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Author's correction:

page 122, in the paragraph Type material examined:
please change the name "*Ascophanus raripilus*"
to "*Ascobolus raripilus*".

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Taxonomic revision of the genus Cheilymenia – 7. A reassessment of the sections Paracheilymeniae and Raripilosae.

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Moravec J. (2003): Taxonomic revision of the genus Cheilymenia – 7. A reassessment of the sections Paracheilymeniae and Raripilosae. – Czech Mycol. 54: 113–133

Two of the sections of the genus *Cheilymenia* Boud., sect. *Paracheilymeniae*, and sect. *Raripilosae*, originally proposed in the infrageneric arrangement published in Moravec (1990) were reassessed. The section *Paracheilymeniae* is newly subdivided into three series: ser. *Paracheilymeniae* (introduced in detail in Moravec 1992), ser. *Raripilosae* (Moravec 1990) stat. n. (originally the separate section *Raripilosae*) and the here newly proposed monotypical ser. *Glabrae* ser. n. Two species of the ser. *Raripilosae*, *Cheilymenia raripila* (W. Phillips) Dennis, *Cheilymenia coprogena* (Berk. et Broome) Rifai and the type species of the new ser. *Glabrae* ser. n., *Cheilymenia bohemica* (Velen.) J. Moravec, are treated in details. All the taxa are illustrated with line drawings, photographs and SEM photomicrographs. Examination of the type material [K(M)] has revealed that *Lasiobolus dubius* Starbäck is a later synonym of *Cheilymenia raripila*.

Key words: *Cheilymenia*, section *Paracheilymeniae*, series *Paracheilymeniae*, series *Raripilosae*, series *Glabrae* ser. n., section *Micropilosae*, taxonomic revision, Discomycetes: Pezizales, Pyronemataceae.

Moravec J. (2003): Taxonomická revize rodu Cheilymenia – 7. Nové hodnocení sekcí Paracheilymeniae a Raripilosae. – Czech Mycol. 54: 113–133

Jsou přehodnoceny dvě ze sekcí rodu *Cheilymenia* Boud., sect. *Paracheilymeniae* a sect. *Raripilosae* původně navržené autorem ve vnitrodruhovém uspořádání rodu (Moravec 1990). Sekce *Paracheilymeniae* je zde rozdělena do tří serií: ser. *Paracheilymeniae* (podrobně představená v revizi Moravec 1992), ser. *Raripilosae* (Moravec 1990) stat. n. (původně samostatná sekce) a zde nově navržená ser. *Glabrae* ser. n. Dva druhy ser. *Raripilosae* – *Cheilymenia raripila* (W. Phillips) Dennis a *Cheilymenia coprogena* (Berk. et Broome) Rifai a typový druh ser. *Glabrae* ser. n. – *Cheilymenia bohemica* (Velen.) J. Moravec jsou detailně představeny. Všechny druhy jsou vyobrazeny kresbami mikroznaků a SEM mikrofotografiemi askospor. Revise typového materiálu odhalila, že *Lasiobolus dubius* Starbäck je pozdější synonymum *Cheilymenia raripila*.

The present paper follows the previously published contributions within the framework of the author's taxonomic revision of the genus *Cheilymenia* Boud. and presents other sections of the genus.

MATERIAL AND METHODS

Material from the herbaria listed below was examined with the usual methods. Only few specimens were examined on fresh material. Their ascospore size measured in vivo was in the range of those examined on the rehydrated material. Dried apothecia were rehydrated in distilled water for studying and measuring of ascospores. Any aggressive liquids which always destroy the outermost ascospore membrane (such as lactophenol), were strictly avoided for the rehydration and staining. As premature ascospores are usually more swollen, only fully mature ascospores (with yellow refractive colour when stained in C^4B) which ripened in normal eight-spored asci were measured. Extremely large mature ascospores, which occasionally are present together with aborted ones within the ascus, were considered abnormal and their size is given in brackets. The ascospores were stained with cotton blue C^4B ("Geigy s. 123" from an old supply) which stains directly without heating the slides. Using C^4B with the direct staining effect is necessary for studying the ascospore ornamentation in *Cheilymenia* in order to avoid destruction of the outermost, easily loosening perisporial membrane. For studying of the anatomy, median sections through apothecia were mostly rehydrated with 1 % of KOH and washed in distilled water before staining with C^4B in lactic acid or trypan blue.

Acronyms:

BHU	Museum für Naturkunde der Humboldt-Universität zu Berlin.
BRA	Botany Department, Slovak National Museum, Bratislava, Slovak Republic
BRNM	Botanical Department of the Moravian Museum, Brno, Czech Republic.
CO (formerly CONC)	Herbier Crouan, Department of Marine Biology, Museum National d'Histoire Naturelle, Concarneau, France.
CUP	Department of Plant Pathology, Cornell University, Ithaca, New York, U.S.A.
LPS	Instituto de Botánica C. Spegazzini, La Plata, Argentina.
K(M)	The Herbarium (Mycological) of the Royal Botanic Gardens, Kew, England, Great Britain.

PRM	Mycological Department of the National Museum, Prague, Czech Republic.
S	Naturhistoriska Riksmuseet, Section for Botany, Stockholm, Sweden.
WAG-W	Herbarium Vadense, Biological Station Wijster, The Netherlands.
WELTU	Herbarium of the Victoria University of Wellington, New Zealand.
J. Mor.	Private herbarium (Discomycetes) of J. Moravec, Adamov, Czech Republic.

TAXONOMIC RESULTS

Nine sections of the genus *Cheilymenia* were established in the infrageneric arrangement originally proposed in Moravec (1990). However, the classification was modified after further studying. Thus, *C. insignis* (H. Crouan et P. Crouan) Boud., which I originally classified as a species of the monotypical section *Insigniae* J. Moravec (1990), was later revised by me and for the presence of rooting apothecial hairs and well differentiated excipular layers proved to be a member of the typical section *Cheilymenia*. Consequently (Moravec 1993), the rank of the formerly separate section was reassessed and was recombined as ser. *Insignes* (J. Moravec) (grammatically correct form of the previously used name *"Insigniae"*) of the typical section *Cheilymenia*. A more recent study of the sect. *Paracheilymeniae* J. Moravec (1990) and sect. *Raripilosae* J. Moravec (1990) has resulted in the following modification of the infrageneric classification.

A synopsis of the two sections treated here – out of the 7 sections of the genus *Cheilymenia* Boud. (previously 9 sections within the infrageneric arrangement in Moravec, Mycotaxon 28: 475, 1990):

Genus **Cheilymenia** Boud., Bull. Soc. Mycol. France. 1: 105, 1885

Sect. 5. **Paracheilymeniae** J. Moravec, Mycotaxon 28: 475, 1990.

Type species: *Ascobolus pulcherrimus* H. Crouan et P. Crouan, Ann. Sci. Nat. (Bot.), ser. 4, 10: 196, 1858.

Ser. a. *Paracheilymeniae*

Species:

Cheilymenia pulcherrima (H. Crouan et P. Crouan) Boud., Hist. Class. Disc. Eur. 63, 1907 (type species of the section and series).

C. lacteoalba Arnolds et J. Moravec in J. Moravec, Czech Mycol. 47: 36, 1993.

C. aurantiacorubra Thind et Kaushal, Indian Phytopath. 33: 428, 1980.

C. lundqvistii J. Moravec, Mycotaxon 44: 67, 1992.

Ser. b. *Raripilosae* (J. Moravec) J. Moravec stat. n.

Basionym: sect. *Raripilosae* J. Moravec, Mycotaxon 28: 475, 1990.

Type species: *Ascobolus raripilus* W. Phillips, Grevillea 7: 23, 1878.

Species:

Cheilymenia raripila (W. Phillips) Dennis, Kew Bull. 14: 428, 1960.

C. coprogena (Berk. et Broome) Rifai, Verh. Koninkl. Nederl. Akad. Wetensch., Nat., Tweede Reeks, 57: 136, 1968.

Ser. c. *Glabrae* J. Moravec ser. n.

Series generis *Cheilymenia* Boud., sect. *Paracheilymeniae* J. Moravec, Mycotaxon 28: 475, 1990. Apothecia habitu *C. raripilae*, sed extus glabra. Typus (species typica et unica): *Fimaria bohemica* Velen., Mon. Discom. Boh. Pragae 1: 332, 2: Tab. 24, fig. 16, 1934.

Species:

Cheilymenia bohemica (Velen.) J. Moravec, Mycotaxon 38: 476, 1990.

The section *Paracheilymeniae* J. Moravec was originally proposed (Moravec 1990) for two species, *Cheilymenia pulcherrima* (H. Crouan et P. Crouan) Boud. and *C. aurantiacorubra* Thind et Kaushal. Later, *C. lundqvistii* J. Moravec (1992) was described and classified as a member of the section *Paracheilymeniae*. Simultaneously, all the species of the section were introduced in detailed descriptions and data (J. Moravec 1992). Finally, I treated *C. lacteoalba* Arnolds et J. Moravec in J. Moravec (1993) based on *Cheilymenia pallida* Arnolds (1982), [illegitimate homonym of *C. pallida* Bell et Dennis (1971)], as a species of the section *Paracheilymeniae*. The section *Paracheilymeniae* thus comprised four species characterised by small, turbinate to columniform apothecia of a simple anatomy and possessing short, superficial hairs. The receptacular hairs are not different from the marginal ones. The excipulum consists of a textura globulosa-angularis throughout, with the medullary layer not clearly differentiated, possessing only occasional hyphoid elements, and a thin hypothecium. The ascospores are small to large, with a nearly smooth to minutely warted perisporium (Moravec 1990, 1992, 1993). Regarding the type species, I have reexamined the holotype (CO) of *Ascobolus pulcherrimus* H. Crouan et P. Crouan, which was transferred into the genus *Cheilymenia* by Boudier (1907), but a detailed description was for the first time introduced by Brummelen (1986). A detailed description based on further material recently examined, as well as illustrations including SEM photomicrographs of the ascospore ornamentation, were given in Moravec (1992).

A further revision of the genus *Cheilymenia* has confirmed that the establishment of the section *Paracheilymeniae* to comprise the above mentioned species is justified but also that three species of the previously independent section *Raripilosae* J. Moravec (1990) – *C. raripila* (W. Phillips) Dennis, *C. coprogena* (Berk. et Broome) Rifai and *C. bohemica* (Velen.) J. Moravec, especially due to their similar excipular structure, should be accommodated in the section *Paracheilymeniae*. Two of them, *C. raripila* and *C. coprogena*, also possess apothecial hairs of a similar shape as those in the sect. *Paracheilymeniae*. Although

the originally separate status of the section *Raripilosae* proved to be superfluous, *C. raripila* and *C. coprogena* are distinguished by the shape of ripe apothecia which are more saucer-shaped and bearing a more distinct marginal collar; *C. bohemica* is, moreover, hairless. Therefore they deserve to be accommodated in two separate series of the section *Paracheilymeniae*.

The series *Raripilosae* is characterised by small apothecia of a simple anatomy, with the ectal layer consisting of a textura globulosa-angularis, whereby the medullary excipulum of a textura angularis is not distinctly differentiated from the ectal layer. The apothecia are sessile, first obconical, cup-shaped to saucer-shaped, possessing gentle but conspicuous, hyaline, indistinctly fissured marginal collar, externally sparsely covered by inconspicuous, pale, short, superficial to hyphoid hairs, a yellow to orange hymenium, large asci and large ascospores. The ascospores bear a separable outermost membrane (perisporium) which is covered by elongate, densely connected cyanophilic warts and crests, forming an incomplete to almost complete irregular reticulum on mature ascospores. The two species of the ser. *Raripilosae* differ from those of the typical series, ser. *Paracheilymeniae*, particularly by more discoid apothecia (they are flatter especially at maturity), in contrast to more constantly turbinate and barrel-shaped apothecia of the species of the ser. *Paracheilymeniae*. The apothecial hairs are of a very similar shape in both sections but those in species of the ser. *Paracheilymeniae* originate from abortive excipular cells, whilst those in the ser. *Raripilosae* are occasionally connected with excipular cells, which resemble short root germs. Unlike the ascospores of the ser. *Paracheilymeniae* which bear an almost smooth or finely isolate-warted perisporium, the ascospores of the species of the ser. *Raripilosae* and ser. *Glabrae* J. Moravec ser. n. bear a perisporium covered with cyanophilic fine crests forming an irregular, mostly very incomplete reticulum.

C. bohemica possesses hairless apothecia and therefore, the new monotypical series *Glabrae* J. Moravec ser. n. is proposed here to accommodate this species.

DESCRIPTIONS

Species of the series a. *Paracheilymeniae* (under a separate section) were treated in Moravec (1992) and supplemented in the additional note in Moravec (1993).

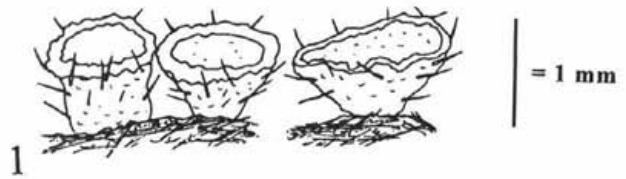
Species of the ser. b. *Raripilosae*

Cheilymenia raripila (W. Phillips) Dennis (Figs. 1–12; 24–25).

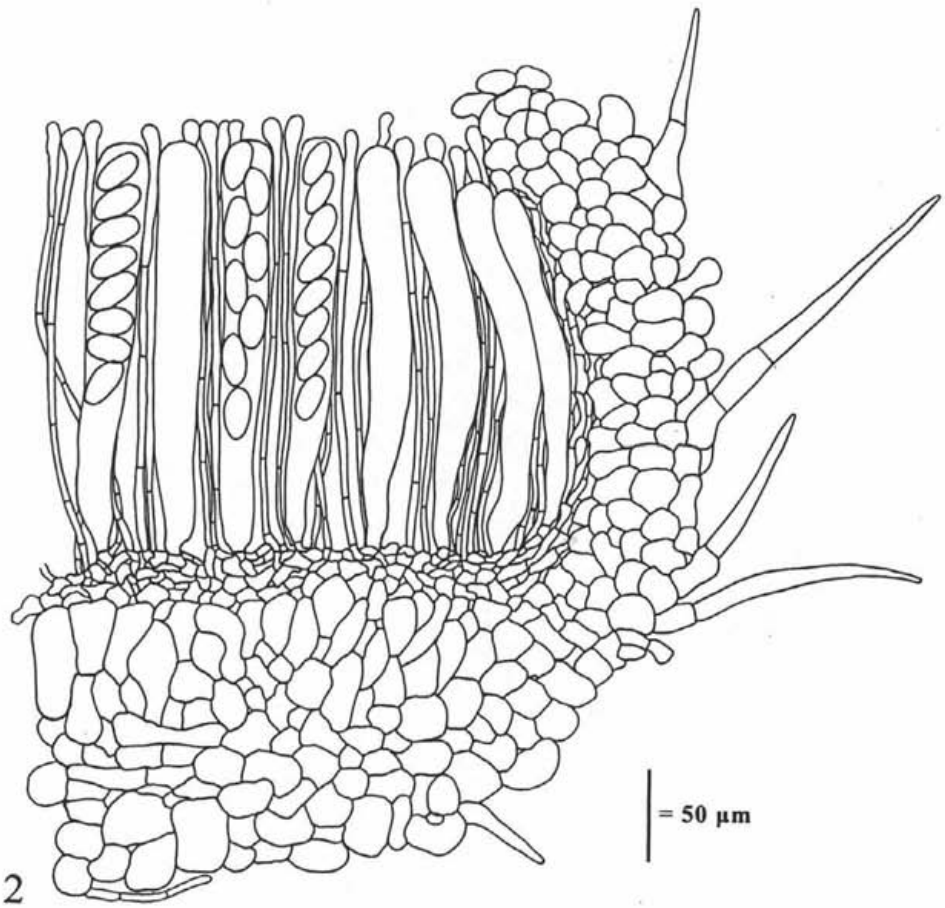
≡ *Ascobolus raripilus* W. Phillips, Grevillea 7: 23, 1878.
≡ *Lasiobolus raripilus* (W. Phillips) Saccardo, Syll. Fung. 8: 537, 1889.
≡ *Patella raripila* (W. Phillips) Seaver, N. Am. cup fungi (Operc.) 173, 1928.

≡ *Cheilymenia raripila* (W. Phillips) Dennis, Kew Bull. 14: 428, 1960.
= *Peziza hyalochaeta* Spegazzini, Ann. Soc. Cient. Arg. 10: 24, 1880.
≡ *Cheilymenia hyalochaeta* (Speg.) Gamundí, Lilloa 30: 326, 1960.
= *Lasiobolus dubius* Starbäck, Ark. Bot. 2 (5): 2, 1904.
= *Cheilymenia notabilispora* J. Moravec, Čes. Mykol. 22: 36, 1968.

Apothecia small, 0.3–1.5(–2.0) mm diam., scattered to densely gregarious, first obconical with a flat hymenium, then saucer shaped with a more or less distinct, slightly fissured membranaceous marginal collar but usually flat hymenium, old apothecia rarely more expanded with elevated margin; hymenium ochre-yellowish to orange-yellow, outer surface paler, sparsely covered with inconspicuous but comparatively long pale hairs. Hairs 90–280(–350) × 10–22(–25) μm (width measured at the base), superficial; marginal hairs of the same shape as the receptacular ones but more rigid and thick-walled (walls 0.7–3.0 μm thick), straight, sometimes with moderately wavy walls, with enlarged to bulbous base which originates from excipular cells; the base simple, rarely with indistinct cell-like appendages; apex blunt, subacute or rarely acute, hyaline to pale brown; towards the base of the apothecia the hairs are shorter and with thinner walls, occasionally feeble, with the apical portion folded downwards. Ectal excipulum not clearly delimited from the medullary layer, composed of a textura globulosa-angularis throughout; ectal layer 40–80 μm thick, narrower at the margin, composed of strongly cyanophilic, polygonal, mostly angular to subglobose cells 8–50(–65) μm diam., which are smaller and more isodiametric at the marginal zone and larger at the apothecial base, fluently passing into the medullary layer. Medulla 50–70 μm thick, consisting of more elongated and irregularly horizontally to mostly vertically oriented polygonal cells 10–25 μm diam., rarely some of them more elongated (up to 35 μm) thus resembling hyphal elements. Hypothecium sharply differentiated, 20–40 μm thick, composed of much smaller, irregular, angular to elongated cells 2–9 μm diam., passing into hyphae towards the lateral margins of the hymenium. Hymenium 200–250(–260) μm thick. Asci 180–230(–260) × 23–30 μm, operculate, broadly cylindrical with rounded to blunt apex, abruptly constricted at base, eight-spored (occasionally some of the ascospores aborted and then the ascus contains only six or four mature ascospores). Ascospores ellipsoid, (21.5–)24.0–29.0(–31.5) × 12.0–16.5(–18.5) μm, mostly 27 × 15 μm, uniseriate to occasionally biseriate, hyaline, eguttulate (with small drops observed in vivo), mature ascospores with yellow refractive colour when stained with C^4B in lactic acid, bearing a loosening perisporium which is ornamented with cyanophilic, irregular fine crests forming a dense irregular and mostly incomplete reticulum. Paraphyses filiform, 3–4 μm thick, unbranched, straight to moderately flexuous, septate, apices moderately to distinctly clavate-dilated, 6–12(–15) μm, occasionally obtusely lanceolate, with yellow to pale orange granular pigment.

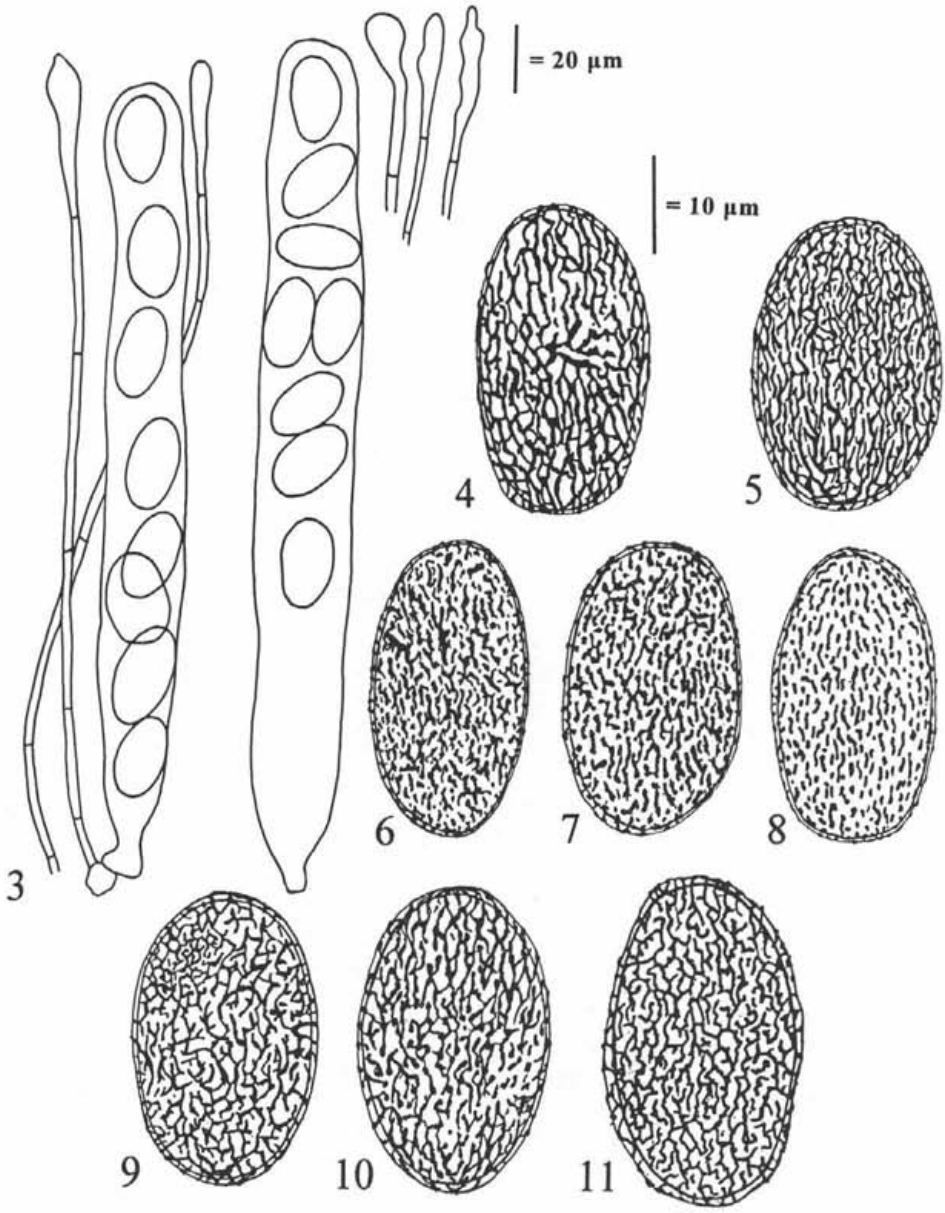


1



2

Figs. 1–2. *Cheilymenia raripila*. 1. apothecia; 2. part of medial section through apothecium (from Wellington, J. Mor.).



Figs. 3–11. *Cheilymenia raripila*. 3. asci and paraphyses [from holotype K(M)]; 4–11. ascospores, under oil immersion, stained with C^4B: 4. from holotype [K(M)]; 5. Wellington (J. Mor.); 6–7. Tucumán [K(M) ex T 3401]; 8. from the same specimen but premature ascospore; 9. from isotype of *C. notabilispora* (J. Mor.); 10. from holotype of *Lasiobolus dubius* [K(M)]; 11. from holotype of *C. hyalochaeta* (LPS).

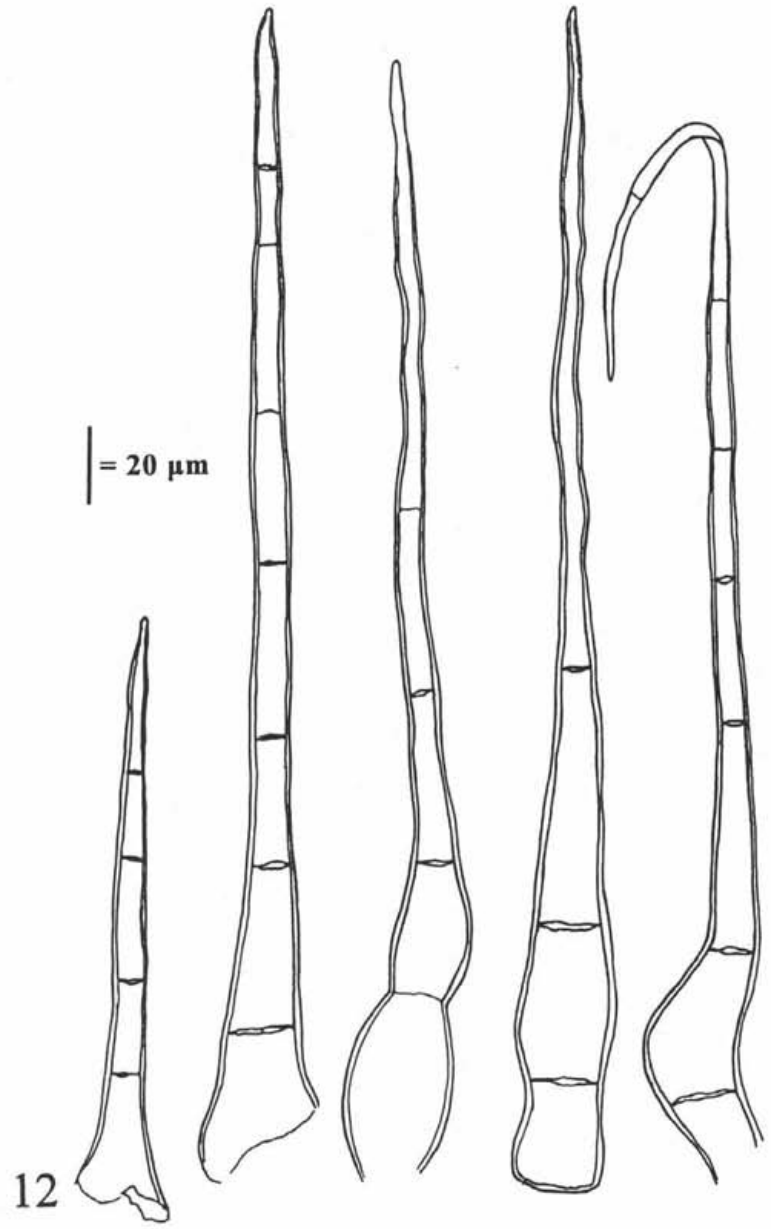


Fig. 12. *Cheilymenia raripila* – apothecial hairs (from Branžež, J. Mor.).

Habitat. On dung of cow, sheep and *Opossum*. Cosmopolitan distribution. Type locality: California. In North America it also occurs in Iowa (Seaver 1928). Besides the countries listed below, *C. raripila* occurs on the British Isles (Dennis 1972, 1979, 1981, Hawksworth 1976, Clarke 1980, Kirk et Spooner 1984) and in Australia (Rifai 1968). It is very interesting that it was not previously recorded from Bohemia neither by Velenovský (1934) nor Svrček (1948). Apothecia usually grow on last year's dung lying on pastures in early spring, sometimes immediately after snow melting; the other main (but less frequent) occurrence is in autumn.

C. raripila does not seem to be so rare as previously considered, it is rather easily overlooked due to its small apothecia.

Type material examined

North America: U. S. A.: California, On cow dung, s. dat., Harkness 509 [K(M), holotype of *Ascophanus raripilus* W. Phillips]. According to Yao and Spooner (1996), the holotype is "apparently exhausted and a neotype may be required". Fortunately, the holotype consists of at least two fully mature apothecia.

Other material examined

Argentina: Rio Cochun, Depto Chicligasta, Tucumán, on dung, 1 VI. 1959 leg. A. Türpe, det. R. Singer [K(M) ex T 3401]; Buenos Aires – Recoleta, sobre estiércol de vaca (in fimo vaccino), 5. V. 1880 leg. Spegazzini, as *Cheilymenia hyalochaeta* (Speg.) Gamundí (LPS 27338 – holotype of *Peziza hyalochaeta* Speg.), rev. J. Moravec; In Delta fluminis Paraná, ad fimum bovinum, 6. IX. 1894 (S – holotype of *Lasiobolus dubius* Starbäck).

New Zealand: South Island: Orinoco Valley near Motueca, on sheep excrements in a margin of pasture and forest, 4. III. 1993 leg. et det. Jiří Moravec (J. Mor., CUP); Marlborough, on cow dung, 12. V. 1978 leg. et det. Ann Bell (WELTU 305); Marlborough, on sheep dung, 4. VI. 1981 leg. et det. Ann Bell (WELTU 329). North Island: near Wellington, on cow dung at a farm, 27. II. 1993, leg et det. Jiří Moravec (J. Mor., CUP); Wellington, Orongorongo Valley, on *Opossum* dung, 20. X. 1970 leg. et det. Ann Bell (WELTU 54); Wellington, near DSIR Field Station, on dung of *Opossum*, 12. III. 1971 leg. et det. Ann Bell (WELTU 64); Wellington, near DSIR Field Station, on dung of *Opossum*, 24. VI. 1971 leg. et det. Ann Bell (WELTU 103); Wainuiomata Road, on cow dung, 22. VIII. 1971 leg. et det. Ann Bell (WELTU 109); Lake Ponuoui, on cow dung, s. dat., leg. et det. Ann Bell (WELTU 151); Wellington, Ohariu Valley Rd., 20. V. 1980 leg. et det. Ann Bell (WELTU 312); Wellington, Ohariu Valley Rd., 26. IV. 1981 leg. et det. Ann Bell (WELTU 335).

Czech Republic: Bohemia, prope Branžež, district Mladá Boleslav, in fimo vaccino in societate *Ascophani brunnescentis*, 17. III. 1967 leg. J. Moravec (holotype of

Cheilymenia notabilispora J. Moravec, PRM 628981, isotype J. Mor., BRNM, BRA, CUP); Branžež, 20. III. 1967, leg. et det. J. Moravec (J. Mor.); Branžež prope Kněžmost, 8. XI. 1987 leg. et det. J. Moravec (J. Mor., CUP); Branžež prope Mnichovo Hradiště, ad fimis vaccinis in prato, 29. X. 1988 leg. et det. J. Moravec (J. Mor., CUP).

Germany: Potsdam: Golmer Luch, Intensivweide nördlich Golm auf Rindermist, 5. XI. 1989 leg. et det. D. Benkert (as "*C.* cf. *pulcherrima*"), det. J. Moravec (BHU); Rügen: Weidefläche am Rande des Schlossparks Ralswiek auf Rindermist, 11. IX. 1988 leg. et det. D. Benkert (as "*C.* cf. *pulcherrima*"), det. J. Moravec (BHU); Demmin: Wiesen im Peenetal bei der Vorwerker Schweiz auf Rindermist, 31. X. 1982 leg. et det. D. Benkert (as "*C.* cf. *pulcherrima*"), det. J. Moravec (BHU).

The Netherlands: Dwingeloo, Noordenveld (illegibly handwritten label), 17. XI. 1976 (determined by Arnolds as "*C. fimicola*" – teste Arnolds (1982), det. J. Moravec (WAG-W, A 3774).

Azores: Monte Brasil, Terceira, in fimo vaccino, 24. III. 1975 [K(M)] – holotype of *Cheilymenia theleboloides* var. *microspora* Dennis (the name based on a mixture of different fungi, the apothecia of *C. raripila* are densely aggregated together with *C. granulata*, *C. insignis*, *Lasiobolus* sp. and other discomycetes.

Remarks

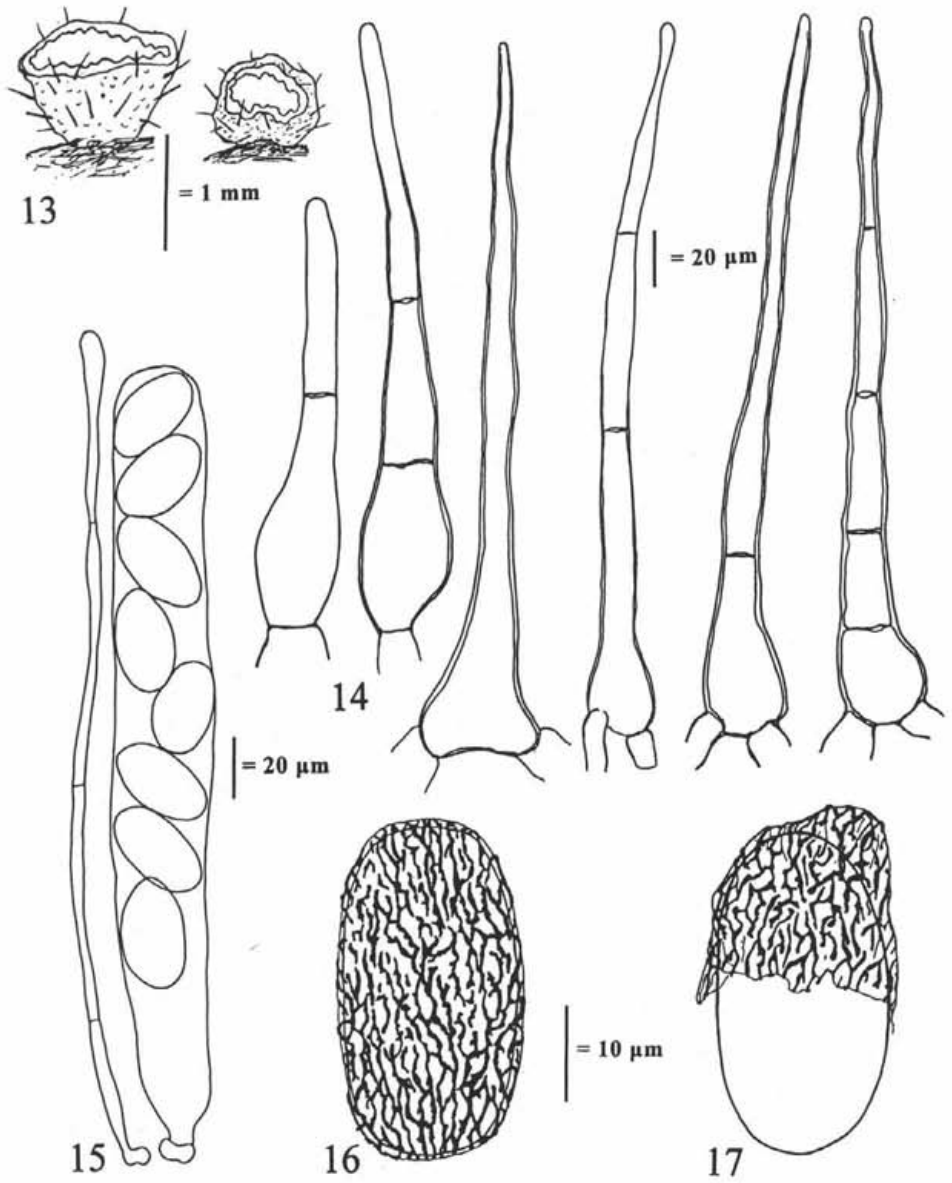
The type species of the series *Raripilosae*, *Cheilymenia raripila* (W. Phillips) Dennis, was for the first time introduced in detail by Rifai (1968) and later by Brummelen (1986). These authors stated the ascospore size somewhat smaller than measured by me in the holotype [K(M)] and all other specimens. They mentioned also the loosening and wrinkling outermost sheath on the ascospores of this species but considered the reticulate ornament on the outermost membrane to be a deformation of the sheath as a consequence of staining in lactic acid or in lactophenol. Nevertheless, although the outermost secondary membrane (perisporium), when heated in lactophenol, is easily deformed and separated from the ascospore, the ornamentation represents an important diagnostic feature of ascospores in *C. raripila* and *C. coprogena* as the cyanophilic ornamentation is reliably recognisable also when the perisporium is evenly stretched, not deformed. The subreticulate ornamentation was already recognised by Gamundí (1960) on ascospores of *C. raripila* (treated by her under the synonym *C. hyalochaeta*), in detail illustrated and also proved by SEM in Moravec (1990) and here. The different pattern of the ascospore ornamentation on non-deformed perisporium in many species of *Cheilymenia* was proved by SEM and discussed many times, lately in Moravec (1998). In contrast, Yao et Spooner (1996: 364) were unable to find the ornamentation and the outermost perisporial membrane (neither in other species of *Cheilymenia* treated by them), and argued that the presence of the

loosening perispore "may prove to have little diagnostic value in *Cheilymenia*". In fact, the loosening outermost membrane is one of the fundamental features of the genus which was recognised and mentioned by all authors who examined species of *Cheilymenia* during the long history of the taxonomy of the genus. As already discussed (Moravec 1998), such a discrepancy in observations is obviously based on inappropriate staining with lactophenol which involves heating of slides and causes the destruction of the perisporium that is afterwards separated from the ascospores, which consequently appear smooth. The perisporial ornamentation is developed in the course of ascospore maturing as the perisporium on premature ascospores bears isolate coarse warts or large pustules that at maturity change into irregular crests forming the subreticulate ornament. When the ascospores are stained with C^4B in lactic acid without heating, the ornament is relatively constant and well recognisable even on ascospores of old dissections after they have repeatedly been stained. Nevertheless, even in properly stained dissections, we can always find some ascospores with a deformed and partly separated perisporium (Fig. 17).

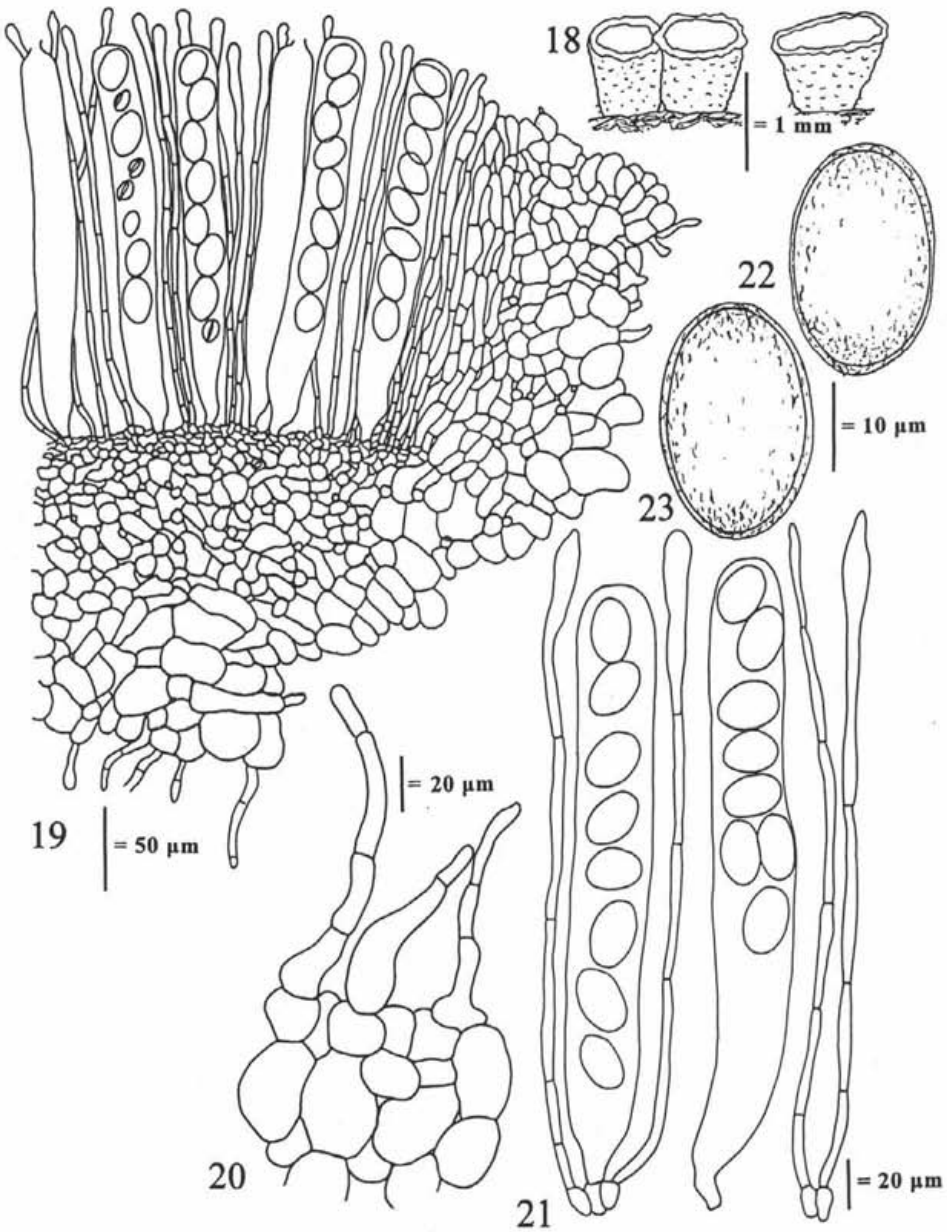
The examination of the holotype (LPS) of *Cheilymenia hyalochaeta* (Speg.) Gamundí (1960), a taxon already supposed to be a synonym of *C. raripila* by Brummelen (1986), as well as the reexamination of the holotype (PRM) of *Cheilymenia notabilispora* J. Moravec (1968), confirmed the identity with *C. raripila* (see also Moravec 1990).

Furthermore, the examination of the holotype of *Lasiobolus dubius* Starbäck (1904) deposited in the S herbarium has revealed that this taxon is also identical with *C. raripila*.

My examination of collection N° 2382 Dennis from the herbaria VEN and K(M), reported by Dennis (1960) from Venezuela under the name *C. raripila* with his new combination of this taxon into the genus *Cheilymenia*, has revealed that the collection paradoxically represents a distinct species of *Cheilymenia*, belonging to the section *Pseudoscutelliniae* J. Moravec (1990). Moreover, my examination of the holotype [K(M)] of *C. theleboloides* var. *microspora* Dennis in Dennis, Reid et Spooner (1977) disclosed that the description of the taxon was based upon apothecia in fact belonging to several different species growing together on a common portion of cow dung (Moravec 1992, 1993). One of the species, the apothecia of which shared the substrate, was identified by me as *C. raripila*. Similarly, one of the collections erroneously reported as *C. fimicola* by Arnolds (1982), A 3774 (WAG-W), represents in fact *C. raripila*.



Figs. 13–17. *Cheilymenia coprogena*. 13. apothecia; 14. apothecial hairs; 15. ascus and paraphysa; 16. ascospore under oil immersion, stained with C^4B; 17. ascospore with partly separated perisporium [all from holotype K(M)].



Figs. 18–23. *Cheilymenia bohemica*. 18. apothecia; 19. part of medial section through apothecium (from WAG-W, A 981); 20. hyphae arising from cells of ectal excipulum; 21. asci and paraphyses; 22. ascospore under oil immersion stained with C^4B (from holotype PRM); 23. ascospore from WAG-W, A 981.

Cheilymenia coprogena (Berk. et Broome) Rifai (Figs. 13–17)

≡ *Peziza* (*Lachnea*) *coprogena* Berk. et Broome, Trans. Linn. Soc. Lond. II. 2: 69, 1882.
≡ *Lachnea coprogena* (Berk. et Broome) Saccardo, Syll. Fung. 8: 181, 1889.
≡ *Scutellinia coprogena* (Berk. et Broome) Kuntze, Rev. Gen. Pl. 2: 869, 1891.
≡ *Cheilymenia coprogena* (Berk. et Broome) Rifai, Verh. Koninkl. Nederl. Akad. Wetensch., Nat., Tweede Reeks, 57: 136, 1968.

Apothecia small, 0.3–1.5 mm diam. (0.2–0.5 mm when dried), scattered, first obconical with broad base and flat to shallowly concave hymenium, distinctly braided with fissured, mostly inward oriented marginal collar, then more broadly saucer shaped; hymenium pale orange ["subaurantiaca" according to Berk. & Broome (1882)], outer surface paler, sparsely covered with inconspicuous pale hairs. Hairs superficial, hyaline to pale brown; marginal and receptacular hairs of the same shape; the receptacular hairs thin-walled (walls 0.5 μm thick), marginal hairs either thin-walled or rarely thick-walled (walls 0.7–1.5(–3) μm thick), sparsely septate, straight, usually with moderately sinuous walls, 80–280(–310) × 10–25(–30) μm (the width measured at enlarged base), arising from excipular cells; the base enlarged to bulbous, simple or usually with elongate cell-like appendages resembling root germs; apex rounded, or narrowed but blunt, rarely subacute, only exceptionally acute. Excipulum composed of a textura globulosa-angularis throughout (similar to that in *C. raripila*), not clearly differentiated from the medullary layer, ectal layer composed of polygonal, mostly angular to subglobose, strongly cyanophilic cells 10–60 μm diam., smaller and more isodiametric in marginal zone, larger at the apothecial base, fluently passing into a medullary layer. Medulla consisting of similar, more elongated and irregularly oriented polygonal cells which measure 10–25 μm diam. but are occasionally more elongated up to 50 μm in length, thus indistinctly resembling hyphal elements. Hypothecium rather sharply differentiated, composed of much smaller irregular, angular to elongated cells 4–10 μm diam., towards the lateral margins passing into marginal hyphae. Hymenium about 180–240 μm thick. Asci (170–)190–235(–240) × (20–)25–30(–34) μm, operculate, broadly cylindrical with rounded apex, abruptly constricted at base into a short stipe, eight-spored, (occasionally some of the ascospores aborted and then one ascus may contain only six or four mature ascospores). Ascospores ellipsoid, (24.0–)25.0–33.5(–36) × (12.0–)13.5–19.0(–20.0) μm, mostly 30.0 × 17.0 μm, uniseriate or occasionally some ascospores within the ascus biseriate, hyaline, eguttulate; mature ascospores with yellow refractive colour when stained with C^4B in lactic acid, bearing a loosening perisporium, which has the same ornamentation as that on the perisporium of *C. raripila*, consisting of cyanophilic, fine irregular

crests which form a dense irregular and mostly incomplete reticulum. Paraphyses filiform, 3–4 μ thick, unbranched, straight to moderately flexuous, septate, apices moderately to distinctly clavate-dilated to 5–8(–10) μm diam.

Habitat. On cow dung in Brisbane, Australia, known only from the holotype.

Type material examined

Australia: Brisbane (Queensland), on cow dung, s. dat., F. M. Balley N° 205 – holotype of *Peziza* (*Lachnea*) *coprogena* Berk. et Broome [K(M)] – about 18 apothecia aggregated on a fragment of cow dung – the apothecia 0.2–0.5 mm diam. when dried.

Remarks

The species was well introduced and properly transferred to the genus *Cheilymenia* by Rifai (1968). He considered it closely related to *C. raripila* but kept it separate on the basis of its much larger ascospores and fewer, more delicate thin-walled hairs. However, as is seen from the description above, the examination of the holotype of *C. coprogena* [K(M)] revealed several differences, especially the presence of thick-walled hairs. The extreme size of the ascospores given by Rifai (1968) probably belongs to those which were developed within the asci with a reduced number of ascospores. Nevertheless, also the ascospores in normally eight-spored asci are larger than those in *C. raripila*. Moreover, although I found the thick-walled hairs also in *C. coprogena*, their apex is usually more rounded, and the paraphyses are of a simpler clavate shape and not so distinctly enlarged and variously shaped as those in *C. raripila*.

The following table shows the variability of the ascospore size based on several collections of *C. raripila* in comparison with the closely related *C. coprogena*. The commonly used term "average size" is not used by me in the statement of the ascospore size. Instead, I used "mostly" which means the mostly occurring ascospore size in each individual collection. This much better reflects the genuine size of most ascospores than the artificially counted "average size".

As showed on the table, the ascospores (normally developed within eight-spored asci) of *C. raripila* mostly do not exceed 29 μm in length whilst those of *C. coprogena* reach 33.5 μm. The exceptional size (in parenthesis) of ascospores (which ripened within asci with a reduced number of ascospores due to the fact that some of them aborted during their development), does not exceed 30 μm in *C. raripila*. An exception is the holotype of the synonymous *C. hyalochaeta*, where such abnormal ascospores reach 31.5 μm of length but do not yet reach the length of such abnormal ascospores in *C. coprogena* (36 μm). Therefore, regarding the different ascospore size and also the other slightly different features mentioned above, I follow Rifai (1968) and keep *C. raripila* as a separate species here.

Table 1.

species and collection	ascospore size
C. raripila, holotype [K(M)]	(22.0-)24.0–28.5(–30.0) × 12.0–16.5(–18.0) μm – mostly 27.0 × 15.0 μm
C. raripila, Tucumán [K(M) ex T 3401]	(22.5-)24.0–28.5(–30.0) × 12.0–16.5(–18.0) μm – mostly 27.0 × 15.0 μm
C. raripila, Wellington (J. Mor.)	(22.0-)23.0–28.5(–30.0) × 12.0–16.5(–18.0) μm – mostly 27.0 × 15.0 μm
C. raripila, holotype of *C. hyalochaeta* (LPS)	(22.5-)24.0–29.5(–31.5) × 12.0–16.5(–18.5) μm – mostly 28.5 × 16.5 μm
C. raripila, holotype of *C. notabilispora* (PRM)	(22.0-)24.0–28.5(–30.0) × 12.0–17.0(–18.0) μm – mostly 27.0 × 16.0 μm
C. raripila, holotype of *Lasiobolus dubius* (S)	(22.0-)23.0–28.5(–30.0) × 13.5–16.0(–17.0) μm – mostly 27.0 × 15.0 μm
C. coprogena, holotype [K(M)]	(24.0-)25.0–33.5(–36.0) × 12.0–19.0(–20.0) μm – mostly 30.0 × 17.0 μm

Humaria coprogena Saccardo (1914) is a species of the genus *Peziza* Dill. ex L: Fr. (teste Brummelen 1995).

Ser. c. *Glabrae* J. Moravec ser. n.

Cheilymenia bohemica (Velen.) J. Moravec (Figs. 18–23; 27).

≡ *Fimaria bohemica* Velenovský, Mon. Discom. Boh. 1: 332, 2: tab. 24, fig. 16, 1934.
≡ *Coprobia bohemica* (Velen.) Svrček, Čes. Mykol. 31: 69, 1977.
≡ *Cheilymenia bohemica* (Velen.) J. Moravec, Mycotaxon 38: 476, 1990.

Apothecia gregarious, small, [according to Velenovský (1934) 1–2 mm diam.], 0.1–0.6 mm diam. when dried, sessile, first columniform-obconical, slightly attenuated towards base, with flat hymenium, thinly braided with whitish membranaceous marginal collar; orange ["tota splendide aurantia" according to Velenovský (1934)], dried apothecia ochraceous ["whitish, almost white" according to Svrček (1979)], outer surface nearly glabrous with only indistinct sparse hyphal outgrowths near the base of apothecia; hyphae 30–80 μm long and 7–15 μm wide at their bulbous base, hyaline. Excipulum composed of a textura globulosa-angularis throughout, not clearly differentiated from the medulla; ectal layer 60–100 μm thick, usually narrower in the marginal zone, composed of polygonal, angular to globose, strongly cyanophilic cells 15–45(–60) μm diam., fluently passing into the medullary layer, which is 60–80 μm thick and consists of similar, but mostly smaller cells 10–25(–35) μm diam. Hypothecium only very indistinctly differentiated, very thin, composed of smaller irregular, angular to elongate cells 4–15 μm diam., towards the lateral margins passing into hyphae which separate the inner margin of the hymenium from the excipular margin. Hymenium 170–220 μm thick. Asci (160–)205(–220) × 22.5–27.0(–30) μm,

operculate, broadly cylindrical with rounded apex, abruptly constricted at base into a short stipe, eight-spored, occasionally some ascospores within the ascus aborted. Ascospores ellipsoid, (20.0–)21.0–27.0(–28.5) × (12.0–)13.5–16.5(–19.0) μm, mostly 23.5 × 15 μm, uniseriate or occasionally some ascospores within the same ascus biseriate, hyaline, eguttulate, mature ascospores with yellow refractive colour when stained with C^4B in lactic acid, bearing a loosening perisporium which under an optical microscope appears almost smooth, with only few recognisable wrinkles; SEM photomicrographs show fine irregular ornaments consisting of low and blunt, delicate to more distinct crests. Paraphyses filiform, 3.5–4.0 μm thick, unbranched, straight to moderately flexuous, septate, apices not to moderately enlarged (4.5–8.0 μm), occasionally the dilated apex is constricted above.

