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CZECH MYCOLOGY

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Fifty years of our Journal

Shortly after the foundation of our Society in 1946, the first issue of "Česká mykologie" (Czech Mycology) appeared, in 1947. Both the Society – named Československý mykologický klub (Czechoslovak Mycological Club) – and the Journal were continuations of the Journal "Mykologia" edited by J. Velenovský in the years 1924–1931 and of the Czechoslovak Mycological Club, founded in 1922 as well by J. Velenovský, which existed up to the Second World War when it terminated its activity.

Česká mykologie started with an editorial committee of the four leading personalities who founded it: Dr. Albert Pilát, Prof. dr. Karel Cejp, dr. Josef Herink and Ivan Charvát. Later the committee was extended and after the death of I. Charvát 1959 dr. Mirko Svrček took up the function of managing editor, a position which he held up to 1992. Dr. A. Pilát worked as editor in chief from 1952 up to his death in 1974. Prof. dr. Z. Urban succeeded him in this position in 1975 and continued until 1993.

The first volumes of the Journal "Česká mykologie" were devoted partly to a popularisation of mycology and partly also to articles with an original scientific content. The Journal has published in this period only in Czech language, articles with a content of broad interest had short summaries in English, German, Latin or French. Česká mykologie gradually started to accept contributions of purely scientific content. From 1963 Česká mykologie became a journal with a mixture of articles of domestic importance and international interest. During the years a number of papers on the discoveries of new species and of new genera appeared, some of which are now in current use in Europe or world-wide. Of little importance were articles on physiology and pathology, as specialists where not accustumed to look for these problems in this journal. Nevertheless, ecology and geographic distribution of fungi, even if only few articles where devoted to this item, attracted the attention of readers. In 1993 the editorial board decided to change its policy and transformed the journal in to an international scientific periodical in which only articles in world languages, preferably in English, are published. With the Volume 46 a new era of Journal started under the title Czech Mycology. We hope to continue fulfilling the expectations of our readers and preserving the scientific level.

Zdeněk Pouzar

CZECH MYCOL. 50 (1), 1997

Fontanospora fusiramosa sp. nov., a hyphomycete from live tree roots and from stream foam

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Marvanová L., Fisher P. J., Descals E. and Bärlocher F. (1997): Fontanospora fusiramosa sp. nov., a hyphomycete from live tree roots and from foam.– Czech Mycol. 50: 3–11

Fontanospora fusiramosa is described from *Alnus* roots and from stream foam. It is based on isolates from the U. K., Canada and the Czech Republic.

Key words: *Fontanospora*, aquatic hyphomycetes, endophytes, streams.

Marvanová L., Fisher P. J., Descals E. a Bärlocher F. (1997): Fontanospora fusiramosa sp. nov., hyfomycet z živých kořenů olše a z pěny z potoků.– Czech Mycol. 50: 3–11

Je popsán nový druh rodu *Fontanospora* (mitosporní houby, hyfomycety), vyskytující se jako endofyt v kořenech olše. Jeho konidie bývají také nalézány v pěně v potocích, zejména na slatinných lokalitách. Druh byl izolován v Anglii, v Kanadě a v České republice.

INTRODUCTION

Fontanospora Dyko (1978) was based on *Tricladium eccentricum* R. H. Petersen, differing from the heterogeneous *Tricladium* Ingold by its subopposite conidial branching. Hitherto three species were described: *F. eccentrica* (R. H. Petersen) Dyko 1978 (type species of the genus), *F. alternibrachiata* Dyko 1978 and *F. minima* Ando 1993. We are describing a fourth species, which appears in stream foam in cold climates and is capable of endophytic existence in submerged *Alnus* roots.

MATERIALS AND METHODS

The ex-type culture of our new species was isolated from roots of *Alnus glutinosa* (L.) Gaertn. growing under water. The root pieces were washed in running water prior to surface sterilization by immersion in 75 % ethanol for 1 min., in a 0.93–1.3 M solution of sodium hypochlorite (3–5 % available chlorine)

for 3 min. and in 75 % ethanol for 5 min. Root segments were then placed onto 1.5 % Oxoid malt extract agar and incubated at 20 °C. Isolations were made from hyphal tips which grew into the agar. The other three cultures were monoconidial isolates from stream foam. Sporulation was observed on submerged pieces of agar cultures in standing sterile distilled water at 15 °C in daylight or diffuse artificial light, or in aerated distilled water at 10 or 18 °C.

TAXONOMY AND DISCUSSION

Fontanospora fusiramosa Marvanová, Fisher et Descals, sp. nov.

Figs 1–3

Fungi mitosporici, hyphomycetes. Teleomorphosis ignota.

Coloniae in agaro maltoso pallide brunneolae, modice crescentes, glabrae, cum mycelio aerio adpresso interdum funiculoso in centro coloniae, nonnullae roseolae si submersae sub aqua in luce. Cellulae inflatae hyalinae, elongatae vel globosae, tenuitunicatae vel crassitunicatae, catenatae vel aggregatae, nonnumquam in sclerotiis minutis in mycelio adsunt. Conidiophora singularia, usque ad c. 600 μm longa, illa curta parce, illa longa valde ramosa, ramis acrotonis. Cellulae conidiogenae incorporatae vel discretae, usque ad ternae, apicales vel postea intercalares, polyblasticae, saepe cum conidiis concurrentes, 17–50 × 3–4 μm. Conidia fasciculata, raro singularia, saepe bina vel terna, in successione crescentia, 'tetraradiata', cum elementis septatis, apicibus subulatis. Axis (30–)45–98(–120) × 2.5–4.5 μm, inter ramos typice flexus et ibidem attenuatus; pars proxima fusoidea vel cylindrica, saepe brevior, extensio basalis abest vel brevissima; pars distalis anguste obclavata. Rami typice duo, laterales, sub centro vel prope partem tertiam inferiorem axis crescentes, subopposite, obclavati, insertione constricta; ramus proximus (10–)25–63(–75) × 2.5–5 μm, ramus distalis (7–)15–48 × 2–5 μm.

Habitat: in radicibus submersis arboris *Alnus glutinosa* in flumine Dart loco Dartmoor dicto, Devon, Anglia.

Holotypus: IMI 374530 (praeparatum e cultura artificiali P. J. Fisher No. 57 = CCM F-10096)

Mitosporic fungi, hyphomycetes. Teleomorph unknown.

Colonies on 2 % malt agar (MA) pale beige, growing moderately fast, glabrous, with appressed aerial mycelium, or slightly funiculose in the centre, pale beige, in some isolates pinkish when submerged under daylight. Inflated cells elongate or globose, thin- or thick-walled, hyaline, c. 12 μm diam., in chains or clusters on the mycelium, in CCM F-12089 aggregated in small colourless soft sclerotia up to 350 μm diam. Conidiophores single, up to c. 600 μm long, branching profuse, acrotonous, or sparse. Conidiogenous cells integrated or discrete, up to three per

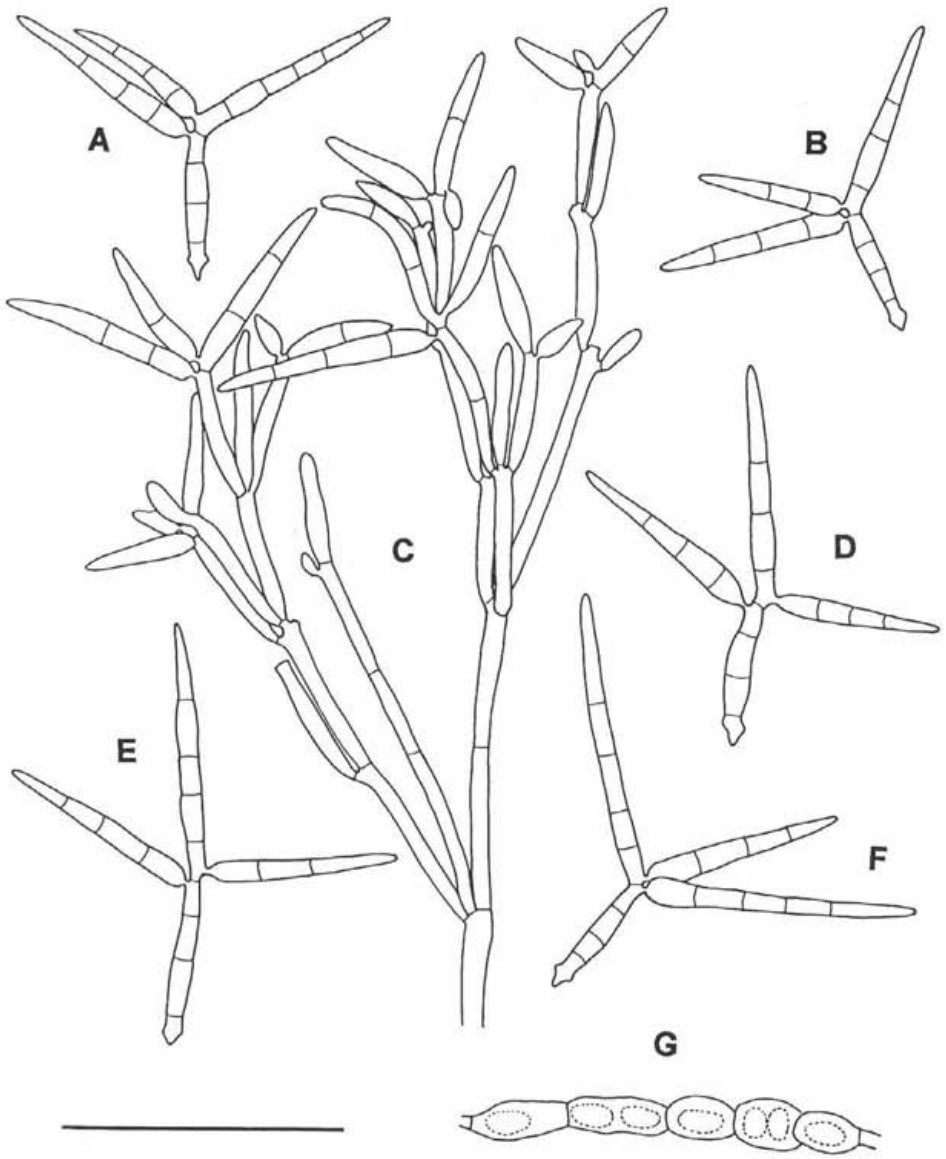

Fig. 1. *Fontanospora fusiramosa*, type. A,B,D-F, conidia. C, conidiophore with developing conidia and spent conidiogenous cells. G, inflated cells. From 10 day old standing water culture. Scale = 50 μm.

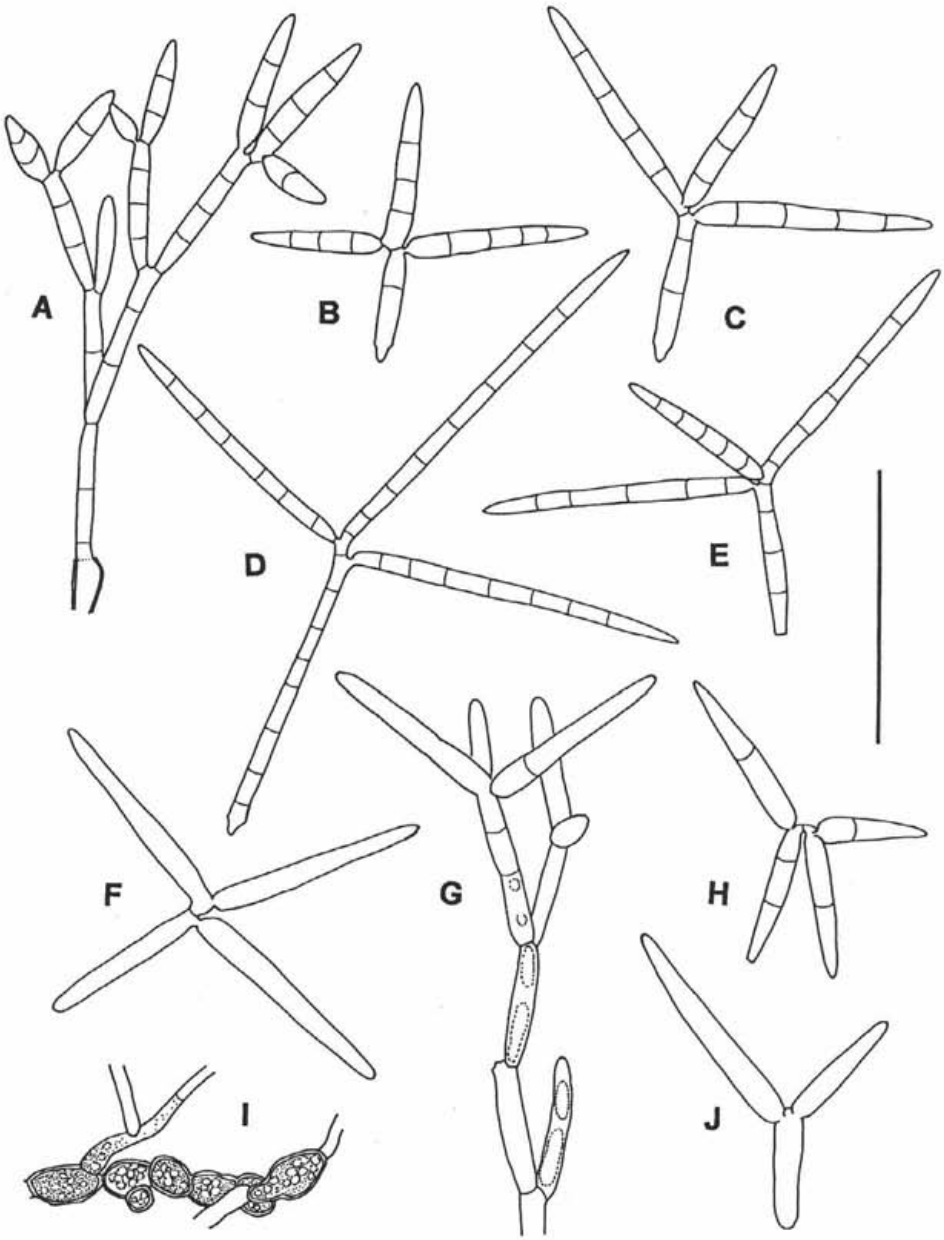

Fig. 2. *Fontanospora fusiramosa*. A-E, CCM F-12089, from standing water culture. A, conidial development. B-E, detached conidia. F-J, E. Descals A212–1–8. G, conidial development. F, H,J, detached conidia. I, inflated cells. Scale = 50 μm.

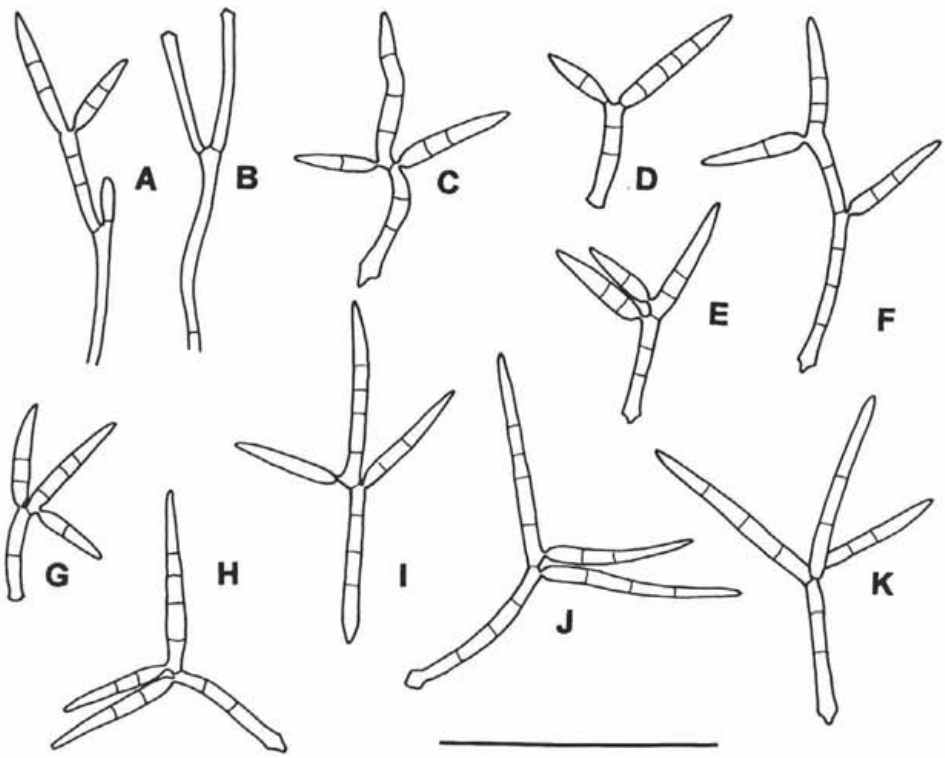

Fig. 3. *Fontanospora fusiramosa* CCM F-21687 after 4 days' aeration. A, conidiophore with developing conidium. B, spent conidiophore. C-K, detached conidia. Scale = 50 μm.

conidiophore branch, apical or becoming intercalary, polyblastic, often concurrent with conidia, 17–50 × 3–4 μm. Conidia in fascicles (rarely single), usually 2–3 per conidiogenous cell, closely sequential, 'tetraradiate', elements septate, ends subulate. Axis (30–)45–98(–120) × 2.5–4.5 μm, typically bent and attenuate between branch insertions, with a septum in the narrowed portion; proximal part fusoid or cylindrical, usually shorter, basal extension lacking or short, typically percurrent; distal part of axis narrowly obclavate. Branches typically two, diverging in different planes, submedian or more often inserted in the lower third of the

axis, subopposite, on either side of the attenuation, obclavate, insertion strongly constricted; proximal branch (12–)25–63(–75) × 2.5–5 μm, distal branch (7–)15–48 × 2–5 μm. Aberrant conidia appear in all our isolates; they may be single-branched (Figs 2 J, 3 D), or with remote branches (Fig. 3 F), or with two subopposite and one remote branche.

Table 1. Lengths of conidial elements (in μm) of five isolates of *F. fusiramosa* (15 conidia of each isolate measured)

Isolate	Axis	Proximal branch	Distal branch
Type	51–88	32–52	24–47
P. Fisher TS	54–74	29–47	17–39
CCM F-21687	30–74	10–37	7–30
CCM F-12089	55–98(–120)	27–63(–75)	20–48
E. D. B12–2–8	45–70	25–40	15–33

Material examined: P. J. Fisher No. 57 (= CCM F-10096), from aquatic roots of *Alnus glutinosa* collected in the River Dart, Dartmoor National Park, Grid Ref. SX 713 711, Devon, U. K., Sept. 1995, P. J. Fisher. P. J. Fisher TS, same data as No. 57 (conidia occurred as admixture in culture of another endophyte). E. Descals A212–1–8, foam from wooded stream flowing off acid moorland, R. Dundonell, nr. Gairloch, N. W. Scotland, U. K., May 1974, E. Descals and J. Webster. CCM F-21687, from foam in a roadside ditch lined with shrubs in a moorland (very slow flowing water), Rock Point, near Sackville, New Brunswick, Canada, Apr. 1987, L. Marvanová and F. Bärlocher. CCM F-12089, from foam in the right tributary of the river Svratka near the road between Herálec and Kadov, in a forest with prevailing *Picea abies*, c. 500 m alt., West Moravia, Czech Republic, May 1989, L. Marvanová.

The ex-type and Czech isolates produce a pinkish water-insoluble pigment in the superficial layer of the colony when submerged and exposed to daylight. The larger conidia of the Czech isolate (Fig. 2 D) have less tapering elements, thus resembling *F. eccentrica*. However, conidial shapes typical of *F. fusiramosa* prevail. The Canadian isolate (Fig. 3) has conidia with narrower elements and the lower part of the axis is often parallel-walled rather than fusoid. However, its conidial branches and the distal part of the axis in developing conidia do have the typical form of *F. fusiramosa*. This isolate sporulated only in an aerated culture and the simpler conidiophores, relatively short conidial branches and frequent single-branched conidia may be a consequence of those conditions. Shorter conidial elements in aerated versus standing culture have been seen also in the ex-type

isolate; under the latter conditions axis and branches longer by 10–20 % could be observed. Thick-walled inflated cells (Fig. 2 I) occurred only in the E. Descals isolate.

The habitats of this species imply its association with aquatic environment. However, the fungus has not been found on substrates common for freshwater hyphomycetes. i.e. submerged leaves or woody debris. The ex-type culture, E. Descals A212–1–8 and the Canadian strain were isolated from moorland habitats, the Czech one was obtained from a stream on acid bedrock.

Conidia of this new species have been recorded from foam in an acidic stream lined by *Alnus* in Gredos Mountains in central Spain (Descals *et al.* 1995, Fig. 3 F, as *Fontanospora eccentrica*). Most probably they also have been depicted by Aimer and Segedin (1985, Fig. 3 H,K, as *F. eccentrica*) from stream foam in New Zealand: Fig. H from a fast clean medium-sized stream flowing through an undisturbed Podocarp-broadleaf forest (370 m alt.) and Fig. K from a moderately fast, small, clean mountain stream (1340 m alt.).

F. eccentrica (Fig. 4) differs from *F. fusiramosa* by the typically cylindrical shape of the conidial elements, and by the blunt, sometimes slightly swollen, conidial ends. *F. eccentrica* and *F. fusiramosa* conidia overlap significantly when we include the extreme values of CCM F-12089 (tab. 1). However, most conidia of *F. fusiramosa* have a 50–100 μm long axis, whereas in *F. eccentrica,* according to our experience, this is frequently over 120 μm long.

F. alternibrachiata, according to the protologue, is similar to *F. eccentrica* but has much larger conidia. It cannot be confused with *F. fusiramosa.*

F. minima was described recently from leaf litter in a terrestrial habitat in Japan. It differs from the generic concept accepted by Dyko in its micronematous conidiophores and in the basipetal sequence of conidial branches. Its conidia are minute, not exceeding 21 μm across. Moreover, Ando (1993) interpreted the branching pattern as an axis and three branches, which would imply the presence of one secondary branch and one retrogressive second primary branch. Even if the branching is perceived as one axis and two laterals, the retrogressive sequence of the laterals still remains. Such branching pattern would be unusual in *Fontanospora.* In our opinion *F. minima* should be excluded from *Fontanospora*, but without having seen the type or authentic material, we hesitate to make any formal changes. We see an overall similarity of *F. minima* with *Articulospora ozeensis* Matsushima (1975), a leaf litter fungus with conidia of similar size, but consisting of an axis and three sequential, primary, coronate branches. According to Ando and

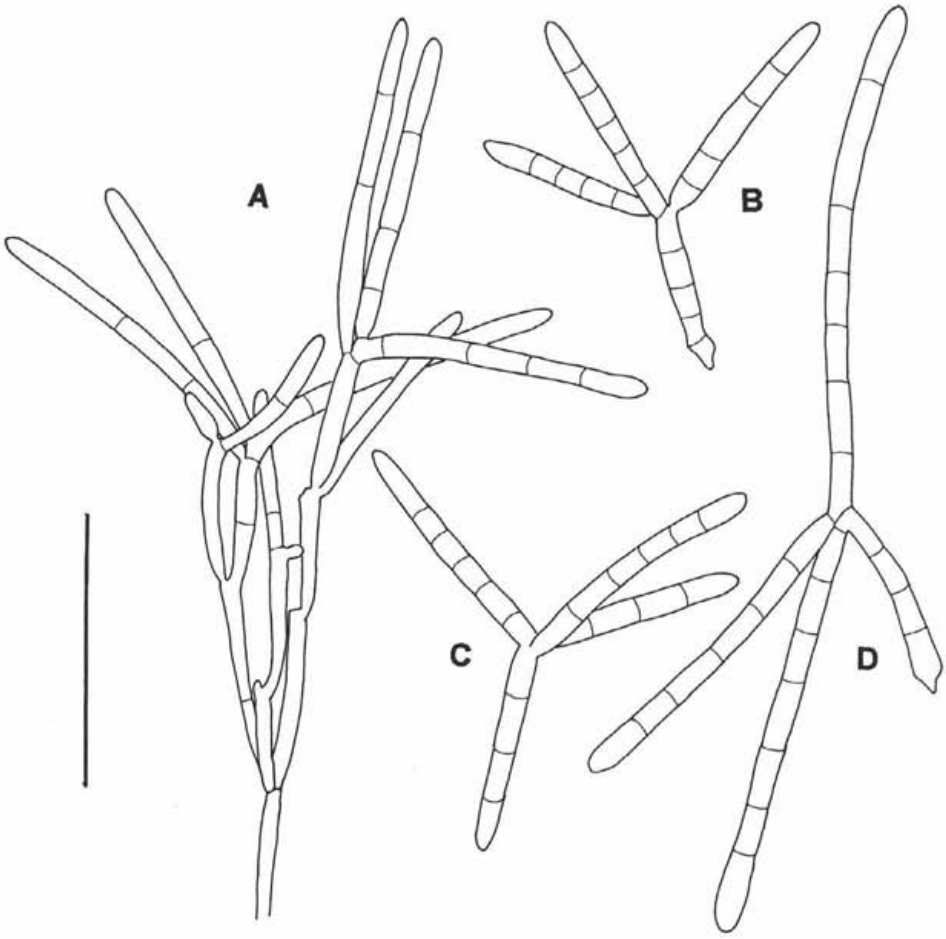

Fig. 4. *Tricladium eccentricum*, type. A, conidial development. B-D, conidia. Scale = 50 μm.

