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# CZECH MYCOLOGY

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## Teleomorph-anamorph connections in Ascomycetes. 1. *Cylindrotrichum* and *Cacumisporium* anamorphs of *Chaetosphaeria*

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Réblová M. and Gams W. (1999): Teleomorph-anamorph connections in Ascomycetes 1. *Cylindrotrichum* and *Cacumisporium* anamorphs of *Chaetosphaeria*. – Czech Mycol. 51: 1–40

The teleomorph-anamorph connections of *Chaetosphaeria* are discussed. On the basis of the revision of the type and other herbarium material, *Zignoëlla crustacea*, *Lasiochaeria britzelmayri* subsp. *fennica*, *Sphaeria decastyla* and *Melanomma macrosporium* proved to be members of *Chaetosphaeria*. The new combinations *Chaetosphaeria crustacea*, *Chaet. fennica* and *Chaet. decastyla*, are proposed, *Melanomma macrosporium* is included in the synonymy of the latter. *Chaetosphaeria crustacea* has an anamorph intermediate between *Chloridium* and *Cylindrotrichum*, *Chaetosphaeria fennica* has a *Chloridium*-like anamorph, and *Chaetosphaeria decastyla* has *Cacumisporium capitulatum* as its anamorph. Two new species, *Chaetosphaeria tulasneorum* associated with the anamorph *Cylindrotrichum oligospermum* and *Chaetosphaeria acutata* associated with a *Cylindrotrichum* anamorph, are described. The teleomorph-anamorph connections of all accepted *Chaetosphaeria* species, except for *Chaet. fennica*, were confirmed by culture studies. *Chaetosphaeria abietis* and *Chaet. fusiformis* are also considered, and the connection with the *Chloridium cylindrosporium* anamorph of the latter is confirmed by culture studies for the first time. The genus *Cylindrotrichum* and its relationship to *Chloridium* and other related genera is discussed. A key to the species of *Chaetosphaeria* with *Cylindrotrichum* and *Cacumisporium* anamorphs is provided.

**Key words:** Ascomycetes, *Chaetosphaeria*, culture studies, wood-inhabiting fungi, systematics.

Réblová M. and Gams W. (1999): Vztahy teleomorfa-anamorfa u askomycetů 1. *Cylindrotrichum* a *Cacumisporium*, anamorfní rody rodu *Chaetosphaeria*. – Czech Mycol. 51: 1–40

Jsou diskutovány teleomorfní-anamorfnní vztahy rodu *Chaetosphaeria*. Na základě revize typového a dalšího herbářového materiálu jsou druhy *Zignoëlla crustacea*, *Lasiochaeria britzelmayri* subsp. *fennica*, *Sphaeria decastyla* a *Melanomma macrosporium* umístěny do rodu *Chaetosphaeria*. Jsou navrženy tři nové kombinace, a to *Chaetosphaeria crustacea*, *Chaet. fennica* a *Chaet. decastyla*. *Melanomma macrosporium* je zařazena do synonymiky druhu *Chaet. decastyla*. Rodové zařazení anamorfy druhů *Chaetosphaeria crustacea* a *Chaet. fennica* je na pomezí rodů *Cylindrotrichum* a *Chloridium*. Anamorfou *Chaetosphaeria decastyla* je *Cacumisporium capitulatum*. Jsou popsány dva nové druhy, *Chaetosphaeria tulasneorum* s anamorfou



*Cylindrotrichum oligospermum* a *Chaetosphaeria acutata* s anamorfoou *Cylindrotrichum* sp. Teleomorfní-anamorfní vztahy všech výše uvedených druhů rodu *Chaetosphaeria* byly ověřeny kultivačními studiemi. Jsou zahrnuty také druhy *Chaetosphaeria abietis* a *Chaet. fusiformis*. Spojení *Chaet. fusiformis* s anamorfoou *Chloridium cylindrosporum* je poprvé potvrzeno kultivačními studiemi. Jsou diskutovány vztahy rodu *Cylindrotrichum* s rodem *Chloridium* a dalšími příbuznými rody. Je vytvořen klíč pro určování druhů *Chaetosphaeria* s anamorfoami *Cylindrotrichum* a *Cacumisporium*.

## INTRODUCTION

The genus *Chaetosphaeria* Tul. et C. Tul. (Tulasne and Tulasne 1863) accommodates lignicolous, saprobic fungi with superficial, dark, glabrous or sometimes setose, non-collapsing perithecia, the perithecial wall is brittle, consisting of thin-walled, opaque, brick-like cells or cells that form a network (*textura epidermoidea*); paraphyses and periphyses are persistent, asci have a distinct non-amyloid, refractive apical annulus and ascospores are transversely 1- to multi-septate, non-fragmenting and hyaline. Associated anamorphs have pigmented conidiophores and phialidic conidiogenesis, and are classified in several genera of hyphomycetes.

Among the synonyms of *Chaetosphaeria*, *Zignoëlla* Sacc. was characterized by hyaline, phragmosporous ascospores (Saccardo 1878), a criterion not recognized here as having generic relevance. *Zignoëlla* is a broadly conceived genus (Saccardo 1878, 1883) whose lectotype species, *Zignoëlla pulviscula* (Currey) Sacc., was selected only by Clements and Shear (1931) and was recombined by Booth (1957) in *Chaetosphaeria*.

Barr (1990) and Eriksson and Hawksworth (1993) placed *Chaetosphaeria* in the broadly perceived Lasiosphaeriaceae Nannf. Recently, *Chaetosphaeria* and six other related genera, viz. *Ascocodinaea* Samuels et al., *Melanochaeta* E. Müll. et al., *Melanopsammella* Höhnelt, *Porosphaerella* E. Müll. et Samuels, *Porosphaerellopsis* Samuels et E. Müll., and *Striatosphaeria* Samuels et E. Müll., were transferred to a new family, the Chaetosphaeriaceae Réblová et al., in the order Sordariales Chadeff. ex D. Hawksw. et O. E. Erikss. (Réblová et al. 1999). The Chaetosphaeriaceae are separated from the more narrowly delimited Lasiosphaeriaceae Nannf. and its core genera, viz. *Lasiochaeta* Ces. et De Not., *Bombardia* (Fr.) P. Karst., *Eosphaeria* Höhnelt, *Cercophora* Fuckel, and *Apiosordaria* Arx et W. Gams, on the basis of characters of perithecium, perithecial wall, asci and ascospores. Réblová et al. (1999) recognized twenty-two species of *Chaetosphaeria* with associated dematiaceous hyphomycetous anamorphs in nine genera, viz. *Catenularia* Grove, *Cylindrotrichum* Bonord., *Chalara* (Corda) Rabenh., *Chloridium* Link: Fr., *Custingophora* Stolk et al., *Dictyochaeta* Speg., *Menispora* Pers.: Fr., *Phialophora* Medlar, and *Zanclospora* S. Hughes et B. Kendrick (Saccardo 1883; Booth 1957, 1958; Müller and von



Arx 1962; Hughes and Kendrick 1968; Gams and Holubová-Jechová 1976; Barr and Crane 1979; Holubová-Jechová 1973, 1982, 1984; Barr 1993; Constantinescu et al. 1995; Réblová 1998 a, b).

The genus *Cylindrotrichum* was discussed by a number of authors (e.g. Hughes 1951; Gams and Holubová-Jechová 1976; Morgan-Jones 1977, 1980; Kendrick 1980; DiCosmo et al. 1983; Cabello and Arambarri 1988; Holubová-Jechová 1990), but no clear and final generic concept is available. The genus comprises species with erect, unbranched conidiophores, terminal conidiogenous cells with multiple or single conidiogenous loci produced by sympodial or percurrent proliferation and hyaline, cylindrical to long ellipsoidal, 1-3-septate conidia. DiCosmo et al. (1983) placed species of the genus *Cylindrotrichum* into two new genera, *Kylindria* DiCosmo et al. and *Xenokylindria* DiCosmo et al., and transferred one species formerly placed in *Cylindrotrichum* to *Uncigera* Sacc., two to *Dictyochaeta* Speg. and five to *Chaetopsis* Greville emend. DiCosmo et al., including the type species, *Cylindrotrichum oligospermum* (Corda) Bonord. These authors emphasized especially the shape of the conidia and the conidiogenous cells, the distinction between mono- and polyphialides being a main differentiating character. They preferred to distinguish several closely related anamorph genera of *Chaetosphaeria*, such as *Chaetopsis*, *Dictyochaeta*, *Kylindria* and *Xenokylindria*, based on details of conidiogenesis, though they share a number of morphological, developmental and ecological characteristics (DiCosmo et al. 1983).

Cabello and Arambarri (1988) considered the concept proposed by DiCosmo et al. (1983) incorrect. They redefined the generic concept of *Cylindrotrichum* and accepted eleven species. The genera *Kylindria* and *Xenokylindria* were reduced to synonyms of *Cylindrotrichum*. According to Cabello and Arambarri (1988), *Cylindrotrichum* accommodates species formerly placed in three closely related genera, viz. *Kylindria*, *Xenokylindria* and *Chaetopsis*, that are associated with one teleomorph genus, *Chaetosphaeria*. Although they did not consider teleomorph-anamorph connections of any of the species accepted in *Cylindrotrichum*, their generic concept of *Cylindrotrichum* justifies the close relationship of the *Chaetosphaeria* anamorphs better than the system proposed by DiCosmo et al. (1983).

In the present paper we describe five species of *Chaetosphaeria*, four of them having *Cylindrotrichum* anamorphs and one a *Cacumisporium* Preuss anamorph. Three species were previously known as *Zignoëlla crustacea* Sacc. (Saccardo 1883), *Lasiosphaeria britzelmayri* Sacc. subsp. *fennica* P. Karst. (Karsten 1887) and *Sphaeria decastyla* Cooke (Cooke 1878) [= *Melanomma macrosporum* Sacc. (Saccardo 1875)]. The respective type specimens were examined. On the basis of perithecial anatomy, ascus, ascospore and hamathecium anatomy, and conidiogenesis of the anamorphs, these three species belong to *Chaetosphaeria*. They are recombined as *Chaetosphaeria crustacea*, *Chaet. fennica* and *Chaet. decastyla*. *Melanomma macrosporum* is included in the synonymy of the latter. These species,

except for *Chaet. fennica*, were recollected on strongly decayed wood of coniferous and deciduous trees in Europe (France, Czech Republic, Ukraine). They are redescribed and illustrated along with notes on previous descriptions and illustrations. The anamorph of *Chaet. crustacea* belongs to *Cylindrotrichum*, though it shows great similarity with *Chloridium*. The anamorph of *Chaet. fennica* is *Chloridium*-like. The anamorph of *Chaet. decastyla* is *Cacumisporium capitulatum* (Corda) S. Hughes. On the basis of conidiogenesis and the structure and function of the phialides, *Cac. capitulatum* may also be included in *Cylindrotrichum*, but we refrain at the moment from making this combination before the anamorph-generic delimitations are further analysed. With the exception of *Chaet. fennica*, conidiophores of all these species were observed on a natural substratum and in living cultures.

Several collections of another fungus associated with a *Cylindrotrichum* anamorph were made on decayed wood of deciduous trees in the Czech Republic, France and Ukraine. It has fusiform, hyaline, at maturity 3-septate ascospores, persistent paraphyses and periphyses, unitunicate asci, black glabrous perithecia with a fragile wall and a dematiaceous hyphomycete anamorph with phialidic conidiogenesis. The anamorph was also obtained in culture. This fungus belongs to *Chaetosphaeria* and is described here as *Chaet. acutata*.

A single collection of the fifth species was made on a dead branch of *Sambucus nigra* in the Czech Republic. The anamorph that was obtained in culture represents *Cylindrotrichum oligospermum* (Corda) Bonord., the type of the generic name *Cylindrotrichum*. On the basis of the black, glabrous perithecia with brittle wall, persistent paraphyses and periphyses, ascospores that are hyaline, 1-3-septate and fusiform, and the anamorph, this recent collection represents an undescribed species of *Chaetosphaeria*, which is described here as *Chaet. tulasneorum*.

*Chaetosphaeria abietis* (Höhnelt) W. Gams et Holubová-Jechová and *Chaetosphaeria fusiformis* W. Gams et Holubová-Jechová (Gams and Holubová-Jechová 1976, 1981) are discussed. A key to the species of *Chaetosphaeria* with *Cylindrotrichum* and *Cacumisporium* anamorphs is provided.

#### MATERIAL AND METHODS

Dry herbarium specimens were rehydrated in 3% (aq.) KOH and subsequently studied in water, Congo Red (aq.) and Melzer's reagent. The abbreviations of herbaria and institutes that kindly lent the material are cited in accordance with the Index Herbariorum (Holmgren et al. 1990).

In the lists of material examined M. R. is the abbreviation for M. Réblová.

Single ascospores of *Chaetosphaeria crustacea*, *Chaet. acutata*, *Chaet. fusiformis*, *Chaet. decastyla* and *Chaet. tulasneorum* were isolated with the aid of a single-spore isolator on cornmeal agar (CMA, Difco). Colonies were grown

on CMA, malt extract agar (MEA), oatmeal agar (OA) and potato-carrot agar (PCA), colony characters were taken from PCA cultures grown for 10 days at 25 °C in darkness and 10 days at 25 °C in cool white fluorescent light. The cultures are maintained in the Institute of Botany, Academy of Sciences at Průhonice and the Centraalbureau voor Schimmelcultures (CBS) at Baarn, the Netherlands.

TAXONOMIC PART

Key to the species of *Chaetosphaeria* with *Cylindrotrichum* and *Cacumisporium* anamorphs

- 1. Occurring on decayed wood and bark of angiosperms.....2
- 1. Occurring on decayed wood and bark of gymnosperms ..... 5
  - 2. Ascospores at maturity 3-5-septate, long cylindrical to fusiform; anamorph *Cacumisporium capitulatum* ..... 6. *Chaetosphaeria decastyla*
  - 2. Ascospores at maturity 3-septate ..... 3
- 3. Ascospores short fusiform, not exceeding 21 μm; anamorph *Cylindrotrichum oligospermum* ..... 7. *Chaetosphaeria tulasneorum*
- 3. Ascospores elongate-fusiform, longer than 30 μm ..... 4
  - 4. Ascospores (34.5-36.5-42(-43) × (3.5-4(-4.5) μm, immediately after the middle septum tapering very strongly towards the ends; anamorph *Chloridium*-like; conidia mid-brown, 1-celled ..... 4. *Chaetosphaeria fennica*
  - 4. Ascospores (28-30.5-38(-44) × 3-4(-5) μm, slightly tapering towards the ends; anamorph *Cylindrotrichum* sp.; conidia hyaline, 2-celled ..... 2. *Chaetosphaeria acutata*
- 5. Ascospores cylindrical-fusiform, 3-5-septate, perithecia setose; anamorph *Cylindrotrichum-Chloridium*-like ..... 3. *Chaetosphaeria crustacea*
- 5. Ascospores cylindrical-fusiform, at maturity 3-septate, perithecia glabrous...6
  - 6. Ascospores asymmetric, tapering towards one end and rounded at the other end; anamorph *Chloridium cylindrosporum* ... 5. *Chaetosphaeria fusiformis*
  - 6. Ascospores symmetric with rounded ends; anamorph *Cylindrotrichum zignoëllae* ..... 1. *Chaetosphaeria abietis*

1. *Chaetosphaeria abietis* (Höhnelt) W. Gams et Hol.-Jech., Stud. Mycol. 13: 53, 1976. Fig. 1a-f.

≡ *Zignoëlla abietis* Höhnelt, in Rehm, Ann. Mycol. 5: 469, 1907; more elaborated in Höhnelt, Sitzungsber. K. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1, 118: 332, 1909.

Anamorph. *Cylindrotrichum zignoëllae* (Höhnelt) W. Gams et Hol.-Jech., Stud. Mycol. 13: 53, 1976.