Habitat. On cow dung in the Czech Republic (type locality) and in the Netherlands.

Type material examined

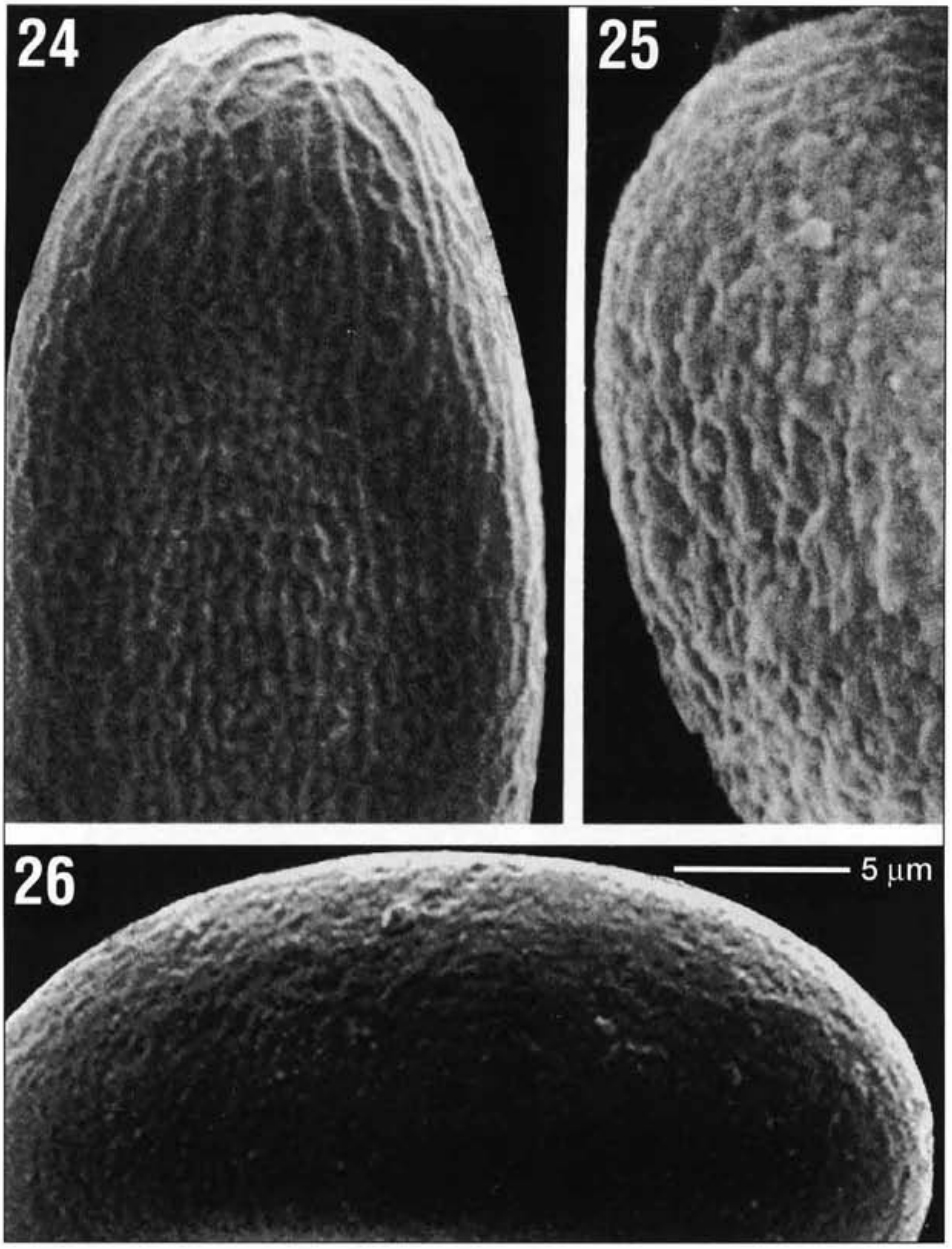
Prope Kostomlaty pod Milešovkou (Bohemia), ad excrementa vaccina, leg. Jan Šimr, det. J. Velenovský (originally labelled with the unpublished invalid herbarium name "*Humaria macrospora* Velen.") – holotype of *Fimaria bohemica* Velen. (PRM 149767). The apothecia are difficult to be recognised on the excrement fragment, they appear to be of an indefinite shape and are probably incomplete.

Other material examined

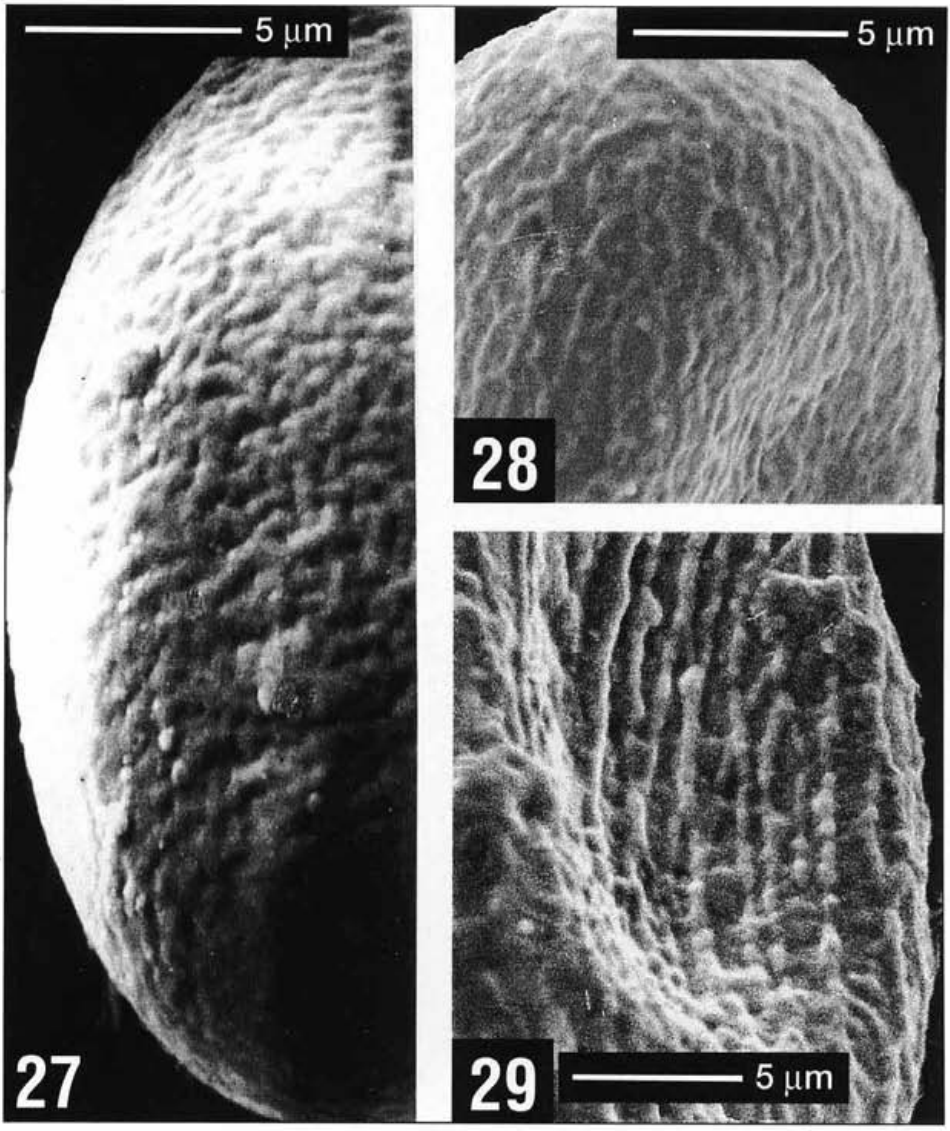
The Netherlands: Beilen, W of Wijster (illegible label), on cow dung, 17. X. 1973 (WAG-W, A 981) – erroneously identified by Arnolds (1982: figs. 244a, e, c) as *C. fimicola*, about 50 dried, ochraceous apothecia, 0.1–0.3 μm diam., densely crowded on a small fragment of cow dung.

Remarks

C. bohemica, type species of the series *Glabrae* J. Moravec ser. n. is certainly extremely rare and was known only from the holotype. Brummelen (1995) treated *Fimaria bohemica* as "very close to, or conspecific with *Cheilymenia raripila*". I managed to find one other collection which possesses hairless apothecia similar to the Velenovský's fungus. The collection was discovered during my examinations of individual specimens recorded by Arnolds (1982) under the name *C. fimicola* (De Not. et Bagl.) as I disclosed that they represented several different species. The collection WAG-W, A 981, illustrated by Arnolds (1982: figs. 244a, e, c) has certainly nothing to do with *C. fimicola* but in fact represent a species of the sect. *Raripilosae*. Especially its hairless apothecia and ascospore size well correspond with those of *C. bohemica*. Nevertheless, although the ascospores of Arnolds'



Figs. 24–26. SEM photomicrographs of ascospores. 24–25. *Cheilymenia raripila*. 24. from Wellington (J. Mor.); 25–26. from isotype of *C. notabilispora* (J. Mor.); 26. *Cheilymenia bohemica* (from holotype (PRM).



Figs. 27–29. SEM photomicrographs of ascospores (details). 27. *Cheilymenia bohemica* (from holotype (PRM). 28–29. *C. bohemica* (from WAG-W, A 981).

collection appear almost smooth under the optical microscope (Fig. 23, like those of the holotype of *C. bohemica*), SEM photomicrographs (Figs. 28–29) show the same subreticulate perisporium as that on the ascospores of *C. raripila*. On the other hand, only two ascospores in a condition suitable for SEM were obtained from the holotype of *C. bohemica* and therefore, the finer perisporial ornamentation on these probably premature ascospores (Figs. 26 and 27) is probably not such a decisive character. The possibility that these two collections represent two distinct species can also be regarded. Nevertheless, herein I consider them conspecific and provisionally distinguish *C. bohemica* as a species which is separated from *C. raripila* merely in having hairless apothecia and slightly smaller ascospores. I have not found (neither in the holotype nor in the WAG-W specimen) ascospores of such an extreme size, 24–30 × (13–)15–19 μm, as stated by Velenovský (1934) and Svrček (1977), but these authors very probably measured premature ascospores which are more swollen and can be recognised by the absence of a yellow refractive colour. Brummelen (1995) stated the ascospore size in the holotype of *Fimaria bohemica* 21.9–26.2 × 13.0–14.7 μm, which corresponds well with my measurements. The species was transferred by Svrček (1977) to the genus *Coprobia* Boud. [=*Cheilymenia* sect. *Coprobiae* in Moravec (1990)] as *Coprobia bohemica* (Velen.) Svrček. However, all the species of the section *Coprobia* (correct form of the previously used name "*Coprobiae*") possess an even much simpler apothecial anatomy and a characteristic longitudinally striate perisporium of the ascospores. The rib-like longitudinal striation was also proved by SEM (Moravec 1987, 1990). Therefore they fundamentally differ from *C. bohemica* as well as from other species of the section *Paracheilymeniae*. *Fimaria bohemica* was transferred to the genus *Cheilymenia* in Moravec (1990).

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Taxonomic revision of the genus Cheilymenia – 8. The section Micropilosae.

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Moravec J. (2003): Taxonomic revision of the genus Cheilymenia – 8. The section Micropilosae. – Czech Mycol. 54: 135–143

The monotypical section *Micropilosae* J. Moravec (1990), originally proposed in the infrageneric arrangement published in Moravec (1990), with the type species *Cheilymenia stercoraria* (Velen.) J. Moravec (= *C. micropila* Svrček et J. Moravec), is introduced in detail. Illustrations such as line drawings, a photograph of apothecia, microphotographs of the apothecial anatomy and SEM photomicrographs of ascospores accompany the paper.

Key words: *Cheilymenia*, section *Micropilosae*, *Cheilymenia stercoraria*, taxonomic revision, Discomycetes, Pezizales, Pyronemataceae.

Moravec J. (2003): Taxonomická revize rodu Cheilymenia – 8. Sekce Micropilosae. – Czech Mycol. 54: 135–143

Je představena monotypická sekce *Micropilosae* J. Moravec (1990) původně navržená autorem v jeho vnitrodruhovém uspořádání rodu (Moravec 1990) a její typový druh *Cheilymenia stercoraria* (Velen.) J. Moravec (= *C. micropila* Svrček et J. Moravec). Článek je doplněn kresbami mikroznaků, fotografií apothecií, mikrofotografiemi jejich anatomie a SEM mikrofotografiemi askospor.

INTRODUCTION

Nine sections of the genus *Cheilymenia* were established in the infrageneric arrangement originally proposed in Moravec (1990). After further examinations, the classification was modified to seven sections (at present). The status of the section *Insigniae* J. Moravec was lowered and treated in Moravec (1993) as series *Insigniae* (= *Insignes* in Moravec 2003) of the typical section *Cheilymenia*. The section *Raripilosae* J. Moravec was lately (Moravec 2003) reassessed as series *Raripilosae* and series *Glabrae* of the section *Paracheilymeniae* J. Moravec.

One of the seven sections of the genus *Cheilymenia* Boud. within the modified infrageneric arrangement, the monotypical section *Micropilosae*, is introduced here.

MATERIAL AND METHODS

Material from various herbaria was examined with the usual methods. The holotype of *C. stercoraria* was examined on rehydrated apothecia. The holotype

(PRM) of the synonymous *C. micropila* was examined partly on fresh material, partly on rehydrated apothecia. The ascospore size measured in vivo was in the range of those examined on the rehydrated material. Dried apothecia were rehydrated in distilled water for studying and measuring of ascospores. Any aggressive liquids, such as alcohol or lactophenol, were strictly avoided in the rehydration, staining, as well as in the treatment of ascospores for the SEM photomicrographs. As premature ascospores are usually more swollen, only mature ascospores were measured. Extremely large ascospores, which occasionally matured together with aborted ones and are consequently present in a reduced number in one ascus were considered abnormal. Their size is given in brackets here. The ascospores were stained with cotton blue C4B ("Geigy s. 123" of an old supply) which stains directly without heating the slides, in order to avoid destruction of the loosening outermost perisporial membrane, as emphasised in my papers (lately in Moravec 1998). The samples for the SEM photomicrographs were taken from pieces of dried hymenium directly coated with gold.

For microscopic examination of the anatomy, median sections through an apothecium were mostly treated with 1 % KOH and washed in distilled water before staining with C4B in lactic acid or trypan blue.

Acronyms:

CUP	Department of Plant Pathology, Cornell University, Ithaca, New York, U.S.A.;
K(M)	The Herbarium (Mycological) of the Royal Botanic Gardens, Kew, England, Great Britain;
PRM	Mycological Department of the National Museum, Prague, Czech Republic;
BRA	Department of Botany, Museum of Natural History, Bratislava, Slovakia;
BRNM	Botanical Department of the Moravian Museum, Brno, Czech Republic;
J. Mor.	Private herbarium (Discomycetes) of Jiří Moravec, Adamov, Czech Republic.

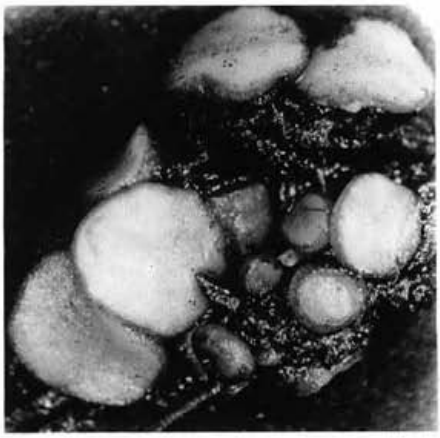
RESULTS

Genus **Cheilymenia** Boud., Bull. Soc. Mycol. France. 1: 105, 1885.

Sect. **Micropilosae** J. Moravec, Mycotaxon 28: 475, 1990.

only one, type species:

Cheilymenia stercoraria (Velen.) J. Moravec, Mycotaxon 28: 475, 1990.



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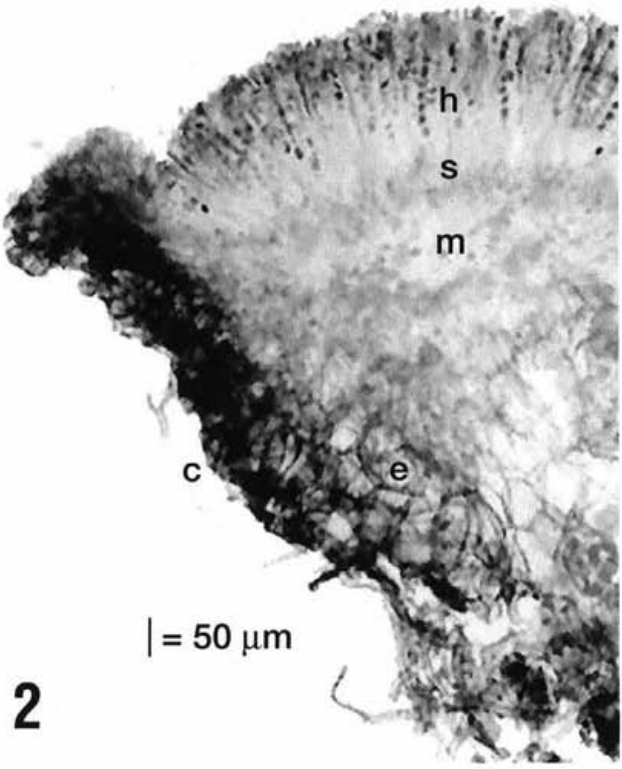

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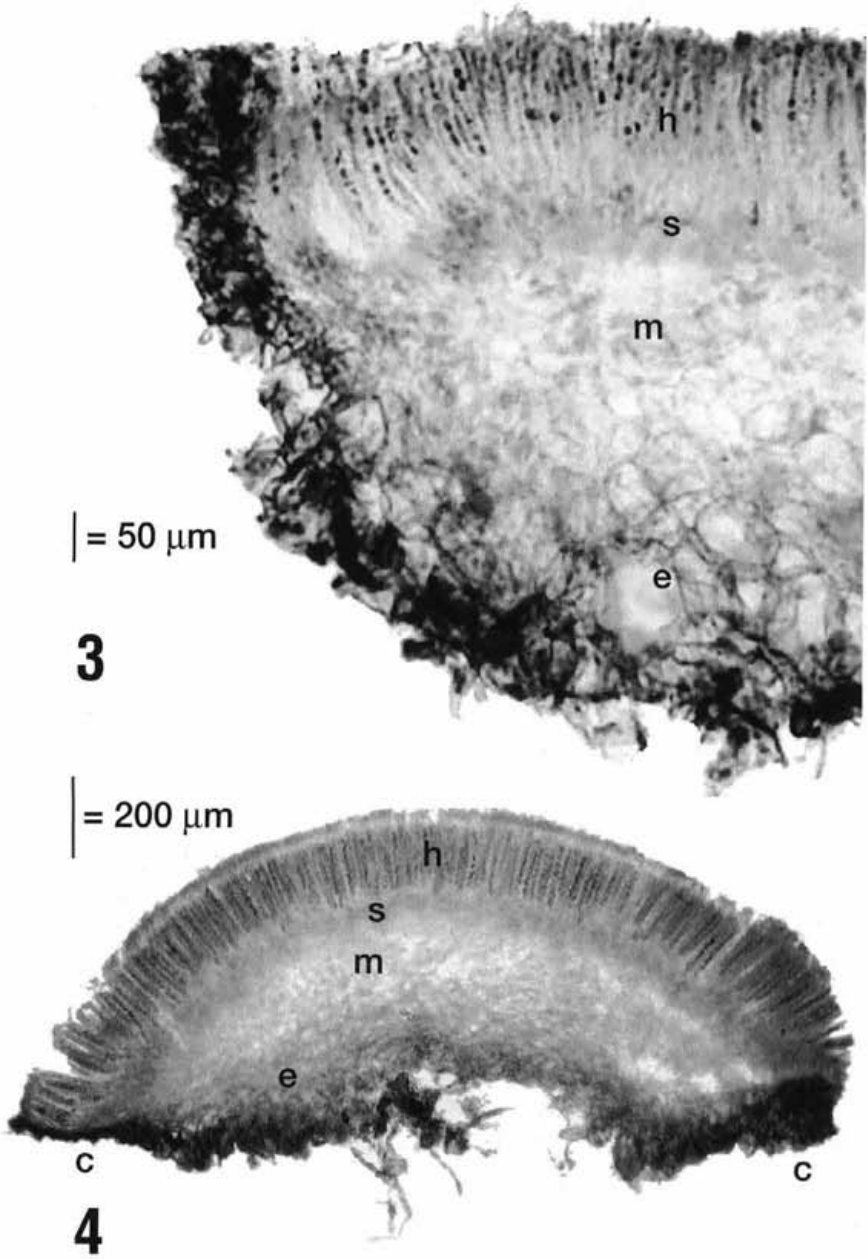
Figs. 1–2. *Cheilymenia stercoraria.* 1. Apothecia (from holotype of *Cheilymenia micropila* Svrček et J. Moravec – PRM 628980). 2. Part of medial section through an apothecium (from isotype of *C. micropila* Svrček et J. Moravec – J. Mor.). Layers – h: hymenium, s: hypothecium (subhymenium), m: medulla, e: ectal excipulum, c: cortex (outermost layer of the ectal excipulum).



Figs. 3–4. *Cheilymenia stercoraria.* 3. Marginal portion of medial section through an apothecium (from isotype of *C. micropila* Svrček et J. Moravec – J. Mor.). 4. medial section through small but mature apothecium; h (from holotype of *Humaria stercoraria* Velen. – PRM 147881). Layers – h: hymenium, s: hypothecium (subhymenium), m: medulla, e: inner layer of ectal excipulum, c: cortex (corticoid outermost layer of the ectal excipulum).

The monotypical section *Micropilosae* was proposed (Moravec 1990) for *Cheilymenia stercoraria* (Velen.) J. Moravec. Simultaneously, my re-examination of the type of *Cheilymenia micropila* Svrček et J. Moravec disclosed that it is identical with *C. stercoraria*.

C. stercoraria is a remarkable species characterised by its comparatively large, discoid to expanded flat apothecia with a bright scarlet-red hymenium and hairless external surface. The apothecial surface is hairless, only hyaline hyphae are present near the base of the apothecia, and modified brown cells resembling hair germs protrude on the distinctly cortical, comparatively thin ectal excipular zone of the apothecium. In the synopsis of the infrageneric arrangement (Moravec 1990), I originally placed the section *Micropilosae* near sect. *Paracheilymeniae*. However, although the apothecia of *C. stercoraria* are nearly hairless and the medullary excipulum composed of textura globulosa to angularis (with indistinct hyphal elements), their flatly expanded shape and brightly scarlet-red hymenium rather resemble species of the section *Pseudoscutelliniae* J. Moravec (1990). Nevertheless, the well-distinguished section *Pseudoscutelliniae* accommodates species with apothecia bearing long, rooting apothecial hairs and a well-delimited medullary layer composed of a textura subintricata to intricata.

The hairless apothecia of *C. stercoraria*, possessing a brown cortical ectal excipular layer, and subglobose ascospores with a minutely warted perisporium, justify the separate classification in the monotypical section *Micropilosae*, as originally proposed in Moravec (1990).

Cheilymenia stercoraria (Velen.) J. Moravec (Figs. 1–13).

≡ *Humaria stercoraria* Velenovský, Mon. Discom. Boh. 1: 330, 2: Tab. 24, fig. 22, 1934.
≡ *Coprobia stercoraria* (Velen.) Svrček, Čes. Mykol. 31(2): 69, 1977.
≡ *Cheilymenia stercoraria* (Velen.) J. Moravec, Mycotaxon 28: 475, 1990.
= *Cheilymenia micropila* Svrček et J. Moravec in Moravec, Čes. Mykol. 22 (1): 37, 1968 pro parte.

Apothecia scattered to densely gregarious, (2.5–)3.5–10.0 mm diam., broadly sessile, at first shallowly saucer-shaped, lenticular, becoming flattened with slightly raised margin to even pulvinate; hymenium dilute-red to conspicuously scarlet ["apothecia igneo coccinea" according to Velenovský (1934)], outer surface paler, dilute-red, large marginal area darkened, irregularly brown-dotted, appearing hairless. Hymenium about 180–220 μm thick with paraphyses conspicuously overlapping asci. Hypothecium about 40–70 μm thick, consisting of small cells 4–12 μm diam. of indefinite shape, indistinctly delimited from the medullary layer. Medulla about 100–180 μm thick, consisting of small irregular globose

and angular cells 15–40 μm diam, vesicular-joint, occasionally forming hyphoid chains (textura globulosa to angularis with hyphal elements). Ectal excipulum about 180–260 μm thick (in marginal zone much thinner, only 50–180 μm thick), at the base composed of a textura globulosa to angularis of very large subglobose to subangular cells 40–160 μm diam.; towards the margin the cells are much smaller (30–60 μm diam.), mixed with brown aborted cells of indefinite shape, forming a cortex with indistinctly protruding outermost cells, which are occasionally elongated into a constricted apex and thus resemble germs of hairs, or occasionally more distinctly protrude as subhyaline or brown, very short clavate hyphae. Hyphae commonly occur at the apothecial base, they are subhyaline and 10–18 μm thick. Asci 160–180(–210) × 15–18(–22) μm, cylindrical, with rounded apex, gradually constricted towards base, eight-spored. Ascospores uniseriate, 14.2–18.0(–19.0) × 11.2–13.5 μm, broadly ellipsoid but commonly also globose-ellipsoid (mostly 16.5 × 12.0 μm) occasionally conspicuously subglobose (14.25 × 12 μm), hyaline; mature ascospores possess a yellow refractive colour; loosening perisporium irregularly covered with cyanophilic irregular warts 0.2–1.5 μm diam. which are occasionally connected. Paraphyses conspicuously overlapping asci, filiform, 2.5–3.7 μm thick, straight, sparsely septate, apices clavate-dilated 4.2–10.2(–12) μm, filled with orange-red guttules.

Habitat. On debris and soil mixed with human excrement, also on cow dung, in forests, known only from Bohemia, Czech Republic.

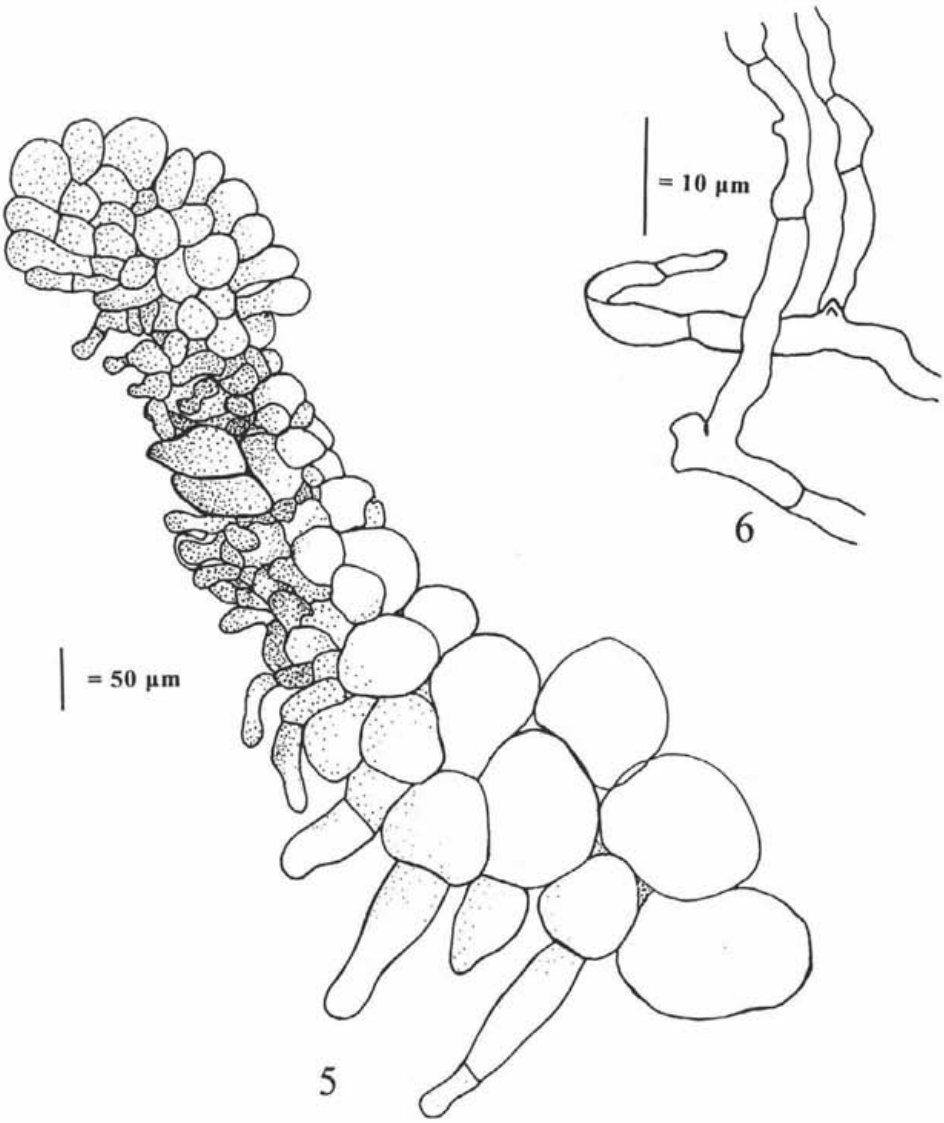
Material examined

1. Holotype (PRM 147881) of *Humaria stercoraria* Velen.: Bohemia centr., Mnichovice, in merda humana in silva humida, 30. VIII. 1922 leg. J. Velenovský, originally under the unpublished herbarium name *Barlaea stercoraria* Velen., the holotype is labelled and its state and substrate are described by Svrček (1989).

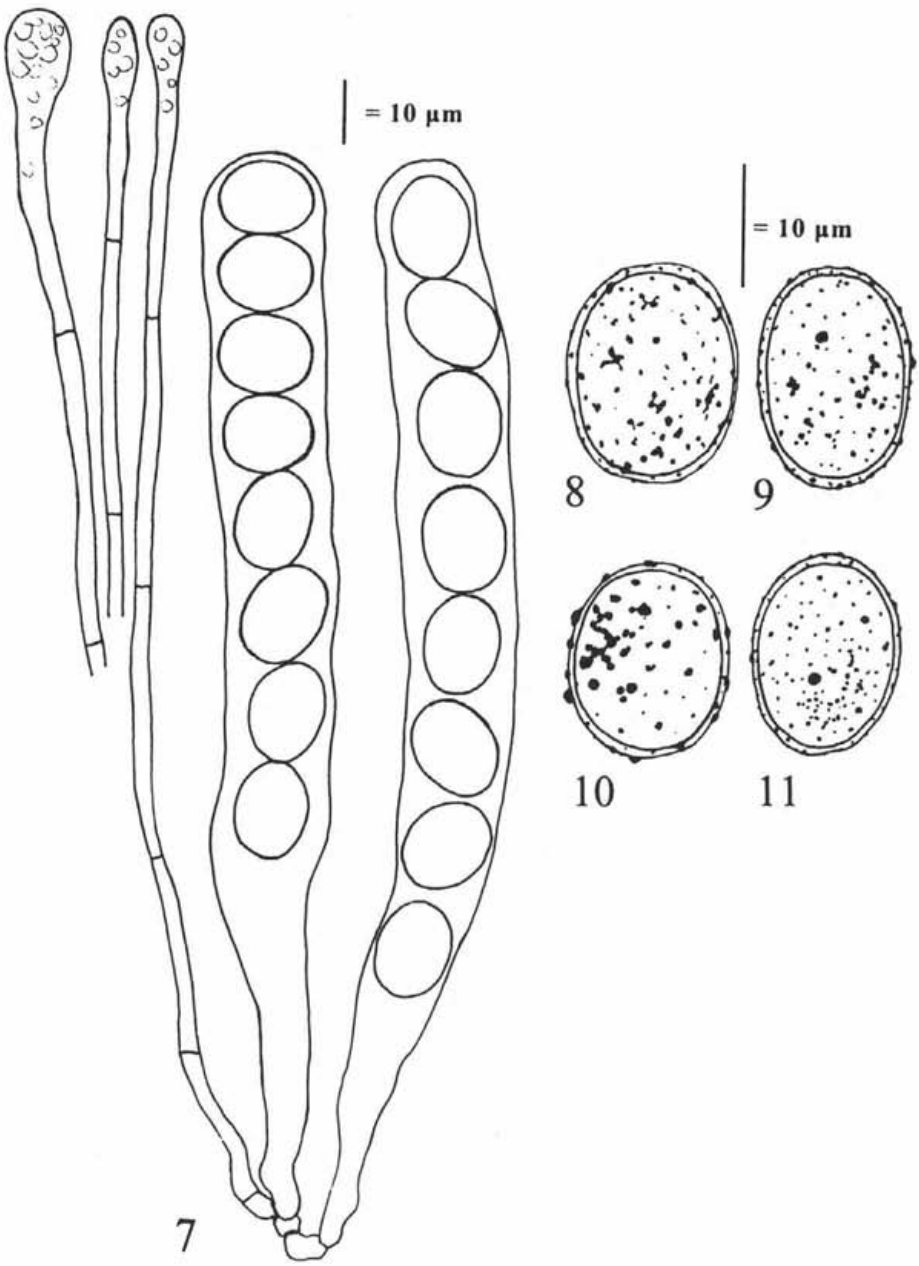
2. Holotype (PRM 628980) of *Cheilymenia micropila* Svrček et J. Moravec: Bohemia: Branžež prope Kněžmost, district. Mladá Boleslav, in fimo vaccino, 15. V. 1966 leg. J. Moravec. Isotypes deposited in CUP, BRA, BRNM, K(M) and J. Mor.

Remarks

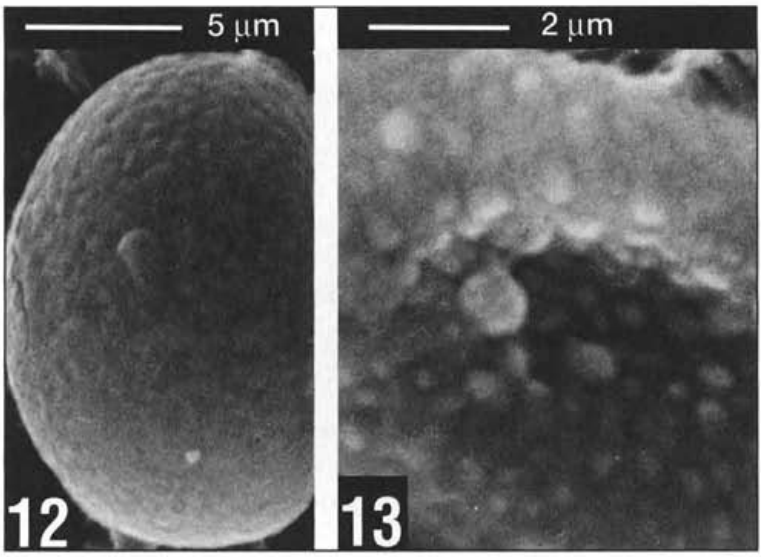
Cheilymenia stercoraria is a very peculiar species, especially for its hairless, conspicuously flat to pulvinate, scarlet coloured apothecia possessing a brown-pigmented cortical layer of the ectal excipulum. Velenovský (1934) described this conspicuous and very rare discomycete as a species of the genus *Humaria*, and accurately illustrated the outstanding shape of the apothecia and ascospores. Svrček (1979) mentioned a smaller ascospore size (13.5–15.5 × 10.0–11.0 μm) than that stated in my description above. My measurements well agree with those



Figs. 5–6. *Cheilymenia stercoraria.* Section of the marginal portion of the cortical layer of the ectal excipulum (from holotype of *Humaria stercoraria* Velen. – PRM 147881). 6. Hyphae at apothecial base.



Figs. 7–11. *Cheilymenia stercoraria*. 7. Asci and paraphyses (from holotype of *Humaria stercoraria* Velen. – PRM 147881); 8–11. Ascospores, oil immersion, stained with C4B in lactic acid (8–10 from holotype of *Humaria stercoraria* Velen. – PRM 147881; 10–11 from isotype of *C. micropila* Svrček et J. Moravec – J. Mor.).



Figs. 12–13. SEM photomicrographs of ascospores. 12. Ascospore; 13. Detail of the ornamentation (from holotype of *Humaria stercoraria* Velen. – PRM 147881).

given by Velenovský (1934) who stated the ascospore length in *Humaria stercoraria* 17–19 µm. The substrate, originally mentioned as human excrement, is in fact a conglomerate. I agree with Svrček (1979), who described the substrate as plant debris, consisting of small roots, mosses, stones, grass, small coniferous twigs and leaves, mixed with soil and covered with Cyanophyta and algae – but in my opinion the stercoraceous character of the substrate is obvious.

The species was insufficiently known as only the type collection existed. Consequently, we additionally described the fungus under the superfluous synonym *Cheilymenia micropila* Svrček et J. Moravec in Moravec (1968). The description of *C. micropila* was based on two separate collections and is therefore misleading. My later examination (Moravec 1990) of the paratype collection (Bohemia, Třeboň, "Dubový rybník", ad terram stercoratam, 23. V. 1964 leg. J. Kubička and M. Svrček – PRM) mentioned in the protologue of *C. micropila* disclosed that it is not this species but in fact *Cheilymenia rubra* (Cooke ex W. Phillips) Boud. The previous misidentification of the collection from Třeboň was mainly caused by the fact that apothecia of the holotype of *C. micropila* grew tightly aggregated together with copious apothecia of *C. rubra* on their common substrate. Consequently, important diagnostic features in the description of *C. micropila* in Moravec (1968) were confused. Thus the hairs, described and illustrated for *C. micropila*, in fact belong to *C. rubra*. The dried apothecia of each of the two species growing densely aggregated and connected on the common substrate of the holotype of *C. micropila*

are difficult to distinguish. Those of *C. stercoraria* are recognisable on the basis of their flat shape, but microscopic examination is necessary. The apothecia of *C. rubra* are microscopically well distinguishable as they possess rigid thick-walled hairs and a different anatomy (medulla consists of a textura intricata), and its ascospores are conspicuously narrower (Moravec 1989).

The hairless apothecia lead Svrček (1977) to transfer *Humaria stercoraria* into the genus *Coprobia* Boud. (= *Cheilymenia* sect. *Coprobiae* (Boud.) J. Moravec 1990). However, species of the section *Coprobia* (correct name of the previously used "*Coprobiae*") possess fundamentally different characters, especially the ascospore perisporium being distinctly longitudinally striate. The striation consists of longitudinal low ribs and was demonstrated also by SEM photomicrographs (Moravec 1987, 1990). Moreover, unlike species of the section *Coprobia*, which possess hyaline cells with sparse hyaline hyphal outgrowths, the ectal excipular layer in *Cheilymenia stercoraria* forms comparatively thin and compact brown cortex with cells changed into brown hair germs.

ACKNOWLEDGEMENTS

I thank Dr. Mirko Svrček and Dr. Jan Holec (Prague) for their kind loan of the type specimens. Special thanks are due to Dr. Vladimír Antonín (Brno), who made the digital microphotographs, to Mr. Jiří Lhotecký (Brno), who performed the SEM photomicrographs of ascospores and to Dr. Zdeněk Pouzar (Prague) who kindly reviewed the manuscript. The Grant Agency of the Czech Republic is greatly acknowledged for financial support of my work on the monograph of the genus *Cheilymenia* (project no. 206/01/1261/B).

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CZECH MYCOL. 54 (3–4), 2003

Notes on three Rimbachia species from the Alps

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Senn-Irlet B. and Moreau P.-A. (2003): Notes on three Rimbachia species from the Alps. – Czech Mycol. 54: 145–154

Rimbachia neckerae is reported from several localities in the Alps. A description is given and the spore size of all *Rimbachia* species hitherto reported from Central Europe is critically reviewed. Special attention is given to the host plants, i.e. pleurocarpous and acrocarpous mosses.

Key words: Basidimycetes, Agaricales, *Rimbachia*, taxonomy, ecology, distribution, Central Europe, moss-inhabiting fungi

Senn-Irlet B. a Moreau P.-A. (2003): Poznámky o třech druzích rodu Rimbachia z Alp. – Czech Mycol. 54: 145–154

Z několika lokalit v Alpách je uveden druh *Rimbachia neckerae*. Je podán popis tohoto druhu a kriticky je vyhodnocena velikost výtrusů všech druhů rodu *Rimbachia* dosud uváděných ze střední Evropy. Zvláštní pozornost je věnována hostitelským rostlinám, to jest pleurokarpním a akrokarpním mechům.

INTRODUCTION

Minute agaricoid species without lamellae and stipe, so-called cyphelloid species, often grow gregariously on mosses, especially in humid places. Despite the small size and sporocarps without any pigments, several species and genera can be recognised based on microscopical characters.

Rimbachia is such a genus with small pleurotoid to cyphelloid sporocarps. According to an emended description of the genus by Redhead (1984) a combination of the following characters is distinctive: the spores are subglobose to broadly subcylindrical, obpyriform or ellipsoid, thin-walled, inamyloid, smooth, and with a prominent apiculus. The trama consists of loosely to compactly arranged, subregular, i.e. slightly interwoven hyphae with clamp connections. Pileipellis a cutis with a transition towards a trichoderm, i.e. often with erect undifferentiated terminal cells. World-wide at least six species are recognised.

Keys in current use for continental and nordic Europe (e.g. Moser 1983, Høiland in Hansen et Knudsen 1992, Kuyper in Bas et al. 1995) list two species, *R. bryophila* and *R. arachnoidea*, whereas Watling & Gregory (1989) report the presence of *R. bryophila* and *R. neckerae* from the British Isles.

We report here the presence of all three *Rimbachia* species from several localities with a high air humidity from the Alps (central Europe) growing on various mosses.

MATERIAL AND METHODS

The study is based on the examination of 17 collections. Fresh carpophores were in most cases documented with colour slides and morphological descriptions. For microscopic study the material was prepared with 5 % ammonia and Congo Red in 1% ammonia. Drawings were made with the aid of a drawing tube. Computations and illustrations of statistical analyses were performed with Microsoft-Excel 97. Mosses were identified using Frahm & Frey (1992) and Smith & Smith (1980).

RESULTS AND DISCUSSION

Key

1. hymenophore consisting of lamellae-like folds or veins, spores 6–7 × 5–6 μm, globose to subglobose, on pleurocarpous mosses ... *R. bryophila*
1* hymenophore smooth, without any veins ... 2
2. spores 4–5 (–6.5) × 4–5 (–5.5) μm, globose to subglobose, on acrocarpous mosses ... *R. arachnoidea*
2* spores 7.5–11(–13) × 5–7 μm, ellipsoid to lacrymoid, on pleurocarpous mosses ... *R. neckerae*

The main distinguishing features to separate the species within the genus *Rimbachia* are the development of the hymenophore (without any lamellae or with lamellae-like folds), and the spore size and shape respectively. Figure 1 illustrates the spore size and the spore shape of 15 collections examined. Clearly two groups can be separated: one group with small, subglobose spores and one with larger, more ellipsoid spores. The first one includes two species, *R. arachnoidea* with slightly smaller spores than *R. bryophila*. In addition *R. arachnoidea* shows a widely observed type of spore variation within fungi: longer spores are also broader, the mean values forming a rotation ellipsoid (linear regression, $r^2 = 0.98$, see Fig. 1). On the other hand the greater variability of the spore size of the larger-spored taxon (linear regression, $r^2 = 0.3$) is more difficult to interpret. Is it due to phenotypic plasticity dependent on microclimatic conditions or is it due to a genetic variability? In the latter case even an additional species may be hidden.

Ecological remark

In most collections sporocarps are produced at the apex of the host moss. Under a binocular lens the mycelium of *Rimbachia bryophila* and *R. neckerae* can usually

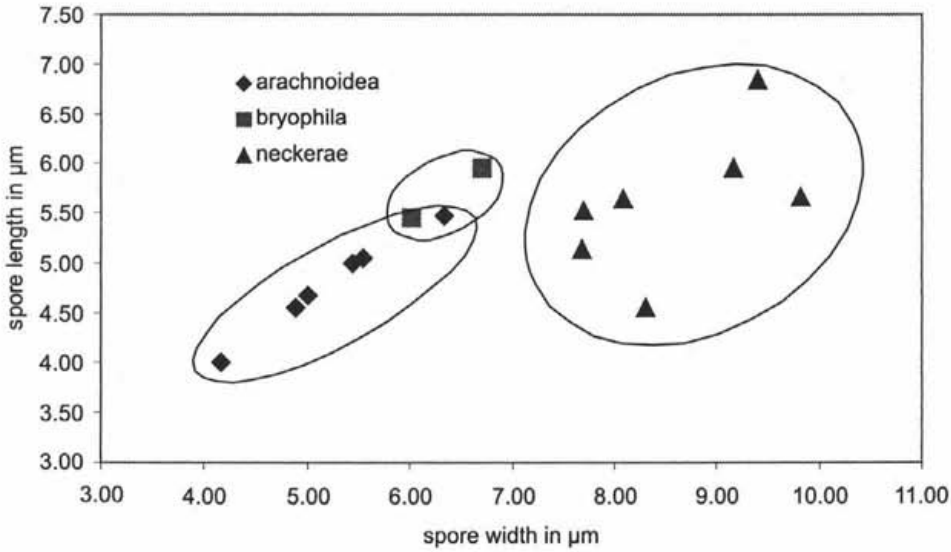

Fig. 1. Spores sizes (mean values) of *Rimbachia* collections from the Alps.

be traced back deep into the moss carpet. Attacked moss plants sometimes show yellowish colours, signs of a distinct parasitism by the fungus.

Notes on the species

Rimbachia arachnoidea (Peck) Redhead Fig. 2

Rimbachia arachnoidea (Peck) Redhead in Can. J. Bot. 62: 878. 1984.
Cyphella arachnoidea Peck in Ann. Rep. N. Y. State Mus. 44: 134. 1891.
Mniopetalum globisporum Donk in Persoonia 2: 332. 1962.

Sporocarps 0.5–3 mm, cupulate, more rarely tubular, soon disciform with small involute margin, at first circular later often lobed and irregular in outline, not hygrophanous, pendant, centrally attached, in late stages at times confluent. Surface felted to silky, white. Hymenophore smooth or at times with some indistinct veins, white, soon cream. When dry firm and brittle, hymenophore pale buff.

Spores 4–5(–6.5) × 4–5(–5.5) μm (see Table 1), globose to subglobose, with truncate, sometimes elongate apiculus. Basidia 18–22 × 7–8.5 μm, (2–3)4-spored, cylindrical, clamped. Subhymenium ramose, dense, with hyphae 10–12 × 4.5–6 μm. Pileipellis a trichoderm, in young stages with long, slender hairs, with equally to

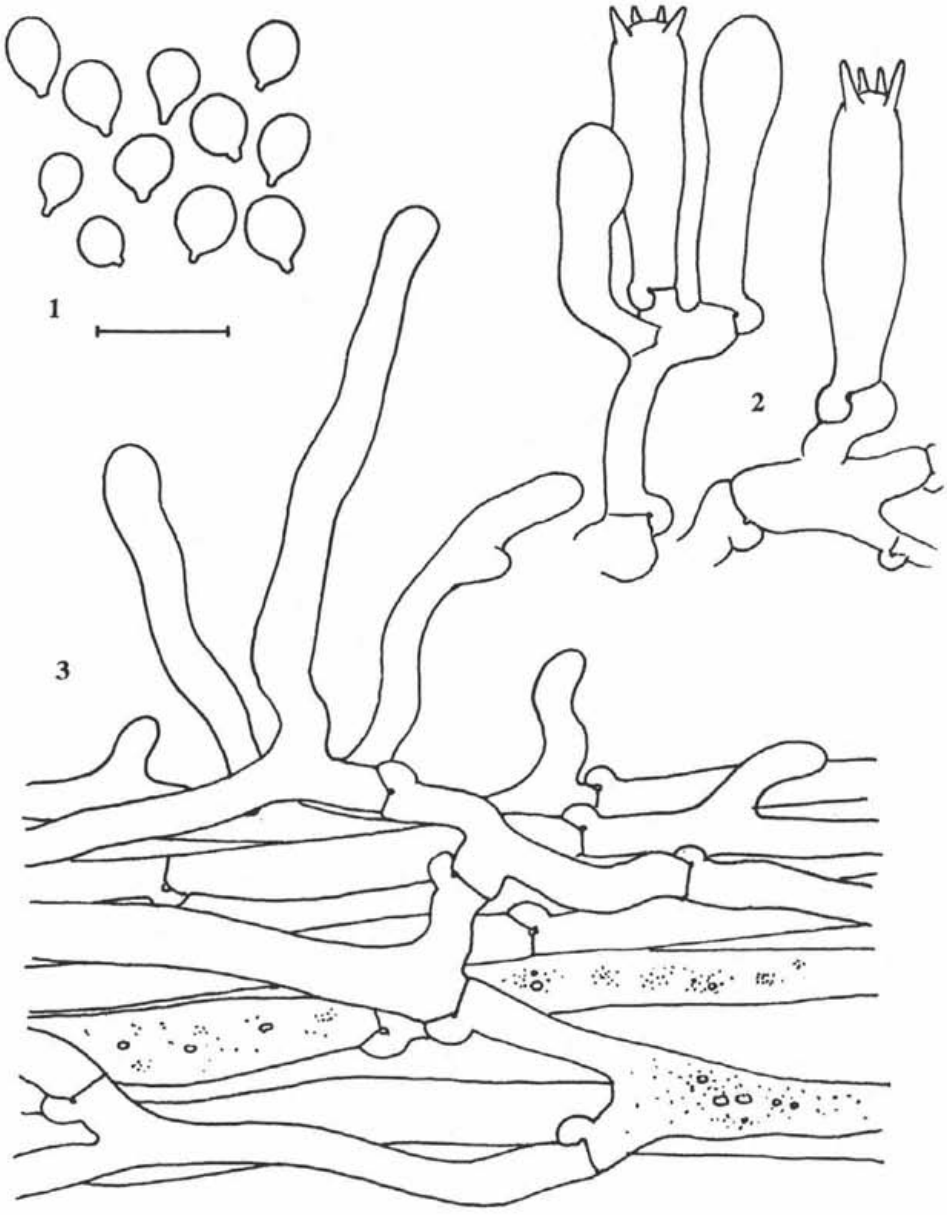

Fig. 2. *Rimbachia arachnoidea*: spores (1), basidia (2), and pileipellis from a young sporocarp (3). Bar = 10 μm

slightly inflated apex, at times ramose, 60–130 × 3–4.5 μm; often broken with age; repent superficial hyphae subparallel, hyaline, 2.5–5 μm wide. Trama with 3.5–6 μm wide hyphae, often inflated up to 15 μm at ramifications, hyaline or with refringent content. Mycelium repent on moss leaves, similar to pileipellis, with 3.5–6 μm hyphae with many free ends similar to the hair-like terminal cells of the pileipellis or shorter. Clamps present at all septae.

Habitat: Pure coniferous and mixed coniferous-deciduous woods, with predominantly herbaceous vegetation or forbs mixed with mosses in the lowest vegetation layer. From the lowlands to the subalpine zone.

Two collections on epiphytic mosses along a road verge.

A population may consist of 10 to over a hundred sporocarps on gametophytes of various acrocarpous mosses.

Collections studied: Germany: Mecklenburg-Vorpommern, Greifswald, Jarmshagen, 21 October 1996, leg. R. Doll (Herb. Doll), 10 November 1996, leg. R. Doll (Herb. Doll).

Switzerland: canton Bern, Wahlern, Dorfwald, 890 m, 12 October 1989, leg. B. Senn-Irlet (Herb. BSI 89/244); canton Fribourg, Charmey, Les Grottes, 980 m, 4 September 1994, leg. B. Senn-Irlet, (Herb. BSI 94/55); canton Jura, Vendlincourt, 450 m, 11 October 1997, leg. B. Senn-Irlet (Herb. BSI 97/192); canton Schwyz, Muothatal, Bödmerenwald, 2 October 1991, leg. B. Senn-Irlet (Herb. BSI 91/160).

Recent descriptions and notes on its distribution are scarce. Kuyper in Bas et al. (1995) suggests primarily *Mnium* species as host plants for the Netherlands. In alpine regions the fungus seems to display a much broader host range. Yet, only acrocarpous mosses have been found attacked (Table 1).

