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Notes on the taxonomy and distribution of Aphyllophorales I.

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Pouzar Z. (2001): Notes on the taxonomy and distribution of Aphyllophorales I. – Czech Mycol. 53: 121–131

Two new species of corticioid Aphyllophorales (Basidiomycetes) are described. *Thanatephorus brevisporus* Pouz. is a species close to *T. fusisporus* (J. Schröt.) Hauerslev et P. Roberts, differing however in shorter, more rounded spores, known from the Czech Republic, Slovakia and Ukraine, growing on rotten wood of broad-leaved trees. *Dendrothele wojewodae* Pouz. is close to *D. acerina* (Pers.: Fr.) P. A. Lemke, but is distinct by its subglobose spores. It is known from the Czech Republic and Ukraine, from bark of living trees of *Acer pseudoplatanus*.

Key words: *Aphyllophorales, Corticiaceae* s.l., new species, *Thanatephorus brevisporus* Pouz., *Dendrothele wojewodae* Pouz.

Pouzar Z. (2001): Poznámky k taxonomii a rozšíření nelupenatých hub I. – Czech Mycol. 53: 121–131

Popisují se dva nové druhy kornatcovitých hub řádu Aphyllophorales (Basidiomycetes): *Thanatephorus brevisporus* Pouz., druh blízce příbuzný druhu *T. fusisporus* (J. Schröt.) Hauerslev et P. Roberts, avšak lišící se kratšími a kulatějšími výtrusy; je znám z České republiky, Slovenska a Ukrajiny z hnijícího dřeva listnatých stromů; *Dendrothele wojewodae* Pouz. je velmi blízký druhu *D. acerina* (Pers.: Fr.) P. A. Lemke, ale liší se skoro kulovitými výtrusy; je znám z České republiky a z Ukrajiny, z kůry živých klenů – *Acer pseudoplatanus*.

INTRODUCTION

During the years 1994–2001 a study of the order Aphyllophorales in the South Bohemian mountains of Šumava (mainly the Šumava National Park) has been carried out. Soon it, however, appeared that these mountainous or even to some degree also boreal species should be compares with those of southern and more thermophilous nature. Hence a study of these fungi started in forests in the vicinity of Prague where such a mycoflora is richly represented. These studies resulted in the recording of several rare species too, some of which appeared to be undescribed. In this first contribution two new interesting species of *Corticiaceae* sensu lato are described and discussed.

Thanatephorus brevisporus Pouz. spec. nov.

Carposomata effusa, hypochnoidea, cremea usque pallide ochracea. Hyphae absque nodis, glabrae, fere tenuiparietales, basales 7–10 μm latae, horizontales in angulos rectos ramificatae, mediae 6–7,5 μm, verticales, laxe contextae. Basidia 14–18 μm longa et 10–12 μm lata in parte distale, leviter vel late clavata, haud constricta, bisterigmatica, nonnumquam tri-, raro tetrasterigmatica; sterigmata 8–11 μm longa et basim 2–3.5 μm lata, solum leviter subarcuata vel fere recta. Sporae 9–12.5 × 7–9.5 μm, subglobosae usque breviter ovoideae, fere tenuiter tunicatae, glabrae, apice cum mamilla distincta, isodiametrica, saepius leviter elongata, seu raro apex sporarum in conum latissimum, brevemque terminatus. Hyphae, basidia, sterigmata sporaeque cyanophilae, sed haud dextrinoideae et haud amyloideae.

Holotypus: Bohemia, Voškov apud Karlštejn, *Carpinus betulus* – ad truncum iacentem, 7. V. 2001, leg. Z. Pouzar, PRM 895056, in Museo Nationale Pragae asservatur.

Paratypus: Slovakia, silva virginea "Badínsky prales" apud Badín prope Banská Bystrica, ad truncum iacentem *Fagi sylvaticae* 4. VIII. 1973, leg. Z. Pouzar PRM 895057.

Fruitbodies resupinate, when young forming very small tufts, soon confluent to become hypochnoid or mucedinoid, comparatively widely spread over the substratum, colour cream to pale ochraceous. Basal hyphae rather sparse, those parallel close to the substratum 7–10 μm wide. Median vertical hyphae loosely arranged 6–7.5 μm broad. Subhymenium of shortly septate hyphae, 7–9 μm broad. Clamp-connections completely missing. All structures without incrustations. Hyphal ends similar to cystidia, scattered in the hymenium of some few specimens (typically developed in PRM 895057), frequently arising from the hymenium close by basidia, straight 27–50(-90) μm long, 6–8 μm broad at the basis and 5–7.5 μm broad in the upper half, cylindrical, sometimes in central part slightly broadened, some 1–3× slightly but abruptly constricted, rounded at the top, 1–2 × (at most 3×) septate, sometimes without septa, filled with strongly cyanophilous content. Basidia 14–18 μm long and 10–12 μm broad in distal part, narrowly to broadly clavate, not constricted, bisterigmatic, only some tristerigmatic and few tetrasterigmatic. Sterigmata only slightly arcuate to almost straight, 9–11 μm long and 2–3.5 μm broad at base. Spores 9–12.5 × 7–9.5 μm, subglobose to shortly ovoid, at the apex with a distinct isodiametric or at most slightly elongate mammiform outgrowth, a few spores broadly coniformly terminated; richly producing spores by repetition. All hyphae, basidia and spores with cyanophilous, but indextrinoid and inamyloid walls.

Distribution and ecology

Thanatephorus brevisporus is so far known from three localities in the Czech Republic (two in Bohemia, one in Moravia), five in Slovakia and one in Ukraine (Transcarpathian region). This species could occur from the lowlands (elevation 152 m in Ranšpurk Virgin Forest) to mountains up to an elevation of about 1000 m in the Slovak Carpathians (Vrátna dolina in Veľká Fatra and Poľana near Detva possibily at 1200 m). It is known only from strongly rotten wood of broad-leaved trees, especially *Fagus sylvatica, Tilia platyphyllos, Carpinus betulus, Acer pseudoplatanus* and also *Ulmus* sp.

Material studied

Czech Republic

1. Bohemia, Voškov ap. Karlštejn, (area tuta); *Carpinus betulus* – ad truncum iacentem 7. V. 2001, leg. Z. Pouzar, PR 895056 (holotype). Ibid. *Tilia platyphyllos* – ad truncum iacentem 7. V. 2001, leg. Z. Pouzar, PRM 894928.
2. Bohemia, Karlštejn, loco "Vodopády" (Bubovické vodopády); ad ligna putrida arboris frondosae iacentis, 29. VII. 1962, leg. Z. Pouzar, PRM 793513.
3. Moravia, silva virginea "Ranšpurk" ap. Lanžhot; in trunco putrido *Ulmi* sp., 28. VII. 1970, leg. Z. Pouzar, PRM 803635.

Slovakia

4. Montes Veľká Fatra, in valle "Vrátna dolina" (sub Ostredok); in cortice *Fagi sylvaticae* (truncus iacens), 4. VII. 1953, leg. F. Kotlaba et Z. Pouzar, PRM 803634.
5. Silva virginea "Badínsky prales" sub monte Laurín prope Badín ap. Banská Bystrica, ca 800 m s.m.; ad truncum iacentem *Fagi sylvaticae*, 4. VIII. 1973, leg. Z. Pouzar, PRM 895057 (paratype).
6. In silvis virgineis montis Poľana ap. Detva, ca 1200 m s.m.; ad ligna *Fagi sylvaticae*, 25. VI. 1952, leg. A. Pilát, PRM 189589, 189599.
7. In silvis Jasov ap. Košice; ad codicem *Aceris pseudoplatani*, 27. VI. 1965, leg. F. Kotlaba, PRM 803633.
8. In monte "Malý Milič" prope Slanská Huta (h.p. Slanec); ad truncum putridum *Fagi sylvaticae*, 17. VII. 1964, leg. F. Kotlaba et Z. Pouzar, PRM 803636.

Ukraine

9. Transcarpathian Region (olim Carpatorossia), Jalinka prope Kosovská Polana; VII. 1930, leg. A. Pilát, PRM 189598.

When studying rather rich material of *Thanatephorus fusisporus* (J. Schröt.) Hauerslev et P. Roberts [*Uthatobasidium fusisporum* (J. Schröt.) Donk], collected in the vicinity of Prague in autumn 2000 and in spring 2001, it appeared

that the variability of spore size and form in this species is more restricted and narrower than presumed earlier. This experience led to the conclusion that short-spored specimens represent an independent species, which is described here as *Thanatephorus brevisporus* Pouz. spec. nov. The main body of the spore is in most spores of this species almost isodiametric, with the length nearly equating the breadth. Only the rather strongly developed apiculus and the striking apical projection on the opposite end of the spore breaks the spore isodiametry.

The original idea of a possible distinguishing of three taxa in the *Thanatephorus fusisporus* complex instead of two, comes from Rogers (1943, p. 107) who, however, came to the opposite solution: "The two, or three, smaller units are easier to define or to insert in a key; but the one species is the natural group." [he named the species *Pellicularia flavescens* (Bonord.) D. P. Rogers]. Our experience with this complex leads nevertheless to the distinction of three species, rather than merging all three morphotypes into one species. Besides the rather rare species *Thanatephorus ochraceus* (Massee) P. Roberts, which is characterised by a rounded spore apex (without a protuberance) there are two taxa in which spores bear an apical outgrowth. One is *Thanatephorus fusisporus* (J. Schröt.) Hauerslev et P. Roberts, in which the spore apex is gradually attenuating towards the terminal protuberance, the other is *Thanatephorus brevisporus* Pouz. spec. nov., in which the spore apex is abruptly outgrowing from the top, which is however rounded or abruptly conically formed. There are some individual spores, in almost every specimen, which display the opposite character viz. attenuated spores in *T. brevisporus* and the apex-rounded (with a terminal protuberance) in *T. fusisporus*, but if taking a representative majority of spores, the result is quite unambiguous. The second character – the number of sterigmata – is of slightly lower value, even if in *T. brevisporus* the basidia have generally two sterigmata, but with a tendency to form three sterigmata and few basidia even four sterigmata. The situation in *T. fusisporus* is quite opposite: generally basidia have four sterigmata, mixed however with basidia bearing three, but some basidia are also bisterigmatic. I am distinguishing three species, being well aware that there could be some objections, as the mentioned transitional spores and basidia exist. But the rather rich available material can easy be split according the criteria cited, hence its recognition at the level of species seems to be substantiated. Nevertheless Roberts (1999) includes both species with apical outgrowth on spores in one broadly defined *Thanatephorus fusisporus* (see his figures 41 and 42).

The nomenclature of this group has been explained by Rogers (1935, 1943), Donk (1958), J. Eriksson (1958) and Roberts (1998). The name *T. fusisporus* could confidently be applied to the species with spores having an attenuated spore apex as in the original diagnosis this character is clearly indicated: "Sporen 11–15 μ

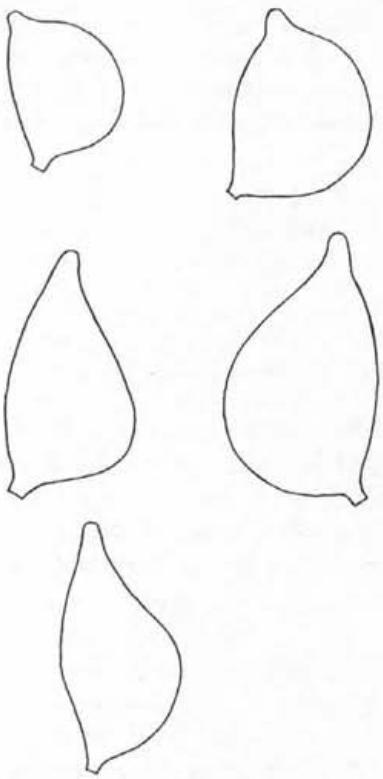

Fig. 1. *Thanatephorus brevisporus* Pouz. – two spores in upper part. – *Thanatephorus fusisporus* (J. Schröt.) Hauerslev et P. Roberts – three spores in lower half.

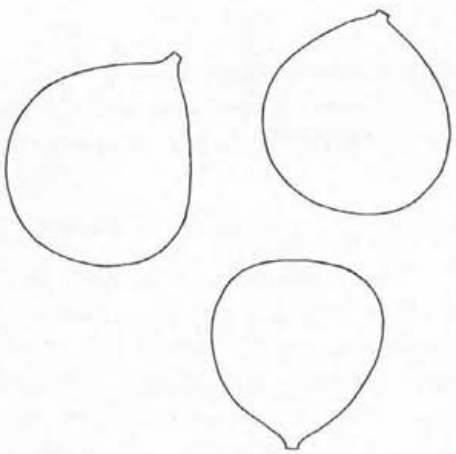

Fig. 2. *Dendrothele wojewodae* Pouz. – spores.

(einzeln bis 17) μ lang, 7–10 μ breit, an beiden Enden stark verschmalert, ..." (Schroeter 1888, p. 416).

Dendrothele wojewodae Pouz. spec. nov.

Carposoma resupinatum, siccum, firme adhaerens, 15–115 μm crassum, maculas irregulares formans, albidum seu pallide griseolum, aliquantulum leviter griseo-rubescens. Hyphae basales 1–3 μm latae, tenuiter tunicatae, haud incrustatae, nodoso-septatae. Cystidia 15–25 × 7–10 μm, elongato ovoidea vel late ellipsoidea, in carposomate inclusa, in cacumine saepe cum appendice hyphali 3–14 μm longo, 1–1.8(-2,5) μm lato, usque 3 × strangulato, haud incrustato.

Dendrohyphidia 1–1.5 μm lata, breviter dendroide ramosa, tenuiter tunicata usque solida, leviter cyanophila, incrustatione crystallina tenuiter saepissime tecta. Basidia 27–40 × 7–11 μm, tetrasterigmatica, late cylindrica, pariete in parte basali leviter incrassata et fortiter dextrinoidea et cyanophila. Sporae 9–12 × 8–11 μm, globosae seu subglobosae, pariete tenui, glabra, cyanophila, haud amyloidea et non dextrinoidea.

Holotypus: Bohemia, montes Šumava, mons "Ždanidla" ap. Prášily; *Acer pseudoplatanus* – ad corticem trunci vivi 8. X. 2000, leg. Z. Pouzar, PRM 895055, in herbario Musei Nationalis Pragae asservatur.

DESCRIPTION

Fruitbody corticioid, resupinate of firm and of hard consistency, rather thin (15–115 μm), forming irregular spots 1–2.5 μm broad, with determinate and adnate margin, colour of hymenium whitish or pale greyish with a slight greyish-rose tinge. Hyphal system monomitic, generative hyphae inconspicuous, 1–3 cm broad, thin-walled, hyaline, with clamps on all septa, inamyloid, indextrinoid, but distinctly cyanophilous, partly incrusted with minute crystals. Cystidia embedded (not projecting) 15–25 × 7–10 μm, elongately ovoid or broadly ellipsoidal to cylindric-ellipsoidal, with wall slightly thickened, hyaline, top rounded or obtuse-conical, sometimes with a hyphal outgrowth (appendix), 3–14 μm long and 1–1.8(-2.5) μm broad, thin-walled, hyaline, when longer up to 3 × strangulated, not incrusted, sometimes twice or three times ramified in the middle part. Dendrohyphidia 1–1.5 μm broad, richly branched, thin– to thick-walled or some also solid, slightly cyanophilous, covered mostly with crystalline incrustations. Basidia 27–40 × 7–11 μm, tetrasterigmatic, broadly cylindric, mostly slightly constricted in the middle part, thin-walled, but in the lower half with a slightly thickened wall and here strongly dextrinoid (especially striking in collapsed ones), strongly cyanophilous, inamyloid. Sterigmata ca. 8–11 × 1.5(-2) μm, only slightly curved. Spores 9–12 × 8–11 μm, globose or subglobose, with distinct basal apiculus, wall comparatively thin, completely glabrous, not amyloid, not dextrinoid, but distinctly cyanophilous.

The name *Dendrothele wojewodae* is dedicated to prof. dr. Władyslaw Wojewoda (Kraków) who contributed substantially to our knowledge of fungi, on the occasion of his forthcoming 70^{th} birthday.

Somewhat similar to our new species is *Dendrothele globulispora* Boidin et Lanquetin, known from one specimen, collected in the Central African Republic, spores of which are smaller viz. 7–8.2 × 6–7(-7.5) μm, compared with those

of *D. wojewodae*, which are 10.5–12 × 9.5–11 μm. Spores of *D. griseocana* (Bres.) Bourd. et Galz. are slightly similar, but in this species clamp-connections on basal hyphae are completely lacking, whereas in *D. wojewodae* they are invariably present. The main difference is, however, the absence of hymenial hyphal pegs (formed of hyphidia) in *D. wojewodae*, contrary to their constant presence in *D. griseocana*. Our new species could be compared with *D. incrustans* (P. A. Lemke) P. A. Lemke, with which it shares the same form and almost also the same size of spores, but our species differs very strikingly by its cystidia, part of which is provided with an apical digitiform outgrowth (cystidia completely lacking in *D. incrustans*). Such cystidia are characteristic of *Dendrothele acerina* (Pers.: Fr.) P. A. Lemke, a species evidently closely related, but distinct in possessing quite different spores. *Dendrothele wojewodae* and *D. acerina* share an important character viz. the presence of clamp-connections on hyphae. Even if Lemke (1964, p. 728) indicated the absence of clamps in *D. acerina*, Boidin et al. (1996) quite correctly treated this species as a clamp-bearing one. To observe clamps in *Dendrothele* it is necessary to study hyphae on the very bottom of the fruitbody, close to the bark tissue (or even the mixture of fungal tissue and cells of bark).

DISTRIBUTION AND ECOLOGY

Dendrothele wojewodae is known from four localities: two in the Šumava Mountains in Southern Bohemia and two in the Transcarpathian Region in Ukraine. On all four localities it was collected in virgin forests at an elevation from 700 to 1200 m s.m.. In all four cases the substrate is the outer side of bark-chips of living trees of *Acer pseudoplatanus*. It is necessary to look for this fungus in other places to verify our tentative supposition that *D. wojewodae* is a species of montane forests at high elevations.