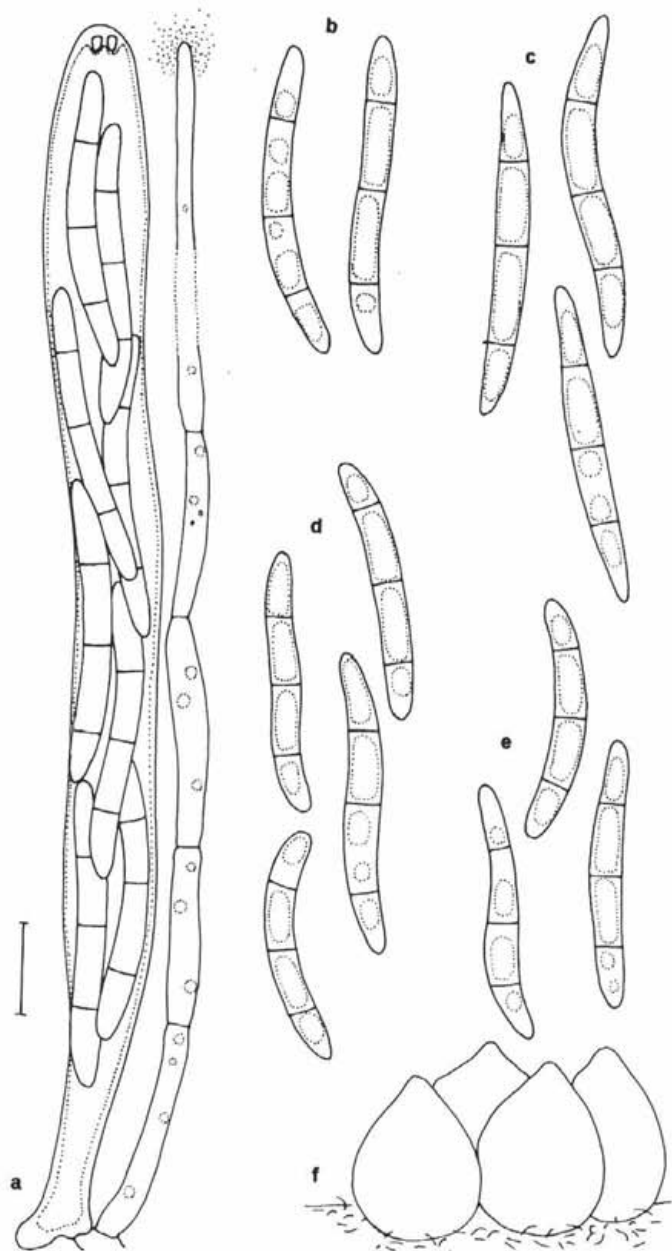


Fig. 1a-f. *Chaetosphaeria abietis*. - a. Ascus with ascospores and a paraphysis. - b-e. Ascospores. - f. Habit sketch of a group of perithecia. - a, b, f from Herb. M. R. 1058/97; c from Herb. M. R. 957/97; d from Herb. M. R. 969/97; e from the type K 49555. - Scale bar: a-c = 10  $\mu$ m.

- ≡ *Acrotheca zignoëllae* Höhnelt, Sitzungsber. K. Akad. Wiss. Wien, Math.-naturw. Abt. 1, Kl. 118: 332, 1909.  
≡ *Kylindria zignoëllae* (Höhnelt) DiCosmo et al., Mycologia 75: 971, 1983.  
= *Cylindrotrichum oblongisporum* G. Morgan-Jones, Mycotaxon 5: 487, 1977.

Material examined. 1) Type material. Rehm Ascom. exsicc. No. 1740 (AUSTRIA. Dürrien, Wiener Wald, on inner side of decayed bark, F. von Höhnelt, July 1907, K 49555 – lectotype of *Zignoëlla abietis*).

2) Additional material. Czech Republic. Southern Bohemia, Šumava Mts., glacial cirque of the lake Čertovo jezero near Železná Ruda, on inner surface of bark of *Abies alba*, 28 Aug. 1997, M. R. (Herb. M. R. 1027/97); *ibid.*, glacial cirque of the lake Černé jezero near Železná Ruda, on bark of *Abies alba*, 27 Aug. 1997, M. R. (Herb. M. R. 1058/97). – France. Pyrenees, Quérigut, Laurenti Lake, on bark of *Abies alba*, 17 July 1997, M. R. (Herb. M. R. 969/97, 973/97). – Ukraine. Eastern Carpathian Mts., Kvasi near Rachiv, on left bank of the river Tisa, on inner side of bark of *Abies alba*, 28 June 1997, M. R. (Herb. M. R. 957/97).

Descriptions and illustrations. Rehm (1907: 469); Höhnelt (1909: 332); Gams and Holubová-Jechová (1976: 53, Fig. 26 a, b).

Habitat. Saprobe on decayed wood and inner surface of bark of conifers.

Known host. *Abies alba*.

Known distribution. Europe: Austria, Czech Republic, France, Ukraine.

Note. The tips of paraphyses were seen enclosed in a small gelatinous cap that was well visible in phase contrast. This character was not seen in any other *Chaetosphaeria* species.

*Chaetosphaeria abietis* has not yet been grown in culture. The associated anamorph *Cylindrotrichum zignoëllae* was regularly observed growing around the perithecia (Gams and Holubová-Jechová 1976; Réblová, unpublished observations). Hawksworth and Minter (1980) reported the anamorph from dead herbaceous material of *Filipendula ulmaria* to be associated with perithecia of a *Chaetosphaeria*-like fungus; its ascospores were described as differing from *Chaet. abietis*, being 1–3-septate, shortly fusiform, 13–17 × 3–4 μm. Hawksworth and Minter (1980) concluded that *Cylindrotrichum zignoëllae* sensu Gams and Holubová-Jechová (1976) was circumscribed too broadly and may accommodate two taxa belonging to distinct teleomorphs. The perithecia found on *Filipendula ulmaria* could not be investigated further, for most of them were immature. No attempt was made to cultivate the specimen (Hawksworth and Minter 1980).

*Chaetosphaeria abietis* occurs rarely. All recent collections were made in regions with natural stands of *Abies alba*. Ascospore germination was not observed.

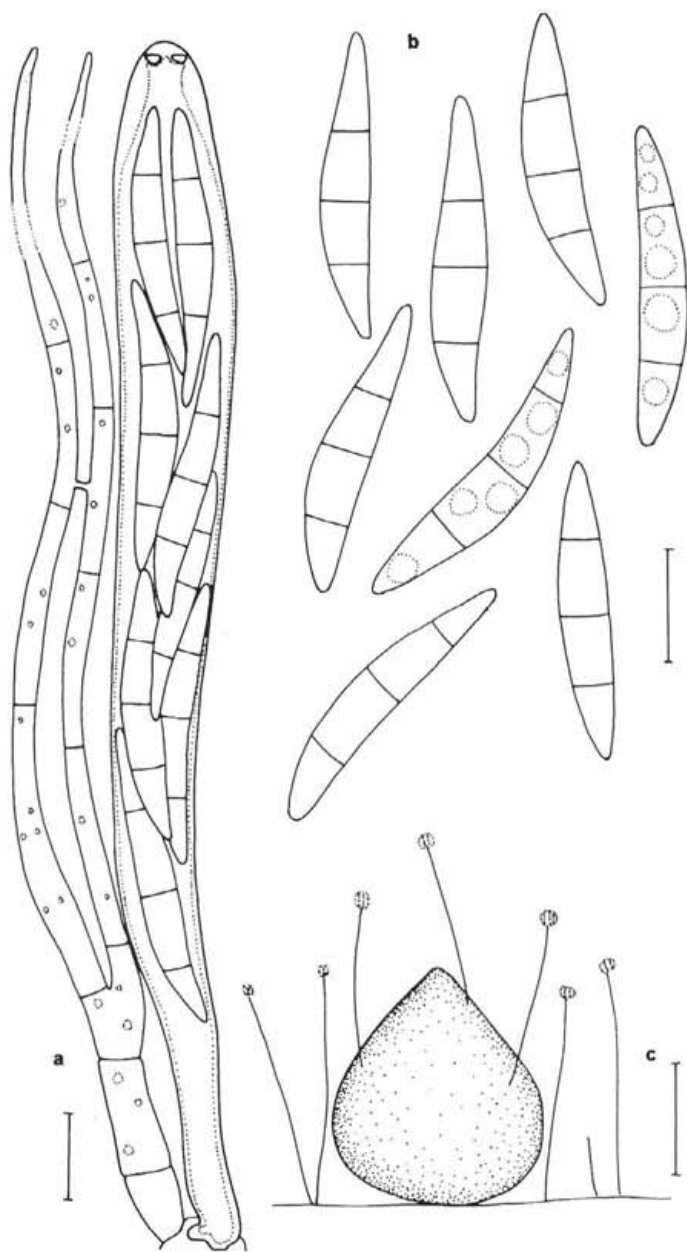


Fig. 2a-c. *Chaetosphaeria acutata*. - a. Ascus with ascospores and paraphyses. - b. Ascospores. - c. Habit sketch of the perithecium and conidiophores. - a-c from the type PRM 842979. - Scale bars: a, b = 10  $\mu$ m; c = 100  $\mu$ m.



2. *Chaetosphaeria acutata* Réblová et W. Gams, sp. nov.

Figs. 2, 3.

Perithecia superficialia, gregaria vel solitaria, subglobosa, 170–220  $\mu\text{m}$  alta, 170–200  $\mu\text{m}$  diam, papillata, ostiolata, fusca vel quasi atra, lucida, glabra vel conidiophoris oblecta, modice asperulata. Canalis ostiolaris periphysatus. Paries perithecii fragilis, ad latus 26–33  $\mu\text{m}$  crassus, bistratosus: stratum exterius e cellulis tenuitunicatis, fuscis, lateriformibus constans, interius e cellulis tenuitunicatis, compressis, subhyalinis. Paraphyses copiosae, intervallis 11–21  $\mu\text{m}$  septatae, ad septa non constrictae, ramosae, anastomosantes, hyalinae, 3–4  $\mu\text{m}$  latae in parte inferiore, ad 1.5–2  $\mu\text{m}$  angustatae, ultra ascorum apices protrudentes. Asci 8-spori, cylindrici vel clavati, (95–)121–147  $\times$  (8.5–)9.5–10.5(–11.5)  $\mu\text{m}$ , breviter stipitati, apex anulo I-, refringente, 3.0–3.5  $\mu\text{m}$  lato et 1.0–1.2  $\mu\text{m}$  alto praeditus, 8-spori. Ascospores fusiformes, utrinque fere acutatae et minime rotundatae, (28–)30.5–38(–44)  $\times$  3–4(–5)  $\mu\text{m}$ , 3-septatae, non constrictae, leves, hyalinae, oblique 1–2(–3)-seriatae inasco, tubis polaribus germinantes.

Anamorphosis *Cylindrotrichum* sp.: Conidiophoris simplicibus, fuscis, sursum pallidioribus, 125–190  $\times$  (4–)5–6.5  $\mu\text{m}$ , sursum ad 3–4  $\mu\text{m}$  angustatis, monophialidicis, saepe semel percurrenter proliferantibus. Phialides supra collare 4–10.5  $\mu\text{m}$  extendentes, quinquies ad dodecies annellatae. Conidia cylindrica vel clavata, recta vel modice curvata, ad basim truncata, ad apicem rotundata, 1-septata, haud constricta, levia, hyalina, (11–)12.5–15.5(–16.5)  $\times$  4–5  $\mu\text{m}$ .

Holotypus. Bohemia meridionalis, montes Šumava, Železná Ruda, ad lignum putridum *Fagi sylvaticae*, 28 Aug. 1997, leg. M. R. 994/97 (PRM 842979).

Etymology. Lat. *acutatus* = pointed, referring to the tapering ends of the ascospores.

Anamorph. *Cylindrotrichum* sp. (described here).

Fig. 3a-d.

Teleomorph. Perithecia superficial, gregarious to solitary, subglobose, 170–220  $\mu\text{m}$  high and 170–200  $\mu\text{m}$  diam, papillate, ostiolate, dark brown to nearly black, glistening, glabrous or covered with conidiophores of the anamorph, slightly roughened. Perithecial wall brittle, lateral wall 26–33  $\mu\text{m}$  thick, consisting of two layers; an outer layer of thin-walled, dark brown, opaque brick-like cells, and an inner layer of thin-walled, compressed, subhyaline cells. Ostiolar canal periphysate. Paraphyses copious, septate at 11–21  $\mu\text{m}$  intervals, non-constricted at the septa, branching, anastomosing, 3–4  $\mu\text{m}$  wide in the lower part, tapering to 1.5–2  $\mu\text{m}$ , protruding beyond the tips of the asci. Asci cylindrical-clavate, (95–)121–147  $\times$  (8.5–)9.5–10.5(–11.5)  $\mu\text{m}$ , shortly stipitate, ascal apex with a J-, refractive, 3–3.5  $\mu\text{m}$  wide and 1–1.2  $\mu\text{m}$  deep apical annulus. Ascospores fusiform, narrowly rounded at the ends, (28–)30.5–38(–44)  $\times$  3–4(–5)  $\mu\text{m}$ , 3-septate, not

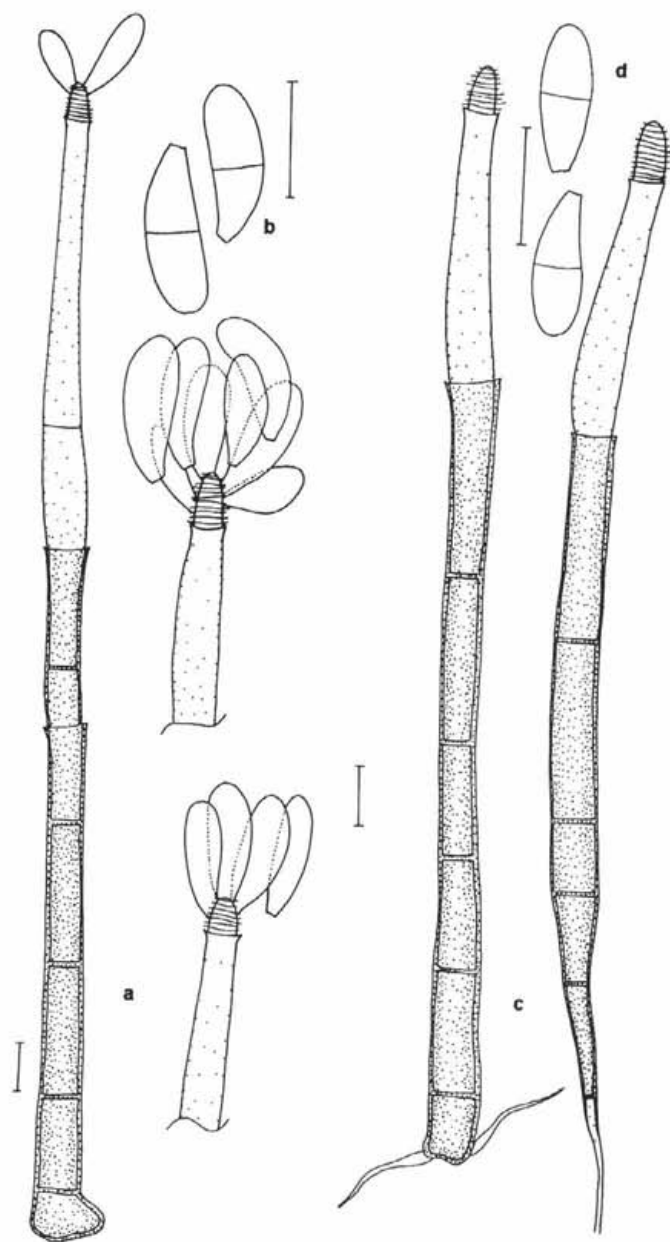


Fig. 3a-d. *Chaetosphaeria acutata*. - a. Conidiophore and sporogenous apices of the conidiophores, from nature. - b. Conidia, from nature. - c. Conidiophores, from PCA culture. - d. Conidia, from PCA culture. - a-d from CBS 101315, PRM 842979. - Scale bar: a-d = 10  $\mu$ m.

constricted, smooth, hyaline, obliquely 1-2(-3)-seriate, partially overlapping in the ascus, germinating by germ tubes at the ends.

Anamorph. Colonies hairy, dark, conidiophores arising from the substratum or covering the perithecia. Setae absent. Conidiophores macronematous, mononematous, solitary, erect, unbranched, cylindrical, 125-190  $\mu\text{m}$  long and (4-)5-6.5  $\mu\text{m}$  wide in the middle, tapering to 3-4  $\mu\text{m}$ , straight or slightly flexuous, dark brown, paler towards the apex, smooth, often with 1 percurrent proliferation. Phialides terminal, cylindrical, the percurrently proliferating part above the collarete 4-10.5  $\mu\text{m}$  long and 3.5-5  $\mu\text{m}$  wide, with 5-12 densely annellate proliferations. Primary collarettes hyaline, 4.5-5.5(-6)  $\mu\text{m}$  wide and 1  $\mu\text{m}$  deep. Conidia (11-)12.5-15.5(-16.5)  $\times$  4-5  $\mu\text{m}$ , cylindrical to clavate, straight or slightly curved, tapering and truncate at the proximal end, rounded at the distal end, 1-septate, not constricted, smooth-walled, hyaline.