Table 1. Spore sizes (mean values) of *Rimbachia arachnoidea*

Coll.	l	w	Q	N	Host plant
DollOct	4.16	4.01	1.04	14	*Mnium hornum*
DollNov	4.89	4.55	1.07	13	*Mnium hornum*
BSI91/160	5.00	4.68	1.07	8	*Mnium undulatum*
BSI89/244	5.45	5.00	1.09	13	*Polytrichum attenuatum*
BSI94/55	5.55	5.05	1.10	11	*Orthotrichum affine*
BSI97/192	6.34	5.48	1.16	12	*Tortula virescens*

l = length, w = width, Q = length/width ratio, N = number of measurements

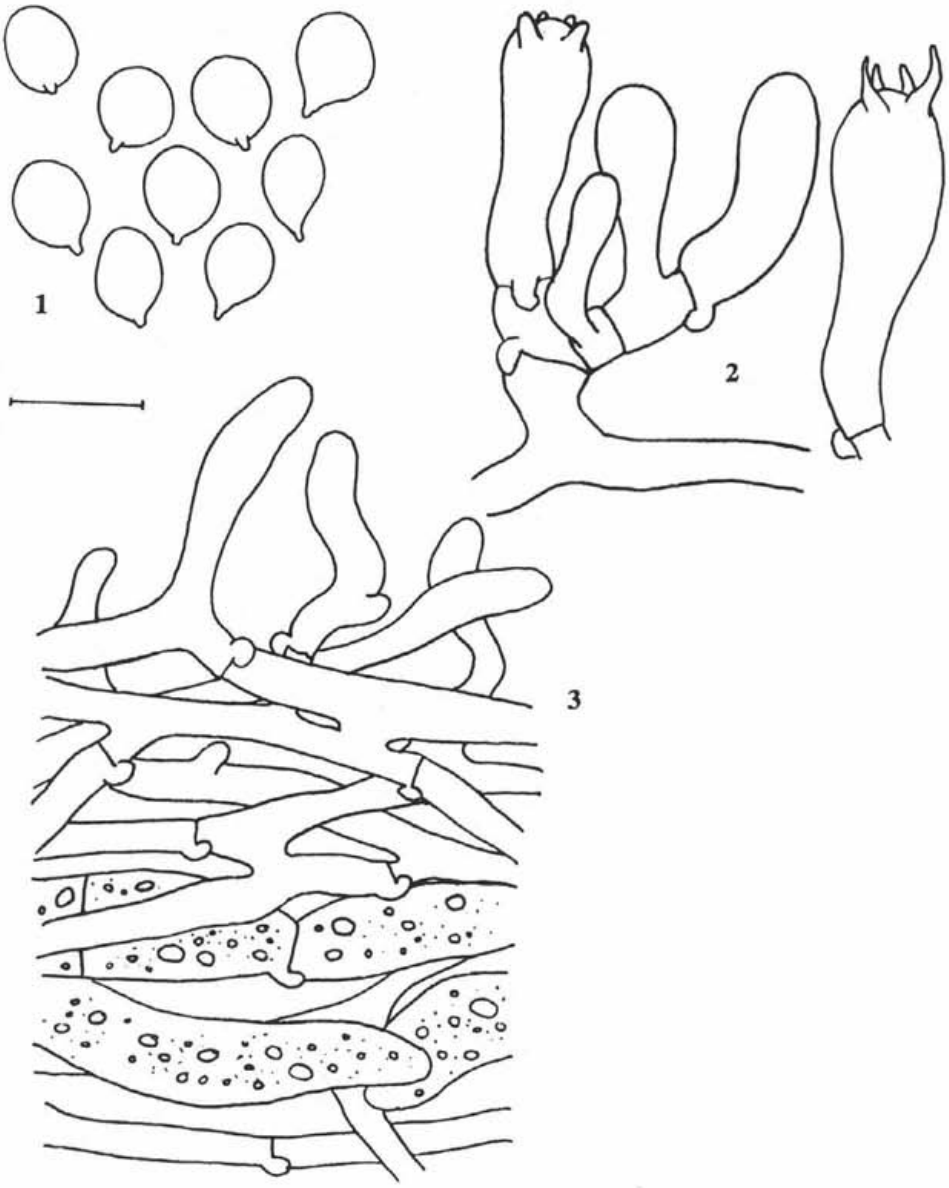

Fig. 3. *Rimbachia bryophila*: spores (1), basidia (2), and pileipellis (3). Bar = 10 μm

Rimbachia bryophila (Pers.) Redhead Fig. 3

Rimbachia bryophila (Pers.) Redhead in Can. J. Bot. 62: 878. 1984.
Agaricus bryophilus Pers.: 8. 1796.
Mniopetalum bryophilum (Pers.) Donk in Persoonia 2: 335. 1962.

Sporocarps up to 5 mm in diam., first cupulate-disciform then conchoid-flabelliform, milk white, with minutely pubescent upper surface; hymenium thinly wrinkled in youngest stage, then vein-like, even with distant lamellae-like folds, concolorous, apricot-cream when dry. Hymenophore facing downwards.

Spores 6–7.5 × 5–6 μm (see Table 2), with truncate apiculus up to 2.5 μm long. Basidia 20–25 × 7–7.5 μm, 4-spored, clavate, clamped. Subhymenium 10–15 μm thick, rather compact, pseudoparenchymatous. Pileipellis a trichoderm especially in young stages, built up of interwoven slender hyphae, with cylindrical to vermiform protruding hairs of 30–40 × 4–5.5 μm. Trama rather regular, with slender hyphae mixed with long and shorter hyphae of 7–11 μm diam., with a diffuse, oily-like content. Thin-walled hyphae throughout. Clamps present at all septa. Pigment absent in all tissues.

Habitat: Pure coniferous and mixed coniferous-deciduous woods, with predominantly herbaceous vegetation and forbs mixed with mosses in the lowest vegetation layer; also on wet saxicolous mosses. From the montane to the subalpine zone. Rather humid to moist microsites.

Collections studied: Switzerland: Canton Bern, Wattenwil, Forst, 620 m, 27 August 1994, leg. B. Senn-Irlet (Herb. BSI 94/41). Canton Luzern, Flüehli, Beichlen, 1500 m, 16 October 1985, leg. B. Senn-Irlet (Herb. BSI 85/266). France: Department Vosges, Rothried, 28 September 1997, leg. G. Corriol (herb. GC 97092815).

Recent descriptions and notes on its distribution are scarce. Kuyper in Bas et al. (1995) suggests primarily *Mnium*-species as host plants, an observation not confirmed with our collections (see Table 2). Only pleurocarpous mosses were found by us as host plants in agreement with observations from Scandinavia by Høiland in Hansen et Knudsen (1992). In collection 85/266 sporocarps were also found attached to litter debris.

Table 2. Spore sizes (mean value) of *Rimbachia bryophila*

Coll.	l	w	Q	N	Host plant
BSI85/266	6.71	5.95	1.11	18	*Rhynchostegium confertum*
BSI94/41	6.02	5.44	1.13	18	*Rhynchostegium megapolitanum*

l = length, w = width, Q = length/width ratio, N = number of measurements

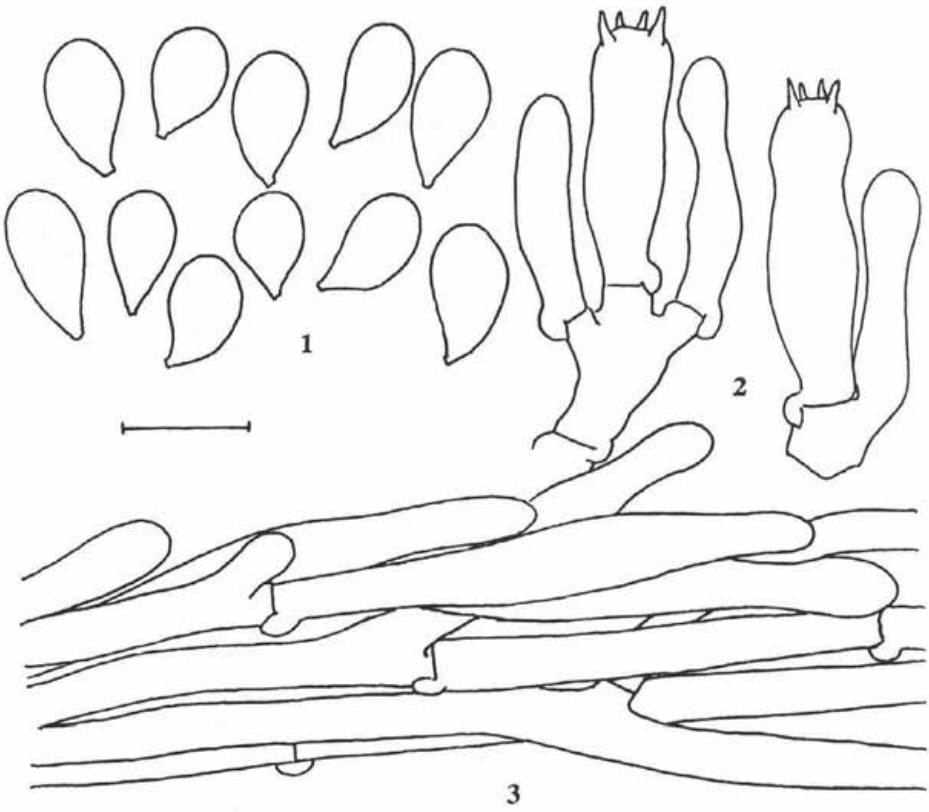

Fig. 4. *Rimbachia neckerae*: spores (1), basidia (2), and pileipellis from a young sporocarp (3). Bar = 10 μm

Rimbachia neckerae (Fr.) Redhead Figs. 4–5

Cyphella muscicola var. *neckerae* Fr.: 202. 1823.
Cyphella neckerae (Fr.) Fr.: 558. 1838.
Leptoglossum candidum Reid in Trans. Br. Mycol. Soc. 48: 514. 1965.

Sporocarps 1–3 mm, at first almost tubular, later cup– to helmet-shaped, circular or irregular in outline, thin-fleshed, not hygrophanous. Surface white, silky, felty under a hand lens, with even, later often undulate, involute margin. Hymenophore smooth, white, chalk-white, later cream-pale buff. Point of attachment dorsal, in young stages with short stipe, later sessile.

Spores 7.5–11 (–13) × 5–7 μm (see Table 3), ellipsoid, hyaline, inamyloid, acyanophilous (however cytoplasma strongly blue in cotton blue), smooth, with

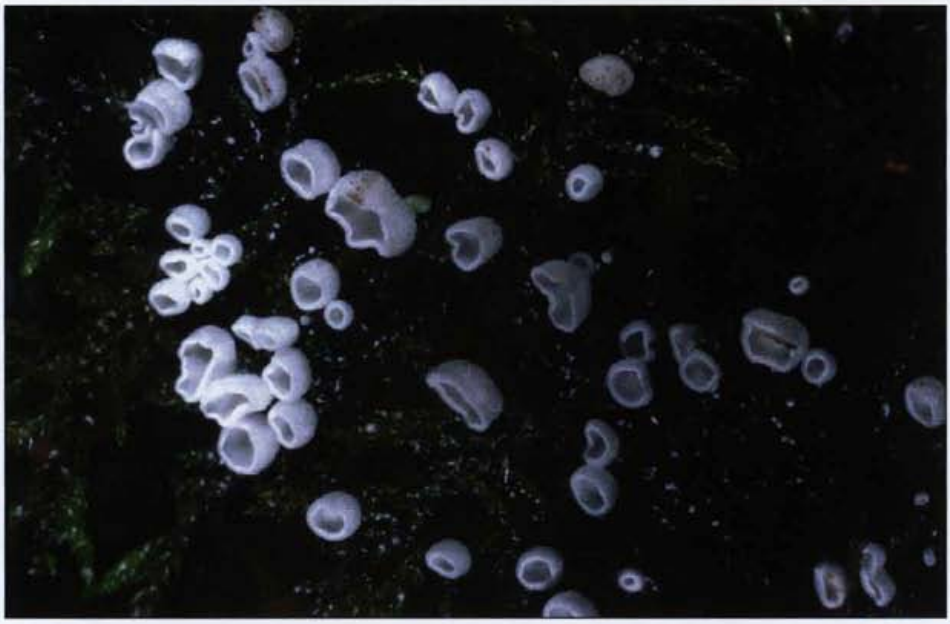

Fig. 5. *Rimbachia neckerae*: coll. PAM95082311, coloured picture (based on a scan).

Table 3. Spore size (mean values) of *Rimbachia neckerae*

Coll.	l	w	Q	N	Host plant
BSI92/52	7.70	5.52	1.50	20	*Ctenidium molluscum*
BSI00/14	9.40	6.85	1.39	20	*Ctenidium molluscum*
BSI01/72	8.30	4.60	1.43	24	*Pseudoleskea incurvata*
PAM95082311	9.17	5.95	1.82	6	*Hylocomium splendens, Scapania spec.*
PAM99042401	9.83	5.66	1.54	16	*Hylocomium splendens*
BSI97/127	8.08	5.63	1.37	12	*Hylocomium splendens*
BSI97/27	7.68	5.13	1.74	16	*Sharpiella seligeri*
PAM 01100301	8.80	5.78	1.67	16	*Ptilium crista-castrensis*

l = length, w = width, Q = length/width ratio, N = number of measurements

prominent apiculus, in one collection germinating spores with 1–2 germ tubes frequent.

Basidia 34–42 × 6–7.5 μm, 4 –spored, cylindrical, clamped. Subhymenium shortly ramose, 10–12 μm thick. Pileipellis a loose cutis with ascending often interwoven hyphae with undifferentiated terminal cells. Pigment not observed. Clamps connections present in all tissues.

Habitat: Calciphilous deciduous wood dominated by beech and oak, green alder shrubs, alpine forb vegetation with larger pebbles or rocks covered with mosses; also on limestone cliffs and mossy rocks close to rivulets. From colline to alpine zones. Humid to wet microsites.

Collections studied: France: Department Savoie, La Motte-Servolex, 450 m, 24 April 1999, leg. P.-A. Moreau (Herb. PAM 99042401); id., 3 October 2001, leg. P.-A. Moreau (Herb. PAM 01100301); Crest-Voland, 1550 m, 23 August 1995, leg. P.-A. Moreau (Herb. PAM 95082311).

Italy: Province Alto-Adige, Pflerschtal, 1140 m, 10 September 1997, leg. B. Senn-Irlet (Herb. BSI 97/127).

Switzerland: Canton Bern, Brienz, Giessbachfälle, 800 m, 20 June 1992, leg. B. Senn-Irlet (Herb BSI 92/52); canton Neuchâtel, Les Brenets, along the Doubs river, 720 m, 20 May 2001, leg. B. Senn-Irlet (Herb BSI 01/14), canton Uri, Unterschächen, Steinboden, 1550 m, 5 August 1997, leg. B. Senn-Irlet (Herb. BSI 97/27); Spiringen, Kinzigpass, 2070 m, 19 August 2001, leg. B. Senn-Irlet & R. Mürner (Herb. BSI 01/72).

This species seems to have been overlooked in Central Europe or not correctly separated from *R. bryophila*. First record for Switzerland.

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CZECH MYCOL. 54 (3–4), 2003

Relationship of Cerebella to Epicoccum and their closest relatives among Dothideales

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Pažoutová S. and Kolínská R. (2003): Relationship of Cerebella to Epicoccum and their closest relatives among Dothideales. – Czech Mycol. 54: 155–160

The Czech isolate of *Cerebella* sp. was confirmed as *C. andropogonis*, as its RAPD patterns were identical to those of Australian and African isolate of this species. Also, rDNA (ITS1–5.8S-ITS2) sequences of African *C. andropogonis* and the Czech isolate (AJ306620 and AJ400905) were identical except for a single transition A-G at position 47 of ITS1. Comparison of the sequence with databases yielded 24 closely related sequences with 96.5–98.9 % identity to *Cerebella*. The highest similarity was found between *Cerebella* and *Epicoccum nigrum/ Phoma epicoccina* isolates, two other related groups were: *Phoma herbarum*, *P. medicaginis*, *Phomopsis* sp., and *P. glomerata/Ampelomyces* sp.

Key words: *Cerebella andropogonis*, *Epicoccum*, phylogeny, rDNA sequence

Pažoutová S. a Kolínská R. (2003): Vztah rodů Cerebella a Epicoccum a jejich nejbližší příbuzní mezi Dothideales. – Czech Mycol. 54: 155–160

RAPD prokázalo, že český izolát *Cerebella* sp. náleží k druhu *C. andropogonis*, zastoupenému australským a africkým izolátem. Sekvence rDNA (ITS1–5.8S-ITS2) afrického a českého izolátu (AJ400905 a AJ306620) byly totožné s výjimkou transice A-G v pozici 47 spaceru ITS1. V databázích bylo nalezeno 24 příbuzných sekvencí rDNA které byly se sekvencí *C. andropogonis* z 96.5–98.9% totožné. Nejpříbuznější byly sekvence *Epicoccum nigrum/Phoma epicoccina*, další příbuzné skupiny tvořily *Phoma herbarum*, *P. medicaginis*, *Phomopsis* sp. a *P. glomerata/Ampelomyces* sp.

INTRODUCTION

Cerebella andropogonis, a hyperparasite colonising sphacelial stages of various *Claviceps* species was once considered a plant pathogen and almost any new collection was named after the grass species where the sporodochium occurred. However, Langdon (1955) after thorough revision of herbarium specimens from the entire world reduced these names to synonyms of *Cerebella andropogonis* Cesati. Schol-Schwarz (1959) suggested transfer of *C. andropogonis* into the genus *Epicoccum*, as *E. andropogonis*, but this was not widely accepted by later authors. One reason for it may be distinct fungal hyperparasitism of *Cerebella* and the fact that the name reflects very vividly the morphology of convoluted sporodochia resembling a brain surface.

Recently, several studies elucidated relationship between *Epicoccum*, *Phoma* and another fungal hyperparasite, *Ampelomyces* using rDNA sequence analyses. Kiss and Nakasone (1998) found that slow-growing isolates of *Ampelomyces* are related to *Leptosphaeria microscopica* and *L. nodorum*, whereas fast-growing isolates were closer to *Epicoccum*. The pycnidia of *Phoma glomerata* and related *Ampelomyces* isolates were sessile, whereas the slow-growing *Leptosphaeria*-related isolates were characterized by stipitate pycnidia. Sullivan and White (2000) identified the rapidly growing isolates as *Phoma glomerata*. These isolates are hyperparasites of powdery mildew fungi and were formerly classified as *Ampelomyces heraclei, A. humuli* and *A. quercinus*. The closest teleomorphic species were *Didymella bryoniae* and *D. lycopersici*. Arenal et al. (2000) confirmed *Epicoccum nigrum* and *Phoma epicoccina* as the same biological species, where the *E. nigrum* isolates probably lost the ability of pycnidium formation. Other rDNA sequences related to *Phoma epicoccina/Epicoccum* were those of *Phoma americana*, *P. macrostoma* and also *Didymella* which places this group among mitosporic Dothideales.

In our previous work (Pažoutová and Kolínská 1999), we described the Czech isolate of dematiaceous hyphomycete *Cerebella* sp. differing slightly in the spore morphology from typical *C. andropogonis* found in Brazil. Obviously, the morphological observations cannot add more to the correct species identification of the Czech *Cerebella* isolate or to the elucidation of *Cerebella-Epicoccum* relationship. Therefore, RAPD fingerprinting which is commonly used for differentiation between isolates of the same species, as well as rDNA sequence comparison, were applied to DNA from the Czech isolate of *Cerebella* sp. and *C. andropogonis* isolates from Africa and Australia. To elucidate the *Cerebella* taxonomical relatedness, its rDNA sequence was compared to fungal sequences from EMBL and GenBank databases.

MATERIAL AND METHODS

Isolates:

Cerebella andropogonis CZ was isolated from the sphacelial stage of *Claviceps purpurea* on *Festuca arundinacea*, in 1998 (Trutnov, Czech Republic) (Pažoutová and Kolínská 1999). *C. andropogonis* AU was isolated from *Sorghum bicolor* colonised by *Claviceps africana* (1999, Warwick, Queensland, Australia, coll. and det. M. Ryley, isol. S. Pažoutová), *Cerebella andropogonis* AF was isolated from *Heteropogon contortus* colonised by *C. pusilla* (2000, Matopos, Zimbabwe, coll. D. Frederickson, isol. and det. S. Pažoutová).

DNA analysis:

Mycelium for DNA preparation was grown for 4–5 days on RK agar plate overlaid with cellophane. Mycelium was then scraped and pulverised in liquid nitrogen

Table 1. Sequences related to *C. andropogonis*

Organism	Accession No.	Reference
Cerebella andropogonis CZ	AJ400905	this paper
Cerebella andropogonis AF	AJ306620	this paper
Epicoccum nigrum strain EP5	AF149926	Arenal et al. (2000)
Epicoccum nigrum strain EP22	AF149927	"
Epicoccum nigrum strain EP27	AF149928	"
Epicoccum nigrum strain EP33	AF149929	"
Epicoccum nigrum strain EP34	AF149930	"
Phoma epicoccina strain PE20002	AF149931	"
Phoma epicoccina strain PE20003	AF149932	"
Phoma epicoccina strain PE20028	AF149933	"
Phoma epicoccina strain PE20044	AF149934	"
Epicoccum nigrum strain CBS 318.8	AJ279448	Wirsel et al. (2001)
Epicoccum sp. isolate A9	AJ279452	"
Epicoccum sp. 5.8S isolate 6/97–74	AJ279463	"
Epicoccum sp. 4/97–60	AJ279486	"
Phomopsis sp. IW-75	AF079770	Rosskopf et al. (2000)
Phomopsis sp. BG-96	AF079771	"
Phomopsis sp. FP1–96	AF079772	"
Phomopsis sp. FP3–96	AF079773	"
Phoma medicaginis	AF079775	"
Phoma herbarum	AF218792	Bradner et al., unpublished
Ampelomyces quercinus	AF035778	Kiss and Nakasone (1998)
Ampelomyces humuli	AF035779	"
Phoma glomerata	AF126816	Sullivan and White (2000)
Phoma glomerata	AF126819	"
Microsphaeropsis amaranthi	AF079774	Rosskopf et al. (2000)

by mortar and pestle. DNA extraction, RAPD analysis, and rDNA amplification were carried out as in Pažoutová et al. (2000). RAPD analysis was performed using primers 8F (GCTCTGAGATTGTTCCGGCT), 5R (TTTGTCCGGCT-CAGAAAC), and 30F (GAGGACGATTCATCAACC). The rDNA of *Cerebella andropogonis* CZ and *C. andropogonis* AF containing the ITS1–5.8S-ITS2 region was sequenced at Microsynth (Balgach, Switzerland) and the sequences deposited in EMBL Nucleotide Sequence Database under the Accession No. AJ400905 and AJ306620, respectively.

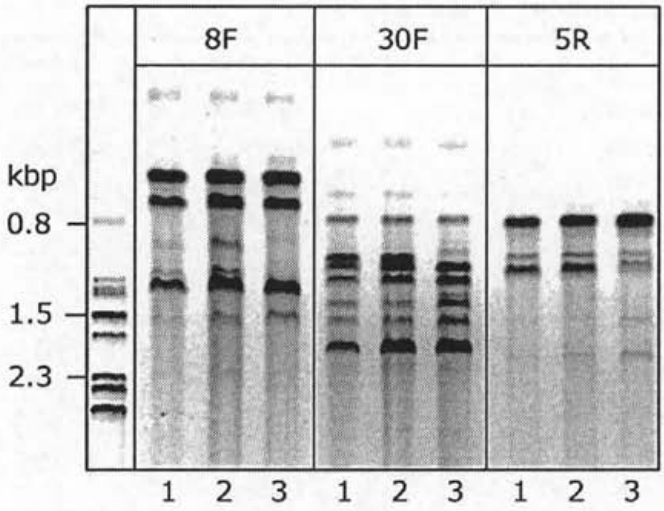

Fig. 1 RAPD patterns of *C. andropogonis* isolates 1 – Australian, 2 – African, 3 – Czech

Phylogenetic methods:

rDNA sequences of *C. andropogonis* were compared with EMBL and GenBank sequence databases. The closest 24 sequences (Tab. 1) were used for further analysis. Sequences were aligned using BioEdit version 4.7.1 (T. Hall, Department of Microbiology, North Carolina State University, Raleigh, NC 27695). The sequence of *Microsphaeropsis amaranthi* (AF079774) was used as an outgroup. Phylogenetic analysis was performed using TREE-PUZZLE 5.0 (©1999–2000, H. A. Schmidt, K. Strimmer, M. Vingron, and A. von Haeseler), which reconstructs phylogenetic trees from molecular sequence data by maximum likelihood.

RESULTS AND DISCUSSION

RAPD analysis of African and Australian *C. andropogonis* and Czech *Cerebella* sp. isolates with three primers (Fig. 1) revealed identical patterns for all three isolates. Therefore, we conclude that, despite small differences in conidial size, all three isolates belong to the same species, *C. andropogonis*. The species identity of the Czech isolate was also confirmed by comparison of its rDNA sequence to that of African *C. andropogonis*. Sequences were identical except for a single transition at position 47 of ITS1 (TAA –TGA).

Alignment of *C. andropogonis* with 24 related database sequences contained 469 sites, 51 of them variable. Quartet trees were based on approximate maximum likelihood values using the HKY model of substitution (Hasegawa et al. 1985) with

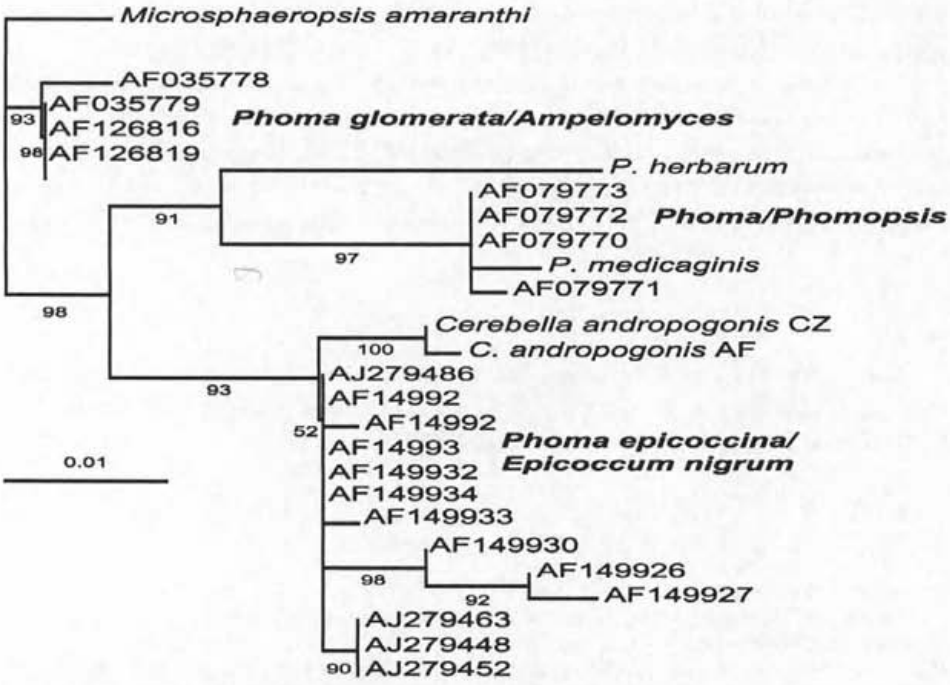

Fig. 2 Phylogenetic relationships of *Cerebella*.
Quartet puzzling tree with maximum likelihood branch lengths and branch support values. Number of puzzling steps: 10000, analysed quartets: 14950, unresolved quartets: 3228 (= 21.6%), log likelihood = –995.40.

uniform rate heterogeneity. Quartet puzzling was used to choose from the possible tree topologies and to simultaneously infer support values for internal branches (Fig. 2). For parameter estimation (substitution process and rate variation), the neighbour-joining tree was used. The transition/transversion parameter estimated from the data set was 2.88 (S. E. 0.87), expected transition/transversion ratio: 2.92, expected pyrimidine transition/purine transition ratio: 1.45.

High sequence similarity (96.5–98.9 % identity) caused that some clades were unresolved. The 5.8S rDNA gene was completely conserved among all taxa. The closest match was found between mycoparasitic *Cerebella* and various *Epicoccum nigrum* or *Phoma epicoccina* isolates which were on a highly supported clade (93 %). However, separation of *Cerebella* and *Phoma epicoccina*/*Epicoccum nigrum* clades was only weakly supported (52 %). Sequence similarity thus supports the placement of *Cerebella* into the genus *Epicoccum*. The second group of related fungi includes phytopathogens *Phoma herbarum* (Bradner et al. unpublished), *Phoma medicaginis* and *Phomopsis* sp. isolates (Rosskopf et al.

2000). The third group consisted of mycoparasitic *Phoma glomerata* and related *Ampelomyces* (Kiss and Nakasone 1998; Sullivan and White 2000).

The similarity of rDNA sequences between *Epicoccum*, *Cerebella*, *Phomopsis* and *Phoma glomerata/Ampelomyces* species is striking when compared to morphological differences and differences in life style of related species. It may reflect recent divergence of these fungi. Among the species related to *C. andropogonis*, saprophytes, necrotrophs, mycoparasites as well as plant pathogens were found.

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CZECH MYCOL. 54 (3–4), 2003

Une réévaluation de Mycena radicifera J. Favre

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Moreau P.-A. et Courtecuisse R. (2003): Une réévaluation de Mycena radicifera J. Favre – Czech Mycol. 54: 161–175

Mycena radicifera J. Favre est étudié sur la base de la récolte-type et de plusieurs récoltes récentes effectuées en France et au Groënland. L'espèce apparaît caractéristique des milieux sablonneux à tendance nitrophile. L'existence de récoltes apogames est reconnue. La variabilité des caractères microscopiques: spores, cheilocystides, piléipellis est examinée. Le nom de *M. radicifera* var. *apogama* est proposé pour dénommer les formes apogames, différant du type par la bisporie des basides, l'absence de boucles et la structure du suprapellis.

Key words: *Basidiomycota, Tricholomatales, Mycena radicifera*, apogamie, variation intraspécifique, milieux xériques, taxinomie.

Moreau P.-A. a Courtecuisse R. (2003): Nové hodnocení druhu Mycena radicifera J. Favre – Czech Mycol. 54: 161–175

Mycena radicifera J. Favre byla studována na základě typového materiálu a několika současných sběrů z Francie a Grónska. Druh je typický pro písčiny s vyšším obsahem dusíku. Byla zjištěna existence apogamických populací. Byla studována variabilita mikroskopických znaků: výtrusů, cheilocystid a pokožky klobouku. Jméno *Mycena radicifera* var. *apogama* je navrženo pro pojmenování apogamických forem, lišících se od typické formy bisporickými bazidiemi, nepřítomností přezek a strukturou svrchní vrstvy pokožky.

INTRODUCTION

Mycena radicifera J. Favre est une espèce peu connue, dont les seules récoltes attestées par la littérature sont deux localités en Suisse (Favre 1957) et une récolte de Winterhoff, en Allemagne (Maas Geesteranus 1991: 459). La seule iconographie connue à présent est la planche originale de Mme J. Favre, conservée au Jardin Botanique de Genève (G) et publiée post mortem par Monthoux (1986: 175).

Nous possédons plusieurs récoltes rapportées d'emblée à *M. radicifera*, mais présentant des caractères a priori différents de la description originale. De légères différences dans les dimensions sporales, la bisporie observée sur plusieurs d'entre elles et le développement inégal des diverticules du suprapellis nous ont conduit à approfondir cette détermination, en étudiant, grâce à l'amabilité de P. Clerc (Conservatoire du Jardin Botanique de Genève), la totalité du matériel original de J. Favre en comparaison de nos récoltes.

MATÉRIEL ÉTUDIÉ

Holotype de *M. radicifera*: "Genolier, canton de Vaud (Suisse), juste en dessous de la colonie de vacances, garide steppique sur sol graveleux à galets calcaires dominants, sur rhizomes d'*Ononis spinosa*, alt. 650 m; 23 novembre 1941", herb. J. Favre (G) n° GK 8082.

Cette unique récolte de Favre est très abondante, représentée par plus d'une trentaine de sporophores qui constituent l'holotype de l'espèce (automatique, non désigné par Favre 1957). Deux spécimens ont été extraits de cette collection par le conservateur et en constituent un "double" disponible pour les prêts. Nous avons eu la possibilité d'étudier la totalité de la collection, afin d'évaluer la variabilité des caractères sur l'ensemble des spécimens.

Nous avons prélevé parmi cette collection 10 spécimens, de taille et d'aspect différents (numérotés de 1 à 10), et nous avons mesuré 30 spores sur chacun d'entre eux. Nous avons également observé la structure du revêtement piléique et les cheilocystides sur chacun. Les dimensions sporales ont été évaluées par extrapolation à une répartition gaussienne et comparées (tab. 1).

Récoltes personnelles:

— Réserve naturelle des îles de Chautagne-Malourdie, Motz (Savoie, France), terrain graveleux aride, sous *Salix eleagnos* avec *Tortula ruralis* et *Trifolium repens*, alt. 320 m, 25 novembre 2000, leg. M. Durand et P.-A. Moreau, herb. P.-A. Moreau n° 00112501.
— Réserve Biologique Domaniale de Merlimont [Forêt de la Côte d'Opale] (Pas-de-Calais, France), fourrés dunaires de l'*Hippophaeion* avec *Tortula ruraliformis*, 22 octobre 2000, leg. P.-A. Moreau, herb. P.-A. Moreau n° 00102204.
— Même lieu et écologie identique, le 1 novembre 2000, leg. R. Courtecuisse & C. Lécuru (échantillon non conservé).
— Salt Lake, Kangerlussuaq (Groënland), végétation subarctique halo-hygrophile dominée par *Salix arctophila, S. herbacea* et *Climacium* sp., 13 août 2000, VI[è] International Symposium of Arctic-Alpine Mycology, leg. C. Cripps, L. Jalink, M. Nauta, B. Senn-Irlet, *Ovibos moschatus* et P.-A. Moreau, herb. P.-A. Moreau n°s GR00.41 et GR00.42.

Pour des raisons pratiques, nous proposons les abréviations suivantes pour les récoltes citées:

Fav: Genolier, J. Favre GK8082 (G, holotype) [spécimens numérotés Fav1 à Fav9]

Mal: Malourdie, P.-A. Moreau 00112501

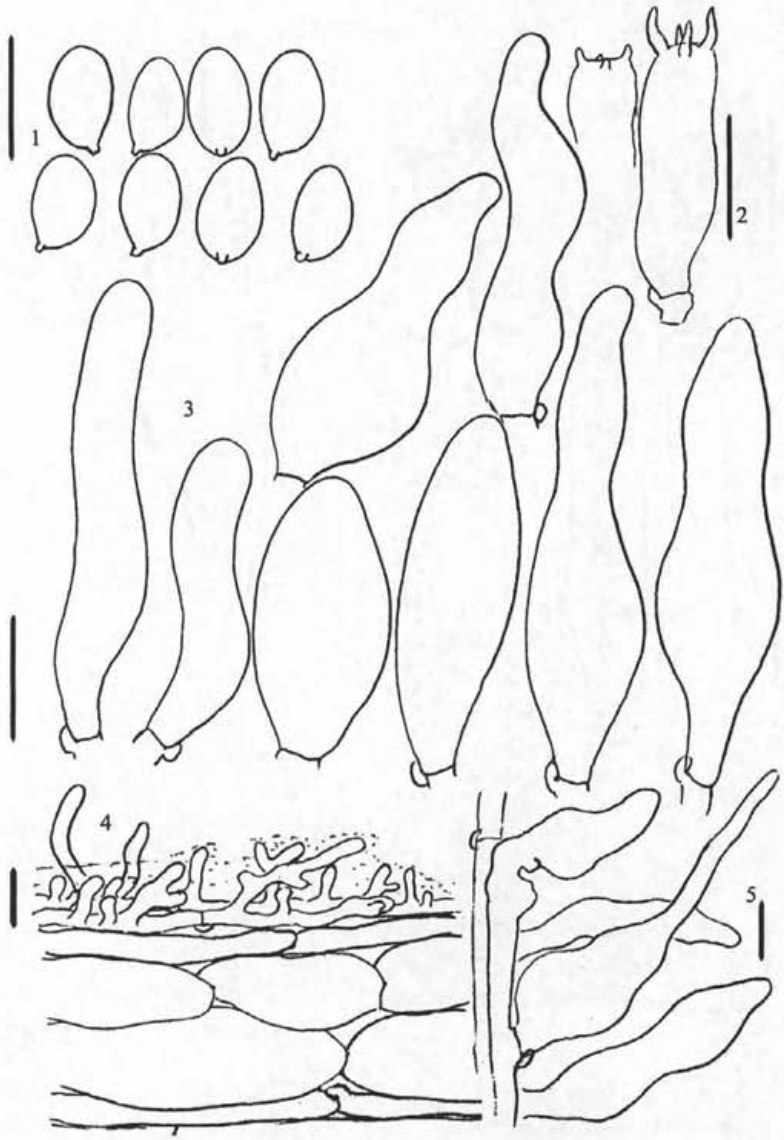


Figure 1: *Mycena radicifera* J. Favre var. *radicifera* (PAM 00112501). 1: spores; 2: basides; 3: cheilocystides; 4: revêtement piléique (coupe radiale); 5: caulocystides. Barre = 10 μm.

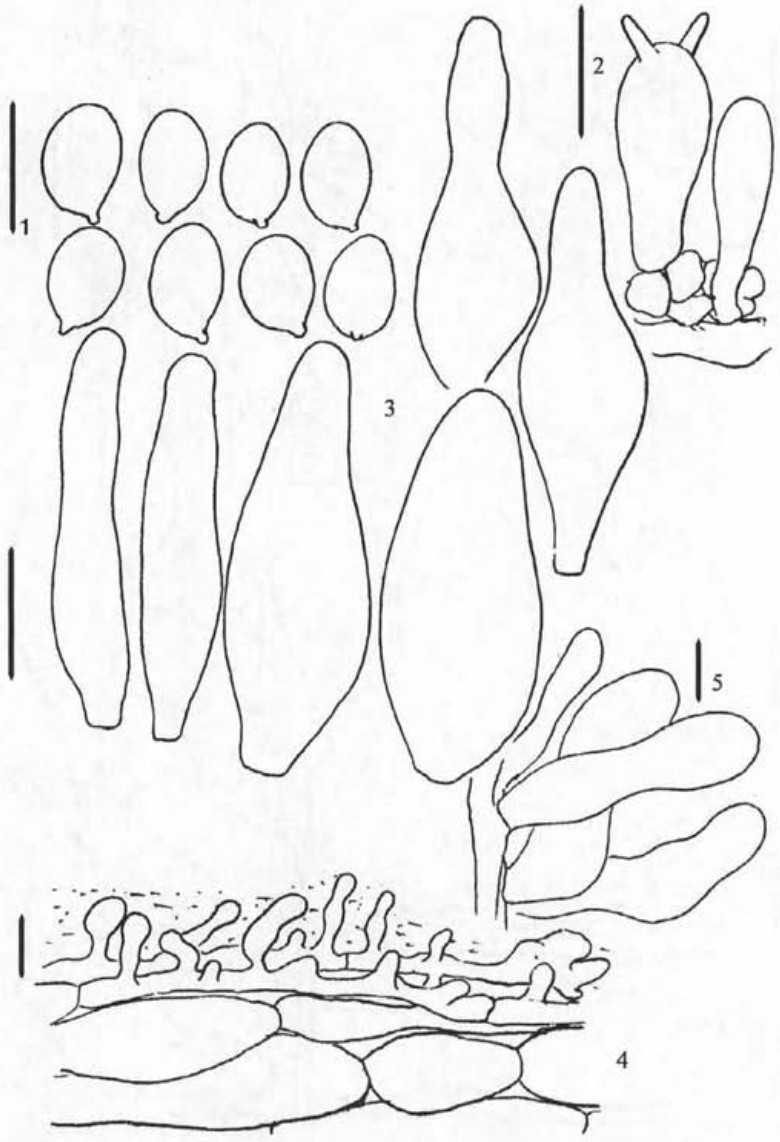


Figure 2: *Mycena radicifera* var. *apogama* var. nov. (PAM 00102204, holotype). 1: spores; 2: basides; 3: cheilocystides; 4: revêtement piléique (coupe radiale); 5: caulocystides. Barre = 10 μm.

Mer: Merlimont, P.-A. Moreau 00102204
Gro1: Kangerlussuaq, Groënland, P.-A. Moreau GR00.41
Gro2: Kangerlussuaq, Groënland, P.-A. Moreau GR00.42

Position systématique de M. radicifera

Intuitivement, *M. radicifera* évoque un *Mycenella*, en raison du pied rigide et pruineux, ou un *Hydropus* sect. *Floccipedes*. En réalité, l'arête stérile par de petites cheilocystides et l'absence de pleurocystides la font naturellement classer dans le genre *Mycena*. L'absence de dextrinoïdie et de métachromasie au bleu de Crésyl dans toutes les parties du sporophore, ainsi que le faible développement du subpellis, conduisent à l'ancien sous-genre *Hemimycena* de Kühner (1938), correspondant actuellement aux sect. *Adonidae* et *Hiemales* du genre *Mycena* (Maas Geesteranus 1980).

La présence d'un subpellis relativement épais et bien différencié, constitué d'éléments larges jusqu'à 35 μm, rappelle la structure pseudoparenchymateuse caractéristique de la plupart des *Mycena* à spores amyloïdes, et habituellement très peu développé chez les *Adonidae* et les *Hiemales*. Ce caractère est présent sur tous les spécimens étudiés, et se montre particulièrement développé sur les récoltes groënlandaises.

Nous suivons donc Maas Geesteranus (1991: 180), en attribuant *M. radicifera* à la section *Hiemales* Konr. & Maubl., sous-section *Hiemales* Maas G., les lames étant non décurrentes; elle représente la plus grande espèce connue de cette section.

Comparaison analytique des collections

Caractères microscopiques communs à tous les spécimens étudiés

Spores non amyloïdes, non cyanophiles, à paroi mince, à apicule étiré et déjeté, long de 0,5–1 μm. Arête hétéromorphe, fertile à cheilocystides dominantes. Sous-hyménium compact, épais de 10–15 μm, pseudoparenchymateux à petits éléments courts × 3–5 μm. Trame régulière, constituée d'hyphes larges elliptiques (× 8–35 μm) et d'hyphes grêles cylindracées (× 3,5–10 μm) en proportion égale, sans hyphes gloeoplères nettement différenciées. Suprapellis épais de 10–15 μm, gélifié, constitué d'1–2 couches d'hyphes grêles × 3,5–4,5 μm, émettant des diverticules dressés. Subpellis épais et différencié, à 2–3 couches d'éléments larges et elliptiques × 9–35 μm. Revêtement du pied à 1 couche d'hyphes grêles × 3–4,5 μm, émettant des bouquets de cystides tantôt basidioloïdes, tantôt difformes, jusqu'à 45–60 μm, plus abondantes dans la partie supérieure. Aucune métachromasie des cellules, même dans la base du pied. Dextrinoïdie nulle, ou très faible et douteuse dans la trame des lames (Favre 1957).

Amyloïdie des spores

Toutes les espèces de ces sections possèdent des spores non amyloïdes. Nos premières observations nous ont fait douter de ce caractère, car les spores dans le Melzer semblent présenter une légère coloration bleutée en coupe optique, et la subtilité de l'amyloïdie chez certaines *Mycena* incite à la prudence. En fait, les amas de spores ne présentent pas de coloration plus intense que les spores isolées, et l'effet de couleur est interprété comme un effet d'optique renforcé par la concavité particulière de la paroi sporique. Nous avons été confortés par les remarques inédites de J. Favre (herb. G), qui doute aussi de ce caractère, pour finalement souligner son affirmation finale: "spores non amyloïdes". Il peut être une cause d'erreur dans la détermination de cette espèce.

Tétrasporie – apogamie

Toutes les récoltes sont très homogènes; sur aucun spécimen nous n'avons observé de basides bisporiques mêlées aux tétrasporiques, ni l'inverse. Les spécimens tétrasporiques présentent des boucles à toutes les cloisons dans l'hyménium et à toutes les hyphes grêles de la trame; elles sont inconstantes aux cloisons des hyphes larges. Les spécimens bisporiques se sont montrés dépourvus de boucles, même au pied des basides.

L'examen des noyaux (observation dans le carmin acéto-ferrique, voir Kühner 1938, 1949) a été conduit sur les basidioles et les spores.

La relation entre la bisporie, l'absence de boucles et la présence d'un unique noyau dans les basidioles permet de conclure à la nature parthénogénétique des récoltes Gro1, Gro2 et Mer (Kühner 1938).

Récoltes tétrasporiques bouclées, normales: Fav, Mal
Récoltes bisporiques non bouclées, apogames: Mer, Gro1, Gro2
Les remarques qui suivent traitent séparément ces deux types de récoltes.

Variabilité sporale

Les dimensions estimées sont calculées sur 30 mesures (Tab. 2), effectuées sur spores déposées sur la surface du chapeau ou du pied (sporée naturelle).

La répartition des récoltes par les dimensions moyennes estimées (Graphe 1) montre l'isolement des 3 récoltes apogames, à spores relativement courtes et plus larges. Les spécimens tétrasporiques macrosporés possèdent un quotient sporal comparable aux autres spécimens tétrasporiques et ne peuvent en être nettement séparés.

– Récoltes tétrasporiques: la variabilité sporale apparaît légèrement supérieure aux mesures fournies par Favre (8,5–10 × 5–6,5 μm) et Maas Geesteranus

Tab. 1: comparaison des caractères anatomiques et cytologiques des récoltes étudiées

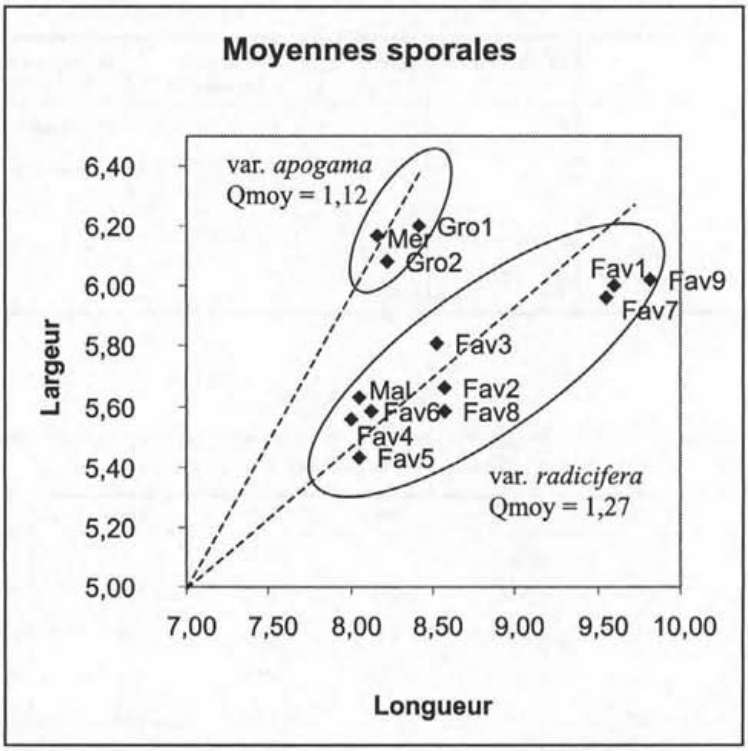
	Nb stérigmates	Boucles	Nb noyaux basidioles	Nb noyaux spores
Fav	4	+	2	2, rarement 1
Gro1	2	0	1	1, rarement 2
Gro2	2	0	1	1
Mal	4	+	2	2, parfois 1
Mer	2	0	1	1

Tab. 2: dimensions estimées des spores (n = 30). Lmoy = moyenne des longueurs; lmoy = moyenne des largeurs; Qmoy = moyenne des quotients (L/l); σ = écarts-types.

	Lmoy	σ(L)	*lmoy*	σ(l)	*Qmoy*	σ(Q)
Fav1	*9,60*	0,66	*6,00*	0,42	*1,60*	0,11
Fav2	*8,57*	0,88	*5,67*	0,41	*1,51*	0,12
Fav3	*8,52*	0,95	*5,81*	0,43	*1,47*	0,10
Fav4	*8,00*	0,58	*5,56*	0,60	*1,45*	0,10
Fav5	*8,05*	0,85	*5,43*	0,29	*1,49*	0,17
Fav6	*8,12*	0,52	*5,58*	0,36	*1,46*	0,11
Fav7	*9,55*	0,67	*5,96*	0,36	*1,61*	0,15
Fav8	*8,57*	0,74	*5,58*	0,61	*1,54*	0,12
Fav9	*9,81*	0,92	*6,02*	0,50	*1,63*	0,10
Gro1	*8,42*	0,80	*6,20*	0,50	*1,36*	0,13
Gro2	*8,22*	0,72	*6,08*	0,60	*1,36*	0,10
Mal	*8,05*	0,46	*5,63*	0,41	*1,43*	0,11
Mer	*8,17*	0,59	*6,17*	0,30	*1,33*	0,10

(8,5–10,7 × 5,5–6,3 μm). La plupart des récoltes se situent dans la fourchette 6,3–10,1 × 4,3–6,9 μm, les trois récoltes macrospores (Fav1, Fav7 et Fav9) se situent vers 7,0–11,0 × 5,1–6,0 μm.

Le diamètre du chapeau est utilisé comme approximation du stade de développement du sporophore. On constate que les spécimens les plus développés ont tendance à produire de grandes spores (Graphe 2), sans qu'une bisporie partielle éventuelle puisse être incriminée.



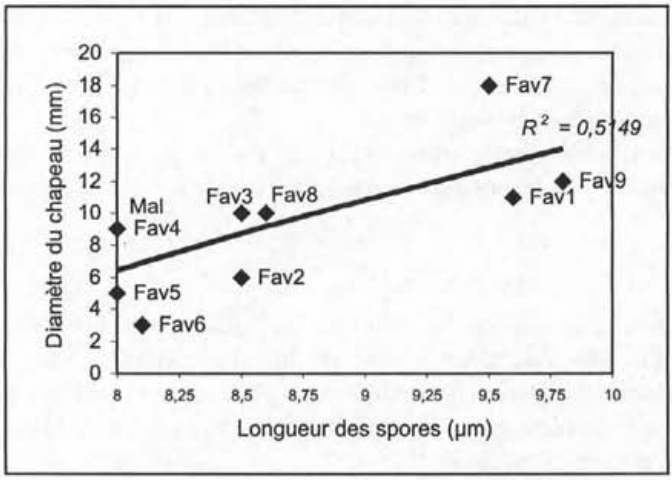
Graphe 1: répartition des récoltes par dimensions sporales moyennes estimées (cf. tab. 2).

– Récoltes bisporiques: les spores des récoltes Gro1, Gro2 et Mer sont nettement plus larges, à face dorsale plus convexe et à tendance amygdaliforme plus accusée que les précédentes; les trois collections montrent des dimensions moyennes très voisines.

Développement du suprapellis

Récoltes tétrasporiques: Le développement des diverticules piléiques est également variable, les plus développés (Fav1, Fav6 et Fav8) étant conformes à l'illustration de Favre, tandis que la majorité des spécimens présente un revêtement nettement moins exubérant, à diverticules plus denses, plutôt courts et non ou peu diversifiés. Ces revêtement sont plutôt homogènes pour un même individu, mais peuvent présenter une variabilité importante d'un individu à l'autre, qui ne semble pas corrélée à des différences d'âge ou de développement, ni aux différences sporales observées.

Récoltes bisporiques: en comparaison des précédentes, ces récoltes présentent des diverticules denses, plus larges et souvent clavés, ondulés ou capitulés. Les



Graphe 2: relation développement du carpophore – longueur des spores sur les récoltes tétrasporiques.

récoltes groënlandaises (fig. 3) se caractérisent par des diverticules très nombreux noyés dans une matrice gélatineuse dense, la gélification des autres récoltes étant diffuse et parfois peu nette.

