isolated from diseased potato (Tucker, 1931; Carnes & Muskett, 1933; Goss, 1949; Rowe & Schmitthenner, 1977). Thus, the precise identification of P. erythroseptica is critical to identification of the pathogen that causes pink rot of potato in the field. However, there have been controversies over the species concept of the pathogen. For the sake of discussion, the published data on P. erythroseptica are summarized in Table 5.

Table 5. Characteristics of Phytophthora erythroseptica recorded in literature chronologically

Ref.	Host	Growth Temperature Min. (C)	Max. (C)	Hyphal swellings	Sporangium Length(µm)	Breadth(µm)	L/B	Oogonium diam(µm)	Oospore diam(µm)	Wall	Antheridium Type	Length (µm)	Width (µm)
41	Potato				32	20	1.6				A		
46					45	28	1.6		36		A		
64	Atropa				44	26	1.7	38	33		A		
1	Atropa				48	30	1.6		29-32		A		
56	Potato	15	30					36	31		A		
8	Potato								30		A		
									32		A		
									31		A		
									32		A		
									32		A		
									33		A		
									32		A		
									32		A		
									31		A		
									31		A		
									31		A		
									32				
	Atropa(a)								33		A		
32									31				
5	Tulip				49	30	1.6	30-37	25-29	3	A		
27	Potato	4.8	34								A		
54	Pink calla	13	28		35	21	1.7	36	29	3-4	A	12-19	12-19
55	White calla				35	20	1.8						
12	Potato(b)			+	29-50	22-36		35	31	2-3	A		
37	Potato(b)				32	20	1.6	33-34	29-30		A		
28	Potato								29-32		A		
51	Sugarcane	8	28	+	61	37	1.6	47					
6		5	35		52	30	1.7	33	26	2.5	A	15	14
		5	35		57	31	1.8	34	26	2.0	A	12	12
		5	35		42	31	1.4	36	27	3.0	A	12	13
		5	35		48	26	1.8	No sex organ produced					

Table 5. (continued)

Ref.	Host	Growth Temperature Min. (C)	Max. (C)	Hyphal swellings	Sporangium Length(µm)	Breadth(µm)	L/B	Oogonium diam(µm)	Oospore diam(µm)	Wall	Antheridium Type	Length (µm)	Width (µm)
	Potato(b)	5	35		53	33	1.6	30	30	3.1		14	12
7	Pea(c)	12	30		86	35	2.5	37	26		A	19	18
					63	29	2.1						
50		5	35						29		A		
57	Potato		35										
62		2.5	34	+	43	26	1.7	30-35			A	14	13
30	Potato		33	+	38	31	1.5	43-48			A	14	13
14	Potato	2.5	33	+	41	29	1.4	35	28		A	15	14
					48	30	1.5						
					51	31	1.6						
58	Potato		34		42	26	1.6	36	31		A	13	13
			34		42	27	1.5	35	31		A	14	12
			34		43	27	1.6	35	31		A	15	13
			34		43	27	1.6	35	30		A	15	13
			34		46	26	1.7	35	31		A	15	13
			32-34		37	26	1.5	35	28		A	13	13
			34		43	26	1.6	35	31		A	15	13
			30-32		42	28	1.5	34	24		A	13	12
			34		43	27	1.6	42	34		A	17	16
40					32	20	1.4-1.6		29-30		A	11-20	10-18
47	Potato		35	+	45-50						A	15	
34	Cineraria				44	27	1.6	35			A	13-14	
52		2.5	34	+	43	26	1.7	30-35			A	14	13
39								28-50	20-35		A	cylindrical	
26	Tomato	8-15	30		28-65	18-38		20-40			A	15-17	
18	Potato		34	+	44	31	1.4	42	39		A	16	12
31	Potato	2	32-35	+	46	30	1.6	37	32	1.5-4	A		
9	Potato				Elliptical						A	Elongated	
20	Wild rice	9	36		64	37	1.8	41	33		A	15	15
35		5-10	30-35										

(a) P. erythroseptica var. atropa (b) P. himalayensis = (P. erythroseptica) (c) P. erythroseptica var. pisi A= Amphigynous

Our study confirmed the findings of previous workers concerning the cultural and hyphal characteristics of P. erythroseptica. Isolates of P. erythroseptica grew well on common agar media, producing non-fluffy to fluffy colonies with uniform and fine hyphae. ATCC 16699 which grew exceptionally slow, showed signs of degeneration probably as a result of long-term storage. In literature, P. erythroseptica var. pisi was unusual in having irregular, tortuous hyphae, whereas the luxuriant, tall aerial mycelium of ATCC 64047 in the present study distinguished itself from the other isolates. Small angular to spherical hyphal swellings, in clusters or in chains, as reported earlier by some researchers, were also produced by some isolates in the present study, usually in water culture. Chlamydospores were never observed. Previous authors also failed to record chlamydospores in P. erythroseptica although Ershad (1971) and Krober (1985) reported chlamydospores, whereas hyaline spherical structures were interpreted as "poorly differentiated chlamydospores" by Tompkins & Tucker (1947) or "chlamydospore-like bodies" by Novotelnova (1974). As noted by previous workers, no isolates of P. erythroseptica grew at 35 C. The sole report of good growth at 35 C by P. erythroseptica (Van der Zwet & Forbes, 1961) needs to be confirmed. Tucker (1931) considered the rather narrow temperature range (15-30 C) a diagnostic character of P. erythroseptica, but this could neither be confirmed in the present study nor by most authors in the literature.

In the species description of P. erythroseptica by Waterhouse (1963) and Stamps (1978), the sporangia were described as variable in shape, ellipsoid or obpyriform (43 - 26 µm; L/B=1.65), often constricted near the middle, sometimes tapering to the sporangiophore. In our study, the overall mean length(L) and breadth(B) of the sporangia for all isolates were 48 ± 7 x 28 ± 3 µm with L/B=1.7 ± 0.2, agreeing well with those reported in literature (46 ± x 28 ± 4 µm; L/B=1.6 ± 0.2). ATCC 64047 produced distinctly larger sporangia that were similar to those reported for P. erythroseptica var. pisi although not as narrow. Median constrictions of sporangia were not mentioned in the original description (Pethybridge, 1913) and have been reported to occur only occasionally by Bywater & Hickman (1959) and Vargus & Nielson (1972). They were observed in some isolates in the present study, and were especially common in ATCC 46725 and ATCC 58104 which produced mostly elongated sporangia with tapering bases. The elongated sporangia of the type culture (ATCC 58104) differed from the obpyriform sporangia diagramed by Pethybridge (1913) for P. erythroseptica. It is not clear if the type culture has undergone changes accounting for the difference which could conceivably be due to different culture conditions. Similarly, ATCC 16699, the sporangia of the type culture of P. himalayensis (=P. erythroseptica) were oval to ellipsoidal instead of

"slipper-shaped" as described by Dastur initially (1948). The broad apex of sporangium of P. himalayensis could be due to its flattening upon mounting.

As reported previously, the sporangiophore of P. erythroseptica showed distinct sympodial branching, but we found that in most cases, the branching was close rather than "lax" (Waterhouse, 1963), and our observation seemed to agree with those of other authors based on their diagrams and/or photographs. Waterhouse & Blackwell (1954), Hickman (1940) and Waterhouse (1963) stated that internal proliferation of sporangia was rare in P. erythroseptica, whereas Gerrettson-Cornell (1981) failed to observe it in an Australian isolate from potato. In our study, the ability of the sporangium to proliferate internally varied with the isolate but was usually not as common as in other related species of Phytophthora with non-papillate sporangia. Many sporangia were plugged at the base after zoospore release. In fact, the sporangia of most isolates of P. erythroseptica, did not release zoospores easily unless the sporangia were chilled (Vujicic & Colhoun, 1966). Pethybridge (1931, 1914) experienced difficulty in inducing zoospore release in P. erythroseptica, whereas Dastur (1948) reported that the sporangia of P. himalayensis (=P. erythroseptica) germinated only "conidially". Neither of them noted internal proliferation of sporangia.

We have also confirmed previous observations that P. erythroseptica produces readily in single culture abundant rather large, smooth spherical oogonia narrowing abruptly to a tubular stalk. Apparently, some isolates of P. erythroseptica have lost their self-fertility due to prolonged storage and/or repeated subcultures. It is interesting to note that ATCC 10924 and ATCC 58104 behaved heterothallically instead, mating successfully with A_1 and A_2 mating types, respectively, of various Phytophthora species. This may be considered a case of "secondary heterothallism" derived from a homothallic precursor, lending support to the hypothesis that heterothallism probably evolved from homothallism (Brasier, 1983; Ho, 1986). Heterothallic isolates of normally homothallic P. megasperma have also been reported (Barr, 1980). However, the oogonia formed by ATCC 10924 and ATCC 58104 as a result of selfing on membranes when crossed with the appropriate mating strain of Phytophthora were smaller and brown in color, different from the larger, usually non-pigmented to pale yellow oogonia formed in single cultures of P. erythroseptica. It is difficult to assess the differences in oogonia characteristics without knowing the changes that have occurred during storage; the heterothallic behavior should not be considered typical for the species. ATCC 16698 never produced any sex organs alone or in pair cultures. The loss of sexuality by isolates of P. erythroseptica in artificial culture was noted by Bywater (1956), Carnes & Muskett (1933) and

Ribeiro et al. (1975). Waterhouse (1963) and Stamps (1978) described the oospores of P. erythroseptica as "nearly filling oogonium", but we found that the oospores in most isolates largely aplerotic. The overall mean values of oogonial diameters and oospore diameters for all isolates in our study were 37 ± 2 and 34 ± 4 μm, respectively, agreeing well with those in the literature (39 ± 3 and 34 ± 5 μm, respectively).

We are also in agreement with those authors who believed that P. erythroseptica produces solely amphigynous antheridia (Hickman, 1958; Tucker, 1931; Waterhouse & Blackwell, 1954; Waterhouse, 1963; Stamps, 1978; Gerrettson-Cornell, 1985). The brief report by Cooper (1928) that P. erythroseptica produced only paragynous instead of amphigynous antheridia should be questioned. It is also our opinion that Phytophthora species identified as P. erythroseptica in the past producing both amphigynous and paragynous antheridia should be reclassified because the identification was either debatable or provisional. Thus, the causal agent of root rot of red raspberry was identified as P. erythroseptica by Converse & Schwartz (1968), P. megasperma by Duncan et al. (1987) and P. fragariae by Wilcox (1989). Pratt (1981) identified some fast growing isolates from arrowleaf clover tentatively as P. erythroseptica, but a thorough search of the literature and a comparative cultural study under similar conditions showed that these isolates are within the range of intraspecific variability for P. megasperma and could be identified as such (Ho, unpublished). Morgan & Johnson (1965) identified the pathogen from vetch as P. erythroseptica because it produced only amphigynous antheridia. However, paragynous antheridia were later found in their isolate (Savage et al., 1968), and it should be re-classified as P. megasperma which it resembled closely based on the published photographs. There is no doubt that the antheridial type, along with the sporangial papillation, is the most important taxonomic criterion in Phytophthora (Tucker, 1931; Waterhouse, 1983; Gerrettson-Cornell 1985). It would be unwise to cloud the species concept of P. erythroseptica by including controversial isolates that produce both amphigynous and paragynous antheridia (Savage et al., 1968; Pratt, 1981). Gerrettson-Cornell (1985) considered the occasional presence of two or three antheridia (amphigynous) to a single oogonium a characteristic feature of P. erythroseptica but we could not confirm this observation in present study.

Since Pethybridge erected P. erythroseptica as a new species (1913) Leonian & Geer (1929) suggested combining P. erythroseptica and mexicana Hotson & Hartge, but this was promptly rejected by Tucker (1931). Bywater & Hickman (1959) proposed that P. cryptogea, P. drechsleri, P. erythroseptica, P. himalayensis and P. richardiae Buis

should be grouped together as one species: P. erythroseptica. Although we agree with Bywater (1956) that these species have much in common especially in growth-temperature relations and sporangial characteristics, P. richardiae seems distinct enough to be retained as a separate species (Ho, unpublished), whereas P. cryptogea (P. drechsleri) can be differentiated based on its heterothallism and usually smaller, brown oogonia with plerotic oospores. The merging of P. drechsleri with P. cryptogea, which has priority, is favored by many workers (Ho & Jong, 1986). Sarejanni (1936) treated P. drechsleri as P. erythroseptica var. drechsleri, but it was not well received. P. himalayensis should be treated as synonymous with P. erythroseptica as suggested by Waterhouse (1963). Thus, P. erythroseptica is characterized by non-papillate sporangia, homothallism and entirely emphigynous antheridia. Based on this broad species concept, the pea isolate was treated as a variety of P. erythroseptica: P. erythroseptica var. pisi (Bywater & Hickman 1959) even though initially it was considered different enough to warrant a new species epithet: P. pisi, (Bywater, 1956). The acceptance of P. erythroseptica var. pisi as a validly published taxon is debatable. Article 37 of the International Code of Botanical Nomenclature (Voss, 1983) stipulates that "publication on or after 1 Jan. 1958 of the name of a new taxon of the rank of family or below is valid only when the nomenclatural type is indicated". Waterhouse (1963) considered P. erythroseptica var. pisi, which was published in 1959 without specifically citing a type specimen according to recommendations 37A and 37B, to be invalid. On the other hand, it is true that the nomenclatural type was clearly indicated in Bywater & Hickman's paper. We believe that this well-defined and well-described taxon should be retained. The isolate from wild rice is of special interest. Although it was identified as P. erythroseptica sensu lato, (Grunnel & Webster, 1988), it is different from the other isolates in its distinctly fluffy colony, larger sporangia, poor growth on malt extract agar medium, inability to hydrolyze starch, sensitivity to HMI and lack of pigment production on casein-hydrolysate medium (Ho, unpublished) and may be considered as a new variety. ATCC 10924, received originally as P. drechsleri (Ho & Jong, 1986), is now re-classified as P. erythroseptica based on its homothallism. The isolate of P. erythroseptica from sugarcane was identified primarily based on the production of oogonia with amphigynous antheridia in single cultures (Steib & Chilton, 1950; Van der Zwet & Forbes, 1961), but a detailed description of the fungus was not published. Based on the data and photographs in Singh's Ph.D. Thesis (1955), the unusually large oogonia (46.75 $\pm$ 4.11 μm diam), the rather large obypyriform sporangia (60.94 $\pm$ 6.25 X 37.38 $\pm$ 3.66 μm; L/B=1.6) and the spherical hyphal swellings spaced evenly in chains suggest greater similarity with the large-spore type of P. megasperma

rather than P. erythroseptica sensu stricto. Perhaps, the antheridial configuration of the sugarcane isolate should be re-examined to determine if it produces any paragynous antheridia. Erwin (1965) initially identified the alfalfa isolate as P. cryptogea on account of its amphigynous antheridia but later renamed it as P. megasperma when paragynous antheridia were found.

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DEUTEROMYCOTINA FROM ANTARCTICA

NEW SPECIES OF HYPHOMYCETES FROM DANCO COAST, ANTARCTIC PENINSULA

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SUMMARY

Three new species of Hyphomycetes have been found recently in the Antarctic Continent, growing on rhizosphere soil of Colobanthus quitensis (Kunth) Bartl. (Caryophyllaceae) and Deschampsia antarctica Desv. (Gramineae).

The new species here proposed are: Acrodontium antarcticum nov. sp., Chalara antarctica nov. sp. and Phialophora dancoii nov. sp.

These new species were found during the "Campaña Antártica Argentina de verano 1989" in Danco Coast, Base Primavera (64º10'S, 60º57'W). The same locality is indicated in a map by Gamundí and Spinedi (1987).

The fungal isolation was carried out using the soil washing technique (Parkinson and Williams, 1961).

The following descriptions concerns observations in pure culture.

ACRODONTIUM ANTARCTICUM Cabello nov. sp. Fig. 1.

Coloniae in vitro post 20 dies 15 mm diametro, floccosae vel funiculosae, primum grisae ad olivaceae, mox nigrescentes. Reversum nigrum. Hyphae hyalinae ad pallide olivaceae, glabrae. Cellulae conidiogenae polyblasticae, integratae vel discretae, sympodiales, pallide brunneae, ad apicem pallidiores, plerumque 9-12 x 2.4-3 um et rachide tenuiter denticulata, recta vel flexuosa, 0.9-1 um crassa, ad 2.5-6.5 longa. Conidia acropleurogena, simplicia, hyalina, laevia, ovoidea basi apiculata, 2.5-3.5 x 1.5-2 um.
HOLOTYPUS: Antarctica, Peninsula Antarctica, Terra de Danco, Base Primavera, leg. M.N.Cabello, 31-I-1989. Ex solo. LPS 44594.

Colonies in vitro growing rather slowly, attaining a diam. of 15 mm in 20 days, appearing floccose or somewhat funiculose, at first grey to olivaceous, soon becoming blackish. Reverse black. Hyphae hyaline or pale olive,

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smooth. Conidiogenous cells polyblastic, integrated or discrete, sympodial, pale brown, paler towards the apex, 9-12 um hight, 2.4-3 um wide at the base,rachis denticulate often not distinctly differentiated from the basal part, straight or flexuous up to 2.5-6.5 um long and 0.9-1 um wide. Conidia acropleurogenous, hyaline, simple, smooth, ovoid, with an apiculate base, 2.5-3.5 x 1.5-2 um.

Our species differs from A. crateriforme (van Beyma) de Hoog (de Hoog, 1972) by the smaller size of the conidiogenous cells and the denticulate rachis which in A. crateriforme can be up to 45 um while in our species is up to 6.5 um. It also differs by the lack of sharp denticles in our species.
Holotypus: Antartic Peninsula, Danco Coast, Base Primavera, leg. M.N.Cabello, 31-I-1989, rhizosfere soil of Colobanthus quitensis. LPS 44594.

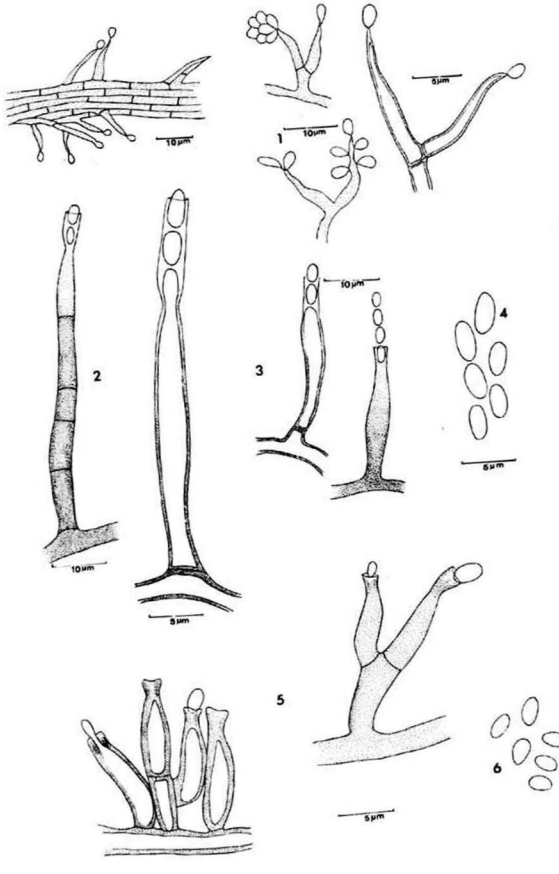
CHALARA ANTARCTICA Cabello, nov. sp. Fig. 2-4.

Coloniae in vitro post 30 dies 50 mm diametro, floccosae vel tomentosae, brunneae. Reversum brunneum. Phialophora erecta, simplicia, 1-4 cellularia, brunnea, cum phialidibus 60 um longa. Phialides elongatae, haut inflatae ad basim, 20-25 x 3 um; cylindraceo vel conico, interne 2-3 conidis munitae, 7-10 um longa. Conidia ellipsoidea, hyalinea, 3-4 x 2-2.1 um. Chlamydosporae absunt.
HOLOTYPUS: Antarctica, Peninsula Antarctica, Terra de Danco, Base Primavera, leg M.N.Cabello, 2-II-1989. Ex solo. LPS 44595.

Colonies in vitro attaining a diameter of 50 mm in 30 days, appearing floccose or tufted, brown. Reverse dark brown. Phialophores erect, simple, bearing phialides. Total lenght of phialophores with phialides 60 um with 1-4 septa. Phialides with a medium brown, slightly or not inflated venter, 20-25 x 3 um, and a cylindrical or obconical, light brown collar, cointaining 2-3 conidia, 7-10 um long. Conidia ellipsoidal, hyaline 3-4 x 2-2.1 um. Aleuroconidia or chlamydospores absent.

The more related species are C. microspora (Corda) Hughes and C. neocaledoniae Dadant ex Kiffer and Delon. Our species differs from C. microspora (Nag Raj and Kendrick, 1975) by the size of the conidia (C. microspora 3-8.5 x 1-1.5 um, C. antarctica 3-4 x 2-2.1 um). It also differs from C. neocaledoniae (Kiffer and Delon, 1983) by the size of the phialide that is larger in this species. Holotypus: Antarctic Peninsula, Danco Coast, Base Primavera, leg. M.N.Cabello, 2-II-1989, rhizosphere soil of Colobanthus quitensis and Deschampsia antarctica. LPS 44595.

ACRODONTIUM ANTARCTICUM. 1.Conidial structures and conidia.
CHALARA ANTARCTICA. 2. Conidiophore, phialide and conidia. 3. Phialides and conidia. 4. Conidia.
PHIALOPHORA DANCOII. 5. Phialides and conidia. 6. Conidia.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PHIALOPHORA DANCOII Cabello nov. sp. Fig. 5-6.

Coloniae modice lente crescunt, olivaceo-grisae, pulverulentae, tomentosae. Reversum olivaceo-nigrum. Hyphae vegetativae modice pigmentatae, 1.2-2 um latae. Phialides simplices vel conidiophora composita ramosa; phialides brunneae, 11-12 x 2-3 um, collare 1-2 um altum divergens. Conidia agregata, ellipsoidea, hyalina, laevia, 2.5-5 x 1.5-2 um.
HOLOTYPUS: Antarctica, Peninsula Antarctica, Terra de Danco, Base Primavera, leg M.N.Cabello, 30-I-1989. Ex solo. LPS 44596.

Colonies reaching 36 mm diam. in 24 days at 20ºC; olivaceous grey, powdery and tufted. Reverse olivaceous black. Vegetative hyphae slightly pigmented, 1.2-2 um wide. Conidiophores consisting of simple phialides, sometimes septate and branched. Phialides brown, 11-12 x 2-3 um, collarette 1-2 um, slightly divergent. Sympodial proliferation absent. Conidia in heads, ellipsoidal, hyaline, smooth-walled, 2.5-5 x 1.5-2 um.

The species most closely related with P. dancoii is P. phaeophora Gams (Gams and Holubová-Jechová, 1976), but differs by the shape of the conidia which are dacryoid with a truncate base in Gams species.
Holotypus: Antarctic Peninsula, Danco Coast, Base Primavera, leg M.N.Cabello, 30-I-1989. Isolated from rhizosphere soil of Colobanthus quitensis. LPS 44596.

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STUDIES ON PHOLIOTA IN CULTURE

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ABSTRACT

Cultural characters are reported for 22 species of *Pholiota* and allied genera. One or several polarity tests have been made for most species in order to determine their mating systems. One species (*P. jahnii*) is bipolar, the others tetrapolar but some are amphithallic. The culture characters reported here add new information of value for intrageneric delimitations. Intercompatibility tests are performed where the species limits are unclear. One new combination is proposed: *Pholiota lignicola*(Peck)S. Jacobss.

INTRODUCTION

In modern fungal taxonomy studies of mycelia in culture play an important role. Culture characters have been described for a great number of species, especially those belonging to Polyporaceae and Corticiaceae s.l. Interfertility tests are increasingly used to delimit species and resolve discussions of synonymy at the species level. Sometimes the use of culture characters and interfertility tests is the only way to solve such problems. In Agaricales s. l. only fragmentary culture data are hitherto available but it is evident that they are valuable for a better understanding of the taxonomy. With earlier known methods only the saprophytic species have been cultured, thus many of the large, mycorrhizal genera are unknown in culture.

Pholiota (Fr.)Kummer is a genus of saprophytic species. Most species in the genus are active wood destroyers and, sometimes, they are parasitic.

Some live on charcoal, soil or humus but no species forms ectotrophic mycorrhiza. Since lignicolous species often are relatively easy to culture members of *Pholiota* were among the first agarics to be studied in that way.

Vandendries (1933 and 1934) and Vandendries & Brodie (1933) studied some species of *Pholiota* in culture and performed polarity tests. Kühner (1946) made morphological and caryological studies on *P. gummosa*. Denyer (1960) studied *Pholiota alnicola* in culture. The mating system in *P. adiposa* was studied by Arita & Mimura (1969) and later Arita (1979) published a comprehensive thesis on the cytology of *P. adiposa* and *P. nameko*. Farr, Miller & Farr (1977) carried out biosystematic studies in the *adiposa* group by using interfertility tests. Hübsch (1978) investigated the different types of conidia (accessory spores) in some species.

However, the published papers based on cultural studies of *Pholiota* are still rather few and only certain species are involved. One is *P. adiposa*, but as this name is interpreted in different ways (cfr Jacobsson 1987) it is not always clear which species had been studied.

The aim of this study is to get additional viewpoints for a better understanding of the systematic relationships within *Pholiota* by the aid of culture characters and interfertility tests. Twentytwo species belonging to *Pholiota* or allied genera have been cultured and studied in different ways.

MATERIAL AND METHODS

Collecting has taken place in various parts of Sweden and Denmark during 1979 - 1988. Basidiospores from collected basidiocarps were, as soon as possible, dispersed on plastic petri dishes with common malt agar for germination. From the germinated spores of each collection a number of monosporous mycelia were isolated. If a sufficient number of single spore (ss-)isolates were obtained, the breeding ability (the ability to form clamps in matings) and the mating-type was investigated by pairing all isolates with each other (polarity tests). The presence or absence of clamps, occurrence of accessory spores and other characters were examined when the mycelia had been in contact with each other for at least three or four weeks.

For some species no Swedish collections were available in culture. Mycelia of *Pholiota albocrenulata* and *P. lucifera* were obtained from CBS Baarn, the Netherlands. Compatibility tests between European and American specimens have been performed between Swedish material and some isolates of *P. alnicola*, *P. limonella* and *P. squarrosoides*, which were obtained from DAOM, Canada.

After the polarity tests, compatibility tests between different strains of the same species were performed. Compatibility tests between different

forms were also made as a method to delimit closely related species or to confirm infraspecific variation. Intercompatibility is accepted as a strong support for conspecificity. Negative results consequently indicate different species but it is well known that there exist other explanations for negative pairings between specimens within a biological species, e.g. a reduction in mating capacity due to degenerative mutations or senescence (cfr Boidin, 1986).

Sometimes only polyspore (ps-) cultures with clamped hyphae were available for compatibility tests. They were then used in di-mon matings. In this type of crossing, a piece of a ps-mycelium is inoculated at the edge of a malt agar dish, while pieces of ss-cultures are inoculated in a half-circle around the ps-culture. After being in contact for four weeksor more the mycelium on the outside of the half-circle is checked for the occurrence of clamps (Buller phenomenon). Occurrence of clamps there is a strong support for conspecificy.

When partial compatibility or unexpected results appeared, the matings were repeated. There is a considerable variation between different species in many characters of the cultured mycelia, e.g. growth rate, appearance of the mycelial mat, width of the hyphae etc and accessory spores (conidia) of various kinds. In Polyporaceae and Corticiaceae a coded system developed by Nobles has been used for a long time to describe the morphology of polyspore cultures. This system is used herein to describe the Pholiotas. The codes are from Nobles (1965) with emendations by Boidin & Lanquetin (1983).

The production of the extra-cellular enzymes laccase and tyrosinase is studied by using several phenolic compounds in drop-tests. This method, the nature, effectiveness and reliability of different reagents is described in detail by Marr (1979). The procedure in this study follows Marr's directions. Small slices of tissue, about 10 mm diam and 5 mm thick, were removed from the mycelial colonies. The slices were placed in cavities of distilled-water rinsed, porcelain, depression plates. Systematically, the six reagents used (Syringaldazine, 1-Naphtol, Guaiacol, Gum Guaiac, P-cresol, L-tyrosine) were added to the tissues, each tissue submerged in several drops of one reagent. Syringaldazine and 1-Naphtol reagents are laccase-specific, p-Cresol and L-tyrosine are tyrosinase-specific.

Also nuclear staining in spores and mycelia has been made with giemsa according to Boidin (1958) in order to study the nuclear behavior in different stages. The terminology in this respect follows Boidin & Lanquetin.

The cultures are stored in the culture-collection at the Department of Systematic Botany, Gothenburg University. The GB-numbers refer to the culture-collection. The original specimens can be identified by the same number and are kept in the herbarium at Gothenburg (GB).

RESULTS

The basidiospores of many *Pholiota* species germinate easily in culture. However, the ability to germinate on common malt agar without any special arrangements varied within the genus. Spores from three species, *P. flammans*, *P. nematolomoides* and *P. astragalina*, never germinated in spite of several attempts. These species also have in common to grow on decayed coniferous wood. Also spores of *P. tuberculosa* were difficult to germinate, but eventually an attempt succeeded. In *P. highlandensis* only a few spores germinated. In most other species numerous spores germinated, but the vitality decreased rapidly after some days in storage. Spores stored more than a week generally did not germinate. In *P. alnicola* and *P. pinicola* the spore-prints had to be stored about two weeks in a freezer (ca - 5 C) before germinating occurred. Some attempts to culture these species without this treatment did not succeed.

Primary mycelia of most species of *Pholiota* grow rather rapidly and usually the two mycelia paired on a dish grew together within two or three weeks. Then they became completely interwoven or formed a more or less distinct barrier. A distinct barrier was usually combined with absence of clamps but exceptions to this rule occurred. Incomplete clamps were sometimes noted, generally in dishes where also genuine clamps were present. False clamps were rarely seen. In certain dishes, clamps were noted only on one side of the confrontation line (unilateral dikaryotization).

The positive pairings were compiled in pairing-tables to determine the mating-systems. However, the interpretation of the pairing tables were often more complicated than expected. Certain tables indicated bipolarity, others tetrapolarity, sometimes for the same species. Frequently irregularities appeard, failures to form clamps where expected but also clamps in combinations which at first sight seemed to be impossible.

CYTOLOGY:

The basidia in all species of Pholiota are normally four-spored. Most basidiospores in all species investigated contained two nuclei, which is the normal condition in Strophariaceae and other chromosporic Agarics (Kühner 1980). Certain spores seemed to contain one or more than two nuclei. Two nuclei in each spore is a result of three successive divisions of the fusion nucleus, the meiosis and two mitotic divisions, which yields eight nuclei. The third division occurs after the sterigmata are formed but the location in which it takes place is variable. Sometimes it occurs in the basidia, during the passage of the nuclei through the sterigmata or in the spores. Arita (1979), who studied the nuclear behaviour in *P. "adiposa"* (=*P. jahnii*?) and *P. nameko*, found that the third division took place in the spores in *P. adiposa* but this varied and frequently took place in the basidia in *P. nameko*.

The results of the polarity tests in this investigation show that it is common for the nuclei in certain basidiospores to contain different mating factors. In most pairing tables there occur positive pairings that can be explained only if the spores contain nuclei with two noncomplementary factors. Such heterocaryotic spores have been recognized in *Psathyrella candolleana* by Galland (1971). She showed that certain monosporic mycelia contained two types of nuclei incompatible because they possess the same allele for one of the mating type factors, A or B. Thus nuclei A2B2 and A2B1 may be found in the same primary mycelium. Undoubtedly the same conditions are common in most species of *Pholiota*. A tendency to "illegitimate copulations" between haplonts of the same strain occur also in *Coprinus* (Lange 1952). Future studies will reveal if this phenomenon is common also in other chromosporic genera.

Kühner (1977) considers that the discrepancies in pairing tables of tetrasporic species at least partly depends on spores borne on occasional basidia with only two or three sterigmata. Such spores may contain one or two additional nuclei which are genetically different. However, the number of spores with more than one mating factor appears to be higher than the corresponding number of basidia with less than four basidiospores. Perhaps spores with more than one mating factor germinate more easily. The occurrence of amphithallism may cause difficulty in interpreting some pairing tables. Mounce (1929) and Vandendries (1933) reported a bipolar mating type in *Pholiota adiposa* (=*P. jahnii*?) and *P. aurivella* respectively. However, Farr et al. (1977) found that although *P. aurivella* and *P. limonella* first appeared bipolar, a careful reexamination showed them to be tetrapolar. As already indicated by Ginns (1974) several cases of reported bipolarity were the results of misinterpretation. Rewriting of the original bipolar tables showed tetrapolarity. The reason for the misinterpretations was that the authors did not attempt to explain the irregularities that showed up in the pairing tables, e.g. the formation of clamps in pairings with presumably identical factors or failures in clamp-formation between assumed compatible pairings. Most of the pairing-tables in this investigation which indicate bipolarity are artificial, but *Pholiota jahnii* and possibly *Pholiota mutabilis* really seem to be bipolar or have bipolar forms.

Isolates from basidiospores of *Pholiota* usually germinate to non-clamped mycelia. The exceptions (approx. 10 - 20 %) may partly depend on dicaryotization between two germlings before they were isolated but probably spores with two compatible nuclei exist. However, such spores are produced only in a low number by chance and no species of *Pholiota* seems to be "secondarily homothallic" (Whitehouse 1949, Raper 1966). This term is applied to a species that regularly produce a high percentage of basidiospores with two compatible nuclei.

In some cases, e.g. in certain isolates of *P. gummosa* and *P. limonella*,

clamps suddenly and unexpected have appeared in ss-mycelia months or years after germination. A spontaneous change of a mating type factor must have taken place. Such changes occur in *Psathyrella candolleana* (Galland 1971). A spontanoeous mutation of a mating factor may exceptionally be the explanation of an unexpected positive mating in a pairing table.

MYCELIA AND HYPHAE:

The appearance of the mycelia varied considerably between different species or groups of species. The surface of the mycelial mat is smooth in many species but distinctly granular in others. In *P. adiposa* and *P. limonella* distinct strands run over the surface. A cottony aerial mycelium is present in most species but there is variation between different mycelia of the same species. The advancing zones are generally even and appressed, with a few exceptions. These characters are described in the species descriptions and photos were taken of the cultures after six weeks growth (Fig. 1 - 3). Also the growth rate appeared to be very different.

Most hyphae in a mycelial colony are generally rather narrow (1 - 5 µm). The widths vary between the species but also between different isolates of the same species. On average the primary are narrower than the secondary mycelia. In all species certain hyphae become consideraby wider and frequently also more irregular in shape. In many species certain hyphae contain a row of short, swollen cells, which give them a moniliform appearance. Such cells appear both apically and intercalary. Also the frequency of oil-drops and other characters is variable (cfr fig. 8, *P. tuberculosa*).

The appearance of mycelia and hyphae seems to be very typical and constant within each species and therefore of a taxonomic value. Closely related species, e.g. *P. adiposa* and *P. limonella*, *P. spumosa* and *P. mixta*, *P. alnicola* and *P. pinicola*, are very similar in these characters.

ACCESSORY SPORES:

Most mycelia of Pholiotas, both ss- and ps-mycelia, form accessory spores in culture. Two types of accessory spores occur in the genus: arthrospores (oidia) and chlamydospores (aleuriospores). The arthrospores are generally formed on short, narrow branches (conidiophores) from certain hyphae on the surface of a mycelial colony. They arise as two or more cells in the apical parts of a branch and start as protoplasmic contraction followed by dissolution of the walls of the emptied parts of the hyphae. The process frequently takes place repeatedly on the same conidiophore. Sometimes the arthrospore-forming branches are coiled, e.g. in certain mycelia of *P. jahnii*. The spore-forming branches appear simple (for instance in *P. spumosa*) or in different-looking ramifications. Possibly also protoplasmic contraction in ordinary hyphae may lead to the formation of arthrospores.

Chlamydospores are formed in several species. They are very frequent,

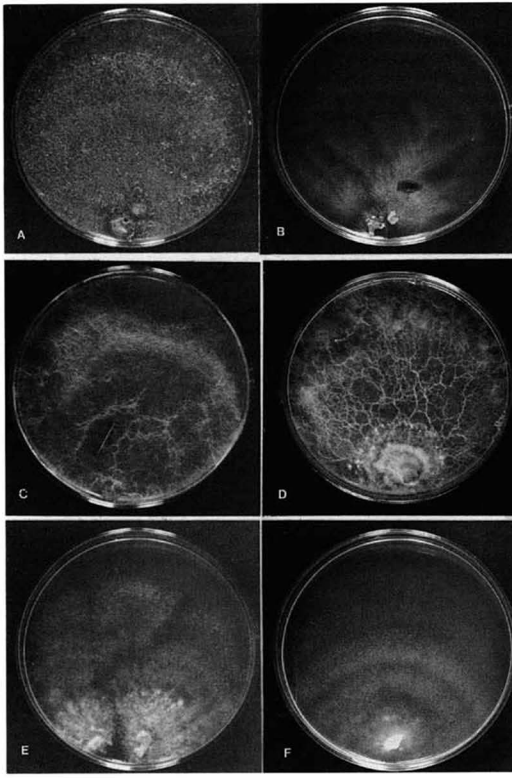

Fig. 1. Cultures after six weeks growth. a) Pholiota squarrosa, GB 1303, b) P. heteroclita, GB 1457, c) P. adiposa, GB 1071, d) P. limonella, GB 1456, e) P. jahnii, GB 1061, f) P. squarrosoides, GB 1458.

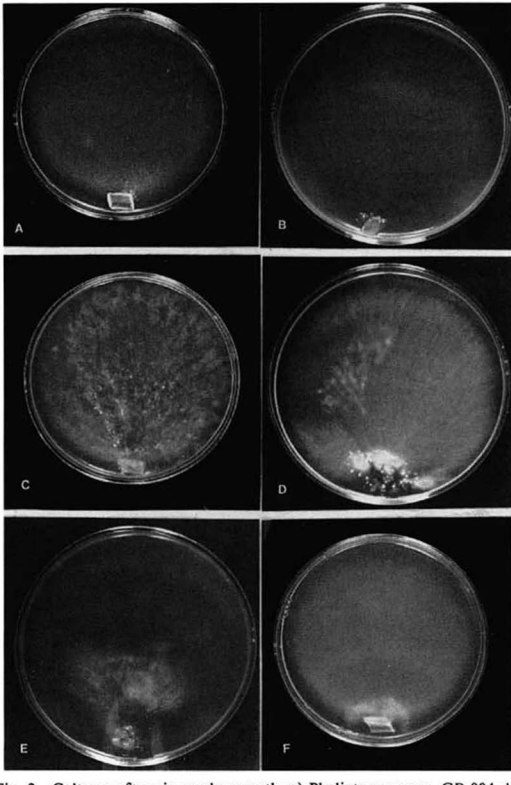

Fig. 2. Cultures after six weeks growth. a) Pholiota spumosa, GB 884, b) P. mixta, GB 948, c) P. lenta, GB 1060, d) P. lubrica, GB 1293, e) P. highlandensis, GB 1328, f) P. scamba, GB 1221.

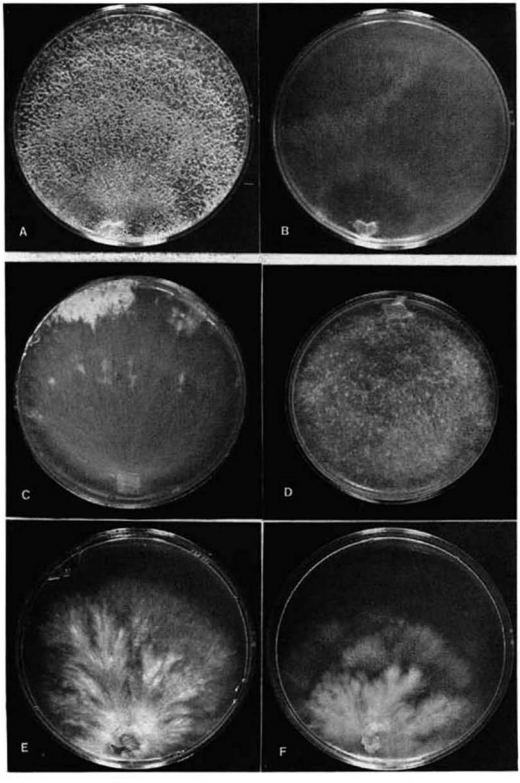

Fig. 3. Cultures after six weeks growth. a) Pholiota gummosa, GB 1295, b) P. graminis, GB 1273, c) P. tuberculosa, GB 1692, d) P. mutabilis, GB 1304, e) P. alnicola, GB 1243, f) P. pinicola, GB 1359.

large and conspicuous (15 - 30 x 10 - 20 μm) in certain species, e.g. *P. alnicola, P. pinicola, P. gummosa, P. graminis* and *P. squarrosa*, but less numerous and rather inconspicuous in others. They are formed both laterally and apically, frequently as the end-cell in a row of moniliform swellings. Chlamydospores occur anywhere in the mycelia but preferably in the submerged parts. Typical chlamydospores are not seen at all in some species, but probably any kind of swellings may loosen and then serve as a chlamydospore. Actually there is no fundamental difference between the two kinds of spores more than the size and shape. Of course chlamydospores are never so numerous as arthrospores, as they are produced for survival and not for dispersal as arthrospores.

The type and shape of accessory spores is very constant within each species and they are a systematically valuable character. However, they are not always formed by all isolates of the same stock. Genetical differences are probably responsible for presence or absence of accessory spores but also environmental conditions may play a role. Many species form only one type of accessory spore but both arthrospores and typical chlamydospores are seen in the same mycelial colonies of *Pholiota squarrosa* and *P. gummosa*. Arthrospores are common e.g. in the *adiposa* and *lubrica* groups and in *P. squarrosa* and *P. gummosa*. Conspicuous, moniliform swellings are especially common in *Pholiota tuberculosa* and *P. albocrenulata*.

Hübsch (1978) described the accessory spores of certain species of *Pholiota* and also illustrated some petri plates to illustrate their formation process. His results correspond well with those of this investigation.

DROP-TESTS:

The results of the drop-tests are presented in table I. The reactions were recorded 5 min., 30 min. and 2 hours after application of the reagents. In all cases where a positive reaction was recorded, it was visible within 30 min.

A positive response is an effect of oxidation and implies occurrence of enzymes. According to the investigations of Marr (1979) and others syringaldazine and 1-naphtol are laccase-specific. L-tyrosine and p-cresol reagents are tyrosinase-specific. Guaiac and guaiacol are nonspecific for laccase and tyrosinase but more effectively oxidized by laccase than tyrosinase.

In this study eleven species contained both laccase and tyrosinase: *jahnii, adiposa, limonella, squarrosoides, graminis, gummosa, scamba, spumosa, mixta* and *mutabilis*. Only tyrosinase is indicated in *squarrosa, heteroclita* and *lenta*. The reactions indicate only laccase in *junonius, highlandensis, lignicola* and *tuberculosa*. Neither laccase nor tyrosinase reaction is noted in the following species: *albocrenulata, alnicola* and *pinicola*.

Table I Result of drop-tests

+ = positive reaction, (+) = weak positive reaction, - = no reaction noted.

	Sy	N	Gl	G	p-C	Ty
adiposa	(+)	-	-	(+)	(+)	-
albocrenulata	-	-	-	-	-	-
alnicola	-	-	-	-	-	-
graminis	+	+	+	+	+	+
gummosa	+	-	(+)	-	-	-
heteroclita	-	-	-	-	+	(+)
highlandensis	-	+	-	(+)	-	-
jahnii	+	+	+	+	-	+
junonius	-	(+)	-	(+)	-	-
lenta	-	-	-	-	-	(+)
lignicola	+	(+)	(+)	+	-	-
limonella	-	(+)	-	(+)	+	-
lubrica	-	-	-	(+)	-	-
mixta	+	+	+	+	+	+
mutabilis	+	+	+	+	+	+
pinicola	-	-	-	-	-	-
scamba	+	+	+	+	+	+
spumosa	+	-	+	+	+	+
squarrosa	-	-	(+)	-	(+)	(+)
squarrosoides	-	-	(+)	-	-	(+)
tuberculosa	-	+	-	(+)	-	-

Abbrevations: Sy = syringaldazine, N = 1-Naphtol, Gl = guaiacol, G = guaiac, p-C = p-cresol, Ty = L-tyrosine.

The results of the spot tests in some species varied from the results in other studies. Kääarik (1970) summarized results of spot tests and other culture characters for a large number of species, among them eight *Pholiota* species. She reported a strong laccase reaction in *P. heteroclita* and *P. squarrosa*, whereas no laccase was indicated in this study. She reported a weak tyrosinase reaction in *P. gummosa* and a strong laccase reaction in *P. adiposa*, which do not correspond with the result of this study. Marr (1979) studied *P. squarrosoides* and *P. lenta*. They were placed in the group of species with tyrosinase only, which corresponds with the results of this study.

Marr concluded that syringaldazine is the best reagent for laccase and L-tyrosinase or p-cresol the better reagents for detecting tyrosinase. It is therefore worth mentioning that laccase in *P. tuberculosa* and *P. high-*

landensis was indicated by a positive reaction with 1-naphtol but not syringaldazine.

The deviating results of different studies may depend on the fact that the localization of laccase and tyrosinase varies either among species or during ontogeny. These variations are associated with other processes, e.g. pigmentation or fruiting. Marr and Käärik performed their tests on tissues from basidiocarps, which may give other results than tests on mycelial colonies in culture. Also environmental factors, such as nutrition, temperature, pH, etc. influence the production of the enzymes. Results of drop-tests therefore must be interpreted carefully and preferably compared with other methods before conclusions can be reached.

CULTURE CHARACTERS FOR THE SPECIES

Pholiota squarrosa (Weigel:Fr.)Kummer

MATERIAL:
GB 165/ *Fagus*/Sweden, Halland, Tjolöholm (SJ 80261).
GB 1249/ *Picea*/Norway, Oppland, Ormtjernkampen (NH 8481).
GB 1299/ *Quercus*/Sweden, Västergötland, V.Tunhem (SJ 84157).
GB 1303/ *Quercus*/Sweden, Västergötland, Göteborg (SJ 84165).
GB 1535/ *Abies*/Romania, Neamt (NH 9201).
POLARITY: Only two polarity tests have been performed, both, however, with a rather small number of available isolates (Tab. 2). In 1249 only two positive pairings were observed. They indicate two different compatibility groups. The remaining isolates may belong to a third and same compatibility group but the negative pairings may be due to reduced fertility. In 1535 three compatibility groups (5 = A1B1; 1, 2, 6 = A2B2; 3, 4, 7 = A1B2) is the best interpretation of the result. Tetrapolarity therefore is the only reasonable mating system in *P. squarrosa*.

GB 1249, GB 1299, GB 1303, GB 1535 were compatible with each other. CYTOLOGY: Astatocoenocytic. The cells of the ss-mycelia contain a variable number of nuclei, at least 2 - 25 have been noted. Multinucleate hyphal cells are distinctly wider than others and occur especially in the terminal part of the hyphae. Multinucleate cells are also noted in a secondary mycelium (GB 1303) though most of them are dicaryotic.
CULTURE CHARACTERS (GB 1303): Growth moderately rapid, the dishes completely covered by mycelia in 3 - 4 weeks. Advancing zone even, appressed. Mycelial mat soon floccose, at first whitish, later yellowish with brown floccules (Fig. 1 A).
Reverse brownish. Hyphae usually 2 - 4 µm wide, regularly branched, with a clamp at all septa. Young, apical cells are frequently somewhat wider (3 - 6 µm) and more irregular in outline. Numerous arthrospores (6

- 10 x 3 - 4 μm) are formed by short branches from certain hyphae in the floccules on the surface. In the submerged part of the mycelium also large

Tab. 2. Polarity tests in *Pholiota squarrosa*.

	GB 1249/2	GB 1249/3	GB 1249/4	GB 1249/5	GB 1249/6
GB 1249/1	–	–	–	–	–
GB 1249/2		+	–	–	–
GB 1249/3			–	+	–
GB 1249/4				–	–
GB 1249/5					–

	GB 1535/2	GB 1535/3	GB 1535/4	GB 1535/5	GB 1535/6	GB 1535/7
GB 1535/1	–	–	–	+	–	–
GB 1535/2		–	–	+	–	–
GB 1535/3			–	–	–	–
GB 1535/4				–	–	–
GB 1535/5					+	–
GB 1535/6						–

chlamydospores (10 - 16 x 8 - 12 μm) are rather common. Fig.4. CODE: 2b, 3c, 26, 34, 35, 37, 39, 43-44, 49, 54, 60, 63. Oxidase reactions: weak reaction with guaiacol, p-cresol and L-Tyrosine, all other tests negative.

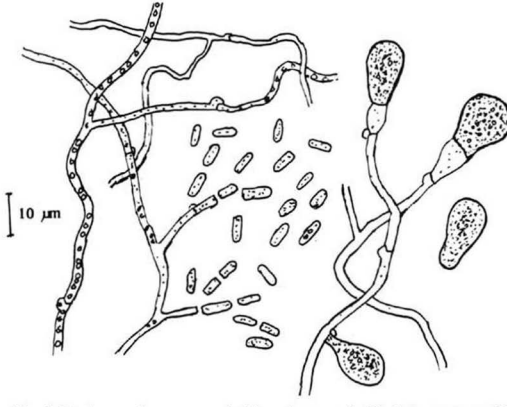

Fig. 4. Hyphae, arthrospores and chlamydospores in Pholiota squarrosa (GB 1303).

The cultural characters of *P. squarrosa* have much in common with *P. gummosa* (e.g. the appearance of the mycelia and the accessory spores) and

indicate a relationship. According to Käärik (1965), *P. squarrosa* belongs to the group of species which produces both laccase and and tyrosinase. In this investigation only tyrosinase is verified. Possibly the weak reaction with guaiacol may be an indication of laccase. Käärik also reports a rather rapid growth rate for this species.

Pholiota limonella (Peck)Sacc.

MATERIAL:
GB 166/ *Alnus* trunk/Sweden, Göteborg, Naturparken (SJ 80286).
GB 1438/ *Betula*/Sweden, Västergötland, Långared (SJ 85056).
GB 1456/ cut trunk of *Quercus robur*/Sweden, Västergötland, Råda (SJ 85092).
GB 1508/ *Abies alba*/Romania, Neamt (NH 9200).
GB 1513/ Deciduous wood/Romania, Iasi (NH 9084).
GB 1597/ Substrate unknown/USA, VT 397 (Blacksburg, Virginia).
GB 1717/ *Populus*/Sweden, Medelpad, Borgsjö.
GB 1736/ *Alnus glutinosa*/Sweden, Uppland, Billudden.

The compatibility between these specimens and with the closely related *Pholiota adiposa* was dealt with in another paper (Jacobsson 1987).
POLARITY: Tetrapolarity is indicated but there are several irregularities, such as failures of clamp formation in supposed compatible pairings and occurrence of clamps in unexpected combinations. In several cases the only explanation for compatible pairings is that certain spores must contain two or more incompatible factors. It is not always easy to interpret a pairing table with total certainty due to irregularities which make more than one solution possible. The interpretation with the lowest number of mating failures and without positive matings between isolates assigned the same factors is considered to be the most probable one, at least theoretically. The pairing tables are showed in tab. 3.

In GB 166 the most reasonable interpretation is: 1 = A1B1; 5, 6 = A2B2; 3, 4, 7 = A1B2; 2 = A1B1+A2B1; 8 = A2B2+A2B1. There are two failures with this explanation (2 x 4, 2 x 7).

GB 1438: 4, 8 = A1B1; 5 = A2B2; 3, 7 = A1B2; 1, 2 = A2B2+A2B1. No 6 is excluded, as it did not result in any positive mating. This isolate is supposed to have a restricted fertility for some reason. With this interpretation there are two failures (1 x 7, 2 x 3), in other interpretations there are more failures.

GB 1456 is tetrapolar without failures: 1 = A1B1; 2, 4 = A2B2; 3, 5, 7 = A1B2; 6 = A2B1.

GB 1513. Amphithallic tetrapolarity is the only possible interpretation. If 1, 2, 3, 7 = A2B1+A1B1; 5 = A1B1; 8 = A2B2; 6 = A1B2; 4 = A2B1, there are no failures. Other interpretations are possible, but in those cases there are at least some failures.

GB 1717: This pairing table is difficult to interpret, but one interpretation indicates amphithallic tetrapolarity without failures: 1, 2 = A1B1; 3, 8 = A2B2; 5 = A1B2; 7 = A2B1; 10 = A1B1+A2B1; 9 = A2B2+A1B2.

Some polarity tests are excluded, because few isolates were available.

Tab. 3. Polarity tests in *Pholiota limonella*.