MATERIAL STUDIED

1. Czech Republic, Bohemia, montes Šumava, in monte Ždanidla, declive merid.-orient. ap. Prášily, ca. 1200 m s.m.; *Acer pseudoplatanus* – ad corticem trunci vivi, 8. X. 2000, leg. Z. Pouzar, PRM 895055 (holotype).
2. Czech Republic, Bohemia, montes Šumava, loco "Dračí skály" sub Čeňkova Pila, in valle rivi Vydra. ca. 700 m s.m., *Acer pseudoplatanus* – ad corticem trunci vivi, 29. IX. 2001, leg. Z. Pouzar, PRM 895172
3. Ukraine, Transcarpathian Region, in valle rivi Liščenka prope vicum Trebušany, in silvis virgineis (*Abies alba, Picea excelsa, Fagus silvatica*), alt. 800–1000m s.m.; matrix: *Acer pseudoplatanus*, VIII. 1936, leg. A. Pilát, PRM 29086.
4. Ukraine, Transcarpathian Region, Žámer prope Kobylecká Polana; ad cortices *Aceris pseudoplatani*, VIII. 1929, leg. A. Pilát, PRM 650787.

Notes on the variability of spore – wall amyloidity in the *Dendrothele acerina* complex

When studying the *D. acerina* complex the most striking phenomenon appears to be the elsewhere not met variability of spore-wall amyloidity in species of this group. Amyloidity of the spore-wall is a rather constant character in fungi, not displaying a more conspicuous unsteadiness. Here we are, however, confronted with the rare feature of fluctuation of spore-wall amyloidity from almost absent to rather strong. In *Dendrothele acerina* material was studied, collected one day (13. VII. 2001) on three different trees of *Acer campestre* in one forest (on the bottom of Radotín Valley near Prague). These specimens were examined the day after collecting. In one collection only collapsed spores were amyloid whereas freshly developed, even fully ripened ones were completely inamyloid. In the collection from another tree amyloidity was very rare, except for a few old, almost nearly collapsed spores (the majority of completely collapsed spores being not amyloid). In the third specimen amyloid spores were very frequent. Not only fully ripened ones, but also those on basidia, and not infrequently also the small, just developing spores were amyloid. Nevertheless, no specimen of *D. acerina* studied completely missed amyloidity. In the closely related species *D. alliacea*, amyloidity of the spore-wall is much stronger and more striking. In all specimens studied some degree of amyloidity was observed (none of the spores was completely inamyloid). In some spores, however, the wall is interpenetrated by diffused, minute amyloid granules, hence the spore-wall is dark nebulous greyish, in other specimens the spore-wall is irregularly spotted by an amyloid substance (mostly in the central part of the spore). In *D. wojewodae* spores are inamyloid, only in one slide four underdeveloped spores were observed on one collapsed basidium with very faintly amyloid walls. A few fully disintegrated, collapsed spores were observed as amyloid.

Amyloidity in the group of *Dendrothele acerina* could be taken into account in taxonomic considerations, but with restraint, having in mind its high degree of variability.

A key to the Central European species of *Dendrothele* (recorded or expected possibly to occur here)

1a Spores with one or several outgrowths or protuberances on their top 2
1b Top of spores rounded or amygdaloid-acuminate (with no outgrowth) 4
2a Spores with three to five outgrowths (on their top) ..... *D. tetracornis*
2b Spores with one outgrowth or protuberance on the top 3
3a Spores up to 7 μm broad ..... see *D. amygdalispora*
3b Spores more than 7 μm broad ..... *D. citrisporella*

4a Spores strongly allantoid (cylindrical and curved) 28–32 × 10–12 μm ..... *D. dryina*
4b Spores not curved (ellipsoidal, subglobose, globose, amygdaloid, ovoid) ... 5
5a Spores amygdaloid (top part a broad cone) ..... *D. amygdalispora*
5b Spores rounded at their top 6
6a Basidia mostly with two or three sterigmata 7
6b Basidia mostly with four sterigmata 8
7a Protruding fascicles of dendrohyphidia in the hymenium, spores almost subglobose ..... *D. griseocana*
7b No protruding fascicles of hyphidia in the hymenium, spores ovoid or short ellipsoidal ..... *D. commixta*
8a Spores prolonged ellipsoidal, more than 14 μm long ..... *D. alliacea*
8b Spores broadly ellipsoidal, ovoid or subglobose to globose, at most 14 μm long 9
9a Cystidia absent, sterigmata 2–3 ..... see *D. commixta*
9b Cystidia present, sterigmata mostly 4 10
10a Spores broadly ellipsoidal to ovoid ..... *D. acerina*
10b Spores globose to subglobose ..... *D. wojewodae*

Notes on species treated in the key

Dendrothele acerina (Pers.: Fr.) P. A. Lemke. Illustr.: Eriksson and Ryvarden (1975) p. 352, fig. 144a: Lemke (1964) p. 728, fig. 1; Boidin et al. (1996) p. 91, fig. 1Ac. Very frequent, especially on bark of living trees of *Acer campestre* and some other trees.

Dendrothele alliacea (Quél.) P. A. Lemke. Illustr.: Eriksson and Ryvarden (1975) p. 352, fig. b-f; Boidin et al. (1996) p. 91, fig. 1 Al; Kotiranta and Saarenoksa (2000) p. 18, fig. 11 a-f. In Europe widely distributed, common on bark of living trees of oaks (*Quercus* spec. div.), but also on *Ulmus, Juglans, Tilia, Salix, Robinia, Acer* and *Alnus*; also in North America and recorded from South Africa. A species very closely related and similar to *D. acerina*, from which it could be distinguished by the slightly narrower and longer spores – the largest spores are mostly longer than 14 μm. The breadth of spores is also different: in *D. alliacea* mostly 6–7 μm, in *D. acerina* 7–8 μm. The smell of fresh fruitbodies is characteristic, reminding of *Allium porrum* L. – hence the appropriate epithet "alliacea".

Dendrothele amygdalispora Hjortst. Illustr.: Eriksson and Ryvarden (1975) p. 361, fig. 147 (as *Dendrothele* sp.); Hjortstam (1987) p. 57, fig. 1B; Kotiranta and Saarenoksa (2000) p. 19, fig. 19a-d. Known only from Norway (on bark of *Prunus padus* = *Padus avium*) and Finland (*Salix, Corylus*), possibly also in

France (see Boidin et al. 1996, p. 93). Besides the spore form, it is characterised by tetrasterigmatic basidia and absence of clamps.

Dendrothele citrisporella Boid. et Duhem. Illustr.: Boidin et al. (1996) p. 100, fig. 3A. Known only from the western part of France on bark of *Salix* and *Arbutus unedo*. Basidia bisterigmatic, clamps and cystidia absent.

Dendrothele commixta (Höhn. et Litsch.) J. Erikss. et Ryv. Illustr.: Eriksson and Ryvarden (1975) p. 356, fig. 145; Boidin et al. (1996) p. 100, fig. 3Co. Known from Sweden, Norway, Poland, Czech Republic and France (also from New Zealand); on bark of oaks (*Quercus* spec. div.). Characteristic by absence of cystidia, complete absence of amyloidity and dextrinoidity, presence of clamp-connections and mainly by its basidia having two to three sterigmata.

Dendrothele dryina (Pers.) P. A. Lemke. Illustr.: Boidin et al. (1996) p. 100, fig. 3D. Known with certainty only from Western Europe, on bark of oaks (*Quercus* spec. div.); the large allantoid spores are diagnostic. The name is probably not definitive, due to uncertainty of the interpretation of the basionym *Thelephora dryina* Pers. 1822.

Dendrothele griseocana (Bres.) Bourd. et Galz. Illustr.: Boidin et al. (1996) p. 102, fig. 4G; Eriksson and Ryvarden (1975) p. 358, fig. 146. Rather rare species growing on bark of living trees like *Salix, Ulmus, Acer* and some other trees. Characteristic by pegs of dendrohyphidia scattered on fruitbody, emerging like teeth from the hymenium, also by the absence of clamps, almost globose spores, bisterigmatic basidia and absence of cystidia.

Dendrothele tetracornis Boid. et Duhem. Illustr.: Boidin et al. (1996) p. 102, fig. 4T. Known only from France, growing on bark of living oaks (*Quercus* spec. div.) and *Populus nigra*.

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CZECH MYCOL. 53 (2), 2001

Remarks to the taxonomy of Gymnopilus josserandii based on records from the Bohemian Forest (Czech Republic)

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Holec J. (2001): Remarks to the taxonomy of Gymnopilus josserandii based on records from the Bohemian Forest (Czech Republic) – Czech Mycol. 53: 133–139

Two records of the rare species *Gymnopilus josserandii* (*Agaricales, Cortinariaceae*) from the Bohemian Forest are thoroughly described and discussed. Line drawings of microcharacters, a colour photograph of fresh fruitbodies and a distribution map of *G. josserandii* in the Czech Republic are provided. The species is better known under the invalid name *G. subsphaerosporus*. A detailed comparison of its characters with those of the American species *G. subbellulus* has shown that the name *G. subbellulus* represents another species differing above all in the presence of pleurocystidia. *Gymnopilus josserandii* seems to prefer strongly decayed wood of conifers in natural or semi-natural forests. At present, five localities are known in the Czech Republic.

Key words: basidiomycetes, *Agaricales*, *Cortinariaceae*, *Gymnopilus josserandii*, *Gymnopilus subsphaerosporus*, taxonomy, Czech Republic

Holec J. (2001): Poznámky k taxonomii druhu Gymnopilus josserandii založené na nálezech ze Šumavy (Česká republika) – Czech Mycol. 53: 133–139

V letech 1997 a 2000 byl na Šumavě dvakrát nalezen vzácný druh *Gymnopilus josserandii* (*Agaricales, Cortinariaceae*), který byl v Evropě dříve znám pod neplatně publikovaným jménem *Gymnopilus subsphaerosporus*. V článku jsou podrobně popsány znaky nalezených plodnic a zveřejněny kresby mikroznaků a fotografie čerstvých plodnic, cheilocystid a výtrusů. Kromě Šumavy byl *G. josserandii* sbírán v Beskydech V. Antonínem a D. Jandou. V České republice je dosud známo 5 lokalit, které jsou zakresleny v mapě rozšíření. *Gymnopilus josserandii* roste na silně zetlelém dřevě jehličnanů, hlavně v přirozených nebo polopřirozených lesích. Srovnání znaků *G. josserandii* se severoamerickým druhem *G. subbellulus* ukázalo, že *G. subbellulus* představuje jiný druh lišící se zejména přítomností pleurocystid.

INTRODUCTION

The genus *Gymnopilus* (*Agaricales, Cortinariaceae*) belongs to the less elaborated genera of fungi in Central Europe. No detailed or monographic study has been published from this area. In Europe, the genus was thoroughly studied only in France (Kühner & Romagnesi 1953), Norway (Høiland 1990), Great Britain (Orton in Watling & Gregory 1993) and Switzerland (Breitenbach & Kränzlin 2000). A monograph of Northern American species was published by Hesler (1969). The present contribution is the first one from a planned series of papers on the taxonomy, ecology and distribution of *Gymnopilus* species in Central Europe. It

is based on collections from the Bohemian Forest (Šumava Mts., protected as the Šumava National Park) typical by the presence of well preserved natural or near-natural forests.

MATERIAL AND METHODS

Description of macro- and microcharacters is based exclusively on my own collections mentioned below. Herbarium specimens are kept in the Mycological Department, National Museum, Prague (herbarium PRM). The colour codes are according to Kornerup & Wanscher (1981). Microcharacters were studied in a 5 % KOH solution. The pigmentation of the pileus and stipe cuticle was studied in pure water. Iodine reaction was studied in Melzer's reagent prepared according to the formula given by Moser (1983), cyanophilous reaction in cotton blue (according to Kotlaba & Pouzar 1964, Singer 1986) after short boiling. For spore size measurements, 20 spores from each collection were randomly selected. Authors' abbreviations are given according to Brummitt & Powell (1992).

Abbreviations: E = length/width ratio of the spores, Q = mean value of E for all spores studied.

RESULTS

Gymnopilus josserandii Antonín, Fungi non delineati 11: 13, 2000

Naucoria subsphaerospora Joss., Bull. Soc. Mycol. Fr. 64: 21, 1948 (invalid name: published without Latin diagnosis). – *Gymnopilus subsphaerosporus* (Joss.) Kühner et Romagn., Fl. anal. champ. supér.: 323, 1953 (invalid combination: based on invalidly published basionym).

Misidentification

Gymnopilus subbellulus Hesler sensu Breitenbach et Kränzlin, Pilze der Schweiz 5: 140, 2000.

Selected icones

Moser in Moser & Jülich, Farbatlas der Basidiomyceten, Gymnopilus 4, figure at the top, 1992 (as *G. subsphaerosporus*). – Antonín in Antonín & Škubla, Fungi non delineati 11: photo no. 5, 2000. – Breitenbach & Kränzlin, Pilze der Schweiz 5: p. 140, photo no. 150, 2000 (as *G. subbellulus*).

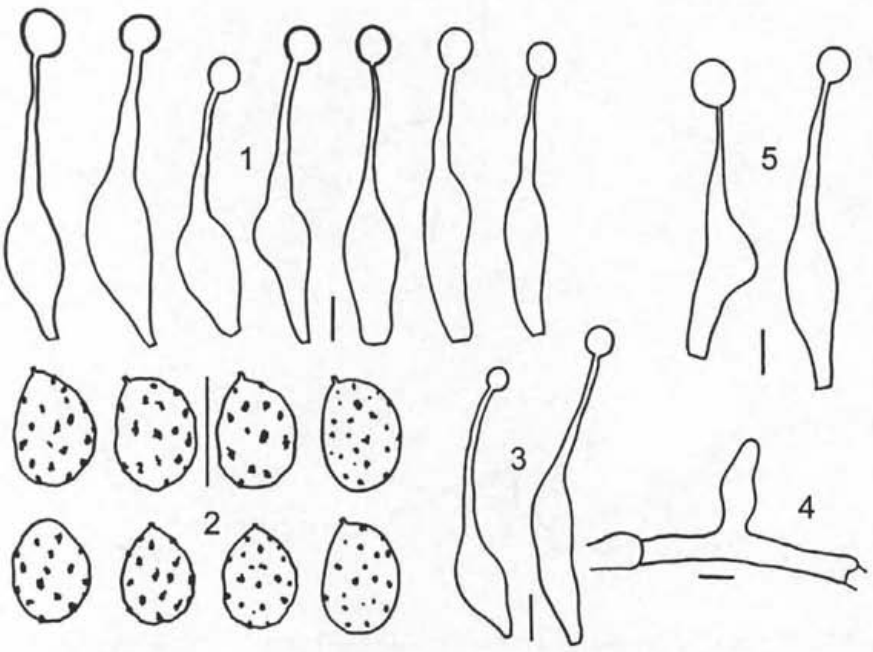

Fig. 1. Microcharacters of *Gymnopilus josserandii*. 1, 5: cheilocystidia, 2: spores, 3: caulocystidia, 4: hypha of the stipe surface with outgrowth. 1–4: nature reserve "Pod kanálem" (PRM 897842), 5: Mt. "Spáleniště" (PRM 891945). Scale bar = 5 μm.

Collections studied

Czech Republic, Southern Bohemia, Bohemian Forest (Šumava Mts.), Prachatice district, 2.2 km NW of the village of Jelení Vrchy near Nová Pec, nature reserve "Pod kanálem" (strictly protected zone of the Šumava National Park), alt. 860 m, mixed natural montane forest dominated by *Fagus sylvatica*, with admixture of *Picea abies, Acer pseudoplatanus*; on decayed stump of *Picea abies* covered with mosses, 30 Sept. 2000, leg. et det. J. Holec, JH 173/2000 (PRM 897842). – Czech Republic, Southern Bohemia, Bohemian Forest (Šumava Mts.), Prachatice district, Lenora, Mt. "Spáleniště" near the village of České Žleby (strictly protected zone of the Šumava National Park), alt. 900 m, mixed natural montane forest (*Fagus, Abies, Picea*) reminding of a virgin forest, on decayed stump of *Abies alba*, 13 Oct. 1997, leg. et det. J. Holec, JH 755/1997 (PRM 891945).

Description

Fruitbodies growing in small groups, not fasciculate. Pileus 0.5–1.2 cm, nearly hemisphaerical to conical-convex with involute margin when young, convex to

Fig. 2. Distribution of *Gymnopilus josserandii* in the Czech Republic (for details on individual collections see chapters "Collections studied" and "Distribution in the Czech Republic"). 1: nature reserve "Pod kanálem", Bohemian Forest (PRM 897842), 2: Mt. "Spáleniště", Bohemian Forest (PRM 891945), 3: "Staré Hamry-Jamník", Beskydy Mts., 2 Aug. 1999, coll. no. 99.36 (Antonín & Škubla 2000: 16, BRNM 648481). 4: "Staré Hamry-Jamník", Beskydy Mts., 23 July 2001, coll. no. 01.148 (BRNM); this locality is about 1–1.5 km far from the previous one. 5: secluded place "Čudácka" near Bílá, Beskydy Mts., coll. no. 01.157 (BRNM).

plano-convex at maturity, in some fruitbodies slightly depressed when old, not hygrophanous, not translucently striate. Surface dry, mat, finely tomentose, rusty-ochre to brown (5D6–7). Lamellae rather sparse, L=30–40, l=1–3, ventricose, adnexed, rusty-ochre to rusty-brown when young, dark rusty-brown (6E8) at maturity, edge concolorous. Stipe 1.5–3 × 0.1–0.2 cm, cylindrical, rusty-ochre, lower part rusty-brown, with white tomentum at base, longitudinally yellow-ochre fibrillose. Taste indistinct (not bitter), smell indistinct.

Spores (4.0-)4.5–6.0(-6.4) × (3.2-)3.5–4.8 μm, E = 1.1–1.4(-1.5), Q = 1.3, rather variable in shape, subglobose, broadly ellipsoid to obovoid in side view, without suprahilar depression but with plane surface near the hilar appendix, in front view subglobose, broadly ellipsoid to broadly lacrymoid; sometimes with a slightly polygonate outline, rusty-ochre in KOH, wall rusty-brown, medium thick, distinctly verruculose, normal spores acyanophilous, those with broken wall cyanophilous, without any reaction in Melzer's reagent or slightly dextrinoid (with reddish-brown hue on mature spores and spores with a broken wall). Basidia 20–30 × 5–6 μm, nar-

rowly cylindrical to narrowly clavate, often with a median constriction, 4(2-)spored, sterigmata long, thin, 5–6 μm. Cheilocystidia 30–40 × 4–8 μm, forming a sterile band on the edge, tibiiform with a narrowly lageniform basal part, long narrow neck (1.0–1.5 μm) and distinct globose head (3.5–5 μm) with a slightly thickened wall. Pleurocystidia absent. Lamellar trama regular, hyphae 4–10(-14) μm broad, near the subhymenium 2–4 μm only, cells cylindrical to slightly inflated (somewhere almost barrel-shaped), with distinct yellow-brown membranal pigment, subhymenium not gelatinous, of densely arranged interwoven hyphae. Pileus cuticle a cutis, not gelatinised, 30–50 μm thick, formed by densely arranged parallel hyphae 2–6 μm broad, with yellow membranal pigment and coarse rusty-brown incrustations, below this layer a hypodermium of parallel to slightly flexuously interwoven hyphae 4–8 μm broad, with the same type of pigmentation, pileocystidia absent. Stipe cuticle 2-layered, lower layer a cutis of parallel, densely arranged, cylindrical hyphae 2–6 μm broad, with yellow-rusty membranal pigment and rusty-brown incrustations, from this layer emerge loosely arranged and interwoven hyphae 2–6 μm broad, cylindrical but with lageniform-fusiform outgrowths or terminal elements and with numerous caulocystidia resembling cheilocystidia in shape but narrower and longer (up to 45×5 μm).