Forme des cheilocystides

Il est surprenant de constater que nos récoltes personnelles montrent toutes des conformations de cheilocystides différentes. Décrites "peu différenciées, étroitement vermiformes" par Favre (1957: 83), "cylindrical to fusiform, simple or somewhat branched apically" par Maas Geesteranus (1991), elles se montrent ainsi sur la plupart des spécimens de la récolte holotype, certains spécimens montrant une tendance (minoritaire) à présenter des éléments à base plus ou moins élargie.

Les autres récoltes présentent les variations suivantes:

Gro1 (2-sp.): une partie d'éléments cylindracés-vermiformes, mêlés à des éléments clavés-subcapités, fusi-lagéniformes, lobés ou digités-ramifiés; certaines portions de l'arête montrent les hyphes radiales émettant des éléments plus courts, larges et non cloisonnés à la base.

Gro2 (2-sp.): majorité d'éléments cylindracés-vermiformes à base non ou peu élargie, parfois légèrement clavés, une partie présentant des digitations sommitales.

Mal (4-sp.): cheilocystides cylindracées mêlées de nombreuses cystides très larges jusqu'à 40 μm, ovoïdes, cylindracées ou fusiformes, non ramifiés.

Mer (2-sp.): identiques au précédent, à éléments un peu moins larges jusqu'à 30 μm, plus volontiers fusi-lagéniformes ou pluriétranglés, non ramifiées.

Ces différences de conformation des cystides n'ont pas pu être reliées à d'autres caractères; nous les considérons donc actuellement comme une variabilité intraspécifique sans valeur taxinomique. Elles sont toutefois spectaculaires, et mériteront d'être réévaluées lors de récoltes futures.

Parmi nos récoltes personnelles, "Mal" ne diffère de la récolte-type de Favre que par la présence de larges cheilocystides.

Habitat

La station originale de Favre (1957) est un milieu xérique continental typique des vallées alluviales, également appelé "garide" (voir Röllin 1996). L'abondance d'*Ononis spinosa* suggère une variante nitrophile de cet habitat. Favre (1957) signale une seconde récolte non documentée dans la garide de l'Allondon (canton de Genève, CH), sur talus aride.

Notre récolte "Mal" provient de la même vallée du Rhône, dans une garide développée sur graviers extraits mécaniquement du Rhône, et en cours de recolonisation par *Salix* spp., *Picris hieracioides, Saxifraga tridactylites* et *Trifolium repens*, avec présence de mousses pionnières comme *Tortula ruralis*. Provenant du même secteur biogéographique que les récoltes de Favre, cette récolte est également la plus proche sur le plan microscopique.

La récolte "Mer" a été effectuée dans les fourrés de l'*Hippophaeion*, sur les dunes fixées de la Côte-d'Opale, parmi la végétation de la dune grise pénétrant sous la lisière des fourrés à *Hippophae rhamnoides*, dominée par *Tortula ruraliformis*.

Les récoltes "Gro1" et "Gro2" proviennent de deux rives voisines d'un lac glaciaire à forte teneur en minéraux, dont la végétation présente à la fois un caractère halophile, subarctique et ± xérophile en raison du substrat limono-sableux drainant; on trouve, outre *Salix arctophila* et *S. herbacea*, des plantes minéralophiles comme *Dryas integrifolia*.

Les points communs de toutes ces récoltes semblent être: un milieu drainant à tendance xérique, alluvionnaire et plutôt sablonneux, à caractère nitrophile. La station groënlandaise est la plus atypique, et pourtant la plus riche en sporophores!

Bien que Lange (1955) ait prospecté cette station du St. Saltsø lors de son inventaire du Groënland, ni ses travaux ni ceux de Watling (1977) ne mentionnent d'espèce de *Mycena* comparable à *M. radicifera*.

Conclusion

La variabilité des caractères anatomiques de *Mycena radicifera* nous semble importante sur les dimensions sporales (apparemment reliables à l'âge du sporocarpe), le développement des diverticules piléiques, et surtout la forme des cheilocystides. Les récoltes bisporiques non bouclées, en outre caractérisées par

un quotient sporal moins élevé et des diverticules piléiques moins allongés et plus nombreux, représentent des individus apogames dont la morphologie ne semble pas significativement différente du type.

Il est délicat d'accorder un rang taxinomique à des spécimens apogames, dont l'origine asexuée est la cause des principales différences observées. Le rang variétal a été proposé à plusieurs reprises pour de tels cas: Kühner & Valla (1972) pour *Hemimycena mauretanica* var. *apogama*, Courtecuisse (1985) pour *Mycenella salicina* var. *bispora*, Bas (1998) pour *Hydropus scabripes* var. *quadrisporus* (la variété-type étant vraisemblablement apogame), etc.

Afin de souligner les différences constatées entre nos récoltes, souhaitons-nous attribuer à ces récoltes un nouveau nom au rang variétal:

Mycena radicifera var. **apogama** var. nov.

A typo differt basidiis bisterigmatibus sporisque latioribus, Q = 1,33–1,36; fibulae desunt. Holotypus in Merlimont (Pas-de-Calais, Gallia) collectus, in herb. LIP (Lille) sub no. PAM00102204 conservatus.

Descriptions

Mycena radicifera J. Favre var. **radicifera**: récolte "Mal" (PAM 00112501, 1 spécimen jeune). Fig. 3.

Chapeau 0,7 cm, bassement conique à petite paille noire au centre, ailleurs gris bistré devenant graduellement ochracé pâle vers la marge, strié sur 2/3 du rayon, mat, finement pruineux sous la loupe. Lames ascendantes-sublibres, ocre pâle, serrées. Pied 4,5 × 0,1 cm, inclus une pseudorrhize de 1,2 cm, blanchâtre à l'apex, graduellement ochracé vers la base, lisse, légèrement strigueux sur la pseudorrhize. Chair blanche, un peu coriace; odeur et saveur nulles.

Spores 7,0–9,0 × 5,5–6,8 μm, Q = 1,52, ovo-elliptiques. Basides 4-sporiques, 18–24 × 6–8,5 μm, cylindracées, à stérigmates longs de 2–3,5 μm, bouclées. Cheilocystides 22–45 × 5–12 μm, cylindracées-vermiformes, ovoïdes à fusi-lagéniformes ± étranglées; arête fertile à basides éparses. Suprapellis épais de 10–15 μm, gélifié, à 1–2 couches d'hyphes grêles × 3–4,5 μm émettant des diverticules dressés jusqu'à 9 (15) μm de longueur, cylindracés ou courtement ramifiés. Subpellis épais de 35–45 μm, à 2–3 couches d'articles larges × 9–30 μm, à pigment vacuolaire grisâtre pâle. Caulocystides en petits bouquets, 18–75 (120) × 4–12 μm, très polymorphes et parfois monstrueuses, cylindro-fusiformes ± ramifiées à longuement appendiculées. Boucles abondantes.

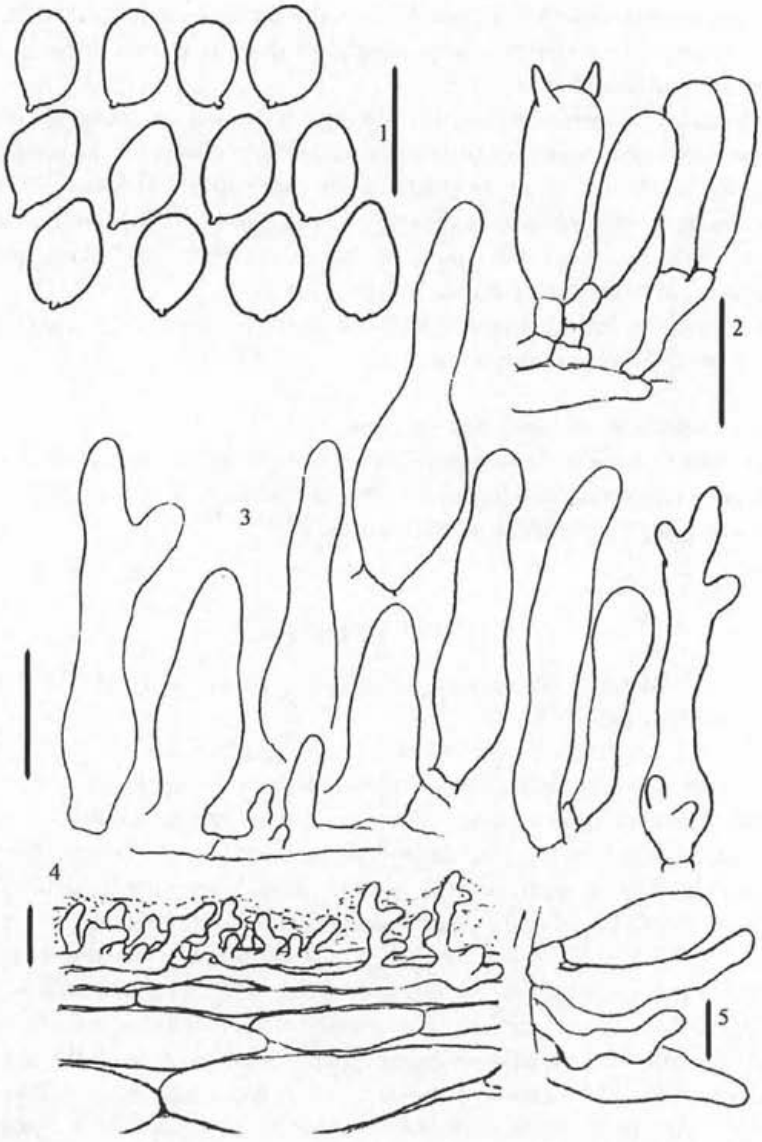


Figure 3: *Mycena radicifera* var. *apogama* var. nov. (PAM GR00–41). 1: spores; 2: basides; 3: cheilocystides; 4: revêtement piléique (coupe radiale); 5: caulocystides. Barre = 10 μm.

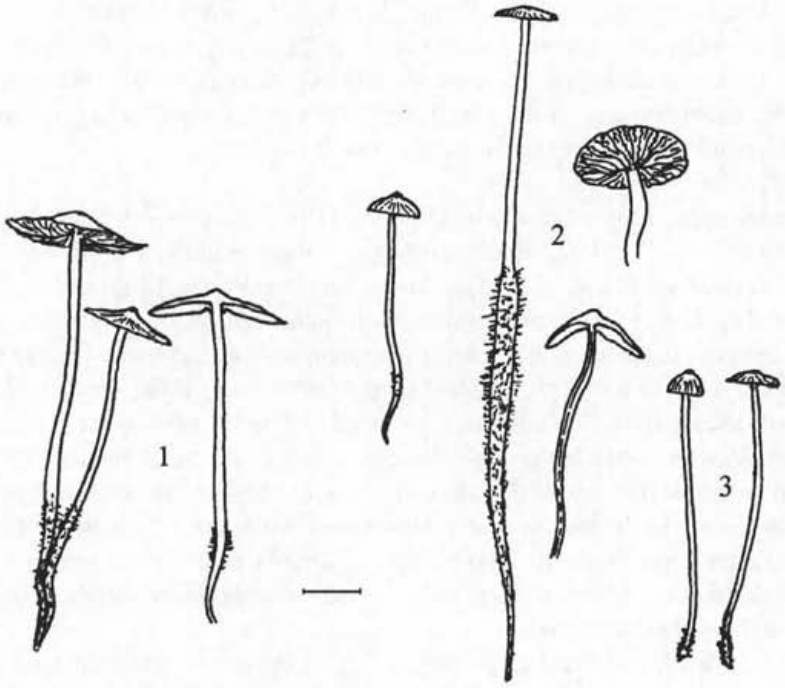


Figure 4: *Mycena radicifera* var. *apogama* var. nov. Sporophores. 1: PAM 00102204 (holotype); 2: PAM GR00–41; 3: PAM G 00–42. Barre = 1 cm.

Mycena radicifera var. **apogama** P.-A. Moreau et Courtecuisse

1) description de la récolte "Mer" (PAM 00102204, 4 spécimens adultes). Fig. 4 et planche 1.

Chapeau 0,8–2 cm, conique-aplani légèrement papillé, mat, lisse, strié jusqu'au centre, gris blanchâtre à bistré clair nuancé d'olivâtre à papille centrale peu marquée, à marge blanche brusquement éclaircie, très hygrophane, pâlissant depuis le centre en gris cendré uniforme. Lames serrées, 24 atteignant le pied, (1) 2 séries de lamellules, sublibres à étroitement échancrées, blanc grisâtre pâle; arête concolore. Pied 3–5 × 0,1–0,2 cm, radicant sur 1–2 cm, lisse dans sa partie aérienne, blanc puis sali d'ochracé depuis la base, plein. Chair blanche. Odeur nulle; saveur légèrement herbacée.

Spores 6,9–9,4 × 5,5–6,8 μm, Q = 1,32, largement ovoïdes à tendance amygdaliforme, à face dorsale très convexe. Basides 18–24 × 5,5–7,5 μm, 2-sporiques, cylindro-clavées, à stérigmates droits jusqu'à 3,5–4 μm de longueur, non bouclées. Cheilocystides 25–40 × 6–16 μm, cylindracées à fusi-lagéniformes fortement ventrus, à col atténué ou sans col, certaines presque ovoïdes; arête fertile à basides éparses. Suprapellis épais de 12–15 μm, gélifié, à hyphes couchées × 3–4,5 μm,

à diverticules dressés jusqu'à 15 µm, la plupart courts, irrégulièrement cylindracés, onduleux ou clavés. Subpellis épais de 25–35 µm, à 1–2 couches d'articles larges × 9–35 µm, à pigment vacuolaire gris pâle. Caulocystides nombreuses, en bouquets, basidioloïdes, 15–45 × 6–9 µm; l'apex est recouvert d'hyménium avec basides fertiles sur 3–4 mm de longueur. Boucles absentes.

2) description de la récolte "Gro1" (PAM GR00–41, 18 spécimens). Fig. 5.

Chapeau 0,8–1,5 (2) cm, digitiforme puis conique-papillé, à la fin campanulé--étalé à mamelon obtus, gris-brun bistré, brun grisâtre, beige grisâtre (code Cailleux J41, F63, C72), légèrement strié jusqu'au centre, hygrophane, pâlissant en gris ochracé uniforme (B61) depuis le centre; lisse et légèrement luisant sur le frais, ridulé sous la loupe en séchant. Lames serrées, 24–28 au pied, 1 (2) séries de lamellules, sublibres, crème pâle puis ochracées sur le tard, briunissant depuis la marge à la fin; arête légèrement fimbriée. Pied 5–12 cm, pseudorrhize incluse pouvant représenter la moitié de la longueur, rigide-cassant, très finement pruineux vers l'apex chez les jeunes, lisse et poli ensuite, blanc puis vite ochracé depuis la base, strigueux sur la partie souterraine. Chair élastique-molle, peu résistante, grisâtre pâlissant à blanchâtre en séchant. Odeur légèrement fruitée à la coupe; saveur acidulée-herbacée fugace.

Spores 6,8–10,0 × 5,2–7,2 µm, Q = 1,36, largement ovo-elliptiques à subglobuleuses, à tendance amygdaliforme-subrhomboïdale fréquente à face dorsale très convexe et apicule fortement déjeté. Basides bisporiques, 18–26 × 5,5–7 µm, cylindro-clavées, non bouclées. Cheilocystides 15–35 (40) × 4,5–9 µm, cylindracés--vermiformes, mêlés à des éléments clavés-subcapités, fusi-lagéniformes, quelques--uns lobés ou digités-ramifiés au sommet ou bourgeonnant à la base; certaines portions érodées de l'arête montrent les hyphes radiales émettant des éléments plus courts, larges et non cloisonnés à la base. Le plus gros spécimen de la collection, en maturité avancée, montre sur ces portions d'arête nue une structure en brosse dense, également développée sur certaines cystides. Suprapellis épais de 10–15 µm, densément gélifié, à 2–3 couches d'hyphes grêles très densément diverticulées, à diverticules dressés, irréguliers, tortueux, lobés ou courtement digités-ramifiés. Subpellis épais de 40–55 µm, à 3–4 couches d'hyphes cylindro-fusiformes larges de 8–25 µm, à pigment vacuolaire gris diffus. Caulocystides 30–35 × 3,5–6 µm, en bouquets surtout abondants à l'apex, clavées basidioloïdes ou vermiformes irrégulières, souvent difformes. Boucles absentes.

Les sporophores sont directement en contact avec les racines mortes des saules rampants; lorsque ces racines sont superficielles, le pied est peu radicant, autrement la pseudorrhize peut atteindre jusqu'à 6 cm de longueur. La récolte "Gro2" (PAM GR00–42, 7 spécimens) ne diffère que par un chapeau plus petit, 0,5–1 cm, beige à papille noire marquée, et un pied 4–6 × 0,05–0,1 cm non ou peu radicant.

Planche 1: *Mycena radicifera* var. *apogama* var. nov. Sporophores PAM 00102204 (holotype) (photo Pierre-Arthur Moreau)

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Russula faginea and similar taxa

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Adamčík S. (2003): Russula faginea and similar taxa – Czech Mycol. 54: 177–191

A selection of five specimens of *Russula faginea* and very similar taxa was made, examined microscopically and compared. The studied material seems to be composed of two groups which differ in spore size, frequency of line connections among the spines of the spores and size and shape of the terminal cells of generative hyphae in the pileus epicutis. I consider the two groups to be taxa at the rank of species. The nomenclature of these two proposed species is discussed. The only valid names available for them are *R. faginea* and *R. abietum*. The types of these species names correspond probably to the two observed groups, but as I have not studied the type of *R. abietum*, I cannot conclude this for certain. *R. faginea* Romagnesi, nom. inval. is validated here.

Key words: macrofungi, Russulales, taxonomy, Russula faginea

Adamčík S. (2003): Russula faginea a podobné taxony – Czech Mycol. 54: 177–191

Mikroskopická štruktúra piatich vybraných položiek *Russula faginea* a podobných taxónov je porovnávaná. Študovaný materiál pozostáva z dvoch skupín, ktoré sa líšia veľkosťou výtrusov, početnosťou lineárnych spojení medzi ostňami výtrusov a tvarom a veľkosťou koncových buniek generatívnych hýf v pokožke klobúka. Predpokladám, že tieto dve skupiny sú taxóny na úrovni druhu. Diskutovaná je nomenklatúra týchto dvoch predpokladaných druhov. Len dve platné mená sú dostupné pre tieto druhy: *R. faginea* a *R. abietum*. Typy týchto druhových mien sa pravdepodobne zhodujú s dvomi študovanými skupinami, ale keďže nebol študovaný pôvodný materiál *R. abietum*, nemožno definitívne urobiť toto rozhodnutie. Neplatne uverejnené meno *R. faginea* Romagnesi, nom. inval. je uvedené do platnosti.

INTRODUCTION

Russula faginea Romagnesi ex Adamčík is a member of *Russula* sect. *Xerampelinae* (Singer) Jul. Schaeff. The section encompasses species that are characterised by a rusty brown coloration of the flesh after bruising, smell of herring when the carpophores are dried or old and green reaction to $FeSO_4$. Although at present different authors have very different opinions on the taxonomy of this section, the majority of them consider *R. faginea* a well-delimited species (e.g. Romagnesi 1967, Einhellinger 1987, Knudsen and Stordal 1992). Romagnesi (1967) for example designated only two taxa among 17 accepted as well-defined: *R. erythropoda* Peltereau and *R. faginea*. He gave the following characters as being typical of *R. faginea*: large fruitbodies, firm flesh, red coloured pileus margin, discoloured pileus centre, large spores with isolated spines, inflated cells in pileus epicutis

and growing in beech forests. However, some authors also accept other taxa with similar characters but associated with different host trees (e.g. *R. graveolens* var. *pseudomelliolens* Singer ex Bon, *R. abietum* J. Blum and *R. barlae* Quél.).

In addition to clarifying the nomenclature of *R. faginea*, the aim of this study is to compare taxa similar to this species with its type and confirm or deny that the species is strictly associated with *Fagus* trees.

MATERIAL AND METHODS

Five specimens were selected to compare the variability among *Russula faginea* and related taxa: the type specimens of *R. faginea* and *R. pseudomelliolens* and three specimens labelled as *R. abietum*, *R. barlae* and *R. faginea* (see Tab. 1). Some traditionally used characters were redefined or modified, especially micromorphological characters of the spores and the pileus epicutis. The number of spines, line connections, amyloid punctations and contact connections were observed in a circle of 3 μm diameter on the spore surface in the upper plane of focus. Width, length and shape of the terminal cells of the pileus epicutis were observed on the margin and in the centre of the pileus. All characters observed on the fruitbodies have been described in Adamčík and Marhold (2000). All micromorphological characters were observed with an oil-immersion lens at a magnification of 1.600 ×. Basidia and pleurocystidia were observed in a solution of Congo Red in ammonia (Fábry 1979), spores were observed in Melzer's reagent (Melzer 1945), hyphae of the pileus epicutis were observed in a solution of sulphovanillin (1 g of vanillin dissolved in 6 ml of distilled water and 5 ml of concentrated sulphuric acid).

Tab. 1. List of studied specimens

1.	***Russula faginea*** (type specimen): [France] Forêt de Compiègne (Oise), hêtraie, 29. VIII.1953, Romagnesi (PC, coll. Romagnesi 53–201).
2.	***Rusula faginea***: [Slovakia] Ondavská vrchovina: "Kuria hora", 2 km NE of the village of Kvakovce, under *Fagus* trees, 280 m a.s.l., 11. VII.1998, Adamčík (SAV).
3.	***Russula barlae* var. *pseudomelliolens*** (type specimen): [France] Desvres (La Poterie), P. de C., taillis charmes, 21. IX.1967, Bon (LILLE, coll. Bon 70921).
4.	***Russula xerampelina* var. *barlae***: [Denmark] Tromnaes, Falster, in fagetis, IX.1934, Møller (K, 57898), det. Jul. Schaeffer.
5.	***Russula abietum***: [France] Bramefarine (Allevard), *Picea* et *Fagus*, 5.IX.1978, Bon (LILLE, coll. Bon 78090503).

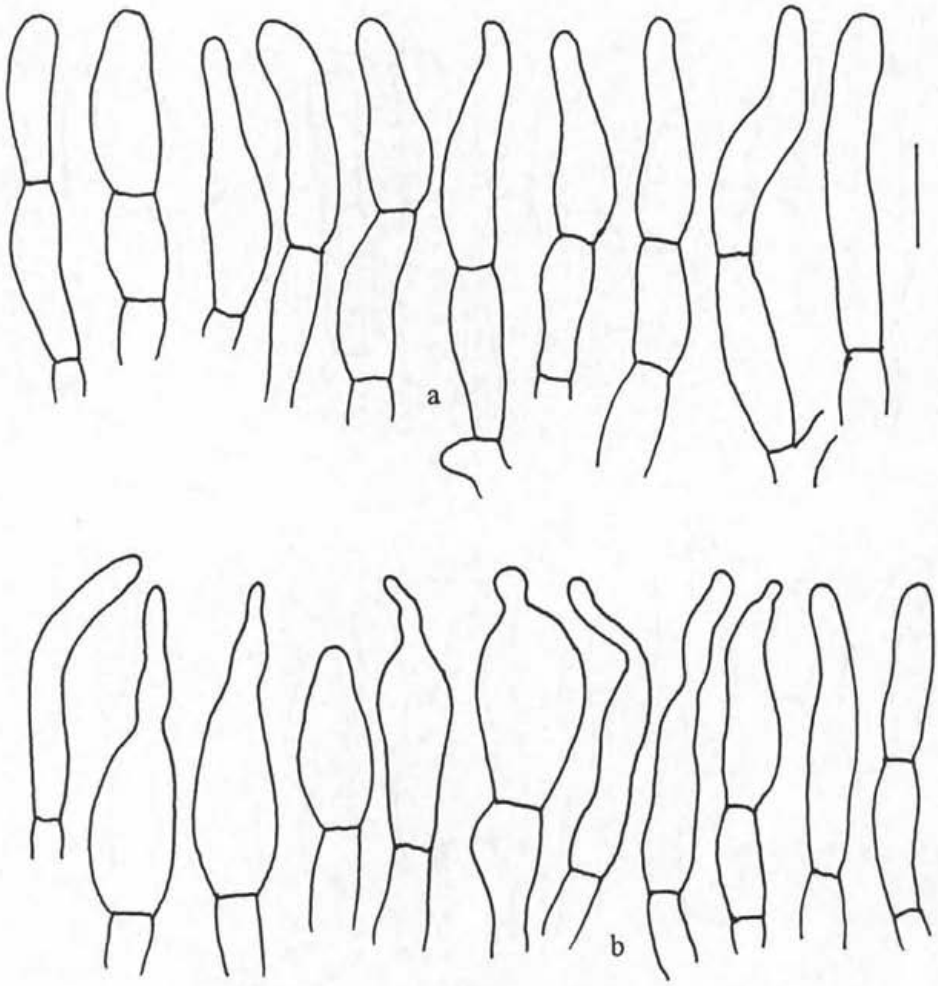

Fig. 1. Terminal cells of generative hyphae of the epicutis at the pileus margin. a. type specimen of *Russula faginea*; b. specimen of *R. faginea* from Slovakia (see Tab. 1). Bar = 10 μm.

RESULTS AND DISCUSSION

Comparison of the selected specimens of *Russula faginea* and related taxa

The microscopical structure of five specimens of *Russula faginea* Romagnesi ex Adamčík and related taxa (Tab. 1) was compared. All five specimens possess a character that differs from the other taxa of *Russula* sect. *Xerampelinae* (Singer) Jul. Schaeff. that grow under deciduous trees: the terminal cells of the generative

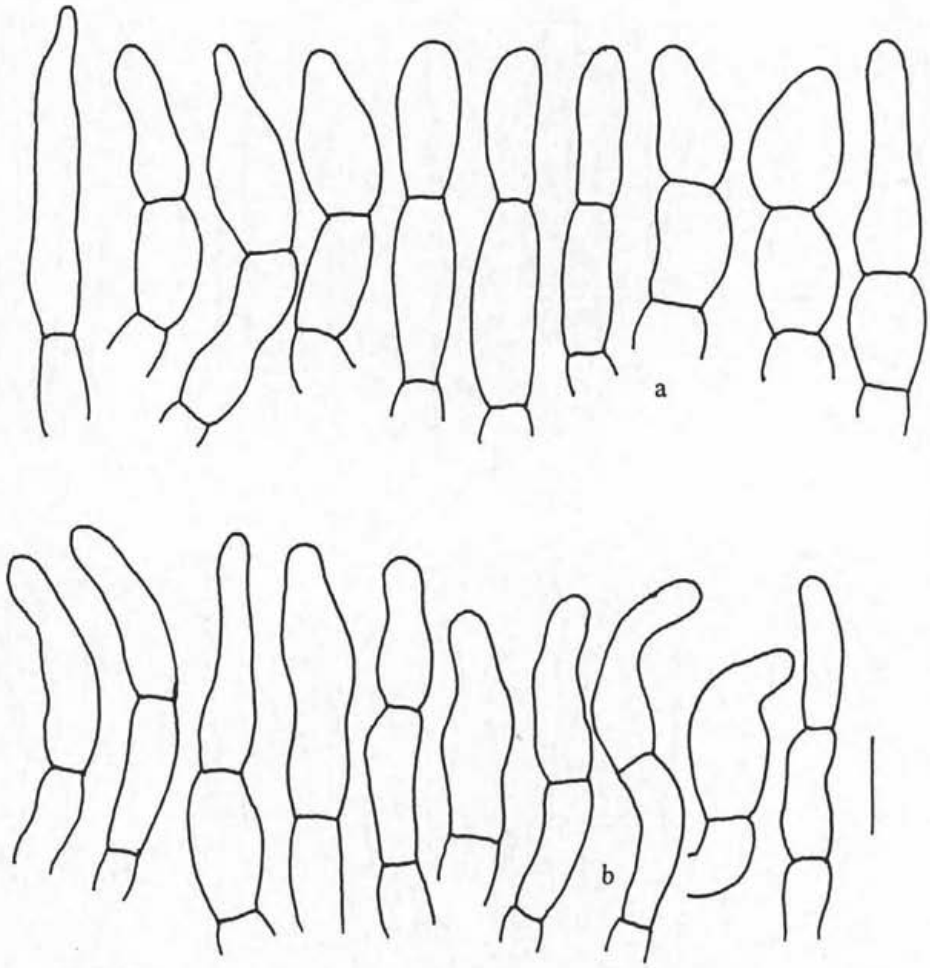

Fig. 2. Terminal cells of generative hyphae of the epicutis at the pileus margin. a. specimen of *Russula xerampelina* var. *barlae* identified by Jul. Schaeffer (see Tab. 1); b. specimen of *R. abietum* identified by Bon (see Tab. 1). Bar = 10 μm.

hyphae of the epicutis on the margin and also in the centre of pileus are often inflated (width 6 μm or more). Only *R. cicatricata* Romagnesi ex Bon has a similar structure of the pileus epicutis but it differs from the examined specimens in spore ornamentation and colour of the pileus (see Adamčík 2002).

Although some of the generative hyphae of the pileus epicutis of all five specimens possess inflated terminal cells (width 6 μm or more), the shape and size of these inflated cells vary among the specimens. The specimens of *R. abietum* identified by Bon and *R. xerampelina* var. *barlae* identified by Jul. Schaeffer have

Tab. 2. Comparison of characters observed on the spores of the selected specimens. 1. – type specimen of *Russula faginea*, 2. – specimen of *R. faginea* with appendiculate terminal cells of the generative hyphae of the epicutis on the pileus margin, 3. – type specimen of *R. barlae* var. *pseudomelliolens*, 4. – specimen of *R. barlae* identified by Jul. Schaeffer, 5. – specimen of *R. cicatricata* identified by Bon. For complete information about the specimens see Tab. 1. The number of spines and line connections were observed in a circle of 3 μm diameter on the surface of the spores. The mean values are calculated as the average of 30 measurements.

		Length of spores [μm]	Width of spores [μm]	Q	Length of spines [μm]	Number of spines	Line connections
	min – 10%	8.2–8.3	6.7–7.3	1.14	1	4.5	0
1. *R. faginea*	mean	**9**	**7.5**	**1.2**	**1.08**	**5.5**	**0.75**
	90% – max	9.5–9.8	8.1–8.3	1.25–1.3	1.2	6.5	2
	min – 10%	7–7.3	5.9–6.2	1.04–1.11	0.8–0.9	3–3.5	0
2. *R. faginea*	mean	**8.2**	**7**	**1.18**	**1.04**	**5.1**	**0.4**
	90% – max	9.4–9.6	7.5–8.1	1.28–1.32	1.2	6	1.5
	min – 10%	7.4–7.7	6.4–6.6	1.15–1.17	1–1.1	2.5	0
3. *R. pseudomelliolens*	mean	**8.7**	**7.1**	**1.23**	**1.18**	**4.1**	**1.88**
	90% – max	9.6–10.1	7.7–7.9	1.29–1.3	1.2–1.6	6	4
	min – 10%	8.3–8.9	6.6–7.4	1.1–1.11	0.8–1	4	1
4. *R. barlae*	mean	**9.6**	**7.8**	**1.24**	**1.18**	**5**	**2.9**
	90% – max	10.3–10.6	8.3–8.4	1.36–1.44	1.3–1.4	6.5–7.5	5–6
	min – 10%	8.3–8.5	7–7.3	1.06–1.11	0.6–0.7	5.5	2–2.5
5. *R. abietum*	mean	**9.7**	**8.1**	**1.2**	**0.9**	**6.9**	**4.6**
	90% – max	10.6–11.1	8.9–9.3	1.3–1.34	1.1–1.2	8–10.5	8–9.5

often very inflated (8 μm or more) terminal cells of the generative hyphae of the epicutis in the pileus centre (Fig. 4). I have found not one such inflated cell in the epicutis of the pileus centre in the other three specimens (Fig. 3). The specimens of *R. abietum* and *R. xerampelina* var. *barlae* are also similar in other characters: the terminal cells of the generative hyphae of the epicutis at the pileus margin are mostly short, pyriform, cylindrical or ampullaceous, they are rarely 3 μm thick or narrower on their tops (Fig. 2), the spores are large (on average 9.6 × 7.8 and 9.7 × 8.1 μm) and the spines are frequently connected with line connections (see Tab. 2 and Fig. 5 d, e). The other three specimens have smaller spores with occasional line connections (Tab. 2, Fig. 5 a, b, c) and the two specimens of *R. faginea* have very often terminal cells of the epicutis at the pileus margin with constricted (3 μm or narrower) tops (Fig. 1). The hyphae of the epicutis on the pileus of the type of *R. barlae* var. *pseudomelliolens* were damaged. It is thus possible to define two distinct groups: the first is composed of specimens of *R. abietum* identified by Bon and *R. xerampelina* var. *barlae* identified by

Jul. Schaeffer and the second is composed of the type specimens of *R. faginea* and *R. barlae* var. *pseudomelliolens* and the specimen of *R. faginea* collected in Slovakia. I suppose that these two groups represent two separate species.

There is also variability within both groups. The specimen of *R. abietum* differs from the specimen of *R. xerampelina* var. *barlae* by its shorter spines on the spores which are strongly connected with line connections. The specimen of *R. faginea* from Slovakia differs from the type by its appendiculate or strongly constricted and more inflated terminal cells of the generative hyphae of the epicutis at the pileus margin and its smaller spores. There are also some differences between the specimen of *R. barlae* var. *pseudomelliolens* and the type of *R. faginea*: the spines of the spores are more connected by line connections and possibly there are some differences in the structure of the epicutis at the pileus margin (the hyphae of the epicutis at the pileus margin of the type specimen of *R. barlae* var. *pseudomelliolens* are damaged). It is necessary to compare more specimens to specify the variability within the two proposed species, but for the moment I am considering this variability as infraspecific.

The pileocystidia of all five compared specimens are rather sparse in occurrence and I did not find any differences between them. Therefore they are not discussed here.

Unfortunately, the macroscopical characters of all five specimens could not be compared, because of the possibility that the description of the specimens was based on collections of both proposed species. Based on the descriptions of the taxa in this group by different authors presented in the studied literature it can be said though, that almost all macroscopical characters are similar. However, I have found a difference in the descriptions of *R. faginea* and *R. barlae* by Bon (1988a) and Einhellinger (1987). Their delimitation is mainly based on the orange colour of the pileus cuticle and the cream to ochre spore print (about IIc according to the scale of Romagnesi 1967) of *R. barlae*. Other authors indicate a large variation in the colour of the spore print of *R. faginea* (for example IIIb-IVa in Romagnesi 1967). Therefore I believe that the two proposed species differ also in spore print colour.

Nomenclature of *Russula faginea*

The name *Russula faginea* Romagnesi, nom. inval. was introduced by Romagnesi (1962) together with other names and the Latin diagnoses were followed by the indication of types: "Typi depositi sunt in Herbario Romagnesi". According to Art. 37.3 of the Code (Greuter et al. 2000), this is not an acceptable indication of a type, because a single element (a specimen) must be designated. Accordingly, *R. faginea* was not validly published by Romagnesi in 1962 (following Art. 37.1 of the Code).

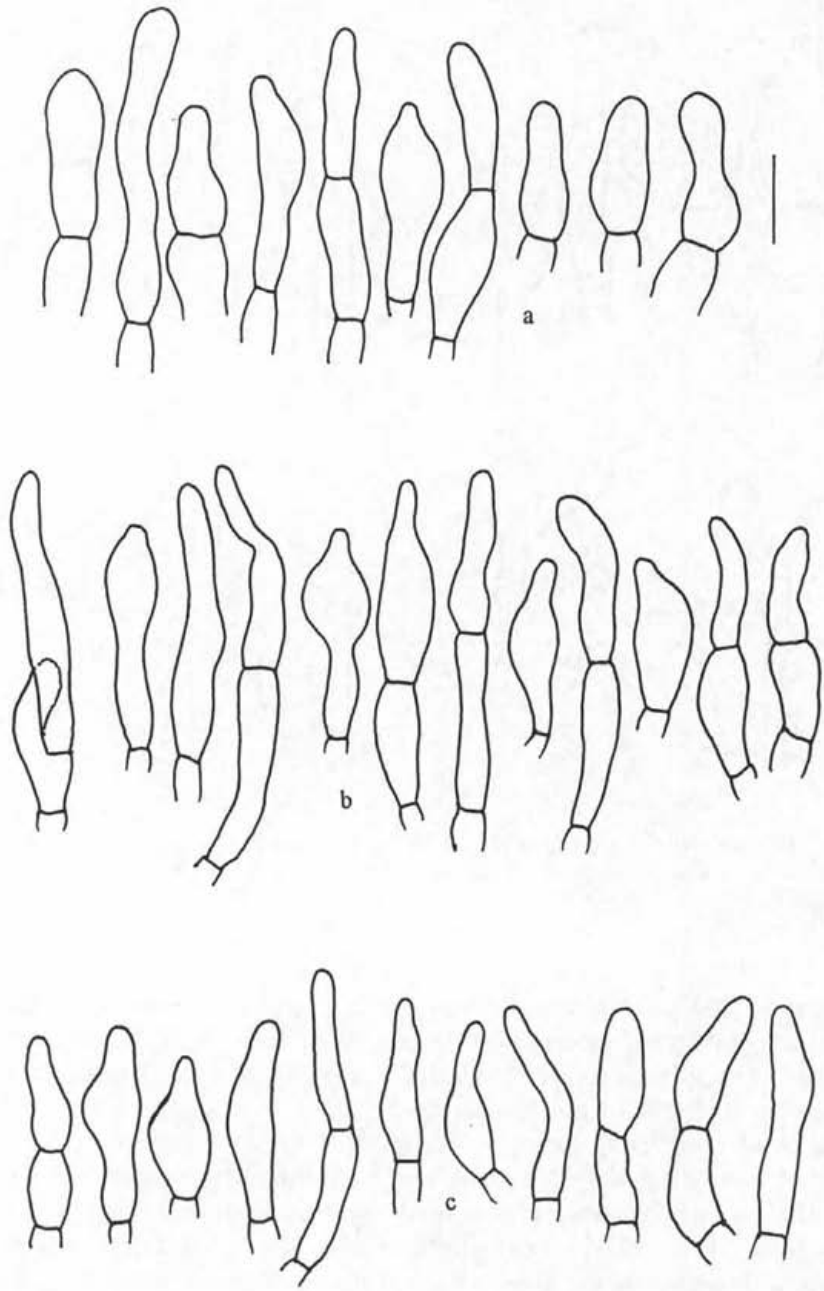

Fig. 3. Terminal cells of generative hyphae of the epicutis in the pileus centre. a. type specimen of *Russula faginea*; b. specimen of *R. faginea* from Slovakia (see Tab. 1); c. type specimen of *R. barlae* var. *pseudomelliolens*. Bar = 10 μm.

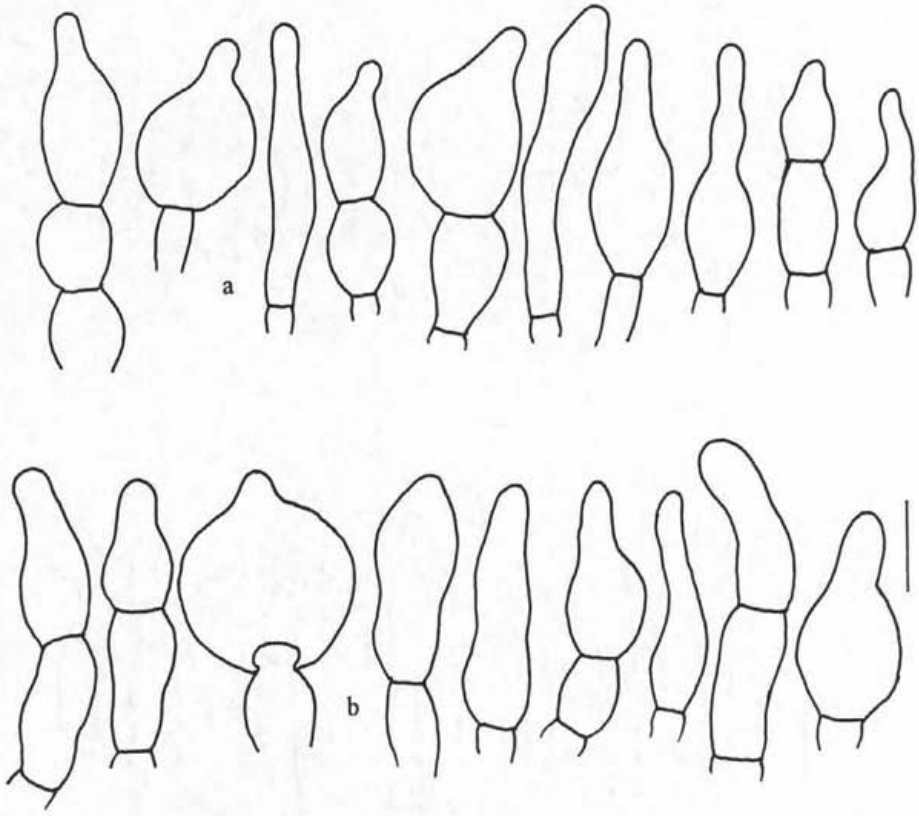

Fig. 4. Terminal cells of generative hyphae of the epicutis in the pileus centre. a. specimen of *Russula xerampelina* var. *barlae* identified by Jul. Schaeffer (see Tab. 1); b. specimen of *R. abietum* identified by Bon (see Tab. 1). Bar = 10 μm.

Kärcher (2000) noticed the invalid publication of the name *R. faginea* by Romagnesi (1962), but he considered Romagnesi's monograph (Romagnesi 1967) the place of valid publication. Romagnesi (1967) added a designation of a type ("Type: no 53–201") to the French description of the species, but it was not accompanied by a Latin description or followed by a reference to a previously published Latin diagnosis (Romagnesi 1962). Kärcher (2000) regarded a mention in the chapter "Références synonymiques des espèces européenes à ce jour décrites" (Romagnesi 1967, p. 926–983) as sufficient reference to the Latin diagnosis for the purpose of valid publication. However, the reference in the chapter is not complete (it refers to another chapter with complete literature citations) and is indirect. Thus, *R. faginea* is not validly published even in the monograph (Romagnesi 1967), because a full and direct reference is required (see Art. 32.3 of the Code).

Conspecifity with some validly published taxa discussed below is possible and therefore a valid publication of the name *R. faginea* seems to be redundant. In spite of that, I am introducing here a valid publication for the name for two reasons: the name is well known and currently used by several authors, and the delimitation and variability of the taxon is still uncertain. The type is designated according to the indication of Romagnesi (1967):

Russula faginea Romagnesi ex Adamčík Fig. 1a, Fig. 5a.

Holotypus (here designated): [France] Forêt de Compiègne (Oise), hêtraie, 29. VIII. 1953, Romagnesi (PC, coll. Romagnesi 53–201).

= ? *Russula barlae* var. *pseudomelliolens* Singer ex Bon, Doc. Mycol. 18 (72): 64, 1988. Holotypus: "Taillis charmes, Desvres (La Poterie), P. de C., 21.9.1967, Bon" [herb. Bon (Lille), 70921]. – *Russula xerampelina* var. *pseudomelliolens* Singer, Ann. Mycol. 34: 424, 1936 [nom. inval.]. – *Russula faginea* Romagnesi, Bull. Soc. Linn. Lyon 31: 176, 1962. [nom. inval.].

Original diagnosis by Romagnesi (1962: 176):

Haec est xerampelina fagetorum. Pileo 6–14 cm, convexo, latius, sed parum alte in senectute depresso, margine obtusa, levi, vel vetustate brevissime sulcata (2–3 mm), sordide rubido, e roseo brunneo, cacaino, medio saepe citrino vel e viridi flavo tincto; cute adnata, mox in ambitu sicca, impolita. Stipite 4–11,5 × 1,5–3,2 cm, duro, pleno, paulum in senectute medullato, albo, flavescente. Carne crassa, alba, flavescente. Odore et notis chemicis typi. Sapore dulci. Lamellis primum satis stipatis, deinde maxime distantibus, crassis, parum furcatis, rotundatis liberis, obtusis, 5–15 mm latis, ex eburneis cremeis, dein clare ochraceis. Sporis in pulvere coloratissimis, clare flavis, 8,5–9,2 × 7,5–8,2 µ, echinulatis, spinis ± longis, parum densis. Basides 42–57 × 9–13,5 µ. Cystidiis 80–120 × 8–13 µ. Cute ex pilis polymorphis, modo obtusis, modo superne attenuatis constante; dermatocystidiis raris, parum manifestis, angustis, 3,2–4,2 µ latis. – In fagetis.

Aditional description of micromorphological characters of the type specimen[1]:

Spores (8.2–)8.3–9.5(–9.8) × (6.7–)7.3–8.1(–8.3) µm, Q = 1.14–1.25(–1.3), broadly ellipsoid. Spines 1–1.2 µm long, sparse (4.5–6.5 in a circle of 3 µm diameter on the spore surface[2]), occasionally connected by line connections (0–2 line connections per circle) and sometimes also merging (0–5 contact connections

[1] I do not present a description of my collection of *R. faginea* from Slovakia, because it is not sure, that it is the same taxon as the type specimen of *R. faginea*. On contrary I am adding the description of microscopic characters of the type specimen of *R. faginea*, because it includes many modified or deviating characters.

[2] For a detailed description of the observed characters see Adamčík and Marhold (2000).

in the circle). Amyloid punctations rather sparse (0–2.5(–5) in the circle). Basidia 46–59 × 11.5–15 μm, clavate. Pleurocystidia 11.5–13.5 μm wide, fusiform to narrowly clavate, with a 2–7 μm long appendage. Terminal cells of generative hyphae of the epicutis at pileus margin 16.5–34 × 4.5–7 μm, often very short (length doesn't exceed 20 μm), pyriform, ampullaceous, subulate or rarely cylindrical, often constricted on their terminal part (3 μm thick or narrower). Terminal cells in centre of pileus epicutis 15–36 × 4–7(–8) μm, often short (length not exceeding 20 μm), ampullaceous, lanceolate, pyriform, cylindrical or rarely also clavate. Pileocystidia very sparse and obscure, their terminal cells up to 9 μm wide, rather short and fusiform.

Taxa similar to *Russula faginea*

Russula barlae

Before *Russula faginea* Romagnesi, nom. inval. (Romagnesi 1962) was described for the first time, several authors had identified it (or related taxa) as *R. xerampelina* var. *barlae* (Quél.) Melzer et Zvára (or *R. barlae* Quél.). For example, specimens of *R. xerampelina* var. *barlae* identified by Singer (collected in 1933) and Schaeffer (collected in 1934) are similar to the type of *R. faginea* according to my observations (the original concept of *R. barlae* and concepts presented by later authors are discussed in Adamčík 2002).

Some later authors distinguished *R. barlae* from *R. faginea* as a separate species. For example, Romagnesi (1967) used this name for a taxon with affinity to *R. cicatricata* Romagnesi ex Bon; Bon (1988a) and Einhellinger (1987) treated it as a species related to *R. faginea* associated with *Quercus* trees with orange tinges on pileus cuticle and a cream spore print.

I am not venturing to estimate the original concept of *R. barlae*, and taking into account the brief original diagnosis and different concepts of later authors, I consider this name to be a nomen dubium. However, the discussion about it will be still legitimate and therefore I think the only solution to this problem is to reject the name following Art. 56.1 of the Code.

Russula abietum

R. abietum (J. Blum) Bon is a taxon similar to *R. faginea*. Blum (1953) described it as variety of *R. xerampelina* (just like other taxa of the section) with robust fruitbodies, brownish-red colour of the pileus cuticle discolouring to ochre, becoming variegated and firm flesh without distinct smell (for original diagnosis see Tab. 3). He accepted *R. xerampelina* var. *barlae* (= *R. faginea* in his sense) and he distinguished it by its darker yellow spore sprint, the spores with lower warts and frequent crest and occurrence under *Abies* trees. He also mentioned, that the combination of these characters is different also in *"pseudomelliolens"*.

He did not choose any specimen as a type and also Bon (1983), who made the combination at the rank of species, did not typify it. Blum (1953) indicated "Sous sapins, région d'Arreau (Htes-Pyr.)" as the place of collection, and all specimens are probably deposited in herbarium G. As I did not search for original material of *R. xerampelina* var. *abietum* collected or identified by Blum, I can not express my opinion about its affinity to *R. faginea*.

Tab. 3. Original diagnosis of *Russula xerampelina* var. *abietum* Blum (1953)

"A typo differt sporis obscurioribus (flavis) et odore obsoleto."

I have studied a collection of *R. abietum* identified by Bon and collected under *Picea* and *Fagus*. This specimen differs from the type specimen of *R. faginea* in the microscopical structure of both spores and pileus epicutis (see comparison of the specimens above). Even if one considers the microscopical features of *R. faginea* and *R. abietum* to be the same, the original description states some differences between the two species: the different habitat (under *Abies* vs. *Picea* and *Fagus*?) and the yellow spore print (IVb-IVc according to the code of Romagnesi 1967). Although it is not possible anymore to check these two characters, I think *R. abietum* is the correct name for the species that differs from *R. faginea*.

Russula barlae var. *pseudomelliolens*

Another taxon with affinity to *R. faginea* is *R. barlae* var. *pseudomelliolens* Singer ex Bon. The epithet "*pseudomelliolens*" was introduced by Singer (1936). He chose the name *R. xerampelina* var. *pseudomelliolens* Singer nom. inval. for a taxon which macroscopically matches *R. melliolens* in the sense of Crawshay (1930). The name was not accompanied by a Latin diagnosis, so it was invalidly published (according to Art. 36.1 of the Code).

It was validated at the rank of variety of *R. barlae* by Bon (1988b), who designated his collection as the type. He distinguishes it from the type variety by the following characters: the fruitbodies are small to medium sized, the colour of pileus cuticle is coppery-red, the spines of the spores are larger and the hyphae of the pileus epicutis are slender (for original diagnosis see Tab. 4). The only microscopical difference I found between the type specimen of *R. barlae* var. *pseudomelliolens* and *R. faginea* is the higher frequency of the line connections between the spines on the spores in *R. barlae* var. *pseudomelliolens* (the hyphae of the epicutis at the pileus margin were damaged and therefore not compared). These two taxa seem to be conspecific and differ only in size of fruitbodies and habitat (*R. barlae* var. *pseudomelliolens* has distinctly smaller fruitbodies and

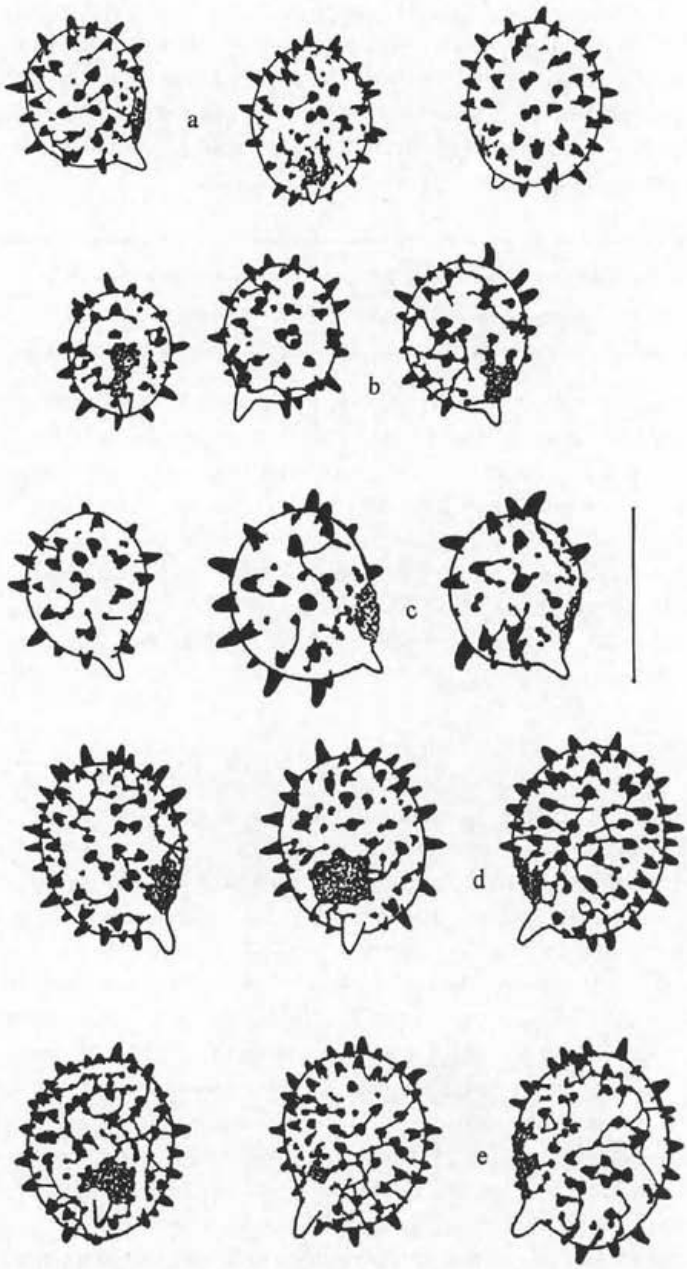

Fig. 5. Spores. a. type specimen of *Russula faginea*; b. specimen of *R. faginea* from Slovakia (see Tab. 1); c. type specimen of *R. barlae* var. *pseudomelliolens*; d. specimen of *Russula xerampelina* var. *barlae* identified by Jul. Schaeffer (see Tab. 1); e. specimen of *R. abietum* identified by Bon (see Tab. 1). Bar = 10 μm.

grows under *Carpinus*). Nevertheless, I am leaving this problem undetermined because I think, that more observations are needed for a conclusion.