Tubaki (1983) it is not properly accommodated in *Articulospora* Ingold. There is a superficial similarity of the conidia of *Fontanospora minima* with those of *Sympodiocladium frondosum* Descals (Descals and Webster 1982), but the latter has a progressive sequence of one primary and one secondary lateral branches and a different conidiogenesis. *Sympodiocladium* is also unique in the strongly restricted, orange, later dark purple colonies, producing a bluish diffusing pigment on 2 % MA.

Acknowledgements

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CZECH MYCOL. 50 (1), 1997

Triparental species hybrids from fused zoospores of Phytophthora

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Érsek T., English J. T. and Schoelz J. E. (1997): Triparental species hybrids from fused zoospores of Phytophthora. – Czech Mycol. 50: 13–20

Genetic exchange among three heterothallic *Phytophthora* spp., *P. nicotianae, P. capsici* and *P. citrophthora* each representing mating type A2, was induced via zoospore fusion. Viable offspring colonies that developed following fusion expressed differential drug resistance of each parental mutant. Detection of DNA with species specific sequences and by means of the polymerase chain reaction confirmed somatic hybrid formation in one of three isolates. By overcoming sexual incompatibility of zoosporic fungi, somatic fusion now improves access to direct study of molecular aspects of population variability.

Key words: Genetic markers, PCR, *Phytophthora*, somatic fusion, zoospores.

Érsek T., English J. T. and Schoelz J. E. (1997): Triparentální mezidruhový hybrid získaný pomocí fúze zoospor Phytophthora spp. – Czech Mycol. 50: 13–20

Genetická výměna mezi třemi heterothalickými druhy *Phytophthora: P. nicotinae, P. caspici* a *P. citrophthora*, z nichž každý reprezentuje párovací typ (mating-typ) A2, byla realizována pomocí fúze zoospor. Vitální kolonie potomstva, které se vyvíjely po fúzi, vykazovaly expresi diferencující rezistence k antibiotikům u každého rodičovského mutantu. Detekce DNA pomocí specifických druhových sekvencí a pomocí polymerázové řetězové reakce potvrdila tvorbu somatických hybridů u jednoho ze tří izolátů. Díky překonání sexuální inkompability zoosporických hub, poskytuje nyní somatická fúze možnost přesného studia molekulárních aspektů populační variability.

INTRODUCTION

Of great interest is the possibility that related species of pathogenic fungi exchange genetic material when they infect a common host. Genetic exchange among *Phytophthora* species via sexual processes has been suggested or described by several authors (Boccas 1981; Brasier 1992; Goodwin and Fry 1994; Sansome et al. 1991). It also has been suggested that somatic hybridization may occur in nature, and that the process may be a means of bypassing the need for sexual reproduction in species that are heterothallic and lack compatible mating types (Brasier 1992; Érsek et al. 1993). Although somatic hybridization might be an important source of variation in some populations of *Phytophthora* species, such hybrids have not been proven to exist in nature; neither have they been created by conventional methods such as hyphal anastomosis or protoplast fusion (Layton and Kuhn 1988).

In a recent study we described a protocol for creating species hybrids between non-compatible mating types of *Phytophthora capsici* Leonian and *P. nicotianae* Breda de Haan (syn. *P. parasitica*) (Érsek et al. 1993, 1995). The approach used was based on the induced fusion of zoospores. The same technique was applied in the present study to create triparental hybrids of *P. capsici*, *P. nicotianae*, and *P. citrophthora* (Sm. et Sm.) Leonian. These are all pathogenic fungal species that have overlapping host ranges.

MATERIALS AND METHODS

Fungal isolates and culture. Isolates W1, 15399, and P1323 of *P. nicotianae*, *P. capsici* and *P. citrophthora*, respectively, were obtained from J. M. Duniway (University of California, Davis). A unique drug resistant mutant of each isolate was derived by chemical mutagenesis and subsequent screening for drug sensitivity, based on modified methods of Joseph and Coffey (1984). These modifications, as well as methods of maintaining and incubating cultures, and inducing zoospore release, were described previously (Érsek et al. 1994a). Each mutant isolate used in these studies expressed a unique and stable drug resistance phenotype. Mutant isolates, *P. nicotianae* Fpa^r10, *P. capsici* Mex^r5 and *P. citrophthora* Gen^r10 were resistant to p-fluorophenylalanine (Fpa), metalaxyl (Mex) and geneticin (Gen), respectively.

Fusion and regeneration of zoospores. Zoospores were fused using a protocol described previously (Érsek et al. 1995). Equal aliquots of zoospore suspensions (10^6 spores/ml) of each mutant isolate were combined in a fusion solution containing 30% polyethylene glycol (PEG 3350) and 50 mM LiCl. To induce encystment, aggregated and fused zoospores were transferred to encystment solution that consisted of 5 mM $CaCl_2$ and 500 mM KCl in 100 mM sorbitol. Spores in encystment solution were dispersed in molten pea-extract agar without drug amendments and incubated at 25 °C.

Selection of hybrids. After 24 h of incubation, the nonamended pea-extract medium containing zoospores was overlaid with the same medium amended with all three drugs of parental resistance at concentrations of 100, 25 and 15 mgL^{-1} of Fpa, Mex and Gen, respectively. Two to three days later, these plates were overlaid with a final layer of the medium supplemented with 200, 50, and 30 mgL^{-1} of Fpa, Mex and Gen, respectively. These drug concentrations were fully inhibitory to each parental mutant isolate. After 8 to 10 days of incubation on this medium, the fastest growing colonies were transferred to V-8 juice agar that contained the three drugs at the highest concentrations. Colonies that showed abnormal growth, sectoring or other indicators of instability, were discarded. The remaining putative somatic hybrids were stable and expressed the triple drug resistance for over one year in the absence of selection pressure. The hybrids were evaluated for

sporulation and pathogenicity on hosts for parental isolates as described previously (Érsek et al. 1994a, 1995).

Molecular evaluation of hybrids. The hybrid nature of selected isolates was confirmed by detection of parental, species-specific DNA sequences. DNAs were digested with appropriate restriction enzymes, electrophoresed in agarose gel, transferred to nitrocellulose membrane, and hybridized with ^{32}P-labelled probes as described by Sambrook et al. (1989). Plasmids pPP33A and pCIT15A (Érsek et al. 1994b), and pCAP12 (Érsek et al. 1995) were used as species-specific probes for *P. nicotianae, P. citrophthora* and *P. capsici*, respectively. Plasmids pPP33A and pCIT15A were derived from pUC18 into which had been subcloned a 1300-bp or 800-bp sequence specific to repetitive chromosomal DNA from *P. nicotianae* or *P. citrophthora*, respectively. Plasmid pCAP12 was derived as pUC18 containing a 2000-bp insert of repetitive DNA specific to *P. capsici*. DNA sequences of *P. nicotianae* and *P. citrophthora* were amplified by PCR using 24-bp primer-pairs derived from the species specific sequences under conditions reported elsewhere (Érsek et al. 1994b, 1995).

Additionally, 10-base oligonucleotides for RAPD-PCR were selected arbitrarily and used for RAPD-PCR (Williams et al. 1990). Primers were designated as follows: OPG-01, OPG-05 and OPG-10 and OPK-03, OPK-04 and OPK-13 (Operon Technologies, Inc.). In a search for polymorphic DNA sequences representing each parental mutant in the hybrid, reactions were cycled with an automated thermal cycler (Hybaid, model HB TR1). The primers (20 pmoles) were mixed with the reaction buffer, $MgCl_2$ (2 mM), dNTPs (200 mM each), Taq DNA polymerase (2.5 units), fungal DNA (100 ng) and sterile, glass distilled water in a total volume of 50 ml. The thermal cycler was programmed for 44 cycles of 94 °C for 1 min, 36 °C for 1 min and 72 ° min, preceded by one cycle with an extended, 5 min denaturation at 94 ° amplification products were resolved by electrophoresis in 1.2% agarose gels and stained with ethidium bromide.

Results

Phenotypic characterization of putative hybrids. Triple-drug resistant colonies were recovered from amended pea-extract agar at a frequency of $5–8 \times 10^{-6}$. No triple-drug resistant colonies were recovered on plates that had been inoculated with spores that had not been treated with the fusion solution.

Three representative isolates, obtained from two fusion experiments and designated H8, H14 and H20, were retained for further analyses. Morphological traits of each isolate were most similar to those of the *P. nicotianae* Fpa^{r}10 (Fig. 1). On drug-free medium, colony growth rates of hybrids varied from 40 to 50% of those of parental species. On medium amended with the three drugs at highest

concentrations, hybrids grew approximately half as fast as did their respective parental species on medium with appropriate selective drug (Table 1).

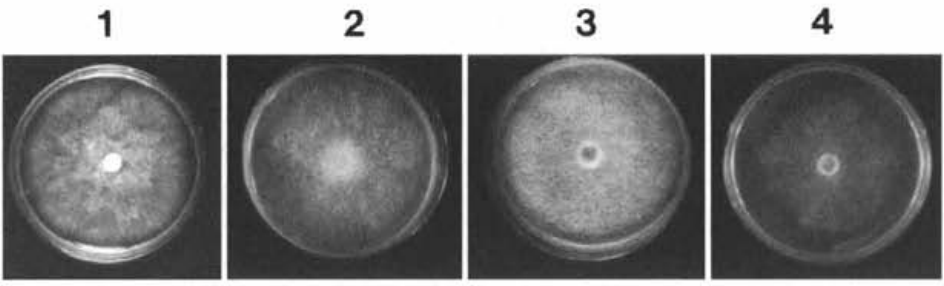

Fig. 1. Colony morphology of parental and hybrid *Phytophthora* isolates. In lanes 1, *P. capsici* Mex^{r}5; 2, *P. nicotianae* Fpa^{r}10; 3, *P. citrophthora* Gen^{r}10 parental mutants and 4, triparental hybrid, H20, after 8 days of growth at 25 °C on V8 medium supplemented with 50 mg l^{-1} of metalaxyl (Mex), 200 mg l^{-1} of fluorophenylalanine (Fpa), 30 mg l^{-1} of geneticin (Gen) and with the three drugs together at the indicated concentrations, respectively.

Table 1. Comparative growth of drug resistant mutants of *Phytophthora* spp. and their triparental hybrids under drug pressure.

Drug, mg l^{-1}	Radial growth (mm) of isolate[a]					
	PpFpa^{r}10	PcMex^{r}5	PciGen^{r}10	H8	H12	H20
None	19[b]	22	18	7	10	10
Fpa, 200	15	0	0	7	8	8
Mex, 50	0	22	0	7	9	9
Gen, 30	0	0	16	7	8	8
Fpa/Mex/Gen[c]	0	0	0	6	8	8

[a]Abbreviations: Pp, Pc and Pci denote *P. nicotianae, P. capsici* and *P. citrophthora*, respectively; H8, H12, H20 are hybrids; Fpa, Mex and Gen denote fluorophenylalanine, metalaxyl and geneticin, respectively.

[b]Growth measurements were made after 4 days of growth at 25 °C on V-8 medium.

[c]Concentration of each drug is the same as that of individual drugs.

None of the hybrids could be induced to produce sporangia, and thus, zoospore progeny could not be evaluated for similarities to parent isolates. The pathogenicity phenotypes of parental mutants were not retained in hybrids. Neither tomato (the common host of all parental microorganisms), or lemon fruit (common host of *P. citrophthora* and *P. nicotianae*), nor the storage taproot of radish (host of

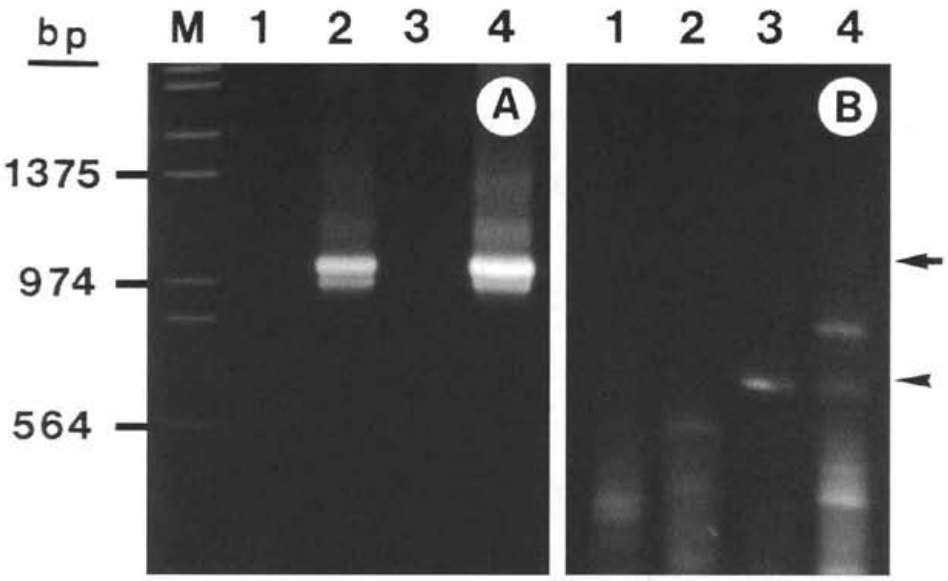

Fig. 2. Parental DNA sequences in triparental hybrid following PCR amplification of 1 μg template (total genomic) DNA with the respective species specific primer pairs. (A) Occurrence of the *P. nicotianae*-specific, 1000-bp DNA sequence (arrow). (B) Occurrence of the *P. citrophthora*-specific, 650-bp sequence (arrowhead). Sources of DNA in lanes 1, *P. capsici* Mex^{r}5; 2, *P. nicotianae* Fpa^{r}10; 3, *P. citrophthora* Gen^{r}10 and 4, hybrid H20.

P. capsici) exhibited any disease symptoms or a hypersensitive resistance response following inoculation with the hybrids.

Genotypic characterization of hybrids. The formation of hybrids was confirmed by detection of parental DNA sequences. When total genomic DNAs from hybrids and parent organisms were digested with EcoRI/XhoI and probed with pPP33A containing the *P. nicotianae*-specific, repetitive DNA sequence, multiple bands were visualized in *P. nicotianae* and, at low intensities, in the hybrid isolates. In contrast, when DNA was digested with PstI and probed with pCIT15A containing the *P. citrophthora*-specific repetitive sequence, hybridization was detected in P. citrophthora Gen^{r}10 only. Similarly, pCAP12 containing the *P. capsici*-specific repetitive sequence hybridized only with total DNA from the *P. capsici* Mex^{r}5 after digestion with HaeIII; hybridization with putative hybrids was not observed (data not shown).

The species-specific primer-pairs derived from pPP33A amplified the 1000-bp, P. nicotianae-specific sequence in all the hybrids, but the primer-pairs from pCIT15A amplified the 650-bp, *P. citrophthora*-specific sequence in the parental isolate and only hybrid isolate H20 (Fig. 2). With two exceptions, all of the

tested 10-base primers that produced various levels of polymorphisms of randomly amplifying DNA sequences of parental isolates, amplified only *P. nicotianae*-characteristic sequences in the hybrids. However, primer OPK-04 amplified DNA sequences characteristic of both *P. nicotianae* and *P. citrophthora* in the hybrids (data not shown). Additionally, amplification of DNA from one of the hybrids, H20, with primer OPG-05 resulted in detectable sequences of *P. nicotianae* and *P. capsici* (Fig. 3).

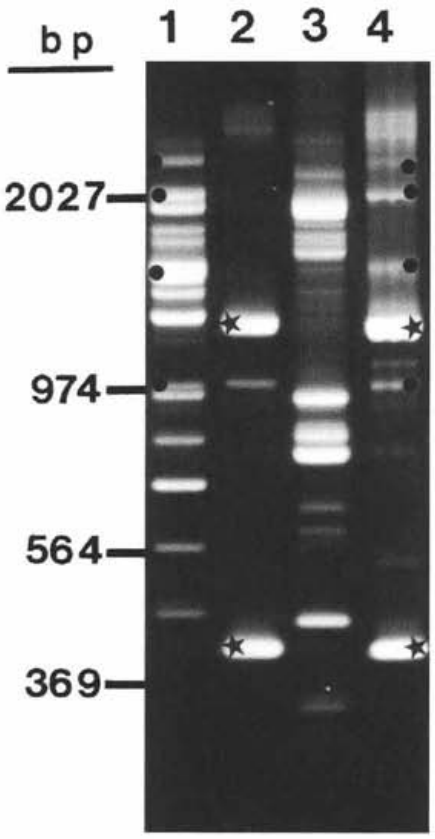

Fig. 3. RAPD patterns of parental and hybrid isolates following PCR with arbitrary 10-base primer OPG-05. Sources of DNA in lanes 1, *P. capsici* Mex^{r}5; 2, *P. nicotianae* Fpa^{r}10; 3, *P. citrophthora* Gen^{r}10 and 4, triparental hybrid H20. DNA bands in the hybrid that correspond to those of *P. nicotianae* and *P. capsici* are differentially marked.

Discussion

Phytophthora species vary in their abilities to reproduce sexually and generate genetic variability. Some species, such as *P. cactorum* or *P. sojae*, are homothallic organisms that reproduce by selfing. Other species, including *P. nicotianae, P. capsici* and *P. citrophthora* are heterothallic and outcross if compatible mating types come into contact with each other. Often in nature, only one mating type of a species will occur at a specific geographic location. Under these circumstances, interspecific somatic hybridization has been suggested as a mechanism of importance in generating genetic variability within a single mating type of a species (Brasier 1992). Evidence for this phenomenon has been provided only recently (Goodwin and Fry 1994; Sansome et al. 1991).

It is not known to what extent somatic hybridization occurs among the species in this study, but we have been able to examine the consequences of the event by creating interspecific hybrids *in vitro*. We reported the first proof that somatic hybrids of this sort between *P. capsici* and *P. nicotianae* can be created via induced fusion of zoospores (Érsek et al. 1993; Érsek et al. 1995). Zoospore fusion was achieved by a novel technique utilizing Li^+ as a key component in the procedure (Érsek et al. 1991). The present study extends the utility of zoospore fusion methods to create hybrids from three parents, *P. nicotianae, P. capsici* and *P. citrophthora*.

On the basis of morphological traits and drug resistance patterns, several putative triparental hybrids were created. Goodwin and Fry (1994) stressed the importance of molecular evidence to confirm the hybrid nature of such organisms. This proved to be an important step in our study, in that only one of three putative hybrids, based on morphology and drug-resistance, contained detectable sequences of all three parental organisms. Specifically, molecular analyses revealed that only DNA sequences specific to *P. nicotianae* and *P. citrophthora* could be detected in the restriction patterns of all hybrids using radiolabelled, species-specific probes, or PCR. This suggested that species-specific DNA sequences of *P. capsici* represented by pCAP12 were lost during hybrid formation. However, in one hybrid organism, other sequences of *P. capsici* were detectable using 10-base random primers in RAPD-PCR. It is apparent that further analyses are required to determine the manner in which genetic material of parent organisms are combined and phenotypic traits are expressed in hybrids.

At present, the reasons for loss of sporulation and virulence in the triparental hybrids are unknown. It is likely that triparental hybrids derived from zoospore fusion represent an array of new genetic combinations. Many of these combinations would be deleterious to the fitness of hybrid individuals and to their abilities to compete with other members of the fungal population. The zoospore fusion technique used in this study may open up new avenues for examination of the

processes that are involved in the success or extinction of widely variable new genotypes in nature.

ACKNOWLEDGMENTS

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Discomycetes of Madagascar — I. Phillipsia ranomafanensis sp. nov. and ascospore sculpture of Cookeina colensoi proved by SEM (Discomycetes, Pezizales, Sarcoscyphaceae)

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Moravec J. (1997): Discomycetes of Madagascar – I. Phillipsia ranomafanensis sp. nov. and ascospore sculpture of Cookeina colensoi proved by SEM. (Discomycetes, Pezizales, Sarcoscyphaceae). – Czech Mycol. 50: 21–33

Results of the author's investigation of discomycetes belonging to the family Sarcoscyphaceae recently collected on Madagascar are presented. *Phillipsia ranomafanensis* sp. nov. is described from Central Madagascar. The new species is distinguished by its large white apothecia with short, inconspicuous thin-walled hyphae on the external surface, and particularly by the small, symmetrical, ellipsoid to attenuate ellipsoid biguttulate ascospores which bear a fine longitudinal striation. The holotype (OSC) of *Phillipsia costaricensis* Denison – a species which also possesses symmetrical ascospores – has been examined. This Central-American species differs clearly by an ochraceous colour of the apothecia which are externally covered by long, rigid, and extremely thick-walled hypha-like hairs, and by uniguttulate, much larger, broadly ellipsoid ascospores bearing a finer and shallower striation and lower and flatter ridges. Type material of several other species of *Phillipsia* Berk. has also been examined and compared. Further collections of *Phillipsia domingensis* (Berk.) Berk. from Madagascar are reported and relations within the genus are discussed. Based on the author's examination of the type material (K) of *Peziza cordovensis* Cooke and *Phillipsia polyporoides* Berk., both are tentatively (as the material is in a poor state) considered synonyms of *P. domingensis*. Ascospore ornamentation of species of the genera *Phillipsia* and *Cookeina* Kuntze has been studied and the author concludes that the ornamentation can truly be recognized by SEM only. The discovery of a very fine "amoeboid"-verrucose ascospore ornamentation in *Cookeina colensoi* (Berk.) Rifai, proved by SEM, is an important result, as the species has commonly been considered a smooth spored one. Illustrations on line drawings and SEM photomicrographs of ascospores of *Phillipsia domingensis* and *Cookeina colensoi* and those of ascospores taken from the type material of *Phillipsia crenulata* Berk. & Br. (K), *P. ranomafanensis* and *P. costaricensis*, accompany the paper.

Key words: *Phillipsia ranomafanensis* sp. nov, *Phillipsia domingensis*, *Cookeina colensoi*, ascospore ornamentation, Discomycetes, taxonomy.