Characteristics in culture. Colonies on PCA slow-growing, reaching 3-4 mm diam in 10 days at 25 °C in darkness, when grown for another 10 days at 25 °C in cool white fluorescent light reaching 7-8 mm diam; felty, greyish brown, aerial mycelium developed, margins fimbriate, no conidiation; reverse dark brown to nearly black. In 20 days: CMA: 4-4.5 mm diam, MEA: 6-7 mm diam; OA: 3.5-4 mm diam; CMA, MEA: felty, greyish brown, aerial mycelium well-developed, margins fimbriate, no conidiation; OA: colonies having a moist appearance, mid-brown, aerial mycelium scant, no conidiation. Colonies sporulated only on PCA, OA and CMA in 2-3-month-old slant cultures preserved at 18 °C under cool white fluorescent light. Mycelium superficial or immersed; hyphae branched, septate, subhyaline, smooth, 2.5-3  $\mu\text{m}$  wide. Setae absent. Conidiophores as on the natural substratum 84-150(-200)  $\mu\text{m}$  long and 5-6  $\mu\text{m}$  wide in the middle, tapering to 3-4.5  $\mu\text{m}$ . Phialides with hyaline primary collarete 4-6  $\mu\text{m}$  wide and 1-1.5(-2)  $\mu\text{m}$  deep. Conidia 10.5-14.5  $\times$  3-4.5  $\mu\text{m}$ .

Material examined. 1) Type material. Czech Republic. Southern Bohemia, Šumava Mts., glacial cirque of the lake Čertovo jezero near Železná Ruda, on wood of *Fagus sylvatica*, 28 Aug. 1997, M. R. 994/97 (PRM 842979 - holotype of *Chaetosphaeria acutata*)

2) Additional material examined. Czech Republic. Southern Bohemia, Šumava Mts., glacial cirque of the lake Černé jezero near Železná Ruda, on wood of *Fagus sylvatica*, 23 Oct. 1996, M. R. (Herb. M. R. 906/96); *ibid.*, 27 Aug. 1997, M. R. (Herb. M. R. 1043/97). - France. Central Pyrenees, Bagnères de Luchon, Lys valley, on wood of *Fagus sylvatica*, 13 July 1997, M. R. (Herb. M. R. 974/97). - Ukraine, Eastern Carpathian Mts., Kvasi near Rachiv, on left bank of the river Tisa, on wood of *Corylus avellana*, 26 June 1997, M. R. (Herb. M. R. 948/97).



Cultures. CBS 101311 (Herb. M. R. 948/97); CBS 101312 (Herb. M. R. 974/97); CBS 101315 (PRM 842979).

Habitat. Saprobe on decayed wood of deciduous trees.

Known hosts. *Corylus avellana*, *Fagus sylvatica*.

Known distribution. Europe: Czech Republic, France, Ukraine.

Note. The species was initially identified as *Lasiosphaeria britzelmayri* Sacc. subsp. *fennica* P. Karst. (Karsten 1887). The redescription of this taxon by Podlahová (1974) seemed to match our fungus perfectly. But the type specimen in H showed that ascospores clearly differ in shape and size. They are rather longer [(34.5–)36.5–42(–43) × (3.5–)4(–4.5) μm], elongate-fusiform and tapering strongly towards the ends immediately after the middle septum. The asci are of a size [(126–)133–152(–168) × (8.5–)9–10.5 μm] comparable to that of *Chaetosphaeria acutata*. The anamorph associated with perithecia on the type material of *Lasiosphaeria britzelmayri* subsp. *fennica* is *Chloridium*-like and entirely different from the *Cylindrotrichum* anamorph of *Chaet. acutata*. The conidia are mid-brown and non-septate. *Lasiosphaeria britzelmayri* Sacc. subsp. *fennica* is therefore another species of *Chaetosphaeria* to be included in the present paper.

*Chaetosphaeria acutata* clearly differs from other species of the genus by its typically long fusiform, at maturity 3-septate ascospores, the *Cylindrotrichum* anamorph and its exclusive occurrence on decayed wood of deciduous trees. In areas with near-natural and natural stands dominated by *Fagus sylvatica* and *Abies alba*, where the fungus occurs, it is not uncommon.

Conidia of the *Cylindrotrichum* anamorph of *Chaet. acutata* are formed successively from the sporogenous apex of the proliferating conidiogenous cells. *Cacumisporium capitulatum* has similarly proliferating conidiogenous cells but differs in having at maturity 4-celled, bicolorous and larger conidia.

### 3. *Chaetosphaeria crustacea* (Sacc.) Réblová et W. Gams, comb. nov. Figs. 4–6.

≡ *Zignoëlla crustacea* Sacc., Syll. Fung. 2: 220, 1883. – basionym.

≡ *Sphaeria crustacea* P. Karst., Fungi fenn. exs. 865, 1869; more elaborated in Bidr. Känn. Finl. Nat. Folk. 23: 95, 1873 [non *Sphaeria crustacea* Sow., Col. Fig. Engl. Fung. 1: Tab. 372, Fig. 3, Pl. 372, 1803 = *Hypoxyylon multifforme* (Fr.: Fr.) Fr.].

Anamorph. Intermediate between *Cylindrotrichum* and *Chloridium* (described here). Fig. 6a–f.

Teleomorph. Perithecia superficial, solitary to densely gregarious, subglobose to globose, 130–250 μm high and 120–220 μm diam, papillate, ostiolate, papilla

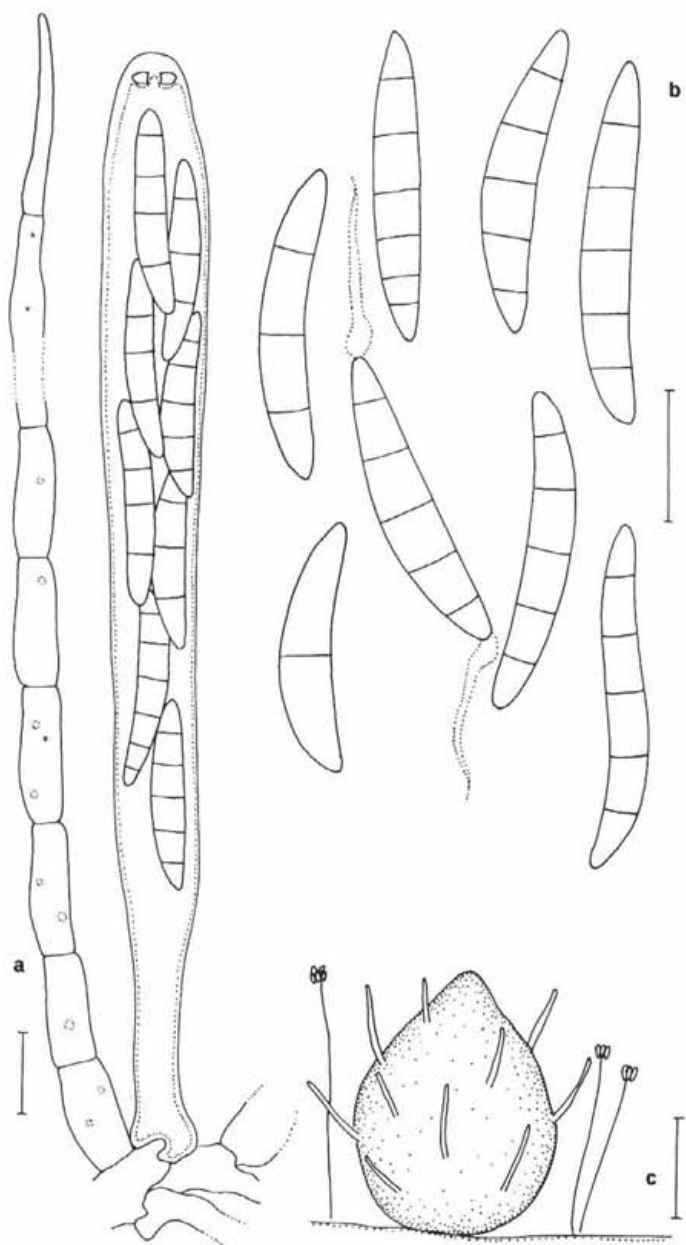


Fig. 4a-c. *Chaetosphaeria crustacea*. - a. Ascus with ascospores and a paraphysis. - b. Ascospores. - c. Habit sketch of perithecium and conidiophores. - a-c from Herb. M. R. 996/97. - Scale bars: a, b = 10  $\mu$ m; c = 100  $\mu$ m.

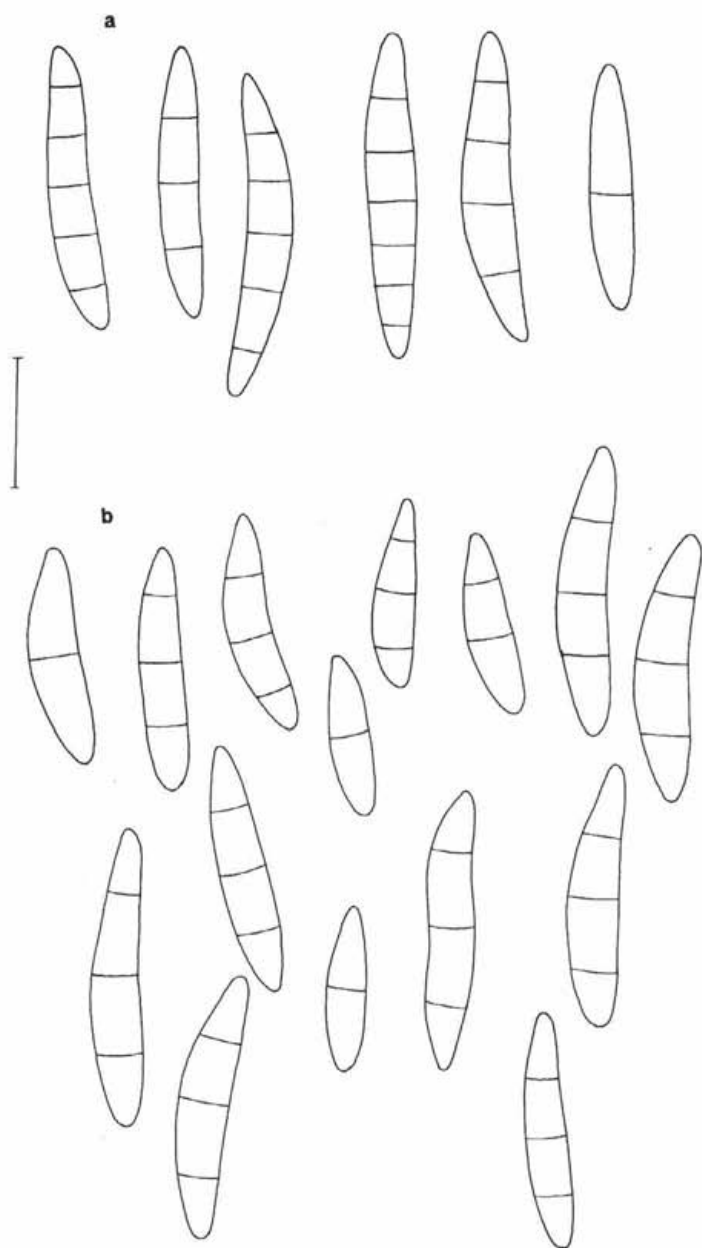


Fig. 5a, b. *Chaetosphaeria crustacea*. - a, b. Ascospores. - a from H; b from Herb. M. R. 1169/97. - Scale bar: a, b = 10  $\mu$ m.

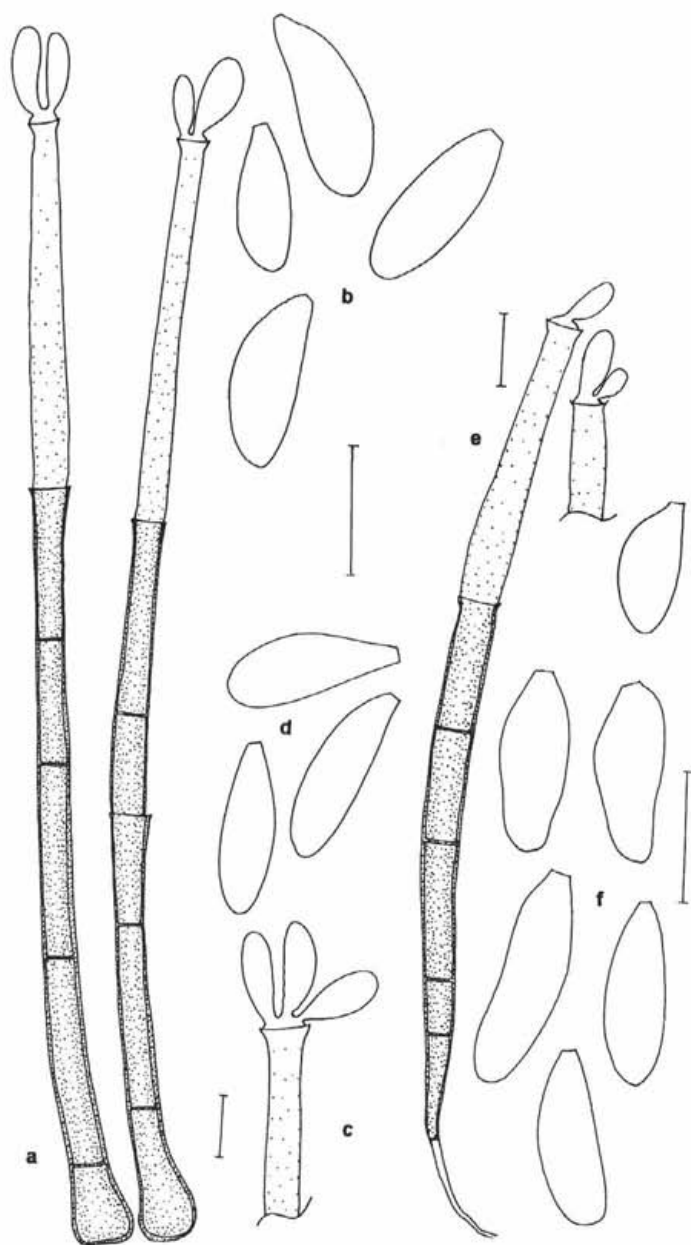


Fig. 6a-f. *Chaetosphaeria crustacea*. - a. Conidiophores, from nature. - b. Conidia, from nature. - c. Sporogenous apex of the conidiophore, from PCA culture. - d, f. Conidia, from PCA culture. - e. Conidiophore, from PCA culture. - a, b, e, f from Herb. M. R. 996/97; c, d from CBS 101321, Herb. M. R. 1169/97; e, f from CBS 101316. - Scale bar: a-f = 10  $\mu$ m.

perforated by a rounded pore in old perithecia, black, glistening, setose, slightly roughened. Setae scattered over the perithecia, erect, dark brown, septate, obtuse, 30–130  $\mu\text{m}$  long and 3–3.5  $\mu\text{m}$  wide in the middle. Perithecial wall brittle, lateral wall 15–22  $\mu\text{m}$  thick, consisting of two layers; an outer layer of thin-walled, opaque, brick-like cells, and an inner layer of thin-walled, subhyaline, compressed cells. Ostiolar canal periphysate. Paraphyses copious, septate at 18–21  $\mu\text{m}$  intervals, slightly constricted at the septa, branching, anastomosing, 4–5  $\mu\text{m}$  wide in the lower part, tapering to 2  $\mu\text{m}$ , protruding beyond the tips of the asci. Asci cylindrical-clavate, 75–105(–115)  $\times$  (8.5–) 9.5–10.5  $\mu\text{m}$ , shortly stipitate, 8-spored, ascus apex with a J-, 2.5  $\mu\text{m}$  wide and 1.5  $\mu\text{m}$  deep apical annulus. Ascospores cylindrical-fusiform, straight or curved, (15.5–)19–26(–28)  $\times$  3–3.5(–4)  $\mu\text{m}$ , predominantly 3-septate, with a delayed formation of the 2–3 additional septa, not constricted, smooth, hyaline, 2–3-seriate, partially overlapping in the ascus, germinating by a germ tube at one or both ends; no tendency of fragmentation.

