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CHEMOTAXONOMIC SIGNIFICANCE OF FATTY ACID COMPOSITION IN THE GENUS MORTIERELLA (ZYGOMYCETES, MORTIERELLACEAE)

NORIHIDE AMANO, YOSHIFUMI SHINMEN, KENGO AKIMOTO
HIROSHI KAWASHIMA and TERUO AMACHI

Institute for Fundamental Research, Suntory Ltd., Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

and

SAKAYU SHIMIZU and HIDEAKI YAMADA

Department of Agricultural Chemistry, Kyoto University, Sakyo-ku, Kyoto 606, Japan

SUMMARY

The fatty acid composition of 18 isolates in *Mortierella* subgen. *Micromucor* and 50 isolates in *Mortierella* subgen. *Mortierella* were analyzed by gas-liquid chromatography to explore the chemotaxonomic significance of fatty acid composition in the taxonomy and the identification of the genus *Mortierella*. The fatty acid composition appeared to be a useful chemotaxonomic marker in the genus *Mortierella* at the subgeneric level. Two subgenera were clearly distinguished from each other by their fatty acid composition; polyunsaturated C20 acids were detected only in *Mortierella* subgen. *Mortierella* isolates. The qualitative similarity in fatty acid composition between *Mortierella* subgen. *Micromucor* and mucoraceous fungi could offer supportive evidence for the determination of the true taxonomic position of the subgen. *Micromucor*. It is very interesting from the taxonomical, phylogenetic, and ecological point of view that polyunsaturated C20 acids were present in *Mortierella* species which is saprophytic and one of the most common soil fungi.

KEYWORDS: *Mortierella* , *Mortierella* subgen. *Micromucor, Mortierella* subgen. *Mortierella* , Zygomycetous fungi, Fatty acid composition, Chemotaxonomy

INTRODUCTION

Following Turner's recognition of *Mortierella isabellina* group (Turner, 1963), Gams (1977) subdivided the genus *Mortierella* into two subgenera, *Micromucor* and *Mortierella* in his key to the species of the genus *Mortierella*. They are distinguished from each other mainly by their morphological and cultural characteristics. The former subgenus is mainly characterized by its rather-slow growing, velvety colony with pigmented sporangia, on the other hand, the latter is mainly characterized by thin, spreading mycelium with hyaline sporangia and a garlic-like odour. His subdivision of *Mortierella* into two subgenera is now widely accepted. Several species of the subgen. *Micromucor* have distinctly *Mucor*-like characteristics (Benjamin, 1979), but they were tentatively placed in the genus *Mortierella*. The true taxonomic position of the subgen.

Micromucor is at present uncertain because of the absence of a known sexual stage, though von Arx (1983) raised the subgen. *Micromucor* to generic rank and placed it in the Mucoraceae sensu von Arx.

Shinmen et al. (1989) briefly mentioned that two subgenera of *Mortierella* clearly differed from each other in their fatty acid composition on the basis of their analysis of fatty acid composition of five isolates in subgen. *Micromucor* and five isolates in subgen. *Mortierella*. Their report suggested the usefulness of fatty acid composition as chemotaxomic characteristic in the taxonomy and the identification of the species of *Mortierella*.

Thus we further determined the fatty acid composition of 18 isolates in subgen. *Micromucor* and 50 isolates in subgen. *Mortierella* to explore the chemotaxonomic significance of fatty acid composition in the genus *Mortierella* .

MATERIALS AND METHODS

Microorganisms: Eighteen isolates assigned to *Mortierella* subgen. *Micromucor* and 50 isolates assigned to *Mortierella* subgen. *Mortierella* were used in this study. Names, culture collection number, and isolated source are listed in Table 1.

Culture media and growth condition: Stock cultures were maintained on slants of YM agar [1% (w/v) yeast extract (Difco), 1%(w/v) malt extract (Difco), 1%(w/v) glucose, 1.6% (w/v) agar, pH 6.0]. To obtain material for fatty acid extraction, the isolates were grown in liquid medium containing 2%(w/v) glucose and 1%(w/v) yeast extract(Difco). The pH of the medium was adjusted to 6.0 before the medium was autoclaved at 15 kgf/cm^2 for 15 min. In all cases 10 ml Erlenmeyer flasks with 2 ml of sterile medium were inoculated with mycelium scraped from the stock slant. After 4 to 7 days of incubation with reciprocal shaking at 28°C, mycelium was harvested by filtration. The mycelium was washed well with distilled water and then dried under vacuum in a centrifugal evaporator (RD-41, Yamato Co., Tokyo, Japan) at 50 to 60° C.

Extraction of fatty acids and preparation of methyl esters: The dried mycelium was suspended in 1 ml of methylene chloride, followed by the addition of 1 ml of 10% HCl in methanol. The mixture was boiled in a water bath for 2 to 3 h. Fatty acid methyl esters were extracted with 4 ml of n-hexane, and then the extract was concentrated under vaccum in a centrifugal evaporator at 30 to 40° C.

Fatty acid analysis: The fatty acid methyl esters were dissolved in 10 µl of acetonitrile and analyzed by gas liquid chromatography (GC09A, Shimadzu Corporation, Kyoto, Japan) on glass column (2m × 3 mm i.d.) packed with 15% diethylene glycol succinate on a 60/80 mesh Neopak 1A (Nishio Kogyo, Tokyo, Japan) equipped with a flame ionization detector. The gas carrier was nitrogen at a flow rate of 40 ml/min. Operation temperature of injection was 250°C with column isothermal at 210°C. Quantitative estimates of the areas under the peaks were obtained with the aid of an integrator (C-R3A, Shimadzu Corporation, Kyoto, Japan). Peak identification was made using fatty acid methyl ester standards and comparing them to the peaks in the sample.

RESULTS

The fatty acid composition of 18 isolates in subgen. *Micromucor* are presented in Table 2. The major fatty acids in all isolates studied were C16:0, C18:1, C18:2, and γ-C18:3. C18:0 was detected as a minor component in all isolates studied. Concentration of C18:1 was the highest of the four major fatty acids ranging from 36 to 46% of the total fatty acids in all isolates except for *M. ramanniana* var. *ramanniana* CBS 112.08. Unlike the other isolates, *M. ramanniana* var. *ramanniana* CBS 112.08 contained γ-C18:3 at 24% of the total fatty acids together with C16:0, C18:1, and C18:2 at 20.3%, 21.4%, and 19.8%, respectively. The polyunsaturated C20 acids were characteristically not detected in all isolates studied.

The fatty acid composition of 50 isolates in subgen. *Mortierella* are presented in Table 3. The polyunsaturated C20 acids were clearly detected in all isolates studied. C20:4 was detected in all isolates studied; C20:3 was also detected in most isolates together with C20:4; moreover, in five isolates traces or small amounts of C20:5 were also detected. In most isolates of subgen. *Mortierella* , either C18:1 and C20:4 or C18:1 and C16:0 represented the major fatty acids, while in *M. alpina* CBS 608.70, C16:0 and C20:4, and in *M. reticulata* CBS 223.29, C18:1 and C18:2 represented the major fatty acids, respectively. It is noted herewith that C20:4 constituted about 70% of the total fatty acids in *M. alpina* CBS 527.72. It is interesting that the level of γ-C18:3 in the isolates of subgen. *Mortierella* was lower than that in the isolates of subgen. *Micromucor* (3-12% vs 10-24%). The fatty acids C22:0 and C24:0 were detected in all isolates in subgen. *Mortierella* , though they are not listed in Table 3. They constituted about 5% of the total fatty acids.

The fatty acid C12 was not detected in all isolates examined in the present study. Traces or small amounts of C14:0 were detected in all isolates, though it is not listed in Tables 2 and 3.

DISCUSSION

Two subgenera of the genus *Mortierella* are distinguished from each other by their morphological and cultural characteristics (Gams, 1977). Shinmen et al.(1989) pointed out that two subgenera also differed from each other in their fatty acid composition; polyunsaturated C20 acids were detected only in the isolates of subgen. *Mortierella* but never detected in those of subgen. *Micromucor*. Our present analysis of the fatty acid composition of 18 isolates in subgen. *Micromucor* and 50 isolates in subgen. *Mortierella* confirmed their results. The difference of their fatty acid composition coincided with that of the morphological and the cultural characteristics.

Gams (1977) grouped the species of subgen. *Mortierella* into nine sections on the basis of the type of sporangiophore ramification. We analysed the fatty acid composition of 50 isolates in subgen. *Mortierella* assigned to seven sections. There appeared to be no correlation between the fatty acid composition and the type of sporangiophore ramification. Fatty acid composition appears to be a useful chemotaxonomic marker in the genus *Mortierella* at the subgeneric level.

Although the subgen. *Micromucor* has *Mucor*-like characteristics, its true taxonomic position is still uncertain because the zygospores have not been discovered in this subgenus (Gams, 1977; Benjamin, 1979). Comparison of the fattty acid composition of mucoraceous fungi so far reported (Lösel, 1988) and that of the isolates in subgen. *Micromucor* obtained in this study showed that fatty acid composition of subgen. *Micromucor* was qualitatively similar to that of mucoraceous fungi: the predominant fatty acids were C16:0, C18:1, and C18:2; γ-C18:3 was presented; polyunsaturated C20 acids were never detected. More detailed chemotaxonomic as well as morphological studies are necessary before coming to a conclusion, but it is considered that the qualitative similarity in the fatty acid composition of the mucoraceous fungi and the isolates of subgen. *Micromucor* offer supportive evidence for the determination of the true taxonomic position of the subgen. *Micromucor*.

In zygomycetous fungi other than *Mortierella* species, the presence of polyunsaturated C20 acids were reported in *Entomophthora* species and *Conidiobolus* species (Tyrrell, 1967, 1971) and *Gigaspora margarita* and *Glomus* species (Jabaji-Hare, 1988). The former are usually parasitic on insects and the latter are symbionts of higher plants; on the contrary, *Mortierella* species are saprophytic and belong to the most common soil fungi. It is very interesting from the taxonomical, phylogenetic, and ecological point of view that all these fungi have the ability to biosynthesize fatty acids longer than C18, although their habitats are remarkably different from one another.

The fatty acid composition of the fungi belonging to the Entomophthorales was shown to be useful as an aid to determining their true taxonomic position (Tyrrell, 1967 , 1971). In this study we also clarified that the fatty acid composition was a useful chemotaxonomic characteristic in the taxonomy and the identification of the genus *Mortierella*. Chemotaxonomic characteristics such as the fatty acid composition, the ubiquinone system, and G+C content of DNA may also provide valuable information to establish a more rational taxonomic scheme of zygomycetous fungi.

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Table 1. List of isolates used in this study

Taxon	Isolate[1)]	Source
Subgenus *Micromucor*		
Mortierella isabellina	CBS 194.28	
Mortierella isabellina	IFO 6336	Soil
Mortierella isabellina	IFO 7824	
Mortierella isabellina	IFO 7873	Wood of *Betula* sp.
Mortierella isabellina	IFO 7874	Germany
Mortierella isabellina	IFO 8286	Soil, Waipoua, New Zealand
Mortierella isabellina	IFO 8308	Soil, Cradle Mountain National Park, Tasmania
Mortierella isabellina	NRRL 1757	
Mortierella nana	IFO 8190	Soil, Cradle Mountain National Park, Tasmasnia
Mortierella ramanniana var. *angulispora*	IFO 5426	Forest soil
Mortierella ramanniana var. *angulispora*	IFO 8186	Soil, Blue Mountain, N.S.W., Australia
Mortierella ramanniana var. *ramanniana*	CBS 112.08	Soil, Scotland, UK
Mortierella ramanniana var. *ramanniana*	CBS 212.72	Forest soil, Sweden
Mortierella ramanniana var. *ramanniana*	IFO 7825	
Mortierella ramanniana var. *ramanniana*	IFO 8184	Soil, Blue Mountain, N.S.W., Australia
Mortierella ramanniana var. *ramanniana*	IFO 8185	Soil, Cradle Mountain National Park, Tasmania
Mortierella ramanniana var. *ramanniana*	IFO 8287	Soil, Te Anau, New Zealand
Mortierella vinacea	CBS 236.82	Root of *Fragaria* sp., Japan
Subgenus *Mortierella*		
Section *Alpina*		
Mortierella alpina	ATCC 16266	Soil, Germany
Mortierella alpina	ATCC 42430	Alpine eutric brunisol, grassy slope, Canada
Mortierella alpina	CBS 219.35	
Mortierella alpina	CBS 224.37	Soil, Hungary
Mortierella alpina	CBS 250.53	
Mortierella alpina	CBS 343.66	Tundra soil, Peters Lake, Alaska, U.S.A.
Mortierella alpina	CBS 527.72	Pasture soil, North Carolina, U.S.A.
Mortierella alpina	CBS 529.72	Pasture soil, North Carolina, U.S.A.
Mortierella alpina	CBS 608.70	Agricultural soil, Netherlands
Mortierella alpina	CBS 754.68	Heavily manured soil, India
Mortierella alpina	IFO 8568	Tundra soil, Peters Lake, Alaska, U.S.A.
Section *Hygrophila*		
Mortierella bainieri	IFO 8569	Tundra soil, C. Thompson, Alaska, U.S.A.
Mortierella beljakovae	CBS 123.72[T]	Soil, USSR
Mortierella beljakovae	CBS 601.68	Bark of *Pinus* stump, North Carolina, U.S.A.
Mortierella clonocystis	CBS 357.76[T]	Soil, Spain
Mortierella dichotoma	CBS 221.35[ST]	Dung of mouse, Germany
Mortierella elongata	CBS 121.71	Soil, Georgia, U.S.A.
Mortierella elongata	CBS 125.71	Soil, Georgia, U.S.A.
Mortierella elongata	NRRL 5513	Soil, Georgia, U.S.A.
Mortierella epigama	CBS 489.70[T]	Municipal waste, Germany
Mortierella epigama	NRRL 5512[T]	Municipal waste, Germany
Mortierella gemmifera	CBS 134.45[T]	Pine forest soil, England, UK
Mortierella hyalina	CBS 654.68	Dung of deer, India
Mortierella hyalina	NRRL 6427	
Mortierella kuhlmanii	CBS 157.71[T]	Stump of *Pinus palustris*, South Carolina, U.S.A.
Mortierella minutissima	CBS 226.35	Germany

Table 1 (Continued)

Mortierella minutissima var. *dubia*	CBS 307.52ST	Soil, Germany
Mortierella minutissima	IFO 8573	Soil, Lake Peters, Alaska, U.S.A.
Mortierella sarnyensis	CBS 122.72^{T}	Soil, Ukraine, USSR
Mortierella selenospora	CBS 811.68^{T}	Mushroom compost, Netherlands
Mortierella zychae	CBS 316.52^{T}	Decaying wood of *Populus tremula*, Germany
Section *Mortierella*		
Mortierella oligospora	CBS 218.72	Greenhouse soil, Netherlands
Mortierella polycephala	IFO 6335	Soil
Mortierella reticulata	CBS 223.29	
Section *Schmuckeri*		
Mortierella camargensis	CBS 221.58^{T}	Sandy soil, France
Mortierella schmuckeri	CBS 295.59ST	Soil under *Opuntia* sp., Mexico
Mortierella schmuckeri	NRRL 2761	
Section *Simplex*		
Mortierella globulifera	CBS 417.64	Soil, Germany
Mortierella rostafinskii	CBS 522.70^{NT}	Soil under *Pinus elliottii* var. *elliottii*, Georgia, U.S.A.
Section *Spinosa*		
Mortierella acrotona	CBS 386.71^{T}	Soil, India
Mortierella cystojenkinii	CBS 456.71^{T}	Agricultural soil, Netherlands
Mortierella pulchella	CBS 440.68	Bark and wood of *Pinus* stump, south carolina, U.S.A.
Mortierella umbellata	CBS124.71^{T}	Cultivated soil, Georgia, U.S.A.
Section *Stylospora*		
Mortierella horticola	CBS 305.52ST	Germany
Mortierella lignicola	CBS 313.52	Soil under *Pinus sylvestris*, Germany
Mortierella stylospora	CBS 211.32^{T}	Sandy loam, Victoria, Australia
Mortierella verticillata	CBS 220.58	Soil under *Betula* sp., France
Mortierella verticillata	IFO 8575	Tundra soil, Umiat, Alaska, U.S.A.
Mortierella verticillata	NRRL 6337	
Mortierella zonata	CBS 228.35^{T}	*Gomphidius glutinosus*, Germany

[1]T, strain derived from the type isolate; ST, strain derived from the syntype isolate, NT, strain derived from the neotype isolate. Culture collection: CBS, Centrralbureau voor Schimmelcultures, Baarn; IFO, Institute for Fermentation, Osaka; NRRL, USDA, Northern Regional Research Center, Peoria.

Table 2. Fatty acid composition of 18 *Mortierella* subgen. *Micromucor* isolates

Strain	16:0	18:0	Fatty acid compositon (%)						
			18:1	18:2	18:3	20:3	20:4	20:5	others
Mortierella isabellina CBS 194.28	15.6	1.9	40.0	17.5	22.9	--	--	--	2.1
Mortierella isabellina IFO 6336	17.5	2.0	39.0	17.7	20.6	--	--	--	3.2
Mortierella isabellina IFO 7824	16.3	2.6	38.3	17.9	21.7	--	--	--	3.2
Mortierella isabellina IFO 7873	21.4	3.3	42.8	13.3	15.4	--	--	--	3.8
Mortierella isabellina IFO 7874	16.6	1.7	50.2	16.1	11.5	--	--	--	3.9
Mortierella isabellina IFO 8286	17.8	4.3	44.1	13.0	17.6	--	--	--	3.2
Mortierella isabellina IFO 8308	18.6	3.2	51.9	11.6	10.0	--	--	--	4.7
Mortierella isabellina NRRL 1757	20.9	3.3	49.5	15.8	6.9	--	--	--	3.6
Mortierella nana IFO 8190	21.1	4.8	45.9	17.5	9.8	--	--	--	0.9
Mortierella ramanniana var. *angulispora* IFO 5426	25.2	5.4	35.7	14.3	17.6	--	--	--	1.8
Mortierella ramanniana var. *angulispora* IFO 8186	19.5	3.8	38.5	23.7	13.6	--	--	--	0.9
Mortierella ramanniana var. *autotrophica* CBS 212.72	16.4	1.7	41.1	25.0	13.2	--	--	--	2.6
Mortierella ramanniana var. *ramanniana* CBS 112.08	20.3	4.4	21.4	19.8	31.4	--	--	--	0.1
Mortierella ramanniana var. *ramanniana* IFO 8184	19.4	3.9	40.1	19.0	15.0	--	--	--	2.6
Mortierella ramanniana var.*ramanniana* IFO 8287	21.2	4.0	39.7	16.1	16.3	--	--	--	2.7
Mortierella ramanniana var.*rramanniana* IFO 7825	17.6	4.0	35.3	24.6	17.2	--	--	--	1.3
Mortierella ramanniana var. *rramanniana* IFO 8185	17.8	3.0	41.7	19.0	15.0	--	--	--	3.5
Mortierella vinacea CBS 236.82	18.1	2.2	46.3	11.4	22.0	--	--	--	--

Table 3. Fatty acid composition of 50 *Mortierella* subgen. *Mortierella* isolates

Strain	16:0	18:0	Fatty acid composition (%)					20:5	others[1)]
			18:1	18:2	18:3	20:3	20:4		
Section *Alpina*									
Mortierella alpina ATCC 16266	10.0	5.9	24.9	7.1	7.7	4.6	39.6	--	0.2
Mortierella alpina ATCC 42430	13.6	7.3	14.3	12.0	7.7	6.5	38.3	--	0.3
Mortierella alpina CBS 219.35	11.2	4.0	30.5	14.4	10.9	4.1	22.4	--	1.7
Mortierella alpina CBS 224.37	14.2	5.9	16.2	10.8	8.9	5.9	38.1	--	--
Mortierella alpina CBS 250.53	14.5	5.9	27.8	11.4	7.4	4.0	27.1	--	1.9
Mortierella alpina CBS 343.66	15.3	6.0	23.9	11.8	8.7	5.7	28.6	--	--
Mortierella alpina CBS 527.72	6.6	8.1	6.9	4.9	3.8	--	69.7	--	--
Mortierella alpina CBS 529.72	12.8	9.9	13.9	7.0	6.0	3.1	47.3	--	--
Mortierella alpina CBS 608.70	20.3	6.7	10.4	7.4	6.3	--	48.0	--	0.9
Mortierella alpina CBS 754.68	13.8	7.4	10.2	6.3	4.7	5.6	52.0	--	--
Mortierella alpina IFO 8568	18.8	7.9	28.1	9.2	7.9	6.5	20.9	--	0.7
Section *Hygrophila*									
Mortierella bainieri IFO 8569	20.8	7.8	31.2	6.6	5.8	5.4	21.5	--	0.9
Mortierella beljakovae CBS 123.72	15.5	11.8	44.3	6.5	5.8	3.7	10.4	--	2.0
Mortierella beljakovae CBS 601.68	10.9	4.2	28.3	16.6	12.4	5.5	19.1	--	3.0
Mortierella clonocystis CBS 357.76	13.6	6.3	42.7	9.9	4.6	2.9	18.7	--	1.3
Mortierella dichotoma CBS 221.35	17.5	8.0	30.5	7.8	3.5	2.5	11.3	--	18.4[a]
Mortierella elongata CBS 121.71	19.0	12.0	33.3	7.3	7.4	4.9	14.3	tr	1.8
Mortierella elongata CBS 125.71	15.4	14.0	30.3	6.7	6.0	3.8	21.7	tr	2.1
Mortierella elongata NRRL 5513	17.1	8.3	32.8	7.4	6.5	3.6	22.8	tr	1.5
Mortierella epigama CBS 489.70	16.8	5.1	27.4	8.2	9.8	4.6	23.0	0.9	4.2
Mortierella epigama NRRL 5512	15.1	5.1	28.7	9.1	12.1	4.5	23.3	0.8	1.3
Mortierella gemmifera CBS 134.45	19.3	8.8	38.7	8.1	6.8	2.5	10.6	--	5.2
Mortierella hyalina CBS 654.68	22.5	6.7	36.5	9.6	7.3	3.1	14.4	--	--
Mortierella hyalina NRRL 6427	17.3	12.9	29.3	7.4	7.5	3.3	11.3	0.2	10.8[b]
Mortierella kuhlmanii CBS 157.71	19.5	14.2	23.9	5.4	5.0	4.0	17.0	tr	11.0[c]
Mortierella minutissima CBS 226.35	16.2	5.2	33.0	13.2	4.7	2.4	24.3	--	1.0

Mortierella minutissima var. *dubia* CBS 307.52	8.1	2.9	22.5	20.1	12.4	4.1	30.0	--	--
Mortierella minutissima IFO 8573	16.1	8.3	23.9	9.6	8.4	4.1	14.3	0.4	14.9[d]
Mortierella sarnyensis CBS 122.72	24.1	9.9	33.0	12.3	3.4	2.0	15.3	--	--
Mortierella selenospora CBS 811.68	18.5	11.5	36.6	5.5	5.7	--	22.2	--	--
Mortierella zychae CBS 316.52	23.2	12.6	29.3	8.7	6.0	3.3	16.3	--	0.6
Section *Mortierella*									
Mortierella oligospora CBS 218.72	22.6	7.4	34.8	9.7	5.9	--	19.7	--	--
Mortierella polycephala IFO 6335	8.2	0.8	18.3	13.0	14.9	3.3	20.1	--	21.3[e]
Mortierella reticulata CBS 223.29	10.4	4.3	38.2	22.6	8.2	--	16.2	--	0.1
Section *Reticulata*									
Mortierella camargensis CBS 221.58	17.5	5.0	36.4	12.2	7.9	2.4	17.6	--	1.0
Mortierella schmuckeri CBS 295.59	26.3	8.8	33.9	8.7	3.1	2.9	15.9	--	0.4
Mortierella schmuckeri NRRL 2761	19.9	12.4	37.1	7.4	4.9	4.9	12.4	tr	1.0
Section *Simplex*									
Mortierella globulifera CBS 417.64	20.9	4.9	51.7	5.4	4.9	1.8	9.4	--	1.0
Mortierella rostafinskii CBS 522.70	21.9	5.1	26.3	13.4	5.9	3.3	22.6	--	1.5
Section *Spinosa*									
Mortierella acrotona CBS 386.71	27.8	8.1	41.9	4.7	4.0	2.7	10.2	--	0.6
Mortierella cystojenkinii CBS 456.71	18.7	6.1	48.2	4.4	3.1	2.6	16.2	--	0.7
Mortierella pulchella CBS 440.68	15.1	2.8	39.9	9.4	3.7	3.1	22.6	--	3.4
Mortierella umbellata CBS124.71	17.3	6.8	23.1	8.1	8.8	5.8	30.1	--	--
Section *Stylospora*									
Mortierella horticola CBS 305.52	18.6	6.5	25.7	13.3	8.1	3.5	24.4	--	--
Mortierella lignicola CBS 313.52	18.8	6.0	38.0	15.1	3.8	2.9	14.0	--	1.4
Mortierella stylospora CBS 211.32	16.7	11.0	33.6	11.6	7.5	3.8	15.8	--	--
Mortierella verticillata CBS 220.58	15.7	4.2	28.2	7.9	11.8	4.7	27.5	--	--
Mortierella verticillata IFO 8575	14.9	5.6	29.7	12.1	6.0	4.8	25.7	--	1.2
Mortierella verticillata NRRL 6337	13.6	6.8	36.4	6.7	6.9	5.4	23.0	--	1.2
Mortierella zonata CBS 228.35	38.0	9.9	24.3	8.0	3.9	2.9	12.9	--	0.1

1)a C15:0 (5.8%), C17:0(9.2%), C17:1(3.4%); b C15:0(3.3%), C17:0(7.5%); c C20:1(10.5%), C20:2(0.5%); d C15:0(4.1%), C17:0(7.3%), C17:1(2.2%), C19:0 (1.3%); e C15:0(4.3%), C17:0(9.8%), C17:1(5.8%), C19:0(1.4%).

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SOME NOTES ON THE TAXONOMY AND NOMENCLATURE OF FIVE EUROPEAN ARMILLARIA SPECIES

HELGA MARXMÜLLER

Zehentbauernstr.15,
D-8000 München 90, Germany

ABSTRACT

Recent nomenclatural publications on the names of the annulate European species A. ostoyae, A. cepistipes and A. gallica are discussed. Reasons for the rejection of the name A. obscura for the species A. ostoyae, of the names A. bulbosa and A. lutea for the species A. gallica and of A. cepistipes forma pseudobulbosa are given.

KEYWORDS: Armillaria ostoyae, A. cepistipes, A. gallica, nomenclature.

INTRODUCTION

In 1978 the Finnish mycologist K. Korhonen demonstrated the existence of five annulate Armillaria species in Europe with the help of biological tests (= mating tests performed in laboratory culture): he designated them the species A, B, C, D and E. However, in the literature more than 50 specific epithets associated with Armillaria mellea s.lat. have been published.

By studies on the morphology of tested basidiomes the five biological species recognised by Korhonen have been identified (Marxmüller 1982, Romagnesi and Marxmüller 1983, Marxmüller 1987) to represent the following species: Armillaria borealis Marxmüller & Korhonen (species A), A. cepistipes Velenovský (species B), A. ostoyae (Romagnesi) Herink (species C), A. gallica Marxmüller & Romagnesi (species E.) and A. mellea (Vahl : Fr.) Kummer (species D).
Below some further comments on questions which have often been asked in relation to our publications on the Armillaria taxonomy and nomenclature.

DISCUSSION

SPECIES C

Which of the two names should be used: Armillaria obscura (Schaeffer 1774) Herink 1973 or Armillaria ostoyae (Romagnesi 1970) Herink 1973 ?

Termorshuizen and Arnolds (1984, 1987) as well as Watling (1987) proposed as a result of extensive historic investigations that A. obscura be rejected for nomenclatural and taxonomic reasons. One of them concerns the interpretation of the plate 74 in Jacob Christian Schaeffer`s "Fungorum Icones ..." (1762), which they considered ambiguous. Several points ought to be considered:
1. The original copper plate was engraved by G. P. Nußbiegel and coloured by S. Loibel (probably later when the book was bound). Considering the time spent on an engraving, it is unlikely that the colours were painted in direct relation to the natural colour.
2. The description accompanying the plate 74 (1762) says : "it is a bicoloured fungus, mostly isolated, big but not very fleshy and not various; with a cap that is initially conic or convex, later rounded (arched), frequently pointed at its center and which presents always hairy scales; with a round (cylindrical?) stipe, somewhat inflated at its base; with a membranous "spore-cover" (veil) and a similar annulus."
3. The globose spores on the picture do not match Armillaria spores. As in this book the spores of other fungi are differentiated

and mostly correspond to the species represented, the argument that they are stylised is not very convincing.

4. Watling (1987) points out the lack of scales on the ring, margin and stipe.

I fully agree that it is not possible to correlate unequivocally Schaeffer`s fungus with Korhonen`s species C, which may perhaps not even be an Armillaria. Therefore, I also propose that A. obscura is to be regarded as a nomen ambiguum and should not be used for any fungus.

SPECIES B

What about A. cepistipes and its forma pseudobulbosa ?

The collections of species B that we found together with H. Romagnesi in Löffingen (Schwarzwald = Black Forest, Germany) on the 21st of September 1982 closely matched with Velenovský`s 1920 description of A. cepistipes. Previously we had examined only a limited number of species B collections which had larger and darker coloured basidiomes, which we had called "A. pseudobulbosa". Therefore the specimens from Löffingen looked on first impressions somewhat abnormal. To assist in the determination we created the two formae (Romagnesi and Marxmüller 1983). Additional collections revealed however that intermediate forms between A. cepistipes forma cepistipes and A. cepistipes forma pseudobulbosa are common. Thus it was necessary to abandon the designation of forms.

As we had been informed by Czechoslovakian mycologists that the type-specimen of A. cepistipes was lost, we proposed a neotype for A. cepistipes (1983). Three years later the holotype specimen was found by Antonín in the Velenovský herbarium in Prague (PRC). It was conserved in a formaldehyde, acetic acid and water solution. Antonín confirmed the identity with the "neotypus" specimen from Löffingen (Antonín 1986).

What is the correct orthography of "Armillaria cepaestipes"?

"Cepaestipes" is an orthographic mistake which should be corrected (Art. 73.8 of the I.C.B.N.; Greuter et al. 1988). According to the rule of compound noun formation in Latin phonetics, each short vowel which is moved from the end into the

middle of a word must be changed into an i when followed by a vowel or one consonant, and (generally) into an e (short) when followed by several consonants (Romagnesi 1986). Therefore the correct orthography should be cepestipes.
However, following the proposal by Prof. T. Ahti, Helsinki (pers. comm.), and using as a pattern the Latin word "lectisternum", provided as an example by Romagnesi, I believe that "cepistipes" is acceptable and has been adopted here. It is also in accordance with the Recommendation 73 G of the Code.

SPECIES E

Why has the name Armillaria bulbosa (Barla) Velenovský been changed ?

Earlier (e.g. in Marxmüller 1982) the species E was referred to A. bulbosa. Barla (1887:143) described Armillaria mellea var. bulbosa and published a colour plate of this fungus (Barla 1888-pl 22, figs. 3 - 7). In addition, he left dried specimens (NICE, Barla herbarium), notes and an original water colour plate painted by Fossat. All these data revealed a greater resemblance of Barla`s fungus to the species B than to the species E. The supposition that var. bulbosa could be the species B was confirmed by ecological information such as the mountain habitat (France, Alpes-Maritimes, Bois de la Mairis, col de Turini, alt.1550 m) and the occurrence under conifers (Holdenrieder 1986, Guillaumin 1986).
If A. mellea var. bulbosa Barla 1887 and A. cepistipes Vel. 1920 are considered as synonymous, Velenovský`s name must have priority over Barla`s at species level.
In 1927 Velenovský raised var.bulbosa to the species level and in 1973 Romagnesi published Armillariella bulbosa (Barla) Romagnesi.
Romagnesi did not know at that time about the existence of two very similar species. His description was documented by specimens he collected near Compiègne (Oise) and Saint Sauveur-le-Vicomte (Manche). These specimens have been shown to be conspecific with species E, because later tested collections from these regions never revealed species B, which seems to occur rarely at low elevations (Guillaumin 1986). However, Romagnesi`s name Armillariella bulbosa was a misapplication for species E

because it is based on Barla`s description. Thus Romagnesi and Marxmüller (1987) proposed for species E the new name Armillaria gallica . Mistakenly we cited Armillariella bulbosa (Barla) Romagnesi as basionym (H. Kreisel, oral communication). It is rather a "pseudobasionym", but as we had clearly mentioned that we accepted neither the typus of Barla, nor Romagnesi`s combination, the mistake does not justify changing the name gallica. (T. Ahti, pers. communication; see also Art. 63.2 of the I.C.B.N. [Kreisel, in litt.]).
Antonín (1990), who studied the Armillaria type specimens of Velenovský herbarium, believes that several species described by Velenovský (A. praecox, A. robliniensis and A. inflata) might be identical with A. gallica or A. cepistipes. However, the material Antonín used for investigation (two field sketches, a specimen later deposited by Velenovský instead of the lost type specimen, and one specimen in poor condition) is not sufficient to allow a reliable determination. Therefore, those names are regarded as nomina ambigua. Even in fresh condition A.gallica and A.cepistipes are often difficult to distinguish.

What is Armillaria lutea Gillet 1874 ?

This name was proposed for the species E by Termorshuizen and Arnolds (1984, 1987). As a precautionary measure they have not corroborated it by neotypification. Watling (1987) also used this name, but later discarded it (Watling, in litt.). Unfortunately two of these publications and mine (Marxmüller 1987), in which the name A. gallica was proposed for the same species, overlapped.
Gillet`s diagnosis describes specimens which were probably deformed at the margin by lack of moisture, as he mentions that the margin is "*fissured*". As we have observed that some Armillaria which usually have cylindrical bases swell during dry periods, the characteristic of an "enlarged" stipe base may not be used with certainty to determine the species. In Gillet`s diagnosis the main characteristic, the annulus, was qualified as "*pointing upwards*" (mistakenly interpreted as "fugaceous" by Termorshuizen), but only A. mellea has a persistent funnel shaped ring. The gills are described as "*decurrent*" (as in A. mellea, A. borealis, A. cepistipes); according to Gillet the "*depressed cap*" (all the species, but least on A. gallica) could be either "ochraceous" (A. borealis, A. ostoyae, A. gallica, A. cepistipes) or "*greenish*

yellow" (so far only seen on A. mellea) or "reddish brown" (A. ostoyae, A. gallica). The stipe is noted as "incurved and covered with ochraceous flocs" (A. gallica, A. cepistipes, A. borealis, A. ostoyae) and the cap "decorated with small brown scales, becoming less numerous at the margin" (observed on A. gallica, A. borealis, A. ostoyae, A. cepistipes) and presenting "yellowish veil flocs on the margin" (A. borealis, A. gallica, A. cepistipes, sometimes A. ostoyae). The diagnosis also mentions: "*solitary or in small groups*" (A. cepistipes, A. borealis, in some cases A. gallica and A. ostoyae).

No further evidence is available to identify the fungus - no specimen, no type locality, no plate and no complementary notes. Romagnesi supposed that it might be a diagnosis of several Armillaria species collected at the same time.

Thus we declared A. lutea Gillet as a nomen ambiguum.

Which of the three names should be taken for species E ?

As A. bulbosa has been rejected as a misapplication and A. lutea has been declared as a nomen ambiguum, A. gallica is the only valid name for Korhonen`s species E.

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CONTRIBUTION TOWARDS A REVISION OF THE GENUS HYPOXYLON S.STR. (XYLARIACEAE, ASCOMYCETES) FROM PAPUA NEW GUINEA.

KATLEEN VAN DER GUCHT AND PAUL VAN DER VEKEN

Laboratory of Plant Morphology, Systematics and Ecology, University of Ghent, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

Abstract

In this study of *Hypoxylon* Bull. s. str. in the rain forests of Papua New Guinea *Hypoxylon archeri, H. crocopeplum, H. bovei* var. *microsporum, H. dieckmannii, H. haematostroma, H. hypomiltum, H.* cf. *investiens, H. macroannulatum, H. nectrioideum, H. oodes, H. rubiginosum, H. sclerophaeum, H. stygium, H. subannulatum, H. subgilvum* and *H. truncatum* are described for the first time for the Papua New Guinean flora. *Hypoxylon retpela* sp. nov. is proposed. Special attention is given to spore ornamentation : the ascospores of *H. haematostroma, H. oodes* and *H. rubiginosum* are provided with transversely oriented fibrils, those of *H. crocopeplum, H. hypomiltum, H. retpela, H. subgilvum* and *H. investiens* are ornamented with transversely oriented ribs. A key to the species is provided.

Introduction

This paper describes some *Hypoxylon* species collected in the rain forests of Papua New Guinea.

Papua New Guinea forms part of one of the largest tropical islands of the world, together with Irian Jaya. It comprises an area of about 460000 km^2 situated between the latitudes 1° - 12° S and longitudes 141° - 160° E. The mainland is characterized by a central cordillera with peaks up to 4600 m and with intra-montane valleys at about 1500 - 1800 m. It lies within the heavy precipitation belt of the humid tropics, most of the country receives over 2000 mm rainfall a year with recordings up to 4000 mm a year. Generally the wet season comes from December to March, with a drier season from May to October. However, it usually rains on both sides of the main cordillera throughout much of the year which makes it one of the largest constantly wet areas of the world. The lowland

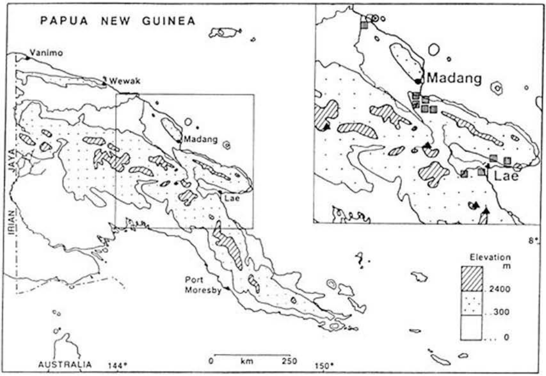


Fig. 1 : Map of the mainland of Papua New Guinea, with collecting spots of the 1989 expedition (see inset) (map modified from Shaw, 1984) : ▥ lowland rain forest (< 1000 m alt.) ; ▲ highland rain forest ; ◌ mangroves ; ⊙ Laing Island (coralligeneous).

rain forest is the most common vegetation type, found up to about 1000 m altitude.

In 1989 we participated in an expedition, supported by the Belgian National Fund for Scientific Research (N.F.W.O.). During this expedition we explored the N.E. side of Papua New Guinea (see fig. 1), where we visited the following habitats : lowland rain forest, mountain rain forest, mangroves and Laing Island, a small coralligeneous Island.
Most collections were made in the lowland rain forests, and a few up to about 2800 m altitude.

Taxonomical delimitation

We recognize *Hypoxylon* Bull. s. str. to include only species with ectostromal pigmentation, spores with a germ slit on the dorsal side (the more convex side of the spore) and a loosening perispore (Pouzar 1979, 1985a, 1985b, 1986). These species constitute the sections *Hypoxylon, Annulata* and *Papillata* subsection *Papillata* as given by Miller (1961). Miller's sections *Applanata* and *Papillata* subsection *Primo-cinerea* are not treated here.

History

Citations of *Hypoxylon* species from Papua New Guinea are very scanty.
The first records of xylariaceous fungi for P.N.G. were noted by Cooke (1886), Cooke & Hennings (1889), Rehm (1889), Hennings (1892, 1893, 1894, 1898a, 1898b, 1899, 1900, 1901, 1905) and Massee (1898). Several *Xylaria* species were mentioned, one *Daldinia*, one *Kretzschmaria*, but no *Hypoxylon*.
The first record of a *Hypoxylon* was apparently made by Cunningham (1952 : 279) in his revision on the Australian and New Zealand species of Thelephoraceae and Hydnaceae of the Kew collection. He stated that collection number Bauerlen 10, Strickland River, New Guinea in the Kew Herbarium filed under *Corticium caeruleum* Fr. by Cooke was a sterile stroma of a *Hypoxylon sp.*
A second record was made by Doi (1971 : 396) who found a *Hypocrea atrogelatinosa* Dingley on "something that looks like a *Hypoxylon sp.*?". This specimen was collected in Rabaul.
Finally, there are records of *Hypoxylon deustum* (Hoffm. : Fr.) Greville, a synonym of *Ustulina deusta* (Hoffm. : Fr.) Lind., not belonging to the genus *Hypoxylon* s. str. It was recorded by Dumbleton (1954), Dwyer (1940) and Mann (1953) (not seen, cit. in Shaw 1984). Shaw (1984) mentioned that it was found on three different substrates : once on *Camellia sinensis* (L.) Kuntze (Theaceae), once on a *Citrus sp.* (Rutaceae) and 3 times on *Hevea brasiliensis* Muell. Arg. (Euphorbiaceae).
Since the first two records of *Hypoxylon* could not be identified more exactly, because of the poor condition of the collected material, and since the third record is an *Ustulina*, we can conclude that so far there is not a single published record of the genus *Hypoxylon* s.str. from P.N.G.

Materials and Methods

Most of the material studied was collected during an expedition to P.N.G. in 1989. Other material was received on loan from the Herbarium of the State University Liège (LG).
Most of our material could be compared with specimens from L, BR, K and specimens from the personal collection of A.J.S. Whalley (cited here as AJSW). The specimens from L have been annotated by J.H. Miller, and those from BR by R.W.G. Dennis.
The specimens were analyzed based on observations with bright field and S.E.Microscopy.
Reagents used with bright field microscopy were Melzer, KOH (10 %) and aqua destillata. Drawings were made with the aid of a camera lucida.
The colour of the stroma was checked by using an acetone extract. Colours are indicated using the Methuen Handbook of Colours by Kornerup & Wanscher (1978).
For the analyses with the S.E.M., material was stuck on tape affixed to an aluminum stub, vacuum coated with gold and examined. The ascospores were air-dried first.

The cited collections have been deposited in the herbarium GENT.

Results

I. Section *Hypoxylon*

Hypoxylon crocopeplum Berk. & Curt., Grevillea 4 : 49 (1875).

Descriptions and illustrations : Miller 1961 : 37-38, figs. 27, 53 ; Martin 1969 : 188-189, pl. II: 13, 14 ; Rogers et al. 1987 : 119.

This specimen corresponds to the descriptions given by Miller (1961), Martin (1969) and Rogers et al. (1987) except for the spore ornamentation which was not mentioned in the previous accounts. The ascospores are inaequilaterally ellipsoid with a straight germ slit running full length on the convex side of the spore (see fig. 2). They are characterized by a conspicuous dehiscent hyaline perispore (see also Rogers et al. 1987 : 119). By light microscopy these perispores appear to be indistinctly transversely striate, especially when examined in KOH (10%), since KOH loosens the perispore. When examined by S.E.M. they seem to be adorned with parallel to anastomosing rope-shaped ornaments, transversely oriented (see fig. 2 & pl. I a).

Specimen examined : PAPUA NEW GUINEA : MADANG PROVINCE : road Madang - Bogia, Nobanob, secondary forest, on dead wood, 6.9.1990, Van der Veken P. 90-674 (GENT).

Reference material examined : UGANDA : Mpanga Forest, on dead wood, Taligoola H.K. 570, determinavit Whalley (AJSW).

Hypoxylon dieckmannii Theiss., Ann. Mycol. 6 : 346 (1908).

Descriptions and illustrations : Miller 1961 : 33, figs. 19, 48 ; Martin 1969 : 170-172, pl. I: 3.

We prefer to consider *H. dieckmannii* as a distinct species (Martin 1969) rather than a small spored variety of *H. rubiginosum* (Miller 1961), this on account of the ascospore characteristics and the pigmentation of the stroma which is very striking violet brown (K. & W. PL. 10F4) at maturity (see table 1, fig. 3 & pl. I b-c).

Figs. 2-7 & 9 : ascospores of members of the section *Hypoxylon* : 2. *H. crocopeplum* (Van der Veken P. 90-674) (inset : perispore ornamented with faint transverse striations) ; 3. *H. dieckmannii* (Van der Gucht K. 89-931) ; 4. *H. haematostroma* (Van der Gucht K. 89-1053a) ; 5. *H. hypomiltum* (Van der Gucht K. 89-1033) (inset : perispore ornamented with faint transverse striations) ; 6. *H. nectrioideum* (Van der Gucht K. & De Meester L. 89-1662) ; 7. *H. oodes* (Van der Gucht K. 89-525) ; 9. *H. rubiginosum* (Van der Gucht K. 89-604a)

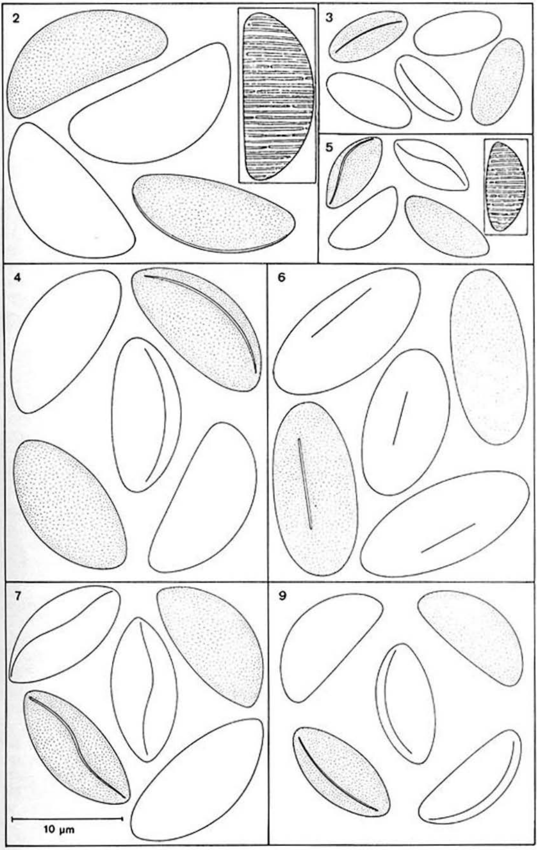

2
3
5
4
6
7
9
10 µm

Table 1 : comparison of the ascospore characteristics of *Hypoxylon rubiginosum* and *H. dieckmannii.*

	H. dieckmannii	*H. rubiginosum*
shape	equilaterally ellipsoid to ovoid	inaequilaterally ellipsoid
perispore	smooth	ornamented with transversely oriented fibrils (only seen by S.E.M.)

Specimens examined : PAPUA NEW GUINEA : MADANG PROVINCE : Hansa Bay, Laing Island, 4°10'S & 144°52'E, sea level, on dead decorticatd wood of *Planchonella obovata* (R.Br.) Pierre (Sapotaceae), 3.10.1989, Van der Gucht K. 89-514 (GENT). Eo loco, 19.8.1990, Van der Veken P. 90-240 (GENT). Bunapas, 4°11'S & 144°47'E, sea level, on dead wood, 20.10.1989, Van der Gucht K. 89-931 (GENT). Jogari, W-side of Manam, 4°05'S & 144°59'E, sea level, on dead decorticated wood, 31.1.1980, Demoulin V. & Smeets L. 5851 (LG, GENT). Alexishafen, bridge River Biges, 1 km to the left (W), on dead decorticated wood, 2.9.1990, Van der Veken P. 90-584 (GENT).

Reference material examined : INDIA : Uttar Pradesh, Asazori, on dead angiospermic stump, 29.8.1973, Dargan J.S. 13153, determinavit Dargan (K).

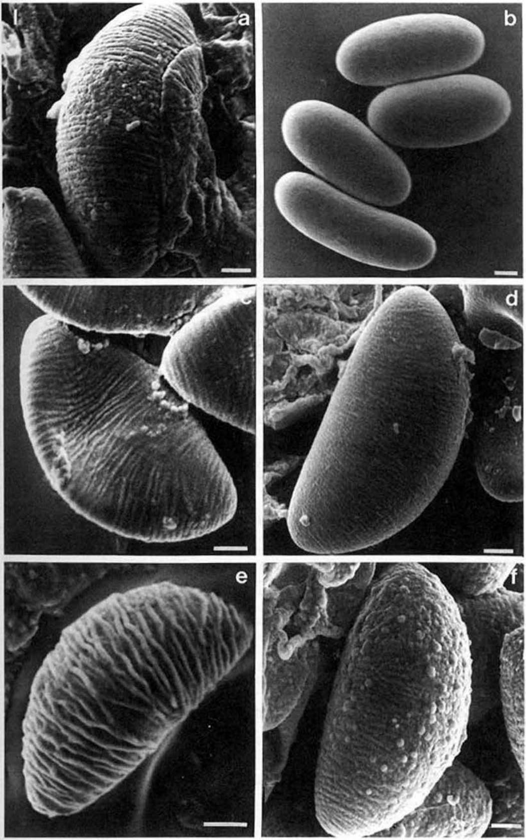
Hypoxylon haematostroma Mont. apud Ramon de la Sagra, Fl. Cubana I : 344 (1842).

Descriptions and illustrations : Miller 1961 : 36-37, figs. 24-26, 52 ; Dennis 1963 : 325, fig. 17D ; Martin 1969 : 195-196.

The ascospores of our specimen are smaller than those given by Miller (1961) and Dennis (1963) and than those measured in the type material : 13-14 x 5.5-8 µm vs 14-18 x 7-9 µm. The dimensions correspond however with those given by Martin (1969) : 10-16.5 x 5-9 µm (avg. 13.4 x 6.8 µm), as well as with those measured in the reference material (Vanderyst s.n. 1908) : 13.5-15.5 x 6-7.5 µm. The ascospores are ellipsoid - inaequilateral with rounded ends and a straight germ slit running on the most convex side of the spore over the whole length (see fig. 4). They appear to be smooth when examined by light microscopy. However when examined by S.E.M. the perispore seems to be ornamented with faint striations oriented perpendicular to the long axis of the spore (see fig. 4 & pl. I d).

Specimen examined : PAPUA NEW GUINEA : MOROBE PROVINCE : Lae, 6°43'S & 146°53'E, elev. 150 m, on dead wood, 24.10.1989, Van der Gucht K. 89-1053a (GENT).

Plate I : S.E.M. photographs of ascospores (scale bar = 1 µm) : a. *Hypoxylon crocopeplum* (Van der Veken P. 90-674) ; b. *H. dieckmannii* (Van der Gucht K. 89-931) ; c. *H. rubiginosum* (Van der Gucht K. 89-604a) ; d. *H. haematostroma* (Van der Gucht K. 89-1053a) ; e. *H. hypomiltum* (Van der Gucht K. & De Meester L. 89-2012a) ; f. *H. oodes* (Van der Gucht K. 89-510)


a
b
c
d
e
f

Reference material examined : CUBA : ex Montagne Herb. (TYPE, K).
ZAIRE : District du Bas-Congo, Sanda, 04°41'S & 15°26'E, on dead wood, 1908, Vanderyst H. s.n., determinavit Dennis (BR).

Hypoxylon hypomiltum Mont., Ann. Sci. Nat. Bot. sér. 2. 13 : 356 (1840). (non sensu J. H. Miller, 1961)

Descriptions and illustrations : Miller 1961 : 39-40, figs. 29, 55 ; Martin 1969 : 196-198 ; Abe 1986.

Our specimens correspond with the description of *H. jecorinum as* given by Miller (1961) and Martin (1969), and with the description of *H. hypomiltum* var. *hypomiltum* (non sensu J.H. Miller, 1961) as given by Abe (1986), but the ascospore ornamentation was not mentioned in these accounts.
Abe (1986), after examining the type specimens, placed *H. jecorinum* into synonymy with *H. hypomiltum* (non sensu J.H. Miller, 1961), a synonymy on which we can agree after examining the type material of *H. hypomiltum* and *H. jecorinum*. The ascospores are navicular to inaequilaterally ellipsoid, 7-9 (11) x 3.5-4.5 (5) µm, with a sigmoid germ slit, full length on the convex side of the spore (see fig. 5). They are characterized by a conspicuous dehiscent hyaline perispore (see also Rogers et al. 1987), which appear to be indistinctly transversely striate by light microscopy. When examined by S.E.M. they seem to be adorned with parallel to anastomosing rope-shaped ornaments, transversely oriented (see fig. 5 & pl. I e).

Specimens examined : PAPUA NEW GUINEA : MOROBE PROVINCE : Lae, 6°36'S & 147°02'E, elev. 300 m, on dead wood, 23.10.1989, Van der Gucht K. 89-1033 (GENT).
MADANG PROVINCE : South Naru, elev. 200 m, on dead wood, 13.11.1989, Van der Gucht K. & De Meester L. 89-2012a (GENT).

Reference material examined : FRENCH GUYANA : Cayenne, Leprieur 371 (HOLOTYPE of *H. hypomiltum*, K).
AMER. BOR. : Ex herb. Berk., on fallen limbs of *Platanus*, 1828 (SYNTYPE of *H. jecorinum*, K).
BRAZIL : Rio Grande do Sul, S. Leopoldo, on dead wood, Theissen, Decades Fungorum Brasiliensium exs. 74, determinavit Miller (L).

Hypoxylon nectrioideum Sacc. & Trott., Bull. Soc. Roy. Bot. Belgique 28 : 160 (1899).

Description and illustration : Dennis 1963 : 322, fig. 17B.

This specimen corresponds completely to the description given by Dennis (1963). The ascospores are oval to equilaterally ellipsoid with rounded ends, smooth, with a straight short germ slit (see fig. 6).

Specimen examined : PAPUA NEW GUINEA : MOROBE PROVINCE : Lae, 6°42'S & 146°51'E, sea level, on dead wood, 8.11.1989, Van der Gucht K. & De Meester L. 89-1662 (GENT).

Reference material examined : ZAIRE : locality unknown, Dewèvre A. s.n., (HOLOTYPUS, BR).

Hypoxylon oodes Berk. & Br., J. Linn. Soc., Bot. 14 : 122 (1873).

Descriptions and illustrations : Miller 1961 : 21, figs. 8, 39 ; Dennis 1963 : 320, fig. 17A ; Martin 1969 : 155-158, pl. I: 10-11.

Our material corresponds completely with the descriptions given by Miller (1961), Dennis (1963) and Martin (1969), but the ascospore ornamentation was not mentioned. The ascospores are inaequilaterally ellipsoid to navicular with a sigmoid germ slit on the convex side of the spore (see fig. 7). They are ornamented with faint transverse striations only seen by S.E.M. (see pl. I f).

Specimens examined : PAPUA NEW GUINEA : MADANG PROVINCE : Laing Island, 4°10'S & 144°52'E, sea level, on dead wood of *Diospyros maritima* Bl. (Ebenaceae), 3.10.1989, Van der Gucht K. 89-510 (GENT). Eo loco, on dead wood, 4.10.1989, Van der Gucht K. 89-525 (GENT). Eo loco, on dead wood of *Excoecaria agallocha* L. (Euphorbiaceae), 4.10.1989 & 6.10.1989, Van der Gucht K. 89-545a & 89-579a (GENT). Eo loco, on dead wood, 6.10.1989, Van der Gucht K. 89-584a (GENT). Eo loco, on dead wood, 17.11.1989, Van der Gucht K. 89-2034 & 89-2039 (GENT).
MOROBE PROVINCE : Lae, 6°42'S & 146°51'E, sea level, on dead wood, 8.11.1989, Van der Gucht K. & De Meester L. 89-1661 (GENT). Lae, 6°36'S & 147°02'E, elev. 200 m, on dead wood, 9.11.1989, Van der Gucht K. & De Meester L. 89-1671a (GENT).

Reference material examined : ZAIRE : District du Bas-Congo, Kisantu, 05°08'S & 15°06'E, on decorticated wood, 31.01.1907, Vanderyst H. s.n., determinavit Dennis (BR).

Hypoxylon retpela[1] K. Van der Gucht & P. Van der Veken, sp. nov.

Stromata superficialia, applanata, 2 x 3 cm x 0.6 mm metientes, superficie cinereo-rosea vel rubiginosa (K. & W. PL. 9 D4), proxime sub superficie et circum partes peritheciorum superiores aurantiaca vel lateritia. Perithecia semiglobosa vel e compressione angularia, 0.25-0.4 mm diametro, emersa vel immersa. Ostiola umbilicata. Asci octospori, cylindrici, 105-130 µm longitudine tota x 6.5-8 µm crassi, partibus sporiferis 80-90 µm longitudine, annulo apicali in liquore iodata Melzeri immerso cyanescenti placentiformi 1 µm alto x 2.5 µm crasso. Paraphyses filiformes, 3 µm diametro, septatae. Ascosporae uni-seriatae inaequilateraliter ellipsoideae, obscure brunneae, 9-12 x 4-5.5 µm (plus minusve 9.8 x 4.7 µm). Rima germinativa recta per totam longitudinem sporae in latere convexo. Perispora conspicua hyalina dehiscens. Sub microscopio luminoso observata superficie laevis vel indistincte transverse striata, sub microscopio electrinoco scrutante simulacrum dantes ornamentorum parallelorum vel anastomosantium funiculiformium in spora transverse positorum.

Stromata superficial, applanate, 2 x 3 cm and 0.6 mm high. Surface greyish rose to reddish brown (K. & W. PL. 9 D4), with orange, brick red particles just beneath

[1] pidgin for pink and red

the surface and between the perithecial vertices. Acetone extract of the stroma reddish orange. **Perithecia** clearly evident to immersed, globose to compressed, 0.25-0.4 mm diam. **Ostiola** umbilicate. **Asci** 8-spored, cylindrical , 105-130 x 6.5-8 µm (the spore bearing part 70-80 µm), apical ring discoid, 1 µm high x 2.5 µm broad, blueing in Melzer's iodine reagent (see fig. 8a). **Paraphyses** filiform, 3 µm diam., septate. **Ascospores** uniseriate, inaequilaterally ellipsoid, dark brown, 9-12 x 4-5.5 µm (avg. 9.84 x 4.71 µm). Germ slit straight, full length, on the convex side of the spore (see fig. 8b). Perispore conspicuous, dehiscent, hyaline, appearing smooth or indistinctly transversely striate by light microscopy, seems to be adorned with parallel to anastomosing rope-shaped ornaments oriented perpendicular to the long axis of the spore by scanning electron microscopy (see fig. 8b & pl. II a).

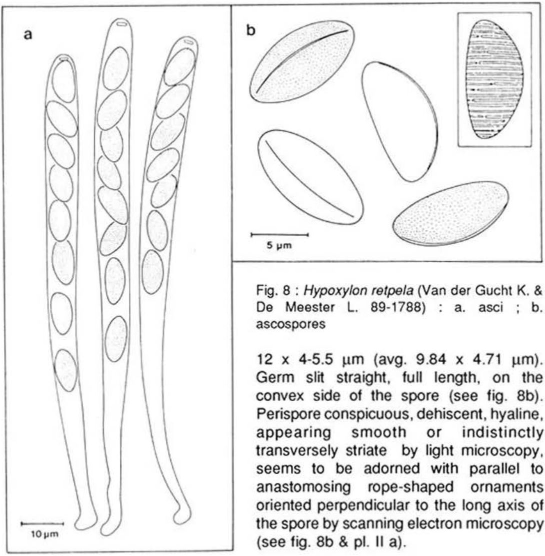


Fig. 8 : *Hypoxylon retpela* (Van der Gucht K. & De Meester L. 89-1788) : a. asci ; b. ascospores

Hypoxylon retpela seems closely related to *H. crocopeplum, H. duranii* (Rogers 1985), *H. gillesii* (Rogers & Candousseau 1982), *H. hypomiltum* and *H. subgilvum*, the only described species with ascospores conspicuously ornamented with transversely oriented ribs.