Ecology

Gymnopilus josserandii was found in natural montane forests in the 1st (strictly protected) zone of the Šumava National Park, in both cases on strongly decayed stumps of conifers (*Picea, Abies*). The records from the Beskydy Mts. (Czech Republic, Moravia) by Antonín (see Antonín & Škubla 2000: 13–16 and unpublished finds in paragraph "Distribution in the Czech Republic") are both from forests almost untached by man as well as from man-made stands. I agree with Antonín that the species prefers natural or semi-natural forests and grows only on strongly decayed wood of conifers, possibly also broadleaved trees (Josserand 1948: *Fagus* with a question mark).

Distribution in Europe

Gymnopilus josserandii is well documented from France (Josserand 1948, as *Naucoria subsphaerospora*), Switzerland (Breitenbach & Kränzlin 2000: 140, as *G. subbellulus*), the Netherlands (Arnolds et al. 1995, as *G. subsphaerosporus*), Germany (e.g. Luschka 1993: 197, as *G. subsphaerosporus*) and the Czech Republic (Antonín & Škubla 2000: 13–16). A colour photograph, probably from Austria (herbarium specimen IB 67/114), was published by Moser (in Moser & Jülich, Farbatlas der Basidiomyceten, as *G. subsphaerosporus*). The species is not reported from Great Britain (Orton in Watling & Gregory 1993) and Norway (Høiland 1990).

Distribution in the Czech Republic

Bohemia: Bohemian Forest, 2 localities, see Collections studied. Moravia: 3 localities: "Staré Hamry-Jamník", near a hunter's cottage, Beskydy Mts., on decaying stump of *Picea abies*, 2 Aug. 1999, leg. D. Janda and V. Antonín, coll. no. 99.36 (Antonín & Škubla 2000: 16, BRNM 648481). – "Staré Hamry-Jamník", Beskydy Mts., semi-natural spruce forest, strongly decayed stump of *Picea abies*, 23 July 2001, leg. V. Antonín and D. Janda, coll. no. 01.148 (BRNM); this locality is about 1–1.5 km far from the previous one. – Secluded place "Čudácka" near Bílá, Beskydy Mts., man-made spruce forest surrounded by rests of semi-natural stand, on strongly decayed stump of *Picea abies*, 25 July 2001, leg. V. Antonín and D. Janda, coll. no. 01.157 (BRNM).

Discussion

This rare species is known as *Gymnopilus subsphaerosporus* (Joss.) Kühner et Romagn. in European literature. Unfortunately, the name is invalid because of a lacking Latin diagnosis. Consequently, Antonín (2000) described the species and named it validly *Gymnopilus josserandii* in honour of Marcel Josserand who recognised it for the first time and published a perfect description with exact line drawings.

The fruitbodies described here are typical by the following characters: very small fruitbodies, up to 1.2 cm broad pileus with finely tomentose surface, brown with rusty tinge, dark rusty-brown lamellae when mature, indistinct (not bitter!) taste, small, subglobose, broadly ellipsoid to broadly lacrymoid, not distinctly dextrinoid spores, cheilocystidia of a typical shape – tibiiform with a narrowly lageniform basal part, long narrow neck and globose head, numerous caulocystidia of a similar shape, no pleuro– and pileocystidia.

My records perfectly agree with the original description by Josserand (1948: 21–23) and the later description and colour photograph by Antonín (2000: 13–16). However, I did not observe so much types of caulocystidia as Antonín did, but only those resembling the cheilocystidia. The fruitbodies found by Josserand and Antonín were larger with pilei up to 2.4 cm broad and stipe reaching up to 5×0.3 cm.

The record photographed by Breitenbach & Kränzlin (2000: 140) and identified as *Gymnopilus subbellulus* Hesler certainly represents *Gymnopilus josserandii*. Almost all characters well agree with the descriptions mentioned above. The only exception represents the bitterish taste given by Breitenbach and Kränzlin. The authors obviously knew the invalid status of the name *G. subsphaerosporus* (which is cited by them as a synonym of *G. subbellulus*) and decided to use the valid name by Hesler. The correctness of this conclusion is discussed below.

Fig. 3. *Gymnopilus josserandii*, fresh fruitbodies, Czech Republic, Southern Bohemia, Bohemian Forest, nature reserve "Pod kanálem", on decayed stump of *Picea* covered with mosses, 30 Sept. 2000, leg. et det. J. Holec, JH 173/2000 (PRM 897842).

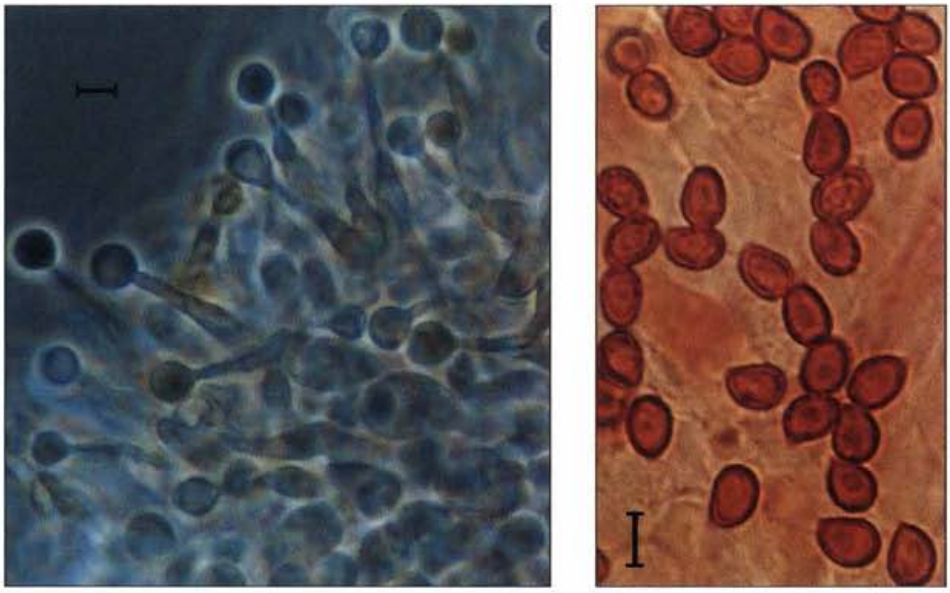

Fig. 4. *Gymnopilus josserandii*, photographs of microcharacters of the fruitbodies from Fig. 3; cheilocystidia (left), spores (right). Scale bar = 5 μm.

Gymnopilus subbellulus Hesler, North American species of Gymnopilus: 46, 1969 (in Mycologia Memoir no. 3) was described as a species of *Gymnopilus* sect. *Microspori*. It is distinguished by the following characters: non-dextrinoid, ellipsoid, ovoid or subglobose spores reaching 3.5–5.0 × 2.4–3.8 μm, pleuro– and cheilocystidia both present, furfuraceous pileus, mild taste, stipe 0.3–0.4 cm thick etc. Many characters are really very close to *G. josserandii* (= *G. subsphaerosporus*) but the presence of pleurocystidia is in conflict with all published descriptions of *G. josserandii* as well as with the finds presented here where no pleurocystidia were observed in spite of long and careful search. Moreover, the spores of *G. subbellulus* seem to be more prolonged and slightly smaller (see e.g. line drawing by Hesler 1969: p. 90, fig. 18) than those of *G. josserandii*. For these reasons I consider *Gymnopilus subbellulus* Hesler a species different from *Gymnopilus josserandii* Antonín.

Acknowledgements

I thank Dr. Vladimír Antonín (Moravian Museum, Brno, Czech Republic) for information on his unpublished finds of *G. josserandii* and the Grant Agency of the Czech Republic for financial support of my work on *Gymnopilus* (project no. 206/01/P050).

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CZECH MYCOL. 53 (2), 2001

Remarks on the distribution of Hymenochaete carpatica in Central and Eastern Europe

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Tomšovský M. (2001): Remarks on the distribution of Hymenochaete carpatica in Central and Eastern Europe – Czech Mycol. 53: 141–148

Hymenochaete carpatica Pilát is an inconspicuous species that was almost completely overlooked until 1988 (Baici and Léger 1988) since its description in 1930. The ecology and distribution of the species in Central and Eastern Europe is described. *Hymenochaete carpatica* grows only on bark chips of old living trunks of *Acer pseudoplatanus* and has not been found on any other host. This species is known from Austria, the Czech Republic, France, Germany and Slovakia. Recently it was also found in Romania and the Ukraine for the first time. Two maps demonstrate the distribution of *Hymenochaete carpatica* in the Czech Republic and Europe to date.

Key words: *Hymenochaete carpatica*, ecology, distribution, Europe

Tomšovský M. (2001): Poznámky k rozšíření druhu Hymenochaete carpatica ve střední a východní Evropě – Czech Mycol. 53: 141–148

Kožovka klenová, *Hymenochaete carpatica* Pilát, je nenápadný druh, který byl popsán v roce 1930, ale až do roku 1988 byl přehlížen (Baici and Léger 1988). Druh roste pouze na odlupujících se kusech borky starých stojících kmenů javorů klenů (*Acer pseudoplatanus*). Na jiném hostiteli nebyl druh dosud zaznamenán. Kožovka klenová byla doposud známa z České republiky, Francie, Německa, Rakouska, Slovenska a Švýcarska. Nyní byla poprvé nalezena v Rumunsku a na Ukrajině. Text je doplněn mapami rozšíření druhu v České republice a v Evropě.

INTRODUCTION

Hymenochaete carpatica was described by Albert Pilát in "Monographie der europäischen Stereaceen" (Pilát 1930). The species description is based on the material collected in the Malé Karpaty Mts. in the former Czechoslovakia, presently Slovakia (PRM 686734). This type specimen dated April 1926 was at first confused with *Hymenochaete subfuliginosa* Bourdot & Galzin (Pilát 1927). Lizoň & Jančovičová (2000) state, that the species was described already in 1927, but this is not correct. Since the time of its description only two specimens found by A. Hilitzer and later identified by A. Pilát (Pilát 1933) and one specimen collected by M. Svrček were known from the former Czechoslovakia. This fungus was then completely overlooked until Baici & Léger (1988) collected *Hymenochaete carpatica* in Switzerland. Rücker & Forstinger (1991) described its distribution

in Austria. Krieglsteiner (1993) summarised records published in literature and supplemented them with his own data from Germany, eastern France and western Bohemia. The search of *Hymenochaete carpatica* in the Czech Republic was initiated by Z. Pouzar who knew the species from literature (Baici & Léger 1988).

DESCRIPTION

According to Baici & Léger (1988).

H. carpatica is characterised by inconspicuous, resupinate basidiocarps with long, smooth setae (50–140 × 6–9 μm) in its hymenium. Basidiocarps firmly attached to the substratum, forming small patches of irregular form. Basidiospores broadly elliptical, hyaline, thin-walled, (5.0)5.5–6.5(7.0) × 3.0–3.5(4.0) μm. Hyphal system monomitic, hyphae simply septate (without clamps). Numerous crystals are present in the trama. The colour of the basidiocarp varies from pale brown to yellowish brown, brown or reddish brown. The species belongs to the section *Gymnochaete* (Léger 1998). The most similar species *H. corrugata* and *H. subfuliginosa* have basidiocarps with a different colour and different microscopic features.

ECOLOGY

The ecology of *H. carpatica* is quite unique among species of the genus *Hymenochaete*. This species grows only on chips of bark (rhytidomata) of old trees of *Acer pseudoplatanus*. Most of the collected fruitbodies were observed on the inner side of the rhytidomata and only a few of them were found on the external or on both sides of the bark. No information about growth on other species of *Acer* or some other tree genera is available (Baici & Léger 1988, Krieglsteiner 1993, Rücker & Forstinger 1991, own observations). The data on the type specimen suggest growth on *Acer platanoides*, but after examination of the type it is clear that the original substrate was *A. pseudoplatanus* (Baici & Léger 1988, own observations).

Hymenochaete carpatica is known from the lowlands to the mountains. The highest known locality is 1700 m a.s.l. in Switzerland (Baici et Léger), the lowest one was recorded at 85 m a.s.l. in Germany (Krieglsteiner 1993). Most records from the Czech Republic were found at an altitude of 700–1000 m a.s.l., the lowest record at 290 m a.s.l., the highest one at 1220 m a.s.l. Most of my records originate from mixed mountain forests (*Eu-Fagion*) or scree and ravine forests (*Tilio-Acerion*) from lower altitudes. *Hymenochaete carpatica* was also recorded on trees in parks, alleys and old maple trees in villages.

Vertical distribution of *Hymenochaete carpatica* in the Czech Republic:

200–300	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	altitude (m a.s.l.)
1	4	3	7	4	12	15	19	5	9	2	number of records

Fig. 1. Distribution of *Hymenochaete carpatica* in Europe.

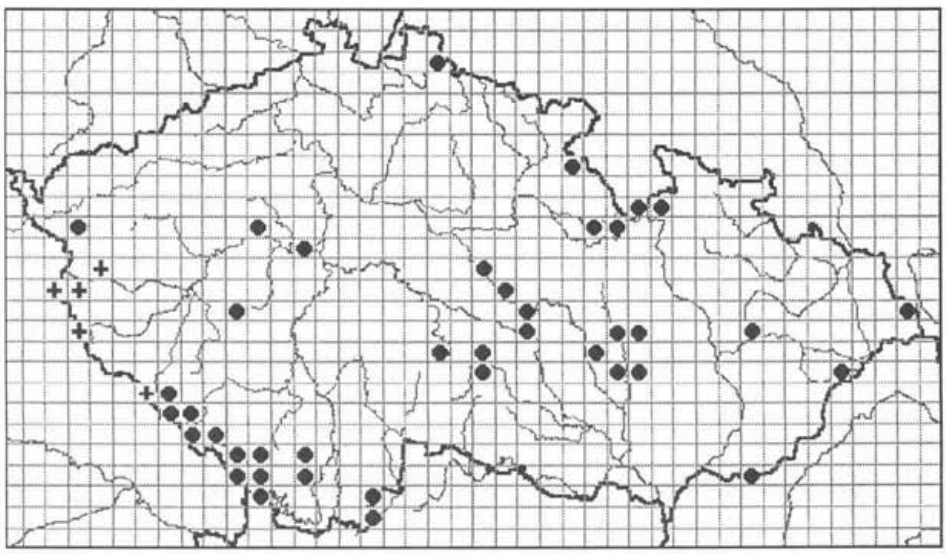

Fig. 2. Distribution of *Hymenochaete carpatica* in the Czech Republic. The crosses demonstrate localities published by Kriegelsteiner (1993).

DISTRIBUTION

Hymenochaete carpatica is known only from Europe (Austria, Germany, former Czechoslovakia, eastern France, Switzerland). I have completed the information with unpublished records from the Czech Republic, Slovakia, Romania and the Ukraine. Most records are documented by specimens deposited in the herbarium of National Museum, Prague (PRM) or Moravian Museum, Brno (BRNM). The collections marked JH (J. Holec) are kept in PRM, but at present they do not yet have the PRM number. All records were collected on *Acer pseudoplatanus*, therefore the substrate is not mentioned at the specimens. The abbreviation M. T. refers to the author.

Czech Republic

Western Bohemia

Lázně Kynžvart, park by the castle, leg. et det. M. Svrček, X. 1998, herbarium M. Svrček.

Jizerské hory Mts.

Hejnice, bank of Velký Štolpich brook, 540 m a.s.l., leg. et det. M. T., 14. IX. 2000, PRM 894251.

Šumava Mts.