Tab. 4. Original diagnosis of *Russula barlae* var. *pseudomelliolens* Singer ex Bon (1988b)

"A typo differt pileo 5-(7) cm, coloribus cupreo-roseis (R. melliolentis instar). Micro sicut in typo sed sporis spinis majoribus usque 1(1,5) μm, haud ampulaceis. In silvis frondosis ± humidis. Holotypus in herbario M. B. (Lille) no 70921, prope Desvres (Pas-de-Calais) lectus."

Reumaux (in Reumaux et al. 1996) has also validated the name *R. pseudomelliolens* Singer ex Reumaux at the rank of species. He did not designate one of Singer's or Bon's specimens as the type, but he chose his own collection for this purpose. I did not study the type of this species, so I can only compare its original diagnosis with *R. faginea*. Reumaux observed only non-inflated hyphal terminations in the pileus epicutis with a width of 3–4 μm, so I not expect, that this species does fit in the range of *R. faginea*.

Russula duportii

Kärcher (2000) has neotypified *R. duportii* W. Phillips (validly published in Phillips and Plowright 1884) with a specimen of *R. xerampelina* var. *duportii* identified by Blum. The figure (drawn by Kärcher) shows numerous inflated terminal cells in the pileus epicutis and in the text Kärcher confirmed that it is similar to *R. faginea*. According to him *R. duportii* has sparser inflated terminal cells in the pileus epicutis, and wider and longer pileocystidia. I do not consider this character of *R. duportii* reliable for delimiting the taxon, because it can vary among fruitbodies of one collection (according to my observations). As I did not study the type specimen of *R. duportii*, I am not able to confirm if these species are conspecific or just similar in some characters (for more details about concepts of *R. duportii* presented by various authors, see Adamčík 2002).

It may seem that the taxa described above cover the complete variability of the *R. faginea* complex, but my collection from Slovakia proves the opposite. In a *Fagus* forest I found fruitbodies which were macroscopically similar to the original description of *R. faginea* by Romagnesi. They differ from the type specimen of the species by their smaller spores and the terminal cells of generative hyphae of the epicutis on the pileus margin with narrowed to appendiculate tops (see the comparison of the specimens above).

Expected taxonomical structure of the studied material

My observations on five selected specimens of *Russula faginea* Romagnesi ex Adamčík and related taxa indicate that the studied material consists of two species.

The type specimen of *R. faginea* and similar specimens have less inflated terminal cells of generative hyphae of the epicutis at the pileus margin and smaller spores with occasional line connections. I prefer not to use the name *R. barlae* Quél. for any taxon of this group, because the original concept of the species is uncertain and is interpreted in various ways by different authors, the only valid names for the taxa of this group are *R. abietum* (J. Blum) Bon, *R. faginea* and *R. barlae* var. *pseudomelliolens* Singer ex Bon. Neither the name *R. pseudomelliolens* is available for taxa of the *R. faginea* group, because Reumaux (in Reumaux et al. 1996) has used it for a taxon related to *R. graveolens* Romell in Britzelm. Thus, only two valid specific names are available for the two species studied here. If observations on the original material of *R. abietum* identified by Blum confirm that it is similar to Bon's concept of the species, the correct name for the species with larger spores will be *R. abietum* and the correct name for the other species with smaller spores *R. faginea*. If the opposite is true, the correct name for the species with the smaller spores will be *R. abietum*, *R. faginea* will be only a synonym.

Unfortunately, the results of my research are not sufficient to delimit the two species and clarify their taxonomy. I just want to draw attention to some problems here. To solve all these problems it is necessary to study more material and to observe the original material of *R. abietum* identified by Blum as well as the type specimen of *R. duportii*.

AKNOWLEDGEMENTS

I wish to thank Nick Legon and Reinhold Kärcher for their nomenclatural notes on *R. duportii*, Marcel Bon and the curators of herbaria K and PC for lending out herbarium material, Jan Holec for help with literature and Jorinde Nuytick for language corrections and other valuable notes. This research was supported by VEGA grant no. 1069.

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Mycorrhizal revival: case study from the Giant Mts., Czech Republic

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Fellner R. and Landa J. (2003): Mycorrhizal revival: case study from the Giant Mts., Czech Republic – Czech Mycol. 54: 193–203

The remarkable trend of revitalization of mycorrhizal mycocoenoses in the spruce forests of the Giant Mts. (Krkonoše National Park and Biosphere Reserve), Czech Republic, recognized in 1999 (Fellner and Landa 2000), is confirmed from the new collection of data obtained in 2000. The increase in abundance and frequency of mycorrhizae-forming fungi and other macromycetes at the end of the 1990s is found to be positively correlated with the distinctive reduction of sulphur dioxide emissions in the last decade. It supported the experience that fungi are highly sensitive bioindicators of air pollution and reflect the deterioration in stability of their host forest (Fellner and Pešková 1995). Analysis of data indicates that the present distribution of mycorrhizal macromycetes in spruce forests in the Giant Mts. could be even greater than it was around 1960 (cf. Nespiak 1971).

Key words: Mycorrhizae-forming fungi, air pollution, bioindicators, spruce forest, Czech Republic

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Pozoruhodný trend revitalizace mykorrhizních mykocenóz zjištěný v krkonošských smrčinách v roce 1999 (Fellner and Landa 2000) je potvrzen na základě nových sběrů z roku 2000. Byla zjištěna pozitivní korelace mezi nárůstem abundance a frekvence mykorrhizních hub i dalších makromycetů koncem devadesátých let a výrazným snížením emisí SO_2 v posledním desítiletí. To potvrdilo zkušenost, že houby jsou vysoce citlivými bioindikátory vzdušného znečištění a narušení stability jejich hostitelských lesních porostů (Fellner and Pešková 1995). Předložená analýza naznačuje, že současné zastoupení mykorrhizních makromycetů v krkonošských smrčinách by mohlo být dokonce vyšší než kolem roku 1960 (cf. Nespiak 1971).

INTRODUCTION

Mycorrhizal fungi are one of the highly sensitive bioindicators of air pollution. An assessment of their presence, abundance and frequency in permanent plots as a part of long-term ecological research (LTER) enables a quite precise diagnosis of deterioration levels of their host forest stands (Schlechte 1986, 1991; Jansen and Dobben 1987; Fellner 1987, 1988; Jansen 1991; Fellner and Pešková 1995; Fellner et al. 1999). It concerns mainly ectomycorrhizae-forming fungi (EMF) because of the essential importance of their symbiotic relationships with many

host forest trees resulting in an improved or more effective uptake of nutrients in the forest (Moser 1967, Singer 1963, Malloch et al. 1980 etc.). Moreover, some species and/or genera of EMF are highly sensitive to many pollutants (Fellner 1989, Fellner and Soukup 1991, Arnolds 1991). Their biodiversity and proportion in relation to other trophic groups of fungi (i.e. to saprophytes and parasites) is substantially reduced even in the case of relatively low levels of air pollution in their host forest stands. It is for this reason why they can be used for bioindication of beginning of the deterioration of the host forest stability even in the situation that there is yet no visually recognizable damage (e.g. defoliation). The recognized level (degree) of the disturbance of ectotrophic forest stability, realized through effective ectomycorrhizal associations, can serve also as a predictive tool, including an assessment of melioration or lime application (Fellner 1993).

METHODS

On the basis of LTER in the Giant Mts. and in some other areas, a model of forest deterioration as a consequence of air pollution was proposed (Fellner 1985, Fellner and Pešková 1995). According to the model the deterioration of the ectotrophic symbiosis as a consequence of air pollution has generally three stages:

(a) latent disturbance of the ectotrophic forest stability, if the percentage of species of ectomycorrhizal fungi in the total count of macromycetes decreases to 40 %, while the percentage of lignicolous species is tending to reach more than 30 %;
(b) acute disturbance of the ectotrophic forest stability, if the ectomycorrhizal species contribute constantly less than 40 % of the total number of macromycetes, while lignicolous species, as a rule, contribute more than 40 %;
(c) lethal disturbance of the ectotrophic forest stability, if the ectomycorrhizal species contribute constantly less than 20 % of the total number of macromycetes, while lignicolous species, as a rule, contribute more than 55 % of all macromycetes.

The three stages are linked directly to particular phases of impoverishment of ectomycorrhizal mycocoenoses and of enrichment of lignicolous mycocoenoses. The latent disturbance is, on the one hand, linked to the inhibition of sporocarp production (accompanied by a decline of highly sensitive species), and on the other hand, to the stimulation of sporocarp production of wood destroying fungi. The acute disturbance is linked, on the one hand, to the reduction of species diversity of ectomycorrhizal fungi (with a continuous inhibition of sporocarp production), and on the other hand, to the increase of species diversity of wood destroying fungi (with a continuous stimulation of sporocarp production). The

lethal disturbance is linked, on the one hand, to the partial or to total destruction of mycorrhizal mycocoenoses, and on the other hand, to the expansion of lignicolous mycocoenoses.

The model of stages of forest deterioration as a consequence of air pollution was shown to be largely applicable in various types of forests not only in the Czech Republic, but also in other regions (Arnolds 1991, Gulden et al. 1992, Pavlík 1999, Ogawa 2000).

To study the decline of ectomycorrhizal fungi and/or mycorrhizae resulting from air pollution, permanent plots were established; in Central Europe their size mostly varied from 1000 to 3000 m^2 (Fellner 1985, 1993; Schlechte 1986, 1991; Jansen 1991; Fellner and Pešková 1995). It was considered that 2500(–3000) m^2 would be a sufficient and suitable size for a minimum area of macromycetes in Central European temperate forest ecosystems (Fellner 1985, 1987; Schlechte 1991). In the case of smaller plots (e.g. 500 m^2 or 1000 m^2) it was recommended that they be reduplicated or clustered into one cumulative minimum area in each locality (Fellner 1993).

For the purpose of carrying out LTER in the Czech Republic (inclusive of the Giant Mts.) the size of permanent plots for mycological monitoring was generally 2500 m^2. Each plot was further subdivided into 25 partial plots being of 100 m^2 in size. Fruit-bodies of all macromycetes were collected during the fructification period at three– to four-week intervals. The data concerning the species diversity of fungi, their abundance in the whole plot and the frequency of findings on 25 partial plots were registered. At yearly intervals the changing ratio of the number of mycorrhizal species of macromycetes to the number of species of other trophic groups was monitored to compare the collected data, to evaluate the stages of the deterioration of ectotrophic forest stability and to prepare a prediction.

RESULTS

In 1999 and in 2000 the situation was checked and evaluated in some permanent plots in the Giant Mts. after a 3-year hiatus. Some surprising results were obtained: many mycorrhizal species fructified much more abundantly in 1999 and 2000, some of them reappearing after many years, and some of them were even unknown from these stands in recent decades. The trend of revitalization of mycorrhizal mycocoenoses can be demonstrated in all spruce plots studied in the Giant Mts. in 1999 and 2000 (for maps of the plots see Fellner et al. 1991). For example the percentage of mycorrhizal species increased by 20–30 % in all plots exposed to air pollution near the locality "Tetřeví boudy" (cluster of plots T-I, T-II, T-III, T-IV), i.e. from 20–35 % in the first half of the 1990's to about 45–55 % in 2000 (Fig. 1) (cf. also Fellner and Landa 2000). It means that they returned from the stage of acute disturbance of the ectotrophic forest stability to the stage of latent disturbance. In

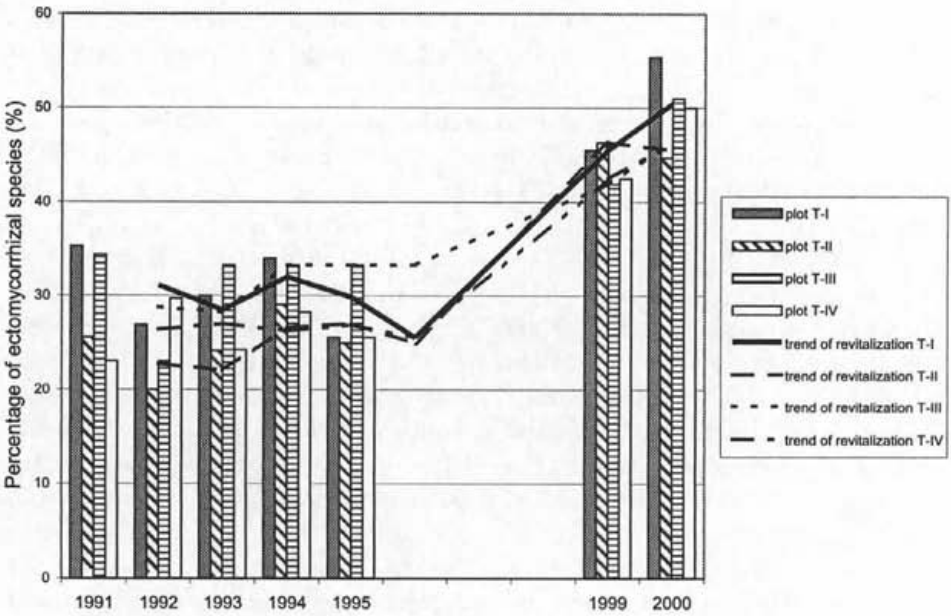

Fig. 1. Ectotrophic forest stability in spruce plots exposed to air pollution (Tetřeví boudy, Giant Mts., Czech Republic)

the plots, which were not directly exposed to air pollution in the bottom of deep valleys, it is not now even possible to recognize the latent disturbance because the situation has improved so much. For example in the bottom of Úpa valley (cluster of plots O-II) the percentage of mycorrhizal species increased as much as 60 % or more, which is equivalent to the standard situation in temperate and boreal forests (Fig. 2).

The reason for the change is recognizable from the diagram showing the reductions in the atmospheric emissions in the Czech Republic in the period 1985–1999 (Horáček 2000), namely of sulphur dioxide, nitrogen oxide and solid substances. In the course of 14 years (1985–1999) sulphur dioxide emissions fell by 88 %, and nitrogen oxide emissions from large sources fell by nearly 79 % from 1989 to 1998. Specifically it means that only after 1995 did the sulphur dioxide emissions fall under one million tons per year and only in 1998 were the emissions of both the most important pollutants under half a million tons per year. A similar trend is recognizable in the whole European context where the sulphur dioxide output fell by about 65 % in the same period (Elvingson 2000). What is important is that both sulphur dioxide and nitrogen oxide depositions are supposed to be two of the most prominent damaging factors for fungi in Europe (Arnolds 1991 etc.).

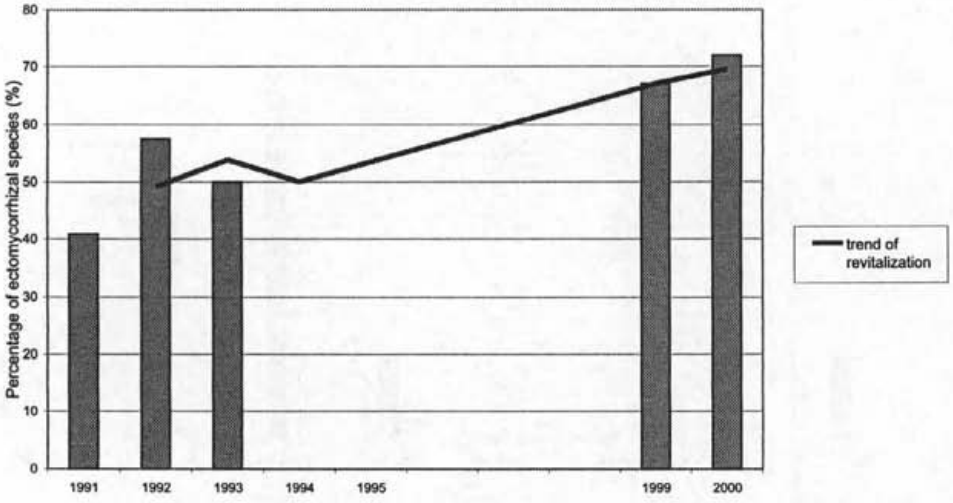

Fig. 2. Ectotrophic forest stability in spruce plots in the bottom of valley (Úpa valley, Giant Mts., Czech Republic)

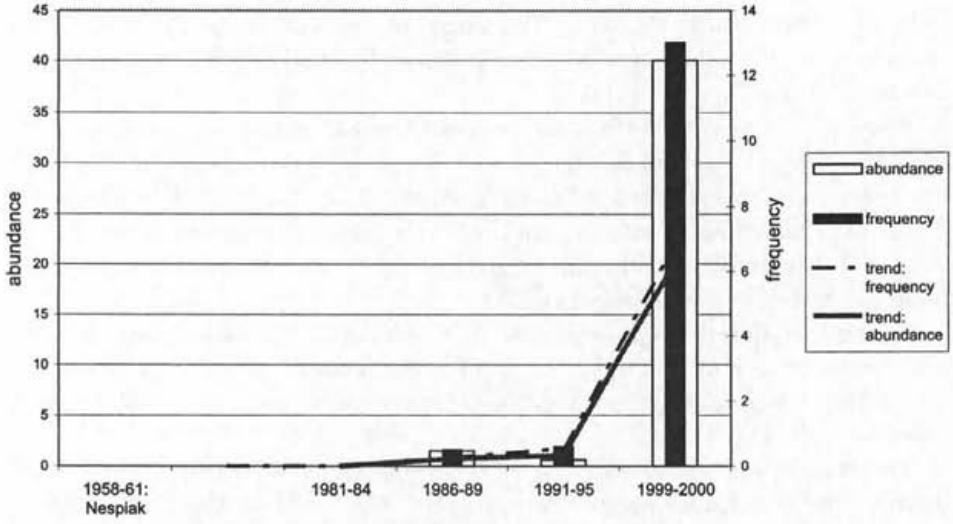

Fig. 3. *Cortinarius anomalus* – abundance and frequency in the permanent plots in spruce forests in the Giant Mts., Czech Republic

By analyzing the long-term distribution of various mycorrhizal fungi in permanent spruce plots in the Giant Mts. we can recognize a very similar pattern of occurrence (both in their abundance and frequency in the plots): generally strongly

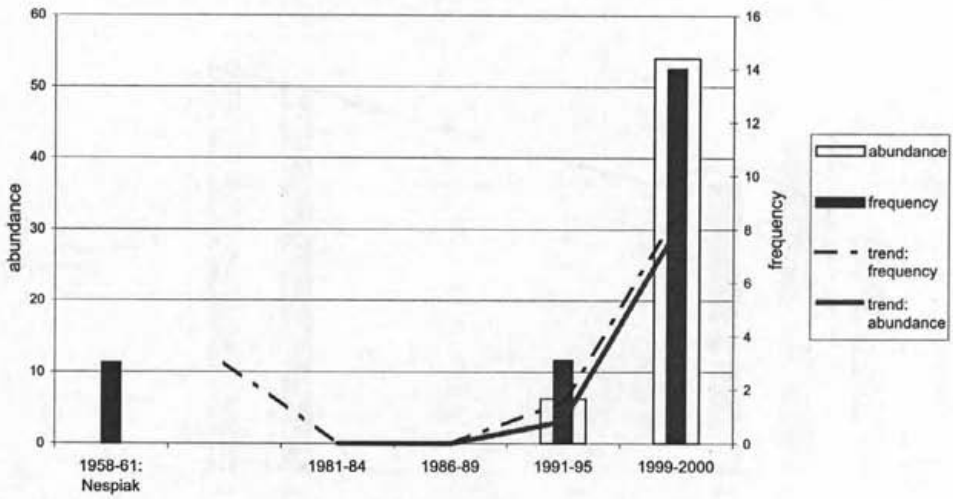

Fig. 4. *Lactarius helvus* – abundance and frequency in the permanent plots in spruce forests in the Giant Mts., Czech Republic

reduced or zero occurrence in the 1980's and the first half of the 1990's and their re-occurrence or multiplied occurrence at the end of the 1990's (e.g. *Cortinarius anomalus*) (Fig. 3).

Some species began to return earlier in the first half of the 1990's in connection with the reduced levels of sulphur dioxide (e.g. *Lactarius helvus*, Fig. 4). Some other species increased their fructification only by the end of the 1990's, which may reflect their higher sensitivity to pollution levels (e.g. *Lactarius lignyotus*, Fig. 5). Increased distribution trends can also be clearly recognized in some saprophytic fungi (e.g. *Clavulina coralloides*, Fig. 6).

In contradiction to the process described above some wood-decaying (lignicolous) species, which at the beginning of the 1990's were abundant, had by the end of the 1990's significantly decreased (e.g. *Gloeophyllum sepiarium* and *Antrodia heteromorpha*, Fig. 7).

For comparison the data from Nespiak's research (Nespiak 1971) on the distribution of fungi in spruce plots on the Polish side of the Giant Mts. in 1958–1961 have been included in the diagrams. Although his methods of gathering data were not fully compatible with our methods, his data give us an idea about the state of fungus flora in the Giant Mts. at the beginning of the 1960's: generally the mycoflora (both the number of species and their frequency) seemed to be already outstandingly inhibited by the industrial air pollution at that time. It is necessary to note that this was the period when the forests in the Ore Mts. in Northwest Bohemia were already seriously damaged by air pollution.

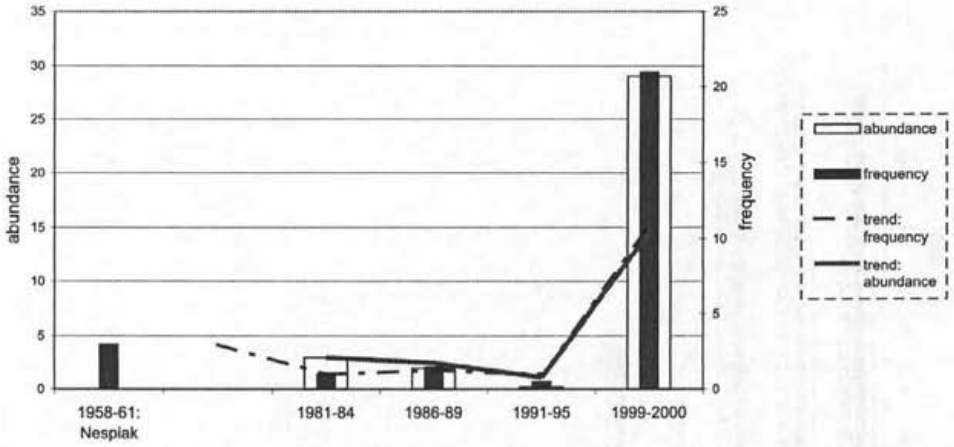

Fig. 5. *Lactarius lignyotus* – abundance and frequency in the permanent plots in spruce forests in the Giant Mts., Czech Republic

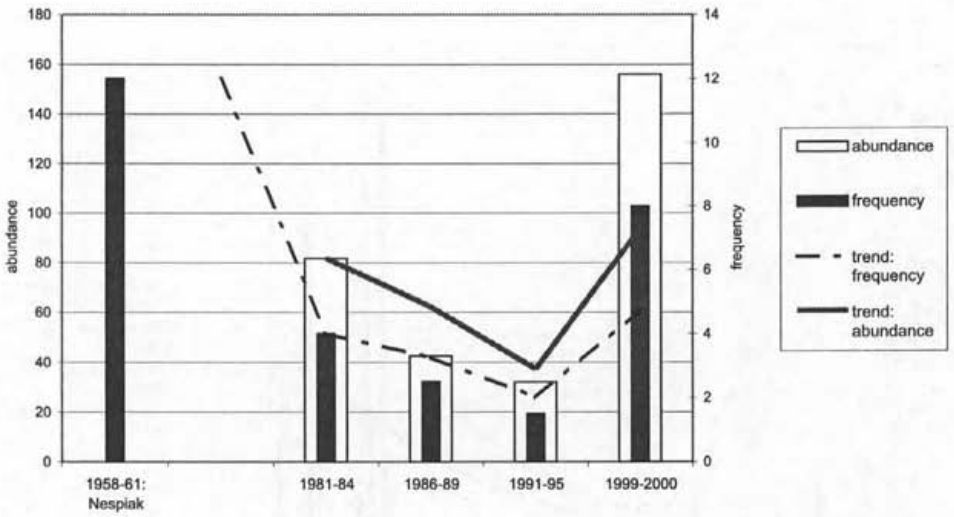

Fig. 6. *Clavulina coralloides* – abundance and frequency in the permanent plots in spruce forests in the Giant Mts., Czech Republic

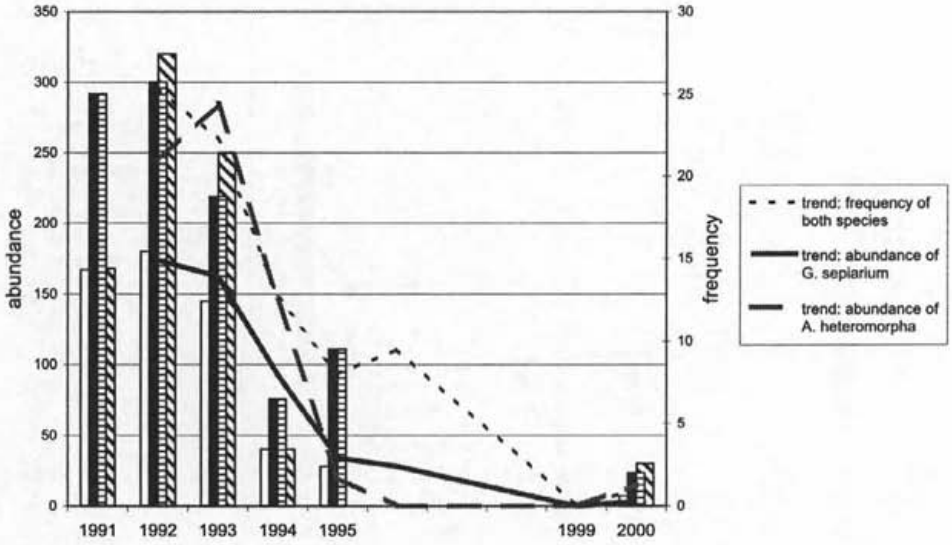

Fig. 7. *Gloeophyllum sepiarium* and *Antrodia heteromorpha* – abundance and frequency in the permanent plots in spruce forests in the Giant Mts., Czech Republic

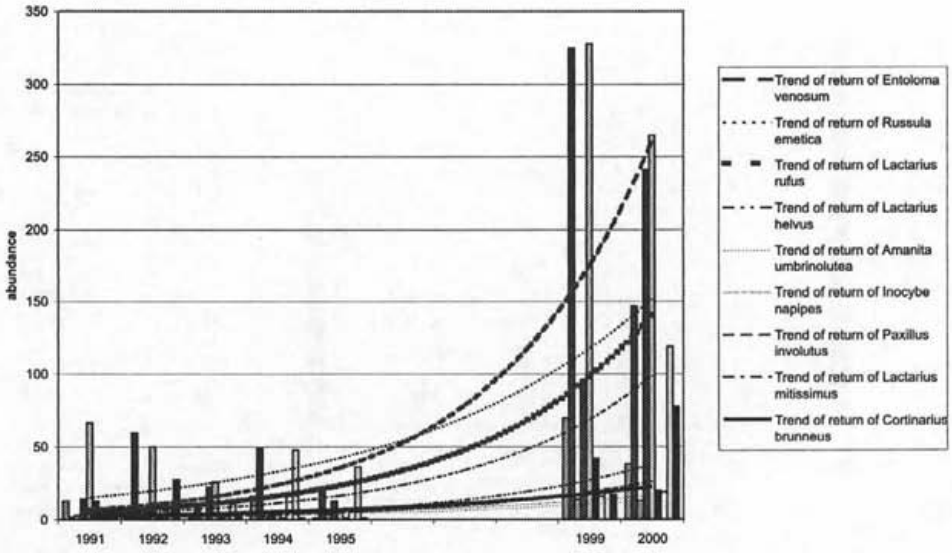

Fig. 8. Examples of mycorrhizal species inhibited in the first half of the 1990's, the Giant Mts., Czech Republic

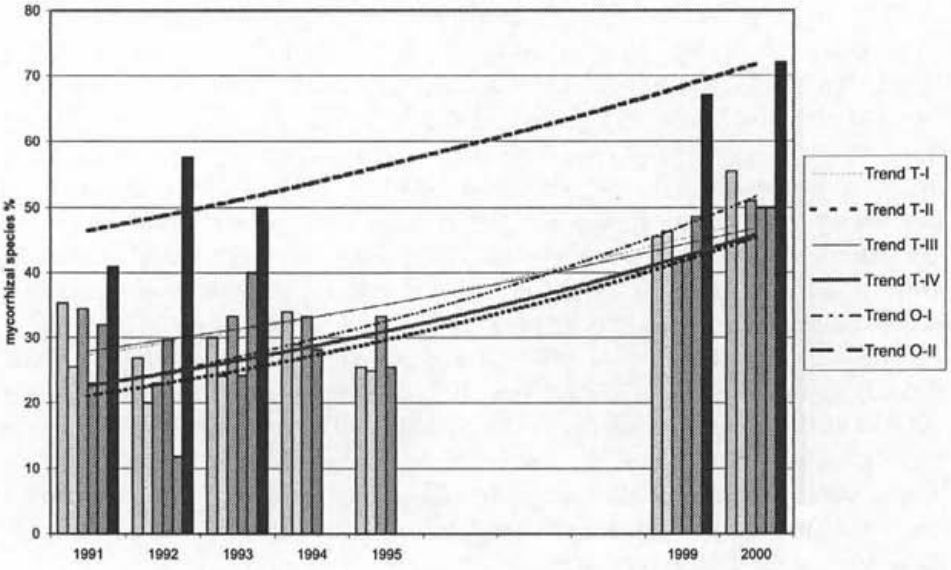

Fig. 9. Percentage of mycorrhizal species in spruce forests of the Giant Mts., Czech Republic

DISCUSSION

By comparing data about fungal biodiversity and their abundance in particular plots in the last two years (1999–2000) with those at the beginning of the 1990's, there are many species of EMF, which were inhibited or almost unknown from the plots before 1995 (Fig. 8). The continuing trend in the increase of the proportion of EMF is illustrated in the cumulative diagram (Fig. 9) both for plots exposed to air pollution (cluster of plots T-I, T-II, T-III, T-IV) and for plots in Úpa valley (cluster of plots O-I, O-II). It is symptomatic that many of these newly occurring species are not even mentioned in Nespiak's research (Nespiak 1971).

To sum up, the positive changes in biodiversity, abundance and frequency of EMF in the last few years are without parallel in the last twenty years, or even in the last half of the century if we also take into account Nespiak's data. If prognoses of a continuing reduction of emissions will be reached (cf. "Analysis of UNECE/EMEP Emission Data, MSC-W Status Report 2000, EMEP, Oslo, 2000"), the mycorrhizal revival will also continue without doubt. A reconstruction of disturbed mycocoenoses is at the same time highly important for the successful reforestation in many parts of the Giant Mts. But the question remains: How many species of EMF in the last half of the century were only inhibited in their fructification (and are able to return) and how many were really completely lost?

By comparing the data from recent decades about fungal occurrence with the data about the damage of trees indicated by the defoliation of spruce in the Giant Mts. (Vacek and Matějka 1999) we can discern that many mycorrhizal fungi (their abundance, frequency), as well as their whole communities-mycocoenoses (percentage of mycorrhizal species or active mycorrhizal tips), are much more dynamic and sensitive to the changes of damaging factors in comparison to tree defoliation. Though in the last period since 1989 some stabilization of the defoliation increase has been observed, the portion of dead trees ("defoliation degree 5") is still very high, mainly at higher elevations. But this improving trend is now convincingly underlined by the fungus bioindication.

The general shift from the stage of acute disturbance of ectotrophic forest stability (quite common before 1995 in all upper parts of the Giant Mts.) to the stage of its latent disturbance and the trend of its yearly continuing improvement – that is a clear indication of a positive global change in mycorrhizal relations in the whole territory of the Giant Mts. As a consequence of this mycorrhizal improvement it is expected that the health of the forest will also improve although there could be a short term delay.

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A revision of the type specimens of new species of Delicatula (Agaricales, Tricholomataceae) described by Josef Velenovský

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Antonín V. (2003): A revision of the type specimens of new species of Delicatula (Agaricales, Tricholomataceae) described by Josef Velenovský. – Czech Mycol. 54: 205–233

The type specimens of 38 new species of the genus *Delicatula* Fayod described by Josef Velenovský were studied. Also the original notes and unpublished pencil drawings from the estate of J. Velenovský were used. All recognised species were taxonomically identical with already known species. As a nomenclatorical result, the following new combination is proposed: *Hemimycena subtilis* (Velen.) Antonín comb. nov. as the older name for a taxon known as *Hemimycena cyphelloides* (P. D. Orton) Maas Geest. (= *Mycena pseudocrispula* Kühner, forme bisporique).

Key words: fungi, Agaricales, Tricholomataceae, *Collybia, Delicatula, Hemimycena, Mycena*, type studies, taxonomy, nomenclature.

Antonín V. (2003): Revize typových položek nových druhů rodu Delicatula (Agaricales, Tricholomataceae) popsaných Josefem Velenovským. – Czech Mycol. 54: 205–233

Bylo studováno 38 typových položek nových druhů rodu *Delicatula* Fayod popsaných Josefem Velenovským. Při jejich identifikaci byly použity rovněž originální popisy a nepublikované tužkové kresby z pozůstalosti J. Velenovského. Všechny taxony, které byly určeny, jsou identické s již dříve publikovanými druhy hub. Nomenklatorickým výsledkem je navržení jedné nové kombinace: *Hemimycena subtilis* (Velen.) Antonín comb. nov. jako starší jméno pro druh známý jako *Hemimycena cyphelloides* (P. D. Orton) Maas Geest. (= *Mycena pseudocrispula* Kühner, forme bisporique).

Josef Velenovský (1858–1949) described 42 new species of *Delicatula* Fayod in his two books "Novitates mycologicae" and "Novitates mycologicae novissimae" (Velenovský 1940, 1947). He did not describe any new *Delicatula* species in his earlier publication "České houby" (1920–1922) and in various papers published in the journal "Mykologia" (1924–1931). Only 38 of them are preserved in the herbarium of the Department of Mycology of the National Museum in Prague (PRM) as herbarium specimens. However, some of them contain no carpophores. Velenovský probably "used" them during his microscopic studies, and let the empty type envelope in his herbarium. Moreover, a lot of specimens contain only

one (often damaged, broken or collapsed) carpophore. In addition, private notes by J. Velenovský to most of the species published in "Novitates Mycologicae Novissimae", including unpublished very illustrative pencil drawings, preserved in the archives of the Dept. of Mycology of the National Museum in Prague, were available to me. They helped me very much in some cases.

Microscopic features are described from the examined material mounted in Melzer's reagent and Congo Red. Authors of fungal names are cited according to Kirk and Ansell (1992).

Delicatula agrostidea Velen., Novit. Mycol. (1939): 97. 1940. (Figs. 1–3)

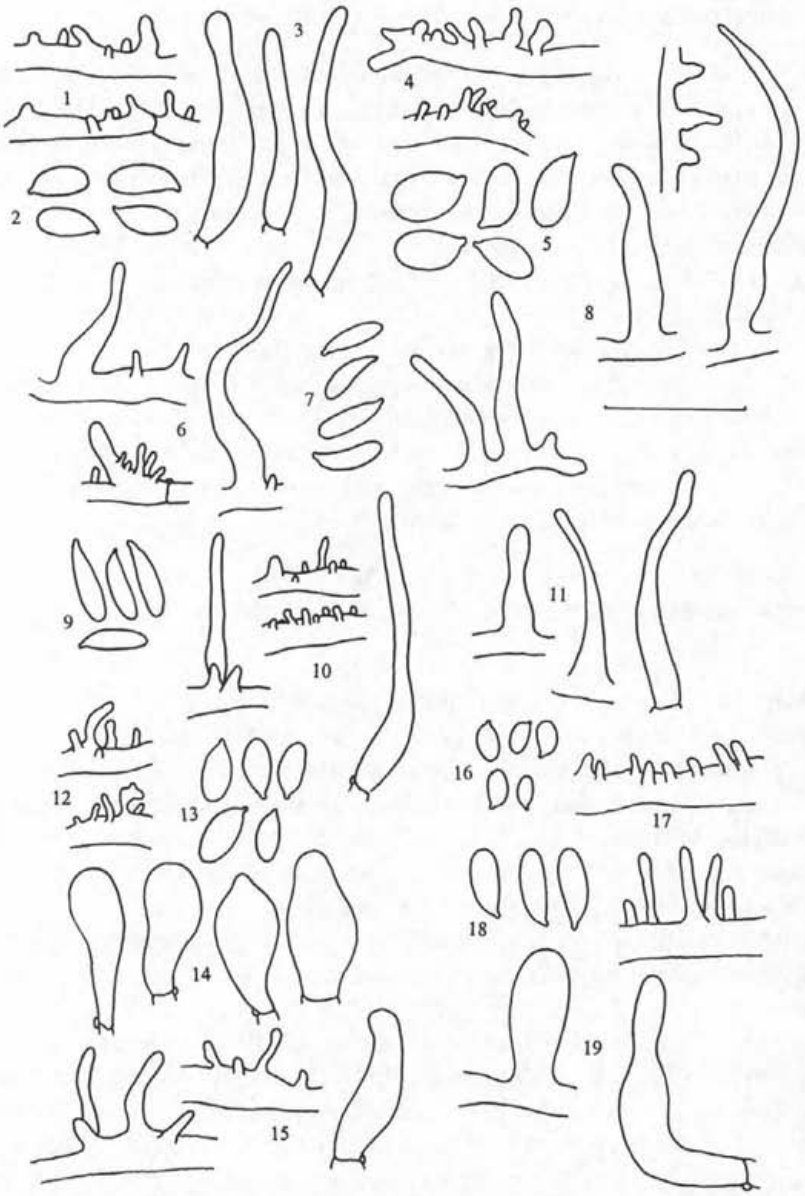
Original description: In caespitibus *Agrostidis* copiosa, tota nivea, gracillima, glabra, pellucida. Pil. 3–5 mm, explanatus, centro depressus, subtiliter membranaceus, leniter distanter sulcatus. St. 3plo longior, basi nuda, truncata insidens. Lam. 10–14, in venas mutatae, ante marginem desinentes, breviter decurrentes, interdum tantum 1–3 evolutae. Sp. oblongo-ellipt., basi attenuatae 5–6 × 2. Cyst. copiosa, tenuiter acicularia, acutissima.

In caespitibus *Agrostidis vulgaris* in colle arido (occid.) pr. Menčice novemb. 1939. Ex affin. *D. ludmilae*. Species pulchella.

Material studied: Mnichovice, Menčice, in caespitibus *Agrostis vulg.*, in colle arido, Nov. 1939 leg. et det. J. Velenovský, PRM 153683 (Holotype). The type specimen consists of four complete carpophores.

Results: Basidiospores 8.0–11 × 3.7–5.0 μm, ellipsoid, subamygdaliform, subfusoid, thin-walled, smooth, non-dextrinoid. Basidia 17–24 × 5.0–6.0 μm, 2-, rarely 4-spored, clavate. Basidioles up to 25 × 4.0–6.5 μm, cylindrical to clavate. Hymenial cystidia 26–36 × 4.0–6.0 μm, ± lageniform, sometimes (sub)capitate, thin-walled. Tramal hyphae of cylindrical to ellipsoid, thin-walled, non-dextrinoid, up to 25 μm wide cells. Pileipellis a cutis, of cylindrical, ± thin-walled, non-dextrinoid, diverticulate, up to 10.0 μm wide hyphae. Pileocystidia absent; very rare cystidioid, e.g. 26 × 5.5 μm, lageniform, capitate elements present. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, non-dextrinoid, smooth (or with scattered single diverticulae), up to 5.0 μm wide hyphae. Caulocystidia absent; scattered adpressed to erect terminal cells present. Clamp-connections absent in all tissues.

Notes: Some features (e.g. 2-spored basidia, clampless hyphae, spore size) agree with *Hemimycena pseudocrispata* (Valla) Maas Geest. On the other hand, the presence of hymenial cystidia and pileocystidioid elements and the absence of distinct caulocystidia contradict it. It remains an unidentified species probably of the genus *Hemimycena*.



Figs. 1–19. *Delicatula agrostidea*: 1. pileipellis hyphae, 2. basidiospores, 3. hymenial cystidia; *D. aristulata*: 4. pileipellis hyphae, 5. basidiospores; *D. conidina*: 6. pileipellis hyphae and pileocystidia, 7. basidiospores, 8. stipitipellis hypha and caulocystidia; *D. crataegi*: 9. basidiospores, 10. pileipellis hyphae and pileocystidia, 11. caulocystidia; *D. fasciata*: 12. pileipellis hyphae, 13. basidiospores, 14. marginal cells, 15. stipitipellis hyphae and caulocystidium; *D. flexuosa*: 16. basidiospores; *D. laevis*: 17. pileipellis hyphae, 18. basidiospores, 19. caulocystidia. Scale bar = 20 μm.

Delicatula alamellosa Velen., Novit. Mycol. (1939): 96. 1940.

Original description: Gracillima, minima, vitreo-pellucida, pure alba, pil. 1–1,5 mm alto, obtuse conico, laevi, margine recto. St. 1–2 cm longus, capillaris (0,4 mm cr.), laevis. Lam. nullae(!), hymenium totam superficiem internam pilei obducens, summopere venae brevissimae circa insertionem stipitis 2–4nae observantur. Sp. lineares, basi oblique constrictae 4–5.

Semper ad folia marcida *Spinosae* locis calidis insolatis pr. Mnichovice raro, autumno 1938. Fungulus mirabilis, vix conspicuus, ex affinitate *D. microscopicae* Fayod (conf. Cejp Mon. II., 86).

Material studied: Type specimen not preserved.

Notes: According to the original description and drawings, it may represent a taxon from the *Hemimycena mauretanica* group. However, the carpophores are described as smooth and, moreover, such small spores are not known from this group. Similar problems arise, when trying to include it in the section *Polyadelphia* of the genus *Mycena*. Therefore, I consider it a nomen dubium.

Delicatula aristulata Velen., Novit. Mycol. Novissimae: 44. 1947.

(Figs. 4–5)

Original description: Zephirea, pellucida. Pil. 2–3 mm diam., conico--convexus, centro obscuro-fuscidulo depressus, sordide pallidus, ad verticem profunde sulcatus, supra aristulis fuscis, falcatis, fragilibus (30–150 × 2,5–3,5) apice et basi acutatis sparse obsitus. Stip. capillaris, praelongus, vix 0,5 mm cr., hyalinus, tremens. Lam. 8–10, crassae, distantes, angustae, cum 4–5nis brevibus ad marginem alternantes, latae, adnatae vel breviter decurrentes. Sp. subgloboso-ellipticae, 3–4, 4sterigm. Cyst. non vidi.

Ad folia marcida quercina in alneto paludoso prope Mirošovice augusto 1944 legi. Species aristulis fuscis in superficie pilei statim agnoscenda. Semper copiosa sporifera.

Material studied: Mnichovice, Mirošovice, Hanzlovka, ad folia *Quercus*, 23 Aug. 1944 leg. et det. J. Velenovský, PRM 153632 (Holotype). This specimen contains one broken carpophore and one stipe part damaged by a parasitic fungus.

Results: Basidiospores 8.5–12 × 4.5–6.0(–6.5) μm, ellipsoid, subfusoid, thin--walled, non-amyloid to slightly amyloid, smooth. Basidia 4-spored, 19 × 6.0 μm (only one found), clavate. Basidioles 8.0–23 × 3.0–6.5 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to ellipsoid, ± thin-walled, non-dextrinoid, up to 15 μm wide hyphae. Pileipellis a dense layer of radially arranged, cylindrical, thin– to slightly thick-walled, diverticulate, up to 5.0 μm wide hyphae, with mostly adpressed terminal cells. Pileocystidia absent. Stipitipellis of parallel, cylindrical, slightly thick-walled, non-dextrinoid, smooth or

rarely with single diverticulae, up to 6.0 μm wide hyphae. Caulocystidia absent. Clamp-connections absent in all tissues.

Notes: According to the at least partly slightly amyloid spores, and the absence of pileo– and caulocystidia, *D. aristulata* Velen. may represent a species of the genus *Mycena*. The spores of the shape described by Velenovský were not found. However, considering the very poor and badly preserved carpophores of the type specimen, I consider it a nomen dubium.

Delicatula barbata Velen., Novit. Mycol. Novissimae: 50. 1947.

Original description: Tota nivea, sporis globosis, cystidiis acicularibus, acutissimis, pileo 3–6 mm, non sulcato insignis. Ad *Stellariam Holosteam. Omph. stellata* Fr. est revera valde affinis, sed sporae ellipticae 6, ad truncum corylinum (Mnichovice 1940), sed itidem ad *Delicatulam* adnumeranda.

Material studied: Type specimen not preserved.

Notes: According to the original description, it may represent several mycenoid species. Therefore, I consider it a nomen dubium.

Delicatula betulina Velen., Novit. Mycol. Novissimae: 50. 1947.

Original description: Sparsa, gracilis, nivea, vitrea, tremens. Pil. 2 mm, campanulatus, non sulcatus, laevis. St. praelongus, capillaris, vitreus, ima basi ciliis longis coronatus. Lam. latae, alternantes, arcuato-decurrentes. Sp. 5–6 × 2,5, ellipticae. Cyst. non vidi.

Ad folia *Betulae* marcida prope Hrusice augusto 1941. Affinis praecedenti (*D. barbata*, not. V. A.)

Material studied: Type specimen not preserved.

Notes: According to the original description and drawings, it probably represents a species of the genus *Mycena*. However, I consider it a nomen dubium.

Delicatula brevipes Velen., Novit. Mycol. Novissimae: 43. 1947.

Original description: Semper dense gregaria vel caespitosa, vitreopellucida, nivea. Pil. 2–3 mm diam., obtuse convexus, margine leniter sulcatus. St. pil. diam. aequilongus, curvatus, vox 1 mm cr. Lam. distantes 10–14, angustissimae, marginem non attingentes, breviter decurrentes. Cyst. 25, copiosa, acicularia, basi crassiora. Sp. globosae 2–3,5, copiosae. Basid. 4sterigm.

Ad radices caulis *Lappae* in frutice prope Mnichovice julio 1940. Ab omnibus vegetatione caespitosa, caule brevi, statione differt.

Material studied: Mnichovice, Tehov, ad radicem *Lappae* in frutico spinosae, 14 July 1940 leg. et det. J. Velenovský, PRM 153627 (Holotype). The holotype specimen consists only of some stipes without pilei.

Results: Stipitipellis a cutis, of parallel, cylindrical, slightly thick-walled, clamped, non-dextrinoid, smooth, up to 5.0 mm wide hyphae. Caulocystidia numerous, 20–45 × 5.0–10.0 μm, adpressed to erect, clavate, cylindrical, sublageniform, thin– to slightly thick-walled, clamped.

Notes: According to its microfeatures, *Delicatula brevipes* probably represents a *Mycena* species.

Delicatula capillipes Velen., Novit. Mycol. (1939): 97. 1940.

Original description: Sparsa, gracillima, alba, vitrea, pil. 2–3 mm diam., plano vel paulisper convexo, albo, glabro. St. 5–10 cm longus, vix 0,5 mm cr., capillaris, laevis, profunde ex humo egrediens. Lam. distantes, sat latae, ante marginem desinentes, decurrentes. Sp. oblongae, basi oblique attenuatae 3–4. Cyst. numerosa, acicularia, recta, obtusa 15–20.

In verrimentis ramis *Crataegi* putridis tectis in colle Plecháč pr. Mnichovice 12, 1938. Sistit transitum ad *Omphaliam*.

Material studied: Mnichovice, collis Plecháč, in verrimentis *Crataegi*, 2 Dec. 1938 leg. et det. J. Velenovský, PRM 153640 (Holotype). The holotype specimen contains no fruitbodies.

Notes: According to the original description, it may represent a large number of mycenoid species. Therefore, I consider it a nomen dubium.

Delicatula citrina Velen., Novit. Mycol. Novissimae: 43. 1947.

Original description: Zephirea, tota citrina, pil. 1–2,5, conicus, non sulcatus. Lam. 4–6, angustae, decurrentes, st. capillaris, basi non incras. insidens. Cyst. e basi lata tenuissime acicularia (25). Sp. globosae 2–3.

Ad strobilos *Struthiopteridis germ.* quotannis frequens. Ne commutatur cum *Omphalia struthiopteris*, quae est multo major, alba, sed in eadem statione ad strobilos.

Notes: See notes on *Delicatula struthiopteridis*.

Delicatula conidina Velen., Novit. Mycol. Novissimae: 45. 1947.

(Figs. 6–8)

Original description: Sparsa, zephirea, nivea. Pil. 1–2 mm, convexus, centro verruca instructus vel etiam depressus, non sulcatus. St. triplo longior, 0,3 mm cr., subtiliter pilosulus, dein glabrescens, basi nuda non incras. insidens. Lam. 3–5, breves, venaeformes, marginem non attignantes. Sp. paucae vel nullae, oblongo-ellipticae 4–5 × 2. Loco earum conidia copiosa globosa 1–2. Cyst. rara, acicularia, basi dilatata, 15.

Ad fructus et folia marcida quercina in m. Kožený vrch prope Mnichovice junio 1940 leg. dom. Ludmila.

Material studied: Mnichovice, infra Kožený vrch, *Quercus* (under willow), 9 June 1940 leg. L. Hostáňová, det. J. Velenovský, PRM 153622 (Holotype). The type specimen consists of only one broken pileus and some stipe parts.

Results: Basidiospores 8.5–10.5 × 2.2–2.7 μm, cylindrical, subcylindrical, narrowly fusoid, sometimes curved, thin-walled, smooth, non-dextrinoid. Basidia 20 × 6.5 μm (only one found), clavate. Basidioles 12–20 × 3.0–6.5 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to ellipsoid, thin-walled, non-dextrinoid, up to 20 μm wide cells. Pileipellis of cylindrical, thin– to slightly thick-walled, mostly diverticulate, up to 6.0 μm wide hyphae. Pileocystidia 19–41 × 3.0–5.5 μm, lageniform, awl-form, subfusoid, rostrate, thin– to slightly thick-walled. Stipitipellis a cutis, of cylindrical, parallel, scatteredly diverticulate, slightly thick-walled, non-dextrinoid, up to 5.0 μm wide hyphae. Caulocystidia 14–45 × 3.0–6.0 μm, awl-form, lageniform, subcylindrical, thin– to slightly thick-walled. Clamp-connections probably present in all tissues.

Notes: The spores of the shape described by Velenovský were not found. According to both the macroscopic and microscopic features, it represents *Hemimycena mauretanica* (Maire) Singer, probably var. *stenospora* (J. E. Lange).

Delicatula crataegi Velen., Novit. Mycol. Novissimae: 47. 1947.

(Figs. 9–11)

Original description: Sparsa, zephirea, nivea, tremens. Pil. 3–4 mm, leniter late convexus, vix umbonatus, non sulcatus, laevis. St. praelongus, vix 0,5 mm cr., laevis, basi nuda non incras. insidens. Lam. 12–18, alternantes, angustae, breviter decurrentes, marginem attingentes. Cyst. ellipsoidea, laevia, vesicaria, 25. Sp. oblongo-cylindricae 8–10 × 2,5, quadristerigmaticae.