It is distinguished by its greyish rose to reddish brown stromatal coloration instead of the typical orange brown to rusty red coloration as is found in the other species.

Specimen examined : PAPUA NEW GUINEA : MADANG PROVINCE, Balek Wildlife Sanctuary, elev. 150 m, on dead wood, 11.11.1989, Van der Gucht K. & De Meester L. 89-1788 (HOLOTYPE, GENT ; ISOTYPE, K).

Hypoxylon rubiginosum (Pers.:Fr.) Fr., Summa Veg. Scand. : 384 (1849).

Descriptions and illustrations : Miller 1961 : 26-31, figs. 13-15, 45 ; Dennis 1963 : 322-325 ; Martin 1969 : 172-175, pl.I: 3 & pl.II: 1-4 ; Rogers 1969 ; Petrini & Müller 1986 : 529-534, abb. 12-14.

Our material corresponds well with the descriptions given by Miller (1961), Dennis (1963), Martin (1969) and Rogers (1969). Petrini & Müller (1986) recognized three different varieties of *H. rubiginosum* based on European material. Our material corresponds to *H. rubiginosum* var. *perforatum* characterized by the short stipes of the ascus : 25-40 µm (total size of the ascus : 95-105 x 6-8 µm). This variety is also known from Brazil (Petrini & Müller 1986).
The ascospores are inaequilaterally ellipsoid, 9.5-11 (12.5) x 4-5.5 µm, with a straight germ slit full length on the convex side of the spore (see fig. 9). The perispore is ornamented with transversely oriented fibrils, only visible by S.E.M. (pl. I c).

Specimens examined : PAPUA NEW GUINEA : EASTERN HIGHLANDS PROVINCE : Ukarumpa, 6°20'S & 146°53'E, elev. 1700 m, on dead wood, 8.10.1989, Van der Gucht K. 89-604a (GENT). MOROBE PROVINCE : Wau, Biaru Raod, 7°30'S & 146°48'E, elev. 1650 m, on dead wood, 5.11.1989, Van der Gucht K. & De Meester L. 89-1549 (GENT).

Hypoxylon sp., a member of the rubiginosum complex.

This fungus differs from typical *H. rubiginosum* as described by Miller (1961) in having brick red granules just beneath the stromatal surface and between the perithecial vertices. The perithecia, asci and ascospores are smaller (see table 2 & fig. 10). The ornamentation of the perispore is less conspicuous than for the typical *H. rubiginosum.*

Table 2 : comparison of *H. rubiginosum* with *H. sp.*, a member of the rubiginosum complex.

	H. rubiginosum	*H. sp.*
perithecia (mm)	0.2-0.3	0.1-0.2
asci (µm)	95-105 x 5-8 (sp.p. 65-75)	60-75 x 6 (sp.p. 50-55)
ascospores (µm)	9.5-12.5 x 4-5.5 (avg. 10.5 x 4.8)	7.8-9 x 3.5-4.5 (avg. 8.4 x 3.9)

Rogers et al. (1987) collected a similar specimen from the rain forests of North Sulawesi. Our material differs from their description primarily in having smaller ascospores 7.8-9 x 3.5-4.5 vs (10)11-12 x 4.5-6 µm. The present material was probably somewhat immature.

Specimen examined : PAPUA NEW GUINEA : SOUTHERN HIGHLANDS PROVINCE : Kaupena, 6°10'S & 144°01'E, elev. 2280 m, on dead branch of *Bambusa sp.*, 11.10.1989, Van der Gucht K. 89-735 (GENT).

Hypoxylon sclerophaeum Berk. & Curt., Exot. Fungi Schw., J. Acad. Nat. Sci. Philadelphia ser. 2 : 285 (1853).

Descriptions and illustrations : Miller 1961 : 40-41, figs. 30, 56 ; Dennis 1963 : 326, fig. 17F ; Martin 1969 : 202, pl.I: 13.

Our specimens correspond completely with the descriptions given by Miller (1961), Dennis (1963) and Martin (1969). The ascospores are inaequilaterally ellipsoid, smooth, with a straight germ slit full length on the convex side of the spore (see fig. 11).

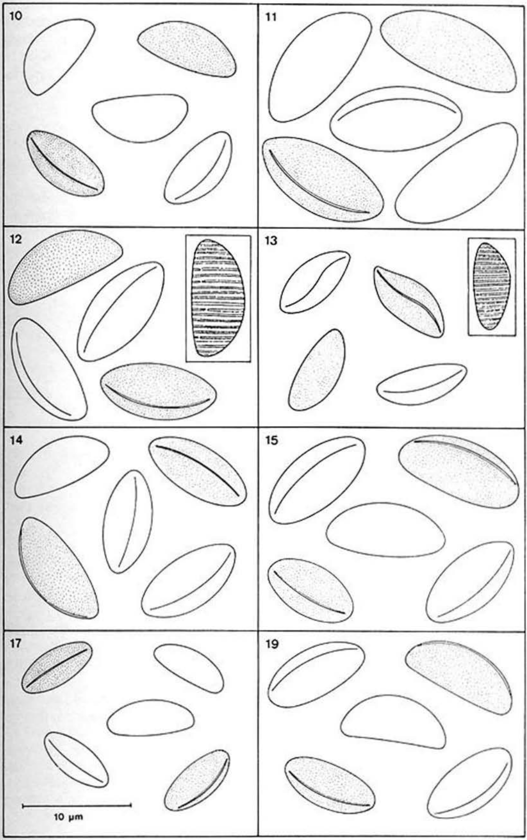
Specimens examined : PAPUA NEW GUINEA : MADANG PROVINCE : Laing Island, 4°10'S & 144°52'E, sea level, on dead wood, 4.10.1989 & 6.10.1989, Van der Gucht K. 89-526 & 89-576 (GENT).

Hypoxylon subgilvum Berk. & Br., J. Linn. Soc., Bot. 14 : 120 (1873).

Descriptions and illustrations : Miller 1961 : 38-39, figs. 28, 54 ; Dennis 1963 : 326, fig. 17E ; Martin 1969 : 193, pl.II: 15.

This material corresponds completely with the description of *Hypoxylon hypomiltum* (non sensu Abe, 1986) as given by Miller (1961), Dennis (1963) and Martin (1969), and with the morphological data of *H. subgilvum* given by Abe (1986), with the exception that ascospore ornamentation, existing of transversally oriented ribs, was not mentioned.
The ascospores of *H. subgilvum* are inaequilaterally ellipsoid with a straight germ slit running full length on the convex side of the spore (see fig. 12). The germ slit

Figs. 10-12 : ascospores of the section *Hypoxylon* : 10. *Hypoxylon sp.*, a member of the rubiginosum complex (Van der Gucht K. 89-735) ; 11. *H. sclerophaeum* (Van der Gucht K. 89-526) ; 12. *H. subgilvum* (Demoulin V. 6902) (inset : perispore ornamented with faint transverse striations) ; section *Papillata* subsection *Papillata* : 13. *H. investiens* (Van der Gucht K. & De Meester L. 89-1646) (inset : perispore ornamented with faint transverse striations) ; section *Annulata* : 14. *H. archeri* (Van der Gucht K. & De Meester L. 89-1722) ; 15. *H. bovei* var. *microsporum* (Van der Gucht K. 89-818) ; 17. *H. stygium* (Van der Gucht K. & De Meester L. 89-1656) ; 19. *H. truncatum* (Van der Gucht K. & De Meester L. 89-1686)


10
11
12
13
14
15
17
19
10 µm

is not always evident. They are characterized by a conspicuous dehiscent hyaline perispore which appears to be indistinctly transversely striate by light microscopy, just like the ascospores of *H. crocopeplum, H. hypomiltum* and *H. retpela.* Examining the spores by S.E.M. we found a similar ornamentation exhibited by those species, existing of parallel to anastomosing rope shaped ornaments, oriented perpendicular to the long axis of the spore (see pl. II b).

A very similar ornamentation of ascospores has already been found within the species *H. gillessii* (Rogers & Candousseau 1982) and *H. duranii* (Rogers 1985), the only other species described with ascospores conspicuously ornamented with transversely oriented ribs.
All these species, *H. crocopeplum, H. subgilvum, H. hypomiltum, H. gillessii* and *H. duranii* are similar in the rusty red color of mature stromata and the umbilicate ostioles. They differ in the size of their ascospores and in the habit of their stromata.

Specimen examined : PAPUA NEW GUINEA : MADANG PROVINCE : Laing Island, sea level, 4°10'S & 144°52'E, on dead wood, 18.4.1986, Demoulin V. 6902 (LG, GENT).

Reference material examined : CEYLON : Dec. 1868, Twaite L.H.K. 1087 (HOLOTYPE, K). ZAIRE : District du Bas-Congo, Kisantu, 05°08'S & 15°06'E, on dead wood, 1907, Vanderyst H. s.n., determinavit Dennis (BR).

II. Section *Papillata* subsection *Papillata*

Hypoxylon* cf. *investiens (Schw.) Curt., Geol. & Nat. Hist. Survey, 3 : 140 (1867).

Descriptions and illustrations : Miller 1961 : 49-52, figs. 65-66, 81 ; Dennis 1963 : 327, fig. 17G ; Martin 1968 : 307.

This fungus looked much like *H. investiens* as described by Miller (1961) and Dennis (1963) but differs in having smaller ascospores i.e. 8-11 x 3.5-5 µm vs 7-9.5 x 3-4 µm. Martin (1968) gives a wide range of spore size i.e. 3-7.5 x 6-18 µm but the average he mentioned is again larger than the average we find cfr. 10.3 x 4.7 µm vs 8.23 x 3.49 µm. Rogers et al. (1987) find the same phenomenon within the specimens they collected in the rain forests of North Sulawesi (Indonesia). The dimensions of the ascospores of their specimen (7-9.5 x 3-3.8 µm) fit completely our spore dimensions.
There is a strong possibility we have here a small spored variety of *Hypoxylon investiens* which can be found in the tropics. Small spored varieties have

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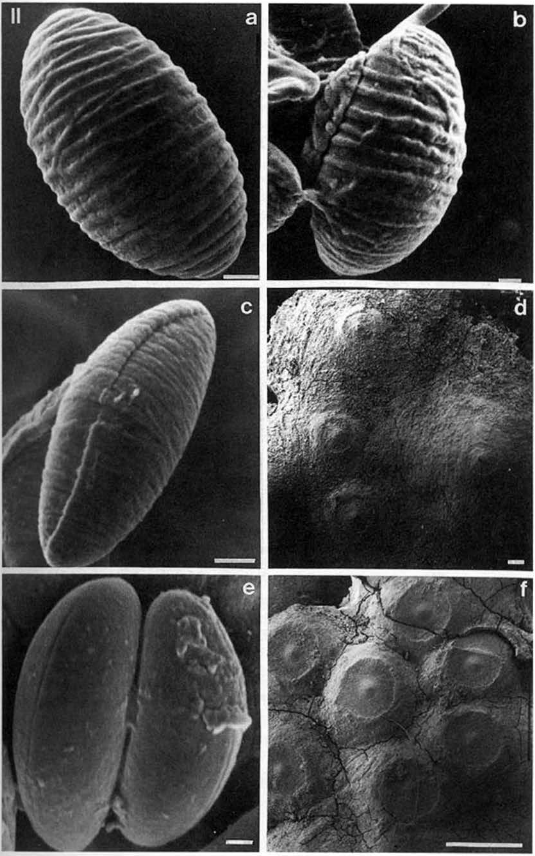
Plate II : S.E.M. photographs : a-b-c-e ascospores (scale bar = 1µm) : a. *Hypoxylon retpela* (Van der Gucht K. & De Meester L. 89-1788) ; b. *H. subgilvum* (Demoulin V. 6902) ; c. *H. investiens* (Van der Gucht K. 89-1051) ; e. *H. macroannulatum* (Van der Gucht K. 89-636a) ; d. stroma of *H. subannulatum* (Van der Gucht K. 89-1221) (scale bar = 100 µm) ; f. stroma of *H. macroannulatum* (Van der Gucht K. 89-636a) (scale bar = 1mm)


II
a
b
c
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f

repeatedly been observed within the genus *Hypoxylon* from different continents e.g. *Hypoxylon weldenii* (Rogers 1980), *Hypoxylon chestersii* (Rogers & Samuels 1985) and *Hypoxylon aeruginosum* (Rogers & Samuels 1985).

Another important characteristic is the spore ornamentation. The ascospores are navicular to inaequilaterally ellipsoid with a sigmoid germ slit full length on the convex side of the spore (see fig. 13). When examined superficially the mature ascospores appear to be smooth. However, faint transverse striations can be seen at high magnification, and can be made more conspicuous by making a slide in KOH (10%).
The true nature of the ascospore ornamentation becomes clear when examined by S.E.M. It is composed of subparallel ribs, transversely oriented (see pl. II c). The ascospore ornamentation of our specimens seems identical to the typical variety.
This is the first species within the section Papillata subsect. Papillata known to have ornamented ascospores.
The presence of more or less conspicuous transversely oriented perispore elements was already seen in some species of the section Hypoxylon (see *H. hypomiltum*, *H. subgilvum*, *H. rubiginosum* and *H. haematostroma*). This ornamentation of the ascospores might indicate a closer relationship of *H. investiens* with the section Hypoxylon.
Whalley and Whalley (1977) suggest that *H. investiens* and its allies form a transitional series between the sections Hypoxylon and Papillata subsect. Primo-cinerea, on account of the colour of the acetone extract of the stroma. They found that only one collection of *H. investiens* yielded pigment, the others remained colourless. We also found coloured as well as colourless extracts of our specimens.

<u>Specimens examined</u> : PAPUA NEW GUINEA : MADANG PROVINCE : Laing Island, 4°10'S & 144°52'E, sea level, on dead wood of *Diospyros maritima* Bl. (Ebenaceae), 3.10.1989, Van der Gucht K. 89-512a (GENT). Eo loco, on dead wood, 6.10.1989, Van der Gucht K. 89-589 (GENT). Bunapas, 4°12'S & 144°49'E, sea level, on dead wood, 30.10.1989, Van der Gucht K. & De Meester L. 89-1311 (GENT). Finisterre Range, 5°28'S & 145°29'E, elev. 500 m, on dead wood, 12.11.1989, Van der Gucht K. & De Meester L. 89-1873, 89-1900 (GENT). The end of the Finisterre Range, 5°45'S & 145°53'E, elev. 150 m, on dead wood, 3-11-1989, Van der Gucht K. & De Meester L. 89-1465 (GENT).
MOROBE PROVINCE : Lae, 6°43'S & 146°53'E, elev. 150 m, on dead wood, 24.10.1989, Van der Gucht K. 89-1051, 89-1056c (GENT). Wau Road, 6°52'S & 146°37'E, elev. 650 m, on dead wood, 8.11.1989, Van der Gucht K. & De Meester L. 89-1646a (GENT). Lae, 6°36'S & 147°02'E, elev. 200 m, on dead wood, 9.11.1989, Van der Gucht K. & De Meester L. 89-1671b (GENT).

<u>Reference material examined</u> : ZAIRE : District Forestier Central, Boende, 00°13'S & 20°52'E, on dead wood, 10-1926, Staner R. 1672, determinavit Dennis (BR).

III. Section *Annulata*

Hypoxylon archeri Berk., Fl. of Tasmania II, in Hook., Bot. Antarctic Voy. II : 280 (1860).

Descriptions and illustrations : Miller 1961 : 91, figs. 155-169 ; Dennis 1964 : 236.

Our material corresponds well with the descriptions given by Miller (1961) and Dennis (1964). The ascospores are inaequilaterally ellipsoid, smooth, with a straight germ slit running full length on the convex side of the spore (see fig. 14).

Specimens examined : PAPUA NEW GUINEA : MADANG PROVINCE : the end of the Finisterre Range, 5°45'S & 145°35'E, elev. 150 m, on dead wood, 3-11-1989, Van der Gucht K. & De Meester L. 89-1521 (GENT).
MOROBE PROVINCE : Lae, 6°43' S & 147°04'E, elev. sea level, on dead wood, 25-10-1989, Van der Gucht K. 89-1157 (GENT). Lae, 6°36'S 147°02'E, elev. 200 m, on dead wood, 9-11-1989, Van der Gucht K. & De Meester L. 89-1722 (GENT).

Reference material examined : ZAIRE : District Forestier Central, Yangambi, 00°46'S & 24°27'E, on *Scorodophloeus zenkeri* Harms, Fassi B. 774, determinavit Dennis (BR).

Hypoxylon bovei Speg. **var. *microsporum*** Mill., Monograph : 95 (1961).

Descriptions and illustrations : Miller 1961 : 95 ; Pérez-Silva 1983 : 11, figs. 16 & 17.

This fungus is very much like *H. bovei* var. *microsporum* as described by Miller (1961), differing in its somewhat larger ascospores i.e. 9-10.5 x 4-5 µm vs 8-10 x 3-4 µm.
Pérez-Silva (1983) mentioned an incomplete germ slit for the spores. We could however clearly observe a germ slit of full spore length (see fig. 15).

Specimen examined : PAPUA NEW GUINEA : EASTERN HIGHLANDS PROVINCE : Ukarumpa, 6°21'S & 145°56'E, elev. 1800-1850 m, on dead wood, 14.10.1989, Van der Gucht K. 89-818 (GENT).

Hypoxylon macroannulatum Ito & Imai, Trans. Sapporo Nat. Hist. Soc. 16 : 137 (1940).

Stromata globose to hemispheric, surface black, 1.5 - 3 cm diam. near the base and 0.8 - 1 cm high, superficial on bark (see fig. 16a). Endostroma dark brown, massive in development, carbonaceous to corky at the base, distinctly radiate - fibrous. Acetone extract of the stroma yellowish brown (K. & W. PL. 5 D5). **Perithecia** peripheral, globose to angular due to compression, 0.8 - 1 mm in diam.
Ostiola papillate each in the center of a slightly sunken, plane annular disc usually with a raised margin, 0.65 - 0.80 mm in diam. (avg. 0.7 mm diam.) (see fig. 16a & pl. II f). **Asci** and **paraphyses** not seen. **Ascospores** inaequilaterally ellipsoid with rounded ends, brown, 9.5-12.5 x 4.5-5.5 µm (avg. 11 x 5 µm). Germ slit straight, full length, on the convex side of the spore (see fig. 16b & pl. II e). Perispore smooth.

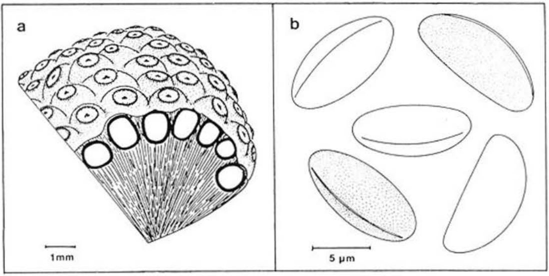


Fig. 16 : *Hypoxylon macroannulatum* (Van der Gucht K. 89-636a) : a. stroma ; b. ascospores

Hypoxylon macroannulatum was described by Ito & Imai (1940 : 137) on a collection from the Bonin Islands. Our material corresponds to their description very well. *H. macroannulatum* was up to now only known from the type locality.

Specimen examined : PAPUA NEW GUINEA : EASTERN HIGHLANDS PROVINCE : 6°22'S & 146°55'E, elev. 1750 m, on bark of unidentified wood, 9.10.1989, Van der Gucht K. 89-636a (GENT).

Hypoxylon stygium (Lév.) Sacc., Syll. Fung. 1 : 379 (1882).

Descriptions and illustrations : Miller 1961 : 91-93, figs. 156, 170 ; Dennis 1963 : 334-335, fig. 18C ; Martin 1968 : 322-328, pl. I: 7.

In the literature we find differences in the descriptions of *Hypoxylon stygium*. Table 4 gives the most important characteristics.
The most striking difference lies within the size of the perithecia i.e. small to minute according to Miller (1961) and Dennis (1963) and clearly larger according to Martin (1968).
Based on those descriptions our material of *H. stygium* could be divided in three groups of specimens. One that corresponds completely with the description given by Miller (1961) and Dennis (1963). Another that fits Martin's description (1968), and finally a third group of specimens that lies in between, which means that they show large perithecia and very small annular discs (< 0.2 mm) or vice versa.
The ascospores are inaequilaterally ellipsoid, smooth with a straight long germ slit, on the convex side of the spore (see fig. 17).

Table 4 : Comparison of the dimensions of the main characteristics of *H. stygium* according to three different authors and our material.

	Miller (1961)	Dennis (1963)	Martin (1968)	our material
Size of perithecia (mm)	100-300	→ 400	500-800 x 750-900	200-800
annular disc (mm)	0.1-0.2	→ 0.3	0.2-0.3	0.1-0.3
asci (µm)	sp.p. 40-60 x 3.5-4 (stipe 15-20)	-	54-170 x 3-5 (stipe 9-105)	60-105 x 3-4.5 (sp.p. 50-60)
ascospore dimensions (µm)	5-8 x 2.5-3	5-8 x 2.5-3	4.5-8.5 x 2-5	5-8 x 2.5-3.5

Specimens examined : PAPUA NEW GUINEA : MADANG PROVINCE : Laing Island, 4°10'S & 144°52'E, sea level, on dead decorticated wood, 4.10.1989, Van der Gucht K. 89-533 (GENT). Finisterre Range, 5°24'S & 145°38'E, elev. 200 m, on dead wood, 22.10.1989, Van der Gucht K. 89-953b (GENT). Finisterre Range, 5°45'S & 145°35'E, elev. 150 m, on dead wood, 3.11.1989, Van der Gucht K. & De Meester L. 89-1480 (GENT). Bunapas, 4°11'S & 144°47'E, sea level, on dead wood, 30.10.1989, Van der Gucht K. & De Meester L. 89-1360 (GENT). Balek Wildlife Sanctuary, 5°20'S & 145°43'E, elev. 150 m, on dead wood, 11.11.1989, Van der Gucht K. & De Meester L. 89-1818 (GENT). South Naru, elev. 200 m, on dead wood, 13.11.1989, Van der Gucht K. & De Meester L. 89-1996 (GENT).

MOROBE PROVINCE : Bulolo, Manki, on *Castanopsis acuminatissima*, 31.1.1973, Horak E. NG 178 (K). Lae, 6°43'S & 147°01'E, sea level, on dead wood, 15.10.1989, Van der Gucht K. 89-826, 89-827a (GENT). Lae, 6°36'S & 147°02'E, elev. 300 m, on dead wood, 23.10.1989, Van der Gucht K. 89-1031 (GENT). Lae, 6°43'S & 146°53'E, elev. 150 m, on dead wood, 24.10.1989, Van der Gucht K. 89-1049a (GENT). Lae, 6°42'S & 146°51'E, sea level, on dead wood, 8.11.1989, Van der Gucht K. & De Meester L. 89-1656, 89-1659 (GENT). Lae, 6°36'S & 147°02'E, elev. 200 m, on dead wood, 9.11.1989, Van der Gucht K. & De Meester L. 89-1667, 89-1669, 89-1720 (GENT).

EASTERN HIGHLANDS PROVINCE : Ukarumpa, 6°20'S & 146°53'E, elev. 1700 m, on dead wood, 8.10.1989, Van der Gucht K. 89-609 (GENT). Ukarumpa, 6°21'S & 145°56'E, elev. 1800 m, on dead wood, 14.10.1989, Van der Gucht K. 89-819 & 89-821b (GENT).

SOUTHERN HIGHLANDS PROVINCE : near lake Kutubu, 6°20'S & 143°17'E, elev. 910 m, on dead decorticated wood, 11.10.1988, Vyverman W. 339 (GENT).

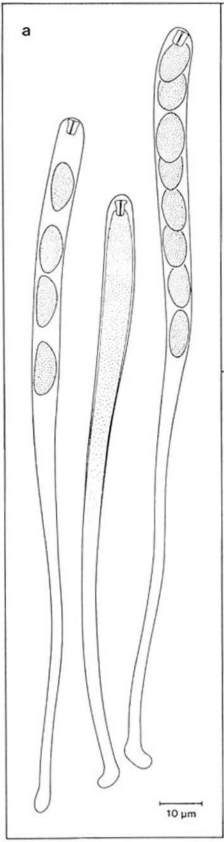
Reference material examined : ZAIRE : District Forestier Central, Yangambi, 00°46'S & 24°27'E, on *Scorodophleus zenkeri* Harms., Fassi B. 764, determinavit Dennis (BR).

Hypoxylon subannulatum Henn. & Nym., Monsunia 1 : 168 (1899).

Description and illustration : Miller 1961 : 93-94, figs. 157, 171.

Our material corresponds to the description of *H. subannulatum* as given by Miller (1961), except for the ascospores which are somewhat smaller : 12-15 x 5.6-7 µm vs (9.5) 10-14 x (4) 4.5-6 µm.

Further characters, not mentioned by Miller (1961) are : asci with a rectangular (3-4 x 2.5 µm), amyloid apical plug (see fig. 18a); ascospores devoid of a



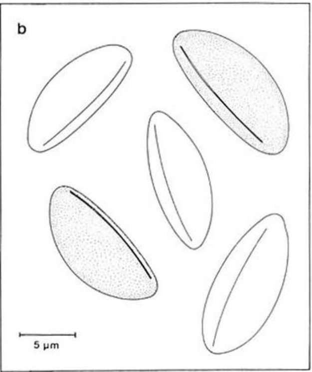


Fig. 18 : *Hypoxylon subannulatum* (Van der Gucht K. 89-1220) : a. asci ; b. ascospores

loosening hyaline perispore and with a straight germ slit running full length on the ventral side of the spore (see fig. 18b) ; the aceton extract of the stroma remains colourless. (see fig. 18a & b ; pl. II d).
Those characters strongly suggest that *H. subannulatum* should not be included in *Hypoxylon* s.str., characterized by asci with a flat or discoid ascal plug, ascospores with a loosening perispore and a germ slit on the dorsal side.
Abe (1984) also mentioned that the tissues of the stroma rather resemble those of *H. serpens* than those of *H. truncatum*, *H. stygium* and *H. archeri*. A photograph of the stroma is shown in Pl. II d.
Provisionally it would better be placed within *Hypoxylon* sect. *Papillata* subsect. *Primocinerea*, a complex of species that needs a redistribution in other genera.

Specimens examined : PAPUA NEW GUINEA :

MADANG PROVINCE : Finisterre Range, 5°27'S & 145°32'E, elev. 250 m, on dead wood, 27.10.1989, Van der Gucht K. 89-1220 & 89-1221 (GENT).

Reference material examined : PHILIPINES : Laguna Province, Mount Maquiling, near Los Banos, on rotting trunks, Baker C.F. 543, determinavit Abe 1986 & Laessoe 1991 (K)

Hypoxylon truncatum (Schw. ex Fr.) Mill., Trans. Brit. Myc. Soc. 17 : 130 (1932).

Descriptions and illustrations : Miller 1961 : 95-98, figs. 160-165, 173-174 ; Dennis 1963 : 335-336, fig. 18D ; Carroll 1964 : 303-304, figs. 6, 12-13 ; Martin 1968 : 317-322, pl. I: 3-6.

Our material corresponds with the descriptions given by Miller (1961), Dennis (1963), Carroll (1964) and Martin (1968). The ascospores are inaequilaterally ellipsoid, smooth, with a straight long germ slit running on the convex side of the spore (see fig. 19).

Specimens examined : PAPUA NEW GUINEA : MADANG PROVINCE : Laing Island, 4°10'S & 144°52'E, sea level, on dead wood, 4.10.1989, Van der Gucht K. 89-527 (GENT). Finisterre Range, 5°24'S & 145°38'E, elev. 200 m, on dead wood, 22.10.1989, Van der Gucht K. 89-953a, 89-971 (GENT). Finisterre Range, 5°27'S & 145°32'E, elev. 350 m, on dead wood, 27.10.1989, Van der Gucht K. 89-1270 (GENT). Bunapas, 4°11'S & 144°47'E, sea level, on dead wood, 30.10.1989, Van der Gucht K. & De Meester L. 89-1353 (GENT). Balek Wildlife Sanctuary, 5°20'S & 145°43'E, elev. 150 m, on dead wood, 11.11.1989, Van der Gucht K. & De Meester L. 89-1824 (GENT).
MOROBE PROVINCE : Bulolo, Manki, on *Castanopsis acuminatissima*, 31.1.1973, Horak E. NG 177 (K). Bulolo, Watut, on unidentified wood, 30.1.1973, Horak E. NG 176 (K). Lae, 6°36'S & 147°02'E, elev. 300 m, on dead wood, 23.10.1989, Van der Gucht K. 89-1026 (GENT). Lae, 6°43'S & 146°53'E, elev. 150 m, on dead wood, 24.10.1989, Van der Gucht K. 89-1102 (GENT). Lae, 6°36'S & 147°02'E, elev. 200 m, on dead wood, 9.11.1989, Van der Gucht K. & De Meester L. 89-1670, 89-1677 & 89-1686 (GENT).
EASTERN HIGHLANDS PROVINCE : N.E. of Kainantu, Kassem Pass, on rotten wood, 16.2.1973, Horak E. NG 185 (K).
SOUTHERN HIGHLANDS PROVINCE : Kaupena, 6°10'S & 144°01'E, elev. 2280 m, on dead wood, 11-10-1989, Van der Gucht K. 89-696 (GENT).

Reference material examined : ZAIRE : District du Bas-Congo, Kisantu, 05°08'S & 15°06'E, on dead wood, 12.1906, Vanderyst H. s.n., determinavit Dennis (BR).

Identification key

1a Perithecial ostioles umbilicate or punctate (section *Hypoxylon*) 2
1b Perithecial ostioles papillate 12
  2a Stromata rosellinioid, the individual perithecia sometimes appearing to be completely free 3
  2b Stromata large, plano-convex or stromata indefinitely effused with the perithecial contours usually not evident 4
3a Surface greyish brown to purple grey (K. & W. pl.7E3) with black umbilicate

or occasionally papillate ostioles ; ascospores inaequilaterally ellipsoid to navicular, 11-13 (15) x (4.5) 5-6 (6.5) µm, with a sigmoid germ slit, full length **H. oodes**

3b Surface cinnamon to brownish orange (K. & W. pl.6C5) with umbilicate ostioles ; ascospores equilaterally ellipsoid to oval, 12.5-15.5 x 6-7 (7.5) µm, with a straight, short germ slit **H. nectrioideum**

4a Stromata large, plano-convex, more than 5 mm thick, surface reddish brown to black with age ; ascospores brown, 10-13 (15) x 4.5-6 (6.5) µm **H. sclerophaeum**

4b Stromata indefinitely effused, the individual perithecia embedded in the stroma 5

5a Stromata lacking bright coloured granules just beneath the surface, surface some shade of grey-brown, red or purple 6

5b Stromata with orange to brick red granules just beneath the surface ; ascospores with a conspicuous dehiscent perispore 7

6a Stromata purple red to greyish brown (K. & W. pl.9E4), often with white periphysate ostiolar mouths ; ascospores inaequilaterally ellipsoid, (9.5) 10-12 x 4-5.5 µm **H. rubiginosum**

6b Stromata deep purple red (K. & W. pl.10F4), ostioles indistinct ; ascospores equilaterally ellipsoid with broad rounded ends, 7-9 x 3-4 µm **H. dieckmannii**

7a Stromatal surface some shade of rose or purple 8

7b Stromatal surface bright red to reddish brown, straw colored or brown, never purple 9

8a Stromata purple red (K. & W. pl.10F5) ; ascospores 7.5-9 x 3.5-4.5 µm, perispore ornamented with faint transverse striations only seen by S.E.M.
H. sp., a member of the rubiginosum complex

8b Stromata greyish rose (K. & W. pl.9D4) ; ascospores 9-12 x 4-5.5 µm, perispore ornamented with transversely oriented ribs **H. retpela**

9a Surface bright red to reddish brown (K. & W. pl.8D7), perithecia tubular, soft, easily separating ; ascospores 13-14 x 5.5-8 µm, perispore ornamented with faint transverse striations, only seen by S.E.M. **H. haematostroma**

9b Surface reddish brown, straw colored or brown, perithecia oval to globose, not easily separating ; ascospores smaller, perispores ornamented with transversely oriented ribs 10

10a Stromata brown with red tones (K. & W. pl.7E4) ; ascospores inaequilaterally ellipsoid to navicular, 7-8 x (3) 3.5-4 µm, with a sigmoid germ slit, full length **H. hypomiltum**

10b Ascospores inaequilaterally ellipsoid, larger, with a straight germ slit, full length on the convex side of the spore 11

11a Stromata brownish orange to rusty red (K. & W. pl.6ED5) ; ascospores 8-11 x 4-5 µm **H. subgilvum**

11b Stromata bright orange (K. & W. pl.5B6) ; ascospores 13.5-15 x 6-7 µm **H. crocopeplum**

12a Ostioles simply papillate, not surrounded by an annular disc (section *Papillata* subsection *Papillata*) ; stromata indefinitely effused, some shade of orange brown, rusty brown when young, later dark purplish red to black (pl.6E8→7E8) ; ascospores (6.5) 7-9.55 (11) x 3-4 (4.5) µm **H. cf. investiens**

12b Perithecial ostioles papillate in a flattened disc (section *Annulata*) 13

13a Stromata with wide ostiolar discs, 0.6-0.8 mm in diameter 14

13b Stromata with ostiolar discs less than 0.6 mm in diameter 15

14a Stromata large, subglobose to hemispherical, 1.5-3 cm in diam. ; ascospores 9.5-12.5 x 4.5-5.5 µm **H. macroannulatum**

14b Stromata pulvinate with forms separating into individual perithecia ; ascospores 9-10.5 x 4-5 µm **H. bovei var. microsporum**

15a Ostiolar disc (0.3) 0.4-0.55 mm diam ; ascospores medium brown, 6.5-9.5 x 3.5-5.5 µm **H. truncatum**

15b Ostiolar disc 0.1-0.3 mm diam 16

16a Perithecia widely dispersed in an undulating stroma, border of discs wide, flat ; ascospores (9.5) 10-14 x (4) 4.5-6 µm **H. subannulatum**

16b Perithecia closely placed in stroma, border of discs sharply defined 17

17a Perithecial discs flat to concave ; ascospores 5-8 x 2.5-3.5 µm **H. stygium**

17b Perithecial discs convex ; ascospores (8) 9-11 x 3.5-5 µm **H. archeri**

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COMPUTER CODING OF STRAIN FEATURES OF THE GENUS PYTHIUM

SHUNG-CHANG JONG[1], HON H. HO[2], CANDACE MCMANUS[3], and MICAH I. KRICHEVSKY[3]

[1] American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 USA, [2] Department of Biology, University of New York, New Platz, NY 12561 USA, and [3] Microbial Systematics Section, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892 USA

ABSTRACT

The fungal genus *Pythium* is a large group of aquatic and terrestrial species that are essentially worldwide in distribution. Identification of *Pythium* isolates to species level is often difficult because species are separated mainly by quantitative differences in the morphology of reproductive structures that are frequently overlapping in some species or absent in others. A data coding system used for computer storage and analysis of microbial strain data was expanded to include features specifically applicable to the identification of *Pythium* species.

BACKGROUND AND DISCUSSION

The genus *Pythium* includes over 180 species that are commonly found in soil and water (Waterhouse, 1967, 1968). *Pythium* species are saprophytic or parasitic on a wide variety of plants, attacking primarily the juvenile or succulent tissues and causing serious seed rot and damping-off of seedlings, root rot, fruit rot, and vegetable rot (Hendrix and Campbell, 1973). Some species also attack algae, fungi, animals, and humans (Middleton, 1943; Bissonnette *et al.*, 1991; Deacon *et al.*, 1991).

Identification of *Pythium* species has always been difficult since keys are based almost exclusively on morphological criteria such as the shape and size of sporangia, oogonia, oospores, and antheridia (Matthews, 1931; Middleton, 1943; Waterhouse, 1967, 1968; Hendrix and Papa, 1974; Van der Plaats-Niterink, 1981; Dick, 1990). However, these characters may be overlapping in some species or absent in others, and the range of variation within a species is often unknown. Van der Plaats-Niterink (1981) used physiological characteristics such as optimum and maximum growth temperatures and daily growth rate as supplementary diagnostic criteria. More recently, other methods have been investigated as additional means of distinguishing between two or more species of *Pythium*. These methods include gel electrophoresis of mycelial proteins (Adaskaveg *et al.*, 1988; Chen *et al.*, 1988, 1991; Yu and Ma, 1989); isozyme analysis (Chen *et al.*, 1989, 1991; Yu and Ma, 1989); serology tests (Krywienczyk and Dorworth, 1980); and analysis of total, ribosomal, and mitochondrial DNA (Martin and Kistler, 1987; Belkhiri and Dick, 1988; Martin, 1991; Chen *et al.*, 1990). Attempts have also been made to develop probes specific for species or isolates of *Pythium* by identifying the unique DNA sequences in mitochondrial DNA (Martin, 1990).

None of the methods that have been described for *Pythium* species can be used as a definitive or universal method of identification for all species. Consequently, identification of a *Pythium* strain to the species level can be difficult and time consuming. A protocol combining all features previously found to be important in the differentiation of *Pythium* species would be of great value. Use of a standardized coding system for these features would facilitate computer storage, retrieval, analysis, and exchange of data on *Pythium* strains as well as development of identification keys or probability matrices. In this communication, we present a comprehensive set of features important in the identification of *Pythium* species. These features have been incorporated into an existing computer coding system, the RKC Code, and can be used for identification purposes, information retrieval, and communication.

The RKC Code (after the original authors -- Rogosa, Krichevsky, and Colwell, 1971) is an open-ended, statement-oriented controlled vocabulary of descriptors of strain characteristics or features. The RKC Code was developed originally for bacterial strains (Rogosa *et al.*, 1971) and was later expanded to include features specific for algae (Van Valkenburg *et al.*, 1977), protozoa (Daggett *et al.*, 1980), and selected groups of fungi (Philpot *et al.*, 1982). In 1986, the expanded RKC Code was published under the auspices of the Committee on Data for Science and Technology (CODATA) (Rogosa *et al.*, 1986). More recently, the RKC Code was further expanded to include characteristics specific for yeasts (Jong *et al.*, 1988), the fungal genus *Phytophthora* (Jong *et al.*, 1989), and the saprolegnian fungi (Jong *et al.*, 1991). The Code currently includes more than 12,000 strain descriptors.

The RKC Code is the standardized vocabulary used in the Microbial Information System (MICRO-IS), a comprehensive system of computer programs for storage, management, and analysis of data on microbial strains (Krichevsky, 1987). MICRO-IS has been used by the staffs of the American Type Culture Collection (ATCC) and the Microbial Systematics Section of the National Institute of Dental Research at NIH for managing and analyzing microbial data and in collaborative efforts with other microbiologists to share information resources. Data are encoded and entered into the computer using the unique code numbers that represent the full statement definitions of individual strain features. A computer-managed set of data encoded using features such as those presented here could enable the investigator to enter data on a new strain and use computer programs to aid in identification by use of keys or probability calculations. Even when computer analysis does not give a definitive identification, the number of species that has to be researched further is reduced, and the results may suggest additional tests that can improve the identification. This is an accurate and more convenient and rapid alternative to doing the entire process manually. Additionally, data encoded in this manner can be used to build computer databases for study of taxonomic relationships and construction of identification keys or probability matrices and for sharing data with collaborators.

The set of characteristics developed for use in the identification of *Pythium* species is presented in the list below. Although some of these features were already part of the RKC Code, many of the descriptors are unique to *Pythium* species and were created specifically for this genus. New features added to the RKC Code for the *Pythium* species are marked with an "*" in the list. The features include morphological descriptions of sporangia, zoospores, sexual organs, hyphae, and chlamydospores; colony characteristics; and growth temperatures. The terms used for morphological descriptions are based on the descriptions given in Hawksworth *et al.*, 1983.

ASEXUAL REPRODUCTION

- Sporangia

*043074: Sporangia are present.
008779: Sporangia are produced on agar medium.
008781: Sporangia are produced in water.
008782: Sporangia are produced in liquid growth medium.
*043075: Sporangia are sessile.
008811: Sporangia are terminal.
008812: Sporangia are intercalary.
008570: Sporangia are produced laterally.
*043076: Sporangia are contiguous.
008566: Sporangia occur singly.
008809: Sporangia proliferate internally.
*043077: Proliferating sporangium forms new sporangium inside the old sporangial wall.
*043078: Sporangia are deciduous (shed at maturity).
008800: Sporangia have papillae.
*043079: Sporangia are filamentous (undifferentiated from vegetative hyphae).
*043080: Sporangia are delimited from hyphae by septa.
*043081: Sporangia are inflated.
*043082: Sporangia are branched.
008553: Sporangia are digitate.
043039: Sporangia are lobate.
008558: Sporangia are spherical (length to breadth ratio is 1.0-1.05).
008815: Sporangia are prolate spheroidal (length to breadth ratio is 1.06-1.15).
008774: Sporangia are ellipsoidal (length to breadth ratio is 1.31-1.6).
008773: Sporangia are ovoid (egg-shaped, attached at broad end).
008816: Sporangia are obovoid (egg-shaped, attached at narrow end).
008817: Sporangia are limoniform (lemon-shaped, citriform).

008818: Sporangia are pyriform (pear-shaped, attached at narrow end).
008819: Sporangia are obpyriform (pear-shaped, attached at broad end).
008552: Sporangia are cylindrical.
008821: Sporangia are bursiform (pouch-shaped).
008555: Sporangia are irregular in shape.
*043083: Sporangia are < 11 μ long.
008574: Sporangia are 11-15 μ long.
008575: Sporangia are 16-20 μ long.
008576: Sporangia are 21-30 μ long.
008981: Sporangia are 31-40 μ long.
008982: Sporangia are 41-50 μ long.
008838: Sporangia are 51-60 μ long.
008839: Sporangia are 61-70 μ long.
008840: Sporangia are 71-80 μ long.
008841: Sporangia are 81-90 μ long.
008842: Sporangia are 91-100 μ long.
008843: Sporangia are > 100 μ long.

- Zoospores

008752: Zoospores (motile spores) are produced.
*043084: Zoospores are < 8 μ in diameter.
*043085: Zoospores are 8-10 μ in diameter.
*043086: Zoospores are 11-13 μ in diameter.
*043087: Zoospores are 14-16 μ in diameter.
*043088: Zoospores are 17-19 μ in diameter.
*043089: Zoospores are > 19 μ in diameter.
*043090: Zoospores are bean-shaped (reniform).
*043091: Zoospores are pear-shaped.
008854: Zoospores of sporangia are released naked (unencysted).
*043092: Zoospore cysts are < 7 μ in diameter.
*043093: Zoospore cysts are 7-9 μ in diameter.
*043094: Zoospore cysts are 10-12 μ in diameter.
*043095: Zoospore cysts are 13-15 μ in diameter.
*043096: Zoospore cysts are 16-18 μ in diameter.
*043097: Zoospore cysts are > 18 μ in diameter.

- Zoospore Flagellation

013381: Zoospores have flagella.
013382: Zoospores are biflagellate.
013384: Whiplash flagella (lacking obvious scales or mastigonemes) are produced.
013385: Tinsel flagella (bearing mastigonemes) are produced.
013386: Anterior flagella are of whiplash type.
013387: Anterior flagella are of tinsel type.
013388: Posterior flagella are of whiplash type.
013389: Posterior flagella are of tinsel type.

- Chlamydospores

008363: Chlamydospores are present.
*043172: Chlamydospores are < 21 μ in diameter.
*043173: Chlamydospores are 21-25 μ in diameter.
*043174: Chlamydospores are 26-30 μ in diameter.
008933: Chlamydospores are 31-35 μ in diameter.
*043175: Chlamydospores are 36-40 μ in diameter.
*043176: Chlamydospores are 41-45 μ in diameter.
*043177: Chlamydospores are 46-50 μ in diameter.
*043178: Chlamydospores are > 50 μ in diameter.
*043179: Chlamydospore walls are 1-2 μ thick.
*043180: Chlamydospore walls are 3-4 μ thick.
*043181: Chlamydospore walls are 5-6 μ thick.

SEXUAL REPRODUCTION

008617: Sexual reproduction occurs.
008618: Strain is homothallic (both mating types on same mycelium).
008619: Strain is heterothallic (mating types on separate mycelia).

- Antheridia

008880: Antheridia are present.
*043005: Antheridia are monoclinous (on oogonial stalk).

043006: Antheridia are diclinous (not on same hypha as oogonium).
008883: Antheridia are paragynous (on one side of oogonium).
043003: Antheridia are hypogynous (directly under oogonium on same hypha).
008890: Antheridia twist around oogonial stalks.
*043098: Antheridia are stalked.
*043099: Antheridia are terminal.
*043100: Antheridia are intercalary.
*043101: Antheridia are lateral.
*043102: Antheridia are cylindrical.
*043103: Antheridia are campanulate (bell-shaped).
008897: Antheridia are clavate (club-shaped).
*043104: Antheridia are crook-necked (curved sharply).
008887: Antheridia are contorted.
008888: Antheridia are lobed.
008889: Antheridia are branched.
*043105: Antheridia are furrowed.
*043106: Antheridia are coralloid (branched like coral).
*043107: Antheridial diameter is uniform.

- Oogonia

008899: Oogonia are present.
*043108: Oogonium has one antheridium.
008903: Oogonium has two antheridia.
008904: Oogonium has three antheridia.
*043109: Oogonium has four antheridia.
*043110: Oogonium has five antheridia.
*043111: Oogonium has 6-10 antheridia.
*043112: Oogonium has 11-15 antheridia.
*043113: Oogonium has 16-20 antheridia.
*043114: Oogonium has more than 20 antheridia.
*043115: Oogonial stalk curves towards the antheridium.
008900: Oogonia occur singly.
*043116: Oogonia are catenulate (in chains).
*043117: Oogonia are sessile.
*043118: Oogonia are terminal.
*043119: Oogonia are intercalary.
*043120: Oogonia are lateral.

008915: Oogonia are spherical (length to breadth ratio is 1.0-1.05).
043016: Oogonia are prolate spheroidal (length to breadth ratio is 1.06-1.15).
043017: Oogonia are broadly ellipsoidal (length to breadth ratio is 1.16-1.30).
043018: Oogonia are ellipsoidal (length to breadth ratio is 1.31-1.6).
*043121: Oogonia are limoniform (lemon-shaped).
*043122: Oogonia are irregular in shape.
*043123: Oogonia are < 11 μ in diameter.
*043124: Oogonia are 11-20 μ in diameter.
*043125: Oogonia are 21-30 μ in diameter.
*043126: Oogonia are 31-40 μ in diameter.
*043127: Oogonia are 41-50 μ in diameter.
*043128: Oogonia are 51-60 μ in diameter.
008921: Oogonia are > 60 μ in diameter.
008905: Surfaces of oogonia are smooth.
043012: Surfaces of oogonia have papillate projections.
043013: Surfaces of oogonia have spiny projections.
*043129: Oogonial projections are branched at the tips.
*043130: Oogonial projections are conical.
*043131: Oogonial projections are cylindrical.
*043132: Oogonial projections are 1-2 μ long.
*043133: Oogonial projections are 3-5 μ long.
*043134: Oogonial projections are 6-8 μ long.
*043135: Oogonial projections are 9-12 μ long.
*043136: Oogonial projections are > 12 μ long.

- Oospores

043026: Oospores are present.
043027: Oogonium has one oospore.
*043137: Oogonium has two oospores.
*043138: Oogonium has more than two oospores.
008922: Oospores are plerotic (fill the oogonium).
*043139: Aplerotic index (oospore/oogonium volume) is < 60%.
*043140: Aplerotic index (oospore/oogonium volume) is 60-65%.
*043141: Aplerotic index (oospore/oogonium volume) is 66-70%.
*043142: Aplerotic index (oospore/oogonium volume) is 71-75%.

*043143: Aplerotic index (oospore/oogonium volume) is 76-80%.
*043144: Aplerotic index (oospore/oogonium volume) is > 80%.
*043145: Surfaces of oospores are smooth.
*043146: Surfaces of oospores are reticulate.
*043147: Surfaces of oospores are papillate.
*043148: Oospores are hyaline.
*043149: Oospores are pigmented.
*043150: Oospores are yellow.
*043151: Oospores are violet.
*043152: Oospores are < 11 μ in diameter.
*043153: Oospores are 11-20 μ in diameter.
008924: Oospores are 21-30 μ in diameter.
008925: Oospores are 31-40 μ in diameter.
008926: Oospores are 41-50 μ in diameter.
*043154: Oospores are 51-60 μ in diameter.
*043155: Oospores are > 60 μ in diameter.
*043156: Oospore walls are < 1 μ thick.
008928: Oospore walls are 1-3 μ thick.
008929: Oospore walls are 4-5 μ thick.
*043157: Oospore walls are 6-8 μ thick.
*043158: Oospore wall index (oospore wall/oospore volume) is < 30%.
*043159: Oospore wall index (oospore wall/oospore volume) is 30-35%.
*043160: Oospore wall index (oospore wall/oospore volume) is 36-40%.
*043161: Oospore wall index (oospore wall/oospore volume) is 41-45%.
*043162: Oospore wall index (oospore wall/oospore volume) is 46-50%.
*043163: Oospore wall index (oospore wall/oospore volume) is 51-55%.
*043164: Oospore wall index (oospore wall/oospore volume) is >55%.

- Ooplasts

*043165: Ooplasts are present.
*043166: Ooplast index (ooplast/oospore volume) is < 20%.
*043167: Ooplast index (ooplast/oospore volume) is 20-25%.

*043168: Ooplast index (ooplast/oospore volume) is 26-30%.
*043169: Ooplast index (ooplast/oospore volume) is 31-35%.
*043170: Ooplast index (ooplast/oospore volume) is 36-40%.
*043171: Ooplast index (ooplast/oospore volume) is > 40%.

HYPHAE

*043182: Hyphae are 1-2 μ in diameter.
008943: Hyphae are 3-4 μ in diameter.
008944: Hyphae are 5-6 μ in diameter.
008945: Hyphae are 7-8 μ in diameter.
008946: Hyphae are 9-10 μ in diameter.
008008: Secondary (aerial) hyphae are produced.
008348: Hyphae have swellings (bodies) (outside diameter varies).
008963: Hyphal swellings (bodies) are terminal.
008964: Hyphal swellings (bodies) are intercalary.
008956: Hyphal swellings (bodies) are catenulate (in chains).
008961: Hyphal swellings (bodies) are irregular in shape.
008958: Hyphal swellings (bodies) are ellipsoidal.
008957: Hyphal swellings (bodies) are spherical.
*043183: Hyphal swellings (bodies) are lobed.
*043184: Hyphal swellings (bodies) are < 11 μ in diameter.
*043185: Hyphal swellings (bodies) are 11-20 μ in diameter.
*043186: Hyphal swellings (bodies) are 21-30 μ in diameter.
*043187: Hyphal swellings (bodies) are 31-40 μ in diameter.
*043188: Hyphal swellings (bodies) are 41-50 μ in diameter.
*043189: Hyphal swellings (bodies) are > 50 μ in diameter.
*043190: Hyphal swellings (bodies) are deciduous (shed at maturity).

COLONY CHARACTERISTICS ON SOLID MEDIA

NOTE: Recommended media for observing colony characteristics for *Pythium* species are corn meal agar and potato/carrot agar (Van der Plaats-Niterink, 1981), incubated at 25°C.

016549: Agar macrocolony has uniformly radiate hyphae.
016576: Agar macrocolony has broad, rounded petal-shaped sectors.

016575: Agar macrocolony has narrow petal-shaped sectors.
*016629: Diameter of colony increases by < 1 mm per day.
016552: Diameter of colony increases by 1-5 mm per day.
016553: Diameter of colony increases by 6-10 mm per day.
*016630: Diameter of colony increases by 11-15 mm per day.
*016631: Diameter of colony increases by 16-20 mm per day.
*016632: Diameter of colony increases by 21-25 mm per day.
*016633: Diameter of colony increases by 26-30 mm per day.
*016634: Diameter of colony increases by 31-35 mm per day.

GROWTH TEMPERATURE AND NUTRITION

017032: Growth occurs at 5°C.
017012: Growth occurs at 10°C.
017013: Growth occurs at 15°C.
017037: Growth occurs at 20°C.
017014: Growth occurs at 25°C.
017033: Growth occurs at 30°C.
017034: Growth occurs at 35°C.
017043: Growth occurs at 40°C.
017017: Growth occurs at 45°C.
017001: The optimum temperature range for growth is 0-10°C.
017002: The optimum temperature range for growth is 11-20°C.
017003: The optimum temperature range for growth is 21-30°C.
017004: The optimum temperature range for growth is 31-40°C.
016114: Thiamine is required for growth.

SOURCE OF ISOLATION

002012: What was the specific source of isolation (e.g., kind of water, soil, etc., species and organ and tissue of plant, animal, etc.)?

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THE TAXONOMY OF THE LIST OF FUNGAL NAMES FOR THE PROPOSED "GENERIC NAMES IN CURRENT USE" MODIFICATION OF THE INTERNATIONAL CODE OF BOTANICAL NOMENCLATURE.

Eric C. Swann
Department of Plant Biology
University of California
Berkeley, CA 94720

Don R. Reynolds
Natural History Museum, LAC
900 Exposition Boulevard
Los Angeles, CA 90007

"A taxonomic system is generally considered to have two functions. The first function is to provide an index to species."..."The second function is to show the phylogenetic relationships among these species. Taxonomy, then, is concerned with the naming, classification, and identification of species and with phylogeny." E.S. Luttrell, 1958 The function of taxonomy in mycology. Mycologia 4: 942-944.

ABSTRACT

The originators of the proposed modification of the International Code of Botanical Nomenclature entitled "Generic Names in Current Use" have invited constructive criticism of the plan to protect, via an approved list, botanical names used in recent literature. The concerns expressed in this paper relate to the taxonomic organization of the "Draft Lists for Fungi" (DLF). Two issues are discussed. (1) Names of phylogenetically non-fungal taxa are included in the DLF, implicit recognition of "Fungal" standing . The Code should reflect a current state of knowledge of the affinities of these taxa. (2) Appendix V, Generic Names in Current Use, should be an alphabetical, non-taxonomic list of generic names applied to members of the polyphyletic "fungal" assemblage. The Index to Appendix V should not be interpreted as part of the Code, and should reference names by taxonomy.

INTRODUCTION

Modification of the International Code of Botanical Nomenclature (ICBN) through the Generic Names in Current Use (GNCU) list, would constitute a starting point that supersedes those already recognized (Art. 13). The listed names are to be given protection from earlier names which do not appear in the lists.

Greuter (1991) gave assurances concerning the Names in Current Use (NCU) effort. *"The listing must not result in any kind of taxonomic censorship; when competing systems of classification or divergent notions of taxon delimitation, position or rank exist, all names reflecting the various opinions are to be listed"* ... *"Approved lists must essentially remain stable once and forever; nevertheless a certain amount of flexibility must be provided in* *order to enable desirable corrections and perhaps additions, and mechanisms to that effect must be provided." ... "The proposed NCU provisions are designed to become an integral part of the Code, not to displace its present provisions." ... "priority will determine the choice between competing protected names...".*

The Draft Lists for Fungi (DLF), prepared under the guidance of an International Association for Plant Taxonomy Secretariat Committee for Fungi and Lichens, is composed of generic names organized using supra-generic taxa. The finalized Lists for Fungi are to be added to the ICBN as Appendix V.

The unprecedented NCU movement could have an unintended influence on taxonomy. While the ICBN modification is related to the names *per se*, the provision of these names in a taxonomic framework is likely to compound ambiguities of the ICBN. Our concern is that the implicit endorsement of a taxonomic framework of the lists will be taken as a codified, stable (non-changing) understanding of the higher relationships of the "fungi".

Here are our thoughts about the GNCU-Draft Lists for Fungi as a taxonomic device for nomenclatural purposes.

1. What are fungi? The naming of what organisms are governed by the ICBN rules?

Preamble 7 states that the ICBN applies to all organisms (historically) treated as plants, including the fungi. The ICBN freely uses the word "fungus" and its grammatical variants, but no definition, except by default and incidental exception, is provided for what organisms are to be regarded as fungi. The ICBN regulation of "fungi" is a matter of what taxa are assigned to that *nomen vulgaris* by the code. The historical basis for defining the fungi is simply that they are those organisms traditionally studied by mycologists. These are "fungi", spelled with a lower case first letter, and are a polyphyletic gathering of organisms that lack chlorophyll. In this respect, the traditional ICBN use of a fungus concept reflects names in "modern languages, the applications of which are often doubtful" (Art. 32.E.1).

One of the alternative bases for a definitive use of fungus (fungi pl.), other than botanical tradition, is natural, phylogenetic classification. A phylogenetic definition of Fungi based on an assessment of current data can be found in Bruns, *et al.* (1991). The kingdom Fungi, spelled with an upper case first letter, is a monophyletic taxon composed of the Basidiomycota, Ascomycota, Zygomycota, and the Chytridiomycota. (The Zygomycota was not included in the assessment for lack of data, but is thought to fall within the "true fungi".)

Some of the Myxomycota are more closely related to protozoan taxa than to the Fungi. The Oomycetes and Hyphochytriomycetes are more closely related to chrysophyte algae (Förster *et al.*, 1990; Lee, 1991). These nonfungal groups of organisms are inadvertently given fungal standing with the formation of higher taxa names using the endings found in ICBN Recommendation 16A.

Patterson and Larsen (1991) noted that taxa such as protists are ambiregnal if their nomenclature legitimately can be regulated by the provisions of the ICBN or by those of the International Code of Zoological Nomenclature. Olive (1975) cited the taxa of the acrasiaceous and acytosteliad organisms in the Protozoa with animal kingdom word terminations.

2. An alphabetical list of generic names should be used in the Lists for Fungi. A taxonomic index to the generic names of this polyphyletic assemblage would be useful.

An alphabetical listing of the generic names in Appendix V will minimize undesired effects of incorporation of taxonomy in a nomenclatural document.

However, to provide for the practical needs of fungal nomenclaturalists, taxonomists, and other "user groups" (Hawksworth and Greuter, 1989) who will consult the GNCU lists, a taxonomic index of the generic names of the "fungi" should be provided. The taxonomy used in the Draft Lists for Fungi is outlined in Figure 1. Major group names used are Ascomycotina, Deuteromycotina, Gasteromycetes, Hymenomycetes, Mastigomycotina, Myxomycetes, Urediniomycetes, and Incertae Sedis.

Unfortunately, the taxonomic model of the Draft Lists, meant to index the codified names, is not especially informative, and in some cases is misleading. If taxonomy is a means of information retrieval, via classification together of organisms of common descent, the taxonomy of the Draft Lists for Fungi contains serious flaws.

The Myxomycetes is composed of phylogenetically unrelated plasmodial, cellular, plasmodiophoroid, and labryinthuloid "slime mold" taxa.

The Mastigomycotina is composed of the Oomycetes, Hyphochytridiomycetes (Hyphochytriomycetes), Chytridiomycetes, Trichomycetes, and Zygomycetes. The first two classes are allied with various algal groups, while the other three are allied with the Ascomycotina and Basidiomycotina in the Fungi.