České Žleby, Kapraď Mt., 980 m a.s.l., leg. et det. M. T., 22. IX. 1998, PRM 893833. – České Žleby, Kostelní cesta pathway, 940 m a.s.l., leg. et det. J. Holec, 3. IX. 1999, JH 139/99. – České Žleby, Radvanovický hřbet Mt., 890 m a.s.l., leg. et det. M. T., 8. X. 1998, PRM 893825; ibid., 900 m a.s.l., PRM 893820; ibid., 900 m a.s.l., leg. et det. J. Holec, 17. X. 1997, JH 866/97; ibid., 930 m a.s.l., JH 877/97. – České Žleby, Spáleniště Mt., 930 m a.s.l., leg. et det. Z. Pouzar, 22. IX. 1998, PRM 893816; ibid., 880 m a.s.l., leg. et det. J. Holec, 13. X. 1997, JH 750/97. – České Žleby, Žlebský kopec hill, 1000 m a.s.l., leg. et det. M. T., 13. IX. 1999, PRM 894010. – Horská Kvilda, Pěnivý potok brook, 950 m a.s.l., leg. et det. J. Holec, 1. VII. 1999, JH 74/99. – Horská Kvilda, Zhůří, 1140 m a.s.l., leg. et det. M. T., 19. IX. 1999, PRM 894022. – Kvilda, Orel, 1140 m a.s.l., leg. et det. J. Holec, 6. X. 1998, PRM 897494. – Modrava, Vchynicko-Tetovský kanál, 920 m a.s.l., leg. et det. M. T., 30. VI. 1999, PRM 893817. – Nová Pec, Chornice, 960 m a.s.l., leg. et det. M. T., 22. VI. 1999, PRM 894182. – Nová Pec, Rakouská cesta pathway – border stone No. 1/10, 1040 m a.s.l., leg. et det. M. T., 22. VI. 1999, PRM 894183.– Nová Pec, Smrčina Mt., 1140 m a.s.l., leg. et det. J. Holec, 25. IX. 1997, PRM 891333. – Nová Pec, Smrčina Mt., 1180 m a.s.l., leg. et det. M. T., 4. VI. 1998, PRM 893830; ibid., 1200 m a.s.l., PRM 893823; ibid., 1010 m a.s.l., PRM 893819. – Nová Pec, village, 795 m a.s.l., leg. et det. M. T., 22. VI. 1999, PRM 893822. – Prášily, Formberg, 950 m a.s.l., leg. et det. M. T., 10. VI. 1999, PRM 894013. Prášily, village, 880 m a.s.l., leg. et det. M. T., 26. VIII. 1998, PRM 893832. – Prášily, village, 950 m a.s.l., leg. et det. F. Kotlaba, 5. VIII. 1998, PRM 8922710. – Prášily, Stodůlky, 850 m a.s.l., leg. et det. M. T., 12. VI. 1999, PRM 894015. – Prášily, Ždanidla Mt., 1220 m a.s.l., leg. et det. M. T., 9. VII. 1998, PRM 894055; ibid., 1190 m a.s.l., PRM 894011; ibid., 1140 m a.s.l., PRM 894017. – Srní, Povydří, 680 m a.s.l., leg. et det. J. Holec, 8. X. 1997, JH 633/97. – Srní, Povydří, 920 m a.s.l., leg. et det. M. T., 28. VI. 1999, PRM 893818; ibid., 750 m a.s.l., 24. IX. 1998, PRM 893826. – Srní, Povydří, Horní Hrádky, 900 m a.s.l., leg. et det. M. T., 30. VI. 1999, PRM 893827. – Srní, Povydří, Hrádecký potok brook, 800 m a.s.l., leg. et det. M. T., 30. VI. 1999, PRM 893828. – Srní, Zadní Paště, 810 m a.s.l., leg. et det. M. T., 14. VI. 1999, PRM 893823. – Stožec, Stožecká

skála rock, 976 m a.s.l., leg. et det. M. T., 23. VI. 1999, PRM 893821. – Stožec, Světlá brook, 860 m a.s.l., leg. et det. M. T., 23. VI. 1999, PRM 893831. – Strážný, village, 840 m a.s.l., leg. et det. M. T., 23. IX. 1999, PRM 894019. – Zátoň, Boubín-Pažení, 1100 m a.s.l., leg. et det. J. Holec, 10. VII. 1998, PRM 896998. – Zátoň, Jilmová skála hill, 960 m a.s.l., leg. et det. M. T., 13. X. 1998, PRM 894023; ibid., 1000 m a.s.l., leg. et det. J. Holec et Z. Pouzar, 16. X. 1996, PRM 889509. – Železná Ruda, Černé jezero lake, VIII. 1926, leg. A. Hilitzer, det. A. Pilát, PRM 686735; ibid., 1926, PRM 686736. – Železná Ruda, Medvědí jámy, 850 m a.s.l., leg. et det. J. Holec, 16. VI. 1997, JH 16/97. – Železná Ruda, Debrník, avenue, 800 m a.s.l., leg. et det. M. T., 24. VIII. 1998, PRM 894021; ibid., 7. VII. 1998, PRM 894020. – Železná Ruda, Debrník, mixed forest, 800 m a.s.l., leg. et det. J. Holec, 15. X. 1997, JH 811/97. – Železná Ruda, Ferdinandovo údolí valley, 740 m a.s.l., leg. et det. M. T., 8. VIII. 1998, PRM 894014; ibid., 850 m a.s.l., leg. et det. J. Holec, 16. VI. 1997, PRM 890902. – Železná Ruda, by the road to Špičák, 760 m a.s.l., leg. et det. M. T., 25. VIII. 1998, PRM 894018. – Železná Ruda, Pancíř Mt., 1110 m a.s.l., leg. et det. M. T., 27. VIII. 1999, PRM 894016. – Želnava, Černý les forest, 900 m a.s.l., leg. et det. M. T., 24. VI. 1999, PRM 893824.

Central Bohemia

Český Kras, Srbsko, Bubovické vodopády waterfalls, 290 m a.s.l., leg. et det. M. T., 31. VIII. 1999, PRM 894465. – Železné hory, Třemošnice, Lovětínská rokle, 750 m a.s.l., leg. et det. M. T., 10. IX. 2000, PRM 894461. – Branov near Křivoklát, valley "V luhu", leg. M. Svrček, 25. VI. 1961, PRM 616218. – Brdy mountains, avenue by Padrťské rybníky pounds, leg. et det. J. Holec, 13. XI. 1997, PRM 891657.

Southern Bohemia

District Prachatice, Křišťanovice, 800 m a.s.l., leg. et det. M. T., 14. IV. 2000, PRM 894249. – Nové Hrady, Terčino údolí valley, 500 m a.s.l., leg. et det. M. T., 4. VIII. 1999, PRM 894240. – Blanský les Mts., Chrášťanský vrch, 750m a.s.l., leg. et det. D. Dvořák, 23. 5. 2001, herbarium D. Dvořák. – Blanský les Mts., Kleť Mt., 950 m a.s.l., leg. et det. D. Dvořák, 23. 5. 2001, herbarium D. Dvořák. – District Český Krumlov, Žofínský prales, 760 m a.s.l., leg. et det. M. T., 21. VI. 2001, PRM.

Czech-Moravian Highlands

Chotěboř, valley of the Doubravka river, 460 m a.s.l., leg. et det. M. T., 31. VIII. 2000, PRM 894238. – Jihlava, Vysoký kámen hill, 650 m a.s.l., leg. et det. M. T., 6. IX. 2000, PRM 894247. – Pelhřimov, top of Křemešník hill, 760 m a.s.l., leg. et det. M. T., 1. IX. 2000, PRM 894244. – Staré Ransko, avenue in the village, leg. et det. M. T., 11. VIII. 2000, PRM 894235. – Třešť, Velký Špičák Mts., 730 m a.s.l., leg. et det. M. T., 7. IX. 1999, PRM 894245. – Žďár nad Sázavou, castle, 560 m a.s.l., leg. et det. M. T., 21. VIII. 1999, PRM 894243.

Eastern Bohemia

District Ústí n. Orlicí, Litice, 430 m a.s.l., leg. et det. M. T., 23. VIII. 2000, PRM 894248. – District Rychnov nad Kněžnou, Potštejn, Modlivý důl, 350 m a.s.l., leg. et det. M. T., 23. VIII. 2000, PRM 894242. – District Rychnov nad Kněžnou, Potštejn, Vochtánka valley, 320 m a.s.l., leg. et det. M. T., 23. VIII. 2000, PRM 894246. – Orlické hory Mts., Sněžné, next to the road to Sedloňov, 590 m a.s.l., leg. et det. M. T., 27. XII. 1999, PRM 894239. – District Ústí n. Orlicí, Velká Morava, Sviní hora Mt., 790 m a.s.l., leg. et det. D. Dvořák, 18. XI. 2000, PRM 894366. – District Ústí n. Orlicí, Horní Morava, Klepý Mt., 660 m a.s.l., leg. et det. D. Dvořák, 14. II. 2001, herbarium D. Dvořák; ibid., 865 m a.s.l., 16. II. 2001.

Central Moravia

Adamov, Jelení skok, leg. A. Vágner, 5. VI. 1999, BRNM 642784. – Černovice u Kunštátu, Hrádky, leg. A. Vágner, 28. VII. 1999, BRNM 648418. – Černovice u Kunštátu, Káčiny, leg. V. Antonín, 27. IV. 2000, BRNM 652783. – Ochoz u Brna, valley of Říčka brook, 25. V. 2000, leg. V. Antonín, BRNM. – Osiky, Horní Židovka, leg. A. Vágner, 28. VII. 1999, BRNM 648414. – Letovice, park at the castle, leg. A. Vágner, 29. VII. 1999, BRNM 648438. – Úsobrno, Durana, leg. V. Antonín, 10. V. 2000, BRNM.

Jeseníky Mts.

Branná near Šumperk, valley of Vrbenský potok, leg. A. Vágner, 7. IX. 1999, BRNM 648832.

Oderské vrchy hills

Hranice, Jezernice valley, leg. et det. Z. Pouzar, 13. VI. 2001, PRM.

Beskydy Mts.

District Frýdek-Místek, Radhošťské Beskydy Mts., Bílá, bank of Salajský potok brook, c. 750 m a.s.l., leg. M. Vašutová, 12. VII. 1999, herbarium M. Vašutová. – District Frýdek-Místek, Slezské Beskydy Mts., Nýdek, village, 420 m a.s.l., leg. et det. M. T., 30. IX. 1999, PRM 894460.

Bílé Karpaty Mts.

Strání, Velká Javořina, *Acer pseudoplatanus*, leg. V. Antonín et A. Vágner, 18. VIII. 2000, BRNM 652866.

Slovakia

Malé Karpaty Mts. (Kleine Karpathen), Sklená huta (Glasshütten), leg. J. Hruby, det. A. Pilát, IV. 1925, PRM 686734 (typus). – Muránska planina plateau, between Ľadová jama and Havranská šopa, leg. Z. Palice, det. M. T., 11. V. 1999, PRM 894462. – Oravské Beskydy Mts, Babia hora Mt., 1000 m a.s.l., leg. et det. M. T., 29. IX. 2000, PRM 894463. – Slovenský raj, Velký Sokol valley, 640 m a.s.l., leg. et det. M. T., 11. VI. 1998, PRM 894464. – Slovenský raj, Pily, bank of the brook, 600 m a.s.l., leg. et det. M. T., 9. VI. 1998, PRM 894466.

Romania

District Suceava, Gura Humorului, in front of the gate of the Humor monastery, leg. M. T. and M. Kolařík, det. M. T., 30. VI. 2000, PRM 894434. – District Suceava, Rarău Mts., by the road from Câmpulung Moldovenesc to the top of Rarău Mt., next to the monastery, leg. et det. M. T., 1. VII. 2000, PRM 894433. – District Suceava, Rarău Mts., top of Rarău Mt., next to mountain hotel, leg. et det. M. T., 1. VII. 2000, PRM 894431. – District Suceava, Rarău Mts., Slătioara, primeval forest, leg. et det. M. T., 1. VII. 2000, PRM 894432. – District Suceava, Caliman Mts., village Gura Haitii, bank of the brook, 1100 m a s. m., leg. et det. M. T., 30. VI. 2000, PRM 894435.

Ukraine

Eastern Carpathians, District Rachov, village Kvasy, Mentchul Mt., 48 °10'52" N, 24 °18'24" E, 900 m a.s.l., leg. et det. J. Holec, 14. VII. 1999, PRM 892898; ibid., 1300 m a.s.l., 13. VII. 1999, PRM 892895. – Eastern Carpathians, District Rachov, village Kvasy, Mentchul Mt., 48 °10'34" N, 24 °18'10" E, 750 m a.s.l., leg. et det. M. T., 11. VII. 1999, PRM 894467; ibid., 48 °10'51" N, 24 °17'52" E, 660 m a s. l., PRM 894468.

Austria

Voralberg, Mühlviertel, Nordtirol, Salzburg, Steiermark (Krieglsteiner 1993, Rücker & Forstinger 1991).

France

Vosges (Krieglsteiner 1993).

Germany

Bavaria (Bayerischer Wald, Bayerische Alpenvorland, Oberpfälzer Wald), Baden-Württemberg, Harz Mts., Schwarzwald Mts. etc. (Krieglsteiner 1993).

Switzerland

Kantons Fribourg, Glarus, Schwyz, St. Gallen, Zürich (Baicin & Léger 1988).

DISCUSSION

The distribution of *Hymenochaete carpatica* is not sufficiently known, despite of the old datum of its description. In past the species was overlooked due to its specific ecology. Many *Aphyllophorales* specialists do not even collect *Hymenochaete carpatica*. According to my correspondence with A. Bernicchia and M. Tortić the species is not known from Italy and countries of the former Yugoslavia, but I think the species can occur there. *Hymenochaete carpatica* has neither been reported from Poland. The fungus may be widely distributed in Europe, where it follows the distribution of the only host – *Acer pseudoplatanus*.

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Lipid, sterol and ergosterol accumulation in isolates of dematiaceous Hyphomycetes

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Mostafa M. E., Zohri A. A. and Kotby R. S. (2001): Lipid, sterol and ergosterol accumulation in isolates of dematiaceous hyphomycetes – Czech Mycol. 53: 149–159

Mycelial dry weight, lipid and sterol contents of fungi tested varied with fungal genus, species and even with isolate of one species. Their dry mass fluctuated between 111.6±10.7–457.0± ±41.5 mg/50 ml medium. Lipids, sterols and ergosterol accumulated by the isolates tested ranged from 4.52± 0.5–29.04±2.76%, 1.23±0.16–10.63±1.24% and 0.43±0.057–7.13± 0.695% of their dry mass, respectively. *Cochliobolus spicifer* isolate No. 35 was the highest lipid-producer while *Ulocladium atrum* No. 90 proved to be superior in the production of sterols and ergosterol. TLC technique and chemical analysis of lipid classes produced by *U. atrum* No. 90 revealed that the lipid fractions are composed of free sterols, free fatty acids, sterol esters, glycolipids, phospholipids and squalene.

Key words: Lipid, sterol, ergosterol, dematiaceous hyphomycetes

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Sušina mycelia, obsah lipidů a sterolů kolísá v rámci různých rodů hub, mezi druhy i mezi izoláty jednoho druhu. *Cochliobolus spicifer* isolát č. 35 byl nejvyšším producentem lipidů a druh *Ulocladium atrum* isolát č. 90 produkoval nejvíce steroly a ergosterol.

Many fungi have a high capacity for lipid production. The lipid content of vegetative fungal hyphae varies between 1% and 50% of their dry mass (Weete 1974). Sterol is a group of lipids that has extensively attracted the attention of biochemists during the past decades. The utilization of sterols, as precursors for certain vitamins (Ellis 1945) and steroid sex hormones (Kieslich 1985), lead to a thorough investigation of their occurrence in nearly all forms of life (animals, plants and microorganisms). Sterols are also used as taxonomical and phylogenetical secondary metabolites (Weete 1974).

Pharmaceutical research has used ergosterol as a target for such antimycotic drugs as amphotericin-B (Rippon 1982). Ergosterol has been also recorded for antirachitic activity (El-Refai and El-Kady 1969). It is a common fungal sterol known to occur in a broad taxonomic range of fungi (Gordon and Webster 1984) and acts as a precursor of vitamins (Ellis 1945).

The first attempt to isolate sterols from fungi was accomplished by Naegeli and Loew (1878). A considerable amount of data has been collected during the

past decades on sterols of fungi. In general, different species of filamentous fungi, especially Aspergilli and Penicilli, have been studied for sterol production (Preuss et al. 1931, El-Refai 1964, Weete 1974, El-Kady et al. 1995). All these studies reported that ergosterol was the principle fungal sterol in the fungi.

There are only a few studies on the lipid and sterol contents of dematiaceous fungi. Therefore, the present study investigated the potential of 100 isolates of 36 species and one variety belonging to 18 genera of dematiaceous hyphomycetes for the production of lipids, sterols and ergosterol. Quantitative analyses of the different lipid classes produced by selected isolates grown on Czapek's medium using thin layer chromatography and chemical analysis were also performed.

MATERIALS AND METHODS

Fungal isolates

One hundred different fungal isolates of dematiaceous hyphomycetes belonging to 18 genera, 36 species and one species variety were collected and used in this study. Sixty– three of these isolates were isolated from medicinal and herbal plants (Youssef 1995, Ragaa Kotby 1996) in our laboratory. The remanining 37 isolates were obtained from Assiut University Culture Collection (AUCC), Botany Department, Faculty of Science, Assiut University, Assiut, Egypt. The numbers and names of these isolates are given in Table 1. Stock cultures were maintained on slants with Czapek's Dox Agar (Smith and Onions 1983). Inocula were prepared from 7 days old cultures as spore suspensions in 0.2% (v/v) aqueous Tween 80.

Cultivation

Each individual isolate was cultivated on Czapek's modified liquid glucose medium of the following composition (g/L of distilled water): sodium nitrate 2.0; potassium dihydrogen phosphate 1.0; magnesium sulphate 0.5; potassium chloride 0.5; ferrous sulphate 0.01; glucose 10.0 and supplemented with yeast extract 1.0 and peptone 10.0. Erlenmeyer flasks (three flasks for each isolate) of 250 ml capacity were used. Each flask contained 50 ml of the medium. The flasks were sterilized at 121 °C for 20 min. and inoculated after cooling with a 2 ml spore suspension (about 10^6 spores/ml) of the prepared inocula of each organism. The cultures were incubated at 28 °C as stationary cultures for 10 days.

Growth determination

Growth was measured as dry mycelial mass. The mass was filtered on Whatman No. 1 filter paper (15 cm diameter), washed three times with distilled water and dried for 24 h at 105 °C.

Analytical methods

Total lipids were extracted and determined according to Fanelli and Fabbri (1980). Total sterols were estimated by the Liebermann-Burchared colour reaction method (Cook 1958). Ergosterol content was estimated using UV spectrophotometric analysis at 282 nm (Maguigan and Walker 1940). Total fatty acids were determined by the phosphovanillin method of Zollner and Kirsch (1962). Glycolipids were determined according to Brown and Dupont (1989). Phospholipids were separated by the method described by Galanos and Kapoulas (1962) and estimated by the method of Rouser et al. (1970).

Fractionation of the lipid classes by TLC

Samples of the lipid matter dissolved in chloroform were used for qualitative fractionation analysis applying thin layer chromatographic techniques (El-Kady 1967, Ragaa Kotby 1996).

Results and discussion

The mycelial dry weight, lipid and sterol contents of the fungi tested varied with fungal genus, species and even with isolate of the same species (Table 1). These results are similar to those previously recorded by several authors (El-Refai 1964, Mumma et al. 1970, Weete 1974).

Dry mass of the fungal isolates varied between 111.6±10.7 and 457.0±41.5 mg/ /50 ml medium. The highest amount of dry mycelium was produced by *Alternaria tenuissima* isolate No. 9 followed by *Cladosporium cladosporioides* No. 15, *Ulocladium tuberculatum* No. 99, *Embellisia didymospora* No. 52 and *Pyrenophora avenae* No. 69, while the lowest dry mass was produced by *Stachybotrys chartarum* No. 82 (Table 1). Similar values were previously recorded by El-Refai (1964) who determined the dry mass of three dematiaceous fungal isolates on six types of synthetic media and found that the dry mass of *Stemphylium consortiale, Alternaria tenuis* Nees and *A. tenuis* acut. ranged from 107–2118, 191–2890 and 87.8–1023 mg/100ml depending on the medium, respectively.