Moravec J. (1997): Discomycetes of Madagascar — I. Phillipsia ranomafanensis sp. nov. a skulptura askospor Cookeina colensoi, prokázaná SEM. (Discomycetes, Pezizales, Sarcoscyphaceae). – Czech Mycol. 50: 21–33

Jsou zveřejněny výsledky studia diskomycetů čeledi Sarcoscyphaceae sbíraných v poslední době na Madagaskaru. *Phillipsia ranomafanensis* sp. nov. je popsána z centrálního Madagaskaru. Nový druh je význačný velkými bílými apothecii s nenápadnými, tenkostěnnými hyfami na jejich zevní ploše a zejména malými, symetrickými, elipsoidními až podlouhle elipsoidními dvoukapénkatými askosporami s jemným podélným rýhováním. Holotyp (OSC) *Phillipsia costaricensis* Denison, která se rovněž vyznačuje symetrickými askosporami byl revidován a srovnáván. Tento středoamerický druh se však zřetelně liší okrově zbarvenými apothecii jejichž zevní plocha je pokryta dlouhými, tuhými, extrémně tlustostěnnými hyfovitými chlupy, a také většími, široce elipsoidními askosporami nesoucími nižší a plošší žebra a mělčí rýhování. Typový materiál několika dalších druhů rodu *Phillipsia* Berk. byl studován za účelem srovnání. Jsou též uvedeny další

nálezy *Phillipsia domingensis* (Berk.) Berk. z Madagaskaru a diskutovány příbuzenské vztahy. Na základě studia skrovného typového materiálu (K) *Peziza cordovensis* Cooke a *Phillipsia polyporoides* Berk. jsou obě provizorně (pro špatný stav materiálu) považovány za synonyma *P. domingensis*. Ornamentika askospor u rodů *Phillipsia* a *Cookeina* Kuntze byla rovněž studována a autor dospěl k závěru, že je správně rozpoznatelná pouze použitím SEM. Důležitým výsledkem je objev velmi jemné „amoeboidně" bradavčité ornamentiky askospor prokázané SEM u *Cookeina colensoi* (Berk.) Rifai, neboť tento druh byl dosud všeobecně pokládán za hladkovýtrusý. Příspěvek je doplněn kresbami a SEM mikrofotografiemi askospor *P. domingensis* a *C. colensoi* a SEM askospor z holotypového materiálu *Phillipsia crenulata* Berk. & Br. (K), *P. ranomafanensis* a *P. costaricensis*.

INTRODUCTION

In the course of scientific forays mostly to countries of the tropical climate zones in which I have participated, a great number of operculate discomycetes including members of the family Sarcoscyphaceae has been found. Many specimens of Sarcoscyphaceae including *Phillipsia* Berk. and *Cookeina* Kuntze have also been found by me in continental Africa and a paper on them is being prepared as these genera appear to be very interesting, and furthermore contain superficially known species. The first part of the results is presented here and covers the genera *Phillipsia* and *Cookeina* recently found in Madagascar.

The genus *Phillipsia* Berkeley, J. Linn. Soc. Bot. 18: 388, 1881, in its modern sense (Boedijn 1933, Le Gal 1953, 1959, Rifai 1968, Denison 1969), is characterised by small to large, sessile to substipitate apothecia the structure of which consists of a textura intricata to subepidermoidea, often forming a tomentose external surface, pink to purple-red or carmineous, orange, yellow, or rarely pure white hymenium, suboperculate asci, ellipsoid, mostly asymmetrical, or only in few species almost symmetrical ascospores which usually bear a longitudinal striation consisting of striae between raised ridges which do not stain in cotton blue in lactic acid (CB), and its occurrence on wood mostly in the tropics.

After my examinations, I agree with Le Gal (1953) and Rifai (1968) that several taxonomic groups (sections or series), but in my opinion not yet clearly delimited, can be recognized within the genus. A paper on species belonging to a group which accommodates species possessing small, substipitate to almost stipitate apothecia of a thin medullary excipulum and a firm consistency, also covering my collections made in continental Africa, is currently under preparation.

Regarding the colour of the hymenium, all fresh apothecia of my two collections of *Phillipsia* possessed a purely white hymenium despite their development under normal light conditions. One species, belonging to the second group mentioned above, comes from Zambia. The other, collected by me in central Madagascar, is treated here as a new species.

TAXONOMY AND DISCUSSION

Phillipsia ranomafanensis J. Moravec sp. nov.

Apothecia solitaria, magna, 12–30 mm in diam. sessilia vix stipitata, leniter patellaria dein paene discoidea, applanata et undulato lobata, tota pure alba, parte externa subtiliter albo-tomentosa, subglabra. Excipulum externum textura dense intricata usque subepidermoidea, parte externa hyphis superficialibus, hyalinis, septatis, tenuiter tunicatis, apice obtusis, laxe singulariterque prominentibus textum. Excipulum internum (medulla) textura dissite intricata, subhymenium textura intricata. Asci suboperculati, 185–225 (–250) × 12–15 μm, cylindracei, deorsum sensim angustati, crasse tunicati, octospori, non-amyloidei. Ascosporae ellipsoideae vel elongato-ellipsoideae, 15–19.5 (–21) × (7.5–) 9–10 (–10.7) μm (plerumque 18.5 × 9.7 μm), guttulis binis magnis instructae, perisporio longitudinaliter sulcato, sulcis simplicibus vel rare anastomosantibus atque costis obtusis (7–10 latere uno visibilibus) instructae. Paraphyses filiformes, 1–1.7 μm, apice non vel sensim dilatatae (1.3–4.5 μm).

Habitat: Ad lignum putridum ad viam publicam in silva pluviali, prope pagum Ranomafana, prov. Fianarantsoa, Madagascar centralis, 28. I. 1995 leg. J. Moravec; Holotypus in herbario mycologico Musei Brunensis (BRNM 612538) et duplicatum in herbario privato J. Moravecii (J. Mor.) asservantur.

Apothecia solitary, 12–30 mm diam., sessile to inconspicuously substipitate as contracted below into a thick and very short central stalk-like base, shallowly cupulate, becoming almost discoid, fleshy but comparatively firm, margin even or often undulate or lobed, hymenium purely white, outer surface whitish, almost smooth, only minutely white tomentose; dried apothecia cream coloured. Excipulum a textura intricata throughout, in the base of the apothecia occasional angular cells (textura subepidermoidea) are present. Ectal excipulum clearly differentiated as a much narrower layer of a compact textura intricata of hyaline hyphae which are 3–8 μm broad, septate or articulate, the articles often of a pyriform shape, with walls 0.2–0.6 μm thick, densely arranged but in the outermost layer occasionally freely and shortly protruding the outer surface in a form of short, mostly isolated, hyaline, thin-walled [the walls 0.2–0.4 (–0.6) μm] hyphae with obtuse tips (Fig. 10); the margin formed by long, thinner septate hyphae. Medullary excipulum thick (about four times thicker than the ectal layer), of looser, interwoven, branched and septate hyphae which are often constricted at their septa, 3–8 μm thick, often inflated up to 11 μm. Subhymenium a textura intricata of smaller interwoven hyphae. Asci suboperculate, 185–225 (–250) × 12–15 μm, cylindrical, constricted towards the simple base, thick-walled, eight-spored, non-amyloid. Ascospores ellipsoid or elongate-ellipsoid 15–19.5 (–21) × (7.5–) 9–10 (–10.7) μm (mostly 18.5 × 9.7 μm), containing two large oil guttules, with a perispore bearing a longitudinal striation which separates the longitudinal ridges rising between the

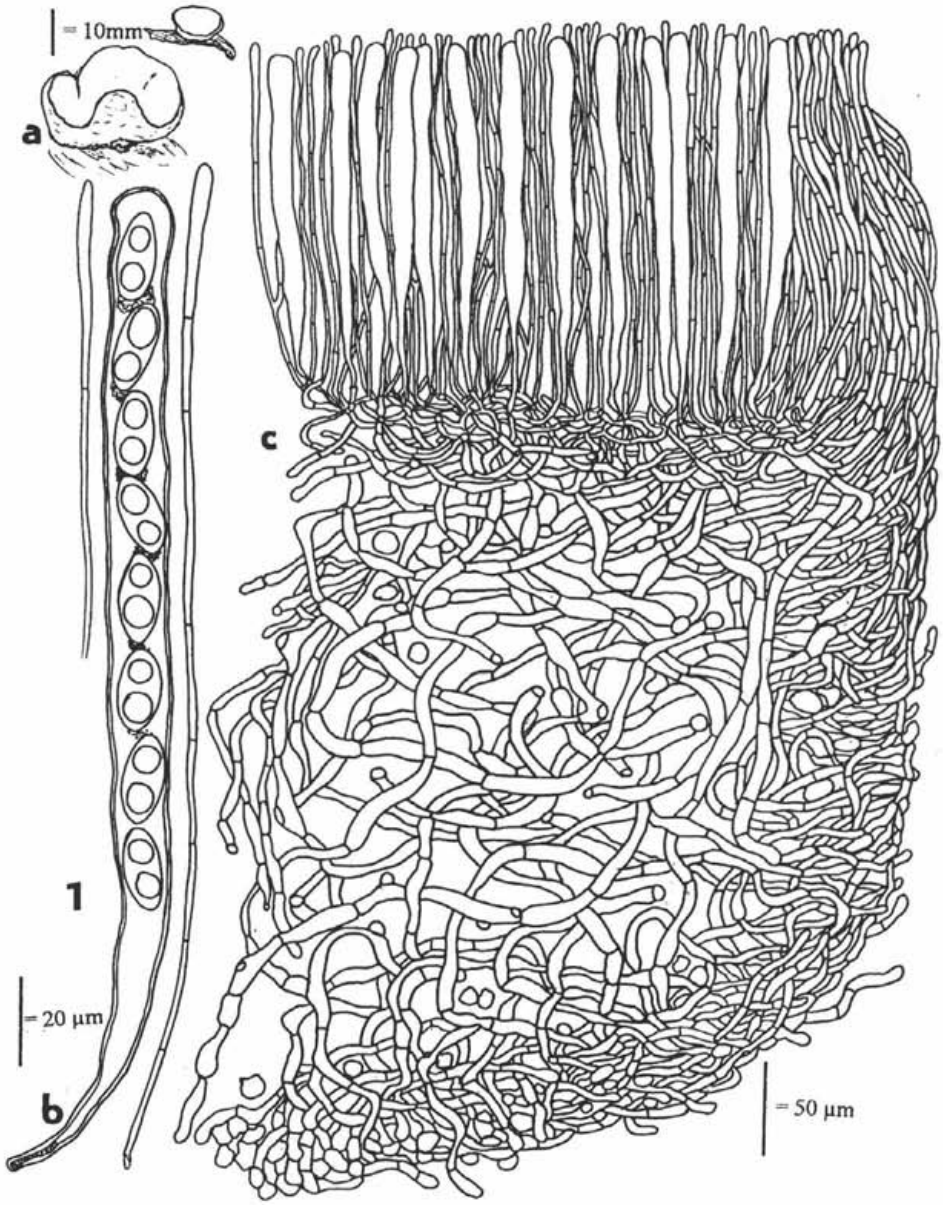


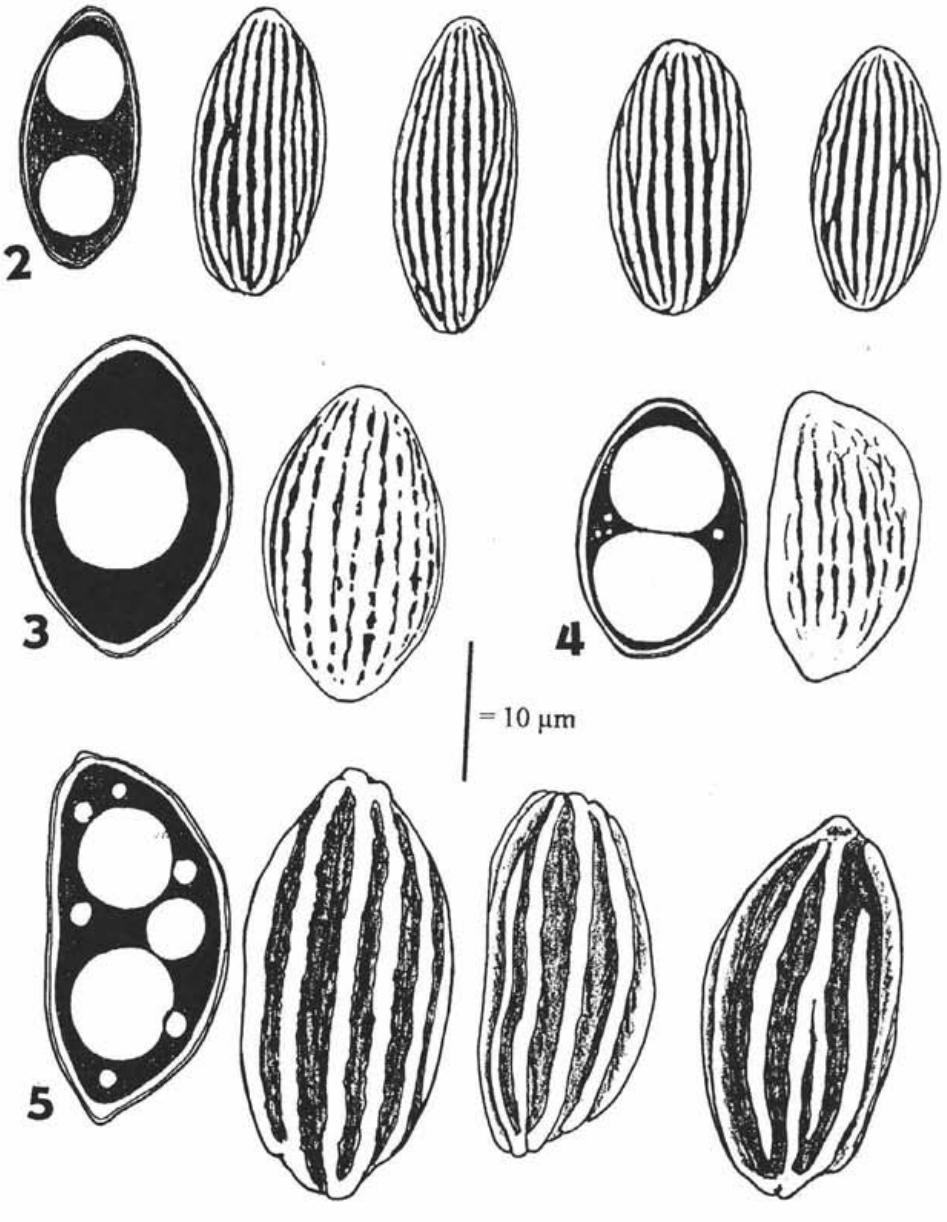
Fig. 1. *Phillipsia ranomafanensis* sp. nov.: a. apothecia, b. ascus and paraphyses, c. section of the marginal part of the apothecium.

striae (7–10 ridges seen on each side of the spore); the risen ridges are blunt, densely arranged, 0.3–0.8 μm broad, separated by the very narrow [0.2–0.3 (–0.6) μm] striae which are simple or rarely subparallelly anastomose or connected (SEM Figs 11–12). Paraphyses straight, filiform, 1–1.7 μm, not or slightly enlarged (1.3–4.5 μm) at their tips, septate, hyaline, with a cyanophilic content.

Habitat: Central Madagascar, prov. Fianarantsoa, near the village of Ranomafana, on decaying wood of a twig laying on an open place at the side of a non-paved road through a partly secondary rain forest, 28. I. 1995 leg. J. Moravec; Holotype BRNM 612538 (Mycological Herbarium of the Moravian Museum, Brno, Czech Republic), isotype in the herbarium of the author (J. Mor.).

P. ranomafanensis differs from other species of *Phillipsia* by the purely white colour of the apothecia (which were developed under normal light conditions), but especially by the shape, size and ornamentation of the ascospores. The ascospores are almost regularly ellipsoid in contrary to species which can be accommodated in a group represented by *Phillipsia domingensis* (Berk.) Berk. which possess ascospores mostly asymmetrical to subcymbiform as unequal-sided and with wider ridges (4–6 seen on each side). Similarly like in most other species of *Phillipsia*, scanning electron micrographs of ascospores of *P. ranomafanensis* show a different picture then that seen by a light microscope under an oil immersion lens (Fig. 2). The SEM (Figs 11–12) revealed that the ridges are blunt and densely arranged and consequently the striae between the ridges are very narrow.

Regarding the symmetrical ascospore shape, the new species is similar to *Phillipsia costaricensis* Denison (1969) described from Costa Rica. However, the examination of the holotype (Costa Rica: forest adjacent to Instituto Interamericano de Ciencias Agricolas, Turrialba, Cartago, alt 520 m., on sticks and old wood, Sept. 1964, Denison 2358, OSC) has revealed that this Central-American species differs by smaller apothecia with a tan, ochraceous to yellow-brown hymenium and a pale ochraceous minutely tomentose external surface which is covered by obtuse, flexuous but rigid hairs – the hairs are 4.5–6 μm in diam. and up to 350 μm long, extremely thick-walled (the walls 1.5–2 μm thick) and consequently the cyanophilic interspace between the walls is very thin (Fig. 9). Also several other important features such as the shape and size of the ascospores separate the two species well. The ascospores of *P. costaricensis* are broadly ellipsoid, usually tapering towards the poles. Denison (1969) stated the ascospore size of (18–) 20–22 (–24) × (11–) 12–14 (–15) μm which is much larger than those of *P. ranomafanensis*. After my reexamination of the holotype (OSC), I have found the size of mature ascospores (18–) 19–23 (–24) × 10.5–13.5 (–14) μm – only immature ascospores up to 16 μm wide. The ascospores of *P. ranomafanensis* are much smaller and conspicuously narrower, 15–19.5 (–21) × (7.5–) 9–10 (–10.7) μm. Their width does not extend 10.7 μm and they usually measure 18.5 × 9.7 μm, whilst the size of most ascospores of *P. costaricensis* is 22 × 12.2 μm. Another feature which can be considered

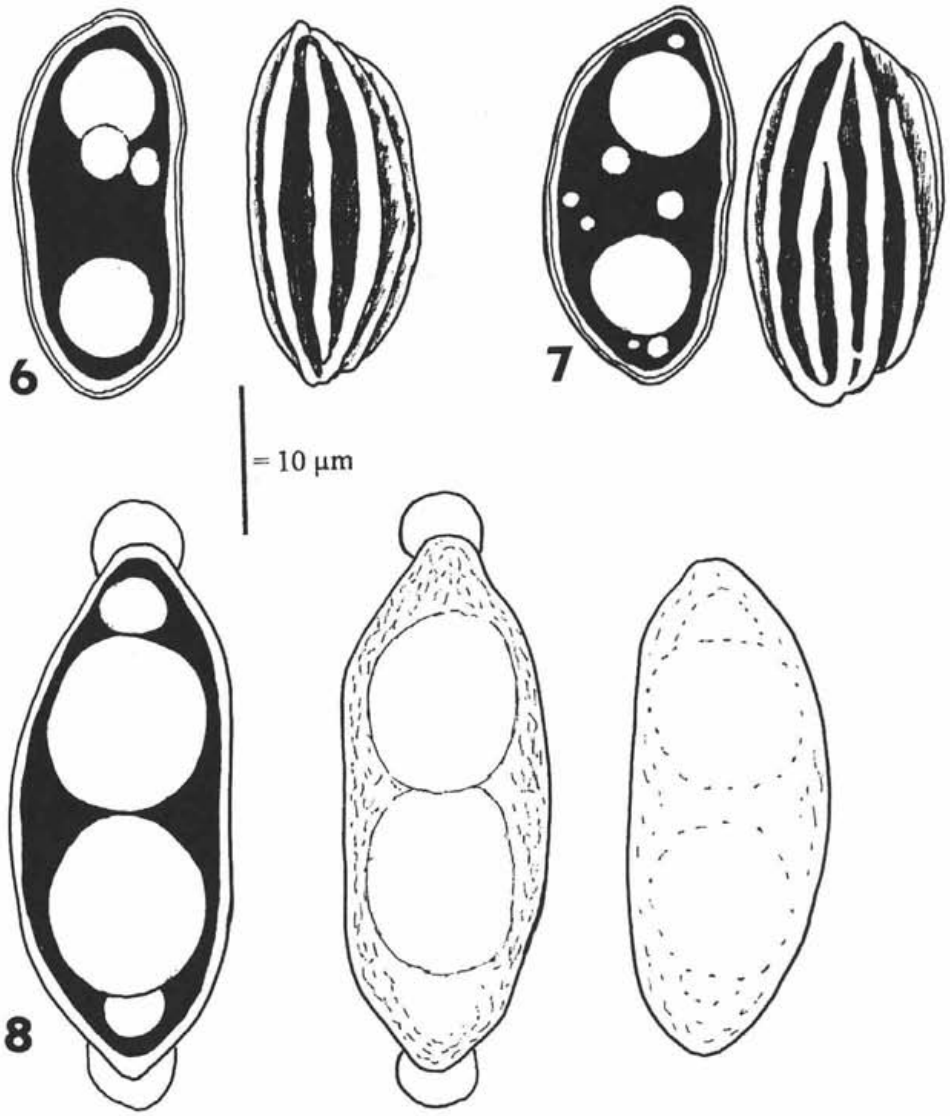


Figs 2–5. Ascospores of *Phillipsia* (oil immersion): 2. *P. ranomafanensis* sp. nov. (Holotype BRNM); 3. *P. costaricensis* Denison (holotype OSC); 4. *P. crenulata* Berk. et Br. (Type K); 5. *P. domingensis* (Berk.) Berk. (Madagascar, Ranomafana, J. Mor.).

a good difference are the biguttulate ascospores of *P. ranomafanensis* whilst those in *P. costaricensis* are regularly uniguttulate, or the large central guttula consists of a number of small ones densely arranged inside one such large central drop. Also, a conspicuous difference in ascospore ornamentation which well separates these two fungi has been revealed by SEM (Figs 13–14). The extremely fine striation on the perispore of ascospores of *P. costaricensis* is very shallow and thus the ridges between the striae are much lower and flatter than those in *P. ranomafanensis*. Last but not least, the asci of the Central-American species are much longer (270–350 μm), and so we can only speculate if these two species, despite their unique symmetrical shape of ascospores, belong to the same taxonomic group within the genus. The thick medullary excipulum indicates an affinity to *Phillipsia domingensis* (Berk.) Berk. – the type species of the genus, but the importance of this character is decreased by other features, especially by shorter asci of *P. ranomafanensis* which are not so flexuous towards their base and by the symmetrical ascospores which are, moreover, uniguttulate in *P. costaricensis*. This shows how complicated and difficult the infrageneric arrangement suggested by the cited authors may appear.

A rather similar ascospore ornamentation was demonstrated by Le Gal (1953) in *Phillipsia crenulata* Berk. et Broome (Journ. Linn. Soc. 14:104, 1875). She claimed the size of the biguttulate ascospores of *P. crenulata* to be 18–24 × 11–15 μm. However, my examination of the type material (labelled *Humaria crenulata*, Ceylon, consisting of 4 dried dirty-orange apothecia, 0.5–2.5 mm diam., K ex herb. C. E. Broome), has revealed that the asymmetrical ascospores measure only 15–19.5 × 9.5–10.5 μm and bear a much finer, denser, incomplete and more irregular ascospore striation (Fig. 4) than that illustrated by Le Gal (1953). The ornamentation is seen completely on SEM photographs only (Fig. 18). Besides the mentioned quite different form of ascospore ornamentation, *P. crenulata* differs clearly by its unequal shape of the ascospores and by much smaller apothecia (5–7 mm diam) possessing an orange hymenium. This species may belong to a different infrageneric taxonomic group of species which could accommodate species characterized by small stipitate apothecia with an orange, pale red to pink hymenium and a thin medullary excipulum of a firm consistence, represented by such species as *Phillipsia hartmannii* (Phill. in Cooke) Rifai (1968) and *Phillipsia carnicolor* Le Gal (1953). I have examined the type (K) of *P. hartmannii*, and in accordance with Rifai (1968) I have found the mature ascospores asymmetrical, smooth under the light microscope, but a fine irregular or even subreticulate ornamentation consisting of "amoeboid" and irregularly arranged wrinkles (without a regular longitudinal striation) was revealed by SEM. A paper on these species is being prepared.