The commonly used sub-division level taxon Basidiomycotina is conspicuously absent from the major group headings. Instead, several of its component class level taxa are used. These are the Gasteromycetes, Hymenomycetes, and Urediniomycetes. Within the Hymenomycetes are the order level taxa Agaricales (gilled mushrooms, excluding the boletes), Aphyllophorales, and a group of fungi under the heading Hymenomycetes, including boletes, the tremelloid, dacrymycetoid, and auricularioid jelly fungi, the tulasnelliod fungi, and various other basidiomycetous fungi.

The class Urediniomycetes is confusingly composed of two other class level taxa, the

Urediniomycetes (rusts), and Ustilaginomycetes (smuts).

How should the taxonomic index be provided?

One model for listing organisms is provided in ICBN Appendix IIIA. *Nomina generica conservanda et rejicienda*. The conserved fungal names are listed alphabetically, without a higher taxonomic framework. This alphabetical listing is a result of regarding fungi as a group of organisms traditionally studied by mycologists rather than as a monophyletic group of organisms. The Index to Appendix IIIA of the ICBN is a separate, alphabetical list of all conserved and rejected names, provided without reference to taxonomy. The generic names for algae, and gymnospermous and angiospermous plants are organized taxonomically in Appendix III. However, there is no taxonomic index of the conserved fungal names; they are included in an alphabetical index containing all of the generic names conserved under the ICBN.

In order to maximize usefulness and biological information content, the index should be consistent with the current state of knowledge at the time of any particular ICBN edition concerning the phylogentic relationships of the taxa named on the lists.

To bring the current Draft Lists for Fungi into current status, the implied taxonomy in Article 13(d) should be disregarded. The Myxomycetes should be distributed into appropriate non-fungal groups which recognize the separate origins of the plasmodial slime molds, cellular slime molds, and the various other disparate taxa included therein. The Mastigomycotina of the lists should be dissolved; the Oomycota and Hyphochytriomycetes names should be listed as separate algal divisions. Under the main heading Kingdom Fungi, division level names should be used. The Trichomycetes, Zygomycetes of the Zygomycota, and Chytridiomycota names should be placed with the Ascomycota and Basidiomycota in the kingdom Fungi. Order level names should be used for each division, as in the Ascomycota list. The Deuteromycetes is a phylogenetic non-concept, and should not be used.

The usefulness of the indexing is not to be measured in its congruence with old-line mycological taxonomic thought, but with the amount of biological information it conveys. A phylogenetic scheme is the best way to organize such information.

CONCLUSIONS

The fungal taxa in Appendix V of the ICBN should be free of taxonomy. A separate, non-codified Index to Appendix V should be taxonomically organized for best practical use. Phylogenetic taxonomic groupings are proposed as the most informative method by which to index the names. Phylogenetically unrelated taxa now regarded under the heading of "fungi" in the ICBN should be indexed to reflect the current state of knowledge as to phylogenetic relationships.

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Figure 1
Outline of the Taxonomic Organization of the GNCU-DLF

I. **Ascomycotina**
- 38 orders of ascomycetes
- Ascomycotina

II. **Deuteromycotina**
- Agaricales
- Aphyllophorales
- Coelomycetes
- Colomycetes (Typographical error)
- Deuteromycotina
- Gasteromycetes
- Hymenomycetes
- Hypocreales
- Leotiales
- Myxomycetes
- Urediniomycetes
- Ustilaginomycetes
- Zygomycetes

III. **Gasteromycetes**

IV. **Hymenomycetes**
- Agaricales
- Aphyllophorales
- Basidomycotina
- Hymenomycetes

V. **Mastigomycotina**
- Chytridiomycetes
- Hyphochytridiomycetes
- Mastigomycotina
- Oomycetes
- Trichomycetes
- Zygomycetes

VI. **Myxomycetes**
- Myxomycetes
- Myxomycotina

VII. **Urediniomycetes**
- Urediniomycetes
- Ustilaginomycetes

VIII. **Insertae Sedis**
- Ascomycotina
- Coelomycetes
- Hyphomycetes
- Incertae Sedis

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NEW COMBINATIONS IN THE GENUS HYMENOSCYPHUS (HELOTIALES)

PAVEL LIZOŇ[1]

Plant Pathology Herbarium, Cornell University
Ithaca, NY 14853, U.S.A.
and
Department of Botany, Museum of Natural History
CS-814 36 Bratislava, Czechoslovakia

KEYWORDS: *Hymenoscyphus*, *H. callorioides*, *H. citrinicolor*, *H. pallide-subolivaceus*, *H. tatrae*, Helotiales, Leotiaceae.

During a comprehensive study of the genus *Hymenoscyphus* S.F.Gray twenty-three species were recognized in Slovakia, Czechoslovakia. In advance of the forthcoming monograph (Lizoň, 1992), I propose following new combinations.

Hymenoscyphus callorioides **(Rehm) comb. n.**
Basionym: *Helotium callorioides* Rehm, Hedwigia 21:98, 1882.

The type material consists of two types of fruit-bodies: about 7 sessile apothecia of *Hymenoscyphus callorioides* and 2-3 stipitate apothecia that are probably *Hymenoscyphus repandus* (Phill.) Dennis. Apothecia of *H. callorioides* are broadly sessile, brownish-orange and to 0.5 mm diam. when dry; disc is plane to plano-convex. Ectal excipulum forms a thin layer up to 40 µm thick, composed of thin-walled cells, lying in rows parallel to the surface. Ascus pore blues in Melzer's reagent. Spores are ellipso-fusoid, nonseptate, hyaline, (8.5)11.0-12.5 x 1.5-2.5 µm.

HOLOTYPE: [Austria] An faulenden *Aconitum varieg.* beim Kartel-Gletscher (Moosthal) im Tyrol, c. 2000 m, 7/1878, Britzlmayer (S, herb. H. Rehm).

Hymenoscyphus pallide-subolivaceus **(Svr.) comb. n.**
Basionym: *Helotium pallide-subolivaceum* Svrček, Čes.Mykol. 12:228, 1958.

I have examined a part of the holotype including a leaf of *Vaccinium* with one fruit-body on the upper side of the leaf blade, a spruce needle with two fruit-

[1] Anna E. Jenkins Visiting Fellow

bodies, and a fir needle bearing no fruit-bodies. Apothecia are apricot-yellow when dry, about 0.3 mm diam., short-stipitate; stipe is concolorous, 0.1 x 0.1 mm. Ectal excipulum is composed at the base of the receptacle of large cells, 15 x 5 µm, with slightly thickened walls, elongated towards the margin and forming a layer of narrow, thin-walled hyphae, 2.0 - 4.0 µm diam. Ascus pore blues only weakly in Melzer's reagent. Spores are ellipso-clavate to fusoid-clavate, some of them irregular, nonseptate, 10.2-12.5 x 1.8-2.5 µm.

HOLOTYPE: [Czechoslovakia] Slovakia, in montibus Belanské Tatry, supra Tatranská Kotlina, 1100 m s.m., Ad acus *Abietis albae*, *Piceae abietis*, folia *Vaccinii myrtilli* etc., 19.V.1958, J. Kubička & M. Svrček (PRM 586641).

Hymenoscyphus tatrae (Svr.) **comb. n.**
Basionym: *Helotium tatrae* Svrček, Čes.Mykol. 12:228, 1958.

I have studied a part of the holotype which contains 3 pieces of larch needles with 3 young (not fully mature) fruit-bodies. Apothecia are yellowish and 0.2 - 0.5 mm diam. when dry, cupulate, short-stipitate. Stipe is paler, about 0.5 x 0.1 mm (1.5x longer than diameter of the receptacle). Ectal excipulum is composed at the base of the receptacle of globular cells, 4.5 - 8.0(14.5) µm diam., towards the margin this structure is replaced by cylindric to clavate cells. Ascus pore blues only weakly in Melzer's reagent. Spores are ellipsoid to ellipso-cylindric, nonseptate, 8.8-11.0 x 1.5-2.5 µm. Fruit-bodies on *Carex flacca* subsp. *claviformis*, as reported in the original description (Svrček, l. c.), have not been available from PRM.

HOLOTYPE: [Czechoslovakia] Slovakia, in montibus Belanské Tatry, supra Tatranská Kotlina, 1250 m s.m., Ad acus *Laricis deciduae* subsp. *polonicae*, 19.V.1958, J. Kubička & M. Svrček (PRM 856642).

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CHAETOPSINA NIMBAE, A NEW SPECIES OF DEMATIACEOUS HYPHOMYCETES

Sergio Merli, Luisa Garofano

Società Farmitalia-Carlo Erba, Via dei Gracchi 35 - Milano.

Angelo Rambelli & Marcella Pasqualetti

Facoltà di Scienze Matematiche, Fisiche e Naturali, Università della Tuscia, Via San Camillo de Lellis, 01100 - Viterbo, Italy.

Abstract

Chaetopsina nimbae, a new species of Dematiaceous Hyphomycetes, collected on fallen leaves of *Lophira alata* Banks ex Gaertn. is described. The morphology and some physiological characters are compared with features of a strain of *Chaetopsina fulva* Rambelli.

Introduction

Chaetopsina fulva Rambelli (1956) is a Dematiaceous Hyphomycete characterized by great variability. The species was subsequently found on litter by several investigators, even if with some morphological differences, in various geographic areas (Tubaki & Saito, 1969; Matsushima, 1975; Samuels, 1985; Kirk, 1982; Rambelli, 1987; Rambelli *et al.*, 1991, 1991a, 1991b).

During some mycological investigations, carried out in 1989 on Guinea Conacry tropical forest litter, a species of *Chaetopsina* was collected on dead leaves of *Lophira alata*. In the same year, in surroundings of Rome, a second strain was found on dead leaves of *Cedrus deodara* (D.Don) G. Don fil. Owing to the great morphological variability of the fungus, the strains were initially included in the species "*fulva*" even if the African one showed some differences in morphology, growth of its colonies and size of its conidia.

In the present work the morphological and some physiological characters of the two strains are investigated in order to verify their specific identity. They are respectively labelled CR1 for the Italian strain and CA2 for the African one.

Materials and methods

Morphological investigations were carried out on natural substrates, in pure cultures (Rambelli *et al.*, 1991b) and on sterilized and inoculated substrates (Kabi & Rambelli, 1990; Rambelli *et al.*, 1991a).

The strains were also tested with respect to their vitamin requirements: a hyphal suspension was inoculated in a agar minimum medium (Czapek) and 0.1 ml of a successive 50% dilution of vitamin complex (composed per ml by vitamin A (u.I. 5000), B1(mg 2), B2 (mg 1.27), Nicotinamide (mg 10), B6 (mg 1), Pantothenol (mg 10), Biotin (mg 0.1), C (mg 50), D2 (u.I. 1000), E (mg 3)) added in a hole cut in the agar plate. Control was carried out with sterilized water.

Slide microcultures were prepared in order to verify the possibility of hyphal connections between the two strains.

Results

CR1 morphological characters.

Microscopic characters on natural substrates, as morphology and sizes, are similar to *C. fulva*. The strain differs only in the strong pigmentation of the walls and in the frequent apical fertility of the conidiophores. This last character seems an anomalous behaviour of the fungus, presumably of nutritional origin (Rambelli *et al.*, 1991). In pure culture the fungus colonizes the medium very fast. The mycelium is prevalently immersed and the surface of the colony is frequently shiny, brown-blackish at the center and red-brown to whitish at the periphery. It grows easily on different media (Mycological, PDA, etc), but not on Czapek agar (Rambelli *et al.*, 1991a).

CA2 morphological characters.

CA2 strain produces, on natural substrates, some unripe globose ascoma like structures (fig. 1a); these structures are spherical, with membranous walls, with evident peridial cells and with only rare hyphae near the base, red-yellow-brown in colour, 118-268 µm in diameter. Some ascogenous hyphae are evident in these structures, but the production of asci has never been observed. The anamorph (fig. 1b, c) is developed from the cells of the wall of this unriped body; it is represented by setiform conidiophores, light yellow, paler towards the apices and with clearly visible septa; they are thick walled, with lighter in colour and chlorine walls wich are coarsely roughened in the last five or six apical cells. The setiform conidiophores are regularly curved on the fertile side. They are 214-392 x 7.5-13 µm. The phialidic conidiogenous cells (Fig. 1b) are 4-5 x 2-3 µm. The conidia are very similar to those of *C. fulva* (*C.fulva* conidia 7.5-10.8 x 1-1.5 µm) and only differ in their size (11-16 x 2-2.5 µm). In Czapek agar pure cultures the growth of the fungus is regular with mycelium prevalently light yellow, immersed only at the center, where unripe globose perithecium like structures and conidiophores of the anamorph are produced; these structures give a granular appearance and a reddish colour to the colony (Rambelli *et al.*, 1991a).

Behaviour on the natural substrates

The morphology and size of the structures of the two strains, inoculated on different sterilized substrates, were compared (Rambelli *et al.*, 1991a). While CR1 presents the same features on the different substrates, CA2 proves a strong variability and, in particular, no characters comparable with the type species *C. fulva.*

Vitamins requirement

CR1, not developing on Czapek agar, grows abundantly on this medium only when vitamin complex is added even at the maximum dilution. CA2, developing on Czapek agar, seems not to be influenced in its growth, compared with the control, by the presence of some vitamins in the medium (fig. 2a, b).

Analysis of hyphal compatibility

By growth analysis of the two strains in microcultures and Petri dishes some little morphological differences in the hyphae of the strains are observed. The growth of mycelia of two strains together do not

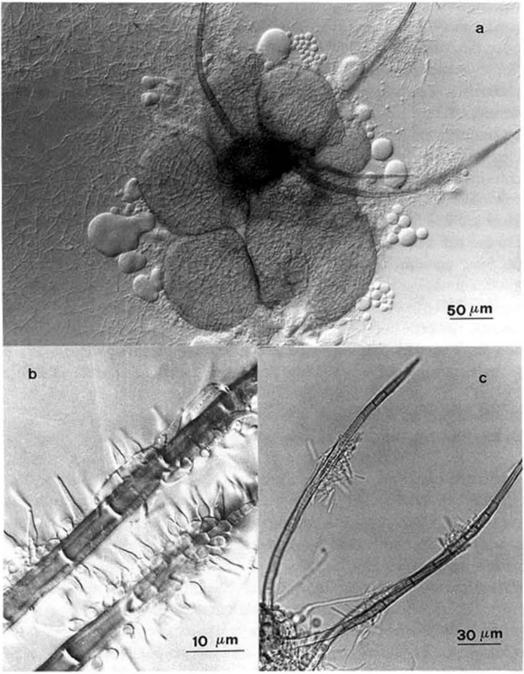

Fig. 1 - *Chaetopsina nimbae*: a, unripe globose perithecium like structures with an anamorph; b, phialidic conidiogenous cells; c, setiform conidiophores.

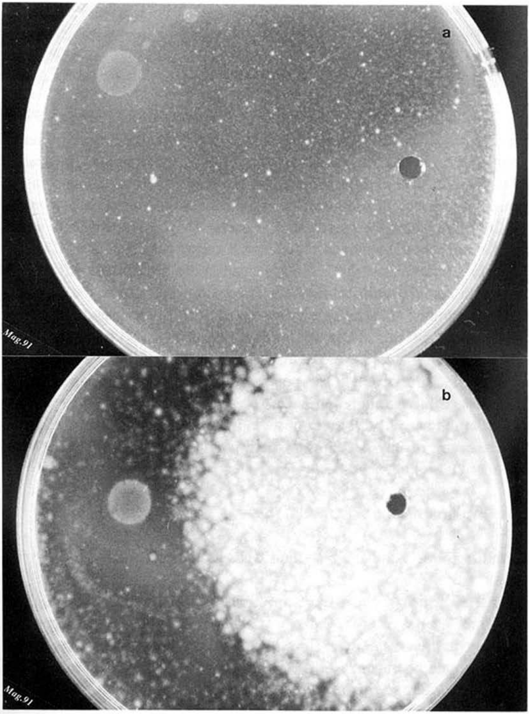

Fig. 2 - a, CA2 strain: limited but uniform development in Czapek not influenced by the presence of vitamins in the medium; b, CR1 strain: influence of a vitamin complex on growth.

present any antagonisms and inhibitions, but no hyphal fusions are observed.

Discussion

Tubaki and Saito (1969) report the finding of *C. fulva* on *Pinus densiflora* Sieb. et Zucc. litter in Japan, but from their description, it seems reasonable to belive that the strain is different from the type species of *C. fulva.* In fact, it differs in conidia and conidiophores dimensions; in conidiophores colour, dark-brown in their strain instead of yellow-red of *C. fulva.* We hadn't the possibility to observe the Tubaki and Saito strain, but, for the above-mentioned characters, it seems very similar to CA2.

In comparison with *C. fulva,* our strain has remarkable differences, mainly in conidia and conidiophore dimensions but also in the colour of fertile structures: chlorine-pale yellow in our *Chaetopsina* and red-yellow in *C. fulva;* in the shape of conidiophores and, with considerable differences, in the colour of the colony. The incompatibility between the mycelia of *C. fulva* (CR1) and our strain (CA2) and the strong diversity of behaviour in vitamin requirements confirm the taxonomic differences.

On the base of all these observations and owing to the absence in the leterature of any description corresponding to CA2, we propose to consider the strain a new species.

Chaetopsina nimbae Rambelli, sp. nov.

Etym.: from Mt. Nimba, where the fungus has been found.

Hyphae pallentes, septatae, leves, protoplasmate granuloso. *Conidiophora* setiformia, erecta, recta vel ad locos conidia producentes modice curvata, septata, crassitunicata, levia, flaventia, pallidiora versus rugosum apicem, protoplasmate homogeneo, 214-392 µm longa et 7.5-13 µm prope basim crassa. *Cellulae conidiogenae* enteroblasticae, monophialidicae, tenuitunicatae, hyalinae, ampulliformes vel lageniformes, copiose productae, 4-5 x 2-3 µm in ramis fertilibus plurimorum ordinum praeditae, *Conidia* hyalina, continua, cylindrica, extremitatibus leniter rotundato, 11-16 x 2-2.5 µm in capitulum mucosum aggregata.

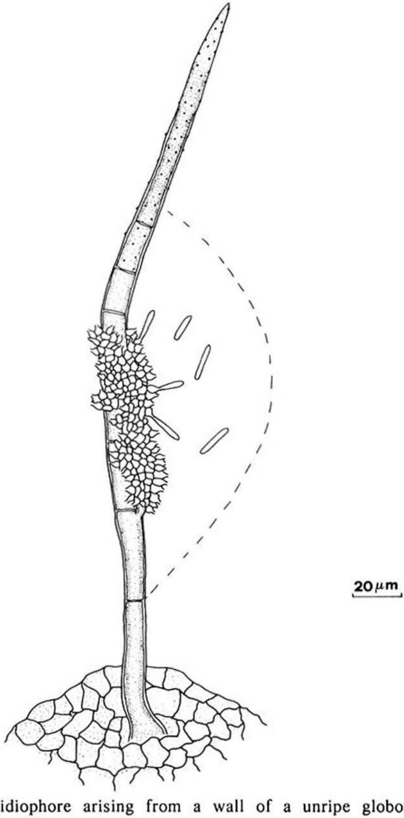

Fig. 3 - Conidiophore arising from a wall of a unripe globose perithecium like structure.

In foliis emortuis *Lophirae alatae* Banks ex Gaertn. Nimba Mountains, Guinea Conacry (SW. Africa), leg. A. Rambelli, Nov. 1989, ROHB 138 A, holotypus.

Hyphae hyaline, septate, with smooth walls, light yellow. *Conidiophores* setiform, slightly curved on fertile side, septate, thick and smooth walled, yellow and paler towards the roughened apex, 214-329 µm long and 7.5-13 µm wide near the base. *Conidiogenous cells* enteroblastic, monophialidic, thin walled, hyaline, ampulliform to lageniform, 4-5 x 2-3 µm, abundantly produced on one up to three (rarely four) branches, originating from the second or the third basal cell of the conidiophore. *Conidia* hyaline, one-celled, rod shaped, with rounded apices, 11-16 x 2-2.5 µm, aggregated in slimy-mucous masses.

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VITAL VERSUS HERBARIUM TAXONOMY:

MORPHOLOGICAL DIFFERENCES BETWEEN LIVING AND DEAD CELLS OF ASCOMYCETES, AND THEIR TAXONOMIC IMPLICATIONS [1]

H. O. BARAL

(Blaihofstr. 42, 7400 Tübingen 9, F. R. Germany)

- in vivo veritas -

ABSTRACT

Micromorphology of fungal cells as observed under the light microscope differs considerably when comparing living with dead cells. Furthermore, different mounting media in current use may highly influence the appearance of fungal cells, and ontogenetic alterations increase the scope of their variability. Taxonomical work must, however, be based on organs in compatible states and development stages. Arguments are presented for diagnoses based on the morphology of living unaltered cells from freshly collected specimens. Living ascocarp cells are to be studied in their fully hydrated state by mounting in aqueous solutions with a very low concentration of ingredients. Tap or rain water is a suitable mountant. Vital taxonomy provides many additional and often new characters of high taxonomic value which are furthermore often more consistent. These can be observed by easy and rapid methods from the living fungus but become obscure or disappear completely in the herbarium. Different kinds of cytoplasmic inclusions are especially concerned. The development stage of a given organ can be much more precisely ascertained in the vital state. Vitality of single cells can be recognized by their

[1]) Based on a poster (IB-67/2) and a video film given at the Fourth International Mycological Congress held in Regensburg, F. R. G., 28th Aug. - 3rd Sept. 1990 (BARAL, 1990).

osmotic response, by the appearance of their cytoplasm, or through staining *in statu vivo* with basic dyes. Striking alterations during the death of fungal cells are, e.g., shrinkage for about 30-50% of the original volume, or expansion of inner wall layers to about 2-5 times their normal thickness. One new combination is proposed: **Allophylaria nervicola** (Velen.) Baral *comb. nov.*

ZUSAMMENFASSUNG

Die Mikromorphologie pilzlicher Zellen ist bei lichtmikroskopischer Analyse in hohem Maße verändert, wenn lebende mit toten Zellen verglichen werden. Außerdem kann das Aussehen von Pilzzellen sehr stark von der Verwendung verschiedener gebräuchlicher Präpariermedien abhängen. Schließlich erhöhen ontogenetische Veränderungen den Grad der zu beobachtenden Variabilität. Taxonomische Arbeit muß sich jedoch auf Organe in kompatiblen Zuständen und Entwicklungsstadien beziehen. Es wird dringend angeraten, Analysen auf die Morphologie lebender, noch unveränderter Zellen frisch gesammelter Fruchtkörper zu gründen. Lebende Ascocarpzellen müssen im Zustand maximaler Wassersättigung unter Verwendung wässriger Lösungen mit sehr niedriger Konzentration gelöster Stoffe untersucht werden. Leitungs- oder Regenwasser sind hierfür geeignete Präpariermedien. Die Vitaltaxonomie ermöglicht den Gebrauch vieler zusätzlicher und oft neuer Merkmale von taxonomisch hoher Relevanz und oft erhöhter Konstanz. Diese können mit einfachen, zeitsparenden Methoden am lebenden Objekt beobachtet werden; sie sind jedoch im Herbar verwischt oder verschwinden gänzlich. Dies betrifft insbesondere verschiedene Typen von Plasmaeinschlüssen. Das Entwicklungsstadium eines Organs kann im Lebendzustand viel präziser erkannt werden. Die Vitalität von Einzelzellen läßt sich am osmotischen Verhalten, am Aussehen des Zellplasmas oder mithilfe der Vitalfärbung durch basiche Farbstoffe erkennen. Auffällige Veränderungen beim Absterben von Pilzzellen sind z. B. das Schrumpfen um etwa 30-50% vom Ursprungsvolumen, oder die Quellung innerer Zellwandschichten um das ca. 2 bis 5-fache ihrer natürlichen Dicke. Eine neue Kombination wird vorgeschlagen: **Allophylaria nervicola** (Velen.) Baral *comb. nov.*

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1. INTRODUCTION

During my taxonomic studies on Ascomycetes (mainly discomycetes) for some 17 years, I soon noticed that fungal cells mostly show a very different micromorphology under the LM (magnification 600-1500x) when fragments of fruitbodies from the herbarium in tap water mounts were compared with those from freshly collected specimens in the same medium. These alterations were also produced when fresh collections were treated without enough care: during transport to the laboratory by prolonged exposure to a dry atmosphere, by mechanical damage of the fresh ascocarps, or by strong pressure on the coverglass during preparation.

The observed differences between fresh and dried or carelessly treated specimens were, e.g. (FIG. 1 abc→def): (1) dramatic shrinkage of spores, asci and paraphyses; (2) spores clustered at the top of the ascus versus dispersed throughout the (shrunken) ascus; (3) many globose refractive guttules (LBs) within the spores versus several large aggregations or none; (4) refractive bodies within the paraphyses versus "empty" paraphyses.

More detailed investigations using basic dyes for staining the cells *in statu vivo*, as well as osmotic tests, clearly proved that the observed differences originate in the living versus dead state of the cells. Since living fungal cells turned out to offer much more valuable and consistent taxonomic characters (with regard to both cytoplasm *and* cell wall), I early restricted my studies intuitively to the morphology of living cells (except for apical ascus wall structures), a method which I finally called *vital taxonomy* (BARAL, 1989a: 120; 1990). This is in contrast to the currently applied method of Ascomycete taxonomy using the LM, which is mainly based on describing dead dried material preserved in herbaria.

STRUGGER (1949) gave a comprehensive summary of this problem for the field of plant physiology. He emphasized the study of the living cytoplasm (*Lebendzytologie*) which, he was convinced, would undoubtedly attain more importance in the future when compared to the classical method of studying fixed material (*Fixierungszytologie*). According to STRUGGER (1949: 2, 143), differences in the structure and organization of the living cytoplasm must not necessarily produce differences in the morphology of the cell wall. STRUGGER's *Lebendzytologie*, however, scarcely influenced the current methods of micromorphological research on fungi in the following decades. Instead, the new technique of electron microscopy which *imposes the restriction of viewing only dead or rapidly dying material* (READ et al., 1982: 2062) started its rapid development. More and more taxonomic work is carried out using this technique.

During my study on Leotiales, I became convinced that many taxonomic conflicts are due to the prevailing absence of careful studies of living cells. Only a few mycologists have emphasized the advantage of microscopic examination of Ascomycetes *in statu vivo*: BOUDIER (1885: 95; 1886: 141; 1914: 54) layed stress on the fact that he never used dried specimens for his descriptions because these nearly always give incorrect results. He consequently considered dried specimens *un obstacle à toute bonne classification*. LAGARDE (1906: 135ff.) wrote: *Les échantillons secs d'herbier (...) donnent toujours des résultats médiocres et exposent à des erreurs. Les organes délicats des Discomycètes charnus subissent par la dessication des altérations profondes, irrémédiables.* In her study on the ontogeny of ascospore ornamentation in the Pezizales, LE GAL (1947: 78) wrote: *(...) seules les observations vitales pouvaient nous donner des résultats satisfaisants.* Even anatomical studies of apothecia are, according to CORNER (1929: 264), better performed with living cells: *The best method of examining the growth of the hyphae and the origin of the tissues was found to be by means of freehand sections of living material.*

Surprisingly few reports of method-dependent alterations in fungal micromorphology have been published. Instead, taxonomists tend to describe either living or (more frequently) dead elements without taking possible alterations into consideration and rarely give detailed indications of their preparative treatments by specifying these for each given measurement or illustrated organ. GRADDON (1951: 693) wrote: *The following descriptions are drawn from fresh material and spore measurements are taken from spores freely thrown by living specimens and collected on coverglasses*, but he sometimes described dead elements as well without commenting on this fact. This can often be concluded from his drawings which, in the case of living specimens, show spores clustered at the top of the asci, containing globose and symmetrically arranged guttules. Personal communication revealed that others are used to ignore the living cells as "abnormal" and to prefer the dead elements for study.

Often, however, one is unable to recognize the state of the measured and described elements in a given publication. Recently, SPAIN (1990) reported drastic alterations induced by reagents applied to fresh spores of Endogonaceae, stating that *it may not be possible to discern from diagnoses whether alterations took place before or after descriptions were made*. He recommended that diagnoses should be given based on both fresh, untreated and reagent-treated specimens. Likewise, HUHTINEN (1990b) wrote: *At least one of the depicted populations should represent living specimens mounted in water*.

My field of work concentrates on the species of the Leotiales. Therefore, most observations of taxonomical importance concerning vital taxonomy were made on this group. A key to the presently known European species is in preparation. This key will be based, as far as possible, using vital taxonomy, and is hoped to allow a rapid identification of fresh collections.

2. MATERIALS AND METHODS

A Zeiss microscope with bright field and phase optics was used for all observations, with phase 100x/1.25 oil immersion objective, and 12.5x or 15x binoculars. The illustrations were made without the aid of a drawing tube. The depicted specimens originate from the following collections (HB = the authors herbarium, A = Austria, CH = Switzerland, D = F. R. Germany, F = France, L = Luxembourg, S = Spain):

Aleuria aurantia (Pers.) Fuck., 25.XI.87, D-Tübingen-Pfrondorf, on clayey ground, leg. HB, HB 3316 -- **Allophylaria nervicola (Velen.) Baral comb.nov.** [2], 24.X.87, D-Tübingen-Pfrondorf, on petioles and veins of **Acer pseudoplatanus**, leg. HB, HB 3292 -- **Brunnipila clandestina** (Bull. ex Mérat : Fr.) Baral in Baral & Kriegl-st., 30.V.88, D-Tübingen-Pfrondorf, on canes of **Rubus idaeus**, leg. HB, HB 3425 -- **Ciboria caucus** (Rebent.) Fuck., 16.III.88, D-Kirchheim-Bonlanden, Breuningsweiler, on male catkins of **Alnus glutinosa**, leg. J. Haedecke -- **Cistella deflexa** (Gradd.) Raitv., 3.XI.85 D-Tübingen-Lustnau, on leaves of **Populus** ? **canadensis**, leg. HB, HB 2951 -- **Conchatium fraxinophilum** Svrček, 24.X.87, D-Tübingen-Pfrondorf, on petioles of **Fraxinus excelsior**, leg. HB, HB 3293a -- **Discina ancilis** (Pers.) Sacc., 26.III.88, D-Tübingen-Pfrondorf, on bark of **Picea abies** trunk, leg. HB -- **Hymenoscyphus** cf. **sazavae** (Vel.) Svrček, ≈27.IX.87, F-Gérardmer, on wood and cone of **Picea abies**, leg. J. Deny, HB 3277 -- **Hymenoscyphus scutula** (Pers. : Fr.) Phill., 24.X.87, D-Tübingen-Pfrondorf, on stems of **Tanacetum vulgare**, leg. HB, HB 3290 -- **Hymenoscyphus consobrinus** (Boud.) Hengstm., 24.VIII. 87, Tübingen-Dettenhausen, on stems of **Anthemis nobilis**, leg. G. Haupter -- **Lachnum controversum** (Cke.) Rehm, 24.VII.87, D-Tübingen, on culms and leaves of **Phragmites communis**, leg. HB, HB 3229 -- **Lasiobelonium corticale** (Pers. : Fr.) Raitv., 15.V.88, L-Tuntange-Hollenfels, on bark of **Populus tremula** stump,

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[2]) Basionym: **Helotium nervicolum** Velenovský, Monogr. Discom. Bohemiae 1934: 206 (≡ **Conchatium nervicolum** (Velen.) Svrček, = **Allophylaria subhyalina** forma b in BARAL & KRIEGLSTEINER 1985: 94)

leg. C. Besch, R. Swart-Velthuyzen & HB, HB 3386 -- **Lasiobelonium variegatum** (Fuck.) Sacc., 28.VII.88, CH-Schaffhausen-Thayngen, on bark of **Salix** ≈ **cinerea** branch, leg. HB & G. Marson, HB 3496 -- **Lecanora conizaeoides** Nyl. ex Crombie, 13.VII.88, D-Tübingen-Pfrondorf, on wood of ? **Picea abies** fence, leg. HB, HB 3459 -- **Melastiza chateri** (W.G. Smith) Boud., 20.XI.87, CH-Schaffhausen-Thayngen, on clayey ground, leg. HB & P. Blank, HB 3317 -- **Mniaecia jungermanniae** (Nees ex Fr.) Boud., 12.II.88, D-Tübingen-Pfrondorf, on **Cephalozia bicuspidata**, leg. HB, HB 3336b -- **Mollisia** spec., 20.VI.88, D-Tübingen-Pfrondorf, on wood of **Quercus** trunk, leg. HB, HB 3441 -- **Nimbomollisia eriophori** (Kirchn.) Nannf., 21.V.88, CH-Zug-Unterägeri, on culms of **Juncus effusus**, leg. J. & L. Rothenbühler -- **Nimbomollisia melatephroides** (Rehm) Nannf., 27.VII.88, CH-Zug-Unterägeri, on leaves & culms of **Molinia coerulea**, leg. HB, P. Blank, J. & L. Rothenbühler, HB 3483 -- **Orbilia auricolor** Blox. ex Berk., 21.VIII.88, D-Tübingen-Pfrondorf, on stems of **Oenothera biennis**, leg. HB, HB 3527 -- **Orbilia delicatula** (Karst.) Karst., 22.VIII.88, D-Tübingen-Pfrondorf, on wood of **Acer pseudoplatanus** stump, leg. HB, HB 3529 -- **Orbilia** ? **rosella** (Rehm) Sacc., 10.VIII.88, D-Tübingen-Pfrondorf, on stem of **Melilotus albus**, leg. HB, HB 3518a -- **Orbilia sarraziniana** Boud., 14.V.88, L-Beaufort, on wood of **Fagus sylvatica** twig, leg. HB -- **Orbilia** spec., 10.VIII.88, D-Tübingen-Pfrondorf, on stem of **Melilotus albus**, leg. HB, HB 3518b -- **Pezicula cinnamomea** (DC. : Pers.) Sacc., 23.VII.87, D-Tübingen-Pfrondorf, on bark of **Carpinus betulus** twig, leg. HB, HB 3239 -- **Pezicula livida** (Berk. & Br.) Rehm, 27.VIII.86 D-Tübingen-Pfrondorf, on bark of **Pinus sylvestris** branch, leg. HB -- **Peziza** ? **fimeti** (Fuck.) Seaver, ≈30.V.88, D-Tübingen-Pfrondorf, on straw and horse dung, leg. P. Zinth & HB -- **Phaeohelotium geogenum** (Cke.) Svrček & Matheis, 18.X.89 A-Grünburg-Steinbach, on leaf & twigs of ? **Quercus**, and apple pressings residue, leg. H. Helm, HB 3907 -- **Polydesmia pruinosa** (Jerdon in Berk. & Br.) Boud., 14.VI.87, D-Tübingen-Pfrondorf, on **Hypoxylon** spec., leg. HB -- **Pyrenopeziza petiolaris** (Alb. & Schw. : Fr.) Nannf., 26.III.88, D-Tübingen-Pfrondorf, on petioles of **Acer pseudoplatanus**, leg. HB -- **Rutstroemia elatina** (Alb. & Schw. : Fr.) Rehm, 15.III.1986 D-Tübingen-Pfrondorf, on bark of **Abies alba** twigs, leg. HB -- **Sarcoscypha austriaca** (Beck ex Sacc.) Boud., 20.V.79, D-Hinterzarten, Feldberg, on wood of **Acer pseudoplatanus** branches, leg. HB, P. & D. Laber, HB 2537 (neotype) -- **S. coccinea** (Scop. : Fr.) Lamb., 12.III.79, D-Karlsruhe-Hambrücken, on wood of **Ulmus carpinifolia** branches, leg. K.H. Waßmuth & HB, HB 2460 (neotype) -- **S. jurana** (Boud.) Baral, 5.III.79, D-Hayingen-Lauterach, on bark of **Tilia platyphyllos** branches, leg. P. Zinth & HB, HB 2461 -- **S. macaronesica** Baral & Korf in Baral, 20.I.82, S-Gomera-Vallehermoso, on wood of ? Lauraceae twigs, leg. P. Zinth, HB 2610 (holotype) -- **Scutellinia scutellata** (L. ex St. Amans) Lamb., (collection of unknown provenance), leg. HB -- **Trichopezizella nidulus** (Fr.) Raitv. s.l., 11.V.88, F-Gérardmer, on stems of **Ranunculus aconitifolius**, leg. J. Deny, HB 3384 -- **Tubeufia cerea** (Berk. & Curt.) Booth, 17.VI.86 D-Murrhardt, on **Diatrype stigma**, leg. L. Krieglsteiner, HB 3039 -- **Tubeufia paludosa** (Crouan & H. Crouan) Rossm., 7.VIII.88, D-Tübingen, on culm of **Phragmites communis**, leg. HB, HB 3507b -- **Tympanis alnea** (Pers.) Fr., 25.V.87, CH-Schaffhausen-Thayngen, on bark of **Prunus avium** twig, leg. P. Blank, HB 3187 -- **Verpa digitaliformis** Pers., 8.V.91 D-Tübingen-Bebenhausen, under **Petasites**, leg. HB, HB 4411 -- **Xanthoria parietina** (L.) Th.

Fr., 7.VI.86 D-Tübingen-Pfrondorf, on bark of **Malus domestica** trunk, leg. HB, HB 3036.

Abbreviations:

✱ = living hydrated state *(in statu vivo et udo/umido)*

† = dead hydrated state *(in statu emortuo et udo/umido)*

LM	= light microscope
TEM / SEM	= transmission / scanning electron microscope

H_2O	= tap water (of medium hardness)
IKI	= Lugol's solution (1% [3] I_2, 3% KI in water)
MLZ	= Melzer's reagent (1.2% I_2, 3.6% KI, 48% chloral hydrate in water)
CRB	= Brilliant cresyl blue (0.1-1% in tap water, not distilled water)
CRB_A	= alkalinized CRB: a small drop of 0.5% KOH or strong NH_4OH is added to a CRB mount
KOH	= potassium hydroxide (0.5-10% in water)
CB	= cotton blue (0.5% in lactophenol, i.e. equal proportions of phenol, glycerol, lactic acid and water)
CZB	= chlorazol black (1% in glycerol buffer)

cytoplasmic structures:		vacuolar structures:	
LB	= lipid body	VB	= refractive vacuolar body
SCB	= KOH-soluble cytoplasmic body	V	= non-refractive vacuole
WB	= Woronin body	MC	= metachromatic corpuscle
N/NO	= nucleus/nucleolus		

DBB = de Bary bubble

3. HOW TO RECOGNIZE LIVING FUNGAL CELLS

Vitality of single cells is defined according to STRUGGER (1949: 170f.) by intact (semipermeable) plasma membranes. Viable cells therefore show turgescence when mounted in hypo-osmotic media (with an osmosity lower than that of the cell sap). Current tests, such as for germination ability or metabolic activity, are here of

[3]) All values in this paper given in % refer to w/w = weight of solute per weight of solution (solvent + solute).

minor value because (1) vitality has to be proved for a single cell, and (2) many types of cells (e.g. asci) are unable to germinate.

Vitality can easily be recognized under the LM (oil immersion) by the following tests: **1.** Cell turgor: adding strongly hyperosmotic media to the water mount provokes shrinkage (not collapse!) of living but not of dead cells (in the case of elastic cell walls). Reversible plasmolysis is also a reliable proof for vitality, occurring especially in vegetative cells. These tests require that the cell wall is freely permeable for the reagent, otherwise both living and dead cells may collapse. In water mounts of multi-celled organs, the septum is strongly curved towards the dead cell if the adjacent cell is intact (FIGs. 2d, 3). Slight constrictions at the septa are typical for living thin-walled cells (FIGs. 2 a-c, 7a, 13a).

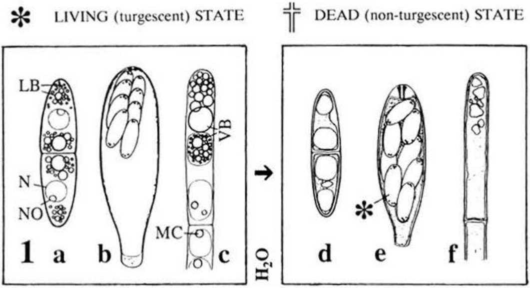

FIG. 1. How to distinguish living from dead cells. Hymenial elements (spore, ascus, paraphysis) of fictitious species of Leotiales in the living (**abc**) versus dead state (**def**). The dead ascus contains still living spores.

2. Structure of the cytoplasm: in water mounts, living fungal tissue shows high transparency and contrast while dead cytoplasm is detached from the cell wall and is usually much more refractive due to dehydration, therefore opaque (FIGs. 1 d,f; 2 d,f; 3; 12b). Cytoplasmic inclusions show aesthetic and symmetrical patterns when intact but irregular distortion or optical absence when dead.

3. Staining *in statu vivo*: basic (alkaline) dyes added to water mounts accumulate (within seconds or minutes) selectively inside intact vacuoles of the living cell without damage to the plasma membranes, either homogeneous (FIG. 30, *VB*) or by forming globose bodies as a result of flocculation of (poly-)phosphates (volutin bodies, metachromatic corpuscles/granules, FIGs. 4, 30, *MC*). The living cytoplasm is thereby not stained. Yet, in dead cells with destroyed plasma membranes (loss of semipermeability), the cytoplasm is deeply stained within seconds while no accumulation and MC-formation occurs in the vacuoles (FIG. 21b; GUILLERMOND, 1941: 129ff.; STRUGGER, 1949: 126ff.; HEINEMANN, 1956: 36ff.; BANCHER & HÖFLER, 1959: 150ff.; HOHL, 1987: 17; ROMEIS, 1989: 27, 302ff., *Trypanblau-Ausschlusstest*). Staining of the vacuoles with basic dyes is *essentially a phenomenon of the living cell* (GUILLERMOND, 1941: 145) and the most valuable test for vitality (BANCHER & HÖFLER, 1959: 153).[4]

The contrast between the unstained (living) and stained (dead) cytoplasm is usually striking and corresponds to fig. 119 in ERB & MATHEIS (1983) showing both living and dead cells in multi-celled spores of **Ophiobolus acuminatus** stained by phloxine. (Similar to basic dyes, phloxine is only able to stain the dead cytoplasm.) Yet, cell contents rich in lipids or vacuoles but poor in cytoplasm are less distinctly stained in the dead state, and thick walls of spores were found to be impermeable to basic dyes in either state.

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[4]) Overstaining must be avoided since concentrations above approx. 0.5% may be lethal. Aqueous CRB solution, slightly alkalinized (CRB_A) in case the stain does not readily penetrate through the plasma membranes, gives good results: blue-violet for MCs, blue-green to violet for homogeneous staining (see CHADEFAUD, 1938: 116; GUILLERMOND, 1941: 143; LE GAL, 1947: 78). The CRB (0.1-1%) is added to the margin of the water mount resulting in a CRB solution of about 0.05-0.5%. The KOH accelerates the process of staining the vacuoles because only undissociated molecules of CRB (which do not exist at a pH lower than 7) pass through the plasma membranes.

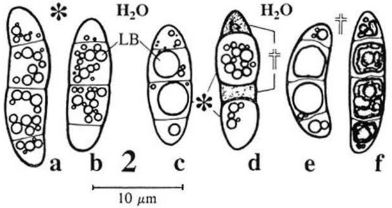
Non-alkalinized CRB is also used for diagnostic violet stains of wall layers in Basidiomycetes (SINGER, 1986: 89). I recommend CRB as a standard reagent for Ascomycete taxonomy because it allows the recognition of mucilage on hyphae or spores by staining deep violet (see chapter 5.c.) while resinous exudates stain deep turquoise-blue.

I found the aqueous solution (in tap water) to be stable for years; yet, CLÉMENÇON (1972) added several ingredients because he found CRB in water to precipitate within a few days. CRB is not considered carcinogenic by ERB & MATHEIS (1983: 28). Other basic dyes dissolved in tap water have been tested in comparison: toluidine blue, used by MOORE (1965: 26) and MATHEIS (1975: 160) as a stain for mucilage, gives results comparable to CRB showing striking turquoise-blue and red-violet metachromatic colors. However, CRB gave more reliable results because it is blue in tap water while toluidine blue is violet in that medium. Contrary to toluidine blue, the color of CRB depends furthermore on the pH (turquoise-blue in acetic acid, red-violet in KOH). Contrary to CRB, toluidine blue did not easily penetrate into the living cell. Neutral red and cotton blue may also be used but do not show striking metachromatic color changes.

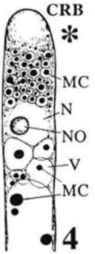
4. HOW TO AVOID MORPHOLOGICAL ALTERATIONS OF LIVING FUNGAL CELLS DURING MICROSCOPIC EXAMINATION

4.a. Mounting medium

STRUGGER (1949: 4) recommended mounting cells of living land plants in *aqua bidest*. Similar to plants, Ascomycetes, developing under natural conditions, are usually exposed to rain water (i.e. approximately distilled water). Drought-



FIGs. 2-4. How to distinguish living from dead cells.
FIGs. 2-3. Two examples of living versus dead cells occurring partly in one organ. Note (1) shape of thin-walled septa (straight between living cells, curved between a living and a dead cell, more or less wrinkled between dead cells), (2) transparency of the living cytoplasm, (3) coalescence of LBs (in FIG. 2c already in the living cells), (4) detachment of the cytoplasm from the cell wall, (5) dramatic loss of volume of the dead cell especially if situated between living cells (FIG. 2d). **FIG. 2.** ascospores of **Polydesmia pruinosa**, **FIG. 3.** hair tip of **Trichopezizella nidulus**. 2000x.



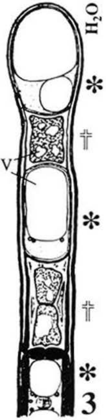


FIG. 4. Upper region of a living immature ascus with fusion nucleus and vacuoles containing MCs which are typical for living cells (stained with neutral red or CRB; from CHADEFAUD, 1938: fig. 2).

tolerant species in particular, which dry out completely during dry weather, rapidly absorb water during rainfall and are most favourably collected immediately afterwards. Aquatic species develop below the water level in rivers and lakes. Rain or tap water is therefore the natural preparation medium for these fungi.

Mounting in media with a high osmosity induces severe shrinkage, especially of asci (see chapter 5.a.). A test with artificial sea water (3.4% sodium chloride [NaCl]) on some species of Leotiales and Lecanorales revealed shrinkage in ascus width for 2-12%. Accordingly, concentrations upto 0.3% NaCl produce shrinkage for max. about 1% and are therefore compatible to distilled water for the purpose of taxonomy. Tap water has an osmosity of roughly 0.0003 to 0.006% NaCl. Hence, it is safe to say that mounting fruit-bodies of Ascomycetes in tap water of any hardness corresponds to the natural situation of these fungi.

Staining *in statu vivo* with basic dyes or with IKI is only lethal after a considerable period of time. However, mounting in currently used media, with either very high (alkaline) or low (acid) pH or other lethal properties, such as MLZ, CB, glycerol buffer (= *L4*), Hoyer's fluid, lactic acid, 2-10% KOH, kills most types of fungal cells within seconds (LINDER, 1929; BARER et al., 1953: 720; HUHTINEN, 1985: 18; BARAL, 1987a: 409 and unpublished data). CLÉMENÇON (1972: 49) worked only with lethal mountants and did not even mention water as a possible medium. The lethal effect of these mountants should be clear but are actually not well-known. Workers are indeed surprised when accidentally encountering it: e.g., LUARD (1983: 529) noted that CB had an *unexpected effect on the appearance of* ***Chrysosporium fastidiosum*** causing a *dramatic contraction of the cytoplasm.*

These mountants have been introduced for the study of herbarium specimens in order to obtain transparency of the cytoplasm, to dissolve trapped air on imperfectly wetted hyphae, to inflate collapsed cells, to avoid movement of floating spores, and to avoid desiccation of the mount during microscopic study (see FLEMING & SMITH, 1944: 17; BARAL, 1987: 408f.). In order to get compatible results, these mountants are often also used for fresh specimens. This led CLÉMENÇON (1972, *L4*) to employ lower concentrations (20% glycerol, ≡ 8.5% NaCl[5]) in order to avoid the collapse of living cells. In my experience, mature spores with rigid walls, e.g. in Pezizales and Xylariales, often survive some hours in media such as MLZ, thus killing by heating of the slides is necessary in order to obtain results compatible with the dead specimen.

Some mycologists try to avoid inflation of fungal cells to an "unnatural" oversize by employing "isotonic media", e.g. 1% glucose (≡ 0.17% NaCl) or 0.85% NaCl (CHADEFAUD, 1938: 116; BRUMMELEN, 1967: 18; ERB & MATHEIS, 1983: 13, 15). The glucose medium is, however, strongly hypo-osmotic and thus gives results corresponding to tap water mounts. H. CLÉMENÇON (in litt. 20.7.87) believed that

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[5]) The given concentration of NaCl solution has the same osmotic pressure (osmosity) as the mentioned solution. An osmosity of 1 g-mol/l = 5.6% NaCl is equivalent to 6×10^{23} ions per litre

the physiological state of fungal cells is usually characterized by a low turgor pressure clearly distinct from the state of full hydration and maximum tugor which is obtained in mounts of distilled or tap water. Since the osmotic pressure of fungal cells strongly varies among species and even organs, each case would then need its special iso-osmotic mountant. The following test proves, however, that maximum turgor and hydration is the natural state for full metabolic activity in ascocarp cells of discomycetes: I compared mounts of fresh ascocarps of several species in tap water with those made instead in oil, e.g. immersion oil (or paraffin oil according to STRUGGER, 1949: 5). The oil prevents a possible uptake of further external water during preparation. No difference in size and appearance of the cells and their contents could be observed. This should hold true for all fungal structures which are naturally exposed to rain water. Cells having a cell wall do not need an iso-osmotic medium: their physiological medium is, in these cases, close to distilled water, i.e. strongly hypo-osmotic.

I therefore use water as a standard medium for obtaining compatible measurements of living fungal cells. Likewise, MAIRE (1926: 44), for example, wrote: *Les dimensions des spores doivent être mesurées autant que possible sur des spores fraiches examinées dans l'eau*, and SPAIN (1990: 71) wrote: *Arguments are presented for diagnoses based on the morphology of fresh spores suspended in water.*

Osmotolerant hyphae, able to grow in media with a high concentration of glucose, and especially marine fungi might represent an exception. LUARD (1983: 533) therefore measured the hyphal diameter of osmotolerant species by mounting in *slightly hyperosmotic KCl*. A similar case is represented by the ascospores while still inside the living ascus: the spores are embedded in the vacuole sap with a high osmosity, and increase considerably in size within about 1/2 minute following discharge into water.

In order to obtain compatible measurements, I recommend to avoid measuring ascospores within living asci. Probably all fungi can be observed and measured in the standard mountant tap water without bursting of the hyphae. This view is supported by the report of JONES et al. (1991) who found fungal cultures to survive several years in distilled water in small phials.

4.b. Preparative techniques in vital taxonomy

The specimens chosen for microscopic examination must be in the fully hydrated living state. Species growing in permanently humid places are usually sensible to drought and must be transported to the laboratory without the loss of intracellular water. Desiccated ascocarps of drought-tolerant species can be rehydrated by spraying with water several minutes prior to preparation. Fragments of the fruit-bodies are gently (!) squeezed under the coverglass. Freehand sections (about 30-100 μm thick, depending upon the diameter of the cells) through the fully hydrated living fruit-body made with a razor blade are superior, allowing excellent study of ascocarp textures (BOUDIER, 1886: 136; CORNER, l.c.), while dead hydrated tissue is difficult to cut freehand because of its flabbiness. Heating or any stronger pressure must be avoided. Very often a certain number of spores, asci, and vegetative cells are already dead prior to preparation (FIGs. 2d, 3), or they die when cut by the razor blade. These cells must be disregarded for the purpose of vital taxonomy.

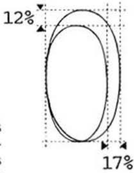
5. ALTERATIONS IN THE CELL WALL

5.a. Cell size: the shrinking effect

As hydrated fungal cells die, they lose turgor and often show a strong decrease in size (without collapsing) due to elasticity of the cell wall and loss of water mainly from the vacuoles. Irreversible shrinkage is induced by lethal substances such as, e.g., 50% chloral hydrate, 1-5% KOH, or simply by mechanical pressure (FIGs. 2, 3, 6, 8, 9, 10, 12, 13, 14, 21, 22, 33, 34, 35), which destroy the semipermeable properties of the plasma membranes. Both length and width are not infrequently reduced for about 10-20%, with the cell volume (including the cell wall) for about 30-50% (TAB. 1). Such variation in measurements is very often employed for differentiation among species! A linear shrinkage in width for about 30-57% was observed in the asci of species of **Lecanora** (FIG. 9), **Xylaria** and **Lasiosphaeria**.

HUHTINEN (1985: 18) wrote: *In numerous taxa of both the Helotiales and Pezizales, I have observed that the mountants commonly in use have a shrinking effect. When dried material is revived with Melzer's reagent or lactic acid, the sections do not always regain their original dimensions. This can be concluded from the results of adding these mountants to natural or water mounts of fresh apothecia. Shrinkage of 5-15% takes place immediately, due to a loss of turgor in the cells.*

decrease in %	ASCI length	ASCI width	ASCI volume	SPORES length	SPORES width	SPORES volume
Mn. jung.	18.5-20.3	17.8-21.7	46-51	5.7- 8.3	13.9-21.5	30-44
Ci. caucus	14.9-19.7	18.7-22.9	44-51	12.1-14.0	20.8-24.6	45-51
Ru. elat.	10.6-17.4	17.2-23.8	40-52	12.1-18.2	13.3-16.7	34-42
Pe. amenti	13.1-20.8	15.1-25.6	37-56	9.8-13.9	7.5- 9.8	24-29



mean linear shrinkage of spores

TAB. 1. Irreversible shrinkage in Leotiales (tap water versus MLZ). **Mniaecia jungermanniae**, **Ciboria caucus**, **Rutstroemia elatina**, **Pezizella amenti**. 4 asci and 4 spores from each species were measured in the living state and after MLZ was added by direct visual monitoring of shrinkage. (Care must be taken in dead spores not to measure the plasma body only).

BECKETT et al. (1984: 93) reported severe dimensional changes during preparative procedures for the SEM: *Although it is logical to expect biological specimens which normally have high water contents to shrink when dried (...), few published results adequately account for this.*

The following literature also discusses the shrinking effect for fungal cells: DE BARY (1887: fig. 43, see FIG. 6), LAGARDE (1906: 135), MAIRE (1926: 44f.), STEINER (1957: 249), HERTEL (1967: 3, 13% linear shrinkage in KOH vs. H_2O), DRING (1971 [in LUARD 1983: 529]), HEIN (1976: 16, about 15% linear shrinkage in CB vs. H_2O), DÖBBELER (1984: 206), BARAL (1987a: 409; 1987b: 121; 1989a: 120; 1989b: 222), HUHTINEN (1990a: 64, 68, shrinkage in width ca. 15%, CB or MLZ vs. *in statu vivo*, H_2O).

The percentage of linear shrinkage during the death of the cells strongly depends upon the taxon, cell type, and cell axis (TAB. 1), but also upon the mountant in which the dead cells are measured (HUHTINEN, l.c.). Furthermore, rehydrating dead cells may result in damaged profiles: e.g., ascus width shows higher variation due to (1) strong pressure on the coverglass which may flatten dead asci, and (2) irregularly arranged spores which swell out the ascus wall (FIG. 9b). Hairs of **Brunnipila** (Hyaloscyphaceae) collapse in dehydrating reagents: their apparent width depends on the angle at which they are lying and can therefore vary from about 1 to 7 μm. The width of the hydrated living hair is about 3-5 μm.

KOH is commonly considered an agent which increases the volume of fungal cells, especially of spores (e.g. HEINEMANN & RAMELOO, 1985), or restores dried plant cells to their "original size" (e.g. CUNNINGHAM, 1969). Such a swelling effect is mainly observed when KOH mounts are compared with mounts of dead cells in water, CB, MLZ etc. When applied to water mounts of living fungal cells, however, KOH often provokes dramatic shrinkage, especially with asci.

On the other hand, living mature spores which pass into the germination phase show a considerable increase in size in many species, partly due to synthesis of new cellular wall material (GARRAWAY & EVANS, 1984: 221). Therefore, even in the dead, shrunken state with a turgor nearly zero this increase in size is obvious. In other species the spores do not change their dimensions during germination. Consequently, measurements which do not indicate (1) the state (living or dead) of the measured cell, (2) the mounting medium, (3) the preparative treatment (heating, mechanical pressure), and (4) the development stage of the cell, are of minor taxonomic value (see also HUHTINEN, 1990a; b).

Decrease of the cell volume with the decrease of the hydrostatic pressure (turgor pressure) is a well-known phenomenon in plant and animal cells and tissue (DAINTY, 1976). The elastic modulus of the cell wall controls the rate of swelling or shrinking: cells with rigid walls do not shrink noticeably if the maximum turgor pressure is brought down to about zero; their elastic modulus is high. Highly elastic cell walls, however, have a very low elastic modulus and the cells shrink considerably in this case. Furthermore, cells shrink to a higher extent if the maximum turgor pressure is rather high, e.g. in mature asci.

It appears therefore incredible that, e.g., CUNNINGHAM (1972) wrote the following incorrect statements about a mountant containing 67% chloral hydrate: *AH*[6] *does not distort spore measurements*, and: *AH sometimes causes slight temporary plasmolysis of certain fresh fungi but normal turgor is totally restored almost always within a few minutes*! CUNNINGHAM misapplied the term *turgor* to cells in a dead, non-collapsed state, their walls showing an even, non-shriveled outline but a very low tension. Likewise, LINDER (1929) and FLEMING & SMITH (1944: 17) wrote that lactophenol *immediately restores the turgor* and *rarely causes either swelling or shrinkage*.

Shrinkage of asci immediately after spore discharge is a well-known phenomenon (FIGs. 23-25; BULLER, 1931: 247: *an ascus reduces its volume to about one half on exploding*; INGOLD, 1986: fig. 1). However, shrinkage to a comparable extent during the death of the mature ascus before *the spores are released* (FIG. 8 a→b, 9 a→b, 10 a→b) was very rarely reported (e.g. by DE BARY, see FIG. 6) though generally occurring. Shrinkage of asci without spore liberation may also be obtained reversibly without killing by using, e.g., 1 M (= 30%) saccharose (≡ 4.4% NaCl) (INGOLD, 1953: 17, fig. 8). With saccharose solutions higher than about 45% (≡ 9% NaCl) I was able to shrink the asci of **Lecanora conizaeoides** in width from 20-21.5 μm (in tap water) to 12-13 μm (i.e., to an extent close to FIG. 9b), and to reverse these asci to their original size and shape by replacing the saccharose solution with water.

Unawareness of shrinkage has resulted in numerous conflicts and misinterpretations. E.g., KORF (1951) found roughly 25% lower measurements of hairs, asci, spores, and paraphyses in some 20 years old type material of two species compared with the original description given by GRELET. Since GRELET usually studied living cells (which can be concluded from his drawings), following the tradition of BOUDIER, the discrepancy discovered by KORF is readily explained by the shrinking effect. KORF, however, concluded that GRELET's measurements were incorrect, *presumably because of an error in the microscope calibration*, and therefore multiplied GRELET's measurements by an assumed error factor of 0.75. Likewise, authors have found BOUDIER's measurements (which he gave in the text) to be usually too high when compared with their own measurements. MAIRE (1926: 47) referred this discrepancy to an error in the construction of BOUDIER's measuring scale, and recommended the subtraction of one-tenth from BOUDIER's values. Clearly, the shrinking effect is at least one of the true reasons for BOUDIER's larger measurements. Indeed, the magnification of *820x* indicated by BOUDIER on his plates *after 1885* is considered by v. BRUMMELEN (1969; and also by me) to be correct while plates *before 1885* are *approx. 840x* instead of "340x" as BOUDIER indicated (BRUMMELEN, l.c.). The values given in BOUDIER's texts after 1885 are, however, highly erroneous in some cases.