Anamorph. Colonies hairy, dark, conidiophores arising from decayed wood and also covering the perithecia. Setae absent. Conidiophores macronematous, mononematous, solitary, erect, unbranched, 170–210  $\mu\text{m}$  long and 4.5–5.5  $\mu\text{m}$  wide above the base, tapering to 3–3.5  $\mu\text{m}$  below the collarette, cylindrical, straight or slightly flexuous, septate, often with 1(–2) distant percurrent proliferation, dark brown, paler towards the apex, smooth-walled. Phialides terminal, cylindrical, 23–32  $\times$  3.5–4.5  $\mu\text{m}$ . Collarettes hyaline, 4–5  $\mu\text{m}$  wide and 1.5–2.5  $\mu\text{m}$  deep. Conidia arising in a sympodial manner on a central dome inside the collarette like in *Chloridium virescens*, cylindrical-clavate, (11.5–)14.5–17(–21)  $\times$  3–4.5  $\mu\text{m}$ , straight or curved, rounded at the tip, tapering and truncate at the base, 1-celled, smooth, hyaline.

Characteristics in culture. Colonies on PCA slow-growing, reaching 4–5 mm diam in 10 days at 25 °C in darkness, when grown for another 10 days at 25 °C in cool white fluorescent light reaching 10–11 mm diam; velvety, centre dark greyish brown due to hyphae of aerial mycelium and conidiophores, dark brown to nearly black at the fimbriate margin, forming a conspicuous dark, sterile zone of submerged mycelium; conidial production copious in 10 days; conidiophores arising in the centre; conidial masses globose, whitish; reverse dark grey; no pigments released. In 20 days: CMA: 9–10 mm diam; MEA 12 mm diam; OA 9–12 mm diam; CMA, MEA, OA: velvety, dark grey, with copious conidial production in 10 days on CMA, OA; on MEA after 20 days. The black sterile zone formed by substrate mycelium at the margins is most pronounced on CMA, less on PCA and OA and is not developed on MEA. Mycelium superficial or immersed; hyphae branched, septate, subhyaline, smooth, 2–3  $\mu\text{m}$  wide. Setae absent. Conidiophores as on the natural substratum, 70–160  $\mu\text{m}$



long, 4–5  $\mu\text{m}$  wide in the middle, tapering to 3–3.5  $\mu\text{m}$  below the collarette. Phialides 23–44  $\times$  3.5–4.5  $\mu\text{m}$ ; collarettes 4–5  $\mu\text{m}$  wide and 1.5–2.5(–3)  $\mu\text{m}$  deep. Conidia (11–)12.5–16.5(–20)  $\times$  3–4(–5)  $\mu\text{m}$ . Chlamydospores absent.

Material examined. 1) Type material. Finland. Ostrobothnia, Vaasa, on *Pinus sylvestris*, P. A. Karsten, Fung. Fenn. exsicc. 865, (H – lectotype of *Zignoëlla crustacea*).

2) Additional material. Czech Republic. Southern Bohemia, glacial cirque of the lake Černé jezero near Železná Ruda, on wood of *Abies alba*, 7 Nov. 1997, K. Prášil (Herb. M. R. 1169/97). – Ukraine. Eastern Carpathian Mts., Kvasi near Rachiv, on left bank of the river Tisa, on wood of *Picea abies*, 26 June 1997, M. R. (Herb. M. R. 996/97).

Cultures. CBS 101316 (Herb. M. R. 996/97); CBS 101321 (Herb. M. R. 1169/97).

Descriptions and illustrations. Karsten (1873: 95); Saccardo (1883: 220).

Habitat. Saprobe on decayed wood and bark of conifers.

Known hosts. *Abies alba*, *Picea abies*, *Pinus sylvestris*.

Known distribution. Europe: Czech Republic, Finland, Ukraine.

Note. The type collection of *Zignoëlla crustacea* made by Karsten in Finland (Karsten 1873) contains densely setose perithecia seated on wood of *Pinus sylvestris*, and 3–5 transversely septate, hyaline ascospores (22–24.5  $\times$  3–3.5  $\mu\text{m}$ ). No anamorph was observed. The perithecia from the recent collections are densely setose in a collection made in the Czech Republic and sparsely setose in a collection made in the Ukraine. In both collections the same *Cylindrotrichum* species was found growing on the substratum and was also obtained in the living culture. Traditionally, *Chaetosphaeria* only contains species with glabrous perithecia. They may, however, become setose when the setae and conidiophores of the associated anamorph arise both from the substratum and the perithecial surface. *Chaetosphaeria crustacea* is an example of a fungus with setose perithecia associated with a *Cylindrotrichum-Chloridium*-like anamorph. The recent finds represent the first records of this fungus since its description.

In the type material both 3-septate and 4–5-septate ascospores were present. Inside the asci only the 3-septate ascospores were seen. The 4–5-septate ascospores were released from the perithecium and seen attached to the perithecial surface and on the substratum. In the specimen from the Ukraine mainly 5-septate ascospores and in that from the Czech Republic only 3-septate ascospores were seen.

*Chaetosphaeria crustacea* is similar in ascospore shape and septation to *Chaet. decastyla* but differs in shorter ascospores and asci, setose perithecia, the *Cylindrotrichum-Chloridium*-like anamorph and the occurrence on decayed wood of conifers. *Chaetosphaeria crustacea* occurs rarely and is known from three localities in Europe only.

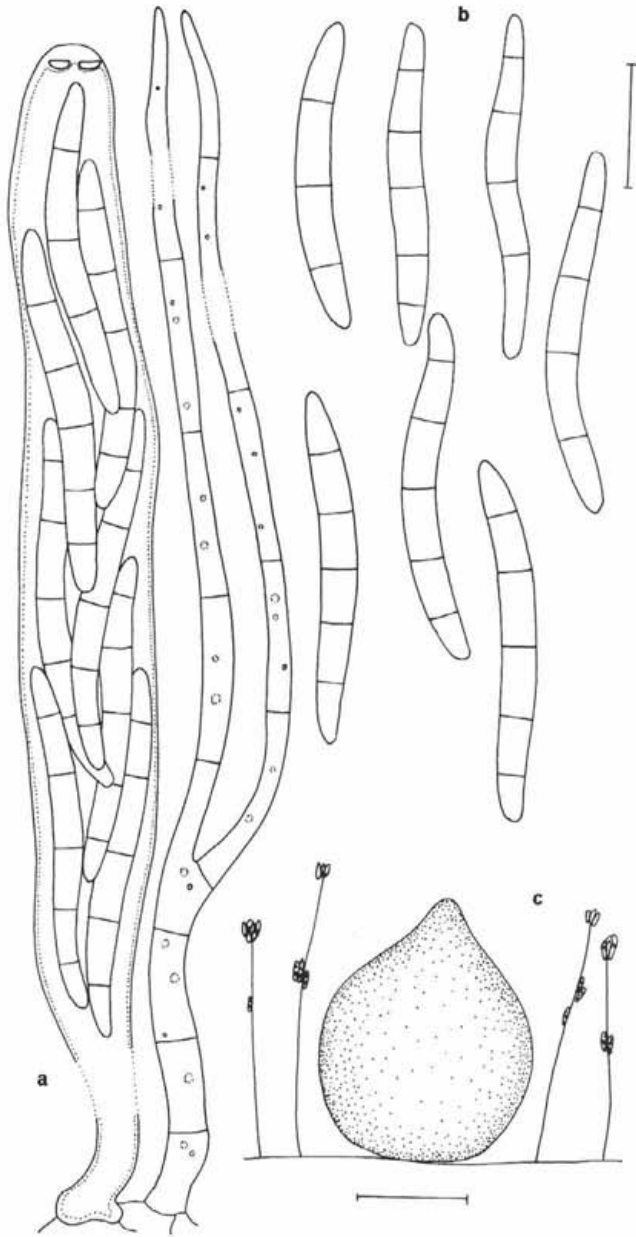


Fig. 7a-c. *Chaetosphaeria decastyla*. - a. Ascus with ascospores and paraphyses. - b. Ascospores. - Habit sketch of perithecium and conidiophores. - a-c from Herb. M. R. 985/97. - Scale bars: a, b = 10  $\mu$ m; c = 100  $\mu$ m.

4. *Chaetosphaeria decastyla* (Cooke) Réblová et W. Gams, comb. nov.

Figs. 7-9.

≡ *Sphaeria decastyla* Cooke, Grevillea 7: 52, 1878. - basionym.

≡ *Acanthostigma decastylum* (Cooke) Sacc., Syll. Fung. 2: 210, 1883.

= *Melanomma macrosporum* Sacc., Hedwigia 14: 73, May 1875; see also Saccardo, Fungi ital. delin. 300, Feb. 1878; Michelia 1: 449, 15 Nov. 1878.

≡ *Zignoëlla macrospora* (Sacc.) Sacc., Michelia 1: 346, 1 July 1878; Syll. Fung. 2: 221, 1883 [non *Chaetosphaeria macrospora* (Kawamura) Hara, J. Pl. Prot. Tokyo 16(2): 16, 1930].

Anamorph. *Cacumisporium capitulatum* (Corda) S. Hughes, Canad. J. Bot. 96: 743, 1958. Fig. 9a-d.

≡ *Helminthosporium capitulatum* Corda, Ic. Fung. 2: 13, 1838.

= *Cacumisporium tenebrosum* Preuss, Linnaea 24: 130, 1851.

Teleomorph. Perithecia superficial, solitary or in groups of 3-5, subglobose to conical, 200-260  $\mu\text{m}$  high and 180-210  $\mu\text{m}$  diam, papillate, papilla perforated by a rounded pore in old perithecia, ostiolate, dark brown to black, glistening, glabrous or covered with conidiophores of the anamorph, slightly rugose. Perithecial wall brittle, 17-22  $\mu\text{m}$  thick, consisting of two layers; an outer layer of thin-walled, dark brown, opaque, polyhedral to brick-like cells, and an inner layer of thin-walled, compressed, hyaline cells. Ostiolar canal periphysate. Paraphyses copious, branching, septate at 18-42  $\mu\text{m}$  intervals, 3-3.5  $\mu\text{m}$  wide in the lower part, tapering to 1.5  $\mu\text{m}$ , protruding beyond the tips of the asci. Asci clavate-cylindrical, 68-90  $\mu\text{m}$  long in the *pars sporifera*  $\times$  10.5-11.5  $\mu\text{m}$ , stipe 30-54  $\mu\text{m}$  long, narrowly rounded at the tip, with a J-, refractive, 3-3.5  $\mu\text{m}$  wide and 1  $\mu\text{m}$  deep apical annulus. Ascospores cylindrical to cylindrical-fusiform or fusiform, occasionally tapering towards one end, (28-)30-42(-46)  $\times$  3-4  $\mu\text{m}$ , at maturity 5-septate, not constricted or very slightly constricted at the septa, smooth-walled, hyaline, 2-3-seriate, overlapping in the upper part of the ascus, germinating by germ tubes at the ends.

Anamorph. Colonies hairy, dark, conidiophores arising from the substratum or covering the perithecia. Setae absent. Conidiophores macronematous, mononematous, solitary, erect, unbranched, cylindrical, up to 200  $\mu\text{m}$  long, 6.5-7.5  $\mu\text{m}$  wide above the base, tapering to 5-6  $\mu\text{m}$  below the primary collarette, straight or slightly flexuous, septate, brown to pale brown, paler towards the apex, smooth, with 1-2 major percurrent proliferations. Phialides terminal, cylindrical, the proliferating part above the primary collarette 10-15  $\mu\text{m}$  long and 5-6  $\mu\text{m}$  wide, with 9-12 narrow annellate proliferations. Primary collarettes almost hyaline, 7-9.5  $\mu\text{m}$  wide and (1-)1.5-2  $\mu\text{m}$  deep. Conidia cylindrical, 15-20  $\times$  5-6.5  $\mu\text{m}$ , straight or slightly curved, rounded at the tip, truncate at the base, 3-septate, not constricted, at

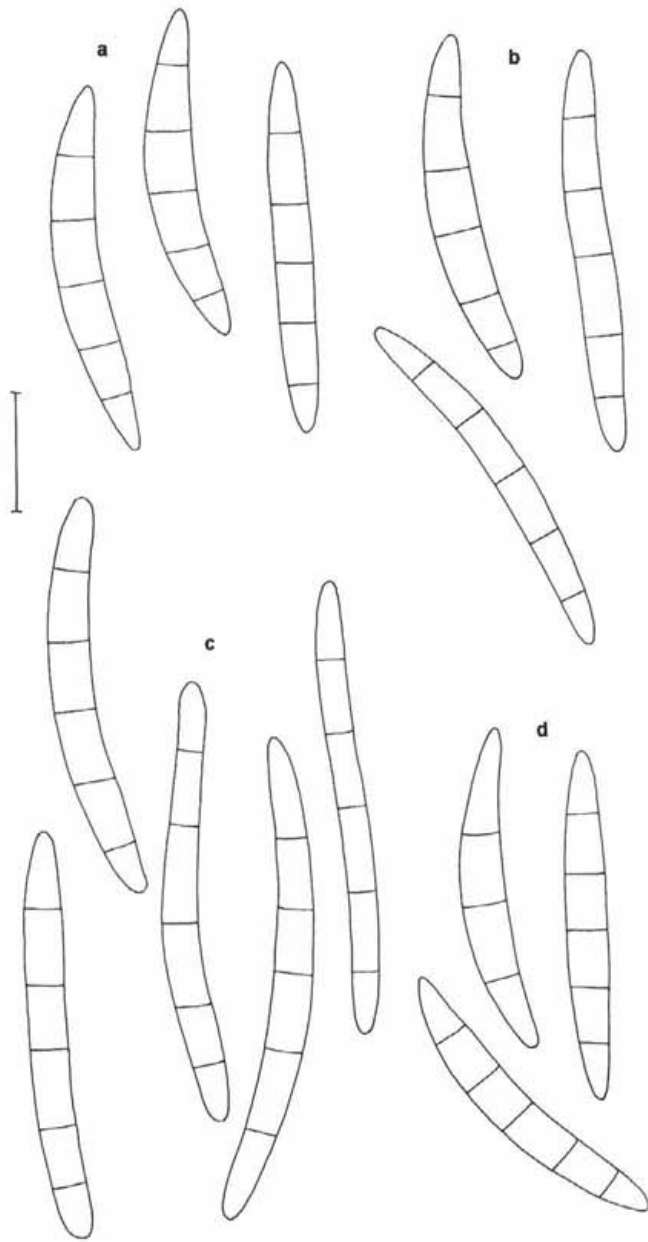


Fig. 8a-d. *Chaetosphaeria decastyla*. - a-d. Ascospores. - a-d from NY: a (USA, New Jersey, Newfield, on bark and wood of *Magnolia* sp.); b (USA, Georgia, Darien, on wood of *Quercus* sp., Ravenel 2420); c (Gloucester County, Newfield, 22 Jan. 1878); d (USA, Louisiana, on a decayed log of oak, 2 Jan. 1886, A. B. Langlois). - Scale bars: a-d = 10  $\mu$ m.

first hyaline, at maturity the two middle cells turning brown and the two end cells remaining hyaline, mature conidia often attached to the middle and lower part of the conidiophore.

Characteristics in culture. Colonies on PCA slow-growing, reaching 4–5 mm diam in 10 days at 25 °C in darkness, when grown for another 10 days at 25 °C in cool white fluorescent light reaching 8 mm diam; velvety, dark grey, aerial mycelium well-developed, margins fimbriate; conidial production scarce after 20 days; conidiophores scattered, conidial masses globose, hyaline; reverse greyish. Colonies sporulating copiously with conidiophores arranged in 2–3 concentric zones in 2 month-old slant cultures preserved at 18 °C under cool white fluorescent light. In 20 days: CMA: 7–8 mm diam; OA: 6–7 mm diam; CMA, OA: velvety, dark grey, conidial production scarce at the margins; MEA: 9–10 mm diam, velvety, greyish to pale brown, no conidial production. Mycelium superficial or immersed; hyphae branched, septate, subhyaline, smooth, 1.5–2.5  $\mu\text{m}$  wide. Setae absent. Conidiophores as on the natural substratum, (100–)127–176  $\mu\text{m}$  long, 5.5–6.5(–7.5)  $\mu\text{m}$  wide in the middle and 13–16  $\mu\text{m}$  wide at the base, tapering to 5–6(–6.5)  $\mu\text{m}$ . Phialides with the proliferating part 5–14  $\mu\text{m}$  long and 5–6(–6.5)  $\mu\text{m}$  wide, with 10–12 proliferations. Conidia 19–22(–24)  $\times$  6–7  $\mu\text{m}$ . In 2-month old slant cultures the conidia became mature, the two middle cells gradually turning brown and the two end cells remaining hyaline or at least paler, mature conidia often sticking to the middle and lower part of the conidiophore.