Phillipsia umbilicata (Penz. et Sacc.) Boedijn (1940), characterized by small (4–10 mm diam.) apothecia with coral red hymenium, short asci and smooth



Figs 6–8. Ascospores of *Phillipsia* and *Cookeina* (oil immersion): 6. Type of *Peziza cordovensis* Cooke (K); 7. Type of *Phillipsia polyporoides* Berk. (K); 8. *Cookeina colensoi* (Berk.) Seaver (Madagascar, Moramanga, J. Mor.).

ascospores, is considered by Rifai (1968) a member of a third group. However, the asci of *P. carnicolor* are short too, and thus the delimitation is not clear.

All these species are well separated from *P. ranomafanensis*. They bear characters which indicate a certain resemblance with the genus *Nanoscypha* Denison (1972) erected for *Cookeina tetraspora* Seaver [= *Phillipsia tetraspora* (Seaver) Le Gal]. *Nanoscypha* can be considered a link between the genera *Phillipsia, Komposcypha* Pfister, *Pseudopithyella* Seaver, and last but not least *Sarcoscypha*. The genus *Komposcypha*, with the type species *K. chudei* (Pat. ex Le Gal) Pfister (1989) based on *Plectania chudei* Pat. ex Le Gal (1953) [= *Sarcoscypha chudei* (Pat. ex Le Gal) Eckblad] is very close to *Nanoscypha* as discussed by Pfister (1989) and its untenable position in the genus *Sarcoscypha* (especially for the quite different apothecial construction) and relations to *Pseudopithyella* were discussed earlier (Moravec 1983, Pfister 1989).

Regarding the difficulties in the ambiguous and not uniform features in species of *Phillipsia* mentioned in the discussion above, we can follow Denison's (1972) separation of *Nanoscypha* by which a division of *Phillipsia* into several genera would become possible after perfecting our knowledge.

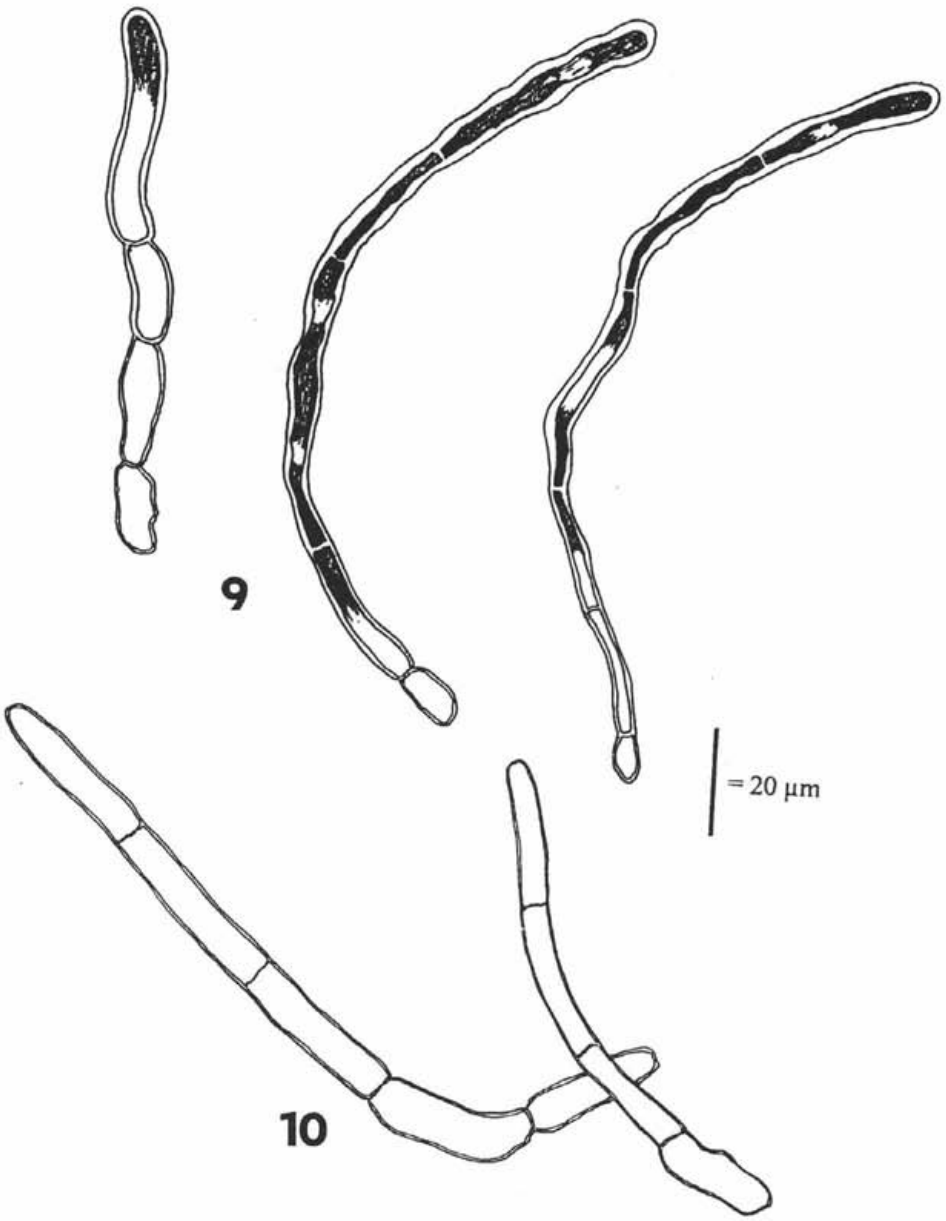
Phillipsia domingensis (Berk.) Berkeley, J. Linn. Soc. London Bot. 18:388, 1881.

Basionym: *Peziza domingensis* Berkeley, Ann. Mag. Nat. Hist. 9:201, 1852.

Central Madagascar: Ranomafana village, prov. Fianarantsoa, On decaying wet bark of a living plant of *Musa* sp. in a secondary forest and a plantation, 29. I. 1995 leg. J. Moravec (J. Mor.); East Madagascar: Moramanga, on bark of a twig in remnants of a partly burnt and destroyed rain forest, 22. I. 1995: Moramanga, on decaying wood on a path through a rain forest, 28. I. 1995 leg. J. Moravec (J. Mor.).

Apothecia of these three Madagascar collections of *P. domingensis* are rather variable in shape, size (up to 30 mm diam.), and especially in the colour of the hymenium, which ranges from pink-red to light pink-violaceous or red – to orange-violaceous. All these features are in accordance with the characters of *P. domingensis* and with the descriptions of this species in Boedijn (1933), Le Gal (1953) and Denison (1969). The ascospores of the Madagascar collections measure 21–27 (–30) × 10.5–14 (–15.5) μm (usually 26 × 12 μm).

Phillipsia subpurpurea Berk. et Br. is recognized by Le Gal (1953) and consequently by Rifai (1968) as a separate species. Rifai (1968) noted that this species differs only critically from *P. domingensis*, whilst Seaver (1928) and Boedijn (1933) united them. After my examination of a number of my collections coming from Sumatra, Zambia and Madagascar, and after reexamination of relevant type material, I am unable to recognize any basic or important feature which can be considered a leading character for such separation. Several features,



Figs 9–10. Hypha-like hairs and hyphae of the external surface of apothecia of *Phillipsia*: 9. Hypha-like hairs of *P. costaricensis* Denison (Holotype OSC); 10. Hyphae of *P. ranomafanensis* sp. nov. (Holotype BRNM).

considered to be distinguishing characters for *P. subpurpurea* stressed by Le Gal (1953) and Rifai (1968) – slightly different size, shape and colour of the apothecia (but always with a red tint), and a slight difference in thickness and number of the ridges of ascospore ornamentation can hardly be taken into consideration. SEM photographs of ascospores (see Figs 15–17) show a variability in thickness, shape and number of these ridges, seen also on individual ascospores which were taken from the hymenium of the same apothecium. Moreover, Le Gal (1953) measured the thickness of the ridges in *P. domingensis* as 0.75 μm, which this is evidently erroneous, as in reality the ridges are much thicker, 1.5–2 μm. This may be explained by the fact that the substances which form the ascospore ornamentation in most species of *Phillipsia* and *Cookeina* do not stain adequately with CB, Melzer reagens, safranin and other sorts of reagens and dyes, and are therefore hardly recognizable under a light microscope oil immersion lens. Consequently, the ridges and striae may be falsely measured and illustrated (e.g. Le Gal 1947, 1953, Rifai 1968). The inaccuracy that occurs when the space between the ridges is illustrated, may be especially caused by the fact that only the upper parts of the ridges are seen under the light microscope and thus the walls of the hyaline ridges merge with the dark striae between the ridges. As was mentioned above in the discussion on ascospore ornamentation of *P. ranomafanensis*, the ridges are in fact much thicker than the striae and the picture seen by the light microscope may be false. The elements which form the ascospore ornamentation are seen clearly only on SEM photomicrographs (see all the SEM figures in this paper). At present I have identified all my collections from Madagascar as *P. domingensis*, and only the fact that I have not examined the type of *P. subpurpurea*, prevents me to consider *P. subpurpurea* definitely a synonym of *P. domingensis*.

I managed to examine the supposedly lost (Le Gal 1953, Denison 1969) type of *Peziza cordovensis* Cooke, Hedwigia 14: 81, 1875 which was synonymized (with a question mark) with *P. domingensis* by Seaver (1928). The type material [Sallé – Mexico, "Cordova" (= Cordoba), Dec. 1874 on rotten wood, K], consists of one incomplete apothecium glued on a piece of paper. The dried apothecium (22 mm in diam.) is flat, brown coloured with dark spots. The ascospore size is in a range of that of *P. domingensis*, and also the ascospore ornamentation (Fig. 6) agrees well with that of this species. The type material is not in a good state. It is especially difficult to examine the construction of the apothecium and judge the possible existence or absence of a gelatinous tissue – the last feature being characteristic of a group of species around *Phillipsia dochmia* (Berk. et Curt. apud Berk) Seaver [= *Aurophora dochmia* (Berk. et Curt. apud Berk.) Rifai (1968)] which also possesses ascospores very similar to *P. domingensis*. Therefore, I only tentatively agree with Seaver (1928) and consider *P. cordovensis* a synonym of *P. domingensis*.

With a certain hesitation caused by similar reasons, but almost with certainty, I also consider *Phillipsia polyporoides* Berkeley, Journ. Linn. Soc. Bot. 18: 388,

1881, a synonym of *P. domingensis*. My examination of the type [labelled *Phillipsia polyporoides* and with the annotation *"Phillipsia expansa B"*, on dead wood, Rockhampton coll. Thozet 852, K ex herb. William Phillips, consisting of a fragment of an apothecium, brownish with a purple tinge (when dried)] revealed ascospores which measure 21–30 × 10.5–14 μm and correspond well with those of *P. domingensis* (Fig. 7).

Cookeina colensoi (Berk.) Seaver, Mycologia 5: 191, 1913.

Basionym: *Peziza colensoi* Berkeley, Hook. f., Fl. Nov. Zealand. 2:200, 1855.

East Madagascar: Moramanga, on a hard wood of dead twigs in a ditch along a path through remnants of a rain forest, 22. I. 1995 leg. J. Moravec (J. Mor.).

A great number of apothecia of the Madagascar collection were of a variable shape – substipitate to with a very long (up to 25 mm long) stipe, and with a beautifully egg-yellow, light yellow to yellow-orange hymenium; the external surface whitish, only very minutely pubescent. The structure of apothecia consists of the pseudoparenchymatous ectal excipulum (textura angularis) and a prosenchymatous medulla of a textura porrecta to intricata, typical of the genus *Cookeina*. The unequally sided ellipsoid to subfusiform apiculate ascospores measure 29–36 (–37.5) × 12–13.5 μm and appear almost smooth or possess occasional fine irregular wrinkles (seen by a light microscope under oil immersion lens). The concept of *C. colensoi* as a smooth-spored species is in accordance with the original sense of Berkeley (1855) adopted also by Le Gal (1953), Gamundí (1957), Rifai (1968) and Denison (1967). Seaver (1928) erroneously applied the name to another species which is now known under the name *Cookeina venezuelae* (Berk. et Curt.) Le Gal and is distinguished by ascospores bearing parallel wrinkles between coarse longitudinal and widely spaced ridges (see Denison 1969). Another related species, *Cookeina indica* Pfister et R. Kaushal (1984) is distinguished by a fine longitudinal ascospore striation.

Although the ascospores of *C. colensoi* appear almost smooth under the light microscope (Fig. 8), SEM revealed that they are ornamented by very fine, densely and irregularly arranged irregular warts of an "amoeboid" shape (SEM Figs 19–20). Such ornamentation, proved for the first time in this species, is quite different from that of *C. venezuelae, C. indica* and all other species of *Cookeina*.

A paper recording recent collections of species of the genus *Cookeina* and on the ascospore ornamentation in *Cookeina* is also under preparation.

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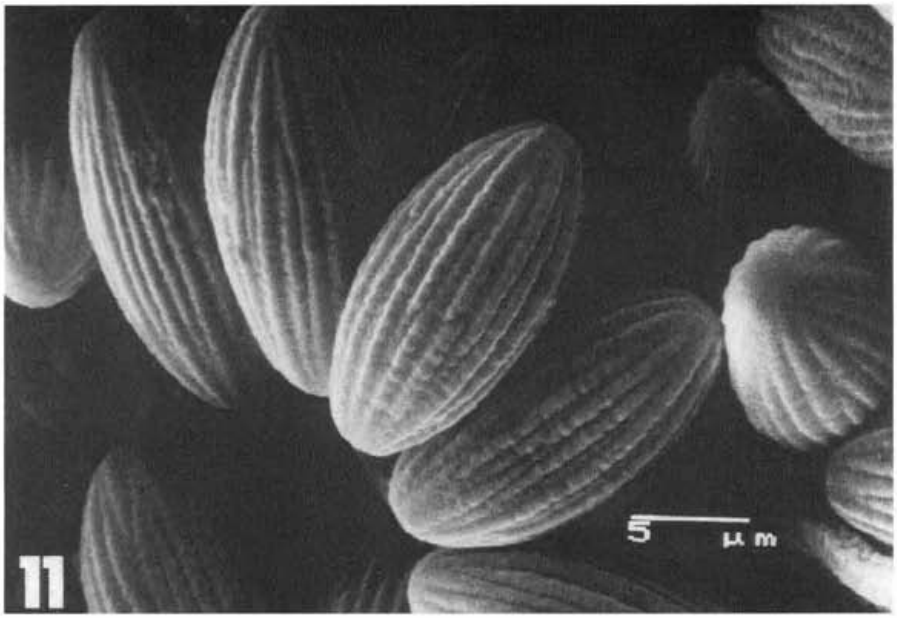
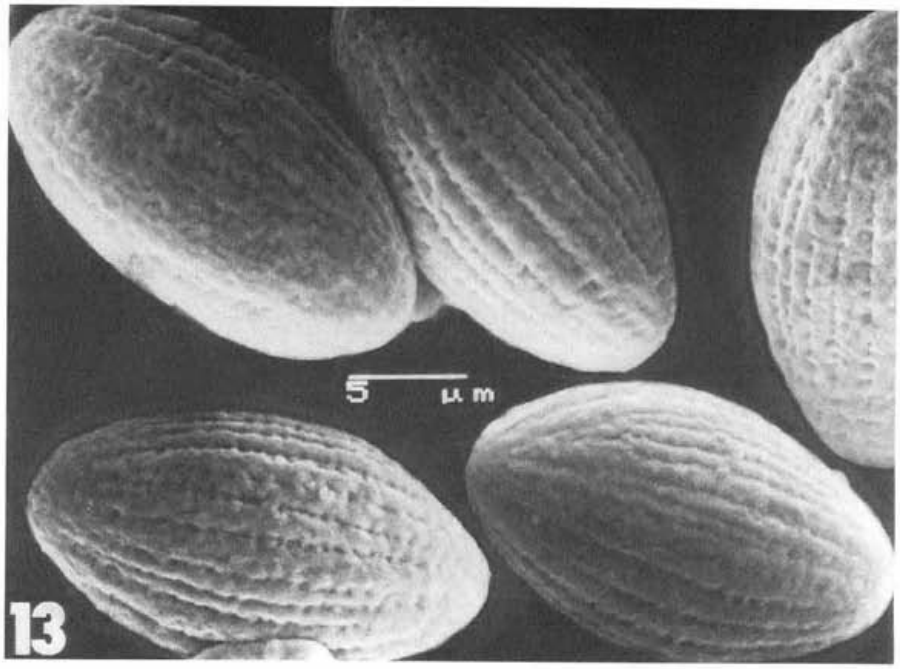
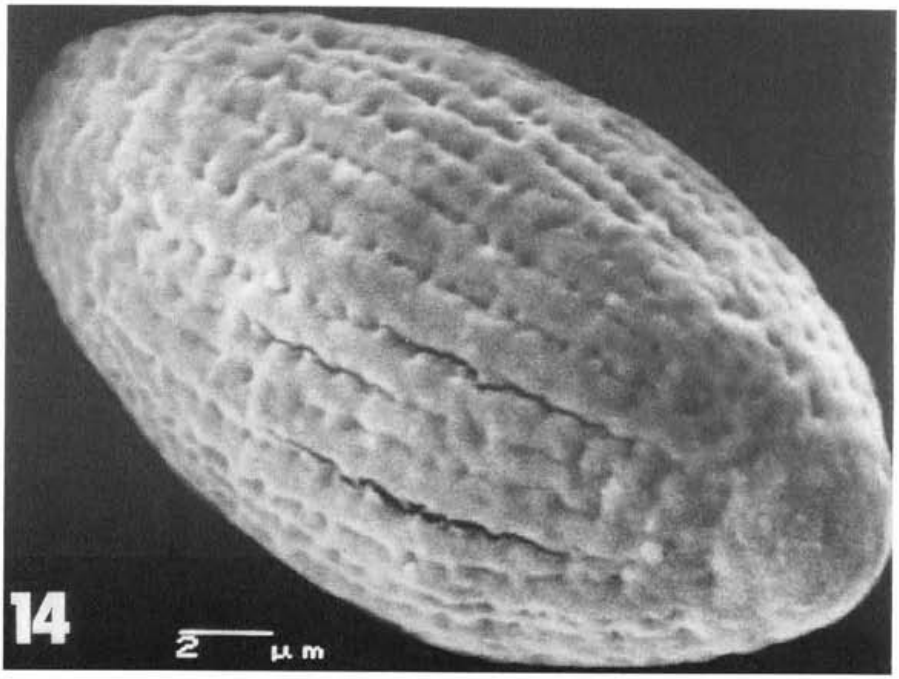


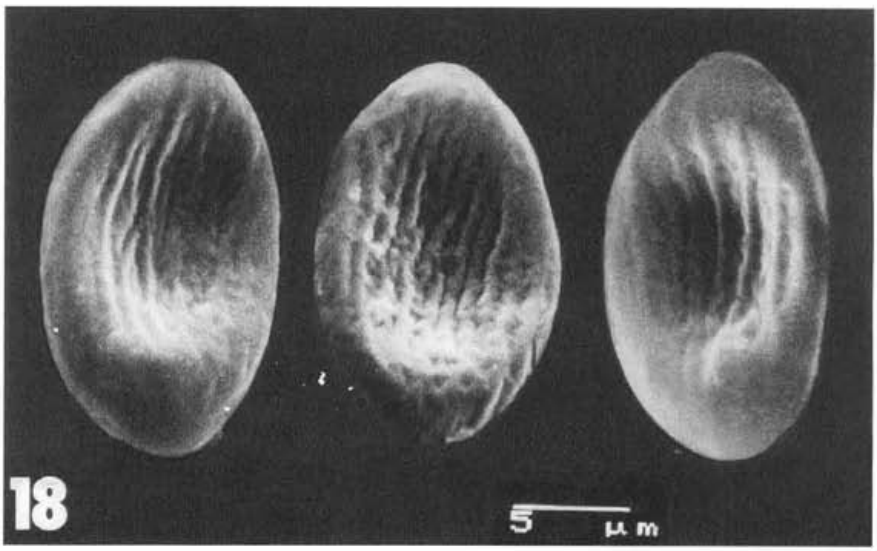
Figs 11–12. SEM photomicrographs of ascospores of *Phillipsia ranomafanensis* sp. nov. (Holotype BRNM).



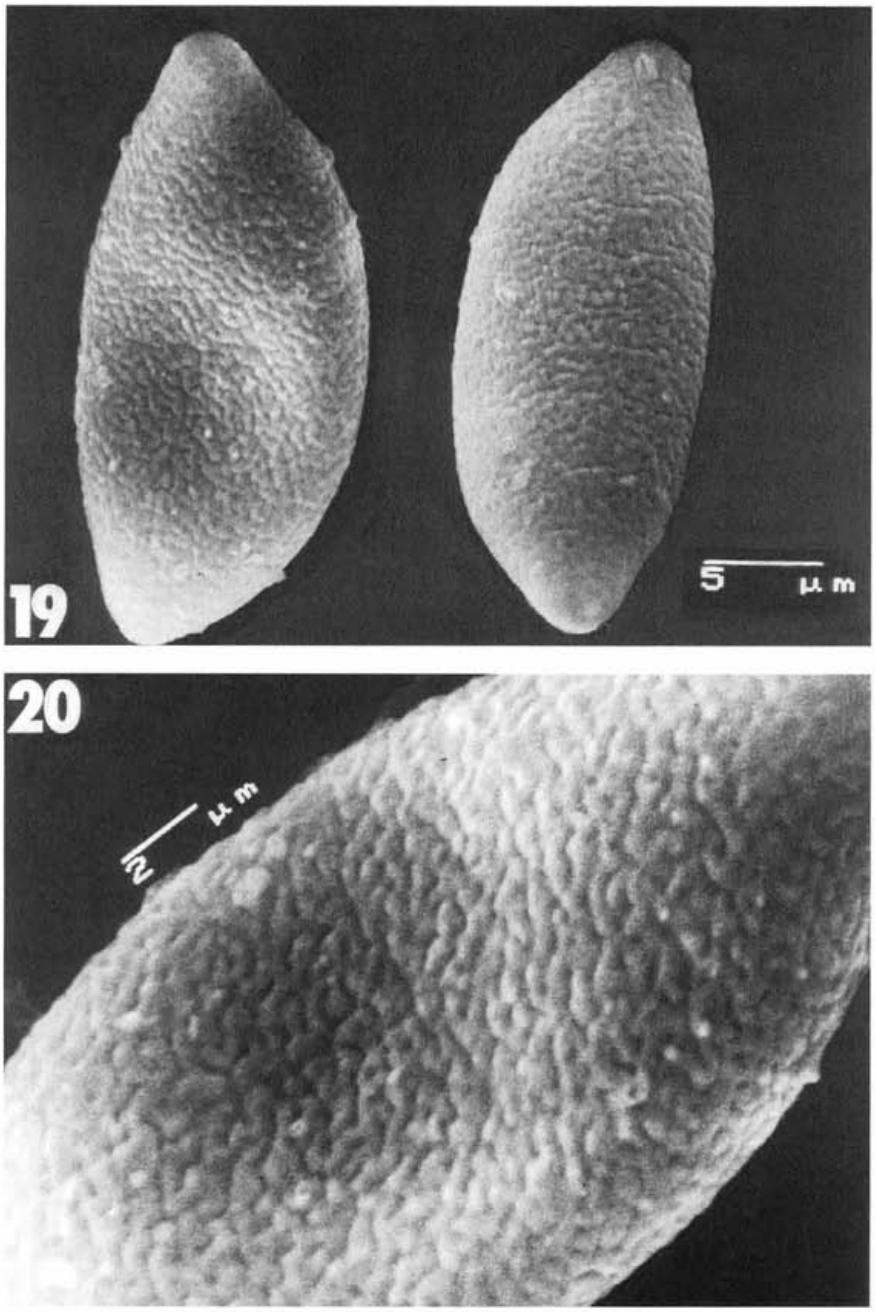


Figs 13–14. SEM photomicrographs of ascospores of *Phillipsia costaricensis* Denison (Holotype OSC).