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[6]) AH = Andre's modification of Hoyer's fluid: arabic gum 15g, chloral hydrate 100g, glycerol 10g, water 25ml

Some striking effects:

1. Spore arrangement: spores conspicuously change their arrangement during the death of the mature ascus due to excessive loss of water from the large vacuole of the ascus. This is of taxonomic importance since the arrangement of spores in the asci is very often mentioned in species descriptions. Three phenomena may be observed:

(1) The spores move towards the base of the ascus, thus the *pars sporifera* increases considerably in length (FIG. 8, **Mniaecia**, from 67 to 135 μm; FIG. 9, **Lecanora**, from 26.5 to 38.5 μm). Strictly biseriate spores thereby often become totally (or only in the lower part) uniseriate (FIG. 8).

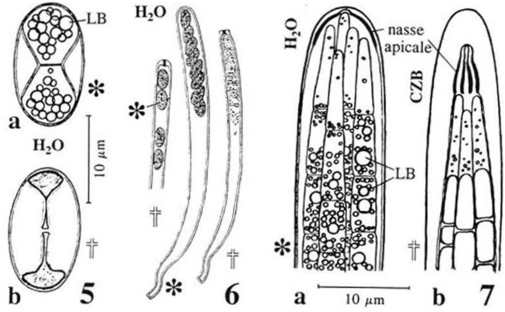
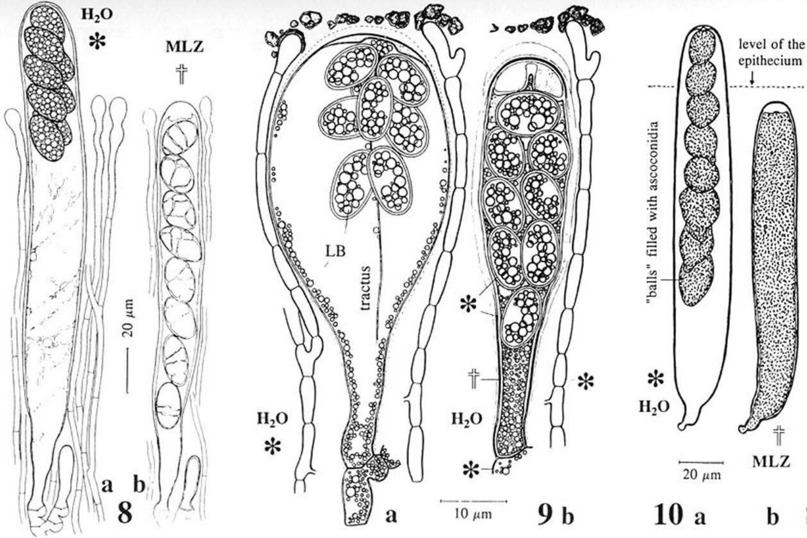


FIG. 5. Expanding wall phenomenon in the ascospores of **Xanthoria parietina**. Note coalescence of single LBs. 2000x.
FIGs. 6-10. Micromorphological alterations of asci (FIGs. 7-10 show one ascus before and few minutes after killing). Note (1) shrinkage of asci and retraction below the tips of paraphyses, (2) loss of most of the vacuole sap, (3) expansion of apical wall layers, (4) movement of spores towards the base of asci. FIG. 9a might be the first published figure of a living Lecanorales ascus! **FIG. 6. Sclerotinia sclerotiorum** (from DE BARY, 1887: fig. 43), **FIG. 7. Tubeufia cerea**, (2000x), **FIG. 8. Mniaecia jungermanniae** (500x), **FIG. 9. Lecanora conizaeoides** (1500x), **FIG. 10. Tympanis alnea** (500x).


H2O
MLZ
20 µm
a b
8
LB
tractus
H2O
10 µm
a
9 b
level of the epithecium
"balls" filled with ascoconidia
H2O
MLZ
20 µm
10 a
b

(2) Filiform spores lying as straight bundles parallel to the long axis of the ascus may become spirally twisted (e.g. in **Vibrissea**).

(3) Ascoconidia packed inside 4-8 "balls" become disarranged and continuously fill the whole ascus (FIG. 10).

2. Living spores within mature living asci mounted in water are narrower when compared with discharged spores due to the ascus turgor (high osmosity of the large vacuole): in some tested species of Leotiales and Rhytismatales, they were 9-15(-30)% narrower within the asci while their length was nearly unchanged.

3. Protuberant asci: asci shrink much more in length compared to paraphyses. In the living state, mature asci often greatly exceed paraphyses in length but retract below the level of the latter on killing or after spore discharge (FIGs. 8, 10, 33; DE BARY, 1887: 87, 92; BULLER, 1931: 247). Statements such as "asci covered by an epithecium" (composed of agglutinated apical cells of the paraphyses) often originate from dead material. Due to their turgor, living Lecanorales asci tear crevices in the epithecium (long before spore discharge takes place) by pushing the paraphyses aside (FIG. 9).

4. Septate thin-walled hyphae or spores show slight constrictions at the septa *in statu vivo* due to the internal turgor, but an even surface *in statu emortuo* (FIGs. 2 ab→ef, 13 a→b, 34 a→b). The septum is often irregularly wrinkled in the dead state (FIG. 2 ef).

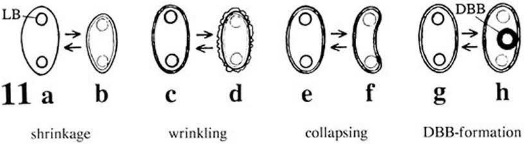


FIG. 11. Four reactions of a living cell on water loss, induced either by desiccation, or by a medium with a high osmosity.

5. False ornamentation: Ascospore walls of Pezizales are often composed of two separable layers, the outer being non-elastic, the inner elastic. As the spores die, the wall loses tension: the inner layer contracts while the outer layer becomes wrinkled by separating more or less from the inner layer. This is, e.g., the case in all studied species of **Sarcoscypha** (BARAL, 1984: fig. 8). Such "false ornamentations" are not stained by CB. ZHUANG (1991) described a new species, **S. striati-**

spora, differing from **S. occidentalis** merely by transverse striations on the spore wall and lower dimensions of spores and asci. Such "characters" are of no use for the delimitation against **S. occidentalis**. LE GAL (1947: 223-238) described a special type of spore ornamentation as *non calloso-pectique*, mainly in members of the Sarcoscyphinae, of longitudinal or transverse striations which are probably also a result of shrinkage of originally smooth spores. READ & BECKETT (1983) reported a reticulate surface texture characteristic of critical point-dried urediospores but a smooth surface in frozen-hydrated material. The authors consider the reticulation to be artificial resulting from shrinkage or the removal of the unfreezable (bound) water from the cell wall.

5.b. Wall thickness: the expanding wall phenomenon (imbibition effect)

Strongly thickened inner wall layers of asci (known as *apical dome/apparatus*, or *tholus*, and approximately corresponding to the endotunica = *Couche D* in REYNOLDS 1989) proved to be very useful in delimiting natural higher taxa. Such thick ascus walls differ fundamentally from solid, constantly thick walls: a thick ascus wall is indicative of the dead state while living asci show dramatically thinner walls (FIGs. 6-10), even in immature asci with a relatively low turgor (DE BARY, 1887: 87, 95; KERR, 1961: 474ff.; BARAL, 1987a: 413; 1987b: 122). Likewise, CHADEFAUD (1944: 9) figured thin and very thick apical apparati in asci of **Leotia**, and correctly referred the strong wall imbibition to the loss of turgor of the ascus vacuoles.

These wall layers represent a swellable, hydrophilous matrix with a considerable but limited expansibility: they become imbibed with water within a few seconds as soon as the asci die, either in water (naturally or by mechanical pressure, FIGs. 6, 9), or by adding lethal reagents (FIGs. 7, 8, 10; see also HOGGAN 1927: 42, pl. V, 6). Increase of about 2-5 times the original thickness usually occurs. The inner contour strongly loses contrast and may severely change its shape (FIG. 9 a→b). KOH is not necessary for the effect as was suggested by HONEGGER (1982: 213, 215) but often produces further expansion when applied to dead asci.

Amyloid layers (except for that part of the amyloid ring which protrudes into the ascoplasm) show this phenomenon also (FIG. 43 b→c). The intensity of the iodine reaction thereby logically decreases with expansion, thus a strong reaction *in statu vivo* changes to a weak or moderate reaction *in statu emortuo*.

TEM-investigations on thickened ascus walls show a loosely fibrillar organization which represents the solid part of the endotunica. The ample space between the microfibrils is clearly only present in the expanded state and should therefore be merely a watery medium; to postulate the presence of an "amorphous matrix" between the fibrils (e.g. in REYNOLDS, 1989: 13) is superfluous.

A more or less severe decrease in wall thickness of apical domes during the ascus ontogeny can be observed in dead asci (and less pronounced in living asci) (BARAL, 1987b: 124). This effect was often misinterpreted to be a result of increased turgor during maturation (e.g. by HONEGGER, 1983: 63, figs. 3a→b, 4b→c, all figured asci were killed by acrolein-glutaraldehyde/osmium-fixation). The difference in thickness is indeed also present in ruptured asci in water mounts. Thus, changes in the fibrillar compactness, which may represent different degrees of polymerisation should have occurred during maturation. Reported variation in thickness of the tholus in dead asci of **Tephromela aglaea** as depicted by HERTEL & RAMBOLD (1985) should partly be referred to this effect. My experience is that living asci show much less variability in their apical structures compared to dead asci in any group of Ascomycetes. Nevertheless, I concur with the common practice of considering the expanded apical apparatus more useful for taxonomic purposes because the amyloid structures are too compressed in the living state to be able to see the important details. The morphology of the living apical apparatus should, however, be simultaneously studied.

Since the observed decrease in wall thickness during maturation of the ascus is the result of a reduced hydration of the microfibrils, the theory of CHADEFAUD (1942: 65) and BELLEMÈRE & HAFELLNER (1982: 272) claiming resorption of wall material during the "regression" of the thickened wall layers is also superfluous.

Variation in thickness is sometimes increased in dead asci by mechanical pressure of the spores against the endotunica, especially in asci with expansible lateral walls. Thus, **Tympanis**, for example, exhibits thick apical and lateral walls in young dead, or in discharged asci but thin lateral walls in mature, non-discharged, dead asci (FIG. 10b) as a result of mechanical pressure of the numerous ascoconidia.

Many accounts of the fissitunicate ("bitunicate") ascus indicate that the wall in the upper region, especially of the immature ascus, is thickened. In the living state, however, fissitunicate asci are thin-walled at any stage of development (DE BARY, 1887: 95; HOGGAN, 1927; KERR, 1961: 475f.). The *banded pattern* or *accordion-like arrangement* of the microfibrils, considered to characterize the non-discharged fissitunicate ascus (REYNOLDS, 1971: 248, fig. 7; MÜLLER, 1981; PARGUEY-LEDUC & JANEX-FAVRE, 1982: figs. 15-20; BELLEMÈRE & HAFELLNER, 1982: fig. 6; BELLEMÈRE & al., 1986: fig. 3), occurs logically only in dead asci (FIG. 7, the fibrils are not seen with the LM). The theory of a "reorientation" of the microfibrils from a banded to a parallel pattern during elongation of the endotunica (e.g. in REYNOLDS, 1971: 254; 1989: 14) is thus superfluous.

Many attempts to reconstruct the process of spore discharge start with the false assumption that the endotunica is thick-walled prior to bursting of the ascus, or that it at least swells prior to discharge. In the Leotiales, numerous observations of spore discharge from asci in water mounts confirm that the apical dome is thin-walled as the asci explode (BARAL, 1987b: 128). The possibility of monitoring the delayed process of successive spore discharge from fissitunicate asci already led

PRINGSHEIM (1858) to the discovery that swelling of the endotunica occurs only after the last spore has been forcibly ejected.

A theory advocated by DUGHI (1957) and CHADEFAUD (1942: 59; 1973: 135f.) claims that tholi in asci of Lecanorales play a role in drought tolerance. The authors correctly refer the variation in thickness to a varying degree of hydration of the ascus wall, but thought that this variation depends upon the atmospheric humidity. The latter is not true: by mounting in oil, I found the endotunica in living asci of **Lecanora conizaeoides** to be always compressed, whether the asci were fully or partly hydrated, or dehydrated by air-drying. Furthermore, remarkable drought tolerance and longevity was found to occur also in asci devoid of wall thickenings (see also chapter 9).

Mounting fresh apothecia in concentrated ethanol, or air-dried apothecia in oil, showed dehydrated and thus thin walls in the ascus apex even in dead asci. This effect was discussed by STEINER & PEVELING (1984: 784) for ascospores with expansible septal walls. In TEM-preparations, different methods of fixation often result in different degrees of expansion (see below).

What is the biological sense of expansible inner ascus wall layers? DUGHI (1957: 13) listed 7 hypothetical functions of the tholi in Lecanorales. From the preceding, I am forced to assert that none of these hypotheses can be maintained. The process of expansion through water absorption prior to discharge must be considered an anomaly in all Ascomycetes with expansible layers: expansion occurs when asci die but never in living asci under natural conditions. Active and complete discharge from dead asci, as indicated by DUGHI, was never observed. Layers capable of expansion (= swelling in thickness) are, however, capable of enormous extension (= elongation in longitudinal direction) directly prior to spore discharge, especially in the fissitunicate ascus (DE BARY, 1887: 96; KERR, 1961: 475; BARAL, 1987b: 128). Apical rings are thereby everted prior to discharge (BARAL, 1987b: figs. 1, 7). Extensibility is presently the only function of expansible layers that I am able to accept.

That the expanding wall phenomenon in asci has almost completely been forgotten in the last decades is obvious from STEINER & PEVELING's (1984) recent account. The authors reported a similar effect for inner wall layers of the thick-walled septa in ascospores of **Caloplaca/Xanthoria** (FIG. 5), and correctly refer the swelling of the septum to the death of the spores. They do not mention, however, that thickened walls of asci show the same effect. I have found comparable expansible septa in (especially immature) spores of **Physcia**, **Rinodina**, **Massaria anomia** and **Nimbomollisia**. NANNFELDT (1983: 297) characterized the latter genus by spores having thick and "refractive" septa (*septo mediano crasso, valde refringente divisae*). Yet, only dead spores have thick septa (FIGs. 12b, 13b) while living spores show thin-walled septa (FIGs. 12a, 13a). Expansible wall layers are said to occur also in spores of Endogonaceae (SPAIN, 1990: 72, induced by acidic mountants), and in septal structures (dolipori) of Basidiomycetes (HOCH & HOWARD, 1981; LÜ & MCLAUGHLIN, 1991). The latter observed under the TEM, that the height of the pore swellings by freeze substitution was 100-184 nm (figs. 2, 4-10) but 370-475 nm by conventional chemical fixation (figs. 3, 11).

5.c. Contrast of cell walls and mucilaginous sheaths

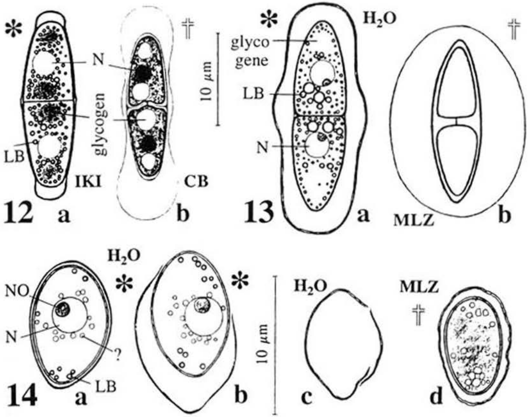
The loss of contrast of the endotunica during the death of the ascus was already mentioned above. The contrast of the spore wall inside asci becomes also often strikingly faint if asci and spores are killed by KOH, CB etc. This is due to the fact that, in the living state, the spores are surrounded by ample transparent (non-refractive) water of the vacuole(s). Therefore, the refractive spore wall markedly contrasts with the surrounding water. In the dead state, however, most of the water has escaped from the vacuole(s), and the spores are now embedded in the dead epiplasm which has a refractive index comparable to that of the spore wall. Dead ascospores inside dead asci of Leotiales can therefore often only be recognized from their LBs (in KOH) if LBs are present, while the spore wall remains quite invisible.

In the species of **Calycellina** with 4-spored asci in the mature stage, 8 spores are formed at first but 4 spores early abort and collapse (BARAL, 1989b). The remaining cell walls of the collapsed spores are clearly visible within living asci (FIGs. 19, 20) but entirely indiscernible in asci from herbarium material. Therefore, this interesting situation has not been reported by other authors.

Coalesced LBs may simulate septa (FIG. 22). In living multiguttulate spores true septa are not easily seen but recognizable by slight constrictions of the spores at the septa and by the nucleus in the middle of each cell (FIGs. 1a, 2ab). True septa often become more obvious when mounting in MLZ, CB etc. (FIG. 7b), but then the asci are killed and, therefore, the degree of maturity of the spores, i.e. the number of septa in the mature spore, is not further recognizable (see chapter 8). BOUDIER (1886: 143f.) recommended to stain *in statu vivo* by IKI in order to see septa more clearly.

The presence or absence of croziers on the ascogenous hyphae, recently re-introduced by HUHTINEN (1990a: 66) as a very important taxonomic character in **Hyaloscypha** and allied genera, is rapidly seen *in situ* in sections through living young apothecia in most taxa of Leotiales (avoid pressure on the cover slip). In dead hymenia, however, the feature is often indiscernible when water, MLZ or CB is used. Mounting in Congo red (gently heated) as HUHTINEN recommended (in litt.), or in KOH as I prefer in the case of herbarium material, usually allows this feature to be seen when separating the tissue by pressure, but it is very often highly time-consuming to be certain about it. The feature has a high significance for the delimitation among the species of Leotiales: 77% of 767 species so far studied by me have croziers but 22% have not. About 1% seem to be variable.

Mucilaginous appendages or sheaths of spores are often overlooked in herbarium material. Sheaths swell considerably after spore discharge (INGOLD, 1978; BRUMMELEN, 1967: 39), or if asci are killed together with their spores, rarely even within the living ascus, and thereby lose refractivity and contrast (FIGs. 12 a→b, 13 a→b). Obviously, a delicate semipermeable membrane covers the exterior of the mucilage. These sheaths have wrongly been thought to supply the internal pressure for spore discharge by several workers. Numerous observations on living material proves, however, that the sheaths are always dehydrated and appressed to the spore wall shortly before and during spore discharge. In dead non-discharged asci they are strongly swollen and fill the space between spore and ascus wall. In some taxa these sheaths stain violet in CRB.



FIGs. 12-13. Spores of **Nimbomollisia** observed in the living versus dead state. The expansion of the mucilaginous sheath occurs when spores are killed but also in the living state: sheaths are compressed inside living asci and expand sooner or later when spores are discharged or when asci die prior to discharge. Note shrinkage of spores and thickened septum. **FIG. 12. N. eriophori, FIG. 13. N. melatephroides.** 2000x.

FIG. 14. Non-mucilaginous sheath (perispore?) in spores of **Ciboria caucus**. The sheath bursts and finally separates due to imbibition of the living spore after discharge (a→b→c). **a.** spore inside living ascus; **b.** spore immediately after discharge; **c.** completely separated sheath; **d.** anomalous separation from the spore wall without bursting due to shrinkage of the spore after killing by MLZ. 3000x.

Other taxa have very thin sheaths of mucilage on the ascospores which become only visible in the case they are stained by CRB showing a weak or deep reddish-violet. This feature of high taxonomic value occurs, e.g., in the species of **Pezicula**, in several species of **Calycellina** (BARAL, 1989b: 212) and **Ombrophila**, in **Calycina alniella**, **Hyaloscypha aureliella**, and **Durella connivens**. **H. aureliella** is easily distinguished from **H. britannica** var. **britannica** by this reaction. The reaction seems so far unreported for the Leotiales. GRUBE & HAFELLNER (1990: 307) found a red-violet stain of the spore wall in some species of **Zwackhiomyces** using aqueous methylene blue. I found this dye to give only a weak bluing to spores reacting deep violet in CRB. CB gave always negative results due to the presence of lactophenol as did CRB + lactophenol. This type of reaction should therefore be distinguished from blue (*cyanophilous*) reactions in CB and is better termed *metachromatic* (see SINGER, 1986: 80, *metachromatism with cresyl blue*).

A delicate, scarcely mucilaginous but inelastic, unstainable sheath (perispore?) was found to occur very frequently in spores of Leotiales. This sheath bursts by separating from the true spore wall after discharge, due to release of ascus turgor and therefore increase in the spore volume (FIGs. 14, 22a, 35a). The spore thereby completely slips out of its sheath. Such sheaths appear not to have been reported in this order by other authors, probably because they are very difficult to see in herbarium material.

6. ALTERATIONS IN THE CELL CONTENTS

Fungal cells often contain more or less refractive cytoplasmic inclusions. BOUDIER (1886: 143; 1907: 28; 1914: 51) was one of the few taxonomists who have emphasized the fact that, in the living state, these inclusions show a strikingly stable and regular image which often serves as an *excellent criterion* in the taxonomy of Ascomycetes. My observations on numerous species confirm BOUDIER's statements. The delimitation among many taxa becomes decidedly facilitated if guttules inside living spores and paraphyses are used as additional features. Dead cytoplasm, however, shows highly variable patterns; its morphology depends furthermore on the mounting medium, and many types of inclusions can no longer be discerned. The variable morphology of the cell contents in different states and development stages observed in water mounts misled many researchers (e.g. KILIAS, 1981: 269) to disregard cytomorphological features and to prefer mounting media which clear the contents and kill the cells. Workers accustomed to studying dead spores sometimes believe the interior of the living spores to represent an anomaly: BENKERT (1976: 632), for example, assumed that the multiguttulate (living) spore in **Melastiza chateri** (FIG. 16f) is an anomalous state while the biguttulate (dead) spore (FIG. 16g) represents the normal case.

Refractive cell inclusions are best visible in the fully hydrated cytoplasm (in water mounts) using bright field optics. The contrast of the inclusions depends on various circumstances: it decreases with increase of the magnification used, and with higher refractive index of the cytoplasm, e.g. by natural dehydration of the spores during maturation in many Pezizales. Applying phase-contrast proved only superior with large cells, but seems useful when mounting in a plasma albumin medium which has a high refraction but a low osmosity (BARER et al., 1953).

6.a. Lipid bodies (LBs, "oil drops")

Lipid forms globose refractive bodies of about 0.2-10 μm diam. within the cytoplasm outside the vacuoles. In germinating spores the lipid usually disappears and serves as an energy and carbon reserve (STEINER, 1957: 242; SUSSMAN & DOUTHIT, 1973: 315; WEETE, 1981: 465). For a review of LBs in plant cells see GURR (1980) and WANNER et al. (1981), for fungi see HESS (1981) and WEETE (1981).

Recognition:

Recognition of lipid can be made by two tests (BARAL, 1989a): (1) 1-5% KOH does not dissolve LBs when added to living or dead cells (LBs remain visible in full strength, even after boiling); (2) staining with CRB is negative while CRB_A stains LBs within dead cells yellowish-amber to deep copper-orange.

Lipophile dyes are commonly used to give more or less specific stains for fatty matters (see KIRK, 1966: 87ff.). Most of these tests are lethal to the cells. I have tested Sudan III in lactic acid in several species: a distinct reddish stain of the LBs was rapidly obtained especially after heating the slide while CRB_A gave the characteristic amber stain. KIRK (l.c.) recommended two tests (using benzapyrene-caffeine, and neutral red) for staining LBs *in statu vivo* by fluorescence.

KORF & ERB (1972) and KORF (1977) found **Trichophaeopsis bicuspis** to differ from **Trichophaea** by ascospores with "non-oleaginous, somewhat resinous inclusions" instead of LBs since the inclusions failed to absorb oil stains such as Sudan IV. My material of **T. bicuspis** showed two polar LBs 3.5-4 μm in size. Indeed, the whole spore content remained unstained by CRB_A or Sudan III for hours, even after boiling, except for immature, and ruptured mature spores, where the LBs stained amber in CRB_A and red in Sudan III. Thus, negative result is clearly due to the impermeability of the thick wall of the mature spore to these dyes.

Formation:

LBs in ascospores either originate from minute precursors (FIGs. 15b, 16b; BARAL, 1984: fig. 7) or are already present in the cytoplasm of the ascus before the spores are formed (FIG. 42a). LBs increase during sporogenesis (without fusing) to a very different size, depending on the species (FIGs. 15, 16, 42).

Distortion:

Coalescence (fusion) of several LBs is frequently found in dead spores in a fresh ascocarp (and also in other groups of fungi with spores with high lipid contents), and is considered an anomaly caused by damage to the limiting membrane of the single LBs (HEINEMANN, 1956: 40ff.; STEINER, 1957: fig. 18; CUNNELL, 1959: 465; FREY-WYSSLING & MÜHLETHALER, 1965: 168ff.; KIRK, 1966: 70; GURR, 1980; WANNER & al., 1981). LBs may thereby lose their spherical shape by forming irregular aggregations with the surrounding cytoplasm (FIGs. 1 a→d, 2 ab→cef, 7 a→b, 16 f→g, 21 a→b, 22 a→b; HUHTINEN, 1990a: fig. 175c, H_2O→MLZ). STEINER pointed out that such state-dependent effects were the reason why cells of **Saccharomyces cerevisiae** have erroneously been thought to vary considerably in oil content (indistinctly multiguttulate *in statu vivo*, one large distinct drop *in statu emortuo*). According to KIRK (l.c.), *no fixatives entirely prevented guttular oil from spreading throughout the spores.*

On the other hand, coalescence does (? regularly) not occur if spores have died in the dried state whilst lying in the herbarium: spores inside asci exhibited undamaged guttule patterns even in about 100 years old dried material when studied in KOH.

Coalescence of the LBs can be induced within a few seconds when killing multiguttulate spores by adding ethanol, HCl (STEINER, l.c.), CB or MLZ to the water mount, or can be observed in spores in water as they die. Thereby, coalescence occurs some seconds or minutes prior to the loss of cell turgor (see FIG. 2c where the small LBs have coalesced in the living spore). The contrary process, the formation of many small drops from one large drop, though reported by researchers, was never observed, either by me or by CUNNELL (l.c.). Note, however, that multiguttulate immature spores may regularly develop into oligoguttulate mature spores, for example in the genus **Octospora**. This effect occurs without fusion of LBs: only one or few of the small LBs grow to a large size so that the remaining small ones are overlooked when viewed at a low magnification. Such a case is figured by JOHNSON (1963: pl. 33, figs. 1-6) for living asci of **Ceriosporopsis**.

A few seconds after coalescence takes place, the lipid often disappears optically (in MLZ, CB, or water): due to (1) the loss of cell turgor and therefore dehydration of the cytoplasm (increase in refraction, FIGs. 15g, 16g), (2) the fact that in shrunken spores with a high relative lipid content the lipid continuously fills the whole cell (FIGs. 2d, 5b), or (3) a high refractive index of the employed mountant which imbibes the cytoplasm around the LBs (FIGs. 8b, 12b, 13b). These effects explain why LBs in spores with rich lipid contents are frequently figured "empty" in many publications (without comment), provided that the contents were not omitted intentionally. HERTEL (1967: 15), KORF (1977), SPOONER & DENNIS (1985: 298) and SVRČEK (e.g. 1989: 72) reported guttules (certainly LBs) "disappearing" from spores after prolonged preservation in the herbarium, or when mounted in MLZ or CB. RAMSBOTTOM (1916) already drew attention to the "disappearance" of guttules in spores when mounted in glycerol: *In no case have guttulae been* *observed in the collection of Discomycete slides preserved in the National Herbarium*. The lipid reappears, however, with more or less strong contrast even in old herbarium specimens if the spores are mounted in 1-5% KOH (KORF, l.c., obtained this effect using KOH-phloxine-glycerol). A varying refractivity of the cytoplasm is also the reason why DODGE (1957) reported the LBs to "disappear" reversibly during DBB-formation (FIG. 11 g→h).

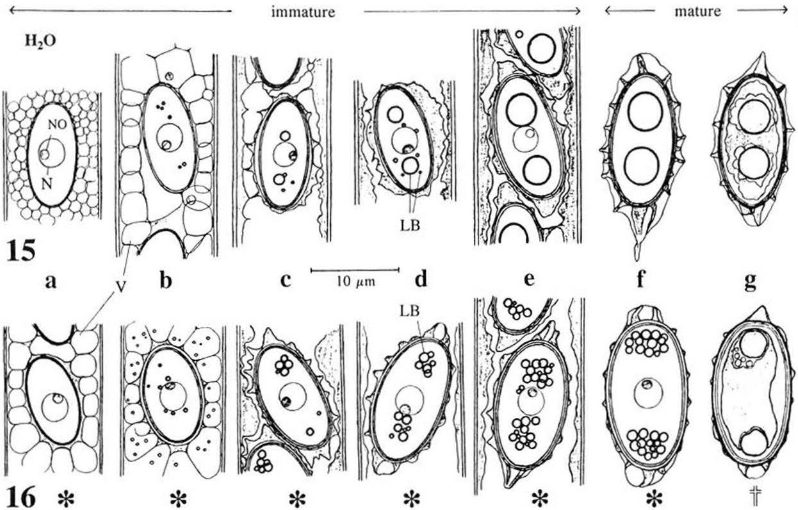
When living spores pass into the germination phase, the lipid is actually broken down and used for energy production and synthesis of new cellular material (DE BARY, 1887: 113; GARRAWAY & EVANS, 1984: 227). It is therefore very important for the purpose of taxonomy to use only mature spores for the study of spore guttulation (see chapter 8).

Taxonomic value:

BOUDIER (1907: 28; 1914) drew attention to the taxonomic importance of LBs in ascospores and regretted the fact that workers have often ignored the guttules in their descriptions. LE GAL (1947) gave a survey of the various types of guttular patterns in the spores of Pezizales. HERTEL (1967: 15) emphasized the taxonomic use of guttules in spores of Lecanorales. KARSTEN (1871) already noted spore guttulation in many species. Many other authors included this feature in their descriptions but few took influences of their methods into account. *The importance of fresh material for species diagnoses, especially for noting ascospore guttulation, cannot be overstated* (HARRINGTON, 1990: 436). Since lipid serves as a nutritive substance, differences among related taxa in the amount of lipid within mature spores seem to reflect differences in ecological adaption in regard to spore germination.

In mature ascospores the total amount of lipid often severely varies between closely related species (**Calycellina**, FIGs. 19-20, **Lasiobelonium**, FIGs. 17-18). Similarly, **Morchella**, **Cheilymenia**, **Tricharina** and **Peziza** p.p. ("**Aleuria**") differ from **Helvella**, **Scutellinia**, **Trichophaea** and **Peziza** p.p. ("**Galactinia**") (BOUDIER, 1885: 101, 104, 105; 1914: 54; KORF, 1977; BARAL, ined.), or **Ciboria** from **Rutstroemia** (BARAL & KRIEGLSTEINER, 1985: 10, 19) in the absence versus abundant presence of LBs in the spores. HUHTINEN (1990a) considered **Calycellina lutea** a possible synonym of **C. lachnobrachya** (as "**Phialina**"). The spores of **C. lutea** are misleadingly depicted eguttulate in the original description. Yet, these contain many LBs in contrast to **C. lachnobrachya** with a low lipid content (FIGs. 19-20). Likewise, **Lasiobelonium variegatum** and **L. corticale** have often been confused because, besides other features, the striking difference in the lipid content was overlooked (FIGs. 17-18). Numerous other such "critical" species can be separated on the basis of the quantities of lipid.

CHADEFAUD (1969: 195) characterized different orders of pyrenomycetes by the lipid content in ascospores (*type «pauvre»* versus *type «riche»*). WEETE (1981: 464) stated that the *lipid content of spores of most fungi generally ranges between*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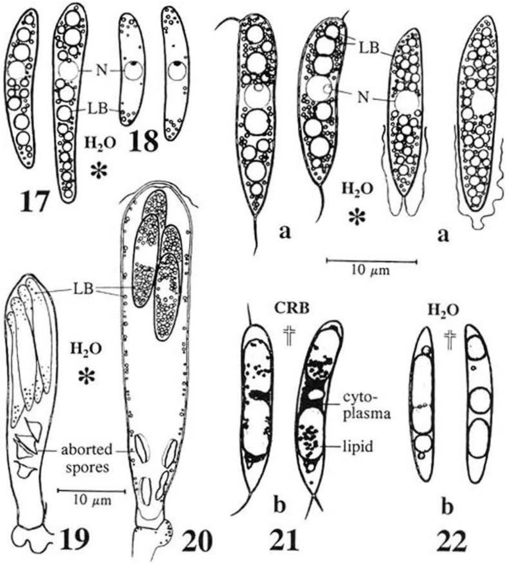
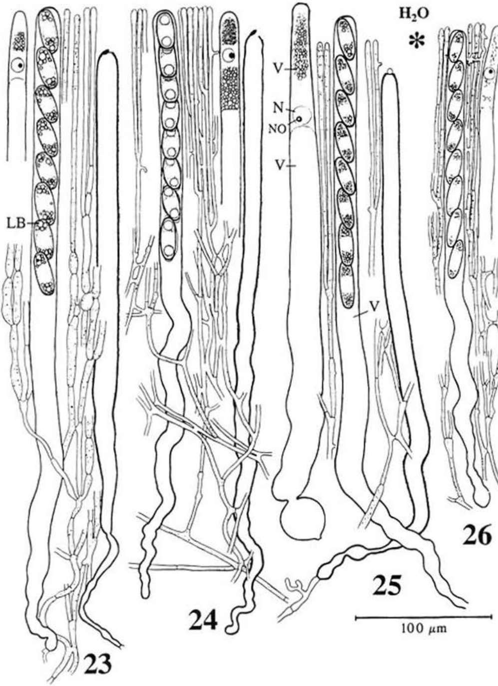


FIGs. 15-22. Four taxon pairs, easily distinguished in the living state by the lipid content of the mature spores (high versus low content: FIGs. 17-20; few large versus many small LBs: FIGs. 15-16, 21-22). Note that (1) coalescence of LBs obscures the distinctive features (FIGs. 15/16 f→g; 21/22 a→b), (2) LBs are absent in the first stage of ascosporogenesis in certain taxa (FIGs. 15a, 16a), but present in others (FIG. 42 abc). **FIG. 15. Aleuria aurantia, FIG. 16. Melastiza chateri** (1500x); **FIG. 17. Lasiobelonium corticale, FIG. 18. L. variegatum** (2000x); **FIG. 19. Calycellina lachnobrachya, FIG. 20. C. lutea** (1500x); **FIG. 21. Hymenoscyphus scutula, FIG. 22. H. consobrinus** (2000x, the dead spores were found in the squash mount together with the living spores). (FIGs. 19-20 from BARAL 1989b).



FIGs. 23-26. The four European species of **Sarcoscypha** distinguished, besides other features, by the size of the lipid bodies in the ascospores (from BARAL, 1984: fig. 4, this figure was unintentionally issued only with its upper half, and is here reproduced in its full extent). **FIG. 23. S. austriaca** (neotype), **FIG. 24. S. jurana**, **FIG. 25. S. coccinea** (neotype), **FIG. 26. S. macaronesica** (holotype). (300x).

5 and 17% of their dry weight, but spores of some species such as rusts may contain up to 35%. I have estimated the total volume of lipid in relation to the volume of the living hydrated spore by the area fraction of the LBs in optical section which is proportional to their volume fraction (WEIBEL et al., 1966). I use the following linear scale: 0 = devoid of lipid; 5 = maximum lipid content. In the ascospores of 815 tested species of Leotiales, these categories were represented in quite equal frequencies, with a slight maximum towards the lower contents: 0-1: 21%; 1-2: 25%; 2-3: 18%; 3-4: 16%; 4-5: 20%. Usually, there is little variation within a species if only living mature ascospores are taken into account.

Differences in the lipid content of asci prior to spore formation are outstanding between certain taxa although, in these case, the spores are finally always rich in lipid: asci of **Pezicula** (FIG. 42) and **Pachyella babingtonii**, for example, have high contents in the stage around meiosis while those of **Sarcoscypha** (FIGs 23-26), **Otidea** and many studied Humariaceae (FIGs. 15a, 16a) have very low contents.

The size of the single LBs in spores (few large versus many small LBs) is a further, usually consistent feature which supports delimitation among many species (FIGs. 15f-16f, 21a-22a). In **Sarcoscypha** (FIGs. 23-26), the size of the larger LBs allows the delimitation among species (BARAL, 1984). Living spores have been studied from 26 collections of **S. jurana**, 30 of **S. austriaca**, 14 of **S. coccinea**, and 4 of **S. macaronesica**. The features were consistent: The first species showed the largest LBs and occurred only on **Tilia**, the second had medium-sized LBs and produced conidia on the ascospores, the two latter differed by small LBs and obtuse spores.

According to DENNIS (1978: 400), Dothioraceae and Dothideaceae usually differ in multiguttulate versus uni- or biguttulate cells of ascospores. HEINEMANN (1956: 41ff.) found **Saccharomyces cerevisiae** to differ *in statu vivo* by cells with many LBs from other Endomycetales having cells with one large and a few small LBs. The Helvellaceae (**Discina**, **Helvella**, **Rhizina**) are characterized by spores with usually one large central LB with some accessory LBs while **Gyromitra** (Morchellaceaea) is strictly biguttulate (BENEDIX, 1966: 360, figs. 1, 2, 4, 5; BARAL, ined.). HERTEL (1967: 15, pl. 15) used different amounts and patterns of lipid in living ascospores of **Lecidea** as a character on the specific level. In the genera **Lecanora** and **Diaporthe** I observed species with multiguttulate spores (FIG. 9) and other species differing by strictly biguttulate spores.

6.b. Refractive vacuolar bodies (VBs)

Normal living fungal vacuoles are totally non-refractive and can be detected under the LM as transparent ("empty") regions within the cytoplasm (FIGs. 27, 28, 31, *V*). CRB or CRB_A gives a homogeneous violet stain to the vacuoles (typical for the single large vacuole in mature asci), or mostly induces flocculation of blue-violet MCs in the vacuole sap (FIGs. 4, 30). A phenomenon characteristic of a major part of the Leotiales (but absent in the other part) is, however, the presence of a specialized type of vacuole side by side with the normal type (FIGs. 27-29, 32-33, *VB*; HUHTINEN, 1990a: fig. 255c, H_2O). This special type contains a colloidal substance of a more or less high refraction within the tonoplast, with the vacuoles appearing "full". Here, CRB never induces the formation of MCs but rather stains the bodies in a homogeneous turquoise-blue. GUILLERMOND (1941: 161, 181ff.) and BANCHER & HÖFLER (1959: 152) described similar vacuoles occurring in vascular plants as opposed to normal vacuoles: highly refractive, more acid, staining *in statu vivo* blue or green by CRB, reducing osmic acid, rich in phenolic compounds (tannin).

Such *refractive vacuolar bodies* (*VBs*), as I term this type of inclusion, occur predominantly towards the surface of the ascocarp: in the top cells of paraphyses, outer excipular cells, or basal part of hairs (FIG. 32). BELLEMÈRE (1958) described them in **Cyathicula coronata** as *granulations réfringentes, brunâtres après coloration par la réaction de A. Prenant* in paraphyses and cortical hyphae. Due to their refraction they have mostly been misinterpreted as lipid bodies (see BARAL, 1989a: 120; 1989b: 225). In certain genera, IKI or MLZ give a reddish-brown reaction to VBs (see BARAL, 1987a: 424; SVRČEK, 1989: 73; HUHTINEN, 1990: 71, as *golden*). The IKI reaction is stable while the MLZ reaction disappears within about 1/2 min. In many mollisiaceous fungi, KOH provokes a deep sulphur-yellow reaction with the VBs (see chapter 7). Hydrophilous (mainly yellow) pigments sometimes occur in VBs (FIG. 32). On the other hand, oxidative color changes to yellow or reddish-brown often occur when cells are injured (this supports the idea that phenolic compounds are involved). Therefore, what HUHTINEN (1990a: 71) described as *yellow pigment* mainly from herbarium specimens and found to be diagnostic for **Calycellina** (as "**Phialina**") is just the same as what I term *VB*. I have found VBs also in vegetative surface cells of Basidiomycetes (**Clavariadelphus**, **Ramaria**). The greenish-blue (turquoise) color of VBs, obtained by staining *in statu vivo* with CRB or toluidine blue, is a purely metachromatic effect and does not indicate a more acid pH because, in the case of toluidine blue, changes in the pH do not affect the color of the dye. According to HARMS (1965, II: 19), basic dyes do not permit a clear evaluation of the pH inside the vacuole.

Recognition:

Delimitation from LBs can be made by two tests: (1) VBs are dissolved instantaneously (complete optical disappearance) by 1-5% KOH (but not by even strong acids) when added to living cells (FIG. 33 a→b, c→d); (2) staining *in statu vivo* with CRB or CRB_A always gives a strong pure turquoise-blue (metachromatic) color to hyaline VBs (BARAL 1989a: 121) within a few minutes.

Numerous taxa have been tested, but only a single exception was found: in species of **Symphyosirinia**, the large conidia of the anamorph are completely filled with strongly refractive VBs which stain deep turquoise in CRB but are not dissolved when killed by a strong KOH-treatment.

Formation:

The refractive substance becomes apparent inside young small vacuoles, e.g. at the tip of paraphyses, as a colloidal solution. During development VBs increase in size. Two main types can be distinguished: (1) multiguttulate type (FIGs. 27, 33): many small vacuoles are formed at the beginning; these later grow in size while the substance sooner or later precipitates within the single vacuole by forming many small globose VBs which show Brownian movement in the transparent vacuole sap (lower part of paraphyses); (2) elongate type (FIGs. 28, 29, 32): few large vacuoles are formed in which the substance remains colloidal in the living state. Intermediate types also occur representing type (1) but with VBs remaining permanently colloidal in the living state.

Due to the mentioned ontogenetic changes in size and shape of the VBs, there is often a slight variation from young to mature apothecia. The feature is, however, highly stable in most taxa if only adult living cells are considered.

Distortion:

Lethal mountants (MLZ, CB, KOH) destroy VBs resulting in their complete invisibility (FIG. 33; see HUHTINEN, 1990a: fig. 255, H_2O vs. MLZ). KOH probably provokes hydrolysis of the refractive molecules. VBs may also disappear within less than 1 sec. if the cells die during examination of a water mount, possibly due to a raised pH when the tonoplast bursts. Herbarium material often lacks any remnants of VBs or shows irregular bodies with often altered pigmentation which are then KOH-insoluble (FIG. 1f; HUHTINEN, 1990a: figs. 212-261, illustrated as black intracellular spots).

Taxonomic value:

VBs in paraphyses and in cortical cells or hairs are of importance in the following examples: **Cyathicula** is easily distinguished by strongly refractive VBs of the multiguttulate type (FIG. 27) and blue reacting apical rings of the **Hymenoscyphus**- or **Bulgaria**-type from **Allophylaria**, which has elongate VBs (FIG. 28) and red reacting apical rings of the **Laetinaevia**-type (BARAL & KRIEGLSTEINER, 1985: 108; BARAL 1987b). Variation in the type of VBs was only seen in 2 of the 13 studied species of **Allophylaria** (showing a tendency to multiguttulation), and in 1 of the 20 studied species of **Cyathicula** (showing a weak refraction). Due to lack of fresh collections there is still no information available about the paraphysis content in the type species of **Crocicreas** which would help in clarifying its relation to **Cyathicula** which was placed in synonymy of **Crocicreas** by CARPENTER (1981). Whilst monographing this large genus, CARPENTER almost completely ignored VBs (but several reports of yellowish contents and a darker stain of the paraphysis plasma by CB or MLZ give indirect indication of their presence). He therefore classified a typical **Allophylaria**, **A. subhyalina**, in **Crocicreas**.

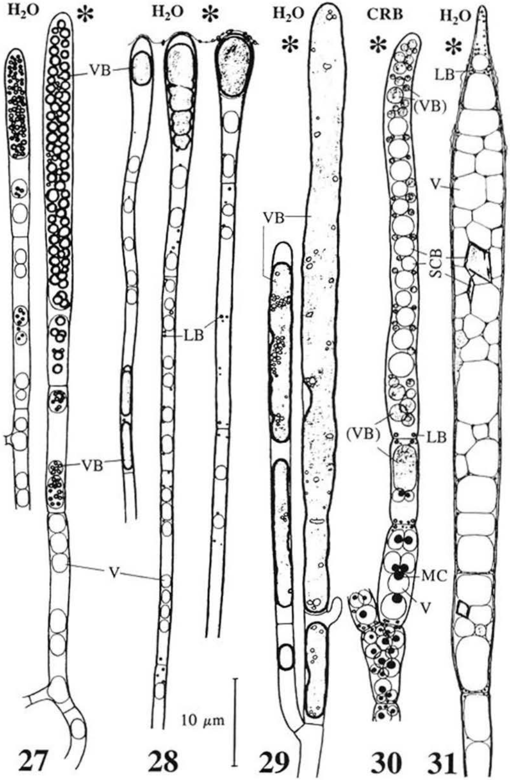
A similar case probably requires a generic split within the genus **Bisporella**: **B. pallescens** and **B. subpallida** have multiguttulate VBs and apical rings of the **Hymenoscyphus**-type, while **"B." citrina**, **"B." lactea**, **"B." sulfurina** and **"B." scolochloae** have elongate VBs (the two former species have apical rings of the **Laetinaevia**-type, the two latter are IKI-).

Mollisia/Tapesia (FIG. 29) and **Calycellina/Phialina** (FIG. 32) are characterized by highly refractive, more or less elongate VBs while **Pyrenopeziza/ Pirottaea** (FIG. 30) and **Hyaloscypha** show globose VBs of very low or vacuoles lacking refraction (BOUDIER, 1885: 119; BARAL & KRIEGLSTEINER, 1985: 35; BARAL, 1989b; HUHTINEN, 1990a: 71). The observed variation was as follows: in **Mollisia** s.l., 44 studied species showed medium to strong refraction while 10 showed low or lacked refraction; in **Pyrenopeziza** s.l., all 27 species showed low or lacking refraction. All 30 studied species of **Calycellina** s.l. had medium to strong refraction but 12 out of 13 studied species of **Hyaloscypha** showed no refraction while only 1 (**H. secalina** var. **paludicola**) differed in having strongly refractive VBs. Refractive VBs of the multiguttulate type are typical in the "**Hysteropezizella**-complex".

In **Lachnum** s.str., 18 out of 46 studied species showed multiguttulate VBs (FIG. 33) whilst 26 lacked VBs (2 are variable), and it is the VBs which are responsible for the reddish color change of the white apothecia (BARAL & KRIEGLSTEINER, 1985: 73). The apices of **Botryotinia** paraphyses (5 studied species) contain ochraceous refractive VBs which are absent in **Sclerotinia** (3 species) and **Myriosclerotinia** (2 species). This seems an unpublished feature which supports the justification of the genus **Botryotinia**, recently put back in synonymy of **Sclerotinia** by SPOONER (1987: 202ff.), who regarded the difference in conidial states as no more than of subgeneric value. **Discina ancilis** and **D. gigas** show ochraceous, strongly refractive VBs of the multiguttulate type (FIG. 40a) which **Gyromitra esculenta** lacks.

Distinct VBs rarely occur in spores. They are characteristic, however, of ascospores of certain taxa, a hitherto overlooked phenomenon, e.g., of most species of **Orbilia** (FIG. 37, showing a highly characteristic shape), of **Hymenoscyphus** cf. **sazavae** (FIG. 35a), and of **Tubeufia paludosa** (FIG. 34c). Vacuoles of low refraction often occur in ascospores of Leotiales (FIG. 36; CHADEFAUD, 1969: 191, figs. 8a, 9b, 10b).

FIGs. 27-31. Five species differing by the contents in their paraphyses (refractive versus non-refractive, multiguttulate versus elongate, restricted to the tip of paraphysis versus reaching down towards the base). Note difference in refraction between non-refractive vacuoles (*V*) and strongly refractive vacuolar bodies (*VB*) (in FIG. 30 the VBs have a very low refraction). The metachromatic corpuscles in FIG. 30 are produced through staining *in statu vivo* with CRB, all other structures are present without any treatment. **FIG. 27. "Conchatium" fraxinophilum** (= **Cyathicula fraxinicola**). **FIG. 28. Allophylaria nervicola**. **FIG. 29. Mollisia** spec., **FIG. 30. Pyrenopeziza petiolaris**, **FIG. 31. Brunnipila clandestina**. 2000x.


H2O
*
VB
VB
V
H2O
*
VB
LB
V
10 μm
H2O
*
VB
CRB
*
(VB)
SCB
(VB)
LB
MC
V
H2O
*
LB
V
SCB
27
28
29
30
31

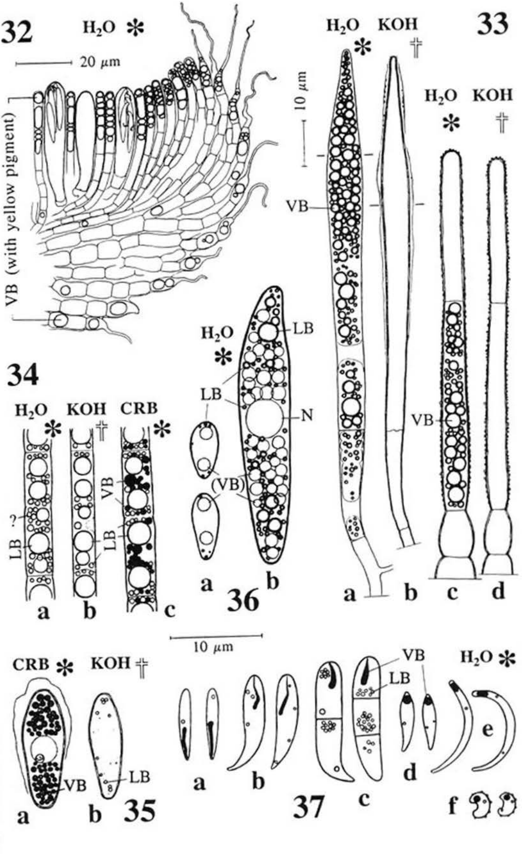
6.c. KOH-soluble cytoplasmic bodies (SCBs)

Vegetative cells of Leotiales may contain globose (FIG. 30) or crystalline (FIG. 31) bodies (here termed *SCBs = soluble cytoplasmic bodies*) of low to high refraction which dissolve in KOH but do not stain with CRB in the living state while adjacent vacuoles are deeply stained. SCBs are localized in the cytoplasm outside the vacuoles. With this set of characters, SCBs resemble WBs but differ in being not associated with septal pores. The contrast of the bodies increases upon staining *in statu vivo* with IKI. Their nature remains unclear; literature reports for Ascomycetes have not been found. GUILLERMOND (1941: 206, fig. 144) described similar crystalloid proteinaceous bodies in cells of lower fungi. Globose SCBs are characteristic of paraphyses of **Pyrenopeziza** (FIG. 30) including "**Pirottaea**", crystalline SCBs occur in paraphyses of **Brunnipila** (FIG. 31) and **Trichopeziza. Hyaloscypha albohyalina** var. **albohyalina** (or **H. vitreola**?) is distinguished by the presence of refractive globose to elongate SCBs in paraphyses and excipular cells from **H. albohyalina** var. **spiralis** where these bodies are absent.

6.d. Woronin bodies (WBs)

Most families of Pezizales are characterized by refractive WBs in the cytoplasm a short distance from the septal pores within the vegetative cells, about 3 to 12 per septum, e.g. two on each side (FIGs. 38-41). Their size is about 0.3-0.8(2.5) μm, their shape either globose or crystalline, and their composition proteinaceous

FIG. 32. Section through the margin of **Calycellina ulmariae** (from BARAL, 1989b). Note that the deep yellow VBs occur preferably at the surface of the apothecium. 600x.
FIG. 33. Paraphysis and hair of **Lachnum controversum**. **a.**, **c.** living state, **b.**, **d.** the same cells a few seconds after killing by KOH. KOH dissolves the VBs instantaneously. Note shrinkage of cells especially in width. The horizontal lines on both sides of the widest part of the paraphysis mark the level of the apex of the mature asci. 1500x.
FIGs. 34-37. VBs in ascospores. Note that LBs and VBs in these examples (except FIG. 36) have the same refraction and color, and partly even the same shape. Yet, CRB stains VBs blue but LBs not (FIG. 34c), and KOH dissolves the VBs instantaneously while the LBs persist (FIG. 34b, 35b). Note shrinkage of spores in KOH. **FIG. 34. Tubeufia paludosa**, **FIG. 35. Hymenoscyphus** cf. **sazavae** (= **Helotium sulphuratum** ss. BARAL & KRIEGLSTEINER 1985: 137), **FIG. 36 a. Cistella deflexa**, **b. Phaeohelotium geogenum**, **FIG. 37 a. Orbilia sarraziniana**, **b. Orbilia** spec., **c. O. septispora**, **d. O.** cf. **rosella**, **e. O. auricolor** (= **O. curvatispora**), **f. O. delicatula** (= **O. xanthostigma** s.auct.). 2000x. (FIG. 37c from BARAL, 1989a). FIGs. 34 and 35 show the same spore in water (a), and after KOH has been added (b).


32
H2O *
20 µm
VB (with yellow pigment)
33
H2O
KOH
10 µm
VB
H2O
KOH
VB
a
b
c
d
34
H2O
KOH
CRB
?
LB
VB
LB
a
b
c
H2O
LB
LB
N
(VB)
a
36
b
10 µm
CRB
KOH
VB
LB
a
b
35
VB
LB
H2O
a
b
37
c
d
e
f

(KIMBROUGH, 1991: 425). I have also seen WBs in species of the Sclerotiniaceae. WBs were thought to function as a pore plugging mechanism, sealing off living from dead cells (KIMBROUGH & CURRY, 1986) but KIMBROUGH (1991: 425) now considers non-proteinaceous structures to be more important in septal plugging. Like SCBs, WBs dissolve in 1-5% KOH (but not in nitric acid) and are not stained with CRB. They have often been observed with the LM (e.g. by CORNER, 1929: 271), but can only be seen in living cells.

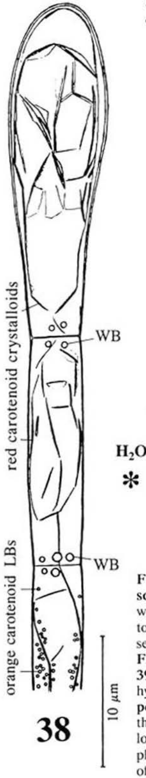
WBs have been used in the systematics of Ascomycetes (e.g. by KIMBROUGH & CURRY, 1986). A special type of WB characterizes **Morchella**, **Verpa**, **Disciotis**, **Gyromitra** (Morchellaceae), and **Discina** (Helvellaceae) (FIGs. 40, 41) but is absent in **Helvella**: several very thin flat crystals of a regular hexagonal outline measuring about 1-2.5/0.2-0.3 μm lie close to the pore. KIMBROUGH (1990; 1991: 422) misleadingly described the WBs of this type as "extremely elongate, rectangular" because, in TEM-sections oriented vertically to the septum, they appear in cross-section as rod-shaped structures. Thicker crystalline hexagonal WBs, however, have been found with the TEM in several non-morchellaceous genera (FIG. 39b; KIMBROUGH 1991: 425).

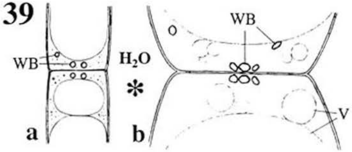
6.e. Pigments

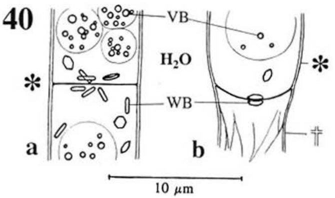
The color of living hymenia may originate from *ectochroic* (= extracellular), *mesochroic* (= cell wall), or *endochroic* (= intracellular) pigments. The first two appear to be typical for long-living discomycetes and are usually unaltered in herbarium specimens. Note, however, that taxonomic problems arise in regard to spore wall pigmentation (see chapter 8). The endochroic pigments are more or less state-dependent and therefore here of special interest: the hymenial color may originate from *cystochroic* (water-soluble, within vacuoles) and/or from *lipochroic* pigments (lipid-soluble, within LBs).

1. Water-soluble pigments (yellow, orange, greenish, bluish or brownish) occur within the VBs of the intact vacuoles of the paraphyses. These pigments may disappear instantaneously (LM, tap water) when cells die during examination in water mounts. On the other hand, a color change of hyaline VBs to yellow, reddish or brownish is often observed and results in deeply colored apothecia which have originally been white. Therefore, HUHTINEN (1990a: 71) describes as *yellow pigment* (often in the dead state) what I differentiate into *hyaline VBs versus yellow VBs* (living state). He consequently merges **Calycellina lachnobrachya** and **C. araneocincta** which have both more or less yellow VBs in the dead state, but differ in the living state (hyaline versus deep yellow VBs; BARAL, 1989b), and occurrence on different hosts. HUHTINEN (l.c.) found, however, that **Hamatocanthoscypha uncipila** is characterized by a yellowish pigment which is *seen clearly only when fresh material is studied in water.*

2. Lipid-soluble yellow to red pigments (carotenoids) occur within globose LBs. These are located in the cytoplasm between the vacuoles, especially in the







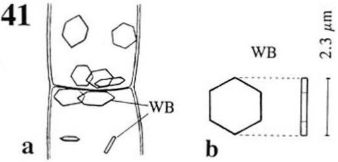


FIG. 38. Pigments and WBs in paraphysis of **Scutellinia scutellata**. Carotenoids form elongate red crystalloids towards the apex but occur also within minute orange LBs towards the base. Globose hyaline WBs occur close to the septa. 3000x.

FIGs. 39-41. WBs close to septa of vegetative cells. **FIG. 39. Peziza** spec., **a.** paraphysis, WBs globose, **b.** medullary hyphae, WBs crystalline. **FIG. 40. Discina ancilis** (= **D. perlata**), **a.** paraphysis, WBs form thin hexagonal crystals, **b.** the pore is plugged by the WBs due to the death of the lower cell. **FIG. 41. Verpa digitaliformis**, medullary hypha, WBs forming very large thin hexagonal crystals (not all of the hyphae contain such large WBs). **a.** 3000x, **b.** 6000x.

paraphyses and in the subhymenium. Yet, in some genera the carotenoids form elongate crystalloid structures, possibly due to a high concentration (FIG. 38; HEIM, 1947; ARPIN, 1968: 430). These crystalloids are inert to KOH. Carotenoids tend to completely lose color in dried specimens (ERB, 1972: 10); consequently, the crystalloids disappear while the LBs are stable except for their pigmentation. Presence or absence of carotenoids in fresh specimens of Pezizales is considered a character of high significance (ERB, l.c.). In my experience, **Scutellinia** (FIG. 38) can be distinguished from **Cheilymenia** in most species by the presence of carotenoid crystalloids in the uppermost 2-4 cells of the paraphyses (LBs which contain carotenoids occur in both genera).