	GB 166/2	GB 166/3	GB 166/4	GB 166/5	GB 166/6	GB 166/7	GB 166/8
GB 166/1	–	–	–	+	+	–	+
GB 166/2		+	–	+	+	–	+
GB 166/3			–	–	–	–	+
GB 166/4				–	–	–	+
GB 166/5					–	–	–
GB 166/6						–	–
GB 166/7							+

	GB 1438/2	GB 1438/3	GB 1438/4	GB 1438/5	GB 1438/6	GB 1438/7	GB 1438/8
GB 1438/1	–	+	+	–	–	–	+
GB 1438/2		–	+	–	–	+	+
GB 1438/3			–	–	–	–	–
GB 1438/4				+	–	–	–
GB 1438/5					–	–	+
GB 1438/6						–	–
GB 1438/7							–

	GB 1508/2	GB 1508/3	GB 1508/4	GB 1508/5	GB 1508/6	GB 1508/7	GB 1508/8
GB 1508/1	–	+	+	–	–	–	+
GB 1508/2		+	+	–	–	–	+
GB 1508/3			–	+	–	–	+
GB 1508/4				+	+	+	+
GB 1508/5					–	–	–
GB 1508/6						–	–
GB 1508/7							–

	GB 1513/2	GB 1513/3	GB 1513/7	GB 1513/6	GB 1513/8	GB 1513/4	GB 1513/5
GB 1513/1	–	–	–	+	+	–	–
GB 1513/2		–	–	+	+	–	–
GB 1513/3			–	+	+	–	–
GB 1513/7				+	+	–	–
GB 1513/6					–	+	–
GB 1513/8						–	+
GB 1513/4							–

	GB 1717/2	GB 1717/3	GB 1717/5	GB 1717/7	GB 1717/8	GB 1717/9	GB 1717/10
GB 1717/1	–	+	–	–	–	(+)	–
GB 1717/2		+	–	–	(+)	(+)	–
GB 1717/3			–	–	–	–	+
GB 1717/5				+	–	–	+
GB 1717/7					–	+	–
GB 1717/8						–	+
GB 1717/9							(+)

	GB 1456/2	GB 1456/3	GB 1456/4	GB 1456/5	GB 1456/6	GB 1456/7
GB 1456/1	+	–	+	–	–	–
GB 1456/2		–	–	–	–	–
GB 1456/3			–	–	+	–
GB 1456/4				–	–	–
GB 1456/5					+	–
GB 1456/6						+

(+) means that only a few clamps were found, restricted to the confrontation line.

CYTOLOGY: Astatocoenocytic. The hyphal cells of a homocaryotic mycelial colony contain a various number of nuclei, 2 - 22 are counted. The highest number of nuclei is found in the youngest, terminal cell of a hypha. Most cells in the secondary mycelia are dicaryotic but multinucleate cells containing as many as 25 nuclei are also seen.
CULTURE CHARACTERS: Growth slow, dishes covered in 5 or 6 weeks. Advancing zone even, appressed. Aerial mycelium cottony-floccose, at first whitish, becoming yellowish brown in old parts. Conspicuous, branched strands run over the surface (Fig. 1 D). Reverse brownish. Hyphae 1 - 4 µm wide, regularly branched, with a clamp at most septa, hyaline or yellowish. Walls in old hyphae brown and incrusted. Numerous arthrospores (5 - 11 x 1,5 - 3 µm), formed by short branches from hyphae on the surface. Chlamydospores (8 - 30 x 5 - 10 µm) occur in submerged mycelia, but not numerous. Old hyphal cells in the submerged mycelia frequently becoming filled with reddish brown necropigments and finally broken in pieces (Fig. 5).
CODE: 2a, 2b, 3c, 16, 26, 34, 35, 37, 39, 45-46, 49, 54, 60, 63.
OXIDASE REACTIONS: Positive reaction with guaiac (very weak), p-Cresol and 1-Naphtol (weak), all other tests negative.

Pholiota limonella is closely related to *Pholiota adiposa* (=*aurivella* auct.). It is not possible to separate the two species in the basidiocarps. A slight difference in the spore size is the only reliable morphological character. Since this species earlier has not been distinguished by any European author, it is quite possible that published culture data (Vandendries 1933, Kääarik 1970, Hübsch 1978 etc) of *Pholiota aurivella* (at least partly also *adiposa* and *squarroso-adiposa*) actually represent *Pholiota limonella*. However, so far no difference in the cultural characters have been found.

Pholiota adiposa (Batsch:Fr.)Kummer

MATERIAL:
GB 167/*Fagus*/Sweden, Halland, Grytsjön (SJ 80307).
GB 1072/*Fagus*/Sweden, Skåne, Silvåkra (SJ 83119).
GB 1291/*Salix* /Sweden, Skåne, Kristianstad (SJ 84131).
POLARITY: GB 167 and GB 1291 were tested (Tab. 4). In GB 167 only one positive pairing was obtained. In GB 1291 the only possible interpretation is amphithallic tetrapolarity: 8, 10 = A1B1; 1, 2, 5, = A2B2; 4, 6 = A1B2; 7 = A2B1; 9 = A2B2+A1B2. This explanation gives no failures.

The three mentioned specimens are intercompatible with each other. *Pholiota adiposa* is very closely related to *Pholiota limonella*. The taxonomy and the differences between them are published in an earlier paper

(Jacobsson 1987).

CYTOLOGY: Astatocoenocytic. Characters identical with those of *P. limonella*.

CULTURE CHARACTERS (GB 1072): Growth rate and general appearance identical with the closely related *P. limonella*. Advancing zone even, appressed. Later a cottony-floccose aerial mycelium is formed. Conspicous, branched strands run over the surface, at first whitish, soon yellowish to brownish (Fig. 1 C). Reverse brownish. Hyphae with a clamp at most septa, regularly branched, 1 - 4 µm wide. Numerous arthrospores (5 - 11 x 1,5 - 3 µm), formed in the same way and of the same appearance as in *P. limonella*

CODE: (2ab), 3c, 16, 26, 34, 35, 37, 39, 45-46, 49, 54, 60, 63.

OXIDASE TESTS: All tests negative in GB 1072. In GB 167 a positive but weak reaction with syringaldazine and p-cresol.

Tab. 4. Polarity tests in *Pholiota adiposa*.

	GB 167/2	GB 167/3	GB 167/4	GB 167/5	GB 167/6
GB 167/1	+	–	–	–	–
GB 167/2		–	–	–	–
GB 167/3			–	–	–
GB 167/4				–	–
GB 167/5					–

	GB 1291/2	GB 1291/5	GB 1291/4	GB 1291/6	GB 1291/7	GB 1291/9	GB 1291/8	GB 1291/10
GB 1291/1	–	–	–	–	–	–	+	+
GB 1291/2		–	–	–	–	–	+	+
GB 1291/5			–	–	–	–	+	+
GB 1291/4				–	+	–	–	–
GB 1291/6					+	–	–	–
GB 1291/7						+	–	–
GB 1291/9							+	+
GB 1291/8								–

This species is better known as *Pholiota aurivella* (Batsch:Fr.)Kummer. However, as this name has been recently questioned (Kuyper & Tjallingii-Beukers 1986) and *Pholiota adiposa* in its original sense undoubtedly is correct (cfr Jacobsson 1987) a name change to *P. adiposa* is unavoidable.

The confusing taxonomy in this group implies that published data on *Pholiota aurivella* should actually be referred to *Pholiota adiposa* or the almost indistinguishable *Pholiota limonella*. On the other hand, published data on *Pholiota adiposa*, at least in recent time (Hübsch 1978, Arita 1979) should be transferred to *Pholiota jahnii*.

Pholiota "aurivella" is one of the most studied species of the genus. Martens & Vandendries (1932) and Vandendries (1933) reports bipolarity for this species, which seems unlikely when compared with later investigations (Farr, Miller and Farr 1977, and those in this paper, cfr *Pholiota jahnii*). Hübsch (1968) noticed the occurrence of arthrospores and Kääarik (1970) a strong reaction both for laccase and tyrosinase together with some

other characters. Only weak reactions for laccase and tyrosinase reaction were noted in this investigation. The difference may depend on the fact that Käärik used basidiocarp tissue for her tests.

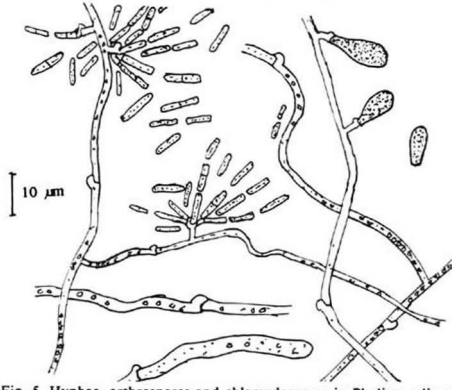

Fig. 5. Hyphae, arthrospores and chlamydospores in Pholiota adiposa (GB 1291).

Pholiota jahnii Tjall. & Bas

MATERIAL:
GB 83/*Fagus*/Sweden, Skåne, Bökebergsslätt (SJ 79231).
GB 164/*Fagus*/Danmark, Själland, Vordingborg (SJ 80210).
GB 1061/*Fagus*/Sweden, Skåne, Silvåkra (SJ 83118).
GB 1305/*Fagus*/Sweden, Blekinge, Sölvesborg (L. Örstadius).
GB 1342/probably *Picea*/Sweden, Södermanland, Överjärna (K.Jaederfeldt).
GB 2021/buried wood, probably *Fagus*/Sweden, Skåne, Silvåkra (SJ88061).
POLARITY. Polarity tests have been performed with the specimens GB 83, GB 1061, GB 1305, GB 1342 and GB 2021 (Tab. 5).
In GB 1342 only two positive pairings were yielded, which indicates a restricted fertility for some reason. The result of the other four polarity tests clearly indicates bipolarity. However, there is one failure in GB 1061 (4 x 9).
All specimens are completely intercompatible with each other (Jacobsson 1987).

Tab. 5. Polarity tests in *Pholiota jahnii*.

	GB 83/2	GB 83/6	GB 83/7	GB 83/3	GB 83/4	GB 83/5	GB 83/8	GB 83/10
GB 83/1	–	–	–	+	+	+	+	+
GB 83/2		–	–	+	+	+	+	+
GB 83/6			–	+	+	+	+	+
GB 83/7				+	+	+	+	+
GB 83/3					–	–	–	–
GB 83/4						–	–	–
GB 83/5							–	–
GB 83/8								–

	GB 1305/2	GB 1305/3	GB 1305/5	GB 1305/7	GB 1305/4	GB 1305/6
GB 1305/1	–	–	–	–	+	+
GB 1305/2		–	–	–	+	+
GB 1305/3			–	–	+	+
GB 1305/5				–	+	+
GB 1305/7					+	+
GB 1305/4						–

	GB 1061/7	GB 1061/8	GB 1061/5	GB 1061/6	GB 1061/9
GB 1061/4	–	–	+	+	–
GB 1061/7		–	+	+	+
GB 1061/8			+	+	+
GB 1061/5				–	–
GB 1061/6					–

	GB 1342/1	GB 1342/2	GB 1342/3	GB 1342/4	GB 1342/5	GB 1342/6	GB 1342/7	GB 1342/8	GB 1342/9	GB 1342/10
GB 1342/1	–	–	–	–	–	–	–	+	–	–
GB 1342/2		–	–	–	–	–	–	–	–	–
GB 1342/3			–	–	–	–	–	–	–	–
GB 1342/4				–	–	–	–	–	–	–
GB 1342/5					–	–	+	–	–	–
GB 1342/6						–	–	–	–	–
GB 1342/7							–	–	–	–
GB 1342/8								–	–	–
GB 1342/9									–	–

	GB 2021/2	GB 2021/3	GB 2021/4	GB 2021/5	GB 2021/6	GB 2021/7	GB 2021/8	GB 2021/9	GB 2021/10
GB 2021/1	–	–	–	–	–	–	+	–	+
GB 2021/2		–	–	–	–	–	+	–	+
GB 2021/3			–	–	–	–	+	–	+
GB 2021/4				–	–	–	+	–	+
GB 2021/5					–	–	+	–	+
GB 2021/6						–	+	–	+
GB 2021/7							+	–	+
GB 2021/8								–	+
GB 2021/9									+

CYTOLOGY (GB 1061): Heterocytic. Terminal cells of ss-mycelia are multinucleate and wider than most intercalary cells, which generally contain two nuclei. In the secondary mycelium only clamped, binucleate cells are found.

CULTURE CHARACTERS (GB 1061): Growth slow, dishes covered in six weeks. Advancing zone even, appressed. Aerial mycelium cottony, whitish, but becoming slightly yellowish brown in patches in old parts (Fig. 1 E). Reverse somewhat brownish. Hyphae generally 1 - 3 μm wide, regularly

branched, with a clamp at all septa. Numerous arthrospores (3 - 6 x 1 - 3 µm) are formed at the surface of the mycelial colony (Fig. 6). Chlamydospores (8 - 45 x 6 - 10 µm) rather rare, only seen in certain mycelia. Many old hyphae are filled with dark reddish-brown necropigments and easily broken to pieces.
CODE: 2ab, 3c, 26, 34, 35, 37, 39, 46, 49, 54, 59, 63.
OXIDASE REACTIONS: Positive reactions noted for all tests except p-cresol.

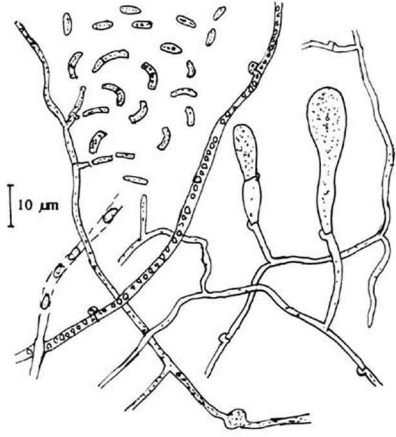

Fig. 6. Hyphae, arthrospores and chlamydospore forming in Pholiota jahnii (GB 1061).

Pholiota jahnii is a recently created new name for the species earlier known as *Pholiota muelleri* (Fr.)Orton. It also includes *Pholiota adiposa* auct. (ss Lange etc, cfr Jacobsson 1987). Therefore most published data on *Pholiota adiposa* (e.g. Arita & Mimura 1969, Arita 1979) probably refer to *Pholiota jahnii*.

The most remarkable character of *Pholiota jahnii* is the bipolarity. Three polarity tests clearly indicate bipolarity, which result corresponds with earlier reports. Arita and Mimura (1969) reported *Pholiota "adiposa"* to be bipolar. Vandendries (1933) reports a perfect bipolarity in a test with 16 isolates of *Pholiota "aurivella"*. The latter species is definitely not bi-

polar and it is more probable that he had collected *P. jahnii*, a species not known at that time.

The strong reaction for both laccase and tyrosinase corresponds well with the report of Käärik (1970).

Pholiota squarrosoides (Peck)Sacc.

MATERIAL:
GB 1458/on dead *Populus* trunk/Sweden, Uppland, Bondkyrka, (S.Ryman 8065).
GB 1680/Canada, Br.Col., Stamp Falls, polysporous & tissue cultures isolated from fruit body (2 cultures, but without clamps, DAOM 22113).
POLARITY: GB 1458 is tested regarding the mating type (Tab. 6). Amphithallic tetrapolarity is the only possible interpretation. If 6, 7 = A1B1; 1, 5 = A1B2; 2 = A2B1; 4 = A2B2+A1B2; 3 = A2B2+A2B1, there is only one failure (1 x 2).

Tab. 6. Polarity test in *Pholiota squarrosoides*.

	GB 1458/2	GB 1458/3	GB 1458/4	GB 1458/5	GB 1458/6	GB 1458/7
GB 1458/1	–	–	+	–	–	–
GB 1458/2		+	–	+	–	–
GB 1458/3			+	–	+	+
GB 1458/4				–	+	+
GB 1458/5					–	–
GB 1458/6						–

The Swedish and Canadian specimens turned out to be incompatible. However, other explanations than species differentiation are possible. The absence of clamps in DAOM 22113, in spite of the fact that it originates from a basidiocarp, indicates that a degenerative mutation has taken place and a restricted mating ability therefore is probable. These cultures were made in 1949.
CYTOLOGY (GB 1458): Probably heterocytic. All hyphae in the secondary mycelium are clamped and binucleate.
CULTURE CHARACTERS (GB 1458): Growth very slow, reaching 50 - 52 mm and not covering the dishes in six weeks. Advancing zone even, appressed. Aerial mycelium almost absent, but the surface of the mat with numerous minute granules, whitish -yellowish (Fig. 1 F). Reverse weakly brownish. Hyphae 1 - 5 μm wide, regularly branched, with a clamp at most septa. Arthrospores (3 - 8 x 1 - 3 μm, frequently curved) are formed by irregular, short branches in the granules on the surface (Fig. 7). A few

rather small and inconspicuous chlamydospores (9 - 15 x 6 - 8 µm) are seen.

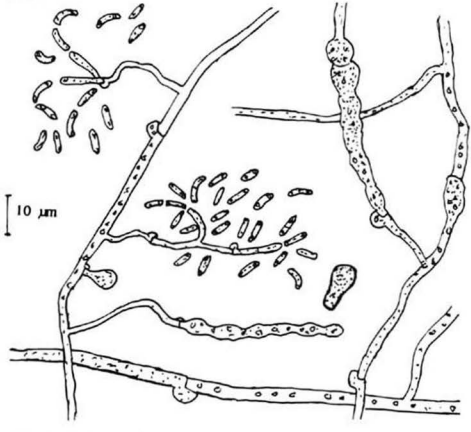

Fig. 7. Hyphae, arthrospores and chlamydospores in Pholiota squarrosoides (GB 1458).

CODE: (2ab), 3c, 26, 34, 35, 37, 39, 47, 49, 54, 60, 63.
OXIDASE REACTIONS: All tests negative.

No data on culture characters for *Pholiota squarrosoides* are published previously. The species apparently belongs to the *adiposa* group, based on its characters, e.g. the occurrence of arthrospores, which are similar to those of the other species in the group. However, it differs from the other species in the negative reactions for laccase and tyrosinase.

Pholiota tuberculosa (Schff.:Fr.)Kummer

MATERIAL:
GB 1692/*Tilia*/Sweden, Västergötland, Trollhättan (SJ 86019).
POLARITY: 7 isolates of the specimen GB 1692 were mated in all possible combinations but no positive pairings appeared. Thus the mating type of this species is still unknown.

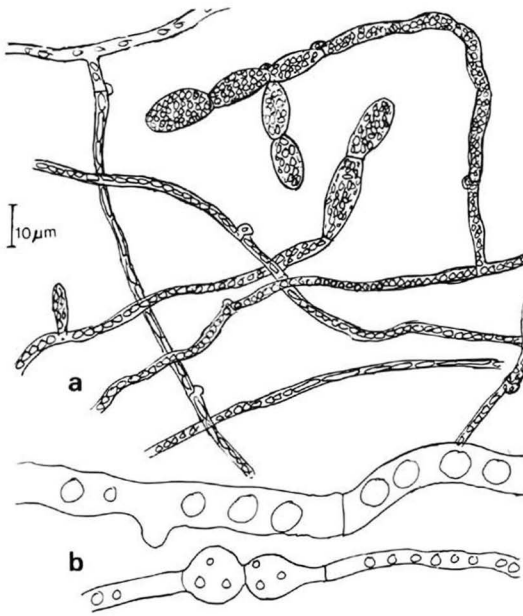

Fig. 8. Hyphae in Pholiota tuberculosa (GB 1692). A) in ps-mycelium, B) in ss-mycelium.

CYTOLOGY: Not studied.

CULTURE CHARACTERS: Growth rapid, the dishes completely covered in two weeks. Advancing zone somewhat fringed. Aerial mycelium cottony, slightly yellowish. Reverse brownish. Normal hyphae 3 - 5 µm wide, regularly branched and with a clamp at all septa, densely filled with oil-drops. Certain hyphae have a moniliform appearance with short and very broad (10 - 30 µm) cells. The ss-mycelia to a great extent consist of very wide (- 20 µm) hyphae, with very big, sometimes strongly yellow, oildrops (Fig. 8). Sometimes bladder-like cells seem to serve as chlamydospores.

CODE: 2a, 3c, 26e, 34, 37, 39, 42, 49, 54, 58.
OXIDASE REACTIONS: Positive but weak reactions with guaiac and 1-Naphtol, otherwise negative.

Pholiota tuberculosa has not been studied in culture before. Some of the cultural characters such as the rapid growth rate and the very wide hyphae densely filled with oildrops differ considerably from other *Pholiota* species and indicate that this species takes a rather isolated systematic position in the genus.

The species seems to be difficult to culture, as spores from one specimen only germinated. Some other attempts have been unsuccessful. It is desirable with more cultures to verify the results.

Pholiota lucifer (Lasch)Quél.

MATERIAL:
GB 1852/*Abies*/Austria/CBS 595.82 (1 ps-culture).
The culture characters of this strain appeared to be almost identical with those of *P. adiposa - limonella* and suggest that *P. lucifer* belongs to this complex. However, this seems unlikely, as the morphological characters are very different and more similar to those of *P. tuberculosa*. Compatibility tests by using Buller's phenomenon have been performed with *P. limonella* (GB 1456) and *P. adiposa* (GB 1072). They were found to be negative. In spite ot that it is possible that the culture descends from a misidentified basidiocarp. No herbarium material is preserved to check the determination. It is desirable to get the culture characters verified by another strain before conclusions.

Pholiota heteroclita (Fr.:Fr.)Quél.

MATERIAL:
GB 1457/*Betula*/Sweden, Halland, Lindome (SJ 85093).
POLARITY: 8 isolates of GB 1457 were mated in all possible combinations. However, the mycelia ceased to grow after a while and only in two combinations were clamps formed and then there were only a few.
CYTOLOGY: Not studied.
CULTURE CHARACTERS: Growth rate very slow for the genus, in six weeks the mycelia had reached only 30 - 37 mm. Advancing zone somewhat uneven and fringed. Aerial mycelium downy, slightly plumose, whitish in young parts, gradually becoming brownish. Reverse somewhat brownish. Hyphae 1 - 3 µm wide, not very differentied, regularly branched, with a clamp at most septa. Numerous arthrospores formed in

the aerial mycelium by short branches in terminal or lateral, brush-like clusters (Fig. 9), rather different-looking compared with those in other Pholiotas. Occasionally small intercalary or terminal swellings appear.
CODE: 2b, 3c, 7, 35, 37, 39, 47, 49, 54, 58.
OXIDASE REACTIONS. Positive reactions with L-Tyrosine (very weak) and p-Cresol, otherwise negative.

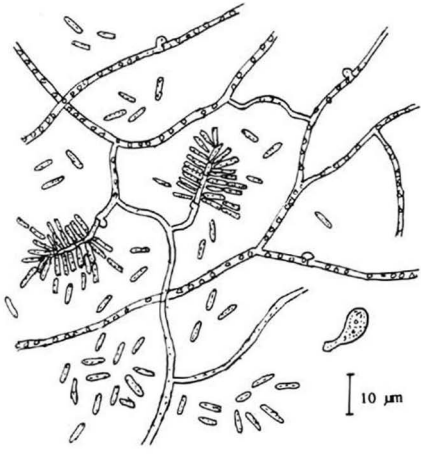

Fig. 9. Hyphae, arthrospores and a chlamydospore in Pholiota heteroclita (GB 1457).

The culture characters, especially the diverging appearance of the arthrospore-forming conidiophores, suggest a rather isolated systematic position of this species. It is often placed in a separate subgenus (*Hemipholiota*) together with some other species. *Pholiota populnea* is generally considered to be a close relative, unfortunately no strain of this species has been available. Hübsch (1978) reports, however, that no accessory spores at all were found in any of three stocks of *P. populnea*.

Pholiota highlandensis (Peck)Smith & Hesler

MATERIAL:
GB 1247/ burnt spot/Sweden, Västergötland, Alingsås (SJ 84091).
GB 1306/ burnt spot/Sweden, Göteborg, Botanical garden (SJ 84168).
GB 1328/ burnt spot/Sweden, Halland, Morup (SJ 84174).
GB 1693/ burnt spot/Finland, PH., Konnevesi, Siikakoski (I.Järvinen).

POLARITY: GB 1247, GB 1306, GB 1693 are tested (Tab. 7). Tetrapolarity is indicated and in GB 1247 and GB 1306 there also occur some positive pairings which could only be explained if spores with more than one factor are present. The following interpretations are made:

Tab. 7. Polarity tests in *Pholiota highlandensis*.

	GB 1247/2	GB 1247/3	GB 1247/4	GB 1247/5	GB 1247/6
GB 1247/1	–	–	+	+	+
GB 1247/2		–	–	–	–
GB 1247/3			+	+	+
GB 1247/4				–	+
GB 1247/5					+

	GB 1306/1	GB 1306/2	GB 1306/3	GB 1306/4	GB 1306/5	GB 1306/6
GB 1306/1		–	–	+	–	–
GB 1306/2			–	+	–	–
GB 1306/3				–	–	–
GB 1306/4					–	+
GB 1306/5						–

	GB 1693/2	GB 1693/3	GB 1693/4	GB 1693/5	GB 1693/6	GB 1693/7
GB 1693/1	–	+	+	–	+	+
GB 1693/2		–	–	–	–	–
GB 1693/3			–	+	–	–
GB 1693/4				+	–	–
GB 1693/5					+	+
GB 1693/6						–

GB 1247: A possible interpretation is 1, 3 = A1B2; 4, 5 = A2B1; 6 = A2B2+A1B2. If nr 2 = A2B2, the table shows no failure. However, this mycelium looked very different compared with the others (more brownish) and a much more clear confrontation line than in all other matings was formed. It therefore seems reasonable that this mycelium was incompatible for other reasons.

GB 1306: 3, 5 = A1B1; 4 = A1B2; 1, 2 = A2B1; 6 =A1B1+A2B1. No failures.

GB 1693: 1, 5 = A1B1; 3, 4, 6, 7 = A2B2; 2 = A1B2 or A2B1. Only

three compatibility groups were detected, which is reasonable with only 7 isolates.

INTERCOMPATIBILITY TESTS: The following tests have been performed: 1247 x 1328, 1247 x 1693, 1306 x 1693. These specimens were found to be intercompatible. Only in one dish were no clamps found (1247/3 x 1693/3). Macroscopically the basidiocarps looked rather different.

CULTURE CHARACTERS (GB 1328): Growth moderately rapid, dishes covered in three weeks. Advancing zone even, appressed. Aerial mycelium downy, whitish but slightly yellow in old parts (Fig. 2 E). Reverse pale yellowish brown. Hyphae rather narrow,
mostly 1 - 3 μm wide, occasionally - 5 μm, regularly branched and with a clamp at all septa. A few hyphae consist of separate or a row of more or less swollen cells (5 - 10 μm wide), which probably may serve as chlamydospores. Occasional slight intercalary swellings also seen (Fig. 10). Arthrospores not seen.

CODE: 2a, 3c, 26e, 34?, 37, 39, 43, 49, 56, 60.

OXIDASE REACTIONS: Positive reaction with syringaldazine, guaiac and guaiacol, otherwise negative.

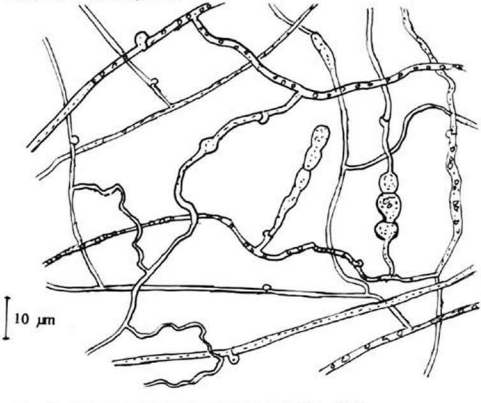

Fig. 10. Hyphae in Pholiota highlandensis (GB 1328).

No data on cultural characters have been previously published for this species. Systematically, it undoubtly belongs to the *lubrica* group (subgenus *Lubricula*). Morphologically it has much in common with *Pholiota spumosa* but in the culture characters they clearly differ.

The basidiocarps of the four stocks in this investigation varied considerably in size and colour but were found to be intercompatible. Some mycologists (cfr Smith & Hesler 1968, Orton 1988) recognize several closely related species growing on charcoal. It is quite possible that more than one species exists but as the morphological characters, especially the pigmentation, vary with the age of the basidiocarp and are influenced by external factors, it should be confirmed by interfertility tests. *Pholiota carbonaria* A.H. Smith is described from the USA and is said to differ from *P. highlandensis* in the distinctly red veil. It is desirable to have interfertility tests made between this species and *P. highlandensis*.

Only a very low number of spores germinate. Probably it depends on a factor connected with the special habitat as basidiospores from all other species of this group are easily germinated on common malt agar. The habitat burned wood indicates that it is adapted to a high concentration of minerals.

Pholiota spumosa (Fr.)Sing.

MATERIAL:
GB 884/*Picea*/Sweden, Medelpad, Borgsjö (SJ 83069).
GB 1245/*Picea*/Sweden, Västergötland, Skepplanda (SJ 84089).
GB 1349/*Picea*/Sweden, Medelpad, Tuna (SJ 84187).
POLARITY: Two polarity tests are performed (Tab. 8).

Tab. 8. Polarity tests in *Pholiota spumosa*.

	GB 884/2	GB 884/3	GB 884/4	GB 884/5	GB 884/6	GB 884/7	GB 884/8	GB 884/9
GB 884/1	+	–	–	+	–	–	–	–
GB 884/2		+	–	+	–	–	+	–
GB 884/3			+	+	–	–	–	–
GB 884/4				–	–	–	–	+
GB 884/5					–	+	–	+
GB 884/6						–	+	–
GB 884/7							–	–
GB 884/8								–

	GB 1245/2	GB 1245/3	GB 1245/4	GB 1245/5	GB 1245/6
GB 1245/1	–	–	(+)	–	–
GB 1245/2		–	–	–	(+)
GB 1245/3			–	–	+
GB 1245/4				–	–
GB 1245/5					–

(+) means only a few clamps seen in the contact zone.

The pairing table of GB 884 is complicated and difficult to interpret. Several explanations are possible, that with the fewest failures is: 9, 7 = A1B1; 4 = A2B2; 8 = A1B2; 6 = A2B1; 1, 3 = A1B1+A1B2; 5 = A2B2+A1B2; 2 = A2B2+A2B1. However, there are 5 failures (1 x 6, 2 x 7, 2 x 9, 3 x 6, 5 x 6). It seems impossible to interpret these results with certainty, but spores with more than one factor must be involved (amphithallic tetrapolarity).

GB 1245 is easily interpreted as normally tetrapolar: 1, 5 = A1B1; 4 = A2B2; 2, 3 = A1B2; 6 = A2B1. However, there is then one failure (4 x 5).

Also GB 1349 is tested but in this specimen (8 isolates) no clamps were formed in any combination.

CYTOLOGY: The spores are binucleate. Ss-mycelia are in terminal cells multinucleate, otherwise generally binucleate (heterocytic behaviour). Only dicaryotic cells seen in ps-mycelia.

INTERCOMPATIBILITY TESTS: Tests have been performed between the specimens 884/1245 and 1245/1349. They proved to be completely interfertile. Clamps were formed in all dishes and no distinct confrontation line between the different mycelia was seen.

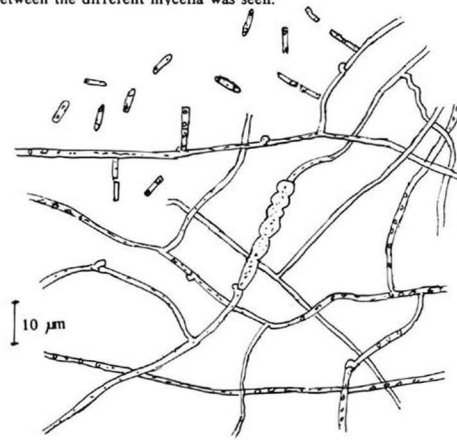

Fig. 11. Hyphae and arthrospores in Pholiota spumosa (GB 884).

CULTURE CHARACTERS (GB 884): Growth slow, dishes covered in five - six weeks. Advancing zone even, appressed (Fig. 2 A). Aerial mycelium

absent. Reverse unchanged. Hyphae narrow, only 1 - 2 µm wide, regularly branched, with a clamp at most septa. Arthrospores (5 - 10 x 2 µm) occur, formed by short, single branches (Fig. 11). No chlamydospores found.
CODE: 2ab, 3c, 7, 35, 36, 38, 45 -46, 49, 55, 60, 63.
OXIDASE REACTIONS: Positive reactions are noted for syringaldazine, guaiac, guaiacol, p-Cresol, L-Tyrosine, but all rather weak.

Pholiota mixta (Fr.)Sing.

MATERIAL:
GB 141/on soil in coniferous forest/Sweden, Vg, Göteborg (SJ 80165).
GB 948/on soil in coniferous forest/Sweden, Vg, Länghem (SJ 83099).
GB 1302/on soil in coniferous forest/Sweden, Vg, St. Mellby. (SJ 84110)

Tab. 9 Polarity tests in *Pholiota mixta*.

	GB 141/2	GB 141/3	GB 141/4	GB 141/5	GB 141/6	GB 141/7	GB 141/8	GB 141/9	GB 141/10
GB 141/1	–	+	+	–	–	+	+	+	–
GB 141/2		–	+	–	+	–	–	–	+
GB 141/3			+	–	–	–	–	–	–
GB 141/4				+	–	+	–	–	+
GB 141/5					–	–	–	–	–
GB 141/6						–	–	–	–
GB 141/7							–	–	–
GB 141/8								–	–
GB 141/9									–

	GB 1302/2	GB 1302/3	GB 1302/4	GB 1302/5	GB 1302/6	GB 1302/7	GB 1302/8
GB 1302/1	+	–	–	–	–	–	+
GB 1302/2		+	–	+	+	–	+
GB 1302/3			+	–	–	+	–
GB 1302/4				–	+	–	+
GB 1302/5					–	–	+
GB 1302/6						+	–
GB 1302/7							+

	GB 948/2	GB 948/3	GB 948/4	GB 948/5	GB 948/6	GB 948/7	GB 948/8	GB 948/9	GB 948/10
GB 948/4	–	+	+	+	+	+	+	+	+
GB 948/7		+	+	+	+	+	+	+	+
GB 948/1			–	–	–	–	–	–	–
GB 948/2				–	–	–	–	–	–
GB 948/3					–	–	–	–	–
GB 948/5						–	–	–	–
GB 948/6							–	–	–
GB 948/8								–	–
GB 948/9									–

POLARITY: Three polarity tests are performed (Tab. 9). The pairing table of GB 141 fits a tetrapolar pattern. However, isolate nr 5 does not form clamps with any of the other isolates. GB 948 seems to be clearly bipolar, without any failures. In GB 1302, however, the pairing table could not be

explained without amphithallic tetrapolarity. If 1, 5 = A1B1; 4, 7 = A1B2; 3, 6 = A2B1; 8 = A2B2+A2B1; 2 = A2B2+A1B2, there are no failures. Other interpretations seem to be improbable.
INTERCOMPATIBILITY: The three specimens have been tested with each other and are found to be completely interfertile.
CYTOLOGY: Heterocytic. The spores are binucleate, terminal cells of ss-mycelia multinucleate, other cells seemingly most binucleate. In a secondary mycelium only dicaryotic cells were seen.
CULTURE CHARACTERS (GB 948): Growth moderately rapid, dishes covered in four weeks. Advancing zone even, appressed. Aerial mycelium absent (Fig. 2 B). Reverse unchanged. Hyphae 1 - 3 µm wide, a few with somewhat wider with slight moniliform swellings, regularly branched, with a clamp at most septa. The mycelium is identical in appearance with that of P. spumosa, but no accessory spores seen.
CODE: 2ab, 3c, 26e, 32, 36, 38, 44, 49, 56, 59, 63.
OXIDASE REACTIONS: Positive reactions are noted in all tests, rapid and strong with syringaldazine, guaiac and guaiacol, but very weak with L-Tyrosine.

Pholiota mixta is very similar to *P. spumosa* in most characters and the two species are undoubtly closely related. Several interfertility tests between them have been performed, but none of the matings led to clamp formation.

Pholiota lenta (Fr.)Sing.

MATERIAL:
GB 82/*Fagus* forest/Sweden, Skåne, Anderslöv (SJ 79228).
GB 1060/litter/Sweden, Skåne, Degeberga (SJ 83127).

Tab. 10. Polarity tests in *Pholiota lenta*.

	GB 82/2	GB 82/3	GB 82/4	GB 82/5	GB 82/6	GB 82/7	GB 82/8	GB 82/9	GB 82/10
GB 82/1	+	-	+	-	-	+	-	-	+
GB 82/2		-	-	+	-	-	-	(+)	-
GB 82/3			-	-	-	-	-	+	-
GB 82/4				+	-	-	-	+	-
GB 82/5					-	+	+	-	+
GB 82/6						-	+	+	-
GB 82/7							-	+	-
GB 82/8								+	-
GB 82/9									-

	GB 1060/2	GB 1060/3	GB 1060/4	GB 1060/5	GB 1060/6	GB 1060/7	GB 1060/8	GB 1060/9	GB 1060/10
GB 1060/1	-	+	-	-	-	-	-	+	-
GB 1060/2		+	-	-	-	-	-	+	-
GB 1060/3			-	-	+	+	-	-	-
GB 1060/4				-	-	-	+	-	-
GB 1060/5					+	-	+	-	-
GB 1060/6						-	-	+	-
GB 1060/7							-	+	-
GB 1060/8								-	+
GB 1060/9									-

(+) means only a few clamps seen.

POLARITY: The two available specimens are tested (Tab. 10). Amphithallic tetrapolarity is indicated in the pairing tables of the two specimens studied:

GB 82. The most probable explanation is: 2, 4, 7, 10 = A1B1; 1, 5 = A2B2; 3 = A1B2; 6 = A1B1+A2B1; 8 = A1B1+A1B2; 9 = A2B2+A2B1. This interpretation yields 5 failures. There may be other interpretations but in that case the number of failures is higher.

GB 1060. If 1, 2, 6, 7 = A1B1; 3, 9 = A2B2; 8 = A1B2; 4, 5, 10 = A2B1, there is a regular tetrapolar pattern without failures.

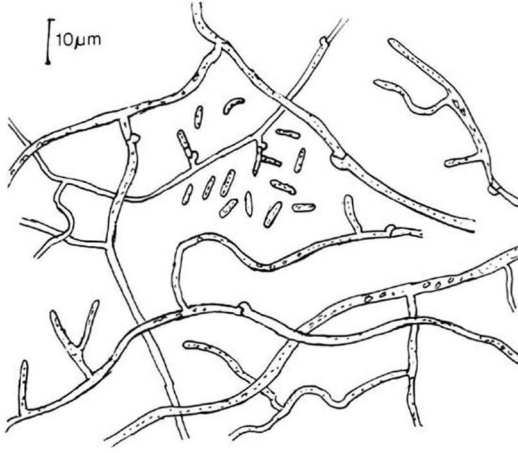

Fig. 12. Hyphae and arthrospores in Pholiota lenta (GB 1060).

INTERCOMPATIBILITY: The two specimens are completely compatible with each other.

CYTOLOGY: Astatocoenocytic behaviour is established. Most cells in ss-mycelia contain two nuclei but some of them, also intercalary cells, are plurinucleate with 5 - 10 nuclei. In a secondary mycelium except normally binucleate, clamped hyphae also pluri- or multinuclear cells in wide hyphae without clamps are seen.

CULTURE CHARACTERS (GB 1060): Growth slow, dishes covered in five weeks. Advancing zone even or somewhat fringed, appressed. Aerial

mycelium absent (Fig. 2 C). Reverse unchanged. Hyphae 1,5 - 4 µm wide, ordinarily branched, with a clamp at most septa. Arthrospores (5 - 10 x 2 µm) occur. Only a few, somewhat wider hyphae with slight irregular swellings seen. No chlamydospores found. (Fig. 12).
CODE: 1 (2b), 3c, 7, 34, 36, 38, 45, 49, 54, 60, 63.
OXIDASE REACTIONS: a weak positive reaction with L-Tyrosine noted, all other tests were negative.

Pholiota lubrica (Pers.:Fr.)Sing.

MATERIAL:
GB 508/litter/Sweden, Västergötland, Halleberg (SJ 82140).
GB 881/*Picea*/Sweden, Jämtland, Mörsil (SJ 83055).
GB 1293/Austria (M.Moser as *Pholiota decussata*).
GB 1495/Sweden, Medelpad, Selånger (J.O.Tedebrand).
POLARITY: Only GB 881 and GB 1495 are tested regarding their polarity. In both specimens amphithallic tetrapolarity is showed (Tab. 11).

Tab. 11. Polarity tests in *Pholiota lubrica*.

	GB 881/2	GB 881/3	GB 881/4	GB 881/5	GB 881/6	GB 881/7
GB 881/1	-	-	-	-	-	-
GB 881/2		-	-	-	-	+
GB 881/3			-	+	-	+
GB 881/4				-	-	+
GB 881/5					-	+
GB 881/6						+

	GB 1495/2	GB 1495/3	GB 1495/4	GB 1495/5	GB 1495/6	GB 1495/7	GB 1495/8
GB 1495/1	-	-	+	+	+	+	+
GB 1495/2		-	+	+	+	+	+
GB 1495/3			+	+	+	+	+
GB 1495/4				-	-	-	+
GB 1495/5					-	-	+
GB 1495/6						-	-
GB 1495/7							-

GB 881: The best interpretation is: 2, 4, 6 = A2B2; 1 = A1B2; 5 = A2B1; 7 = A1B1+A1B2; 3 = A2B2+A1B2. One failure (1 x 5).

GB 1495: 1, 2, 3 = A1B2; 4, 5, 6, 7 = A2B1, 8 = A1B1+A1B2. Two failures with this interpretation (8 x 6, 8 x 7).
INTERCOMPATIBILITY: GB 508, 881, 1293 are tested with each other and were found to be completely interfertile. However, GB 1495 was not compatible with any of the others. The basidiocarps of this collection appeared strikingly yellow ("forma *lutea*") and it seems probable that it represent a separate species. This will be discussed later.
CYTOLOGY: Astatocoenocytic. In ss-mycelia the number of nuclei is very variable (2 - 15 are noted). Also in secondary mycelia with clamps a number of multinucleate (5 - 15) cells are found. These are wider than normal cells and lack clamps at the septa.
CULTURE CHARACTERS (GB 1293): Growth moderately rapid or slow, dishes covered in four or five weeks. Advancing zone even, appressed.

Aerial mycelium absent (Fig. 2 D). Hyphae 2 - 4 μm wide, regularly branched, with a clamp at most septa, in appearance identical with *P. lenta*. Arthroconidia (5 - 10 x 2 μm) occur. No chlamydospores seen.
CODE: 1 (2), 3c, 7, 34, 36, 44-45, 49, 54, 60, 63.
OXIDASE REACTIONS: A weak positive reaction noted with guaiac, otherwise negative.

Tab. 12. Interfertility test between *P. lubrica* and *P. lenta*.

		GB 1289			
		1	2	3	4
GB 881	1	-	-	+	-
	2	-	-	-	+
	3	-	-	-	-
	4	-	-	+	-

In most characters, both morphological and in culture studies, *Pholiota lubrica* is identical with *P. lenta* and without doubt the two species are very closely related. The most evident difference seems to be the pigmentation of the basidiocarps. Some intercompatibility tests have been performed between different strains of *P. lubrica* and *P. lenta*. Four isolates of *lenta* (GB 507) were mated with four each from GB 508 and GB 881. Clamps did not appear in any dish and a distinct barrier was always formed. But in an interfertility test between GB 881 and another specimen of *P. lenta* (GB 1289) clamps were formed in three cases (Tab. 12). This confirms their close relatioship but is not a reason enough to consider them conspecific. Interfertility tests have also been made between *P. lubrica* and *P. mixta* but they were, as expected, negative.

Pholiota scamba (Fr.)Mos.

MATERIAL:
GB 1221/*Picea*/Sweden, Jämtland, Mörsil, Sällsjö (SJ 84057).
GB 1710/*Picea*/Sweden, Västergötland, Hindås (SJ 86043).
POLARITY: A polarity test was performed with GB 1221. The pairing table fits a tetrapolar pattern: 1, 5 = A1B1; 3, 6 = A2B2; 2 = A1B2; 4 = A2B1 (Tab. 13).
INTERCOMPTATIBILITY: The two specimens are completely compatible.
CYTOLOGY: Heterocytic. Several cells of ss-mycelia are multinucleate (- 15 nuclei) and wider than other cells. In ps-mycelia only binucleate cells are seen.

Tab. 13. Polarity test in *Pholiota scamba*.

	GB 1221/2	GB 1221/3	GB 1221/4	GB 1221/5	GB 1221/6
GB 1221/1	–	+	–	–	+
GB 1221/2		–	+	–	–
GB 1221/3			–	+	–
GB 1221/4				–	–
GB 1221/5					+

CULTURE CHARACTERS (GB 1221): Growth slow, dishes covered in six weeks. Advancing zone even, appressed. Aerial mycelium absent.(Fig. 2 F). Reverse unchanged. Hyphae 1 - 4 µm wide, regularly branched, with a clamp at all septa. Irregular or moniliform swellings (- 10 µm wide) fre-

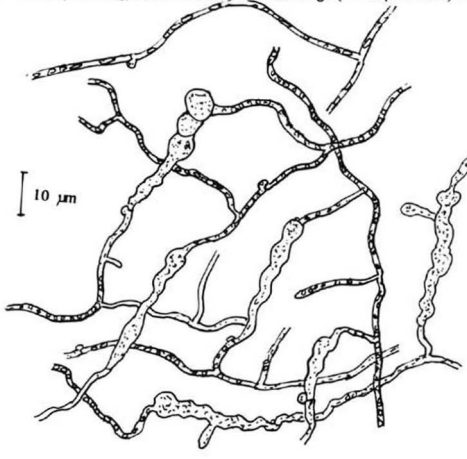

Fig. 13. Hyphae in Pholiota scamba (GB 1221).

quently occur (Fig. 13), probably some of them may serve as chlamydospores.

Arthrospores not seen in any isolate.

CODE: 2ab, 3c, 26, 34?, 36, 38, 46, 49, 55, 60.

OXIDASE REACTIONS: A strong positive reaction was noted in all tests.

Pholiota gummosa (Lasch)Sing.

MATERIAL:
GB 1254/buried wood/Sweden, Halland, Tjolöholm (SJ 84095).
GB 1295/buried wood/Sweden, Västergötland, Angered (SJ 84163).
GB 1300/*Pinus* bark/Sweden, Västergötland, Bergum (SJ 84164).
GB 1327/buried wood/Sweden, Halland, Kungsbacka (SJ 84180).

POLARITY: The first three specimens are tested regarding their polarity (Tab. 14). The pairing tables show several irregularities and are difficult to interpret with certainty. Amphithallic tetrapolarity is the only possible explanation.

Tab. 14. Polarity tests in *Pholiota gummosa*.

	GB 1254/2	GB 1254/3	GB 1254/4	GB 1254/5	GB 1254/6	GB 1254/7
GB 1254/1	–	–	+	+	–	–
GB 1254/2		–	–	–	+	+
GB 1254/3			+	+	–	–
GB 1254/4				–	+	–
GB 1254/5					+	–
GB 1254/6						+

	GB 1295/2	GB 1295/3	GB 1295/4	GB 1295/5	GB 1295/6	GB 1295/7	GB 1295/8
GB 1295/1	(+)	+	–	–	–	+	+
GB 1295/2		–	(+)	–	+	–	–
GB 1295/3			+	–	+	–	–
GB 1295/4				–	–	+	+
GB 1295/5					+	–	–
GB 1295/6						+	–
GB 1295/7							–

	GB 1300/2	GB 1300/3	GB 1300/4	GB 1300/5	GB 1300/6	GB 1300/7	GB 1300/8	GB 1300/9
GB 1300/1	+	–	+	–	(+)	–	+	–
GB 1300/2		+	–	–	–	+	–	+
GB 1300/3			–	–	+	–	+	–
GB 1300/4				+	–	+	–	+
GB 1300/5					–	–	+	–
GB 1300/6						–	–	+
GB 1300/7							+	–
GB 1300/8								+

(+) means only a few clamps seen, limited to the confrontation line.

GB 1254 is best interpreted as follows. 2 = A1B1; 1, 3 = A1B2; 4, 5 = A2B1; 6 = A2B2+A1B2; 7 = A2B2+A2B1. In this interpretation two failures exist:(1 x 7, 3 x 7). Other interpretations yield more failures.

There is one interpretation of GB 1295 without failures: 5 = A1B1; 6 = A2B2; 1, 4 = A1B2; 8 = A2B1; 2, 3, 7 = A1B1+A2B1. However, it is remarkable that three mycelia have the same combination of factors.

GB 1300 is difficult to interpret. The most reasonable interpretation

yields five failures: 5 = A1B1; 4 = A2B2; 6 = A1B2; 2 = A1B1+A1B2; 1, 3, 7, 9 = A1B1+A2B1; 8 = A2B2+A2B1.

The failures are 2 x 4, 3 x 4, 2 x 8, 6 x 7, 6 x 8. The interpretation must be considered as theoretical and uncertain, others are possible but yield more failures.

INTERCOMPATIBILTY: Tests have been made between all possible combinations of the 4 specimens. They appeared to be competely intercompatible.

CYTOLOGY: Boidin (1971) reported an astatocoenocytic behaviour in this species. In this investigation, ss-mycelia were found to have a varying

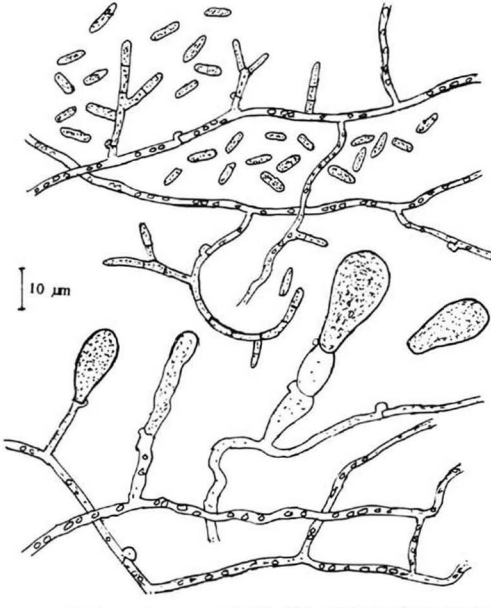

Fig. 14. Hyphae, arthrospores and chlamydospores in Pholiota gummosa (GB 1295).

number of nuclei in the cells, frequently two but many were multinucleate. No multinucleate cells seen with certainty in ps-mycelia.
CULTURE CHARACTERS (GB 1295): Growth moderately rapid, dishes completely covered in three weeks. Advancing zone even, appressed. Mycelium floccose, whitish with brown floccules (very similar to that of *P. squarrosa*) (Fig. 3 A). Reverse somewhat brownish. Hyphae 1,5 - 5 µm wide, regularly branched, with a clamp at most septa, frequently with oil-rich, terminal swellings (chlamydospores, 15 x 30 x 12 - 20 µm), single or in chains. The brown floccules contained numerous arthrospores, 4 - 10 x 2 - 4 µm (Fig. 14).
CODE: 2a, 3c, 26e, 34, 35, 37, 39, 43, 49, 56, 60, 63.
OXIDASE REACTIONS: A weak positive reaction was noted with syringaldazine and guaiacol, other tests were negative.

Pholiota gummosa has been studied in culture. Kühner (1946) made a careful investigation of cultured mycelia and describes the formation of both types of accessary spores. Käärik (1970) states a strong laccase but weak tyrosinase reaction. In this investigation a weak laccase but no tyrosinase reaction was found. The different results may depend on the fact that Käärik used basidiocarp tissue for her tests.

Pholiota graminis (Quél.)Sing.

MATERIAL:
GB 1273/moist ground, close to *Salix* scrub/Sweden, Västergötland, Österplana (SJ 84102).
POLARITY: Unknown, only one ss-mycelium received.
CULTURE CHARACTERS: Growth moderately rapid, the dishes covered in three weeks. Advancing zone even, appressed. Aerial mycelium slightly cottony, brownish in old parts (Fig. 3 B). Reverse brownish. Hyphae 2- 5 µm wide, regularly branched, with a clamp at most septa. Frequent chlamydospores, 15 - 20 x 10 - 15 µm (Fig. 15). No arthrospores seen.
CYTOLOGY: Only ps-mycelia studied. All hyphal cells seemingly binucleate.
CODE: 2ab, 3c, 26e, 34, 37, 39, 43, 49, 56, 58.
OXIDASE REACTIONS: Positive reactions were noted with all reagents.

The culture characters are very similar to those of *P. gummosa*, for instance the conspicuous chlamydospores, which indicate that the species are closely related. Certainly the mycelia on the dishes look very different, very smooth compared with distinctly floccose in *P. gummosa*, but this character seems to be connected with presence or absence of arthrospore formation. The two species also have morphological similarities, especially

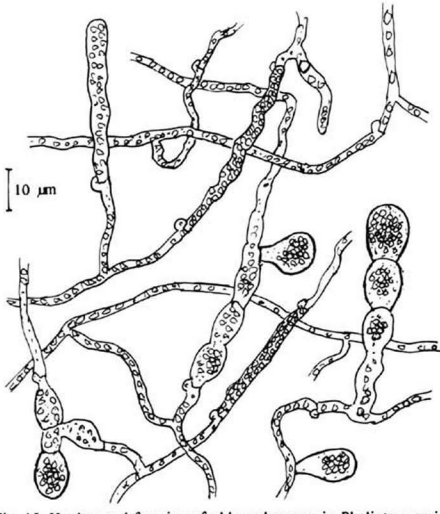

Fig. 15. Hyphae and forming of chlamydospores in Pholiota graminis (GB 1273).

in the shape of the spores. Interfertility tests between GB 1273 and some strains of *P. gummosa* by Buller's phenomen indicate incompatiblity between them.

Pholiota alnicola (Fr.)Sing.