Figs 15–16. SEM photomicrographs of ascospores of *Phillipsia domingensis* (Berk.) Berk. (Madagascar, Ranomafana, J. Mor.).



Figs 17–18. SEM photomicrographs of ascospores of *Phillipsia*: 17. *P. domingensis* (Berk.) Berk. (Madagascar, Moramanga, J. Mor.); 18. *P. crenulata* Berk. et Br. (type K).



Figs 19–20. SEM photomicrographs of ascospores of *Cookeina colensoi* (Berk.) Seaver (Madagascar, Moramanga, J. Mor.).

CZECH MYCOL. 50 (1), 1997

Specific responses of some phytopathogenic fungi to fungicides

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Abdel-Mallek A. Y., Mazen M. B., Allam A. D. and Hashem M. (1997): Specific responses of some phytopathogenic fungi to fungicides. – Czech Mycol. 50: 35–44

Laboratory experiments were carried out to examine the effect of four fungicides on spore germinating potentialities, radial growth and survival of viable propagules in soil of five phytopathogenic fungal species. The test organisms were achieved from infected roots of wheat plants cultivated in the Assiut area, Egypt. These were: *Alternaria alternata, Cochliobolus sativus, Drechslera halodes, Fusarium moniliforme* and *F. oxysporum*. The fungicides reduced germ tube production and radial growth of all fungi, and the reduction increased with increase in concentration. The maximal reduction was recorded at 50 μg ml^{-1}. At this concentration, Homai prevented spore germination of all test species. Neither *F. moniliforme* nor *F. oxysporum* can grow on agar medium supplemented with 50 μg ml^{-1} of either Benlate or Homai. The suppressive effect of fungicides on spore survival in soil cultures was also noticed but seemed to be lower than in agar application. In certain treatments, the numbers of viable propagules of tested fungi were not significantly affected in autoclaved nor non-autoclaved soil.

Key words: fungicides, phytopathogenic species, Egypt

Abdel-Mallek A. Y., Mazen M. B., Allam A. D. a Hashem M. (1997): Specifické reakce některých fytopatogenních hub na fungicidy. – Czech Mycol. 50: 35–44

K zjištění účinků čtyř fungicidních látek na schopnost klíčení výtrusů, růstu mycelia a schopnosti přežívání v zemi byly prováděny laboratorní experimenty s pěti fytopatogenními druhy hub. Testované organismy byly získány z infikovaných kořenů pšenice pěstované v oblasti Assiut v Egyptě. Jednalo se o houby *Alternaria alternata, Ochliobolus sativus, Drechslera halodes, Fusarium moniliforme* a *F. oxysporum*. Fungicidy redukovaly tvorbu klíčních hyf a růst mycelia všech těchto druhů hub a tato redukce byla přímo úměrná koncentraci. Nejvyšší redukce byla zaznamenána při 50 μg ml^{-1}. Při této koncentaci fungicid homai zabránil klíčení výtrusů u všech zkoušených druhů. Jak *Fusarium moniliforme* tak *F. oxysporum* nerostou na agarovém mediu pokud je tam přidáno 50 μg ml^{-1} fungicidu benlate nebo homai. Supresivní efekt fungicidů na přežívání výtrusů byl též zaznamenán, ale zdá se být nižší než na agarových půdách. Při některých experimentech nebyly zaznamenány žádné významné rozdíly v počtu rozmnožovacích částic při použití sterilizované nebo nesterilizované zeminy.

Fungicides are designed to protect economic plants against pathogenic fungi. However, some cases of increased disease severity have been documented by the disturbance of natural antagonists such as *Trichoderma viride*, an antagonist to root pathogens (Baker and Cook 1974).

The present investigation was planned to test the responses of some phytopathogenic fungi to fungicides using the following parameters: a) spore germination, b) radial growth and c) survival of viable propagules in soil cultures.

MATERIALS AND METHODS

Organisms. Five phytopathogenic fungal species, viz. *Alternaria alternata* (Fr.) Keissler, *Cochliobolus sativus* (Ito et Kuribayashi) Drechsler ex Dastur, *Drechslera halodes* (Drechsl.) Subram. et Jain, *Fusarium moniliforme* Sheldon and *F. oxysporum* Schlecht.: Fr. were isolated from roots of symptomatic root rot wheat plants cultivated (season 1993/1994) in the Assiut area. The isolates were maintained on 2% potato-dextrose agar medium (PDA) at 28 ± 1 °C.

Fungicides. Four fungicides which commercially used in Egypt were selected. Their active substances are as follows: Benlate, 50% methyl-N-(1-butyl carbomolyl)-2-benzimidazole carbamate; Homai, 50% dimethyl 4,4, (0-phenylene) bis (3-thioallophonate) +30% bis (dimethylthio carbamyl) disulfide; Rhizolex-T 50% 0–2,6-dichloro-4-methyl phenyl 0,0-dimethyl-phosphorothioate + TMTD (thiram); Vitavax-Captan, 37.5% 5–6 dihydro-2-methyl-1,1,4 oxathine-3- carboxanilide) + 37.5% N-trichloromethyl mercapt-4-cyclohexene 1,2 (carboxymide).

Effect of fungicides on spore germination. Essentially the method described by Michailides and Spotts (1991) was essentially used. PDA was amended with 1, 10, 20 and 50 μg ml^{-1} of the fungicide. All fungicides were added after autoclaving the agar medium and just before pouring on the plates. For each fungus, a spore suspension containing 3–4 × 10^4 spores/ml was prepared and 100 μl was spread on the agar surface of four PDA plates. Unamended PDA controls were included for each fungus. The plates were incubated at 28 ± 1 °C, and spore germination was recorded after 6, 12 and 24 hours. Spore germination was determined by counting 50 spores in all four microscopic fields. Spores were considered germinated if germ tube lengths were at least half the diameter of the spores.

Effect of fungicides on radial growth. This was established by the method described by Spalding (1980). Aqueous suspensions of the fungicides were prepared and then added to the PDA medium to obtain the following concentration: 0, 1, 10, 20 and 50 μg a.i. ml^{-1}. The plates were inoculated with mycelial discs (5 mm diam.) taken from the periphery of actively growing colonies on PDA plates. The discs with the fungal mycelium were placed in contact with the agar surface. Three replicates were prepared for each concentration and species. The inoculated plates were incubated at 28 ± 1 °C for 7 days and the average diameter of growth was measured.

Effect of fungicides on spore survival in soil. Spore survival in soil treated with fungicides was studied according to the method described by Michailides and Spotts (1991). Survival of the spores was tested in both autoclaved and non-autoclaved soil. The soil (clay) was screened through a 2 mm screen and 2 g were placed in each test tube. Test tubes containing the soil were autoclaved at 121 °C and 1.1 kg/cm^2 pressure for 45 min. in two consecutive cycles with a two-day interval between the cycles. 0.5 ml of a spore-fungicide suspension containing

$3–4\times10^5$ spores/ml of each pathogen was added to each tube to reach a fungicide concentration of 20 and 50 $\mu g\ g^{-1}$ dry soil. Spores in water without fungicide were added to the soil in a set of control tubes. The tubes were sealed with a laboratory film (Parafilm M) and incubated at 28 ± 1 °C. After 1, 6 and 12 weeks, three tubes of each treatment were sampled. Surviving colonies were determined by the dilution plate technique of a 1:99 dilution of soil from the tube with sterile distilled water. A 100-μl sample of the diluted suspension was spread on three replicate plates of PDA per test tube. Plates were incubated at 28 ± 1 °C for 7 days. Counted colonies represented viable spores were expressed in colonies (per g dry soil).

Statistical analysis. Data were subjected to statistical analysis and means were compared using the L. S. D. test (Snedecor 1962).

RESULTS AND DISCUSSION

Spore germination

The results in Table 1 show the effect of fungicides for each one incorporated into PDA cultural medium at different concentrations on the germinability of conidiospores of the tested fungi.

Irrespective of fungus, the fungicides exerted a progressive reduction in the number of germinated spores that increased with the increase in concentration. However, this effect diminished with the length of the experimental periods.

The inhibitory effect of Benlate was more noticeable in the two fusarial species than the three dematiaceous ones. While the maximum inhibition recorded in *Cochliobolus sativus* (Dematiaceae) was 23.8% at 50 $\mu g\ ml^{-1}$, this rate reached 80% and 100% (at the same concentration) in the case of *Fusarium oxysporum* and *F. moniliforme*, respectively.

The conidiospores of all tested fungi were unable to germinate in culture medium supplemented with 50 $\mu g\ ml^{-1}$ Homai after all experimental durations. The same response was also noticed at 20 $\mu g\ ml^{-1}$ in *F. oxysporum*.

The parameter of spore germinability showed that Rhizolex-T seems to be the most toxic fungicide used in the present investigation. Its inhibitory impact on conidiospore germination was even more prominent at 1 $\mu g\ ml^{-1}$, especially during the earlier experimental periods. At 10 $\mu g\ ml^{-1}$, the inhibition percentage was 97% in the case of *Drechslera halodes* and reached 100% in *Cochliobolus sativus, Fusarium moniliforme* and *F. oxysporum*. At 20 $\mu g\ ml^{-1}$, the number of germinated spores of *Alternaria alternata* was reduced, while spores of the remaining species were unable to germinate only after 6 hours.

The phenomenon of spore germination was also observed in spores seeded on agar medium supplemented with Vitavax-Captan. The degree of inhibition increased with concentration and reached its maximum value at 50 $\mu g\ ml^{-1}$. At

Table 1. Effect of fungicides on spore germination. Data are presented in percentage (in relation to control) of germinated spores.

	Incubation					Fungicide concentration (μg ml^{-1})											
Fungi	Period	Benlate				Homai				Rhizolex				Vitavax-Captan			
	(h)	1	10	20	50	1	10	20	50	1	10	20	50	1	10	20	50
Alternaria	6	98	94	88*	85*	67	20*	4*	0*	91	31*	23*	0*	89*	27*	25*	0*
alternata	12	96	94*	90*	88*	60*	34*	6*	0*	92*	55*	39*	0*	95	64*	6*	0*
	24	99	89*	84*	83*	100	52*	31*	0*	94	82*	63*	0*	93*	84*	76*	14*
Cochliobolus	6	96	88*	83*	76*	94*	18*	3*	0*	84*	0*	0*	0*	98	80*	76*	2*
sativus	12	92*	87*	84*	80*	93*	87*	11*	0*	85*	22*	11*	0*	100	92*	83*	13*
	24	94	86*	80*	70*	100	89*	75*	0*	81*	60*	14*	0*	94	90*	86*	76*
Drechslera	6	95	88*	88*	73*	92	82*	4*	0*	94*	3*	0*	0*	92	73*	73*	8*
halodes	12	92*	82*	79*	78*	90*	77*	5*	0*	98	9*	2*	0*	92*	83*	81*	14*
	24	100	93*	85*	80*	100	92*	81*	0*	100	66*	7*	0*	95*	90*	84*	76*
Fusarium	6	86*	26*	23*	0*	100	15*	25*	0*	37*	0*	0*	0*	56*	16*	4*	0*
moniliforme	12	94*	37*	20*	6*	84*	44*	12*	0*	87*	23*	9*	0*	82*	9*	9*	0*
	24	93*	80*	70*	62*	68*	34*	11*	0*	87*	16*	14*	0*	94*	86*	82*	8*
Fusarium	6	59*	47*	30*	20*	67*	36*	0*	0*	73*	40*	0*	0*	86	16*	6*	0*
oxysporum	12	90*	35*	32*	31*	83*	19*	0*	0*	50*	16*	0*	0*	96	5*	3*	0*
	24	95	70*	63*	38*	100	26*	0*	0*	94	33*	3*	0*	92*	83*	84*	2*

Asterisked values mean significant difference compared with control values, at 0.05 of probability.

this concentration, conidiospores of *Alternaria alternata, Fusarium moniliforme* and *F. oxysporum* could not germinate during the 6 and 12 h periods. This effect was partially alleviated the 24 h later.

Studies on fungal spore germination are usually considered to be one of the most sensitive test (Strzelezyk 1976). According to the available literature, pesticides when incorporated into a medium (agar or liquid) may have either an inhibitory or stimulatory effect on spore germinability. In this respect, Duncan (1985) immersed strawberry roots containing oospores of *Phytophthora fragariae* for up to 60 days in solutions (1000 μg ml^{-1}) of Captafol, Dichlofluanid, Fostyl-Aluminium and Metalaxyl in water and buffer (pH 6.5) to test the effect of these fungicides on survival, infectivity and germination of oospores. He found that, oospores extracted from untreated roots and placed on agar incorporating the fungicides showed reduced germ tube production with Metalaxyl (3 μg ml^{-1}), Captafol (10 μg ml^{-1}) and Dichlofluanid (30 μg ml^{-1}). The results obtained by Zawahry

et al. (1991) indicated that there is a critical dose of the insecticide Nuvacron (1000, 1200, 1400 and 1600 ppm) above which germination of conidiospores of *Alternaria humicola, Fusarium sporotrichoides, Aspergillus candidus, Aspergillus niger* and *Penicillium notatum* could not be achieved. This phenomenon was also noticed by Ashour (1975). On the other hand, Afifi and Abdulla (1977) observed that the insecticide Thiolane increased the germination percentage of *Aspergillus niger, Fusarium solani* and *Penicillium frequentans.* Georgopulos (1963) and Strezelezyk (1976) suggested that strains of fungi resistant to fungicides were mutants containing in their cells different genes responsible for this resistance.

Mycelial growth

As expected, fungicides inhibited the mycelial growth of the tested fungi but in various degrees (Fig. 1). Growth inhibition was minimal at 1 μg ml^{-1} Benlate. Less than 50% inhibition in growth of both *Alternaria alternata* and *Drechslera halodes* was obtained by 10, 20 and 50 μg ml^{-1}. *Cochliobolus sativus* was inhibited by over 50% at 20 and 50 μg ml^{-1}. Both *Fusarium moniliforme* and *F. oxysporum* seemed to be sensitive to Benlate. Their growth was completely inhibited even at 10 μg ml^{-1}. These results seem to be similar to those obtained by Moubasher et al. (1984) when they tested the effect of Benlate on the mycelial growth of some fungi. They reported that while *Alternaria alternata* could survive Benlate but its growth was lower than in the control treatment, both *Fusarium oxysporum* and *F. moniliforme* were completely inhibited.

Homai exerted a significant inhibition of the mycelial growth of all tested fungi even at 1 μg ml^{-1}. The lowest inhibition rate (9%) was noticed in *Alternaria alternata* at 1 μg ml^{-1}, while 100% inhibition was observed in the case of *Fusarium moniliforme* and *F. oxysporum* at least at 50 μg ml^{-1}. The depressive effect exerted by Rhizolex-T was over 50% (at 10–50 μg ml^{-1}) in *Alternaria alternata, Cochliobolus sativus, Drechslera halodes* and *Fusarium moniliforme,* and at 50 μg ml^{-1} in *F. oxysporum.* Vitavax-Captan inhibited all tested fungi grown on media supplemented with 10 μg ml^{-1} or more and the greatest inhibition (75%) was at 50 μg ml^{-1} recorded for *F. oxysporum.* These results confirm those of El-Maraghy et al. (1993) who noticed that Vitavax-Captan retarded the mycelial growth of 5 (out of 8) fungal species, while Rhizolex-T retarded 4. Also earlier investigators concluded that Carboxin, Captan and Rhizolex inhibited the mycelial growth of different fungal species (Ekundayo 1984; Asenov 1986; Sharma and Gupta 1986; Singh and Sethunathan 1987). Trying to explain the sensitivity of several fungal species to Vitavax, it was found that Vitavax blocks the tricarboxylic acid cycle in sensitive fungi and thus causes shortage of necessary TCA intermediates required for growth. The main site of inhibition is at succinate oxidation (Sijpesteijn 1977).

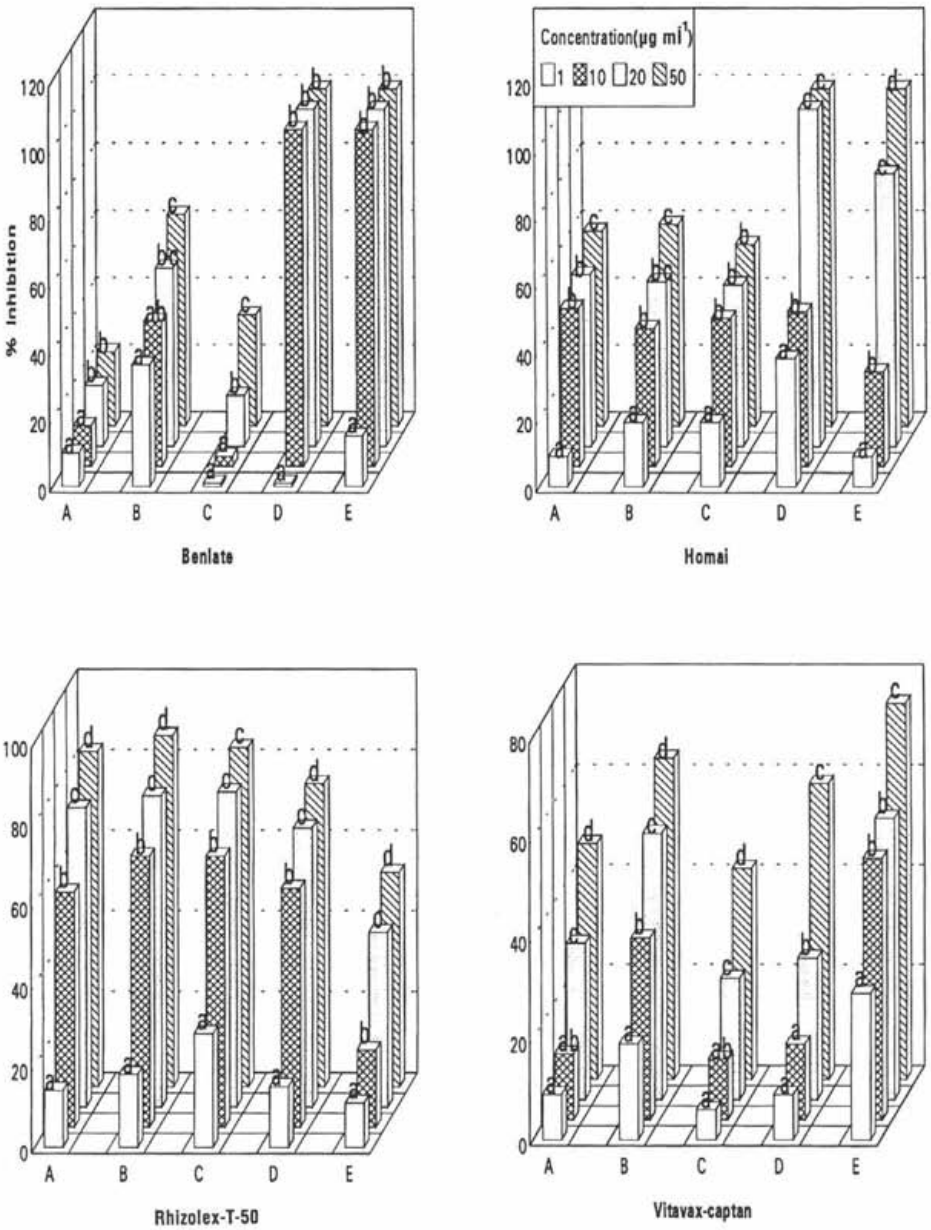

Fig. 1. Effect of fungicides om mycilial growth (calculated as % inhibition compared with control) of some phytopathogenic fungi.
A = *Alternaria alternata*, B = *Cochliobolus sativus*, C = *Drechslera halodes*, D = *Fusarium moniliforme*, E = *Fusarium oxysporum*.
Columns having the same letter for each species are not significantly different at L.S.D. = 0.05.

Spore survival in soil culture

The results collected in Table 2 show that treatment of soil with fungicides generally reduced to some extent the number of viable propagules. However, none of the species were significantly affected or even affected at all in certain treatments. Changes in the fungal population of Egyptian soil treated with fungicides have also been documented earlier (e.g. Moubasher et al. 1984; Abdel-Mallek et al. 1992; Abdel-Kader et al. 1993). Rana and Gupta (1984) studied the effect of fungicides on the viability of *Phytophthora cactorum* propagules in soil. They reported that out of eleven fungicides, Ridomil, Aliette and Euparen M were most effective in activating the fungus mycelium in the soil within 2 days. They also found that these fungicides and Antracol reduced the viability (50–100%) of sporangia in soil as well.

It is worth mentioning that two main facts have been established:

1. The response of a given fungal species to a fungicide when incorporated directly into agar medium (as in spore germination and radial growth experiments) did not accurately reflect its response to the same chemical in soil treatment experiment. This was obvious in the case of both *Fusarium moniliforme* and *F. oxysporum* with Benlate. The number of viable propagules of the two fusarial species obtained from Benlate-treated soil (even at 50 μg ml^{-1}) reached up to nearly 100% compared with untreated soil after a 6 wk incubation. On the other hand, application of Benlate to agar medium at the same dose completely inhibited the mycelial growth of the two fusarial species. Also, the average of germinated spores of the two species on agar medium ranged between 0–60% only. Such variation in fungal response to the pesticide has also been demonstrated before (Greaves 1987, Wardle and Parkinson 1990).

2. The counts of colonies forming units of a given fungal species (the data in Table 2 were expressed in % of viable propagules) recovered from autoclaved soil cultures were higher than those recovered from non-autoclaved ones. This may be attributed to the competition between the tested species and other soil fungi. This explanation is supported by the findings of Michailides and Spotts (1991). They reported that the levels of surviving propagules of *Botrytis cinerea, Mucor piriformis* and *Penicillium expansum* were generally lower in both autoclaved and non-autoclaved soil amended with herbicide than in non-amended soil. They found that this effect was greatest in non-autoclaved soil, suggesting the involvement of microbial antagonists.

The preceding results and discussion show that these fungicides have a promising effect in reducing the inoculum potential of pathogens in soil. However, the expected undesirable effect of these fungicides on non-target microorganisms must be taken into consideration.

Table 2. Effect of fungicides on survival of spores (number of viable propagules per mg dry soil) of some phytopathogenic fungi in soil cultures*.