In the living fungal cell, the cytoplasm outside vacuoles, is generally (? always) without pigmentation, except for LBs and carotenoid crystals. Yet, in dead cells of species with pigmented exudates one often observes a coloration of the cytoplasm (reddish, olivaceous, bluish, brown, yellow etc.) similar to the exudates. Obviously, a colorless precursor molecule occurs in the living cytoplasm of these species which becomes colored either outside the cell wall by active exudation, or inside the cells as they die. This situation leads to differing reports of cytoplasmic color depending upon the living versus dead state of the cells.

6.f. Nuclei (N)

Nuclei can often be discerned in living unstained cells by their nucleoli (NO) and nuclear membrane (FIGs. 1a, 4, 12-18, 21-26, 35, 42-43) allowing a rapid evaluation of cell nucleation. For example, species of Sclerotiniaceae differ in the number of nuclei per ascospore. Staining *in statu vivo* with IKI usually strongly enhances contrast of the nuclei (CORNER, 1929: 264) whilst CRB_A, applied to living cells, often stains the nucleolus pale blue. Nuclei are indiscernible in dead, unstained cells and shrink considerably as they die (FIG. 12 a→b). CB often stains them a darker blue than the cytoplasm (FIG. 12b).

6.g. Tracti and "nasse apicale"

A tractus to which the spores are attached occurs in several groups (BARAL, 1987b: fig. 17; 1989b: pl. 3, D), e.g. in **Lecanora** (FIG. 9a, perhaps the first report in this genus). A *nasse apicale* was observed in asci of several species of both fissitunicate and unitunicate Ascomycetes (CHADEFAUD, 1942; BARAL, 1987b: figs. 17, 25), e.g. in **Tubeufia** as a quadripartite structure (FIG. 7). The contrast is enhanced by staining *in statu vivo* with IKI, or it is stained greyish by chlorazol black. Tracti are masked in dead asci by the cytoplasm (LM, FIG. 9b), but have been reported in TEM-investigations. The existence of the *nasse apicale* is doubted, e.g., by REYNOLDS (1971) who interpreted CHADEFAUD's structure as striae on the inner surface of the endotunica resulting from the banded pattern of the microfibrils. My observations in both Leotiales and Dothideales, however, clearly indicate that the *nasse apicale* is a cytoplasmic structure able to detach from the endotunica.

6.h. De Bary bubbles (DBBs)

Living cells with non-elastic walls compensate for water loss by (FIG. 11 c-h) wrinkling, by collapsing or, in cells with rigid thick walls, by forming DBBs, a gas of presumably water vapour (INGOLD 1956). DBBs are consistently absent in water mounts of living hydrated spores and soon disappear if dry living spores are rehydrated: dry spores of **Hypoxylon serpens**, collected recently and 15 years ago, when placed in water lose DBBs within about 0.5-2 min. Then, DBBs are rapidly induced anew within about 10-15 sec by adding CB, MLZ etc. to the water mount (see also DODGE, 1957). DBBs are also seen by the common practice of mounting fresh or dried ascocarp fragments directly in CB or MLZ. On boiling MLZ slides of **H. serpens** several times, DBBs do not disappear. They disappear only when the MLZ is removed by water. Likewise, HUHTINEN (1983) found DBBs in various genera to be present in MLZ or heated lactophenol but to disappear by gentle heating of a water, Congo red, or KOH mount.

Yet, after having brought an MLZ or a water mount of **H. serpens** to boiling, it proved impossible to induce DBBs anew by adding MLZ (the heated MLZ must be replaced with water until the DBBs have disappeared), which indicates that heating has killed the spores. Collapsing in MLZ occurred in not fully mature spores of this species, including both living and dead spores. This clearly indicates that, in this species, the spore wall is impermeable to the chloral hydrate of MLZ.

DBBs have been introduced as a taxonomic feature by workers who are used to study herbarium material or to mount in CB, MLZ etc. Those, however, who usually mount fresh ascocarps in water wonder why they never see DBBs. From the above, the formation of DBBs within some seconds by adding MLZ or the like to a water mount can serve as a test for vitality.

7. ALTERATIONS IN THE CHEMICAL REACTIONS

7.a. KOH-reaction of VBs

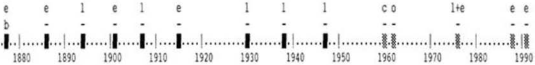
A sulphur-yellow reaction of hyaline VBs to KOH characterizes many mollisiaceous fungi, e.g., **Nimbomollisia eriophori, N. melatephroides, Mollisia phalaridis, Tapesia rosae, T. prunicola, T. fusca, T. hydrophila, T. retincola, Obtectodiscus aquaticus.** This reaction has been observed in 22 out of the 45 species with VBs so far studied, with some showing variations. SVRČEK's (1986) description of **Mollisia alcalireagens** is mainly based on the yellow KOH reaction, which he thought to be exceptional in this "new species", and thus needs critical revision. The reaction in **Obtectodiscus** supports the idea that this genus is close to the Dermateaceae as was supposed by SCHEUER (1988: 128).

The following method is used: KOH is added to the water mount. The yellow color instantaneously appears around the ascocarp as soon as the KOH reaches the paraphyses, but is rapidly diluted in the medium and soon becomes invisible. The KOH concentration may vary from 2% to 10% while 0.5-1% is much less efficient. The reaction is often absent in senescent ascocarps but was still present in full strength in 0.5 and 4 years old **M. phalaridis** and in 14 years old **T. rosae**. Perhaps, old material fails to react or gives inconsistent results since NANNFELDT (1986: 197) wondered why he could not obtain the reaction in any species. Yet, DENNIS (1950: 182) observed a sulphur-colored KOH reaction in the type material of **M. junciseda** collected in 1868. It is therefore surprising that this reaction has very rarely been reported although KOH is in current use for mounting dried fungi, perhaps because the yellow color had disappeared before the KOH-mount was studied. **Scutomollisia russea** differs in having deep orange VBs which reversibly turn deep violet by KOH.

7.b. Hemiamyloidity

The red (hemiamyloid) reaction of certain wall layers of lichen asci observed in IKI but not in MLZ (BARAL, 1987a; COMMON, 1991: 96) is converted to a uniformly blue reaction not only when KOH-pretreated, but also by preservation in the herbarium for at least about one century (KILIAS, 1981: 256, 410; RAMBOLD, 1989: 37). Conversion from types RR and RB (hemiamyloid) to BB (euamyloid) means that the different types of hemiamyloidity which serve as features of high taxonomic value are irreversibly lost in old herbarium specimens. This conversion is not related to the living versus dead state of the asci. It can now also be reported for the apical rings of the Leotiales:

Tests were made in the summer of 1991 on the genera **Pezicula** and **Ocellaria** (Leotiales), which are defined by apical rings reacting hemiamyloid (type RR). Three species of **Pezicula** and one of **Ocellaria** were studied. 15 collections made between 1877 and 1991 were examined, 11 of which (1877-1963) were received from the Staatsherbarium Munich (M): IKI without KOH-pretreatment gave a red reaction (type RR) in 0-31 years old material, while 44-114 years old material consistently showed a blue reaction which only sometimes turned grey to reddish-grey at a high IKI-concentration. The material in M was repeatedly treated in a freezer (-20°C for 2 h) since about 15 years and poisoned prior to this date (D. TRIEBEL, pers. comm.). This valuable generic feature of **Pezicula** therefore disappears in herbarium specimens much earlier than observations on lichens indicate. The MLZ-reaction without pretreatment was, however, still negative in all collections except for the one from 1877 which reacted MLZ+ pale blue. This situation (blue in 1% IKI but MLZ-) corresponds to that obtained by a weak KOH-pretreatment (BARAL, 1987a: 421, tab. 4) and offers a certain opportunity to recognize hemiamyloidity in old material.



TAB. 2. Iodine reaction (without KOH-pretreatment) of apical rings in 15 collections of **Peziculoideae**, depending on the age of the herbarium material. ▒ = IKI-red, ▌ = IKI-blue. 1st line: l = **P. livida** (asci 8-spored), e = **P. eucrita** (here understood as a 4-spored species), c = **P. cinnamomea**, o = **Ocellaria ocellata**; 2nd line: b = MLZ-pale blue, - = MLZ-negative.

8. ALTERATIONS DEPENDING UPON THE DEVELOPMENT STAGE OF THE CELLS

The development stage of fungal cells can be determined more precisely in the living state. This is of special importance with ascospore characters: number of septa, wall pigmentation, ornamentation, size, and lipid content often markedly differ when comparing the stages of immaturity, maturity, first and second phase of germination. The first phase of germination (GARRAWAY & EVANS, 1984: 221) differs from the stage of maturity by morphological changes, and may either lead directly to the protrusion of a germ tube (second phase of germination), or may persist for a long period of time (dormancy; such spores often show a light brown wall pigmentation though having been hyaline at maturity).

In vital taxonomy, maturity of ascospores can be understood in a very narrow sense: I term ascospores *mature* when actively discharged by the internal pressure of the living ascus, either in a humid atmosphere, or in a water mount during microscopic examination, without applying external pressure. A few hours after natural discharge, however, the spores may already have passed into the first phase of germination, provided that the water mount was protected from evaporation.

BOUDIER (1885: 94) emphasized the fact that spores are often less variable than usually believed when examined in the mature state, i.e. *sorties naturellement de la thèque*. Spore diagnoses should therefore consequently be based on mature spores which have been naturally liberated by explosion of the asci in water mounts. Spore characters in the phases of germination should be evaluated separately and kept apart from those of the mature spore as was done, e.g., by WOLLENWEBER (1939).

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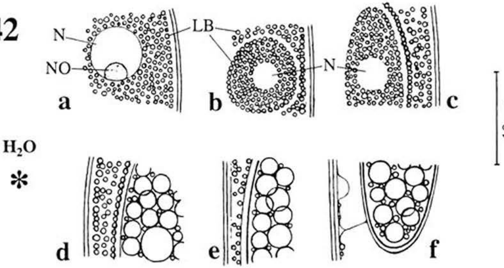

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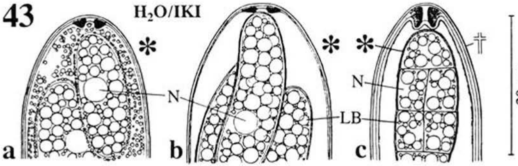

FIGs. 42-43. Ascosporogenesis in **Pezicula**. Note changes in spore width and septation (FIG. 43 b→c), and in lipid content. Living asci are required in order to determine the development stage of spores. Septate spores never occur inside living asci of **Pezicula**. **FIG. 42. P. cinnamomea**, **a.** fusion nucleus, **b.-e.** spore formation, **f.** mature spore; **FIG. 43. P. livida**, **a.** immature spore, **b.** mature spore, **c.** spore in the first phase of germination. FIG. 43 ab from BARAL (1987b). 2000x.

Ascospores are often 1-celled when mature but 2- to multi-celled in the first phase of germination. Thus, in certain species, a collection of young mature apothecia shows consistently 1-celled spores both within and outside asci while a collection of senescent apothecia shows predominantly multi-celled spores. Other taxa, however, show 2- or multi-celled spores already in the stage of maturity. It is therefore unwise to consider spore septation a variable feature with little reliability, as is often done. Usually, there is little variability if we carefully separate the different development stages of ascospores. This is only possible if we study living asci and ascospores.

The species of **Pezicula** discharge their spores consistently in the 1-celled stage while muriform spores occur only in the germination phase (FIG. 43). Likewise, **Velutarina rufoolivacea** discharges consistently hyaline spores. These may later become brown (delayed pigmentation), either outside asci or within dead asci but never within living asci. Other taxa, however, have septate, or brown spores inside mature living asci.

A further example is the phenomenon of ascosporal phialidic conidia born directly on ascospores: The term *ascoconidia* has often been misapplied to conidia produced inside dead asci by the budding of ascospores; it should be restricted to conidia which are produced from ascospores in a premature stage of development of the ascus (true secondary polyspory). Ascoconidia are actively discharged in aggregations of 4-8 "balls" which at first glance simulate mature ascospores (FIG. 10a). BREFELD (1891: 293, pl. XI, fig. 41,3) described these balls in **Tympanis truncatula**. Species of **Claussenomyces** can easily be divided into two larger groups, one with true ascoconidia (e.g., **C. olivaceus**), the other with conidia produced only on ascospores outside asci or within dead asci but never within living asci (e.g., **C. prasinulus**). Likewise, **Tympanis**, **Rhamphoria** and **Nectria coryli** produce ascoconidia, while, e.g., **Ascocoryne** and **Pezicula** produce conidia only on ascospores following natural discharge or within dead asci, and are therefore not polysporous. MARTENS (1936: 385) wrongly considered the asci of **C. prasinulus** and **Ascocoryne cylichnium** as secondarily polysporous. Likewise, HAFELLNER & BELLEMÈRE (1983) were unable to distinguish between true secondary polyspory and conidia produced on naturally discharged ascospores. They reported conidial formation within (dead) asci of **Brigantiaea**, leaving the question open whether or not the spores are ejected prior to budding.

Since fresh ascocarps mostly contain both living and dead asci, and since it is impossible to see from the dead specimen which asci have been alive before desiccation, one is completely unable with herbarium material to decide whether true ascoconidia were present, or whether the spores were hyaline or brown, 1-celled or septate in the mature living ascus. Thus, taxonomists working only with herbarium material are often not prepared to refer such a "variation" to certain development stages of the spores. HAFELLNER & BELLEMÈRE (1983: 175) and HUHTINEN (1990a: 70) have stressed this problem: the authors were only able to consider intuitively "good-looking" spores as "mature", and to ignore "bad-looking" spores as "very old" or "senescent". This subjective method, however, leads, in my experience, to unreliable results.

When drying discomycetes for the herbarium, we should be aware that the dry air often induces most of the mature asci to shoot their mature spores, which represent the standard condition for spore descriptions, rapidly into the air. Herbarium specimens then exhibit predominantly immature spores within the asci, or discharged spores in different phases of germination lying on the hymenium, although the apothecia showed full maturity when collected.

The precise development stage can be recognized by the appearance of the living cytoplasm of spores and asci: immature asci show many small vacuoles and hydrated cytoplasm (FIGs. 15ab, 16ab; CHADEFAUD, 1938), which may contain many small LBs in the *pars sporifera* (FIGs. 42 a-e, 43a); CRB induces the formation of MCs in these vacuoles (FIG. 4). The cytoplasm of the spores shows an increase in number and/or size of LBs during sporogenesis (FIGs. 15, 16, 42 c→d; see also JOHNSON, 1963). Mature asci show strongly dehydrated cytoplasm and a high percentage of "free space" represented by one large vacuole which is filled with transparent water (FIGs. 8a, 9a, 10a, 19, 20, 43b); CRB gives a homogeneous violet stain to the large vacuole, while no MCs are formed; the *pars sporifera* reaches its minimum length.

9. DROUGHT TOLERANCE (= POIKILOHYDRY)

When herbarium material collected within the last few years is studied in water mounts, living cells may still be met along with dead cells in a quantity of species. Spores, especially, are known to survive in some cases for many years (SUSSMAN, 1968). Drought tolerance of fruit-bodies is well-known in a few Agaricales and in many Polyporales and Tremellales (INGOLD, 1986: 578).

Fungal cells usually lose a considerable amount of water when passing into the dry, dormant (still living) state. We must therefore distinguish between *in statu vivo* (living state) versus *in statu emortuo* (dead state) on the one hand, and *in statu umido/udo* (hydrated or moist state) versus *in statu sicco* (dry state) on the other hand. Only few fungi perform a strategy of drought avoidance (e.g. **Daldinia**; INGOLD, 1954: 101). In most pyrenomycetes I found little or no structural check on evaporation: the hymenium desiccates and recovers repeatedly, and the perithecial cavities are completely filled with air in the dried dormant state.

Since vitality of single ascocarp cells can easily be recognized under the LM, I carried out tests on tolerance to dehydration: asci and paraphyses of some selected species of Leotiales and Pezizales did not survive even a few minutes or hours in the dry-air conditions of the herbarium while others survived several months. About 50% of either asci or paraphyses were found to be viable after preservation *in statu sicco* at 18-25°C and about 60% relative humidity for the following period of time: (paraphyses {P} were often more tolerant than immature asci, and these more than mature asci {A})

< 1 day: **Ciboria caucus** {AP}, **Peziza succosa** {AP}, **Hymenoscyphus fructigenus** {AP}, **H. rhodoleucus** {AP}, **Ombrophila violacea** {AP}

1-2 days: **Lachnum pudicellum** {A}, **L. subvirgineum** {AP}, **Dasyscyphella nivea** {A}, **Sarcoscypha coccinea** {P}

1-2 weeks: **Trichopeziza mollissima** {AP}, **Brunnipila clandestina** {AP}, **Dasyscyphella crystallina** {P}

3-5 months: **Encoelia furfuracea** {AP}, **Lachnellula occidentalis** {P}, **Capitotricha rubi** {P}
8 months: **Lecanora conizaeoides** {AP}

INGOLD (1954: 97) erroneously assumed that fungal spores are "normally without vacuoles" and therefore drought-tolerant. From the above we must conclude, however, that even cells with large vacuoles (asci, paraphyses) are able to withstand strong and prolonged desiccation. Small vacuoles are often present in mature spores (FIGs. 34-37). From the results on paraphyses, I conclude that even wall thickness is unimportant for a cell to be drought-tolerant.

Fungal cells, including mature spores, mostly have very high water contents (60-90%; READ et al., 1982: 2072) in the state of full vitality and hydration (if ample external water is available). Measurements of low total water contents in spores (YARWOOD, 1950; SUSSMAN, 1968; 5-25% for conidia and ascospores) refer to spores which have been in equilibrium with the dry laboratory environment (*in statu sicco*, 22-24°C, 42-51% relative humidity). The spores have *probably lost a considerable portion of their water in the process of collection* (YARWOOD, l.c., often by collapsing), having thereby passed into a state of dormancy. *Naturally collapsed, viable fungal spores are probably of common occurrence in nature* (BECKETT et al., 1984: 94), owing to their inability to check evaporation. *On wetting, they imbibe water and rapidly swell* by regaining their original size (BECKETT et al., 1984: 87). This phenomenon of low water content is called *anhydrobiosis*. SUSSMAN (1966: 740) believed that anhydrobiosis plays no major role in the dormancy of fungal spores, probably because he thought the wall of the mature spore to be quite impermeable to water.

10. CONCLUSIONS

Studying dead cells from fungi preserved in herbaria has very often led to erroneous taxonomic conclusions. It means disregarding many features of high taxonomic importance which have become obscure or have completely disappeared during the death of the cells. Being unaware of the numerous method-induced alterations presented in this paper means working with incompatible observations made on different states or development stages of the cells. Many taxa, even on generic level (e.g. **Mollisia** s.l. versus **Pyrenopeziza** s.l.), are easily distinguished with fresh material but can often hardly be recognized in the dead state.

Many published theories on ascus function and the mechanism of spore discharge which were based on the study of dead asci turned out to be in part erroneous, while DE BARY's (1887) observations on living asci have still high importance and validity. TEM-investigations should routinely be accompanied by LM-studies of the living cell in order to be aware of the changes affecting both cell wall and cytoplasm.

If taxa concepts have been worked out on the basis of abundant fresh collections, as I have done, e.g., in the "**Sarcoscypha coccinea**-complex" (BARAL 1984), one is more or less able to recognize these taxa also from dried material on the basis of correlated features being unaffected by the death of the fruit-body. Yet the process of getting new ideas and arriving at new taxonomic concepts heavily depends upon the applied method: the species of **Sarcoscypha** clearly differ in the size of the lipid bodies inside the living mature ascospores (FIGs. 23-26, the numerous minute accessory LBs occur in all 4 species). This is the most conspicuous feature in that group (apart from striking ecological differences) which independently led BOUDIER and me to the idea that different taxa are involved. In a part of the dead spores of all these species, however, the lipid forms large variable aggregations of comparable size, so this distinction is strongly obscured. HARRINGTON (1990: 436) confirmed this experience: *although I had examined material (dried herbarium specimens) from western North America I was not prepared to recognize that group as a species distinct from the two, large eastern North American species until I saw fresh (living) material.* LE GAL (1941) was unable to distinguish between **S. jurana** and **S. coccinea** in European material because she studied only herbarium specimens.

Those who are not skilled in recognizing vitality of single cells may even be unimpressed when examining cell contents of fresh specimens because, very often, living and dead cells can be found in a single preparation. Thus, one observes a broad scope of morphological and cytological "variation". BOUDIER (1886: 143) stressed this *relative variability* concerning lipid bodies in spores, a variability which is only manifest if one does not carefully separate living from dead cells, and mature cells from those in other development stages.

I have now studied about 9000 collections of Ascomycetes in the fresh, living state (nearly all of them were admittedly collected in Central Europe), and a further 900 in the dried, mainly dead state. Personal communications revealed, however, that mycologists usually consider that, for various reasons, they are not always able to study a major portion of their specimens in the living state. *A monographer receives material from all over the world, most of which is in the dead dried state. It is mainly his personal collecting effort that will enable him to study fresh material* (S. HUHTINEN, in litt.). Some monographers have not even macroscopically seen some or most of their treated species in the fresh state. The method of studying fungi preserved in herbaria seems fairly convenient and highly advantageous at first sight due to the possibility of comparing critical taxa simultaneously. The present study, however, presents a lot of arguments for a precise taxonomy based on the study of living cells. The results obtained by this method are considered so superior and the conflicts resulting from ignorance of the facts presented here are so important, that it is urgent for everybody to reflect on the methods practiced so far. Even describing "clear" new species which are thought to be easily recognizable from persistent characters is of limited taxonomic value since critical unknown taxa close to every such "clear" taxon may be discovered in the near future.

Vital taxonomy means to be ready for study whenever a species is collected or is received from a colleague by post. Ascomycetes, or even Basidiomycetes (KOIVURINTA, 1978), can be stored in the refrigerator at about +5-10°C for several days or even weeks without any ill effects. The great value of vital taxonomy is that a relatively large amount of microscopic data can be gathered in a reasonable period of time and with higher efficiency concerning the value of the results. SVRČEK (1976: 116) wrote: *In fact, the study of dried specimens as such is much more difficult and more time-consuming than work with fresh material.* Furthermore, vital taxonomy means frequent and regular field work. Herbarium taxonomy has resulted in a deficiency in our knowledge of ecological preferences of a species. Many species are known only from the type collection, the host on which they grew being often unknown, and many species are said to be difficult to find. The experience of G. MARSON (pers. comm.) and me is, however, that many species are of more common occurrence and are consequently available for a study *in statu vivo* at a much higher frequency than is usually believed.

A problem arises in the typification of taxa being mainly distinguished by transitory characters (which are only visible in the living state). According to Art. 9.1, ICBN (GREUTER et al., 1988), the type of a species must be a dried specimen or microscope slide). Living plants or cultures are not permitted (Art. 9.5, ICBN). Illustrations rank second and are only accepted as types if specimens are lacking or cannot be preserved as dried specimens (Art. 9.3, ICBN). This method requires the recognition of the species from the dead state. Should we therefore consider characters which are visible only in the living fungus to be less valuable for taxonomic purposes?

According to BRESINSKY (1964), exsiccata especially in Agaricales, exhibit much less distinctive macroscopical differences when compared with the living basidiocarp. In order to emphasize the value of microscopic features in this group and to facilitate the study of type material, he produced keys to exsiccata which either allow the determination of species, or only taxa of somewhat higher level. I suppose, however, that these keys are predominantly based on pre-existing taxa concepts which had been prepared on the basis of macroscopical characters of fresh, living basidiocarps. Thus, ORTON (1960: 161) recommended to study fresh Agaricales and Boletales both *macro- and microscopically, before pronouncing a verdict on the dried material.* Concerning the Pezizales, BENEDIX (1972: 163) stated that dried material alone is insufficient for taxonomic decisions on critical, mainly macroscopically defined species.

Nevertheless, monographers have probably very often prepared their taxa concepts on the basis of dried material. In the higher plants, for example, LEENHOUTS (1968: 26ff.) recommended to find out taxonomical entities by carefully comparing a large number of herbarium specimens ("herbarium taxonomy").

We have to acknowledge that dried specimens do not reflect either the macro- or the micromorphology of the living state. Since there exist many state-dependent features, it is necessary to describe these features from living cells in water and note anomalies caused by the death of the cells or by reactions to mountants (this was also recommended by SPAIN, 1990: 75). No satisfactory method for the fixation of transitory characters for permanent preparations is known. According to READ et al. (1982), *all preparative procedures that all necessitate the elimination of constituent water used in SEM produced artifacts*. It is therefore highly desirable that drawings, photographs, and descriptions of living fungal organs are deposited together with the dried specimens. Microscopic measurements should mainly be taken from living cells in water (although these data are incompatible with those obtained from herbarium material) since the stage of development of the organs cannot clearly be recognized after mounting in lethal media (Note that HUHTINEN, 1990, gave measurements of spores and asci, whenever possible, *when fresh in water* along with those in MLZ or CB).

In order to prepare more precise taxonomic concepts, both extended field work and immediate microscopic study is necessary. Monographers are urged to make as many personal fresh collections as possible. Due to the substantial loss of valuable vital characters in dead fungi, descriptions of type material *in statu vivo* have high importance. If an Ascomycete taxon cannot satisfactorily be recognized from dried specimens, the protologue, if based on living specimens, must automatically rank first in the typification of its basionym. New taxa should therefore be described from living specimens whenever possible.

11. HOW TO MAKE VITAL TAXONOMY

11.a. Method

1. How to collect:
- drought-intolerant Ascomycetes:
  - use boxes of watertight material (no paper boxes)
  - produce a humid atmosphere inside by adding fresh moss etc.
  - avoid mechanical pressure
- drought-tolerant Ascomycetes:
  - can be collected in dry or fresh condition
  - rehydrate dry fruit-bodies a few minutes prior to preparation by spraying with water

2. How to make preparations for the LM:
  - mount in tap water
  - avoid long-time exposure of ascocarps to dry air or warm light
  - place the fungal fragment immediately into the water drop on the microscope slide in order to avoid critical desiccation
  - cut freehand through the hydrated ascocarp under the dissecting microscope
  - stain *in statu vivo* by adding CRB or IKI to the edge of the coverglass

- add toxic (lethal) reagents to the edge of the cover glass to observe alterations
- note whether each described cell was alive or dead
- employ (osmotically inert) viscous solutions of albumin for photomicrography in order to prevent movement of spores
- keep immature collections for some days in the box to obtain mature hymenia
- allow some apothecia to deteriorate in order to study ascospore germination and possible production of conidia on the ascospores

11.b. Important vital characters

1. Asci:
   - measure living, mature asci first because these readily liberate the spores in many genera
   - observe presence or absence of croziers which can readily be seen in sections of living young apothecia (apply no pressure on the coverglass!)
   - study the apical apparatus prior to discharge in both the living and the dead state; employ IKI for diagnosing blue versus red reactions

2. Spores:
   - take measurements and observe spore characters from living mature spores recently shot into the medium by active spore discharge (spores inside living asci are often distinctly narrower though mature due to the ascus turgor, and spore characters like pigmentation, septation etc. strongly depend upon the development stage)

3. Sterile tissue:
   - study excipular structures from living sections which are not too thin (approx. 30-100 μm)
   - recognize imbibition of water by intercellular gel in the dead state when applying CB or MLZ to the section (textura oblita in the dead state may look like textura prismatica in the living state!)
   - observe cell contents in living paraphyses, hairs, and excipular cells, especially properties of the VBs: refraction, shape, size, color, location

11.c. How to study dead herbarium specimens

- use water first in order to prohibit dissolution of wall deposites or exudates, and to test the IKI reaction for hemiamyloidity
- use 2% KOH for the observation of the lipid in the cells, especially in the spores (the lipid in dead cells is often masked in nearly all other

mountants in use)

- the ascogenous hyphae can be studied for croziers by mounting in KOH or in Congo red (strong pressure on the coverglass and heating may be necessary)

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NOTEWORTHY SPECIES OF *COLLYBIA* FROM MEXICO AND A DISCUSSION OF THE KNOWN MEXICAN SPECIES

GASTON GUZMAN, VICTOR M. BANDALA and **LETICIA MONTOYA**

Instituto de Ecología, Apartado Postal 63, Xalapa, Veracruz 91000, Mexico

SUMMARY

A check list of the 20 reported species of ***Collybia*** from Mexico since 1910 is presented, of which 4 belong to other genera. The differences between ***Collybia alkalivirens*** Sing. and ***C. fuscopurpurea*** (Pers. : Fr.) Kumm. are discussed. New localities are presented from the States of Morelos, Oaxaca, Tlaxcala, and Veracruz for the former, and from the States of Mexico, Nuevo León, and Veracruz for the latter. ***C. alkalivirens*** is distinguished by the smaller spores and coarse incrusted hyphae of both lamellar and stipe tramas, and ***C. fuscopurpurea*** by its larger spores, and punctate hyphae in the lamellar trama and coarsely incrusted on the stipe trama. New localities from the States of Hidalgo, Oaxaca, Tamaulipas and Veracruz of ***C. iocephala*** (B. & C.) Sing. are presented. Moreover ***C. polyphylla*** (Peck) Sing. ex Halling and ***C. butyracea*** (Bull. : Fr.) Kumm. are described; they have a widespread distribution in the country but have not been studied microscopically.

RESUMEN

Se presenta una lista de las 20 especies de ***Collybia*** registradas en México, desde 1910 al presente, de las cuales 4 se adscriben a otros géneros. Se discuten las diferencias entre ***Collybia alkalivirens*** Sing. y ***C. fuscopurpurea*** (Pers. : Fr.) Kumm. y se dan nuevas localidades de los Estados de Morelos, Oaxaca, Tlaxcala y Veracruz para la primera y de los Estados de México, Nuevo

León y Veracruz para la segunda. ***C. alkalivirens*** se distingue por sus esporas pequeñas y por las incrustaciones grandes en las hifas tanto de la trama laminar como en del estípite y ***C. fuscopurpurea*** tiene esporas grandes, hifas de la trama laminar solamente punteadas y las hifas de la trama del estípite fuertemente granulosas. Se presentan nuevas localidades de ***C. iocephala*** (B. & C.) Sing. de los Estados de Hidalgo, Oaxaca, Tamaulipas y Veracruz y descripciones de ***C. polyphylla*** (Peck) Sing. ex Halling y ***C. butyracea*** (Bull. : Fr.) Kumm., especies de amplia distribución en el país, pero que no habían sido estudiadas microscópicamente.

INTRODUCTION

A revision of species of ***Collybia*** collected in forests of the State of Veracruz by the authors and several exsicati identified as ***Collybia alkalivirens*** Sing. in XAL, FCME and ENCB herbaria was made. Some of the materials could be ascribed to that species concept, mainly because of the instantaneous green reaction with 5% KOH and by hymenial and stipe hyphal incrustations as observed in water. Other collections discussed below did not fit this species concept and agree with ***C. fuscopurpurea*** (Pers. : Fr.) Kumm.

Also it was noted that ***C. polyphylla*** (Peck) Sing. ex Halling and ***C. butyracea*** (Bull.: Fr.) Kumm. are poorly known in Mexico, in spite of their widespread distribution, and ***C. iocephala*** (Berk. & Curt.) Sing., previously studied by the authors (Montoya-Bello *et al.*, **1987**) is reported from new localities.

Considering the present status of the genus in Mexico, it was concluded that a revision of the known species of ***Collybia*** in Mexico is needed, and this is a first attempt towards the analysis of the genus in the country. Microscopic mounts were made in 5 % KOH, congo red, cotton blue and water.

SPECIES OF *COLLYBIA* REPORTED FROM MEXICO
Table 1

Murrill (**1910, 1915, 1916**) was the first to study ***Collybia*** (most of them as ***Gymnopus***) from Mexico. He considered ***C. dryophila*** (Bull. : Fr.) Kumm., ***C. fimetaria*** (Murr.) Murr., ***C.***

orizabensis (Murr.) Murr., ***C. roseilivida*** (Murr.) Murr., ***C. velutipes*** (Curt.: Fr.) Kumm. and ***C. xuchilensis*** (Murr.) Murr., of which the second and the two latter species are now placed in ***Mycena, Flammulina*** and ***Hydropus***, respectively. On the other hand, ***C. orizabensis*** and ***C. roseilivida*** are known only from Mexico. Murrill (1915) also described ***Marasmius subcyathiformis*** Murr., which Pegler (1977) considered as ***Collybia subcyathiformis*** (Murr.) Pegler.

Sharp (**1948**), Guzmán (**1961**, **1972**, **1977**), Welden *et al.* (**1979**) and Martínez-Alfaro *et al.* (**1983**) recorded several additional species of ***Collybia*** . Table I presents the 20 species reported from Mexico from 1910 to 1990. As previously noted, 3 of them are actually in other genera and ***C. platyphylla*** reported by Sharp (**1948**) is treated in ***Tricholomopsis*** following Singer (1986) or also in ***Oudemansiella*** or ***Megacollybia*** following others.

At present only 16 species of ***Collybia*** are known to ocurr in Mexico, but a critical taxonomic study is necessary, because some of them are not treated in the modern concept of the genus, i.e. example, ***C. roseilivida*** (Murr.) Murr. should be a ***Mycena*** in the modern sense, and others are known only from the type specimens. Moreover, ***C. peronata*** (Bolt. : Fr.) Kumm. seems to be represented in the New World by three taxa involved (Halling, pers. comm.)

Among the most common species considered in Mexican literature are ***C. dryophila***, ***C. polyphylla***, ***C. butyracea***, ***C. confluens*** (Pers. : Fr.) Kumm. and ***C. maculata*** (Fr.) Kumm., which are distributed mainly in temperate forests of the country (Guzmán, **1977**).

NEW RECORDS OF *COLLYBIA ALKALIVIRENS*

Figs. 1-9 & 20-21

Collybia alkalivirens is easily distinguished by the green reaction of all parts of the basidiocarp in contact with KOH, and also by the brown hyphal incrustations of the pileal, hymenial, and stipital tramas observed in water or in Melzer's solution. Specimens examined had spores (4-) 5-8 (-9) x (2.4-) 3.2-4 (-4.8) μm and polymorphic cheilocystidia (15-)20-80 x 3-9 (-11) μm, light brown to yellowish brown in water, and stain green with KOH. The same staining reaction with KOH was observed in caulocystidia [29-54 x 4-6 (-10) μm] and in the hymenium and stipe hyphae.

The six Mexican studied materials, agree well with the descriptions of Halling (**1979**, **1981**, **1983**) and also with two collections from the U.S.A. identified by him (Halling 3726, New York, Tomkins Co., and Halling 4425, from Massachusetts, Franklin Co., both at NY).

For the differences between ***C. alkalivirens*** and ***C. fuscopurpurea*** see a discussion below. This species was originally described from a deciduous forest in the U.S.A. (Singer, **1948**). In Mexico it has been reported from temperate forests (Guzmán, **1977**), particularly from the States of Durango (Quintos *et al.*, **1984**) and Morelos (López *et al.*, **1985**). Here it is recorded from five new localities in *Pinus-Quercus*, mesophytic (subtropical) and *Abies religiosa* (H.B.K.) Schl. & Cham. forests.

Material studied. STATE OF MEXICO: Parque Nacional Nevado de Toluca, junction of the roads Temascaltepec-Sultepec, Colón 56 (ENCB). STATE OF MORELOS: km 55 old road from Tres Marías to Cuernavaca, Colonia Atlixco, Guzmán 5681; 12104 (ENCB). OAXACA: San Pedro Macuiltianguis, Alvar González, s.n., Jun. 5, 1984 (XAL, IBUG). STATE OF TLAXCALA: Municipio de Ixtenco, Parque Nacional La Malintzi, ladera Xalapasco, González-Fuentes 983 (XAL, FCME). STATE OF VERACRUZ: Cofre de Perote Region, Municipio de Xico, N of Ingenio El Rosario, Los Gallos, Villarreal 2892 (XAL).

ADDITIONS TO THE KNOWLEDGE OF *COLLYBIA FUSCOPURPUREA*

Figs. 10-19 & 22-23

Collybia fuscopurpurea (Pers. : Fr.)Kumm. was redescribed recently by Halling (**1990**) based on materials from Europe, the U.S.A., and a collection made by Singer in Mexico (Region of Río Frío). Coincidental with Halling's study, the authors of the present paper were studying some Mexican materials related to ***C. alkalivirens*** which tentatively were considered to be a new species, but later it was concluded that the materials agree well with ***C. fuscopurpurea***.

Halling differentiated ***C. fuscopurpurea*** from ***C. alkalivirens***, by spore size and by the geographical distribution. The former grows in the U.S.A. Rocky Mountains during Autumn, and the latter grows in the east of the Great Plains in early Summer. The distribution and seasonal influence in the U.S.A. cannot be applied to Mexico. But in addition to spore size, the hyphal incrustations is another good microscopic feature to separate the species.

C. fuscopurpurea have the hymenial trama hyphae slightly but constantly granulose, or just punctated, and those of the stipe trama have big granular incrustations (figs. 22 & 23), in contrast with ***C. alkalivirens*** having both stipe and hymenial trama hyphae distinctly ornamented with big granulose incrustations (figs. 20 & 21). These features were also observed in material of ***C. fuscopurpurea*** collected by Halling from Belgium (Halling 4888, Prov. Namir Bois Resteigne, at NY). However a collection studied by Halling (Desjardin 464, from California, Marin Co., Lake Bon Tempe, at NY) and considered by Halling (**1990**) as ***C. fuscopurpurea***, presents features which relate it with ***C. alkalivirens*** (medium size spores and both stipe and hymenial trama hypha strongly granulose). Then the size of the hyphal incrustations is a good feature to separate ***C. fuscopurea*** from ***C. alkalivirens***, because is a constant feature, as the authors observed in the several studied specimens of both species.

Mexican materials of ***C. fuscopurpurea*** studied by the authors present the following characters:

Pileus (5-) 10-30 (-40) mm diam., convex to nearly plane or subumbilicate, smooth, margin translucent striate, lubricous, hygrophanous, dark reddish brown to reddish straw or cinnamon color, with irregular blackish stains. Lamellae subdecurrent to adnexed, subdistant, more or less thick and wide, sometimes intervenose, concolorous with the pileus or light chocolate brown color to blackish in the dried state. Stipe 40-70 x 1-3 (-4) mm, uniform, sometimes applanate, fibrillose, slightly longitudinally sulcate, smooth or slightly pruinose mainly at the base, concolorous with pileus or vinaceous red, to nearly black in the dried state. Context whitish to reddish brown, thin and subleathery, odor and taste mild. Surfaces instantaneously green olivaceous in contact with KOH.

Spores (5-) 6.4-9 (-10) x 3-4.8 (-5) μm, lacrymoid or subellipsoid, hyaline or pale yellowish in KOH and in water, inamyloid. Basidia 24-30.4 x 4.8-6.4 μm, tetrasporic, hyaline, clavate, frequently clamped at the base. Pleurocystidia lacking. Cheilocystidia (14-) 18-45 (-60) x 4-6 (-8) μm, numerous, cylindric, flexuous, irregularly lobulated, sometimes apically dichotomously or trichotomously lobulated, hyaline, yellowish or yellowish brown in water and greenish in KOH, frequently clamped at the base. Caulocystidia 22-65 (-90) x 3-5 μm, common, cylindric, flexuous, sometimes irregularly lobulated, similar in color to cheilocystidia. Pileus cuticle not gelatinous; elements (3-) 5-8 (-10) μm wide, subglobose, short and irregularly bifurcate, not radially arranged, punctate, incrustations brown when observed in water. Pileus context with hyphae 3.2-4.8 μm wide, hyaline or yellowish in water, sometimes with short granulations observed in water. Hymenial trama subregular; hyphae (3-) 4-10 (-16) μm wide, yellowish to yellowish brown when observed in water, brown in mass, with very short and obscurely conspicuous granulations when observed in water. Stipe hyphae (3-) 5-15 (-18) μm wide, hyaline to yellowish in water, surface covered with very distinct strong granulations (bigger than those of the hymenial and pileus trama), brown when observed in water. All granulations mentioned above, disappear in contact with KOH and hyphae appear smooth and stain greenish. Clamp connections common.

Habitat and distribution of Mexican materials. Common in humus in coniferous forests composed of ***Pinus patula*** Schl. & Cham., ***P. montezumae*** Gord., ***P. hartwegii*** Lindl. and other species or of ***Pseudotsuga macrolepis*** Flous. It is known from the States of Mexico, Veracruz and Nuevo León.

Material studied. STATE OF MEXICO: road Mexico to Puebla, Río Frío Region, Parque Nacional Llano Grande, Guzmán 7516; Frias-Neve 37 (both in ENCB). Parque Nacional Nevado de Toluca, km 16.5 carretera a Sultepec, Rancho Casas Viejas, Colón 705

(ENCB; XAL). STATE OF NUEVO LEON: Municipio de Galeana, road 18 de marzo to Torre de Microondas, Cerro El Potosí, Girón 19; Galván 230 (both in ENCB). STATE OF VERACRUZ: Cofre de Perote Region, 1 km S of Tembladeras, El Revolcadero, Villarreal 421, 429, 1866, 2404-C (all in XAL).

Halling (**1990**) described the species with spores 6.7-8.5 x 3.3-4.8 µm, pleurocystidia absent, cheilocystidia 20-25 µm long, hymenial trama hypha with scattered brown encustring pigment, pileus trama hyphae 5-10 µm wide, with "granular encrusting pigment" and caulocystidia 3.5-7 µm diam. In the material from Belgium (Halling 4888 NY) the present authors found spores 6.4-8.8(-9.6) x 3.2-4.8 µm, cheilocystidia similar to Mexican materials, hymenial trama hyphae punctate or with fine granulose incrustations and stipe hyphae with big granulose incrustations. Therefore, Mexican specimens fit the European concept of this species.

NEW LOCALITIES OF *COLLYBIA IOCEPHALA*

This species is characterized by the distinct violet color of basidiocarps, also by its strong odor which resembles that of garlic or gunpowder and by the instantaneous blue turquoise reaction of the surfaces in contact with KOH, which can also be demonstrated in the dried state. The pileipellis is a layer of cylindric hyphae radially arranged, 3-4(-5) µm wide (figs. 24-25), which distinguish this species of those of alkalivirens group, which present analogous reaction in contact with KOH, but have short, subglobose and irregularly bifurcate pileus hyphae not radially disposed.

Collybia iocephala is common in deciduous and subtropical forests in the U.S.A. including Florida (Halling, **1983**). In Mexico, it is known from tropical rain forests (in Uxpanapa region) to the mesophytic (subtropical) forests in SE of the State of Veracruz (Welden *et al.*, **1979**; Montoya-Bello *et al.*, **1987**). Five new records of this species are reported. They were collected in mesophytic forests in the States of Veracruz (central part), Hidalgo and Tamaulipas.

Material studied. STATE OF HIDALGO: Municipio de Molango, Laguna Atesca, Cifuentes 675 (FCME, XAL). STATE OF OAXACA: SE de Huautla de Jiménez, San Agustín, Vargas 253

(ENCB). STATE OF TAMAULIPAS: Municipio de Gómez Farías, Reserva de la Biósfera El Cielo, Bandala 1436 (ITCV). STATE OF VERACRUZ: 2 km SW from Xalapa, near Coapexpan River, Bandala, 1369 (XAL), 1333 (XAL, IBUG), 1345 (XAL, ENCB). Municipio de Banderilla, SW Banderilla, Cerro La Martinica, Montoya 549 (XAL, FCME), Rancho La Pomarrosa, Anell 394 (XAL). Old road Xalapa-Coatepec, km 2.5, Casa Asistencial CONECALLI-DIF, Ochoa 28 ; Murrieta 12, 477; Tapia 594, 643; same road, Parque Ecológico Francisco J. Clavijero, Anell 484, 434, Montoya 1295; Mata 325; El Haya, Bandala 1536 (all in XAL).

COLLYBIA POLYPHYLLA IN MEXICO
Figs. 26-28

Collybia polyphylla is widespread in Mexico (Guzmán, **1977**; Bandala *et al.*, **1988**), but still has not been studied microscopically in the country and as noted in herbaria materials it has been commonly missunderstood with ***C. dryophila***, but they differ microscopically and by the strong odor of garlic in the former.

Mexican materials have the following microscopic fectures: spores (3.2-) 4-6.4 (-7.2) x 2.4-3.2 (-4) µm, cylindric-elliptic, inamyloid, asymmetric, with a conspicuous hilar appendix. Basidia 16-24 x 5.6-6.4 µm, tetrasporic, sometimes bisporic, clavate, frequently clamped at the base. Pleurocystidia absent. Cheilocystidia (16-)20-56 (-72) x (2.4-) 3.2-5.6 µm, numerous, but obscurely conspicuous, hyaline, irregularly cylindric-flexuous, irregularly ramified with long or short prolongations, clamped at the base. Hymenial trama with parallel hyphae, 4-11.2 µm diam., thin walled and clamped. Pileus hyphae more or less parallel to the surface and radially oriented, hyphae 3.2-9.6 (-11.2) µm, hyaline, thin walled, clamped, with subcylindric, irregularly ramified or bifurcate elements, which are sometimes erect.

The materials studied agree well with the description of Halling (**1983**), who described the species with spores 5.6-7(-7.6) x 2.8-3.4(-4.4) µm and cheilocystidia 35-56 µm long. Halling recorded ***C. polyphylla*** from eastern deciduous forests of the U.S.A.

Habitat and distribution. Subgregarious in humus, in *Pinus-Abies* or in subtropical and tropical forests, from nearly sea level to mountains at 3000 m altitude. It has been reported from the States of Durango, Hidalgo, Jalisco, Michoacan, Morelos, Quintana Roo and Veracruz.

Material studied. STATE OF VERACRUZ, Cofre de Perote Region, Municipio de Xico, N of Ingenio El Rosario, Los Gallos, Murrieta 2, 24; Bandala 71, 1859; Montoya 1935, 1636; Chacón 4226, 4227, 4252, 4269; Villarreal 405, 1876 (all in XAL).

COLLYBIA BUTYRACEA IN MEXICO

Figs. 29-31

This species has commonly been considered in Mexican literature (Guzmán, **1977**; Bandala *et al*, **1988**) but like ***C. polyphylla***, Mexican specimens have not been studied microscopically and frequently have been missunderstood with ***C. dryophila***, as noted in herbaria materials. The species is common in coniferous and ***Quercus*** forests, and is sold in popular markets. It can be distinguished from ***C. dryophila*** by the lubricous or oily and fleshy pileus, and by the rosy cream spore print, unramified pileipellis hyphae as well as by the cyanophilous and dextrinoid spores. The Mexican specimens agree with Halling's description (1983).

The material studied have spores (4.8-) 6.4-8 x (2.4-) 3.2-4 µm, subelliptic, with an acute asymmetric appendix, hyaline, cyanophilous and dextrinoid, appearing surrounded except at apex by a sheet when observed in Melzer's solution, in congo red or sometimes in KOH. Basidia 21.6-25.6 x 4.4-6.4 µm, tetrasporic, hyaline, clavate, frequently clamped at the base. Pleurocystidia absent. Cheilocystidia (16-)21.6-28(-32) x 2.4-3.2 (-4) µm, hyaline, numerous but not conspicuous, subcylindric-moniliform, sometimes irregularly lobulated or subramified, clamped at the base. Hymenial trama subregular, hyphae 4-8 µm wide, thin walled and smooth. Pileus cuticle with radially arranged cylindric hyphae, (2.4-) 4-9.6 (-12) µm, hyaline, smooth or with brown and short granulations observed in KOH. Context hyphae 6.4-12 µm diam., hyaline, smooth. Stipe trama hyphae 6.4-12 µm diam., hyaline, smooth. Caulocystidia not observed.

TABLE 1. SPECIES OF *COLLYBIA* REPORTED FROM MEXICO

C. acervata (Fr.) Kumm. (Guzmán, 1961) *
C. alkalivirens Sing. (Guzmán, 1977)
C. butyracea (Bull. : Fr.) Kumm. (Guzmán, 1972)
C. confluens (Pers. : Fr.) Kumm. (Guzmán, 1977)
C. distorta (Fr.) Quél. (Martínez-Alfaro *et al.* 1983)
C. dryophila (Bull. : Fr.) Kumm. (Murrill, 1910)
C. fimetaria (Murr.) Murr. (Murrill, 1916)
[actually ***Mycena fimetaria*** (Murr.) Sing.]
C. fibrosipes (B. & C.) Dennis (Guzmán, 1977)
C. fuscopurpurea (Pers. : Fr.) Kumm. (Halling, 1990)
C. fusipes (Bull. : Fr.) Quél. (Guzmán, 1961)
C. iocephala (B. & C.) Sing. (Welden *et al.*, 1979)
C. maculata (Alb. & Schw. : Fr.) Kumm. (Guzmán, 1977)
C. orizabensis (Murr.) Murr. (Murrill, 1916)
C. platyphylla (Pers. : Fr.) Kumm. (Sharp, 1948)
[actually ***Tricholomopsis platyphylla*** (Pers. : Fr.) Sing.]
C. peronata (Bolt. : Fr.) Kumm. (Guzmán, 1977)
C. polyphylla (Peck) Sing. ex Halling (Guzmán, 1977)
C. roseilivida (Murr.) Murr. (Murrill, 1916)
C. subcyathiformis (Murr.) Pegler (Murrill, 1915)
C. velutipes (Curt. : Fr.) Kumm. (Murrill, 1916)
[actually ***Flammulina velutipes*** (Curt. : Fr.) Sing.]
C. xuchilensis (Murr.) Murr. (Murrill, 1916)
[actually ***Hydropus xuchilensis*** (Murr.) Sing.]

*** Only the first reference to each species from Mexico is annotated.**

Habitat and distribution. Solitary or subgregarious, in subtropical or coniferous forests. It has been recorded from the States of Durango, Hidalgo, Jalisco, Mexico, Michoacan, Morelos, Oaxaca, Puebla, Veracruz and Zacatecas.

Material studied. STATE OF PUEBLA, Mercado de Teziutlán, Guzmán 29265. E from Teziutlán, Cerro de Techachapa, Bandala 1790. STATE OF VERACRUZ, Cofre de Perote Region, Municipio de Xico, N of Ingenio El Rosario, Los Gallos, Bandala 71, 1911, 1934, 1947; Chacón 4332-A; Tapia 112, 139; Ochoa 97. W of Tembladeras, Atopa, Bandala 1519 (all in XAL).

ACKNOWLEDGMENTS

The authors are indebted to the authorities of Instituto de Ecologia, CONACYT and Sistema Nacional de Investigadores at Mexico City by the support given to their researches. They also appreciate the collaboration of the directors or curators of ENCB, FCME, ITCV, and NY Herbaria, particularly to R. Valenzuela, J. Cifuentes, J. García and R. Halling. Drs. Harry D. Thiers from San Francisco State University and Roy E. Halling from the New York Botanical Garden kindly revised this paper.

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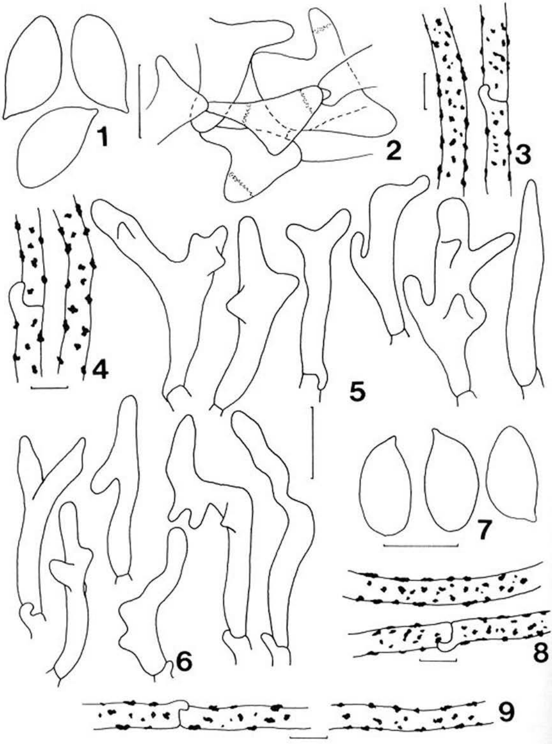
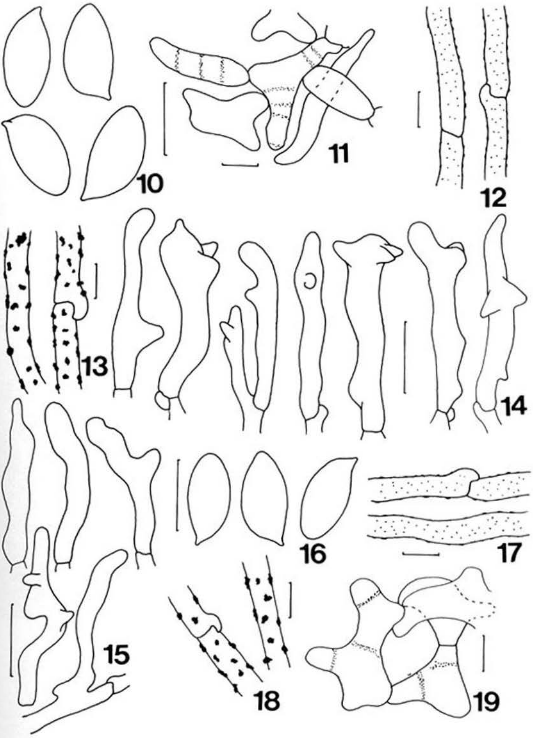
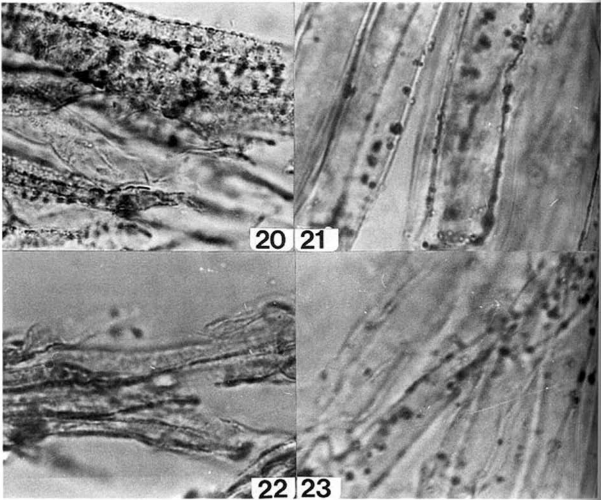


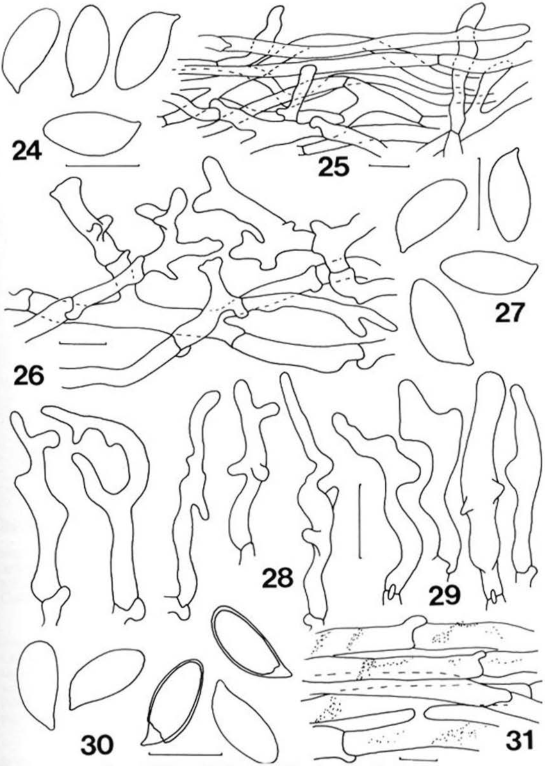
Figs. 1-9.- ***Collybia alkalivirens***, 1: spores; 2: pileus cuticle; 3: hymenial trama hyphae; 4: stipe trama hyphae; 5: cheilocystidia; 6: caulocystidia (Alvar González, s. n., Jun. 5, 1984); 7: spores; 8: hymenial trama hyphae; 9: stipe trama hyphae (Desjardin 464). (Scale bar, 1 & 7 = 5.3 μm; 2, 3-4, 6 & 8-9 = 16 μm & 5 = 20 μm).



Figs. 10-19.- ***Collybia fuscopurpurea***, 10: spores; 11: pileus cuticle; 12: hymenial trama hyphae; 13: stipe trama hyphae; 14: cheilocystidia; 15: caulocystidia (Villarreal 1866); 16: spores; 17: hymenial trama hyphae; 18: stipe trama hyphae; 19: pileus cuticle (Halling 4888). (Scale bar, 10-11, 16 & 19 = 10 μm; 12-13 & 17-18 = 16 μm; 14 = 15 μm & 15 = 23 μm).



Figs.- 20-23.- ***Collybia alkalivirens*** and ***C. fuscopurpurea***: 20-21: ***C. alkalivirens***, 20: hymenial trama hyphae; 21: stipe trama hyphae. 22-23: ***C. fuscopurpurea***, 22: hymenial trama hyphae; 23: stipe trama hyphae (all mounted in water; 400 x) (20-21; Alvar González, s. n., Jun. 5, 1984; 22-23: Villarreal 1866).



Figs. 24-31.- 24-25: ***Collybia iocephala***, 24: spores; 25: pileus cuticle (Montoya 549). 26-28: ***C. polyphylla***, 26: pileus cuticle (Montoya 1636); 27: spores; 28: cheilocystidia (Bandala 1859). 29-31: ***C. butyracea***, 29: cheilocystidia; 30: spores; 31: pileus cuticle (Chacón 4332-A). (Scale bar, 24, 27 & 30 = 4.8 μm; 25-26 = 10 μm; 28 = 16 μm; 29 = 9 μm & 31 = 11.8 μm).

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THE CHEMISTRY OF FOLIICOLOUS LICHENS. 1. CONSTITUENTS OF SPOROPODIUM VEZDEANUM AND S. XANTHOLEUCUM

JOHN A. ELIX and CAROLINE E. CROOK

Department of Chemistry, The Faculties, Australian National University, GPO Box 4, Canberra, ACT, 2601, Australia

H. THORSTEN LUMBSCH

Fachbereich 9/Botanik, Universität Essen, Postfach 103 764, D-4300 Essen 1 Federal Republic of Germany

ABSTRACT : An investigation of the chemical constituents present in the lichens *Sporopodium vezdeanum* and *S. xantholeucum* has revealed that both species contain the depsidone pannarin and the triterpene zeorin together with a chemosyndrome of chloro-xanthones. This is one of the few reported chemical studies of foliicolous lichens and indicates that secondary metabolite chemistry may well provide important characters in the taxonomy of such genera. A new name *Sporopodium vezdeanum* Lumbsch and Elix has been proposed for *S. phyllocharis* var. *flavescens*. 5,7-Dichlorolichexanthone is reported for the first time from a lichen.

Introduction

Natural product chemistry has played an important role in lichen taxonomy ever since Nylander (1866) introduced chemical reagents as an aid for the identification of lichen species. Current concepts in lichen chemotaxonomy and the alternative views on the taxonomic value of chemical characters have been reviewed by Egan (1986) and Culberson (1986).