Ad ramulos *Crataegi* inter vegetationem ad rivum loco calido, tecto, occid. prope Mnichovice junio 1941.

Material studied: Mnichovice, Potočiny, ad rivum, 9 June 1941 leg. et det. J. Velenovský, PRM 153616 (Holotype). This specimen consists of one broken carpophore.

Results: Basidiospores 9.0–12 × 2.0–3.0(–3.5) μm, narrowly fusoid to (sub)-cylindrical, thin-walled, smooth, non-dextrinoid. Basidioles 12–22 × 3.0–7.0 μm, clavate to cylindrical. Cheilo– and pleurocystidia not found. Tramal hyphae cylindrical, thin-walled, non-dextrinoid. Pileipellis of cylindrical, ± thin-walled, diverticulate, up to 10.0 μm wide hyphae. Pileocystidia 24–32 × 3.5–5.0 μm, awl-form, lageniform, ± thin-walled, often (sub)capitate. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, scatteredly diverticulate, up to 5.0 μm

wide hyphae. Caulocystidia 15–30 × 4.0–5.0 μm, subcylindrical, awl-form, sublageniform, thin-walled. Clamp-connections present in all tissues.

Notes: According to both the macroscopic and microscopic features, it probably represents *Hemimycena mauretanica* (Maire) Singer var. *stenospora* (J. E. Lange).

Delicatula cyathea Velen., Novit. Mycol. Novissimae: 48. 1947.

Original description: Sparsa, zephirea, vitrea, gracillima, nivea. Pil. vix 0,5 mm diam., cito anguste et profunde cyatheus, margine deorsum flexo, in stipitem sensim attenuatus. St. praelongus, capillaris, laevis, basi nuda insidens. Lam. pauceae venaeformes, longe decurrentes. Sp. oblongo-cuneate 5–7. Cyst. non vidi.

Ad culmos graminum minorum in junceto prope Mirošovice augusto 1941 leg. dom. Ludmila. Ad culmos *Caricis remotae* m. Kožený vrch 1942 legi ipse.

Material studied: Mnichovice, Mirošovice, in junceto paludosa, 2 Aug. 1941 leg. L. Hostáňová, det. J. Velenovský, PRM 153631 (Holotype?). The holotype specimen contains no carpophores.

Notes: According to the original description, it may represent several mycenoid species. Therefore, I consider it a nomen dubium.

Delicatula dumetorum Velen., Novit. Mycol. Novissimae: 51. 1947.

Original description: Sparsa, gracilis, laevis, tremens. Pil. 3–5 mm, campanulatus, sulcatus, vertice minute umbonatus vel depressus, umbroso-fusciduLus vel ochraceus, ad marginem pallidus. St. praelongus, capillaris, vitreus, basi corona ciliarum instructus. Lam. latae, numerosae, alternantes, pure albae, breviter decurrentes. Cyst. acicularia, obtusa 15–25. Sp. late ellipticae vel fere obovato-ellipticae 8–12.

In dumetis udis inter verrimenta aestate sat frequens.

Material studied: Mnichovice, in palude Hanzlovka, *Salix caprea,* 6 Oct. 1942 leg. et det. J. Velenovský, PRM 153629 (Holotype?). The type specimen contains no carpophores.

Notes: According to the original description and drawings, it belongs to the genus *Mycena* s. str. and may represent a lot of species. Therefore, I consider *D. dumetorum* Velen. a nomen dubium.

Delicatula faginea Velen., Novit. Mycol. Novissimae: 44. 1947.

Original description: Sparsa, zephirea, nivea, laevis. Pil. 1 mm diam. obtuse campanulatus, leniter sulcatus. St. capillaris, strictus, vix 0,3 mm cr.,

basi nuda insidens. Lam. 10–14, angustae, marginem non attingentes, breviter decurrentes. Cyst. copiosa, globosa (15–20), longe echinulata. Basid. clavata, 15, 1–2sterigm. Sp. globosae 4–5.

Ad folia marcida *Fagi* prope Všesimy octob. 1941.

Material studied: Mnichovice, Všesiny, ad folia *Fagi*, 9 Oct. 1941 leg. et det. J. Velenovský, PRM 153619 (Holotype). The envelope labelled as the type material contains no carpophores.

Notes: According to the original description, *D. faginea* may represent several mycenoid species. I consider *Delicatula faginea* Velen. a nomen dubium.

Delicatula fasciata Velen., Novit. Mycol. (1939): 96. 1940. (Figs. 12–15)

Original description: Plerumque in fasciculis 2–4 cephalis, gracillima, nivea, pil. 1 mm alto, obtuse conico, dein explanato obtuse umbonato. St. capillaris, minute puberulus, p. d. 2–3plo longior. Lam. paucae (5–7), aequilongae, veniformes. Sp. 4sterigm. anguste lineares, deorsum sensim tenuissime acutatae 12–14 × 1.

Ad ramulos putridos *Pruni spinosae* pr. Kunice (Mn.) in dumetis, 8, 1938, *Omph. cuspidata* Quél. secundum diagnosin mire differt.

Material studied: Mnichovice, Mirošovice, viaductus, ad ramul. *Pruni spinosae*, July 1940 leg. et det. J. Velenovský, PRM 153593 (Lectotype, designated by M. Svrček ad schedam). This specimen consists of three minute carpophores.

Results: Basidiospores 7.0–9.0 × 3.0–4.0 μm, ellipsoid, subfusoid, lacrymoid, thin-walled, smooth, non-amyloid. Basidia 18–19 × 5.5–6.0 μm, 4-spored, clavate or subfusoid. Basidioles 13–19 × 4.0–7.0 μm, clavate to cylindrical. Marginal cells (?) 18–23 × 7.0–10.0 μm, clavate to broadly fusoid, thin-walled. Pileipellis made up of cylindrical, thin- to slightly thick-walled, diverticulate, non-dextrinoid, up to 7.0 μm wide hyphae. Pileocystidia not found. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, non-dextrinoid, diverticulate, up to 7.0 μm wide hyphae; diverticulae up to 15 × 2.0(–3.0) μm, digitate, obtuse. Caulocystidia scattered, 16–21 × 6.0–8.0 μm, fusoid, (sub)cylindrical, thin-walled. Clamp-connections present in all tissues.

Notes: According to the original description, this fungus may represent *Hemimycena mauretanica* (Maire) Singer. On the other hand, carpophores preserved as the lectotype specimen showed quite different microscopic features which more or less agree with the genus *Mycena*. Spores as described by Velenovský were not found. I am not able to identify this species exactly.

Delicatula flexuosa Velen., Novit. Mycol. Novissimae: 43. 1947. (Fig. 16)

Original description: Sparsa, zephirea, pil. 2–3,5 mm diam., obtuse late conico, sordide albo, vertice fere fulvello, vix leniter sulcato. St. praelongus,

flexuosus, ca 0,1 mm cr., pellucido-vitreus, luteus(!), infra subtiliter pannosus, sed non ciliatus nec incrassatus. Lam. numerosae, alternantes, postice attenuatae et decurrentes. Sp. globosae 2–3. Cyst. acicularia, acuta 25.

In caespitibus *Nardi* et *Festucae ovinae* in decliv. calidis occid. pineti juvenilis Mirošovice octob. 1940 ipse legi. In contextu copia crystallorum luteorum.

Material studied: Mnichovice, Mirošovice, in caespitibus graminum, 8 Oct. 1940 leg. et det. J. Velenovský, PRM 153630 (Holotype). The type specimen consists of three partly mouldy carpophores.

Results: Basidiospores 4.0–6.0 × 2.5–3.0 μm, ellipsoid, thin-walled, non-dextrinoid, smooth. Basidia 15–18 × 4.0–5.5 μm, 4-spored, clavate to subcylindrical. Basidioles up to 18 × 2.5–6.0 μm, clavate to cylindrical. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to ellipsoid, thin-walled, non-dextrinoid, up to 15 μm wide hyphae. Pileipellis a cutis, of radially arranged, cylindrical, slightly thick-walled, smooth, non-dextrinoid, up to 5.0(–6.0) μm wide hyphae. Pileocystidia not found, but adpressed to erect, cylindrical to clavate, irregular to regular cystidioid elements present. Stipitipellis a cutis, of parallel, cylindrical, slightly thick-walled, non-dextrinoid, up to 6.0 μm wide hyphae. Caulocystidia absent; scattered, e.g. 35 × 5.0 μm, cylindrical terminal cells present. Clamp-connections present in all tissues.

Notes: The spores of the shape described by Velenovský were not found. Except for the absence of caulocystidia, *Delicatula flexuosa* Velen. both macro– and microscopically well agrees with the genus *Collybia* (Fr.) Staude s. str. (= *Microcollybia* Métrod ex Lennox). It certainly does not belong to the *Mycena/Hemimycena* group.

Delicatula gossypina Velen., Novit. Mycol. Novissimae: 47. 1947.

Original description: Sparsa, gracillima, zephirea, nivea, glabra, pil. 2 mm diam., obtuse campanulato, margine breviter sulcato. St. 10–13 mm longus, vix 0,3 mm cr., tremens, pallidus, infra gossypio albo tectus et ex hypothallo albo gossypino excedens. Lam. angustae, confertae, breviter arcuato-decurrentes, marginem attingentes. Sp. 8–10 × 2, oblongae. Cyst. non reperi. In contextu corpuscula crystallina copiosa.

Ad radicem *Alni glutinosae* in palude silvatico prope Mnichovice aug. 1942.

Material studied: Mnichovice, Alnetum near Břečkovský pivovar brewery, ad radicem *Alni glutinosae*, Aug. 1942 leg. et det. J. Velenovský, PRM 153628 (Holotype). The holotype specimen consists of only one stipe part, complete carpophores are absent.

Results: Stipitipellis of cylindrical, parallel, clamped, smooth, slightly thick-walled, dextrinoid, up to 5.0 μm wide hyphae. Stipe medulla hyphae

thin-walled, clamped, dextrinoid, up to 10.0 μm wide. Caulocystidia absent; scattered, adpressed to suberect terminal cells present.

Notes: *Delicatula gossypina* probably represents a species of *Mycena* s. str. I consider it a nomen dubium.

Delicatula graminicola Velen., Novit. Mycol. Novissimae: 49. 1947.

Original description: Sparsa, zephirea, nivea. Pil. permanenter conico--tubulosus, obtusus, 1–2 mm longus, 1 mm cr., margine leniter paucisulcatus. St. praelongus, tremens, basi paulo puberula, non incrassata insidens, vix 0,2 mm crassus. Lam. venaeformes, 4–6, brevissimae, ad marginem pilei (!) tantum evolutae. Sp. sparsae, late ellipticae, 5 × 2,5. Conidia copiosa, globosa 2.

Ad vaginas graminum prope Mnichovice julio 1940 legi.

Material studied: Mnichovice, Tehov, ad vaginas graminis, 3 July 1940 leg. et det. J. Velenovský, PRM 153625 (Holotype). The preserved material is very poor. The holotype specimen consists of two stipes and a part of a collapsed (decayed) pileus sticked to the envelope.

Results: Stipitipellis a cutis, of parallel, cylindrical, slightly thick-walled, clamped, smooth, probably slightly dextrinoid, up to 5 mm wide hyphae. Caulocystidia absent, scattered cylindrical to clavate terminal cells present.

Notes: This species may represent many mycenoid species. I consider the name *D. graminicola* Velen. a nomen dubium.

Delicatula juliana Velen., Novit. Mycol. Novissimae: 43. 1947. (Figs. 20–23)

Original description: Zephirea, nivea, sparsa. Pil. 2–3,5 mm, conicus, vertice depressus, margine crenulatus, sulcatus. St. capillaris, vitreus, nudus et non incrassatus. Lam. 10–15, angustatae, marginem non attingentes, arcuato-decurrentes. Cyst. sparsa, e basi latiori sensim longe attenuata, clavula terminata, 25–30. Sp. perfecte globosae, 3–5, quadristerigm.

In *Calluna* ad marginem pineti prope Ondřejov sat frequens, julio 1940.

Material studied: Mnichovice, ad viam Ondřejov, in caespitibus *Callunae* in soc. *Nardi*, 20 July 1940 leg. et det. J. Velenovský, PRM 153596 (Holotype). It consists of two incomplete broken carpophores.

Results: Basidiospores 6.0–7.5 × 2.5–3.0(–3.5) μm, ellipsoid, cylindrical-ellipsoid, smooth, thin-walled, non-dextrinoid, forming tetrads. Basidia e.g. 24 × 7.0 μm (only one found), 4-spored, clavate. Basidioles 10–22 × 3.0–8.0 μm, cylindrical to clavate. Hymenial cystidia 30–41 × 5.0–9.0 μm, lageniform, fusoid, rostrate, often subcapitate, thin-walled. Tramal hyphae of cylindrical to ellipsoid, ± thin-walled, non-dextrinoid, up to 25 μm wide cells. Pileipellis of radially arranged, cylindrical, ± thin-walled, non-dextrinoid, diverticulate, up to 10.0 μm wide hyphae. Pileocystidia not found. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled,

smooth, non-dextrinoid, up to 5.0 μm wide hyphae. Caulocystidia 42–65 × 7.0–13 μm, lageniform, subfusoid, frequently rostrate, sometimes (sub)capitate, ± thin-walled. Clamp-connections present in all tissues.

Notes: The type material is too poor for an exact identification of this taxon. The spores of the shape described by Velenovský were not found. Some microscopic features are similar to *Mycena* sect. *Adonidae* (e.g. the combination of cheilo– and caulocystidia shape and the absence of pileocystidia), some of them to *Hemimycena* (however, such a combination of microfeatures is not known in this genus). I propose to consider *D. juliana* a nomen dubium.

Delicatula lacrimans Velen., Novit. Mycol. (1939): 96. 1940.

Original description: Sparsa, gracillima, pellucida, pure alba, pil. 1–2 mm, subtiliter puberulo, campanulato, dein leniter convexo, rugoso, glabro. St. capillaris, tenax, vitreus, 2–3duplo p. d. longior, 0,2 mm cr., albus, sine tubere basali, totus guttulis aquosis conspersus. Lam. 3–4 aequilongae, veniformes, valde distantes. Sp. tenuisime lineares, 3–5 × 0,2, falcatae. Basid. 15–17 × 3–4.

Ad ramulos frondosos putridos in dumetis humidis, umbrosis maio, junio et julio sat frequens. Gracilitate, colore niveo, nervis 3–4nis notabilis.

Material studied: Mnichovice, in horto nostro, in trunco *Pruni avii*, June 1939 leg. et det. J. Velenovský, PRM 153644 (Lectotype, designated by M. Svrček ad schedam). The lectotype specimen contains no carpophores.

Notes: Velenovský (1947: 46) described this fungus for the second time. He did not mention the type locality again. The second specimen preserved in the herbarium (Mnichovice, Stráncice, *Rosa* sp., 10 Oct. 1940 leg. et det. J. Velenovský, PRM 154150) contains very poor material, which probably represents *Hemimycena mauretanica*. According to the original decription (Velenovský 1940), this species may really represent *Hemimycena mauretanica* (Maire) Singer.

Delicatula laevis Velen., Novit. Mycol. (1939): 96. 1940. (Figs. 17–19)

Original description: Sparsa, gracillima, vitreo-pellucida, nivea. Pil. 1 mm diam., campanulatus, marginibus involutis, supra et subtus laevis, sine lamellis et venis, sensim in stipitem (0,3 mm cr.) attenuatus. St. basi puberulus, ima basi paulisper incrassatus longeque ciliatus. Sp. 3–7 × 2, anguste cylindr., 4 sterigm. Bas. 25, clavata, septata. Cyst. non vidi.

Fungulus minimus, pileo prorsus allamelloso, forma plene diversa ac in praecedenti (*D. allamelosa*, not. V. A.), vertice paulisper depresso.

Material studied: Mnichovice, Jidášky, ad gramina, 1939 leg. et det. J. Velenovský, PRM 153677 (Holotype). The holotype specimen consists of only one damaged (mouldy) carpophore.

Results: Basidiospores 9.0–11.5 × 3.0–4.0 μm, narrowly ellipsoid, (sub)lacrymoid, thin-walled, smooth, non-dextrinoid, often in tetrads. Basidia 21–23 × 7.5–8.0 μm, 4-spored, clavate. Basidioles up to 22 × 4.0–8.0 μm, clavate to cylindrical. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to ellipsoid, ± thin-walled, non-dextrinoid, up to 20 μm wide cells. Pileipellis made up of cylindrical, non-dextrinoid, diverticulate, up to 10.0 μm wide hyphae. Pileocystidia absent. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, smooth, non-dextrinoid, up to 5 μm wide hyphae. Caulocystidia scattered, single or in small groups, 18–30 × 6.5–10.0 μm, cylindrical, clavate, fusoid, slightly thick-walled. Clamp-connections present in all tissues.

Notes: The type specimen of *Delicatula laevis* Velen. represents *Hemimycena pseudogracilis* (Kühner et Maire) Singer, which is the correct name for this taxon.

Delicatula ligustrina Velen., Novit. Mycol. Novissimae: 51. 1947.

Original description: Sparsa, zephirea, nivea, pil. tantum vertice paulo umbroso. Pil. 1,5–2,5 mm, convexus, vertice umbone solide terminatus, remote sulcatus. St. praelongus, capillaris, albus, basi ciliis brevibus coronatus. Lam. numerosae, latae, albae, breviter decurrentes. Sp. oblongo-ellipticae 6–11. Cyst. 25, columniformia, obtusa. Conidia globosa copiosa 3–4.

Ad ramulos *Ligustri* prope Stráncice octob. 1941. Affinis praecedenti (*D. dumetorum*, not. V. A.)

Material studied: Mnichovice, Skalky near Stráncice, in dumeto *Ligustri*, 12 Sept. 1941 leg. et det. J. Velenovský, PRM 153675 (Holotype?). The holotype specimen contains no carpophores.

Notes: According to Velenovský's original diagnose, it may represent a lot of mycenoid species. I consider *Delicatula ligustrina* Velen. a nomen dubium.

Delicatula longispora Velen., Novit. Mycol. Novissimae: 47. 1947.

(Figs. 24–25)

Original description: Sparsa, zephirea, pellucida, alba, tremens, nuda. Pil. 4–5 mm diam., convexo-campanulatus, apice leniter umbonatus, vix manifeste sulcatus, membranaceus. Stip. praelongus, vix 0,5 mm cr., basi nuda insidens. Lam. numerosae, alternantes, angustae, ante marginem desinentes, breviter decurrentes. Sp. rectae, cylindricae, basi sensim attenuatae, 12–14 × 2,5. Basid. 25–30, quadristerigm.

Ad folia marcida quercina in palude *Ulmaria* obsito prope Mirošovice aug. 1944, sat frequens.

Material studied: Mnichovice, Mirošovice, in ulmeto paludoso, Aug. 1944 leg. et det. J. Velenovský, PRM 153618 (Holotype). This specimen consists of two partly broken and partly collapsed carpophores.

Results: Basidiospores 8.0–10.0 × 3.0–4.0 μm, ellipsoid, subfusoid, thin-walled, smooth, seemingly slightly amyloid. Basidia 25–30 × 5.5–7.5 μm, 4-spored, (narrowly) clavate. Basidioles up to 33 × 3.5–7.0 mm, cylindrical to (narrowly) clavate. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to ellipsoid, ± thin-walled, non-dextrinoid, up to 25 μm wide cells. Pileipellis made up of cylindrical, ± thin-walled, non-dextrinoid, diverticulate, rarely almost smooth hyphae. Pileocystidia absent. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, smooth, sometimes with scattered diverticulae, non-dextrinoid, up to 5.0 μm wide hyphae. Caulocystidia absent; scattered terminal cells present. Clamp-connections present in all tissues.

Notes: Having probably slightly amyloid spores, *D. longispora* Velen. may belong to the genus *Mycena* s. str. However, their amyloidity is very slight. The spores of the size described by Velenovský (12–14 × 2.5 μm) were not found.

Delicatula ludmilae Velen., Novit. Mycol. (1939): 97. 1940.

Original description: Gracillima, tota glabra, alba, vitreo-pellucida pil. 3–5 mm convexo vel plano, pellucido. St. 0,5 mm cr., basi glabra, non incras., supra in pileum sensim incrassatus, hic nonnunquam compressus, pellucidus. Lam. distantes, nanae, decurrentes, aequilongae, ante marginem desinentes. Sp. anguste cylindricae, basi attenuatae 5–6. Cyst. e basi lata acuminata 25–30.

Ad culmos *Brachypodii silvatici* pr. Mnichovice (Jidáš.) junio 1939, in comitatu Ludmilae. Aff. *D. gracillimae* Vel. (*Omphal.*).

Material studied: Mnichovice, Jidášky, *Brachypodium silvaticum*, 25 June 1939 leg. et det. J. Velenovský, PRM 153641 (Holotype). The type specimen consists only of one half-decayed carpophore covered by hyphae and conidia of a mould.

Results: Basidiospores 7.0–9.5 × 3.0–4.0 μm, ellipsoid to pip-shaped, smooth, thin-walled, non-dextrinoid. Basidia 24 × 7.0 μm (only one found), clavate, clamped. Tramal hyphae of cylindrical to ellipsoid, thin-walled, non-dextrinoid, clamped, up to 25 μm wide cells. Pileipellis of cylindrical, diverticulate, up to 10 μm wide hyphae. Stipitipellis of cylindrical, parallel, slightly thick-walled, smooth, non-dextrinoid, clamped, up to 5.0 μm wide hyphae. Other structures collapsed.

Notes: In his drawing, Velenovský depicted a small omphalioid fungus with decurrent lamellae; he also drew awl-form cystidia. However, the type material is too badly preserved to decide which species it may represent. Therefore, I consider it a nomen dubium.

Velenovský described this specimen twice (as *D. ludmilae* and *D. vernalis*); for details see notes on *D. vernalis*.

Delicatula luzulae Velen., Novit. Mycol. Novissimae: 48. 1947.

(Figs. 27–31)

Original description: Zephirea, vitrea, nivea. Pil. 2 mm diam., campanulatus, leniter sulcatus. St. triplo longior, 0,5 mm cr., laevis, basi nuda insidens. Lam. 8–10, angustae, breviter decurrentes. Sp. 6–8 × 2,5, cylindricae, bisterigmaticae. Ad superficiem lamellarum cystidia longe elliptica, obtusa, laevia (25–35). In sporis guttula oleosa magna e membrana evanida elapsa in aqua fluit.

Ad sqamas basales *Luzulae albidae* in colle Plecháč julio 1940.

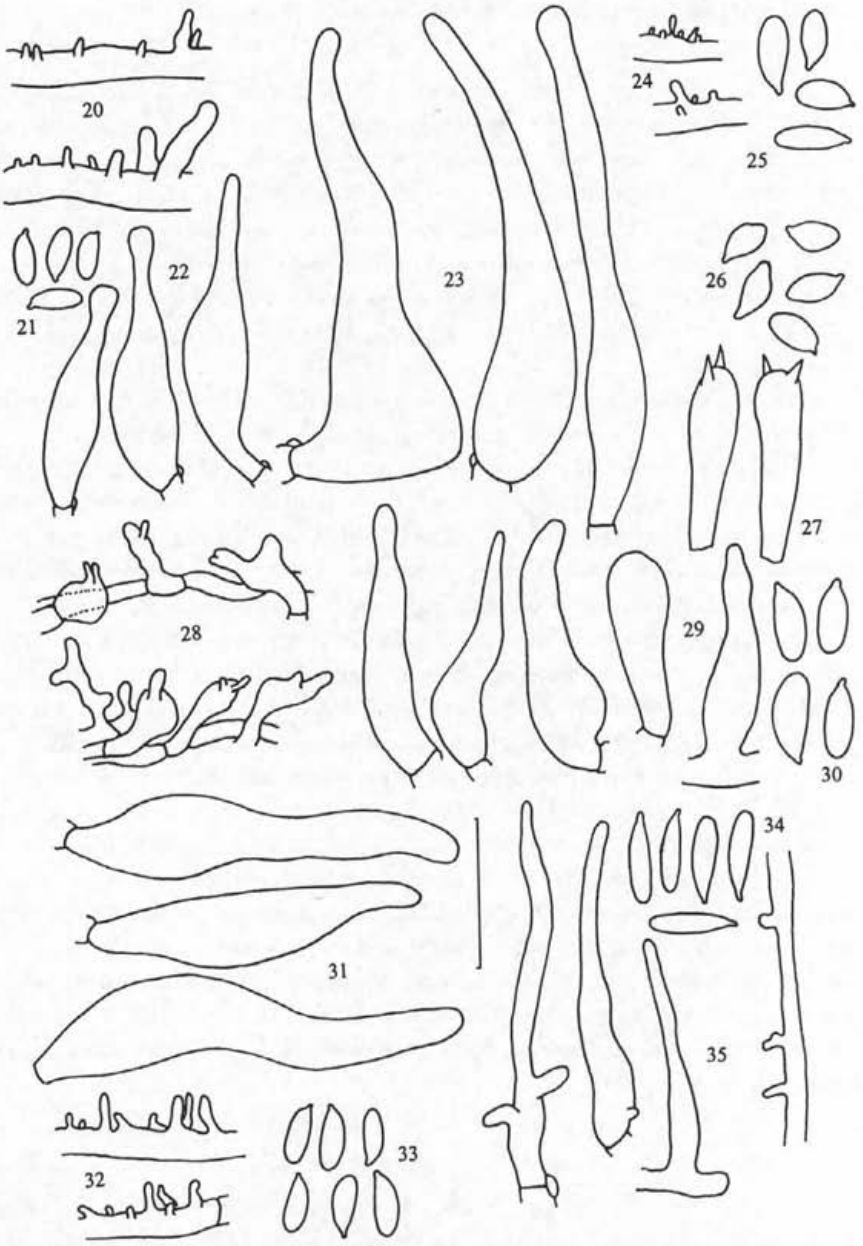
Material studied: Mnichovice, collis Plecháč, *Luzula*, 2 July 1940 leg. et det. J. Velenovský, PRM 153681 (Holotype). The type specimen consists of one broken carpophore.

Results: Basidiospores 9.0–11 × 3.5–4.5 μm, ellipsoid, sublacrymoid, subfusoid, thin-walled, smooth, non-dextrinoid. Basidia 20–23 × 5.5–8.0 μm, 2-spored, clavate. Basidioles up to 23 × 3.5–8.0 μm, cylindrical to clavate. Cheilocystidia 32–57 × 8.0–10.0 μm, lageniform, fusoid, subcylindrical, sometimes rostrate, obtuse, thin-walled. Tramal hyphae of cylindrical to ellipsoid, thin-walled, non-dextrinoid, up to 25 μm wide hyphae. Pileipellis a cutis, of cylindrical, thin-walled, non-dextrinoid, hyaline, up to 6.0 μm wide hyphae; with adpressed to erect, cylindrical, clavate or sublageniform, irregular to coralloid, up to 6.0 μm wide terminal cells. Stipitipellis a cutis, made up of cylindrical, parallel, slightly thick-walled, non-dextrinoid, smooth, up to 5.0 μm wide hyphae. Caulocystidia numerous, (12-)23–31 × (4.0-)5.0–9.0 μm, lageniform, awl-form, clavate, (sub)fusoid, mostly rostrate, regular, obtuse, thin-walled. Clamp-connections absent in all tissues.

Notes: With regard to the pileipellis structure with irregular to coralloid terminal cells, only a few *Hemimycena* species could be taken into account. *H. persimilis* (Malençon ex Redhead) Antonín et Noordel. has longer and narrower spores and 4-spored basidia, *H. pithyophila* (Malençon ex Redhead) Antonín et Noordel. has quite different caulocystidia and not developed hymenial cystidia, and *H. delectabilis* (Fr.) Singer var. *bispora* (Kühner) Antonín has a nitrous smell, distinctly larger spores and different caulocystidia. Moreover, all of them have well-developed rhizoids at the stipe base. Therefore, *D. luzulae* represents probably a species of *Mycena* s. str.

Delicatula major Velen., Novit. Mycol. Novissimae: 45. 1947. (Fig. 26)

Original description: Saepe 2–4fasciata, nivea, vitrea, glabra. Pil. 5–10 mm diam., cito explanatus, centro leniter depressus, subtiliter sulcatus, membranaceus, margine undulatus. St. 2–3plo longior, tenuissimus, primum paleaceus, dein glabratus, basi nuda, non incras., insidens. Lam. distantes, angustae, alternantes, decurrentes. Sp. 5–7, obovato-ellipticae, basi attenuatae. Cyst. pauca, e basi latiori acuminatae, acutae, 25.



Figs. 20–35. *Delicatula juliana*: 20. pileipellis hyphae, 21. basidiospores, 22. cheilocystidia, 23. caulocystidia; *D. longispora*: 24. pileipellis hyphae, 25. basidiospores; *D. major*: 26. basidiospores; *D. luzulae*: 27. basidiospores, 28. pileipellis hyphae, 29. caulocystidia, 30. basidiospores, 31. cheilocystidia; *D. picea*: 32. pileipellis hyphae, 33. basidiospores; *D. phyllophila*: 34. basidiospores, 35. stipitipellis hypha and caulocystidia. Scale bar = 20 μm.

In cavitate trunci betulini "Bílá Skála" prope Božkov julio 1940.

Material studied: Mnichovice, Bílá Skála infra Božkov, in cavitate trunci *Betulae*, 12 July 1940 leg. et det. J. Velenovský, PRM 153679 (Holotype). The holotype specimen contains one larger broken carpophore.

Results: Basidiospores 6.5–8.5 × 3.5–4.0 μm, fusoid to ellipsoid-fusoid, amyloid, smooth, thin-walled. Basidia collapsed. Basidioles 18–27 × 3.5–7.0 μm, cylindrical to clavate. Tramal hyphae of ellipsoid to cylindrical, ± thin-walled, non-dextrinoid, up to 40 μm wide hyphae. Pileipellis a cutis, of parallel, cylindrical, thin-walled, up to 10 μm wide hyphae, covered by fusoid, ± rostrate, slightly thick-walled velar remnants. Stipitipellis a cutis, of parallel, cylindrical, up to 6.0 μm wide hyphae, covered by e.g. 150 × 15 μm large velar remnants similar to pileipellis. Clamp-connections present in all tissues.

Notes: *Delicatula major* Velen. represents *Delicatula integrella* (Pers.: Fr.) Pat.

Delicatula picea Velen., Novit. Mycol. Novissimae: 47. 1947. (Figs. 32–33)

Original description: Sparsa, zephirea, vitrea, nivea. Pil. 2–2,5 mm, convexus, vertice umbone acuto terminatus, leniter sulcatus. St. triplo longior, 0,2 mm cr., basi nuda, non incras. insidens. Lam. 8–10, angustae, ante marginem desinentes, decurrentes. Sp. oblongae, basi acutatae, 5–6 × 2,5. Basid. 1–2sterigm. Cyst. non reperi.

Ad aciculos piceos ad marginem piceti octob. 1940 prope Mnichovice.

Material studied: Mnichovice, Jidášky, ad aciculos *Piceae*, 16 Oct. 1940 leg. et det. J. Velenovský, PRM 153588 (Holotype). The holotype specimen consists of one carpophore with an incomplete pileus.

Results: Basidiospores 6.5–8.0 × 2.5–3.2 μm, lacrymoid, ellipsoid, subfusoid, amyloid, thin-walled, smooth. Basidia 23–27 × 7.0–8.0 μm, 2– and 4-spored, clavate. Basidioles 15–25 × 3.5–5.0 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to fusoid, ± thin-walled, non-dextrinoid, up to 20 μm wide cells. Pileipellis a cutis, of radially arranged, cylindrical, thin– to slightly thick-walled, non-dextrinoid, diverticulate; diverticulae digitate, up to 8.0 × 1.5 μm. Pileocystidia absent. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, smooth or scatteredly diverticulate, up to 6.0 μm wide hyphae. Caulocystidia absent. Clamp-connections present in all tissues.

Notes: *Delicatula picea* Velen. represents a species of *Mycena* s. str.

Delicatula phyllophila Velen., Novit. Mycol. Novissimae: 49. 1947.

(Figs. 34–35)

Original description: Sparsa, zephirea, vitrea, nivea, tota minutissime pilosula, pilis 25, e basi latiori sensim acuminatis, membranis crassis. Pileus

0,5–1 mm, convexus, non umbonatus, non sulcatus, margine involuto. St. 3plo longior, capillaris, basi non incras. insidens. Lam. paucae (3–4), venaeformes, ad stipitem tantum brevissime et hic breviter decurrentes. Sp. globosae, 2–3, semper spora unica in sterigmate longo basidium aequilongum terminans. Cyst. pauca acicularia, acutissima, 15–20.

In foliis marcidis carpineis et quercinis prope Mnichovice locis calidis, tectis passim. Fungulus minutus, sporis suis eodem jure inter Globisporas locum tenere posset.

Material studied: Mnichovice, Hubačov, *Carpinus, Quercus*, 15 July 1940 leg. et det. J. Velenovský, PRM (Lectotype, designated here). The type specimen consists of one broken carpophore, sticked to the envelope paper.

Results: Basidiospores 10.5–12 × 2.5–3.0 μm, cylindrical to narrowly lacrymoid, thin-walled, non-dextrinoid, smooth. Hymenium partly collapsed. Basidia 4-spored. Basidioles 10–18 × 3–8 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae made up of ellipsoid, thin-walled, non-dextrinoid, up to 25 μm wide cells. Pileipellis collapsed, of cylindrical, diverticulate, up to 6.0 μm wide hyphae. Remnants of pileocystidia present. Stipitipellis a cutis, of parallel, cylindrical, slightly thick-walled, non-dextrinoid (to slightly dextrinoid?), smooth to diverticulate, up to 5.0 μm wide hyphae. Caulocystidia 25–48 × 3.5–6.0 μm, sublageniform, awl-form, obtuse to subacute, thin– to slightly thick-walled, often with diverticulate base. Clamp-connections present in all tissues.

Notes: Velenovský did not mention the type locality. From two preserved envelopes labelled as *D. phyllophila*, the above mentioned specimen was selected as the lectotype, because the second one (Mnichovice, Myšlín, ad folia quercina, 6 Oct. 1940 leg. L. Hostáňová, det. J. Velenovský, PRM 153669) contains no carpophores.

Although partly collapsed, the type specimen distinctly represents *Hemimycena mauretanica* (Maire) Singer. According to the very small carpophores, the small number of lamellae, the length of the cystidia and the large basidiospores, it represents var. *megaspora* (Kühner).

Delicatula platyphylla Velen., Novit. Mycol. (1939): 97. 1940. (Figs. 36–37)

Original description: Sparsa, summopere gracilis, fragilis, pure alba, vitreo-pellucida, glabra. Pil. 1–2 mm diam., obtuse conicus, non sulcatus. St. capillaris 3plo longior, basi non incrassatus. Lam. 10–12, alternantes, decurrentes. Sp. ovato-globosae, hyalinae, basi constrictae, 3–5. Cyst. nulla.

In verrimentis stratus humosi humidi inter formationem *Melampyri nemorosi* collis Plecháč pr. Mnichovice julio 1939 sat frequens. Sed faciliter praetervidetur. Propter lamal. latas potius ad *Omphaliam* referenda esset, sed consistentia omnesque characteres pro *Delicatula* testantur.

Material studied: Mnichovice, collis Plecháč, ad verrimenta in formatione *Melampyri*, July 1939 leg. et det. J. Velenovský, PRM 153671 (Lectotype, designated by M. Svrček ad schedam). The type specimen consists of two minute carpophores.

Results: Basidiospores 6.5–7.5 × 2.7–3.7 μm (only two spores found), ellipsoid or pip-shaped, thin-walled, smooth, non-dextrinoid. Basidioles 10–18 × 3.0–6.0 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical to ellipsoid, thin-walled, non-dextrinoid, up to 15 μm wide cells. Pileipellis made up of cylindrical, thin-walled, diverticulate, up to 6.0 μm wide hyphae; diverticulae up to 6.0 × 1.0(–2.0) μm, digitate, obtuse. Pileocystidia not found. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, non-dextrinoid, smooth, up to 5.0 μm wide hyphae. Caulocystidia 19–23 × 6.0–7.0 μm, cylindrical, fusoid, sublageniform, clavate, thin-walled. Clamp-connections present in all tissues.

Notes: The microscopic features of the type specimen more or less agree with *Hemimycena pseudogracilis* (Kühner et Maire) Singer, except for the shorter spores. Moreover, *H. pseudogracilis* has well-developed distinct rhizoids at the stipe base which are neither described nor drawn by Velenovský in *D. platyphylla*. The second preserved specimen (Mnichovice, in colle saxoso, in formatione *Brachipodii* copiose, 20 Sept. 1940 leg. et det. J. Velenovský, PRM 153726) contains no carpophores. Therefore, I consider it a nomen dubium.

Delicatula polyphylla Velen., Novit. Mycol. Novissimae: 44. 1947.

Original description: Sparsa, zephirea, nivea. Pil. 3 mm diam., convexus, vertice leniter solide umbonatus, sulcatus. St. vitreus, tremens, basi nuda insidens, capillaris. Lam. numerosae, angustissimae, marginem non attingentes, longe decurrentes. Sp. globosae et ovato-globosae, magnitudine variabili, nunc 5–6, nunc 8–11. Cyst. non reperi.

Ad ramulum *Salicis capreae* prope Menčice in dumeto, septemb. 1941.

Material studied: Mnichovice, Menčice, *Salix caprea*, 10 Sept. 1941 leg. et det. J. Velenovský, PRM 153652 (Holotype). The type specimen contains no carpophores, only small twigs are present in the envelope.

Notes: *Delicatula polyphylla* may represent many mycenoid species according to the original diagnose and drawings by Velenovský. Therefore, I consider it a nomen dubium.

Delicatula quercina Velen., Novit. Mycol. Novissimae: 46. 1947.

Original description: Gregaria et sparsa, summopere gracilis, vitrea, alba, nuda. Pil. 0,5–1 mm diam., obtuse campanulatus, sulcatus, lam. 12–18,

alternantes ad marginem procurrentes, breviter decurrentes. St. capillaris, vitreus, saepe flexus, praelongus. Sp. cylindricae, 3–5 × 1. Cyst. numerosa, pyriformia, dense breviter spinosula, 15–20 × 8–10.

Ad folia marcida quercina locis calidis (mer.) in gramine sicco "Bílá Skála" prope Božkov. Quotannis autumno lego statione indicata. Basin stipitis cingit annulus tenuis (non discus!). A *D. polyadelpha*, quae autumno in foliis quercinis ubique vulgaris apparet statura minori, lamellis numerosis, fere confertis, sat latis parum decurrentibus, annulo basali bene differt.

Material studied: Mnichovice, Bílá Skála near Božkov, ad folia *Quercus*, 2 Nov. 1942 leg. et det. J. Velenovský, PRM 153661 (Holotype). The holotype specimen consist of only one stipe part.

Results: Stipitipellis of cylindrical, slightly thick-walled, dextrinoid, diverticulate, clamped, up to 4.0 μm wide hyphae; diverticulae 1.0–6.0 × 1.0 μm, digitate. Stipe medulla hyphae thin-walled, cylindrical to slightly inflated, dextrinoid, up to 15 μm wide.

Notes: *Delicatula quercina* Velen. probably represents a species of the genus *Mycena* s. str.

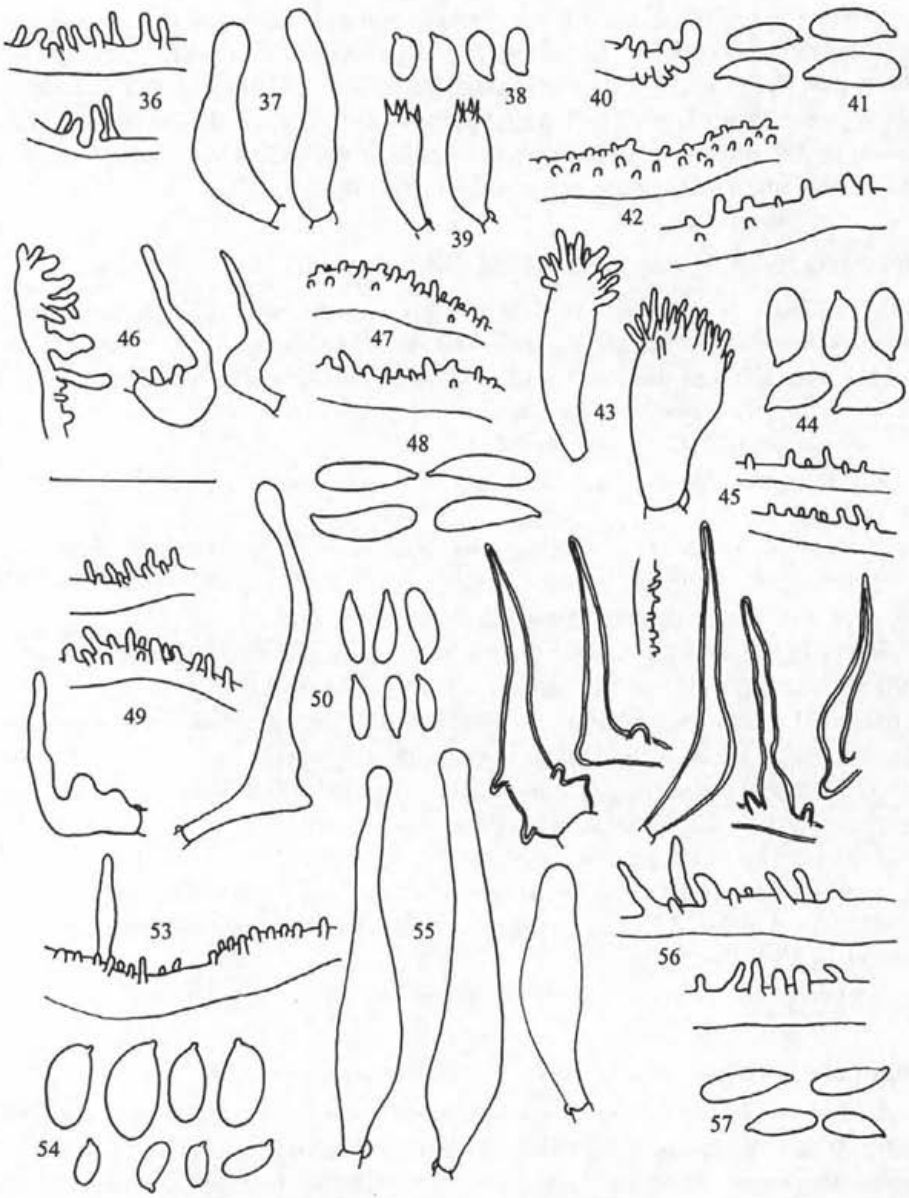
Delicatula ramosa Velen., Novit. Mycol. Novissimae: 45. 1947. (Figs. 38–39)

Original description: Sparsa, zephirea, nivea. Pil. 1–2 mm diam., convexo-conicus, tandem explanatus et centro depressus, non sulcatus. St. capillaris, praelongus, vix 0,1 mm cr., saepe ramosus, 2–3cephalus (!), basi truncata, barbata insidens. Lam. numerosae, alternantes, breviter decurrentes, angustae. Sp. ovato-ellipticae, 3–5. Cyst. non vidi.

Ad terram nudam in societate *Aleuriae aurantiacae* loco calido merid. inter dumeta prope Božkov septemb. 1940. Species curiosa, ad sectionem *Barbatae* transiens.

Material studied: Mnichovice, Bílá Skála near Božkov, 19 Sept. 1940 leg. et det. J. Velenovský, PRM 153654 (Holotype). This specimen consists of two complete minute carpophores.

Results: Basidiospores 5.0–7.0 × 3.0–4.0 μm, ellipsoid or cylindrical-ellipsoid, thin-walled, smooth, non-dextrinoid. Basidia 13–17 × 4.0–6.0 μm, 4-spored, clavate. Basidioles 10–18 × 3.0–6.0 μm, (broadly) clavate or cylindrical. Cheilo– and pleurocystidia not found. Tramal hyphae cylindrical, thin– to slightly thick-walled, non-dextrinoid, up to 10.0 μm wide. Pileipellis a cutis, of radially arranged, cylindrical, slightly thick-walled, smooth, up to 6.0 μm wide hyphae. Pileocystidia absent. Stipitipellis of cylindrical, parallel, thick-walled, non-dextrinoid, smooth, up to 6.0 μm wide hyphae. Caulocystidia scattered as clavate to cylindrical terminal cells. Clamp-connections present in all tissues.



Figs. 36–57. *Delicatula platyphylla*: 36. pileipellis hyphae, 37. caulocystidia; *D. ramosa*: 38. basidiospores, 39. basidia; *D. scirpina*: 40. pileipellis hypha, 41. basidiospores; *D. rigida*: 42. pileipellis hyphae, 43. cheilocystidia, 44. basidiospores, 45. stipitipellis hyphae; *D. spinosae*: 46. stipitipellis hyphae and caulocystidia, 47. pileipellis hyphae, 48. basidiospores; *D. struthiopteridis*: 49. pileipellis hyphae, 50. basidiospores; *D. subtilis*: 51. pileipellis hypha and pileocystidia, 52. caulocystidia; *D. terrestris*: 53. pileipellis hypha, 54. basidiospores (two types), 55. cheilocystidia; *D. umbonata*: 56. pileipellis hyphae, 57. basidiospores. Scale bar = 20 μm.

Notes: According to the microscopic features and also the nice original drawing by Velenovský in his notes, *D. ramosa* Velen. represents a species from the genus *Collybia* (Fr.) Staude s. str. (Antonín et Noordeloos 1997), probably *C. cirrhata* (Pers.) Quél. The unusual feature of the branched stem described and drawn by Velenovský, is an individual aberration and differs morphologically from the lateral outgrowths which are present in *C. racemosa* (Pers.: Fr.) Quél.

Delicatula rigida Velen., Novit. Mycol. Novissimae: 46. 1947. (Figs. 42–45)

Original description: Sparsa, nivea, gracilis. Pil. 4–5 mm, convexus, membranaceo-pellucidus, glaber, non sulcatus. St. praelongus, 0,5 mm cr., strictus, rigidus, non tremens, deorsum sensim crassior, basi nuda insidens, nudus. Lam. 12–16, latae, ante marginem desinentes, longe decurrentes. Sp. ovato-ellipticae, basi attenuate, 6–8. Cyst. non reperi.

Ad folia marcida et verrimenta quercina sub quercubus prope Mnichovice novemb. 1940.

Material studied: Mnichovice, Mirošovice (viaductus), in verrimentis *Quercinis*, Nov. 1940 leg. et det. J. Velenovský, PRM 153665 (Holotype). The holotype specimen consists of two complete carpophores.

Results: Basidiospores 8.0–10.0 × 3.7–5.0 μm, ellipsoid to broadly ellipsoid, thin-walled, amyloid, smooth. Basidia 23–29(–32) × 8.0–10.0 μm, 4-spored, subcylindrical to clavate. Basidioles 15–25 × 6.0–10.0 μm, clavate to broadly clavate. Cheilocystidia 15–20 × 6.0–8.0 μm, subcylindrical to clavate, thin-walled, with numerous apical projections. Tramal hyphae of cylindrical to ellipsoid, thin-walled, dextrinoid, up to 20 μm wide cells. Pileipellis a cutis, of cylindrical, thin-walled, diverticulate, up to 8.0 μm wide hyphae. Pileocystidia absent. Stipitipellis a cutis, of parallel, cylindrical, dextrinoid, diverticulate, up to 4.0 μm wide hyphae; diverticulae digitate, 5.0–10.0 × 1.0 μm. caulocystidia absent. Clamp-connections present in all tissues.

Notes: This species represents a species of *Mycena* s. str.

Delicatula rostellata Velen., Novit. Mycol. Novissimae: 44. 1947.

Original description: Solitaria, zephirea, nivea. Pil. 2 mm altus, basi 1 mm latus, oblongo-conicus, acute rostellatus, leniter paucisulcatus. St. capillaris, laevis, basi nuda insidens. Lam. 3–4, venaeformes, breviter decurrentes. Sp. globosae, 2–2,5.

Ad lignum putridum in cavitate trunci frondosi prope Božkov septemb. 1941.

Material studied: Mnichovice, Bílá Skála near Božkov, in cavitate trunci humidi sub *Pado*, 28 Sept. 1941 leg. et det. J. Velenovský, PRM 153590 (Holotype). The holotype specimen consists only of one stipe part, complete carpophores are absent.

Results: Stipitipellis of parallel, cylindrical, slightly thick-walled, clamped, non-dextrinoid, smooth or scatteredly diverticulate, up to 6.0 μm wide hyphae. Caulocystidia absent.

Notes: According to the original description, it may represent several species of the genera *Mycena* or *Hemimycena*. I consider it a nomen dubium.

Delicatula scirpina Velen., Novit. Mycol. Novissimae: 44. 1947. (Figs. 40–41)

Original description: Sparsa, gracillima, vitrea, nivea. Pil. 3–4 mm, permanenter semiglobosus, atomatus, non sulcatus, non umbonatus. St. capillaris, praelongus, vix 0,1 mm cr., infra pileum incrassatus, flavidus, subtiliter pannosus, basi non ciliatus. Lam. 10–15, latae, marginem attingentes, longe decurrentes. Sp. perfecte globosae, 2,5–3,5. Cyst. maxima (30–50) ellipsoidea, vesicaria, nuda.

Ad folia marcida *Scirpi silv.* in palude prope Mnichovice octob. 1940. Haec species notis additis memorabilis est.

Material studied: Mnichovice, Zíta, ad folia marcida *Scirpi silv.*, 26 Oct. 1940 leg. et det. J. Velenovský, PRM 153598 (Holotype). The type specimen consists of two carpophores partly damaged by a mould.

Results: Basidiospores of two sizes, (6.5-)7.5–12.5(–14.5) × 3.0–4.5 μm and 14–15 × 4.5–7.0 μm, subfusoid, sublacrymoid, narrowly ellipsoid, thin-walled, smooth, non-amyloid, the smaller ones mostly in pairs. Basidioles up to 19 × 3.0–6.0 μm, cylindrical to clavate. Cheilo- and pleurocystidia not found. Tramal hyphae of cylindrical to mostly ellipsoid to subglobose, thin-walled, non-dextrinoid, up to 35(–40) μm wide cells. Pileipellis made up of cylindrical, ± thin-walled, diverticulate, non-dextrinoid, up to 8.0 μm wide hyphae. Pileocystidia not found. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, non-dextrinoid, smooth, up to 5.0 μm wide hyphae. Caulocystidia absent. Clamp-connections present in all tissues.

Notes: Macroscopically (Velenovský 1947 and in notes), *D. scirpina* is very similar to *Hemimycena pseudogracilis* (Kühner et Maire) Singer. Velenovský did not describe the presence of basal rhizoids on the stipe. However, he drew them in his notes. Microscopically, the clavate cystidia as well as globose spores (mould?) described and drawn by Velenovský were not found. The presence of such clavate cystidia do not correspond with any *Hemimycena* species. Therefore, I consider it a nomen dubium.

Delicatula spinosae Velen., Novit. Mycol. Novissimae: 50. 1947. (Figs. 46–48)

Original description: Gracillima, zephirea, tremens, pervia, nivea, semper dense gregaria, plerumque 5–12 specimine in unico folio. Pil. leniter convexus, 1–1,5 mm, non sulcatus, subtus laevis, sine lamellis vel venis. St. praelongus,

capillaris (0,1 mm), nudus, basi nuda insidens. Sp. numerosae, tenuiter lineares (8–12 × 1), rectae, supra obtusae, infra sensim acutissimae, bisterigmaticae. Cystidia et conidia non reperi.

Ad folia Spinosae in dumetis autumno frequens. Ad folia marcida *Spinosae* in ejusdem frutice in valle merid. calido infra Struhařov in copia magna octob. 1941. Apparitione sua revocat *D. polyadelpham*, sed multo minor et prorsus sine lamellis. Fungulus pulchellus, pervius.

Material studied: Mnichovice, in dumeto *Pruni spinosae*, ad ejus folia, 14 Oct. 1941 leg. et det. J. Velenovský, PRM 153650 (Holotype). This specimen consists of three minute carpophores.