Fungi	Conc.	Incubation periods (weeks)											
		Benlate			Homai			Rhizolex			Vitavax-Captan		
	($\mu g\ ml^{-1}$)	1	6	12	1	6	12	1	6	12	1	6	12
Alternaria alternata	0	12.3a	5.3a	10.0a	35.7a	28.3a	32.3a	27.0a	35.0a	12.3a	17.3	15.3a	13.0a
	AS 20	10.7a	2.0b	5.0b	29.3b	16.7b	10.0b	20.0b	21.7b	14.7a	8.0	8.0b	7.7b
	50	4.3b	0.7b	3.7b	20.0b	23.7a	8.3b	7.7c	19.0b	6.0b	6.7	1.3c	0.0c
	0	7.3a	1.7	2.3	5.3a	3.3	3.7a	4.3a	4.3	2.2a	23.3a	1.7	3.7
	NS 20	2.7b	1.0	1.7	3.7b	1.3	2.3a	3.3ab	3.7	0.0b	7.0b	0.7	2.3
	50	1.3c	1.3	0.0	0.0c	0.7	0.3b	1.0b	1.7	0.0b	4.3b	0.7	2.3
Cochliobolus sativus	0	27.0a	33.0	30.0a	10.3a	15.7a	32.3a	15.0a	25.0a	5.3	9.7	6.0	4.7
	AS 20	22.0a	26.3	25.3a	6.0b	10.7b	10.3b	7.7b	18.0b	9.0	8.3	4.7	3.7
	50	14.3b	32.3	19.0b	2.7b	6.7b	7.3b	5.0b	14.0b	7.3	7.0	5.0	2.7
	0	7.0	2.7	2.7	10.3a	4.3	3.3a	6.7a	5.7a	3.3	17.3	6.3a	3.0
	NS 20	5.0	1.3	1.3	3.7b	1.0	1.7ab	3.7b	4.3a	2.3	16.7	3.0b	1.7
	50	3.0	1.0	1.0	1.3c	1.0	0.0b	0.3c	1.7b	0.7	8.7	1.3b	0.3
Drechslera halodes	0	30.0a	21.0	20.0a	39.7a	33.6a	39.3a	34.7a	17.7a	28.0a	12.0	6.0	8.3a
	AS 20	16.3b	16.3	20.0a	19.3b	23.0b	18.7b	15.3b	11.7b	13.7b	7.7	3.0	7.7b
	50	8.3c	10.7	9.3b	12.7c	17.3b	9.7c	9.0b	6.3c	12.7b	7.0	2.3	0.0c
	0	4.0	3.3	3.3a	13.7a	7.7a	5.0a	11.7a	4.7	6.3a	15.7	3.3a	1.7
	NS 20	3.0	4.7	3.3a	12.7a	5.3b	2.7b	4.7b	4.3	3.3b	11.7	1.7a	1.0
	50	3.0	4.0	0.7b	6.7b	2.7b	2.7c	4.3b	3.0	0.0c	5.3	4.7b	0.3

Table 2. Effect of fungicides on survival of spores (number of viable propagules per mg dry soil) of some phytopathogenic fungi in soil cultures*. (Continued).

Fungi	Conc.	Incubation periods (weeks)											
		Benlate			Homai			Rhizolex			Vitavax-Captan		
	($\mu g\ ml^{-1}$)	1	6	12	1	6	12	1	6	12	1	6	12
Fusarium moniliforme	0	113.3a	55.3a	39.3a	83.3a	69.3a	64.7a	37.0a	60.0a	39.0a	35.3a	96.7a	33.7a
	AS 20	54.1b	80.0b	31.7b	61.7b	52.7b	37.7b	27.7b	45.0ab	28.0b	19.0b	73.3ab	30.0b
	50	33.7b	60.0b	18.0c	34.0c	30.0c	29.0c	20.3c	35.0b	16.7c	13.0b	55.0b	25.7b
	0	20.7a	15.0	8.0a	57.3a	32.7a	12.0a	7.0a	24.3a	13.0a	11.0a	15.3	18.0
	NS 20	9.3b	9.0	2.7b	10.0b	16.3b	10.0a	4.7ab	15.7b	5.3b	8.3a	12.7	14.7
	50	3.6c	10.3	1.7b	8.7b	3.7c	3.7b	4.0b	15.0b	3.3c	2.7b	8.7	16.0
Fusarium oxysporum	0	116.7a	14.3	18.0a	39.0a	30.0	22.3a	88.3a	39.3a	36.3a	44.3	64.3a	20.0
	AS 20	80.0b	12.7	13.0b	19.7b	28.3	12.3b	47.7b	25.3b	20.0b	23.3	46.0b	19.7
	50	63.3b	10.3	6.7c	19.3b	22.0	5.0c	19.3c	19.0b	11.7c	35.0	33.3c	19.3
	0	33.3a	4.6	4.7a	15.7a	5.0a	9.0a	34.7a	9.7	11.3a	19.3	13.7a	26.7a
	NS 20	10.3b	1.3	2.3b	6.0b	2.3b	2.3b	12.7b	5.0	6.7b	13.7	14.3a	16.7b
	50	4.0b	2.0	2.7b	3.3b	1.0b	1.0b	8.7b	4.3	6.0b	10.0	5.3b	10.7b

Values followed by the same letter in the same column under each treatment separately are not significantly different at 5%.

* AS = autoclaved soil; NS = non-autoclaved soil.

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CZECH MYCOL. 50 (1), 1997

First records of Pholiota subochracea and Pholiota elegans in the Czech Republic

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Holec J. (1997): First records of Pholiota subochracea and Pholiota elegans in the Czech Republik. – Czech Mycol. 50: 45–56

The rare species *Pholiota subochracea* (= *P. nematolomoides*) was found on three localities in south Bohemia (Šumava Mts. and Novohradské hory Mts.) in the year 1995. These records represent the first data on its occurrence in the Czech Republic. The recently described species *Pholiota elegans* Jacobsson 1990 was found in south Bohemia (Šumava Mts., Spáleniště hill) in the year 1996. It is the first record of this fungus outside the Nordic countries (Sweden, Norway, Finland). Thorough descriptions of macro- and microcharacters based on the author's own collections are given together with drawings of important microcharacters, colour photographs and a discussion on ecology, distribution and taxonomy of both species.

Key words: *Pholiota subochracea, Pholiota elegans*, Czech Republic, first records, taxonomy, ecology, distribution.

Holec J. (1997): První nálezy druhů Pholiota subochracea a Pholiota elegans v České republice. – Czech Mycol. 50: 45–56

Vzácný druh šupinovky *Pholiota subochracea* (= *P. nematolomoides*) byl v roce 1995 nalezen na třech lokalitách v jižních Čechách, ležících na Šumavě a v Novohradských horách. Jde o první nálezy tohoto druhu v České republice. Nedávno popsaný druh *Pholiota elegans* Jacobsson 1990 byl sbírán v roce 1996 na lokalitě Spáleniště u Českých Žlebů na Šumavě. Je to nejen první nález pro Českou republiku, ale zároveň i pro Evropu mimo Švédska, Norska a Finska, kde byl druh rozeznán a popsán. U obou druhů jsou uvedeny podrobné popisy makro- a mikroznaků, založené na studiu plodnic nalezených autorem článku a M. Beranem. Popisy jsou doprovozeny kresbami důležitých mikroznaků, barevnými fotografiemi a diskusí o ekologii, rozšíření a taxonomii obou druhů.

INTRODUCTION

In the period 1992–1996 I studied the taxonomy of *Pholiota* species growing in the Czech Republic as a subject of my doctoral thesis. Preliminary results were published in two small contributions (Holec 1995, 1996) and doctoral thesis (Holec 1997), all written in Czech. During the field work many interesting, critical or extremely rare *Pholiota* species were found. Some of these species were new for the Czech Republic. The finds of *Pholiota subochracea* (A. H. Smith) A. H. Smith et Hesler and *Pholiota elegans* Jacobsson belong to the most interesting ones due to their rare occurrence in Europe. Therefore, records of these two species are published in the present paper.

MATERIAL AND METHODS

Descriptions of macrocharacters are based on the author's own finds, the given microcharacters are based on all specimens mentioned in the paragraphs "Specimens studied". Microcharacters were analysed using a 5% solution of KOH and an aqueous solution of Congo Red. Fruitbodies collected by the author are deposited in the PRM herbarium (Mycological Department, National Museum, Praha). Several specimens were kindly provided by Mgr. M. Beran from the CB herbarium (Museum of South Bohemia, České Budějovice) and Prof. M. Moser from IB (Herbarium, Institut für Botanik, Universität Innsbruck).

RESULTS AND DISCUSSION

Pholiota subochracea (A. H. Smith) A. H. Smith et Hesler

Hypholoma subochraceum A. H. Smith, Mycologia 36: 250, 1944. – *Pholiota subochracea* (A. H. Smith) A. H. Smith et Hesler, The North American species of Pholiota: 153, 1968.

Syn.: *Nematoloma subochraceum* (A. H. Smith) A. H. Smith, Mycologia 38: 502, 1946. – *Dryophila nematolomoides* Favre, Schweiz. Z. Pilzk. 36: 67, 1958. – *Pholiota nematolomoides* (Favre) M. Moser, Röhrlinge und Blätterpilze, ed. 3: 243, 1967 (in Gams H., Kleine Kryptogamenflora, vol. 2b/2).

Selected icones: Favre (1958): Table 5. – Moser and Jülich, Farbatlas der Basidiomyceten, part III: Pholiota 1. – Dähncke (1993): p. 627. – Breitenbach and Kränzlin (1995): Fig. 435.

Illustrations: Fig. 1, Fig. 4

Description (according to my collection: PRM 890574). Fruitbodies grew in a small fascicle. Pileus 1–3.5 cm, almost hemisphaerical when young, with involute margin, convex at maturity, with a low obtuse umbo, flesh thin, surface smooth, slightly viscid when moist but not apparently glutinous. Pileus cuticle ochre-brown in young fruitbodies, light yellow et margin at maturity, towards centre becoming darker, yellow-ochre. The pileus is slightly hygrophanous, the moist pileus margin having an olivaceous tinge. Pileus surface covered with unregularly distributed minute ochre-brown to red-brown patches. Lamellae very dense, with lamellulae, 0.3–0.5 cm broad, even or slightly ventricose, emarginate, dull yellow when young, light yellow-brown at maturity. Stipe 4–7 × 0.3–0.4 cm, cylindric, hollow at maturity, often curved, smooth in upper part, light yellow, towards base ochre-brown, covered with white-yellow remnants of velum forming fissile floccose patches; base of stipe white tomentose, velum light yellow in young fruitbodies, later missing. Context light yellow to yellow in pileus, yellow in upper part of stipe, rusty brown in lower part. Taste mild, later slightly bitter, smell none. Spore print brown.

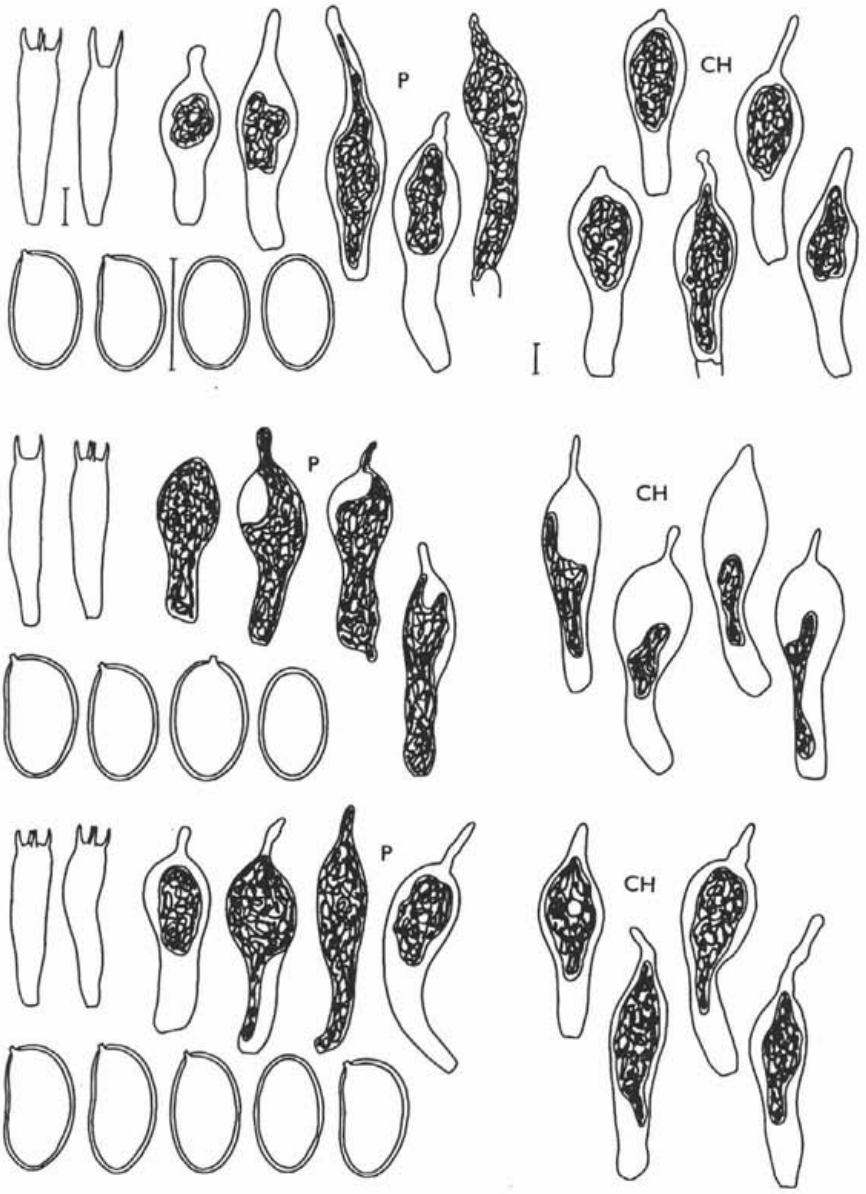

Fig. 1. *Pholiota subochracea* (spores, basidia, cheilocystidia, pleurocystidia).
– Šumava Mts., margin of Mrtvý luh peat bog, 22. VIII. 1995, leg. J. Holec (PRM 890574).
– Novohradské hory Mts., Žofínský prales virgin forest, 26. VIII. 1995, leg. M. Beran (CB, as *P. nematolomoides*).
– Šumava Mts., 0,5 km SSE of Černý Kříž railway station, 27. VIII. 1995, leg. M. Beran (CB, as *P. nematolomoides*).
Explanations: P: pleurocystidia, CH: cheilocystidia. Scale bar = 5 μm. Ill. J. Holec.

Spores 5–5,8(–6) × (2,7–)3–3.7(–4) μm, ellipsoid to ovoid-ellipsoid, in side view some of them slightly phaseoliform; wall thin, yellow-brown, smooth; germ pore absent. Basidia 21–27 × 4.5–6 μm, cylindric to narrowly clavate, clamped, 4–(2–)–spored, sterigmata 3–6 μm. Cheilocystidia of the chrysocystidia type, filled with a refractive inclusion colouring yellow in KOH or NH_4OH, 31–49 × 8–12 μm, clavate, cylindric-clavate, clavate-fusiform, in upper part mostly with narrow cylindric protuberances (mucronate cystidia), clamped. Pleurocystidia of the same character as the cheilocystidia. Lamellar trama regular, made up of parallel to subparallel hyphae 4.5–15 μm broad, individual cells cylindric or slightly inflated, clamped, subhymenium non-gelatinous, formed by densely packed interwoven hyphae 3–4.5 μm broad. Pileus cuticle a cutis, 2-layered, upper layer made up of densely arranged parallel to subparallel hyphae 2–3 μm broad, with membranal pigment and yellow incrustations not soluble in KOH, lower layer made up of sparsely arranged hyphae 6–9 μm broad, without incrustations. Clamps present. Stipe cuticle a cutis of densely arranged cylindric hyphae 3–6 μm broad, brown-ochre coloured, with prominent membranal pigment and incrustations, sometimes also with a vacuolar pigment, clamps present.

Ecology. *Pholiota subochracea* was found on dead wood of *Picea abies* and in one case on strongly decayed wood of a conifer (*Picea abies* or *Pinus sylvestris*). The species prefers wood in later stages of decay and occurs on fallen trunks and branches. All finds from the Czech Republic originate from the montane belt (elevation 740–780 m a.s.l.). Concerning the vegetation, *P. subochracea* was found in a montane mixed wood (*Fagus, Abies, Picea*) with the character of a virgin forest (Žofínský prales), in a spruce forest on humid soil (Černý Kříž in the Šumava Mts.) and in a wood stand with *Pinus rotundata, Pinus sylvestris, Picea abies* and *Betula pubescens* (at the margin of Mrtvý luh peat bog). In other European countries the species is reported from wood of *Picea abies* (Jacobsson 1990; herbarium specimens from IB: see Specimens studied), *Pinus cembra* (Favre 1958, as *P. nematolomoides*) and probably also *Pinus mugo* (Breitenbach and Kränzlin 1995, as *P. nematolomoides*). The records in North America originate from decaying conifer logs (Smith and Hesler 1968). According to the finds from the Czech Republic, the species prefers stands with presence of dead wood of *Picea abies* in later stages of decay, especially forests of natural or seminatural character where fallen trunks and logs are present. Besides, *P. subochracea* was found in the montane belt where the climate is relatively humid. This fact agrees well with the conclusions of Jacobsson (1990) that *P. subochracea* "seems to prefer a humid climate..., ... in central Europe only found in mountainous areas".

Distribution. *Pholiota subochracea* seems to be very rare in the Czech Republic. The first data on its occurrence in the Czech Republic are included in my previous paper (Holec 1996). There are no specimens of this species in Czech herbaria collected in the past. The three finds reported in this paper originate from

mountainous areas in south Bohemia – Šumava Mts. and Novohradské hory Mts. In my opinion, the species may be found on other localities in the above areas where habitat conditions are suitable (especially humid climate, high amount of decaying wood of conifers) and probably also in other mountainous regions of the Czech Republic.

Pholiota subochracea is relatively common in southwestern Sweden and isolated records are known from other parts of Sweden as well as from Norway and Finland (Jacobsson 1990; distribution map included). In other European countries *Pholiota subochracea* is reported under the name *Pholiota nematolomoides* (Favre) M. Moser. The species was found in Switzerland at an altitude of 1350 m (Breitenbach and Kränzlin 1995: Berner Voralpen) and in the alpine belt at an altitude of 2000 m (Favre 1958: Alps). It is also known from Germany where the finds are located in mountains too – the Schwarzwald and Bayerischer Wald (Krieglsteiner 1982, 1991). The finds from Bayerischer Wald are located close to the Czech records from the Šumava Mts. – both Czech and German finds were made in one mountain range on the border between the two countries. Altitude and habitat of the finds from Bayerischer Wald are also similar to Czech records (Luschka 1993: 740–780 m a.s.l., spruce forest on peaty soil: "Aufichtenwald"). *Pholiota subochracea* is known from montane regions of Austria (Krieglsteiner 1991; herbarium specimens from Tirol: IB 80/703, IB 82/317 collected by M. Moser). These data clearly show that *Pholiota subochracea* has a boreal-montane to boreal-subalpine distribution pattern in Europe. In North America the species is reported from the Pacific Northwest (Idaho, Oregon, Washington; see Smith and Hesler 1968).

Discussion. *Pholiota subochracea* is a rare fungus and only few mycologists had an oppurtunity to see it in nature. Moreover, the species is rather inconspicuous and probably overlooked due to its resemblance to some *Hypholoma* species. However, *P. subochracea* is recognized by small fruitbodies, brown spore print, yellow-brown lamellae at maturity and relatively small spores having no germ pore. The presence of numerous chrysocystidia (both pleuro- and cheilocystidia) places the species within *Pholiota* subg. *Pholiota* sensu Jacobsson (1990) where it has a rather isolated position due to the appearance of its fruitbodies. The species is known as *Pholiota nematolomoides* (Favre) M. Moser in Europe. Favre described his species according to fruitbodies found in Switzerland at an altitude of 2000 m on wood of *Pinus cembra*. It is rather interesting that no *Pholiota* species described by Fries fits it, although according to Jacobsson (1990) the species is common in Femsjö.

After careful study of some herbarium specimens and type material of *Pholiota subochracea* (A. H. Smith) A. H. Smith et Hesler from the MICH herbarium, Jacobsson (1990) came to the conclusion that this American fungus is identical with *Pholiota nematolomoides* (Favre) M. Moser. As the description of *Pholiota*

subochracea by Smith and Hesler (1968) agrees well with finds from the Czech Republic, I agree with Jacobsson's opinion. Thus, the correct name for this species is *Pholiota subochracea* (A. H. Smith) A. H. Smith et Hesler that was published already in 1944 (as *Hypholoma subochraceum*).

Specimens studied. Czech Republic: Šumava Mts., margin of Mrtvý luh peat bog near Černý Kříž railway station, 740 m a.s.l., strongly decayed wood of a conifer (*Picea abies, Pinus sylvestris*?), 22. VIII. 1995, leg. J. Holec, JH 184/95 (PRM 890574). – Šumava Mts., 0,5 km SSE of Černý Kříž railway station, 750 m a.s.l., decayed log of *Picea abies*, 27. VIII. 1995, leg. M. Beran (CB, as *P. nematolomoides*). – Novohradské hory Mts., Žofínský prales virgin forest, 750–780 m a.s.l., decayed stump of *Picea abies*, 10. IX. 1995, leg. M. Beran (CB, as *P. nematolomoides*); decayed log of *Picea abies*, 26. VIII. 1995, leg. M. Beran (CB, as *P. nematolomoides*). Austria: Tirol, Gnadenwald, *Picea abies*, 30. VIII. 1980, leg. et det. M. Moser (IB 80/703, as *P. nematolomoides*). – Tirol, Angerberg, *Picea abies*, 10. IX. 1982, leg. et det. M. Moser (IB 82/317, as *P. nematolomoides*).

Pholiota elegans Jacobsson

Pholiota elegans Jacobsson, Windahlia 19: 72, 1990.

Illustrations: Fig. 2, Fig. 5

Description (according to my collections: PRM 889476, 889455). Fruitbodies are growing in fascicles or small groups. Pileus 2–7 cm, hemisphaerical with involute margin when young, then convex, in some fruitbodies with a low obtuse umbo, margin covered with fine and loosely arranged tomentose velum when young, the velum later missing. Pileus cuticle strongly viscid to glutinous in moist weather, white-yellow or light yellow at margin, yellow to yellow-ochre towards the centre, almost yellow-orange when young, innately radially striate, surface covered with irregularly distributed, minute and innate scales that are rusty-ochre to cinnamon-brown, the scales present in some fruitbodies only, sometimes swollen up in the gelatinous covering of the pileus or removed by rainfall. Lamellae dense, with lamellulae, 0.4–0.8 cm broad, even or slightly ventricose, emarginate and decurrent with a small tooth, yellow-white to light yellow when young, then light yellow or light yellow-ochre, at maturity light ochre-brown, edge even, somewhat yellower than the lamellae surface, almost lemon-yellow when young. Stipe 2.5–6 × 0.3–0.9 cm, cylindric, base sometimes slightly swollen, connected with pileus margin by white arachnoid velum later forming an almost indistinct annular zone, the zone missing and absent at maturity; above the velum zone the stipe is white or yellow-white, smooth or finely floccose, below it is white or whitish, at the base slightly ochre with an orange flush, finely yellow-rusty floccose to fibrillose, the upper part light yellow at maturity, the lower part ochre to rusty-ochre, finely rusty-yellow to rusty-ochre floccose-fibrillose, becoming rusty-ochre after touching the stipe

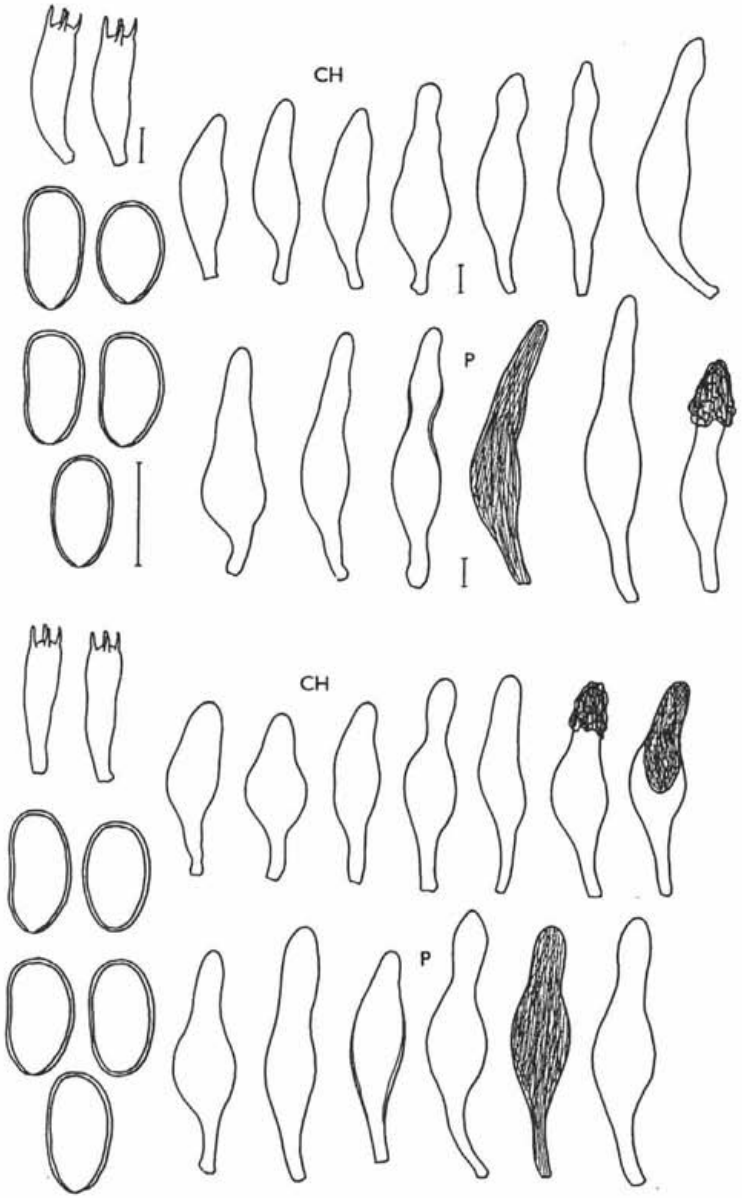

Fig. 2. *Pholiota elegans* (spores, basidia, pleurocystidia, cheilocystidia).
– Šumava Mts., Spáleniště hill near the village of České Žleby, 11. X. 1996, leg. J. Holec, (PRM 889455).
– Šumava Mts., Spáleniště hill near the village of České Žleby, 11. X. 1996, leg. J. Holec, (PRM 889476).
Explanations: P: pleurocystidia, CH: cheilocystidia. Scale bar = 5 μm. Ill. J. Holec.

surface. Context light yellow in pileus, sometimes with a grey flush, deeper yellow below the pileus surface, in stipe yellow-white to lemon yellow below the surface, in central part yellow, in stipe base yellow-ochre to rusty-brown. Taste mild, smell indistinct or slightly "fleshy-gummose". Spore print brown (Moser 1978: B7).