Although the large majority of lichen groups have been the subject of such chemical investigation (Culberson, 1969, 1970; Culberson & Elix, 1989; Culberson, Culberson & Johnson, 1977; Elix, Whitton & Sargent, 1984) there have been very few studies of foliicolous lichens (Santesson, 1970). Undoubtedy the lack of material accounts for this deficiency, but with more sensitive methods for detection and identification, in particular high performance liquid chromatography (HPLC) and lichen mass spectrometry (LMS) (Culberson & Elix, 1989) this should no longer be the case.

Following our recent investigation on some xanthone-containing chemosyndromes in the genera *Lecanora, Lecidella, Micarea* and *Pertusaria* (Elix, Chappell & Jiang 1991; Elix & Crook, 1992), we have turned our attention to several yellow members of the foliicolous genus *Sporopodium*. Two species were studied, *Sporopodium vezdeanum* Lumbsch & Elix nom. nov. [Basionym: *Sporopodium phyllocharis* (Mont.) Mass. var *flavescens* R. Sant., Symb. Bot. Upsal. 12:518 (1952)] and *Sporopodium xantholeucum* (Müll. Arg.) A. Zahlbr. The secondary metabolites present in these two species and their relationships are discussed.

Materials and Methods

The lichen fragments were freed as far as possible from obvious organic substrate material and extracted with warm acetone for thin layer chromatography (TLC) or with warm methanol for HPLC. Compounds were identified by TLC using the methods standardized for lichen products (Culberson & Ammann, 1979; Culberson & Johnson 1976, 1982; Elix, Johnston & Parker, 1987) and by high performance liquid chromatography (HPLC) (Elix, Jenkins and Lumbsch, 1988; Elix & Crook, 1992; Elix, Chappell & Jiang, 1991; Feige, Lumbsch & Mies, 1992; Lumbsch & Elix, 1985) with retention index values (RI) (Huovinen, Hiltunen & Schanz, 1985) calculated from salazinic acid and atranorin controls. Two HPLC systems were used. One used a Perkin-Elmer HS-5C18 column and a LC-85 spectrometric detector operating at 254 nm. Elution was effected with 90% water-methanol containing orthophosphoric acid (20ml/100ml) with a flow rate of 0.6 ml min^{-1}. The second HPLC system used a Kontron Li-Chrosorb RP-18 column. Two solvent systems were used; 1% orthophosphoric acid (A) and methanol (B). The run started with 30% B and was raised to 70% B within 15 min., then to 100% B in 30 min. and isocratic elution in 100% B for a further 20 min. Lichen mass spectra (LMS) (Santesson 1969) were recorded at a VG micromass 7070F mass spectrometer at 70eV linked online to Finnigan Incos data system.

The following lichens were studied.

Sporopodium vezdeanum **Lumbsch & Elix**

AUSTRALIA, Queensland. On leaves, Sylvesters Lookout rainforest, Mistake Mts., Goomburra State Forest, ca. 50 km NE of Warwick, 27°58'S, 152°31'E, *H.T. Lumbsch 5685c & R.W. Rogers*, 26. ix. 1987 (herb. Lumbsch). New South Wales. On leaves in remnant rainforest Yatteyattar, Currowar Creek, 35°16'S, 150°25'E, *H. T. Lumbsch 8941 & T. S. Henshall*, 8. viii. 1991(ANUC).

NORFOLK ISLAND. On palm leaves in mixed subtropical rainforest, Mt. Pitt Reserve, Filmy Fern Trail, 29°01'S, 167°57'E, 130m, *J.A. Elix 18400 & H. Streimann, J.A. Elix 18410 & H. Steimann*, 3.xii. 1984 (ANUC).

Sporopodium xantholeucum **(Müll. Arg) A. Zahlbr.**

AUSTRALIA. Queensland. On leaves in tropical rainforest, Sonita waterfalls, Atherton Tableland, *H.T. Lumbsch 5437/17E*, 16. viii. 1987 (herb. Lumbsch); on leaves of *Lomandra* sp. in subtropical rainforest, Mistake Mts. 28°19'S, 152°22'E, *H.T. Lumbsch 5701i & R.W. Rogers, H.T. Lumbsch 5705a & R.W. Rogers,* 29. ix. 1987 (herb. Lumbsch); on leaves, Sylvesters Lookout rainforest, Mistake Mts., Goomburra State Forest, *ca.* 50 km NE of Warwick, 27°58'S, 152°31' E, *H.T. Lumbsch 5687a & R.W. Rogers,* 26.ix. 1987 (herb. Lumbsch).

Authentic pannarin was isolated from *Pannaria elatior* Stirton while zeorin (Elix, Whitton & Jones, 1982) was available from previous work. Authentic 2,5-dichloronorlichexanthone and thiophanic acid were supplied by Dr S. Huneck (Huneck 1966, Huneck & Höfle, 1978). 2,7-Dichlorolichexanthone was prepared by the literature method (Sundholm, 1978). The following have been synthesised or isolated previously: 2,7-dichloronorlichexanthone and asemone (Elix, Jiang & Wardlaw, 1990); arthothelin and isoarthothelin (Elix, Jiang & Portelli, 1990); 5,7-dichloro-3-*O*-methylnorlichexanthone and 2,5,7-trichloro-3-*O*-methylnorlichexanthone (Elix & Jiang, 1990); 5,7-dichlorolichexanthone and 2,5,7-trichlorolichexanthone (Elix, Crook et al., 1992).

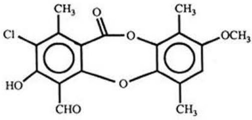
Results and Discussion

A total of 12 identifiable lichen substances were identified in the specimens examined. These included the β-orcinol depsidone, pannarin (1); the triterpene, zeorin (2); the chloro-xanthones, 2,7-dichlorolichexanthone (3), 5,7-dichlornorlichexanthone (4), 5,7-dichloro-3-*O*-methylnorlichexanthone (5), 5,7-dichlorolichexanthone (6), arthothelin (7), isoarthothelin (8), asemone (9), 2,5,7-trichloro-3-*O*-methylnorlichexanthone (10), 2,5,7-trichlorolichexanthone (11) and thiophanic acid (12). The known lichen compounds were readily identified by comparison with authentic materials, but this is the first reported natural occurrence of 5,7-dichlorolichexanthone (6) in lichens. The identity of 5,7-dichlorolichexanthone was confirmed by direct comparison with a synthetic sample of (6) (by TLC, HPLC, and LMS). 2,5,7-Trichloro-3-*O*-methylnorlichexanthone (10) was reported previously as occurring in *Sporopodium phyllocharis* v.*flavescens* (Santesson, 1970). The standardized chromatographic data for these compounds are listed in Table 1.

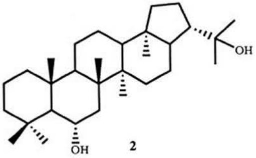
Table 1: **Standardized Chromatographic Data for *Sporopodium* Metabolites**

Standard R_F values (x 100) were determined in five independent t.l.c. solvent systems: (A) toluene / dioxane / acetic acid (180 : 45 : 5); ((B*) hexane / *t*-butyl methyl ether / formic acid (140 : 72 : 18); (C) toluene / acetic acid (170 : 30); (E) ethyl acetate / cyclohexane (25 : 75); (F) ethyl acetate / cyclohexane (50 : 50). HPLC retention index (RI) values (x 100) are relative to salazinic acid and atranorin.

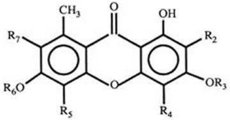
Compound R_F	(A)	(B*)	(C)	(E)	(F)	*RI*
Pannarin (1)	73	63	79	40	64	96
Zeorin (2)	52	43	43	19	74	--
2,7-Dichlorolichexanthone (3)	77	70	80	24	57	163
5,7-Dichlornorlichexanthone (4)	44	48	33	11	43	60
5,7-Dichloro-3-*O*-methyl-norlichexanthone (5)	67	67	59	16	40	140
5,7-Dichlorolichexanthone (6)	80	81	90	72	90	275
Arthothelin (7)	43	40	37	15	32	61
Isoarthothelin (8)	45	44	36	6	18	71
Asemone (9)	47	55	37	7	20	78
2,5,7-Trichloro-3-*O*-methyl-norlichexanthone (10)	64	56	56	6	16	136
2,5,7-Trichlorolichexanthone (11)	87	74	85	58	90	235
Thiophanic acid (12)	55	52	49	2	9	122
Atranorin (Standard)	75	73	79	57	85	100
Chloroatranorin (Standard)	74	73	81	30	60	126
Norstictic Acid (Standard)	40	32	30	0	0	13



1



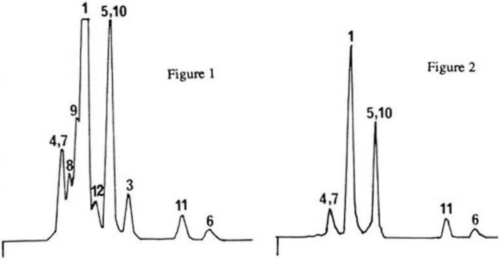
2



	R_2	R_3	R_4	R_5	R_6	R_7
3,	Cl	Me	H	H	Me	Cl
4,	H	H	H	Cl	H	Cl
5,	H	Me	H	Cl	H	Cl
6,	H	Me	H	Cl	Me	Cl
7,	Cl	H	Cl	Cl	H	H
8,	Cl	H	H	Cl	H	Cl
9,	H	H	Cl	Cl	H	Cl
10,	Cl	Me	H	Cl	H	Cl
11,	Cl	Me	H	Cl	Me	Cl
12,	Cl	H	Cl	Cl	H	Cl

The two species studied, *Sporapodium vezdeanum* and *S. xantholeucum* showed remarkedly similar chemical profiles (compare Figures 1 and 2). The only apparent difference concerned the relative intensities of some of the minor xanthones e.g. 2,7-dichlorolichexanthone. An analogous chemosyndrome of related chloro-xanthones has been identified previously in the lichens *Lecanora broccha* Nyl. (Elix, Chappell & Jiang, 1991; Elix & Crook, 1992) and *Lecidella meiococca* (Nyl) Leuckert & Hertel (Elix & Crook, 1992), but not in combination with the depsidone pannarin. *S. xantholeucum* has previously been reported to contain the pulvinic acid derivatives pulvinic dilactone, vulpinic acid and calycin (Santesson, 1970) but these substances were not detected in the collections examined.

Morphological descriptions and a discussion of the distribution of both species of *Sporopodium* are given by Santesson (1952). The two species can be distinguished morphologically by the margins of the apothecia. The apothecia of *S. xantholeucum* have thick margins (at least when young), while in *S. vezdeanum* the margins are not prominent. Santesson treated *S. vezdeanum* as a variety of the pantropical species, *S. phyllocharis*. However *S. vezdeanum* differs in morphology (Santesson 1952) and distribution, being confined to Australia, Norfolk Island and New Caledonia. Hence we are convinced that this taxon should be regarded as a distinct species and have introduced the new name *vezdeanum* in honour of our friend Dr. Antonin Vězda, for his many contributions to the knowledge of foliicolous lichens.



Figures 1-2. H.p.l.c. traces of *Sporopodium* sp. : 1, *S. vezdeanum* (*H. T. Lumbsch 8941 & T. S. Henshall* in ANUC); 2, *S. xantholeucum* (*H.T. Lumbsch 5701i & R.W. Rogers* in herb. Lumbsch).

Index to H.p.l.c. Peaks: pannarin (1); 2,7-dichlorolichexanthone (3), 5,7-dichlornorlichexanthone (4), 5,7-dichloro-3-*O*-methylnorlichexanthone (5), 5,7-dichlorolichexanthone (6), arthothelin (7), isoarthothelin (8), asemone (9), 2,5,7-trichloro-3-*O*-methylnorlichexanthone (10); 2,5,7-trichlorolichexanthone (11); thiophanic acid (12).

ACKNOWLEDGEMENTS

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CORALLICOLA NANA GEN. & SP. NOV. AND OTHER ASCOMYCETES FROM CORAL REEFS

BRIGITTE VOLKMANN-KOHLMEYER AND JAN KOHLMEYER

Institute of Marine Sciences
University of North Carolina at Chapel Hill
Morehead City, North Carolina 28557, USA

ABSTRACT

Corallicola nana (Ascomycotina, Halosphaeriaceae) is described from a subtidal dead coral slab in Belize (Central America) and compared with a similar genus, *Arenariomyces* Höhnk. Evaluation of new and old collections of *Koralionastes* adds considerable information on the biogeography of the five coral-inhabiting species. *K. angustus* and *K. giganteus* appear to be restricted to Belize (Caribbean), where the first is a frequent representative, while *K. giganteus* is found only rarely. Both *K. ellipticus* and *K. ovalis* occur in Belize, Australia, and Fiji; *K. ellipticus*, moreover, in St. Croix (U.S. Virgin Islands). At this point, *K. violaceus* has been found only in the Pacific (Australia, Fiji).

INTRODUCTION

The regular occurrence of filamentous higher fungi on coral reefs has been discovered only recently (Kohlmeyer & Volkmann-Kohlmeyer 1987, 1988, 1989, 1990, 1992). In the course of searches for fungi on corals and coral rubble of the Caribbean and the Pacific, we found a new genus and species of ascomycetes that is described in the following. New records of *Koralionastes* spp. are also reported.

1. A new ascomycetous genus from corals

Corallicola Volkm.-Kohlm. & Kohlm., *gen. nov.*

Etymology: From the Latin *corallum* = coral and *-cola* = dweller, in reference to the substrate.

Genus Halosphaeriacearum. **Ascomata** subglobosa, superficialia, ostiolata, breve papillata vel epapillata, subiculata, coriacea, brunnea, singularia vel gregaria; **peridium** leptodermum, texturam angularem formans; **centrum** cellulis pseudoparenchymaticis, leptodermis, deliquescentibus; **asci** leptodermi, deliquescentes; **ascosporae** ellipsoideae, uniseptatae, hyalinae, appendiculatae; ad apices ambos aliquot appendiculis terminalibus, gibbosis ad basem, complanatis ad centrum, gradatim contractis ad apicem.

Typus generis: *Corallicola nana* Volkm.-Kohlm. & Kohlm.

A genus of Halosphaeriaceae with one obligately marine species. **Ascomata** subglobose, superficial, ostiolate, short papillate or epapillate, subiculate, coriaceous, brown, single or gregarious. **Peridium** thin-walled, forming a *textura angularis.* **Centrum** of immature ascomata filled with pseudoparenchymatous, hyaline, deliquescent cells. **Asci** thin-walled, deliquescing. **Ascospores** ellipsoidal, one-septate, hyaline, appendiculate; at both apices with several terminal appendages, gibbous at the base, flattened in the middle and gradually tapering towards the tip.

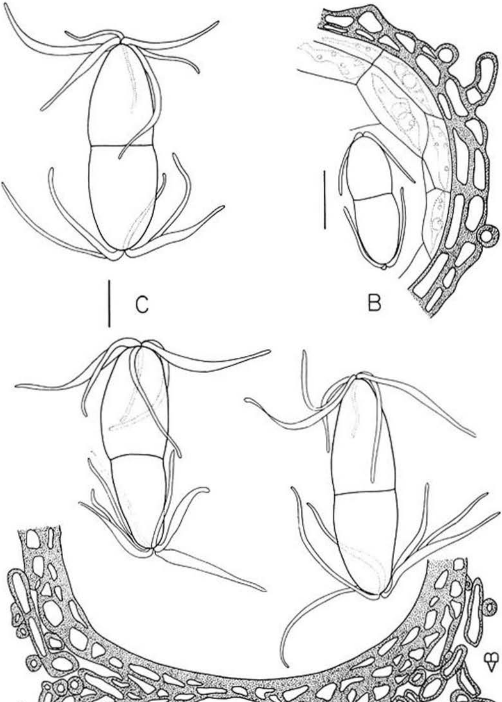
Corallicola nana Volkm.-Kohlm. & Kohlm, *sp. nov.*

Etymology: From the Latin *nanus* = dwarf, in reference to the small ascomata.

Ascomata 80-95 μm diametro, subglobosa, superficialia, ostiolata, breve papillata vel epapillata, subiculata, coriacea, atro-brunnea, interdum hyphis brevibus brunneis tectis, singularia vel gregaria; **peridium** 5-7 μm crassum, 1-2 stratis cellularum multangularium, texturam angularem formantia; **paraphyses** absentes, centra ascomatum immaturorum cellulis pseudoparenchymaticis, hyalinis, leptodermis, deliquescentibus; **asci** leptodermi, deliquescentes ante maturitatem ascosporarum; **ascosporae** 21.0-26.5 x 7.0-8.5 μm ($\bar{x}$ = 23.5 x 7.7 μm; n = 37), ellipsoideae, uniseptatae, ad septum leniter constrictae, hyalinae, appendiculatae; ad apices ambos 5-7 appendiculis terminalibus, 15-18 μm longis, 1.4-1.7 μm latis ad centrum, 0.5 μm diam ad apicem.

SUBSTRATUM: Saxa corallinarum mortuarum
DISTRIBUTIO: Oceanus Atlanticus (Belize)
HOLOTYPUS: J. K. 5004 (IMS)

Fig. 1. *Corallicola nana.* (A) Section through peridium with basal subiculum (scale = 10 μm). (B) Section through lateral part of peridium with thin-walled pseudoparenchyma (scale = 10 μm). (C) Ascospores (scale = 5 μm).


C
B
A

Ascomata 80-95 μm in diam., subglobose, superficial, ostiolate, short papillate or epapillate, subiculate, coriaceous, dark brown, sometimes covered by short brown hyphae, solitary or gregarious. **Peridium** 5-7 μm thick, composed of one or two layers of polygonal cells, forming a *textura angularis*, dark brown (Fig. 1B); at the base attached to the substrate with a thin subiculum that is more or less hyphoid (*textura intricata*) or dense (*textura angularis*) (Fig. 1A). **Pseudoparenchyma** of thin-walled polygonal cells filling the centrum of young ascomata, deliquescing at ascospore maturity; no indication of pit-connections in the walls (Fig. 1B). **Asci** thin-walled, deliquescing before ascospore maturation. **Ascospores** 21.0-26.5 x 7.0-8.5 μm ($\bar{x}$ = 23.5 x 7.7 μm; n = 37), ellipsoidal, 1-septate, slightly constricted at the septum, hyaline, appendaged (Fig. 1C); at each end with 5-7 terminal appendages, 15-18 μm long, round and swollen at the base, 1.4-1.7 μm wide and flat in the middle, and tapering to the thin (0.5) μm round tip.

SUBSTRATE: Lower side of lose dead coral slab.

RANGE: Caribbean (known only from Belize, Central America).

Material examined: Subtidal coral slab, back reef of South Water Cay, Belize, 16°49'N, 88°04'45"W, 24 Nov. 1986, J.K. 5004 (HOLOTYPE, IMS).

Corallicola nana appears to be a rare fungus, because we found only one colony among hundreds of dead coral slabs that we examined in the Caribbean and the Pacific Ocean while looking for *Koralionastes*. Ascomata were attached to the lower side of the coral slab in a rusty-brown area, some of them partly covered by a thin, white crustaceous sponge. *Corallicola nana* is possibly associated with sponges, like species of *Koralionastes* which live in the same kind of habitat (Kohlmeyer & Volkmann-Kohlmeyer 1987, 1990). It must be clarified in this context that the corals are dead, broken off pieces of rubble of variable size; the fungus is not responsible for the damage or death of the coral.

At first sight, *C. nana* appears quite similar to members of the genus *Arenariomyces* Höhnk. Examination of ascomata and ascospores under oil immersion clearly show crucial differences between the new fungus and the type species of *Arenariomyces*, viz. *A. trifurcatus* Höhnk. In *C. nana* the pseudoparenchyma of the centrum has no pit-like connections where the contracted cytoplasm of adjoining cells touches the walls (Fig. 1B) as is the case in *A. trifurcatus* (Kohlmeyer & Kohlmeyer 1968, Pl. 73, Fig. 2). Furthermore, the 5-7 terminal appendages at each ascospore end of *C. nana* are swollen at the base, flat and wide in the middle and tapering to a thin round tip (Fig. 1C); whereas *A. trifurcatus* has 3 subterminal appendages with bulbous bases at each end. The appendages are round in cross section, taper towards the tip and terminate in an apical thickening or bifurcated structure (Jones et al. 1983). Appendages in *A. trifurcatus* grow directly from the spore wall (Jones et al. 1986). The ontogeny of appendages in *C. nana* is conceivably similar, but can be determined with certainty only by electronmicroscopic studies. Finally, there is a major ecological difference between *C. nana* and *A. trifurcatus*. The former grows subtidally offshore on dead coral rocks, whereas *A. trifurcatus* is a member of the intertidal arenicolous beach mycota (Kohlmeyer & Kohlmeyer 1979; Kirk 1983).

2. New records of *Koralionastes* spp.

The initial biogeographic records of five species of *Koralionastes* presented in Kohlmeyer & Volkmann-Kohlmeyer (1987, 1990) are supplemented with numerous additional data based on the evaluation of old and new collections. The collecting sites in the list have the following symbols:

AUSTRALIA

H = Heron Island, Queensland, 23°27'S, 151°55'E
O = One Tree Island, Queensland, 23°30'S, 152°03'E

FIJI

S = Suva, Viti Levu, 18°11'S, 178°27'E
K = near Korolevu, Viti Levu, 18°15'S, 177°42'E

BELIZE, CENTRAL AMERICA

CB = Carrie Bow Cay, 16°48'N, 88°05'W
GNE = Glovers Reef, NE Cay, 16°45'N, 87°45'30"W
GS = Glovers Reef, SW Cay, 16°42'N, 87°49'30"W
SW = South Water Cay, 16°49'N, 88°04'45"W
TO = Tobacco Cay, 16°54'30"N, 88°03'30"W

ST. CROIX, U.S. VIRGIN ISLANDS

CR = Grass Point, 17°44'06"N, 64°36'40"W

Koralionastes angustus Kohlm. & Volkm.-Kohlm.

BELIZE - **CB**: 21 May 1987, J.K. 5024; 15 Oct. 1988, J.K. 5191; **GNE**: 24 Oct. 1988, J.K. 5196, 5204; **SW**: 22 May 1987, J.K. 5025, 23 Oct. 1988, J.K. 5195; 25, 26 May 1989, J.K. 5265, 5267; **TO**: 24, 25, 29 May 1987, J.K. 5013, 5014, 5016, 5020; 2 June 1987, J.K. 5037; 28, 30 May 1989, J.K. 5271, 5273; 3 June 1989, J.K. 5278; 13, 16, 19 March 1990, J.K. 5365, 5366, 5367.

This species had been reported only once before from Carrie Bow Cay, Belize (Kohlmeyer & Volkmann-Kohlmeyer 1987). The 19 collections made on three additional islands in Belize show that *K. angustus* is relatively frequent and has been found in this area in March, May, June, October, and November.

Koralionastes ellipticus Kohlm. & Volkm.-Kohlm.

AUSTRALIA - **H**: 24, 29 Feb., 1 Mar. 1988, J.K. 5127, 5183, 5221, 5222; **O**: 13 Oct. 1989, J.K. 5347. FIJI - **K**: 5 Oct. 1990, J.K. 5424. BELIZE - **CB**: 15, 18, 23 Oct. 1988, 5 June 1989, 18 Mar. 1990, J.K. 5197, 5198, 5199, 5282, 5283, 5359; **GNE**: 24 Oct. 1988, J.K. 5187, 5200, 5201, 5202; **GS**: 31 May 1987, 24 Oct. 1988, J.K. 5012, 5189, 5203; **SW**: 29 May 1989, J.K. 5272; **TO**: 24, 29 May 1987, 30 May, 3 June 1989, 13, 16, 19 Mar. 1990, J.K. 5018, 5019, 5275, 5276, 5279, 5357, 5358, 5360. ST. CROIX - **CR**: 24, 25 Sept., 6 Oct. 1987, J.K. 5045-5048.

Until now, *K. ellipticus* was known only from one island in Belize (South Water Cay; Kohlmeyer & Volkmann-Kohlmeyer 1987). In the Caribbean we collected it on four additional Belizean islands; it is new for the U.S. Virgin Islands, and for the Pacific where we found it on two Australian islands in the Great Barrier Reef and in Fiji. Collections of mature ascomata were made in the Caribbean from March until November (no trips were made in July and August), in the Pacific in February, March and October.

Koralionastes giganteus Kohlm. & Volkm.-Kohlm.

BELIZE - **CB**: 18 Mar. 1990, J.K. 5363; **SW**: 11, 14, 20 Mar. 1990, J.K. 5354, 5355, 5364.

Records of *K. giganteus* existed for two Belizean islands, Southwater and Tobacco Cays (Kohlmeyer & Volkmann-Kohlmeyer 1990); a third, on Carrie Bow Cay, is added herewith. It appears to be much rarer than the other species of *Koralionastes* and mature ascomata were found so far only in March and May.

Koralionastes ovalis Kohlm. & Volkm.-Kohlm.

AUSTRALIA - **H**: 26, 28 Feb. 1988, J.K. 5225, 5228. FIJI - **K**: 5 Oct. 1990, J.K. 5423. BELIZE - **CB**: 23 May 1987, 15, 18 Oct. 1988, 5 June 1989, 18 Mar. 1990, J.K. 5015, 5017, 5029, 5030, 5031, 5205, 5208, 5281, 5368; **GNE**: 24 Oct. 1988, J.K. 5214, 5215, 5216; **GS**: 24 Oct. 1988, J.K. 5217, 5218, 5219; **SW**: 22 May 1987, 16, 17, 22, 23 Oct. 1988, 25, 27 May 1989, 17 Mar. 1990, J.K. 5025, 5182, 5206, 5207, 5210, 5211, 5212, 5213, 5266, 5268, 5362; **TO**: 24, 25 May, 2 June 1987, 21 Oct. 1988, 28, 30 May, 3 June 1989, 13, 16, 19 Mar. 1990, J.K. 5032, 5033, 5037, 5209, 5269, 5274, 5277, 5280, 5356, 5361, 5369.

This species was known before only from two collections in Belize on Curlew Bank and South Water Cay (Kohlmeyer & Volkmann-Kohlmeyer 1987). The numerous collections of *K. ovalis* made on four additional islands in Belize, as well as in Australia and Fiji indicate that it is nearly as common and widely distributed as *K. ellipticus*. We found mature ascomata in the Caribbean from March to June and October and November, in Australia in February and in Fiji in October.

Koralionastes violaceus Kohlm. & Volkm.-Kohlm.

FIJI - **S:** 3, 6 Oct. 1990, J.K. 5422, 5425.

So far, *K. violaceus* is known only from the Pacific Ocean, the Australian Great Barrier Reef and Fiji. Ascospores from Fiji are mostly 5-septate and 86-127 x 27-40 µm ($\bar{x}$ = 103 x 34 µm; n = 60), compared to the slightly thinner, mostly 4- to 5-septate spores from Australia that are 85-130 x 25-34 µm ($\bar{x}$ = 107 x 30 µm; n = 105; Kohlmeyer & Volkmann-Kohlmeyer 1990).

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A NEW PHOMOPSIS WITH LONG PARAPHYSES

F. A. UECKER

Systematic Botany and Mycology Laboratory
USDA, Agricultural Research Service
Beltsville Agricultural Research Center
Beltsville, Maryland 20705

and

KER-CHUNG KUO

Taiwan Agricultural Chemicals and Toxic
Substances Research Institute
189 Chung Cheng Road, Wufeng,
Taichung Hsien, Taiwan 41301
Republic of China

ABSTRACT

A new species, *Phomopsis longiparaphysata*, is described from grapes in Taiwan. This fungus is distinctive because of its long, narrow, branched paraphyses and is the second *Phomopsis* described with paraphyses. It has also been isolated from fruits of *Spondias* sp. from Jamaica and from *Anacardium occidentale* from Kenya. Illustrations of all three isolates of *P. longiparaphysata* are provided along with illustrations of type material of *P. anacardii*.

KEY WORDS: *Phomopsis longiparaphysata* sp. nov., *Phomopsis javanica, Phomopsis anacardii, Spondias* sp., *Anacardium occidentale, Vitis sp. Cv. Black queen*

During a study of *Phomopsis* on asparagus *P. javanica* Uecker & Johnson (1991) was described as new because it exhibited sterile hypha-like structures extending from conidiophores or arising between conidiophores. Sutton (1980) used the term paraphyses for similar sterile hyphae in his descriptions of other genera of phialidic coelomycetes in which the sterile hyphae are found. Such structures had not previously been noted in *Phomopsis* despite the existence of more than 800 different epithets already published in this genus (Uecker, 1988). The earliest indication of paraphyses is found in an illustration of *Phomopsis theae* Petch (Punithalingam and Gibson, 1972), which showed a single paraphysis-like component. No reference to it occurred either in the text or in the legend to the figure. Another species, *P. anacardii* Early & Punithalingam (1972), was described with simple or branched conidiophores that sometimes were up to 75µm long, apparently referring to the components that we call paraphyses. However, Punithalingam (in litt., 1990) and co-workers believed that conidiomata of some species only occasionally have such structures, which they further believed are derived from sterile or underdeveloped conidiophores.

Two recently acquired isolates along with a specimen from IMI were found to possess long, narrow, branched paraphyses. This paper describes and illustrates a new species, *Phomopsis longiparaphysata,* that is morphologically distinct because of its unique paraphyses.

MATERIAL AND METHODS

The first isolate of *Phomopsis longiparaphysata* that came to our attention was designated ELP by K.-C. Kuo. The fungus originally came from fruit of grape (*Vitis sp. Cv. Black Queen*) collected in Taiwan in May, 1989. The original specimens have been lost but cultures have been maintained continuously on autoclaved pieces of stems of grape, asparagus (*Asparagus officinalis* L.), and alfalfa (*Medicago sativa* L.) on water agar (WA) as FAU-488. Methods for fixation, imbedding in paraffin, staining, rehydration of herbarium specimens, and study of conidia and conidiogenous apparatus have been detailed previously (Uecker and Johnson, 1991). To determine whether the conidiogenous apparatus and paraphyses varied morphologically at any stage of development from those at other stages and from those on the other host substrates, *P. longiparaphysata* was grown on stem pieces of alfalfa, asparagus, and grape on WA plates for 40 days. The conidiogenous apparatus and paraphyses were observed and photographed on days 5,6,7,8,12,13,15,18,22,26,28,32, and 34 after plating.

RESULTS

Phomopsis longiparaphysata Uecker et Kuo, sp. nov.

Mycelium hyalinum, immersum, ramosum, septatum; conidiomata brunnea vel atris, simpliciter eustromatica, immersa, plerumque dissita raro confluentia, ampulliformia vel complanata, loculo solitari interdum convoluto, 175-430(-550)µm longo x 157-275µm lato, paries fuscus apicem versus, ad latera et infimum juventute pallidior, textura angularis; ostiolum papillatum, plerumque solitarium, circulare, 15-20µm diam; conidiophora hyalina, brevia vel elongata, plus minusve decrescentia, et basi et super septata, praeter terminalem ramo laterale longo vel breve infra septum omnes cellulae conidiophori conidiogenentes, 10-40 x 2-4µm; cellulae conidiogenae enteroblasticae, phialidicae, integratae vel discretae, hyalinae, apertura in ramo laterali apicali, canale et collulo minutis, spissitudine periclinali crassa vel non, 10-20 x 2-4µm; conidia acropleurogena; conidia alpha hyalina, aseptata plerumque biguttulata, elliptica vel fusiformia-elliptica, (5-)6-7(-11) x 2-2.5(-3.5)µm; conidia beta non visa; paraphyses hyalinae, septatae, ramosae, e cellula terminale conidiophori vel e cellulas iisdem atque conidiophoris, usque 130 x 1-2µm, in conidiomatibus plerumque abundantes.

Mycelium hyaline, immersed, branched, septate; conidiomata (Fig. 1) eustromatic, immersed, usually separate but sometimes confluent, brown to black, nearly globose to elliptic, ampulliform or flattened, 175-430 (-550) x 157-275µm wide, unilocular (Fig. 2), walls of the locule often convoluted, wall of textura angularis; usually one ostiole but sometimes more, usually papillate, circular, 15-20µm diam; conidiophores (Figs. 3,4) 10-40 x 2-4µm, hyaline, short or elongate, more less tapered toward the apex, septate both at base and above, each cell of the conidiophore except the terminal one producing a short or long lateral branch just below the septum and becoming conidiogenous; conidiogenous cells (Figs. 3,4) 10-20 x 2-4µm, enteroblastic, phialidic, integrated or discrete, hyaline, aperture apical on the lateral branch, channel and collarette minute, periclinal thickenings of variable thickness; conidia acropleurogenous; alpha conidia (Fig. 5) hyaline, aseptate, usually biguttulate but sometimes with one large or several small guttules, elliptic or fusiform-elliptic, (5-)6-7(-11) x 2-2.5(-3.5)µm; beta conidia not seen; paraphyses (Fig. 3,4) hyaline, septate, arising from the terminal cell of the conidiophore or from the same cells that give rise to conidiophores, usually branched, free at tips, to 130 x 1-2µm, generally abundant.

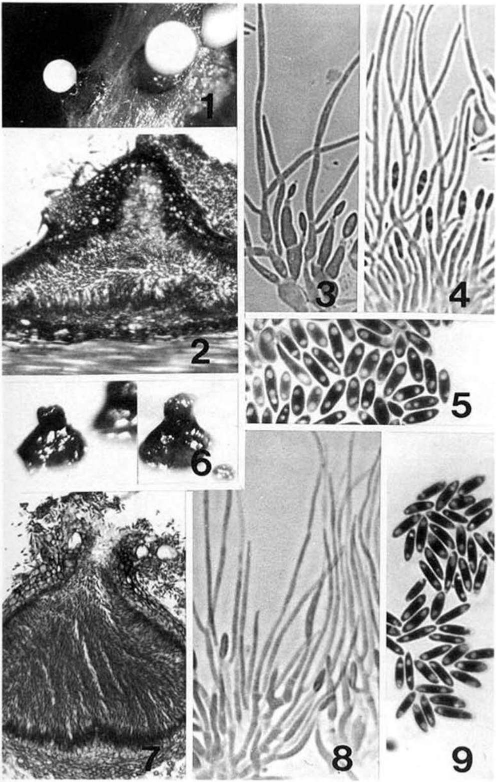
HOLOTYPE: US 1108873, on sterilized stems of alfalfa (*Medicago sativa* L.) in BPI. Isotypes in NY, DAOM, and IMI (abbreviations from Holmgren et al., 1990). Isolated from fruits of *Vitis sp. Cv. Black Queen* at Er-lin, Chang-Hwa Hsien, Taiwan.

The series of photographs taken 5-34 days after inoculation on stems of alfalfa, asparagus and grape on WA showed that five days after inoculation paraphyses were present on asparagus and alfalfa but not on grape. On the sixth day longer, branched paraphyses were present on all three hosts and by day seven some paraphyses were up to 90um long. Long, thin, branched paraphyses were present at all sampling times through 34 days. They were more numerous on day eight and thereafter than on days five to seven. The longest paraphyses, up to 130µm, were observed on and after day 18.

Two other isolates are considered to belong to this species. The first of these, designated as FAU-500, was isolated from fruit of *Spondias* sp. from Jamaica. Fruits were intercepted by inspectors from U.S. Animal and Plant Health Inspection Service at J.F. Kennedy International Airport on XI/16/1989, seq. no. 100 for that date. Conidiomata (Fig. 6) fall within the same size range as those of the type. The wall of the conidioma (Fig. 7) is typical textura angularis. Dimensions of the paraphyses and of the conidiophores (Fig. 8) are likewise similar. The isolate from *Spondias* has longer alpha conidia (6.5-)8-9(-10) than does the type (5-)6-7(-11)µm. Otherwise there is little to distinguish it from the type. Beta conidia have not been found either in the isolate from *Spondias* or in the type.

The second isolate that we consider conspecific with *Phomopsis longiparaphysata* is IMI 136470, labeled *Phomopsis* sp. on *Anacardium occidentale* L., coll. R. Prasad, det. M.P. Early, from Min. of Agriculture, Nairobi, Kenya, August, 1968. Conidiomata (Figs. 10,11) were black, ostiolate, papillate, (238-)370-520

Figs. 1-9. *Phomopsis longiparaphysata*. 1-5 from type culture on alfalfa stem on WA. 1. Habit, X46. 2. Section through conidioma, X193. 3. Portion of conidiogenous layer showing septate conidiophore with conidiogenous branch emerging from below septum and branched paraphyses arising from same cell as the conidiophore, X1000. 4. Portion of conidiogenous layer showing conidiophores and long, branched paraphyses, X1000. 5. Alpha conidia, X1000. 6-9 from isolate FAU-500 on *Spondias* from Jamaica grown on alfalfa stem on WA. 6. Habit, X50. 7. Section through conidioma, X280. 8. Portion of conidiogenous layer showing conidiophores and long, branched paraphyses, X1000. 9. Alpha conidia, X1000.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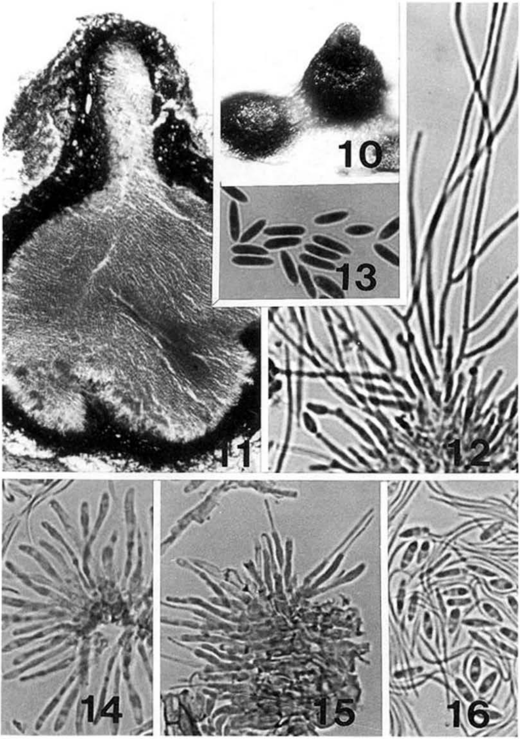
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x (213-)390-620µm high. Conidiophores (Fig. 12) were 10-30 x 1.5-2µm, more or less tapered near the apex. Conidiogenous cells (Fig. 12) were 10-20 x 1.5-2µm. Alpha conidia (Fig. 13) were (6-)7-9 x 2-2.5µm. Beta conidia were not seen. Of 16 specimens sent in response to a loan request for specimens of *Phomopsis anacardii*, this was the only one that showed long, narrow, branched paraphyses. This isolate is considered distinct from *P. anacardii* (Type: IMI 144866), which was described from leaves of *Anacardium occidentale* from Coast Province of Kenya. Punithalingam (1985) further reported *P. anacardii* from Africa (Gambia, Guinea, Mozambique, Nigeria, and Zambia); Asia (Bangladesh, Burma, India, Malaysia); Central America; and West Indies (Cuba, Jamaica). Conidiomata of *P. anacardii* were described as up to 600µm wide, black or blackish brown, numerous, stromatic, solitary or aggregated, unilocular or multilocular, ostiolate. Conidiophores were hyaline, simple or branched, septate or non-septate, cylindric to obclavate, straight, 10-18 x 3-5µm, sometimes up to 75µm long. Such long conidiophores were not illustrated either in the original illustrations or in the photographs provided by Punithalingam (1985). The portion of the type specimen that we examined had neither long conidiophores up to 75µm in length nor any other elements extending above the usual height of the conidiogenous cells. Young conidiogenous cells (Fig. 14) were up to 24µm long and 3µm wide with rounded apices. Beta conidia later developed from such conidiogenous cells (Fig. 15). The few conidiomata present on this specimen contained many beta conidia but considerably fewer alpha conidia. Conidiogenous cells with alpha conidia still attached were not seen. Alpha conidia were described as 6-8(-10) x 2-2.5(-3)µm. The ones we saw (Fig. 16) were 5-6(-9) x 2-2.5µm, with 87% in the 5-6µm range. Beta conidia were 20-26 x 1µm.

Figs. 10-16. 10-13 *Phomopsis longiparaphysata*, IMI 136470 on dried agar. 10. Habit, X50. 11. Section through conidioma, X137. 12. Portion of conidiogenous layer showing conidiophores and long branched paraphyses, X1000. 13. Alpha conidia, X1000. 14-16 *P. anacardii*, IMI 144866 ex type. 14. Young conidiogenous cells, X1000. 15. Portion of conidiogenous layer producing mostly beta conidia, X1000. 16. Alpha and beta conidia, X1000.

DISCUSSION

Little work has been done with coelomycetes that possess paraphyses. Sutton and Sellar (1966) pointed out that there are few references in the literature to such sterile hyphae in Sphaeropsidales and Melanconiales. Several terms have been used for them. Clements and Shear (1931) used the term pseudoparaphyses for such structures in *Lichenophoma* Keissler, *Pleosphaeropsis* Died., *Cytoplea* Bizz. & Sacc., *Camarographium* Bubak, *Lagynodella* Petrak, *Gloeodes* Colby, and *Michenera* Berk. & Curtis. Bender (1934) used the term paraphyses in *Pleosphaeropsis, Camarographium*, *Lagynodella*, *Gloeodes*, *Plectophomella* Moesz, *Sphaeronaemopsis* Speg., *Naemosphaera* (Sacc.) Karst. and *Macrophomopsis* Petrak. Petrak and Sydow (1927) referred to pseudoparaphysoids in *Coleophoma* Hoehnel. Sutton (1980) employed the term paraphyses to refer to sterile hyphae in *Amerosporium* Speg., *Aschersonia* Mont., *Coleophoma, Massariothea* Sydow, *Phaeocytostroma* Petrak, *Plectophomella, Pseudorobillarda* Morelet, and *Titaeospora* Bubak. Further studies are needed to determine if all these structures are homologous. We found no mention of any of them becoming conidiogenous, although Sutton (1980) in the description of *Titaeospora* mentioned that paraphyses formed from the acervular tissue and from conidiogenous cells.

It seems problematical that paraphyses sometimes become conidiogenous and that they arise from the innermost layer of cells that also gives rise to conidiophores. Whether paraphysis is the appropriate term to apply to these structures is uncertain. If they usually become conidiogenous, then "immature conidiophores" might be appropriate. The term "paraphysis" implies that they are sterile structures with questionable function. It seems reasonable to use the latter term as long as the structure is sterile and call it a conidiophore when conidium formation begins.

This is the second species of *Phomopsis* that is considered distinctive because of its paraphyses. The first, *P. javanica,* has much shorter, broader, mostly unbranched paraphyses, has distinctly larger alpha conidia, and produces beta conidia. No teleomorph is known for either.

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We thank the director of herbarium IMI for sending critical specimens and James S. Plaskowitz for photographic assistance. Mary E. Palm, Martin M. Kulik, and James F. White, Jr. reviewed the manuscript.

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PODOSORDARIA INGII SP. NOV. AND ITS LINDQUISTIA ANAMORPH

Jack D. Rogers
Department of Plant Pathology
Washington State University
Pullman, Washington 99164-6430

and

Thomas Laessøe
Royal Botanic Gardens
Kew, Richmond
Surrey TW9 3AE
England

ABSTRACT

Podosordaria ingii sp. nov. is described and illustrated from a frond of *Phoenix dactylifera* collected in the Canary Islands. Its *Lindquistia* anamorph is described on features of synnemata produced in nature and in culture.

A collection of a new species of *Podosordaria* Ellis & Holw. was kindly made available to us by the collector, Bruce Ing, Chester College, Chester, UK. We have named this fungus in honor of the collector. Fortuitously, the anamorph was present in close association with the teleomorph. The anamorph was likewise produced in cultures initiated from ascospores, allowing the anamorph-teleomorph connection to be made unequivocally.

Podosordaria ingii J. D. Rogers & Laessøe, sp. nov. Figs. 1-16.

Capitula stromatum rotunda, irregulariter compressa, usque ad 1 cm lata x 4 mm crassa, stipitibus usque ad 2 cm longitudine x 2 mm diam, extus fulva cum ostiolis atris, intus alba. Textura satis dura, strato carbonaceo destituto. Superficies asperata a ambitibus peritheciorum et rugis. Perithecia 0.3-0.5 mm diametro. Ostiola papillata. Asci octospori, cylindrici, stipitati, 110-130 μm longitudine tota x 7-9 μm crassi, partibus sporiferis 65-72 μm longitudine, annulo apicali in liquore iodato Melzeri cyanescente, cuneato, 1.5 μm alto x 2.9 μm crasso. Paraphyses simplices, copiosae. Ascosporae brunneae, unicellulares, ellipsoideo-inaequilaterales, leves, (8-) 9-10.5 x 4.5-5 (-6) μm, rima germinativa recta ventrali longa praeditae.

Status agamicus ad *Lindquistiam* pertinet. Synnemata consociata cum statu sexuali. Pars fertilis clavata, nivea, usque ad 1 mm diam, in stipite atros ca. 0.5 mm diam, usque ad 3 mm longitudine tota. Conidia et apparatus conidicus ut in genere.

Stromatal heads rotund, irregularly compressed, up to 1 cm broad x 4 mm thick, with stipes up to 2 cm long x 2 mm diam (Fig. 1), externally tawny with black ostioles, internally white. Texture fairly hard, lacking carbonaceous layer. Surface roughened by perithecial contours and wrinkles (Fig. 8). *Perithecia* 0.3-0.5 mm diam. Ostioles papillate. *Asci* eight-spored, cylindrical, stipitate, 110-130 µm total length x 7-9 µm broad, with the spore-bearing part 65-72 µm long, with apical ring bluing in Melzer's iodine reagent, cuneate, 1.5 µm high x 2.9 µm broad (Fig. 5). Paraphyses simple, abundant. *Ascospores* brown, unicellular, ellipsoid-inequilateral, smooth, (8-) 9-10.5 x 4.5-5 (-6) µm, with straight ventral germ slit spore-length (Figs. 6 and 7).

Asexual state belongs to genus *Lindquistia* Subram. & Chandrashekara. Synnemata associated with sexual state. Fertile part clavate, whitish, up to 1 mm diam, on blackish stipe ca. 0.5 mm diam, up to 3 mm total length (Fig. 3). Conidia and conidiogenesis as described for the genus.

SPECIMEN EXAMINED: CANARY ISLANDS: Gomera, San Sebastian de la Gomera, 17°03'W, 28°04'N, cultivated zone, 14.I.1990, Ing, B., rotten frond of *Phoenix dactylifera* L. (K:HOLOTYPE).

Lindquistia anamorphic state and culture of *P. ingii*. Figs. 2 - 4, 9-16.

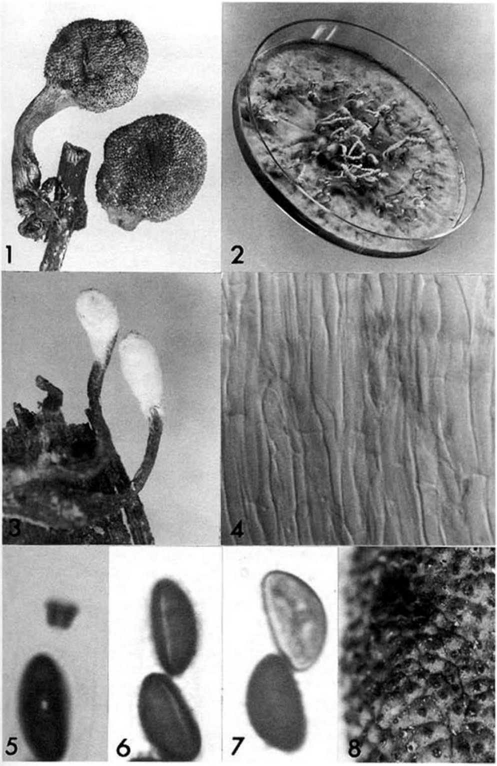
Colonies on 2% oat meal agar at ca. 20° C under 12 h fluorescent light covering 9 cm diam Petri plate in 2 wk, with mycelium more or less tomentose, tawny to tan to brown. Reverse yellowish. Synnemata produced in 3-4 weeks (Fig. 2). Synnemata producing light to deep rose-colored to purplish pigmentation in 2% KOH.

Synnemata cylindrical to clavate, up to 2 cm high x 1-2 mm diam, tawny, with the stipe bearing conidia overall except for a basal portion. Hyphae of central stipe (2-) 2.5-4.5 (-5) µm diam, hyaline to yellowish. *Conidiophores* loosely arranged, branched, indeterminate in length, (1.5-) 2-4 (-5) µm diam, hyaline to yellowish, with many hyphal cells bearing one to several more or less globose,

Figs. 1-8. *Podosordaria ingii* and its *Lindquistia* anamorph. 1. Teleomorphic stromata, X 2. 2. Culture showing synnemata near center, X 0.6. 3. Synnemata on natural substrate, X 12. 4. Orientation of hyphae in synnematal stipe, X 1000. 5. Ascus tip above ascospore, X 2200. 6. Ascospores showing germ slits, X 2200. 7. Ascospores, X 2200. 8. Detail of teleomorphic stromatal surface showing perithecial contours and ostioles, X 16.

Figs. 1-3, 8 by photomacrography. Fig. 4 by differential interference contrast micrography. Figs. 5-7 by brightfield microscopy.

Fig. 5 from material mounted in Melzer's reagent. Figs. 6 and 7 from material mounted in water.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sessile to subsessile conidiogenous cells (Fig. 16). *Conidiogenous* cells produced holoblastically, 3-4 µm diam, each cell producing one to several conidia holoblastically (Figs. 9, 10, 13-16). Conidiogenous cells eventually bearing one to several inconspicuous secession scars. *Conidia* hyaline, smooth, ellipsoid, obovoid, or globoid, with flattened bases, (2-) 2.5-4.5 (-5) x 2-3 µm (Figs. 11, 12, 14-16).

Cells of conidiophores and conidiogenous cells eventually disarticulating, forming a dusty mass composed of conidia, conidiogenous cells, and hyphal fragments (Figs. 12, 16).

Culture deposited in American Type Culture Collection as number 76588. Dried culture deposited in WSP and K.

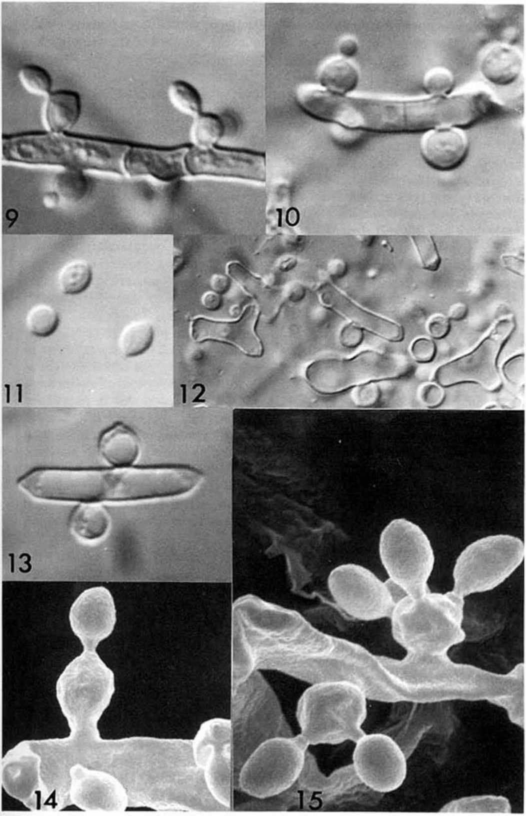
Podosordaria ingii has some features reminiscent of *P. jugoyasan* (Hara) Furuya & Udagawa. Conidiogenous cells and conidia of these species are very similar (Furuya and Udagawa, 1977). However, the latter species has much smaller stromata with more discoid fertile parts, occurs on hare dung, has ascospores with the germ slit conspicuously less than spore-length and a gelatinous sheath and, probably, bears its anamorph on the immature teleomorphic stroma as discussed later herein (see Furuya and Udagawa, 1977, for a description of *P. jugoyasan*). *Podosordaria ingii* likewise differs from *P. hircinia* (Tai & Wei) Krug & Cain, a species that has somewhat larger and darker ascospores and occurs on goat dung (Krug and Cain, 1974). The anamorph of *P. hircina* apparently has not been described.

Several taxa of *Podosordaria* and *Poronia* Willd.:Fr. have been reported from plant materials. *Poronia johorensis* (Morgan-Jones & Lim) Morgan-Jones has an anamorph with conidiogenous features much like those of *Podosordaria ingii*, but a teleomorph that differs greatly (Morgan-Jones and Hashmi, 1973; Morgan-Jones and Lim, 1968). *Poronia ustorum* Pat. has ascospores of the size range of *Podosordaria ingii*, but examination of type material [Patouillard no. 49, 1887. (FH)] corroborates Dennis' description (1957) [as *Xylaria ustorum* (Pat.) Dennis] of a fungus with much smaller white stromata. Some additional taxa that are probably related to *Podosordaria ingii*, but with much different ascospore characteristics are discussed elsewhere (Rogers et al., 1992).

Because of its *Xylaria*-like aspect attempts were also made to equate it with a named *Xylaria*. Biologically and taxonomically, however, its assignment must be to either *Poronia* or *Podosordaria*, based on the distinctive and characteristic *Lindquistia* anamorph (Rogers, 1985). Unfortunately, two influential publications have listed the anamorphs of *Poronia* species as *Xylocladium* Syd. (Carmichael et al., 1980; Kendrick and DiCosmo, 1979), a form-genus unlike *Lindquistia* in most important respects and inevitably associated with *Camillea* Fr.

Figs. 9-15. *Lindquistia* state of *Podosordaria ingii*. 9 and 10. Hyphae bearing conidiogenous cells, some of which bear conidia, X 1800. 11. Conidia, X 1800. 12. Disarticulated hyphae and conidiogenous cells and conidia, X 1000. 13. Hyphal cell bearing two conidiogenous cells, X 1900. 14 and 15. Conidiogenous cells bearing one to several conidia, X 7,000.

Figs. 9-13 by differential interference contrast microscopy. Figs. 14 and 15 by scanning electron microscopy.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species (Rogers, 1985). Distinctions between *Poronia* and *Podosordaria* are not clearly demarcated. *Podosordaria* was originally separated from *Poronia* on the convex surface of the fertile stroma of the former genus and the more or less plane surface of the latter. Martin (1970) greatly redefined and extended *Podosordaria* on the supposed lack of an ectostroma, including a number of traditional *Xylaria* species and some other lignicolous and graminicolous taxa. Krug and Cain (1974) restricted *Podosordaria* to coprophilous taxa and included two uniperitheciate species.

In general, we accept the Krug and Cain concept of *Podosordaria*, but include taxa that occur on substrates other than dung. Ironically and inconveniently, the type species of both *Poronia* and *Podosordaria* were described from dung and both genera include species that were collected from non-dung substrates (Morgan-Jones & Hashmi, 1973; Rogers et al., 1992). Limited cytological data suggest that *Poronia* and *Podosordaria* might be separated on nuclear condition of mature ascospores--binucleate or quadrinucleate in two investigated *Poronia* species (Rogers, 1970) and uninucleate in one investigated *Podosordaria* species (Rogers, 1973). It is likewise possible that further investigations will support uniting all taxa under the older name, *Poronia*, as already suggested by Koehn & Cole (1975).

Most *Podosordaria* and *Poronia* species grown in culture produce the *Lindquistia* state on the immature ascigerous stroma. The dusty products of conidiogenesis and disarticulation blow away and perithecial ostioles begin to appear on the upper stromatal surface. In *Podosordaria jugoyasan* stromata more or less morphologically typical of ascigerous stromata produced in nature are formed in culture, but these produce only the anamorph (Furuya & Udagawa, 1977). With this fungus, however, it is suspected that the teleomorph could be induced to develop from such stromata. In *Podosordaria ingii*, however, synnemata are produced along with the teleomorph in nature (Fig. 3). It is not known if synnemata are, in reality, incipient teleomorphs. Cultural evidence suggests, however, that the *Lindquistia* state is separate from the teleomorphic state in that it does not develop structures indicative of a teleomorph, even an immature one.

The synnemata produced in culture can be classified, as follows, using the recent anatomical system proposed by Seifert and Okada (1990). Synnemata are *indeterminate* in that the stipe continues to grow after sporulation begins. The central stipe is of *parallel* hyphae (Fig. 4), becoming of textura intricata as conidiophores diverge toward the periphery. The hyphal system is *monomitic.* The sporulating zone or capitulum is *divergent* (*loose*) to apparently *random.* This contrasts with the *hymenial* type of capitulum in many *Xylaria* species including the type where the conidiogenous cells are in a palisade.

The conidia of *Lindquistia* germinate readily, as do those conidiogenous cells and disarticulated hyphal cells that have not become devoid of cytoplasm

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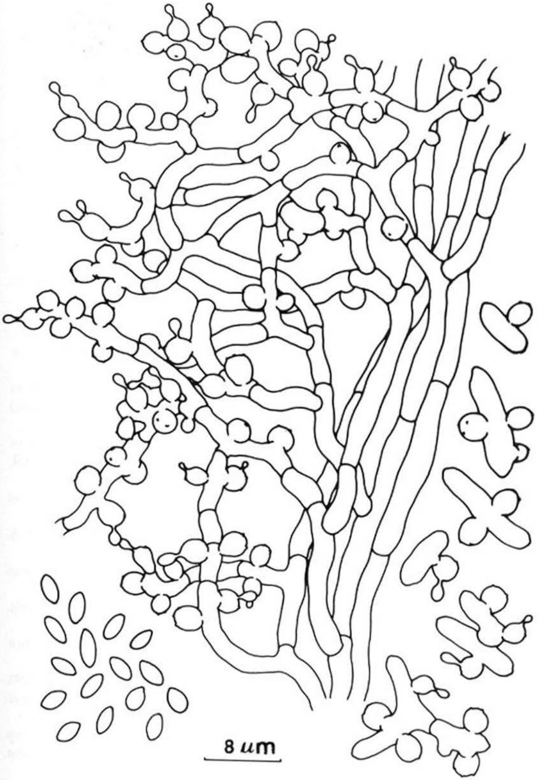
Fig. 16. *Lindquistia* state of *Podosordaria ingii.* Camera lucida depiction of conidiophores, conidiogenous cells, and conidia from culture. Drawing by Y.-M. Ju. Line = 8 μm.



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(Figs. 12 and 13). Ascospores likewise germinate readily without a requirement for heat activation as is usual for some other *Podosordaria* and *Poronia* species.

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ARTHROBOTRYS FEROX SP. NOV., A SPRINGTAIL-CAPTURING HYPHOMYCETE FROM CONTINENTAL ANTARCTICA

Silvano Onofri and Solveig Tosi

Facoltà di Scienze Matematiche Fisiche e Naturali, Università della Tuscia, via S. Camillo de Lellis, 01100 Viterbo, Italy

Abstract

In studies of the mycoflora in Victoria Land of Continental Antarctica, a species of *Arthrobotrys*, which has not been described previously, was discovered. This Hyphomycete, proposed here as a new species under the name of *A. ferox*, produces aerial predaceous organs consisting of ovoidal cells surrounded by an adhesive secretion and supported by a 2-celled stalk. Frequently, it was observed capturing springtails belonging to the Antarctic species *Gressittacantha terranova* Wise by means of these organs.