Results: Basidiospores 11–13 × 2.5–3.5 μm, subcylindrical, subfusoid, thin-walled, non-dextrinoid, smooth. Basidia 21–25 × 7.0–8.5 μm, 4-spored, clavate. Basidioles up to 25 × 4.0–8.0 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae non-dextrinoid. Pileipellis a cutis, of cylindrical, ± thin-walled, diverticulate, up to 8.0 μm wide hyphae. Pileocystidia absent. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, diverticulate, 1.0–5.0 μm wide hyphae. Caulocystidia 15–21 × 3.5–7.0 μm, awl-form, subfusoid, ± slightly thick-walled, sometimes diverticulate at base. Clamp-connections present in all tissues.

Notes: According to the microscopic features (except for the absence of pileocystidia) and the original drawings by Velenovský, it is very similar to *Hemimycena mauretanica* (Maire) Singer var. *stenospora* (J. E. Lange).

Delicatula struthiopteridis Velen., Novit. Mycol. (1939): 97. 1940.

(= *D. citrina* Velen., Novit. Mycol. Novissimae: 43. 1947.)

Original description: Sparsa, gracillima, laevis, citrina. Pil. 1–2 mm diam., conicus, leniter umbonatus, non sulcatus. St. 5–7 mm l. et 0,2 mm cr., nitens laevis, basi glabra adnatus (raro 3–4 pili breves). Lam. 4–6, distantes, angustae, decurrentes. Pili pilei sparsi, acute aciculares 15–20. Sp. globosae 2–3. Cyst. non vidi.

Ad squamas strobilorum *Struthiopteridis german.* in nostro horto Mnichovice octobr. 1939. Cum *Omphalia struthiopteridis* vulgo non affinis nec similis.

Material studied: Mnichovice, in nostro horto, ad squamas *Struthiopteridis*, 4 Oct. 1939 leg. et det. J. Velenovský, PRM 153645 (Holotype). The type specimen contains no carpophores.

Notes: It is very difficult to identify this taxon. According to Velenovský original drawings it may represent a *Mycena* species. The drawn carpophores and cystidia may remind of *Mycena flavoalba* (Fr.) Quél. (which was also transferred to *Hemimycena* by Singer) but its lamellae are too distant and the spores were descibed as round. However, Velenovský also described spores as round in some

other of his *Delicatula* species but I never saw such spores in my type revisions. Nevertheless, I consider *D. struthiopteridis* Velen. 1940 (as well as *D. citrina* Velen.) a nomen dubium. For other notes see under *D. struthiopteridis* Velen. 1947 below.

Delicatula struthiopteridis Velen., Novit. Mycol. Novissimae: 45. 1947.

(Figs. 49–50)

(= *Omphalia struthiopteridis* Velen., Novit. Mycol.: 94; *Delicatula vitrea* Velen. in litt.)

Original description: Gregaria, vitrea, nivea, pil. 6–11 mm, convexo, dein explanato, centro depresso, sulcato. St. duplo longior, vix 1 mm cr., in pileum dilatatus, subtiliter pilosulus, basi nuda insidens. Lam. distantes, venaeformes, nonnulae furcatae et anastomosantes. Cyst. et pili filiformes 30–45, apice clavula terminata. Sp. 6–8 × 3, cylindricae, basi attenuatae, curvatae.

Ad squamas strobilorum *Struthiopteridis* Mnichovice julio, augusto quotannis.

Material studied: Mnichovice, in nostro horto, *Struthiopteris germ.*, 4 July 1940 leg. et det. J. Velenovský, PRM 153651 (Holotype).

Results: Basidiospores 7.0–9.0 × 2.2–3.0 μm, narrowly fusoid, ellipsoid-fusoid, sublacrymiform, non-dextrinoid, smooth, thin-walled. Basidia 20–21 × × 6.0–7.0 μm, 4-spored, clavate. Basidioles up to 22 × 2.5–7.0 μm, cylindrical to clavate. Cheilo– and pleurocystidia not found. Tramal hyphae of cylindrical, fusoid to ellipsoid, thin-walled, non-dextrinoid, up to 25 μm wide cells. Pileipellis of cylindrical, ± thin-walled, diverticulate, non-dextrinoid, up to 10.0 μm wide hyphae; diverticulae digitate, obtuse, up to 5.0 × 2.0 μm. Pileocystidia 22–55 × 3.5–6.0 μm, lageniform, (sub)capitate, sometimes with subdiverticulate or irregular base, thin-walled. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, scatteredly diverticulate, non-dextrinoid, up to 5.0 μm wide hyphae. Caulocystidia e.g. 45 × 5.0 μm, lageniform, capitate, thin-walled. Clamp-connections present in all tissues.

Notes: *Delicatula struthiopteridis* Velen. undoubtedly represents *Hemimycena mauretanica* (Maire) Singer var. *mauretanica*.

Velenovský described this fungus twice from the same locality: in 1940 as *Omphalia struthiopteridis* Velen. for the first time (holotype PRM 154536!), and in 1947 as *Delicatula struthiopteridis* Velen. for the second time. According to my type revisions, they really represent the same taxon.

However, in 1940 Velenovský already used the name *Delicatula struthiopteridis* for a yellow coloured fungus found in his private garden again. Later (Velenovský 1947), he renamed it *Delicatula citrina* Velen. (see above). This type specimen is missing. Therefore, the name *D. struthiopteridis* Velen. 1947 is a later homonym of it.

Delicatula subluteola Velen., Novit. Mycol. Novissimae: 50. 1947.

Original description: Gracilis, vitrea, pil. 4–5 mm, niveo tinctu luteolo, convexo, non umbonato, leniter remote sulcato. St. praelongus, capillaris, tremens, vitreus. Lam. 10–12, latae, albae, breviter decurrentes. Cyst. 15–17, copiosa, crasse columniformia, obtusa vel subclavata. Sp. 8–11, late ellipticae, basi attenuatae, 1–2 guttulatae.

Ad ramulos putridos *Spinosae* in frutice infra Klokočná octob. 1941. Stipes basi ciliatus.

Material studied: Type specimen not preserved.

Notes: According to the original description, it may represent several mycenoid species. Therefore, I consider it a nomen dubium.

Delicatula subtilis Velen., Novit. Mycol. Novissimae: 50. 1947. (Figs. 51–52)

Original description: Zephirea, vitrea, nivea, gracillima, pervia, glabra. Pil. 0,5–1 mm, leniter convexus, dein horizontaliter explanatus, subtus laevis, sine lamellis. In superficie interna tantum cystidia acicularia (12–20 × 2), sensim tenuissime acuminata, inter eas conidia copiosa, globosa 1 μ diam.

Ad folia quercina in 6 speciminibus in gramine sicco ad marginem dumeti junio 1941 legi.

Material studied: Mnichovice, Hrusice, lateritia, in gramine sub *Pruno spinosa*, 11 June 1941 leg. et det. J. Velenovský, PRM 153656 (Holotype). The holotype material is very poor, it consists of only one broken carpophore.

Results: Basidiospores (7.0-)11–13 × 3.0–3.5 μm (only three spores found), lacrymoid, thin-walled, smooth, non-dextrinoid. Basidia and basidioles collapsed. Pileipellis of cylindrical, ± thin-walled, diverticulate hyphae. Pileocystidia 23–42 × × 4.0–8.0 μm, awl-form to fusoid, slightly thick-walled, obtuse. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, smooth or scatteredly diverticulate, non-dextrinoid, up to 6.0 μm wide hyphae. Caulocystidia 28–45 × × 4.0–5.5 μm, awl-form to setoid, slightly thick-walled, often with diverticulate base. Clamp-connections absent in all tissues.

Notes: Although the type material is very poor and, moreover, partly collapsed, the microfeatures found (especially the large spores, the presence of pileo– and caulocystidia, the diverticulate pilei– and stipitipellis) as well as the macroscopic features (except for the carpophore surface described as smooth) show that it represents a species known as *Hemimycena cyphelloides* (P. D. Orton) Maas Geest. (= *Mycena pseudocrispula* Kühner, forme bisporique). It represents the older name for this taxon, therefore, the following new combination is proposed:

Hemimycena subtilis (Velen.) Antonín comb. nov.

Basionym: *Delicatula subtilis* Velen., Novit. Mycol. Novissimae: 50. 1947.

Delicatula terrestris Velen., Novit. Mycol. Novissimae: 49. 1947. (Figs. 53–55)

Original description: Gracilis, zephirea, nuda, pellucida, tremens. Pil. 5 mm diam., convexus, centro depresso, leniter sulcatus albus, sed tinctu mellino. St. praelongus, vix 0,2 mm cr., hyalinus. Lam. numerosae, latae, alternantes, breviter decurrentes. Sp. oblique ovato-ellipticae, 8–12 × 3–4. Cyst. 50–80, e basi lata sensim acutissima, attenuata, membranis crassis.

In caespite *Dicrani* stipitis basi nuda excedens. Sistit transitum ad *Omphaliam*, sed gracilitate potius sub *Delicatula* enumeranda est.

Material studied: Mnichovice, Koloděje, in *Dicrano*, 3 Oct. 1944 leg. et det. J. Velenovský, PRM 153655 (Holotype). The type specimen consists of one complete carpophore.

Results: Basidiospores – two types found: 1) 5.0–7.0 × 2.5–3.5 μm, ellipsoid, cylindrical-ellipsoid, non-dextrinoid, thin-walled, smooth, and 2) 9.0–12.5 × 4.5–6.0 μm, ellipsoid to broadly ellipsoid, amyloid, thin-walled, smooth. Basidia 14–15 × 4.0–5.0 μm, 4-spored, clavate. Basidioles 10.0–15 × 2.0–6.5 μm, cylindrical to clavate. Cheilocystidia 30–55 × 6.0–11 μm, lageniform, subfusoid, (sub)capitate, ± thin-walled. Tramal hyphae of cylindrical to fusoid, thin-walled, non-dextrinoid, up to 25 μm wide cells. Pileipellis a cutis, of radially arranged, cylindrical, ± thin-walled, diverticulate, up to 10.0 μm wide hyphae. Pileocystidia 40–55 × (4.5-)7.0–13 μm, lageniform, (sub)capitate, subfusoid, thin-walled. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, non-dextrinoid, up to 6.0 μm wide hyphae. Caulocystidia 50–60 × 10.0–15 μm, lageniform to (sub)fusoid, thin-walled. Clamp-connections present in all tissues.

Notes: It is uncertain if both types of spores belong to the same fungus. More common are the larger ones. However, they are rather large in comparison with the basidium size. Regarding the other microscopic and also macroscopic features I nevertheless, consider *D. terrestris* a species of *Mycena* s. str.

Delicatula umbonata Velen., Novit. Mycol. Novissimae: 48. 1947.

(Figs. 56–57)

Original description: Sparsa, gracillima, nivea. Pil. 1–2 mm diam., permanenter campanulatus, non sulcatus, vertice umbone acuto solido terminatus. St. quadruplo longior, 0,2 mm cr., supra basin breviter, subtiliter pilosulus. Lam. 10–13, latae, marginem attingentes, decurrentes. Sp. 5–7, oblongae, basi constrictae. Cyst. acute acicularia, basi dilatata, 15–20.

Ad folia marcida graminum in colle arido, insolato prope Stráněice octob. 1940.

Material studied: Mnichovice, Stráněice, hill above a gravel-pit, ad gramina, 10 Oct. 1940 leg. et det. J. Velenovský, PRM 153653 (Holotype).

Results: Basidiospores 7.5–10.0 × 2.5–3.5 μm, narrowly ellipsoid to ellipsoid-fusoid, sublacrymoid, thin-walled, smooth, non-dextrinoid. Basidia 21 × 8.0–8.5 μm,

4-spored, clavate. Basidioles up to 21 × 3.0–8.5 μm, cylindrical to clavate. Hymenial cystidia (only two found) 27–30 × 4.0–8.0 μm, lageniform, (sub)cylindrical. Tramal hyphae of cylindrical to ellipsoid, thin– to slightly thick-walled, non-dextrinoid, up to 20 μm wide cells. Pileipellis a cutis, of radially arranged, cylindrical, ± thin-walled, diverticulate, non-dextrinoid, up to 8.0 μm wide hyphae; diverticulae up to 12 × 2.0 μm, digitate. Pileocystidia absent. Stipitipellis a cutis, of cylindrical, parallel, slightly thick-walled, smooth, non-dextrinoid, up to 6.0 μm wide hyphae. Caulocystidia absent; scattered terminal cells present. Clamp-connections present in all tissues.

Notes: According to the original description, drawings and type studies, it belongs to the group of *Hemimycena crispata* (Kühner) Singer.

Delicatula vernalis Velen., Novit. Mycol. Novissimae: 48. 1947.

Original description: Zephirea, nivea, vitrea, sparsa. Pil. 1–2 mm, cito explanatus, centro depressus, non sulcatus, glaber. St. triplo et ultra longior, sursum in pileum sensim incrassatus, basi nuda insidens, vix 0,5 mm cr. Lam. latae, numerosae, alternantes, sensim decurrentes. Sp. oblongo-ellipticae, bisterigmaticae, 5–7 × 2–3. Cystidia sparsa, e basi latiori sensim attenuata, 25.

Ad relicta graminum in collibus insolatis primo vere ubique sat frequens (aprili-maio).

Material studied: Mnichovice, guarries above the mill near Menčice, May 1940 leg. et det. J. Velenovský, PRM 153649 (Holotype?). The type specimen contains no carpophores.

Notes: According to the original description, it may represent several mycenoid species. Therefore, I consider it a nomen dubium.

Velenovský desribed this fungus twice as a new species. For the first time, he called it *D. ludmilae* (Novit. Mycol.: p. 97, see above). Later (Novit. Mycol. Novissimae: 48) he described it as *D. vernalis* and synonymised it with *D. ludmilae*. However, he made a complete description and, therefore, both names are validly published.

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CZECH MYCOL. 54 (3–4), 2003

The first record of Amyloflagellula inflata from Benin, West Africa

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Antonín V. (2003): The first record of Amyloflagellula inflata from Benin, West Africa. – Czech Mycol. 54: 235–238

A collection of *Amyloflagellula inflata* Agerer et Boidin from Benin, West Africa, with a detailed description, drawings of microscopic features and a discussion is given. It represents the first record from Benin and the fourth one from West Africa.

Key words: *Amyloflagellula*, Tricholomataceae, Basidiomycota, Africa

Antonín V. (2003): První nález druhu Amyloflagellula inflata v Beninu (západní Afrika). – Czech Mycol. 54: 235–238

Je publikován nález druhu *Amyloflagellula inflata* Agerer et Boidin z Beninu v západní Africe. Je podán jeho detailní popis s kresbami mikroskopických znaků. Publikovaný nález představuje první nález v Beninu a čtvrtý v západní Africe.

The small genus *Amyloflagellula* Singer was proposed by Singer (1966), and contains small cyphelloid or marasmioid fungi known from tropical regions of South America, Africa and Asia (Agerer and Boidin 1981, Dennis and Reid 1959, Petch 1924, Singer 1966). It is characterised (Singer 1966) in having stipitate or non-stipitate carpophores and white rhizomorphs, and terminal cells of the subiculum (hypotrichium) are dextrinoid with flagelliform projections. In other features it is similar to the genera *Crinipellis* Pat. and *Chaetocalathus* Singer but has narrow, sometimes absent lamellae and a very short and curved stipe (if present). The type species is *Amyloflagellula pulchra* (Berk. et Broome) Singer (= *Cyphella pulchra* Berk. et Broome).

During my excursion to Benin, West Africa, an interesting small marasmioid-cyphelloid fungus growing on dead twigs in a marshy forest with *Raffia* was found. Its microscopical study and a comparison with literature showed that it belongs to the genus *Amyloflagellula* Singer, and represents *A. inflata* Agerer et Boidin.

A macroscopical description is given according to the author's personal notes. Microscopical features are described from the material examined, mounted in Melzer's reagent, Congo-Red and KOH. For the basidiospores the following factors are used: E (quotient of length and width in any one spore); Q (mean of E-values). Authors of fungal names are cited according to Kirk and Ansell (1992).

DESCRIPTION OF THE COLLECTION FROM BENIN:

Amyloflagellula inflata Agerer et Boidin

Carpophores single. Pileus 0.5–2 mm broad, attached to the substrate laterally, rarely on top, rounded flabelliform, reniform to convex, almost cyphelloid when young, broadly convex in old specimens, with straight to slightly involute, non-striate margin, entirely pruinose-tomentose, smooth or finely rugulose, white to whitish. Lamellae absent; hymenium smooth to slightly rugulose, rarely veined, white to whitish. Stipe absent. A greyish-ochraceous mycelial layer (subiculum) covers large parts of the substrate.

Basidiospores (7.0-)7.5–9.0(–9.5) × (3.5-)4.0–4.5(–5.0) μm, E = 1.7–2.2, Q = 2.0, ellipsoid, sublacrymiform, ellipsoid-fusoid, thin-walled, inamyloid, hyaline. Basidia collapsed. Basidioles 14–28 × 4.0–8.0 μm, clavate, subfusoid, subcylindrical. Hyphae ± cylindrical, ± smooth and thin-walled to incrusted ("zebred") and slightly thick-walled, branched, hyaline, non-dextrinoid, up to 5.0(–6.0) μm wide; hyphae near attachment to the substrate thin– to slightly thick-walled, cylindrical to sometimes inflated (up to 10 μm broad), smooth to incrusted. Hymenial cystidia 13–27 × 4.5–10.0 μm, clavate, broadly clavate, (sub)utriform, lobate, irregular to subcoralloid, thin-walled, hyaline. Pileipellis a hymeniderm, consisting of broom-cells of the Siccus-type, 10.0–17 × 5.5–9.0 μm, clavate to (sub)cylindrical, thin-walled at base, slightly thick-walled above, sometimes branched in upper part, hyaline in KOH, slightly yellow-brown in Melzer's reagent in thick-walled parts; projections up to 15 × 1 μm, rather numerous (10–20), acute, slightly thick-walled, yellow-brown in Melzer's reagent. Pileus margin with flagelliform broom-cells of the Siccus-type, 8.0–20 × 3.5–7.0 μm, cylindrical, ± clavate, ± thin-walled at base, slightly to distinctly thick-walled at apex; projections up to 10.0 × 2.0 μm, rather numerous (3–15), subacute to acute, rarely obtuse, slightly to distinctly thick-walled. Subiculum of thick-walled, up to 5.0 μm wide hyphae and broom-cells of the Siccus-type (flagelliform) almost without bodies (star-shaped), dextrinoid. Clamp-connections present in all tissues.

Ecology: on dead twigs in a marshy forest with *Raffia*.

Locality: Benin, Province Oueme, Agongo, coordinates: 6 °23.361' N, 2 °37.416' E, 17 Aug. 1997 leg. V. Antonín B97.42 (BR 101096–22).

Amyloflagellula inflata Agerer et Boidin was described in 1981 from Gabon (Agerer and Boidin 1981). It is characterised in having densely crowded carpophores growing on a distinct subiculum, and moreover by the presence of inflated hyphae in the base of the carpophores, which represents a unique feature among the known *Amyloflagellula* species.

Only four species have been described or combined in the genus *Amyloflagellula*. *Amyloflagellula pseudoarachnoidea* (Dennis) Singer differs especially in having

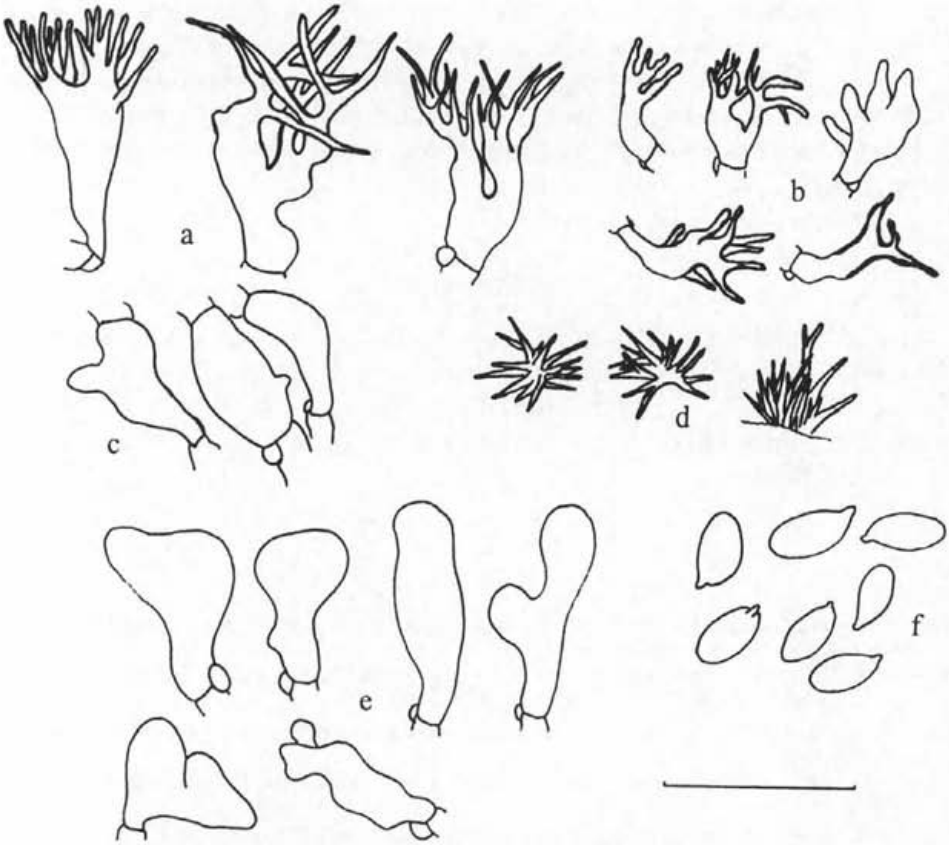

Fig. 1. *Amyloflagellula inflata* (Antonín B97.42). a) pileipellis broom-cells; b) broom-cells at the pileus margin; c) inflated to subinflated hyphae near the base of the carpophore; d) star-shaped broom-cells of the subiculum; e) hymenial cystidia; f) basidiospores. Scale bar = 20 μm.

large spores (18–19 × 4 μm) and growing on prominent white rhizomorphs. Moreover, it has well-developed typical lamellae (therefore, it looks like an agaricoid fungus). All other species have a developed subiculum and are typically cyphelloid. The species *A. verrucosa* Agerer et Boidin also known from West Africa (Gabon) has very small symmetrical cup-shaped carpophores (up to 0.3 mm broad), a white subiculum, slightly larger basidiospores [(8.5-)9–10(–10.5) × (3.5-)4.0–4.5(–5) μm], ± warty cells (never with long projections) on the margin of the carpophores, and no inflated hyphae in the carpophore base; *A. pulchra* (Berk. et Broome) Singer forms ± asymmetric carpophores which seat in a layer of thick-walled hyphae which are absent in *A. inflata*; according to Petch (1924), this

species should have lamellate carpophores while Dennis and Reid (1959) mentioned it as non-lamellate [under the name *Marasmius pulcher* (Berk. et Broome) Petch].

In West Africa, *Amyloflagellula inflata* is known from two records from Gabon and one from Guadeloupe (Agerer and Boidin 1981); therefore, the collection published here represents the first record from Benin and the fourth one from West Africa.

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CZECH MYCOL. 54 (3–4), 2003

The occurrence of yeasts in grass-grown soils

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Sláviková E. and Vadkertiová R. (2003): The occurrence of yeasts in the grass-grown soils – Czech Mycol. 54: 239–247

One hundred and fifty six yeast strains were isolated from 160 grass-grown soil samples collected in four different localities in Bratislava, Slovakia. The collection of soil took place in March, May, August, and October. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Sporobolomyces salmonicolor*, and *Trichosporon cutaneum* were the most frequently isolated species from the samples taken in the unpolluted localities Rusovce and Dúbravka. These species represented 92.1 % of total yeast counts found in these soil samples. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Debaryomyces castellii*, and *Rhodotorula glutinis* were the most frequently isolated species from the samples taken in the polluted localities Polianky and Mlynská Dolina. These species represented 93.3 % of total yeast counts there.

Yeast densities ranged from 400 to 80.000 CFU/g soil. We found that yeasts occurred unevenly in soils during the year. The lowest average number of yeasts was found in August and the highest one in May.

Key words: yeast community, total yeast counts, Slovakia, grass-grown soil

Sláviková E. and Vadkertiová R. (2003): Výskyt kvasiniek v zatrávnených pôdach. – Czech Mycol. 54: 239–247

Zo 160 vzoriek zatrávnenej zeme, ktoré boli odobraté v štyroch rôznych oblastiach Bratislavy, bolo izolovaných 156 kvasinkových kmeňov. Zber vzoriek sa robil v marci, máji, auguste a v októbri. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Sporobolomyces salmonicolor* a *Trichosporon cutaneum* boli najčastejšie izolovanými druhmi zo vzoriek zeme odobratej v neznečistených oblastiach Rusoviec a Dúbravky a reprezentovali 92,1 % z celkového počtu kvasiniek zistených v odobratých vzorkách zeme. *C. laurentii, C. albidus, Cystofilobasidium capitatum, Debaryomyces castellii* a *Rhodotorula glutinis* boli najčastejšie izolovanými druhmi zo vzoriek zeme odobratej v znečistených oblastiach Polianky a Mlynskej doliny a tvorili 93,3 % z celkového počtu kvasiniek z týchto lokalít.

Počet kvasiniek tvoriacich kolónie (CFU) v 1 g vzorky sa pohyboval v rozmedzí od 400 do 80 000. Výskyt kvasiniek v pôde bol v priebehu roka nerovnomerný, pričom najnižší priemerný počet kvasiniek bol zistený v auguste a najvyšší v máji.

INTRODUCTION

Microorganisms form the basis of the ecological balance of the biosphere. The composition of the microbial communities influences the transformation of plant residues into soil organic matter and plant available nutrients, and also stabilises soil aggregates, reduces erosion and maintains the water-holding capacity (Beare et al. 1993, Kennedy & Gewin 1997). Yeasts are important organisms in many

ecosystems and form a significant contribution to biodiversity (Fleet 1998). The soil is the ultimate repository for storage and an even development of certain species of yeasts (Phaff & Starmer 1987).

The occurrence of yeasts in soil has been studied in various parts of the world (Jensen 1963, Vishniac 1996, Dmitriev et al. 1997). Yeasts were found in tropical (Mok et al. 1984) as well as antarctic soils (Baublis et al. 1991). A number of interesting species of yeasts have been isolated from soil (Kurtzman & Fell 1998). Soil biodiversity is generally high in forests, which may represent "biodiversity hot spots" in agricultural landscapes. Forest soils tend to be species-rich and represent stable and often old environments (Hägvar 1998).

The presence of certain species in soil depends on many factors, e.g. type of soil, rainfall, climate, the presence of plants or animals. It is evident that the ability of yeasts to survive in this habitat plays a fundamental role. The yeast density may range from none or a few to several thousand cells per gram of soil (Phaff & Starmer 1987).

Only little information on yeasts associated with soil on the territory of Slovakia and neighbouring countries is available. This work is part of a broader survey of the occurrence of yeasts in various types of soil in the Záhorská nížina lowlands, Slovakia. In our previous investigations we studied the occurrence of yeasts and yeast-like species in forest soil (Sláviková & Vadkertiová 2000). The aim of this work was to study yeast populations occurring in grass-grown soil with reduced plant species richness.

MATERIAL AND METHODS

Study sites

The soil samples came from four different localities: Rusovce, Dúbravka, Mlynská Dolina, and Polianky. The localities Mlynské Dolina and Polianky are in the city centre of Bratislava and the soil samples were taken from zones adjacent to the main road (in the distance about 3 m), where air pollution by exhaust fumes is very high. The grass on these places was mowed very irregularly and before our sampling it was not mowed for a long time. The localities Rusovce and Dúbravka are located in the vicinity of Bratislava (about 20 km from the city centre) and we considered them not or less polluted in comparison with the two foregoing localities. The grass on these places was never mowed. The collection of soil was carried out in March, May, August, and October. In each locality, ten samples were collected from grass-grown soil. In total, this resulted in 160 samples from which yeasts and yeast-like organisms were isolated. The samples were taken from a depth of 5 cm and put into the sterile bottles, transported to the laboratory, and processed within 2 hours after collection.

Methods

The soil pH was measured by the method described by Rump & Krist (1992). The average pH values of the soil samples from the localities Mlynská Dolina and Polianky were equal, namely 7.6, from the localities Rusovce and Dúbravka 7.5 and 6.5, respectively.

Five grammes of the soil sample were suspended in 50 ml of sterile tap water and shaken on a rotary shaker for 1 h. The suspension was diluted to a 1 % solution. An inoculum of 0.5 ml was put on each 10 cm plate with agar and spread with a glass spreader. For cultivation, malt-extract agar and glucose agar (2 % glucose, 0.3 % yeast extract, and 0.1 % $(NH_4)_2SO_4$, 0.02 % K_2HPO_4, 0.01 % KH_2PO_4, 0.02 % $MgSO_4.7H_2O$, 0.02 % NaCl, 0.01 % K_2SO_4, 2 % agar, water) were used. Both media contained 80 $\mu g.ml^{-1}$ streptomycin. The plates were incubated at 25 °C (2–3 d) and 7 °C (14 d). Sodium propionate (0.25 %) reduces the growth of hyphal fungi and was added to the part of medium used for cultivation at 25 °C. Colonies of different appearance were counted in both medium variants, and their representatives were purified according to Slávikovᡠ et al. (1992). Yeast counts were calculated as the number of colony-forming units (CFU) per dry gramme of soil sample. Dry weights for soil samples were determined gravimetrically by subsampling c. 1 g of soil and drying in a drying oven at 105 °C to constant weight.

The morphological and physiological characteristics of isolates were examined by the methods described by Van der Walt & Yarrow (1984). Strains were identified to species according to Kurtzman & Fell (1998) and Kocková-Kratochvílová (1990).

Results and discussion

One hundred and fifty six yeast strains belonging to 8 genera and 11 species were isolated from the 160 soil samples. Table 1 provides a list of the species isolated. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Sporobolomyces salmonicolor*, and *Trichosporon cutaneum* were the most frequently isolated species from the samples taken in the localities Rusovce and Dúbravka. These species represented 92 % of total yeast counts found in the soil samples there. *Cryptococcus laurentii, C. albidus, Cystofilobasidium capitatum, Debaryomyces castellii*, and *Rhodotorula glutinis* were the most frequently isolated species from the samples taken in the polluted localities Polianky and Mlynská Dolina and represented 93 % of total yeast counts obtained there.

The dominant species *Cryptococcus laurentii*, as well as *C. albidus*, occurred in soil samples from all four localities, and formed up to 26.4–45.1 % and 10.2–28.2 % of total yeast counts found in unpolluted and polluted zones, respectively (Table 1). Investigations of various soils indicate that these species were the most frequently

Table 1: The occurrence of individual species isolated from soil samples collected from studied areas

Species	Occurrence of individual species related to the total yeast counts (%) at each locality			
	Rusovce	Dúbravka	Ml. Dolina	Polianky
Aureobasidium pullulans	0.4	0	0	3.0
Candida maltosa	3.0	0	0	0
Cryptococcus albidus	26.4	35.4	10.2	25.9
Cryptococcus laurentii	45.1	34.5	28.2	18.9
Cystofilobasidium capitatum	8.5	9.7	23.9	12.2
Debaryomyces castellii	1.1	3.7	30.8	24.9
Debaryomyces hansenii	0	1.3	0	0
Rhodotorula glutinis	2.9	0	0.5	11.1
Sporobolomyces salmonicolor	5.0	9.7	4.6	2.3
Trichosporon cutaneum	7.2	2.5	1.5	0.2
Trichosporon pullulans	0.4	3.2	0.3	1.5

encountered in tundra ass on these places 1980), Antarctic (Vishniac 1996), and prairie (Spencer & Spencer 1997) soils. *C. laurentii* constituted on average 15 % of total yeast counts found in forest soil (Sláviková & Vadkertiová 2000). On the other hand, *C. laurentii* and *C. albidus* formed just 6.0 % of the total yeast population occurring in eutrophised Morava river water (Sláviková & Vadkertiová 1997). Species of *Cryptococcus* belong to the group of capsulated yeasts, which survive better in habitats poor in nutrients and during periods of desiccation. Capsules may serve to protect cells from physical and biological stresses encountered in their natural habitat and may influence the ability of the cells to survive low moisture conditions (Spencer & Spencer 1997, Golubev 1991).

The proportion of carotenoids producing species varied seasonally. They were widely distributed in October and May (Fig. 1). The majority belonged to the species *Cystofilobasidium capitatum,* which formed 8.5–9.7 % and 12.2–23.9 % of total yeast counts found in unpolluted and polluted zones, respectively. The occurrence of this species could be affected by its ability to grow at lower temperatures. Nearly all strains of this species grew well at 5 °C. *C. capitatum* was very frequently found also in forest soils (Sláviková & Vadkertiová 2000). *Sporobolomyces salmonicolor* occurred in smaller proportions. Its mean percentage was 2.3–9.7 %. This species forms ballistoconidia and is often associated with the phyllosphere of plants. During periods of bright sunlight, carotenoids protect

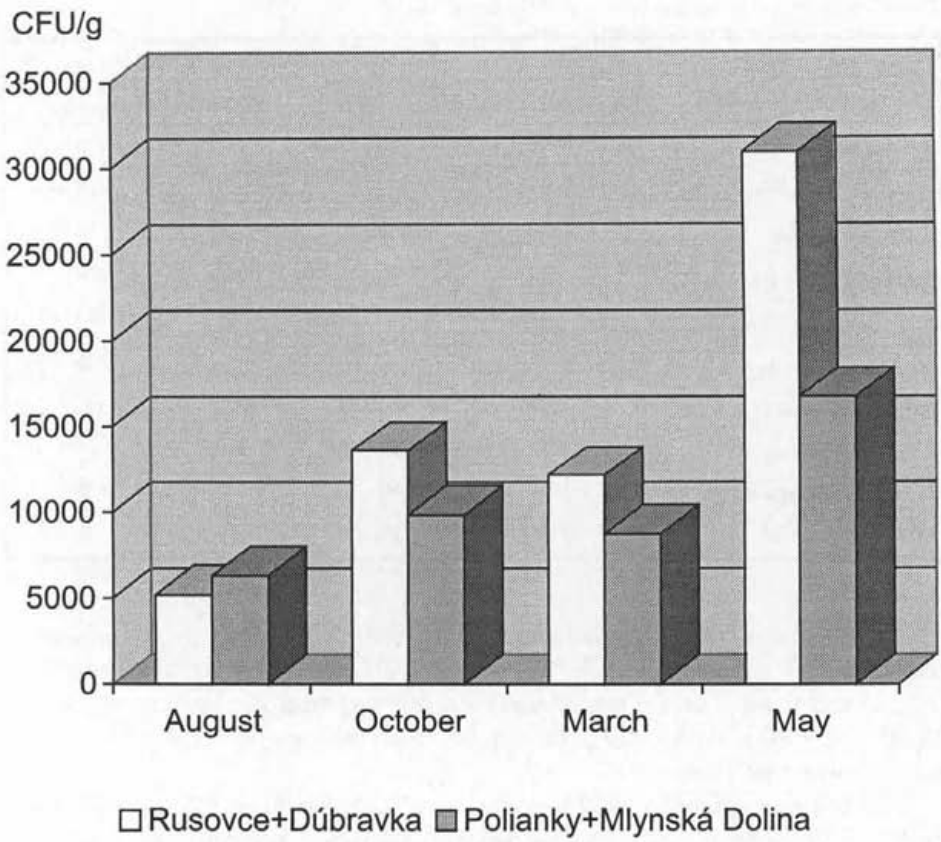

Fig. 1 Changes in counts of the most frequently isolated yeasts through the year

the photosynthetic apparatus of plants against photodestruction (Goodwin 1981). Similarly, they may also protect the vital structures and processes of red yeasts in upper layers of soil. *Rhodotorula glutinis* constituted at most 2.9 % of the total yeast population in three localities, only in the (polluted) locality of Polianky it constituted up to 11.1 %. The high proportion of red yeasts in soil is in an agreement with results obtained by other authors (Golubev 1986, Babyeva & Belianin 1966, Dmitriev et al. 1997).

The other yeast-like species, *Trichosporon cutaneum* and *T. pullulans,* characterised by the formation of true hyphae and arthrospores, were present in lower quantity; they formed only 0.2–7.2 % of the total yeast population, contrary to forest soils, where the species *T. cutaneum* formed up to 30 % and *T. pullulans* 9.8 % of the population. The frequent occurrence of the above yeast species in soil was also observed by other authors. Spencer & Spencer (1997) isolated numerous cultures of the species *T. cutaneum* from Californian and Florida soils.

Table 2: Survey of some features of yeast population

Feature	% of the occurrence of individual feature			
	Rusovce	Dúbravka	Ml. Dolina	Polianky
Presence of urease	96	95	69	75
Fermentation of saccharides	4	5	31	25
Assimilation of nitrate	44	58	39	56
Assimilation of D-xylose	100	100	100	100
Assimilation of L-arabinose	97	100	100	100
Assimilation of cellobiose	95	90	96	98
Assimilation of trehalose	100	100	100	100
Assimilation of lactose	89	90	95	87
Assimilation of soluble starch	92	90	96	98
Assimilation of inositol	88	86	64	61

Debaryomyces castellii appeared to be the only common ascosporogenous species in the grass-grown soils. We recorded its presence particularly in May (Fig. 1) and in soil samples taken from both polluted localities, making up around 25–30 % of yeast counts found in the soil. *D. hansenii* occurred only in one locality and in a small proportion.

The last two species, *Candida maltosa* and the "black yeast" *Aureobasidium pullulans,* were found only in one and two localities, respectively. Their proportion in the total yeast counts did not exceed 3 % but they were the most frequently isolated species from river water (Slávikovᡠ& Vadkertiovᡠ1997, Slávikovᡠ& Vadkertiovᡠ1997a).

The yeast communities isolated from the grass-grown soils consisted predominantly of species with an ability to assimilate not only glucose, saccharose and maltose but also xylose, arabinose, cellobiose, trehalose, lactose, and soluble starch (Table 2). A substantial part of the species was able to use also inositol as a sole carbon source. Approximately one half of yeasts was capable to utilise nitrate as the sole nitrogen source. The marked prevalence of basidiomycetous yeasts in the populations is interesting; they represented 95–96 % in unpolluted zones. These yeasts lacked fermentative abilities and thus depend on aerobic metabolism for their growth. The occurrence of basidiomycetous yeasts in polluted zones is lower (69–75 %), because up to 25–31 % of the yeast population fermented saccharides and belonged to ascomycetous species or anamorphs. In comparison, the presence of ascomycetous and basidiomycetous yeasts in water taken from lakes situated in this area was approximately equal (Slávikovᡠet al. 1992).

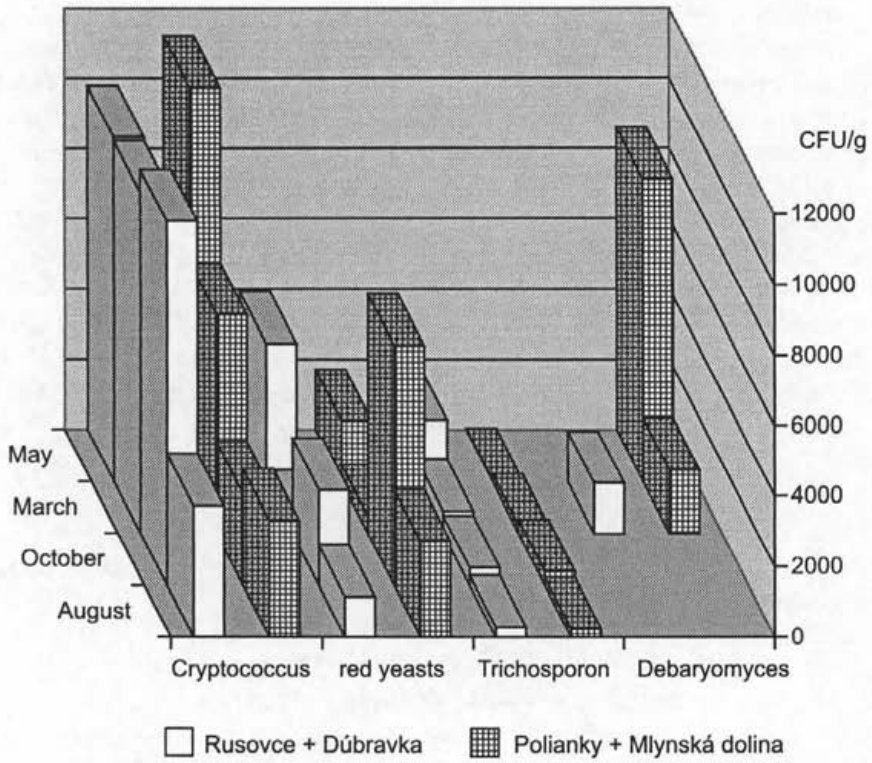

Fig. 2 The average number of yeasts (CFU/g) isolated from soil samples

The number of yeasts in the grass-grown soils ranged from 4×10^2 to 8×10^4 CFU/g soil and the average number reached approximately 1.4×10^4 CFU/g soil. The samples were gathered in March, May, August, and October. We found that the yeasts occurred unevenly in the soil from a quantitative point of view during the year (Fig. 2). The samples collected in May contained the highest number of yeasts, presumably due to the very suitable temperature and moisture in this season. The importance of abiotic environmental factors, especially soil moisture and temperature, on the abundance and biomass of microflora was supported also by Berg et al. (1998). The lowest number of yeasts was found in August, when probably sunshine (temperatures were many times higher than 30 °C) and drought had an inhibitory effect on yeasts. We have found also quantitative differences between yeast counts occurring in polluted and unpolluted localities. A noticeable difference was observed in the May collection (Fig. 2).

We can conclude that the species composition was practically the same in both types of grass-grown soils. The differences were found in species frequency. In soil

samples taken from zones adjacent to a main road (where air pollution by exhaust fumes is very high), *Debaryomyces castellii* and *Cystofilobasidium capitatum* were much more frequent than in samples taken from unpolluted soil (Table 1). On the other hand, the lower occurrence of *Cryptococcus* sp. in these zones was evident. However, on the whole it can be stated that capsule-forming organisms formed the majority. These capsules may act as an extracellular buffer system preventing rapid loss of water and cause efficient rehydration of the cells (Golubev 1991).

Yeast species abundance in the grass-grown soil was lower than in forest soil (Slávíková & Vadkertiová 2000). This may be influenced by the fact that grassland soil contains a higher percentage of less decomposed material (Tate et al. 1988) and by a reduced plant species richness, because it has been shown that the plant community may have some effect on the organism community colonising soil (Spehn et al. 2000, Wardle & Giller 1996).

ACKNOWLEDGEMENTS

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CZECH MYCOL. 54 (3–4), 2003

The effect of excluding plant litter on the aquatic hyphomycete conidia in a headwater stream

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Gulis V. and Suberkropp K. (2003): The effect of excluding plant litter on the aquatic hyphomycete conidia in a headwater stream. – Czech Mycol. 54: 249–260

The concentrations and community structure of aquatic hyphomycete conidia in water were followed over a two-year period in two headwater streams at Coweeta Hydrologic Laboratory, NC, USA using the membrane filtration technique. Litter input into one stream was excluded for 6 years prior to and during the course of our study whereas the reference stream received natural litter inputs during this time. This whole-stream substrate manipulation resulted in seasonal differences in maximum conidia concentrations in the two streams and shifts in dominant species or their rankings. However, total conidia concentrations were not significantly affected by the litter-exclusion treatment.

Key words: freshwater fungi, leaf litter, conidia concentration, community structure, seasonal patterns.

Gulis V. a Suberkropp K. (2003): Důsledek odstranění rostlinného opadu na konidie vodních hyfomycetů v horním toku. – Czech Mycol. 54: 249–260

Koncentace a struktura společenstva konidií vodních hyfomycetů byla sledována po dobu dvou let na dvou horních tocích potoků v Coweetské hydrologické laboratoři (Severní Karolína, USA) za použití techniky membránové filtrace. Vliv opadu na jeden z toků byl vyloučen po dobu 6 let a dále po dobu výzkumu, zatímco do kontrolního potoka se opad dostával přirozenou cestou. Výsledky zásahů znamenaly rozdíly v maximu koncentrace konidií mezi těmito dvěma potoky a v dominanci jednotlivých druhů. Avšak celková koncentrace konidií nebyla podstatně ovlivněna odstraněním rostlinného opadu.

INTRODUCTION

Aquatic hyphomycetes are recognized as important intermediaries of energy flow from allochthonous plant material to higher trophic levels in freshwater lotic ecosystems (Bärlocher & Kendrick 1981, Suberkropp 1992). They convert a substantial portion of leaf material into mycelial biomass and conidia. Fungal biomass can account for as much as 18% of the total detrital mass (Suberkropp 1995) and up to 80% of fungal production may be allocated to conidia (Suberkropp 1991).

Most aquatic hyphomycetes form tetraradiate (predominant), branched or filiform conidia which are adapted for dispersal in flowing water (Webster & Descals 1981). Conventionally shaped conidia are less frequent. Tetraradiate conidia can often be easily identified to species due to their unique conidial morphology. Iqbal

and Webster (1973) proposed a simple technique to enumerate the conidia suspended in water by filtering an aliquot of stream water through a membrane filter, followed by staining, identifying, and counting the conidia trapped on the filter surface. This technique has since been widely used to assess aquatic hyphomycete conidia assemblages in transport (e.g. Bärlocher & Rosset 1981, Thomas et al. 1989, Fabre 1998, Bärlocher 2000). It is believed that species composition of conidia in water is generally in agreement with aquatic hyphomycete communities developing on submerged substrates in a particular stream (Bärlocher 1982). However, some conidia may be produced outside the stream and then introduced from terrestrial leaf litter (Bandoni 1981, Sridhar & Bärlocher 1993), phylloplane of trees overhanging streams (Tubaki et al. 1985) or even soil and groundwater.

The objective of this study was to examine how an ecosystem-level manipulation that excluded plant litter from a headwater stream would affect conidia concentrations in the water transported out of the reach as well as to compare the species composition and community structure of aquatic hyphomycete conidia in transport with those in a reference stream that continued to receive natural litter inputs.

METHODS

The study was conducted at two headwater streams draining catchments 53 (C 53) and 55 (C 55) in the Coweeta Hydrologic Laboratory, Macon county, North Carolina, USA. These streams are small (avg. discharge about 2 L s^{-1}), circumneutral, softwater and contain low nutrient concentrations (NO_3-N + NH_4-N <7 μg L^{-1}, soluble reactive phosphorus <3 μg L^{-1}). They drain mixed deciduous forest with a dense understory of rhododendron (*Rhododendron maximum* L.) in the southern Appalachian Mountains at an elevation of *ca.* 800 m a.s.l. Physical and hydrochemical characteristics of the two streams are very similar (Wallace et al. 1999). Water temperature during the study period was monitored with StowAway temperature probes (Onset Computer Corp.) that recorded temperature every 30 min.

Our study was a part of a larger project started in August 1993 in which leaf litter inputs were excluded along the upper 170 m of C 55 by means of 1.2 cm mesh netting placed over the stream and lateral fences along each bank. This resulted in 95% reduction in leaf litter input and 94% decline in leaf detritus standing crop (Wallace et al. 1999). In addition, in 1996 and 1997 small woody debris ($\leq$10 cm diameter) was removed that led to a decrease of the standing crop of small woody debris from an average of 443 g m^{-2} to 121 g m^{-2} (Wallace et al. 1999). Since we started sampling for conidia of aquatic hyphomycetes in late 1999, average monthly standing stock of benthic particulate organic matter had presumably declined even more than it was in 1997. As a result, organic substrates available

for fungal colonization in C 55 included very limited amounts of leaf litter (e.g. some needles of *Pinus strobus* L.), woody debris, dead and living roots of riparian trees, mosses, and plant parts (e.g. stipules, bud scales) that were smaller than the mesh. C 53 served as the reference stream and contained a variety of types of leaf litter and woody substrates for colonization.

To determine concentrations and species composition of aquatic hyphomycete conidia, water samples were taken monthly over a 2-year period (December 1999 – November 2001) at flumes located 135 and 170 m downstream from the source of streams C 53 and C 55, respectively. Triplicate samples of stream water (500 mL) were filtered through membrane filters (5 μm pore size, Millipore) at streamside (Iqbal & Webster 1973), and conidia were stained with trypan blue in lactic acid (0.1%). Filters were taken to the laboratory where conidia were identified and counted (50–75 fields, Leitz Laborlux, 160×).

Aquatic hyphomycete conidia concentrations in water of the litter-exclusion and reference streams were compared by randomized intervention analysis (RIA, Carpenter et al. 1989) using pretreatment conidia concentrations reported by Suberkropp and Wallace (1992). Similarity indexes (SI) between fungal assemblages of different streams [Sorensen's quantitative index = Czekanowski's quantitative index = Pielou's percentage similarity (Magurran 1988) = Bray-Curtis similarity index] were calculated as

$$SI = 2\frac{\sum_{k=1}^{S} min(x_{ik}, x_{jk})}{\sum_{k=1}^{S} min(x_{ik} + x_{jk})},$$

where S is the number of species, x_{ik} is the relative abundance of species k in stream i. Evenness of conidia distribution among taxa and Shannon-Weaver diversity index were calculated as

$$E = \frac{H}{H_{max}} = \frac{-\sum_{i=1}^{S} p_i \ln p_i}{\ln S},$$

where E is evenness, H is Shannon-Weaver index, S is the number of species, p_i is the relative abundance of species i in the community.

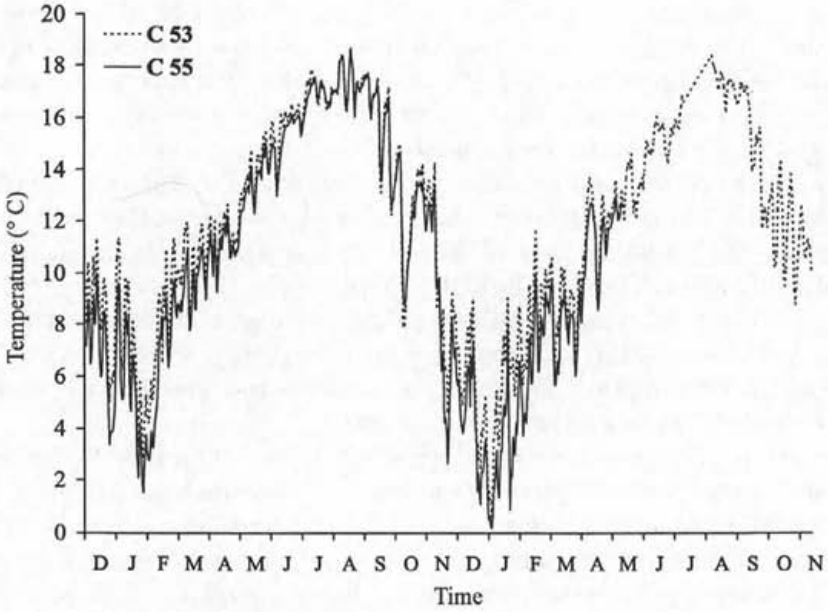


Fig. 1. Average daily water temperatures of C 53 and C 55 during the study period.

RESULTS

The mean daily water temperatures in both streams were quite similar during the study period (Fig. 1). Conidia concentrations in the litter-exclusion stream (C 55) were generally lower than those in the reference stream (C 53) during autumn and winter, but were somewhat higher in C 55 than in C 53 during the spring and summer (Fig. 2). The litter-exclusion treatment, however, did not significantly affect conidia concentrations (p=0.096, RIA).