Spores 5–6(–6.5) × (2.7–)3–3.5(–3.7) μm, ellipsoid to ovoid-ellipsoid, in side view slightly but distinctly phaseoliform, wall ochre-brown, smooth, with minute and narrow germ pore (at most 0.4–0.6 μm broad), the pore is indistinct in some spores. Basidia 16–25 × 6–8 μm, cylindric or narrowly clavate, sometimes slightly narrower in the middle part, 4- or 2-spored, with clamps at base. Cheilocystidia (27–)31–54 × 8–12 μm, forming a sterile band on the edge, clavate when young, then lageniform-fusiform to narrowly utriform with prolonged basal part, in upper part often slightly broadened, thin-walled, hyaline or filled with a regularly distributed yellow pigment, sometimes covered with prominent yellow-ochre incrustation ("cap") in the upper part, clamped. Pleurocystidia numerous, 45–61 × 9–12 μm, mostly lageniform-fusiform but also cylindric-fusiform or narrowly utriform, thin-walled but exceptionally with slightly thickened wall (up to 1.5 μm) in the middle part, hyaline or filled with a regularly distributed yellow pigment, sometimes covered with prominent yellow-ochre incrustation ("cap") in the upper part, clamped. Lamellar trama regular, made up of parallel hyphae, individual cells cylindric or slightly inflated, in the middle part 4–13 μm broad, near the subhymenium only 2–3 μm broad, subhymenium distinctly gelatinous, consisting of loosely arranged interwoven and branched hyphae. Clamps present. Pileus cuticle an ixocutis, 3-layered, upper layer thin, made up of densely arranged, parallel, 2–4.5 μm broad hyphae, distinctly yellow coloured, with a membranal and incrusting pigment; middle layer relatively thick, strongly gelatinous, formed by loosely arranged, parallel to subparallel, 1–3.5 μm broad hyphae, with hyaline content but distinctly yellow incrusted; lower layer thin, yellow coloured, made up of densely arranged parallel to subparallel, 3–5 μm broad hyphae, densely covered by yellow-rusty incrustations. Clamps present. Stipe cuticle a cutis consisting of densely arranged cylindric and parallel 2–4 μm broad hyphae, with membranal and incrusting pigment; clamps present.

Ecology. Only two finds of *Pholiota elegans* are known from the Czech Republic, both from the same locality (Šumava Mts., Spáleniště Hill near the village of České Žleby). In one case the fruitbodies grew in decaying needles and leaves under *Picea abies, Acer pseudoplatanus* and *Fraxinus excelsior* (montane scree wood), in the second case on a fallen trunk of *Fagus sylvatica* in later stage of decay (mixed montane wood with predominance of *Fagus*). The vegetation has a virgin forest character and the area is protected as the first (strictly natural) zone of the Šumava National Park. The Spáleniště locality is characterised by a great amount of fallen trunks of *Fagus sylvatica, Acer pseudoplatanus, Ulmus glabra, Fraxinus excelsior, Abies alba,* and *Picea abies*. The altitude of both finds amounts 900–920 a.s.l.

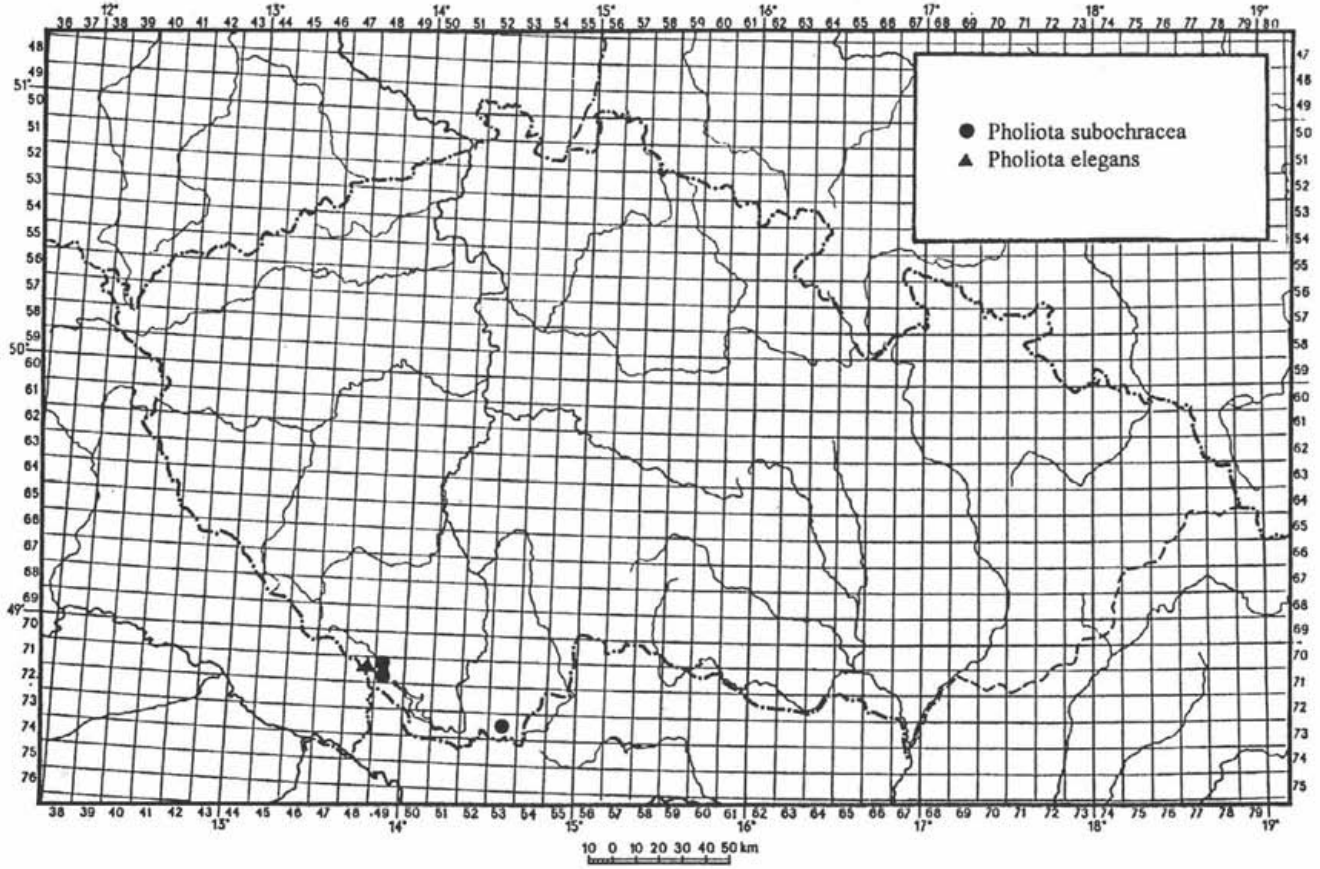

Fig. 3. Localities of *Pholiota subochracea* and *Pholiota elegans* in the Czech Republic.

According to Jacobsson (1990), *P. elegans* occurs on "old logs, branches and other wood debris, bark etc. on the ground, generally of deciduous wood but also *Picea*".

Distribution. Up to now, *Pholiota elegans* is known only from 14 localities scattered throughout Sweden, Norway and Finland (see distribution map published by Jacobsson 1990) and occurs abundantly at many of them. As there are no literature data on its occurrence in other countries, my finds seem to be the first ones outside the Nordic countries. Both Czech finds originate from the same locality (south Bohemia, Šumava Mts., NE slope of the Spáleniště hill near the České Žleby village) and their distance is about 400 m. *Pholiota elegans* is included into the fungi guide by Courtecuisse and Duhem (1994: 352). However, R. Courtecuisse confirmed me that the species is not known from France.

Discussion. *Pholiota elegans* was recently described as a new species (Jacobsson, Windahlia 19: 72, 1990) and its status was confirmed by negative results of compatibility tests with monosporic strains of *P. lenta* and *P. lubrica* (Jacobsson 1990: 74). According to Jacobsson (1990), *P. elegans* differs from the closely related and similar species *P. lubrica* in having smaller spores (see Tab. 1) and the mostly yellow colour of the pileus, and from *P. spumosa* by its spore shape (*P. spumosa*: ovoid, *P. elegans*: slightly phaseoliform in side view).

Table 1. Comparison of spore size in *P. lubrica* and *P. elegans*

	Pholiota lubrica	*Pholiota elegans*
Jacobsson (1990)	6–7.5 × 3–4 μm	5–6.5(–7) × 3–3.5(–4) μm
Holec (1997)	(5.3–)5.8–7.5 × (3–)3.3–4(–5) μm	5–6(–6.5) × (2.7–)3–3.5(3.7) μm

All macro- and microcharacters of fruitbodies found in the Czech Republic agree well with Jacobsson's description (Jacobsson 1990). To be sure, I sent several fruitbodies to S. Jacobsson for revision, who unambiguously confirmed that my fruitbodies represent his species *Pholiota elegans*.

The finds of *P. elegans* in the Czech Republic are important from several points of view. They represent the first records outside Scandinavia and confirm that *P. elegans* is a good species, because fruitbodies found in the Czech Republic differ from all *Pholiota* species known in Central Europe (see Holec 1996, 1997). The species seems to be very rare and is probably also overlooked or confused with other species, especially *P. lubrica*. In the PRM herbarium, where a rich collection of *Pholiota* species found in several European countries is deposited, *P. elegans* was not represented although I revised all specimens labelled *P. lubrica* or *P. lenta*, which the species could have been filled under. Moreover, I have studied the mycoflora of natural woods in the Šumava mountains as well as other areas

of the Czech Republic for more than 10 years and I never found such a fungus before. Therefore, during investigation of natural forests with a great amount of dead wood of deciduous trees (especially in the montane belt), attention should be paid to this nice species that could be commoner than we think on the basis of few recent records. I expect that *P. elegans* will be found in other countries of Central, West and East Europe too.

Specimens studied. Šumava Mts., Spáleniště hill near the village of České Žleby, in fallen leaves and needles under *Picea abies, Acer pseudoplatanus* and *Fraxinus excelsior*, 920 m a.s.l., 11. X. 1996, leg. J. Holec, JH 669/96 (PRM 889476); fallen decaying trunk of *Fagus sylvatica*, 900 m a.s.l., 11. X. 1996, leg. J. Holec, JH 682/96 (PRM 889455).

ACKNOWLEDGEMENTS

I thank Mr. M. Beran of the South Bohemian Museum in České Budějovice for loaning me his specimens of *Pholiota subochracea* and Prof. M. Moser for the loan from the herbarium IB. The field work was supported by grants from the Agency of the Nature and Landscape Protection of the Czech Republic (contract no. M 44/11/95) and the Ministry of Culture of the Czech Republic (project no. PK96M05OP124), the final elaboration by the Grant Agency of the Czech Republic (project no. 206/97/0273).

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Fig. 4. 1) *Pholiota subochracea*, Šumava Mts., margin of Mrtvý luh peat bog, 22. VIII. 1995, leg. J. Holec (PRM 890574). Detail of mature fruitbodies.

Fig. 5. *Pholiota elegans*, Šumava Mts., Spáleniště hill near the village of České Žleby, 11. X. 1996, leg. J. Holec, (PRM 889476). Young fruitbodies. Photo J. Holec.

CZECH MYCOL. 50 (1), 1997

The effect of chloroform extracts of micromycete biomass on the movement of tracheal cilia in one-day old chickens in vitro

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Piecková E. and Jesenská Z. (1997): The effect of chloroform extracts of micromycete biomass on the movement of tracheal cilia in one-day old chickens in vitro. – Czech Mycol. 50: 57–62

The ciliostatic effect of metabolites from mycelia and spore biomass of 185 micromycete strains extractable with chloroform on tracheal epithel cilia was investigated in 1-d old chickens *in vitro*. The strains were isolated from cotton or flax. Extracts of 54 strains (29 %) displayed ciliostatic activity: 16 (9 %), 6 (3 %), and 32 (17 %) strains stopped the movement of cilia after 24, 48, and 72 hours, respectively. There may be relationships between these results and respiratory tract illnesses in people living in mouldy dwellings, working with mouldy materials, or with sick building syndrome.

Key words: Micromycete, biomass, chloroform extract, tracheal cilia.

Piecková E. a Jesenská Z. (1997): Vplyv chloroformových extraktov biomasy mikromycét na pohyb tracheálnych cílií jednodňových kurčiat in vitro. – Czech Mycol. 50: 57–62

Sledoval sa ciliostatický účinok chloroformom extrahovateľných metabolitov z biomasy mycélia a spór 185 kmeňov mikromycét na tracheálnom epiteli jednodňových kurčiat *in vitro*. Mikromycéty boli izolované z bavlny a ľanu. Ciliostatickú aktivitu mali extrakty 54 kmeňov (29 %): 16 (9 %), 6 (3 %) a 32 (17 %) extraktov zastavilo pohyb cílií po 24, 48, resp. 72 h. Možno uvažovať o vzťahu medzi týmito výsledkami a ochoreniami dýchacích ciest u ľudí žijúcich v plesnivých bytoch, pracujúcich s plesnivými materiálmi, resp. trpiacimi tzv. sick building syndrome.

There are many micromycete particles, such as intracellular secondary metabolites, or dust contaminated with extracellular mycotoxins in the air of working and indoor environments. Aflatoxin B_1, ochratoxin A, zearalenone, secalonic acid D and deoxynivalenol were detected in the working environment. Some trichothecenes were found in the atmosphere of dwellings and offices (Hendry and Cole 1993, Jesenská 1993, Jesenská et al. 1990, Pasanen et al. 1993, Verhoeff et al. 1994).

Tracheal and bronchial illnesses affect people, especially children, living in damp and mouldy dwellings to a higher degree (Smoragiewucz et al. 1993). People working in air-conditioned offices may suffer from sick building syndrome – nonspecific respiratory complaints of uncertain aetiology (Jaakkola et al. 1994, Marasm et al. 1994, Mishra et al. 1991). Increased morbidity from chronic bronchitis in textile and agricultural workers is also known (Jaroš 1989, Summerbell et al. 1992, Zejda

and Dosman 1991, Zuskin et al. 1991). The negative influence of micromycetes and their secondary metabolites, mainly mycotoxins, on respiratory organs in connection with the mentioned illnesses is well-known.

The aim of our work was to contribute to the explanation of the possible aetiology of the above illnesses referring to our former results (Jesenská and Bernát 1994, Piecková and Jesenská 1994, 1995). We studied the ciliostatic effect of chloroform extracts from micromycete mycelia and spores on tracheal cilia in a model system of 1-day old chicken organ cultures. Micromycete strains were isolated from cotton and flax, cultivated stationary on a liquid medium with sucrose and yeast extract during 10 days.

MATERIAL AND METHODS

Biomass extracts of micromycetes. 185 strains of filamentous fungi were isolated from samples of cotton and flax. The isolated strains were cultivated on slant Sabouraud agar (IMUNA, Co., Šarišské Michaľany, Slovakia) at 25 °C during 14 days. The culture of each strain growing in 3 tubes was scratched into 200 ml of a liquid medium with yeast extract (2 %) and sucrose (10 %) in 500 ml Erlenmayer flasks and stationary cultivated at 25 °C during 10 days. Biomass of each culture was extracted twice by 200 ml of chloroform after filtration of the cultivation medium. The united extract was dried with Na_2SO_4 without water and evaporated in a water bath.

The ability of isolated strains of *Aspergillus flavus* to produce aflatoxin B_1 and G_1 was investigated by their cultivation on liquid medium with 20 % sucrose and 2 % yeast extract, at pH 5.5 ans 25 °C during 14 days (Abarca et al. 1988).

Cultivation medium for organ cultures, tracheal organ cultures of 1-day old chickens and test evaluation were described in our previous studies (Piecková and Jesenská 1994, 1995).

RESULTS

The biomass of 54 (29 %) out of 185 investigated micromycete strains contained chloroform-extractable secondary metabolites with ciliostatic activity against tracheal cilia of the 1-day old chickens *in vitro*:

sixteen strains (9 %) stopped the movement of cilia already after 24 hours, these were strains of *Aspergillus flavus* (2 strains, 1 of them produced aflatoxins *in vitro*), *A. glaucus* group (2 strains), *A. nidulans* (1), *A terreus* (1), *Fusarium* sp. (7) and *Penicillium* sp. (3);

six strains (3 %), namely *A. flavus* (1 strain), *Fusarium* sp. (1) and *Penicillium* sp. (4), stopped the ciliary movement after 48 hours;

thirty-two strains (17 %), namely *A. flavus* (4 strains, 3 of them produced aflatoxins *in vitro*), *A. fumigatus* (2), *A. glaucus* group (3), *A. nidulans* (6), *A. niger* group (1), *A. ochraceus* (2), *A. terreus* (3), *A. versicolor* (3), *Cladosporium* sp. (1), *Fusarium* sp. (1), and *Penicillium* sp. (6), stopped the ciliary movement after 72 hours.

The ciliary movement was not affected by metabolites of the other 131 studied micromycete strains and in the reference media within the experiment (Table 1).

Discussion

Cells of microscopic filamentous fungi may contain various mycotoxins, e. g. citreoviridin, cyclopiazonic acid, luteoskyrin, penitrem A, stachybotryotoxins, sterigmatocystin, verruculogen, viomellein, xantomegnin, etc. (Filtenborg et al. 1983, Pasanen et al. 1993). Airborne fungal particules can become a transfer vehicle of mycotoxins to the human organism (Burg and Shotwell 1984). Aflatoxin B_1can be stored in spores and the mycelium of toxinogenic strains of *Aspergillus flavus* and *A. parasiticus* (Shih and Marth 1975). It is known that aflatoxins negatively affect the function of tracheal cells of hamsters, rabbits and monkeys *in vitro* (Coulombe et al. 1986, Wilson et al. 1990), and are carcinogenic for lung cells (Autrup et al. 1979). Aflatoxin B_1 transforms the metabolism of tracheal epithelia by its binding to the cell DNA (Daniels et al. 1993).

Ciliostatic activities of some mycotoxins on 1-d old chickens tracheal organ cultures *in vitro* were described in the previous studies (Jesenská and Bernát 1994). Ciliostatic activities of chloroform-extractable and heat-stable metabolites of some micromycetes isolated from cotton, flax, straw and sorghum were published by us, too (Piecková and Jesenská 1994, 1995). In this part of our study we have been concentrating on the problem of ciliostatic activities of chloroform extracts of micromycete biomass *in vitro* in this part of our study. It was found that 29 % of the investigated strains were able to produce metabolites which stopped the movement of tracheal cilia under conditions of the described model system in 24, 48, and 72 hours.

Destroyed ciliary movement in the airways may be the first step in the development of human chronic respiratory illnesses with major health losses and they need to be studied further.

Table 1. The effect of chloroform extracts of micromycete biomass isolated from flax and cotton on the movement of tracheal cilia in 1-day old chickens *in vitro*

Micromycetes	Number of strains	Time [h] 24	48	72	Number of strains total	+
		Movement of cilia				
Acremonium sp.	1	+	+	+	1	0
Alternaria sp.	7	+	+	+	7	0
Aspergillus candidus	1	+	+	+	1	0
A. flavus	19 (5*)	+	+	+		
	4 (3*)	+	+	−		
	1	+	−	−	26	7
	2 (1*)	−	−	−		
A. fumigatus	9	+	+	+	11	2
	2	+	+	−		
A. glaucus group	12	+	+	+	17	5
	3	+	+	−		
	2	−	−	−		
A. niger group	9	+	+	+	10	1
	1	+	+	−		
A. nidulans	2	+	+	+		
	6	+	+	−	9	7
	1	−	−	−		
A. ochraceus	12	+	+	+	14	2
	2	+	+	−		
A. terreus	8	+	+	+		
	3	+	+	−	12	4
	1	−	−	−		
A. versicolor	7	+	+	+	10	3
	3	+	+	−		
A. wentii	1	+	+	+	1	0
Cladosporium sp.	11	+	+	+	12	1
	1	+	+	−		
Fusarium sp.	15	+	+	+		
	1	+	+	−	24	9
	1	+	−	−		
	7	−	−	−		

Table 1. The effect of chloroform extracts of micromycete biomass isolated from flax and cotton on the movement of tracheal cilia in 1-day old chickens *in vitro* (Continued).

Micromycetes	Number of strains	Time [h] 24	48	72	Number of strains total	+
		Movement of cilia				
Gliocladium sp.	1	+	+	+	1	0
Penicillium sp.	14	+	+	+		
	6	+	+	–		
	4	+	–	–	27	13
	3	–	–	–		
Sporotrichum sp.	1	+	+	+	1	0
Torula sp.	1	+	+	+	1	0
Strains with ciliostatic activity		16	6	32	185	54
	%	9	3	17	100	29

Note: * aflatoxin B_1 and G_1 producing strain of *Aspergillus flavus*

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Close encounters with Clathrus ruber, the latticed stinkhorn

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Stijve T. (1997): Close encounters with Clathrus ruber, the latticed stinkhorn – Czech Mycol. 50: 63–70

Considerable variation in the height of the carpophores of *Clathrus ruber* Mich.: Pers. was observed, ranging from a mere 8 cm for Spanish and French collections to more than 20 cm among the Clathri growing in a park at Ouchy (Lausanne) on Lake Geneva. Chemical investigation of collections from that site confirmed that *C. ruber* accumulates manganese, just as other stinkhorns do. In all probability, this metal plays a role in the biochemistry of the fungus, notably in the enzymatic liquefaction of the gleba with simultaneous formation of odorous compounds. *Clathrus* eggs were subjected to multi-element analysis in which the gelatinous outer layer, the embryonal receptaculum and – gleba were separately investigated. The gelatinous layer proved most rich in potassium, calcium, manganese and iron. Calcium undoubtedly stabilizes the polysaccharide gel protecting the embryonal carpophore from drying out during the growth of the egg. The superior concentrations of the other elements (compared to those in the developing carpophore) suggest a placenta-like function of the gelatinous layer. The significance of the various elements in the biology of the *Clathrus* is briefly discussed.