Introduction

During the Italian Antarctic Expeditions, a species of *Arthrobotrys* was isolated from the moss species *Bryum algens* Card. and *Ceratodon purpureus* (Hedw.) Brid., collected in Kay Island, Edmonson Point and Baker Rocks (Wood Bay, Victoria Land, Antarctica) (Onofri & Tosi, 1989; Onofri & Tosi, 1990; Tosi *et al.*, 1990). This Hyphomycete produces interwoven aerial hyphae that carry ovoidal cells supported by 2-celled stalks and surrounded by an adhesive secretion. These vesicles are able to capture relatively large springtails of the species *Gressittacantha terranova* Wise (about 1.2 mm in length). Arthropods are seldom captured by predaceous fungi. Among Hyphomycetes only *Arthrobotrys entomopaga* Drechsler produces stalked adhesive vesicles, sorrounded by an adhesive mucilage, which are able to capture small springtails (0.35 mm in length) of the genus *Sminthurides* (Drechsler, 1944). This species was neotypified (van Oorschot, 1985) by the type isolate of *A. pauca* J.S. McCulloch described as producing adhesive spherical knobs without a mucous secretion surrounding it and capturing nematodes (McCulloch, 1977).

Some species of other genera also have adhesive vesicle-like capture organs; among them *Dactylella*, *Monacrosporium* and *Nematoctonus* (the latter is characterized by clamp connections): none of the species in these genera is known as springtail predator.

The species here described frequently presents branched conidiophores; within *Arthrobotrys* only *A. arthrobotryoides* (Berlese) Lindau, *A. cladodes* Drechsler, *A. robusta* Duddington (Haard, 1968) and *A. botryospora* Barron (Van Oorschot, 1985) possess branched conidiophores, but they have conidia of different shape *(A. arthrobotryoides)* , dimensions *(A. cladodes)* and both shape and dimensions *(A. robusta)*, conidia produced only at the apex of the conidiophore and its branches *(A. cladodes)* , different conidiophore shape *(A. robusta)* or typically aseptate conidia *(A. botryospora)*. Moreover, some of them are known to be nematophagous and form adhesive loops as organs of capture. Among the species with unbranched conidiophores, the conidia of the present species have some morphological affinities with *A. superba* Corda, (but the latter possesses conidia not constristricted at the septum) and with *A. oligospora* Fresenius which captures nematodes by means of a tridimensional network, lacks vesicles and has conidia with the distal cell distinctly longer than the proximal one. The vesicles of this Antarctic Hyphomycete are similar to those of *A. entomopaga*, which measure 6-10 µm (Roxon & Jong, 1975), but they are larger and normally supported by 2-celled, instead of 1-celled stalks. Moreover, it differs from the species here described, in the unbranched conidiophores that present peg-like sterigmata, in producing conidia tapering to a protruded base and not constricted at the septum. On the base of these observations, we therefore propose a new species in *Arthrobotrys* Corda emend. Schenck, Kendrick & Pramer (1977) to accommodate our isolates.

This species is the first predaceous hyphomycete collected in Continental Antarctica, the first springtail-predaceous one in the Antarctic Continent (Gray, 1982; Gray *et al.*, 1982; Gray & Lewis Smith, 1984) and the second springtail-predaceous Hyphomycete known.

Arthrobotrys ferox Onofri & Tosi, sp. nov.
Etym.: *ferox*, ferocious.

Coloniae in CYA albae vel deinde tarde luteo-roseae. Mycelium hyalinum; hyphae repentes et aeriae, 4.5-6.5 µm crassae, intervallis 22-35(-45) µm septatae. Conidiophora macronematosa, mononematosa, erecta, septata, saepe ramosa, (33.5-)44-144(-466) µm longa, prope basim 5-7 µm crassa et apicem versus 3-4 µm, (2-)4(-10) conidia e denticulis 2-

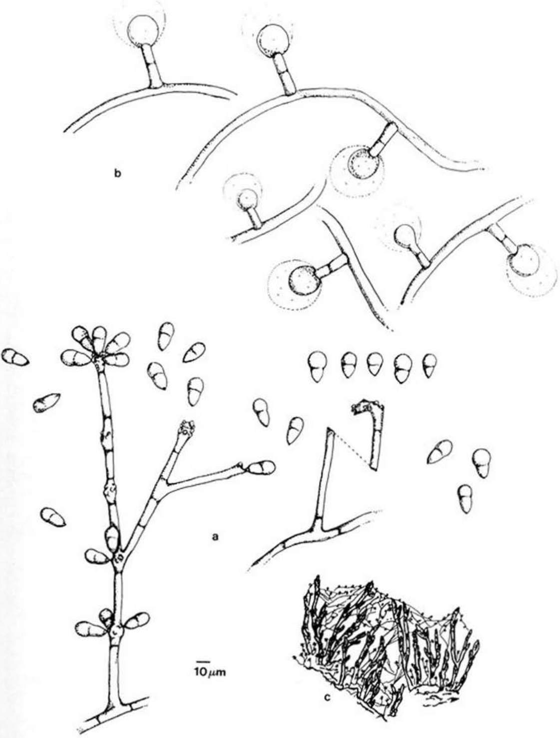

Fig. 1. *Arthrobotrys ferox* ROHB 400 A. a) Conidiophores and conidia. b) Aerial hyphae with predaceous organs. c) Habit sketch (on natural substratum).

4.5 μm longis formantia. Conidia hyalina, obovata-clavata, in medio 1-septata, ad septum modice constricta, (13-)15-18(-24.5)x(5-)6-8(-9) μm.

Collembola (*Gressittacantha terranova* Wise) depraedans; ex hyphis aereis ramuli bicellulares oriuntur qui vesiculam ovoideam adhaesivam (18-)20-25(-31)x(16.5-)17-21(-24.5) μm formant, involucro glutinis circumdatam.

In musco *Bryum algens* Card., Edmonson Point, Wood Bay, Terra Victoria, Antarctica (quo continentem attinet), G. Del Frate, 23 Feb. 1988, ROHB 400A, holotypus, cultus CBS 245.91.

Colonies on Czapek yeast agar white to pale pink-orange, mycelium hyaline; repent and aerial hyphae, 4.5-6.5 μm wide, septate at intervals of 22-35(-45) μm. Conidiophores macronematous, mononematous, erect, septate, often branched, (33.5-)44-144(-466) μm long, 5-7 μm wide at the base and 3-4 μm farther upward, producing (2-)4(-10) conidia on 2-4.5 μm long denticles. Conidia hyaline, obovoidal to clavate, 2-celled, 1-septate, slightly constricted at the septum which is usually in the middle, (13-)15-18(-24.5)x(5-)6-8(-9) μm. Predatory on springtails *(Gressittacantha terranova)* ; aerial predaceous organs consisting of ovoidal cells, (18-)20-25(-31)x(16.5-)17-21(-24.5) μm, surrounded by an adhesive secrection and supported by a commonly 2-celled stalk, (9-)14-24(-34)x4.5-7 μm. It grows well at room temperature.

Specimens examined. All were collected on mosses of *Bryum algens* Card. and *Ceratodon purpureus* (Hedw.) Brid., in Wood Bay, Victoria Land, Continental Antarctica: ROHB 400A (holotype), Edmonson Point, 23 Feb. 1988, G. Del Frate; ROHB 401A and ROHB 402 A, Baker Rocks, 26 Dec. 1988, S. Onofri; ROHB 403A, Edmonson Point, 29 Dec. 1988, S. Onofri; ROHB 404A, 5 Jan. 1989, G. Carchini; ROHB 405A, Kay Island, 16 Jan. 1989, S. Onofri.

Two strains are deposited in the CBS culture collection: CBS 245.91 ex holotype of ROHB 400A; CBS 137.91 ex ROHB 404 A.

Discussion

Among the predaceous fungi only species of *Arthrobotrys* are known to capture springtails (Drechsler, 1944). *Arthrobotrys* contains about 25 predaceous species; and vesicles are produced only by *A. entomopaga*.

It is very difficult to induce the production of predaceous organs of *A. ferox* in pure cultures. In fact we obtained the production of vesicles in pure culture only once, observing a predaceous organ produced in a pure culture obtained from the specimen ROHB 404A of *A. ferox*, growing on cornmeal agar, after one year of cultivation (fig. 2 d). On the Antarctic mosses, directly observed just after collection or after maintenance in a moist chamber, it was possible to find conidiophores and aerial hyphae

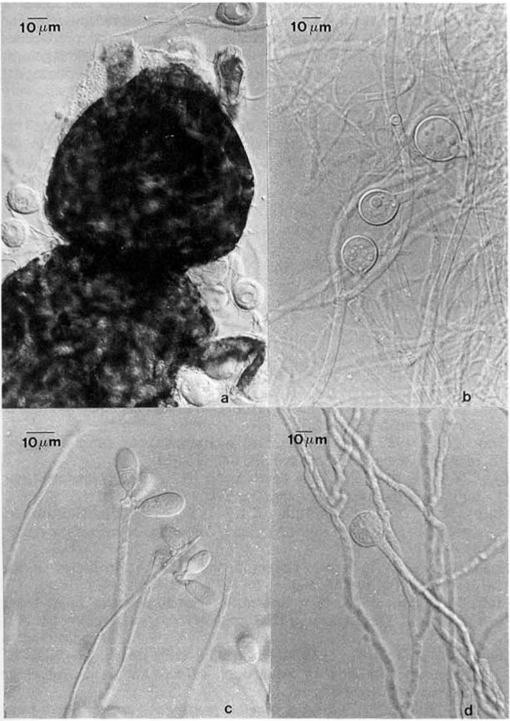

Fig. 2. a) Fore-end of the springtail *Gressittacantha terranova* sorrounded by the vesicles of *Arthrobotrys ferox.* b) Vesicles from the natural substratum. c) Conidiophores and conidia of *Arthrobotrys ferox* in pure culture on CYA. d) Vesicle in pure culture on cornmeal agar.

bearing vesicles closely associated. A constant pattern of two kinds of mycelium was observed on the moss: the interwoven reproductive hyphae were always supported by a repent mycelium, whilst the predaceous structures were only seen on the aerial hyphae. This spatial arrangement appears to be most efficient for the capture of springtails. Morphological analysis shows that the repent and aerial hyphae are microscopically very similar.

On this evidence we conclude that the vesicles belong to *A. ferox*. This suggestion seems to be further corroborated by the fact that in the investigated area, no other species of the predaceous genera has ever been found.

Acknowledgments

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RIMULARIA CAECA, A CORTICOLOUS LICHEN SPECIES FROM NORTH AMERICA

G. RAMBOLD & CH. PRINTZEN

Botanische Staatssammlung München, Menzinger Str. 67, D-8000 München 19, F.R.G.

ABSTRACT: On examination the North American corticolous *Lecidea caeca* was found to be a member of the genus *Rimularia* (Rimulariacae, Lecanorales). The species is described there in detail.

KEYWORDS: *Lecidea*, Lecanorales, lichens, flora of North America, *Rimularia caeca*.

INTRODUCTION

Since ascus structures have been found to be highly valuable diagnostic characters in lecideoid lichens, many changes have occured in the taxonomy of this unnatural group of species. This, however, have mostly concerned the saxicolous members of *Lecidea* s.l., presently placed in several new or re-established genera (see e.g. HERTEL 1984, HERTEL & RAMBOLD 1987, 1990).

The actual knowledge about the taxonomy of the corticolous, terricolous and muscicolous taxa is still very poor. No modern monograph of this very heterogenous group yet exists. In recent years, the relationships of just a few corticolous or terricolous lecideoid taxa have been discussed within only a few smaller contributions, e.g. in COPPINS & JAMES (1984), HINTEREGGER & al. (1989), or TØNSBERG (1990).

It recently became clear, that *Lecidea* s.str. is an exclusively saxicolous genus, restricted to calciferous and siliceous rock, and does not grow on organic substrates. Some of the corticolous lecideoid species belong to *Biatora*, *Lecanora*, *Lecidella* and other genera of the Lecanoraceae s.l. like *Protoparmelia* or *Pyrrhospora*. There is also a high number of corticolous lecideoid species, which belong to genera of the suborder Cladoniineae sensu RAMBOLD et al. (1992). They have different ascus structures and belong to families like the Micareaceae, Agyriaceae (incl. Trapeliaceae) and Rimulariaceae. A member of the latter group is the North American species *Lecidea caeca*, which was found to belong to the world-wide distributed genus *Rimularia*.

We would like to thank Prof. Dr. H. Hertel (München) for revising the manuscript and various help. Particular thanks are due to Dr. C.M. Wetmore (Minnesota), the first lichenologist after LOWE who recognized *R. caeca*, for his comments on the ecology of this species. He made available to us numerous (all correctly determined) collections, which form the basis of our description and discussions. We thank Dr. A. Taylor (München) for improving the English text and Miss B. Rambold (München) for making the habit drawings. We are also most grateful to the curators of the herbaria M, MICH and MIN. For financial support we gratefully acknowledge grant He 953/5-1 from the Deutsche Forschungsgemeinschaft (DFG).

THE SPECIES

Rimularia caeca (Lowe) Rambold & Printzen **comb. nova**

≡ *Lecidea caeca* Lowe, *Lloydia* **2**(4): 244-245 (1939). - Type: U.S.A.: New York, Essex Co., Adirondack region, Chapel Pond (near St. Huberts), 1600 ft, on white pine on talus slope, *J. L. Lowe* 5533 (MICH! - holotype).

Description: **Thallus** crustose, mostly epiphloeodic, up to 2 cm diam., white to sordid olive, composed of rounded, weakly convex, confluent verrucules, sometimes combining to form small areolae. *Verrucules* c. 0.1-0.2 mm diam. Thallus occasionally sorediate (observed in about 25 % of the specimens). *Soredia* dark brown and somewhat glossy, 15-25 µm; soredial hyphae short-celled, brown pigmented. *Soralia* rounded, mostly small, 0.1-0.25 mm diam. and ± confluent, rarely 0.2-0.45 mm diam. and well-delimited. Thallus margin often indistinct and overgrowing adjacent thalli of other crustose lichens. In section, thallus c. 60-150 µm thick, poorly differentiated. Epinecral layer 5-20 µm, sometimes lacking; uppermost cell layer sometimes brownish; algae trebouxioid, 8-18 µm diam., densely entangled by more or less isodiametric hyphae. **Apothecia** sessile, 0.25-0.45(-0.6) mm diam., round to strongly flexuose, single, rarely in groups of 2-3, up to more than 300/cm^2, mostly regularly distributed over the thallus. *Disc* flat to weakly convex, black, with matt, epruinose surface. *Margin* persistent, 0.02-0.05 mm thick, black, matt. *Excipulum* 20-30 µm, max. 40 µm, pseudoparenchymatic; ectal zone dark brown, 10-20(-30) µm, with hyphae of 2.5-6 µm diam. and lumina of 1.5-4 µm diam.; inner zone colourless and more or less plectenchymatic. *Hypothecium* colourless, 40-60(-70) µm, with densely interwoven, short-celled hyphae of 2-4 µm diam. *Hymenium* colourless to sordid greenish, 40-60 µm, I_{Lugol} + greenish-blue to sordid-brown, $I_{Lugol\ 1:6}$ + blue, K- or K+ rose red to violet; epihymenium dark brown, sometimes with an olive tinge, 5-15(-20) µm. *Paraphyses* frequently branched and anastomosing, short-celled, moniliform, (1.5-)2-3 µm diam., lumina 1-2 µm; apical cells (3-)3.5-5.5 x 2.5-5.5 µm, lumina 2-4 µm. *Asci* of *Rimularia*-type, 8-spored, 30-40 x 9-14 µm; length-width-index: 1:2.5-3.5(-4); amyloid wall layer c. 0.5 µm thick, I_{Lugol} + greenish-blue to orange-brown, $I_{Lugol\ 1:6}$ + blue,; non-amyloid wall layer c. 1.0 µm thick; tholus max. 6-8,5 µm, min. 2-6 µm high. *Spores* ellipsoid, colourless, non-septate, 7.5-**10.5**-13.5 x 4.5-**5.5**-7.5 µm; length-width-index 1:1.5-2 (-2.4), wall c. 0.5 µm thick. **Pycnidia** not observed.

Chemistry: TLC method according to CULBERSON & AMMANN (1979): 1) 16 of 32 specimens examined containing unidentified substance 'C-1' as major substance (often in low concentrations). [Unidentified substance 'C-1': R_f-classes A:2; B:3; C:2; DL: not visible; H_2SO_4 + pale yellow; AS-; UV_{254} +; UV_{350} + whitish blue; after spraying with H_2SO_4 and charring, UV_{350} + olivaceous.] 2) 16 specimens containing no detectable substances.

Ecology and distribution: *R. caeca* is hitherto known only from the temperate part of eastern North America, and was collected mainly in the Great Lakes area (see also WETMORE 1981). This probably often overlooked species grows on the bark of conifers like *Abies balsamea* (balsam fir), *Larix laricina* (tamarack), *Picea glauca* (white spruce), *Picea mariana* (black spruce), *Pinus banksiana* (jack pine), *Pinus rigida* (pitch pine), and *Pinus strobus* (white pine). One specimen was found to grow on a birch snag (*Betula* sp.).

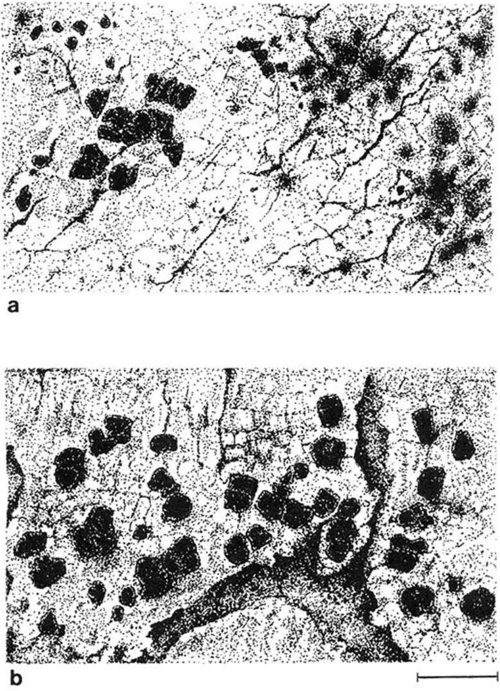

Fig. 1: *Rimularia caeca* (habitus) on bark **a**) specimen with apothecia (left) and sorediate parts of the thallus (right): *C.M. Wetmore* 22850, MIN. **b**) non sorediate specimen: *C.M. Wetmore* 58728, MIN. Scale: 1 mm.

Selected specimens: CANADA: NEWFOUNDLAND, 1.6 km SW of Conne River Pnd (35 km N of Miltown), [c. 48°10'N, 55°47'W], in sloping bog with pools, on tamarack, 26 VI 1981, *C.M. Wetmore* 42915 (MIN).

NEW BRUNSWICK, Chance Harbour, 35 km SW of St John, 1.6 km S of Chance Harbour in small open bog, [c. 45°05'N, 66°25'W], on tamarack, 6 VII 1981, *C.M. Wetmore* 43403 (MIN).

U.S.A.: MINNESOTA, St Louis Co., Voyageurs National Park, N of Agnes Lake E of Lost Bay on Kabetogama Lake, [c. 48°26'N, 93°01'W], on rocky ridges with jack pine and thick young balsam fir, on jack pine, 17 VI 1978, *C.M. Wetmore* 33613 (MIN). – Boundary Waters Canoe area, just E of Bezhik Creek, SE of Seranade Lake, 17 mi NW of Ely, [48°23'N, 92°05'W], black spruce bog, with a few tamarack, balsam fir, and paper birch along the border, on white pine, c. 1400 ft, 7 IX 1986, *T.D. Trana* 13407, 13446 (MIN). – E of Tomahawk Camp, 5 mi SE of Babbitt, [c. 47°35, 91°48'W], in middle age jack pine stand, on spruce, 11 VI 1977 *C.M. Wetmore* 27267 (MIN). – Near St Louis River, 3 mi S of Hoyt Lakes, white spruce plantation planted in 1940, on white spruce, 8 IX 1977, *C.M. Wetmore* 30255B (MIN). – Cook Co., Seagull Creek near end of Gunflint Trail, 40 mi N of Tofte, [c. 48°10'N, 90°50'W], around shaded rock outcrop, on jack pine, 2 VII 1974, *C.M. Wetmore* 22650 (MIN). – Lake Co., 13 mi E of Ely, on Hwy 18 (Fernberg Rd), [c. 47°50'N, 92°05'W], around rock outcrop and mixed conifer hardwood forest, on jack pine, 25 VIII 1973, *C.M. Wetmore* 21893 (MIN). – S of Stony Creek, 17 mi SSE of Ely, [c. 47°45'N, 91°48'W], in mature jack pine stand on ridgetop, on fallen branch, 10 VI 1977, *C.M. Wetmore* 27206A (MIN). – N of Stony River, 9 mi E of Babbitt, [c. 47°40'N, 91°40'W], in jack pine plantation planted in 1959, on jack pine, 12 VI 1977, *C.M. Wetmore* 27433B (MIN). – 7 mi ESE of Babbitt, Tomahawk Rd, [47°40'N, 91°51'W], in tamarack swamp with young trees, on tamarack, 14 VI 1977, *C.M. Wetmore* 27633 (M, MIN). – Hubbard Co., 1 mi N of Lake George, in jack pine area in open second growth pines, on jack pine, 24 VII 1974, *C.M. Wetmore* 22850 (MIN).

MICHIGAN, Alger Co., Pictured Rocks National Lakeshore, N side of Grand Sable Lake, 2 mi W of Grand Marais, [c. 46°39'N, 86°05'W], on ridges with jack pines and openings, on jack pine, 10 VII 1987, *C.M. Wetmore* 58728 (MIN). – Pictured Rocks National Lakeshore, 0.5 mi S of Twelvemile Beach Campground, in jack pine forest near junction of campground road and Hwy 58, on jack pine, 13 VII 1987, *C.M. Wetmore* 58951 (MIN).

MAINE, Washington Co., Machias bog, 10 mi SE of Machias, [c. 44°40'N, 67° 35'W], in bog with shrubs and black spruce and tamarack, on black spruce, 20 VI 1981, *C.M. Wetmore* 42635 (MIN). – Hancock Co., Acadia National Park, Mt Desert Isl. E of Great Meadow Marsh (1 mi S of Bar Harbour), [c. 44°19'N, 68° 14'W], at base of cliff in maple birch, oak woods along rock outcrops with oak and pitch pine, on pitch pine, 5 VII 1983, *T.J. Sullivan* 1302 (MIN). – Acadia National Park, Mt Desert Island, S of Upper Hadlock Pond, in maple spruce and *Thuja* woods with some balsam fir, birch and aspen, on birch snag, 3 VII 1983, *T.J. Sullivan* 1172 (MIN).

DISCUSSION

R. caeca is a further corticolous representative of the genus *Rimularia*, which hitherto comprises species growing on siliceous rocks (lichenicolous or saxicolous), bryophytes, and bark. It is a small and inconspicuous lichen.

R. caeca is easily identified as a species of the genus *Rimularia* by its flexuose apothecia, the short-celled paraphyses and hyphae of the excipulum, hypothecium, the vegetative thallus, and the distinctive asci of the *Rimularia*-type (see HAFELLNER 1984: 332, fig. 77). Dark brown soralia with glossy soredia are developed only facultatively (nine out of 38 specimens examined). Soralia of this type are also typical for other sorediate species of *Rimularia*, like *R. furvella*. Sorediate specimens without apothecia, which may occur in nature, are not known to us. Within the genus, the species is characterized by persistently hyaline, ellipsoid spores, the contents of the unidentified substance "C-1" and its occurrence on conifer bark.

Because there are no corresponding anatomical features, sorediate and non-sorediate specimens, are regarded as conspecific. In our opinion, the lack of detectable amounts of the substance "C-1" in 50 % of the examined specimens does also not justify the separation of two taxa.

The species strongly resembles the sorediate *R. fuscosora*, recently described by MUHR & TØNSBERG (1989) from northern Europe, but differs in having smaller spores (*R. fuscosora*: (9.5-)11-16(-20) x (5-)7-11 µm) and a positive K-reaction of the hymenium. The rose red to violet colour reaction is often very weak and sometimes missing at all. However, hymenia with a sordid green tinge give a strong and lasting violet reaction. The two species are also separated by their chemistry, *R. caeca* contains the unidentified substance "C-1", while *R. fuscosora* has norstictic acid. In addition to their different distribution, chemistry, and spore size, they prefer different substrates: the European *R. fuscosora* seems to be restricted to the bark of deciduous trees (*Alnus incana*, *Betula* sp.), whereas the North American *R. caeca* (with one exception) was found on conifers, mostly on *Pinus banksiana* (jack pine).

No closer relations are assumed with the tropical *Rimularia globulispora* Aptroot & Sipmann, described from Papua New Guinea, which is a muscicolous (type found on *Frullania* sp.) or corticolous species. It has globose spores and contains lobaric acid (APTROOT & SIPMAN 1991).

R. caeca is the eighth species of *Rimularia*, recognized for the North American flora. From there, reports exist already for the two lichenicolous taxa *R. furvella*, *R. insularis*, for the saxicolous *R. badioatra*, *R. gibbosa*, *R. gyrizans*, *R. impavida*, and the muscicolous *R. sphacelata* (EGAN 1987, 1989, 1991).

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COMPARATIVE MORPHOLOGICAL STUDIES OF DISCOSIA ARTOCREAS AND DISCOSIA FAGINEA

SIMEON G. VANEV
Institute of Botany, 1113 Sofia, Bulgaria

ABSTRACT. Comparative morphological studies on the original specimens of Discosia artocreas (Tode) Fr. and D. faginea Lib. were carried out. The results proved that D. artocreas and D. faginea are two separate species. D. artocreas is a type of genus Discosia.

Genus Discosia was described by Libert in 1837 and it comprises imperfect fungi belonging to order Sphaeropsidales of class Coelomycetes. In the diagnosis of the original species Discosia faginea, Libert (1837) indicates Sphaeria artocreas Tode (a species described by Tode in 1791) as a synonym of D. faginea. Later Fries (1849), considering S. artocreas an individual species from genus Discosia, suggests the new combination Discosia artocreas (Tode) Fr.

While we revised taxonomically genus Discosia in the period 1975-1991, a number of obscure questions arose on the taxonomic status and the nomenclature of D. faginea and D. artocreas, bearing a direct relation to the determination of the type species of the genus Discosia. For example, in case Libert's position is accepted that D. faginea and D. artocreas are synonymous names of a common species, then, under article 55 of the International Code of the Botanical Nomenclature, priority is given to the older epithet of the species, i.e. "artocreas". On the other hand, Fries (l. c.), referring S. artocreas to genus Discosia was obviously acquainted with Libert's work, but in the original description of the new combination there is no mention of the indicated relation between D. faginea and D. artocreas,therefore it might be assumed he considers the two species to be independent.

Subramanian and Reddy (1974) have not studied the original specimens of D. faginea and D. artocreas, therefore they have no position as regards the taxonomy and the nomenclature of the two species.

The main difficulty when elucidating the taxonomic status of D. faginea and D. artocreas is due to the fact the original specimen of Sphaeria artocreas is considered to be destroyed or lost, that preventing the investigators, so far, from expressing an opinion on the problem of whether the two species are good ones, or we have to do with synonyms (Sutton, 1980).

In 1981, while revising herbarium materials from the Herbarium at the Royal Botanical Gardens, Kew, England (K), we came across the original specimen of S. artocreas from Fries's collection, marked: "Scleromyceti Sueciae No 151. Sphaeria artocreas Tode" (Fig. 1).

Having that material, together with the original specimen of D. faginea from Libert's Mycological collection, kindly placed at our disposal by the Herbarium at the National Botanical Garden in Brussels, Belgium (BR), we carried out comparative morphological studies on both specimens, the result of which provided us with a possibility to take a definite position with respect to the question discussed.

MATERIAL AND METHODS

In conformity with the modern taxonomic criteria, accepted in the systematics of the imperfect pycnidial fungi from class Coelomycetes (Sutton, l. c.) we based our comparative investigations on the morphology of the conidiogenous apparatus (pycnidia, conidiogenous cells), and the conidia of the original herbarium specimens of D. faginea (ex BR) and D. artocreas (sub Sphaeria artocreas-ex K).

The dimension, the colour and the shape of the conidia as well as the exact position of the conidial septa and appendages were used as the basic systematic features in the comparative morphological characteristics of the studied specimens. The cited morphological elements were studied in lacto-phenol semistable preparations under Amplival light microscope, after the passing-light method. Conidiogenous cells and conidia were observed and photographed using Leitz-AMR-1000 A scanning electron microscope. For the precise investigation of the conidiogenous process and the structure of the generative organs, using a freezing microtome, thin cuts of pycnidia were made (having a thickness of about 10 µm), covered in paraffin.

Studied were also live liophylized specimens of D. faginea (strain CBS 443.67) and D. artocreas (strain CBS 241.66), preserved in the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The live specimens were cultivated setting conidia cultures on oatmeal agar in Petri-dishes, and then exposed at a constant temperature of 24°C. The outward appearance and the diameter of the colonies were compared 3, 6, 9 and 12 days after setting the culture.

Traced out was the influence of the temperature on the growth of the colonies of both strains on oatmeal agar in Petri-dishes at a temperature of 3, 6, 15, 21, 24, 30, 33 and 36°C in a serial thermostat, as a result of which the temperature requirements were determined for the growth and spore-formation of the strains studied.

All the variants of the experimental investigations were carried out three times repeatedly.

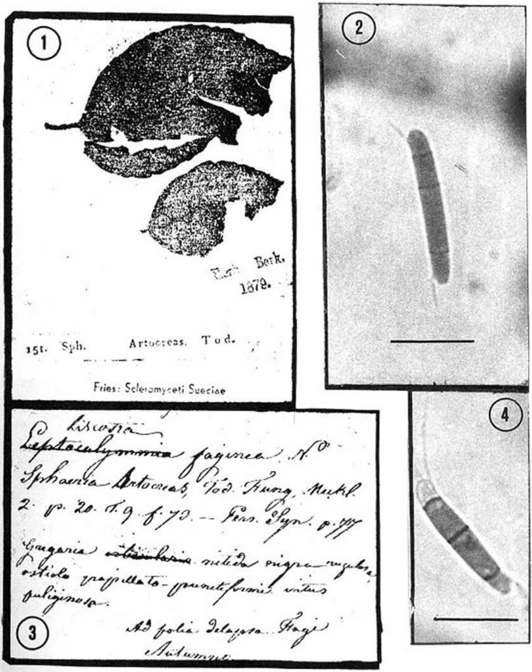
RESULTS AND CONCLUSIONS

As a result of the comparative investigations carried out on the original herbarium specimens of D. faginea and D. artocreas, as well as on live cultures of these fungi, we established considerable morphological, cultural and physiological differences between them, to be considered successively.

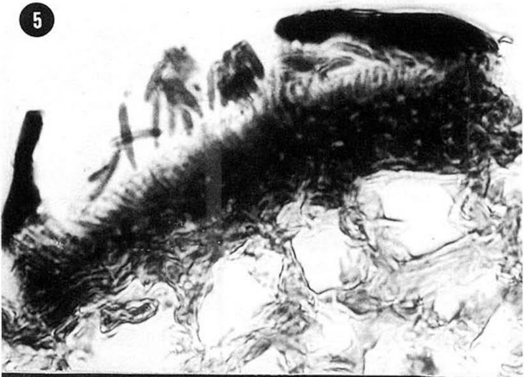
1. MORPHOLOGY OF THE CONIDIA. The conidia of the fungi of genus Discosia have characteristic shape, structure and dimensions, the genus being well differentiated as a separate group on the base of those features. On the other hand, within the limits of the genus to be observed are considerable differences in the structure of the conidia, the fact providing a possibility for the differentiation of intra-generic taxa of various ranks.

According to the position of the conidial appendages and the relative length of the conidial cells, 6 sections were differentiated within the limits of the genus (Vanev, 1991). It was established that the original specimens of D. faginea and D. artocreas share common features, referring them to the common Section I. Discosia, more particularly: the conidial appendages are adjacent to the apex and the base of the conidia, the two middle cells being of different length - the cell, adjacent to the base is always longer than the one adjacent to the apex. Regardless of the common features cited, others exist, in which the conidia of the studied specimens differ considerably from each other. On fig. 2 it is obvious that D. faginea has considerably wider conidia than the ones of D. artocreas. Differences are also to be observed in the shape - with D. artocreas predominating are cylindrical conidia having cells of equal width and colour, while with D. faginea the majority of the conidia are spindle-shaped, the two middle cells being wider and darker in colour than the two end cells. In the conidia of D. artocreas the middle cell adjacent to the base is always twice or more times longer than the other middle cell, adjacent to the apex, while in the conidia of D. faginea the difference in the length of the two middle cells never reaches 2 : 1 (Fig. 4).

2. MORPHOLOGY OF THE CONIDIOGENOUS CELLS AND THE PYC-



Figs. 1-2. <u>Discosia</u> <u>artocreas</u>: 1. Original specimen. 2. Conidium. Figs. 3-4: <u>Discosia</u> <u>faginea</u>: 3. Original description. 4. Conidium. Scale bars = 10 µm.



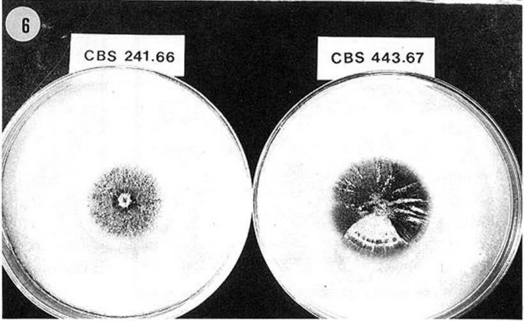


Fig. 5. Longitudinal pycnidial cut of Discosia faginea.
Fig. 6. Colonia of Discosia artocreas (CBS 241.66) and Discosia faginea (CBS 443.67) at 24°C.

NIDIA. The conidiogenous cells of the fungi from genus Discosia form on a stromatic base within the pycnidia (Fig. 5). They are of a varying shape and length, altering within rather wide limits, due to which their taxonomic value is relatively limited. Studying completely developed pycnidia, certain differences were established in the structure of the conidiogenous cells of D. artocreas and D. faginea: in the pycnidia of the former species relatively short (up to 8 µm) conidiogenous cells are formed, most often cone-shaped, while with the latter species those cells are longer (up to 15 µm), being predominantly cylindrical or bottle-shaped.

Studying longitudinal pycnidial cuts from both species, it was established that with D. artocreas the pycnidia are most often pluriloculate, flat or slightly concave at the centre, with a convex margin and a relatively thin stromatic base, while with D. faginea the pycnidia are monoloculate, disc-shaped, convex in the middle and having a thicker stromatic base.

3. CULTURAL CHARACTERISTICS. The data from fig. 6 show that the colonies of D. artocreas and D. faginea on oatmeal agar at 24°C have a varying rate of growth and a rather different outward appearance. The colony of D. artocreas (strain CBS 241.66) has a more retarded growth - on the 12th day after setting the culture it has a diameter of 48.5 mm (average for the 3 repetitions), while that of D. faginea (strain CBS 443.67) within the same time period reaches a diameter of 58 mm. Considerable are also the differences in the outward appearance of the colonies of both strains. Twelve days after setting the culture D. artocreas forms an indistinctly marked out yellowy-brown colony, having no concentric zonation and no radial rays, secreting a yellow pigment in the nutrient environment around the colony, in the form of a nimbus; the aerial mycelium is sparse, greyish-whity, cotton -like, placed predominantly in the centre; the formation of pycnidia is to be observed not earlier than nine days

after setting the culture. The colony of D. faginea is dark-olive-green to almost black, sharply outlined, having a number of concentric rings and well-seen whity radial rays; the aerial mycelium is cobweb-like, grey, predominantly in the centre; no pigmentation of the environment surrounding the colony is to be observed; the formation of pycnidia starts after the 6th day following the setting of the culture.

4. TEMPERATURE REQUIREMENTS. It was experimentally proved that D. artocreas and D. faginea have different temperature requirements related to their growth and development. At 3°C, on the 12th day after setting the culture D. faginea forms a well shaped colony having a diameter of 3.1 mm (average for the 3 repetitions), while D. artocreas forms no colony at the same temperature and within the same time period. At 24°C both strains form the largest colonies, their conidiogenesis being most intensive. Considerable differences are to be observed in the requirements of both species towards high temperatures: at 30°C the growth of the colony and the formation of pycnidia of D. artocreas are almost normal, while at the same temperature the grows of the colony of D. faginea is highly suppressed, no pycnidia forming at that. At 33°C D. faginea forms no colony while D. artocreas develops successfully even at those relatively high temperatures.

The conclusion that should be drawn out these investigations is, the two species have different temperature requirements: D. artocreas develops more successfully at higher temperatures.

One of the goals of the experimental investigations carried out was to establish how and what an extent certain basic factors of the environment (nutrient substratum and temperature) effect the variability of the morphological features, on the base of which our classification scheme of genus Discosia is developed. For that purpose, the two strains were cultivated on different nutrient substrata (oatmeal agar, potato-dextrose agar and sterilized lupine stems) at different temperatures after the methods described.

The generalised results show that the position of the conidial appendages and the relative length of the conidial cells remain unchanged, i.e. they are not influenced by the composition of the nutrient environment and the changes in the temperature, while the dimention of the conidia vary within the limits established for each species.

Table 1. Comparison between D. artocreas and D. faginea.

Species	Dimensions of conidia (µm)
D. artocreas	(16,3-)18±1,2(-20) X (1,8-)2,1±0,2(-2,5)
D. faginea	(13,8-)18±2,7(-23) X (2,5-)2,9±0,2(-3,5)

It ensues from the cited results that the basic morphological features, on which we have founded the intrageneric classification of the fungi from genus Discosia, are characterized by insignificant variability amplitudes under changing environmental conditions, due to which their taxonomic value is relatively high.

In fine, the generalized conclusions may be drawn out that D. faginea and D. artocreas are two separate species having the right to independent existence. The cited conclusion provides us grounds to propound Discosia artocreas (Tode) Fr. (basionym Sphaeria artocreas Tode) as a type species for genus Discosia.

ACKNOWLEDGEMENTS

This research was carried out at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands and was supported financially from the International Agricultural Centre, Wageningen, The Netherlands. The author wishes to thank the Curators of K and BR for the loan of the specimens examined.

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DISCOSIA SUBRAMANIANII, SP. NOV.

SIMEON G. VANEV

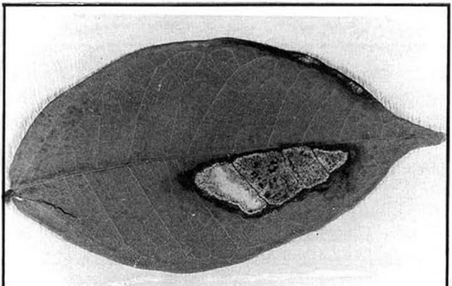
Institute of Botany, 1113 Sofia, Bulgaria

ABSTRACT. Discosia subramanianii - a new species of genus Discosia Lib. (Deuteromycotina, Coelomycetes), parasiting on leaves of Ficus pumila L. (Moraceae) in India is described and illustrated.

During a taxonomic revision of genus Discosia Lib. (Deuteromycotina, Coelomycetes) a herbarium specimen labelled "Discosia artocreas" received from the Herbarium of the International Mycological Institute, Kew, England (IMI) was examined. The fungus is a parasite on leaves of Ficus pumila L. (as F. repens Rottler) in India. In the present paper a new Discosia species, belonging to Section III. Clypeata Vanev (Vanev, 1991) is described and illustrated.

DISCOSIA SUBRAMANIANII VANEV, SP. NOV. (Figs. 1, 2)

Maculae magnae (1,5-5 cm in diam.), orbiculares vel angulatae, amphigenae, solitariae, pallido-brunneae, atrobrunneo-marginatae. Conidiomata pycnidialia 175-280 µm in diam., epiphylla, solitaria, sparsa vel gregaria, rotundata, globoso-complanata vel discoidea, nigra, ostiolis 28-63 µm in diam., rotundatis vel plus minusve angularis. Cellulae conidiogenae 8.5-30 X 1.5-2 µm, cylindricae, rectae vel leniter curvatae, hyalinae. Conidia holoblastica (12.5-)14.5±1.21(-17.5) X (2.8-)3.16±0.24(-3.5) µm, fusiformia vel elliptica, apice rotundata, basi truncata, arcuata, rarius recta, dorsiventralia, biappendiculata, hy-



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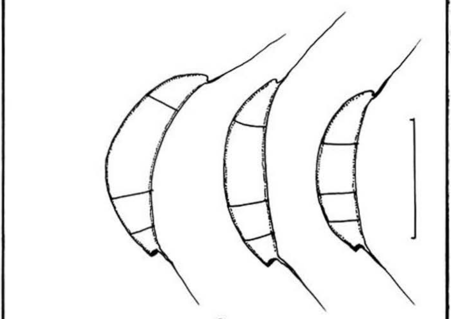

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Figs. 1-2. Discosia subramanianii: 1. Leaf spot. 2. Conidia. Scale bar = 10 µm.

alina, 3-septata, semper brevior cellula media, vicina basis, quam cellula media, vicina apicis; appendiculae filiformes, ventrales, hyalinae, proxime apicem basimque conidii formantur.

In foliis vivis Fici pumilae L. (sub Fici repentis Rottler), India, Solan, Martius 1965, G. K. Gupta, IMI No 114375, holotypus, SOM, isotypus (slide).

In speciebus e familia Moraceae Discosia primum observatur.

Leaf spots large (1.5-5 cm in diam.), single, rounded or angular, amphigenous, pale-brown, surrounded by a dark-brown halo. Conidiomata pycnidial, 175-280 µm in diam., ostiolate, epiphyllous, separate, gregarious or scattered, subcircular in outline, depressed-globose or discoid, black; ostioles 28-63 µm in diam., central, circular or irregular, sometimes surrounded by a darker rim. Conidiogenous cells 8.5-30 X 1.5-2 µm, cylindrical, straight or slightly curved, hyaline. Conidia holoblastic (12.5-)14.5±1.21(-17.5) X (2.8-)3.16±0.24(-3.5) µm, mostly fusiform, sometimes ellipsoidal, tappered to the both ends, with a truncate base and an obtuse apex, dorsiventrally curved, hyaline, clearly 3-euseptate, the two middle cells unequal in length: the middle cell adjacent to the apex always longer than the other middle cell, adjacent to the base; conidial appendages two, hair-like, single, unbranched, hyaline, arising just at the apical and the basal extremity of the ventral side of the conidium.

D. subramanianii is the only Discosia species, parasiting on plants of family Moraceae.

ACKNOWLEDGEMENTS

This research was carried out at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands and was supported financially from the International Agricultural Centre, Wageningen, The Netherlands. I wish to thank the Curator of IMI for the loan of the specimen examined.

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FIRST RECORDS OF JELLY FUNGI (DACRYMYCETACEAE, AURICULARIACEAE, TREMELLACEAE) FROM SONORA, MEXICO

Evangelina **Pérez-Silva***

and

Martín **Esqueda Valle****

* Laboratorio de Micología, Instituto de Biología, UNAM, México, D.F. 04510.

** CESUES, Escuela Superior de Ecología, Ap. Postal A-126 Hermosillo, Sonora, México, 83190.

ABSTRACT

Twelve taxa of jelly fungi growing mainly with **Pinus** and **Quercus** are new records from Sonora, México. They were found in the Municipios of Yécora, Alamos and Nácori Chico. **Tremella fibulifera** and **Dacryopinax yungensis** are reported for the first time from México.

INTRODUCTION

This report is concerned with new records of twelve taxa of jelly fungi from Sonora State, two of them new to México (Table 1). The descriptions are based on material collected by the authors and on several previous publications (Kennedy, 1958; Lowy, 1971; Binyamini, 1983; Bandoni and Oberwinkler, 1983; Courtecuisse and Lowy, 1990). These are common species inhabiting decomposing conifer and oak logs of tropical rain forests throughout the Neotropics (Lowy, 1971). They appear to be confined to the central parts of México (Herrera and Guzmán, 1961), but information about jelly fungi is scarse in the predominantly desertic State of Sonora. The collections in this report were obtained on several field trips during 1990-1991. The source of each record is indicated at the end of each description. All the specimens have been deposited in the National Herbarium of the Instituto de Biología, UNAM (MEXU); the herbarium numbers are indicated in parenthesis.

DACRYMYCETACEAE

Dacrymyces deliquescens (Mérat) Duby, var. **deliquescens**

This species is recognized by its small pulvinate basidiocarps (1 - 4 mm in diam.), yellow color when fresh, drying reddish-brown; hyphae with clamp connections and early 3 - septate basidiospores, 9.5 - 12 x 3 - 5 µm, although these were slightly larger than those described by Kennedy (1958), Lowy (1971) and Pacioni (1981).

HABITAT: On logs and fallen branches of **Quercus** sp.

DISTRIBUTION: Municipio of Yécora: Mesa Grande. Leg. M. Esqueda, M. Coronado. 6.08.1990 (MEXU 22650). Known only from La Marquesa, Mex. (Lowy, 1971) (Table 1).

Dacrymyces dictyosporus Martin Fig. 1.

Basidiocarps yellowish-orange to pale yellow, cerebriform, up 8 -12 mm wide, drying to an inconspicuous, pale yellow horny film, with a root like base; hyphae without clamp connections, from 1.7 - 2 µm; basidia 68 - 75 x 13.6 - 15.3 µm, clavate at first, becoming bifurcate. Basidiospores 21.1 - 26.2 x 11.9 - 15.3 um broadly ellipsoid, muriform, with 5 - 7 transverse septa and several short longitudinal septa; with a prominent apiculus.

HABITAT: On dry branches of **Pinus** sp.

DISTRIBUTION: It was known from Distrito Federal, Chiapas, Oaxaca, Estado de Mexico, Jalisco, Morelos, Michoacán and Nuevo León (Lowy, 1980) (Table 1), and it is here reported from Municipio of Nácori Chico: Km 45, Nácori Chico to Mesa Tres Ríos road. Leg. M. Coronado, M. Esqueda. 27.05.1990. (MEXU 22649). Municipio of Yécora: Mesa Grande. Leg. M. Coronado, M. Esqueda and E. Pérez-Silva. 15.08.1991. (MEXU 22900).

Dacrymyces palmatus (Schw.) Bres. Figs. 2-3.

Basidiocarps pale yellowish when fresh, gelatinous, lobed, 13 mm long x 3 mm wide, drying to a brown, horny film; contextual hyphae 3.4 µm, with thin walls; some clamp connections; probasidia clavate; becoming bifurcate.Basidiospores 18 - 20 x 5.7 µm with 7 septa, mostly curved, hyaline, with apiculus.

HABITAT: On conifer logs.

DESCRIPTION: It was reported from Distrito Federal, Estado de México, Coahuila, Durango, Morelos, Hidalgo (Lowy, 1980), (Table 1), and it is here reported from Municipio of Nácori Chico: Km 45, Mesa Tres Ríos road. Leg. M. Esqueda, M. Coronado. 14.07.1990 (MEXU 22651).

Dacrymyces punctiformis Neuhoff Fig. 4.

The characteristic feature of this species is its attachment to the substratum by a point, forming small yellow patches, 1-3 mm diam., becoming dark blackish - brown on drying; forming a gelatinous mass on bark when fresh; contextual hyphae with scarse clamp connections, thin walled in lactophenol; metabasidia bifurcate 34 x 1.7 - 2 µm; basidiospores cylindrical, 10 - 15 x 3 - 3.5 µm, 3 septate.

HABITAT: On gymnosperm logs.

DISTRIBUTION: Known only from Estado de México, Chiapas and Hidalgo (Lowy, 1980) from Municipio of Nácori: Km. 14 Nácori Chico to Mesa Tres Ríos road. Leg. M. Esqueda, M. Amaya. 14.07.1990 (MEXU 22653).

Dacryopinax yungensis Lowy Figs. 21, 22.

Basidiocarps stipitate-pileate, yellow when fresh, drying to a horny film; stipe central, 10 - 15 mm tall x 1 - 3 mm diam.; with shorter hairs on abhymenial surface; probasidia subclavate 40 - 45 x 2.5 - 3 µm, metabasidia bifurcate; sterigmata cylindrical. Basidiospores curved-cylindrical, 12.5 x 5 µm, 3 septate.

HABITAT: On dry branches of **Pinus** sp.

DISTRIBUTION: This species was previously known only from Bolivia (Lowy, 1971), and now from Municipio of Yécora: Mesa Grande. Leg. G. Tapia **et al.** 15.08.1991. (MEXU 22901).

AURICULARIACEAE

Auricularia auricula (Hook.) Underwood

Basidiocarps up 5.5 - 8.5 cm x 3 cm wide, auriform, sessil; abhymenium pilose with hairs up to 93 - 100 x 5 µm; hymenium brownish to black on drying; probasidia cylindrical; metabasidia becoming triseptate 56 x 5 µm; hyphae 1.7 µm diam. with clamp connections, interwoven in a gelatinous matrix. Basidiospores curved-cylindrical, 12.6 x 5.1 µm.

HABITAT: Isolated or gregarious, on logs of **Quercus** spp.

DISTRIBUTION: Municipio of Alamos: Arroyo Cuchujaqui-Navojoa road. Leg. G. Yanes. 9.06.1990 (MEXU 22661). Municipio of Yécora Mesa Grande to Santa Rosa road. Leg. M. Esqueda, M. Amaya. 10.07.1990 (MEXU 22662). Municipio of Nácori Chico: Km 45 to Mesa Tres Ríos road. Leg. T. Quintero, M. Esqueda. 14.07.1990 (MEXU 22665).

This species was collected only in the Edo. de México (Lowy, 1965, 1971) where it is common each year, and Chiapas, Distrito Federal, Hidalgo, Jalisco, Morelos (Mendiola and Guzmán, 1973). (Table 1). This species is eaten in the central part of México (Herrera y Guzmán, 1961).

Auricularia delicata (Fries) Henn.

Basidiocarps solitary, orbicular up to 2 cm diam., sessil; abhymenium pilose, brown hairs up 100 x 5 - 6.1 µm with a parenchymatous layer of more or less isodiametrical cells 18 µm; probasidia cylindrical; metabasidia transversaly triseptat, 50-56 x 5 - 7 µm; basidiospores cylindrical to allantoid, 12.6 x 5- 6 µm. Hyphae with clamp connections 2 - 4 µm, interwoven in a gelatinous matrix.

HABITAT: Gregarious on logs of **Quercus** spp.

DISTRIBUTION: Lowy (1971, Courtecuisse and Lowy 1990) recently reported it from French Guiana, but it was not reported from Municipio of Yécora: Yécora to Mesa Grande to Santa Rosa road. Leg. M. Coronado, M. Esqueda. 10.07.1990 (MEXU 22664). Municipio of Nácori Chico, Km 45 Nácori Chico to Mesa Tres Ríos. Leg. M. Esqueda, M. Coronado. 14.07.1990 (MEXU 22666).

This species is more frequent than **A. auricula.** It has been collected several times in Veracruz, México (Lowy, 1965, 1971, 1980). (Table 1). This fungus is eaten in the central part of México.

Auricularia mesenterica Pers.

Basidiocarps gelatinous when fresh, drying coriaceous up to 5 cm wide, sessil; abhymenium pilose, greyish; hymenium with purple tints, smooth to multiveined. Probasidia cylindrical, 56 x 5-8 µm; metabasidia triseptate up 60 µm; sterigmata cylindrical; basidiospores 12.6 x 5 - 5.5. µm, allantoid, germinating by repetition.

HABITAT: Saprobic on wood of broad leaf trees. Gregarious.

DISTRIBUTION: This species is known from Veracruz, Guerrero, Chiapas and Morelos (Lowy, 1971), and Campeche, Colima, Hidalgo, Jalisco, Michoacán, Oaxaca, Puebla (Pérez-Silva **et al.** 1987) it is reported here for the first time from Municipio of Yécora: Yécora Santa Rosa road. Leg. M. Esqueda, M. Coronado. 6.08.1990 (MEXU 22663), (Table 1).

TREMELLACEAE

Tremella fibulifera A. Möller. Figs. 5-7, 18.

Basidiocarps soft gelatinous, pulvinate, lobate, hyaline, white with yellow tints when fresh, up to 2.5 cm in diameter and height; drying, they are reduced to horny effused films. Hyphae 2 - 2.5 µm diam. with clamp connections. Probasidia 10.8 x 10 µm, subspherical to elliptical. Metabasidia cruciate septate, 4-spored. - Fig 5 shows a basidium with well developed sterigmata. Basidio spores 10 x 7.5 µm, elliptical. No hymenial conidia observed. Clamps present at bases of some basidia.

HABITAT: Solitary on logs from **Quercus** sp.

DISTRIBUTION: This species was previously known from Brazil, Colombia, Costa Rica, Panamá (Lowy, 1971; Bandoni and Oberwinkler, 1983) and French Guiana (Courtecuisse and Lowy, 1990) and now from Municipio of Yécora: Yécora. Leg. A. Aparicio. 10.06.1990 (MEXU 22657, 22660). It is reported here for the first time from México.

Tremella fimbriata Fr.: Fr. Figs. 8-10, 19.

Basidiocarps soft gelatinous, light brownish when fresh, imbricate - foliose up to 3 cm broad x 1 cm height, drying to a black film; hyphae 2 - 4 µm wide with clamp connections and bulbous septa. Probasidia subglobose; metabasidia cruciate septate; sterigmata cylindrical up to 2.5 µm wide. Basidiospores subglobose to ovoid, 8 - 10 x 5 - 6 µm, germinating by repetition.

HABITAT: Saprobic, solitary on logs of **Quercus** sp.

DISTRIBUTION: This species was previously known from Colombia, Cuba, Guatemala. In México: Chiapas, San Cristóbal de las Casas (Lowy, 1971), Estado de México (Lowy, 1980), Veracruz (Mendiola y Guzmán, 1973) and French Guiana (Courtecuisse and Lowy, 1990). It is reported here from Municipio of Yécora: Km 11 to Santa Rosa road. Leg. M. Coronado and M. Esqueda. 10.06.1990 (MEXU 22659), (Table 1).

Tremella fuciformis Berk. Figs. 11-13, 20.

This species is recognized by its foliose basidiocarps, with simple lobes whitish - yellow when fresh and drying horny, brownish - yellow 3 - 5 cm x 1 cm height; hyphae with clamp connections and bulbous septa; metabasidia 25 - 30 x 2 - 3 µm wide. Basidiospores ovoid 10 - 12 x 5 - 6 µm, germinating by repetition.

HABITAT: Solitary, on logs of **Quercus** sp.

DISTRIBUTION: Previously known from Argentina, Bolivia, Brazil, Chile, Cuba, Guyana, Jamaica. In. México: Durango, Venezuela (Lowy, 1971); Veracruz (Lowy, 1980) and Distrito Federal (Pérez-Silva **et. al.** 1987); French Guiana (Courtecuisse and Lowy, 1990). It is reported here from Municipio of Yécora: Km 13 Yécora to Santa Rosa road. Leg. A. Aparicio. 10.07.1990 (MEXU 22656), (Table 1).

Tremella lutescens Fr. Figs. 14 - 17.

Basidiocarps cerebriform to lobate, gelatinous, orange - yellow; on drying becoming a film with red tints. Hyphae with clamp connections up to 2.5 µm wide. Probasidia globose; metabasidia cruciate septate; sterigmata 2 - 4 µm wide. Conidia produced on slender conidiophores; basidiospores 5 - 7 x 3.5 - 4.5 µm, germinating by repetition.

This species is found very frequently on branches and trunks of **Quercus** sp.

HABITAT: On decomposing logs of gymnosperm wood in tropical rain forest.

DISTRIBUTION: Previously known from Estado de México (Lowy, 1971), Morelos, Hidalgo, Jalisco and Veracruz (Lowy, 1980); Baja California (Ayala and Guzmán, 1984); Zacatecas (Acosta and Guzmán, 1984). Chiapas, Distrito Federal, Durango. Estado de México (Pérez-Silva **et. al.** 1987), and now from Municipio of Nácori Chico, Km 45 Nácori Chico to Mesa Tres Ríos road. Leg. M. Coronado, M. Esqueda. 14.07.1990, (MEXU 22652). Municipio of Alamos, Km 15 Alamos to Navojoa road. Leg. G. Yanes 29.07.1987 (MEXU 22655), (Table 1).

DISCUSSION

Because of the different ecological conditions prevalent in the Sierra Madre Occidental of Sonora, it is quite probable that in the future the reports on Tremellales of this state will increase. In this paper twelve taxa are registered, two of them, **Tremella fibulifera** and **Dacryopinax yungensis**, are reported for the first time for the Mexican mycobiota.

Until recently, field trips in Sonora have been scarse. In the pertinent literature (Lowy, 1965, 1971; Mendiola y Guzmán, 1963), these two taxa had not been previously reported from México.

Most of the reported taxa in this paper are saprobic - lignicolous on **Pinus** spp. and **Quercus** spp., and their major role in nature is the degradation of lignin and cellulose from stems, bark and debris. The degradation process is indispensable for the maintenance of the carbon balance in nature (Hudson, 1980). Nutrient mobilization into vegetative fungal mycelium is only possible when the complex lignocellulosic medium is degraded (Lu **et al.**, 1988).