The total lipids accumulated by the different isolates of dematiaceous hyphomycetes tested ranged from 45.2±5.02 to 290.40±27.63 mg/g mycelial dry mass (= 4.52±0.5–29.04±2.76% of their dry weight) as shown in Table 1. These results are in close agreement with the findings of many workers. In early studies, Preuss et al. (1934) cultivated 24 moulds for lipid and sterol production on two types of media and found that the total lipid content of the mycelium varied from 1.1–19.9% and 1.5–24.4% of their dry mass on glucose-inorganic salts and glucose-malt-sprouts media, respectively. Prill *et al.* (1935) recorded that the

total lipid contents of *Aspergillus fisheri* reached 37% of the dry weight. Mumma et al. (1970) found that the total lipids of *Chaetomium globosum, Penicillium chrysogenum, Sporotrichum thermophile, Malbranchea pulchella* and *M. pulchella* var. *sulfurea* were 54.1%, 9.8%, 15.5%, 26.5% and 24.8% of their dry weight, respectively. Weete (1974) reported that the lipid contents of different fungi varied between 1 and 50% of their dry weight depending on the species, stage of growth development, and culture conditions. Weete et al. (1985) recorded that the lipid content comprised 3.4% of the cellular dry matter of *Mucor rouxii* cells. Rawia Saad (1992) found that the total lipids of *Aspergillus amstelodami* and *A. repens* were 51.1% and 18.2% of their dry masses, respectively. Leobardo et al. (1992) determined the total lipid accumulation of two filamentous fungi (*Trichoderma harzianum* and *T. viride)* and found that lipid accumulation was about 17% and 32% (w/w) of dry weight for the two species, respectively. Recently, El-Kady et al. (1995) recorded that the total lipids of 57 fungal isolates (capable to grow on whey) ranged from 6 ±1 to 38 ±8% of their dry mass.

The highest lipid-producer isolates (Table 1) were *Cochliobolus spicifer* No. 35 (produced 29.04±2.76% lipid of dry weight), *Stachybotrys elegans* No. 84 (28.48± 2.85%), *Monodictys castaneae* No. 61 (24.58±2.29%), *Embellisia didymospora* No. 52 (23.94±2.45%), *Stachybotrys chartarum* No. 81 (23.40±2.27%), *Cladosporium cladosporioides* No. 14 (21.50±2.38%) and *Setosphaeria rostrata* No. 72 (21.12±1.96%). El-Refai (1964) found that the total lipids of *Stemphylium consortiale* and *Alternaria tenuis* reached 18.18% and 23.27% of their dry weight, respectively. The lipid contents of the different species of dematiaceous hyphomycetes tested in the present study are similar to those recorded by different fungal groups such as *Penicillium* species (Ward et al. 1935, Ghanem et al. 1990); Phycomycetes, Ascomycetes, Basidiomycetes (Weete 1974, Weete et al. 1985); *Eurotium* species (Rawia Saad 1992, El-Kady et al. 1995); *Trichoderma* species (Leobardo et al. 1992), as well as different species of *Aspergillus, Emericella* and *Fennellia* (El-Kady et al. 1995).

The average values of total sterols produced by the different isolates tested fluctuated between 12.31±1.60 and 106.3±12.35 mg/g dry weight (1.23±0.16% and 10.63±1.24% of their dry mass; 9.08% and 80.71% of their total lipids). The most sterol-producing isolate was *Ulocladium atrum* No. 90 (Table 1). These results are in accordance with those previously recorded by several workers (Preuss et al. 1934, El-Refai 1964, Ghanem et al. 1990, El-Kady et al. 1995). Fungi appear to differ quantitatively in their sterol contents (Ratcliffe 1937). El-Refai (1964) found that the total sterols of *Stemphylium consortiale* and *Alternaria tenuis* Auct. grown on Bills medium were 1.66 and 1.40% of their dry weight, respectively. Also, he recorded that the level of sterol reached 4.68% of *Aspergillus fumigatus* dry weight in presence of 1.0 μg/ml pantothenic acid in the basal medium. Ghanem et al. (1990) found that the total sterol yields of *Penicillium crustosum* grown

on a beet medium containing molass reached 8.4% of dry mass at pH 7.0 after 8 days of incubation. El-Kady et al. (1995) investigated the total sterol contents of 57 isolates belonging to *Aspergillus* (10 species), *Emericella* (two species varieties), *Eurotium* (four species and one variety) and *Fennellia* (two species), which were capable of growing on cheese whey. They noted that their total sterol contents ranged between 2±0.6% and 20±4% of their dry mass.

The mean values of ergosterol produced by the different isolates of dematiaceous hyphomycetes tested ranged from 4.31±0.57 to 71.3±6.95 mg/g dry weight (= 0.43±0.057% –7.13±0.695% of their dry weight, 3.35% –42.44% of their lipids and 4.71% –80.64% of their total sterols). *Ulocladium atrum* No. 90 proved to be the best ergosterol producer (Table 1). Few investigations have determined the production of ergosterol as a metabolite of the different genera and species of dematiaceous hyphomycetes such as *Stemphylium consortiate* (El-Refai 1964) in addition to *Drechslera graminea* and *D. teres* (Gordon and Webster 1986).

Ergosterol was found to be the principle sterol in the isolates screened (Table 1). These results are in accordance with the results previously reported by several workers (El-Refai 1964, Weete 1974, Weete et al. 1985, Fernando and Bean 1986, Ellis et al. 1991). Moreover, Fiore (1948) reported that ergosterol was the only sterol produced by *Fusarium lini, F. lycopersici* and *F. solani*. El-Refai (1964) found that ergosterol formed about 92% of the total sterols of *A. fumigatus* when the organism was cultivated on molasses at a concentration equivalent to 10% sugars and corn steep liquor at a level of 2% solids. Recently, El-Kady *et al.* (1995) recorded that ergosterol production by 57 fungal isolates belonging to 10 species of *Aspergillus*, two species varieties of *Emericella,* four species and one variety of *Eurotium* in addition to two species of *Fennellia* which could be grown on whey, ranged from 15% to 65% of their total sterols.

An attempt has also been made to fractionate and determine the different classes of lipids produced by *Ulocladium atrum* No. 90 which proved to be superior in the production of sterols and ergosterol. Thin layer chromatographic techniques and chemical analysis of the lipid classes produced by *U. atrum* No. 90 cultivated on Czapek's medium revealed the following lipid fractions: glycolipids, phospholipids, free sterols, free fatty acids, sterol esters and squalene (Table 2). These lipid fractions have been reported from different fungi (El-Refai and El-Kady 1969, Fanelli and Fabbri 1980, Weete et al. 1985, Naim and Saad 1986, Leobardo et al. 1992). El-Kady (1967) found that the fractionation of yeast lipids by the thin layer chromatographic technique resulted in: phospholipids, free sterols, fatty acids, fatty acid esters, triglycerides, sterol esters and squalene. The presence of squalene was expected as it was elucidated by many workers (Corwn et al. 1956, El-Kady 1967).

Table 1. Isolates of dematiaceous hyphomycetes screened for total lipid, total sterols and ergosterol production (the values are the average ±standard deviation based on three replicates).

No.	Species	Dry wt. (mg/50ml)	Total lipid (mg/g dry wt.)	Total sterol (mg/g dry wt.)	Ergosterol (mg/g dry wt.)
	Alternaria				
1	*A. alternata* (Fr.) Keissler	234.5±21.6	123.80±11.72	74.78±8.43	19.00±2.00
2	*A. alternata* (Fr.) Keissler	281.0±26.2	135.00±12.51	70.72±7.65	35.70±3.62
3	*A. alternata* (Fr.) Keissler	200.9±20.5	140.00±13.71	88.00±8.60	26.20±2.23
4	*A. chlamydospora* Mouchacca	173.9±16.3	127.50±10.63	55.50±6.48	9.80±1.05
5*	*A. chlamydospora* Mouchacca	269.3±24.1	120.60±12.46	78.06±8.62	16.00±1.52
6*	*A. chlamydospora* Mouchacca	170.2±15.6	103.60±11.20	61.80±5.47	10.70±1.35
7*	*A. chlamydospora* Mouchacca	182.4±16.8	93.80±8.97	68.11±7.41	17.00±1.47
8	*A. macrospora* Zimm.	184.7±16.7	66.00±7.48	24.60±3.27	7.55±0.85
9	*A. tenuissima* (Kunze) Wiltshire	457.0±41.5	199.60±20.85	30.12±2.80	6.69±0.64
10	*A. tenuissima* (Kunze) Wiltshire	189.4±17.4	96.40±7.82	59.99±6.00	14.27±1.23
	Bipolaris				
11*	*B. neergaardii* (Danquah) Alcorn	187.8±16.9	45.20±5.02	44.53±3.54	6.48±0.70
	Bispora				
12	*B. betulina* (Corda) Hughes	248.3±22.1	63.20±5.63	27.64±3.42	6.09±0.58
	Cladosporium				
13	*C. cladosporioides* (Fres.) de Vries	261.7±23.9	134.20±14.25	39.29±3.58	10.38±9.54
14	*C. cladosporioides* (Fres.) de Vries	151.7±14.3	215.00±23.82	19.52±2.66	8.27±1.02
15	*C. cladosporioides* (Fres.) de Vries	399.5±36.5	105.20±10.64	39.95±4.44	11.79±1.31
16	*C. herbarum* (Pers.) Link	197.0±17.5	133.20±14.06	60.64±5.35	9.71±0.87
17	*C. oxysporum* Berk. et Curt.	218.0±19.7	163.80±15.90	24.38±2.64	5.99±0.64
18	*C. oxysporum* Berk. et Curt.	226.3±20.4	153.00±16.18	53.00±6.00	35.30±2.76
19	*C. sphaerospermum* Penzig	183.6±15.9	146.60±15.51	64.30±6.52	18.12±2.01
20	*C. sphaerospermum* Penzig	238.7±22.1	185.00±19.04	19.52±2.07	9.59±1.05
	Cochliobolus				
21	*C. australiensis* (Tsuda et Ueyama) Alcorn	253.0±23.5	110.00±10.63	81.99±7.98	11.30±1.21
22	*C. australiensis* (Tsuda et Ueyama) Alcorn	165.7±15.1	69.00±7.12	28.29±3.05	13.14±1.40
23	*C. australiensis* (Tsuda et Ueyama) Alcorn	225.4±21.0	165.60±16.82	50.82±4.15	9.55±1.00
24	*C. australiensis* (Tsuda et Ueyama) Alcorn	213.6±19.6	120.20±13.00	51.47±6.02	6.41±0.71
25	*C. lunatus* Nelson et Haasis	287.5±26.0	159.80±16.67	27.77±2.53	6.75±0.69
26	*C. lunatus* Nelson et Haasis	332.8±29.8	176.80±16.93	44.01±4.09	21.13±1.95
27	*C. lunatus* Nelson et Haasis	302.0±26.4	148.00±13.92	27.11±3.00	13.87±1.62
28	*C. lunatus* Nelson et Haasis	250.0±23.2	160.20±17.00	31.70±3.42	10.69±1.02
29	*C. lunatus* Nelson et Haasis	273.8±25.3	176.23±17.23	33.28±3.55	13.53±1.60
30	*C. lunatus* Nelson et Haasis	225.5±21.6	154.41±15.60	42.96±3.86	9.41±1.05
31*	*C. pallescens* (Tsuda et Ueyama) Sivan.	238.3±23.0	74.40±6.98	49.90±5.10	12.97±1.22

Table 1. Cont.

No.	Species	Dry wt. (mg/50ml)	Total lipid (mg/g dry wt.)	Total sterol (mg/g dry wt.)	Ergosterol (mg/g dry wt.)
32*	*C. pallescens* (Tsuda et Ueyama) Sivan.	243.3±25.1	76.40±7.07	34.71±3.67	11.81±1.25
33*	*C. pallescens* (Tsuda et Ueyama) Sivan.	220.2±20.6	130.82±12.55	58.42±5.46	14.59±1.43
34	*C. spicifer* Nelson	297.4±31.4	94.63±10.34	38.90±4.00	9.83±1.16
35	*C. spicifer* Nelson	253.4±23.7	290.40±27.63	45.08±4.66	12.71±1.50
36	*C. spicifer* Nelson	214.0±22.0	154.40±16.50	36.94±4.05	17.60±2.02
37	*C. spicifer* Nelson	287.1±30.4	151.80±15.66	47.41±4.85	9.48±1.18
38	*C. spicifer* Nelson	279.4±25.5	93.42±8.78	13.10±1.53	4.31±0.57
39	*C. spicifer* Nelson	262.5±25.6	170.60±16.90	27.37±2.90	12.16±1.31
40*	*C. tuberculatus* Sivan.	285.2±27.8	70.40±8.04	56.82±4.78	10.71±1.35
41*	*C. tuberculatus* Sivan.	211.9±22.1	162.60±15.69	44.92±4.80	15.55±1.54
42*	*C. tuberculatus* Sivan.	152.2±13.6	79.00±8.56	63.19±6.54	13.33±1.50
43*	*C. tuberculatus* Sivan.	218.5±19.8	89.00±1.00	59.59±6.11	16.01±1.33
	Curvularia				
44	*C. clavata* Jain	124.0±15.2	96.00±1.08	39.29±4.27	16.78±2.01
45	*C. clavata* Jain	228.7±22.9	75.20±7.25	19.52±2.70	6.73±0.75
46	*C. oryzae* Bugnicourt	238.5±22.5	172.00±18.12	34.40±3.25	15.36±1.72
47*	*C. oryzae* Bugnicourt	278.3±30.0	110.00±10.50	27.90±3.01	10.56±1.00
48*	*C. oryzae* Bugnicourt	249.9±25.3	149.00±15.00	41.65±3.85	7.02± 0.61
49	*C. ovoidea* (Hiroe et Watan.) Muntanola	281.6±26.5	102.00±10.81	24.23±3.10	9.90±0.86
50	*C. ovoidea* (Hiroe et Watan.) Muntanola	215.5±20.7	73.20±6.77	17.33±1.56	6.50±0.55
51*	*C. ovoidea* (Hiroe et Watan.) Muntanola	164.2±15.6	105.10±9.87	21.90±1.84	6.74±0.64
	Embellisia				
52*	*E. didymospora* Muntanola-Cvetkovic	371.6±35.8	239.40±24.51	48.07±5.55	11.46±0.95
53*	*E. didymospora* Muntanola-Cvetkovic	150.8±16.0	67.11±7.63	57.92±5.48	6.42±0.75
54*	*E. didymospora* Muntanola-Cvetkovic	229.9±24.4	88.60±9.05	66.01±6.51	10.69±0.99
	Epicoccum				
55	*E. nigrum* Link	217.2±20.8	119.60±12.56	21.48±2.00	17.32±1.91
56	*E. nigrum* Link	240.1±26.4	125.83±15.20	12.31±1.60	8.02±0.95
	Humicola				
57	*H. grisea* Traaen	237.7±22.6	127.61±15.07	46.33±5.30	15.55±1.38
58	*H. grisea* Traaen	325.5±35.2	92.84±8.97	66.01±5.99	37.36±4.06
	Memnoniella				
59	*M. echinata* (Riv.) Galloway	321.2±33.5	62.80±7.00	25.93±2.80	9.93±1.10
	Monodictys				
60	*M. castaneae* (Wallr.) Hughes	185.9±20.4	126.63±13.65	69.42±5.85	31.15±2.84
61*	*M. castaneae* (Wallr.) Hughes	287.8±26.8	245.81±22.86	20.83±1.86	5.49±0.71
62*	*M. castaneae* (Wallr.) Hughes	190.1±21.5	90.22±10.43	39.55±4.65	11.87±1.20

Table 1. Cont.

No.	Species	Dry wt. (mg/50ml)	Total lipid (mg/g dry wt.)	Total sterol (mg/g dry wt.)	Ergosterol (mg/g dry wt.)
	Mycosphaerella				
63*	*M. tassiana* (de Not.) Johans.	276.3±29.3	131.66±13.50	49.94±5.45	11.67±0.96
64*	*M. tassiana* (de Not.) Johans.	315.3±29.6	131.66±11.95	54.48±5.56	12.74±1.36
	Pleospora				
65	*P. herbarum* (Fres.) Rabenh. ex Ces. et de Not.	254.0±26.5	137.00±12.09	62.23±5.80	25.11±2.25
66	*P. herbarum* (Fres.) Rabenh. ex Ces. et de Not.	248.7±25.8	106.60±11.54	79.17±8.66	26.73±3.02
	Pyrenophora				
67	*P. avenae* Ito et Kuribayashi	164.5±18.2	32.00±2.86	27.64±2.57	5.97±0.60
68	*P. avenae* Ito et Kuribayashi	260.3±24.8	72.80±8.00	38.77±4.05	7.61±0.85
69*	*P. avenae* Ito et Kuribayashi	349.1±35.6	93.41±8.80	39.29±4.30	6.83±0.76
	Setosphaeria				
70	*S. rostrata* Leonard	336.9±35.6	146.60±14.51	18.73±1.56	9.47±1.11
71	*S. rostrata* Leonard	304.6±31.2	137.40±14.30	40.73±4.10	12.00±1.30
72	*S. rostrata* Leonard	285.9±30.9	211.20±19.64	49.51±4.78	12.06±1.25
73	*S. rostrata* Leonard	290.4±30.5	75.22±6.99	28.29±3.08	12.93±1.52
74	*S. rostrata* Leonard	281.1±26.7	49.61±5.56	18.07±1.68	7.89±0.69
75	*S. rostrata* Leonard	287.6±30.4	64.43±8.00	13.62±1.65	7.87±0.75
76	*S. rostrata* Leonard	230.8±22.6	138.48±14.57	33.63±3.53	8.73±0.95
77	*S. rostrata* Leonard	149.1±13.8	110.25±9.88	16.45±2.00	4.69±0.50
78	*S. rostrata* Leonard	237.9±25.5	126.20±15.31	40.08±3.83	4.31±0.47
	Stachybotrys				
79*	*S. atra* var. *microspora* Mathur et Sankhla	250.3±27.6	62.22±5.89	38.91±3.65	6.97±0.58
80*	*S. atra* var. *microspora* Mathur et Sankhla	185.3±20.5	118.00±12.60	72.95±6.50	17.30±2.00
81	*S. chartarum* (Ehrenb.) Hughes	231.4±21.8	234.00±22.67	38.77±4.04	16.14±1.70
82	*S. chartarum* (Ehrenb.) Hughes	111.6±10.7	160.37±14.75	50.33±5.48	38.71±3.55
83*	*S. elegans* (Pidopl.) Gams	209.9±18.8	122.68±12.55	45.90±5.04	14.13±1.38
84*	*S. elegans* (Pidopl.) Gams	236.8±25.6	284.81±28.52	78.59±8.00	36.65±3.80
	Torula				
85	*T. graminis* Desm.	213.7±20.9	120.60±14.00	79.29±7.60	29.51±3.09
86*	*T. graminis* Desm.	177.2±20.1	84.00±9.56	42.83±3.45	6.95±0.65
87*	*T. graminis* Desm.	259.8±27.3	193.80±18.90	77.81±8.40	13.74±1.27
	Ulocladium				
88*	*U. alternariae* (Cooke) Simmons	185.0±16.9	189.00±20.03	55.50±4.65	16.81±1.50
89	*U. atrum* Preuss	299.6±31.5	169.81±20.00	75.05±8.60	14.08±1.64
90	*U. atrum* Preuss	219.0±21.4	168.00±16.54	106.30±12.35	71.30±6.95
91	*U. botrytis* Preuss	221.0±21.6	128.42±11.95	57.63±6.38	7.25±0.88
92	*U. botrytis* Preuss	255.8±28.5	105.00±11.55	63.13±7.00	12.35±1.52

Table 1. Cont.