Material examined. 1) Type material. Italy. Cansiglio (Treviso), on decorticated wood of *Fagus sylvatica*, Oct. 1874, P. A. Saccardo (PAD – holotype of *Melanomma macrosporum*). – USA, Georgia, Darien, on wood of *Quercus* sp., Ravenel 2420, (K 59126 – holotype of *Sphaeria decastyla*; NY – isotype).

2) Additional material. France, Central Pyrenees, Bagnères de Luchon, Lys valley, on decayed wood of *Fagus sylvatica*, 13 July 1997, M. R. (Herb. M. R. 985/97, 988/97). – USA, New Jersey, Newfield, on bark of *Magnolia* sp., 10 July 1887 (NY); New Jersey, Gloucester County, Newfield, 22 Jan. 1878 (NY); *ibid.*, on a bark of *Magnolia* sp., 29 June 1882 (NY); Louisiana, on a decayed log of oak, 2 Jan. 1886, A. B. Langlois (NY).

Cultures. CBS 101313 (Herb. M. R. 985/97); CBS 101314 (Herb. M. R. 988/97).

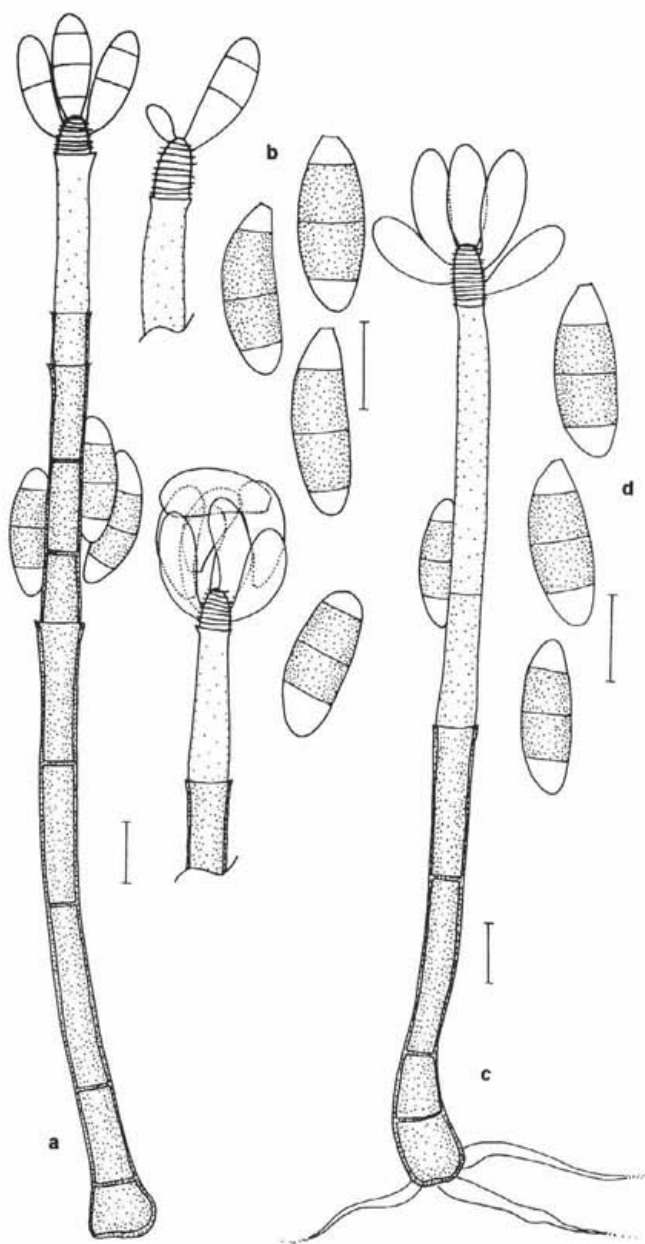
Descriptions and illustrations. Saccardo (1875: 73; 1878: 449; 1883: 210, 221); Cooke (1878: 52); Ellis and Everhart (1882: 155); Berlese (1894: 100, Tab. 94, Fig. 1).

Habitat. Saprobe on decayed wood of deciduous trees.

Known hosts. *Fagus sylvatica*, *Magnolia* sp., *Quercus* sp.

Known distribution. Europe: France, Italy; North America: USA (New Jersey, Louisiana).





**Fig. 9a-d.** *Chaetosphaeria decastyla*. - a. Conidiophore with conidia and sporogenous conidiophore apices, from nature. - b. Conidia, from nature. - c. Conidiophore with conidia, from PCA culture. - d. Conidia, from PCA culture. - a-d from Herb. M. R. 985/97; c, d from CBS 101313. - Scale bar: a-d = 10  $\mu$ m.

Nomenclatural note. The name *Chaetosphaeria decastyla* is based on *Sphaeria decastyla* Cooke 1878, even though the name *Melanomma macrosporum* Sacc. 1875 would have priority. If the epithet 'macrosporum' were transferred to *Chaetosphaeria* this would result in a homonymous combination (Art. 53.1, Greuter et al. 1994) with *Chaetosphaeria macrospora* (Kawamura) Hara [Hara, J. Pl. Prot. Tokyo 16(2): 16, 1930] that is based on *Miyoshiella macrospora* Kawamura [Kawamura, J. Coll. Sci. Imp. Univ. Tokyo 23(2): 295, 1929]. *Miyoshiella macrospora* does not belong to *Miyoshiella* Kawamura, a member of the Trichosphaeriaceae G. Winter (Réblová 1998c). *Miyoshiella macrospora* is a pyrenomycetous ascomycete forming black spots on bamboo culms having transversely 7-septate, blackish brown ascospores and unitunicate asci. Therefore, the second available name is chosen and the new combination *Chaetosphaeria decastyla* is proposed.

Note. The holotype and isotype and other collections of *Sphaeria decastyla*, including those cited by Ellis and Everhart (1882), are preserved in K and NY. They possess perithecia accompanied by conidiophores of the *Cacumisporium capitulatum* anamorph. Ascospores of North American collections, except for one specimen (USA, New Jersey, Gloucester County, Newfield, 22 Jan. 1878), differ in shape and size from European material that is represented by Saccardo's type material of *Melanomma macrosporum* and two recent collections made in France. Specimens from North America have rather fusiform and shorter ascospores (Fig. 8) [(28-)30-34.5(-36.5)  $\times$  3-4  $\mu$ m] than those of the European material, in which the ascospores (Fig. 7) are cylindrical to cylindrical-fusiform and longer [(31.5-)35.5-42(-46)  $\times$  3-4  $\mu$ m]. The specimen collected in USA, New Jersey, Gloucester County, Newfield, has ascospores much like those of the European collections, cylindrical, measuring (27-) 30.5-39(-42)  $\times$  3-4  $\mu$ m.

Ellis and Everhart (1882) described perithecia sparsely clothed with obtuse, septate setae. These setae (50-115  $\times$  3-4.5  $\mu$ m) were found only on several perithecia of the holotype of *Sphaeria decastyla* and other material (USA, New Jersey, Newfield, on bark of *Magnolia* sp., 10 July 1887, NY). The majority of perithecia were glabrous and bore remnants of conidiophores. The obtuse setae were not found in European material. Ellis and Everhart (1882) noted that the substratum surface in both collections was clothed with hairs similar to those growing on the perithecia. Our revision revealed that the so-called hairs are conidiophores of the *Cacumisporium capitulatum* anamorph.

Ellis and Everhart (1882) cited in a synonymy of *Acanthostigma decastylum* three names, viz. *Sphaeria cariosa* Cooke et Ellis, *Sphaeria atriella* Cooke et Ellis and *Lasio-sphaeria subvelutina* Ellis et Everhart. *Sphaeria cariosa* (Holotype: USA, New Jersey, Newfield, on bark of decayed oak, 15 Feb. 1877, J. B. Ellis 2789, K 59125, NY - isotype) is a species of *Chaetosphaeria*, known as *Chaet. ovoidea* (Fr.) O. Constant. et al.; the *Menispora glauca* Pers. anamorph is abundantly present on the type. *Sphaeria atriella* Cooke et Ellis (Isotype: USA, New Jersey,

Newfield, Gloucester County, on rotten wood of *Acer* sp., Dec 1876, NY) was combined by Barr (1993) under *Chaetosphaeria*, as *Chaet. atriella* (Cooke et Ellis) M. E. Barr. We do not believe that this is a true *Chaetosphaeria* species, for it differs in several characters that would characterize a *Chaetosphaeria*. The perithecial wall is leathery, three-layered, ca. 35–48  $\mu\text{m}$  thick, composed of non-opaque, thick-walled cells; paraphyses were not present and the asci were enclosed in a hyaline matrix that could be formed of dissolved paraphyses. No anamorph was associated. The wall of perithecium of *Chaetosphaeria* is different; it is thinner, ca. 15–25  $\mu\text{m}$ , comprising thin-walled, opaque brick-like cells or cells that form a network, paraphyses are always persistent and do not dissolve. *Lasiosphaeria subvelutina* is another possible synonym of *Chaetosphaeria decastyla*, but the type could not be examined (not available in NY).

The type material of *Melanomma macrosporum* contained several mature perithecia and conidiophores of the *Cacumisporium capitulatum* anamorph arising sparsely from the substratum. Mature bicolorous conidia were found attached to the substratum and to the perithecial walls.

The conidiogenesis of *Cac. capitulatum* was described in detail by Goos (1969). The conidia have a flat basal scar and are formed successively from multiple growing points on the apex of the conidiogenous cell. The conidiogenous cells proliferate conspicuously 5–14  $\mu\text{m}$  above the shallow collarete. A similarly proliferating apex of the conidiogenous cells is also found in the *Cylindrotrichum* anamorph of *Chaetosphaeria acutata*, which differs by smaller, hyaline, 2-celled conidia. The measurements of conidia of *Cac. capitulatum* are given from the original culture obtained immediately after isolation from the ascospores. Conidia are generally larger in vitro than on material from nature. After several transfers, *Cac. capitulatum* produces much smaller conidia in vitro [(14–)16–22(–24)  $\times$  5–7  $\mu\text{m}$ ] than in nature.

The ascospores of *Chaetosphaeria decastyla* are somewhat similar in shape to those of *Chaet. crustacea*. *Chaet. decastyla* can be clearly distinguished from *Chaet. crustacea* by the exclusive occurrence on wood of angiosperms, longer ascospores and asci, and the *Cac. capitulatum* anamorph.

##### 5. *Chaetosphaeria fennica* (P. Karst.) Réblová et W. Gams, comb. nov.

Figs. 10, 11.

- ≡ *Lasiosphaeria britzelmayri* Sacc. subsp. *fennica* P. Karst., Rev. Mycol. Toulouse 9: 160, 1887. – basionym.
- ≡ *Acanthostigma fennicum* (P. Karst.) Berlese, Icon. Fung. 1: 102, 1894.
- ≡ *Zignoëlla abietis* Höhnelt var. *fennica* (P. Karst.) Höhnelt, Sitzungsber. K. Akad. Wiss., Wien, Math.-naturw. Kl., Abt. 1, 118: 332, 1909.

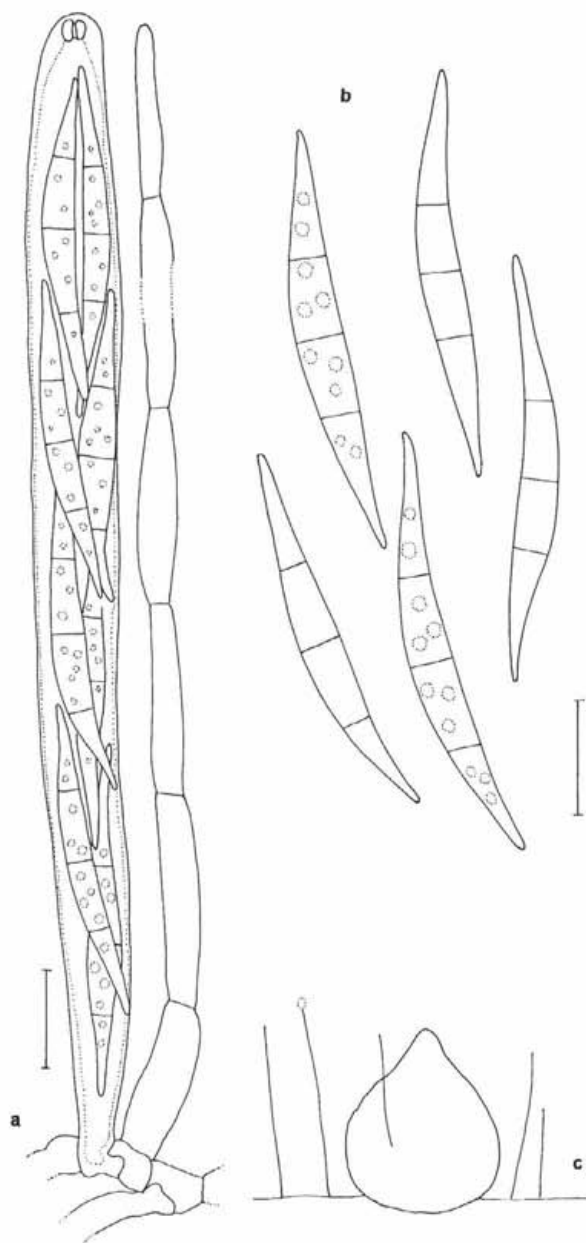


Fig. 10a-c. *Chaetosphaeria fennica*. - a. Ascus with ascospores and a paraphysis. - b. Ascospores. - c. Habit sketch of perithecium and conidiophores. - a-c from H 929. - Scale bars: a, b = 10  $\mu$ m.

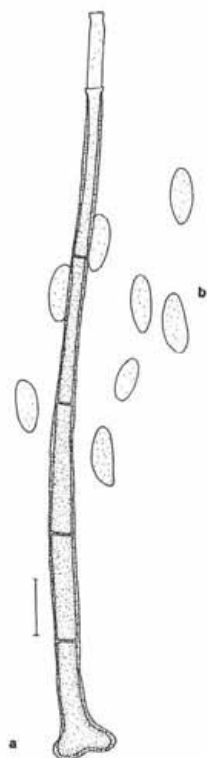


Fig. 11a, b. *Chaetosphaeria fennica*. – a. Conidiophore, from nature. – b. Conidia, from nature. – a, b from H 929. – Scale bar: a, b = 10  $\mu$ m.

Anamorph. *Chloridium*-like (described here).

Fig. 11a, b.

Teleomorph. Perithecia solitary, scattered, subglobose, 150–200  $\mu$ m high and 200–220  $\mu$ m diam, papillate, papilla perforated by a rounded pore in old perithecia, ostiolate, dark brown to black, glabrous or covered with conidiophores of the anamorph, smooth. Perithecial wall brittle, 22–26  $\mu$ m thick, consisting of two layers; an outer layer of thin-walled, dark brown, opaque, polyhedral to brick-like cells, and an inner layer of thin-walled, compressed, hyaline cells. Ostiolar canal periphysate. Paraphyses copious, branching, anastomosing, septate, 4–5  $\mu$ m wide in the lower part, tapering to 2.5  $\mu$ m, protruding slightly beyond the tips of the asci. Asci long-cylindrical, (126–)133–152(–168)  $\times$  (8.5–)9–10.5  $\mu$ m, narrowly rounded at the tip, with a J-, refractive apical annulus, 2.5  $\mu$ m wide and 1–1.5  $\mu$ m deep. Ascospores elongate-fusiform and tapering strongly towards the ends immediately after the middle septum, (34.5–)36.5–42(–43)  $\times$  (3.5–)4(–4.5)  $\mu$ m, at maturity 3-septate, not constricted at the septa, smooth-walled, hyaline, 2-seriate, overlapping partially in the upper part of the ascus.