Species composition of aquatic hyphomycete conidia in transport from both streams is presented in Table 1. Species richness over the two-year period was lower in the litter-exclusion stream in comparison to the control stream (36 *vs.* 43 species, Table 1). However, at each particular sampling date the differences in species richness were small and fluctuations occurred in a similar manner (Fig. 3). No clear temporal pattern was observed. Five species assumed dominance (more than 5% of total conidia over the study period) in each of the streams. The relative abundances of the dominant species in each stream during the study period are illustrated in Figs. 4, 5. The main differences between litter-exclusion and reference streams are shifts in dominant species or their ranking. The top ranked species in both streams was *Alatospora acuminata* (26 and 31% of total conidia in C 53 and C 55,

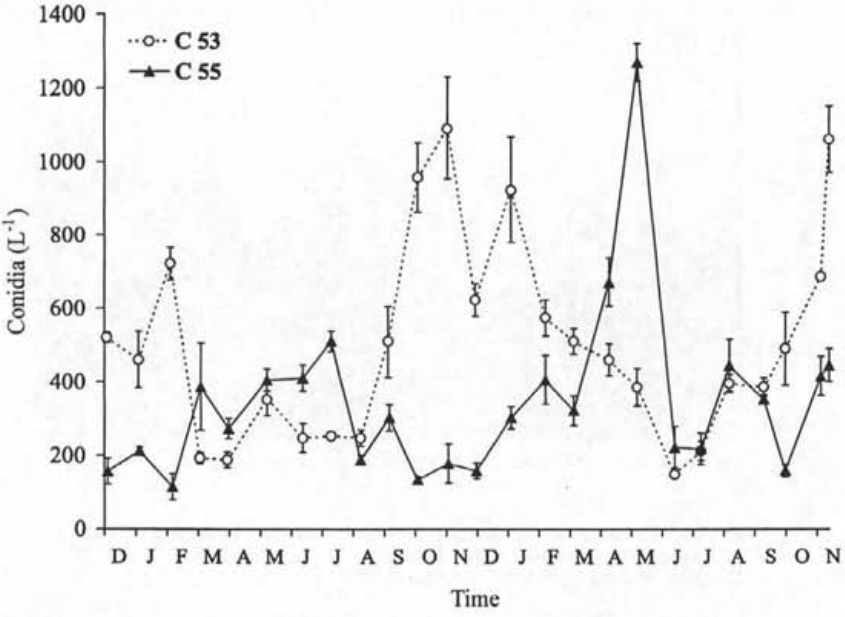


Fig. 2. Conidia concentration of aquatic hyphomycetes in the water of C 53 and C 55. Symbols indicate mean ± SE.

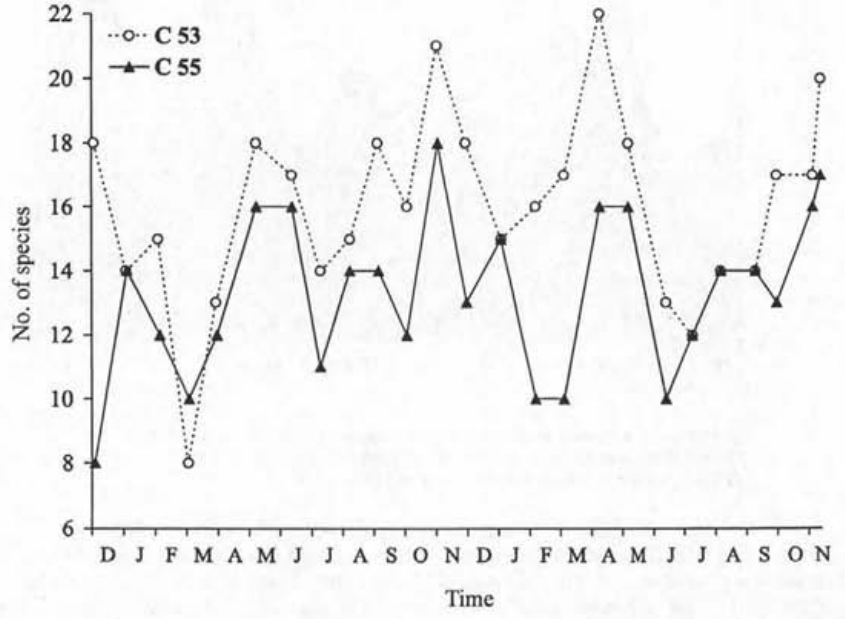
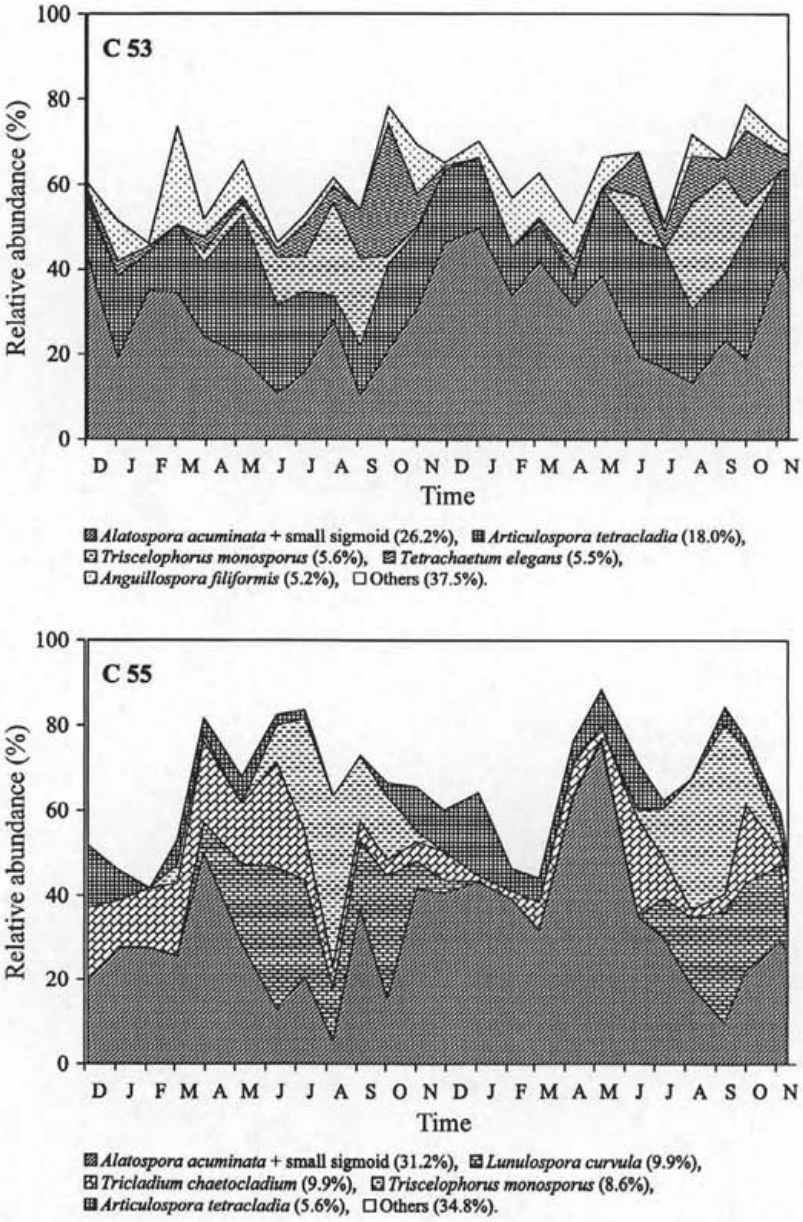


Fig. 3. Species richness of aquatic hyphomycete conidia in transport in C 53 and C 55 during the study period.



Figs. 4, 5. Relative abundance of aquatic hyphomycete conidia in transport in C 53 and C 55. *Note.* Numerous isolates obtained from small sigmoid conidia later appeared to belong to *Alatospora acuminata* s. s. (L. Marvanová, pers. comm.); for this reason we combined the data on the graphs for these two conidia types even though species from genera *Flagellospora, Sigmoidea*, etc. might have been present but could not be positively identified on the basis of detached conidia.

Table 1. Aquatic hyphomycete conidia assemblages from water from the control (C 53) and litter-exclusion (C 55) streams (mean of percent contributions at each sampling date).

Species	Streams	
	C 53	C 55
Alatospora acuminata Ingold aggreg.	13.6	8.5
Alatospora pulchella Marvanová	0.02	1.2
Anguillospora crassa Ingold		0.2
Anguillospora filiformis Greath.	5.2	0.5
Anguillospora cf. *furtiva* J. Webster et Descals	0.3	0.4
Anguillospora longissima (Sacc. et Syd.) Ingold		0.2
Anguillospora cf. *rosea* J. Webster et Descals	0.1	0.1
Articulospora tetracladia Ingold	18.0	5.6
Casaresia sphagnorum Gonz. Frag.	4.1	1.9
Clavariopsis aquatica De Wild.	0.1	1.2
Clavatospora longibrachiata (Ingold) Marvanová et Sv. Nilsson	0.1	1.9
Dactylella microaquatica Tubaki	0.1	
Dendrospora erecta Ingold	0.1	0.2
Dimorphospora foliicola Tubaki	+	+
Dwayaangam sp.	0.1	
Flagellospora curvula Ingold	4.1	3.6
Fontanospora alternibrachiata Dyko	2.3	
Fontanospora eccentrica (R. H. Petersen) Dyko	0.02	
Geniculospora inflata (Ingold) Marvanová et Sv. Nilsson	0.4	0.03
Heliscella stellata (Ingold et V. J. Cox) Marvanová	0.2	
Heliscina antennata Marvanová	0.1	
Heliscina campanulata Marvanová	0.4	0.1
Heliscus lugdunensis Sacc. et Thérry	1.0	1.6
Lateriramulosa uniinflata Matsush.	1.1	0.8
Lemonniera aquatica De Wild.	0.2	0.3
Lemonniera pseudofloscula Dyko	1.2	0.3
Lemonniera terrestris Tubaki	0.2	
Lunulospora curvula Ingold	0.04	9.9
Mycofalcella calcarata Marvanová, Om-Kalth. et J. Webster	0.2	0.6
Pleuropedium tricladioides Marvanová et S. H. Iqbal	0.1	

Table 1. Aquatic hyphomycete conidia assemblages from water from the control (C 53) and litter-exclusion (C 55) streams (mean of percent contributions at each sampling date). (Continued)

Species	Streams	
	C 53	C 55
Taeniospora gracilis var. *enecta* Marvanová et Stalpers	4.9	4.0
Tetrachaetum elegans Ingold	5.5	3.9
Tricladium chaetocladium Ingold	1.5	9.9
Trinacrium sp.	0.3	0.4
Tripospermum myrti (Lind) S. Hughes	0.5	
Tripospermum cf. *prolongatum* R. C. Sinclair et Morgan-Jones	0.3	0.1
Tripospermum sp.	0.2	0.2
Triscelophorus konajensis K. R. Sridhar et Kaver.	4.2	4.2
Triscelophorus monosporus Ingold	5.6	8.6
Sigmoid (<60 μm)	14.7	22.7
Sigmoid (60–120 μm)	3.1	2.2
Sigmoid (>120 μm)	3.1	1.3
Unidentified # 1 (H-like)	0.2	0.1
Unidentified # 2 (pentaradial)	0.3	0.1
Unidentified tetraradiates	2.4	3.0
Sum	100.0	100.0
Total no. of species	**43**	**36**

+ = was not counted because of similarity to propagules of other organisms (ellipsoid conidia)

respectively, but see note to Figs. 4, 5). In the reference stream, *Articulospora tetracladia, Triscelophorus monosporus, Tetrachaetum elegans* and *Anguillospora filiformis* were the next most dominant species and accounted for 34 % of total conidia whereas in the litter-exclusion stream, *Lunulospora curvula, Tricladium chaetocladium, T. monosporus* and *A. tetracladia* were the next most abundant species and also accounted for 34% of total conidia. The most apparent pattern on a temporal scale is the greater contribution to the total conidia pool of *Lunulospora curvula* in the litter-exclusion stream and *Triscelophorus monosporus* in both streams during summer and early autumn (Figs. 4, 5).

DISCUSSION

The effects of whole-stream substrate manipulations on aquatic hyphomycetes have not been previously studied. However, it is known that aquatic hyphomycetes

in streams lacking woody riparian vegetation and hence considerable inputs of leaf litter and woody debris may be affected. Iqbal & Webster (1977) reported very low conidia concentrations and unusual taxa from moorland streams. Metwalli and Shearer (1989) found higher conidia concentrations, species richness and diversity in wooded in comparison to clear-cut reaches of an Illinois stream. Shearer and Webster (1985a) reported lower conidia concentrations and species richness for the moorland reach of the River Teign than for wooded sites. However, it is not clear whether the differences observed were the result of substrate limitation or represented a longitudinal pattern.

Although we anticipated that litter exclusion would have a drastic effect on reproduction of aquatic hyphomycetes, conidia concentrations fluctuated over similar ranges in both streams (114–1269 conidia L^{-1} in C 55 *vs.* 148–1091 in C 53, Fig. 2) and were not significantly affected by the treatment in C 55 (RIA). Important substrates remaining in the litter-exclusion stream included large woody debris and roots of riparian trees. A number of aquatic hyphomycetes have been reported to grow as endophytes of living roots (Fisher et al. 1991, Sridhar & Bärlocher 1992, Iqbal et al. 1995). Since the proportion of total plant litter composed of woody substrates and roots in C 55 was higher than in the reference stream, this perhaps favoured some species of aquatic hyphomycetes that are adapted for growth on wood and changed their relative abundances in the conidia pool. In addition, the almost complete absence of leaf litter and reduced woody debris in the litter-exclusion stream should have resulted in lower retention that allowed more conidia to remain suspended, thus elevating conidia concentrations.

Maximum conidia concentrations observed in C 53 and C 55 are relatively low in comparison to some published values [up to 22000 (Shearer & Webster 1985a), 18000 (Suberkropp 1997) or 11000 conidia L^{-1} (Gönczöl & Révay 1999)] but similar to concentrations found in some mountain or low-nutrient streams (Fabre 1998, Bärlocher 2000). Part of the explanation for these low concentrations could be that we sampled only 135 and 170 m downstream from the sources of C 53 and C 55, respectively. Consequently it is somewhat surprising that the concentrations we observed were achieved in such short reaches. In addition, N and P have been found to stimulate conidia production in aquatic hyphomycetes (Sridhar & Bärlocher 2000, Grattan & Suberkropp 2001) and the streams examined in the present study had extremely low nutrient concentrations that negatively affected fungal reproduction. It is also possible that inputs of conidia from groundwater and terrestrial plant litter played a significant role in these streams since headwater streams are known as having high aquatic-terrestrial interface.

The unexpected difference we observed was that conidia concentrations exhibited maxima at different times in the two streams. The reference stream, C 53 exhibited a pattern that is typical for temperate forested streams with maximum

conidia concentrations in autumn and winter. Such a response to seasonal substrate input is well documented (e.g. Iqbal & Webster 1973, Bärlocher & Rosset 1981, Shearer & Webster 1985b, Suberkropp 1997, Gönczöl & Révay 1999, Bärlocher 2000). In contrast, conidia concentrations in the litter-exclusion stream exhibited maxima in the spring-summer (Fig. 2). We speculate that in the absence of the influence of seasonal litter inputs, activity of aquatic hyphomycetes in C 55 was controlled more by temperature (cf. Figs. 1 and 2).

Shredders are known to compete with aquatic hyphomycetes for available substrates and to feed on fungi, thereby lowering fungal biomass and conidia production (Bärlocher 1980). After four years of litter exclusion in C 55, shredder biomass was only 12–40% of pretreatment level (Wallace et al. 1999). It is not clear, however, whether fungi in C 55 partially escaped the pressure of competition with and grazing by invertebrates because the reduced availability of leaf detritus should, in contrast, intensify these interactions. Shredders may also selectively feed on certain fungal species (Bärlocher & Kendrick 1973, Arsuffi & Suberkropp 1989, Suberkropp 1992), so changes in shredder activity may also have led to shifts in community structure of aquatic hyphomycetes.

The streams examined in the present study were previously sampled for aquatic hyphomycetes using the same technique during a one-year period in 1988–1989 when they both served as reference streams in another study (Suberkropp & Wallace 1992). Conidia concentrations determined in the present study are similar to those determined 11 years ago. We used relative abundances of fungal taxa from both studies to calculate some indices (Table 2). The similarity index between fungal assemblages of C 55 before and after litter exclusion exhibited the lowest value (0.49) and suggests that changes in community structure of aquatic hyphomycetes occurred after litter exclusion. The lower similarity index between C 53 and C 55 in 1999–2001 in comparison to 1988–1989 also supports the idea that changes in community structure took place in response to litter exclusion. Higher species richness and less even distribution of conidia between taxa in 1999–2001 in both streams (Table 2) appear to be due to the higher number of relatively rare species found over two years (1999–2001) in contrast to the one-year period (1988–1989).

The eight-year long litter exclusion from C 55 resulted in changes in the timing of maximum concentrations of aquatic hyphomycete conidia in transport in comparison to the reference stream, C 53. It also caused shifts in community structure. However, in this headwater stream, litter exclusion did not appear to have a major effect on the annual output of conidia.

ACKNOWLEDGEMENTS

We dedicate this paper to Ludmila Marvanová on the occasion of her seventieth birthday and thank her for confirming the identification of some *Alatospora*

Table 2. Comparison of aquatic hyphomycete communities of C 53 and C 55 sampled with 11-year interval.

Statistics	1988–1989		1999–2001	
	C 53	C 55	C 53	C 55-exclusion
Number of species	26	25	43	36
Shannon-Weaver index	2.03	2.20	1.85	1.88
Evenness	0.62	0.68	0.49	0.53
Similarity index	*C 53 – C 53*	*C 55 – C 55*	*C 53 – C 55 (1988–1989)*	*C 53 – C 55 (1999–2001)*
	0.60	0.49	0.73	0.64

isolates. We are grateful to Holly Weyers for help with sample collection. We thank J. B. Wallace, J. L. Meyer, and J. R. Webster for allowing us to sample the litter-exclusion stream and providing temperature data for this stream. This work was supported in part by the North Atlantic Treaty Organization under NSF-NATO Postdoctoral Fellowship in Science and Engineering awarded to VG and by an NSF grant DEB 9806610.

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Note of the Editorial Board: this article is printed anew because of printing error (missing table) in its original edition (Czech Mycol. 53(4): 275–284, 2002).

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Physiological reactions of Aspergillus niger isolates from different heavy metal polluted sites in Slovakia

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Vizárová G., Bacigálová K. and Tamás L. (2003): Physiological reactions of Aspergillus niger isolates from different heavy metal polluted sites in Slovakia – Czech Mycol. 54: 261–272

The paper is focused on the dominant *Aspergillus niger* Tiegh. strain isolated from three different locations in Slovakia polluted by heavy metals. The stress factors (ions of Hg^{2+} and Cu^{2+}) of some physiological reactions of fungus isolates in "in vitro" conditions (mycelium growth, biomass formation, cytoplasmic protein content in mycelium, as well as morphology and development of conidiophores) were observed. To compare physiological reactions of these different *Aspergillus niger* strains during "in vitro" cultivation showed, that *Aspergillus niger* strains isolated from locations with heavy metals (Krompachy and Rudňany) showed reactions which tolerated lower concentrations of Hg^{2+} and Cu^{2+} ions in medium (mycelium growth), they contained cytoplasmic proteins with a higher molecular weight (29,34 and 40 kD), and showed irregular growth and ripening of spores in the heads of conidiophores. After one year of "in vitro" cultivation, all the *Aspergillus niger* isolates (from polluted and non-polluted sites in Slovakia), showed the same physiological reactions to Hg^{2+} and Cu^{2+} ions. All fungus cultures showed sensitivity to ion concentration with an inhibitory effect on growth and development.

Key words: *Aspergillus niger*, heavy metals, cytoplasmic proteins, morphology, ecology.

Vizárová G., Bacigálová K. a Tamás L. (2003): Fyziologické reakcie izolátov huby Aspergillus niger izolovaných na Slovensku z lokalít rôzne zaťažených ťažkými kovmi. – Czech Mycol. 54: 261–272

V práci sme sledovali vplyv stresových faktorov prostredia (prítomnosť ťažkých kovov) na niektoré fyziologické a morfologické reakcie izolátov huby *Aspergillus niger* z troch rôzne zaťažených lokalít ťažkými kovmi na Slovensku. Počas kultivácie in vitro sme sledovali vplyv iónov Cu^{2+} a Hg^{2+} na rast mycélia, zmeny v obsahu cytoplazmatických proteínov v mycéliu ako aj morfológiu a dozrievanie spór v hlavičkách konídiofórov huby. V porovnaní s izolátmi huby z nezaťaženej lokality sme zistili, že izoláty huby zo zaťažených pôd vykazovali reakcie ktoré možno charakterizovať ako tolerantné k nižším koncentráciam iónov Cu^{2+} a Hg^{2+} v substráte (rast mycélia), bol zistený aj zvýšený obsah cytoplazmatických bielkovín s vyššou molekulovou hmotnosťou v bunkách mycélia (29,34 a 40 kD), ako aj nepravidelný rast a dozrievanie spór v hlavičkách konídioforov. Po jednoročnej kultivácii všetkých izolátov huby (zo zaťažených aj nezaťažených lokalít) v podmienkach „in vitro" neboli zistené odlišnosti v reakciách húb k iónom Hg^{2+} a Cu^{2+}, čo potvrdilo tzv. indukovanú „toleranciu" huby *Aspergillus niger* k iónom Hg^{2+} a Cu^{2+}. Všetky izoláty huby boli senzitívne k uvedeným ionom, čo sa prejavilo aj rovnakou inhibíciou rastu mycélia.

INTRODUCTION

In the last years much attention has been paid to the effect of heavy metals on the ecology of terrestrial microorganisms. Most works were focused on their general impact on processes of soil respiration, nitrification, mineralisation, ammonisation, cellulose decomposition, activity of soil enzymes and total abundance of soil microorganisms (Tyler et al. 1989, Valsecchi et al. 1995, Výbohová et al. 1999).

Aspergillus niger is a typical soil micromycete, identified from various types of soils (WRB 1994) in Slovakia (Bernát 1981, Šimonovičová 1980, Benková 1999), but also from soils with high heavy metal concentrations brought about by mining industry. Studies of heavy metal effects on different species of micromycetes under "in vitro" conditions show inhibiting effects on the growth of *Aspergillus niger* strains. However, the strains of *Aspergillus niger* isolated from the Spišsko-Gemerský region were more resistant to lower concentrations of Hg^{2+} and Cu^{2+} ions in comparison with others (Chorvátová & Vizárová 1999).

Due to the mentioned fungus reactions, we focused our attention on the stress reactions of *Aspergillus niger* isolates from different locations, permanently contaminated with heavy metals from mining activities since the 17th century, on different concentrations of Cu^{2+} and Hg^{2+} ions in comparison with reactions of isolates obtained from non-contaminated areas in "in vitro" conditions. In order to obtain more information for understanding the mentioned reactions, we investigated also changes of cytoplasmic proteins in mycelium, mycelium growth and morphology of fungus conidiophores during cultivation. The same experiments were studied after one year of cultivating *Aspergillus niger* isolates under "in vitro" conditions to confirm the introductive "tolerance" of fungus isolates to Hg^{2+} and Cu^{2+} ions.

MATERIAL AND METHODS

As a fungus model we used the micromycete *Aspergillus niger* Tiegh., because only this fungus was isolated from Spiš-Gemer Region locations during each season of the year.

The locations from which we obtained the soil cultures were forests created by man and still maintained. They are located in regions heavily influenced by mining and emissions from metallurgical industry (Krompachy and Rudňany) and in a non-polluted region around the dam Gabčíkovo.

Characteristics of locations

1. Soil contaminated with heavy metals were taken at Krompachy approximately 600 m from the factory.

2. At Rudňany soil from a mining waste heap was sampled.

Chemical characteristics of the investigated soil cultures: pH H_2O 3.5–6.5, pH KCl 2.8–3.2, % C_{ox} 0.6–6.75, % $CaC0_3$ 8.1–11.5, % N_{tot} 0.252–1.232, C:N 2.4–7.1, % humus 1.03–11.64.

Concentrations of heavy metals in soils from which we isolated *Aspergillus niger*:

Krompachy: 343.8 $mg.kg^{-1}$ Cu, 132.5 $mg.kg^{-1}$ Pb, 2.7 $mg.kg^{-1}$ Hg, 1.5 $mg.kg^{-1}$ Cd

Rudňany: 618.8 $mg.kg^{-1}$ Cu, 75.0 $mg.kg^{-1}$ Pb, 187.6 $mg.kg^{-1}$ Hg, 1.5 $mg.kg^{-1}$ Cd (Bučková et al. 2000).

The soil samples were taken from the organic horizon at a depth of about 5–10 cm.

3. Soil obtained from a floodplain forest located around the dam Gabčíkovo area from the meadow Kráľovska lúka.

Chemical characteristics of investigated soil samples: pH H_2O 7.3–7.7, pH KCl 7.1–7.4, % C_{ox} 5.0–10.2, % $CaCO_3$ 11.6–15.2, % N_{tot} 19.7–23.4, C:N 2.4–7.1, % humus 8.6–18.0 (Šimonovičová & Kožuch 1995).

The soil samples were taken from the organic horizon at a depth of about 5–10 cm.

Isolation of *Aspergillus niger* from the soil

The dilution plate method was used for the isolation of *Aspergillus niger*. We used the ratio $1{:}10^{-5}$ KTJ (CFU). The basic suspension which a ratio of 1:10 was prepared from 10 g of fine soil dissolved in 90 ml of sterile water. Cultivation was carried out using 1 ml of soil suspension with nutrient media. Each soil sample was cultivated five times in two different agars: Czapek-Dox (Cz-D) and soil agar with Bengal Red (Fassatiová 1979). After 10 days of cultivation in a thermostat at 25 ± 1 °C, the various fungus colonies were isolated (Gilman, 1957). For identification of the soil fungus isolates both visual and anatomical morphological characteristics of the fungi were used. An evaluation was made by means of a Zeiss "Amplival" microscope with microphotographic equipment, and with the aid of diagnostic literature (Fassatiová 1979, Domsch et al. 1980). Isolates of *Aspergillus niger* were transferred to slope agar. For our experiments we used strains of *Aspergillus niger* obtained from the localities Krompachy and Rudňany, which were cultivated on slope agar only once after the isolation.

The strains of *Aspergillus niger* from Gabčíkovo were isolated and identified by Dr. A. Šimonovičová, Department of Pedology, Comenius University, Bratislava, and used as a control.

Study of the effect of Hg^{2+} and Cu^{2+} ions on the growth of *Aspergillus niger* under "in vitro" conditions

The fungus isolates were cultivated in liquid Cz-D medium (Fassatiová 1979) with various concentrations of Hg^{2+} and Cu^{2+} ions supplemented in the form of $HgCl_2$ and $CuSO_4$. To each Erlenmayer flasks containing 20 ml of liquid Cz-D medium $HgCl_2$ solutions were added the following concentrations: 0.001 $mg.l^{-1}$, 0.005 $mg.l^{-1}$, 0.025 $mg.l^{-1}$, 0.125 $mg.l^{-1}$, 0.625 $mg.l^{-1}$ and 2.7 $mg.l^{-1}$. The $CuSO_4$ solutions were added the following solutions: 0.001 $mg.l^{-1}$, 0.005 $mg.l^{-1}$, 0.025 $mg.l^{-1}$, 0.125 $mg.l^{-1}$, 0.625 $mg.l^{-1}$ and 2.7 $mg.l^{-1}$.

The fungus isolates were cultivated at a temperature of 27 °C in the dark. The Cz-D medium without addition of Hg^{2+} and Cu^{2+} were used as a control. Spore suspensions were prepared from 14 days old cultures. For each experiment 1 ml of suspension was used. After 14 days of cultivation of the samples we determined the dry matter of the fungi. The samples were dried at a temperature of 105 °C. The individual series concerning the effect of different concentrations of $HgCl_2$ and $CuSO_4$ on fungus growth were statistically evaluated according to Lamoš & Potocky (1989).

The same experiments were prepared with isolates of *Aspergillus niger* after one year cultivation under "in vitro" conditions (one year old fungus isolates).

Determination of cytoplasmic proteins

The Sodium Dodexyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method was used to monitor changes in cytoplasmic proteins isolated from mycelium of three various isolates of *Aspergillus niger*.

The fungus isolates (Gabčíkovo, Krompachy and Rudňany) were cultivated in liquid Cz-D medium (100 ml) in the dark at 25 °C + 1 °C during 25 days.

The 25 day old cultures (mycelium with spores) of fungus isolates were harvested and frozen immediately in liquid nitrogen. Samples were ground to a fine powder in a cold mortar in liquid nitrogen and the resulting powder was extracted with 100 $mmol.l^{-1}$ Tris buffer, pH 8.0. The homogenate was centrifuged at 10.000 × g for 15 min. and the resulting supernatant was centrifuged again at 100.000 × g for 30 min. in a Beckman L 8-M ultracentrifuge. The supernatant was considered to be the cytoplasmic fraction. After passing through Sephadex G-25, the proteins were precipitated overnight at –20 °C with 4 volumes of ice-cold acetone. Proteins were solubilised and separated under denaturing conditions on 10–20 % gradient polyacrylamide slab gels using the discontinous buffer system (Leammli 1970) and silver stained (Heukesloven & Dernick 1985). Protein concentrations were determined by the slightly modified method of Lowry et al. (1951) with BSA as the standard. The apparent molecular masses of polypeptides were calculated based on mobilities of protein standards (Serva).

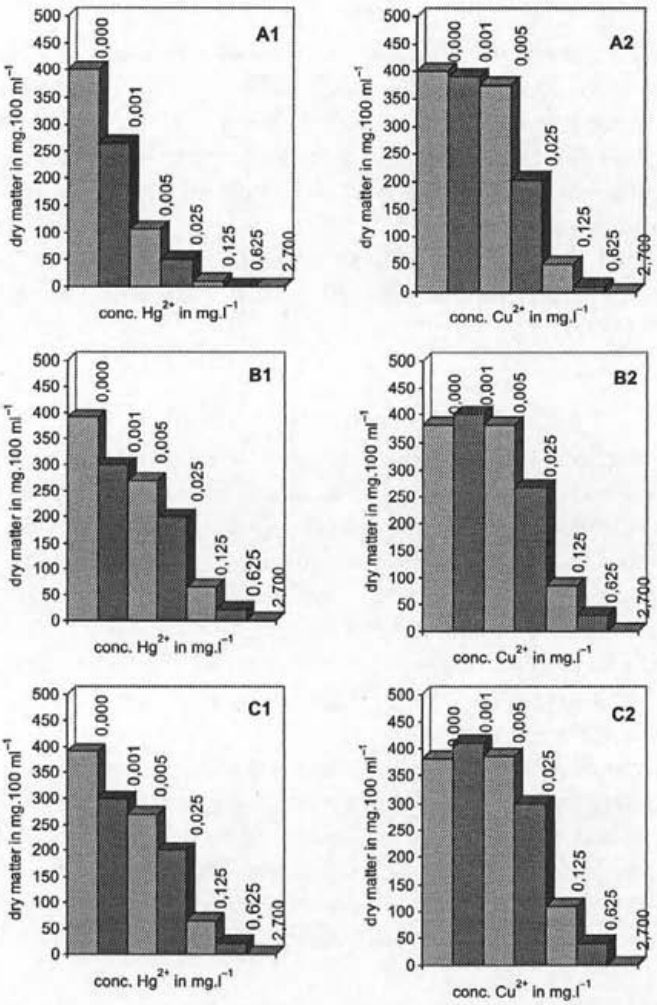

Fig. 1. Effect of Hg^{2+} and Cu^{2+} ions on the mycelium growth of *Aspergillus niger* isolates from contaminated and non-contaminated locations in Slovakia under "in vitro" conditions.
A1 = Effect of Hg^{2+}on the mycelium growth of isolates from the non-contaminated region Gabčíkovo
A2 = Effect of Cu^{2+}on the mycelium growth of isolates from the non-contaminated region Gabčíkovo
B1 = Effect of Hg^{2+} on the mycelium growth of isolates from the contaminated region Krompachy
B2 = Effect of Cu^{2+} on the mycelium growth of isolates from the contaminated region Krompachy
C1 = Effect of Hg^{2+} on the mycelium growth of isolates from the contaminated region Rudňany
C2 = Effect of Cu^{2+} on the mycelium growth of isolates from the contaminated region Rudňany
Ordinata: dry weight of mycelium in mg pro 100 ml of liquid medium
Abscissa: ion concentration of Hg^{2+} in $mg.l^{-1}$
ion concentration of Cu^{2+} in $mg.l^{-1}$
n = 20, significance P = 0.01, SE = 0.3 or 0.27

Morphological study of *Aspergillus niger* strains

The mycelial growth and visual morphology of the strains of *Aspergillus niger* was observed after 25 days of "in vitro" cultivation on Cz-D Agar (Fassatiová 1979). For the experiments we used the strains which were cultivated only once after isolation on slope agar, and one year old strains (one year cultivated under "in vitro" conditions). During the year we inoculated the strains on slope agar in a frequency of once a month.

We evaluated mycelium growth and morphological differences in ripening conidiophores of fungus strains after 25 days of cultivation, using the methods of Bacigálová (1982).

RESULTS AND DISCUSSION

Our results showed that cultures of *Aspergillus niger* isolated from permanently polluted soils grown in medium with low concentrations of Hg^{2+} and Cu^{2+} ions caused little inhibition or stimulation of the growth, compared with strains from the non-polluted location. The fungus cultures isolated from fluvisoils in the locality Gabčíkovo (non-polluted soil) were sensitive to all concentrations of Hg^{2+} and Cu^{2+} ions and showed inhibition of fungus growth under "in vitro" conditions. Isolates from the polluted regions Krompachy and Rudňany showed reactions, which are in literature described as "tolerance" for Hg^{2+} ions much as 0.005 $mg.l^{-1}$ and for Cu^{2+} until 0.25 $mg.l^{-1}$ (Fig. 1).

However, the data from literature show a negative effect of heavy metals on many microorganisms and soil micromycetes (Bisessar 1982, Valsecchi et al. 1995). After one year of "in vitro" cultivation no cultures of *Aspergillus niger* from Krompachy and Rudňany showed different grow reactions to Cu^{2+} and Hg^{2+} ions compared with isolates from the non-polluted locality Gabčíkovo. All cultures were sensitive to ion concentrations, which showed an inhibitory effect on the growth of the fungus (Fig. 2).

At the study of the content of cytoplasmic proteins in the mycelium and spores of *Aspergillus niger* we found, that fungus isolates from soils from regions permanently polluted with heavy metals represent other cytoplasmic proteins than cultures from soils of the non-polluted region. The fungus isolates from the locations Krompachy and Rudňany showed the presence of cytoplasmic proteins with a molecular weight of 29 and 34 kD, and also 40 kD in the isolates from the location Rudňany. These cytoplasmic proteins were not found in the fungus isolates from soils of the location Gabčíkovo (Fig. 3).

The qualitative and quantitative changes of cytoplasmic proteins were found after one year *Aspergillus niger* cultivation under the same "in vitro" conditions. In all *Aspergillus niger* cultures the total content of cytoplasmic proteins was lower and had a smaller molecular weight (Fig. 4).

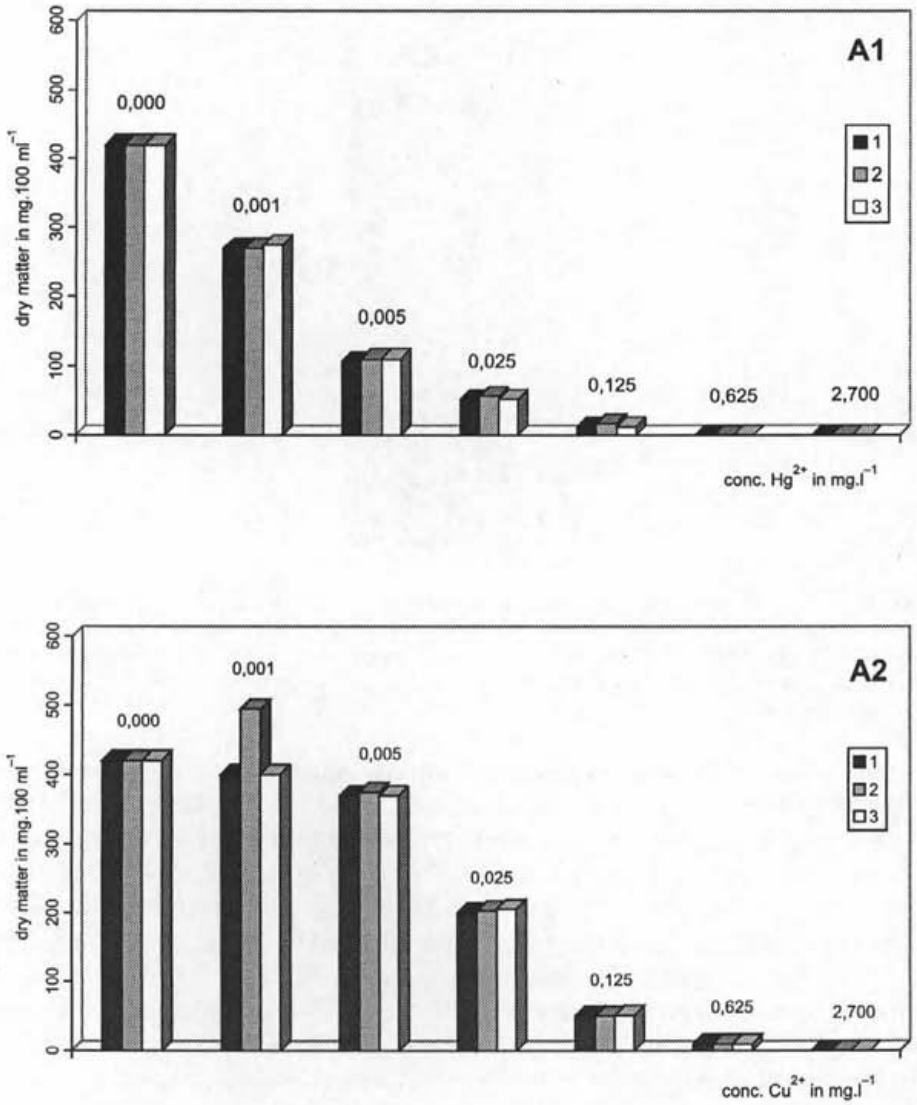

Fig. 2. Effect of Hg^{2+} and Cu^{2+} ions on mycelium growth of *Aspergillus niger* isolates from contaminated and non-contaminated locations in Slovakia. Isolates after one year cultivation "in vitro" conditions.
A1 = Effect of Hg^{2+} ions on mycelium growth of fungus isolates
A2 = Effect of Cu^{2+} ions on mycelium growth of fungus isolates
Ordinata: dry weight of mycelium in mg. 100 ml^{-1} liquid medium
Abscissa: ion concentration Hg^{2+} in $mg.l^{-1}$
ion concentration Cu^{2+} in $mg.l^{-1}$
locations: 1 = Gabčíkovo, 2 = Krompachy, 3 = Rudňany
n = 20, significance P = 0.01, SE = 0.3 or 0.37

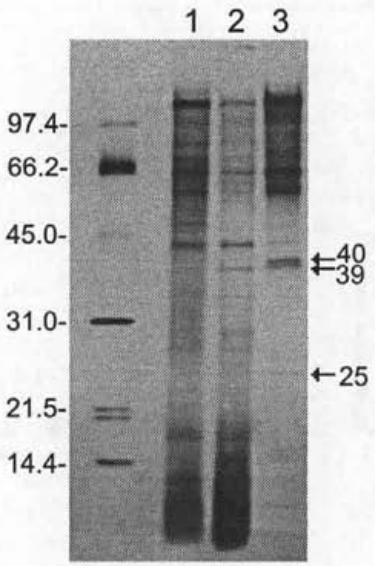

Fig. 3. SDS-PAGE analysis of cytoplasmic proteins from 25 day old cultures (mycelium and spores) of *Aspergillus niger* isolates from non-contaminated (1 = Gabčíkovo) and contaminated soils (2 = Krompachy, 3 = Rudňany) cultivated in liquid Cz-D medium. The molecular mass of marker proteins are indicated on the left (in kD). Arrows indicate heavy metal induced polypeptides n = 5

Our results correspond to the literature data about "stress proteins" by the bacteria *Escherichia coli* after pollution with Cd^{2+} ions (Andreoni et al. 1991, Ferianc et al. 1998). Similarly, in plant roots after pollution with Al^{3+} ions "stress proteins" were identified (Mistrík et al. 1997; Tamás et al. 1997, 1999). It is likely, that bacteria, plants, as well as soil micromycetes produce "stress proteins" after pollution with metals, as a reaction which is responsible for starting mechanisms that eliminate the effect of stress factors. It is likely, that this phenomenon is reversible at micromycetes as showed by the *Aspergillus niger* correlation between tolerance to heavy metal ions and content of high molecular cytoplasmic proteins. We suppose, that *Aspergillus niger* like other microorganisms exposed to heavy metals under "in situ" conditions produce proteins which play an important role in its entire biological activity.

The mycelium growth of isolates from Krompachy and Rudňany on liquid and agar Cz-D medium revealed inhibition of mycelium growth and production of mycelium biomass compared with isolates from the non-polluted region Gabčíkovo (Table 1). The isolates from permanently polluted soils showed also irregular growth of conidiophores and ripening of spores manifested mainly as a grey and grey-brown colour of the upper mycelium (conidiophores) on Cz-D agar fungus culture.

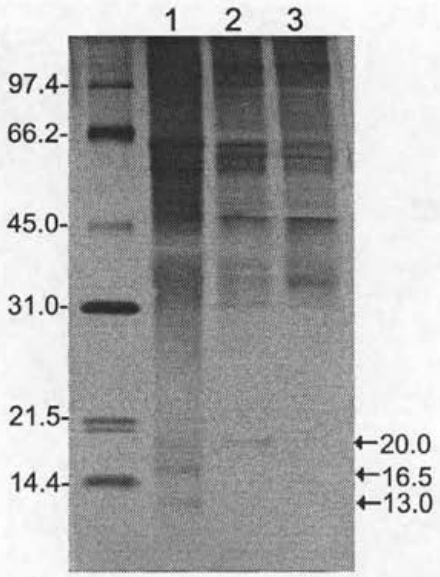

Fig. 4. SDS-PAGE analysis of cytoplasmic proteins from 25 day old cultures (mycelium and spores) of one year old *Aspergillus niger* isolates from non-contaminated (1 = Gabčíkovo) and contaminated soils (2 = Krompachy, 3 = Rudňany) cultivated in liquid Cz-D medium. The molecular mass of marker proteins are indicated on the left (in kD). Arrows indicate reversibly induced polypeptides n = 5

After one year cultivation of fungus cultures under "in vitro" conditions we did not find irregular growth and development of conidiophores and ripening of spores in the heads of the conidiophores. A little irregularity of spore ripening was shown as black spots in the heads of the conidiophores in fungus cultures from the location of Krompachy (Fig. 5). The mycelium growth and biomass formation of fungus isolates from polluted soils and the non-polluted location showed the same result after one year "in vitro" cultivation (Table 1).

Table 1. Mycelium growth and visual sporulation of *Aspergillus niger* isolates after 25 days cultivation under "in vitro" conditions.
n = 20 p = 0.01

Location	Mycelium growth on Cz-D (mm)		Sporulation (%)	
	After isolation	After 1 year	After isolation	After 1 year
Gabčíkovo	70 × 70	71 × 70	85	85
Krompachy	65 × 55	70 × 71	75	85
Rudňany	60 × 50	70 × 71	75	85

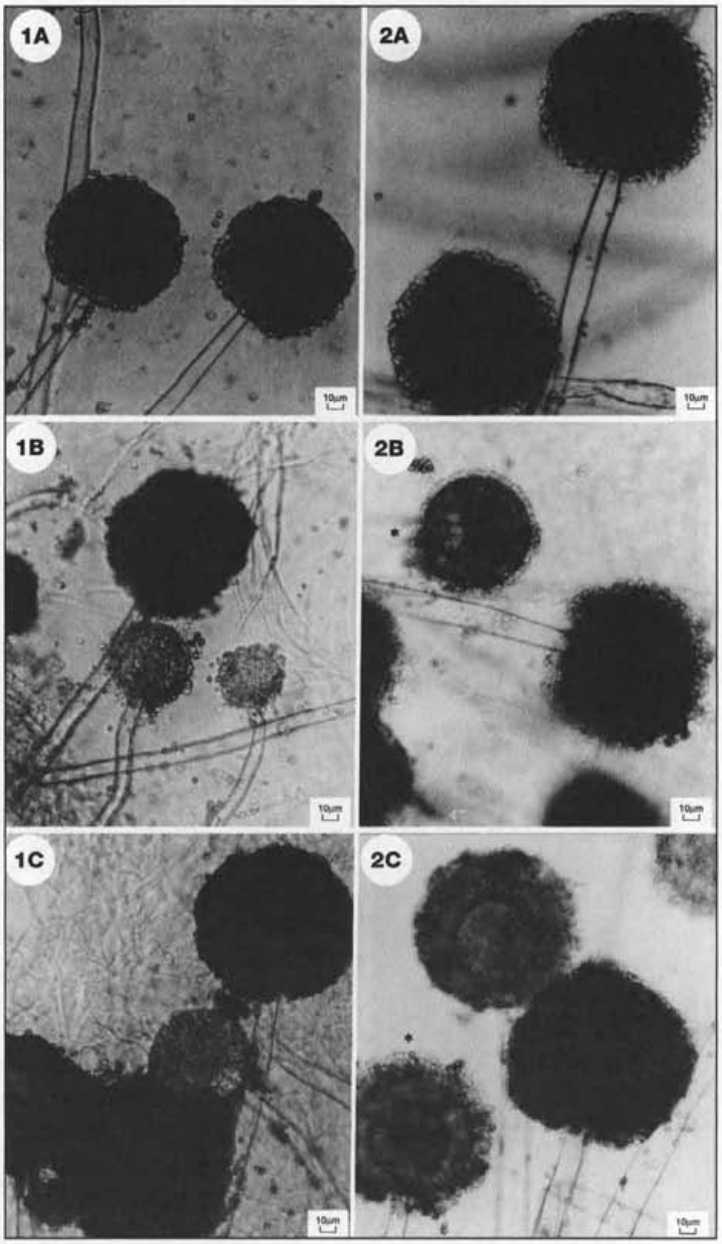

Fig. 5. Mycelium growth and development of conidiophores of *Aspergillus niger* isolates from non-contaminated and contaminated soils on Cz-Dox agar medium.
1. 25 days of cultivation after fungus isolation; 2. 25 days of cultivation of one year old fungus isolates. 2. A = Gabčíkovo, B = Krompachy, C = Rudňany
* black spots (irregular spore ripening) on heads of conidiophores

Anatomical-morphological deformation of *Aspergillus niger* conidiophores under Hg^{2+} and Cu^{2+} influence has been observed by Kocianová & Streško (1999) in the location Nálepkovo. The results are in correlation with the results published by Ashworth & Anin (1964), who examined the mechanism of *Aspergillus niger* tolerance to Hg^{2+} ions.

We assume that our confirmation of introductive "tolerance" of *Aspergillus niger* to heavy metal ions, contribute to the answer on some ecological problems in biological diversity of soil micromycetes (Filip 1999).

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CZECH MYCOL. 54 (3–4), 2003

Doc. RNDr. Zdeněk Hubálek sexagenarian

JIŘÍ KUNERT and MIROSLAV NĚMEC

Zdeněk Hubálek

Dr. Zdeněk Hubálek was born on 22th August 1942 in Brno, Moravia. Since 1959 he attended J. E. Purkinje University (now again Masaryk University) in Brno where he studied biology and chemistry, and graduated in 1964. While still an undergraduate he took great interest in microbiology and participated in research.

He started his professional career at the Fodder Research Institute at Pohořelice near Brno but since 1966, he has continuously worked at the institutes of the Academy of Sciences, namely the Institute of Parasitology, Institute of Vertebrate Research, Institute of Systematic and Ecological Biology, Institute of Landscape Ecology and Institute of Vertebrate Biology. Presently, Dr. Hubálek is senior scientist and deputy director of the Institute of Vertebrate Biology. Since 1999, he is also head of the Institute of Microbiology at the Faculty of Sciences, Masaryk University in Brno.

In 1966, he became a member of the Mycoparasitology Group at the CSAS Institute of Parasitology in Pardubice, together with MUDr. J. Dvořák DSc. and RNDr. M. Otčenášek DSc. In this period, he participated in mycological examinations of human patients and domestic animals. He continued to study microfungi when he later moved to the Laboratory for Natural Focality of Diseases of the Institute of Parasitology at Valtice.

During his PhD work (1969–1971) he began to investigate the ecology of microfungi associated with free-living birds, especially keratinolytic fungi. He also mycologically examined hundreds of small mammals trapped during five expeditions of the Institute of Parasitology in Czechoslovakia and abroad. The results of this research were submitted as the PhD thesis "Dispersal of microfungi by free-living birds" and published in two monographs and 22 papers.

Dr. Hubálek's work on keratinolytic fungi led to the description of a new dermatophyte species – *Microsporum ripariae*, isolated from sand martins (*Riparia riparia*). He also studied the ecology of cellulolytic fungi of the genus *Chaetomium* associated with wild birds and mammals and detected the presence of an opportunistic pathogenic fungus – the basidiomycetous yeast *Cryptococcus neoformans* in pigeon droppings for the first time in Moravia.

The studies by Dr. Hubálek on the ecology of microfungi in soil, wild birds and small mammals were highly appreciated by the scientific community. His critical evaluation of coefficients of association and similarity in fungal and general ecology was published in Biological Reviews (United Kingdom). According to the Science Citation Index, this paper has been cited 55 times.

In cooperation with the Institute of Chemistry of the Slovak Academy of Sciences in Bratislava (Dr. A. Kocková-Kratochvílová DSc.) he developed an efficient technique for long-term cryopreservation of yeasts. His broad experience in methods of preservation of various strains of microorganisms was summarised in the book "Cryopreservation of microorganisms at ultra-low temperatures" that appeared in 1996.

Dr. Hubálek devoted much effort to the biology of *Emmonsia crescens* and the disease adiaspiromycosis, caused by this opportunistic soil fungus mainly in rodents but occasionally also in humans. The presence of *E. crescens* in small animals was studied in many areas of Czechia and Austria and ecological variables determining its distribution have been revealed.

Dr. Hubálek's scientific interests were very broad and not limited to mycology. At present his interests focus on the ecology of arthropod-borne pathogenic bacteria and viruses, medical zoology, and cryobiology. However, he is still investigating the ecology of the dimorphic pathogenic fungus *Emmonsia crescens*.

As a renowned scientist he published over 220 scientific papers, one book and contributed to eight international monographs. A list of congresses and conferences held abroad in which he took an active part would be very long. In this way he stayed in seven European microbiological laboratories and participated in several field expeditions to study natural foci of diseases in Austria, Bulgaria and the former Yugoslavia.

Dr. Hubálek has been a member of the Czech Scientific Society for Mycology since 1967 and participated in the activities of several other Czech scientific societies. He is also a regular member of the International Society for Human and Animal Mycology. The results of his scientific research were appreciated many times. He received the Purkinje medal for achievements in biological sciences (1988) and three awards of the Czechoslovak Academy of Sciences for collections of papers (the first two collections being mycological).

At present, he is a member of the Scientific Council of the Academy of Sciences of the Czech Republic, Scientific Council of the Faculty of Sciences of Masaryk University, and commissions for postgraduate studies at three universities.

Our contribution, focusing on Dr. Hubálek's mycological activities does not mention further fields of his scientific interest, his like and hobbies. It is our sincere wish that he will, in the many years ahead, retain his subtle sense of humor and above all, his appetite for life and desire to do creative work.

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Errata

Czech Mycology 53(4), 2002, article by J. Gönczöl and L. Marvanová:
p. 311, bottom line: 50 μm (instead of 50 mm: printing error)
plate between pages 312 and 313, bottom line: 30 μm, 50 μm (instead of 30 mm, 50 mm: printing error).

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Preparation of manuscripts. Manuscripts are to be submitted in English, German or French. The text of the manuscript should be written on one side of white paper (A4, 210 × 297 mm) with broad margins (maximum 30 lines per page). Each manuscript must include *an abstract* (in English) not exceeding 100 words and a maximum of five key words. The paper will be followed by an abstract in Czech (or Slovak). The journal is responsible, however, for the translation of abstracts into Czech for foreign authors. Please send *two copies* of the typescript. The authors are asked to submit diskettes with *the accepted manuscripts* prepared on personal computers. The files should be in ASCII format, graphs in Excel. Both HD and DD/3.5″ and 5.25″ diskettes are acceptable.

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Nomenclature. Latin names should conform to the International code of botanical nomenclature. New taxa must be substantiated by a Latin diagnosis including a reference to the public herbarium where the type specimen is deposited. The authors are asked to use only the acronyms listed in the Index Herbariorum.

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Moravec J. (1984): Two new species of Coprobia and taxonomic remarks on the genera Cheilymenia and Coprobia (Discomycetes, Pezizales). – Čes. Mykol. 38: 146–155. (*journal article*)

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