No.	Species	Dry wt. (mg/50ml)	Total lipid (mg/g dry wt.)	Total sterol (mg/g dry wt.)	Ergosterol (mg/g dry wt.)
93*	*U. chartarum* (Preuss) Simmons	215.1±23.3	125.81±13.40	80.09±7.50	10.98±1.50
94*	*U. chartarum* (Preuss) Simmons	268.3±30.1	105.27±9.76	60.23±6.31	11.07±1.21
95*	*U. chartarum* (Preuss) Simmons	273.6±29.0	102.22±11.32	25.67±2.60	4.50±0.38
96*	*U. consortiale* (Thwm.) Simmons	152.5±13.8	143.63±16.71	29.33±2.84	17.27±1.60
97*	*U. consortiale* (Thwm.) Simmons	228.4±23.6	153.41±14.90	67.98±6.51	10.85±1.23
98*	*U. tuberculatum* Simmons	244.6±26.0	171.16±16.56	38.47±4.03	8.03±0.65
99*	*U. tuberculatum* Simmons	381.9±35.6	153.40±15.59	21.48±1.88	7.29±0.56
100*	*U. tuberculatum* Simmons	189.4±20.0	154.41±17.10	63.52±5.55	27.93±2.85

* Fungal isolates (37 isolates) obtained from Assiut University Culture Collection (AUCC), Botany Department, Faculty of Science, Assiut University, Assiut, Egypt. The other isolates (63 isolates) were isolated from medicinal and herbal plants in our laboratory (Youssef 1995, Ragaa Kotby 1996).

They concluded that cyclisation of squalene leads to the formation of sterol compounds.

The total lipid fractions were about 17.0% of the fungal dry mass while the total sterols were about 63.3% of the total lipids on Czapek's medium. Free sterols were represented by about 7.9%, while ergosterol formed about 64.6% of total sterols, respectively. On the other hand, free fatty acids, glycolipids and phospholipids formed about 4.17%, 17.5% and 5.4% of total fungal lipids, respectively (Table 2). El-Kady (1967) reported that free sterols (only ergosterol and zymosterol) contributed to about 5.8% of total sterol, while the combined sterols fraction in the form of esters represent 94.2% of the total sterols detected. Weete et al. (1985) reported that *Mucor rauxii* cells contained 3.4% of dry mass as lipids, of which phospholipids, sterols (25% ergosterol) and squalene contributed to 35%, 4% and 2% of dry weight, respectively.

These conclusions concerning both chemical and thin layer chromatographic analysis of *Ulocladium atrum* lipid and sterol fractions may throw some light on the information needed for the possible utilization of this organism for the microbiological production of sterols and ergosterol (a precursor of vitamin D).

Table 2. Lipid constituents of *U. atrum* No. 90 grown on Czapek's medium at 28±2 °C for 10 days (the values are the average ±standard deviation based on three replicates).

Dry weight	Total Lipid	Total sterols	Free sterols	Ergosterol	Free fatty acids	Glycolipids	Phospho-lipids	Squalene
(mg/50 ml)	(mg/g dry weight)							
221.5 ±20.5	170.3 ±15.8	107.8 ±10.9	8.5 ±1.0	69.6 ±7.1	7.1 ±0.8	29.8 ±2.6	9.2 ±0.8	6.4 ±0.5

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The role of some saprophytic micromycetes and the fungus Micromucor ramannianus var. ramannianus in forest soils

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Different saprophytic micromycetes were isolated from the humic horizon (H-A 02) of different types of forest soils (barren land of reforested waste dumps, cambisol of spruce, birch, European mountain ash, and blue spruce forests) in several areas (at Most in the Krušné hory (Ore Mts.), Jizerské hory (Izera Mts.)). Besides the spectrum of common species of soil micromycetes (*Penicillium* spp., *Humicola* spp., *Trichoderma* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Aureobasidium* spp., *Mucor* spp., *Absidia* spp.), the fungus *Micromucor ramannianus* (Möller) Arx var. *ramannianus* (*Mortierella ramanniana* (Möller) Linneman, *Mucor ramannianus* Möller) was regularly isolated from all types of soils, except barren soils of waste dumps. The biological quality of forest soils in connection with other biological characteristics was evaluated in relation to the presence and quantity of this fungus in forest soils. Basic biological processes (basal and potential respiration, ammonification, nitrification) show an increased intensity in forest soils in which the proportion of *Micromucor ramannianus* v. *ramannianus* was not present in the soil of the worst biological quality (lower values of biological soil parameters), e.g. in of waste dumps. It is a topic for discussion whether this fungus can also be an indicator of environmental pollution.

Key words: humic horizon, soil fungi, saprophytic micromycetes, *Micromucor ramannianus* var. *ramannianus*, biological soil functions, respiration, ammonification, nitrification

Hýsek J. a Brožooová J. (2001): Role některých saprofytických mikromycetů a houby Micromucor ramannianus var. ramannianus v lesních půdách – Czech Mycol. 53: 161–171

Z humusového horizontu (H-A02) různých lesních typů (neplodná půda výsypek, kambisol různých typů – smrk, bříza, jeřáb, stříbrný smrk) v několika místech (výsypky v Mostu, půdy v Krušných a Jizerských horách) byly izolovány různé saprofytické mikromycety. Vedle spektra běžných půdních mikromycetů (*Penicillium* spp., *Humicola* spp., *Trichoderma* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Aureobasidium* spp., *Mucor* spp., *Absidia* spp.) byla pravidelně izolována ze všech typů lesních půd houba *Micromucor ramannianus* (Möller) Arx var. *ramannianus* (*Mortierella ramanniana* (Möller) Linneman) kromě půd výsypek. Biologická kvalita lesních půd ve spojení s jinými biologickými charakteristikami byla stanovena společně s přítomností a kvantitou této houby v lesních půdách. Základní biologické procesy (bazální a potenciální respirace, amonifikace a nitrifikace) ukazují zvýšenou intenzitu v lesních půdách, ve kterých celkový počet nálezů houby *Micromucor ramannianus* v. *ramannianus* přesahuje 50 % všech přítomných mikromycetů. Na druhé straně houba *Micromucor ramannianus* v. *ramannianus* nebyla přítomna v půdách horší biologické kvality (nižší hodnoty půdních biologických charakteristik) např. v půdách výsypek. Je otázkou diskuse, zda tato houba může být také indikátorem znečištění nebo čistoty životního prostředí.

Introduction

Decomposition of organic matter into humus, gases and inorganic salts is a continuous process in forest soils. Soil micromycetes are extraordinary important participants in this process. Forest soils are habitats of mainly soil-borne saprophytic and some pathogenic fungi. The genus *Penicillium* (Dyr 1940) and its teleomorphs, e.g. *Eupenicillium* (Takada and Udagawa 1983), are the most frequently isolated genera from forest soils. Several new species of *Penicillium* from forest soils have been described. E.g. the new species *Penicillium kananaskense* (Seifert et al. 1994) was isolated from forest soil under *Pinus contorta* v. *latifolia*. New species of the perfect stage of *Eupenicillium* were isolated e.g. from Nepalese forest soils – *E. angustiporcatum* (imperfect stage: *Penicillium angustiporcatum*) and *E. nepalense* (imperfect stage: *Penicillium nepalense*) (Takada and Udagawa 1983). *Talaromyces unicus* and its anamorph were isolated from Taiwanese soil. The anamorph is characterised by mono– or biverticillate penicilli with long-necked lanceolate phialides (Tzean et al. 1992). The genus *Talaromyces* is also dominant in soil in which heat-resistant fungi are present (Jesenská, Piecková and Bernát 1992).

Differently broad spectra of micromycetes are usually isolated from forest soils. Grunda and Marvanová (1982) identified the following genera of micromycetes from soil of the forest type group *Ulmi-Fraxinetum carpineum* with *Quercus robur* predominating in the stand: 1) (at a depth of 1–2 cm – horizon F) *Absidia*, *Alternaria*, *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Humicola*, *Mucor*, *Paecilomyces* abd *Penicillium*, 2) the same genera as in horizon F were isolated at a depth of 2–7 cm (horizon Am), but besides them *Sporothrix* and *Trichoderma* were identified. *Cephalosporium*, *Mortierella*, *Phialophora*, *Stachybotrys* and *Trichocladium* were isolated from a depth of 15–25 cm (horizon Btg). Besides these the following genera were determinated at a depth of 35–45 cm (horizon Btg): *Bispora*, *Phoma* and *Rhizopus*. It is interesting that the fungus *Micromucor ramannianus* v. *ramannianus* (*Micromucor ramannianus*) was not detected in that soil, although it is present in almost all types of forest soils (Dyr 1940). Grunda (1981) investigated methods of research on soil microflora when he studied the floodplain forest soil in Moravia. The following genera of micromycetes were identified in floodplain forest soil: *Bispora*, *Calcarisporium*, *Chrysosporium*, *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Humicola*, *Mortierella*, *Mucor*, *Paecilomyces*, *Phialophora*, *Phoma*, *Penicillium*, *Rhizopus*, *Sporothrix*, *Stachybotrys*, *Trichocladium* and *Trichoderma*. Not even in this case the fungus *Micromucor ramannianus* v. *ramannianus* (*Mucor ramannianus* Möller) was detected, even if it is regularly present in forest soils (Dyr 1940).

Saprophytic micromycetes in forest soils were studied by Vláčilíková (1978), who described the presentation of genera of micromycetes in forest soils. Av-

erage proportions (in percent) of fungal genera in four types of forest soil were as follows: *Absidia* (7.5), *Circinella* (0.35), *Mucor* (16), *Rhizopus* (0.1), *Zygorhynchus* (0.6), *Cunninghamella* (0.1), *Mortierella* (9.0), *Gymnoascus* (2.7), *Thielavia* (2.3), *Chaetophoma* (0.1), *Truncatella* (0.2), *Aspergillus* (2.0), *Cephalosporium* (4.0), *Gliocladium* (2.0), *Monocillium* (0.5), *Paecilomyces* (0.9), *Penicillium* (51.5), *Spicaria* ((2.1), *Sporotrichum* (0.9), *Trichoderma* (6.4), *Verticillium* (0.4), *Botryotrichum* (0.1), *Cladosporium* (1.9), *Hormiscium* (0.4), *Humicola* (0.9), *Masoniella* (0,4), *Pullularia* (0.1), *Stachybotrys* (1.9), *Cylindrocarpon* (0.6), *Fusarium* (0.3), *Volutella* (0.3), sterile mycelium (0.6), *Myrothecium* (0.3). *Mortierella ramanniana* (Möller) Linneman ranked second among all species of the genus *Mortierella* in forest soils (0, 1.5 %, 13.7 %, 5.1 %). *Penicillium nigricans* (Bainier) Thom was the species with the highest representation in the different soils (%): 2.5, 49, 18.1, 1.1.

Micromucor (*Mortierella*) is the best known genus of forest soil zygomycetes. A relationship of *Endogone* to *Mortierella* (*Mortierellaceae*) has been suggested since in some species, especially *M. nigrescens* and *M. renispora*, the zygospores are surrounded by hyphae, much like the hyphae developed by zygospores in sporocarps of *Endogone* (Benny 1982).

Micromucor ramannianus is a fungus with one of the highest frequencies in forest soils. The genus is classified in the genus *Mucor* by Dyr (1942) as *Mucor ramannianus* A. Möller 1903 (Zeitschr. F. Forst. und Jagdw. 35, p. 330). It differs from other species by the colour of its colonies beeing deep red to soil pink. The cultures reached a height 1.6–2.5 mm only, fungus growth is very dense and velvety. Sporangiophores are usually not ramified. Sporangia are small, 18–35 μm and not ramified, they are deep red in colour and have a slightly melting membrane. The columella is round or disk-shaped, colourless, 10.8–12.7 μm in height and 11–14 μm in width. Sporangiospores are shortly eliptic to round (1.2×2.3, $2.7 \times 3.3 \times 3$ μm). The species was isolated in Germany (Johann 1932), France (Ling-Young 1930), Austria and Yugoslavia (Pispek 1929), Russia (Raillo 1929), Northern America (Povah 1917), Australia (Dale 1914) and Norway (Hagen 1908).

This species is generally present in forest soils, its frequency being the highest of all species of micromycetes and it could probably be isolated at every forest site. The population density of this species is regularly enormous amounting to more 50 % of micromycetes at the site. The ratio of *Mucor ramannianus* to other micromycetes was 25:1 in forest soils of the Jevany district. Sites with the highest population density of the fungus had the highest concentration of sporangiospores in the soil. The fungus population density was low at two forest sites only – in the environs of Dobřichovice (right bank of Berounka river) and in Brdy forest. Different types of forest showed different rates of the fungus (out of the total number of micromycetes). It was 64.2 % in beech forest soils, 39.8 % in spruce forest soils, 37.4 % in pine forest soils, 26 % in oak forest soils, 20 % in alder forest

soils, 32.5 % in birch grove soils. This species had modest environmental demands with respect to the extreme soil factors in broad-leaved and coniferous forest stands. Fungus growth in nutrient media in Petri dishes makes up a continuous coating of a typical colour. Fungus growth is very fast. The species is usually at a soil depth of up to 20 cm, in the upper humus layer under it. Kubátová et al. (1998) reporting on the biodiversity of soil microfungi of the Šumava Mts., Czech republic, confirmed that among the most frequent species *Micromucor ramannianus* v. *ramannianus* was. They determined soil micromycetes from 12 localities, including peat-bogs, Norway spruce forest, beech forests, mixed forests in glacial cirques in the period 1993–1996 (121 soil samples). The most frequent species were: *Trichoderma viride* (in 57 % of all samples), *Penicillium spinulosum* (55.4 %), *Micromucor ramannianus* v. *ramannianus* (33.1 %) and *Mucor hiemalis* f. *hiemalis* (24.8 %).

Micromucor ramannianus has not been described as a typical fungus of forest soils in many papers, or its population density is characterised as very low. E.g. Grunda and Vorel (1996) reported a high density of micromycete species with some differences in various types of soils (H-horizons). The following micromycetes were present in the H-horizon of humic acidic Cambisol: *Penicillium* sp., *Aspergillus* sp., *Gliocladium* sp., *Trichoderma* sp., *Cladosporium* sp., *Sporotrichum* sp., *Alternaria* sp., *Humicola fuscoatra* and *Humicola grisea*. Acidic Cambisol contained also these fungi: *Botrytis* sp., *Mortierella nana*, *Chaetomium* sp. The fungi were determined in deep acidic Cambisol in comparison with acidic Cambisol: *Mortierella ramanniana* (*Micromucor ramannianus* at present) and *Verticillium albo-atrum*. In addition, *Absidia glauca* was found in gley acidic Cambisol.

Many of the soil micromycetes showed intensive metabolic activity, e.g. lignolytic activity in *Penicillium chrysogenum* (Rodriquez et al. 1994). This metabolic activity can last very long. Marfenina (1991) studied the morphological development of microscopic fungi in soil. The life cycle (mycelium and conidium formation) is usually very long.

Many species of *Mortierella* produce a white oily substance in large drops among the aerial hyphae. One group of species, characterised by velvety odourless colonies, is considered by some mycologists to represent a separate genus, *Micromucor* (Benny 1982). The objective of this study was to determine the importance of *Micromucor ramannianus* v. *ramannianus* in soil humic horizons of Czech forests.

MATERIAL AND METHODS

Forest soil samples were taken from the humic horizon (H-horizon) after the F-horizon (fermentation horizon) was disclosed. Soil was sampled into platic bags under sterile conditions, and was processed as soon as it was brought into

a laboratory. It was ground through a fine sieve. 5 grams of ground sample was added under sterile conditions into 500 ml of a sterile physiological saline, it was left to clarify for 2 min. 1 ml was pipetted under sterile conditions onto the bottom of sterile Petri dishes and overlaid with cooling down agar (Czapek-Dox agar). After coolong down, Petri dishes were placed in a termostat controlled room at a temperature 24 °C for 10 days. The number of micromycete colonies was determined quantitatively. Qualitative analyses were made and micromycete colonies were determined in microscopic preparations in lactophenol with methylene blue and examined under a microscope. Basic soil characteristics were determined by methods described in e.g. Schilling and Blume's book (1966). The species were typified with the use of different literature (e.g. Domsch and Gams 1993).

RESULTS

The H-horizon (humic) was examined microbiologically in all cases together with F (fermentation) and A (mineral) horizons. Tab. 1 shows the localities of soil sampling. The results of identification of cultivable microscopic fungi (Tab. 2) demonstrate that the fungus *Micromucor ramannianus* v. *ramannianus* is present in spruce, beech, birch and mountain ash soils but not in soils of waste dumps in the Most district, even though they have been reforested. The fungus was also isolated from F and A horizons, but the population density were lower and less regular. The H-horizon was taken as the basic component of forest soils because it contains typical humus (humic acids and fulvoacids). It is evident from Table 2 that the fungus was present in the H horizon of all productive soils. Its rates were higher than 50 % of all cultivable CFU (Colony Forming Units) in soils of higher biological activivity.

Table 1. Localities of soil sampling.