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TABLE 1. DISTRIBUTION IN MEXICO OF THE JELLY FUNGI FOUND IN SONORA

	BAJA CALIFORNIA	CAMPECHE	COAHUILA	CHIAPAS	COLIMA	DISTRITO FEDERAL	DURANGO	ESTADO DE MEXICO	GUERRERO	HIDALGO	JALISCO	MICHOACAN	MORELOS	NUEVO LEON	OAXACA	PUEBLA	SAN LUIS POTOSI	SONORA	TABASCO	VERACRUZ	ZACATECAS
AURICULARIA AURICULA				2		6		8		1			2					3*			
A. DELICATA				4						1			1		2		2	2*		10	
A. MESENTERICA		1		1	4				3	3	3	3	8		4	1		1*	1	7	
TREMELLA FIBULIFERA																		** 2			
T. FIMBRIATA				1				2								1		1*		3	
T. FUCIFORMIS						3	1											1*		3	
T. LUTESCENS	1			1		4	1	7		2	1	1	7		2		1	2*		2	1
DACRYMYCES DELIQUESCENS VAR. DELIQUESCENS								2										1*			
D. DICTYOSPORUS				1				4			1	1	3	1	1			2*			
D. PALMATUS			1			1	1	1	1	2			1					1*		1	
D. PUNCTIFORMIS				1				2		1								1*			
DACRYOPINAX YUNGENSIS																		** 1			
TOTAL OF SPECIES	1	1	1	7	1	4	3	7	2	6	3	3	6	1	4	2	2	12	1	6	1

DATA FROM THE BIBLIOGRAPHY. THE NUMBERS INDICATE THE LOCALITIES. * = NEW RECORDS FROM SONORA

** = FIRST RECORD IN THE MEXICAN MYCOBIOTA

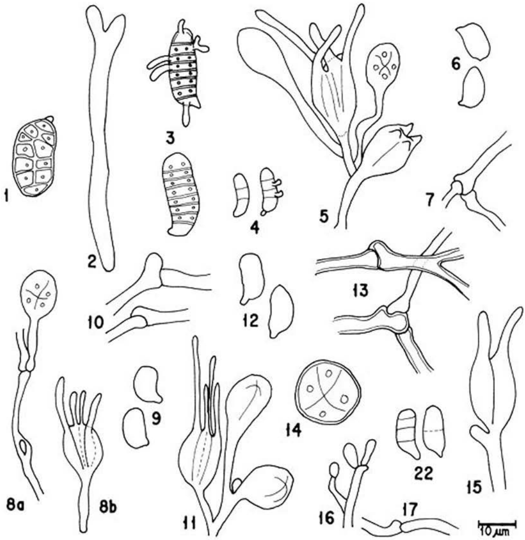
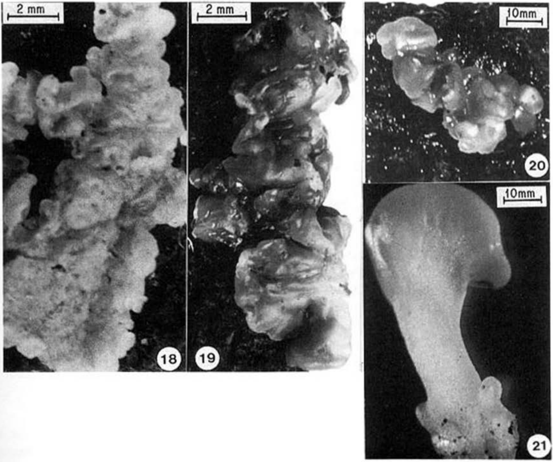


Fig. 1. **Dacrymyces dictyosporus.** 1. Basidiospore. Figs. 2-3 **Dacrymyces palmatus.** 2. Basidium. 3. Basidiospores. Fig. 4. **Dacrymyces punctiformis.** 4. Basidiospores. Figs. 5-7 **Tremella fibulifera.** 5. Section trough basidiocarp lobe showing peripheral hymenium. 6. Basidiospores. 7. Variation in clamp connections. Figs. 8-10 **Tremella fimbriata.** 8a. Probasidium. 8b. Metabasidium. 9. Basidiospores. 10. Clamp connections. Figs. 11-13 **Tremella fuciformis** 11. Section trough basidiocarp lobe showing basidial ontogeny. 12. Basidiospores. 13. Variation in clamp connections. Figs. 14-17. **Tremella lutescens.** 14. Probasidium. 15. Meta basidium. 16. Conidiophore with conidia. 17. Clamp connection. 22. **Dacryopinax yungensis.** Basidiospores: Dibujos F. Villegas.



Figs. 18-21. Habitat of preserved basidiocarps. 18. **Tremella fibulifera.** 19. **Tremella fimbriata.** 20. **Tremella fuciformis.** 21. **Dacryopinax yungensis.**

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ADDITIONAL DATA ABOUT THE GENUS NEPHROMOPSIS (LICHENES, PARMELIACEAE)

TIINA RANDLANE and ANDRES SAAG

Laboratory of Bioindication, Tartu University

EE-2400 Tartu, Estonia

Abstract. A synopsis of the species (16) of the genus Nephromopsis is presented. The new combinations N. endoxanthoides (Awasthi) Randl. et Saag, N. isidioidea (Räsänen) Randl. et Saag, N. komarovii Elenk.) Randl. et Saag and N. yunnanensis (Nyl.) Randl. et Saag are proposed. Information about the lichen substances in all species is provided according to literature as well as the original data obtained by means of thin-layer chromatography.

The lichen genus Nephromopsis was described by Müller Argoviensis (1891) to accommodate N. stracheyi, which was said to have a thallus like in Cetraria but the position of apothecia like in Nephroma. In contemporary lichenology the genus was not usually recognized until in recent times. Räsänen (1952) was the last author to draw several species with nephromoid apothecia on the underside of cetrarioid thallus together into a separate genus under the name Nephromopsis. After a period of almost thirty years the treatment was taken up again by Lai (1980). Meanwhile some of the species had been dealt with under the section Nephromopsis (Müll. Arg.) Rassad. of the genus Cetraria (Rassadina, 1948; Poelt, 1968). Still, the infrageneric systematics of Cetraria has always been poorly developed.

Nowadays homogenous evolutionary lineages are emphasized in the delimitation of genera. Thus the resurrection of the genus Nephromopsis is highly motivated. There are other important characters of this group besides the unusual position of apothecia: presence of laminal pseudocyphellae over the lower surface of the thallus; absence of soredia or isidia; presence of marginal as well as laminal pycnidia, frequently on emergent projections; occurrence of diagnostic medullary compounds (orcinol depsides and depsidones, anthraquinonic pigments and higher aliphatic acids, probably excluding caperatic acid). All the species of this genus are distributed in East and South-East Asia only (including the islands of Japan, Taiwan and Indonesia).

Till now 14 species have been referred as belonging to the recircumscribed genus Nephromopsis. According to the characters mentioned above some more

Cetrariae must be added to the genus*. Below we present a list of all species of Nephromopsis, including some new combinations. Information about the chemical compounds in each species is added, as well as a few remarks on morphology.

1. Nephromopsis asahinae (Sato) Räsänen, Kuopion Luonnon Ystäväin Yhdistyksen Julkaisuja B 2(6):50, 1952. -- Cetraria asahinae Sato. The species is reported to contain fumarprotocetraric and protocetraric acids (Yoshimura, 1979). Usnic acid is an accessory compound in the cortex (Randlane and Saag, 1991) and physodalic acid in the medulla (Lai, 1980). Pseudocyphellae occur over the upper as well as the lower surface.

2. N. ectocarpisma (Hue) Gyeln., Ann. Cryptog. Exot.4:173, 1931. -- N. stracheyi f. ectocarpisma Hue; Cetraria nephromoides (Nyl.) Vainio. Usnic acid in the cortex and some fatty acids in the medulla (lichesterinic, protolichesterinic, nephromopsinic or even caperatic acids have been reported).

3. N. endocrocea Asah., Journ. Jap. Bot. 11:24, 1935. -- Cetraria endocrocea (Asah.) Sato; N. endoxantha sensu Hue (pro parte). The species contains endocrocin and two fatty acids of nephrosteranic and nephrosterinic type (Culberson, 1969; Lai, 1980). Endocrocin is an anthraquinone pigment causing the orange colour of the medulla.

4. N. endoxanthoides (Awasthi) Randl. et Saag, comb. nov. Basionym: Cetraria endoxanthoides Awasthi, Bull. Bot. Surv. India 24:9, 1982. Fumarprotcetraric and protocetraric acids and traces of lichesterinic and protolichesterinic acids have been demonstrated in this species (Awasthi, 1982). The unidentified pigment, colouring medulla yellowish and causing its K+ yellow reaction, might be secalonic acid. This species is probably closely related to N. endocrocea, N. ornata and especially N. asahinae; N. endoxanthoides has pseudocyphellae also over the upper surface similarly to N. asahinae.

5. N. globulans (Nyl. ex Hue) Lai, Quart. Journ. Taiwan Museum 33:222, 1980. -- Cetraria globulans (Nyl. ex Hue) Zahlbr. Lai (1980) reports only the anthraquinonic pigment (secalonic acid C) that colours the medulla yellowish. TLC of the type specimen (China, Yunnan, 1885, Delavay, H-NYL) has also indicated usnic acid in the cortex and lichesterinic and protolichesterinic acids in the medulla.

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* We think that the generic position of Cetraria kurokawae Shibuichi et Yoshida and C. laureri Kremplh. needs futher studies. Therefore we do not include them into the genus Nephromopsis yet, though this was done by Kurokawa (1991). The species without pseudocyphellae over the lower surface (e. g. Nephromopsis ciliaris (Ach.) Hue) are included into the genus Tuckermannopsis Gyeln. in accordance with Lai (1980) and other recent authors.

6. N. isidioidea (Räsänen) Randl. et Saag, comb. nov. Basionym: Cetraria isidioidea (Räsänen) Awasthi, Bull. Bot. Surv. India 24:10, 1982. Lichesterinic and protolichesterinic acids have been reported by Awasthi (1982). Usnic acid, secalonic acid C and endocrocin have also been tested in the holotype (East Himalayas, Darjeeling district, 1948, D.Awasthi 179, H).

7. N. komarovii (Elenk.) Randl. et Saag, comb. nov. Basionym: Cetraria komarovii Elenk., Bull. Jard. Imp. Bot. St.-Petersbourg 3:51, 1903; C. perstraminea Zahlbr. The species contains usnic acid in the cortex and protolichesterinic and fumarprotocetraric acids in the medulla (Huneck et al., 1984). The latter seems to be an accessory compound in the material from eastern Siberia (17 specimens from TU analysed).

8. N. laxa (Zahlbr.) Sato, Journ. Jap. Bot. 14:783, 1938. -- Cetraria laxa (Zahlbr.) Sato; C. daibuensis Räsänen. Contains also usnic acid in addition to the lichesterinic - protolichesterinic type fatty acids reported by Lai (1980). This is the only species in the genus that bears marginal cilia.

9. N. morrisonicola Lai, Quart. Journ. Taiwan Museum 33:223, 1980. Usnic acid, lichesterinic - protolichesterinic type fatty acids and unidentified pigments have been reported (Lai, 1980) for this species.

10. N. nipponensis (Asah.) Lai, Quart. Journ. Taiwan Museum 33:223, 1980. -- Cetraria nipponensis (Asah.) Culb. Contains protolichesterinic acid as the main substance and physodic and conphysodic acids as accessory compounds (Yoshimura, 1979; Lai, 1980).

11. N. ornata (Müll. Arg.) Hue, Nouv. Arch. Mus. Hist. Nat., 4:90, 1900. -- Cetraria ornata Müll. Arg.; N. delavayi Hue; N. endoxantha sensu Hue (pro parte). Anthraquinonic pigments secalonic acid A (Park, 1990; Randlane and Saag, 1991) or secalonic acid C and the traces of endocrocin (Yosioka et al., 1972) are the substances in the medulla that cause its yellow colour. Fumarprotocetraric and usnic acids appear to be accessory.

12. N. pallescens (Schaer.) Park, Bryologist 93:122, 1990. -- Cetraria pallescens Schaer.; C. citrina Tayl.; C. teysmannii Mont. et Bosch. Contains usnic acid in the cortex and lichesterinic and protolichesterinic acids in the medulla (Yoshimura, 1979; Awasthi, 1982; Park, 1990).

13. N. pseudocomplicata (Asah.) Lai, Quart. Journ. Taiwan Museum 33:224, 1980 -- Cetraria pseudocomplicata Asah. Alectoronic acid is the main compound and α-collatolic and usnic acids are the accessories (Culberson, 1969; Lai, 1980).

14. N. rugosa Asah., Journ. Jap. Bot. 11:12, 1935. -- Cetraria rugosa (Asah.) Sato. Contains usnic acid in the cortex and either physodic or olivetoric acids in the medulla (Yoshimura, 1979; Lai, 1980; Randlane and Saag, 1991). It might be reasonable to describe these chemotypes as separate species.

15. N. stracheyi (Church. Bab.) Müll. Arg., Flora 74:374, 1891. -- Cetraria stracheyi Church. Bab. This species has apparently also two chemotypes: one with olivetoric (Lai, 1980; Awasthi, 1982) and the other with anziaic acid (Kurokawa, 1967). Usnic acid is an accessory substance. A specimen tested by us (Himalayas, R.Strachey and J.E.Winterbottom, LE) contained olivetoric acid as a major compound and anziaic acid as a minor one plus usnic acid.

16. N. yunnanensis (Nyl.) Randl. et Saag, comb. nov. Basionym: Cetraria yunnanensis (Nyl.) Zahlbr. Contains usnic acid in the cortex and lichesterinic and protolichesterinic acids in the medulla (one specimen tested: China, Yunnan, 1855, Delavay, H-NYL 36134). Pseudocyphellae occur over both the upper and lower cortex like in N. asahinae and N. endoxanthoides.

It may be concluded that in the genus Nephromopsis usnic acid is an accessory substance in most of the species. Therefore the presence or absence of it has no practical diagnostic value. Fatty acids occur quite frequently (in 11 species out of 16) in the genus. Lichesterinic - protolichesterinic type fatty acids appear to be the major substances in many cases and thus worthy of determination. Orcinol depsides (olivetoric and anziaic acids) and depsidones (alectoronic, α-collatolic, physodic and physodalic acids) are often accessory compounds, and therefore of lower value in the identification of species. Two species (N. rugosa and N. stracheyi) consist of two chemotypes, which contain either olivetoric or physodic/anziaic acids. The anthraquinone pigments (endocrocin, secalonic acid A and C) represent the most interesting group of chemical constituents in Nephromopsis. There are not many cetrarioid lichens that contain them. N. endocrocea, N. globulans, N. endoxanthoides, N. isidioidea and N. ornata form apparently quite a homogeneous group characterized by yellowish or ochraceous medulla. Still, some further studies of these rare eastern species are needed to make a correct identification key and show the possible derivation patterns inside the genus.

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NEW COMBINATIONS OF SOME CETRARIOID LICHENS (PARMELIACEAE)

TIINA RANDLANE and ANDRES SAAG

Laboratory of Bioindication, Tartu University

EE-2400 Tartu, Estonia

Abstract. The following new combinations are proposed on the basis of morphological, anatomical and chemical data: Allocetraria cucullata (Bellardi) Randl. et Saag, A. nivalis (L.) Randl. et Saag and A. potaninii (Oxn.) Randl. et Saag.

The same process that was initiated and successfully carried out by M. E. Hale in the genus Parmelia s. lat. is now proceeding in the large heterogeneous genus Cetraria. Although more than ten new or recently proposed small and homogeneous genera (Allocetraria Kurok. et Lai, Asahinea W. Culb. et C. Culb., Cetrariopsis Kurok., Cetrelia W. Culb. et C. Culb., Cetreliopsis Lai, Esslingeriana Hale et Lai, Masonhalea Kärnefelt, Nephromopsis Müll. Arg., Parmelaria Awasthi, Platismatia W. Culb. et C. Culb., Tuckermannopsis Gyeln.) have been separated from Cetraria, it still includes about five clearly quite different groups of species. One of such groupings is formed by Cetraria cucullata, C. nivalis and C. potaninii. They all are yellow, subfruticose or almost foliose lichens with usnic acid in the cortex. Therefore they do not suit well into the genus Cetraria s. str. represented by the type species C. islandica. Their anatomical structure of the cortex is also totally different from that of the brown fruticose Cetrariae (pachydermatous paraplectenchyma often overlying a thin prosoplectenchymatous tissue of the inner cortex) (Kärnefelt, 1979). When cortical structures in lichens are studied one must bear in mind that the terms "paraplectenchyma" and "prosoplectenchyma" are being used in two different meanings. We accept the terminology by Hale (1976) that determines first of all the hyphal orientation in the cortex and not the form of the lumina. The hyphae in the cortices of C. cucullata, C. nivalis and C. potaninii seem to be oriented anticlinally and thus ought to be considered a palisade plectenchyma. The hyphae are quite short-celled (with frequent septations) and densely conglutinated. In such circumstances the long and cross sections of the cortex are fairly similar in the light microscope to those of the paraplectenchymatous tissue. Still, the palisade plectenchymatous cortex is anticlinally striate in the general appearence and the cells are situated in considerably regular columns. This type of cortex is not very usual in the Parmeliaceae; it is known by now in the parmelioid genus Parmotrema and also in a newly described cetrarioid genus Allocetraria (Kurokawa and Lai, 1991). At present the latter includes three very rare species endemic to the Himalayan region (A. ambigua, A. isidiigera and A. stracheyi). Besides this anatomical and general morphological similarity several other common characters can be noticed between the species of Allocetraria and the yellow subfruticose Cetrariae. C. cucullata and C. nivalis, although often growing in the suberect or erect form, may have sparse rhizines along the margins or on the lower surface. This has also been mentioned for the species of Allocetraria but it is totally lacking in the group of brown Cetrariae. All the six named species have tiny pseudocyphellae on the lower surface, either marginal and/or laminal. Cortical as well as medullary chemistry of all these species show remarkable similarity. We cannot agree with Kurokawa and Lai (1991) who considered the chemistry of Allocetraria very unique. All of them produce usnic acid in the cortex. Lichesterinic and protolichesterinic acids appear to be the main medullary compounds in this group. Only in two species -- C. nivalis and A. isidiigera -- the fatty acids have not yet been demonstrated. None of these species contains any depsides or depsidones which are the most widely distributed lichen substances. The presence of anthraquinones (secalonic acid A and related pigments) in A. isidiigera and A. stracheyi is not quite unusual among the cetrarioid lichens. The same compound has been demonstrated in Nephromopsis ornata already (Randlane and Saag, 1991) as well as other closely related anthraquinonic pigments in several species of Nephromopsis. Endocrocin, the precursor of secalonic acid (Lai, 1980) together with other red and orange pigments, is also known in the basal part of C. cucullata (Krivoshchekova et al., 1982).

On all these considerations we propose to transfer C. cucullata, C. nivalis and C. potaninii into the genus Allocetraria Kurok. et Lai.

1.Allocetraria cucullata (Bellardi) Randl. et Saag, comb. nov.
Lichen cucullatus Bellardi, Obs. Bot. 1788:54.
Cetraria cucullata Ach., Meth. Lich. 1803:293.

2.Allocetraria nivalis (L.) Randl. et Saag, comb. nov.
Lichen nivalis L., Spec. Pl. 1753:1145.
Cetraria nivalis (L.) Ach., Meth. Lich. 1803:294.

3.Allocetraria potaninii (Oxn.) Randl. et Saag, comb. nov.
Cetraria potaninii Oxn., Journ. Cycle Bot. l'Acad. Sci. d'Ukraine, 1933:168.

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MYCOTAXON

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A NEW SPECIES OF THE LICHEN GENUS PUNCTELIA FROM THE MIDWESTERN UNITED STATES

Gerould Wilhelm & Douglas Ladd
The Morton Arboretum
Lisle, Illinois 60532, USA

Recent field work in the tall grass prairie and savanna provinces of the midwestern United States has revealed the presence of a distinct species of *Punctelia* Krog that is widely distributed and common in portions of the region. The species has been collected rarely because, until recently, relatively little collecting has occurred in this part of the country. The few older specimens which exist were determined as *P. subrudecta* (Nyl.) Krog. Although both taxa have pale lower cortices, lecanoric acid, and soredia, they are very different morphologically and ecologically.

Punctelia missouriensis Wilhelm and Ladd *sp. nov.*
Thallus ut in Punctelia subrudecta (Nyl.) Krog, *sed sorediis grossis granularibus, paucioribus quam decem soredia propria omni soralio; soralia erumpentes pseudocyphellis consociata; cortex plana superne, interdum lobulis clavatis complanatis vel teretiusculus, hi saepe cum soraliis immixis, sed aliquando tegentibus partem amplas corticis superi.*

Thallus foliose, typically 5 cm or more in diameter, the lobes mostly more than 1 mm wide; lower cortex pale to light tan; rhizines white to pale, mostly sparse and diminishing to incipient punctae near the lobe margins; upper cortex sometimes lobulate, gray, lustrous, the margins often brunnescent and weakly reticulate; pseudocyphellae numerous, minutely punctate in the lobe areas, enlarging to 0.2-0.3 mm in diameter and often with 1 or 2 elongate cracks, erupting into into fewer than 10 granular or lobuliform soredia, the soralia remaining discrete or coalescing into masses and sometimes associated with cortical cracks; cortex C-, K+ yellow (atranorin); medulla and soredia C+ red, K- (lecanoric acid). Apothecia very rare; microconidia not seen.

Type collection: MISSOURI. **Crawford Co.**, Onondaga Cave State Park, Vilander Bluff, on *Juniperus virginiana*; NW¼ NW¼ Sec.15 T39N R2W; Ladd & Wilhelm 15879, 22 DEC 1991 (Holotype: MOR; Isotypes: COLO, BAFC, F, IMI, LSU, MICH, NY, O, OMA, US).

Punctelia missouriensis is distinguished from *P. subrudecta* by its coarser, more granular to often lobuliform, sometimes partly corticate soredia occurring in small clusters of 10 or fewer per soralium; these soralia are almost always associated with pseudocyphellae or cracks in the upper cortex. *Punctelia subrudecta* has farinose to finely granulose soredia occurring in large numbers in each soralium; the soralia are often diffusely laminal and marginal as well as emanating from the pseudocyphellae, and the soralia average larger than in *P. missouriensis*. Additionally, *P. missouriensis* often has flattened, corticate lobules; these are absent in *P. subrudecta*. Plates I - III illustrate the differences between these taxa.

Two other diasporous taxa of *Punctelia* occur in the Midwest. *Punctelia rudecta* (Ach.) Krog is characterized by fine, cylindrical isidia, these often with darkening tips and sometimes branched. *P. perreticulata* (Räs.) Wilhelm & Ladd is a usually smaller lichen with a foveolate-ridged upper cortex and finely farinose marginal and laminal soredia.

Riefner (1989) has recently reported *Punctelia punctilla* (Hale) Krog from southern California. This is a smaller-lobed, coarsely isidiate species superficially similar to *P. missouriensis*. The cortical pseudocyphellae of *P. punctilla*, although sometimes appearing insipiently erumpent, do not develop into aggregated groups of well-defined soredia. *P. punctilla* is characterized by cylindrical to somewhat flattened, darkly apiculate isidia, as contrasted with the flattened, sorediose lobules sometimes occuring in *P. missouriensis*. The isidia in *P. punctilla* are frequently coralloid-branched; in *P. missouriensis* the lobules are infrequently simply-branched.

In her key to the genus, Krog (1982) implied that *P. bolliana* is consistently lobulate with isidioid to squamiform lobules, apparently on the basis of Mueller's (1877) type description of the species, which mentioned lobules. The vast majority of Midwestern collections of this taxon we have examined are without lobules. In rare specimens with a few lobulate processes, these are foliose and clearly not confuseable with isidia.

While the diaspores of *P. missouriensis* are not typical soredia as seen in *P. subrudecta*, they certainly represent medullar eruptions in the cortex which coalesce into discrete soralia. The diaspores of *P. punctilla* are clearly isidiate, and more closely related to those of *P. rudecta*. A revised key to North American *Punctelia* is included below.

An apparent analogue of *P. missouriensis* occurs in South America [*i.e.* Montes 12038G, Paraguay (NY)]. This evidently undescribed lichen has nearly identical diaspores, but a black lower cortex and contains gyrophoric acid. It has previously been confused with *P. constantimontium* Sérusiaux, a species with abundant lobuliform squamules. Conidiospores in *P. constantimontium* are uncinate and about 5-7 μ long, while in the analogue of *P. missouriensis*, the conidia are filiform and about 10-12 μ long. We have been unable to locate pycnidia on any specimens of *P. missouriensis*. Krog (1982) mentioned that the prevailing conidial morphology in the genus is unciform.

In North America, *Punctelia missouriensis* appears to be a common component of the lichen flora of the Tallgrass Prairie/Interior Highlands savanna biomes. It occurs regularly on exposed older trunks of oaks and other trees in areas of former prairie and open savanna vegetation, even when most of the ambient landscape has been converted to agriculture. It is largely absent from the more closed timbers currently existing in much of the Midwest, except that it occurs with some regularity on lightly shaded siliceous rock faces in the unglaciated districts. It occurs regularly where anthropogenic activity has rendered the landscape more open, such as in parks, along roadsides, and in cleared agricultural areas. About half of the nearly 200 specimens we have seen were collected on a species of *Quercus*, about 75% of which were in the section Erythrobalanus. About 10% of the specimens are from *Juniperus* and another 10% are saxicolous. The 169 known corticolous specimens occurred on 43 different tree species. We have also seen a specimen from the interior temperate region of Argentina, although no effort has been made to examine South American material.

It would appear that the center of distribution of this species is in the Interior Highlands region in North America. Significant portions of this region remain unexplored by lichenologists. Based upon our field observations, additional localities for *P. missouriensis* certainly occur in the largely unexplored region between the Mississippi River and the Appalachian Mountains. *Punctelia missouriensis* is common in the lower Midwest, especially through Missouri, northern Arkansas, southern Illinois and Indiana, western Kentucky, and Tennessee. It is known from 10 states, with approximately equal distribution in glaciated and unglaciated regions (Figure 1).

In the Interior Highlands, *P. missouriensis* occurs most commonly in high-quality natural areas, including savannas, glade margins, and bluff systems. In this region, it regularly occurs on saxicolous substrates as well as on trees. Elsewhere in its range, it occurs in remnant savannas and prairie border timbers, and in anthropogenically altered sites where older trees remain. In this latter habitat, *P. rudecta,* and often *P. bolliana,* are consistent associates.

Punctelia subrudecta, on the other hand, is a widely distributed lichen evidently tolerant of more closed woodlands. In the regions where its range overlaps *P. missouriensis,* it is restricted to remnant sites of fairly high natural quality. Typical substrates there include *Juniperus virginiana* and *Quercus alba,* as well as *Pinus echinata* and several other deciduous tree species. Judging from existing herbarium records, *P. subrudecta* is more common in eastern, northern, and western North America, where it occurs on a wide variety of corticolous substrates as well as on rocks. It is absent from the Tall Grass Prairie districts of the central United States. We have also seen specimens from Central America, Europe and Africa. In those areas where it is a consociate of *Punctelia missouriensis, P. subrudecta* is not as tolerant of agricultural activity or woodland clearing.

It is interesting to speculate whether *P. missouriensis* has spread recently with the advent of a plethora of anthropogenically created corticolous habitats, such as parks, pastures, landscaped areas, fencerows and farm borders. With perturbations to the landscape wrought by European settlement, a more or less continuous "bridge" of corticolous substrates has developed across the region while the presettlement timbers of the area, where they still exist, have become more closed-canopied as a result of fire suppression, and at the same time fragmented and interspersed with cleared areas. These conditions might have allowed an eastward spread of *P. missouriensis;* the Kentucky, Tennessee, and Alabama populations are all from widely spaced trees in anthropogenically altered landscapes. Many of the host trees in the glaciated, silt-loam districts of the prairie biome are also in artificial landscapes.

Key to the North American Species of *Punctelia*

Thallus without diaspores, though sometimes lobulate.
- Lower surface black or darkening.
  - Medulla C-, fatty acids only; eastern . . . . *P. appalachensis*
  - Medulla C+ rose, gyrophoric acid; Texas . . . . *P. subpraesignis*
- Lower surface pale to light tan.
  - Medulla C-, fatty acids only; almost always corticolous . . . . *P. bolliana*
  - Medulla C+ red, lecanoric acid; corticolous or saxicolous.
    - Microconidia (8)10-14μ; southwestern . . . . *P. hypoleucites*
    - Microconidia 4-8μ; widespread . . . . *P. semansiana*

Thallus isidiate or sorediate, lobulate or not.
- Lower surface prevailingly dark to black; medulla C- or C+ rose (gyrophoric acid).
  - Medulla C-, fatty acids only; pseudocyphellae large and easily distinguished as pore-like openings; soredia coarse and subisidiate . . . . *P. reddenda*
  - Medulla C+ rose; pseudocyphellae small, appearing as tiny white dots; soredia farinose.
    - Thallus brown and lustrous, on exposed rocks at high altitudes or along shorelines . . . . *P. stictica*
    - Thallus mineral gray, not notably lustrous; prevailingly corticolous; eastern . . . . *P. borreri*
- Lower surface pale to light tan; medulla C+ red (lecanoric acid).
  - Thallus with simple to coralloid isidia.
    - Isidia fine and cylindrical, generally with a shiny well-developed cortex; widespread on a variety of substrates . . . . *P. rudecta*
    - Isidia papilliform, dull, with a poorly developed cortex; saxicolous in southern California . . . . *P. punctilla*
  - Thallus sorediate, sometimes with lobules.
    - Soredia mostly fewer than 10 per soralium, coarsely pustular to lobulate, the soralia often appearing as eruptions from the pseudocyphellae . . . . *P. missouriensis*
    - Soredia farinose to finely granular, in well-developed marginal and laminal soralia; soredia numerous.
      - Upper cortex foveolate-ridged; lobes rarely more than 2 mm wide . . . . *P. perreticulata*
      - Upper cortex smooth or weakly reticulate; lobes typically more than 2 mm wide . . . . *P. subrudecta*

Representative Specimens

All specimens are deposited at the Morton Arboretum, Lisle, Illinois (MOR) unless indicated. Only one specimen is cited from each county.

ARGENTINA: CORDOBA. **San Alberto:** *sobre espinillos*, Estrabou 45037 (COLO).

UNITED STATES: ALABAMA. **Limestone:** on oak, Ladd 14435.

ARKANSAS. **Benton:** on *Quercus velutina*, Ladd 14632; **Carroll:** on *Juniperus ashei*, Ladd 14723; **Clay:** on *Quercus rubra*, Ladd 16109; **Greene:** on *Carya*, Ladd 15959; **Lee:** on *Fagus grandifolia*, Ladd 15693; **Madison:** on shaded, cherty limestone, Ladd 14785; **Monroe:** on *Quercus stellata*, Ladd 15669; **Montgomery:** Hale 6 (ILL); **Pike:** on shaded rock, Ladd 14960; **Prairie:** on *Quercus rubra*, Ladd 14868; **Stone:** on *Quercus falcata*, Ladd 15498.

ILLINOIS. **Carroll:** on *Quercus*, Jones 2679 & 2695; **Edgar:** on *Quercus rubra*, Wilhelm & Wetstein 17998; **Effingham:** on *Quercus velutina*, Wilhelm & Ladd 16453; **Fayette:** on *Quercus velutina*, Wilhelm & Wetstein 17077; **Gallatin:** on dry sandstone wall, Parker 2327; **Hardin:** on *Juglans nigra*, Wilhelm 16834; **Jackson:** on *Quercus*, Wilhelm 8300; **Jo Daviess:** on *Gleditsia triacanthos*, Wilhelm & Wetstein 19972; **Johnson:** on *Juniperus virginiana*, Wilhelm & Wetstein 19041; **Lee:** on *Quercus rubra*, Jones 1193a; **McLean:** on *Ostrya virginiana*, Wilhelm 16409; **Massac:** on *Quercus velutina*, Wilhelm & Wetstein 18784; **Piatt:** on *Quercus velutina*, Wilhelm & Wetstein 18020; **Rock Island:** on *Quercus macrocarpa*, Jones 1528; **Saline:** on *Juniperus virginiana*, Wilhelm & Johnson 16553; **Stephenson:** on fallen branch, Jones 1336; **Union:** on sandstone, Winterringer 1832 (ILL); **Wabash:** on *Quercus rubra*, Wilhelm & Wetstein 18157; **Warren:** on planted *Quercus palustris*, Wilhelm & Wetstein 19667; **Williamson:** on *Quercus rubra*, Wilhelm & Wetstein 19163.

INDIANA. **Bartholomew:** on *Fagus grandifolia*, Wilhelm 20063; **Gibson:** on *Acer saccharinum*, Wilhelm 20114; **Jasper:** on *Quercus velutina*, Wilhelm 14174; **Knox:** on *Quercus palustris*, Wilhelm 20115; **Montgomery:** on *Quercus alba*, Wilhelm *et al.* 18678; **Newton:** on *Quercus velutina*, Wilhelm 13254; **Sullivan:** on *Platanus occidentalis*, Wilhelm 20120; **Vanderburgh:** on *Quercus velutina*, Wilhelm 20113.

IOWA. **Clayton:** on *Pinus strobus*, Imshaug 28050 (MICH, MSC).

KANSAS. **Cherokee:** on *Quercus marilandica*, Ladd & Heuman 15563.

KENTUCKY. **Butler:** on *Ulmus alata*, Wilhelm 20108; **Daviess:** on *Quercus falcata, Wilhelm 20110*; **Franklin:** on *Malus pumila*, Ladd 11460; **Hardin:** on *Diospyros virginiana*, Wilhelm 20064; **Hart:** on *Quercus imbricaria*, Wilhelm 20067; **Henderson:** on *Quercus palustris*, Wilhelm 20112; **Ohio:** on *Quercus velutina*, Wilhelm 20109; **Simpson:** on *Carya ovata*, Wilhelm 20105; **Warren:** on *Quercus falcata*, Wilhelm 20068.

MICHIGAN. **Berrien:** on *Quercus velutina*, Wilhelm & Wetstein 19276.

MISSOURI. **Andrew:** on *Quercus macrocarpa*, Ladd 11264; **Audrain:** on *Quercus velutina*, Ladd & Wilhelm 9924; **Barry:** on *Juniperus*, Egan 12880 (Egan Herbarium); **Benton:** on shaded chert boulder, Ladd 9041; **Bollinger:** on *Quercus velutina*, Wilhelm & Ladd 11061; **Boone:** on *Gleditsia triacanthos*, Berry 219 (UMC); **Buchanan:** on *Quercus rubra*, Ladd 12296; **Butler:** on *Taxodium distichum*, Ladd 14183; **Callaway:** on *Quercus marilandica*, Ladd 11162; **Camden:** on *Cercis canadensis*, Ladd 15609; **Cape Girardeau:** on *Quercus velutina*, Skinner 846; **Carroll:** on *Quercus macrocarpa*, Ladd 8328; **Cedar:** on *Carya*, Ladd & Ladd 7830; **Clinton:** on *Quercus alba*, Ladd 15210; **Cole:** on *Fraxinus americana* Ladd 11992; **Cooper:** on *Celtis laevigata*, Ladd 10841; **Dade:** on *Quercus velutina*, Ladd 14860; **Dallas:** on *Prunus*, Ladd & Wilhelm 7424; **Daviess:** on *Acer* *saccharum*, Ladd 11231; **DeKalb:** on *Gleditsia triacanthos*, Ladd 11278; **Douglas:** on shaded sandstone ledge, Wilhelm 10870; **Dunklin:** on *Quercus stellata*, Summers 3153; **Gasconade:** on shaded sandstone boulder, Ladd 12646; **Greene:** on *Quercus marilandica*, Ladd & Ladd 8484; **Grundy:** on *Quercus palustris*, Ladd 15211; **Henry:** on fallen tree trunk, Ladd 13909; **Hickory:** on cherty sandstone boulder, Ladd & Ladd 7675; **Holt:** on *Quercus velutina*, Wilhelm & Wilhelm 15943; **Howell:** on *Quercus stellata*, Wilhelm 10981; **Iron:** on shaded rhyolite face, Ladd 14431; **Jasper:** on *Ulmus rubra*, Ladd 9340; **Jefferson:** on *Quercus velutina*, Wilhelm 11215; **Laclede:** on *Juniperus virginiana*, Ladd 10154; **Lafayette:** on rotting stump, Ladd 11493; **Lawrence:** on *Quercus stellata*, Ladd 10490; **Lewis:** on *Prunus serotina*, Ladd 14842; **Lincoln:** on *Fraxinus americana*, Ladd 15156; **Livingston:** on *Quercus macrocarpa*, Ladd 11057; **McDonald:** on *Ulmus alata*, Ladd 9241; **Macon:** on *Quercus velutina*, Wilhelm & Ladd 12354; **Madison:** on shaded red granite, Ladd & Schuette 12955; **Maries:** on *Quercus velutina*, Ladd 12597; **Marion:** on *Gleditsia triacanthos*, Ladd 10431; **Mercer:** on *Tilia americana*, Ladd 11288; **Mississippi:** on *Celtis*, Wilhelm 13377; **Moniteau:** on *Acer saccharinum*, Wilhelm & Ladd 15739; **Monroe:** on *Quercus rubra*, Ladd & Wilhelm 9851; **Montgomery:** on *Bumelia lanuginosa*, Ladd 13660; **Morgan:** on *Quercus imbricaria*, Ladd 10030; **New Madrid:** on *Quercus*, Wilhelm 11967; **Newton:** on *Quercus palustris*, Ladd 9285; **Nodaway:** on *Quercus velutina*, Wilhelm & Wilhelm 15919; **Osage:** on shaded sandstone, Ladd & Wilhelm 12514; **Ozark:** on *Carya*, Ladd & Wilhelm 7500; **Pemiscot:** on *Liquidambar styraciflua*, Ladd 10169; **Pettis:** on *Quercus alba*, Wilhelm 15209; **Phelps:** on mossy shaded sandstone wall, Ladd 12990; **Pike:** on *Quercus velutina*, Schuette 325; **Platte:** on *Quercus velutina* and *Q. alba*, Wilhelm & Wilhelm 15931; **Polk:** on *Quercus marilandica*, Ladd 10784; **Pulaski:** on *Juniperus virginiana*, Ladd 12573; **Ralls:** on *Juniperus virginiana*, Schuette 916; **Randolph:** on *Quercus velutina*, Wilhelm & Ladd 12373; **Reynolds:** on *Quercus velutina*, Ladd 11094; **St. Clair:** on exposed sandstone, Ladd & Ladd 6461; **St. Francois:** on *Quercus rubra*, Ladd & Summers 12089; **Ste. Genevieve:** on shaded sandstone wall, Ladd 10557; **St. Louis:** on *Carya*, Wilhelm 10716; **Scott:** on *Prunus serotina*, Wilhelm 10463; **Shannon:** on *Pinus echinata*, Ladd *et al.* 8671; **Shelby:** on *Juniperus virginiana*, Ladd & Wilhelm 9814; **Stoddard:** on *Quercus palustris*, Ladd & Wilhelm 7605; **Stone:** on *Quercus marilandica*, Ladd & Wilhelm 12362; **Sullivan:** on *Quercus palustris*, Ladd 11025; **Taney:** on *Juniperus virginiana*, Ladd & Ladd 9405; **Texas:** on *Juniperus virginiana*, Ladd 9222; **Vernon:** on shaded sandstone wall, Ladd 13142; **Warren:** on chert boulder, Ladd 10615; **Washington:** on *Juniperus virginiana*, Ladd 9788; **Webster:** on *Quercus velutina*, Ladd 6518; **Wright:** on *Ulmus alata*, Ladd 15016.

TENNESSEE. **Dickson:** on *Quercus rubra*, Ladd 14866; **Dyer:** on *Quercus lyrata*, Ladd 10181; **Giles:** on *Liquidambar styraciflua*, Wilhelm 20094; **Madison:** on *Quercus*, Ladd 14867; **Marshall:** on *Quercus alba*, Wilhelm 20098; **Maury:** on *Quercus velutina*, Wilhelm 20100; **Montgomery:** on *Quercus rubra*, Ladd 14862; **Robertson:** on *Acer rubrum*, Wilhelm 20069.

Acknowledgments

Appreciation is expressed to Richard Harris of the New York Botanical Garden for his review and comment; Webster Crowley of the Morton Arboretum and Marty Cano and Linda Johnson of the University of Nebraska Medical Center for electron microscopy, and Robert Egan of the University of Nebraska at Omaha for electron microscopy and reveiw and comment. Sherry Pittam of the Smithsonian Institution and Tim Hogan of the University of Colorado provided herbarium material.

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Riefner, R. E., Jr. 1989. *Punctelia punctilla* (Hale) Krog, new to North America. Phytologia 67: 254-257.

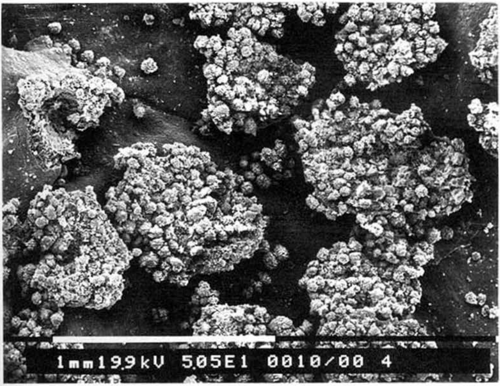


PLATE I. ***Punctelia subrudecta*** **[Ladd 14413, Missouri], 50X.**

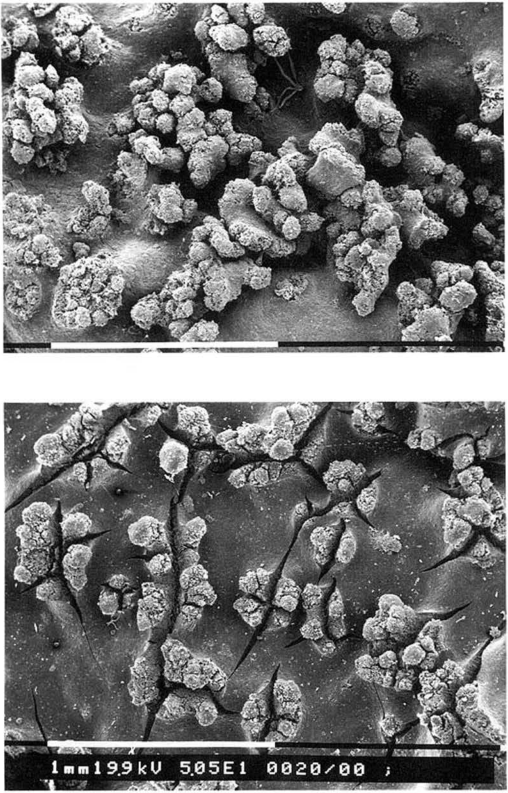
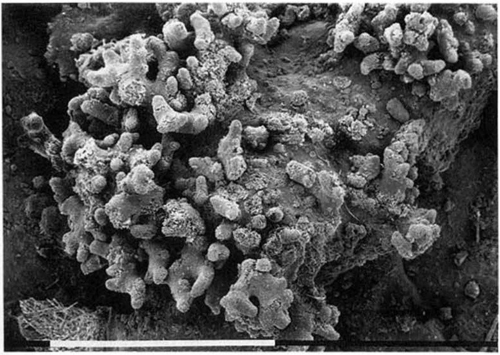


PLATE II. **Top:** ***Punctelia missouriensis*** **[Ladd 14189, Missouri], 50X; note lobules, some of which are sorediate. Bottom:** ***P. missouriensis*** **[Ladd 14652, Arkansas], 50X; note soredia associated with cracks in upper cortex.**



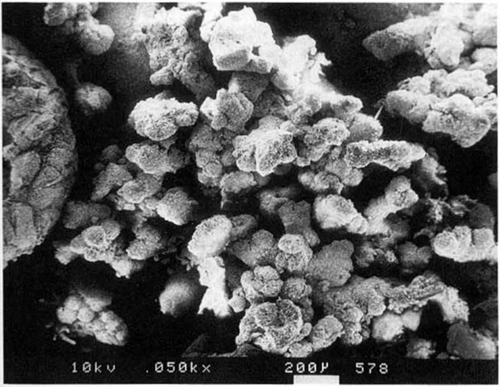


PLATE III. Top: *Punctelia rudecta* [Ladd 11169, Missouri], 50X. Bottom: *P. punctilla* [Almborn 8941, South Africa, Isotype (US)], 50X.

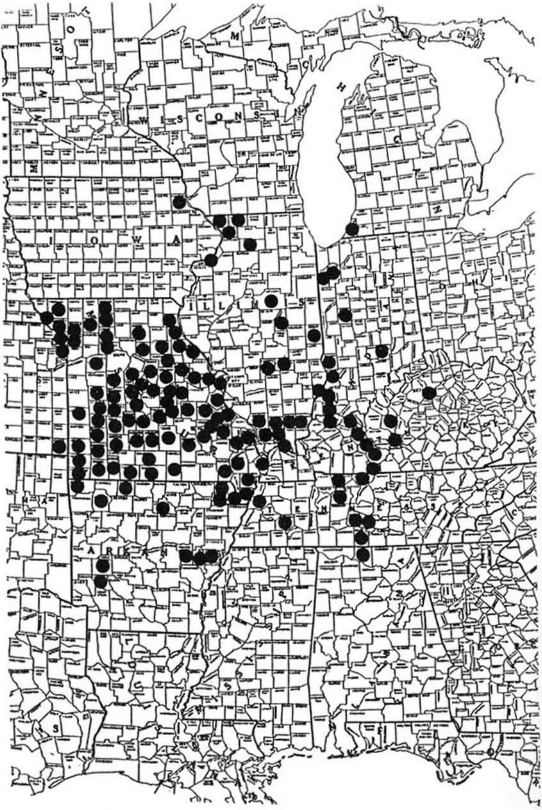


Figure 1. Range map of *Punctelia missouriensis* in North America.

MYCOTAXON

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BOOK REVIEWS

L. M. Kohn, Book Review Editor

Ascomyceten im Bild, by I. and H. Schmid. 1 Serie, Tafel 1-50, 16 (unnumbered) pp., 50 pl. in ring binder 20x22 cm, 1990. DM 88.-- US$55.-- 2 Serie, Tafel 51-100, 18 (unnumbered) pp., 50 pl., 15x21 cm, looseleaf for inclusion in binder provided with Serie 1, 1991. DM 86.-- US$53.75. IHW-Verlag, Bert-Brecht-Str. 18, D-8057 Eching, Germany. ISBN 25481072

Elegant, often gorgeous, colored photographs (two of each fungus illustrated, on opposite sides of each looseleaf sheet) are accompanied by clear macroscopic, microscopic, and ecological notes (students would be advised to brush up on their German), and by line drawings of important microscopic characters. Scales of magnification are clearly indicated, but the individual illustrations are not sub-numbered, a minor inconvenience in citing these figures.

Illustrated in these two series are 13 members of the Pezizales, 35 of Helotiales, 5 of Rhytismatales, 4 of Clavicipitales, 9 of Hypocreales, 1 of Polystigmatales, 2 of Ophiostomatales, 4 of Sordariales, 3 of Sphaeriales, 2 of Diaporthales, 1 of Elaphomycetales, 1 of Erysiphales, 1 of Gymnoascales, and 19 of Dothideales. Each fungus is assigned to a currently used family in an overview table provided at the beginning of each series. A sheet of important synonyms for the illustrated species is provided for each series, a literature listing for the citations on each plate is provided for each series, and indices to the species names (Gattungsindex) and to the species epithets (Artenindex) are provided for series I, with those indices being cumulative for both fascicles in series II.

This reviewer can scarcely wait for additional series to be published, and was pleased to see illustrated many Discomycetes (but also other Ascomycetes) that are relatively uncommon, and not well-illustrated elsewhere. That alone makes these plates essential to any library hoping to be comprehensive. In addition, the authors have a good grasp of modern taxonomy, and I find little to fault in their choice of names or their nomenclature. Typographical errors are very few. These plates are a delight to own. The quality is equivalent to that of Breitenbach and Kränzlin's *Fungi of Switzerland*, which is to say of a very high standard indeed. The format here allows for larger photographs, two for each species. And as in that book, the full collection data and place of deposit of the specimen for the illustrated material is provided. Bravo! *Richard P. Korf, Plant Pathology Herbarium, Cornell University, Ithaca, New York, USA.*

Russula-Monographie Romagnesis [,] Zum Studium von Täublingen unentbehriche Schlüssel und Tablellen aus der Russula-Monographie Romagnesis unter Berücksichtigung der Ergänzungen Romagnesis von 1985 und 1987. Paper, 66 pp. IHW-Verlag, Bert-Brecht-Str. 18, D-8057 Eching, Germany. DM 32.-- US$20.--

This booklet is intended for those who would find useful a German translation of the infrageneric descriptions and species keys from Henri Romagnesi's magnum opus, LES RUSSULES D'EUROPE ET D'AFRIQUE DU NORD (Éditions Bordas, Paris, 1967), as revised to include additional species and taxonomic and nomenclatural changes published later by Romagnesi. Also, descriptions of eight alpine species of Russula treated by Rober Kühner in 1975 are appended. The booklet illustrates neither fruit-bodies nor microscopic structures such as spores and pileus cuticular elements, whose characters are essential in identifying russulas but difficult to convey in words. In partial compensation for this the translator provides Romagnesi's keys to spore ornamentational units and patterns, which may help somewhat. Spore color is another significant taxonomic character in Russula, and a plate meant to reprduce Romagnesi's ten spore-color samples is also included. Unfortunately, in the review copy of the booklet "mittlecreme", "intensiv creme", and "blass ocker" are indistinguishable, as are "intensiv ocker" and "blass gelb"; only four of the samples are identical or reasonably close to their normal equivalents in my copy of Romagnesi's book itself. All things considered, I doubt that the booklet will give much satisfaction to someone struggling to identify a russula. *Robert L. Shaffer, University of Michigan Herbarium, Ann Arbor, Michigan, USA.*

Lichens Selecti Exsiccati Upsalienses. Fascicle 4, by R. Moberg. Thunbergia No. 14. Paper, 12 pp., 1991. Distributed by the Botanical Museum, Uppsala University. ISSN-0283-2275. Skr. 30.-- + postage [ca. US$6.--].

This publication documents with complete data the specimens distributed in the fourth fascicle of an exsiccati now containing 100 numbers and material from 17 countries. This fascicle includes 26 specimens, representing 25 taxa distributed in 21 genera, of which two are isotypes and one is from near the type locality. Most numbers are from Sweden but 9 are extra-limital collections. Critical comments are provided for several taxa. An alphabetical listing of the taxa included in the first 100 numbers is included of which 10 are types and 2 represent new combinations. Although of limited general usefulness, most herbaria with lichenological holdings will want to have a copy. *J. C. Krug, Dept. of Botany, University of Toronto, Canada.*

An Annotated List of Peronospora Names, by O. Constantinescu. Thunbergia No. 15. Paper, 110 pp., 1991. Distributed by the Botanical Museum, Uppsala University. ISSN-0283-2275. Skr. 110.-- + postage [ca. US$20.--]

This list includes 787 specific and subspecific names referred to Peronospora with 653 correctly placed in the genus of which 551 are valid and legitimate. Among the excluded names, 88 belong to other genera of the Peronosporales. Two new combinations are proposed. As the author indicates, the practice of considering one host to be infected by one species has resulted in a large number of uncritically described and sometimes invalid taxa. Each entry includes author, bibliographical citation, host, original locality, location of authentic specimens, and in some instances comments on taxonomic position. A simple coding, explained in the introduction, is used for the location of the specimen, nomenclatural status and taxonomic correctness. In most instances location of the original speciment and reference has been checked. On account of the large number of taxa little attempt is made to typify names but rather the compilation is restricted to bringing together and clarifying the information on taxa described in Peronospora. Only 36 taxa are typified based on examination of material and comparison with the protologue. A host-fungus index of all valid and invalid names is included. Since no modern treatment exists, this is an important compilation that will be essential for anyone dealing with this group of fungi. *J. C. Krug, Dept. of Botany, University of Toronto, Canada.*

The Genus Clavariadelphus in North America, by Andrew S. Methven. Bibliotheca Mycologia Band 138. Softcover, 143x233 mm, 192 pp., 1990. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Johannesstr. 3 A, D-7000 Stuttgart 1, Germany. (U.S. Agent: Lubrecht & Cramer, R.D. 1, Box 244, Forestburgh, NY 12777) ISBN 3-443-59039-X. DM 80.-- US$62.50

This monograph on Clavariadelphus constitutes an intensive study of the genus in North America, and a preliminary study of the genus worldwide. In this work new species are described, new characteristics are used, and the genus is redefined. While most of the work consists of keys and descriptions to 24 species or varieties, this information is augmented with tables of spore or chemical data, line drawings of hyphae and spores, six black and white plates of fruiting bodies, appendices, and an index of taxa. I particularly appreciated: 1) the extensive comparison of taxa, 2) the greater emphasis on hyphal structure, 3) the attempts to determine relative taxonomic value of the characteristics used, and 4) the systematic inclusion of pistillarin and phenoloxidase data. Breadth and depth are reflected by the many collections examined from 57 herbaria, representing most states and provinces of North America, and 20 other countries beyond the borders of the U.S. and Canada. Moreover, every taxon is represented by a type study, and neotypes are designated when necessary. Dr. Methven's work effectively updates monographs by Corner (1950, 1970) and Kempton & Wells (1968), and his work represents a major advancement in securing taxonomic stability. Graduate science libraries, undergraduate libraries supporting classes in mycology, and anyone interested in coral and cantharelloid fungi should consisder purchasing this book. *C. D. Marr, SUNY-Oneonta, New York, USA.*

Studies on Amanita (Amanitaceae) from Andean Columbia, by R. E. Tulloss, C. L. Ovrebo, and R. E. Halling. Memoirs of The New York Botanical Garden Volume 66. Paper, 46 pp., 17x25.4 cm, 1992. The New York Botanical Garden, Scientific Publications Department, Bronx, NY 10458-5126, USA. ISBN 0-89327-371-6. U.S. Orders $18.60; Non-U.S. Orders $19.90 (All orders prepaid and include postage and handling).

This is a treatise on amanitas occurring in a specific, significant habitat: Columbian oak forests on steep Andean slopes above 1800 meters, which are especially rich in Amanita. According to the publisher's blurb, "This habitat, which is seriously endangered from encroaching pastureland, is interesting phytogeographically because it represents the southern-most distribution of oak (Quercus) in the Americas, and sometimes contains Colombobalanus (Trigonobalanus) excelsa, a genus of Fagaceae that is endemic to Andean Columbia." Ironically, neither the Abstract (which is also provided in Spanish) nor the Introduction, while both concise and businesslike, deliver this compelling rationale. This study increases the number of taxa of Amanita recorded for Columbia from six to thirteen; nine new species are described, plus two new varieties of A. flavoconia. A type study of A. humboldtii is provided and misapplications of European names to Columbian collections by previous authors are critically examined. Line drawings of critical characters are provided and eight of the new taxa are illustrated by black and white photographs. Keys to subgenera and sections of Amanita and to the species from Columbia are provided. Clearly this treatment is an important step towards understanding the mycogeography of Amanita and will be most helpful to monographers and phytogeographers. It is also helpful in highlighting the limits of species concepts based on European collections in interpreting neotropical (and other extra-limital) specimens. *L.M.K.*

Frontiers in Mycology, edited by D. L. Hawksworth. Hardcover, 15x24 cm, 290 pp., 1991. C.A.B. International, Wallingford, Oxon 0X10 8DE, UK (U.S. Agent: 845 North Park Avenue, Tucson, AZ 85719). ISBN 0-85198-698-6. £40.-- US$76.-- (Americas only)

This book consists of edited versions of the key general lectures delivered at the Fourth International Mycological Congress held in Regensburg in 1990. It includes the following: Molecular aspects of ageing... (K. Esser), Fungal growth and development: a molecular perspective (J.G.H. Wessels), Importance of siderophores in fungal growth, sporulation and spore germination (G. Winkelman), Neoteny in the phylogeny of eumycota (H. Kreisel), Homologies and analogies in the evolution of lichens (J. Poelt), Mycorrhizas in ecosystems... (D. J. Read), The significance of Mycology in medicine (O. Male), Aerobiology and health... (J. Lacey), Lichens and Man (D.H.S. Richardson), Modified amatoxins and phallotoxins for biochemical, biological, and medical research (H. Faulstich), Mycology, mycologists, and biotechnology (J.D. Miller), Mycologists and nature conservation (E. Arnolds), and The teaching of Mycology (J. Webster). Abstracts of each chapter are provided and chapters are illustrated with halftone plates and line drawings, although the deftness of illustration varies among chapters. Though the readability of the chapters also varies, with some authors more creative in their synthesis than others, clearly this volume will provide stimulation to educators and their students in the fields of Mycology and Microbiology. As supplementary readings, many chapters are perfect introductions to applications of Mycology for upper level undergraduate students; the bibliographies are for the most part quite extensive. Although everyone will have their favorites, my students and I have found the chapter by E. Arnolds on nature conservation to be a refreshing and much-needed examination of the relevance of mycology (and how we as Mycologists can contribute) to solving some pressing environmental problems. Educators and their libraries will want to own this volume. *L.M.K.*

MYCOTAXON

Volume XLIV, no. 2, pp. 511 July-September 1992

NOTICE

A MAJOR CHANGE IN MYCOTAXON EDITORIAL POLICY CONCERNING OFFPRINTS

The cost of offprints has made it difficult or impossible for some of our authors, particularly in developing countries, to obtain these for their use. A new editorial policy addresses this problem:

Beginning with volume 45, a major change is being instituted by MYCOTAXON in regard to offprints. In an effort to be as ecologically sound as possible, the journal will now print only 100 extra copies to serve as offprints for authors. These will be provided *free of charge*, but authors are asked to pay postage and handling charges for shipping these. (If authors cannot obtain the US funds to pay the nominal shipping charges, the journal will ship them without payment, as a courtesy.) One hundred copies will be shipped to the address of the senior author, and *it will no longer be possible to order additional offprints beyond the 100 offprints we provide*, nor to ship to more than one address. For papers with joint authorship, the senior author will be expected to distribute offprints to co-workers on receipt.

Authors of book reviews will receive 25 offprints of their reviews, without charge.

As in the recent past (for articles but not for book reviews), we shall return one set of tear sheets of the article, the original manuscript, and figures to the senior author. *Additional reprints can be made by the author's local printer* from such a manuscript or from the tear sheets. Alternatively, authors who know they will want reprints in addition to the 100 offprints we provide may prefer to receive in addition the original photo-offset negatives used in production of the journal, for use by their local printer. This can be a much more expensive printing procedure. The negatives can be ordered when replying to the letter of acceptance of a manuscript (a shipping and handling fee for preparing the negatives for shipment will be charged).

MYCOTAXON

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NOTICE

THE HOLOMORPH CONFERENCE
A CONSIDERATION OF MITOTIC, MEIOTIC & PLEOMORPHIC SPECIATION IN FUNGI

AUGUST 4-7, 1992, NEWPORT, OREGON, U.S.A.

A Symposium, The Holomorph Conference: A Consideration of Mitotic, Meiotic and Pleomorphic Speciation in Fungi, will be held on August 4-7, 1992 in Newport, Oregon. The conference is being organized by Don R. Reynolds, Natural History Museum, Los Angeles, California 90007, and John W. Taylor, University of California, Berkeley, CA 94720 with the support of the National Science Foundation.

Topics to be considered are:

✓ *The Asexual Potential for Evolution.*
✓ *Integration of Mitotic and Meiotic, Morphological and Molecular Studies on Fungi.*
✓ *Nomenclature and Molecules.*
✓ *What are the Consequences of Abandoning the Deuteromycetes?*
✓ *How Could Mitotic, Meiotic and Pleomorphic Species be Classified Together?*

Reports from the conference are planned for the 1992 meetings of the Mycological Society of America together with the American Phytopathological Society in Portland, Oregon, August 8-12, 1992, which immediately follow the conference; the 2nd International Association for Lichenologists Symposium, Lund, Sweden; and the XIth Congress of European Mycologists, Kew, United Kingdom. Furthermore, CAB International has expressed an interest in publishing the conference proceedings.

For additional information contact:

Don Reynolds ☎ 213 744-3379 FAX 213 746-2999

MYCOTAXON

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NOTICE

6^{TH} INTERNATIONAL CONGRESS OF PLANT PATHOLOGY

JULY 28 - AUGUST 6, 1993

PALAIS DES CONGRÈS DE MONTRÉAL (CANADA)

SCIENTIFIC PROGRAM.

Symposia. The program will consist of five symposia running concurrently each morning. In addition there will be a plenary session on Sustainable Agriculture.

Posters/Discussion Sessions. The discussion sessions will be organized among groups of contributed posters on related topics. Time has been allotted in the afternoons to view the posters, followed by several concurrent discussion sessions, with time remaining to visit the posters for renewed dialogue with the authors.

Satellite Meetings. Persons interested in organizing a satellite meeting are requested to contact the Program Chair: Dr. M. C. Heath, Dept. of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario, Canada M5S 3B2. FAX: (416) 978-5878.

CONGRESS SECRETARIAT. *Mail all correspondence to:*

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6th International Congress of Plant Pathology
National Research Council Canada
Ottawa, Ontario, Canada K1A 0R6

Attention: Doris Ruest (Mrs.)
☎ (613) 993-9228
Telex: (613) 053-3145
FAX: (613) 957-9828

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The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers appearing in this volume:

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