MATERIAL:
GB 174/*Alnus*/Sweden, Göteborg, Botanical Garden (SJ 80194).
GB 1243/*Betula*/Sweden, Åsele Lappmark, Stenbithöjden (SJ 84073).
GB 1341/*Sorbus*/Sweden, Medelpad, Sundsvall (SJ 84097).
GB 1366/*Salix*/Sweden, Scania, Kristianstad (SJ 84132 A).
GB 1763/*Fagus*/Sweden, Scania, Vegeholm (SJ 86071).
POLARITY: GB 174 was tested, but clamps did not appear in any dish. In GB 1763 6 ss-mycelia were tested. It proved to be normally tetrapolar (Tab. 15).

Tab. 15. Polarity test in *Pholiota alnicola*.

	GB 1763/3	GB 1763/4	GB 1763/5	GB 1763/6	GB 1763/7
GB 1763/1	–	–	–	–	+
GB 1763/3		–	–	+	–
GB 1763/4			–	+	–
GB 1763/5				+	–
GB 1763/6					–

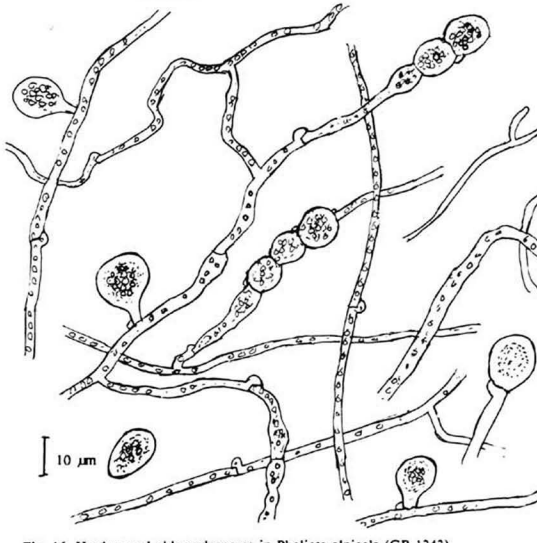

Fig. 16. Hyphae and chlamydospores in Pholiota alnicola (GB 1243).

INTERCOMPATIBILITY: The specimens mentioned have been tested in most possible combinations and were found to be completely intercompatible. It is especially notable that specimens growing on *Sorbus* and *Fagus* were compatible with specimens growing on *Alnus* or *Betula*, as substrate

specificity is sometimes used in discussions on species delimitations (cfr *Flammula apicrea* ss. Lge.).
CYTOLOGY (GB 1243): Heterocytic. Cells of ss-mycelia frequently pluri- to multinucleate. All hyphae of the secondary mycelium are regularly clamped.
CULTURE CHARACTERS (GB 1243): Growth very slow, in six weeks three mycelia had reached only 42 - 50 mm from the place of inoculation. Advancing zone appressed, fringed. Aerial mycelium downy - cottony (Fig. 3 E). Reverse somewhat yellowish brown. Hyphae 2 - 5 µm wide, regularly branched, with a clamp at all septa. Numerous large chlamydospores, 15 - 30 x 12 - 15 µm, i all parts of the mycelium (Fig. 16). They arise both apically and as lateral inflations.
CODE: 1, 3c, 26, 34, 37, 39, 47, 49, 54, 60, 61.
OXIDASE REACTIONS: No positive reaction noted.

Pholiota alnicola and *P. pinicola* diverge morhologically from other Pholiotas and therefore appear to take a rather isolated systematic position, and the culture characters emphasize this. The mycelia look rather different from most other species by the rich occurrence of large chlamydospores. *P. alnicola - pinicola* appear to be normally tetrapolar contrary to others, which mostly are amphithallic.

According to Käärik (1965) *P. alnicola* belongs to the group of fungi producing laccase but not tyrosinase.

Pholiota pinicola S.Jacobss.

MATERIAL:
GB 1356/*Pinus*/Sweden, Göteborg, V.Frölunda (SJ 84169).
GB 1359/*Pinus*/Sweden, Västergötland, Sätila (SJ 84158).
POLARITY: 10 ss-mycelia of GB 1359 are tested (Tab. 16). The specimen was normally tetrapolar.
INTERCOMPATIBILITY: The two specimens in culture were found to be compatible.
CYTOLOGY: Not studied but probably heterocytic like *P. alnicola*. The hyphae of the secondary mycelium are not very variable in wideness and always clamped.
CULTURE CHARACTERS (GB 1359): Growth very slow, in six weeks the mycelia only had reached 35 - 37 mm from the place of inoculation. Advancing zone appressed, bayed - fringed. Aerial mycelium downy and whitish in distal (young) parts, patch-wise brownish and subfelty in old parts. (Fig. 3 F). In all other respects, chlamydospores etc, identical with those of *P. alnicola*.
CODE: 1, 3c, 26e, 34, 36, 39, 47, 49, 55, 60, 61.

Tab. 16. Polarity test in *Pholiota pinicola*.

	GB 1359/2	GB 1359/3	GB 1359/4	GB 1359/5	GB 1359/6	GB 1359/7	GB 1359/8	GB 1359/9	GB 1359/10
GB 1359/1	–	–	–	+	–	–	–	–	+
GB 1359/2		–	–	–	–	–	+	+	–
GB 1359/3			–	–	–	–	+	+	–
GB 1359/4				–	–	–	+	+	–
GB 1359/5					–	+	–	–	–
GB 1359/6						–	+	+	–
GB 1359/7							–	–	+
GB 1359/8								–	–
GB 1359/9									–

OXIDASE REACTIONS: No positive reaction noted.

The culture characters of this species are very similar to those of *P. alnicola* and confirm their close relationship.

Pholiota mutabilis (Schaeff.:Fr.)Kummer

MATERIAL.
GB 1304/*Betula*/Sweden, Västergötland, Göteborg (SJ 84172).
POLARITY: 10 ss-mycelia of the specimen GB 1304 were mated in all possible combinations (Tab. 17). The most reasonable interpretation suggests bipolarity, in that case with two failures (1 x 8, 2 x 7).

Tab. 17. Polarity test in *Pholiota mutabilis*.

	GB 1304/2	GB 1304/3	GB 1304/4	GB 1304/5	GB 1304/6	GB 1304/7	GB 1304/8	GB 1304/9	GB 1304/10
GB 1304/1	–	–	+	+	–	+	–	–	+
GB 1304/2		–	+	+	–	–	+	–	+
GB 1304/3			+	+	–	+	+	–	+
GB 1304/4				–	+	–	–	+	–
GB 1304/5					+	–	–	+	–
GB 1304/6						+	+	–	+
GB 1304/7							–	+	–
GB 1304/8								+	–
GB 1304/9									+

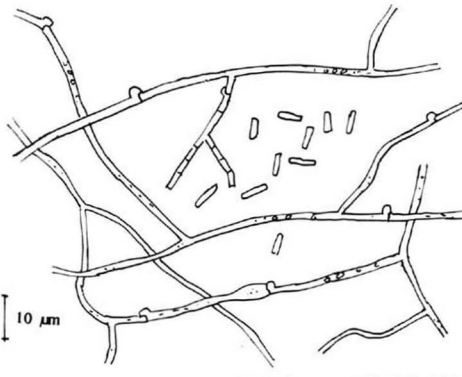

Fig. 17. Hyphae and arthrospores in Pholiota mutabilis (GB 1304).

CULTURE CHARACTERS: Growth slow, dishes covered in five weeks. Advancing zone even, appressed. Aerial mycelium cottony, white (Fig. 3 D). Reverse only weakly brownish. Hyphae 1 - 3 (-4) µm wide, regularly branched, with a clamp at all septa. A rather low number of arthrospores (5 - 10 x 2 µm) are formed in the aerial mycelium. The hyphae are very little differeniated, only occasionally slight intercalary swellings (2 - 5 µm wide) appear (Fig. 17).
CODE: 2ab, 3c, 7, (26), 35, 36, 38, 45, 49, 54, 59.
OXIDASE REACTIONS. Strong positive reaction noted with all reagents.

Pholiota mutabilis has long been placed in the genus *Kuehneromyces*. However, Kühner (1980) has pointed out that there actually are no real differences between this and other species of *Pholiota* and therefore proposes a reunion of *Kuehneromyces* with *Pholiota*. The culture characters noted are normal for the genus and do not support generic separation. The bipolarity must be confirmed by additional investigations, it may be an artifact. Vandendries & Brodie (1933) reported this species to be tetrapolar. Bipolarity is apparently uncommon in *Pholiota*, with certainty only known for *P. jahnii*.

The strong reaction both for laccase and tyrosinase was noted also by Käärik (1970).

Pholiota lignicola (Peck)S.Jacobss. comb. nov.
(basionym: *Agaricus lignicola* Peck, N.Y.State Cab., Ann.Rep. 23:91, 1872)

MATERIAL:
GB 1908/coniferous wood/Sweden, Göteborg, Skatås (SJ 87001).
POLARITY: The polarity test indicates that the species is normally tetrapolar (Tab. 18). If 1, 2 = A1B1; 4, 5, 8 = A2B2; 3 = A1B2; 7 A2B1; 6 = A2B2+A1B2, there are no failures.

Tab. 18. Polarity test in *Pholiota lignicola*.

	GB 1908/2	GB 1908/3	GB 1908/4	GB 1908/5	GB 1908/6	GB 1908/7	GB 1908/8
GB 1908/1	–	–	+	+	–	–	+
GB 1908/2		–	+	+	–	–	+
GB 1908/3			–	–	–	+	–
GB 1908/4				–	+	–	–
GB 1908/5					+	–	–
GB 1908/6						+	+
GB 1908/7							–

CYTOLOGY: Not studied. Only clamped hyphae are seen in the secondary mycelium.
CULTURE CHARACTERS: Growth slow, dishes covered in six weeks. Advancing zone even, appressed. Aerial mycelium sparse, downy. Reverse pale yellowish. Normal, straight hyphae 1,5 - 4 μm wide, regularly branched and with a clamp at most septa, but a great part of the hyphae are very irregular in shape and (mostly 5 - 10 μm wide) with numerous swellings, often with a moniliform appearance (Fig. 18). Many of the swellings serve as chlamydospores. No arthrospores are seen.
CODE: 2a, 3c, 26e, 34, 36, 39, 46, 49, 55, 60, 63
OXIDASE REACTIONS: Positive but rather weak reactions were noted with syringaldazine, 1-naphtol and guaiac, otherwise negative.

This species was earlier known as *Kuehneromyces vernalis* (Peck)Singer & Smith, which name, however, is illegitimate because the basionym *Agaricus vernalis* Peck is a later homonym of *Agaricus vernalis* Bolton(Redhead 1984). It is frequently placed in *Kuehneromyces* close to *mutabilis*, depending on similarities in microscopical characters as the prominent germ-pore in the spores and the absence of chrysocystidia. The cultural characters of *P. lignicola* are very different from those of *P. mutabilis* and it is obvious that the two species are not closely related.

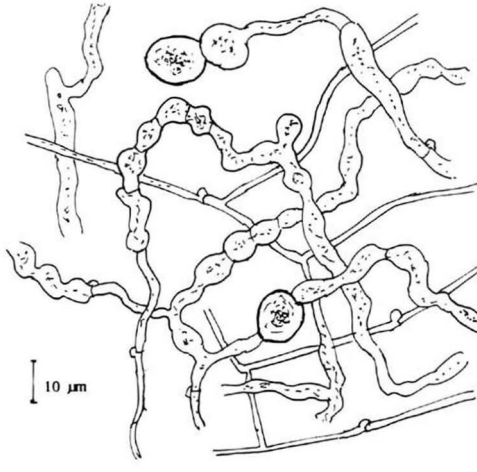

Fig. 18. Hyphae in Pholiota lignicola (GB 1908).

Pholiota albocrenulata (Peck)Sacc.

MATERIAL:
GB 1851/*Acer*/USA (CBS 228.30).
POLARITY: Unknown.
CULTURE CHARACTERS: Growth slow, dishes covered in five weeks. Advancing zone even, appressed. Aerial mycelium absent. Hyphae generally 3 - 6 µm wide, regularly branched, without clamps. Actively growing, terminal hyphae frequently moniliform with 5 - 10 µm wide swellings (Fig. 19). No accessory spores seen.
CODE: 1, 6, 26, 32, 37, 45, 49, 54.
OXIDASE REACTIONS: No positive reaction noted with any reagent.

The secondary mycelium of *Pholiota albocrenulata* is normally clamped. The culture CBS 228.30 is apparently very old (originally collected by I.Mounce) and lacks clamps. The reason for the absence of

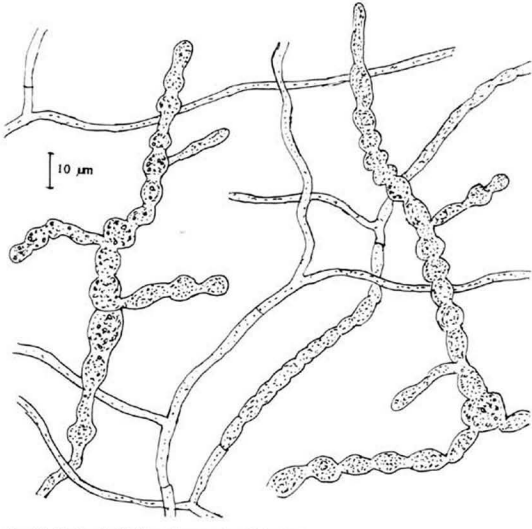

Fig. 19. Hyphae in Pholiota alnocrenulata (GB 1851).

clamps is probably senescence. P. albocrenulata (often placed within Stropharia) differs in some characters, e.g. pigmentation and the spores, from other species of Pholiota and might perhaps better be transferred to a genus of its own. However, there are no special microcharacters noted which give additional support for this.

Gymnopilus junonius (Fr.)Orton

MATERIAL:
GB 1311/*Betula*/Sweden, Bohuslän, Hönö (SJ 84074).
POLARITY: Unknown, only one ps-mycelium studied.
CULTURE CHARACTERS: Growth moderately rapid, dishes covered in four weeks. Advancing zone somewhat fringed. Aerial mycelium downy, whitish. Hyphae 2 - 5 µm wide, regularly branched, with lamps at most septa. Piriform to globose chlamydospores (10 -20 x 7 - 15 µm) are rather

numerous (Fig. 20). No arthrospores seen.
CODE: 2a, 3c, 26e, 34, 36, 44, 49, 54.
OXIDASE REACTIONS: A weak positive reaction noted with guaiac and 1-Naphtol, otherwise negative.

The culture characters noted correspond well with those of species belonging to Pholiota. Gymnopilus is separated from Pholiota by having rough spores, which is easily seen also in a light microscope, and the absence of chrysocystidia. The two genera have the pigments and ecology in common and are undoubtly related.

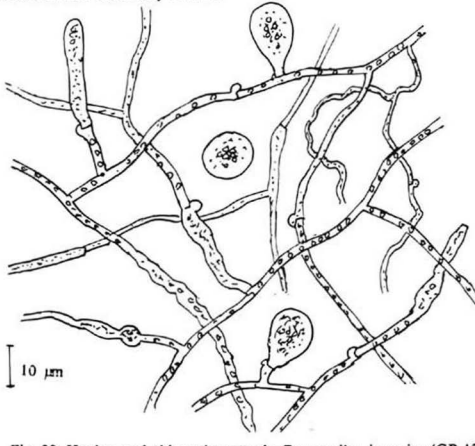

Fig. 20. Hyphae and chlamydospores in Gymnopilus junonius (GB 1311).

CONCLUSIONS

The culture characters of this investigation are part of a taxonomic work on Pholiota in northern Europe which will include morphological and ecological data. Culture characters, until now, have been rarely used in the taxonomy of agarics, though regularly used by mycologists in Aphyllophorales. No doubt cultural studies add many valuable characters for a better understanding of relationships between species, groups of species or genera, but of course, the results must be used with care, as the infra-

specific variation is not well documented.

In *Pholiota* and allied genera there are clear differences between species in several characters as the general appearance of the hyphae, growth rate, type of and frequency of accessory spores, presence or absence of laccase/tyrosinase etc. The reliability of a character increases with the number of studied isolates. It is desirable to sample several cultures of each species, which, however, not always has been possible.

Some natural groups of closely related species are easily distinguished in *Pholiota*, e.g. the *adiposa* and the *lubrica* groups. That the species in these groups are closely related is quite obvious as they have many basidiocarp characters in common. As expected closely related species in culture also have most characters in common. For instance, all members of the *adiposa* group have a slow growth rate and all form numerous arthrospores in aerial mycelia and chlamydospores in submerged parts of the mycelia. The *lubrica* group is characterized by moderately to slow growth rate and a rather simple appearance of the hyphae. Arthrospores are formed in some species but chlamydospores are absent or rare.

However, there are also divergences. *Pholiota highlandensis*, judging from basidiocarp morphology a typical member of the lubrica group, differs from the others in some respects. It is rather difficult to culture, only a very low number of spores germinate on common malt agar (the other species are easily cultured) but when germinated, the growth is rather rapid. The differences are probably connected with the specialized ecology of this species.

Some species, e.g. *P. tuberculosa* and *P. heteroclita*, deviate in many characters from others and therefore appear isolated systematically. *Pholiota squarrosa* has some characters, e.g. the chlamydospores and growth rate, similar to *P. gummosa* - *P. graminis* and may therefore be related to these species, though they generally are placed in a different subgenus (*Flammula*) based on their morphological characters. In any case, it is obvious that the old division of *Pholiota* into two subgenera *Pholiota* and *Flammula* is not natural. *P. mutabilis* and *P. lignicola* are earlier generally placed in a separate genus, *Kuehneromyces*. The characters in culture reveal that the two species are not closely related and apparently there do not exist any tenable reason to maintain *Kuehneromyces*. *Gymnopilus junonius* has characters which correspond with many species of *Pholiota* and the culture study supports a close relationship between the two genera. Owing to the rough spores *Gymnopilus* often is supposed to be related to *Cortinarius* rather than *Pholiota*.

The result of the oxidase drop tests indicate differences also between closely related species regarding occurence of laccase and tyrosinase. It is difficult to distinguish any pattern. Some results do not correspond with earlier published reports and it is probable that the occurence of the enzymes vary depending on environmental circumstances. Reports on absence

or presence of laccase or tyrosinase therefore seem to be of restricted taxonomic value.

Several polarity tests were in *Pholiota* difficult to interprete, due to unexpected irregularities. Tetrapolarity is the normal state within the genus, only one species (*P. jahnii*) seems to be bipolar. Amphithallism is a common reason to irregularities in the polarity tests, which corresponds with earlier results (cfr Ginns 1974). Many species have an astatocoenocytic behaviour in their cytology.

The intercompatibility tests have proved to be very valuable for taxonomical studies in *Pholiota*. Several species complexes investigated with this method included some which were, on the basis of morphological characters, essentially unresolvable.

In most cases matings between isolates of the same species have resulted in the formation of clamps in almost 100 % of the crosses, because of the multiple allele effect. Sometimes positive matings do not occur in all crosses. A reasonable explanation is deficient monokaryons. In many cases collections with slight morphological differences which possibly could be united within the same species, were completely incompatible and, hence, regarded as distinct species.

Generally all crosses between closely related species are negative. In some cases a few positive pairings have appeared between closely related species, e.g. between *P. lenta* and *P. lubrica*. This suggests that the two taxa involved have not been completely genetically isolated. Also between *P. adiposa* from the USA and *P. limonella* from Sweden some positive pairings have appeared. However, the species concepts in the *adiposa* complex is rather complicated (Jacobsson 1987).

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Scientific Names in the Endogonales, Zygomycotina

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ABSTRACT

Analysis of scientific names of 150 species in the Endogonaceae suggested correction of one generic name and 10% of the specific epithets that were not in accordance with the rules of the International Code of Botanical Nomenclature. It is suggested that authors be more cognizant of etymology and pay more attention to the rules of the Code when naming new species in order to have more uniformity in nomenclature of this important group of fungi.

The Endogonales, with one family, Endogonaceae, is composed of seven genera and 150 described species. Although some species probably will be relegated to synonyms, there probably will be a proliferation of new species in future years. To describe new fungal species, knowledge of nomenclature, a word of Latin origin or its Greek equivalent, onomatology (Leal, 1972), is necessary. Nomenclature mainly involves studies on the interpretation of the International Code of Botanical Nomenclature, rules of Greek and Latin etymology and Latin grammar. Etymology (Gr. *etymon*, the true, original or literal meaning of a word and Gr. *logos*, science of) is a branch of linguistics that studies the origin or derivation of words.

Under Principle V. of the International Code of Botanical Nomenclature (ICBN) (Stafleu et al., 1983), scientific names of plants and fungi are Latin or treated as Latin regardless of their derivation. The name of a genus is a singular noun and the first letter is always capitalized. It may be taken from many sources but its derivation is based on regulations established by ICBN (Stafleu et al., 1983).

Genera

Excluding *Glomus*, (Latin, a ball of yarn, gender neuter) all other genera of the Endogonaceae are feminine and are compound or hybrid words: *Endogone*, Gr. *endo*, inside, and Gr. *gone*, seed; *Sclerocystis*, Gr. *skleros*, hard, and Gr. *kystis*, bladder; *Gigaspora*, Gr. *gigas*, giant, and Gr. *spora*, spore; *Acaulospora*, Gr. *a*, without, Gr. *kaulos*, stem, and Gr. *spora*, spore; *Entrophospora*, Gr. *en*, within, Gr. *trophos*, nourished or reared, and Gr. *spora*, spore; *Scutellospora*, L. *scutellum*, small shield, and Gr. *spora*, spore. *Scutellospora* is a hybrid word formed from two

different languages. According to recommendation 75A.2 of ICBN (Staffleu et al., 1983), the gender in generic compound names is determined by the gender of the last word. If the termination is altered, however, the gender should agree with it. In the formation of compound names, the recommendation 73G should be followed also (Stafleu et al., 1983). The prefixes a– and en– are connected to the stem of the first word. In *Scler-o-cystis*, *Acaul-o-spora* and *Entroph-o-spora*, a connecting vowel –o– is inserted before a consonant in the second word if the first word stem is Greek and ends in a consonant (*scler–*, *acaul–* and *entroph–*). In *Gigaspora*, the stem of the first word is *gigas*. In this case the last vowel of the stem may be preserved just as it was in the prefix *Endo–*to form *Endogone*. In *Scutellospora*, the recommendation is that an –i–, and not an –o– be inserted between the two consonants since *scutellum* is a Latin word. Thus, the correct spelling for the genus *Scutellospora* is *Scutellispora*. Article 73.8 (Stafleu et al., 1983) stipulates that the use of an incorrect compounding form in an epithet is treated as an orthographic error to be corrected (see Rec. 73G).

Specific Epithets

Most of the specific epithets in the Endogonaceae are adjectives (Table 1) and are found in Schenck & Pérez (1988), the *Endogone* species in Gerdemann & Trappe (1974) and Tandy (1975), and other new species in Blaszkowski (1988), Sieverding, Chaverri & Rojas (1988), Sieverding (1988), and Spain, Sieverding & Schenck (1989). Most Greek and Latin words in the etymology portion of Table 1 were obtained from Lewis and Short (1907), Brown (1956), Nybakken (1960), Ayers (1977) and Simpson (1979). Examples of compound specific epithets in the Endogonaceae that were formed in a similar manner to *Scutellispora*, with the first word in Latin, are *Sclerocystis clav-i-spora*, *Glomus magn-i-caule* and 18 other specific epithets (Table 1). In *Sclerocystis pachy - caulis* and *Acaulospora myrio-carpa*, the last vowels of the Greek stem were maintained as in compounding the word *Giga-spora*. For *Glomus microaggregatum*, the recommendation is to form the epithet *micraggregatum*, as in the genus *Micranthus*, small flowered, because the second word starts with a vowel. The specific epithet of *Gigaspora alborosea* is a pseudocompound formed by two adjectives and the adjective in a non final position (*albo*) appears as a word with a case ending not as a modified stem, *alborosea*, meaning pink with white.

The specific epithet may be an adjective, a present participle, a noun, or a combination of an adjective and noun or two adjectives. It may be taken from any source but must be based on regulations (Stafleu et al., 1983). Present participles follow the normal declension rules and must modify nouns like any adjective. According to Houg (1953), all present participles conform to one–ending third–declension and may be compared in the same fashion as adjectives. When specific epithets are adjectives they must agree grammatically – gender, number and case – with the generic name. Specific epithets, when they are nouns, do not necessarily agree grammatically with a generic name. These epithets may be either 1) a noun in apposition or in the nominative case, singular number, or 2) a noun in the genitive case, singular or plural (Stafleu et al. 1983, Article 23.5).

A noun in apposition or a noun in the nominative case, singular number, is found in Acaulospora appendicula, Endogone flammicorona, Glomus pansihalos, G. deserticola, Scutellispora savannicola and *Glomus citricola*, instead of *citricolum*, as found in the original description. The nouns ending in *–cola* as *deserticola, savannicola* and *citricola*, used as specific epithets do not have to change their endings in order to agree with the gender of the genus. Snell & Dick (1971) point out that *Cronartium ribicola* is correct but *Pythium graminicolum* is incorrect. In

Gigaspora margarita, the specific epithet can be either a noun in apposition, *margarita, ae*, pearl or an adjective *margaritus, a, um*, pearly (Gledhill, 1985). The specific epithet of *Scutellispora auriglobа* is formed by L. *aurum* and L. *globus*. Used as a noun, it must be *Scutellispora auriglobus*, not *auriglobа* in order to agree grammatically with *Scutellispora*. As an adjective it is formed by L. *aurum*; L. *globosus, a, um* giving *Scutellispora auriglobosa* as the suggested name for this species (Table 1).

Fifteen specific epithets in the Endogonaceae, which honor individuals, are in the genitive case (Table 1). According to Stafleu et al. (1983) when the patronym ends in a vowel, substantive epithets are formed by adding the genitive inflection appropriate to the gender of the person honored as in *Acaulospora trappei*, for Trappe (masculine) and in *Glomus mosseae*, for Mosse (feminine). When the personal name ends in a consonant, substantive epithets are formed by adding *–i–* (stem augmentation) plus the genitive inflection appropriate to the gender of the person honored as in *Entrophospora schenckii*, for Schenck (masculine) and *Scutellispora weresubiae*, for Weresub (feminine). Stafleu et al. (1983) do not recommend the dedication of genera to individuals not connected with botany or the natural sciences. This recommendation should be followed also when naming a species.

Glomus manihotis is an example of a noun in the genitive case used to name a species, referring to the host *Manihot*. According to Stafleu et al. (1983) Boehmer and Adamson failed to indicate the gender of *Manihot* when they described the genus. The first author to supply a specific epithet to *Manihot* was Crantz who in 1766 proposed the name *Manihot esculenta*, thus indicating that *Manihot* was feminine. Stearn (1966) stated that *Manihot* is best treated as indeclinable, i.e, as being the same as the nominative in all cases. Based on this recommendation, the correct name for this species is *Glomus manihot* similar to that applied to *Meliola manihot* by Stevens & Tehon (1926). The epithet for *Glomus invermaium* was derived from the locality Invermay. Recommendation 73D (Stafleu et al., 1983) proposes that an epithet derived from a geographical name is preferably an adjective and usually takes the termination *–ensis*, *–(a)nus*, *–inus*, or *–icus*. Art. 73.4 adds that the letters w and y, foreign to classic Latin and k, rare in that language, are permissible in Latin fungal names. Based on this , a more appropriate name for *Glomus invermaium* would be *Glomus invermayanum*. This epithet provides a better notion of the location from which the specific epithet was derived. Similarly, in *Acaulospora gedanensis*, a more appropriate name would be *Acaulospora gdanskensis* named for the Gdansk area. The words formed with the Latinized Greek ending *–oides*, as in the word *bryoides* (moss like), have in nominative singular the same ending in all genders: *bryoides* (m.), *bryoides* (f.), *bryoides* (n.) (Stearn, 1966; Gledhill, 1985). In the Endogonaceae (Table 1), the specific epithets of *Sclerocystis coremioides*, *Glomus botryoides*, *G. claroides*, instead of *claroideum*, and *Scutellispora coralloides*, replacing *coralloidea*, are formed with the invariable ending *–oides* regardless of the gender of the genus. Stearn (1966) gives the neuter name *sporocarpium*, sporocarp, that could form the adjective *sporocarpius, a, um* and justify the specific epithet of *Acaulospora sporocarpia*. The specific name of *Acaulospora sporocarpia* is formed from Gr. *spora*, spore and Gr. *karpos*, fruit, giving *spor-o-carpa* (Table 1). Based on etymology the suggested name for *A. sporocarpia* is *A. sporocarpa* formed in the same way as in *Acaulospora myriocarpa*, G. *macrocarpum* and *G. microcarpum* compounded with the adjective *carpus, a, um*. *Glomus intraradices* (Table 1) has the

Table 1. Scientific names in the Endogonaceae: Species names as described, etymology, accentuation and suggested names.[1]

Species name as described	Etymology	Suggested name and accentuation
Acaulospora		*Acaulóspora*
A. appendicula Spain, Sieverding & Schenck	L. *appendicula*, little appendage	*A. appendícula*
A. bireticulata Rothwell & Trappe	L. *bi*; two, twice; L. *reticulatus, a, um*, reticulate	*A. bireticulàta*
A. delicata Walker, Pfeiffer & Bloss	L. *delicatus, a, um*, soft, tender	*A. delicàta*
A. denticulata Sieverding & Toro	L. *denticulatus, a, um*, provided with small teeth	*A. denticulàta*
A. dilatata Morton	L. *dilatatus, a, um*, dilated, expanded	*A. dilatàta*
A. elegans Trappe & Gerdemann	L. *elegans, ans, ans*, elegant	*A. élegans*
A. foveata Trappe & Janos	L. *foveatus, a, um*, pitted	*A. foveàta*
A. gdanensis Blaszkowski	Place Gdansk; *ensis, is, e*	*A. gdanskénsis*
A. gerdemanni Schenck & Nicolson	Honor Dr. Gerdemann, noun m.; *ii*, gen.	*A. gerdemánnii*
A. lacunosa Morton	L. *lacunosus, a, um*, full of hollows	*A. lacunósa*
A. laevis Gerdemann & Trappe	L. *levis, is, e*, smooth (*laevis* erroneously)	*A. láevis*
A. longula Spain & Schenck	L. *longulus, a, um*, dim. of *longus*, long	*A. lóngula*
A. mellea Spain & Schenck	L. *melleus, a, um*, of or belonging to honey	*A. méllea*
A. morrowae Spain & Schenck	Honor Dr. Morrow, noun f.; *iae*, gen.	*A. morrówiae*
A. myriocarpa Spain, Sieverding & Schenck	Gr. *myrios*, numerous, Gr. *karpos*, fruit	*A. myriocárpa*
A. nicolsonii Walker, Reed & Sanders	Honor Dr. Nicolson; noun m.; *ii*, gen.	*A. nicolsónii*
A. polonica Blaszkowski	Place Poland; *icus, a, um*	*A. polónica*
A. rehmii Sieverding & Toro	Honor Dr. Rhem; noun m.; *ii*, gen.	*A. réhmii*
A. rugosa Morton	L. *rugosus, a, um*, wrinkled	*A. rugósa*
A. scrobiculata Trappe	L. *scrobiculatus*, a, um, minutely pitted	*A. scrobiculàta*
A. spinosa Walker & Trappe	L. *spinosus, a, um*,full of thorns	*A. spinósa*
A. splendida Sieverding, Chaverri & Toro	L. *splendidus, a, um*, sparkling, splendid	*A. spléndida*
A. sporocarpia Berch	Gr. *spora*, spore; Gr. *karpos*, fruit	*A. sporocárpa*
A. thomii Blaszkowski	Honor author's son; noun m.; *ii*, gen.	*A. thómii*

[1] *Scientific names are not accented. Accentuation marks provided as a suggestion for pronunciation; m. (masculine); f. (feminine); gen. (genitive), L. (Latin); Gr. (Greek); (/) stressed syllable.*

A. trappei Ames & Linderman	Honor Dr. Trappe; noun m.; *i*, gen.	*A. tráppei*
A. tuberculata Janos & Trappe	L. *tuberculatus, a, um*, with small rounded projections	*A. tuberculáta*
A. undulata Sieverding	L. *undulatus, a, um*, undulatory, wavy	*A. undulăta*
Endogone		*Endógone*
E. acrogena Gerdemann, Trappe & Hosford	Gr. *akros*, at the end; Gr. *genes*, to be produced	*E. acrógena*
E. aggregata Tandy	L. *aggregatus, a, um*, clustered	*E. aggregáta*
E. alba (Petch) Gerdemann & Trappe	L. *albus, a, um*, white, whitish	*E. álba*
E. crassa Tandy	L. *crassus, a, um*, thick	*E. crássa*
E. flammicorona Trappe & Gerdemann	L. *flamma*, a flame; L. *corona*, crown	*E. flammicoróna*
E. incrassata Thaxter	L. *incrassatus, a, um*, thickened	*E. incrassáta*
E. lactiflua Berkeley & Broome	L. *lac, lactis*, milk; L. *fluo*, to flow	*E. lactíflua*
E. multiplex Thaxter	L. *multiplex* (*multus*/*plico*), having many folds	*E. múltiplex*
E. oregonensis Gerdemann & Trappe	Place Oregon; *ensis, is, e*	*E. oregonénsis*
E. pisiformis Link ex Fries	L. *pisum*, pea; L. *forma*, shape	*E. pisifórmis*
E. reticulata Tandy	L. *reticulatus, a, um*, reticulate	*E. reticulăta*
E. stratosa Trappe & Gerdemann	L. *stratosus, a, um*, layered	*E. stratósa*
E. tuberculosa Lloyd	L. *tuberculosus, a, um*, provided with small swellings	E. *tuberculósa*
E. verrucosa Gerdemann & Trappe	L. *verrucosus, a, um*, warty	*E. verrucósa*
Entrophospora		*Entrophóspora*
E. colombiana Spain & Schenck	Place Colombia; *anus, a, um*	*E. colombiána*
E. infrequens (Hall) Ames & Schneider	L. *infrequens, ens, ens*, rare	*E. infrequens*
E. schenckii Sieverding & Toro	Honor Dr. Schenck; noun m.; *ii*, gen.	*E. schénckii*
Gigaspora		*Gigáspora*
G. albida Schenck & Smith	L. *albidus, a, um*, whitish	*G. álbida*
G. candida Bhattacharjee, Mukerji, Tewari & Skoropad	L. *candidus, a, um*, of a shinning, dazzling white	*G. cándida*
G. decipiens Hall & Abbott	L. *decipiens, ens, ens*, deceiving, deceptive	*G. decípiens*
G. gigantea (Nicol. & Gerd.) Gerdemann & Trappe	L. *giganteus, a, um*, of or belonging to the giants	*G. gigantéa*

G. margarita Becker & Hall	*L. margaritus, a, um*, pearly	*G. margarita*
G. ramisporophora Spain, Sieverding & Schenck	L. *ramus*, branch; Gr. *spora*, spore; Gr. *phoros*, bear	*G. ramisporóphora*
G. rosea Nicolson & Schenck	L. *roseus, a, um*, pink	*G. rósea*
Glomus		*Glómus*
G. aggregatum Schenck & Smith emend. Koske	L. *aggregatus, a, um*, grouped	*G. aggregátum*
G. albidum Walker & Rhodes	L. *albidus, a, um*, whitish	*G. álbidum*
G. ambisporum Smith & Schenck	L. *ambi*, both; Gr. *spora*, spore	G. *ambísporum*
G. arborense McGee	L. *arbor*, a tree; *ensis, is, e*	*G. arborénse*
G. australe (Berk.) Berch	L. *australis, is, e*, southern	*G. austrále*
G. boreale (Thaxter) Trappe & Gerdemann	L. *borealis, is, e*, northern	*G. boreále*
G. botryoides Rothwell & Victor	Gr. *botrys*, cluster of grapes; Gr. *oides*, like	*G. botryoides*
G. caledonium (Nicol. & Gerd.) Trappe & Gerdemann	L. *caledonius, a, um*, pertaining to Scotland	*G. caledónium*
G. callosum Sieverding	L. *callosus, a, um*, horny skin, callose	*G. callósum*
G. canadense (Thaxter) Trappe & Gerdemann	Place Canada; *ensis, is, e*	*G. canadénse*
G. cerebriforme McGee	L. *cerebrum*, the brain; L. *forma*, form	*G. cerebriforme*
G. cinicolum Tang & Zang	*L. cinis*, citron tree; L. *colo*, to inhabit, to dwell	*G. cinícola*
G. claroideum Schenck & Smith	L. *clarus, a, um*, clear; Gr. *oides*, like	*G. claroides*
G. clarum Nicolson & Schenck	*L. clarus, a, um*, clear, transparent	*G. clárum*
G. constrictum Trappe	L. *constrictus, a, um*, constricted	*G. constrictum*
G. convolutum Gerdemann & Trappe	L. *convolutus, a, um*, convolute	*G. convolútum*
G. delhiense Mukerji, Bhattacharjee & Tewari	Place Delhi; *ensis, is, e*	*G. delhiénse*
G. desercticola Trappe, Bloss & Menge	L. *desertum*, desert; L. *colo*, to dwell, to inhabit	*G. desercticola*
G. diaphanum Morton & Walker	L. *diaphanus, a, um*, colorless	*G. diáphanum*
G. dimorphicum Boyetchko & Tewari	Gr. *di*, two, twice; Gr. *morphe*, shape	*G. dimórphicum*
G. dominikii Blaszkowski	Honor Prof. Dominik; noun m.; *ii*, gen.	*G. dominikii*
G. etunicatum Becker & Gerdemann	L. *e*, deprived of; L. *tunicatus, a, um*, clothed in a tunic	*G. etunicátum*
G. fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	L. *fasciculatus, a, um*, clustered	*G. fasciculátum*
G. fecundisporum Schenck & Smith	L. *fecundus, a, um*, prolific; Gr. *spora*, spore	*G. fecundísporum*
G. flavisporum (M. Lange & Lund) Trappe & Gerdemann	L. *flavus, a, um*, gold-colored; Gr. *spora*, spore	*G. flavísporum*
G. formosanum Wu & Chen	Place Formosa; *anus, a, um*	*G. formosánum*

G. fragile (Berk. & Broome) Trappe & Gerdemann	L. *fragilis, is, e,* fragile	*G. frágile*
G. fuegianum (Spegazzini) Trappe & Gerdemann	Place Tierra del Fuego; *ianus, a, um*	*G. fuegiánum*
G. fulvum (Berk. & Broome) Trappe & Gerdemann	L. *fulvus, a, um,* yellowish brown	*G. fúlvum*
G. geosporum (Nicol. & Gerd.) Walker	Gr. *ge,* earth; Gr. *spora,* spore	*G. geósporum*
G. gerdemannii Rose, Daniels & Trappe	Honor Dr. Gerdemann; noun m.; *ii,* gen.	*G. gerdemánnii*
G. globiferum Koske & Walker	L. *globus,* sphere; L. *fero,* to bear	*G. globíferum*
G. glomerulatum Sieverding	L. *glomerulatus, a, um,* clumped	*G. glomerulátum*
G. halonatum Rose & Trappe	L. *halonatus, a, um,* haloed	*G. hálon*
G. heterosporum Smith & Schenck	Gr. *heteros,* different; Gr. *spora,* spore	*G. heterósporum*
G. hoi Berch & Trappe	Honor Dr. Ho; noun m.; *i,* gen.	*G. hói*
G. intraradices Schenck & Smith	L. *intra,* within; L. *radix,* root	*G. intrarádix*
G. invermaium Hall	Place Invermay; *anus, a, um*	*G. invermaýdnum*
G. lacteum Rose & Trappe	L. *lacteus, a, um,* milky, milk-white	*G. lácteum*
G. leptotichum Schenck & Smith	Gr. *leptos,* thin; Gr. *teichos,* wall	*G. leptotíchum*
G. macrocarpum Tul. & Tul.	Gr. *makros,* large; Gr. *karpos,* fruit	*G. macrocárpum*
G. maculosum Miller & Walker	L. *maculosus, a, um,* full of spots	*G. maculósum*
G. magnicaule Hall	L. *magnus, a, um,* large; L. *caulis,* stem	*G. magnicáule*
G. manihotis Howeler, Sieverding & Schenck	referring to the host, *Manihot; is,* gen.	*G. mánihot*
G. melanosporum Gerdemann & Trappe	Gr. *melanos,* black; Gr. *spora,* spore	*G. melanósporum*
G. microaggregatum Koske, Gemma & Olexia	Gr. *mikros,* small; L. *aggregatus, a, um,* grouped	*G. microaggregátum*
G. microcarpum Tul. & Tul.	Gr. *mikros,* small; Gr. *karpos,* fruit	*G. microcárpum*
G. monosporum Gerdemann & Trappe	Gr. *mono,* one; Gr. *spora,* spore	*G. monósporum*
G. mosseae (Nicol. & Gerd.) Gerdemann & Trappe	Honor Dr. Mosse; noun f.; *ae,* gen.	*G. mósseae*
G. multicaule Gerdemann & Bakshi	L. *multus, a, um;* many, L. *caulis,* stalk	*G. multicáule*
G. multisubstensum Mukerji, Bhattacharjee & Tewari	L. *multus, a, um,* many; L. *subtensus, a, um,* subtended	*G. multisubténsum*
G. occultum Walker	L. *occultus, a, um,* hidden	*G. occúltum*
G. pallidum Hall	L. *pallidus, a, um,* pale	*G. pállidum*
G. pansihalos Berch & Koske	L. *pandere* (*pansus*), to spread out; L. *halos,* halo	*G. pansihálos*
G. pubescens (Sacc. & Ellis) Trappe & Gerdemann	L. *pubescens, ens, ens,* pubescent	*G. pubéscens*
G. pulvinatum (P. Henn.) Trappe & Gerdemann	L. *pulvinatus, a, um,* cushion shaped	*G. pulvinátum*
G. pustulatum Koske, Friese, Walker & Dalpe	L. *pustulatus, a, um,* having pustules	*G. pustulátum*
G. radiatum (Thaxter) Trappe & Gerdemann	L. *radiatus, a, um,* radiate	*G. radiátum*

G. reticulatum Bhattacharjee & Mukerji	L. *reticulatus, a, um*, reticulate	*G. reticulátum*
G. scintillans Rose & Trappe	L. *scintillans, ans, ans*, sparkling	*G. scintíllans*
G. segmentatum Trappe, Spooner & Ivory	L. *segmentatus, a, um*, segmented	*G. segmentátum*
G. tenebrosum (Thaxter) Berch	L. *tenebrosus, a, um*, dark	*G. tenebrósum*
G. tenerum Tandy emend. McGee	L. *tener, era, erum*, soft-textured	*G. ténerum*
G. tenue (Greenhall) Hall	L. *tenuis, is, e*, thin	*G. ténue*
G. tortuosum Schenck & Smith	L. *tortuosus, a, um*, full of windings	*G. tortuósum*
G. tubaeforme Tandy	L. *tuba*, trumpet; L. *forma*, shape	*G. tubaefórme*
G. versiforme (Karsten) Berch	L. *versiformis, is, e (versoforma)*, changing shape	*G. versifórme*
G. vesiculiferum (Thaxter) Gerdemann & Trappe	L. *vesicula*, small vesicle; L. *fero*, to bear	*G. vesiculíferum*
G. warcupii McGee	Honor Dr. Warcup; noun m.; *ii*, gen.	*G. warcúpii*
Sclerocystis		*Sclerocýstis*
S. clavispora Trappe	L. *clava*, club; Gr. *spora*, spore	*S. clavíspora*
S. coccogena (Pat.) v. Hohn.	Gr. *kokkos*, berry; Gr. *genes*, to be produced	*S. coccógena*
S. coremioides Berk. & Broome	Gr. *korema*, coremium; Gr. *oides*, like	*S. coremioídes*
S. dussii (Pat.) v. Hohn.	Honor Dr. Duss; noun m.; *ii*, gen.	*S. dússii*
S. indicus Bhattacharjee & Mukerji	Place India; *icus, a, um*	*S. índica*
S. microcarpus Iqbal & Bushra	Gr. *mikros*, small; Gr. *karpos*, fruit	*S. microcárpa*
S. pachyculis Wu & Chen	Gr. *pachys*, thick; L. *caulis*, stem	*S. pachyculis*
S. pakistanica Iqbal & Bushra	Place Pakistan; *icus, a, um*	*S. pakistánica*
S. rubiformis Gerdemann & Trappe	L. *rubus*, blackberry; L. *forma*, shape	*S. rubifórmis*
S. sinuosa Gerdemann & Bakshi	L. *sinuosus, a, um*, sinuous	*S. sinuósa*
Scutellospora		*Scutellíspora*
S. alborosea (Ferr. & Herr.) Walker & Sanders	L. *albus, a, um*; white; L. *roseus, a, um*, pink	*S. alborósea*
S. auriglobа (Hall) Walker & Sanders	L. *aurum*, gold; L. *globus*, sphere	*S. auriglobósa*
S. calospora (Nicol. & Gerd.) Walker & Sanders	Gr. *kalos*, beautiful; Gr. *spora*, spore	*S. calóspora*
S. coralloidea (Tarppe, Gerd. & Ho) Walker & Sanders	Gr. *corallion* or L. *corallum*, coral; Gr *oides*, like	*S. coralloídes*
S. dipapillosa (Walker & Koske) Walker & Sanders	Gr. *di*, two; L. *papillosus, a, um*, having papilla	*S. dipapillósa*
S. dipurpurescens Morton & Koske	Gr. *di*, two; L. *purpurascens, ens, ens*, becoming purple colored	*S. dipurpurascens*

S. erythropa (Koske & Walker) Walker & Sanders	Gr. *erythros*, red; Gr. *trope*, turn, affinity for	*S. erýthropa*
S. fulgida Koske & Walker	L. *fulgidus, a, um*, shinning	*S. fúlgida*
S. gilmorei (Trappe & Gerd.) Walker & Sanders	Honor Dr. Gilmore; noun m.; *i*, gen.	*S. gilmórei*
S. gregaria (Schenck & Nicol.) Walker & Sanders	L. *gregarius, a, um*, associating together	*S. gregária*
S. heterogama (Nicol. & Gerd.) Walker & Sanders	Gr. *heteros*, different; Gr. *gamos*, gametes	*S. heterógama*
S. minuta (Ferr. & Herr.) Walker & Sanders	L. *minutus, a, um*, small	*S. miníta*
S. nigra (Redhead) Walker & Sanders	L. *niger, gra, grum*, black	*S. nígra*
S. pellucida (Nicol. & Schenck) Walker & Sanders	L. *pellucidus, a, um*, transparent	*S. pellúcida*
S. persica (Koske & Walker) Walker & Sanders	L. *persicus, a, um*, peach	*S. pérsica*
S. reticulata (Koske, Miller & Walker) Walker & Sanders	L. *reticulatus, a, um*, reticulate	*S. reticulàta*
S. savannicola (Herr. & Ferr.) Walker & Sanders	Latinization *savanna*, savannah; L. *colo*, to dwell, to inhabit	*S. savannícola*
S. tricalypta (Herr. & Ferr.) Walker & Sanders	Gr. *tri*, three; Gr. *kalypto*, cover	*S. tricalýpta*
S. verrucosa (Koske & Walker) Walker & Sanders	L. *verrucosus, a, um*, warty	*S. verrucósa*
S. weresubiae Koske & Walker	Honor Dr. Weresub; noun f.; *iae*, gen.	*S. weresúbiae*

specific epithet formed by L. *intra*, within, L. *radix*, root. The noun *radix*, *–icis* is third declension feminine and according to Stearn (1966), when used as an adjective, has the same nominative singular for all genders. The specific epithet of *G. intraradices*, as found in the species description, is in nominative plural of the noun and nominative plural masculine and feminine of the adjective. The suggested name for *G. intraradices* is *Glomus intraradix*, the epithet being either a noun in apposition or an adjective (Stafleu et al., 1983, Article 23.5).

According to Stafleu et al. (1983) in Article 32.5, names published with an incorrect Latin termination, but otherwise in accordance with this Code, are regarded as validly published. They are to be corrected without change of the author's name or date of publication. Trappe (1982) changed Latin endings of specific epithets in many *Glomus* species to agree grammatically with the neuter generic names. According to Morton (1988) such minor modifications are mildly irritating to researchers for a time, but they are necessary to preserve consistency in nomenclature.

Based on etymology typographic errors are found in the specific epithets of *Glomus multisubstensum*, that should be corrected to *G. multisubtensum*, and in *Scutellispora dipurpurescens*, corrected to *S. dipurpurascens*. The original spelling of a name or epithet is to be retained, except for the correction of typographic or orthographic errors, Article 73.1. Although Stafleu et al. (1983) recommend that names not be made by combining words of different languages, they established in example 2 from Article 62.1 that *Ardisia quinquegona* should not be changed to *A. pentagona*, even though the specific epithet *quinquegona* is a hybrid word of Latin and Greek. Similar words are found in the Endogonaceae, e.g. in the specific epithets of *Scutellispora dipapillosa*, Gr. *di*; L. *papillosa*, and *Sclerocystis pachycaulis*, Gr. *pachy*, L. *caulis*. Better names for these epithets would be *bipapillosa*, L. *bi*; L. *papillosa*, and *Sclerocystis pachycaula* or *pachycaulos* (Gledhill, 1985), Gr. *pachy*; Gr. *kaulos*, respectively. With *regard to the etymology of Glomus halonatum*, the word halonate, haloed was found in Snell & Dick (1971) and Hawksworth et al. (1983), but not in English dictionaries. Stearn (1966) gives the Latin adjective *halonatus, a, um*, haloed, from *halos*, halo, which is not found in Latin dictionaries. The word halo, L. *halos*, was found in Lewis & Short (1907) and in Cash (1965). Gledhill (1985) showed that the corresponding adjective in nominative singular for L. *halos* is *halos* (m.), *halos* (f.), *halon* (n.). In my opinion based on etymology, *Glomus halos* (the specific epithet as a noun in apposition, e.g., in *Glomus pansihalos*) or *Glomus halon* (the specific epithet as an adjective) are better and shorter names than *Glomus halonatum*.

Pronunciation of Scientific Names

According to Alcock (1876), it would be impossible to lay down absolute rules for the correct pronunciation of scientific names, and absurd to insist upon the accuracy of a certain pronunciation when another may be more customary. Latin is a "dead" language, and each nation pronounces these words according to the usage of its own language. The pronunciation of Verónica is much more common and is accepted by most, but according to Alcock (1876), based on its derivation, the correct accentuation is Veronícа and it is the pronunciation given by the older authorities. Lindsay (1923) points out that scientific names can be pronounced in any way preferred. There is no rule, and it is equally correct to say *Pittósporum* or *Pittospórum*. Although there are many exceptions, there are some rules that apply to the pronunciation of Latin words. According to Nybakken (1960) a Latin word has as many syllables as it has vowels and diphthongs (ae, au, ei, eu, oe). In Latin every vowel, including the final one, is pronounced, hence co–to–ne–as–ter and not cot–on–easter. The same rule applies to the Latinized Greek ending o–i–des (not oi–

des). The quantity of a syllable is either long or short. A syllable is long if it contains a long vowel or a diphthong or if it has a short vowel followed by two consonants or by x or z. The last syllable is never accented. If the next to the last syllable is long it receives the accent; otherwise, the accent falls on the preceding syllable, the antepenult. Also, Nybakken (1960) provides the vowels and diphthongs in Latin with their respective pronunciations in English: a (as in arch and father), e (met, prey), i (pit, machine), o (obey, hole), u (full, rule), ae (aisle), au (out) ei, (freight), eu (feud) and oe (boil). The Latin consonants have the same sounds as they have in English except that: c is pronounced like cap; g like gas, v like w in window and s like son.

In a survey on pronunciation of the genera in the Endogonaceae conducted at the University of Florida, Gainesville, involving 30 individuals working in biological sciences, with many working on VA mycorrhizal fungi, the following results were obtained: *Glómus* (100%), *Sclerocýstis* (100%), *Endógone* (80%), *Endogóne* (20%), *Acaulóspora* (40%), *Acaulospóra* (60%), *Gigaspóra* (60%), *Gigáspora* (40%), *Entrophospóra* (66.6%), *Entrophóspora* (33.3%) and *Scutellóspora* (80%), *Scutellospóra* (20%). *Pittosporum* is a compound name, Gr. *pitta*, pitch, and Gr. *spora*, seed, *Pitt-o-sporum*, formed in the same way as most genera in the Endogonaceae, and having the last Greek word–*spora*. Bayley (1963), Lindsay (1923), and Nybakken (1960) applying the rules mentioned previously for pronunciation would prefer *Pittósporum*. Nybakken's recommendation for accentuation of the names applied to the genera of the Endogonaceae that have two consonants following one vowel would be *Sclerocýstis*, *Gigáspora*, *Scutellóspora*, *Acaulóspora* and *Entrophóspora*. Stearn (1966) indicates that for *–gone*, reproductive organs, used in Greek compounds, the correct pronunciation is *Endógone*. In order to have a more uniform pronunciation, based on these observations, for the genera in the Endogonaceae the following accentuation is suggested: *Glómus, Sclerocýstis, Endógone, Acaulóspora, Entrophóspora, Gigáspora* and *Scutellóspora*.

The author would be pleased if this paper contributed to a more uniform nomenclature for species in the Endogonaceae. Authors should give more attention to etymology and to the rules of the International Code of Botanical Nomenclature, when naming new species in this important family of fungi. To accomplish this the suggested references for reading and consulting are Stafleu et al. (1983), Stearn (1966), Gledhill (1985), Ayers (1977), Nybakken (1960), Brown (1956), and Latin and Greek dictionaries or glossaries.

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MYCOTAXON

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CONTRIBUTION TO THE LICHEN FLORA OF BRAZIL.XXIII. LICHENS FROM SAO PAULO CITY.