Locality	Mountain range	Stand	Soil type
Jizera river basin	Jizera Mts.	Spruce	Cambisol
Nová Ves v Horách	Ore Mts.	Spruce	Cambisol
		Blue spruce	Cambisol
		Birch	Cambisol
		Mountain ash	Cambisol
Velebudice	Most waste dump	Maple	Artificial ground
		Lime	

Table 2. Micromycetes isolated from H-horizons of cambisol types of forest soils

Locality/Stand	Micromycetes	Proportion / frequency
Jizera river basin/Spruce	*Micromucor ramannianus* var. *ram.*	55%
	Trichoderma viride	20%
	Penicillium expansum	10%
	Paecilomyces farinosus	5%
	Paecilomyces niveus	5%
	Scopulariopsis brevicaulis	2,5%
	sterile mycelium	2,5%
Nová Ves v Horách/Spruce	*Micromucor ramannianus* var. *ram.*	52%
	Trichoderma viride	15%
	Penicillium italicum	15%
	Paecilomyces varioti	7%
	Paecilomyces fulvus	3%
	Scopulariopsis brevicaulis	5%
	sterile mycelium	3%
Nová Ves v Horách/Blue spruce	*Micromucor ramannianus* var. *ram.*	58%
	Scopulariopsis brevicaulis	12%
	Absidia corymbifera	10%
	Paecilomyces fulvus	8%
	Trichoderma viride	5%
	sterile mycelium	7%
Nová Ves v Horách/Birch	*Micromucor ramannianus* var. *ram.*	65%
	Paecilomyces farinosus	8%
	Penicillium expansum	6%
	Scopulariopsis brevicaulis	6%
	Gilmaniella humicola	5%
	Trichoderma viride	2%
	sterile mycelium	8%
Nová Ves v Horách/Mountain ash	*Micromucor ramannianus* var. *ram.*	59%
	Aureobasidium pullulans	10%
	Cladosporium resinae	8%
	Paecilomyces fulvus	6%
	Trichoderma viride	1%
	sterile mycelium	16%
Velebudice/Most waste dump	*Mucor hiemalis*	65%
	Mucor piriformis	16%
	Humicola fuscoatra	15%
	sterile mycelium	4%

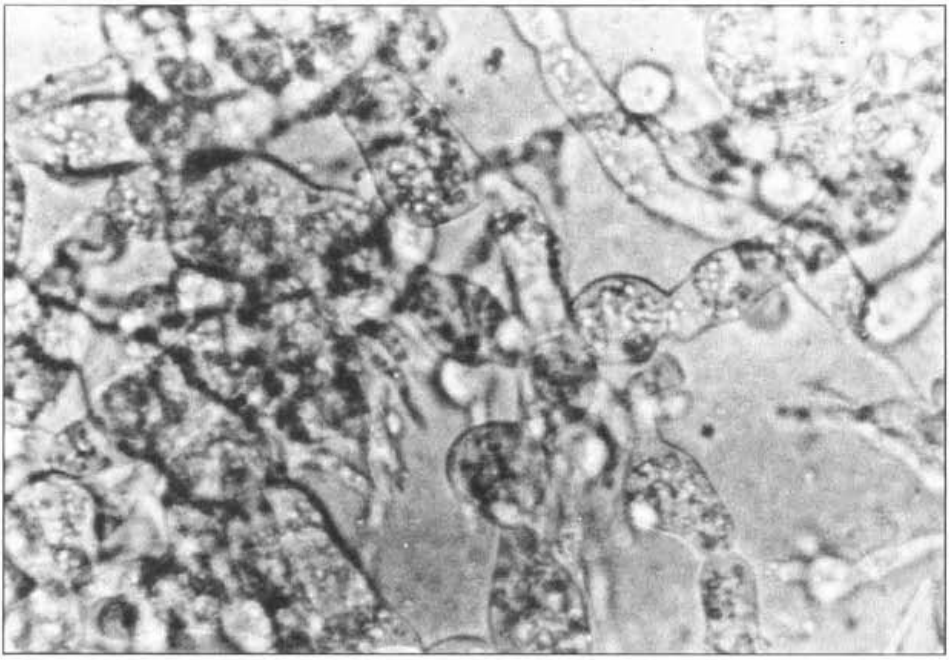

Fig. 1 *Micromucor ramannianus* var. *ramannianus*, mycelium with oil droplets (× 500).

The growth of *Micromucor ramannianus* var. *ramannianus* was satisfactory in artificial media (Czapek-Dox agar) at a temperature of 24 °C. Most colonies produced a red pigment. There exist differences in isolate colour, some isolates are cream-coloured, others are from white to pink, many are brown-red in colour. Sporangia reddish in colour and containing several sporangiospores were exceptionally discovered in the preparats. A major part of the cultures produced a system of sacs containing high amount of oily droplets (Fig. 1).

Table 3 shows the the values of biological soil characteristics from different H-horizons. It is evident from this figure that soils with higher biological activities have higher counts of *Micromucor ramannianus* var. *ramannianus* germs.

Discussion

This paper has many features identical with the papers by Dyr (1942), Grunda and Marvanová (1982), Grunda and Vorel (1996), Kubátová et al. (1998). These authors determined *Micromucor ramannianus* var. *ramannianus* as the basic microfungus of forest soils. The basic paper by Dyr (1942) contains a relatively successful description of its morphology, although the fungus illustration is not

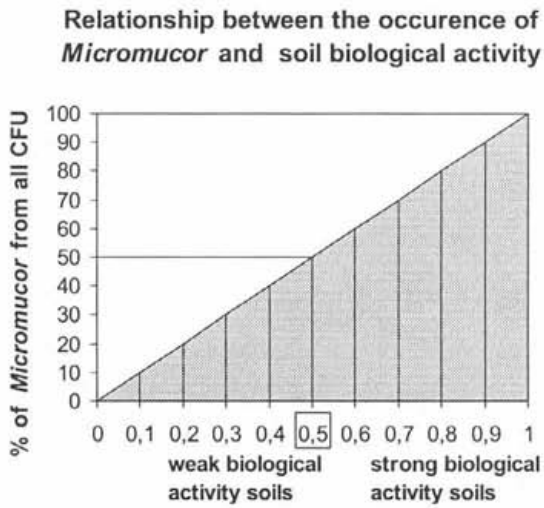

Fig. 2 Incidence of *Micromucor ramannianus* var. *ramannianus* in percent (all microfungi) in relation to soil fertility (scale from 0, 0.1.....0.9, to 1.0).

very satisfactory. Sporangia were discovered rarely during our studies. Dyr (1942): "Of all micromycete species, the frequency of this species is highest in forest soils, and it is likely to be isolated from every forest site." We observed during our studies that the fungus was not present in soil of reforested waste dumps and that its population density varied in different types of soils. *Micromucor ramannianus* var. *ramannianus* is dominant in "physiological" ("helthy") forest soils while soil germ counts of this fungus decrease in soils exposed to degradation processes. A worldwide distribution is typical of this fungus (Johann 1932, Ling-Young 1930, Pispek 1929, Reillo 1929, Povah 1917, Dale 1914, Hagen 1908). Dyr (1942) reported different frequencies in forest soils (beech 64.2 %, spruce 39.8 %, pine 37.4 %, oak 26 %, alder 20 %, birch 32.5 %), but he did not take into into account environmental and soil pollution that was substantially lower sixty years ago than (recently or) at present. Air pollutants (mainly sulphur dioxide) had negative impacts on the environment and forest soils while bacterial counts, especially of the genus *Thiobacillus,* increased (Lettl 1984) and total counts of micromycetes in forest soils decreased. The population densities of *Micromucor ramannianus* v. *ramannianus* are also likely to fluctuate snd decrease, although it has not been verified experimentally in the last sixty years. Since this fungus is not present in less nutritive soils, it is to deduce that it needfs forest soil humus for its life. Some researchers did not discover this fungus in forest (Grunda 1981). Due to its high frequency in forest soils, it is possible to anticipate the fungus' high

Table 3. Types of forest soil according to biological characteristics.

	High fertile soils	Moderately fertile soils	Weakly fertile soils	Non-fertile soils
pH (H_2O)	6–7	5–6	4–5	4–3
pH (KCl)	5–6	4–5	3–4	3–2
% humus	20	15–19	10–14	5–9
% C	20–15	15–10	10–5	5–0
% N	2–1,5	1–1,5	1–0,5	0,5–0
C: N	20	19–15	10–15	5–10
		20–25	25–50	30–40
Basal respiration	> 1000	> 500	> 250	< 250
Potential respiration	4×	3×	2×	< 1×
Ammonification	> 50	> 25	> 10	< 10
Nitrification	100–80	80–50	25–50	25–0
Number of aer. bact.	> 5 mil.	> 0,5 mil.	> 10 000	> 1000
Number of am. bact.	> 1 mil.	> 0,5 mil.	> 10 000	> 1000
% *Micromucor* from CFU	80–100	50–80	< 50	

Values: Basal respiration – in mg CO_2 released after 24 h at 21°C
Potential respiration – how many times is higher than basal respiration
Ammonification – in mg ammonia nitrogen after 14 days at 21°C
Nitrification – in mg nitrate nitrogen after 14 days at 21°C

metabolic activity like e.g. in *Penicillium chrysogenum* (Rodriquez et al. 1994). The genus *Penicillium* was isolated from many types of forest soils and there dominates (Tzean et al. 1992). Even new species of *Penicillium* and their perfect stages were isolated (Takade and Udagawa 1983) A very broad spectrum was isolated by Vláčilíková (1978), although *Mortierella ramanniana* ranked second among all isolated species in her study (*Penicillium* was a dominant genus), the rate of the fungus appeared to be low. Many authors did not indicate the horizons where the fungus was detected. The spectra of soil micromycetes were determined at different depths (1–2 cm, 2–7 cm, 7–15 cm, 15–25 cm) (Grunda and Marvanová 1982). The genus *Mortierella* was isolated from a depth of 15–25 cm, although it was not explicitly described as *Micromucor ramannianus*. The spectrum of micromycetes detected in our study was not so broad as reported in above paper. Mutual interactions of many of the reported genera are possible in vitro as well as in soil. It would also be interesting to study their antagonistic effects (Veselý 1997). Soils can undergo development during the year, and micromycetes along them, as was investigated by Marfenina (1991). They can have fungistatic effects on soil micromycetes. Kubátová, Váňová and Prášil (1998) determined the soil micromycetes from Šumava Mts. (Bohemian Forest). Amongst 139 fungal

species *Micromucor ramannianus* v. *ramannianus* was on the third place from most frequent species (33.1 %) and this species occurred on all localities.

This paper was exclusively focused on the species spectrum of soil micromycetes that were associated with the presence of a fungus typical of forest humic horizons – *Micromucor ramannianus* v. *ramannianus*. The fungus appears to be a basic micromycete of forest soils underlying biological functions of soil (respiration, ammonification, nitrification).

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Supplement to the Checklist of non-vascular and vascular plants of Slovakia. The species of microscopic fungi of the order Eurotiales

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Šimonovičová A. (2001): Supplement to the Checklist of non-vascular and vascular plants of Slovakia. The species of microscopic fungi of the order Eurotiales. – Czech Mycol. 53: 173–188

Submited are 50 species of microscopic fungi of the order Eurotiales, which are not listed in the Checklist of non-vascular and vascular plants of Slovakia, part Fungi (Lizoň and Bacigálová 1998). The first group is presented by those microscopic fungi which were isolated only once so far. So we consider them to be scarce or rare. From among 30 species of microscopic fungi more than a half belongs to the genus *Penicillium* (16 species) or genus *Aspergillus* (6 species). The genera *Eupenicillium, Eurotium* and *Paecilomyces* have two new species, *Emericella* and *Merimbla* only one species. The second group is presented by more frequently isolated species of microscopic fungi. From among 20 species the genus *Penicillium* dominates with 8 species, followed by the genus *Aspergillus* with 4 species. Other genera (*Byssochlamys*, *Dichotomomyces*, *Eupenicillium*, *Eurotium*, *Fennellia, Paecilomyces* and *Talaromyces*) are presented with one or two species. From the total number of 50 species of microscopic fungi the prevailing part was isolated from different soils (73.3–75.0 %), from different foodstuffs (3.3–30.0 %) and from other sources (23.3–45.0 %), including drinking water, dwellings and different materials in depositories and archives.

Key words: new records of microscopic fungi (Eurotiales), Checklist of non-vascular and vascular plants of Slovakia.

Šimonovičová A. (2001): Doplnok k Zoznamu nižších a vyšších rastlín Slovenska. Druhy mikroskopických húb radu Eurotiales. – Czech Mycol. 53: 173–188

Uvádzame 50 druhov mikroskopických húb radu Eurotiales, ktoré nie sú uvedené v Zozname nižších a vyšších rastlín Slovenska, časť Huby (Lizoň a Bacigálová 1998). Prvú skupinu predstavujú mikroskopické huby, ktoré boli doteraz na Slovensku izolované iba jedenkrát, a preto ich považujeme za vzácne, resp. zriedkavé. Z 30 druhov viac ako polovicu tvoria druhy rodu *Penicillium* (16), potom nasledujú druhy rodu *Aspergillus* (6), *Eupenicillium, Eurotium* a *Paecilomyces* (2), *Emericella* a *Merimbla* (1). Druhú skupinu tvoria častejšie izolované druhy. Z celkového počtu 20 opäť dominuje rod *Penicillium* zastúpený 8 druhmi a *Aspergillus* zastúpený 4 druhmi. Ostatné druhy (*Byssochlamys*, *Dichotomomyces*, *Eupenicillium*, *Eurotium*, *Fennellia, Paecilomyces* a *Talaromyces*) sú zastúpené 1–2 ×. Z celkového počtu 50 druhov mikroskopických húb bola prevážna časť izolovaná z rôznej pôdy (73.3–75.0 %), z rôznych potravín (3.3–30.0%) a z iného zdroja (23.3–45.0 %), ktorý predstavuje napr. pitná voda, vnútorné steny bytov, archivovaný materiál rôzneho pôvodu a pod. Druhy, doplnené o synonymá a bibliografické údaje, sú uvedené v abecednom poradí. Nomenklatúra je uvedená podľa Pitt et al. (2000). Autorské skratky sú upravené podľa Brummitt a Powell (1992).

The order Eurotiales (Ascomycetes – Ascomycota) includes 52 genera of microscopic fungi (Hawksworth et al. 1995), from which in Slovakia have been isolated only 13 genera. Among them, the most frequent were species of the genus *Aspergillus* and the genus *Penicillium* isolated from different soils (Bernát 1954, 1958, 1965, Braunová 1981, Pavličková 1994, Šimonovičová 1980, 1992, 1993, Vláčiliková 1978), from the phyllosphere of agricultural plants and from corn (Bernát et al. 1983, Bernát et al. 1984, Dubovská 1981, 1984, Dubovská et al. 1982, 1986), but from atypical biotopes and ecotopes, too (Franková and Šimonovičová 1999a, b, Franková et al. 1999, Šimonovičová and Franková 1998, Šimonovičová et al. 2000). For humans a very important and dangerous source of species of microscopic fungi of the order Eurotiales are contaminated foodstuffs, cereals and meal, cotton and flax (Jesenská and Poláková 1978, Jesenská and Šepitková 1984a, b, Jesenská et al. 1988, Jesenská and Piecková 1990b, Piecková et al. 1994, Piecková et al. 1992, 1996).

We submit a number of species of microscopic fungi of the order Eurotiales which are not listed in the Checklist of non-vascular and vascular plants of Slovakia, part Fungi (Lizoň and Bacigálová, 1998).

The species are arranged alphabetically, and synonyms and bibliography are included. Nomenclature is according to Pitt et al. (2000). The abbreviations of authors' names follow Brummitt and Powell (1992).

Order Eurotiales

Aspergillus Fr.:Fr.

Aspergillus chevalieri (L. Mangin) Thom et Church → *Eurotium chevalieri* L. Mangin.

Aspergillus clavatus Desm.

Isolated from garden soil in Bratislava and in Poprad, from compost, maize corn, peeled wheat, from maize flour, from foodstuff and dust from a mill (Piecková and Jesenská 1999b), from dried milk products for sucklings and baby foods (Jesenská and Poláková 1978), from meal, semolina and bread-crumbs (Jesenská et al. 1984, Jesenská and Šajbidorová 1984), from the inner mycoflora of malt, barley malt and from the surface of barley malt (Šepitková and Jesenská 1985a,b,c, 1986, 1988, Šepitková et al., 1987a, Šepitková et al. 1988, 1990, Šepitková and Jesenská 1990, 1991), from the surface of corn (Šajbidorová et al. 1988), from samples of substrates and air from different types of factories (Jesenská 1988), from cereals of domestic provenience (wheat corn and different sorts of flour) (Jesenská et al. 1988), from samples of roasted coffee-beans (Jesenská et al. 1989).

Aspergillus duricaulis Raper et Fennell

Isolated from different material (tonsils, stools, sputum, nose, vagina, dissecting material) from immunosuppressive patients with oncological diseases (Trupl et al. 1992).

Aspergillus fischeri Wehmer → *Neosartorya fischeri* (Wehmer) Malloch et Cain

Aspergillus flavus Link var. *columnaris* Raper et Fennell

Isolated from dried milk products for sucklings and baby foods (Jesenská and Poláková 1978).

Aspergillus glaucoaffinis Samson et W. Gams → *Eurotium pseudoglaucum* (Blochwitz) Malloch et Cain

Aspergillus janus Raper et Thom

Isolated from the inner mycoflora of malt (Šepitková and Jesenská 1985a), from malt-barley and malt (Šepitková and Jesenská 1988).

Aspergillus melleus Yukawa

Isolated from 45 cotton samples of different origin (Uzbekistan, U. S. A., China, Egypt, Russia) and from 24 domestic flax samples (Piecková and Jesenská 1996b).

Aspergillus niger var. *cinnamomeus* (Schiemann) Thom et Raper

Isolated from agricultural soils (Braunová 1981).

Aspergillus parasiticus Speare

Isolated from different sorts of foodstuff (Jesenská et al. 1980), from imported foodstuffs, such as roasted and non-roasted peanuts in chocolate, salt, sugar, or as a part of wafers, grated coconut and rice (Jesenská et al. 1988), from drinking water (Franková and Šimonovičová 1999b).

Aspergillus parvulus G. Sm.

Isolated from forest soil in the neighbourhood of Bratislava (Krakovská et al. 2000).

Aspergillus penicillioides Speg.

Isolated from wooden sculptures, picture canvases from depositories and from indoor air of the Slovak National Museum (Franková et al. 1999, Šimonovičová and Franková 1998).

Aspergillus reptans Samson et W. Gams → *Eurotium repens* de Bary

Aspergillus rugulosus Thom et Raper → *Emericella rugulosa* (Thom et Raper) C. R. Benj.

Aspergillus violaceo-fuscus Gasperini

Isolated from Albic Luvisols (Bernát 1965).

Byssochlamys Westling

Byssochlamys fulva Olivier et G. Sm.

Isolated from garden, forest and agricultural soils in different parts of Bratislava and from a flower bed in a private garden (Jesenská and Piecková 1994a), from non-specified soil (Jesenská and Piecková 1994b, Jesenská et al. 1994), from conserved fruit and fruit-juice (Jesenská et al. 1983).