Anamorph. Remnants of conidiophores with broken apices were observed growing copiously from the substratum surface and occasionally from the outer wall of the perithecia. Conidiophores macronematous, mononematous, erect, solitary, unbranched, septate, opaque, paler to subhyaline towards the top, 250–270  $\mu\text{m}$  long, 5.5–6.5  $\mu\text{m}$  wide above the base and 20–22  $\mu\text{m}$  wide at the base. Once, a conidiophore with an inconspicuously proliferated apex ending in a hyaline collarete 3  $\mu\text{m}$  wide and 2  $\mu\text{m}$  deep was observed. A lot of conidia were seen attached to the conidiophore. Conidia mid-brown, ellipsoidal, straight or inequilateral, slightly truncate at the base, 8.5–9.5(–10)  $\times$  3–4.5  $\mu\text{m}$ .

The teleomorph-anamorph connection could not yet be proved by culture studies and is only suggested by the joint occurrence of perithecia and conidiophores.

Material examined. Type material. Finland, Mustiala Myllyperä, on decayed bark of *Betula* sp., on old stromata of *Eutypa* sp., 28 July 1887, K. Starbäck, herb. P. Karsten 929 (H – holotype of *Chaetosphaeria fennica*).

Descriptions and illustrations. Karsten (1887: 160); Berlese (1894: 102, Pl. 119, Fig. 3); Saccardo (1891: 852); Podlahová (1974: 149, Tab. 42).

Habitat. Saprobe on stromata of *Eutypa* sp.

Known host. *Eutypa* sp. on *Betula* sp.

Known distribution. Europe: Finland, known only from the type locality.

Note. Podlahová (1974) revised the type material and her observations agree well with ours. Although Karsten (1887) and Berlese (1894) described and illustrated the perithecia as sparsely setose, Podlahová (1974) found them being glabrous. The present revision of the type material showed that the presumed setae were conidiophores that are usually quite firm but only visible as remnants in old herbarium material.

*Lasiosphaeria britzelmayri* Sacc. (Saccardo 1883) differs from *Chaetosphaeria fennica* in having shorter asci (90–100  $\times$  9–10  $\mu\text{m}$ ) and shorter (28–35  $\times$  3.5–4  $\mu\text{m}$ ), 6–11-celled, cylindrical-fusiform ascospores. Winter (1887) considered this fungus to have bitunicate asci and classified it as *Trematosphaeria paradoxa* G. Winter.

On the basis of ascospore anatomy, *Chaetosphaeria fennica* is closest to *Chaet. acutata*. The ascospores of the latter, though also long-fusiform, are much less tapering towards the ends and are rather shorter. The *Cylindrotrichum* anamorph of *Chaet. acutata* with hyaline and 1-celled conidia at maturity is entirely different from the *Chloridium*-like anamorph associated with *Chaet. fennica*.

6. *Chaetosphaeria fusiformis* W. Gams et Hol.-Jech., Mycotaxon 13: 257, 1981.

Figs. 12, 13.

$\equiv$  *Chaetosphaeria fusispora* W. Gams et Hol.-Jech., Stud. Mycol. 13: 45, 1976. (illegitimate, Art. 53.1.) [non *Chaetosphaeria fusispora* (Kawamura) Hino,

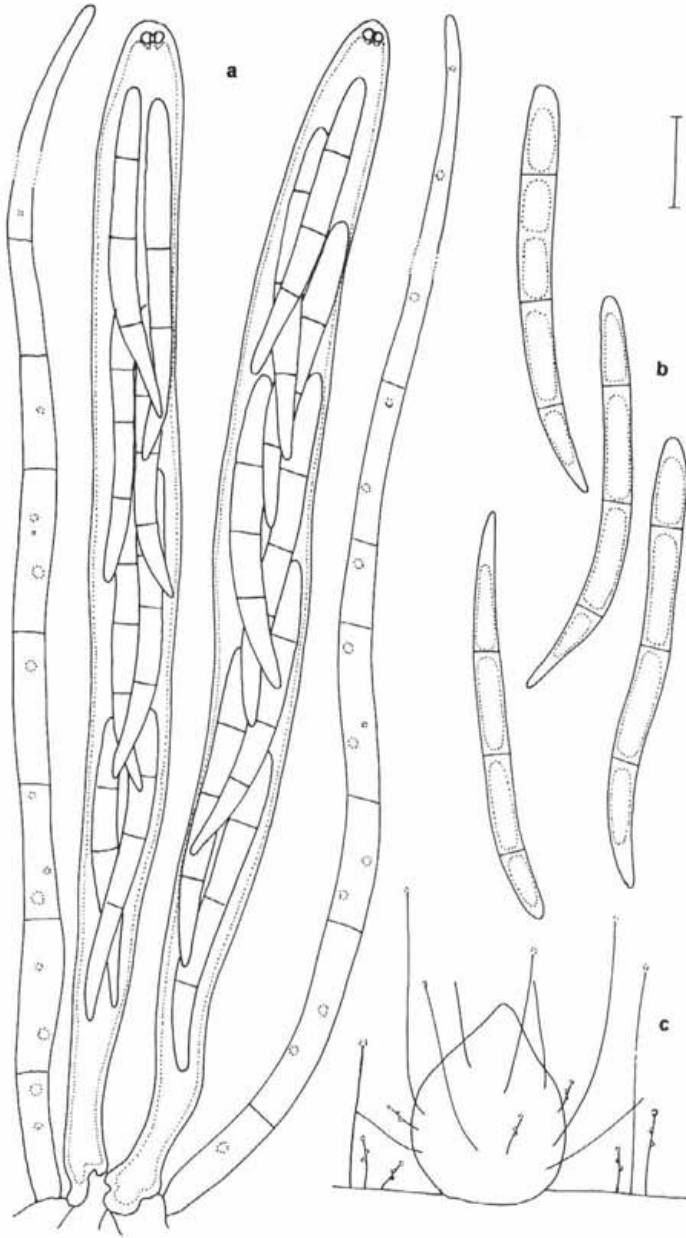


Fig. 12a-c. *Chaetosphaeria fusiformis*. - a. Asci with ascospores and paraphyses. - b. Ascospores. - c. Habit sketch of the perithecium and conidiophores. - a-c from Herb. M. R. 862/96. - Scale bar: a, b = 10  $\mu$ m.

Bull. Miyazaki Coll. Agr. For. 4: 191, 1932, nec *Chaetosphaeria fusispora*  
P. Larsen, Dansk Bot. Ark. 14(7): 7, 1952.]

Anamorph. *Chloridium cylindrosporum* W. Gams et Hol.-Jech., Stud. Mycol.  
13: 46, 1976. Figs. 13a-c.

≡ *Chaetopsis cylindrospora* (W. Gams et Hol.-Jech.) DiCosmo et al., Mycologia  
75: 962, 1983.

Teleomorph. Perithecia superficial, densely gregarious or in small groups, globose, 170–250  $\mu\text{m}$  high and 160–240  $\mu\text{m}$  diam, papillate, ostiolate, black, glistening, glabrous, covered with conidiophores of the anamorph, slightly rugose. Perithecial wall brittle, lateral wall 18–28  $\mu\text{m}$  thick, consisting of two layers; an outer layer of dark brown, thin-walled, opaque, brick-like cells, and an inner layer of thin-walled, subhyaline, compressed cells. Ostiolar canal periphysate. Paraphyses copious, septate at 10–21  $\mu\text{m}$  intervals, slightly constricted at the septa, branching, 3–5  $\mu\text{m}$  wide in the lower part, tapering to 2–2.5  $\mu\text{m}$ , rounded at the top, protruding beyond the tips of the asci. Asci cylindrical-clavate, (89–)99–126(–146)  $\times$  8.5–10.5(–11.5)  $\mu\text{m}$ , shortly stipitate, ascus apex with a J-, refractive, 2.5–3  $\mu\text{m}$  wide and 1–1.5  $\mu\text{m}$  deep apical annulus that is situated ca. 1  $\mu\text{m}$  below the apex. Ascospores fusiform, tapering at one end and rounded at the other, rarely tapering to both ends, (34.5–)39–53.5(–62)  $\times$  2.5–3(–4)  $\mu\text{m}$ , 3-septate, not constricted at the septa, smooth-walled, hyaline, 2–3-seriate, overlapping in the ascus.

Anamorph. Colonies hairy, dark, conidiophores arising from the substratum or covering the perithecia. Setae absent. Conidiophores macronematous, mononematous, solitary, erect, unbranched, forming two layers. Conidiophores of the lower layer cylindrical, 33–55  $\mu\text{m}$  tall and 3.5–4  $\mu\text{m}$  wide in the middle, tapering to 1.5–2 (–2.5)  $\mu\text{m}$  below the collarete, straight or slightly flexuous, 0–2-septate, pale brown, sympodially proliferating. Conidiophores of the upper layer cylindrical, (88–)116–230  $\mu\text{m}$  tall and 6–7  $\mu\text{m}$  wide above the base, tapering to 2.0–2.5  $\mu\text{m}$  below the collarete, straight, 6–10-septate, thick-walled, brown to dark brown, paler upwards, usually with 1 percurrent proliferation, ending in a monophialide. Phialides terminal, integrated or intercalary, polyblastic, with 1 apical or 1–3 lateral phialidic openings arising from the sympodial proliferation. Collarettes hyaline, 4–5  $\mu\text{m}$  wide and 2–3  $\mu\text{m}$  deep. Conidia phialidic, cylindrical, centrally slightly constricted, tapering towards the ends, truncate at the base, 10.5–13.5  $\times$  3.5–5  $\mu\text{m}$ , aseptate when young, later forming 2–3 additional septa, smooth-walled, hyaline, in dry, irregular or star-like heads.

Characteristics in culture. Colonies on PCA slow-growing, reaching 3 mm diam in 10 days at 25 °C in darkness; when grown for another 10 days at 25 °C in

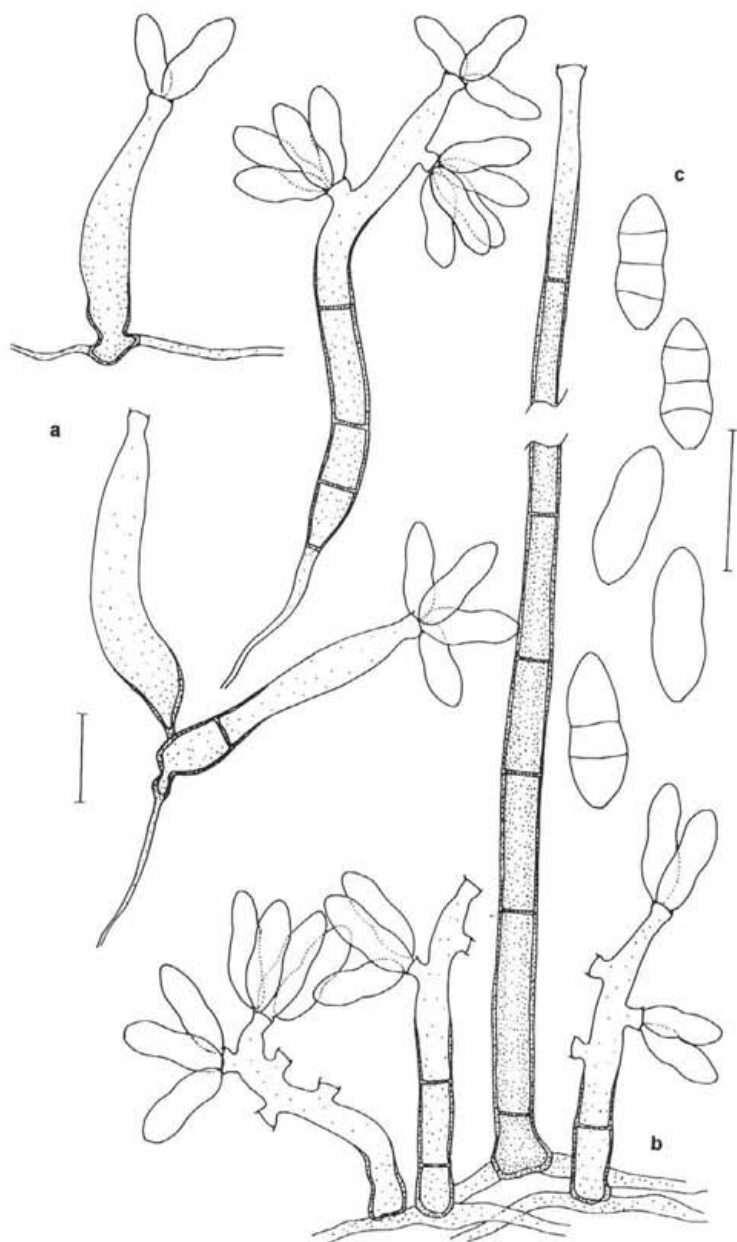


Fig. 13a-c. *Chaetosphaeria fusiformis*. - a. Conidiophores with conidia, from PCA culture. - b. Taller and shorter conidiophores with conidia forming two distinct layers, from nature. - c. Conidia, from nature. - a from CBS 101430; b, c from Herb. M. R. 862/96. - Scale bar: a-c = 10  $\mu$ m.

cool white fluorescent light reaching 4–5 mm diam; velvety, mid-brown, zonate, aerial mycelium developed, margins fimbriate; conidial production copious in 20 days, conidiophores scattered, conidial masses globose, hyaline; reverse pale brown. CMA, OA: 4–5 mm diam, velvety, zonate, aerial mycelium developed, conidial production copious in the centre in 10 days; CMA: ochraceous to pale brown; OA: mid-brown; MEA: 2–2.5 mm diam, felty, not zonate, mid-brown, aerial mycelium scant, no conidiation in 20 days. Mycelium superficial or immersed; hyphae branched, septate, subhyaline, smooth-walled, 1.5–2.5  $\mu\text{m}$  wide. Setae absent. Conidiophores as on the natural substratum, conidiophores of the lower layer (24–)30–59(–65)  $\mu\text{m}$  tall and 5–6(–7)  $\mu\text{m}$  wide above the base, tapering to 1.5–2.5  $\mu\text{m}$ ; conidiophores of the upper layer up to 136  $\mu\text{m}$  tall and 6–7  $\mu\text{m}$  wide above the base, tapering to 2.5–3  $\mu\text{m}$ . Phialides with collarettes 4–5  $\mu\text{m}$  wide and 2–3  $\mu\text{m}$  deep. Conidia 11.5–13.5(–17)  $\times$  4–5  $\mu\text{m}$ .

An upper layer of longer conidiophores was only seen in 4-months old slant cultures on PCA preserved at 18 °C under cool white fluorescent light.

Material examined. 1) Type material. Czech Republic. Moravia, Hrubý Jeseník Mts., on slopes of Mt. Mravenečník near Loučná nad Desnou, on decayed branch of *Abies alba*, 3 Aug. 1971, V. Holubová-Jechová (PRM 794008 – holotype of *Chaetosphaeria fusispora* ( $\equiv$  *Chaetosphaeria fusiformis*)).

2) Additional material. Czech Republic. Southern Bohemia, Šumava Mts., glacial cirque of the lake Černé jezero near Železná Ruda, on the inner surface of bark of *Abies alba*, 23 Oct. 1996, M. R. (Herb. M. R. 862/97, 863/97, 866/96, 867/96, 868/96, 874/96, 889/96); *ibid.*, 27 Aug. 1997, M. R. (Herb. M. R. 1042/97, 1048/97, 1049/97); *ibid.*, glacial cirque of the lake Čertovo jezero near Železná Ruda, on bark of *Abies alba*, 22 Oct. 1996, M. R. (Herb. M. R. 864/96). Moravia, Hrubý Jeseník Mts., on slopes of Mt. Mravenečník near Loučná nad Desnou, on decorticated wood of *Abies alba*, 3 Aug. 1971, V. Holubová-Jechová (PRM 794111). – Ukraine. Eastern Carpathian Mts., Kvasi near Rachiv, on the left bank of the Tisa River, on the inner side of bark on a stump of *Abies alba*, 26 June 1997, M. R. (Herb. M. R. 949/97, 958/97).

Cultures. CBS 101429 (Herb. M.R. 1048/97), CBS 101430 (Herb. M.R. 1049/97).

Descriptions and illustrations. Gams and Holubová-Jechová (1976: 45; Figs. 21a, b, 22).

Habitat. Saprobe on decayed wood and inner surface of bark of gymnosperms.

Known host. *Abies alba*.

Known distribution. Europe: Czech Republic, Ukraine.