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SUMMARY: Seventeen lichens gathered in two forested localities within Sao Paulo City, Brazil, are listed.

During November 1968 the author was able to collect lichens in two forested areas located in Sao Paulo City, Brazil.
One of them was the park surrounding the well known "Instituto Butantan" (IB). The other collection site was the "Horto Florestal" (HF) composed of remnants of tropical forest in the zone named Cantareira, 15 km far from the central part of Sao Paulo City.
Both localities are situated inside the urbanized area of Sao Paulo City and surrounded by a high density of population.
I do not know the present status of both places,but the increased pollution occurring in the last 20 years in Sao Paulo led us to suppose possible damage to the lichen flora.
Notwithstanding the small number of collections, the occurrence of some taxa rarely reported in the literature dealing with the studied area encouraged the author to publish the present list. The numbers between parentheses belong to my numbering system and are preserved in my private herbarium.

Bulbothrix tabacina (Mont. & v.d. Bosch) Hale
IB.: on trunk of a tree (Leguminosae) (4920),det. M. Hale
Candelaria concolor (Dicks.) Arn.
IB.: on trunk of Melia azedarach (4917).
Catinaria versicolor (Fée) Sipman
HF.: on trunk of a tree (4895).
Chiodecton sanguineum (Sw.) Vain.
IB.: on trunk of trees, locally common (4923).
HF.: on trunk of a tree (4894, 4899).
Cladonia subradiata (Vain.) Sandst.
IB.: on bark of a tree (Myrtaceae) (4924), det. T. Ahti
HF.: on soil, inner part of the forest (4902), det. T. Ahti
Dirinaria picta (Sw.) Clem. & Shear
IB.: on trunk of Melia azedarach (4918)
Glyphis cicatricosa (Ach.) Vain. f. confluens (Zenk.)Zahlbr.
HF.: on trunks of shrubs (4913 pro parte).
Parmelina pilosa (Nyl.) Hale
HF.: on trunk of a tree (4903).

Parmotrema sancti-angelii (Lynge) Hale
HF.: on trunk of a tree (4898).
Parmotrema tinctorum (Nyl.) Hale
IB.: on trunk of a tree (Leguminosae) (4921).
HF.: on trunk of trees (4893, 4897, 4906).
Phaeographina caesiopruinosa (Fée) Müll. Arg.
HF.: on trunk of Melia azedarach (4908), on trunk of shrubs (4913 pro parte, 4914, 4915).
Phlyctella brasiliensis (Nyl.) Nyl.
HF.: on trunk of a tree (4909).
Pseudoparmelia caroliniana (Nyl.) Hale
IB.: on trunk of a tree (Leguminosae) (4925), det. M. Hale
Pseudoparmelia texana (Tuck.) Hale
IB.: on trunk of a tree (Leguminosae) (4919, 4926), det. M. Hale
Pseudopyrenula diluta (Fée) Müll. Arg.
HF.: on trunk of a shrub (4911), det. R. Harris
Punctelia rudecta (Ach.) Krog
HF.: on trunk of a tree (4896), conf. M. Hale
Tylophoron protrudens Nyl.
HF.: on bark of a tree, N slope of a small hill (4904), det. L. Tibell.

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A NEW SPECIES AND FIRST RECORD OF GYMNOPAXILLUS (HYMENOGASTRALES) FROM ARGENTINA

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SUMMARY

Gymnopaxillus crubensis is described from North-Western Patagonia. The new species grows sub-hypogeously,on soil, under Nothofagus pumilio and N.dombeyi. Gymnopaxillus morchellaeformis Horak is reported for the first time from Argentina. Up to now the genus Gymnopaxillus Horak was monospecific and previously recorded only from Tierra del Fuego, Chile.

DESCRIPTION

Gymnopaxillus crubensis Calvelo & Lorenzo. Fig.1

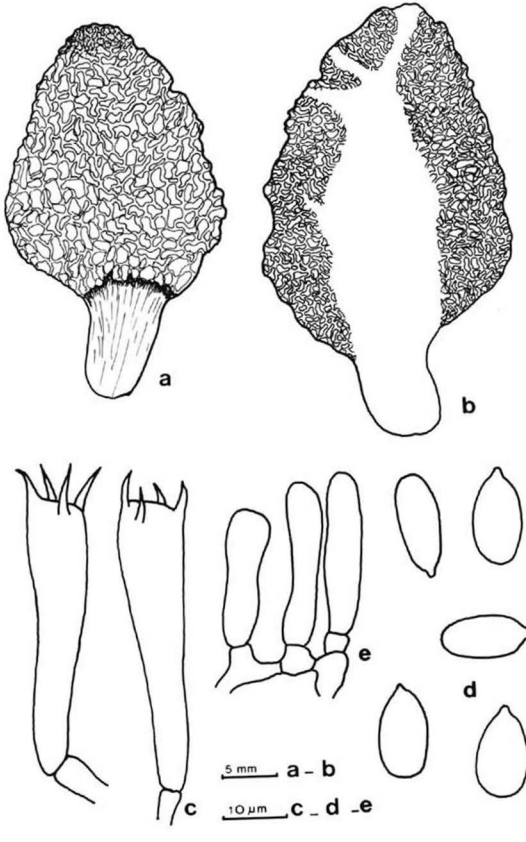
Carposomatis subhypogaeis, habitu morchelliforme. Pileo 12-22 mm lato, 20-26 mm alto, ellipsoideo vel subgloboso, ferrugineobrunneo, peridio nullo. Gleba exposita et denudata, morchelaeforme, loculis 0.5-1.5 mm, sicca. Stipite 3-8 mm lato, 8-15 mm alto, cylindraceo vel ad basin versus attenuato,albidus, sicco. Columella ramosa, plena,

deinde vetustate subcava. Odore et sapore gratus vel fortis aromaticus, Solanum tuberosum assus simile. Sporis (8-) 10-14 (-15) x (4-) 5-7 µm, symmetricis, ovoideis vel subfusoideis, auriantio luteus, numquam episporio perforato, poro germinativo nullo. Basidia 33-42 x 6-9 µm, tetrasterigmata. Cystidiis nullis. Fibulae absentes. Ad terram in Nothofagetis (Nothofagus pumilio). Holotypus in Herbario BAFC 31767 conservatum est.

Carpophore fleshy, pileate, caespitose and gregarious. Pileus 12-22 mm wide, 20-26 mm height, broadly ellipsoidal to subglobose; brownish-ferrugineous; hymenium morchellaeform, irregularly wrinkled in small chambers 0.5-1.5 mm, not following a regular pattern of distribution; with rest of peridium present only at the apex of the pileus. Stalk 3-8 mm wide, 8-15 mm height, cylindrical, slightly tapering to the base, white, dry. Columella irregularly branched, up to the apex of the pileus, solid, partially hollow after desiccation. Smell and flavour delicious, resembling roasted potatoes. Chemical reactions of context: KOH negative, ClH negative, KOH-ClH negative. Spores (8-) 10-14 (-15) x (4-) 5-7 µm, bi-radial symmetric, ovoid to subfusiform, appendix apical, smooth, golden-yellowish, without germinative pore. Spores in mass ferrugineous. Basidia 4-spored, cylindrical, 33-42 x 6-9 µm, sterigmata 4-6 µm long. Basidiole cylindrical, unbranched, 21-30 x 4-5 µm. Without cystidia. Tramal hyphae cylindrical, 2-6 µm diam.,smooth, not jellified, without clamp connections.

Holotype: Argentina, Prov. de Río Negro. Parque Nacional Nahuel Huapi, headwaters of Río Nireco, 1560 m above sea level. On soil, Nothofagus pumilio forest, S.Calvelo, 26-II-1989. BAFC 31767. Isotype: BCRU 00140

Fig.1 Gymnopaxillus crubensis
a- Carpophore
b- Carpophore, longitudinal section.
c- Basidia
d- Basidiospores
e- Basidioles


a
b
c
e
d
5 mm
a - b
10 µm
c - d - e

Additional specimens examined: Argentina, Prov. de Río Negro, Parque Nacional Nahuel Huapi, Puerto Alegre, 820 m above sea level. On soil, Nothofagus dombeyi forest, L. Lorenzo, 15-III-1985. BCRU 00141.

REMARKS

The genus appears to be endemic of southern South-American Nothofagus forests.The species is strongly water dependent, since the collections were made in places with very high precipitations levels, headwaters of Río Ñireco: 1900 mm/year, and Puerto Alegre: 3000 mm/ year. Furthermore, on soil with high content of water, one a few meters away from a bog and the other from a lake.

Gymnopaxillus crubensis grows subhypogeously and inconspicuously on humus under fallen leaves of Nothofagus sp.

The new species is close to G.morchellaeformis Horak with which our specimens were compared; the results are shown on Table 1.

The name of the new species refers to CRUB, Centro Regional Universitario Bariloche, were the authors develop their research work.

NEW RECORD

Gymnopaxillus morchellaeformis Horak,described from Tierra del Fuego, Chile, is reported for the first time from Argentina, Lago Roca, Prov. de Santa Cruz.

Specimens examined: Argentina, Prov. de Santa Cruz, Lago Roca, Leg. ?, III?- 1986. BAFC 30668.

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Table 1. Differential features between Gymnopaxillus species.

	Gymnopaxillus crubensis (BAFC 31767)	Gymnopaxillus morchellaeformis (LPS 38227)
Columella and stalk	white, not concolourous with the pileus	concolorous with the pileus
Hymenium chambers	0.5-1.5 mm, without a regular pattern of distribution	6 mm, distributed along longitudinal ribs
Smell-flavour	delicious	disagreeable
Context chemical reactions (KOH-ClH)	negative	positive
Spores	10-14 µm golden-yellowish appendix apical bi-radially symmetric ovoid to subfusiform	12-15 µm brownish-yellow appendix lateral asymmetric fusiform to ellipsoid
Basidia	33-42 x 6-9 µm	48-65 x 8-10 µm
Sterigmata	4-6 µm	3 µm
Basidioles	21-30 x 4-5 µm, unbranched	20-45 x 4-7 µm, branched

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A TAXONOMIC REVISION OF THE GENUS CHEILYMENIA - 1.

SPECIES CLOSE TO CHEILYMENIA RUBRA.

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ABSTRACT

Type material of Cheilymenia rubra (Cooke ex Phill.) Boud. and Cheilymenia humarioides (Rehm) Gamundí has been examined. Two new taxa are described: Cheilymenia pseudohumarioides Dissing,J.Moravec et Sivertsen based on collections from Greenland, and Cheilymenia liskae J.Moravec,Fellner et Landa spec. nov. based on a collection from Svalbard, West Spitsbergen. One new combination, Scutellinia alleghenensis (Denison) comb.nov., based on Cheilymenia alleghenensis Denison (1964) is proposed. Descriptions, line drawings and SEM photomicrographs of ascospore ornamentation accompany the paper.

INTRODUCTION

Taxonomic revision of the genera Coprobia Boud. em. J.Mor. and Cheilymenia Boud. has revealed that these are large genera with many species. It is intended that the results of the examination of almost all relevant type material will be published in several parts, culminating in a monograph of these genera. In the present paper, the results of an examination of material of Cheilymenia rubra (Cooke ex Phill.) Boud. and Cheilymenia humarioides (Rehm) Gamundí are presented, and two new taxa are described.

MATERIAL AND METHODS

Relevant type material from the herbaria of the Royal Botanic Gardens, Kew (K), Instituto de Botánica "Spegazzini", La Plata (LPS), The New York Botanical Gardens, New York (NY) and Oregon State University, Corvallis (OSU) were examined. Dried specimens were revived in 4 % NH_4 OH. Sections were stained in Cotton Blue in lactic acid (CB) and fuchsin acid. In the case of C. pseudohumarioides, ultramicrotome sections were prepared and sent to me by Dr. H. Dissing, Kobenhagen. Ascospores were observed and measured under an oil immersion objective at a magnification x 1600. The perisporial ornamentation of ascospores was studied on sections stained with CB Geigy S. 123, which stains promptly without heating the slides. This is important as heating may easily destroy the

perisporium . A scanning electron microscope TESLA BS 300 was used and the photomicrographs (SEM) were prepared from dried specimens in the usual way.

TAXONOMIC RESULTS

Cheilymenia rubra (Cooke ex Phill.) Boud.

Peziza theleboloides Alb.et Schw. var. rubra Cooke, Fungi Britt. ser. 1 N° 572, 1872; Grevillea III, fig 119, 1874.
Peziza (Sarcoscypha) rubra Cooke, Mycographia 83,1876 (nomen abortivum).
Lachnea rubra Cooke ex Phillips, British Discom. 225, 1887.
Scutellinia rubra (Cooke ex Phill.) Kuntze, Rev. Gen. Plant. 2:869, 1891.
Cheilymenia rubra (Cooke ex Phill.) Boudier, Hist. class. Discom. Eur. 63, 1907.

Apothecia 2-5 mm diam., sessile, scattered to crowded, subglobose and closed when young, becoming shallow cupulate to discoid, fleshy; hymenium orange-red (the dried apothecia are dark reddish-brown), externally concolorous or paler, covered with short, brownish hairs of a very irregular size; these are densely aggregated at the margin of the apothecia where long hairs are mixed with small hairs or pyriform cells with thickened, brownish walls, giving a brownish colour to the marginal zone. Rooting marginal hairs 80-320 x 12-28 µm, pale brown to reddish-brown, rigid, thick-walled (walls 1.5-4 µm thick), usually pointed above, irregularly more or less curved, with a simple attenuated base or, rarely, with a shortly bifurcate base, arising among the excipular cells. Hairs of the lower surface of the excipulum are smaller, 40-160 x 10-25 µm, with thinner walls and occasionally with hyaline tips. Ectal excipulum of textura globulosa-angularis, consisting of 4-5 rows of large cells, which become smaller and angular towards the margin of apothecia, and very large and globose near the base, (15-)45-80 (-100) µm diam. Medulla composed of compact, interwoven, often inflated, hyaline hyphae, which are 7-12 µm thick, forming a textura intricata. Hypothecium poorly differentiated,consisting of smaller hyphae. Asci 135-170 x 14-21 µm, cylindrical with rounded tips and attenuated below towards a simple or inconspicuously bifurcate base, 8-spored. Ascospores uniseriate, ellipsoid, (15-)16.5-21(-22.5) x (8-) 9-11(-12) µm, mostly 19 x 10.5 µm, subhyaline, without guttules, with a yellow refractive colour when stained with CB and with a loosening perisporium covered by fine, low, irregular cyanophilic warts and crests 0.1-1.0 µm diam. and up to 0.3 µm high. Paraphyses filiform, 3-4 µm thick, with apex slightly enlarged to 4-6(-7) µm diam., clustered together.
Habitat: On spent decaying hops and vegetable debris, straw mixed with dung and soil.
Material examined: England, Batheaston, on spent hops, April 1872 leg. E.C. Broome, labelled "P. theleboloides red form" (K ex Cooke, holotype); England, Batheaston, s.date (NY ex herbarium of G. Massee, isotype); Ed.1, N° 572 and N° 186 of Fungi Brittanici Exsiccati, labelled Peziza (Sarcoscypha) rubra (K); Switzerland, Zürich, "on the ground", April 1888

leg G. Winter, Fungi Helvetici Suppl. 78, labelled Lachnea rubra (K).
This taxon was first described as a variety of Peziza theleboloides and later described at specific rank by Cooke (1876) under the invalid name Peziza (Sarcoscypha) rubra Cooke (a later homonym of Peziza rubra Peck 1872).
It was redescribed by Phillips (1887) under the new name Lachnea rubra Cooke. As Phillips used Cooke's epithet and attributed it to the author, the correct name for this species, following the combination in Cheilymenia by Boudier (1907), is Cheilymenia rubra (Cooke ex Phill.) Boud.
All packets of the type material contain the same species. The holotype must be the specimen collected by C.E. Broome and labelled "P. theleboloides red form", which includes about eight apothecia growing on spent hops. Material, which is apparently a part of the same collection is deposited in NY herbarium and must be considered to be isotype, though Denison (1964) referred to it as "holotype". The two other collections deposited in K as well as Winter's collection (NY) represent the same species. However, the substrate of Winter's collection is not just soil ("on the ground" as annotated by Winter) but in fact consists of soil mixed with straw and chaff (probably from horse dung) and the apothecia are growing on the vegetable debris.
It should be noted that another specimen deposited in NY labelled as "Sarcoscypha rubra Cooke (Sur les feuilles pourrissantes dans les bois, au voisinage du type, Shrewsbury (Angleterre), Mars 1882 leg. W. Phillips) and with a diagnose identical to that of Cooke,is, in my opinion, Coprobia theleboloides (Alb.et Schw.) J.Mor. I have found only apothecia with superficial, pale hairs and immature asci without developed ascospores. According to Cooke (1876), C. theleboloides often grows together with C. rubra on the same substrate (spent hops), and this may explain the error.
The most conspicuous features of C. rubra are the short but rigid thick-walled brownish hairs, which have a simple or shortly bifurcate base, and the irregular arrangement of the hairs, which are densely mixed with copious, very short hairs or even pyriform or irregularly-shaped cells with brownish thickened walls, giving a brownish colour to the marginal zone. The size and shape of the hairs, their arrangement, and also the larger ascospores clearly distinguish C. rubra from Cheilymenia coprinaria (Cooke) Boud. as has already been stated by Denison (1964). The type of C. coprinaria (K) has also been examined (J.Moravec 1989).

Cheilymenia humarioides (Rehm) Gamundí

Lachnea humarioides Rehm, Bih. Kon. Sv. Vet. Ak. Handl 25; Afd. III, N° 6:11, 1899.
Cheilymenia humarioides (Rehm) Gamundí, Bull. Soc. Argent. 14 (3):168, 1972.

Apothecia 1-3 mm diam., sessile, gregarious, at first subglobose, becoming cupulate to discoid with an undulate margin, fleshy, hymenium bright orange-red when fresh, becoming orange-red when dry, with a pale yellowish margin, external surface paler, covered with scattered curved hairs; Hairs brown, becoming hyaline in their upper part (as seen

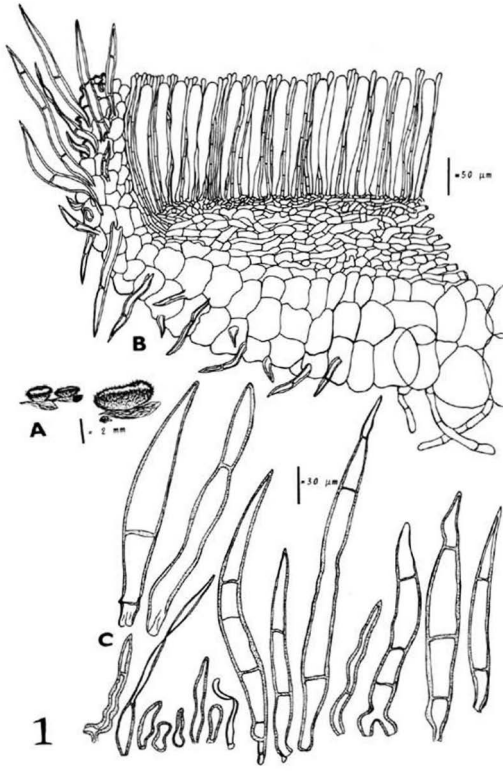

Fig. 1: Cheilymenia rubra. A. apothecia; B. section of the marginal part of the apothecium; C. hairs.(Holotype K).

with the naked eye). Rooting marginal hairs 90-300(-400) x 11-25 µm, rigid, yellow-brown to brownish, apically colourless, obtuse to pointed at the apex, sparsely septate or rarely lacking septa, thick-walled,(the walls 1-2 µm thick) with a bifurcate or simply attenuated base, arising deeply within the ectal excipulum. Hairs towards the base of the excipulum yellowish or subhyaline, septate, with an obtuse apex, 90-180 x 8-15 µm. Ectal excipulum a textura globulosa-angularis composed of pale yellowish cells, 20-75 µm diam., which are more angular and smaller towards the margin of apothecia and become subglobose and angular towards the base. Medullary excipulum clearly differentiated, comprising a textura intricata composed of interwoven, often inflated, mostly horizontally arranged 4-7 µm thick hyphae. Hypothecium poorly developed, composed of smaller hyphae and cells. Asci 150-240 x 16-26 µm, cylindrical with subtruncated tips, shortly attenuated towards a simple or shortly bifurcate base, inamyloid, 8-spored. Ascospores uniseriate, ellipsoid, (19.2-)21-26(-28.5) x 12-14.5(-15.7) µm, mostly 24 x 13.8 µm, without guttules, subhyaline, with a dark yellow refractive colour when stained with CB. The ascospore wall consists of three layers, the perisporium bearing conspicuous,cyanophilic rounded or irregular warts 0.3-1.5(-2.5) µm diam. and 0.2-1 (-1.5)µm high (measured under oil immersion + CB). Paraphyses filiform, simple, septate, 3-4 µm thick, the upper cells somewhat enlarged (4-9 µm) containing yellowish to orange pigment.
Habitat: coprophilous, collected on cow-dung and horse excrements in Argentina and Chile.
Material examined: Argentina, Tierra del Fuego,Depto.Ushuaia, alrededores de Ushuaia, "malin" al NE del pueblo, 20.I.1964 leg. I.J.Gamundí (Neotype LPS 33427a).
Cheilymenia humarioides is a distinct species which differs from C. rubra in having larger ascospores with a three layered wall. This unusual feature has already been noted by Gamundi (1972,1975). The ornamentation of the perisporium (the external delicate easily loosening coat) consists of much larger and higher warts than that in C. rubra (compare also SEM photomicrographs). Moreover, the apothecia of C. humarioides are smaller and not so densely covered with hairs, the hairs being usually colourless in their upper parts and with thinner walls. For a detailed illustration of apothecia, their anatomy and other microfeatures see Gamundí (1972,1975). The ascospore perisporium was described and illustrated as smooth by Gamundi, perhaps because the slides with sections stained in lactophenol were heated, and the perisporium consequently destroyed.
C . humarioides is known from 7 other collections from Argentina (Tierra del Fuego) and 2 collections from Chile examined by Gamundí (1972,1975). Holotype material does not exist and the neotype selected by Gamundi (1972) is from the same area as the original collection.

Cheilymenia pseudohumarioides Dissing, J.Moravec et Sivertsen **spec.nov.**:

Apothecia 2-3 mm diam., gregaria, breviter turbinata, usque discoidea, margine tenui, albido, disco aurantiaco usque

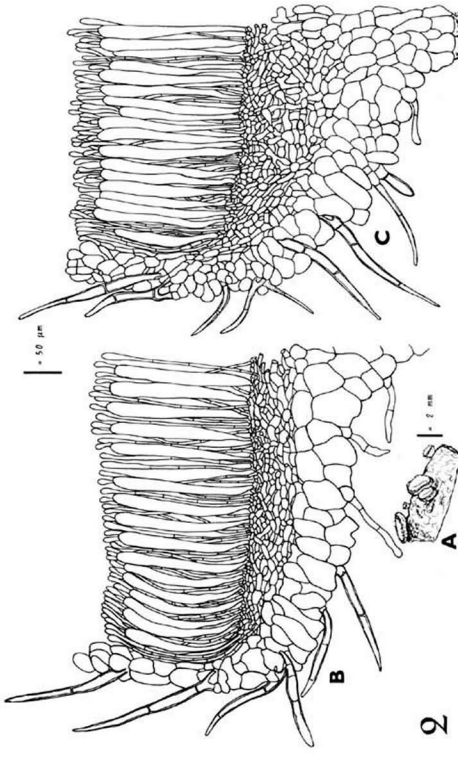

Fig. 2: Cheilymenia pseudohumarioides. A. apothecia; B. section of apothecium of the holotype (C); C. section of apothecium of the paratype Gr.8387 (C).

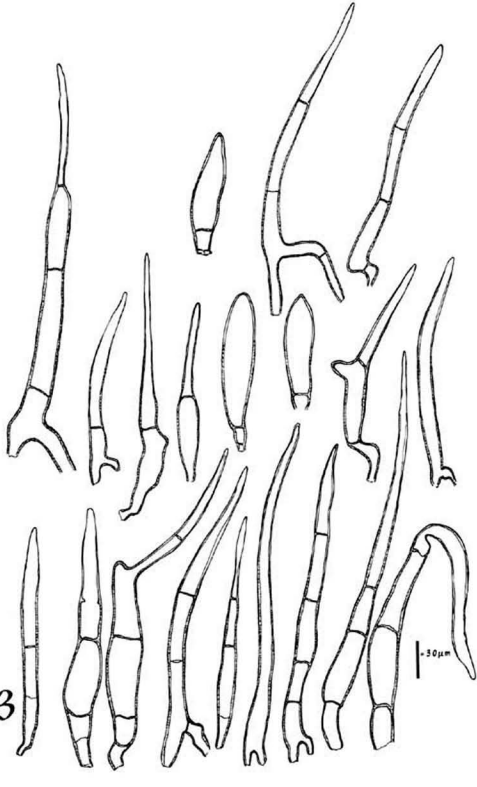

Fig. 3: Cheilymenia pseudohumarioides. Hairs. (Holotype C).

laete aurantiaco-rubro, extus pallide aurantiaca, pilis pallidis disperse obsita. Pili 80-300 x 8-27 µm, luteofusci, parte superiori ecolorati, membranis 1-2 µm crassis, sparse septati, curvati, apice acuti vel obtusi, infra attenuati, basi simplices vel interdum bifurcati. Excipulum externum textura angulari usque globuloso-angulari. Excipulum internum (medulla)e textura intricata constat. Asci cylindracei, 187-250 x 16-22 µm, apice obtusi, infra angustati, basi nonnumquam breviter bifurcati, octospori. Ascosporae monostichae, late ellipsoideae, (18.2-)19-22.8 (-24) x (10.8-)12-13.8(-15) µm, creberime 20 x 12.5 µm, eguttulatae, perisporio verrucis cyanophilis, 0.1-0.3 µm diam., 0.1 µm altis. Paraphyses filiformes, (1.3-)2.5-4 µm crassae, apice 5-10 µm dilatatae, granulis vel fibrillis aurantiaco-coloratis impletae.
Habitat: fimicola.
Holotypus: Greenland, prope Nyhavn, Metsersvig, in fimo Brantae berniclae, 5.VIII. 1983 leg. H. Dissing. (Holotypus in herbario C, isotypus BRA, Paratypus PRM.

Apothecia 2-3 mm diam., low turbinate to discoid with a pale margin, hymenium light orange to bright orange-red, dried apothecia yellow ochraceous to ochraceous, outer surface concolorous, covered with pale hairs, which are sparsely scattered. Rooting marginal hairs 80-300 x 8-27 µm, straight or often curved or wavy, yellow-brown, apically colourless, sparsely septate, usually bifurcate bellow or with a simple attenuated base; excipular hairs near the base of the apothecia shorter, with thinner walls (up to 1.5 µm thick). Excipulum clearly differentiated: Ectal excipulum a textura angularis of two rows of cells, which become subglobose or vertically elongated towards the base of the apothecia, smaller and angular towards the margin, (15-)25-45 (-75) µm diam.,with orange pigment. Medullary excipulum a textura intricata composed of interwoven, inflated hyphae occasionally mixed with globose to irregularly angular elements, the hyphae 5-12 µm thick, septate, hyaline. Hypothecium not clearly differentiated, composed of smaller hyphae and cells. Asci 187-250 x 16-22 µm, cylindric with rounded or broad pleurorhynchous base, eight-spored, inamyloid. Ascospores uniseriate, globose-ellipsoid to broadly ellipsoid, (18.2-)19-22.8(-24) x (10.8-)12-13.8(-15) µm, hyaline, without guttules, with a refractive yellow colour when stained with CB, the loosening perisporium covered with inconspicuous fine cyanophilic warts 0.1-0.3 µm diam. and 0.1 µm high. Paraphyses filiform, straight, sometimes branched, (1.3-)2.5-4 µm thick, enlarged to 5-10 µm thick at the apex, with orange granular to fibrous content. In Melzer's reagent the carotenoids in the paraphyses and excipulum stain at first bluish for a very short while, than become reddish-brown to violet and finally dark greenish.
Habitat: On dung of Barnacle goose and summer dung of musk-ox.
Holotype: East Greenland, 72°15'N,24°W near Nyhavn, Metsersvig, on old dung of Barnacle goose, 5.VIII.1983 leg. Henri Dissing (Gr.83.96-holotype C, isotype BRA).
Paratypes: East Greenland, Along Gåsesøen, near the aeroport in Mestersvig, Kong Oscar Fjord, on dung of Barnacle goose, 9.VIII.1982 leg. H.Dissing et S.Sivertsen (Gr.82.134,C,BRA);

East Greenland, Near Nyhavn, Metsersvig, on summer dung of musk-ox, 2.VIII. 1983 leg. H.Dissing (Gr.8387, C, BRA).

This new species has several features which are virtually identical to those exhibited by C. humarioides, notably the size and shape of the hairs, which are colourless in their upper part, the broadly ellipsoid ascospores which are of a similar size, the shape and size of asci, and excipular construction. However, the ascospore wall in C. humarioides consists clearly of three layers and the perisporium is covered by much larger and higher warts,whilst the ascospore wall in C. pseudohumarioides consists of only two layers and the perisporium has a finer ornamentation of much smaller and lower warts giving it a punctate appearance. In both species the perisporium is easily separable, as in other species of the genus. C. rubra clearly differs from the new species in having larger apothecia with much more densely arranged hairs, especially near the margin, giving a dark brown colour to the marginal zone. In contrast, apothecia of C. pseudohumarioides are only sparsely covered with paler hairs, which are scarcely visible to the naked eye.
In addition, marginal hairs in C. rubra are entirely brownish, without a hyaline apex; only hairs near the base of apothecia occasionally have a colourless apex.
In C. pseudohumarioides, C. humarioides and C. liskae, the marginal hairs often possess a hyaline apex, which extends approximately one third of the hair length. Moreover, the ascospores of C. rubra are narrower than the ascospores of these three species.
All the apothecia of examined material of C. pseudohumarioides are yellow-ochraceous when dried, with only scattered pale hairs as seen on the revived apothecia. The above description of fresh apothecia is based on observations made in the field laboratory in Mestersvig by Dr. H.Dissing, accompanied with colour slides, and sent to me together with the material. The collection from musk-ox excrement has more reddish apothecia (when fresh,according to the colour slide) and slight differences in excipular construction (see fig.2), but, based on its other features, I consider it identical with the holotype.

Cheilymenia liskae J.Moravec, Fellner et Landa spec.nov.

Apothecia 1-5 mm diam., primum subglobosa dein subpatellaria usque discoidea, disco aurantiaco, extus concolor, pilis brevibus, saepe curvatis, pallidis, solum basi brunneocoloratis obsita. Pili 90-240 x 10-30 µm, rigidi, recti vel curvati, luteo-fusci, parte superiori ecolorati, apice acuti, sparse septati (septis 1-4 instructis), membranis 1.5-3 µm crassis, ad bases radicantes, saepe basi bifurcati vel trifurcati (rarior quadrifurcati) sed etiam simplices, attenuati; praeterea pili breves, clavati, luteo-fusci adsunt.
Excipulum externum textura globuloso-angulari. Excipulum internum (medulla) e textura intricata constat. Asci 150-180 x (18-)19.5-25(-27) µm, crasse cylindracei, sursum saepe attenuati, apice obtusi vel truncati, deorsum breviter angustati, basi simplici, octospori. Ascosporae mono - vel distichae, ellipsoideae, (18-)19.5-21(-22.5) x 10.5-12(-13.6) µm, subhyalinae, eguttulatae, perisporio separabili, verrucis

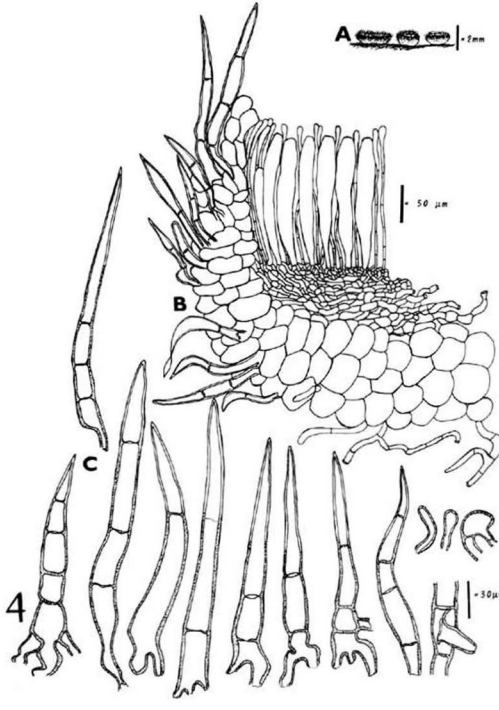

Fig. 4: Cheilymenia liskae. A. apothecia; B. section of the marginal part of the apothecium; C. hairs. (Holotype PRM).

cyanophilis, rotundatis vel elongatis usque irregularibus, sed etiam saepe reticulum haud completum formantes; verrucae 0.1-1.5 µm diam., 0.1-0.5 µm altae. Paraphyses filiformes, 3 µm crassae, septatae, apice 6-9 µm dilatatae, succo aurantiaco impletae.
Habitat: fimicola.
Holotypus: Spitsbergen occidentalis, Svalbard, Kongress, in fimo, 21 VII. 1988 leg. J. Liška et Z. Soldán. (Holotypus PRM, isotypus BRA asservantur).

Apothecia 1-5 mm diam. (0.75-3.50 mm when dried), at first subglobose and closed, becoming shallow cupulate to flattened, hymenium orange (orange-olivaceous when dried), external surface concolorous, covered with short, densely aggregated, irregularly curved pale hairs, which are brownish at their base, giving a brownish colour to the apothecial margin. Rooting marginal hairs 90-240 x 10-30 µm, rigid, straight or curved, with few (1-4) septa, pointed above, thick-walled (walls 1.5-3 µm thick) , yellow-brown but usually with the upper third colourless, the base often rooting, bifurcate or with three, rarely four roots, sometimes simply attenuated, arising deep amongst the excipular cells. Hairs towards the base of the apothecia shorter, with thinner walls (0.7-1.5 µm thick), mixed with juvenile, clavate yellow-brown hairs. Ectal excipulum a textura globulosa-angularis, composed of 3-4 rows of cells which are slightly clavate at the margin of the apothecia, larger and subglobose near the base, 45-65 µm diam.,subhyaline. Medullary excipulum composed of hyaline, septate, interwoven, mostly horizontally arranged hyphae which are (4-)7-9 µm diam., occasionally inflated up to 15 µm diam. Hypothecium not clearly differentiated, composed of narrower hyphae and smaller cells. Asci 150-180 x (18-)19.5-25(-27) µm, eight spored, broadly cylindric, usually attenuated above, with apex obtuse or truncated, the base shortly attenuated, simple, Ascospores uniseriate or biseriate, ellipsoid, (18-)19.5-21(-22.5) x 10.5-12(-13.6) µm, subhyaline, without guttules, with a yellow refractive colour when stained in CB, perisporium loosening, covered by cyanophilic irregular crests, which occasionally form a fine, incomplete reticulum, or with rounded warts 0.1-1.5 µm diam. and 0.1-0.5 µm high. Paraphyses filiform, 3 µm thick, septate, with yellow-orange apices enlarged to 6-9 µm.
Habitat: coprophilous.
Holotype: West Spitsbergen, Svalbard, Kongress, on dung (probably of reindeer), 21 VII. 1988 leg. J.Liška and Z. Soldán. (Holotype PRM, isotype BRA).

The present species resembles C. rubra especially with regard to the densely arranged hairs near the margin and ascospore characters. However, the marginal hairs of C. rubra are brownish throughout, with a much simpler base and usually thicker walls. The marginal hairs of C. liskae are hyaline in their upper part. Moreover, the ascospores of C. liskae are broader and bear higher, coarser warts. In addition, the cells of the ectal excipulum of C. rubra are much larger, the asci are shorter and narrower, the ascospores uniseriate and the apices of the paraphyses narrower. The species also differ in habitat.

C. humarioides differs clearly from C. liskae in having much larger ascospores with three-layered walls and larger warts on the perisporium. C. pseudohumarioides differs in having broader ascospores with a more finely ornamented perisporium (the warts are much lower and smaller) and, especially, in the appearance of the apothecia, which are only sparsely covered by hairs. Moreover, the hairs in C. pseudohumarioides have thinner walls and a simpler base and the asci are narrower and contain uniseriate ascospores.
All taxa discussed in the present paper are quite distinct from C. coprinaria, especially with regard to the shape, colour and length of the hairs. In the course of the investigation of the type and other material I have found that C. coprinaria, as commonly interpreted, is, in fact, a complex of several species. These include Cheilymenia magnipila J.Moravec (1969), C. megaspora (Gamundí) J.Moravec (1988) and an undescribed species for which a description is currently being prepared.
Cheilymenia aurea Boud. may also be related to C. coprinaria. However, it is insufficiently known as the type collection contains no apothecia and no other material is preserved in Boudier's herbarium in PC. According to Boudier (1905-1910), C. aurea has long, narrower (270-680 x 10-15 µm), brown hairs. Cheilymenia coprinaria sensu Boudier (1905-1910) somewhat resembles C. rubra. However, no material of the collection illustrated by Boudier is preserved in PC.
The type of Arhenia fimicola de Not.et Bagl., identified with C. coprinaria by Dennis (1978), has not been examined. However, it may not be identical with the type of C. coprinaria (see J.Moravec 1989) as it is described as having larger ascospores (Dennis 1978).
Cheilymenia fraudans (Karst.)Boud., which has excipular structure and hairs similar to those of the species under discussion, in fact differs from all these taxa in having smaller and more subglobose ascospores which bear a dense subreticulate ornamentation of the perisporium, narrower asci and hairs. Furthermore, due to the absence of the yellow refractive colour of mature ascospores when stained with CB (see also the description and drawings in J.Moravec 1989), it seems likely that C. fraudans belong to Scutellinia sect. Minutae Svrček (1971).
During the course of this study, isotype material (OSU) of Cheilymenia alleghenensis Denison (1964) was examined. Mature ascospores of C. alleghenensis are without the yellow refractive colour when stained with CB and this species proves to belong in the genus Scutellinia (Cooke) Lamb. sect. Minutae Svr. I, therefore, propose the new combination:
Scutellinia alleghenensis (Denison) J.Moravec **comb. nov.**
Basionym: Cheilymenia alleghenensis Denison, Mycologia 56: 732, 1964.
The ascospores of S. alleghenensis have a similar ornamentation to those of a similar Scutellinia convexa (Velen.) Svr. but are much smaller, 12-16.5 x (7.5-)8-9(-10) µm. Indeed, they are smaller than measured and published by Dennison (1964). They are similar in size to those of Scutellinia minutella Svr.et J.Mor. but their ornamentation consists of warts and crests which are much coarser than in that species.
The species of Scutellinia which have ascospores with a

separable perisporium and are referred to the sections Minutae and Pseudocheilymeniae Svr. (including Scutellinia crucipila (Cooke et Phill.in Cooke) J.Moravec (1984)) may, in my opinion, represent a distint genus.
The revision has confirmed my opinion (J.Moravec 1984) that the genus Cheilymenia as commonly interpreted is a complex of several distinct genera and a proposal for a new division of this genus is being prepared.

ACKNOWLEDGEMENTS

I thank Dr.Cailleux (Paris),Dr.I.Gamundí de Amos (La Plata), Prof.Dr.R.P.Korf (Ithaca) and the curators of herbaria NY and OSU for kind arrangement of loans of the type and other material. Dr. B.M.Spooner (Kew) kindly corrected the English and Dr. M.Svrček (Prague) the latin diagnosis. I am obliged to Mr. J.Lhotecký (Brno) for the preparation of the SEM photomicrographs. Dr. H.Dissing collaborated in the description of C. pseudohumarioides.

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Figs 5-8: Microfeatures of Cheilymenia. 5: C. rubra. A. ascus and paraphyses; B. ascospores under oil immersion + CB; C. an optical section of the ascospore. 6: C. liskae. A. asci and paraphyses; B. ascospores. 7: C. pseudohumarioides. A. asci and paraphyses; B. ascospores; C. an optical section of the ascospore. 8: C. humarioides. A. ascospores; B. an optical section of the ascospore.- Holotypes.

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B
C
6
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7
A
B
C
8
A
B
dark reddish
ochraceous
colour

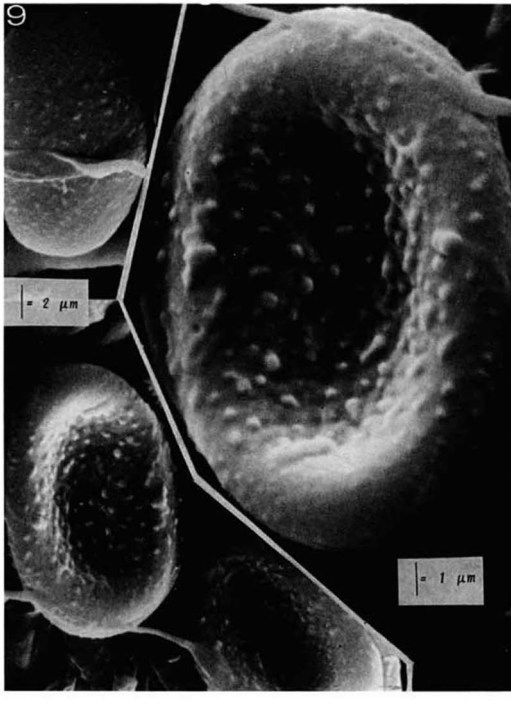

Fig. 9: SEM of ascospores of Cheilymenia rubra, Holotype (K).

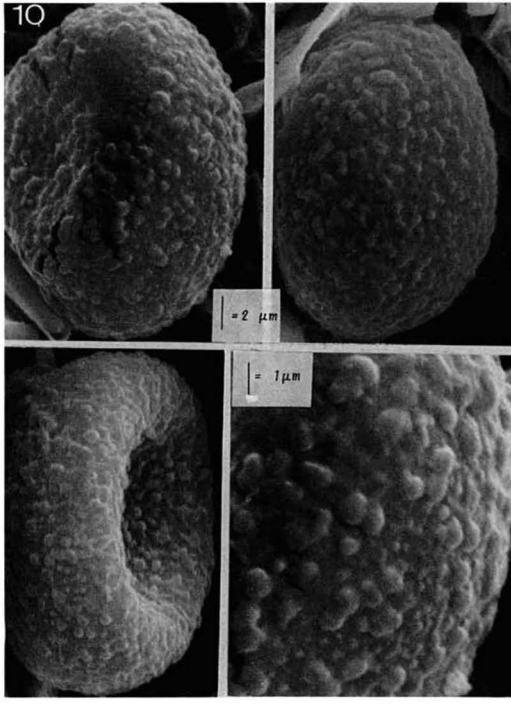

Fig. 10: SEM of ascospores of Cheilymenia humarioides. Holotype (LPS).

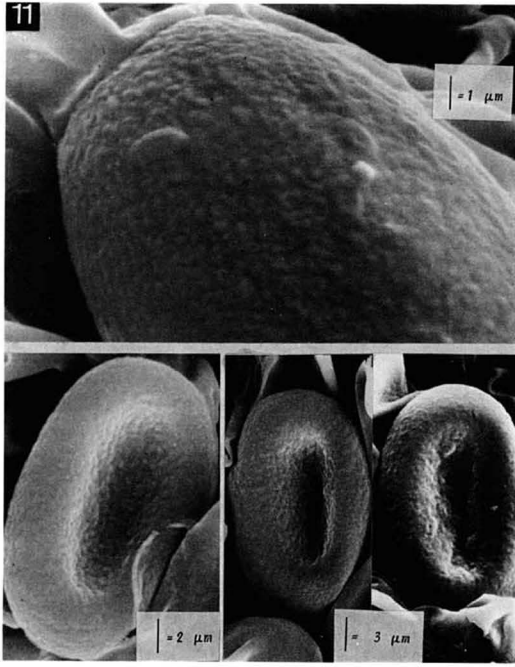

Fig. 11: SEM f ascospores of Cheilymenia pseudohumarioides. Holotype (C).

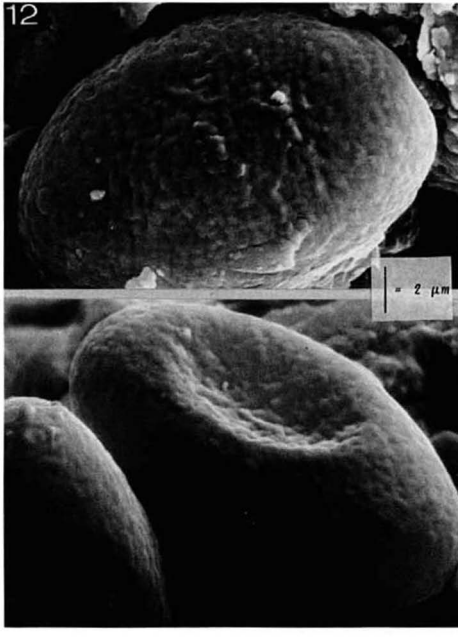

Fig. 12: SEM of ascospores of Cheilymenia liskae. Holotype (PRM).

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SIZE AND SHAPE OF UREDINIOSPORES AS INFLUENCED BY AMBIENT RELATIVE HUMIDITY*

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ABSTRACT

Urediniospores of 21 rust species were examined under natural and/or artificial conditions to determine their size and shape when hydrated or dehydrated. Urediniospores under room conditions were 70-80% of their hydrated size and were indented or collapsed. Shape of dehydrated urediniospores varied among species depending on several factors, primarily the distribution and number of germ pores. Urediniospores of Puccinia graminis, P. recondita, and Uromyces appendiculatus were manipulated experimentally to determine the relative humidity (r.h.) at which they would assume their expanded or collapsed shape. The three species responded differently to the r.h. levels studied. Urediniospores of

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P. graminis were expanded at 92.0% r.h. and above, were partially collapsed at 80.3% r.h. and were entirely collapsed at 75.8% r.h. P. recondita urediniospores were expanded at 92.0% r.h. and above, but were collapsed at 80.3% r.h. U. appendiculatus urediniospores were expanded at 100 and 96.9% r.h., were only partially collapsed at 92.0% r.h., and were entirely collapsed at 80.3% r.h. When placed into 100% r.h. collapsed urediniospores of these species absorbed moisture and regained their expanded size and shape usually within 1-2 min.

INTRODUCTION

Most descriptions of rust urediniospores are based on spores mounted in aqueous solutions such as chloral hydrate or lactophenol (Arthur, 1934; Cummins & Hiratsuka, 1983). Such descriptions most commonly characterize urediniospores as globoid to ellipsoid, although fusiform, lanceolate, reniform, or other configurations are not uncommon. Distribution and number of germ pores have also been described for most urediniospores. Germ pores may be scattered, bizonate, equatorial, supraequatorial, subequatorial, or basal near the hilum, with both their numbers and distribution varying among and sometimes within species (Cummins, 1936).

Seldom have dehydrated urediniospores been described. If collapsed or indented areas on dry urediniospores are observed they are often attributed to dehydration damage and dismissed as artefacts of preparation (Littlefield & Heath, 1979). Hardwick et al. (1975), however, contended that the collapsed, "doughnut" form of Uromyces appendiculatus is natural and occurs under normal atmospheric conditions. Cummins (1935) noted that when hydrated urediniospores of Puccinia spp. exposed to an alcohol solution collapsed into characteristic configurations that were related to the number and arrangement of germ pores. Jones (1971) suggested that depressions in the surface of Uromyces dianthi urediniospores are associated with underlying weak germ pore regions of the wall. In a survey of 150 collections of Melampsora medusae, M. occidentalis and M. abietis - canadensis the length of dehydrated urediniospores examined by scanning electron microscopy was noted to be

39% less than that of hydrated spores (Stack and Ostry, 1989). Strobel (1965) noted that dehydrated urediniospores of Puccinia striiformis were about 50% the size of hydrated spores. The reduced size and weight of Puccinia graminis f. sp. tritici urediniospores caused by dehydration, and their effects on rate of fall in still air were studied by Weinhold (1955). The collapse and resulting characteristic shape of dehydrated smut teliospores has been used to help differentiate Tilletia species in contaminated grain samples (Trione & Krygier, 1977).

The objectives of this study were threefold: 1) to determine the size and shape of the urediniospores of various rusts under natural atmospheric conditions; 2) to determine if the pattern of wall collapse, if any, is related to the number and/or distribution of germ pores or any other factors; and 3) to determine the relative humidity levels at which selected rust urediniospores will assume either their collapsed or their expanded shape.

MATERIALS AND METHODS

Urediniospores of 21 rust species in 8 genera, either dry or mounted in aqueous chloral hydrate solution, were examined and measured. For some photographs enhanced contrast was required. This was achieved by mounting urediniospores in lactophenol plus .05% (w/v) cotton blue. Fresh urediniospores from glasshouse-grown plants (6 species) and urediniospores from herbarium material (15 species) were used (Table 1). All herbarium specimens came from the North Dakota State University Plant Pathology Department herbarium. Specimens were selected to provide a variety of germ pore arrangement, urediniospore shape and size, and wall thickness. Length and width dimensions were recorded for 30 urediniospores of each species examined, both in hydrated and dehydrated states. Patterns of collapse and general shape of the dry urediniospores were determined. An Olympus interference contrast microscope, model BH-2, and Kodak Plus X Pan film were used throughout the study.

To determine the relative humidity levels at which various urediniospores would assume their collapsed or expanded condition, three rust species were utilized, Puccinia graminis, Puccinia recondita and Uromyces

appendiculatus. Small humidity chambers were constructed on 25 x 75 x 1 mm glass microscope slides to allow light microscopic examination of spores without disturbing the controlled relative humidity environment within. Each chamber, 2.5 X 5.0 cm, was constructed from four pieces of 5mm outer-diameter glass tubing cemented together and to an underlying microscope slide with clear epoxy cement. Within each chamber two short pieces of 4 mm diameter glass tubing were also cemented to the microscope slide. These pieces provided the base for a small (10 X 12 mm) observation platform (a piece of a coverglass) onto which the urediniospores were sprinkled. To prevent condensation on that platform, each side of it was coated with a thin film of an anti-fog agent (Anti-Fog®, World Optical, Arlington, TX) prior to adding the urediniospores. Urediniospores freshly collected from glasshouse grown plants were used exclusively.

Solutions of K_2SO_4, KNO_3, $(NH_4)_2SO_4$, and NaCl were used to establish relative humidity (r.h.) levels of 96.9, 92.0, 80.3, and 75.8%, respectively, at 25°C within the chambers (Wexler & Hasegawa, 1954). Distilled water was used to establish a humidity level of 100%. A small amount of these liquids (0.8 ml) was added to the humidity chambers containing the urediniospores. The humidity chambers were then covered with a 25 x 60 mm coverglass coated with anti-fog agent. The coverglass was sealed to the chamber with petroleum jelly to prevent loss of moisture.

Humidity chambers were held at 25°C on a temperature controlled hotplate in a 20-22°C room for at least 24 h to achieve the desired humidity levels. Following that equilibration urediniospores were examined microscopically and photographed. The temperature under the light microscope was also held constant at 25°C by use of heat absorbing blue filters placed between the light source and the condenser. Light intensity and iris diaphragm settings were predetermined in order to maintain a constant temperature among experiments. The temperature was monitored with a calibrated glass bead thermistor (accurate to ± 0.1°C) placed on the field diaphram of the microscope light source. To approximate the percentage of urediniospores expanded, collapsed, or partially collapsed, 100 spores were counted and the individual configuration of each spore was recorded for each humidity

chamber. Three replicate trials were made with all three rust fungi at all humidity levels, each time using fresh urediniospores collected from glasshouse-grown material.

Table 1. List of species examined

Urediniospores obtained from fresh glasshouse-grown material

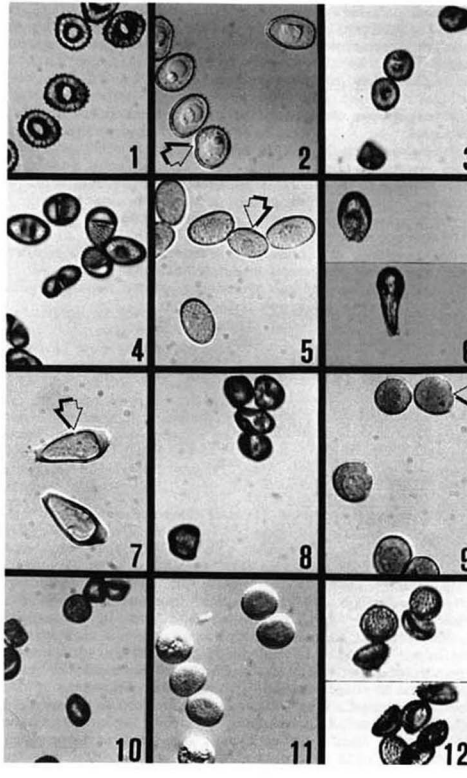
- Melampsora lini (Ehrenb.) Lev.
- Puccinia coronata Cda.
- Puccinia graminis Pers.
- Puccinia recondita Rob. ex Desm.
- Uromyces appendiculatus (Pers.) Unger
- Uromyces striatus Schroet.

Urediniospores obtained from herbarium specimens

- Hemileia vastatrix Berk. & Br.
- Melampsora medusae Thuem.
- Pileolaria brevipes Berk. & Rav.
- Puccinia caulicola Tracy & Gall
- Puccinia helianthi Schw.
- Puccinia minussensis Thuem.
- Puccinia purpurea Cke.
- Puccinia sporoboli Arth.
- Puccinia tanaceti DC.
- Puccinia tumidipes Pk.
- Tranzschelia pruni-spinosae (Pers.) Diet.
- Uredinopsis osmundae Magn.
- Uromyces plumbarius Pk.
- Uromyces silphii Arth.
- Uropyxis amorphae (Curt.) Schroet.