Key words: *Clathrus ruber*, multi-element analysis.

Stijve T. (1997): Důvěrné setkání s Clathrus ruber, mřížovku červenou. – Czech Mycol. 50: 63–70

Byly pozorovány značné rozdíly velikosti plodnic *Clathrus ruber* Mich.: Pers. pohybující se od 8 cm u sběrů z Francie a Španělska až do více než 20 cm plodnic rostoucích v parku Ouchy (Lausanne) u Ženevského jezera. Chemické výzkumy sběrů z těchto oblastí potvrdily, že *C. ruber* koncentruje v plodnicích mangan stejně jako ostatní hadovkovité houby. Tento kov hraje pravděpodobně roli v biochemii houby, zvláště v enzymatickém zkapalnění gleby za současné tvorby vonných sloučenin. Vajíčka mřížovky byla podrobena multielementární analýze a samostatně byla studována vnější gelatinosní vrstva, embryonální receptakulum a gleba. Gelatinosní vrstva se ukázala jako nejbohatší na draslík, vápník, mangan a železo. Vápník nepochybně stabilizuje polysacharidový gel, který chrání mladé plodnice před vysušením během růstu vajíčka. Je krátce diskutován význam různých prvků v biologii *Clathrus*.

Clathrus ruber is undoubtedly one of the most beautiful representatives of the large family of stinkhorns and allies. It was already described by the 16th century botanist Charles de l'Escluse, better known as Carolus Clusius. In fact, in his large work on the fungus flora of Austria/Hungary, "Fungorum in Pannoniis observatorum brevis historia (1601)", he gives a full description of the species as *Fungus coralloeides cancellatus*, complete with an illustration that is reproduced here. In all European literature *Clathrus ruber* is presented as a warmth-loving species that is rather common in countries surrounding the Mediterranean. It is

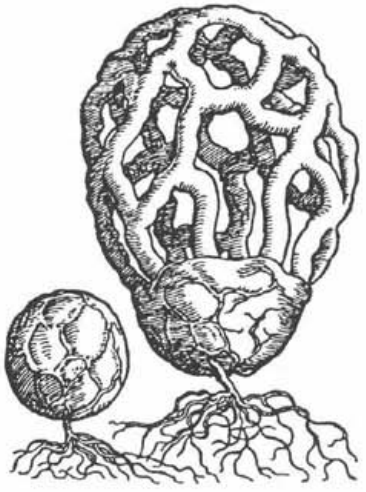

Fig. 1. *Clathrus ruber* Mich: Pers. (from Clusius C. (1601): Fungorum in Pannoniis observatorum brevis historia)

virtually absent in Holland and the Scandinavian countries, rare in Germany and Switzerland, but, surprisingly, not uncommon in the British isles, especially on the South Coast.

As a Dutch chemist with a keen interest in mycology, I published my first studies on the "flavour" of the big stinkhorn (*Phallus impudicus*) in the mid-sixties. At that time, I would have loved to extend my modest research to the *Clathrus,* but alas, this species proved extremely rare in my country. From the literature I learned that it had been found in 1735 by the great Linnaeus (the founding father of the modern botanical nomenclatural system) along a road between Amsterdam and Haarlem. After that it had only been observed a few times in gardens and hothouses, presumably introduced with soil or leafmould. For a very long time I knew *Clathrus* only from pictures and photos until I found it in a garden on the Spanish island of Mallorca. It formed a small colony there, and the eggs had only the size of a ping-pong ball. Of course, I was thrilled to watch those eggs burst, and see the beautiful red receptacle emerge. This process took only a few hours.

French authors describe the fruitbody as a "fenêtre treillisée" (window with bars), which is about the translation of the Greek word *Clathrus.* English and American mycologists speak of a latticed stinkhorn, which amounts to the same thing.

For those readers who are not familiar with the *Clathrus,* it may be useful to give a brief description. The egg of this particular mushroom can already be recognised by the network markings that become more pronounced during development. The wall of the egg consists of three layers, the inner and outer ones are thin, the middle is a thick gelatinous mass that protects the embryonal mushroom from drying out. This mass also contains the minerals and chemical compounds necessary for the development of the stinkhorn. Upon eclosion the holes in the emerging lattice are still small, but they rapidly grow bigger upon expansion of the receptacle. Finally, the pink to coral red *Clathrus* stands upright, somewhat loosely connected to the remainders of the egg.

Subsequently, the olive brown spore mass on the inner side of the receptacle starts liquefying, whereupon a particular fetid smell is produced, which readily attracts flies which feed on the sugar-containing mucus, thus assuring the dissem-

ination of the spores. After about 24 hours the lattice structure collapses, but by this time the spore mass has been completely removed and the offensive smell greatly diminished.

I observed that my Spanish collection had indeed a cadaverous smell, but it was not as strong as that produced by a mature *Phallus impudicus*. This was also the case with the *Clathrus* I found some years later in a neglected garden in the French town of Lyon. The owner of the garden had rather negative feelings about these "clathres en réseau". He looked on with disgust, while I dug out a few eggs to take them along, assuring me that it was dangerous even to touch those fungi. Indeed, Ramsbottom in his classic "Mushrooms and Toadstools" (London, 1953) mentions that *Clathrus* has a bad reputation in France, e.g. people in Gascogne believe that – what they call – the Cancru causes cancer when handled. If they find one, they bury it carefully and deep. In other French departments touching the *Clathrus* is supposed to give you eczema, or even convulsions! In Spain the population does not love the *Clathrus* either. Folknames like "Gita de bruixa" (witches' egg) and "Cranc" (cancer) speak volumes...

Although in several countries eggs of *Phallus impudicus* are eaten and sometimes considered a delicacy, I have not found any information about culinary or medicinal use of *Clathrus ruber*.

My third encounter with this fungus took place in 1987 during a visit to the Barla Museum in Nice (South of France). Jean Baptiste Barla (1817–1890), a well-known mycologist, had not only written a voluminous guide to the fungi found in the Nice area, but had also made a series of most realistically looking wax models of the *Clathrus* in all stages of development and with variously shaped receptacles. I noticed that these models were far bigger than my collections of this particular mushroom, and I asked myself if this reflected reality. In 1988 this question was positively answered when I found in the parc d'Elysée in Ouchy (Lausanne, Switzerland) some ghost eggs being as big as an average apple. At first I thought that these were eggs of *Phallus impudicus*, which is a common species in this country. However, imagine my surprise and joy when one of those eggs – which I had taken home for further study – produced after a while a most beautiful *Clathrus*! The colour of the lattice work was not as red as that of my earlier finds, but the receptacle measured not less than 5 inches which was twice as big as that observed in the Spanish and French collections.

Finding *Clathrus ruber* in Ouchy can probably be explained by the almost Mediterranean climate there. The park is situated on the side of Lake Geneva that receives most sunshine. Clearly, *Clathrus* must feel itself at home there, since further investigation at the site revealed two more colonies, which produced carpophores two to three times a year. The occurrence of *Clathrus* here is probably just a manifestation of what is called " the advance of the stinkhorns in Europe." These highly specialised, non-mycorrhizal gasteromycetes are apparently not affected by

environmental degradation. Svrček (1983) has pointed out that during the last 30 years *Phallus impudicus* has been widely diffused, even to the South of Sweden, whereas in the beginning of the century it was a fairly rare mushroom there. *Clathrus ruber* may also be conquering new territories. Indeed, about ten years ago, it was repeatedly reported in the Berlin area. Even more exotic stinkhorns turn up with increasing frequency in Europe, e.g. *Anthurus archeri*, the octopus stinkhorn, which was accidently brought into France by the Australian army during WW I. It is now already a common species in Ticino, the Italian-speaking part of Switzerland. Recently, it was even observed as far north as Holland.

I learned from further observations in Ouchy that the dimensions of the latticed stinkhorn are variable, but it was clear that Barla had not exaggerated when making his wax models. Late autumn 1993, at a temperature of 4 °C, with a strong wind blowing, I found a colony that was really thriving: when approaching the park from a 100 yards distance I saw a really enormous red receptacle. It was 8 inches high and 5 inches broad, and it was accompanied by half a dozen large eggs. Two of those, weighing 110 and 195 gms were taken along and put in a bin of garden soil under glass jars of respectively 0.6 and 1 litre (to avoid being surprised by the stench of the expanded carpophore). The skin of the biggest egg was already torn during the evening of the next day showing the orange-red colour of the embryonal *Clathrus*. During the next 24 hours it emerged as a bulging sphere with holes, which permitted to see the olive-black gleba on the inside. The typical latticed form was only achieved on the 4th day: the 1 litre jar proved too small and was removed whereupon the receptacle proceeded to grow into a fine orange-red lantern, measuring 4 × 4 × 6 inches! Somewhat surprisingly, the cadaverous odour proved weak enough to permit measuring and photographing the fungus, and to show it to interested persons. When placed outside, the smell still proved strong enough to attract flies, in spite of the low temperature. After one day the lattice work collapsed, and was dried to be preserved as an herbarium collection. The other egg only opened after 8 days producing a pink receptacle of 2.8 × 3.3 × 4.5 inches, which was also too big for the glass jar covering it. The dimensions of the receptacles proved about proportional with the weight of the eggs. This specimen also had a rather weak odour.

Chemical investigations

Stinkhorns are not only characterised by their peculiar Jack – in – the – box way of growth, but they also have in common that, after eclosion, a number of chemical reactions are initiated to liquefy the gleba and produce the cadaverous odour. *Phallus impudicus* has repeatedly been the subject of chemical investigations, which even resulted (during the 60ies) in two doctoral theses. The German scientist Johannes Schmitt found that during eclosion of *Phallus impudicus* and *Anthurus*

archeri a considerable amount of carbon dioxide gas is produced, simultaneously with the carrion-like stench. Carbon dioxide and the "flavour" components (methyl sulfides, aldehydes and amines) are probably produced by enzymatic decarboxylation of keto- and amino acids, but such a process will work only in presence of certain metals, such as manganese. Now every mushroom contains detectable amounts of this trace element, but in most gilled fungi, boletes and puffballs the concentration seldom exceeds 60 mg kg^{-1} on dry matter. Interestingly, Schmitt found in a number of Hysterangia, and especially in stinkhorns, exceptional high levels of manganese. The concentrations of this metal were even higher than those of the closely related essential element iron. See Table 1.

Table 1. Manganese and iron concentrations in Hysterangiales and Phallales (as reported by Schmitt et al., 1977)

Species	Manganese in mg kg^{-1}	Iron mg kg^{-1}	Ratio Fe: Mn
Hysterangiales			
Hysterangium coriaceum	100	557	5.6
Hysterangium stoloniferum	13–25	75–78	3.2–5.8
Hysterangium nephriticum	14–46	393–702	15.3–28.1
Hysterangium rubricatum	225	116	0.5
Hysterangium calcareum	18	295	16.4
Gauteria otthii	10	138	13.8
Phallogaster saccatus	448	135	0.3
Phallales			
Clathrus ruber	447	573	1.3
Anthurus archeri Egg	1956	226	0.1
Receptacle	538	297	0.6
Mutinus caninus Egg	230	335	1.5
Phallus impudicus Egg	218	224	1.0
Gelatinous layer	447	270	0.6
Egg without outer layer	168	132	0.8

All values expressed on dry matter

These interesting results invite a number of comments. Among the Hysterangia there are species with a low as well as a high manganese content. Some of these subterranean gasteromycetes apparently exclude the element, since the soil contains on the average 1000 mg kg^{-1} (0,1 %), whereas the iron content fluctuates

between 1 and 6 percent. The above — ground growing *Phallogaster saccatus*, a rare fungus representing a bridge to the "true" stinkhorns and which contains, just as them, more manganese than iron! Some of the stinkhorns, e.g. *A. archeri* (bio) concentrates manganese, since its content, 2000 mg kg^{-1}, is higher than that of the average soil.

Our *Clathrus ruber* contains both much manganese and iron, but since Schmitt examined herbarium material, it is not clear what part of the fungus he analysed. The figures listed for the different parts of the big stinkhorn indicate that the outer part of the egg contains more manganese than the embryonal gleba! Such differences are also observed in the results for the various parts of *A. archeri*.

Table 2. Manganese and iron concentrations in dried eggs of two Phallales species compared to soil levels (values expressed on dry matter)

	Manganese [mg kg^{-1}]	Iron [mg kg^{-1}]
Phallus impudicus from la Foret de Jorat, Lausanne, CH	725 —1118	108–143
Soil samples	430–1220	24000–36000
Clathrus ruber from the Parc d'Elysée, Ouchy, CH	450–1900	180–570
Soil samples	650–1250	13500–50000

Table 3. Essential chemical elements in *Clathrus ruber* (values expressed on dry matter)

	Na mg kg^{-1}	K %	P %	Ca mg kg^{-1}	Mg mg kg^{-1}	Zn mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}	Cu mg kg^{-1}
Whole eggs after drying & grinding	170	4.03	0.72	1137	1953	13	488	229	20
Gelatinous layer and outer skin	413	8.65	0.51	3490	2045	37	1454	261	17
Receptaculum	431	5.62	0.82	289	2230	20	621	97	22
Spore mass	223	2.84	0.62	111	2094	23	236	127	26

To check these interesting findings, we decided to analyse a few stinkhorns in our own laboratory. For this purpose, comparative analyses of dried eggs of both *Clathrus ruber* and *Phallus impudicus*, as well as corresponding soil samples were carried out. Table 2 shows that both stinkhorns prefer manganese. The much more abundant iron is only taken up in minor quantities. To study the distribution of these metals and those of other essential elements in the different parts of *Clathrus*

ruber, we gathered a number of eggs in July 1993 of the afore-mentioned colony in Ouchy. Half a dozen were cut in thin slices, whereupon we isolated with a sharp knife the reddish embryonal receptacle and its blackgreen gleba, and dried these parts overnight separately in a draft oven set at 55 °C. The remaining gelatinous layer and its adhering skin were treated in the same way. Subsequently, the dried parts were ground to a fine powder, sieved and stored in glass vials until carrying out the multi-element analyses of which the results are given in Table 3.

The concentrations listed for the various elements should not be taken too absolutely, since we analysed biological material, subject to considerable variation. However, the high levels of potassium, calcium, manganese and iron in the gelatinous layer are striking. These are undoubtedly those elements that are most essential to the fungus. Potassium is a component of the cells regulating their osmotic pressure. It is foremost necessary for the growth of the carpophore. There is not only a correlation between the potassium concentration and the water content of the fungi, but also the velocity of growth depends on the metal. The slowly growing polypores contain seldom more than 2 percent potassium, but in the rapidly evolving Coprinaceae 10 to 12 percent is found (Stijve 1996). The gelatinous layer contains 8.65%. It is therefore not unthinkable that the receptacle obtains its potassium from this source.

The calcium concentration of 3490 mg kg^{-1} is much higher than that reported in literature for gilled fungi and puffballs (Seeger and Hüttner 1981). Calcium plays a role in the metabolism of the mushroom, stabilising intercellular membranes. In our *Clathrus* calcium undoubtedly stabilises the gelatinous layer which protects the embryonal carpophore during the growth of the egg, which takes between 2–4 weeks for its full development. The concentrations in the receptacle and gleba are rather modest. It has been established (Bindler 1967) that the gelatinous layer consists of polysaccharides just as the vegetable gums that are used as thickeners in the food industry. Indeed, the slimy part of the egg has characteristics similar to those of alginic acid and pectine that also need calcium to produce a gel. The amount of manganese in the gelatinous layer suggests again that this part plays the role of a reservoir, even as a placenta, because the receptacle as well as the gleba contain more than average concentrations of the metal. The level in the spore mass (236 mg kg^{-1}) suggests the presence of manganese-containing enzymes that produce the sugars and odorous compounds necessary to attract the flies. Although the ratio iron: manganese in Stinkhorns is smaller than 1, it cannot be said that these fungi are poor in iron. In our *Clathrus* the amount in the gelatinous layer is well above the average value of 158 mg kg^{-1} reported by Manfred Lupper in 1988 who examined not less than 500 fungi. An antagonism between the two metals – as observed in animal metabolism – does not seem to exist in higher fungi. In all stinkhorns analysed so far, manganese predominates, but the iron content is also appreciable.

The other elements listed in Table 3 do not invite much comment. The sodium content is less than 1 percent of the potassium concentration. It apparently does not play a role in the fungal metabolism. It is curious that the levels of zinc and copper are significantly lower than those measured in many other mushrooms (Mutsch et al. 1979). Perhaps the uptake of these metals is inhibited in presence of much manganese. Magnesium is evenly distributed among the different parts of the *Clathrus* and its levels are in agreement with those reported in literature for other stinkhorns (Seeger and Beckert 1979). The reader having some knowledge of biochemistry will not be surprised that the metals are accompanied by a considerable amount of phosphorus (P), just as is the case in green plants. The element is largely present as phosphate (quantitatively the major anion) and it plays a key role in the transport of metals through the cell membranes. Of course, phosphate is also necessary for buffering the acid compounds formed during the metabolism of *Clathrus*.

There is little doubt that the chemistry of *Clathrus* is interesting enough to be investigated more thoroughly. We know now that the mushroom takes up much manganese, but the supposed role of this metal in the enzymatic reactions occurring during the liquefaction of the gleba has still to be elucidated. The isolation and characterisation of the manganese-containing enzymes would be a fine subject for a doctoral thesis, especially for a biochemist having an interest in mycology.

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Book review

PAUL STAMETS:

Psilocybin mushrooms of the world – an identification guide.

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Towards the end of the 70 ies, when interest in the possible occurrence of psilocybin mushrooms in Europe was just awakening, there was hardly any literature on the subject except for Roger Heims' now classic treatise on "Les champignons toxiques et hallucinogènes". In this book Heim presented *Psilocybe semilanceata* and *Panaeolus subbalteatus* as psilocybin-containing mushrooms which could be found in Europe, but analytical data were still lacking then.

On the other hand, in the USA a whole subculture surrounding the recreational use of at least half a dozen of these mushroom species existed already. A stream of pamphlets and field guides, often of poor quality, provided information on the identification and location of the hallucinogenic fungi growing in North America.

A book that really distinguished itself favourably from all those amateurish publications was Paul Stamets' "Psilocybe mushrooms and their allies", edited by the Homestead Book Company in Seattle (Wa.). This guide did not only give user-friendly keys for the genera *Stropharia, Psilocybe* and *Panaeolus*, but also excellent descriptions of the individual species, illustrated with very good colour prints. This book has been most helpful to those European mycologists who wanted to find out whether these mushrooms could also be found in their respective countries. Now, some twenty years later, not only the number of known hallucinogenic Psilocybes and Panaeoli has increased dramatically, but on both sides of the Atlantic ocean it was discovered that psilocin and psilocybin also occur in representatives of unrelated genera, such as *Conocybe, Gymnopilus, Pluteus, Inocybe,* and even in *Galerina.*

It was therefore time to bring out a new, updated book on the subject, and it was again Paul Stamets who has taken the initiative in editing a worldwide guide. After short introductory chapters on e.g. history, ecological aspects, world-wide distribution of psilocybin mushrooms, the various types of their habitats, the greater part of the book – characterised by yellow pages – is devoted to major – and minor psilocybin genera. The part on *Psilocybe* and *Panaeolus* in which most psilocybin-containing species are found is undoubtedly the most interesting. Not only are there good descriptions of macroscopic and microscopic characteristics, but also high-quality colour prints which are not to be found elsewhere. The illustrations are definitely better than those of Guzmáns' well-known guide to the genus *Psilocybe.* As far current knowledge permits, the contents of the active principles psilocybin, psilocin and baeocystin are listed for each species. There are also some descriptions of inactive species which are often erroneously considered hallucinogenic, e. g. *Panaeolina foenisecii* and *Psilocybe coprophila.*

Of course, many of the 63 Psilocybes described by Stamets are tropical or subtropical species, and there are even a few which have only been discovered quite recently, for example, *P. samuiensis* Guzmán, Allen et Merlin discovered on the Thai island Koh Samui, and a strongly blueing species, *P. natalensis,* which was reported from South Africa by Gartz et al.

The chapter on "minor psilocybin genera" is, as far as the hallucinogenic *Inocybe* species are concerned, mostly based on the papers by Drewitz, Gartz, and Stijve and Kuyper. For somebody familiar with the literature there are no surprises. The lack of photos of the said Inocybes is somewhat disappointing. The occurrence of psilocin/psilocybin in some *Gymnopilus* species is still a matter of conflicting reports. According to the reviewers' experience, a positive or negative result could well depend on the time interval between collecting the mushrooms and their chemical analysis. For example, fresh, strongly blueing *G. purpuratus* contains much psilocin, which can disappear completely in about two weeks, even from dried material.

In contrast to his earlier book, Stamets is now making propaganda for the recreational use of psilocybin mushrooms, although there is the usual disclaimer from the editor who "does not advocate violating the law." It is, however, significant that Stamets' first book was prefaced by

the mycologist Gastón Guzmán, whereas it has now a foreword by medical doctor Andrew Weil, who has gained some notoriety by his mystic and pseudoscientific writings. We should therefore not be surprised that this book subscribes to certain far-fetched theories, e.g. the faculty to biosynthesize psilocybin is seen as a competitive evolutionary advantage, because the consumers help in disseminating the spores, thus propagating the species. Moreover, the author states that psilocybin mushrooms are carriers of messages from Nature about the health of the Planet: their widespread consumption in the 70 ies prompted the ecological movement! Furthermore, it is repeatedly emphasized that, during the last 20 years in the USA, Psilocybe mushrooms are increasingly found in places wherever people congregate: in parks, lawns by housing developments, schools, churches, etc. Admittedly, Stamets also mentions the role that the growing use of wood-chips plays in creating a suitable habitat for lignicolous species as *P. stuntzii* and *P. cyanescens* in parks and gardens.

The author rightly points out the need to properly identify the psilocybin mushrooms one wants to collect. Indeed, severe cases of poisoning have occurred in people who were foolhardy enough to randomly ingest little brown mushrooms. Amateur collectors should be able to distinguish the highly poisonous amatoxin-containing *Galerina* species from Psilocybes. For this purpose, the chapter on the dangers of mistaken identification shows a very good photograph, depicting *Galerina autumnalis* and *Psilocybe stuntzii* growing side by side.

In the chapter "Good tips for great trips" the reader finds – as usual in this kind of literature – much talk about the great experiences offered by psilocybin mushrooms. The dangers of actually ingesting these conscious-altering fungi, especially to nervous persons, are played down. Stamets even cites a psychiatrist who in 20 years of medical practice never had a patient complaining of a bad mushroom session. We should, however, give the author credit for suggesting a number of valid precautions to minimize bad experiences and maximize the positive. For example, he emphasizes the importance of time and setting for the actual trip. Much attention is also paid to the right dosage by supplying tables and histograms based on comparative potency of the principal hallucinogenic Psilocybes.

The book has a literature list that is updated to 1996. It largely covers the relevant publications on the subject. This 12 page list is wrongly called "Works cited", because many a paper is not mentioned in the text.

Summarising it can be said that Stamets' book is by far the best and most complete guide to psilocybin mushrooms. Even if one is only mycologically interested in the genus *Psilocybe*, the purchase is still warmly recommended. Considering the quality and quantity of the information provided, the price of the book is really low.

Tjakko Stijve

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Ryvarden L. (1978): The Polyporaceae of North Europe, Vol. 2. Inonotus-Tyromyces. – 507 p. Oslo. (book)

Tommerup I. C., Kuek C., and Malajczuk N. (1987): Ectomycorrhizal inoculum production and utilization in Australia. – In: Sylvia D. M., Hung L. L., and Graham J. H. (eds.), Proceedings of the 7th North American Conference on Mycorrhizae, p. 93–295, Gainesville.

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