Note. Although the conidia of *Chloridium cylindrosporum* were described as non-septate (Gams and Holubová-Jechová 1976), 3-septate conidia were seldom observed on recent material from nature. The 3-septate conidia can easily escape our



attention because they are usually attached to the lower part of the conidiophores, the surface of the perithecia or the substratum. The presence of conidial septation, although the septa are formed much later, would argue for placement of the *Chloridium cylindrosporium* anamorph of *Chaet. fusiformis* in *Cylindrotrichum*. Because no applicable and final generic concept of *Cylindrotrichum* is available and the relationships between *Cylindrotrichum* and *Chloridium* require further analysis, we refrain at the moment from making this combination. However, *Chaetosphaeria fusiformis* is considered another member of a group of related species of *Chaetosphaeria* with *Cylindrotrichum* anamorphs.

*Chaetosphaeria fusiformis* is highly host-specific and occurs seldom, exclusively on decayed wood and bark of coniferous trees. All recent collections were made in regions with natural stands of *Abies alba*.

Of *Chaetosphaeria* species with *Cylindrotrichum* anamorphs, *Chaet. abietis* and *Chaet. crustacea* also occur exclusively on coniferous wood and bark. *Chaet. fusiformis* differs from these two species in having longer, at maturity 3-septate and slightly asymmetrical ascospores that taper towards one end and are rounded at the other end, and the *Chloridium cylindrosporium* anamorph. The ascospores of *Chaet. abietis* and *Chaet. crustacea* are symmetrical and cylindrical-fusiform, 3-septate in the former and 3-5-septate in the latter.

This is the first report of successful cultivating of *Chaet. fusiformis*. The anamorph-teleomorph connection, previously suggested on the basis of the regular joint occurrence, is herewith confirmed.

7. *Chaetosphaeria tulasneorum* Réblová et W. Gams, sp. nov. Figs. 14-16.

Perithecia superficialia, ad basim modice immersa, solitaria vel pauca aggregata, subglobosa vel conica, deorsum applanata, 190-220  $\mu\text{m}$  alta, 185-210  $\mu\text{m}$  diam, minute papillata, ostiolata, fusca, glabra, modice verrucosa. Canalis ostiolaris periphysatus. Paries perithecii fragilis, ad latus et apicem scleroticus, deorsum attenuatus; paries lateralis 26-39  $\mu\text{m}$  crassus, bistratosus: stratum exterius e cellulis tenuitunicatis, fuscis, lateriformibus compositum, interius e cellulis tenuitunicatis, compressis, subhyalinis. Paraphyses copiosae, filiformes, raro septatae, ad septa haud constrictae, ramosae, anastomosantes, reticulum formantes, hyalinae, 12  $\mu\text{m}$  latae in parte inferiore, ultra ascorum apices protrudentes. Asci cylindrici vel clavati, 93.5-115  $\times$  8.5-10.5(-12.5)  $\mu\text{m}$ , sursum modice truncati, breviter stipitati, apex anulo I-, refringente, 2  $\mu\text{m}$  lato et 0.5-1  $\mu\text{m}$  alto praeditus, 8-spori. Ascosporae fusiformes, 15.5-20(-21)  $\times$  4-5(-6)  $\mu\text{m}$ , plerumque bicellulares, sero 2 alteris septis divisa, haud vel paene constrictae in medio, leves, hyalinae, 1-2-seriatae inasco.

Anamorphosis *Cylindrotrichum oligospermum* (Corda) Bonord.

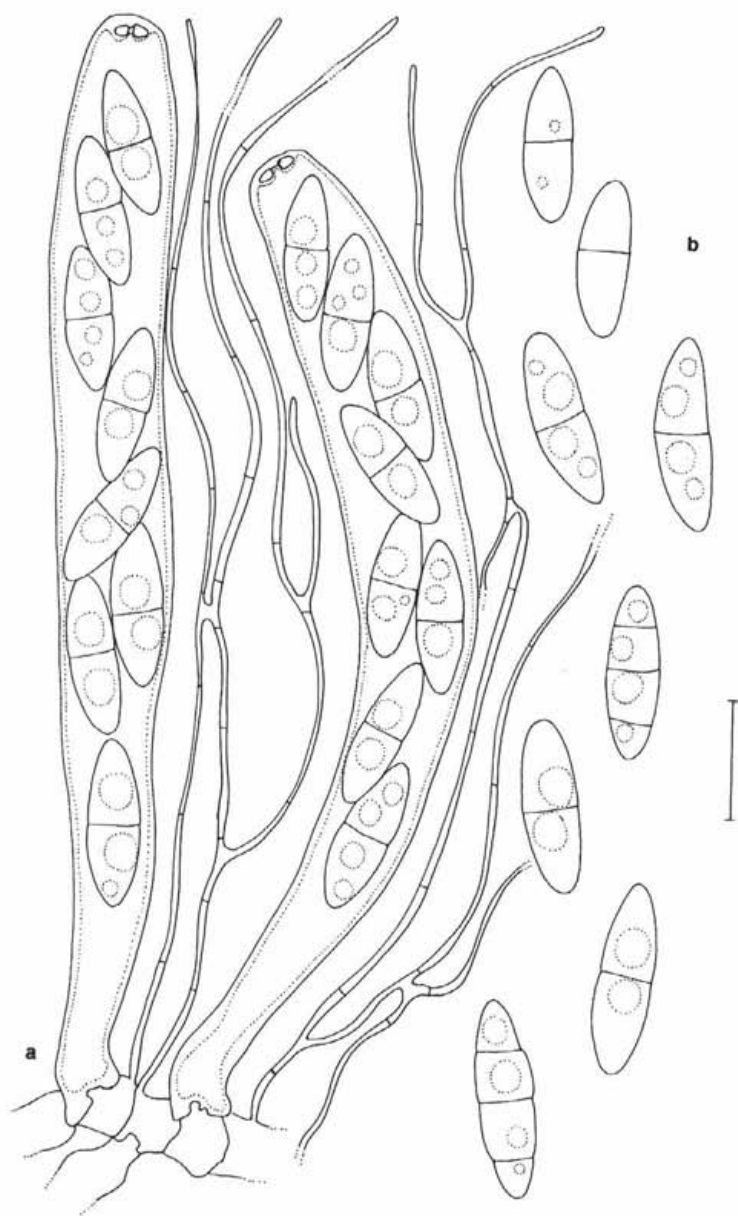


Fig. 14a, b. *Chaetosphaeria tulasneorum*. - a. Asci with ascospores and paraphyses. - b. Ascospores. - a, b from PRM 842978. - Scale bar: a, b = 10  $\mu$ m.

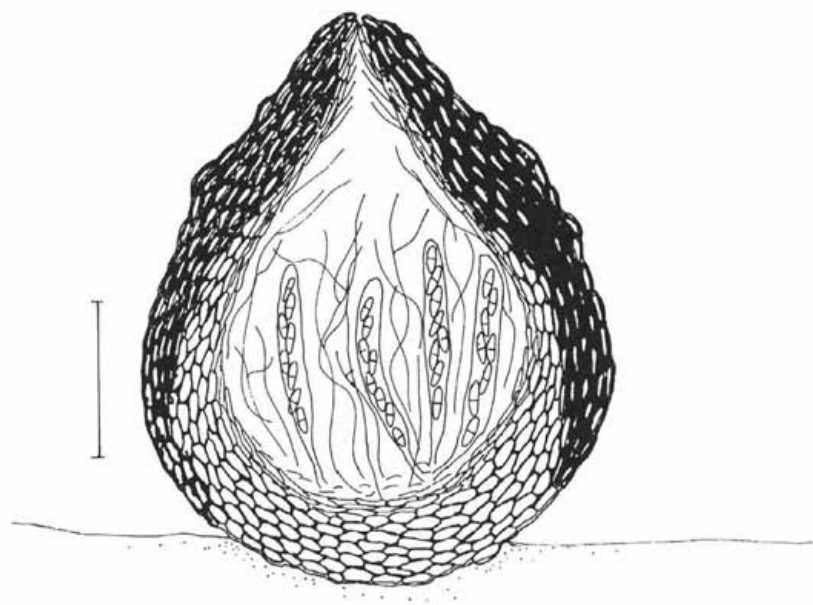


Fig. 15. *Chaetosphaeria tulasneorum*. – Median, longitudinal section of perithecialium, note the sclerotisation of the lateral perithecial wall. – From PRM 842978. – Scale bar: 50  $\mu\text{m}$ .

Holotypus. Bohemia meridio-occidentalis, montes Javornická hornatina, Strašín apud Sušice, ad lignum putridum *Sambuci nigrae*, 21 Oct. 1997, leg. M. Svrček (PRM 842978).

Anamorph. *Cylindrotrichum oligospermum* (Corda) Bonord., Handb. allg. Mykol. p. 88, 1851. Fig. 16a–e.

≡ *Menispora oligosperma* Corda, Icon. Fung. 2: 12, 1838.

= *Acrothecium deliculatum* Berk. et Broome, Ann. Mag. nat. Hist., Ser. 3, 15: 402, 1865.

≡ *Cordana deliculata* (Berk. et Broome) O. Kuntze, Rev. Gen. Pl. 2: 850, 1891.

Etymology. Honouring the Tulasne brothers, L.-R. Tulasne and C. Tulasne, who described the genus *Chaetosphaeria*.

Teleomorph. Perithecia superficial, at the base slightly immersed, solitary or in small groups, subglobose to conical, base flattened, 190–220  $\mu\text{m}$  high and 185–210  $\mu\text{m}$  diam, minutely papillate, ostiolate, dark brown, glabrous, slightly verrucose. Perithecial wall brittle, the whole wall heavily sclerotised in the upper part, sclerotisation disappearing towards the bottom and absent at the base.

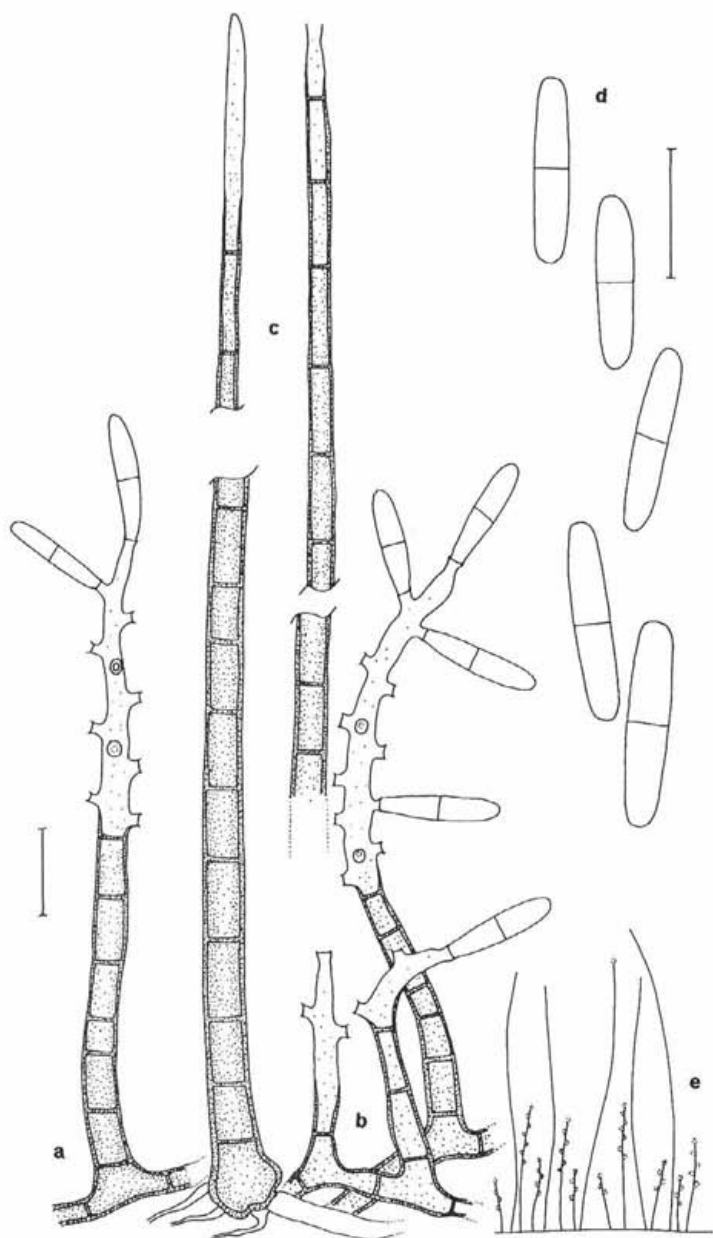


Fig. 16a-e. *Chaetosphaeria tulasneorum*. - a-c. Taller and shorter conidiophores with conidia forming two distinct layers and setae, from PCA culture. - d. Conidia, from PCA culture. - e. Habit sketch of conidiophores and setae. - a-e. from CBS 101319, PRM 842978. - Scale bar: a-d = 10  $\mu$ m.

Lateral wall 26–39  $\mu\text{m}$  thick, consisting of two layers; an outer layer of thin-walled, dark brown, brick-like cells, and an inner layer of thin-walled, compressed, subhyaline cells. Ostiolar canal periphysate. Paraphyses copious, filiform, sparsely septate, not constricted at the septa, forming a branching network, hyaline, 1–2  $\mu\text{m}$  wide, protruding beyond the tips of the asci. Asci cylindrical to clavate, 93.5–115  $\times$  8.5–10.5(–12.5)  $\mu\text{m}$ , slightly truncate at the top, shortly stipitate, ascal apex with a J-, refractive, 2  $\mu\text{m}$  wide and 0.5–1  $\mu\text{m}$  deep apical annulus, 8-spored. Ascospores fusiform, 15.5–20(–21)  $\times$  4–5(–6)  $\mu\text{m}$ , mostly 2-celled, with a delayed formation of the 2 additional septa, not constricted or slightly constricted at the median septum, smooth, hyaline, 1–2-seriate, partially overlapping in the ascus.

Anamorph. Remnants of shorter and longer conidiophores and setae with broken apices were found growing from the perithecia and from the substratum surface on a decorticated branch of *Sambucus nigra*.

Characteristics in culture. Colonies on PCA slow-growing, reaching 27 mm diam in 10 days at 25 °C in darkness, when grown for another 10 days at 25 °C in cool white fluorescent light reaching 15–16 mm diam; velvety, greyish-yellowish in the centre due to crowded conidiophores, yellowish at the margins, margins fimbriate, thin, aerial mycelium scant, conidiophores scattered, copious conidial production in 10 days, conidial masses whitish; reverse yellow-greyish. CMA, OA: 21–25 mm diam; MEA: 18 mm diam; CMA, OA, MEA: velvety, aerial mycelium scanty, conidial production copious in 10 days; CMA, OA: yellowish to pale brown in the centre due to crowded conidiophores, whitish sterile zone at the margins; MEA: whitish to pale brown in the centre due to crowded conidiophores, whitish sterile zone at the margins. Mycelium superficial or immersed; hyphae branched, septate, subhyaline, smooth, 2–3  $\mu\text{m}$  wide. Setae straight, cylindrical, 180–560  $\mu\text{m}$  tall and 3–4.5  $\mu\text{m}$  wide in the middle, tapering to 1.5–2  $\mu\text{m}$ , 5–23-septate, dark brown, paler towards the apex, narrowly rounded at the top or some developing into a monophialide. Conidiophores macronematous, mononematous, solitary, erect, unbranched, arising from both aerial and substrate mycelium, forming two layers. Conidiophores of the lower layer 33–66(–104)  $\mu\text{m}$  tall and 3.5–4.5  $\mu\text{m}$  wide in the middle, tapering to 1.5–2.5  $\mu\text{m}$  below the collarette, cylindrical, straight or slightly flexuous, 0–4-septate, subhyaline to pale brown, sympodially proliferating. Conidiophores of the upper layer 180–280  $\mu\text{m}$  tall and 3–4.5  $\mu\text{m}$  wide in the middle, paler towards the apex, tapering to 1.5–2  $\mu\text{m}$  below the collarette, cylindrical, straight or slightly flexuous, 10–12-septate, mid-brown to dark brown, less commonly sympodially proliferating at the top. Phialides integrated, terminal or intercalary, with 6–11 lateral phialidic openings arising from a sympodial elongation, fertile apices 23–47(–57)  $\times$  3.5–4(–4.5)  $\mu\text{m}$ . Collarettes hyaline, disappearing early, 2–2.5  $\mu\text{m}$  wide and 2–3  $\mu\text{m}$  deep. Conidia phialidic,

cylindrical to clavate, (9-)11-14.5(-17) × (2-) 2.5-3(-4) μm, 1-septate, not constricted, rounded at the tip, tapering and truncate at the base, smooth, hyaline.