RESULTS

Dimensions of rust urediniospores examined in both hydrated and dehydrated states and the percentage reduction in size of dehydrated spores are given in Table 2. As would be expected, the dehydrated spores were nearly always smaller than those mounted in chloral hydrate. Urediniospores typically dehydrated to 70-80% of their hydrated size. Extremes in shrinkage observed were 67.9% width and 71.1% length, compared to hydrated spores. It appeared that in some rusts, e.g. Uredinopsis osmundae and Pileolaria brevipes, the width of dehydrated urediniospores did not change, or even increased, while the length was reduced.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Urediniospores of M. lini, P. coronata, P. graminis, P. recondita, U. appendiculatus and U. striatus that had fallen free of the uredinia onto the leaf surface of

FIGURE LEGENDS

Note: All micrographs of dehydrated spores were made from dry spores with no mounting medium or coverglass applied. All hydrated spores were mounted in aqueous, saturated chloral hydrate, and covered with a coverglass. Experimentally dehydrated and rehydrated spores (Figs. 14-21) were photographed unmounted on dry coverglasses located within controlled humidity microchambers. All figures X325.

Fig. 1. Dehydrated urediniospores of Uromyces appendiculatus. Note their characteristic "doughnut" shape, the result of wall collapse in the region of the two equatorial germ pores.

Fig. 2. Hydrated urediniospores of Uromyces appendiculatus. Note their typical ovoid shape and larger size compared to dehydrated spores, Fig. 1. Germ pore (arrow).

Fig. 3. Dehydrated urediniospores of Uromyces striatus. Note collapsed areas which apparently are centered around the equatorial germ pores.

Fig. 4. Dehydrated urediniospores of Puccinia graminis. Note the equatorial "valley" that results from coalescence of collapsed wall areas around the 3-4 equatorial germ pores.

Fig. 5. Hydrated urediniospores of Puccinia graminis. Germ pore (arrow).

Fig. 6. Dehydrated urediniospores of Tranzschelia pruni-spinosae.

Fig. 7. Hydrated urediniospores of Tranzschelia pruni-spinosae. Note the thickened apical wall areas that apparently prevent collapse of the cell wall in those same regions close to the germ pores (arrow).

Fig. 8. Dehydrated urediniospores of Puccinia recondita. Note the localized regions of cell wall collapse around the germ pores.

Fig. 9. Hydrated urediniospores of Puccinia recondita. Germ pore (arrow).

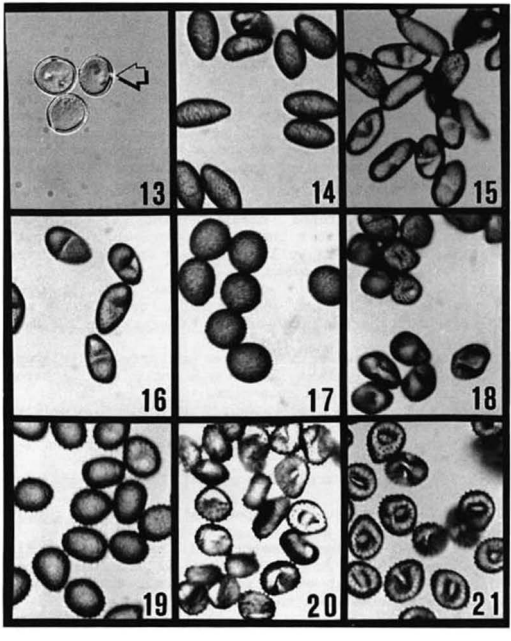

Fig. 10. Dehydrated urediniospores of Melampsora lini. Note the localized regions of cell wall collapse around the scattered germ pores.
Fig. 11. Hydrated urediniospores of Melampsora lini.
Fig. 12. Dehydrated urediniospores of Puccinia caulicola. The bowl shape of these spores results apparently from wall collapse around the basally located germ pores.

Fig. 13. Hydrated urediniospores of Puccinia caulicola. Germ pore (arrow).
Fig. 14. Experimentally rehydrated urediniospores of Puccinia graminis at 100% r.h. Note the expanded ellipsoid shape and the absence of wall depression.
Fig. 15. Partially dehydrated urediniospores of Puccinia graminis at 80.3% r.h. Note the partial collapse of the cell wall in distinct regions, but the lack of coalescence of those depressions to form an equatorial "valley". Compare to totally collapsed spores, Fig. 16.
Fig. 16. Totally collapsed, dehydrated urediniospores of Puccinia graminis at 75.8% r.h. Compare to the naturally dehydrated urediniospores in Fig. 4.
Fig. 17. Experimentally rehydrated urediniospores of Puccinia recondita at 100% r.h.
Fig. 18. Experimentally dehydrated urediniospores of Puccinia recondita at 80.3% r.h. Compare to the naturally dehydrated urediniospores in Fig. 8.
Fig. 19. Experimentally rehydrated urediniospores of Uromyces appendiculatus at 100% r.h.
Fig. 20. Partially dehydrated urediniospores of Uromyces appendiculatus at 92% r.h. Note the small depressions on the surface of several spores.
Fig. 21. Collapsed, dehydrated urediospores of Uromyces appendiculatus at 80.3% r.h. Compare to the naturally dehydrated urediniospores in Fig. 1.

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glasshouse grown plants were always collapsed when the leaves were viewed by epi-illumination light microscopy. Urediniospores within uredinia were also typically collapsed except for those deeply enmeshed in the central areas of uredinia.

Urediniospores with two opposite, equatorial germ pores (Puccinia helianthi, Uromyces appendiculatus, U. plumbarius) typically collapsed into the 'doughnut' shape as described by Hardwick et al. (1978) for U. appendiculatus (Figs. 1,2). U. silphii, which has two supraequatorial germ pores, appeared to behave somewhat differently. Although the "doughnut" shape commonly occurred in the urediniospores of U. silphii, other configurations, e.g. bowl-shaped, or simply irregular patterns of collapse were also noted.

Table 2. Average dimensions (um) of urediniospores studied[a].

Species	Hydrated state; mounted in chloral hydrate	Dehydrated state; on dry glass slides	Percentage value dehydrated spores compared to hydrated spores: Width[a]	Percentage value dehydrated spores compared to hydrated spores: Length[a]
Urediniospores with 2 equatorial germ pores[b]				
Puccinia helianthi	26.0 X 32.5	24.9 X 27.5	98.5	84.6
Uromyces appendiculatus	20.4 X 27.5	17.6 X 21.0	93.1	86.9
Uromyces plumbarius	19.0 X 23.7	18.1 X 20.2	95.3	85.2
Urediniospores with 2 supraequatorial germ pores				
Uromyces silphii	19.5 X 23.6	17.6 X 21.0	90.3	88.9
Urediniopores with >2 equatorial germ pores				
Puccinia graminis	18.5 X 32.2	14.8 X 22.9	80.0	71.1
Puccinia tanaceti	24.5 X 32.6	18.3 X 26.2	74.7	80.4
Tranzschelia pruni-spinosae[a]	19.1 X 34.8	16.0 X 32.5	83.8	93.4
Uromyces striatus	17.7 X 19.3	15.9 X 18.6	89.8	96.4

Table 2 continued...

Urediniospores with numerous, scattered germ pores				
Melampsora lini	18.2 X 21.5	14.2 X 19.2	78.0	89.3
Melampsora medusae	20.1 X 32.6	13.9 X 25.4	69.1	77.9
Puccinia coronata	21.6 X 26.1	15.2 X 19.0	70.4	72.8
Puccinia minussensis	22.6 X 24.6	17.0 X 21.2	75.2	86.2
Puccinia purpurea	26.6 X 34.7	22.9 X 28.8	86.1	83.0
Puccinia recondita	21.3 X 25.3	16.6 X 19.9	77.9	78.7
Uropyxis amorphae	16.2 X 20.8	11.0 X 15.5	67.9	74.5
Urediniospores with 2-6 germ pores near base				
Pileolaria brevipes	21.9 X 44.1	23.5 X 37.5	108.0	85.0
Puccinia caulicola	21.4 X 23.8	18.6 X 20.9	86.9	87.8
Puccinia sporoboli	26.0 X 27.3	20.8 X 22.7	80.0	83.2
Urediniospores with 8 germ pores, 4 in each of 2 transverse bands				
Puccinia tumidipes	22.8 X 33.7	7.7 X 27.8	77.6	82.5

continued on next page...

Table 2 continued...

Urediniospores with 4 germ pores; 2 much above and 2 much below equator				
Uredinopsis osmundae	12.9 X 45.4	13.7 X 40.2	106.0	88.5
Urediniospores reniform to pyramidal, with 3 or 4 germ pores typically at the acute-angle regions of the spore				
Hemileia vastatrix[f]	20.9 X 29.7	17.7 X 23.8	84.7	80.1

[a]All size measurements based on 30 urediniospores per species.
[b]Germ pore descriptions based on Arthur (1934), Cummins (1936), or our observations.
[c]Length is considered the side with the greater dimension, width the side with the smaller dimension. In a collapsed state, a urediniospore may measure smallest on one side where it would not when expanded.
[d]Some germ pores supraequatorial; a few subequatorial.
[e]Typically 2 germ pores on apical shoulders; occasionally with 1 more near the base.
[f]Germ pores are extremely difficult to observe in the thick walled urediniospores of *H. vastatrix*. Germ pore locations were assumed to be restricted to the locations of germ tubes illustrated by Ward (1882) and Harr and Guggenheim (1978).

Urediniospores of species with more than 2 equatorial germ pores tended to collapse around the equator where the pores are located. Urediniospores with 3-5 equatorial germ pores showed variations in collapse depending on the number of germ pores as well as wall thickness and spore shape. Puccinia tanaceti, with three equatorial pores, appeared more on less elliptical, with 3 indented areas, giving a triangular configuration in optical section. A somewhat similar appearance, i.e., 2-4 localized depressions around the equator, typified the globoid spores of Uromyces striatus (Fig. 3). Some urediniospores of that species, however, collapsed into a "doughnut" configuration, which suggests those particular spores had only two equatorial germ pores. P. graminis with 4-5 equatorial germ pores collapsed mostly at the equator, forming a distinct "valley" across the middle of the spore with noncollapsed areas on either side (Figs. 4,5). The configuration of dehydrated urediniospores in Tranzschelia pruni-spinosae may be more dependent on spore wall thickness than on germ pore arrangement. Spores of this species collapsed throughout, including the germ pore regions, except in the apical areas where a thickened cell wall is present (Figs. 6,7).

Urediniospores with scattered, numerous germ pores tended to have a more irregular pattern of collapse, e.g. P. recondita and Melampsora lini (Figs. 8-11). Some showed small indentations across and around the spore, and others had larger collapsed areas on one side. The irregularity of collapse seen in these urediniospores is consistent with what would be expected from the scattered arrangement of germ pores.

Urediniospores with pores near the hilum, e.g. Puccinia caulicola and Puccinia sporoboli, collapsed into a 'bowl' shape, with one large indentation, upon dehydration (Figs. 12,13). Presumably the indented area occurred in the hilum region, although this was not proven.

The patterns of collapse in Hemileia vastatrix, Melampsora medusae and Uredinopsis osmundae were difficult to categorize. In these rusts urediniospores were variously shrunken when dehydrated, M. medusae more so than the other two. The shrinkage was more or less uniform throughout, with no marked depressions around germ pore regions.

When maintained under the controlled humidity conditions P. graminis urediniospores did not collapse at 100, 96.9, and 92.0% r.h., (Fig. 14). At 80.3% r.h., 81% of the urediniospores were collapsed to the extent that the characteristic "valley" could be seen across the spore; 19% were partially collapsed to the extent that indentations in the wall were evident but no "valley" was visible (Fig. 15). Nearly all urediniospores of P. graminis were collapsed at 75.8% r.h. (Fig. 16). At 100, 96.9, and 92.0% r.h. P. recondita urediniospores remained expanded with no visible signs of collapse (Fig. 17). All urediniospores of P. recondita were collapsed at 80.3% r.h. and appeared as irregularly collapsed spheres (Fig. 18). U. appendiculatus urediniospores were not collapsed at 100 and 96.9% r.h. (Fig. 19). At 92.0% r.h. those urediniospores showed various configurations, being 2% fully expanded, 21% partially collapsed but not "doughnut" shaped, and 77% collapsed to the characteristic "doughnut" configuration (Fig. 20). All urediniospores of U. appendiculatus were collapsed into the "doughnut" shape when held at 80.3% r.h. (Fig. 21).

Expanded, freshly collected urediniospores from leaf pustules assumed their characteristic dehydrated configuration in as little time as 30 sec to 1 min when placed onto a microscope slide at laboratory, ambient r.h.; conversely, dehydrated spores regained their characteristic hydrated configuration within 1-2 min when placed in a saturated atmosphere. These times were typical of spores individually situated on the microscope slide; spores in clusters required greater lengths of time to respond, particularly to rehydrate to their fully expanded state.

DISCUSSION

Light microscopy showed that rust urediniospores do indeed assume collapsed configurations under certain natural environmental conditions. Those shapes can differ markedly from the shapes of hydrated spores, which are generally used to study spore morphologoly. Urediniospores can change from their hydrated to their dehydrated configuration, or vice versa, within 1-2 min when placed in the appropriate atmospheric environment. Results of this study suggest that several aspects of

rust taxonomy, morphology and aerobiology may be significantly influenced by the effects of atmospheric relative humidity.

Urediniospore size is a major criterion when identifying rust species. The use of scanning electron microscopy in such determinations can be inaccurate, or misleading at best, if the shrinkage effect is not taken into account. Identification of Melampsora spp. on Populus spp. by scanning electron microscopy of urediniospores required that shrinkage factors be determined and calculated into the data when species were being determined (Stack and Ostry, 1989); otherwise, serious errors would result.

Urediniospore germ pore regions are considered to be areas where the spore wall is weakened (Jones, 1971; Bennell and Henderson, 1978); it is thus reasonable to assume that spores would collapse at these weaker areas upon dehydration. If this is true, urediniospores with similar germ pore distribution and number should show a similar pattern of collapse, other factors being equal. Indeed, certain patterns of collapse could be noted which seem to correspond to germ pore number and arrangement. Species with 2 equatorial germ pores generally collapsed to the "doughnut" configuration; those with more than 2 equatorial germ pores collapsed around those pores to form a series of depressions, or when coalesced those depressions formed an equatorial "valley" across the spore. Those with basally located pores collapsed to form a "bowl" configuration. Spores with numerous, scattered germ pores collapsed in a more random, nonuniform manner. Exceptions such as H. vastatrix, M. medusae, U. osmundae, T. pruni-spinosae exist. Those urediniospores have either nonuniformly thick spore walls, or have germ pores restricted to rigid "corners" of the spores. It can be concluded from this study that the shape of dry urediniospores of many species is determined to a large extent by germ pore number and distribution, wall thickness, and possibly spore shape.

This study indicates that at humidity levels below 75-80% many urediniospores normally occur in their dehydrated configurations. Consequently, in many areas of the world those urediniospores are disseminated, not

as round to oval spores, but as irregularly shaped, indented structures. Some of the theoretical advantages of this fact were discussed by Hardwick et al. (1975). The state of the urediniospores on the leaf surface, whether hydrated or dehydrated, would depend upon the microclimate in the immediate vicinity. Urediniospores will attain complete expansion at 100% r.h. within only a few minutes after being held at 53% r.h. for 24 h.

The configuration of urediniospores could be significant in spore dispersal. The terminal velocity of falling spores, as defined by Stoke's Law (Gregory, 1973), is positively correlated to spore diameter. Consequently spore diameter can influence the horizontal distance that spores can travel in moving air. Most calculations of theoretical terminal velocity have been based on spore dimensions obtained from measurements of liquid-mounted spores examined by standard light microscopy. The smaller size and the variously convoluted shapes of dehydrated spores could affect their rate of fall. Results consistent with Stoke's Law were obtained by Ferrandino and Aylor (1984) for U. appendiculatus urediniospores. They observed that air-dried spores and fresh spores released into a settling chamber directly from the leaf surface settled at a rate of 0.86 and 1.08 cm s^{-1}, respectively. Presumably the faster rate of descent of fresh spores was related to their larger diameter, and perhaps a greater percentage of inflated, oval shaped spores compared to the percentage of dehydrated spores in their typical "doughnut" shape. Little is known, however, about the precise applicability of Stoke's Law to nonspherical spores or the effects of spore surface ornamentation on the rate of descent of spores in air (Gregory, 1973).

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NUCLEAR DNA, AN ADJUNCT TO MORPHOLOGY IN FUNGAL TAXONOMY

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ABSTRACT

Estimates of nuclear DNA in the haploid nuclei of two species of *Neurospora* (Ascomycetes) and 70 smut fungi (Ustilaginales) were calculated by microfluorometry. Haploid and diploid nuclei stained with Schiff reagent (pararosaniline) consistently fluoresced in proportion to DNA content. In using *Saccharomyces cerevisiae* Meyen ex Hansen as a bench mark organism (= 1.5×10^{10} daltons of DNA per haploid nucleus) it was estimated that haploid DNA among the 72 species ranged from 13.5×10^{9} to 95.8×10^{9} daltons. In nine species tested the average amounts of DNA in haploid and diploid nuclei closely approximated ratios of 1:2, respectively. The estimated amount for microconidia of *Neurospora crassa* Shear & Dodge by chemical analysis (Horowitz & Macleod, 1960) was confirmed by microfluorometry. Moreover, DNA replication occurred *after* karyogamy in fusion nuclei of both asci and teliospores. The potential use of genome size for taxonomic purposes as an adjunct to morphology is discussed.

INTRODUCTION

Thus far chromosome counts have had little application in fungal taxonomy, probably because the chromosomes of fungi generally are extremely small, they are hard to separate, and hence are difficult to count. For example, nuclei of smut fungi generally are so small that chromosome counts have yet to be categorically demonstrated with convincing photomicrographs. Consequently, the taxonomy of Ustilaginales likely will continue to be principally based on spore morphology, the way spores germinate, soral characteristics, and host specialization (Durán, 1988). However, Bosshard (1964) and Ruch (1966) have shown that the desoxyribonucleic acid (DNA) content of individual nuclei can be accurately measured with cytofluorometry, if an organism in which DNA has been determined by some other means is used as an internal standard, such as *Saccharomyces cerevisiae* Meyen ex Hansen. Since the chromosome number has been demonstrated only in random fungal taxa from time to time, it has thus far failed to figure prominently in the taxonomy of any of the major groups of fungi. As an alternative to the chromosome number, and/or in addition to it, the genome size (along with classical morpho-

logical criteria) should help the taxonomist to distinguish more critically between morphologically similar taxa, especially if the amounts of nuclear DNA can be shown to differ significantly.

Recently Cavalier-Smith (1985) summarized genome sizes for a large number of bacteria, protozoa, higher animals, plants, etc.; however, few estimates were indicated for fungi, apparently because thus far there have been relatively few quantitative studies of fungal DNA.

S. cerevisiae has been used for many years as a eukaryote of choice in genetic research, yet estimates of the genome size have varied considerably. Researchers have estimated the genome size by renaturation kinetics with very different results. Consequently, what genomic size to attribute to *S. cerevisiae* which was used as an internal standard in the present study was given careful consideration because it presented a dilemma. Based on the rate of DNA renaturation Bicknell & Douglas (1970) reported a minimum genome of 9.2 X 10^9 daltons, whereas Lauer, Roberts, & Klotz (1977) using both the diaminobenzoic acid and ethidium bromide methods to calculate amounts of DNA per cell concluded that a haploid nucleus contained 1.0 X 10^{10} ± 0.1 x 10^{10} daltons of DNA, adding ... 'recent DNA per cell studies from two other laboratories yielded a nuclear DNA content for *S. cerevisiae* of 8 x 10^9 to 9 x 10^9 daltons (Whitney & Hall, personal communication; Kaback, personal communication) in agreement with our values.' In contrast, Galeotti et al. (1981) reported 5.42 X 10^9 daltons for the haploid genome. With the exception of the latter estimate those indicated above agree with the recent one of Mortimer & Schild (1985) who calculated that recombinant lengths for the 16 chromosomes of *S. cerevisiae* totaled 3891 centimorgans (cM), i.e. about 9.34 x 10^9 daltons of DNA based on the DNA content of intervals of known genetic length. Their regression line showed a general correlation between chromosome size and recombination lengths.

Using orthogonal field alternating gel electrophoresis (OFAGE) to separate large chromosomal DNA's Carle & Olson (1987) estimated that chromosome XII, the largest in the genome, consisted of about 2.06 X 10^9 daltons of DNA, a figure which fell within a range of DNA values earlier reported for the same chromosome by Lauer et al. (1977). Using microfluorometric techniques to measure nuclear DNA and C. C. Lindegren's haploid alpha strain of *S. cerevisiae* (ATCC #10275) as an internal standard, we present herein estimates of nuclear DNA in two species of *Neurospora* (Ascomycetes) and 70 species of smut fungi (Ustilaginales).

MATERIALS AND METHODS

Considering that the estimates of Bicknell et al. (1970), Lauer et al. (1977), and Mortimer et al. (1985) were in large regard comparable, and since some decision had to be exercised in assigning a genome value to the internal standard,

we settled for a genome size of 1.5 X 10^{10} daltons of nuclear DNA which is the mean for dividing cultures (1.0 x 10^{10} daltons being a 1 C value for non-dividing cultures reported by Lauer and co-workers in their 1977 paper.

Teliospores were streaked on 2% potato dextrose agar or 2% water agar. Those that germinated and others presumably in very early stages of the germination process but not showing germ-tubes, as well as the products of germination which consisted mostly of uninucleate sporidia, were smeared onto glass slides coated with Haupt's adhesive (Johansen, 1940) and fixed.

A microconidial strain of *Neurospora crassa* (stock #FGSC 2179) with mutant genes for peach/fluffy (Barratt & Garnjobst, 1949) was grown on neurospora culture agar (NCA). It was selected for study because the mutant genes eliminate production of macroconidia; moreover, uninucleate haploid microconidia are produced in profusion which auto-arrest as soon as formed. Material studied consisted of microconidia and mycelium removed from subcultures 4 days old, smeared onto slides coated with Haupt's adhesive and subsequently fixed. *Neurospora tetrasperma* (ATCC #38097) was subcultured on corn meal agar (CMA). Perithecia, mostly immature, were removed with microforceps from cultures 5-8 days old, squashed between two glass slides coated with Haupt's adhesive, fixed, and dried on a warming table at 45°C. Haploid and diploid strains of *S. cerevisiae*, ATCC #10275, and ATCC #26109, respectively, were subcultured on yeast malt agar (YMA). All subculturing was done at room temperature. Except for the teliospores of *Tilletia controversa* which were germinated at 4°C with 8 h of light and 16 h of darkness, those of all the other smut fungi were germinated at room temperature. All materials were fixed for 24 h or more in FAA (formalin/glacial acetic acid/ 50% ethanol (1:1:18, by vol.).

After fixation the specimens were brought down to water through 70% ethanol and hydrolyzed in 3.5 M HCl (37°C) for 20 min. Nuclei were optimally stained with this hydrolysis schedule (Fand, 1970) and hence it was used throughout the study (Fig. 1). After hydrolysis the specimens were rinsed in water and stained 3.5 h in Schiff reagent (Leuchtenberger, 1958). Pararosaniline was used as the stain of choice because 1) nuclei stained vividly and were very clearly resolved with fluorescence microscopy 2) unlike other fluorochromes it quenched very slowly upon excitation and 3) it fluoresced in green light in proportion to DNA content (Bosshard, 1964; Ruch, 1966; Böhm & Sprenger, 1968).

To prepare the nuclear stain 0.5 g pararosaniline hydrochloride (Harleco basic fuchsin, lot #60801, certified by the Biological Stain Commission) was dissolved in 100 ml of boiling water, cooled to 50°C and filtered. To the filtrate were added 10 ml of 1 M HCl and 1.0 g of $K_2S_2O_5$. The stain was stored in the dark for 18 h; subsequently, 0.25 g of decolor-

izing carbon was added, the material shaken 1 min and then rapidly filtered. Stained specimens were differentiated 10 min in each of three sulfite rinses of deionized water/10% aqueous potassium meta-bisulfite/1 M HCl (18:1:1, by vol.), dehydrated in 50%, 70%, 95%, and 100% ethanol, cleared in ethanol/xylene (1:1, by vol.), three changes of xylene, and mounted in Entellan, a nonfluorescent resin.

Microfluorometry with epifluorescence was used to estimate nuclear DNA because it is much more sensitive than absorbance microphotometry, light scattering is less troublesome, and the light quanta emitted by specimens are uniformly measured by the photomultiplier, even when DNA in the nucleus is unevenly distributed (Bosshard, 1964; Ruch, 1966). Also, the laborious calculations associated with the two-wave method (Patau, 1952) required to overcome distributional errors were avoided.

A Dialux 20 microscope equipped with a Leitz MPV photometer, fixed measuring diaphragms, a 1X Ploemopak vertical illuminator, and a 100 W Hg vapor lamp were used to measure fluorescence intensities of individual nuclei. The lamp routinely was allowed to burn 15-20 min before fluorescence intensities were read. A Leitz MPV control panel interfaced with a Hewlett-Packard 86 B computer was used to record and

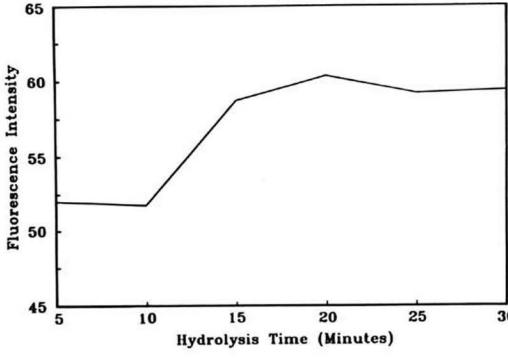

Fig. 1. *Saccharomyces cerevisiae*. The relationship between fluorescence of haploid nuclei stained with pararosaniline-Schiff and hydrolysis periods of from 5 to 30 min in 3.5 M HCl at 37°C. Each point is the mean of 30 fluorescence measurements.

statistically analyze fluorescence data. An A filter block (excitation range 340-380 nm, barrier filter 430 nm) and a uranium glass slide (Schotts-Mainz GG-17) were used to standardize the panel. With the iris in the illuminator open, and a fixed 0.2 mm circular measuring aperture in place, a X100 oil immersion objective (N.A.1.32) was focused on the slide for maximum fluorescence and the potentiometer adjusted to give a digital display reading of 99.9-100. During standardization the preselector was set for minimum sensitivity (X1), the measured-value integration for continuous measurement and display (nx), and the high-tension amplification adjusted to 700 V. To isolate incident light and record fluorescence intensities of nuclei an N2 filter block (excitation range 530-560 nm, barrier filter 580 nm) was used, the sensitivity range increased to X100, and the measured-value integration adjusted for readings averaging 1.0 sec (T2).

To standardize exposure of the stained nuclei to the excitation source microscopic fields were selected in which the nuclei were separated by distances several times their diam. Using the X100 objective with the illuminator iris closed provided a field 22.0 μm in diam. A fixed aperture 0.2 mm in diam was used to isolate single nuclei. The aperture provided a field 2.0 μm in diam or slightly larger than the nuclei. Fluorescence intensities were recorded after each nucleus was exposed to 1 min of excitation because this much time was required for the intensity/time curve to stabilize. After each measurement a background reading of the accompanying cytoplasm was subtracted from the fluorescence intensity of each nucleus. Since other nuclei in the field unavoidably were exposed to excitation, only one intensity reading was taken per field.

Specimens consisting of 6 to 10 species were simultaneously processed with specimens of the internal standard in each series of experiments. In each experiment the fluorescence intensities of 30 nuclei of the standard were averaged, the means ranging from 55.7 to 61.0 fluorescence units, the standard errors of the means from 2.2 to 3.1. To estimate the average amount of DNA of each species the fluorescence intensities of 30 to 60 nuclei were likewise averaged, the means multiplied by 1.5×10^{-10} daltons, and the product divided by the mean fluorescence of the corresponding standard.

Diploid values for the smut fungi were based on measurements of the single nucleus contained in each teliospore, haploid values mostly on uninucleate sporidia. Haploid nuclei of *N. tetrasperma* were measured in immature ascospores at the 2- and 4-nucleate stage. Lacking melanin at this time, the measurements were made without frustration. Because the nuclei in ascospores were often closely spaced, only one nucleus per ascospore was measured. Diploid values were measured in young developing asci, all of which contained one nucleus. Haploid values for *N. crassa* were based on measurements of pre-S microconidia and vegetative mycelium in which cells were dividing.

RESULTS AND DISCUSSION

The average amounts of haploid DNA for the various species were plotted against fluorescence intensity. A linear relationship was shown, (Fig. 2) the slope of the line was 5.137, and r = 0.982. The average amounts for most species fell between 13.5 X 10^9 and 30.0 X 10^9 daltons. No general correlation of genome size and generic affinities was suggested, although nine species of *Tilletia* and *Schroeteria delastrina* contained the most DNA. The average haploid DNA content of each species and other data are summarized in Table 1.

The average amounts of nuclear DNA, the range of values, and the C-values for haploid and diploid nuclei of the nine species tested were summarized (Table 2). Since all materials were unarrested the values of haploid nuclei ranged from 1 to 2 C, those of diploid nuclei from 2 to 4 C. The values

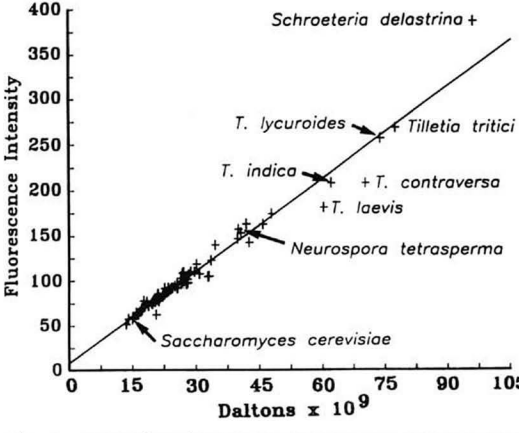

Fig. 2. Proportionality between fluorescence and DNA content in haploid nuclei of 70 species of smut fungi and *Neurospora tetrasperma*. Each point is the mean of 20-38 fluorescence measurements. The slope of the line is 5.137 and r=0.982. [Genome size assigned the internal standard (*Saccharomyces cerevisiae*) was 1.5 x 10^{10} daltons of DNA]. See Table 1 for statistical data.

are graphically shown in Figure 3. Especially noteworthy were values of young asci of *N. tetrasperma* because the single nuclei contained therein showed intergrading values which ranged from 51.9-129.6 X 10^9 daltons (Fig. 4). The few

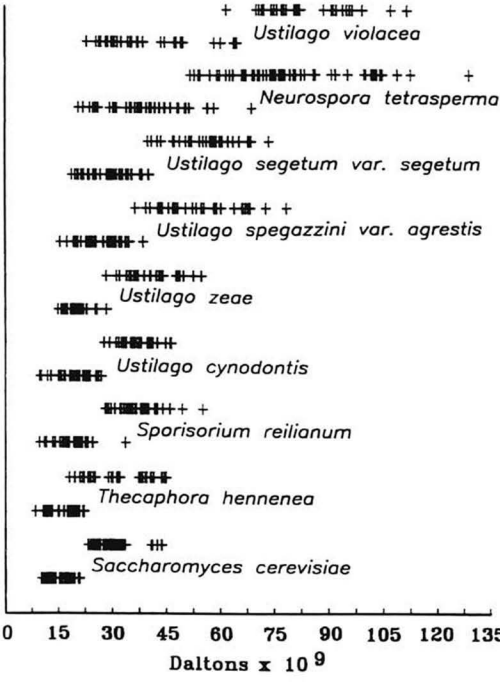

Fig. 3. Ranges of nuclear DNA in haplo- and diplophases of seven species of smut fungi, and in *Neurospora tetrasperma* and *Saccharomyces cerevisiae*. (Average amounts and 1 and 2 C values are summarized in Table 2.)

nuclei in primary asci which gave values around 4 C (Fig. 4) might have resulted from replication in the croziers, although the data mostly suggested that the diploid nuclei were in varying stages of post-karyogamy synthesis. This contrasts what has been described in *Neottiella rutilans* (Fr.) Dennis by Rossen & Westergaard (1966), namely, that meiotic chromosome replication is completed *before* karyogamy in the croziers, i.e. before primary asci form. The diploid nuclei of teliospores, like those of asci, were likewise in varying stages of post-karyogamy replication, as evidenced by values which ranged in general from 2 C to 4 C (Table 2).

The haploid genome of *N. crassa* has been variously estimated over the years and hence merits brief discussion. From chemical extractions Horowitz & Macleod (1960) estimated that microconidia averaged 27.7 X 10^9 daltons of DNA. By renaturation kinetics Krumlauf & Marzluf (1980) calculated that haploid nuclei of mycelia contained about 2.7 x 10^7 base

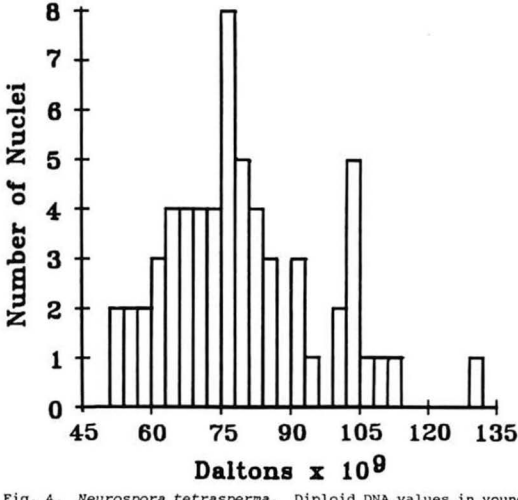

Fig. 4. *Neurospora tetrasperma*. Diploid DNA values in young asci, all with one nucleus. Mean = 79.3 x 10^9 daltons; s.e.m. = 2.16; the range 51.9-129.6 x 10^9 daltons; n = 60.

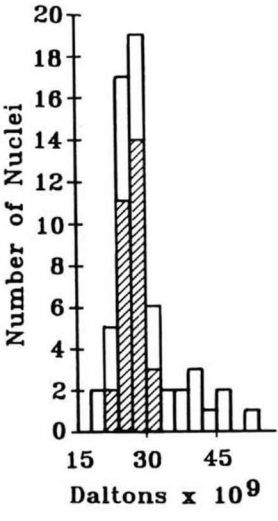

Fig. 5. *Neurospora crassa*. Haploid DNA values in microconidia (hatched bars). Mean = 27.0 x 10^9 daltons; s.e.m. = 0.39; the range 23.9-31.6 x 10^9 daltons; n=30. Mycelia (open bars). Mean = 31.6 x 10^9 daltons; s.e.m. = 1.54; the range 20.7-51.0 x 10^9 daltons; n=30. Chromosomal DNA in microconidia mostly unreplicated, some in mycelia replicated and hence somewhat greater.

pairs. Microconidia of the peach/fluffy strain we used were 99% uninucleate and averaged 27.0 X 10^9 daltons per nucleus. Mycelia, however, averaged more DNA than microconidia, apparently because some nuclei were pre-S and some post-S. Since *N. crassa* microconidia mostly were unreplicated (Fig. 5), the mean and 1 C values were virtually the same. If the estimate of Krumlauf & Marzluf (op. cit.) is a 1 C value for non-dividing cultures, multiplying it by 1.5 would yield also a theoretical mean of about 27.0 X 10^9 daltons.

Using an alternating-field gel electrophoretic system which employs contour-clamped homogeneous electric fields (CHEF), Orbach, Volbrath, Davis & Yanofsky (1988) estimated the molecular karyotype of *N. crassa* to be around 31.3 x 10^9 daltons. This exceeds the estimates indicated above but equals the amount indicated herein for mycelium (Table 1; Fig. 5).

Estimates of nuclear DNA should be included in descriptions of fungi when possible, either in lieu of the chromosome number or in addition to it. Such information could prove to be very useful in taxonomic studies of fungi, especially among fungi with a dearth of definitive characteristics. Durán and Fischer (1961), for example, concluded that *Tilletia horrida* Tak. on rice was a synonym of *Tilletia barclayana* (Bref.) Sacc. which parasitizes range grasses. However, this study showed that *T. horrida* contains 24% more DNA than *T. barclayana* hence the two probably are distinct species. Furthermore, the protocol for the quantification of nuclear DNA as described herein was

simple, inexpensive, and consistently yielded reproducible results.

The amount of DNA ascribed to *S. cerevisiae* for purposes of estimating the DNA content of the fungi included in this study deserves additional mention because it relates to the veracity of the data presented herein. The decision to use 1.5×10^{10} daltons in the calculations stemmed from a number of sophisticated researches, namely, those of Bicknell et al. (1970), Lauer et al. (1977), and Mortimer et al. (1985), all of which provided estimates of the haploid genome which hovered around 1.0×10^{10} daltons. Since this figure probably closely approximated the 1 C value of haploid nuclei, the estimates of DNA presented here were considered near absolute amounts for dividing cells, exclusive of mitochondrial DNA.

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Table 1. Estimates of nuclear DNA in haploid nuclei of 72 species of fungi.

Species	daltons x 10^9 mean	range	s.e.m.
Ustilago succisae	13.5[1/]	9.1-21.6	0.61(30)
Tolyposporium penicillariae	13.9	5.5-36.1	1.26(30)
Sphacelotheca hydropiperis	15.0	7.9-26.7	0.76(30)
Sphacelotheca cruenta	15.3	9.7-22.6	0.70(31)
Thecaphora hennenea[2/]	15.9	8.5-22.3	0.70(31)
Sporisorium anthistirae	15.9	10.2-24.9	0.69(30)
Ustilago betonicae	16.3	8.5-24.6	0.69(30)
Tolyposporium bullatum	16.9	9.6-23.4	0.79(30)
Sporisorium puellare	16.9	9.0-28.6	0.79(30)
Urocystis colchici	17.1	11.5-24.1	0.58(30)
Cintractia taubertiana	17.1	9.7-31.6	1.14(30)
Ustilago convertere-sexualis	17.2	10.5-29.7	0.99(30)
Sorosporium consanguineum	17.7	8.7-24.7	0.88(30)
Sorosporium penuriasorus	18.0	8.4-32.4	1.08(30)
Sporisorium reilianum	18.3	9.6-33.4	0.88(30)
Ustilago cynodontis	18.7	9.7-26.8	0.84(30)
Ustilago zeae	19.5	14.8-28.3	0.57(30)
Ustilago scitaminea	19.8	11.5-27.6	1.00(30)
Ustilago aschersoniana	19.9	13.8-28.2	0.69(30)
Ustilago spermophora	20.4	12.1-34.9	1.18(30)
Ustilago tricophora	20.5	11.2-30.9	1.02(30)
Ustilago buchloës	20.7	15.4-29.1	0.61(30)
Sporisorium rhynchelytri	20.8	11.2-28.6	0.79(30)
Sporisorium sorghi	21.0	11.2-33.9	0.99(30)
Ustilago ixophori	21.1	12.1-28.8	0.88(30)
Tilletia barclayana	21.1	13.8-28.8	0.96(30)
Sphacelotheca diplospora	21.4	12.7-32.5	1.03(30)
Sorosporium caledonicum	22.2	11.8-36.7	1.21(30)
Sphacelotheca pamparum	22.5	9.6-34.8	1.18(30)
Sorosporium cenchri	22.6	12.1-33.1	1.29(30)
Ustilago bethelii	22.8	14.1-31.5	0.99(30)
Sphacelotheca monilifera	23.2	14.2-34.6	1.08(30)
Ustilago opiziicola	23.4	12.1-32.8	1.24(30)
Sphacelotheca nealii	23.5	13.6-38.5	1.35(30)
Sphacelotheca andropogonis-hirtifolii	24.0	16.0-34.6	0.79(30)
Ustilago neglecta	24.3	13.3-31.8	0.64(30)
Sorosporium confusum	24.4	13.2-39.3	1.29(30)
Tilletia tuberculata	24.9	17.7-40.3	1.23(30)
Tilletia trachypogonis	25.5	14.1-32.4	1.29(20)
Tilletia rugispora	25.5	17.1-29.5	0.45(30)
Tolyposporium junci	26.4	14.2-33.0	0.88(30)
Sorosporium mixtum	26.4	13.3-35.1	1.26(30)

Ustilago elegans	26.7	19.0-36.6	0.88(30)
Ustilago tricuspidis	26.7	16.0-41.8	1.32(30)
Ustilago spegazzini var. *agrestis*[1/]	27.0	15.4-38.4	1.02(30)
Ustilago minor	27.3	15.0-50.2	1.84(30)
Ustilago enneapogonis	27.7	15.1-47.8	1.42(30)
Tilletia horrida	27.9	18.9-46.9	1.68(30)
Melanopsichium pennsylvanicum	27.9	16.5-47.4	1.38(30)
Ustilago bullata	28.3	18.6-37.6	0.88(30)
Ustilago segetum var. *segetum*	28.6	18.7-40.0	1.08(35)
Ustilago aegopogonis	29.5	18.1-44.7	1.56(30)
Ustilago segetum var. *avenae*	30.1	17.2-49.6	1.86(30)
Tilletia narasimhanii	31.0	17.2-45.9	1.24(30)
Neurospora crassa	31.6	20.7-51.0	1.54(30)
Tilletia obscura-reticulata	32.7	25.0-54.9	1.78(30)
Tilletia bouteloua	33.0	24.4-43.2	0.99(30)
Tilletia buchloeana	33.6	25.0-43.0	0.79(30)
Tilletia muhlenbergiae	34.5	18.0-46.5	1.60(30)
Tilletia narayanaraoana	39.7	23.5-54.1	1.74(30)
Neurospora tetrasperma[3/]	39.9	20.5-68.7	1.77(38)
Ustilago violacea	40.5	23.2-64.8	2.28(30)
Sorosporium saponariae	41.8	22.5-59.1	2.22(30)
Tilletia durangensis	42.6	30.9-73.5	1.77(30)
Tilletia aegopogonis	45.7	25.6-63.6	1.98(30)
Tilletia brunkii	47.8	25.6-79.3	2.65(30)
Tilletia laevis	60.1	35.5-80.1	2.28(30)
Tilletia indica	61.9	41.2-81.6	1.98(30)
Tilletia contraversa	70.2	38.4-99.9	3.16(30)
Tilletia lycuroides	73.6	27.0-127.8	4.93(30)
Tilletia tritici	77.2	40.5-117.7	4.48(30)
Schroeteria delastrina	95.8	43.9-164.8	6.10(30)

1/ The means shown are 1.5 times the 1 C value and are relative to nuclear measurements in dividing cultures of the standard, *Saccharomyces cerevisiae*, in which 1 C = 1.0 X 10^{10} daltons of DNA, and the mean for dividing cultures therefore = 1.5 X 10^{10} daltons. Data plotted in Figure 2.

2/ Measurements for *Thecaphora hennenea, Urocystis colchici, Ustilago spegazzini* var. *agrestis*, and *Neurospora crassa* from mycelial nuclei; all other measurements of smut fungi from secondary sporidia.

3/ From 2- and 4-nucleate ascospores.

Table 2. Average amounts of nuclear DNA estimated for haploid and diploid nuclei of nine fungi and their corresponding C values.[1/]

Species	daltons X 10^9 n nuclei mean	s.e.m.	range	1C value	2n nuclei mean	s.e.m.	range	2C value
Ustilago violacea	40.5	±2.28(30)	23.2-64.8	27.0	83.8	±2.14(30)	61.9-112.0	55.8
Neurospora tetrasperma[2/]	39.9	±1.77(38)	20.5-68.7	26.5	79.3	±2.16(60)	51.9-129.6	52.9
Ustilago segetum var. *segetum*	28.6	±1.08(35)	18.7-40.0	19.0	56.1	±1.63(30)	40.0-73.6	37.3
Ustilago spegazzini var. *agrestis*[3/]	27.0	±1.02(30)	15.4-38.4	18.0	54.1	±1.99(30)	36.3-78.3	36.0
Ustilago zeae	19.5	±0.57(30)	14.8-28.3	12.9	41.1	±1.18(30)	28.2-54.6	27.3
Ustilago cynodontis	18.7	±0.84(30)	9.7-26.8	12.4	36.7	±0.91(30)	27.7-46.2	24.4
Sporisorium reilianum	18.3	±0.88(30)	9.6-33.4	12.1	37.5	±1.14(30)	28.0-55.0	24.9
Thecaphora hennenea	15.9	±0.70(31)	8.5-22.3	10.5	31.0	±1.48(31)	18.1-45.0	20.7
Saccharomyces cerevisiae[4/]	15.0	±0.57(30)	10.3-21.0	10.0	30.1	±0.99(30)	23.5-43.8	20.1

1/ See Swift (1950) for C-value terminology.

2/ Haploid nuclei measured in ascospores at the 2- and 4-nucleate stage. Diploid nuclei measured in developing asci with one nucleus. Measurements based on ATCC #38097.

3/ Haploid nuclei of the smut fungi measured in uninucleate sporidia, diploid nuclei in uninucleate teliospores. (In Ustilago spegazzini var. agrestis and Thecaphora hennenea haploid nuclei measured in young thalli formed by germinating teliospores.)

4/ Haploid measurements based on C. C. Lindegren's 2333 haploid mating type alpha strain (ATCC #10275). For diploid measurements ATCC #26109 was used (E. Cabib x 2180 & R. K. Mortimer, NCYC 826).

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MELIOLACEOUS FUNGI FROM THE STATE OF KERALA, INDIA I.

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SUMMARY

This paper reports in part the results of a survey undertaken during 1981-1984 of the meliolaceous fungi found in the Idukki Hydroelectric Project Area in the State of Kerala, India. Two hundred fifty eight collections were made, resulting in the identification of 103 species and infra- specific taxa. Of these, 32 are undescribed species, and 13 have been determined to be new varieties. These fungi will be described in subsequent papers. Twenty-seven taxa were recorded from India for the first time. The taxa were distributed in five genera, as follows: Amazonia (4), Armatella (2), Asteridiella (9), Irenopsis (4), and Meliola (84).

Species belonging to genera other than Meliola are treated in this paper, and include four species of Amazonia, with three new species A. acronychiae, A. actinodaphnis and A. syzygii; two species of Armatella, nine species of Asteridiella, including the four new species A. clerodendricola, A. crotonis, A. macarangicola, and A. turpiniicola; four species of Irenopsis, including the new species I. eriolaenae, and a new variety I. leeae Hansford var. indica.

Keywords: Meliolaceae, Amazonia, Armatella, Asteridiella, Irenopsis, India, Kerala, black mildews.

The Meliolaceae (Order Meliolales), commonly known as the 'black mildews' or 'dark mildews', are epiphyllous parasites on a broad range of host plants. As a group, they show many parallels with the 'powdery mildews' (Order Erysiphales) (Alexopoulos & Mims, 1979) and have been considered by some authors (Wellman, 1972) to be a tropical

counterpart of that group. They are sometimes erroneously referred to as "sooty moulds", which are saprobic fungi associated with scale insects or honey dew producers and which are placed in another order of fungi (Stevens, 1931; Hughes, 1976). In contrast to the sooty moulds, the black mildews are parasites, penetrating their hosts by means of haustoria that arise from the characteristic superficial hyphopodiate mycelium. The production of the bulbous haustoria from the lower surface of the head cells of the capitate hyphopodia has been schematically illustrated by Doidge (1921) Roger (1953) and Luttrell (1989). The black mildews are most abundant in the tropics, although some species occur in temperate regions.

As with Erysiphales and the Uredinales, the Meliolales show a high level of host specialization, making it essential to know the host species before any attempt is made to identify these fungi to the species level. The probability of accurately identifying these fungi to the species level or of recognizing a new taxon without first identifying the host are remote. Although attempts have been made to culture these fungi, both in the laboratory and on host plants (Bal, 1919; Hansford, 1961; Thite, 1975; Goos, 1978), no one has yet succeeded in doing so.

Hansford's (1961) monumental monograph of the group gives an account of 1814 taxa, known from throughout the world. About 100 taxa, including homonyms and synonyms, have been reported from India (Bilgrami et. al., 1979, 1981; Hosagoudar, 1985).

To learn more about the occurrence of the Meliolaceae in India, a survey was made in the region of the Idukki Hydroelectric Project Area in Kerala. This study was carried out during the period 1981-84, when twenty well-planned collecting trips, covering all seasons, were conducted. These surveys resulted in 258 collections. There are no prior collection records for this region, and several of the collections are new records for India.

THE STUDY AREA

Idukki, the largest hilly district in Kerala State, is located in the Western Ghats, between 9^{o} 15' and 10^{o} 21' of north latitude and 76^{o} 37' and 77^{o} 25' of east longitude. The district extends 115 km north to south and 67 km east to west (Fig. 1). The important feature of this district is the Idukki Hydroelectric Project. The reservoir combines the courses of the Cheruthoni and Periyar rivers and is spread over an area of 59.8 sq km. The catchment area of the reservoir is 649.3 sq km and is situated at an altitude of 695 m.

The forest area bordering the reservoir, an area estimated to contain 57,312 hectares including the reservoir and the catchment area, was chosen for study. (see Fig. 1). Climatological data for the area are summarized in Fig. 2.

The following types of forests, as classified by Chandrasekharan (1962), Champion and Seth (1962) and Mohanan (1985), are found in the study area: (1) West Coast Tropical Evergreen Forests, (2) West Coast Semi-Evergreen Forests, (3) Southern Moist Mixed Deciduous Forests, and (4) South Indian Subtropical Hill Savanna (grassland with exposed rocks and scattered trees). The understory of the evergreen forests has been cleared and cash crops such as cardamom, coffee, and ginger are now grown.

METHODS AND MATERIALS

Identification of the host plant is an essential step in the identification of these fungi; hence, it was necessary to collect specimens of the host, preferably with reproductive parts, when making collections of the fungi. Each specimen collected was assigned a collection number, and data regarding pathogenicity, nature of the colonies, nearby infected host plants and other relevant information was recorded. Following collection, the leaf material was dried between blotters, changed daily for several days (Jain and Rao, 1977). Host identity was confirmed with the help of experts and through comparison with specimens deposited in the Madras Herbarium, Coimbatore, (M.H.). For rapid temporary mounts, cellophane tape worked well. For permanent mounts, use of clear nail polish, which is both cheap and readily available, was preferred. With this method, a drop of the nail polish was applied to the fungal colonies, spread carefully with the tip of a fine brush so as not to disturb the colonies, and allowed to dry in a dust free chamber for about half an hour. A "flip" was formed, with the fungal colonies firmly embedded in it. This was easily eased off the leaf with the help of a razor blade or scalpel. A drop of mounting medium, such as Canada balsam or D.P.X. was spread on a clean slide, and the flip carefully placed upon it so as to avoid air bubbles. Two more drops of the mounting medium were placed over the flip, and a clean cover glass gently applied. The slides were allowed to dry for 2 to 3 days in a dust free chamber, after which the excess mounting medium was removed.

In some fungi, septa may not be visible because of the heavy pigmentation. In such cases, fungal material was scraped from the leaf and mounted in 10% KOH solution. After 30 minutes, the KOH was removed and replaced with clear lacto-phenol (prepared according to Rangaswamy,

1975). Both KOH and lacto-phenol are good clearing agents, making the septa visible for study. Camera lucida drawings were made of all specimens.

TAXONOMIC REVIEW

Meliola and its associated genera were formerly considered under the tribe Meliolineae (Stevens, 1927, 1928; Hansford, 1961). Martin (1941) proposed the family Meliolaceae in the Order Meliolales; the description of the family was emended and validated by Hansford (1946). Alexopoulos and Mims (1979) followed this arrangement. Yarwood (1973), however, considered all of the genera of the family Meliolaceae under the Perisporaceae of the Order Erysiphales. Müller and von Arx (1973) considered the Meliolaceae under the unitunicatae of the Order Meliolales. Hawksworth et al. (1983) and Eriksson (1982) placed the Meliolaceae under a broadly conceived Order Dothideales. Hawksworth and Eriksson, in Eriksson and Hawksworth (1986), emended the description of the Order Meliolales proposed by Gaumann (1964), treating it under the bitunicatae, and compared the group with the Microthyriales. Luttrell (1989) concluded that the Meliolales belong in the Pyrenomycetes (in the narrow sense) and should not be placed with the Loculoascomycetes, thus essentially agreeing with Müller and von Arx (1973).

For many years, Meliola amphitricha Fries was considered to be the type species of the genus. Arnaud (1918) was the first to question its validity, and subsequent study has shown it to be a nomen confusum. The situation was reviewed by Toro (1952) who resolved the problem by selecting M. trichostroma (Fr.) Toro as the lectotype species. Hansford (1961) accepted Toro's views and segregated from the "catch-all" species, M. amphitricha Fr., more than 100 species, relegating the name M. amphitricha to his list of Species Excludendae, with the comment: "that ephithet is discarded".

The number of genera assigned to the family Meliolaceae varies from 5 to 50, depending on the limits established for the family. Stevens (1927, 1928) considered seven genera; Hansford (1961) gave an account of five genera; Ainsworth (1971) recognized about fifty genera; Müller and von Arx (1973) included seven genera, while Eriksson and Hawksworth (1986) included twenty-two genera, three of which were doubtful. To keep the group homogeneous, we are following Müller and von Arx (1973) in treating six genera, namely: Amazonia, Appendiculella, Armatella, Asteridiella, Irenopsis and Meliola. The saprobic, rhizophyllous, monotypic genus Diporotheca Gordon and Shaw is excluded.