Byssochlamys nivea Westling

Isolated from non-specified soil from a depth of 0–5 cm in the neighbourhood of Bratislava and Poprad (Jesenská and Piecková 1990a, 1991), from non-specified soils of Slovakia (Jesenská et al. 1992b, Jesenská et al. 1994, Jesenská and Piecková 1994b, 1995a, Piecková et al. 1994), from garden, forest and agricultural soils in different parts of Bratislava (Jesenská et al. 1992c), from soil of a private garden which had been fertilised with organic fertilisers (Jesenská et al. 1993), from a flower bed in a private garden (Jesenská and Piecková 1993, 1994a), from non-specified soils in different parts of Slovakia (Jesenská et al. 1992a, Jesenská and Piecková 1995a, Piecková and Jesenská 1997b), from soil samples after heating at 70 °C for 60 min. in a physiological solution (Piecková and Jesenská 1996a, 1997b, Piecková et al. 1996), from conserved fruit and fruit-juice (Jesenská et al. 1983), from stewed fruit and juice (Jesenská and Petríková 1985), from juice and the surface of stewed fruit, which had some signs of sensorical depreciation (Petríková et al. 1985), from equipment used for conserving fruit and vegetables and final products (Šepitková et al. 1987b, 1989), from mouldy stewed fruit (Jesenská and Piecková 1990a), from apricots (Kubátová et al. 1996).

Dichotomomyces D. B. Scott

Dichotomomyces cejpii (Milko) D. B. Scott

Isolated from non-specified soil from a depth of 0–5 cm in the neighbourhood of Bratislava and Poprad (Jesenská and Piecková 1990a, b, 1991), from garden, forest and agricultural soils in different parts of Bratislava (Jesenská et al. 1992c), from soil of different beds of a private garden which had been fertilised with organic fertilisers (Jesenská et al. 1993), from a flower bed in a private garden (Jesenská and Piecková 1993), from non-specified soils in different parts of Slovakia (Jesenská et al. 1992a,b; Jesenská and Piecková 1994b, 1995a, Piecková et al. 1992, Piecková et al. 1994), from garden, forest and agricultural soils in Bratislava and in Poprad (Piecková and Jesenská 1997a), from soil samples after heating at 70 °C for 60 min. in a physiological solution (Piecková and Jesenská 1997b).

Emmericella Berk.

Emmericella rugulosa (Thom et Raper) C. R. Benj

Anam.: *Aspergillus rugulovalvus* Samson et W. Gams
Syn.: *Aspergillus rugulosus* Thom et Raper
Isolated from Albic Luvisols (Bernát 1965) as *Aspergillus rugulosus*.

Eupenicillium F. Ludw.

Eupenicillium javanicum (J. F. H. Beyma) Stolk et D. B. Scott
Anam.: *Penicillium indonesiae* Pitt
Syn.: *Penicillium javanicum* J. F. H. Beyma
Isolated from Albic Luvisols (Bernát 1965), from floodplain forest soils near Gabčíkovo (Bučková and Bacigálová 1999) as *Penicillium javanicum*.

Eupenicillium pinetorum Stolk
Isolated from meadow soils in the locality Kaltwasser-Turček (Marvanová 1998).

Eupenicillium shearii Stolk et D. B. Scott
Anam: *Penicillium asperum* (Shear) Raper et Thom
Syn: *Penicillium glaucum* Link after Bref.
Isolated from meadow soils in the locality Kaltwasser-Turček (Marvanová 1998).

Eurotium Link: Fr.

Eurotium chevalieri L. Mangin
Anam.: *Aspergillus chevalieri* (L. Mangin) Thom et Church
Isolated from Albic Luvisols (Bernát 1965) as *Aspergillus chevalieri*.

Eurotium pseudoglaucum (Blochwitz) Malloch et Cain
Anam.: *Aspergillus glaucoaffinis* Samson et W. Gams
Syn.: *Aspergillus pseudoglaucus* Blochwitz
Isolated during the storage of wheat corn (Dubovská et al. 1986) as *Aspergillus pseudoglaucus*.

Eurotium repens de Bary
Anam.: *Aspergillus reptans* Samson et W. Gams
Syn.: *Aspergillus repens* (Corda) de Bary
Isolated from forest soils in Tichá, Krížná, Kôprová and Furkotská valleys in the Vysoké Tatry Mts. (Šimonovičová 1992, 1993), from agricultural soils (Braunová 1981), during the storage of wheat corn (Bernát et al. 1983, Dubovská et al. 1986), from the phyllosphere of maize leaves and other agricultural plants (Dubovská 1984) as *Aspergillus repens*.

Fennellia B. J. Wiley et E. G. Simmons

Fennellia flavipes B. J. Wiley et E. G. Simmons

Anam.: *Aspergillus flavipes* (Bainier et Sartory) Thom et Church

Isolated from agricultural soils (Bernát et al. 1984, Braunová 1981), from forest soils in the neighbourhood of Bratislava (Krakovská et al. 2000) and from non-specified soil (Piecková and Jesenská 1999a) as *Aspergillus flavipes*.

Hamigera Stolk et Samson

Hamigera avellanea (Thom et Turesson) Stolk et Samson

Anam.: *Merimbla ingelheimensis* (J. F. H. Beyma) Pitt

Syn.: *Talaromyces avellaneus* (Thom et Turesson) C. R. Benj., *Penicillium avellaneum* Thom et Turesson

Isolated from an altar canvas in a gothic church in Okoličné (Gódyová 2000) as *Merimbla ingelheimensis*.

Merimbla Pitt

Merimbla ingelheimensis (J. F. H. Beyma) Pitt → *Hamigera avellanea* (Thom et Turesson) Stolk et Samsom

Neosartorya Malloch et Cain

Neosartorya fischeri (Wehmer) Malloch et Cain

Anam.: *Aspergillus fischerianus* Wehmer

Isolated from conserved fruit and fruit-juice (Jesenská et al. 1983), from stewed fruit and juice (Jesenská and Petríková 1985), from juice and the surface of stewed fruit which had some signs of sensorical depreciation (Petríková et al. 1985), from equipment used for conserving and final products (Šepitková et al. 1987b), from wooden sculptures and picture canvases from depositories and from indoor air of the Slovak National Museum (Franková et al. 1999, Šimonovičová and Franková 1998), from prefabricated dwellings and wooden substrates (Franková and Šimonovičová 1999a), from drinking water, dwelling and from different materials in depositories and archives (Franková and Šimonovičová 1999b) as *Aspergillus fischeri*.

Paecilomyces Bainier

Paecilomyces carneus (Duché et R. Heim) Brown et Smith

Isolated from soil in Strážovské vrchy Mts. (Kubátová et al. 1996).

Paecilomyces farinosus (Holmsk.) Brown et Smith

Isolated from wood in Pieniny (Kubátová et al. 1996), from mouldy wall in a gothic church in Okoličné (Šimonovičová et al. 2000).

Paecilomyces marquandii (Massee) S. Hughes
Isolated from soil, Magurka hill, Oravská Magura Mts. (Kubátová et al. 1996).

Penicillium Link: Fr.

Penicillium arenicola Chalab.
Isolated from sepulchral monuments of stone in the Crypt of Chatam Sófer in Bratislava (Gódyová 2000, Šimonovičová et al. 2000).

Penicillium atramentosum Thom
Isolated from forest soil in Nízke Tatry Mts. (Kubátová 1990a).

Penicillium brasilianum Bat.
Isolated from spruce forest soil in Nízke Tatry Mts., from soil under alder trees in Oravská Magura Mts. (Kubátová et al. 1996).

Penicillium clavigerum Demelius
Isolated from agricultural soils (Braunová 1981b), from culture contaminant of an antibiotics factory in Slovenská Ľupča (Kubátová et al. 1996).

Penicillium coprobium Frisvad
Isolated from soil in Strážovské vrchy Mts. (Kubátová 1993–1994, Kubátová et al. 1996) as *Penicillium coprophilum*.

Penicillium cyaneum (Bainier et Sartory) Biourge
Isolated from the phyllosphere of maize corn and maize leaves (Dubovská et al. 1982).

Penicillium cyclopium Westling
Syn: *Penicillium verrucosum* Dierckx var. *cyclopium* (Westling) Samson et al.
Isolated from floodplain forest soils in Gabčíkovo (Bučková and Bacigálová 1999) as *Penicillium verrucosum* var. *cyclopium*.
Isolated from the phyllosphere of maize corn and maize leaves (Dubovská, 1981; Dubovská et al., 1982), from Cambizem Stagnogleyic (Pavličková 1994), from different cereals (flour, fine groats, rye, oat and barley corn (Jesenská and Šepitková 1984a, b), from the surface of corn (Šajbidorová et al. 1988), from collections of historical costumes and from the air in depositories of the Slovak National Museum (Šimonovičová and Franková 1998), from wooden sculptures and picture canvases in depositories and from indoor air of the Slovak National Museum (Franková et al. 1999, Šimonovičová and Franková 1998), from drinking water, dwelling and from different objects and materials in depositories and in archives (Franková and Šimonovičová, 1999b) as *Penicillium cyclopium*.

Penicillium flavovirens Cooke et Massee [according to Pitt 1979 an indeterminate name]

Isolated from agricultural soils (Ondrišová and Gašpieriková 1982).

Penicillium fellutanum Biourge

Syn: *Penicillium charlesii* G. Sm.

Isolated from Albic Luvisols (Bernát 1965) as *Penicillium charlesii*.

Isolated from meadow soils in the locality Kaltwasser-Turček (Marvanová 1998) as *Penicillium fellutanum*.

Penicillium geophilum Oudem. apud Oudem. et Koning [according to Pitt 1979 not a *Penicillium*]

Isolated from spruce forest soils (Bernát 1958).

Penicillium griseofulvum Dierckx

Isolated from sepulchral monuments of stone in the Crypt of Chatam Sófer in Bratislava (Šimonovičová a kol. 2000).

Penicillium herquei Bainier et Sartory

Isolated from Albic Luvisols (Bernát 1965).

Penicillium implicatum Biourge

Isolated from Albic Luvisols (Bernát 1965).

Penicillium lanosum Westling

Isolated from soil in Nízke Tatry Mts. (Kubátová et al. 1996).

Penicillium luteum Sopp [according to Pitt 1979 an indeterminate name]

Isolated from forest soils (Vláčiliková 1978), from agricultural soils (Braunová 1981a, Šimonovičová 1980), from the phyllosphere of maize (Dubovská et al. 1986), from Mollic Fluvisol (Ondrišová 1976).

Penicillium megasporum Orpurt et Fennell

Isolated from forest soils (Vláčiliková 1978).

Penicillium melinii Thom

Isolated from soil in Nízke Tatry Mts. (Kubátová et al. 1996), from meadow soils in the locality Kaltwasser-Turček (Marvanová 1998).

Penicillium montanense M. Chr. et Backus

Isolated from humus of peat-bog in the Horná Orava region, from soil under *Pinus mugo* in Nízke Tatry Mts. (Kubátová et al. 1996).

Penicillium nalgiovense Laxa

Isolated from picture canvases (Franková and Šimonovičová 1999a), from different objects and materials in depositories and archives (Franková and Šimonovičová 1999b).

Penicillium purpurogenum Stoll var. *rubrisclerotium* Thom
Isolated from agricultural soils (Braunová 1981).

Penicillium sacculum E. Dale
Syn: *Eladia saccula* (E. Dale) G. Sm.
Isolated from hardwood forest soils in Spišská Magura (Kubátová 1990b) as *Eladia saccula*.

Penicillium smithii Quintan.
Isolates from soil in Chabenec, in Nízke Tatry Mts. (Kubátová et al. 1996).

Penicillium sulfureum Sopp [according to Pitt 1979 a possible synonym of *Penicillium purpurogenum*]
Isolated from spruce forest soils (Bernát 1954, 1958).

Penicillium verruculosum Peyronel
Isolated from agricultural soils (Braunová 1981, Ondrišová and Gašpieriková 1982).

Talaromyces C. R. Benj.

Talaromyces avellaneus Thom et Turesson → *Hamigera avellanea* (Thom et Turesson) Stolk et Samson

Talaromyces bacillisporus (Swift) C. R. Benj.
Anam.: *Geosmithia swiftii* Pitt
Isolated from non-specified soil from a depth of 0–5 cm in the neighbourhood of Bratislava and Poprad (Jesenská and Piecková 1990a, b, 1991), from non-specified soils of Slovakia (Jesenská et al. 1992a, 1994, Jesenská and Piecková 1994a, b, 1995a, b, Piecková et al. 1992, Piecková et al. 1994), from garden, forest and agricultural soils in the neighbourhood of Bratislava (Jesenská et al. 1992b), from soil of a flower bed in a private garden (Jesenská and Piecková 1993, 1994a, Jesenská et al. 1994, Piecková et al. 1994) as *Talaromyces bacillisporus*.

Talaromyces emersonii Stolk
Anam.: *Geosmithia emersonii* (Stolk) Pitt
Isolated from non-specified soils of Slovakia (Jesenská et al. 1992a), from garden, forest and agricultural soils in the neighbourhood of Bratislava (Jesenská et al. 1992c), from soil of a flower bed in a private garden (Jesenská and Piecková 1993), from three different beds of a private garden which had been fertilised with organic fertilisers (Jesenská et al. 1992c) as *Talaromyces emersoni*.

Table 1. Species of microscopic fungi with a scarce or rare occurrence.

Fungi	Occurrence of species		
	Soil	Food-stuffs	Other sources
Aspergillus duricaulis Raper et Fennell			*
A. flavus Link var. *columnaris*		*	
A. melleus Yukawa			*
A. niger var. *cinnamomeus* (Schieman) Thom et Raper	*		
A. parvulus G. Sm.	*		
A. violaceo-fuscus Gasperini	*		
Emericella rugulosa (Thom et Raper) C. R. Benj.	*		
Eupenicillium pinetorum Stolk	*		
E. sheari Stolk et D. B. Scott	*		
Eurotium chevalieri L. Mangin	*		
Eurotium pseudoglaucum (Blochwitz) Malloch et Cain			*
Merimbla ingelheimensis (J. F. H. Beyma) Pitt			*
Paecilomyces carneus (Duché et Heim) Brown et Smith	*		
P. marquandii (Massee) S. Hughes	*		
Penicillium arenicola Chalab.			*
P. atramentosum Thom	*		
P. brasilianum Bat.	*		
P. coprobium Frisvad	*		
P. cyaneum (Bainier et Sartory) Biourge			*
P. flavovirens Cooke et Massee	*		
P. geophilum Oudem. apud Oudem. et Koning	*		
P. griseofulvum Dierckx			*
P. herquei Bainier et Sartory	*		
P. implicatum Biourge	*		
P. lanosum Westling	*		
P. megasporum Orpurt et Fennell	*		
P. montanense M. Chr. et Backus	*		
P. purpurogenum Stoll var. *rubrisclerotium* Thom	*		
P. sacculum E. Dale	*		
P. smithii Quintan.	*		
Σ 30	22	1	7
	73.3 %	3.3 %	23.7 %

Other sources: patients with oncological diceases, cotton, stored wheat, stone monuments, phyllosphere of agricultural plants.

Table 2. Species of microscopic fungi not listed in the Checklist of non-vascular and vascular plants of Slovakia, part Fungi (Lizoň, Bacigálová, 1998).

Fungi	Occurrence of species		
	Soil	Food-stuffs	Other sources
Aspergillus clavatus Desm.	*	*	*
A. janus Raper et Thom		*	*
A. parasiticus Speare		*	*
A. penicillioides Speg.			*
Byssochlamys fulva Olivier et Smith	*	*	
B. nivea Westling	*	*	
Dichotomomyces cejpii (Milko) D. B. Scott	*		
Eupenicillium javanicum (J. F. H. Beyma) Stolk et D. B. Scott	*		
Fennellia flavipes B. J. Willey et E. G. Simmons	*		
Paecilomyces farinosus (Holmsk.) Brown et Smith			*
Penicillium clavigerum Demelius	*		*
P. cyclopium Westling	*	*	*
P. fellutanum Biourge	*		
P. luteum Sopp	*		*
P. melinii Thom	*		
P. nalgiovense Laxa			*
P. sulphureum Sopp	*		
P. verruculosum Peyronel	*		
Talaromyces bacillisporus (Swift) C. R. Benj.	*		
T. emersonii Stolk	*		
Σ 20	15	6	9
	75.0 %	30.0 %	45.0 %

Other sources: air in factory, different material in depositories and archives (wooden sculpture, picture canvases, frames), drinking water, dwelling, phyllosphere of agricultural plants, stored wheat, culture contaminant from an antibiotics factory.

DISCUSSION

Due to its ecological features (suitable amount of organic matter, moisture, temperature, protection from sunshine) soil is a natural biotope of microscopic fungi and is therefore the richest in them, too.

The total number of 50 species of microscopic fungi which are not listed in the Checklist of non-vascular and vascular plants of Slovakia (Lizoň and Bacigálová 1998) has been divided into two groups.

The first group (Tab. 1) represents those microscopic fungi, which have been isolated in Slovakia only once. So we can consider them to be scarce

or rare. From the 30 species 22 were 22 isolated from different soils (forest, agricultural and meadow), 1 species from foodstuffs and 7 species from other sources (such as material from patients with oncological diseases, cotton, stored wheat, stone monuments, phyllosphere of different agricultural plants). More than half of them (16 species) belongs to the genus *Penicillium* (*P. arenicola, P. atramentosum, P. brasilianum, P. coprobium, P. cyaneum, P. flavovirens, P. geophilum, P. griseofulvum, P. herquei, P. implicatum, P. lanosum, P. megasporum, P. montanense, P. purpurogenum* var. *rubrisclerotium, P. sacculum* and *P. smithii*). Scarce or rare species of the genus *Aspergillus* can be considered *A. duricaulis, A. flavus* var. *columnaris, A. melleus, A. niger* var. *cinnamomeus, A. parvulus,* and *A. violaceo-fuscus*. The list is completed with the species *Emericella rugulosa, Eupenicillium pinetorum, E. sheari, Eurotium chevalieri, E. pseudoglaucum, Merimbla ingelheimensis, Paecilomyces carneus* and *P. marquandii.*

The second group (Tab. 2) represents species of microscopic fungi which are isolated more often than in the first case. Among the 20 species of microscopic fungi again the genus *Penicillium* dominates with 9 species (*P. clavigerum, P. cyclopium, P. fellutanum, P. luteum, P. melinii, P. nalgiovense, P. sulphureum* and *P. verruculosum*) and the genus *Aspergillus* with 4 species (*A. clavatus, A. janus, A. parasiticus,* and *A. penicillioides*). Species of the genera *Byssochlamys*, *Dichotomomyces*, *Eupenicillium*, *Eurotium*, *Paecilomyes* and *Talaromyces* are represented once or twice. Of the 20 species 15 were isolated from soil, 6 species from foodstuffs and 9 species from other sources (such as air in a factory, different material in depositories and archives, drinking water, dwelling, phyllosphere of different agricultural plants and corn, stored wheat, culture contaminant in an antibiotics factory). Out of all species of microscopic fungi (Tab. 1 and 2) only the species *Aspergillus clavatus* and *Penicillium cyclopium* were isolated from soil, foodstuffs as well as other sources.

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