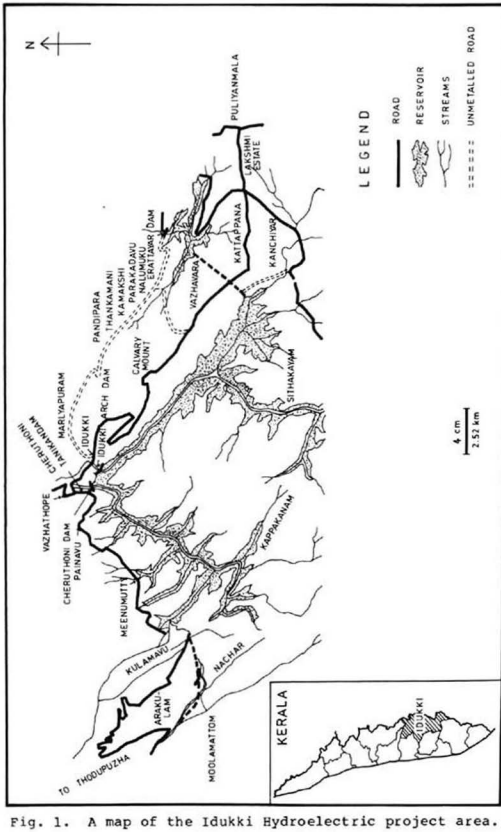


Fig. 1. A map of the Idukki Hydroelectric project area.

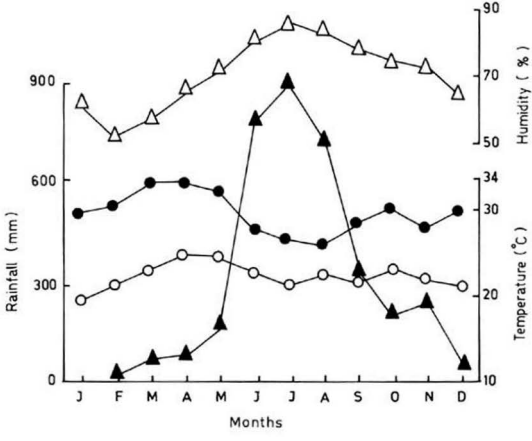


Fig. 2. Climatological data for the Idukki Project Area, based on the average of five years (1978-1982).

● Maximum temperature

○ Minimum temperature

▲ Rainfall

△ Humidity

We are following Hansford (1961) and Luttrell (1989) in using the term mucronate hyphopodia for the phialide-like branches found on the mycelium of many members of the Meliolaceae. Hughes (1981), following the examination of several species, concluded that these structures do indeed function as phialides, but Luttrell (1989) did not find this to be the case in Meliola floridensis Hansf. Until further evidence is brought forth, it seems advisable to continue use of the established terminology.

ORDER MELIOLALES: with a single family.
FAMILY MELIOLACEAE Martin ex Hansford. CMI Paper 15:23. 1946.

Poliicolous ectoparasites; mycelium superficial, brown, septate, branched, hyphopodiate; thin, penetration hyphae arising from the apical (head) cells of the capitate hyphopodia penetrating the underlying host epidermis and forming haustoria within the epidermal cells; mucronate hyphopodia often present, mycelial setae present or absent; ascomata superficial, globose, dark, with parenchymatous wall of one or more layers, usually without ostioles, setae and appendages often present on ascomatal wall; asci borne in hymenium, 2 to 8 spored, evanescent; ascospores 1, 3, or, 4 septate, brown at maturity.

KEY TO THE GENERA OF THE MELIOLACEAE: (Sensu Müller & von Arx, 1973)

1. Ascospores 0-1 septate . . . . . Armatella
1. Ascospores 3-4 septate 2
2. Mycelial setae present . . . .Meliola
2. Mycelial setae absent 3
3. Setae present on ascomata . . . .Irenopsis
3. Setae not present on ascomata 4
4. Appendages present on ascomata . . . . . Appendiculella
4. Appendages not found on ascomata . . 5
5. Ascomata below a shield of radiating mycelium . . . . . Amazonia
5. Ascomata lacking shield . . .Asteridiella

Description of the genera

1. Amazonia Theissen, Ann. Mycol. 11:499. 1913.
= Actinodothis Sydow & Sydow, Philipp. J. Sci. 9:174. 1914.
= Meliolaster Doidge, Trans. Roy. Soc. South Africa 8:123. 1920. (non Meliolaster Hoehnel, 1918)

Mycelium superficial, brown, septate, branched, hyphopodiate. Ascomata globose, perithecioid, in a shield of radiating mycelium. Asci 2-4 spored, evanescent. Ascospores 3 or 4 septate, brown.

Type species: A. psychotriae (P. Henn.) Theissen, based on Meliola asterinoides Winter var. psychotriae P. Henn.

2. Appendiculella Hoehnel, Sitzb. K. Akad. Wiss. Wien, Math. - naturw. Kl. 128:556. 1919.
= Irene Theiss. & Sydow sensu Stevens, Ann. Mycol. 25:420. 1927. (non Irene Theissen & Sydow, 1917).
Mycelium superficial, brown, septate, branched, hyphopodiate, without setae. Ascomata superficial, globose, perithecioid, bearing larviform appendages, setae lacking. Asci 2 - 4 spored, evanescent. Ascospores 3-4 septate, brown.
Type species: A. calostroma (Desm.) Hoehnel, based on Sphaeria calostroma Desm.

3. Armatella Theissen & Sydow, Ann. Mycol. 13:235. 1915.
= Artallendea Bat. & Maia, Atas Inst. Micol., Univ. Recife 1:222. 1960.
Mycelium superficial, brown, septate, branched, hyphopodiate, lacking setae. Ascomata superficial, globose, lacking appendages and/or setae. Asci 4-8 spored, evanescent. Ascospores initially non-septate and hyaline, becoming brown and 1 septate at maturity. On germination, the upper cell enlarges to from a capitate hyphopodium; the other empties and collapses.
Type species: A. litseae (P. Henn.) Theiss. & Sydow.

4. Asteridiella McAlpine, Proc. Linn. Soc. New South Wales, 1897, p. 38.
= Irene Theiss. & Sydow, Ann. Mycol. 15:194. 1917.
=Irenina Stevens, Ann. Mycol. 25:411. 1927.
Mycelium superficial, brown, septate, branched, hyphopodiate, lacking setae. Ascomata superficial, globose, lacking appendages and/or setae, cells protruding. Asci 2-4 spored, evanescent. Ascospores 3-4 septate, brown.
Type species: A. solani McAlpine.

5. Irenopsis Stevens, Ann. Mycol. 25:411. 1927.
Mycelium superficial, brown, septate, branched, hyphopodiate, setose. Ascomata superficial, globose, perithecioid. Asci 2-4 spored, evanescent. Ascospores 3-4 septate, brown.
Type species: I. tortuosa (Winter) Stevens, based on Meliola tortuosa Winter.

6. Meliola Fries emended Bornet, Ann. Sci. Nat. III, 16:267. 1851.
=Meliola Fries, Syst. Orb. Veg. 1825, p. 111.
=Amphitrichum Nees ex Spreng, Pl. Crypt. Trop. 1820. p. 46, pro parte

=Sphaeria Fries, Syst. Myc. 2:513. 1823. pro parte.
=Myxothecium Kunze in Fries, Syst. Mycol. 3:232. 1829.
=Couturea Cast. in Fries, Summa Veg. Sand. 1846, p. 407.
=Asteridium Sacc., Syll. Fung. 1:49. 1882.

Mycelium superficial, brown, septate, branched, hyphopodiate, setose. Ascomata superficial, globose, perithecioid, lacking setae and/or appendages. Asci 2-4 spored, evanescent. Ascospores 3-4 septate, brown.

Lectotype species: M. trichostroma (Kunze) Toro, based on Sphaeria? trichostroma Kunze.

RESULTS

Meliolaceous fungi collected and identified in this survey include 103 taxa, distributed among five genera, as follows: Amazonia (4), Armatella (2), Asteridiella (9), Irenopsis (4), and Meliola (84). Of these taxa, 32 are new species, 13 are new varieties, and 27 taxa are reported from India for the first time (Hosagoudar, 1987). Formal descriptions of new taxa will be presented in subsequent papers. The highest incidence of meliolaceous fungi occurred at the end of the rainy season.

Twenty-four of the host plants are endemic to the Western Ghats (Ahmedullah & Nayar, 1987). Of these, Apodytes benthamiana (Icacinaceae), Atylosia lineata (Papilionaceae), Cinnamomum malabatrum (Lauraceae), Ixora elongata (Rubiaceae), Litsea coriacea, L. stocksii var. glabrescens (Lauraceae), Mucuna hirsuta (Papilionaceae), Nilgirianthus heyneanus (Acanthaceae), Otonephelium stipulaceum (Sapindaceae), Premna glaberrima (Verbenaceae), Wendlandia notoniana (Rubiaceae) are the hosts of undescribed taxa. The remainder of the endemic species are hosts of meliolaceous fungi previously unrecorded from India, but known from other tropical countries.

The large number of taxa of meliolaceous fungi found in the small area included in this study illustrates their abundance in the tropics. About one-third of the total taxa encountered have proven to be new. Is this due to a general lack of exploration in the tropics for the meliolaceous fungi, or is it due to the exploration of a area that has previously been totally unstudied? The answer can only be determined by further collecting in unexplored areas.

Results of the present study reveal the affinity of the meliolaceous fungi of India with those of North and South America, tropical Africa, China, and the islands of

Santa Domingo, Puerto Rico, Trinidad, Sri Lanka, Sumatra, Java, The Philippines, New Guinea, New Caledonia and Taiwan. In a paper on an Indian species of Meliolina, Hughes and Pirozynski (1985) stated: "To students of Indian fungi . . . puzzled by consistent similarities of the mycota of India, intertropical Africa and Australo-Papua/New Zealand, we offer a reminder: the mycological road from Ootacamund winds its way to Mysore through Gudalur, Brisbane and Entebbe."

The Genera Amazonia, Armatella, Asteridiella and Irenopsis

1. **Amazonia acronychiae** Hosagoudar, sp. nov. Fig. 3

Plagulae amphigenae, plerumque epiphyllae, subdensae, ad 3 mm diam., confluentes. Hyphae brunneae, subrectae, opposite lateque ramosae, dense reticulatae, cellulis 22-30 x 8-10 µm. Hyphopodia capitata alternata, antrorsa, recta vel curvula, 24-44 µm longa; cellula basali cuneata, 10-22 µm longa; cellula apicali ovata, clavata, angulosa vel irregulariter sublobata, 18-22 x 14-18 um. Mucronate hyphopodia numerosa, illis capitatis commixta, alternata vel opposita, conoidea vel ampullacea, 22-30 x 8-10 µm. Perithecia dispersa, applanate-globosa, ad 110 µm; sporae obovoidae, 4-septatae, constrictae, 42-46 x 20-22 µm.

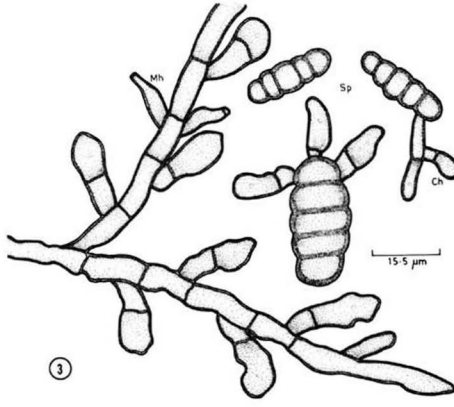
Colonies amphigenous, mostly epiphyllous, subdense, up to 3 mm in diameter, confluent. Hyphae substraight, branching opposite at wide angles, closely reticulate, cells 22-30 x 8-10 µm. Capitate hyphopodia alternate, closely antrorse, straight to curved, 24-44 µm long; stalk cells cuneate, 10-22 µm long; head cells ovate, clavate, angular to irregularly sublobate, 18-22 x 14-18 µm. Mucronate hyphopodia numerous, mixed with capitate hyphopodia, conoid to ampulliform, 22-30 x 8-10 µm. Perithecia scattered, flattened globose, up to 110 µm; spores obovoidal, 4-septate, constricted, 42-46 x 20-22 µm.

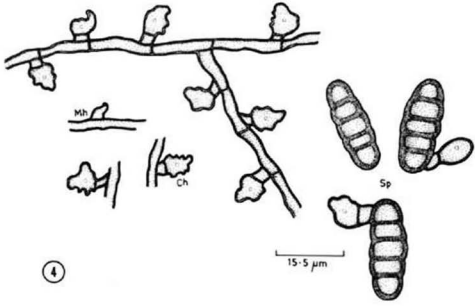
Holotype: On leaves of Acronychia pedunculata (L.) Miq. (Rutaceae), Lakshmi Estate, June 12, 1983, V.B. Hosagoudar HCIO 40463.

There is no record of the genus Amazonia on the members of the family Rutaceae (Hansford, 1961).

2. **Amazonia actinodaphnis** Hosagoudar, sp. nov. Fig. 4

Plagulae epiphyllae, densae, ad 5 mm diam., confluentes. Hyphae subrectae vel leniter undulatae, alternatim acuteque vel lateque ramosae, laxe reticulatae, cellulis 26-36 x 3-5 µm. Hyphopodia capitata alternata,





Figs. 3-11. New taxa of the Meliolaceae. Key to abbreviations: Ch = capitate hyphopodia. Mh = mucronate hyphopodia. Sp = ascospore. Pc = perithecial cell. Ps = perithecial setae.

Fig. 3 Amazonia acronychiae Hosagoudar. Fig. 4 Amazonia actinodaphnis Hosagoudar.

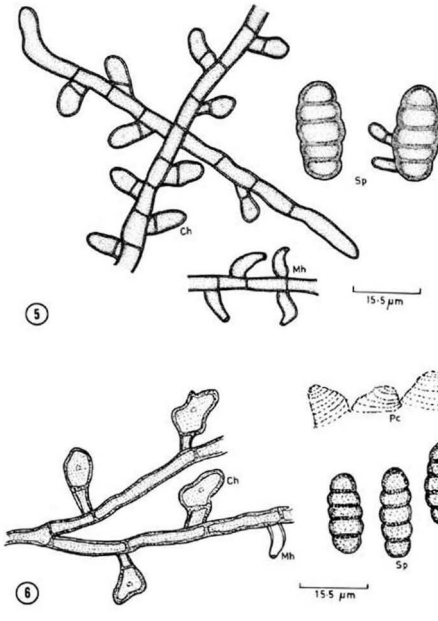


Fig. 5. Amazonia syzygii Hosagoudar. Fig. 6. Asteridiella clerodendricola Hosagoudar.

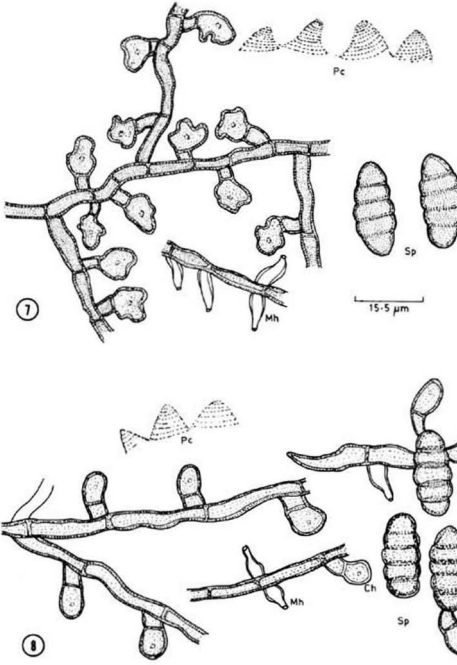


Fig. 7. Asteridiella crotonis Hosagoudar. Fig. 8. Asteridiella macarangicola Hosagoudar (drawn to the same scale as Fig. 7).

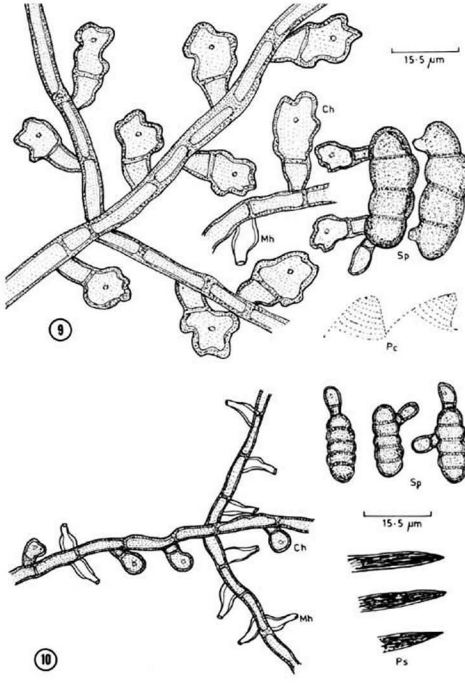


Fig. 9. Asteridiella turpiniicola Hosagoudar. Fig. 10. Irenopsis eriolaenae Hosagoudar.

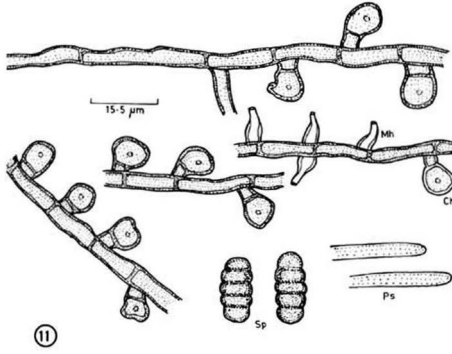


Fig. 11. Irenopsis leeae Hansford var. indica Hosagoudar.

dispersa, antrorsa, patentia, recta vel curvula, 16.5-20 um longa; cellula basali cylindracea vel cuneata, 3-8 um longa; cellula apicali ovata, globosa, piriformia, stellate lobata, apice rotundata, 10-15 x 10-16.5 um. Mucronate hyphopodia pauca, illis capitatis commixta, alternata, ampullacea, 13-26.5 x 6-10 um. Perithecia acuteque dispersa, applanate-globosa, verrucosa, ad 165 um; sporae cylindraceae, 4-septatae, constrictae, 43-46 x 15-16.5 um.

Colonies epiphyllous, dense, up to 5 mm in diameter, confluent. Hyphae straight to slightly undulating, branching alternate at acute to wide angles, loosely reticulate, cells 26-36 x 3-5 um. Capitate hyphopodia alternate, scattered, antrorse, spreading, straight to curved, 16.5-20 um long. Stalk cells cylindrical to cuneate, 3-8 um long; head cells ovate, globose, pyriform, stellately lobate, rounded at the apex, 10-15 x 10-16.5 um. Mucronate hyphopodia few, mixed with capitate hyphopodia, alternate, ampulliform, 13-26.5 x 6-10 um. Perithecia closely scattered, flattened-globose, up to 165 um; spores cylindrical, 4-septate, constricted, 43-46 x 15-16.5 um.

Holotype: On leaves of Actinodaphne hookeri Meissn. (Lauraceae), Idukki, Oct. 11, 1982, V.B. Hosagoudar HCIO 40465; isotype: HCIO 40466.

A single species of Amazonia, viz. A. philippinensis Theiss. has been recorded on Ullolitsea villosa from the Philippines (Hansford, 1961). The present species differs from it in having substraight to undulating mycelia, stellately lobed head cells of the capitate hyphopodia, smaller perithecia and ascospores. Further, there is no record of the genus Amazonia on Actinodaphne hookeri.

3. **Amazonia peregrina** Sydow & Sydow, Ann. Mycol. 15:414, 1927; Hansford, Sydowia Beih. 2:507, 1961.

On leaves of Maesa indica (Roxb.) DC. (Myrsinaceae), Idukki, Jan. 10, 1982, V.B. Hosagoudar MH 72647; HCIO 40467.

This species occurs mostly on the leaves also infected with Meliola groteana Syd. but A. peregrina can be easily distinguished by its crustose colonies.

4. **Amazonia syzygii** Hosagoudar, sp. nov. Fig. 5

Plagulae amphigenae, subdense, crustosae vel leniter velutinae, ad 2 mm diam., raro confluentes. Hyphae subrectae vel leniter undulatae, plerumque opposite lateque ramosae, densae reticulatae, cellulis 16-20 x 6-8 um. Hyphopodia capitata alternata, recta, antrorsa vel patentia, 18-20 um longa, cellula basali cylindracea vel

cuneata, 4-8 µm longa; cellula apicali ovata vel subglobosa, integra, 10-14 x 8-10 µm. Mucronate hyphopodia illis capitatis commixta, opposita vel alternata, conoidea vel ampullacea, 20-24 x 8-10 µm. Perithecia applanate-globosa, dispersa vel aggregata, ad 180 µm; sporae obovatae, 4-septatae, leniter constrictae, 44-48 x 16-20 µm.

Colonies amphigenous, subdense, crustose to slightly velvety, up to 2 mm in diameter, rarely confluent. Hyphae substraight to slightly undulating, branching mostly opposite at wide angles, closely reticulate, cells 16-20 x 6-8 µm. Capitate hyphopodia alternate, straight, antrorse to spreading, 18-20 µm long; stalk cells cylindrical to cuneate, 4-8 µm long; head cells ovate to subglobose, entire, 10-14 x 8-10 µm. Mucronate hyphopodia mixed with capitate hyphopodia, opposite to alternate, conoid to ampulliform, 20-24 x 8-10 µm. Perithecia flattened-globose, scattered to grouped, up to 180 µm; spores obovate, 4-septate, slightly constricted, 44-48 x 16-20 µm.

Holotype: On leaves of Syzygium cumini (L.) Skeels (Myrtaceae), Idukki, Dec. 13, 1982, V.B. Hosagoudar HCIO 40469. Isotype: MH 75742.

So far there is no record of the genus Amazonia on members of the family Myrtaceae (Hansf., 1961).

5. **Armatella cinnamomicola** Hansf., Reinwardtia 3:87, 1954.
On leaves of Cinnamomum malabatrum (Burm.f.) Blume (Lauraceae), Idukki, April 18, 1982, V.B. Hosagoudar MH 72696.

Hansford (1954) described this species from Indonesia. The present collection shows variations in having smaller capitate hyphopodia, larger perithecia and smaller ascospores.

6. **Armatella litseae** (P. Henn.) Theiss., Sydow & Sydow, Ann. Mycol. 13:235, 1915.

On leaves of Neolitsea zevlanica Merr. (Lauraceae), Lakshmi Estate, Dec. 6, 1983, V.B. Hosagoudar MH 78177, 78190; HCIO 40474.

7. **Asteridiella clerodendricola** Hosagoudar, sp. nov. Fig. 6

Plagulae amphigenae, plerumque epiphyllae, densae, ad 10 mm diam., raro confluentes, maculae halonate, folia infecta corrugata. Hyphae mycelii tortuosae, alternatae vel oppositae lateque ramosae, densae reticulatae, cellulis 18-38 x 6-8 µm. Hyphopodia capitata alternata vel

unilateralia, recta vel curvula, patentia vel antrorsa, 22-30 µm longa; cellula basali cylindracea vel cuneata, 8-16 µm longa; cellula apicali globosa, angulosa, integra vel sublobata, 14-18 x 12-16 µm. Mucronate hyphopodia pauca, illis capitatis commixta, opposita vel alternata, ampullacea, 20-22 x 8-10 µm. Perithecia plerumque aggregata, ad 245 µm; cellulis parietis irregulariter protrudo, 30-36 µm longis; sporae ellipsoidae, 4-septatae, recta vel leniter curvulae, 36-42 x 14-18 µm.

Colonies amphigenous, mostly epiphyllous, dense, scattered, up to 10 mm diameter, rarely confluent, causing stretching of the surrounding leaf surface with a yellow halo surrounding the spots. Hyphae strongly adpressed to the leaf surface, not easily separable, tortuous, branching alternate to opposite at wide angles, strongly reticulate, cells 18-38 x 6-8 µm. Capitate hyphopodia alternate to unilateral, straight to curved, antrorse to spreading, 22-30 µm long; stalk cells cylindrical to cuneate, 8-16 µm long; head cells globose, angulose, entire to sublobate, 14-18 x 12-16 µm. Mucronate hyphopodia few, mixed with capitate hyphopodia, opposite to alternate, ampulliform, 20-22 x 8-10 µm. Perithecia mostly aggregated, up to 245 µm; perithecial surface cells irregularly protruded, 30-36 µm long; spores ellipsoidal, 4-septate, straight to slightly curved, 36-42 x 14-18 µm.

Holotype: On leaves of Clerodendrum viscosum Vent. (Verbenaceae), Idukki, Dec. 22, 1983, V.B. Hosagoudar HCIO 40475. Isotype: MH 78998. Paratypes: Lakshmi Estate, Dec. 25, 1983, V.B. Hosagoudar MH 78998.

The infection was restricted to the young growing leaves. Two to many such infected spots on the leaves resulted in hypertrophy of the leaf, giving a peculiar appearance to the growing plant parts.

Twelve species of the genus Asteridiella have been recorded on various members of the family Verbenaceae. The present species differs in producing a pathogenic effect on the host plant.

8. **Asteridiella combreti** (Stev.) Hansf. var. **leonensis** Hansf., Sydowia Beih. 20:160, 1961.

On leaves of Terminalia paniculata Roth (Combretaceae), in the savanna of Idukki, Dec. 13, 1982, V.B. Hosagoudar HCIO 40476; V.B. Hosagoudar MH 75727, Jan. 24, 1983; V.B. Hosagoudar MH 75824; Dec. 27, 1983, V.B. Hosagoudar MH 78990; Oct. 4, 1983, V.B. Hosagoudar MH 78142.

9. **Asteridiella confragosa** (Sydow & Sydow) Hansf., Sydowia 10:47, 1957.

On leaves of Trichosanthes palmata Roxb. (Cucurbitaceae), Idukki, Oct. 8, 1983, V.B. Hosagoudar HCIO 40477; MH 78904; Dec. 12, 1983, V.B. Hosagoudar MH 79042.

10. **Asteridiella crotonis** Hosagoudar, sp. nov. Fig. 7

Plagulae hypophyllae, densae, ad 5 mm diam. Hyphae subrectae vel undulatae, opposite laxe ramosae, laxe vel densae reticulatae et solidae, cellulis 18-24 x 6-8 µm. Hyphopodia capitata alternata vel unilateralia, patentia, antrorsa vel reflexa, 22-26 µm longa; cellula basali cylindracea vel cuneata, 6-8 µm longa; cellula apicali ovata, integra vel sublobata, 16-20 x 12-18 µm. Mucronate hyphopodia pauca, illis capitatis commixta, opposita vel alternata, ampullacea, 16-18 x 6-8 µm. Perithecia dispersa, ad 196 µm; cellulae parietis conoidae, 20-26 µm longae; sporae ellipsoideae, 4-septatae, constrictae, rectae vel curvulae, 44-48 x 16-20 µm.

Colonies hypophyllous, dense, up to 5 mm in diameter. Hyphae substraight to undulating, branching opposite at wide angles, loosely to closely reticulate and forming a solid mass of mycelia, cells 18-24 x 6-8 µm. Capitata hyphopodia alternate and unilateral, spreading, antrorse to reflexed, 22-26 µm long; stalk cells cylindrical to cuneate, 6-8 µm long; head cells ovate, entire to imperfectly lobate, 16-20 x 12-18 µm. Mucronate hyphopodia few, mixed with capitate hyphopodia, opposite to alternate, ampulliform, 16-18 x 6-8 µm. Perithecia scattered, up to 196 µm; perithecial cells conoid, 20-26 µm long; spores ellipsoidal, 4-septate, constricted, straight to slightly curved, 44-48 x 16-20 µm.

Holotype: On leaves of Croton reticulatus Heyne (Euphorbiaceae), Pamba, Oct. 10, 1983, V.B. Hosagoudar HCIO 40478.

Note: Six species of Asteridiella have been reported on members of the family Euphorbiaceae, having the Beeli formula 3101.4220 (Hansford, 1961). Of these, A. antidesmatis Hansf. and A. drypeticola Hansf. are closest to the present species. However, A. crotonis differs from A. antidesmatis in having dense hypophyllous colonies and larger capitate hyphopodia. It differs from A. drypeticola in the morphology and arrangement of the capitate hyphopodia, and in having larger ascospores. Further, there is no record of the genus Asteridiella on this host genus.

11. **Asteridiella cyclopoda** (Stev.) Hansf., Sydowia 10:47, 1957 and Sydowia Beih. 2:619, 1961.

On leaves of Vernonia monosis Clarke (Asteraceae), Idukki, Oct. 6, 1983, V.B. Hosagoudar HCIO 40479; MH 78174.

The present collection varies slightly from the species description (Hansford, 1961) in forming hypophyllous colonies, and in having larger capitate hyphopodia and smaller perithecial cells.

12. **Asteridiella formosensis** (Yamam.) Hansf., Sydowia 10:48, 1957 and Sydowia Beih. 2:686, 1961.

On leaves of Callicarpa tomentosa (L) Murray (Verbenaceae), Pamba, Oct. 10, 1983, V.B. Hosagoudar MH 78929.

13. **Asteridiella macarangicola** Hosagoudar, sp. nov. Fig. 8

Plagulae epiphyllae, tenues, indistinctae, ad 2 mm diam. Hyphae tortuosae, opposite vel alternate ramosae, laxe reticulatae, cellulis 38-44 x 6-8 µm. Hyphopodia capitata alternata, recta vel curvula, patentia, plerumque antrorsa, 20-28 µm longa; cellula basali cylindracea vel cuneata, 8-12 µm longa; cellula apicali globosa, ovata, integra, raro leniter angulosa, 12-16 x 6-10 µm. Perithecia dispersa, ad 180 µm; cellulis parietis conoideis, usque ad 14 µm longis; sporae ellipsoideae, 4-septatae, constrictae, 38-40 x 16-18 µm.

Colonies epiphyllous, thin, indistinct, up to 2 mm diameter. Hyphae tortuous, branching opposite to alternate, loosely reticulate, cells 38-44 x 6-8 µm. Capitate hyphopodia alternate, straight to curved, spreading, mostly antrorse, 20-28 µm long; stalk cells cylindrical to cuneate, 8-12 µm long; head cells globose, ovate, entire, rarely slightly angulose, 12-16 x 6-10 µm. Perithecia scattered, up to 180 um; perithecial cells conoid, up to 14 µm long; ascospores ellipsoidal, 4-septate, constricted, 38-40 x 16-18 µm.

Holotype: On leaves of Macaranga peltata Muell.-Arg. (Euphorbiaceae), Calvary Mount, Dec. 24, 1983, V.B. Hosagoudar HCIO 40481. Isotype: MH 75050. Paratype: Lakshmi Estate, Dec. 25, 1983, V.B. Hosagoudar MH 79054.

The present species is similar to A. erythrococcae Hansf. and A. hansfordii (Stev.) Hansf. but differs from both in forming inconspicuous colonies, tortuous mycelia, larger capitate hyphopodia, and in having entire head cells of the capitate hyphopodia and distinctly broader ascospores. Further, there is no record of the genus Asteridiella on this host genus.

14. **Asteridiella malloti** (Hansf. & Thirum.) Hansf., Sydowia 10:49, 1957.

On leaves of Mallotus philippinensis (Lam.) Muell. (Euphorbiaceae), Lakshmi Estate, Dec. 25, 1983, V.B. Hosagoudar HCIO 40482; MH 79098. On leaves of M. tetracoccus (Roxb.) Kurz, Pamba, Oct. 12, 1983, V.B. Hosagoudar MH 78950.

15. **Asteridiella turpiniicola** Hosagoudar, sp. nov. Fig. 9

Plagulae amphigenae, plerumque hypophyllae, densae, ad 3 mm diam. Hyphae rectae vel subrectae, alternate vel opposite lateque ramosae, laxe vel densae reticulatae vel reticulato-intertextae, cellulis 16-32 x 8-12 µm. Hyphopodia capitata alternata, patentia, antrorsa, 26-30 µm longa; cellula basali cylindracea vel cuneata, 6-10 µm longa; cellula apicali globosa, stellate sublobata, 18-20 x 16-24 µm. Mucronate hyphopodia pauca, illis capitatis commixta, opposita vel alternata, 20-24 x 8-10 µm. Perithecia aggregata vel dispersa, ad 360 µm., appendiculae peritheciales larviformae, flexuosae, fulvus, simplices, patentiae, ad 196 µm longae et 7-8 um crassae, rotundae ad apicem, rectae vel tortuosae ad apicem; sporae fusiformae, plerumque curvulae, 3-septatae, constrictae, 44-56 x 16-20 µm.

Colonies amphigenous, mostly hypophyllous, dense, up to 3 mm in diameter. Hyphae straight to substraight, branching alternate to opposite at wide angles, loosely to closely reticulate and forming an almost solid mass of mycelia; cells 16-32 x 8-12 µm. Capitate hyphopodia alternate, spreading, antrorse, 26-30 µm long; stalk cells cylindrical to cuneate, 6-10 µm long; head cells globose, stellately sublobate, 18-20 x 16-24 µm. Macronate hyphopodia few, mixed with capitate hyphopodia, alternate to opposite, ampulliform, 20-24 x 8-10 µm. Perithecia aggregated to scattered, up to 360 µm; perithecial appendages larviform, wavy, golden-brown, simple, spreading, up to 196 µm long and 7-8 µm broad, tip obtuse, straight to twisted; spores fusiform, predominently curved, 3-septate, constricted, 46-56 x 16-20 µm.

Holotype: On leaves of Turpinia malabarica Gamble (Staphyleaceae), Idukki, April 4, 1982, V.B. Hosagoudar HCIO 40483. Isotype: MH 73701. Paratypes: Oct. 11, 1982, V.B. Hosagoudar MH 73623, 73630; Pamba, Oct. 10, 1983, V.B. Hosagoudar MH 78928; Idukki, Dec. 21, 1983, V.B. Hosagoudar MH 78961.

Note: Appendiculella turpiniae (Yamam.) Hansf. has been recorded on Turpinia formosana Nakai and T. pomifera DC. from Formosa and the Philippines (Hansford, 1961),

having repent, abnormal mycelial setae and the perithecial appendages. The present collection differs from it in lacking the perithecial appendages, and in having smaller capitate hyphopodia, perithecia and ascospores.

Yamamoto (1941) described Irenina turpiniae. Later, Hansford (1961) transferred this species to Appendiculella as A. turpiniae (Yaman.) Hansford, noting two characteristics:

1. No species of Meliola is hitherto known with perithecial appendages.

2. The "mycelial setae" are quite different from those commonly found in the species of Meliola, where they are erect, but are closer to those found on the perithecia of species of Irenopsis. They differ in being produced around the base of the perithecium, on mycelial hyphae, and in being repent, as well as being longer than in the so far known species.

In the present material, also, the repent appendages (termed here as perithecial) arise from the subicle just below the perithecia. Such appendages have also been seen on the mycelia where there were no perithecia. These repent setae/appendages are neither perithecial nor mycelial setae. Hence the present material has been placed under the genus Asteridiella.

16. Irenopsis benquetensis Stev. & Rold. ex Hansf., Sydowia 26:311, 1963.

On leaves of Ficus asperrima Roxb.(Moraceae), Calvary Mount, Oct. 12, 1982, V.B. Hosagoudar MH 73639; Oct. 4, 1983, V.B. Hosagoudar MH 78155. On leaves of Ficus gibbosa Bl., Calvary Mount, Feb. 21, 1983, V.B. Hosagoudar MH 75890; Lakshmi Estate, Dec. 12, 1982, V.B. Hosagoudar MH 79073, 79078; Oct. 12, 1982, V.B. Hosagoudar MH 73639; Oct. 4, 1983, V.B. Hosagoudar MH 78155. On leaves of Ficus hispida L.f., Idukki, Oct. 11, 1983, V.B. Hosagoudar MH 78943.

17. Irenopsis eriolaenae Hosagoudar, sp. nov.
Fig. 10

Plagulae epiphyllae, tenues, dispersae, ad 3 mm diam., confluentes. Hyphae subrectae vel undulatae, alternatae vel oppositae lateque ramosae, laxe reticulatae, cellulis 30-34 x 6-8 µm. Hyphopodia capitata alternata vel unilateralia, recta, antrorsa vel patentia, 14-16 µm longa; cellula basali cylindracea vel cuneata, 4-5 µm longa; cellula apicali ovata, clavata, integra vel leniter angulosa, 10-12 x 8-10 µm. Mucronate hyphopodia illis capitatis commixta vel in hyphis distinctis evoluta,

alternata vel opposita, ampullacea, 12-20 x 6-8 um. Perithecia dispersa, verrucosa, ad 110 um; setae peritheciales 8-12, rectae, simplices, olivaceo-brunneae, septatae, ad apices acutae vel obtusae, ad 72 um longae et 6-8 um crassae; sporae obovoidae, 4-septatae, constrictae, 32-38 x 10-14 um.

Colonies epiphyllous, thin, scattered, up to 3 mm in diameter, confluent. Hyphae substraight to undulating, branching alternate to opposite at wide angles, loosely reticulate, cells 30-34 x 6-8 um. Capitate hyphopodia alternate to unilateral, straight, antrorse to spreading, 14-16 um long; stalk cells cylindrical to cuneate, 4-5 um long; head cells ovate, clavate, entire to slightly angular, 10-12 x 8-10 um. Mucronate hyphopodia mixed with capitate hyphopodia and borne on a separate mycelial branch, alternate to opposite, ampulliform, 12-20 x 6-8 um. Perithecia scattered, verrucose, up to 110 um; perithecial setae 8-12, straight, simple, septate, olivoceous brown, acute to obtuse at the tip, up to 72 um long and 6-8 um broad; spores obovoidal, 4-septate, constricted, 32-38 x 10-14 um.

Holotype: On leaves of Eriolaena quinquelocularis (Wight & Arn.) Wight (Sterculeaceae), Idukki, Dec. 23, 1983, V.B. Hosagoudar HCIO 40487. Isotype: MH 79027.

The present species is close to I. tiibodense Hansf., recorded on Pterospermum javanicum Fungh., and P. niveus Vidal from Java and the Philippines (Hansford, 1961), but differs in having smaller capitate hyphopodia, perithecia and perithecial setae; absence of radiate exhyphopodiate mycelia below the perithecia, in having mucronate hyphopodia mixed with capitate hyphopodia which are also borne on separate mycelial branches. Further, there is no record of meliolaceous fungi on this host genus.

18. **Irenopsis leeae** Hansf. var. **indica** Hosagoudar, var. nov. Fig. 11

Differt a I. leeae Hansf. var. leea hyphopodiis capitatis alternatis et myceliis subrectis ad undulatis. Differt a I. leeae Hansf. var. javensis Hansf. Plagulis tenuibus, hyphis subrectis ad undulatis lateque ramificantibus.

Colonies epiphyllous, very thin, up to 3 mm in diameter. Hyphae straight to undulating, branching opposite to alternate at wide angles, loosely reticulate, cells 18-28 x 6-8 um. Capitate hyphopodia scattered, alternate to unilateral, closely antrorse, 18-24 um long; stalk cells cuneate, 6-10 um long; head cells ovate, globose, entire to irregularly sublobate, 10-18 x 16-20 um. Mucronate hyphopodia numerous, mixed with capitate

hyphopodia, alternate to opposite, ampulliform, 16-22 x 8-10 µm. Perithecia scattered to grouped, verrucose, up to 150 um; perithecial setae 3-8, straight to flexuous, spreading, dark at the base and paler towards the apex, tip obtuse, 84-150 x 8-10 µm; spores obovoidal, 4-septate, constricted, 30-36 x 12-16 µm.

Holotype: On leaves of Leea indica (Burm. f.) Merr. (Leeaceae), Kanchiar forest, Dec. 17, 1982, V.B. Hosagoudar HCIO 40488. Isotype: MH 75795.

Paratypes: Kanchiar forest, Dec. 17, 1982, V.B. Hosagoudar MH 75795; Dec. 23, 1983, V.B. Hosagoudar MH 79032; Reservoir side of Calvary Mount, Dec. 24, 1983, V.B. Hosagoudar MH 79069; Lakshmi Estate, Dec. 25, 1983, V.B. Hosagoudar MH 79088.

The new variety indica differs from var. leea in having alternate capitate hyphopodia and substraight to undulating mycelia. It also differs from I. leeae Hansf. var. javensis Hansf. in having thin colonies, substraight to undulating mycelia and branching at wide angles.

19. Irenopsis triumfettae (Stev.) Hansf. & Deight., Mycol. pap. 23:14, 1948.

On leaves of Triumfetta pilosa Roth (Tiliaceae), Lakshmi Estate, Dec. 14, 1982, V.B. Hosagoudar, HCIO 40489; MH 75746; Calvary Mount, Dec. 15, 1982, V.B. Hosagoudar MH 75779; Dec. 28, 1983, V.B. Hosagoudar MH 80322; Oct. 6, 1983, V.B. Hosagoudar MH 78175, 78176. On leaves of Triumfetta rhomboides Jacq., Pamba, Oct. 10, 1983, V.B. Hosagoudar MH 78935.

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CULTURAL, ENZYMATIC AND CYTOLOGICAL STUDIES IN THE GENUS PHOLIOTA

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SUMMARY

Mycelial cultures of 14 species of the genus **Pholiota** Kummer **(Basidiomycotina, Agaricales)** together with **Kuehneromyces mutabilis** (Schaeff.: Fr.) Sing. et Smith species have been studied. The morphological, microscopical, cultural and cytological characteristics of the species and their enzymatic activity are presented. Emphasised are the features applicable for the taxonomy of the genus. These results have been used to arrange the dichotomic key for determination af all the species.

INTRODUCTION

Study of **Basidiomycetes** in pure cultures is one of important but still little spread taxonomic methods. Nobles (1965) suggested the use of cultural characters in developing a taxonomy of the **Polyporaceae** that reflects natural relationship and phylogeny. Study of **Basidiomycetes** in pure cultures has mostly been pursued on wood-decaying basidiomycetes. Nobles (1948) provided an 11-character key pattern and description based on cultural information to 126 basidiomycetes that decay wood. Other studies were done, e. g., by Boidin (1958), Siepmann and Zycha (1968), Siepmann (1969), Boidin and Languetin (1963), Job (1968), Adaskaveg and Gilbertson (1989). Some **Agaricales** in pure cultures were studied by Lyman (1907), Kühner (1946, 1947), Semerdžieva (1965), Pantidou et al. (1983), Buchalo (1988).

The genus **Pholiota** that includes mostly wood-decaying species, has been studied relatively often (Sawyer, 1917; Martens and Vandendries, 1933; Smith and Brodie, 1935; Deneyer, 1960; Farr et al., 1977; Hübsch, 1978; Arita, 1979; Arita et al., 1980) but the studies concerned only a few generally occurring species. No comprehensive comparative study of a larger number of species including rare ones has so far been undertaken.

MATERIAL AND METHODS

The culture used were from the collection of macromycetes cultures, Institute for Toxicology, Charles University in Prague (Klán and Štípek, 1987). The species under study are as follows: **Pholiota destruens** (Brond.) Quél. s. l., (year of isolation 1965)

- Sect. **Hemipholiota**; **Ph. squarrosa** (Müll.: Fr.) Kumm., (strain I-1985, II-1986) - Sect. **Pholiota**; **Ph. adiposa** (Batsch: Fr.) Kumm., (1983); **Ph. conifera** (Karst.) Karst. (1984); **Ph. flammans** (Fr.) Kumm. (1982); **Ph. jahnii** Tjall. et Bas (1979); **Ph. lucifera** (Lasch) Quél., (1978); **Ph. squarroso-adiposa** Lange (1986) - Sect. **Adiposae**; **Ph. alnicola** (Fr.) Sing. (1983); **Ph. flavida** (Schaeff.: Fr.) Sing. (I-1980, II-1983) - Sect. **Flammula**; **Ph. gummosa** (Lasch) Sing. (I-1975, II-1982) - Sect. **Subsiccae**; **Ph. carbonaria** (Fr.: Fr.) Sing. (I-1986, II-1986, III-1977); **Ph. lenta** (Pers.: Fr.) Sing. (1983); **Ph. spumosa** (Fr.) Sing. (1983) - - Sect. **Lubricae**; **Kuehneromyces mutabilis** (Schaeff.: Fr.) Sing et Smith (1983).

All isolates were cultivated on malt extract agar - MEA (agar 15 g, malt extract (Difco) 10 g, demineralized water 1000 ml, pH 6 - 6.5) in Petri dish 90 mm in diameter, with 20 ml medium. They were inoculated by means of strike cork borer 8 mm in diameter. Incubation was carried out in the dark in a thermostat at 24° C ± 0,5° C.

Cultural characteristics were examined after 14-21 day cultivation. The following features were examined: colony colour (after Kornerup and Wanscher 1984), the texture of the mycelial mat, margin of the colony, growing zones, colour change in the agar induced by the fungus, odour, guttation. Growth rate was evaluated as well. Cultural characteristics were examined microscopically (oil immersion magnif. 1000 x using Melzer reagent and phloxine). Macro and micro characteristics were completed by changes after one, 3 and 9 months cultivation. Spontaneous fructification is mentioned wherever observed. The HCl--Giemsa method (fixation: ethanol-glacial acetic acid 3:1, hydrolyse 7' at temperature 60° C in 1 M HCl, staining 30' at 30° C) was used for caryological study.

Asexual reproductive structures. As some authors call certain structures of the genus **Pholiota** by different names, we are describing the structures observed as follows: arthrospores (according to other authors "oidia, arthroconidia") - - reproductive structures formed seriately by fragmentation of the hyphae, thin walled, 1-2 (or more) nuclei. Conidia - - reproductive structures formed on conidiophores, often vertically on vegetative hyphae, thin-walled, 1-2 (or more) nuclei. Aleurospores (according to some authors "chlamydospores") - - terminally forming structures, often on conidiophore, thick walled. Allocysts - swollen cells with thin or slightly thick wall. They are not reproductive structures.

Enzymatic activity has been studied. Oxidoreductases: 1. Spot tests (laccase - as substrate used syringaldazine; peroxidase - p-phenylendiamine tartrate and 3 % H_2O_2; catalase - 10 % H_2O_2). 2. Plate diffusion method (tyrosinase - L-tyrosin) all oxidoreductases according to Klán and Baudišová (1989 b). Hydrolases 1. Plate diffusion methods (lipase - Tween 80; lecithinase - soybean lecithin; amylase - starch and Lugol solution; protease - gelatine or dried milk; milk clotting enzymes - - dried milk; urease - urea and phenol red; according to Klán

and Baudišová, 1989 a) and cellulase - 1.5 % agar, 1 % pepton, 1 % microcrystalline cellulose, pH 6.5. After six day incubation, the Petri dishes were overlaid with 1 ml 1 M HCl and 5 ml 0,5 % Iodine solution in 2 % KI. 2. Cytochemical methods (α -glucosidase - 6-brom-2-naphtyl- α -D-glucopyranoside, ß-galactosidase - 6-brom-2-naphtyl-ß-D-galactopyranoside; Klán et al., 1989).

RESULTS

Macroscopic and microscopic descriptions are given of cultures of 14 species of the genus **Pholiota** and the species **Kuehneromyces mutabilis**, including their enzymatic activities (see Tab. 1). A key is suggested for determining mycelial cultures of the species under study according to characteristic features.

Pholiota adiposa (Batsch: Fr.) Kumm.

The culture grows well, the colony attaining a 80 mm diameter in 15 days. The growth does not alter the colour of the nutrient medium. Aerial mycelium is sparsely woolly, with outlined advancing zones and radial filaments. The profile is flat. The colony margin is regular, inconspicuosly demarcated, finely ciliate; marginal hyphae do not grow into the substrate. The colour is whitish: during ageing the culture becomes yellow in the direction from the centre. (Tab. 4, 5A according to the colour scale of Kornerup and Wanscher 1984). The odour is inconspicuous; no guttation has been observed.

Aerial hyphae are thin-walled, 1.2 - 3.3 μm diam. Clamp connections are very frequent, on nearly every septum. Anastomoses are rare, branching very rare. Conidia 5.2 - 10.5 x 2.1 - 3.0 μm, bullet-shaped, elongate or cylindrical, observed already from day 14 of the culture age (Fig. 1). After 28 days conidia very frequent, as a rule separated from conidiophores.

A 9-month old culture observed to contain, in addition to conidia, also aleurospores of a cylindrical (7 x 12 μm) to more or less spherical shape, with slightly thickened wall (Fig. 1).

Hyphae dikaryotic, conidia most often dikaryotic, rarely 1, 3 or 4-nucleate.

Ref.: Arita (1979), Arita et al. (1980), Buchalo (1988), Buchalo et al. (1971, 1985), Hashioka and Arita (1978), Hübsch (1978), Kääřik (1965), Nerud et al. (1982), Nobles (1948), Mišurcová (1987), Rypáček (1957).

Pholiota alnicola (Fr.) Sing.

The culture grows very slowly, the colony attaining a diameter of 62 mm after 44 days. The growth does not alter the

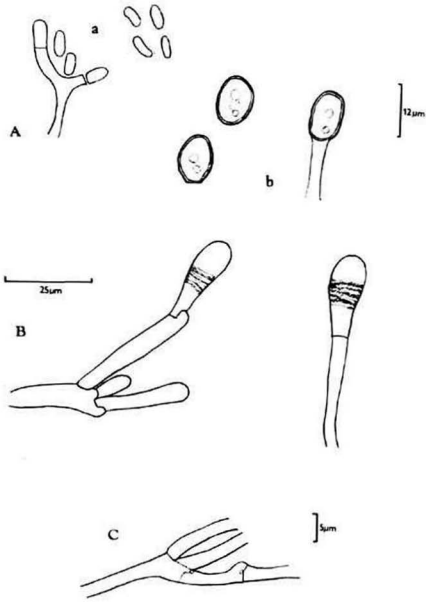

Fig. 1 A- **Pholiota adiposa**, a) conidia on conidiophore b) aleurospores, B- **Ph. jahnii**, club-like terminal cells with incrusted wall, C- **Ph. spumosa**, branching on hyphae

medium hue. Aerial mycelium is very finely woolly, low, with demarcated growth zones. Profile flat, colony margin irregularly shallowly lobate with fine ciliate. Colour pastel yellow (Tab. 3, 4A), most intensive in the inoculum, becoming more intensively yellow on ageing. Odour conspicuous; guttation not observed.

Aerical hyphae are thin-walled, 2.1 - 3.0 µm wide. Clamp connections on each septum. Anastomoses present, branching relatively frequent. Allocysts 10-15 um in diameter branch off the main hypha (usually at an angle of 90°), the branching hypha usually carries a clamp connection (Fig. 2). Intercalary allocysts also observed (a hypha 2.5 µm in diameter extends after the septum with a clamp connection to up to 5-7 µm wide swelling).

9-month old culture without change.
Hyphae and allocysts dikaryotic.

Ref.: Deneyer (1960), Kääarik (1965), Kühner (1947), Nobles (1965).

Pholiota carbonaria (Fr.: Fr.) Sing.

The culture grows very well (colony diameter 80 mm in 7 days). The growth does not alter the colour of the medium. Aerial mycelium finely and sparsely filamentous, no advancing zones observed. Profile flat. Colony rim even, inconspicuously demarcated, finely ciliate. Peripheral hyphae do not grow into the substrate. Colour whitish, no changes observed in the character of the mycelium on ageing. The culture produces readily fruiting bodies under laboratory conditions.

Aerial hyphae thin-walled, 1.2 - 3.1 µm diam. Clamp connections frequent, on nearly every septum. Anastomoses noticed, branching not too frequent, hyphae originate at a 90° angle. Anamorphs not observed.

A 9-month old culture without change.
Hyphae dikaryotic.

Ref.: Hübsch (1978).

Pholiota conifera (Karst.) Karst.

Syn.: **Pholiota aurivella** (Batsch: Fr.) Kumm.

The culture grows well (colony diameter 80 mm in 18 days). The growth does not alter the colour of the medium. Aerial mycelium finely woolly with perceptible radial filaments, growth zones not observed. Profile disc-shaped. Colony margin finely ciliate. Colour white, turning yellow from the inoculum with ageing (Tab. 4, 6A). Odour musty, guttation not observed. Prolonged culturing in a refrigerator on malt agar produces primordia with partial differentiation.

Aerial hyphae thin-walled, 1.2 - 3 µm diam. Clamps frequent. Anastomoses present, lateral branches originate at the site of the septum as well as outside it, at an angle of 60 - 90°. Be-

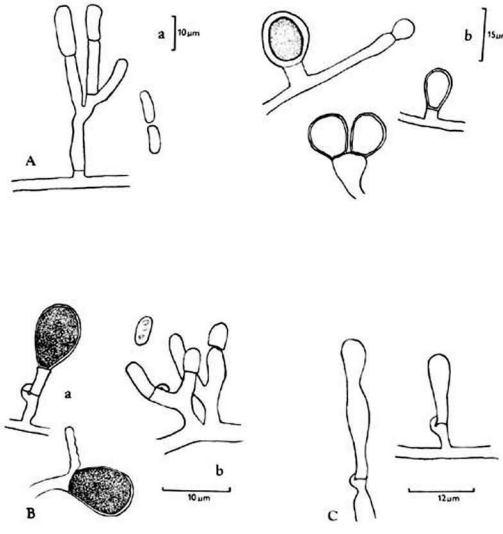

Fig. 2 A- **Pholiota conifera**, a) conidia on conidiophore b) aleurospores, B- **Ph. gummosa** a) aleurospores, b) conidia on conidiophore, C- **Ph. alnicola**, allocysts

ginning on day 10 cylindrical conidia 5-12x2.1-7 µm observed (Fig. 2). Conidiophores 35-40 µm long, multiple branching.

A 9-months old culture contains cylindrical (5x15 µm) to spherical aleurospores with thickened walls (Fig. 2).

Hyphae dikaryotic, conidia most often dikaryotic, rarely 1, 3, 4 or 5-nuclear.

Ref.: Buchalo (1988), Buchalo et al. (1971, 1985), Hashioka and Arita (1978), Hübsch (1978), Käärik (1965), Nerud et al. (1982); Nobles (1965), Pantidou et al. (1983), Rypáček (1957), Watling (1983).

Pholiota destruens (Brond.) Quél. s.l.

The culture grows very slowly (colony diameter 80 mm after 52 days). The growth does not alter the colour of the medium. Aerial mycelium finely powdery with coarser sprinkling, very low, growing zones not observed. Profile flat. Colony margin sharply demarcated, very finely ciliate. Colour yellowish white (Tab. 3, 2A). Odour inconspicuous, guttation yellowish.

Microscopic description available only for submerged mycelium. Hyphae thin-walled, 1.8 - 2.2 µm diam. Clamp connections frequent, septa without clamp connections also observed. Anastomoses rare, lateral hyphae originate at an angle of 30-60° most often at the site of the septa. Anamorphs not observed.

A 9-month old culture without changes.

Ref.: Hübsch (1978), Käärik (1965), Kühner (1946), Rypáček (1957).

Pholiota flammans (Fr.) Kumm.

The culture grows very well (colony diameter 80 mm after 18 days). The growth does not alter the colour of the medium. Aerial mycelium finely woolly, low, with radially extending hyphae. Advancing zones not observed. Profile flat. Colony margin even, inconspicuously demarcated, marginal hyphae do not grow into the substrate. Colour pale yellowish white (Table 2, A2), mycelial character does not change during ageing. Odour faint; guttation not observed. One-year culturing in a refrigerator on an apple-carrot medium produces well developped primordia.

Aerial hyphae thin-walled, 1.1-3.0 µm diam. Clamp connections very frequent, on almost every septum. Anastomoses very frequent, projections very rare, branching fairly frequent. No anamorphs observed after a 1-month cultivation, after 3 months discernible aleurospores with a thin wall which becomes thickened on ageing. They are 15x30 µm in size, round to pyriform (Fig. 3).

A 9-month old culture contains as a rule thick-walled aleurospores.

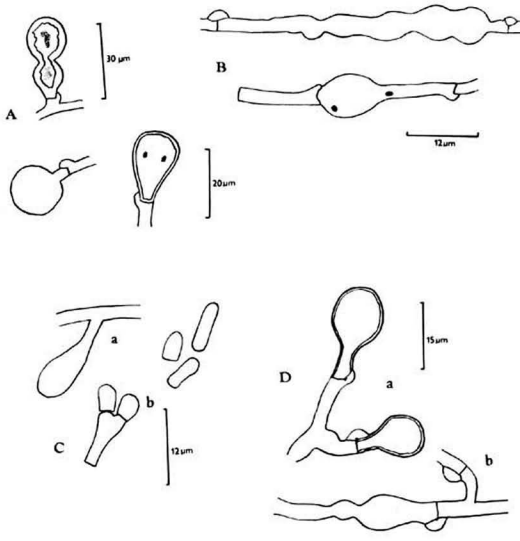

Fig. 3 A- **Pholiota flammans**, aleurospores, B- **Ph. lucifera** allocysts, C- **Ph. squarroso-adiposa**, a) allocyst, b) conidia, D- **Ph. flavida** a) aleurospores, b) intercalary thickened hyphae

Hyphae most often dikaryotic but also monokaryotic, aleurospores mono- or dikaryotic.

Ref.: Käärik (1965).

Pholiota flavida (Scheff.: Fr.) Sing.

Strain I grows very slowly (colony diameter 65 mm after 50 days), strain II grows moderately (80 mm after 28 days). Medium turns dark during growth. Aerial mycelium powdery, at certain sites finely flocculate, very low, with perceptible radial arrangement of hyphae. Colony margin delineated, finely ciliate. Colour yellowish, at the site of the inoculum more intensively light yellow (Table 4, A5). Odour pleasant but inconspicuous, guttation not observed.

Aerial hyphae thin-walled, 1.1 - 2.5 µm diam. Clamp connections frequent, on almost every septum. Anastomoses very frequent, branching frequent, hyphae of uniform diameter branch off usually at an angle of 90°. Frequent terminal coils on hyphae 35-80 µm in diameter. Aleurospores 15-25x15-11 µm, pyriform or bottle-shaped, are formed on a hyphae with a clamp connection (Fig. 3), in a Melzer reagent they are filled with a yellow substance. Intercalary thickened hyphae 65-66 µm also rarely present (Fig. 3).

A 9-month old culture without changes.

Hyphae dikaryotic, aleurospores also dikaryotic, rarely monokaryotic.

Ref.: Hübsch (1978).

Pholiota gummosa (Lasch) Sing.

The culture grows well (colony diameter 80 mm after 9 days). The growth does not alter the colour of the medium. Aerial mycelium very sparsely woolly, surface grosty. Profile flat. Colony margin even, inconspicuously demarcated, peripheral hyphae do not grow into the substrate. Colour whitish, ageing produces fine powdery to groaty sprinkling, greyish orange to yellowish brown (Table 5, B5 - Table 5, D8), in the direction from the inoculum. Odour pleasant. Guttation yellow-brown.

Hyphae thin-walled, 2.5 - 3.1 µm diam. Clamp connections very frequent, on almost every septum. Anastomoses observed, branching very frequent, hyphae branch of at an angle of 60-90°. Aleurospores 7-13x3-5 µm, bottle-shaped, pyriform to round (Fig. 2). In Melzer reagent they are filled with an amorphous yellow substance. After aleurospores there appear conidia 1.5-3x5-10 µm in size, oval or bullet-shaped, sometimes with sharp or rounded edges (Fig. 2). Yellowish droplets observed in Melzer reagent.

A 9-month old culture without changes.

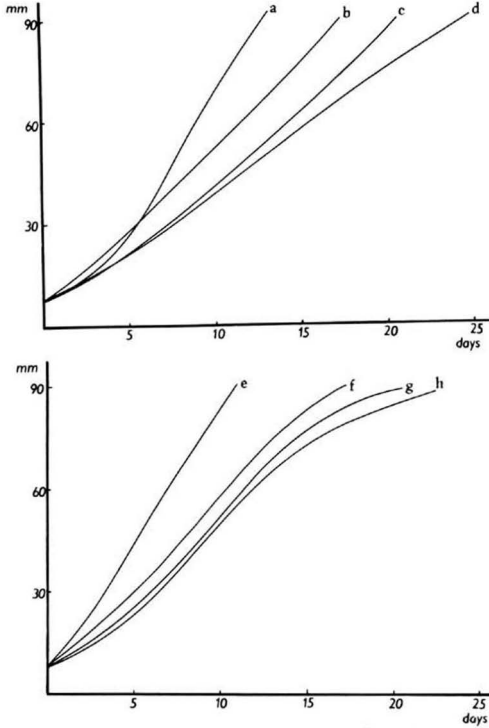

Fig. 4 Growth curves of the species, a) Pholiota gummosa, b) Ph. adiposa, c) Ph. squarroso-adiposa, d) Ph. conifera, e) Ph. carbonaria, f) Kuehneromyces mutabilis, g) Ph. flammans, h) Ph. lucifera

Hyphae and conidia dikaryotic, aleurospores observed to have 2 or 4 nuclei, more rarely 1 or 3.

Ref.: Deneyer (1960), Hübsch (1978), Käärik (1965), Kühner (1946).

Pholiota jahnii Tjall. et Bas

Syn.: Pholiota muelleri (Fr.) Orton

The culture grows very slowly (colony diameter 20 mm after 90 days). Aerial mycelium velvety. Profile high, convex, irregularly puckered. Peripheral hyphae grow into the substrate. Colour pale yellow (Table 3, A3). Odour inconspicuous, guttation not observed. A 1-year incubation in a refrigerator on malt agar yielded partially developed primordia.

Hyphae thin walled, 1.0-3.5 µm diam. Clamp connections frequent. Anastomoses not observed, hyphal branching rare.

After 9 months frequent club-like terminal cells of hyphae, with incrusted wall (Fig. 1).

No references.

Pholiota lenta (Pers.: Fr.) Sing.

The culture grows moderately well (colony diameter 80 mm after 28 days). The growth does not alter the colour of the medium. Aerial mycelium is very finely powdery, with tiny groat-like sprinkling in the region of the inoculum. No advancing zones were observed. Profile flat, colony margin even, without marked delineation. Colour whitish, with central yellowing. Odour inconspicuous, guttation not observed.

Hyphae thin-walled, 2.5-4 µm diam., with numerous septa. Clamp connections very frequent. Anastomoses not observed, branching frequent, especially in submerged mycelium.

Anamorphs not observed.

A 9-month old culture without change.

Hyphae dikaryotic.

Ref.: Hashioka and Arita (1978), Hübsch (1978), Kühner (1946), Marr et al. (1979).

Pholiota lucifera (Lasch) Quél.

The culture grows moderately well (colony diameter 80 mm after 24 days). The growth does not alter the colour of the medium. Aerial mycelium with marked radial filamentation. The mycelial mat is denser in the centre and looser at the periphery, with tree-like arrangement. Colony margin with long filaments, hyphae do not grow into the substrate. Profile flat. Mycelium colour white, with greyish-orange stripes spreading radially

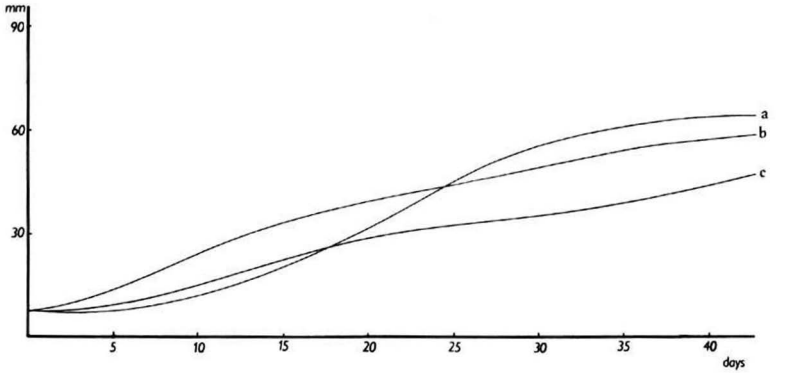

Fig. 5 Growth curves of the species, a) Pholiota destruens, b) Ph. alnicola, c) Ph. squarrosa

(Table 5, B5). Odour fungal, guttation not observed.

Aerial hyphae are thin-walled, 1.0-3.3 µm diam. Clamp connections very frequent, septa without clamps observed as well. Anastomoses present, branching very frequent. Hyphae sometimes with club-like ends, sometimes with terminal coils, hyphal wall often corrugated, hyphal cells always bonded by two clamps. Frequent allocysts round to ovoid, 12x17 µm (Fig. 3).

A 9 month old culture without change.

Both hyphae and allocysts dikaryotic.

Ref.: Kühner (1946).

Pholiota spumosa (Fr.) Sing.

The culture grows very slowly (colony diameter 80 mm after 60 days). Discolouration of nutrient medium during growth. Mycelium for the most part densely woolly, compact, at places the mycelium thinner. Profile flat. Colony margin markedly lobate. In places the margin irregularly demarcated with a woolly flucculose character, sometimes with circular islets separated from the main colony. Colour white. Odour inconspicuous, guttation not observed.

Hyphae thin-walled, 1.2x4.8 µm diam. Clamp connections frequent, septa without clamps also observed. Anastomoses observed, branching abundant (Fig. 1), frequent connections via clamps.

A 9-month old culture without change.

Hyphae dikaryotic.

Ref.: Hashioka and Arita (1978).

Pholiota squarrosa (Müll.: Fr.) Kumm.

The culture grows very slowly (colony diameter 74 mm after 57 days). Nutrient medium turns darker during growth, more conspicuously in strain II. Aerial mycelium woolly to flocculose, finely groaty in the centre. Profile flat. Colony margin delineated. Colour whitish, on ageing turning gradually darker - - light yellow (Table 4, A4) to greyish orange (Table 5, B5). Odour unpleasant, musty, guttation very fine, colourless. Fructification recorded on malt agar in a refrigerator.

Aerial hyphae thin-walled, 1.0-2.0 µm diam. Clamp connections frequent, septa without clamps also frequent. Anastomoses observed, branching not very frequent. After 3 months conidia observed very rarely, belonging to two types: cylindrical 8-3 µm, and pear-shaped or club-like, 6-12x4-7 µm; mostly unicellular but also bi- or tricellular. Both types of conidia arise often on a single conidiophore.

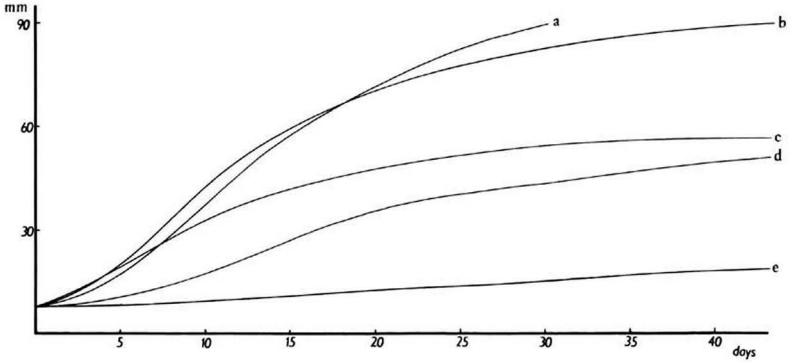

Fig. 6 Growth curves of the species, a) **Pholiota flavida** (strain II), b) **Ph. lenta**, c) **Ph. spumosa**, d) **Ph. flavida** (strain I), e) **Ph. jahnii**

After 9 months abundant conidia.

Both hyphae and conidia dikaryotic.

Ref.: Buchalo (1988), Buchalo et al. (1971), Hashioka and Arita (1978), Hübsch (1978), Käärik (1965), Kühner (1946), Lyr (1958), Mišurcová et al. (1987), Watling (1983).

Pholiota squarroso-adiposa Lange

The culture grows well (colony diameter 80 mm after 15 days). The growth does not change the colour of the medium. Aerial mycelium finely filamentous, in places subtly flocculose. Profile flat. Colony margin delineated, finely woolly, colour white, turning yellow on ageing. Odour pleasant, yellowish guttation appears after 3 weeks in the region of the inoculum.

Aerial hyphae thin-walled, 2-3 µm diam. Clamp connections frequent, on almost every septum. Anastomoses observed, lateral hyphae originate at the site of the septum as well as in other places, most often at a 90° angle. Aerial mycelium rarely features club-like allocysts 7x12 µm.

The occurrence of cylindrical to bullet-shaped conidia 5x12 µm observed after 9 months (Fig. 3).

Hyphae dikaryotic, allocysts and conidia also dikaryotic.

Ref.: Hübsch (1978).

Kuehneromyces mutabilis (Scheff.: Fr.) Sing. et Smith

Syn.: **Pholiota mutabilis** (Fr.) Kumm.

The culture grows well (colony diameter 80 mm after 16 days). The growth does not alter the colour of the medium. Aerial mycelium sparsely velvety with suggestion of radial filaments. Advancing zones regular and about 0,7 mm wide. Profile flat. Colony margin even, demarcated, very finely woolly. Colour white. Odour inconspicuous, guttation not observed. A one-year incubation in a refrigerator on malt agar yielded moderately developed primordia.

Aerial hyphae thin-walled, 1.3x4.2 µm diam. Clamp conections frequent, septa without clamps also relatively frequent. Anastomoses very frequent, branching very rare. Anamorphs not observed.

A 9-month old culture without change.

Hyphae dikaryotic.

Ref.: Boidin (1959), Bresinski and Besl (1979), Buchalo (1988),

Table 1: Results of enzymatic activities

	Pholiota adiposa	Ph. alnicola	Ph. carbonaria I	II	II	Ph. conifera	Ph. destruens	Ph. flammans	Ph. flavida I	II	Ph. gummosa I	II	Ph. jahnii	Ph. lenta	Ph. lucifera	Ph. spumosa	Ph. squarrosa I	II	Ph. squarroso-adiposa	Kuehneromyces mutabilis
laccase	++	++	+	++	+	+	-	+	+++	++	+++	+++	+++	+	+++	++	++	++	++	+++
catalase	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+
peroxidase		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
tyrosinase	+++	-	-	-	-	++	++	-	+	+	+	+	+	+	-	+	++	++	++	+
lipase	-	-	+	+	+	-	+	-	-	+	-	-	+	+	++	+	-	-	+	++
lecithinase[x]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
amylase	+	+	++	++	+	++	+	+	++	++	++	++	++	++	+	++	-	-	-	+
cellulase	+	+	+	+	+	+	+	+	+	+	+/-	+/-	+	+	+	+	+/-	-	-	+
β - galactosidase[x]	-	-	+	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	+
α - glucosidase[x]	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+
proteases - gelatinase	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
- milk peptidase		+	-	-	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
milk clotting enzymes	+++	-	+	+	+	+	-	+	-	-	+	+	+	+	+	++	++	+	+	+
urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Explanatory notes to Table 1: - no activity, + disputable activity, + - +++ semiquantitative demonstration of activities;
x activities very weak

Buchalo et al. (1971), Käärik (1965), Kühner (1946), Lyr (1958), Mišurcová et al. (1987), Semerdžieva (1965).

KEY FOR DETERMINATION OF SPECIES OF THE GENUS PHOLIOTA ACCORDING TO MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF MYCELIAL CULTURES

1a. amylase positive 2

2a. tyrosinase positive 3

3a. peroxidase positive ..... Pholiota spumosa

3b. peroxidase negative 4

4a. urease positive ..... Ph. lenta

4b. urease negative 5

5a. growth rate more than 90 mm in 24 days 6

6a. conidia or aleurospores present 7

7a. aleurospores present after 21 day cultivation, cellulase activity very weak ..... Ph. gummosa

7b. aleurospores absent after 21 day cultivation, strong cellulase activity 8

8a. positive ß-galactosidase, culture turns yellow ..... Ph. adiposa

8b. negative ß-galactosidase, culture does not turn yellow . Ph. conifera

6b. anamorphs absent ..... Kuehneromyces mutabilis

5b. growth rate is remarkably slower 9

9a. aleurospores present, lipase negative, the profile of the colony flat ..... Ph. flavida

9b. aleurospores absent, lipase positive, the profile of the colony convex ..... Ph. jahnii

2b. tyrosinase negative 10

10a. laccase positive 11

11a. growth rate more than 90 mm in 24 days, gelatinase positive ..... Ph. squarroso-adiposa

11b. growth rate is remarkably slower, gelatinase negative ..... Ph. squarrosa

10b. laccase negative ..... Ph. destruens

1b. amylase negative 12

12a. urease positive ..... Ph. lucifera

12b. urease negative 13

13a. growth rate more than 90 mm in 24 days 14

14a. aleurospores present after 3 month cultivation, milk peptidase positive, fructification very rare ..... Ph. flammans

14b. aleurospores absent, milk peptidase negative, fructification frequent Ph. carbonaria

13b. growth rate remarkably slower ..... Ph. alnicola

DISCUSSION

Summary treatment of a larger set of species of the genus **Pholiota** has so far been lacking, with literature sources bringing only partial data on individual species. **Pholiota jahnii** has not yet been cultivated. Most of the species were found to have common cultural characters: thin-walled dikaryotic hyphae with anastomoses, positive laccase, catalase, amylase, milk-clotting enzymes, cellulase, milk proteinase and negative peroxidase, urease, lecithinase, α-glucosidase, and ß-galactosidase.

The mat colour and mat texture studied as macroscopic features differed in individual species (see Results). As to the rate of growth, the set can be divided into two more or less homogeneous groups (see growth curves) - species envincing rapid growth on MEA (**Pholiota adiposa, Ph. carbonaria, Ph. conifera, Ph. flammans, Ph. gummosa, Ph. lucifera, Ph. squarroso-adiposa, Kuehneromyces mutabilis**) and species with slow growth (**Ph. alnicola, Ph. destruens, Ph. flavida, Ph. jahnii, Ph. lenta, Ph. spumosa, Ph. squarrosa**).

Among microscopic characters a number of authors observed conidia in species **Pholiota adiposa, Ph. conifera, Ph. squarrosa** and **Ph. squarroso-adiposa**, in keeping with our results (e.g. Kühner, 1946; Nobles, 1948, 1965; Semerdžieva, 1965; Arita et al., 1980; Pantidou et al., 1983; Watling, 1983). Hübsch (1978) observed conidia in the **Pholiota adiposa** species in all strains, in the **Ph. squarrosa** species only in three out of six strains, in the **Ph. squarroso-adiposa** species no conidia were observed. We have found that species **Pholiota squarrosa** and **Ph. squarroso-adiposa** form conidia only after a prolonged cultivation. It is therefore necessary to state with individual characteristics also the culture age and cultivation conditions.

Anamorphs or allocysts are produced abundantly when the mycelia are transferred to a fresh medium as when they have been growing for a long time. Aleurospores were observed in species **Pholiota adiposa, Ph. gummosa, Ph. flammans.** In **Pholiota adiposa** our observations of aleurospores were in keeping with the observations of Hübsch (1978) and Arita (1979), in the **Ph. gummosa** species with the data of Hübsch (1978); the same author observed these formations also in **Ph. squarrosa** where we failed to record them. Other authors (Nobles, 1948; Semerdžieva, 1965) did not observe aleurospores in the **Pholiota adiposa** species. According to our observations, the **Pholiota adiposa** species forms aleurospores only after a prolonged cultivation. Allocysts were observed in species **Pholiota alnicola, Ph. lucifera** and **Ph. flavida.** In the **Pholiota alnicola** species our positive observations were in accordance with the data of Kühner (1947), Nobles (1948), and Deneyer (1960). In **Pholiota flavida** Hübsch (1978) termed formations of the same shape and size chlamydospores.

Enzymatic activity. Studies of oxidase in the **Pholiota** genus have been relatively frequent (Boidin, 1951; Lyr, 1958; Käärik, 1965; Nobles, 1965; Koenigs, 1971; Bresinski and Besl, 1979; Marr, 1979). The results of tests of peroxidase, catalase and tyrosinase activity published by other authors are in keeping with our results. Laccase tests of the **Pholiota destruens** species performed with syringaldazine or guiacol were negative; however, the species oxidized within 24 h α-naphtol (guiacol and α-naphtol are low-specificity agents for determination of activity of phenol oxidases which include also laccase). Marr (1979) failed to detect laccase in **Pholiota lenta** by using syringaldazine; however, he worked with fruit-bodies, not with mycelial cultures. Hydrolases have been much less studied. No studies of the genus **Pholiota** have been done with respect to the activity of lecithinase, amylase, α-glucosidase, ß-galactosidase and urease. Nerud et al.(1982) found lipase in **Pholiota adiposa** and **Ph. conifera** species with triolein as substrate. On using Tween 80 and 20 we have detected no lipase in these species. Proteinases and milk clotting enzymes have been more thoroughly studied. Buchalo et al. (1971) found a slight activity during casein decomposition (submerged cultivation) in **Pholiota adiposa** (in our studies milk peptidase likewise positive) and, again similar to our data, a negative proteolytic activity in **Kuehneromyces mutabilis.** In contrast to their findings, however, we found slight peptidase activity in **Pholiota squarrosa** and **Ph. conifera** species. No gelatinase was detected in **Pholiota squarrosa.** Similar to Mišurcová et al. (1978) we have found activity of milk clotting enzymes in **Pholiota adiposa,** but in contrast to these authors also in **Ph. squarrosa** while it was absent in **Kuehneromyces mutabilis.**

The culture of the **Kuehneromyces mutabilis** species, which is related to the genus **Pholiota,** did not differ appreciably from cultures of **Pholiota** either morphologically or biochemically.

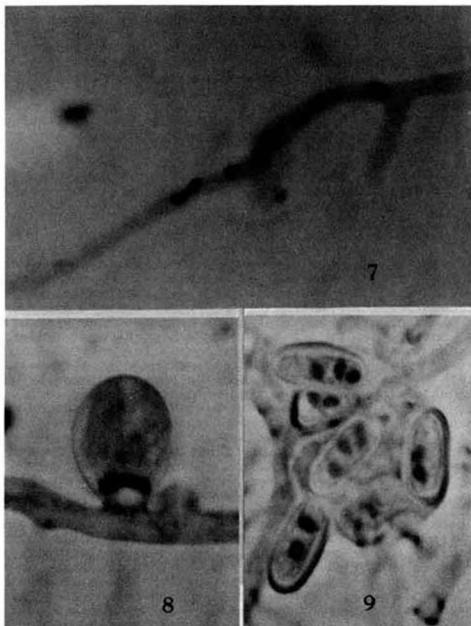

Fig. 7. **Pholiota gummosa.** A position of a multinucleate tip cell with the nonseptate clamp owing to lack of septal formation as well as repeated division of nuclei Fig. 8. **Pholiota gummosa.** Aleurospore with two homologous nuclei. Fig. 9. **Pholiota adiposa.** Conidia. Each cell of contains two nucleus or three homologous nuclei.

Our results made it possible to select characters for determination of species in mycelial culture. These include growth rate, formation of anamorphs, production of tyrosinase, laccase, amylase, urease and peroxidase. Other, less distinct markers in which the species differed often only quantitatively, were used as auxiliary, mostly in combinations.

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TWO NEW *GLOMUS* SPECIES FROM ARABLE LAND

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SUMMARY

The vesicular-arbuscular mycorrhizal fungi *Glomus fistulosum* and *Glomus fragilistratum* are described. The former is characterized by a fistular laminated wall, the latter by a unit wall which breaks into flakes and strips when spores are broken.

INTRODUCTION

Investigations into the ecology and agronomic importance of vesicular-arbuscular mycorrhizal (VAM) fungi in cultivated soils have been carried out in Denmark for several years (cf. *e.g.* Jensen & Jakobsen, 1980; Jakobsen & Nielsen, 1983; Jakobsen, 1986). A number of VAM fungi were collected. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe predominated in five soils and *G. caledonium* (Nicol. & Gerd.) Trappe & Gerd. in one. Further, one sixth of the spores isolated from one soil were identified as *Gigaspora calospora* (Nicol. & Gerd.) Gerd. & Trappe (syn. of *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders). Some isolates (Jensen & Jakobsen, 1980) were regarded as new species, and two of these are described in this paper.

MATERIALS AND METHODS

Isolates of VAM fungi were collected in 1978 at Askov, southern Jutland, at Hillerslev, northern Jutland, and at Jullerup, Funen, Denmark. The fungi, taken from soil suspensions, initially were propagated on white clover (*Trifolium repens* L.). Similar-looking spores then were selected and reinoculated on maize (*Zea mays* L.). Homogeneous cultures were obtained by five repeats of this procedure.

The spores were examined in several different mountants: PVLG using the recipe of Omar, Bolland & Heather (1979), and prepared according to the method of Koske & Tessier (1983); Shear's mounting fluid (2% CH_3COOK in 0.2M

pH 8 McIlvaine's buffer: glycerol: ethanol (95%) in a 5:2:3 proportion; Punithalingam, 1971); 3% KOH for clearing (cf. *e.g.* Baral, 1987); 1M sucrose (osmoticum; cf. *e.g.* Villanueva, 1966), or Melzer's reagent.

Identification of the isolates were based mainly on the INVAM species guide (Schenck & Pérez, 1988) and by consideration of details discussed by Morton (1988). For each species, spore size was based on more than 200 measurements made with an ocular screw micrometer.

RESULTS

***Glomus fistulosum* Skou & Jakobsen sp. nov.**

Etymology: The Latin epithet '*fistulosum*' means fistular and refers to the pronounced fistular laminated wall of the spores.

Descriptio: *Sporocarpia ignota. Sporae in terra singulatim efformatae, luteae, globosae, 78 - 137 - 200 µm magnae, inter quas 11 pro 100 late ellipsoideae vel pyriformes, 67 - 121 - 166 x 94 - 138 - 178 µm magnae. Sporae complexis tunicis, 5.5 - 8.9 - 13 µm crassae, quinque stratis (1-5) instructae, in duo turmis (A-B) formatae. Stratum externum evanescens et stratum unitum (sequens), haec duo strata adherentia, tenuibus, non plus quam 1 a 2 µm, et hyalina. Stratum tertium laminatum, luteum, variabiliter crassum, fistulas habens, quo aditus tenuiter declivis ad aperturam angustam, quae plerumque 0.5 µm. Strata membranacea duabus in turma B, hyalina, non plus quam 1 a 2 µm crassa. Hyphae affixae, hyalinae, non occlusae, non septatae, 6-10 µm crassae ad basim sporae pariete 2.7 µm plerumque. Mycorrhizas vesicular-arbusculares formans.*

Habitat in terra ad Askov et Hillerslev, Jutlandia, Dania.

Holotypus increvit ad Zea mays L. anno 1989, in Museo et Herbario Hauniensi (C), Dania depositus. Isotypus aeque ac cultura ad INVAM Universitas Florida, Gainesville, U.S.A. depositus.

Description: Sporocarps unknown. Spores formed singly in soil, pale yellow and yellow in reflected and transmitted light, respectively, globose, 78 - 137 - 200 µm diam. with 86% between 120 and 160 µm; 11% broadly ellipsoid or pyriform, 67 - 121 - 166 x 94 - 138 - 178 µm diam. The spore wall consists of five walls in two groups, with composite wall 5.5 - 8.9 - 13 µm thick (Fig. 1).

WALL GROUP A consists of three walls. Outermost, two thin, hyaline, adhering walls, each not more than 1-2 µm. Wall 1 is evanescent and wall 2 is a rigid unit wall (Fig. 2g). Wall 3 is a yellow, laminated and fistular wall which increases in thickness and number of laminar fissures with spore age (Fig. 2a-i). The openings of the fistules in wall 3 gently slope into an aperture of 0.5 µm on an average (Fig. 2h, i). The fistules appear as points at the focal plane. As the focus is lowered, the fistules incline and appear as diffuse radial lines until they are in horizontal position and clearly visible. This characteristic appear-

ance results from the degrees of inclination of the fistules across the spores as they are seen in the microscope.

The laminated wall 3 breaks rather easily along the laminar fissures. The fractures take on a denticular appearance at the broken edge of this wall, and thus considered ornamented (Fig. 2i).

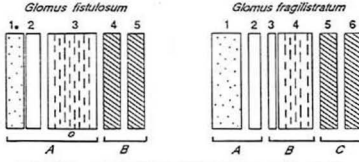


Figure 1. Micrographs of the spore wall structure of *G. fistulosum* and *G. fragilistratum*. Muronyms are A(E$_{p}$UL$_{o}$), B(MM), and A(EU), B(UL), C(MM), respectively.

WALL GROUP B consists of two thin, hyaline membranaceous walls, each not more than 1-2 µm thick. The innermost wall 5 is most easily seen when the cell contents contract on plasmolysis in 1M sucrose (Fig. 2a, e).

A subtending hypha appears to be inserted in the spore walls. It is 6-10 µm wide at the spore base. Hyphal walls are hyaline and with an averaged thickness of 2.7 µm. The pore in the subtending hyphae is open without occlusion or septum (Fig. 2a, b). Spore contents are colourless and appears as variable-sized globules.

G. fistulosum forms vesicular-arbuscular mycorrhizas on several cultivated plants.

Distribution. *Glomus fistulosum* was collected in two habitats by I. Jakobsen in July 1978. Isolate No. 21 was collected in a crop of winter wheat (*Triticum aestivum* L.) on a long-term experimental field with sandy loam at Askov (Askov lermark) in southern Jutland, Denmark. Isolate No. 22 was collected in a crop of spring barley (*Hordeum vulgare* L.) on a loamy soil at Hillerslev in northern Jutland, Denmark (cf. Jensen & Jakobsen, 1980).

Mycorrhizal associations. *G. fistulosum* was collected under conditions that suggest associations with wheat and barley in the field. Further, the fungus (Nos 21 and 22) formed VA mycorrhizas in pot cultures with leek (*Allium porrum* L.), maize (*Zea mays* L.), and white clover (*Trifolium repens* L.).

Types. For purification of *G. fistulosum* (Nos 21 and 22), ten alike spores were separated under the microscope and added to pre-sterilized soil seeded with maize in pot cul-

tures. The holotype (No. 21) was selected in 1989 after five repeats of this procedure and deposited at the Botanical Museum and Herbarium, Copenhagen, Denmark (C). The isotype and a living culture from No. 21 are deposited at the International Culture Collection of VA Mycorrhizal Fungi (INVAM), University of Florida, Gainesville, U.S.A.

***Glomus fragilistratum* Skou & Jakobsen sp. nov.**

Etymology: The Latin epithet, '*fragilistratum*' from *fragilis* = fragile, easily shattered, and *stratum* = layer, refers to the third spore wall which is characteristically fragile.

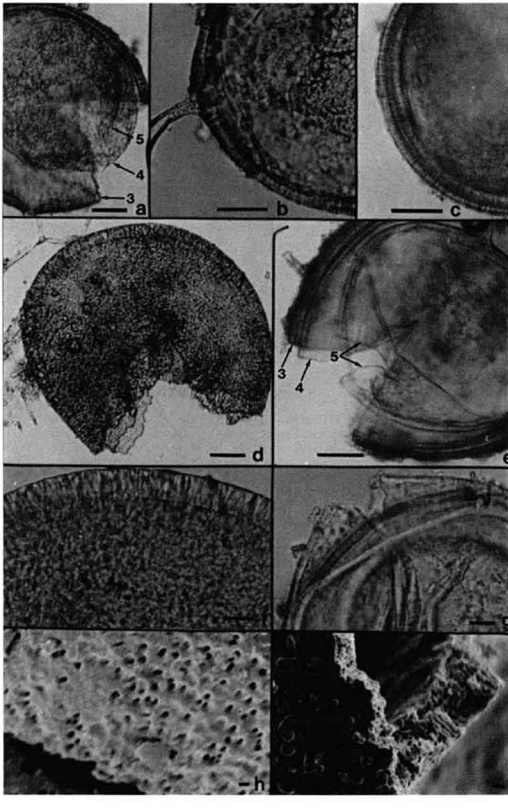
Descriptio: *Sporocarpia ignota. Sporae in terra singulatim efformatae, luteae vel pallide aurantiacae, globosae, 108 - 146 - 191 µm magnae inter quas 18 pro 100 late ellipsoideae vel irregulares, 108 - 142 - 181 x 121 - 169 - 231 µm magnae. Sporae complexis tunicis, 7 - 9 - 12 µm crassae, sex stratis (1-6) instructae, in tres turmis (A-C) formatae. Stratum externum hyalinum, gelatinosum cum granulis, et evanescens, variabiliter crassum attingentia 4.5 µm. Stratum secundum unitum, hyalinum, rigidum, 1.4-3.0 µm. Stratum tertium item unitum, hyalinum, vitri instar, in rimis et vittatis fragilibus, ca. 1 µm crassum, plus minusve ad stratum laminatum adhaerens. Stratum quartum luteum vel pallide aurantiacum, laminatum, variabiliter crassum, plerumque 5.1 µm. Stratum quintum hyalinum, membranaceum, leniter alveolatum, non plus quam 1 µm et stratum sextum hyalinum, membranaceum et granulatum, non plus quam 1 µm. Stratum primum usque ad quartum declive ad hyphas affixas, quae habentes diametrum 9-15 µm ad basim sporarum. Mycorrhizas vesicular-arbusculares formans.*

Habitat in terra ad Jullerup, Fionia, Dania.

Holotypus increvit ad Zea mays L. anno 1989, in Museo et Herbario Hauniensi (C), Dania depositus. Isotypus aeque ac cultura ad INVAM Universitas Florida, Gainesville, U.S.A. depositus.

Description: Sporocarps unknown. Spores formed singly in soil, yellow and bright yellow or pale orange in reflected and transmitted light, respectively, globose, 108 - 146 -

Figure 2. *Glomus fistulosum*. **a.** Broken spore in 1M sucrose. Arrows indicate the laminated wall (3) and the two innermost walls (4 and 5). Note the subtending hypha with the open pore. x 300, bar 25 µm. **b.** Spore showing the open pore to the subtending hypha. The fistules appear as radial stripes on the laminated wall. In PVLG. x 425, bar 25 µm. **c.** The laminated wall with radial lines of the fistules. In PVLG. x 425, bar 25 µm. **d.** Broken spore cleared in 3% KOH. The fistules appear as small points on the inside (note the fractures) as well as on the outside, and as radial lines at the spore periphery. x 300, bar 25 µm. **e.** Broken spore. Arrows point to the laminated wall 3 and the two innermost walls (4 and 5). In PVLG. x 400, bar 25 µm. **f.** Cross section of the fistular, laminated wall in 3% KOH. x 650, bar 10 µm. **g.** Spore in 3% KOH with the outer, hyaline walls (1 and 2) broken. x 550, bar 10 µm. **h.** SEM micrograph of the surface of the laminated wall with fistule openings. x 2500, bar 1 µm. **i.** SEM micrograph of fractures of the laminated wall with the fistules in longitudinal section. x 2500, bar 1 µm.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191 µm diam. with 84.5% between 120 and 170 µm; 18% broadly ellipsoid or irregular, 108 - 142 - 181 x 121 - 169 - 231 µm diam. The spore wall consists of six walls in three groups (A-C), with the composite wall 7 - 9 - 12 µm thick (Fig. 3).

WALL GROUP A consists of two walls. Wall 1 is gelatinous, hyaline and evanescent. It may have a gritty content that is most easily seen in Melzer's reagent. When present, this wall may be up to 4.5 µm thick (Fig. 3a-c). Wall 2 is a rigid, hyaline unit wall, 1.4-3.0 µm thick (Fig. 3e, h, i).

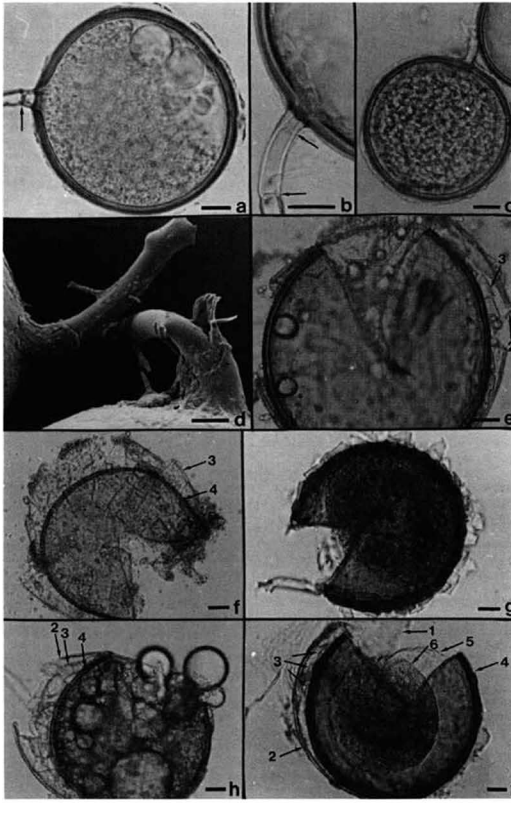
WALL GROUP B consists of two walls (3 and 4). Wall 3 a vitreous hyaline unit wall which rarely exceeds 1 µm on thickness. It breaks into flakes and strips on broken spores. Cracks radiate from the point where the spores are broken with a tapering instrument. This wall sometimes is adherent to the laminated wall (Fig. 3e-i). Wall 4 is a yellow or pale orange laminated wall of varying thickness, 5.1 µm on an average. This wall becomes reddish-brown in Melzer's reagent (Fig. 3a-i).

WALL GROUP C consists of two hyaline membranaceous walls, neither more than 1-2 µm thick. Wall 5 is weakly alveolate and the innermost wall 6 appears granular. The latter wall is most clearly visible when the cell contents contract on plasmolysis (Fig. 3h, i). The spore content consists of differently sized oil globules that become viscid with age (Fig. 3h).

Walls 1-4 extend as walls of the subtending hyphae (Fig. 3d) for a short distance and then tapes off (Fig. 3a, b). Diameter of subtending hyphae at the spore base is 9-15 µm, usually with 1-2 hyphal septa positioned close to the spore. Occasionally, the hypha has an angular bend between the first and the second septum, and narrow branch hyphae may occur at this region (Fig. 3a, b, d).

G. fragilistratum forms vesicular-arbuscular mycorrhiza on several cultivated plants.

Figure 3. *Glomus fragilistratum*. **a.** Spore with subtending hypha and remains of the exterior, gelatinous, hyaline wall. Arrow points to a hyphal septum. x 250, bar 25 µm. **b.** Part of spore with subtending hypha. Arrows point to hyphal septa. Note the thick hyphal wall due to the extension of the four outer walls of the spore. x 400, bar 25 µm. **c.** Spore with the exterior, gelatinous, hyaline wall. x 225, bar 25 µm. **d.** SEM micrograph of subtending hyphae. Note the continuation of spore outer walls onto hypha. x 800, bar 10 µm. **e.** Broken spore showing two outer rigid, hyaline unit walls. Wall 3 is broken into radiating sections. x 225, bar 25 µm. **f.** Crushed spore more clearly showing the separation of the third wall in to radial segments. x 175, bar 25 µm. **g.** Hyaline flakes broken off the third wall of a spore in Melzer's reagent. x 225, bar 25 µm. **h.** Broken spore with wall 2-4 and the oily to viscid content visible. x 175, bar 25 µm. **i.** Broken spore with all six wall layers visible. x 175, bar 25 µm. **a-c, e** and **f** in PVLG; **h** and **i** in 1M sucrose.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Distribution. *Glomus fragilistratum* was collected by I. Jakobsen after harvest in July 1978. The fungus (isolate No. 33) was collected in a crop of spring barley (*Hordeum vulgare* L.) on sandy loam at Jullerup (Statens gård), Funen, Denmark (cf. Jensen & Jakobsen, 1980).

Mycorrhizal associations. *G. fragilistratum* was collected under conditions that suggest association with barley in the field. Further, the fungus (No. 33) formed mycorrhizas in pot cultures with leek (*Allium porrum* L.), maize (*Zea mays* L.), and white clover (*Trifolium repens* L.). Inoculation with this fungus improved uptake of phosphorus (P) and growth of barley, maize, and white clover in P-deficient irradiated soils (Jakobsen, unpublished).

Types. After five inoculations on maize in pot cultures, the holotype of *G. fragilistratum* (No. 33) was selected in 1989 and deposited at the Botanical Museum and Herbarium, Copenhagen, Denmark (C).

Isotype and a living culture from No. 33 are deposited at the International Culture Collection of VA Mycorrhizal Fungi (INVAM), University of Florida, Gainesville, U.S.A.

DISCUSSION

We have used the conventional wall-grouping (Walker, 1983) though in reality only the two outermost walls of spores in *G. fistulosum* may be difficult to separate, and as flakes or segments of the vitreous wall 3 may occasionally adhere to the laminated fourth wall of the spores in *G. fragilistratum*.

The spores of *G. fistulosum* and *G. fragilistratum* have more complex wall structures than those of other described *Glomus* species. The five-walled spores of *G. gerdemannii* Rose, Daniels & Trappe is closest in wall complexity, but the sequence of wall types, hyphal attachment, and spore size are different (Rose et al., 1979).

The fistular or pored structure of the laminated wall in spores of *G. fistulosum* is unique. The narrow (about 0.5 µm), fistules pass through the laminar fissures. They are uniformly distributed over the spore wall and are confined to the laminated wall. For this reason, the fistules are not considered artifacts caused by bacterial or fungal activities.

The vitreous unit wall (wall 3) in spores of *G. fragilistratum* is diagnostic for this species and does not compare to any other wall in described *Glomus* species. It is visible, however, only when spores are broken with a pointing instrument. It does not flake as Rose et al. (1979) report for the outermost walls of *G. gerdemannii*.

The outer evanescent, gelatinous wall is clearly thicker on spores of *G. fragilistratum* than on those of *G. fistulosum*. This wall resembles the outer wall of *G. clarum* Nicol. & Schenck (Nicolson & Schenck, 1979), *G. manihotis* Howeler, Sieverding & Schenck (Schenck, Spain, Sieverding

& Howeler, 1984), and probably *G. intraradices* Schenck & Smith (Schenck & Smith, 1982).

The unit wall 2 of both species is hyaline and rigid rather than gelatinous. Therefore, this wall cannot be just a second layer of the outer wall.

The presence of two inner membranaceous walls in spores of *G. fistulosum* and *G. fragilistratum* is unique for *Glomus* species described to date. This wall structure suggests a phylogenetic relationship with members of *Acaulospora* (J.B. Morton, pers. comm.).

Walls 1-4 of *G. fragilistratum* spores extend on or past the closing septum in the subtending hyphae. In that respect, they resemble several other *Glomus* species such as *G. clarum* (Nicolson & Schenck, 1979) and *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe (Nicolson & Gerdemann, 1968).

Globular to pear-shaped, thin-walled, hyaline, vesicle-like cells, 8-15 µm in diameter, occur scattered on the mycelium of *G. fistulosum* between soil particles. Their function, if any, is unknown.

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THE GENUS PEZIZELLA I.: NOMENCLATURE AND HISTORY.

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Summary

The nomenclature and the history of the exploration of the genus ***Pezizella*** *Fuckel and of genera connected with it (****Allophylaria*** *Karst., ****Calycina*** *(Nees) Gray, ****Cystopezizella*** *Svr., ****Eubelonis*** *Clem., ****Eubelonis*** *Höhnel, ****Pezizella*** *subgenus* ***Ctenoscypha*** *Starb., and* ***Pseudohelotium*** *Fuckel) are discussed. The "typus generis" of* ***Pezizella*** *is* ***P. sordida*** *(Fuckel) Fuckel. In this connection some systematic consequences are pointed out.* ***Eubelonis*** *Clem. is a synonym of* ***Pezizella*** *but neither* ***Allophylaria*** *nor* ***Calycina*** *is. The supposed synonymy of* ***Peziza sordida*** *Fuckel with* ***Peziza avellanae*** *Lasch and* ***Peziza vulgaris*** *Fr. is discussed. The typification of* ***Calycina*** *with* ***Peziza herbarum*** *Pers. is dubious.*

> Natürlich System, ein widersprechender Ausdruck. Die Natur hat kein System, sie hat, sie ist Leben und Folge aus einem unbekannten Zentrum, zu einer nicht erkennbaren Grenze. Naturbetrachtung ist daher endlos, man mag ins einzelnste teilend verfahren oder im ganzen nach Breite und Höhe die Spur verfolgen.
>
> Goethe, Probleme

I. Introduction

In the present work I want to try to represent the history of the exploration and the nomenclature of the genus **Pezizella** (Ascomycetes, Leotiales) and of some further genera, which in this connection are of special interest. If this is perhaps done in more detail than necessary, I take the following considerations as a basis for the style of presentation:
Firstly: I want to point out troubles which arise by treating these organisms - many of which are smaller than 0.5 mm in diameter - and which are exclusively caused by nomenclatural problems of this order.
Secondly: The more detailed representation should increase the legibility of a subject, which generally is looked at as dry as dust and scarcely readable.

The interest in these organisms is no child of our time, but already our forefathers struggled with them more than 200 years ago. First of all they used their eyes for their examinations or, to enhance their efficiency, a hand-lens, and only in rarer cases the compound microscope. But not until the 19th century was the application of this device used regularly. Accordingly the descriptions - usually written in Latin - were limited to such characters as colour, shape, consistency, etc. of the apothecia. Anatomical characters were completely absent or were only considered insufficiently.

Many species, the names of which we still use today, were named by the originators of systematic mycology, e.g., ALBERTINI and SCHWEINITZ, BATSCH, FRIES, NEES von ESENBECK, PERSOON, to mention only some of them. The present use of these names is not arbitrarily, but is governed by definite rules, which are laid down in the ICBN. This "law" shall contribute to the nomenclatural stability, if it is obeyed. Often an inconsiderable sacrifice of time is necessary to find a reliable nomenclatural basis for the species and genera, before the "true" work can start.

This working method reminds us of a criminologist's or a historian's work, rather than of the work of a biologist, who is supposed to use modern and promising methods, as usually employed, e.g., in molecular biology or gene technology. This "screening" of old names is by no means an end in itself: at the same time the name of any taxon is its "address," where all characters and qualities are filed, and, what is much more important, can be found again; it serves for storing information (data).

In the herbaria of many authors we often find specimens to which the names are tied ("Datenträger"), so that we have the possibilty to study many of those old species with a multitude of modern methods. The "finger-print", however, which is obtained by such studies, is sufficient to identify species, which have been found today, even if in some cases doubts can remain . The working up, i.e., the check up of the "old" species, has still not been completed to this day. Nevertheless again and again species or genera are pushed to and fro in the system according to descriptions which frequently do not meet the minimal demand, which we need for the systematic evaluations, or species and genera are sometimes described far too thoughtlessly.

As long as this "homework" has not been finished to a greater extent than till now, there can scarcely be nomenclatural stability and a reliable natural system, what ever we may understand by it for the fungi, for the at present third-largest order of the Ascomycetes.

Consequently at the moment it is only possible to deduce

reliable information, such as distribution of species and genera, interdependences of substrata or further ecological data, the occurence of secondary metabolic products, as far as these are known at all, with great reservation, quite apart from such "simple" jobs as determination of the families, genera and species, which often reveal inconsistencies and absurdities within this system.

These comments do not imply that the application of biochemical and physiological methods in taxonomy and systematics is useless, but only that these have no sound basis as long as the "classical" ones have led to stable opinions.

If it is right that the quality of a system reflects the acquaintanceship with a group of organisms, then we do not know - nearly at the end of the 20th century - very much more about the Leotiales than REHM had at its beginning.

II. The typification of the genus Pezizella

FUCKEL (1870 p. 299) (1) founded the genus **Pezizella** with the following diagnosis:
"Cupulae gregariae, minutae, ceraceae, subdiaphanae, apertae, stipitatae, totae glabrae: Discus subhemisphaericus. Asci elongati, oblongi, linearsve, 8spori. Sporidia cylindracea, oblongave (?), plerumque curvata, continua, hyalina: Paraphyses simplices, filiformes". In his genus he placed six species in the order listed below:

1. **Pezizella avellanae** (Lasch) Fuckel
2. " **sordida** (Fuckel) Fuckel
3. " **pulchella** (Fuckel) Fuckel
4. " **juncina** (Pers.) Fuckel
5. " **rubella** (Pers.) Fuckel
6. " **dilutella** (Fr.) Fuckel

Over the period of its more than one hundred-year-old history more than 400 (2) species have been added to this genus till today. FUCKEL, as was the custom in the last century, did not designate a "typus generis." For that reason the few or insignificant microscopic characters had inevitably to lead to different interpretations of the genus by subsequent mycologists, as far as they accept **Pezizella** at all; for the majority of the mycologists of

(1) Concerning the publication date of FUCKEL's opus cf. ROGERS (1954) and STAFLEU & COWAN (1976).

(2) About one quarter (107) of the "species" were exclusively described by VELENOVSKÝ (1934, 1939, 1947), who even founded a family on this name: "12. Fam. **Pezizellaceae**" (1934, p. 154). Unfortunately I had not the privilege to study any of VELENOVSKÝ's species. Within the **Leotiales** (CARPENTER 1988 = **Hyaenoscyphales** nom. nud. BELLEMÈRE (1978) = **Helotiales** auct.) this number is only exceeded in the genus **Hyaenoscyphus** with more than 560 "species."

the late 19th century did not use it at all (3) or only as a subgenus (4). SACCARDO (1889), a few others (5), and REHM (1892) made use of FUCKEL's generic name. Both, SACCARDO and REHM described FUCKEL's genus as having sessile apothecia, which was emphasized by both authors: SACCARDO, l.c. p. 276: "Est **Phialea** sessilis v. **Mollisia** laeticolor..." and REHM, l.c. p. 653: "Diese Gattung umfaßt eine große Anzahl zumeist winziger Arten mit sitzenden Apothecien...". The two authors did not designate a type species, either. REHM (l.c.) did not even retain **any** of the "foundation species" in **his** genus Pezizella (6) , whereas SACCARDO (1.c.) retained at least **P. juncina** and **P. dilutella.**

Only in the 20th century a typification was made and that just four times, although CANNON et al. (1985) stated: "Typus: Not designated.", a statement which is only right if it refers to the original author:

1. BACHMAN (1909): **Pezizella sordida** (Fuckel) Fuckel
2. v. HÖHNEL (1926): **Pezizella avellanae** (Lasch) Fuckel
3. CLEMENTS & SHEAR (1931): **Pezizella granulosella** (Karst.) Rehm
4. SVRČEK (1983): **Pezizella pulchella** (Fuckel) Fuckel

Let us have a close look at these typifications in their chronological order and then analyze the results referring to their nomenclatural significance.

BACHMAN (1909 p. 55) wrote - without going into detail: "Type species, **Pezizella sordida** Fuckel." In the diagnosis of the genus she also described the apothecia as "sessile," surely influenced by REHM's flora.

v. HÖHNEL (1926) designated **P. avellanae** (Lasch) Fuckel as the type specimen ("Gründungsart"), without adequate reasons, too. From his publication about fungi it can be shown that he used the obsolete "first species rule" for his typifications: "Als erste, also Typusart, erscheint der Name Naevia scripta Fr." (1917, p. 300) (7).

CLEMENTS & SHEAR (1931) selected **P. granulosella** (Karst.)

(3) E.g. KARSTEN (1871, 1885), BRESADOLA (1881), PATOUILLARD (1883), BOUDIER (1885), QUELET (1886), GILLET (1887), PHILLIPS (1887) and MASSEE (1895).

(4) SCHROETER (1893), LINDAU (1894).

(5) HAZSLINSKY (1886) and FELTGEN (1898 ff) mentioned species of **Pezizella** in their floras, too; as did BOMMER & ROUSSEAU (1890); but these two also characterized the apothecia as "sessile ou subsessile".

(6) As REHM placed all of the original species of **Pezizella** elsewhere, he founded a later homonym (cf. also v. HÖHNEL 1926). In other words this was already emphasized by STARBÄCK (1895, p. 28): "... die von Fuckel (Symb. p. 299) aufgestellt wurde, jetzt aber von REHM ganz anders begrenzt wird,..." Though also VELENOVSKÝ (1934) did not retain any of the original species in his treatment of **Pezizella**, nevertheless he did not create a second homonym, because he only dealt with **P. juncina, rubella** and **pulchella** in his opus.

(7) Cf. also NANNFELDT (1932, p. 7).

Rehm as the type species, which, however, was not among the six original species. Probably with their choice they intended to contribute to stability and uniformity in using the names of genera, as they also emphasized in the preface of their opus. (8) I presume, they took REHM's influential publications as a basis for their typification, for he was the most recognised and appreciated discomycete scientist world-wide until his death 1915.

According to SVRČEK's (1983) considerations, "Fuckel's **Pezizella pulchella** is the only one species agreeing perfectly with all essential features in the original generic diagnosis." (l.c. p. 68), for **"Pezizella avellanae** (Lasch) Fuckel and **P. sordida** Fuckel are identical with **P. vulgaris** (Fr.) Höhnel and the last three belong to other genera (**P. juncina** (Pers.) Fuckel, **P. rubella** (Pers.) Fuckel, **P. dilutella** (Fr.) Fuckel)" (l.c. p. 68).

Thus we have four typifications with four different lectotype species:
1. **P. sordida** (Fuckel) Fuckel
2. **P. avellanae** (Lasch) Höhnel
3. **P. granulosella** (Karst.) Rehm and
4. **P. pulchella** (Fuckel) Fuckel,
but only **one** of these can represent the genuine type or, none of the four, conceivably, typifies the genus.

In compliance with the rules of the ICBN the "typus generis" has to be selected from among the six species which FUCKEL assigned to his genus, so that CLEMENTS & SHEAR's typification is invalid. V. HÖHNEL's choice has to be excluded, too, because it resulted from the "first species rule" and BACHMAN's typification antedates it by 17 years. Hence BACHMAN's and SVRČEK's typification remain to be considered.

On the strength of nomenclatural considerations there is nothing to argue against BACHMAN's choice, even if it was made without advancing arguments:
1. **P. sordida** belongs among the six original species of the genus.
2. The description of the genus, which is based rather on physical habits and gross morphology, is ± compatible with **P. sordida**, at least grave contradictions do not arise.
3. The type was distributed by FUCKEL in his Fungi rhenani # 2078, thus the species is easily available.

Consequently her typification is the first one which corresponds with the rules of the ICBN. According to Art. 8.1 it is obeyed and the use of the name **Pezizella** can

(8) "...This in many cases necessitates the choice of a species not (underlined by the author) included by the original author of the genus." (l.c., p. 15). This typification corresponds to the use of the name by REHM and other mycologists following him, but it contradicts the rules of the ICBN.

only be annulled by conservation (Art. 14) or rejection (Art. 69) or "if (b) it can be shown that it is in serious conflict with the protologue and another element is available which is not in conflict with the protologue" (ICBN 1988, Art. 8.1, p. 11).

Only in the latter case SVRČEKS' s typification has not to be rejected. Then, however, **Pezizella** becomes a synonym of **Cistella** (= **Clavidisculum**), **Lachnum** (= **Dasyscyphus**), respectively; for the study of a syntype specimen of **P. pulchella** (Fuckel) Fuckel proved without doubts its identity with **Cistella acuum** (Alb. & Schw.) Raitv. (= **Dasyscyphus acuum** (Alb. & Schw.) Sacc. (Cf. also ARENDHOLZ & RAITVIIR 1988). In each case the nomenclatural problem of "PEZIZELLA" is solved in any event: since BACHMAN's typification cannot be ignored, we can say with v. HÖHNEL (1926): "Es steht nun fest, was Fuckel unter Pezizella verstand."

III. Allophylaria - a Synonym of Pezizella or vice versa?

NANNFELDT (1932), too, dealt with the **Pezizella** problem thoroughly and concluded: "Es scheint mir, als ob der Standpunkt v. Höhnels, **P. vulgaris** (9) als Typusart einer emendierten Gattung zu betrachten, nicht zweckmäßig sei: Eine Stütze seiner Ansicht ist auch nicht vorhanden. Es dürfte zweifellos das Richtigste sein, den ein Jahr jüngeren Namen, **Allophylaria**, beizubehalten, mit dem ihr Auctor es verstanden hat, eine höchst natürliche Einheit zu umgrenzen und zu beschreiben" (l.c. p. 290).

His argumentation is not absolutely reasonable (what is the meaning of: "nicht zweckmäßig" ?) and contradictory: on the one hand he rejected the typification and emendation with **P. avellanae** with scarcely convincing arguments on the other hand he exactly synonymized this genus **Pezizella**, using v.HÖHNEL's typification, with Allophylaria (10) (l.c. p. 289: "Syn. **Pezizella** Fuckel. Symb. Myc. 299 emend. v. Höhnel .... (Pseudotypus: **Peziza avellanae** Lasch (= **P. vulgaris** Fr.)"). Furthermore his proceeding is not logical: before a non-typified genus can be synomized, a "typus generis" must be designated.

His typification of the genus **Allophylaria** seems to be without contradiction. If we, however, pursue the use of this name in somewhat more detail, some absurdities arise: for the first time in 1869 KARSTEN used the name as section VI of the (collective) genus **Peziza.** In this section he placed two species: **P. eucrita** Karst. and **P. sublicoides** (11).

(9) = Pezizella avellanae (Lasch) Fuckel.

(10) The genus is absent in FARR et al. (1979) and CLEMENTS & SHEAR (1931).

(11) In the index KARSTEN himself (1869, p. 205) changed the epithet : "**Peziza sublicoides** in **P. sublicaeformen** est emendanda". If the index is published at the same time as the main part, **P. sublicaeformis** is valid, otherwise this name is il-

In the following year (1870, p. 243) he raised this section to generic rank and included four species within it: **A. eucrita** (Karst.) Karst., **A. sublicaeformis** (Karst.) Karst., **A. clavuliformis** (Karst.) Karst. and **A. byssacea** (Karst.) Karst. He affixed a question mark to the last three mentioned species: I supposed he was in the dark about the membership of these to **Allophylaria**.

Again one year later (1871) he considered his genus as a synonym of **Pezicula** Tul. and described in the following order six species:
1. **P. subliciformis** (Karst.) Karst., 2. **P. clavuliformis** (Karst.) Karst., 3. **P. byssacea** (Karst.) Karst., 4. **P. myrtillina** Karst., 5. **P. eucrita** (Karst.) Karst. and 6. **P. phyllophila** (Desm.) Karst. Nearly 15 years later (1885) he restored the genus to life, this time with four species, i.e. **A. subliciformis**, **A. clavuliformis**, **A. byssacea** and **A. phyllophila**. Until its resuscitation by NANNFELDT (1932) the name sank in a long sleep, apart from three new species descriptions by KARSTEN (1889) (12), CLEMENTS (1903) (13) and SPEGAZZINI (1926) (14) and the synonymizing with **Hyaloscypha** by BOUDIER (1907) (15).

KARSTEN himself never designated a "typus generis." According to the ICBN the type has to be selected from among the two species mentioned in the protologue. Doing this it would be wise not to fall back on a species which one year later (1870) was labelled by him with a question mark. From this viewpoint **P. eucrita** Karst. (= **Pezicula eucrita** (Karst.) Karst.) would be a suitable choice, especially as he himself synonymized his genus with **Pezicula** in 1871. In this case **Allophylaria** fide KARSTEN 1870 would become a synonym of **Pezicula** (16). Thus I cannot help feeling that in 1885 KARSTEN unconsciously created a later homonym, on which NANNFELDT (1932) founded his interpretation of **Allophylaria**, for he stated: "Sichere Arten dieser Gattung sind die Karstenschen **A. subliciformis**, **A. clavuliformis**, **A. byssacea** und **A. phyllophila...**," although he apparently did not study a single of these species, which KARSTEN regarded as belonging to **Allophylaria**; at least he did not mention any

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legal (Art. 63.1 ICBN). According to STAFLEU & COWAN (1979) KARSTEN's work was published between "2 Oct - 6 Nov 1869". I interpret this to the effect that the exact date of publication is unknown, and the article itself was published as one complete unit.

(12) **Allophylaria terrigena** Karst.

(13) **Allophylaria senecionis** Clem., although this species was described by CLEMENTS, he did not mention the generic name neither as a genus of its own or as a synonym in his "Genera of Fungi," published in 1909.

(14) **Allophylaria cordobensis** Speg.

(15) This synonymy is without any foundation, especially since none of KARSTEN's species is synonymized.

(16) **Peziza eucrita** Karst. was mentioned by NANNFELDT (1932) as a synonym of **Pezicula livida** (B. & Br.) Rehm.

examination in his work. The listed order of the species coincides exactly with KARSTEN's (1885) and probably was adopted from him.

NANNFELDT surely endeavoured to maintain KARSTEN's order, for it is very improbable that he got this sequence by chance. He expanded the genus to include six additional species, which I interpret as an emendation. Consequently he should have designated the type species of **Allophylaria** as "Neotypus," (17) but he only wrote "Typus."

The above mentioned statement offers a further, surprising interpretation to the typification of **Allophylaria**: under the Codes in effect from 1969 to 1987, KARSTEN could be said himself to have typified (1885) the genus by means of of "schizotypification," for he excluded all (**Peziza eucrita** Karst., = **Pezicula eucrita** (Karst.) Karst.) but one (**Peziza sublicoides** Karst.) of the original taxa. If this is accepted as the first valid and uncontradicted typification, which would coincide with NANNFELDT's choice, **Allophylaria** represents a genus of its own, which because of its anatomical characters (especially the jodine-positve asci and the large spores) cannot be united with **Pezizella**. But the use of "schizotypification" was outlawed at the Berlin Congress in 1987, with the result that **Allophylaria** could still be considered a synonym of **Pezizella** if NANNFELDT's neotype is not accepted. Finding no difficulty with NANNFELDT's conclusion, I accept that neotypification and believe that **Allophylaria** and **Pezizella** are not synonyms. Hence it will hardly be necessary to ascertain which of the names enjoys priority, because the two were both published in 1870, a problem which only would have to be solved if **Allophylaria** and **Pezizella** are synonyms.

IV. Peziza sordida Fuckel and Peziza avellanae Lasch - Synonyms of Peziza vulgaris Fr. ?

The lectotype species of **Pezizella** is **Pezizella sordida** (Fuckel) Fuckel, which was synonymized with **Pezizella avellanae** (Lasch) Fuckel (v. HÖHNEL 1926, NANNFELDT 1932, DENNIS 1956). **P. avellanae** itself was synonymized by KARSTEN (1870, 1871, 1885) with **Peziza vulgaris** Fr. (as **Helotium albellum** (With.) Karst.). This synonymy was accepted by REHM (1893), v. HÖHNEL (1926), NANNFELDT (1932) and DENNIS (1956), so that we have to discuss the above mentioned synonyms.

I studied several syntype specimens of Peziza avellanae and additional specimens filed under this name, (18) but never could find either asci, basidia or spores, although

(17) "Den Terminus 'Neotypus' habe ich für diejenigen Arten angewendet, die bei der Emendierung einer Gattung als Typusart zu betrachten ist." NANNFELDT (1932, p. 8).

(18) Fungi rhen. # 2079, Rehm Asc. # 63.

fruiting bodies of different size and of different stages were cut. Thus the argument of overripe fruting bodies being studied is excluded. The question, whether an ascomycete is "hidden" behind this name, could not be clarified by light microscopical studies, and hence its solution is reserved for ultrastructural examinations, which, however, requires living specimens or for new technologies which allow one to do gene sequencing from herbarium specimens. Still not today, but in a few years; this will put a whole new light on just how valuable herbarium specimens are.

But nevertheless there are some significant differences between **Peziza avellanae** and **P. sordida**, even without the characters of the hymenium:

1. In **P. avellanae** the hyphae of the ectal excipulum, which are embedded in a gelatinous matrix in both species are clearly narrower in diameter and arranged more irregularly.

2. Macroscopically the receptacle of P. sordida shows a somewhat downy surface under the stereo- and/or reflected-light microscope, whereas **Peziza avellanae** looks smooth.

3. In 3% to 40% KOH dried fruting bodies of **P. avellanae** - oddly enough - never soak up completely, even being allowed to react up to 24 hours (a character which I also noticed in studying fruting bodies of **Guepiniopsis**, a basidiomycete). The apothecia of **P. sordida** reach their "life size" at the latest after 15 seconds under such tests.

On the basis of the above mentioned characters and qualities, I do not consider, in contrast to REHM (1893), v. HÖHNEL (1926), NANNFELDT (1932) and DENNIS (1956) - **P. avellanae** conspecific with **P. sordida**, which is in fact what FUCKEL also noted, since he distributed the two separately.

Because of the outlined obscurities **P. avellanae** is scarcely suitable to serve as a type species for the genus **Pezizella**, as v. HÖHNEL (1926) chose, quite apart from the fact that his typification was antedated by BACHMAN's in 1907 and from his use of the "first species rule" (cf. p. 286).

KARSTEN (1870) was the first to state the synonymy of **P. avellanae** with **P. vulgaris** which he also maintained in 1871 and 1885. SACCARDO's (1889) opinion is confused: on the one hand he quoted **P. avellanae** as a synonym of **Pezizella albella** (With.) Sacc. (about **Peziza albella** see below) (l.c. p. 280), a species which he, following FRIES (1823), considered as identical with **P. vulgaris** Fr. On the other hand (l.c. p. 278), he turned the tables and

synonymized **Peziza albella** with **Pezizella vulgaris** (Fr.) Sacc. non **Pezizella vulgaris** (Fuckel) Sacc. (19). This is nomenclaturally untenable because a taxon "...with a circumscription, position and rank can bear only one correct name" (ICBN 1988, Art. 11, p. 14). REHM (1881) quoted **P. avellanae** as **Helotium albellum.** In 1893, however, he transfered this species as **Phialea vulgaris** (Fr.) Rehm to **Phialea.**

According to the short diagnosis one cannot recognize unquestionably which fungus FRIES meant by this name. In his herbarium in Upsala only one collection made by ROBERGE in France was found, which, however, did not contain any apothecia, and which judged by the label appears to be identical with DESMAZIÈRES's Pl. crypt. France ed. I # 1065. As FRIES (1823 p. 147) stated having seen **P. vulgaris** alive, I suppose DESMAZIÈRES's specimen could be perhaps identical with it, whereby a solution of the problem **P. vulgaris** could be offered.

But a study of the specimen from Paris was disillusioning: the sample only showed poor remains of fruting bodies, a duplicate from Genf was in better condition indeed, but no asci or spores could be found. The macroscopic and anatomical characters and the reaction in KOH were identical with **P. avellanae**, so that you can suppose with some probability that **P. vulgaris** Fr. sensu Desm. is conspecific with **P. avellanae** Lasch. This presumption does not clear either the identity of **P. avellanae** or that of **P. vulgaris**: whether DESMAZIÈRES's interpretation is really in accordance with FRIES's fungus has to remain unsettled, because - as already mentioned - it is not possible to tell from the brief description which fungus he had in mind, especially since FRIES classified it with the tribus "XI **Mollisia.**" **P. vulgaris** could be a **Mollisia** according to today's interpretation. Because of the sketchy facts, I refrain from designating a neotype for **P. vulgaris** at the moment, especially since this name has no

(19) **Pezizella vulgaris** (Fuckel) Sacc. and **P. vulgaris** (Fr.) Sacc. are simultaneous homonyms which have equal priority, so that "the first of them that is adopted ... by an author who simultaneously rejects the other(s) is treated as having priority" (ICBN Art. 64.5, p. 67, 1988). This has not been done hithereto. Acccording to to my studies **Niptera vulgaris** Fuckel is identical with **Pezizella conorum** Rehm (cf. also v. HÖHNEL, 1926), the epithet of which is of doubtful validity on which already KEISSLER (1912) called attention to. In a further paper I shall return to this problem. These specimens are not congeneric with **Pezizella sordida** and until further notice they will stand as **Cystopezizella conorum** (Rehm) Svr. (= **Niptera vulgaris** Fuckel, = **Pezizella vulgaris** (Fuckel) Sacc. nom. illeg. ICBN 1988, Art. 64.5).
As Pezizella vulgaris (Fr.) Sacc. is "the homonym for the taxon that is not renamed (it) is treated as having priority" (l.c. p. 67). V. HÖHNEL (1926), by the way, created a third homonym **P. vulgaris** (Fr.) HÖHNEL, although MASSEE (1895) used **P. vulgaris** (Fr.) Sacc. as a synonym of **Pseudopeziza albella** (With.) Massee. MÜLLER (1977) classified **Peziza vulgaris** Fr. with **Hymenoscyphus: H. vulgaris** (Fr.) Raschle et Müller. This is a later homonym of **H. vulgaris** (Fr.) Lindau.

significance concerning the typification of the genus **Pezizella.**
FRIES (1823) synoymized **P. vulgaris** with **P. albella** With. (20).Indeed WITHERING never published a fungus with this name, but the one to which FRIES referred, is called **P. albida** With. (WITHERING 1796, p. 350). Already STEUDEL (1824) (21) called attention to this error and was followed by PHILLIPS (1890), NANNFELDT (1939), and SEAVER (1951). WITHERING's fungus is clearly larger, found on a different substrate, and undoubtedly not identical with FRIES's **P. vulgaris.** According to NANNFELDT it is identical with, or closely allied to **Peziza Adae** Sadl." (1.c., p. 244).

V. Calycina Nees - an older name for Pezizella Fuckel and Cystopezizella Svrček?

Recently BARAL (in KRIEGELSTEINER & BARAL 1985) dealt with the genus **Pezizella**, which he synonymized with **Calycina** (Nees) Gray: "**Pezizella** Fuckel 1870 ss. Dennis pp." At the same time he considered **Cystopezizella** Svr. (SVRČEK 1983) as congeneric with **Calycina**, consequently **Calycina** must be congeneric with **Pezizella**, too.

A brief statement and explanation of my position is imperative: it is safe to say that **Pezizella** is **not** congeneric with **Cystopezizella** Svr. (type: **Pezizella conorum** Rehm). The two types differ clearly from each other not only in the characters of the excipulum but also in those of the hymenium. If these characters were not accepted as differences, no classification in genera and accordingly no systematics of the Leotiales would be realized. The same holds for the differences of **Calycina** and **Pezizella.**

The problem **"Calycina = Cystopezizella"** is somewhat more complicated: The name **Calycina**, which was raised by GRAY (1821) to generic rank is traced back to NEES von ESENBECK (1817). The genus itself was lectotypified with **Peziza herbarum** Pers. by DUMONT (1972) (22). This species was

(20) CLEMENTS & SHEAR (1931) lectotypified **Phialea** (Pers.: Fr.) Gillet with this name; cf. also CARPENTER (1981).

(21) Thus already one year after the publication of the Systema: "Vitiose in Friesio: albella" (l.c. p.330). In the "Index alphabeticus" (FRIES, 1832) "albella" is missing, but "albida" is listed and with that sanctioned. Even KUNTZE (1898) used the "phantom:" **Hyaenoscypha albella** (Fr.) Ktze.; the combination **Hyalinia albella** (With.) Boud. is just as absurd.

(22) "Erste Sippschaft. Familia prima ... (Calycinae)." NEES ranked with this "Sippschaft" four species: **P. sphaerioides** Roth., **P. urceolus** Alb. & Schw. **P. herbarum** Pers. and **P. pallescens** Pers. The first and the third were studied by himself and accampanied by illustrations. Furthermore he ranked with "Calycinae alle gestielten (Arten) aus Persoons 5ter Abtheilung (E.) Coriaceae, siccae" (l.c. p. 264). Thus DUMONT's statement, "he (NEES) included four species" (DUMONT l.c. p. 913) is incorrect and an additional 15 species have to be taken into consideration as a possible lectotype. It is, however, right to reject SEAVER's

described by PERSOON (1794), who also listed it in his later publications (e.g. 1797, 1801, and 1822, however not 1796/1799). As I often stumbled on **P. herbarum** during my studies of the genus **Pezizella**, and, as I agree with KORF (KORF in KRIEGELSTEINER & BARAL 1985) that this species is out of place in **Hymenoscyphus**, I wanted to study PERSOON's specimen, which was made available to me from Leiden generously. I got six collections with this name. As PERSOON did not designate a holotype a lectotype specimen has to be choosen from these:
Three specimens could be excluded from the beginning, according to the hand-writing two are collected and labeled by himself: 1. "**Peziza** affinis **P. herbarum** (substrate is not named, fragments of twigs) and 2. "**Peziza herbarum** var." (fragments of twigs, too). In addition both gatherings are named with the entry "Hb. Pers." The third is entitled "Desm. in Hb. Pers., No. 9 **Peziza herbarum** ? Pers."

The remaining three specimens which according to the substrate were suitable as a type are labeled "Hb. Pers., Chaill. in Hb. Pers., Moug. in hb. Pers.," respectively. The first mentioned specimens ("Hb. Pers., Prope Gottingen (sic!) lecta"), would be first quality, from which you can conclude with some probability that it was collected by himself, for PERSOON (born 1761/62 in South Africa) studied medicine and natural history in Göttingen from 1787 to 1802. Unfortunately he did not specify the date of collection. Hence it is uncertain whether he described **P. herbarum** by means of this specimen, for just as well it is possible that he only collected it after 1794, so that, in my opinion, this does not appear qualified as a lectotype, and I disregard it as a neotype, too, because the material is rather scanty.

The remaining specimens, collected by CHAILLET and MOUGEOT, certainly got into PERSOON's hands only after 1794, for, according to the hand-writing the lettering does not date from him, but the label already bears the name "**Peziza herbarum** Pers." Thus neither of the two specimens can be used as lectotype specimen, too, even though PERSOON did not add critical notes and hence surely accepted them as identical with "his" **P. herbarum**. I select MOUGEOT's gathering (# 910.261-431) as the neotype of **P. herbarum** Pers., because the substrate is designated and the original description of the apothecia corresponds with the specimen. The microscopic study of this and of PERSOON's and CHAILLET's specimens showed that the excipu-

typification, who typified this genus with **Peziza firma** Pers. in 1934, because this species was not mentioned even among the 15 additional ones. PERSOON (1822) considered NEES's **P. herbarum** as a synonym of his **P. scutula** Pers. In this interpretation he was followed by all later authors, e.g. FRIES (1823), WALLROTH (1831) and Rabenhorst (1844). Thus, if PERSOON's interpretation is right, the typification of **Calycina** with **Peziza herbarum** Pers. nec Nees becomes questionable, too, because this species does not belong to the "Gründungsarten."

lum is not composed of rectangular cells (textura prismatica to textura angularis), as it is delineated by DENNIS (1956), but of extended, narrow and strongly interwoven hyphae, the arrangement of which is difficult to follow not only in longitudinal sections but also in crush mounts. Also the downy structure of the surface of the receptacle can hardly be seen. The spores correspond well in form and size (14-16 x 2.5 µm) as in the number of the septa (two-celled) with those represented by DENNIS (1956) and BREITENBACH & KRÄNZLIN (1981). The asci, however, are clearly shorter (not exceeding 60 x 6 µm).

If these species belong to **P. herbarum**, in spite of the outlined differences, the value of the excipulum as a systematic character again would be made dubious. It is rather more probable that the name "herbarum" was and is used by different authors for different species, which also my own gatherings on **Urtica** indicate. However, studies hereto are not closed . If this presumption will be corroborated in the last analysis, **Calycina** and **Cystopezizella** surely are not congeneric and the placement of **P. herbarum** remains vague, too.

VI. The generic names Eubelonis Clem. and Eubelonis Höhnel

CLEMENTS (1909) founded the genus **Eubelonis** with **Helotium drosodes** Rehm. V. HÖHNEL (1918) transferred this species to **Belonioscypha**. In 1926 he placed a fungus in **Eubelonis** which was determined by SACCARDO (Mycoth. veneta # 1509) as **Helotium subcarneum** (Schum.) Sacc., and which he named **Eubelonis albosanguinea** Höhn. According to the rules of the ICBN v. HÖHNEL thus created a later homonym, **Eubelonis** Höhnel, the use of which as a name of a genus is invalid (cf. also CARPENTER 1981). GRADDON (in CLARK, 1980) thought this species belongs to **Pezizella**. This was not confirmed by my investigations of the above mentioned specimen. **E. albosanguinea** Höhnel seems to be a member of a genus of its own, for no suitable place in an alraedy existing genus has been found until now. My efforts on this are still incomplete.

The study of **Helotium drosodes**, the type species of **Eubelonis** Clem., produced a surprising result: a free and easy classification with **Pezizella** is allowed not only by the anatomical characters of the excipulum but also by the structures of the asci, so that we regard **Eubelonis** Clem. as a synonym of **Pezizella** until further notice.

In this connexion we have to examine a further synonym, which was mentioned by v. HÖHNEL (1926), viz. **Ctenoscypha**, more detailed **Pezizella** subgenus **Ctenoscypha** Starb. Within the scope of a short treatment of **Pezizella** this subgenus was founded by STARBÄCK (1895) together with the subgenus **Eupezizella**. He placed into **Ctenoscypha** -in the following order - **Pezizella dilutelloides** Rehm, **P. helotioides**

Starb. and, with some doubt, **P. punctiformis** (Grev.) Rehm. V. HÖHNEL (1.c.) regarded P. **dilutelloides** as the "Grundart" of **Ctenoscypha** (once again according to "the first species rule") because in his opinion it is constructed completely as in **P. vulgaris**, thus **Ctenoscypha** would be identical with **Pezizella.**

Although some similarities in structure of the excipulum are recognizable between **P. sordida** and **P. dilutelloides**, the structure of the asci (the apices of the latter are ± thickened and with and without KOH pretreatment iodine-positive) argues against v. HÖHNEL's position: in my opinion these differences are adequate to do not classify **P. dilutelloides** with **Pezizella**, i.e. **Pezizella** Fuckel subgenus **Ctenoscypha** is not a synonym of **Pezizella** Fuckel (23).

VII. The genus Pseudohelotium Fuckel

A further genus which is frequently mentioned in connexion with **Pezizella**, is **Pseudohelotium** Fuckel (24)(e.g. DENNIS 1960, 1968, 1978).(25) It was also founded by FUCKEL (1870) with:

1. **Pseudohelotium pineti** (Batsch) Fuckel
2. **Pseudohelotium puberulum** (Lasch) Fuckel
3. **Pseudohelotium hyalinum** (Pers.) Fuckel.

Again he did not designate a "typus generis," this only being done by v. HÖHNEL (1923) and CLEMENTS & SHEAR (1931). In each case the authors selected the first species: v. HÖHNEL anew used the "first species rule"(26). Whether CLEMENTS & SHEAR adopted his typification, or whether they typified the genus themselves, is unclear. In the bibliography of their book, in any case, v. HÖHNEL's posthumous published work cannot be found. Together with their typification CLEMENTS & SHEAR synonymized **Pseudohelotium** with **Belonium** Sacc. and typified this genus with exactly the same species. As **Peziza pineti** does not belong to the "Gründungsarten" of **Belonium** it is out of question as a type for this genus (cf. also KORF, 1978). If we, following KORF, accept the typifications by CLEMENTS & SHEAR as not arbitrarily, i.e., as a result of the "first species rule" (27) and, as far as they do not violate other rules of the ICBN, **Pseudohelotium pineti**

(23) Already NANNFELDT (1932) and ARENDHOLZ (1979) expressed doubts about the membership of this species to **Pezizella.**
SEAVER (1951) synonymized **Pezizella** together with **Myridium** Clem. (Type: **Calloria myriospora** Phill. & Hark. = **Laetinaevia myriospora**; cf. HEIN, 1976) and **Orbilia** Fr. This is entirely unfounded.

(24) FARR et al. (1979): "T.(ypus): non designatus".

(25) "The general aspect is that of a **Pezizella** with acicular spores" (1.c., 1978, p. 126).

(26) "Diese Gattung (**Pseudohelotium**) stellt Fuckel (Symb. myc. 1869, p. 298) auf Grund von **Peziza Pineti** Batsch 1786 auf" (1.c., p. 113).

(27) "In no manner of thinking can these designations be considered 'first cited species as type' example" 1.c., p. 494.

(Batsch) Fuckel is the "typus generis" of **Pseudohelotium**, for which a neotype has to be designated, because in BATSCH's herbarium in Jena no specimen remains.

VIII. Some systematic consequences

If we use **Pezizella** in the sense of **Pezizella sordida**, some akward consequences arise:

1. Nearly all species bearing the name **Pezizella** (e.g. in CANNON et al., 1985, ENDERLE in KRIEGELSTEINER, 1983 etc.) have to be removed from the genus and to be transfered to existing and/or many times in newly-to-be-founded genera.

2. The second consequence, which does not arise from the typification of the genus **Pezizella** itself, and which is connected with it only indirectly, viz. by using the characters of the genus to delimit it from neighbouring ("related") ones, is of greater significance, because, used for other genera and families of the Leotiales, it could change the system (classification) of the order greatly: the systematic value of the characters, which we learn from the species, are at stake.

The delimitation and independence of Pezizella from "related" genera (e.g., **Bisporella**, **Stamnaria**, **Crocicreas** etc.) is not realized totally by the structure of the excipulum. In addition to the excipulum I directed my attention to the structures of the asci, which in **Pezizella** are rounded at the apex. The lateral walls and the apex itself are of equal thickness. They are jodine-negative in MELZER's reagent (and also in LUGOL's solution) both without or with KOH pretreatment (in contrast to e.g. **Bisporella** Sacc.), a character which is predicted by light optic analysis: the often linear wall thickening, which is recognized exceptionally clearly in differential interference contrast microscopy according to Nomarski, but it is also observed by trained eyes in bright field microscopy, is missing in **Pezizella sordida.**

In other words, if these (linear) light-optic discernible thickenings always exist at the ascus apex, the asci of the Leotiales are jodine-positive. Because of the smallness of these structures, a statement about their structure is impossible, for true images of structures are only possible if these reach a dimension of 1-4 µm, hence roughly five- to tenfold of the lateral resolution. For less than 1-4 µm it can only be ascertained whether "something is there," and morphological definitions of light microscopic images disappear in useless speculations (cf., e.g., ABBE 1873, ANONYMUS 1971, KRAMMER 1979, 1981) (28).

(28) "...weil immer wieder die Auflösungsgrenze des Lichtmikroskops... mit der Auflösbarkeit morphologischer Strukturen gleichgesetzt wird." (KRAMMER 1979, p. 70); "Distinctions should always be made between resolving power and what can be

If these facts are not borne in mind errors and misunderstandings easily can arise by analyzing very small structures in the light microscope: e.g., as when BARAL (1987) stated that he saw four layers of even different thickness in a roughly 1 µm thick ascus wall in inoperculate asci (surely with reference to BELLEMÈRE's (1977) ultrastructural studies).

Similar considerations are put forward if structures the size of which touches the resolution of the light microscope should be found again in the TEM. A request such as: "Es sollte doch möglich sein, diese Strukturen (29) elektronenoptisch nachzuweisen und exakt zu lokalisieren!" (BARAL 1987, p. 123) is of little help, for it would be difficult to fix and embed a "structure", which is, e.g., due to diffraction and to prepare from that ultra-thin sections.

The non-observance of the above mentioned considerations may have contributed to the result, that the (three) "categories of asci" of the French school (cf., e.g., CHADEFAUD 1942, 1960, 1973) have not been accepted by many mycologists, because they could not reconstruct the observations of the corresponding characters. (30)

Nevertheless for a long time the structures of the asci have been used as an important (most important?) criterion in classifying the ascomycetes into higher taxa. Also at the level of families and genera these characters should yield reliable "tools" to distinguish between these, especially in correlation with other characters as, e.g., excipulum, spores, etc. Therefore we agree with HAFFELNER, who wrote (1984, p. 255): "Verschiedene Ascustypen dürfen in der Regel in einer Gattung (Familie) nicht vorkommen. Grundsätzlich ist also mit dem Ascustyp die Gattung (Familie) definiert." In my opinion, a further important principle is defined by this statement, which is often violated, although it is a commonplace, viz., that the characters defining, e.g., a genus are valid in all taxa, which are classified with in this genus. In other respects the circumscriptions of the genera would be watered down in such a manner that we cannot speak of genera any more, a situation which holds true for many genera of the Leotiales today. Yet I am absolutely aware that the species of a genus have to differ from the type species in definite characters, for otherwise at the end the number of the genera becomes nearly as great as the number

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seen with the microscope. Such differentiation is seldom clear to the microscopists" (NEEDHAM 1958, p. 223).

(29) Certain structures of the apical apparatus of the asci are meant.

(30) "A demonstration set up by Professor M. Chadefaud at the 1957 9th International Botanical Congress in Paris, France, especially to show the various ascus types failed to convince other ascomycete workers of the reality of the configurations seen in the ascus wall (pers. comm. with various mycologists)." (REYNOLDS, 1989, p. 15)

of the species. But a genus, as, e.g., described by CARPENTER (1981) for **Crocicreas**, in which the majority of the variations of morphological and anatomical characters recognized throughout the order are used, seems not very "natural" to me.

In my opinion only increased and joint efforts and co-operation of many mycologists in as many countries as possible will perhaps lead to a more natural system of the Leotiales, possibly with a change of the "paradigm." But I think it is still a long journey to that time.

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THREE NEW SPECIES IN THE LICHEN GENUS PARMELIA (PARMELIACEAE, ASCOMYCOTINA) FROM SOUTHERN AFRICA, WITH FURTHER NOTES

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ABSTRACT

Three new species of *Parmelia* (Parmeliaceae, Lichenized Ascomycetes) are described from southern Africa. They are: *Parmelia festiva* Brusse, *P. infausta* Brusse and *P. ponderosa* Brusse. Two new combinations are made: *Parmelia areolata* (Hale) Brusse and *Parmelia colensoica* (Nash, Elix & Johnston) Brusse. One new lichen record for southern Africa is reported, and one is deleted. Notes on three *Parmelia* species are given.

NEW SPECIES

Parmelia festiva Brusse, *sp. nov.* Fig. 3

Thallus minute foliosus, saxicola, usque ad 5 cm diametro, sat vel laxe adnatus. *Lobi* sublineares, 0,1–0,8 mm lati, 115–400 μm crassi. *Thallus superne* flavo-viridis, nitidus, pustuli-soraliatus, epicortice poroso. *Soralia* viridia, capitata, 0,2–0,8 mm diametris. *Soredia* granularia, 35–80 μm diametris. *Cortex superior* 15–25 μm crassus. *Stratum gonidiale* 35–75 μm crassum, algis *Trebouxiis* 5–18 μm diametris. *Medulla* albida, 20–230 μm crassa. *Cortex inferior* 5–15 μm crassus. *Thallus inferne* piceus, sat rhizinatus. *Rhizinae* simplices, 60–95 μm crassae. *Apothecia* adnata, usque ad 1,2 mm diametris. *Hypothecium* hyalinum, 20–55 μm crassum, J −. *Subhymenium* hyalinum, 10–35 μm crassum, J −. *Hymenium* hyalinum, 50–60 μm crassum, J + caeruleum. *Asci* clavati, tholis J + caeruleis (figura 1). *Ascosporae* octonae, hyalinae, simplices, ellipsoideae, 8,5–11,5 × 5,5–7,0 μm. *Pycnidia* globosa, hyalina, 100–120 μm profunda, 80–100 μm lata. *Pycnidiosporae* longe aciculares, hyalinae, 12–22 × 0,8 μm, rectae vel subrectae. *Thallus* acidum usnicum, acidum rhizocarpicum, acidum gyrophoricum, acidum protoconstipaticum et acidum constipaticum continens.

TYPUS: SOUTH AFRICA, CAPE PROVINCE—**3319** (Worcester): Summit of Jona's Kop in the Riviersonderend Mountains near Villiersdorp. On Table Mountain Sandstone rocks on N slope. Alt. 1630 m (**−DC**). *F. Brusse 5454*, 21. iii. 1988 (**PRE**, holo-; **ANUC, BM, LD, US**, iso-). Figura 3.

Thallus minutely foliose, saxicolous, up to 5 cm across, moderately to loosely adnate. *Lobes* sublinear, 0,1–0,8 mm wide, 115–400 μm thick. *Upper surface* yellow-green, glossy,

pustular-soraliate, epicortex pored. *Soralia* green, capitate, 0,2–0,8 mm across. *Soredia* granular 35–80 μm diam. *Upper cortex* 15–25 μm thick. *Algal layer* 35–75 μm thick, algae *Trebouxia*, 5–18 μm diam. *Medulla* whitish, 20–230 μm thick. *Lower cortex* 5–15 μm thick. *Lower surface* black, moderately rhizinate. *Rhizines* simple, 60–95 μm thick. *Apothecia* adnate, up to 1,2 mm across. *Hypothecium* hyaline, 20–55 μm thick, J –. *Subhymenium* hyaline, 10–35 μm thick, J –. *Hymenium* hyaline, 50–60 μm thick, J + blue. *Asci* clavate, eight-spored, tholus J + blue (figure 1). *Ascospores* hyaline, simple, ellipsoid, 8,5–11,5 × 5,5–7,0 μm. *Pycnidia* globose, hyaline, 100–120 μm deep, 80–100 μm wide. *Pycnidiospores* long hyaline needles, 12–22 × 0,8 μm, straight to slightly curved. *Chemistry*: Usnic and rhizocarpic acids in the upper cortex, gyrophoric, protoconstipatic and constipatic acids in the medulla.

The occurrence of rhizocarpic acid has no precedent in the genus *Parmelia*, and causes the upper surface to fluoresce dull orange in long wave ultra-violet light. This unique species is also pustular-soraliate, which is quite uncommon among the *Xanthoparmeliae*, so far being known only in *P. ganymedea* Brusse (1988b) and *P. geckonalis* Brusse (1989; = *Xanthoparmelia saleruptens* Hale 1989) for southern Africa.

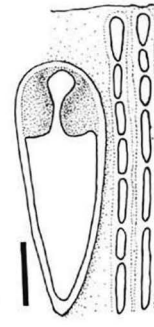

FIGURE 1. —*Parmelia festiva* Brusse, ascus and paraphyses. *F. Brusse 5454*, holotype. Bar = 10 μm.

Parmelia festiva could also be confused with *Xanthoparmelia olivetorica* Hale (1986), which it resembles both in general appearance and in chemistry, but is easily distinguished by the presence of pustular-soralia and the dull orange colour in long wave ultra-violet light, due to the presence of rhizocarpic acid in the cortex. The medullary chemistry of these two species is the same, both containing gyrophoric, protoconstipatic and constipatic acids. In fact, these two species grow together in the same spot on Jona's Kop, where the type material of *P. festiva* was obtained.

This new species is thus far known only from the type locality, the summit of Jona's Kop in the Riviersonderend Mountains closest to the town of Villiersdorp, in the south-western Cape.

Parmelia infausta Brusse, *sp. nov.* Fig. 4

Thallus minute foliosus, saxicola, usque ad 4 cm diametro, arcte adnatus. *Lobi* elongati, 0,2–1,0 mm lati, 70–220 μm crassi. *Thallus superne* viridis, nitidus, isidiatus, epicortice poroso. *Isidia* globosa vel sphaerica, simplicia (Fig. 5). *Cortex superior* 9–14 μm crassus. *Stratum gonidiale* 15–70 μm crassum, algis *Trebouxiis*, 6–23 μm diametris. *Medulla* alba, 10–120 μm crassa. *Cortex inferior* 8–10 μm crassus. *Thallus inferne* pallide brunneus, sat rhizinatus. *Rhizinae* parvae. *Apothecia* adnata, plana, usque ad 0,7 mm diametris. *Hypothecium* hyalinum, 10–50 μm crassum, J –. *Subhymenium* hyalinum, 10–20 μm crassum, J + pallide caeruleum. *Hymenium* hyalinum, 40–50 μm crassum, J + caeruleum. *Asci* clavati, tholis J + caeruleis (figura 2). *Ascosporae* octonae, hyalinae, simplices, ellipsoideae, 8,0–11,0 × 5,5–6,5 μm. *Pycnidia* globosa, hyalina, 90–110 μm profunda, 70–90 μm lata. *Pycnidiosporae* aciculares, hyalinae, 5–8 × 0,8 μm. *Thallus* acidum usnicum et norlobaridonum continens.

TYPUS: SOUTH AFRICA, TRANSVAAL—**2428** (Nylstroom): 5 km from the main Potgietersrus-Naboomspruit road to Sterk River, farm Waterval 297 KR. On talus rocks at base of SE Waterberg Sandstone cliffs. Alt. 1190 m (−**BD**). *F. Brusse 5613*, 31. v. 1989 (**PRE**, holo-; **ANUC, BM, LD, US**, iso-). Figura 4.

Thallus minutely foliose, saxicolous, up to 4 cm across, tightly adnate. *Lobes* elongate, 0,2−1,0 mm wide, 70−220 μm thick. *Upper surface* green, glossy, isidiate, epicortex pored. *Isidia* globose to spherical, simple (Fig. 5). *Upper cortex* 9−14 μm thick. *Algal layer* 15−70 μm thick, algae *Trebouxia*, 6−23 μm diam. *Medulla* white, 10−120 μm thick. *Lower cortex* 8−10 μm thick. *Lower surface* pale brown (tan), moderately rhizinate. *Rhizines* small. *Apothecia* adnate, plane (flat), up to 0,7 mm across. *Hypothecium* hyaline, 10−50 μm thick, J −. *Subhymenium* hyaline, 10−20 μm thick, J + pale blue. *Hymenium* hyaline, 40−50 μm thick, J + blue. *Asci* eight-spored, clavate, tholus J + blue (figure 2). *Ascospores* hyaline, monolocular, ellipsoid, 8,0−11,0 × 5,5−6,5 μm. *Pycnidia* globose, hyaline 90−110 μm deep, 70−90 μm wide. *Pycnidiospores* hyaline needles, 5−8 × 0,8 μm. *Chemistry*: usnic acid in the cortex, norlobaridone and an unidentified substance in the medulla.

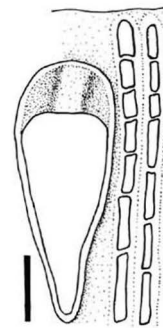

FIGURE 2.—*Parmelia infausta* Brusse, ascus and paraphyses. *F. Brusse 5613*, holotype. Bar = 10 μm.

This new species is similar to *Parmelia exillima* Elix (1981), but the latter is an even smaller species with lobes up to 0,5 mm wide and cylindrical isidia (Elix 1981, Elix, Johnston & Armstrong 1986). *P. infausta* has globose to spherical isidia (figure 5) and has lobes up to 1 mm broad. The ascospores of *P. infausta* seem slightly larger (8−11 × 5,5−6,5 μm) than those of *P. exillima* (7−9 × 4−5,5 μm. Elix, Johnston & Armstrong 1986).

Another similar species is *Xanthoparmelia lynii* Elix & Johnston (1988), but this is a larger and looser lichen with imbricated and rolled lobes. *X. lynii* contains a minor amount of loxodin with the norlobaridone, whereas *P. infausta* contains no trace of loxodin, but an unidentified substance instead.

At present this new species is known only from the type locality near Potgietersrus.

Parmelia ponderosa Brusse, *sp. nov.* Fig. 6

Thallus minute foliosus, saxicola, usque ad 4 cm diametro, sat vel laxe adnatus. *Lobi* sublineares, 0,1−0,8 mm lati, 70−220 μm crassi. *Thallus superne* viridis, nitidus, isidiis sorediisque destitutus, valde nigromarginatus, epicortice poroso. *Cortex superior* 18−30 μm crassus. *Stratum gonidiale* 30−65 μm crassum, algis *Trebouxiis*, 4−17 μm diametris. *Medulla* albida, 10−140 μm crassa. *Cortex inferior* 10−20 μm crassus. *Thallus inferne* piceus, sat rhizinatus. *Apothecia* non visa. *Pycnidia* globosa, hyalina, 100−110 μm profunda, circa 100 μm lata. *Pyniodiosporae* hyalinae, aciculares, 12−23 × 0,8 μm. *Thallus* acidum isousnicum, acidum gyrophoricum et duo pigmenta ignota continens.

TYPUS: SOUTH AFRICA, CAPE PROVINCE—**3419** (Caledon): Summit of Galgeberg near Greyton (but accessed via McGregor), farm Galge Berg. On S face of Table Mountain Sandstone cliff on steep S slope. Alt. 1410 m (−**BA**). *F. Brusse 5490*, 22. iii. 1988 (**PRE**, holo-; **LD**, iso-). Figura 6.

Thallus minutely foliose, saxicolous, up to 4 cm across, moderately to loosely adnate. *Lobes* sublinear, 0,1–0,8 mm broad, 70–220 μm thick. *Upper surface* green, glossy, non-isidiate and non-sorediate, strongly black-margined, epicortex pored. *Upper cortex* 18–30 μm thick. *Algal layer* 30–65 μm thick, algae *Trebouxia*, 4–17 μm diam. *Medulla* whitish, 10–140 μm thick. *Lower cortex* 10–20 μm thick. *Lower surface* black, moderately rhizinate. *Apothecia* not seen. *Pycnidia* globose, hyaline, 100–110 μm deep, about 100 μm wide. *Pycnidiospores* hyaline needles, 12–23 × 0,8 μm. *Chemistry*: isousnic acid in the cortex, gyrophoric acid and the two "schenckiana pigments" in the medulla.

This new species resembles *Parmelia endochromatica* (Hale) Brusse (1988b) rather uncannily, but contains isousnic acid instead of usnic acid and has much longer pycnidiospores. The occurrence of isousnic acid has a precedent in *P. lurida* Brusse (1988a), but this lichen contains stenosporonic acid as the major medullary substance (see below), and does not have strongly blackened lobe margins.

Although the original description of *P. endochromatica* (Hale) Brusse states that pycnidia are lacking (Hale 1986), pycnidia were in fact found on the isotype housed at PRE. The pycnidiospores of *P. endochromatica* are the usual hyaline needles, but they are only 6–8,5 μm long, compared to the 12–23 μm length of those of *P. ponderosa*.

For the rest, the two species are very similar, with strongly blackened margins, which give the internodes a constricted appearance, and the overall lichen a subsquamulose appearance. The medullary chemistry of *P. endochromatica* and *P. ponderosa* is the same.

Thus far, this new species is known only from the type collection, from the summit of Galgeberg in the Riviersonderend Mountains, near the village of Greyton.

NOTES AND NEW COMBINATIONS IN *PARMELIA*

Parmelia areolata (Hale) Brusse, *comb. nov.*

Basionym: *Xanthoparmelia areolata* Hale, Mycotaxon **29**: 251. 1987.

More material of this lichen collected from the northern Transvaal, shows that this is clearly a pustular species, and the use of the term 'isidia' in the original description is misleading. The isotype at PRE has warty dactyls up to 1 mm across, which are not yet pustulate, however. The additional material also shows that this is not a small species, and the lobes are between 1 and 2 mm broad, as can be seen on the lower part of the original photograph of the type.

Xanthoparmelia centralis Elix & Johnston is clearly not a pustular species (Elix, Johnston & Armstrong 1986, fig. 7).

Parmelia colensoica (Nash, Elix & Johnston) Brusse, *comb. nov.*

Basionym: *Xanthoparmelia colensoica* Nash, Elix & Johnston, Mycotaxon **33**: 355. 1988.

This is a rather similar species to *Parmelia stenosporonica* (Hale) Brusse, but contains colensoic and norcolensoic acids as major constituents instead of stenosporonic acid. This species has now been collected on Table Mountain near Cape Town some 80 km SW of the type locality, Bain's Kloof. So far, *P. stenosporonica* has not been recovered from the south-western Cape, and is only known from the Swartberg Mountains at high altitude.

SOUTH AFRICA. CAPE PROVINCE—**3318** (Cape Town): Cape Town, south side of summit of Table Mountain. On S face of Table Mountain Sandstone outcrop. Alt. 980 m (**–CD**). *F. Brusse 5418*, 19. iii. 1988 (**BM, LD, PRE**).

Parmelia inuncta Brusse, *Mycotaxon* **27**: 187. 1986.

The range of this lichen has been increased from the original Robinson's Pass to Table Mountain at Cape Town.

As mentioned by Brusse (1988b) the type material and description of *Xanthoparmelia olivetorica* Hale (1986) is based on a mixture of *Parmelia astricta* Brusse and a new lichen containing usnic and gyrophoric acids. However, this lichen is not at all related to *P. endochromatica*, as suggested (Brusse 1988b), but to *P. inuncta*. More material of *Xanthoparmelia olivetorica* was collected from Table Mountain (*F. Brusse 5441*, **BM, PRE**) and this material contains usnic, gyrophoric, protoconstipatic and constipatic acids. The paratype material of *X. olivetorica* (*Hale 72080*, **PRE**) has no detectable amounts of protoconstipatic and constipatic acids. These aliphatic γ-lactones show-up as white spots on TLC plates in long wave ultra-violet light, after developing with sulphuric acid and heat. These two names (which were published simultaneously) may therefore refer to the same species.

SOUTH AFRICA, CAPE PROVINCE—**3318** (Cape Town): Cape Town, east side of summit of Table Mountain near Maclear's Beacon. On low Table Mountain Sandstone pavement on gentle NE slope. Alt. 1040 m (**–CD**). *F. Brusse 5426*, 19. iii. 1988 (**ANUC, BM, LD, PRE, S, US**).

Parmelia lurida Brusse, *Mycotaxon* **31**: 157. 1988.

The chemistry of this species has been determined as isousnic acid in the cortex, and stenosporonic (major), divaronic (minor) and colensoic (minor) acids in the medulla, by Dr J. A. Elix, G. A. Jenkins and Jen Johnston (Canberra). This occurrence of isousnic acid was unprecedented in the *Xanthoparmeliae*, but has been found since in the unrelated *P. ponderosa* Brusse (see above).

Parmelia lurida must therefore be closely related to *P. stenosporonica* Hale, with the identical medullary chemistry and similar morphology. More material is required to clarify the status of this species.

Parmelia squamatica Brusse, *Mycotaxon* **27**: 242. 1986.

An isotype of this lichen, with a beautiful white fluorescent medulla in long wave ultra-violet light, has been deposited at **PRE**. The holotype is at **J**.

MISCELLANEOUS NOTES

Phyllopsora haemophaea (Nyl.) Müll. Arg.

This species was recently cited as a new record for the flora of southern Africa (Brusse 1988b), but this determination has been amended to *Ph. pannosa* Müll. Arg. (see below) on the basis of chemistry, and is hereby deleted.

Phyllopsora pannosa Müll. Arg.

The chemistry of a lichen cited as *Ph. haemophaea* (Brusse 1988b), was recently checked by TLC, and found to lack the 'haemophaea unknown', but to contain atranorin and a major terpene (R_f classes 6: 7–8: 6) as does *Phyllopsora pannosa* (Swinscow & Krog 1981). The determination of this material has therefore had to be amended to *Ph. pannosa*, despite the rather pale hypothecium. *Phyllopsora pannosa* is also a new record for the flora of southern Africa, and is herewith enstated as such.

Further material (*F. Brusse 1846*) has been distributed to **BM, CBG, COLO, E, O, S, UPS, UC, US**, over and above the original **LD** and **PRE**.

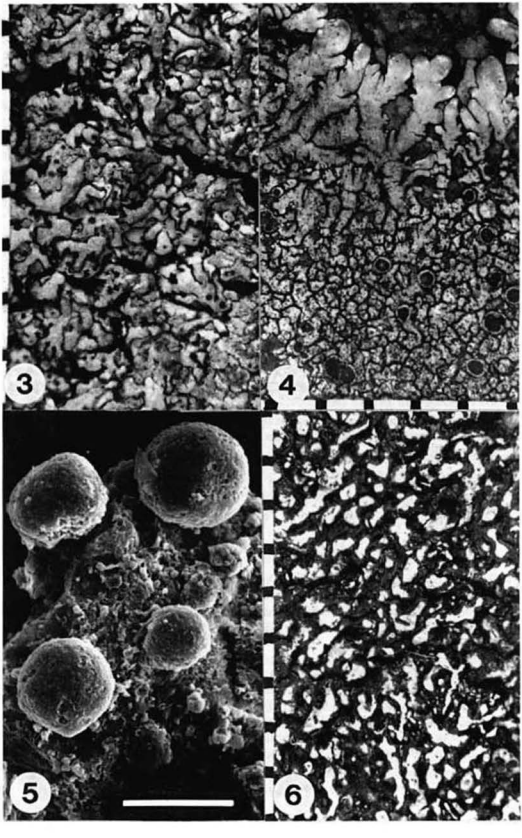
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FIGURE 3.—*Parmelia festiva* Brusse, habit. *F. Brusse 5454*, holotype. Scale in mm.

FIGURE 4.—*Parmelia infausta* Brusse, habit. *F. Brusse 5613*, holotype. Scale in mm.

FIGURE 5.—*Parmelia infausta* Brusse, scanning electron micrograph of the isidia. *F. Brusse 5613*, holotype. Bar = 100 μm.

FIGURE 6.—*Parmelia ponderosa* Brusse, habit. *F. Brusse 5490*, holotype. Scale in mm.

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