Material examined. Type material. Czech Republic. South-western Bohemia, Javornická hornatina Mts., Strašín near Sušice, on wood of a dead branch of *Sambucus nigra*, 21 Oct. 1997, M. Svrček (PRM 842978 - holotype of *Chaetosphaeria tulasneorum*).

Culture. CBS 101319 (Herb. M. R. 1164/97).

Habitat. Saprobe on decayed wood of a shrub.

Known host. *Sambucus nigra*.

Known distribution. Europe: Czech Republic; known only from the type locality.

Note. Shape and septation of the ascospores of *Chaetosphaeria tulasneorum* resemble those of *Chaet. innumera* Berk. et Broome ex Tul. et C. Tul., *Chaet. pulviscula* (Currey) C. Booth and *Chaet. ovoidea* (Fr.) O. Constant. et al., but the ascospores are longer than those of the first and shorter than those of the two latter species. *Chaet. tulasneorum* is also distinct in the *Cylindrotrichum oligospermum* anamorph and the occurrence on decorticated wood of an angiosperm shrub. The lateral perithecial wall has a very characteristic sclerotisation that was not observed in any other *Chaetosphaeria* species.

We are not aware of any report of a *Cylindrotrichum oligospermum* anamorph isolated from ascospores of any pyrenomycetous ascomycete. The connection to the teleomorph is reported here for the first time by a culture study. The conidiophores obtained in the living culture formed two layers of taller and shorter conidiophores accompanied by much longer setae. The upper layer of conidiophores was observed neither on the natural substratum nor in culture by Gams and Holubová-Jechová (1976). In all other respects our isolate of *Cyl. oligospermum* agrees well with the observations of Gams and Holubová-Jechová (1976) and Barron (1968).

The size of setae, shorter conidiophores, phialides, collarettes and conidia are given from the original culture of *Cyl. oligospermum* obtained immediately after the isolation from ascospores. The description of taller conidiophores that have 8-11 lateral phialidic openings is given from an older culture after several transfers. In the original culture fertile apices with well-developed lateral phialidic openings were hardly seen.

#### DISCUSSION

The division of *Cylindrotrichum* into five genera, viz. *Chaetopsis*, *Dictyochaeta*, *Kylindria*, *Xenokylindria* and *Uncigera*, and their delimitation proposed by DiCosmo et al. (1983), seems to be rather schematic, apparently disregarding

natural units. *Chaetopsis grisea* (Ehrenb.) Sacc. has holoblastic conidiogenesis and a predominantly lateral branching pattern of the conidiophores unlike *Cylindrotrichum oligospermum* and is regarded as representing a distinct genus. *Uncigera cordae* Sacc. cannot be separated from *Cylindrodendrum album* Bonord. (W. Gams, unpublished), as redescribed by Buffin and Hennebert (1984). The remaining genera are very closely related indeed. *Cylindrotrichum* is closest to *Chloridium* Link: Fr., from which it is merely separated by conidial septation and shape. Conidia in *Cylindrotrichum* are 1- to multi-septate, usually cylindrical to long-ellipsoidal, straight or slightly curved and truncate at the base. Conidia of *Chloridium* are non-septate, generally shortly ellipsoidal, rarely fusiform to clavate (*Chloridium codinaeoides* Pirozynski) or shortly catenate to dacryoid [*Chloridium clavaeforme* (Preuss) W. Gams et Hol.-Jech.] or reniform (*Chloridium reniforme* Matsushima). But longer ellipsoidal conidia have also been described, e.g. in *Chloridium matsushimae* W. Gams et Hol.-Jech. (1976: 29). Thus the anamorphs of *Chaetosphaeria crustacea* and *Chaet. fusiformis* might be classified in either genus. *Cylindrotrichum* accommodates species with both sympodial and percurrent proliferation of the conidiophores (Cabello and Arambarri 1988); *Cyl. oligospermum* (Corda) Bonord., the type species of the genus, has sympodially proliferating conidiophores with numerous lateral phialidic openings, the conidiophores are accompanied by sterile setae and the conidiogenesis is multiple from different loci within a collarete. The presence of percurrent besides sympodial conidiophore proliferation would argue for distinguishing two sections within *Cylindrotrichum*, comparable with the situation in *Chloridium*. *Chloridium* was divided into three sections (Gams and Holubová-Jechová 1976), viz. species with sympodial proliferation of conidiophores with single conidiogenous loci (sect. *Psilobotrys*), and percurrent proliferation of conidiophores with multiple (sect. *Chloridium*) or single conidiogenous loci (sect. *Gongromeriza*). Species of both *Cylindrotrichum* and *Chloridium* are currently associated with *Chaetosphaeria* and *Melanopsammella* Höhnelt species (Booth 1957, 1958; Gams and Holubová-Jechová 1976; Réblová et al. 1999).

The conidiogenesis of the *Cacumisporium capitulatum* anamorph of *Chaetosphaeria decastyla* is identical to that of the *Cylindrotrichum* anamorph of *Chaet. acutata*, the *Cylindrotrichum* anamorph of *Chaet. crustacea*, and the *Chloridium virescens* (sect. *Chloridium*) anamorph of *Melanopsammella vermicularioides* (Sacc. et Roum.) Réblová et al. (Réblová et al. 1999). However, the conidiophores of *Cac. capitulatum* proliferate percurrently and the conidia are 3-septate and bicolorous. The conidia are formed successively from multiple conidiogenous loci on the sporogenous apex of the percurrently proliferating conidiogenous cell. The conidia mature after being liberated and usually remain attached to the conidiophores separately or in groups of 2-3. In the *Cylindrotrichum* anamorph of *Chaet. acutata* and the *Cac. capitulatum* anamorph of *Chaet. decastyla* the



sporogenous apex of the conidiogenous cell has a similar function and structure as in *Xenokylindria* if a generic separation were required (DiCosmo et al. 1983).

Nine genera have been described for anamorphs of *Chaetosphaeria* (Réblová et al. 1999). In the present paper *Cacumisporium* is introduced as another anamorph genus. The anamorph genera of *Chaetosphaeria* are more or less closely related to each other and require further analysis. Until this has been done, we retain the Saccardoan treatment for them. In Saccardo's (1886) scheme conidial septation was a major criterion and thus we retain *Chloridium*, *Cylindrotrichum*, and *Cacumisporium* for the time being as separate though very closely related taxa.

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## Revision von Velenovskýs Galera-Arten, die den Gattungen Conocybe und Pholiotina angehören

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Hausknecht A. (1998): Revision of Velenovský's species of the genus *Galera* which belong to the genera *Conocybe* and *Pholiotina* – *Czech Mycol.* 51: 41–70

All species of *Galera* described by Velenovský and belonging to the genera *Conocybe* and *Pholiotina* are critically revised. Of 31 species cited in Velenovský's papers many are considered dubious, the herbarium material being in a too bad state to allow a correct interpretation; in a number of cases such material is even not existing. Two species are described as new, nine new combinations are proposed and six species are reduced to synonyms.

**Key words:** Agaricales, Bolbitiaceae, *Galera*, *Conocybe*, *Pholiotina*, Velenovský, J. – Mycoflora of the Czech Republic.

Hausknecht A. (1998): Revision von Velenovskýs Galera-Arten, die den Gattungen *Conocybe* und *Pholiotina* angehören – *Czech Mycol.* 51: 41–70

Alle von Velenovský als *Galera* beschriebenen Arten, die den Gattungen *Conocybe* oder *Pholiotina* zuzuordnen sind, werden revidiert. Von den 31 in seinen Arbeiten aufgeführten Arten werden viele – meist weil das Herbariummaterial in zu schlechtem Zustand für eine korrekte Interpretation ist oder fehlt – als zweifelhaft eingestuft; zwei neue Arten werden beschrieben und neun Neukombinationen werden vorgeschlagen, sechs Arten werden als Synonyme erkannt.

Hausknecht A. (1998): Revize Velenovského druhů rodu *Galera* náležejících do rodů *Conocybe* a *Pholiotina* – *Czech Mycol.* 51: 41–70

Byly revidovány všechny druhy, které popsal Velenovský v rodu *Galera* a které dnes patří do rodů *Conocybe* nebo *Pholiotina*. Z 31 druhů, které Velenovský uvádí ve svých publikacích, mnohé autor pokládá za pochybné vzhledem k tomu, že dokladový materiál je v příliš špatném stavu, aby umožnil správnou interpretaci; v mnoha případech takový materiál vůbec neexistuje. Dva druhy jsou zde popsány jako nové, je navrženo 9 nových kombinací a 6 druhů je považováno za synonyma.

In seinen drei wichtigsten Werken (Velenovský 1920–1922, 1940, 1947) hat Velenovský über 2000 neue Arten von Basidio- und Ascomyceten beschrieben, darunter 74 Arten in der Gattung *Galera*, die in der aktuellen Systematik den Gattungen *Agrocybe*, *Conocybe*, *Galerina*, *Phaeogalera*, *Pholiotina* und in Einzelfällen sogar noch anderen Gattungen zugeordnet werden. In der vorliegenden Arbeit werden all jene Taxa, bei denen auf Grund der Beschreibung Velenovskýs, von Literaturzitatzen basierend auf früheren Revisionen oder infolge sonstiger Hinweise der Verdacht bestand, daß sie in die Gattungen *Conocybe* und *Pholiotina* gehören, kritisch untersucht. Bei einigen Herbarbelegen lagen bereits Revisionszettel von Singer und auch Svrček bei, wobei allerdings nur wenige Ergebnisse von Singer

(1989) bzw. Singer und Hausknecht (1988) bisher publiziert worden sind. Die von Svrček erzielten Resultate wurden mit den Analysen des Autors abgestimmt und sind in der vorliegenden Arbeit integriert.

Es war zu erwarten, daß der überaus fleißige und vielseitige Sammler und Neubeschreiber Velenovský auch die häufigsten Arten von *Bolbitiaceae*, die in seinem Sammelgebiet vorkommen, in Händen gehabt und sie zum Großteil als neue Arten angesehen haben mußte. Nachdem Kühner (1935) in seiner grundsätzliche *Galera*-Arbeit fast alle *Conocyben* ungültig beschrieben hat (ohne lateinische Diagnose) und diese oft erst viel später validiert wurden, haben in den meisten Fällen die von Velenovský publizierten Taxa Priorität. Die Frage war in den Einzelfällen daher vor allem, ob das derzeit noch vorhandene Material, in Abstimmung mit der makroskopischen Typusbeschreibung, eine vertretbare Interpretation zuläßt. Die von Velenovský publizierten mikroskopischen Daten sind bekannterweise zu ungenau und die Meßangaben oft unrichtig, sodaß nur die vom Autor selbst am Originalmaterial vorgenommenen mikroskopischen Untersuchungen (in Abstimmung mit den Messungen von Svrček) berücksichtigt werden durften.

Zusammenfassend ist zu bemerken, daß in der vorliegenden Arbeit zwei Arten, *Conocybe microrrhiza* Hauskn. und *Conocybe pragensis* Hauskn., neu beschrieben werden, und weiters folgende Neukombinationen vorgeschlagen werden: *Conocybe brachypodii* (Velen.) Hauskn. et Svrček, *Conocybe dumetorum* (Velen.) Svrček var. *laricina* (Kühn.) Hauskn., *Conocybe microrrhiza* var. *tetraspora* (Singer et Hauskn.) Hauskn., *Conocybe microrrhiza* var. *parvispora* (Hauskn.) Hauskn., *Conocybe pulchella* (Velen.) Hauskn. et Svrček, *Conocybe rostellata* (Velen.) Hauskn. et Svrček, *Conocybe velutipes* (Velen.) Hauskn. et Svrček, *Pholiotina rimosa* (Velen.) Hauskn. et Svrček und *Pholiotina velata* (Velen.) Hauskn. Für *Conocybe microspora* (Velen.) Dennis wird ein Neotypus benannt. Zu allen untersuchten Kollektionen werden Mikrozeichnungen vom Originalbeleg beigegeben.

#### **Galera albipes** Velen., *Novitates mycologicae* 1940: 128. Abb. 1a-d

Material: Böhmen, auf dem Hügel Budíkov, 16. 8. 1939 (PRM 153767, Holotypus).

Mikroskopische Eigenschaften: Sporen: 6,8–8,7 × 4,0–4,8 µm, im Mittel 7,8 × 4,4 µm, ellipsoidisch, nicht lentiform, dünnwandig mit deutlichem, teils über 1 µm breitem Porus, hell gelbbraun in KOH, teilweise in Tetraden. Basidien: 4-sporig, 13–18 × 6–7,5 µm. Cheilozystiden: 15–22 × 6–11 µm, mit 4–5,3 µm breitem Köpfchen. NH<sub>3</sub>-Reaktion auch nach 10 Stunden negativ. Stielbekleidung: fast nur aus lecythiformen Zystiden ähnlich den Cheilozystiden bestehend. Huthaut: hymeniform aus rundlich-gestielten Elementen.

*Galera albipes* ist konspezifisch mit *Galera brachypodii* und hätte gegenüber letzterer Priorität. Das Epitheton *albipes* kann aber in der Gattung *Conocybe* nicht verwendet werden, da *Bolbitius albipes* Otth ein früherer Name für *Conocybe lactea*

(Lange) Métrod ist und bereits gültig in *Conocybe albipes* (Othth) Hausknecht umkombiniert wurde (Hausknecht 1998). Bezüglich weiterer Bemerkungen hierzu siehe bei *Galera brachypodii*.

**Galera apala** Fr. 1821, České houby 1921: 539 (mit SW-Abbildung)

Material: nicht mehr vorhanden in PRC.

Die Beschreibung und vor allem die dazugehörige Zeichnung lassen keinen Zweifel offen, daß dies *Conocybe albipes* (Othth) Hauskn. [= *C. lactea* (Lange) Métrod] ist.

**Galera brachypodii** Velen., Novitates mycologicae novissimae 1947: 67. Abb. 1 e-h

Material: Böhmen, Mnichovice, Jidášky, 2. 9. 1941 (PRM 153778, Holotypus).

Mikroskopische Eigenschaften: Sporen: 6,8–10,3 × 4–4,8 µm, im Mittel 8,2 × 4,5 µm, ellipsoidisch, nicht plattgedrückt, dünnwandig mit deutlichem Porus (unter 1 µm breit), in KOH gelbbraun. Basidien: 4-sporig, 15–20 × 7–9 µm. Cheilozystiden: lecythiform, 17–23 × 6–12 µm, mit 3,5–5 µm breitem Köpfchen. Stielbekleidung: fast nur aus lecythiformen Zystiden ähnlich den Cheilozystiden zusammengesetzt. Huthaut: hymeniform aus rundlich-pedunculaten Elementen.

Diese Sippe ist dem Autor gut bekannt, er hat sie bisher als "zarte Form" von *Conocybe excedens* Kühn. und Watling var. *pseudomesospora* Singer und Hausknecht aufgefaßt, obwohl einige makroskopische Abweichungen vorhanden sind (siehe dazu Singer und Hausknecht 1992). Jedenfalls ist die gültige Publikation von *Galera brachypodii* älteren Datums, ganz egal ob sie sich in Zukunft als eigenständige Art, als Varietät bzw. Form oder sogar konspezifisch mit der bisherigen *C. excedens* erweisen sollte. Folgende Neukombination wird vorgeschlagen:

*Conocybe brachypodii* (Velen.) Hausknecht und Svrček, comb. nova

Basionym: *Galera brachypodii* Velen., Novitates mycologicae novissimae: 1947: 67

Synonym: *Galera albipes* Velen., Novitates mycologicae: 1940: 128

**Galera bulbosa** Velen., České houby 1921: 543. Abb. 2 a-c

Material: Böhmen, Roblín, Juli 1918 (PRC, Holotypus, ausgesondert aus Flasche 440).

Mikroskopische Eigenschaften: Sporen 7,2–10,5 × 4,4–6 µm, im Mittel 9,0 × 5,4 µm, bei einem Fragment 6,5–7,5 × 4–4,7 µm, im Mittel 7,0 × 4,4 µm, ellipsoidisch, nicht lentiform, mit leicht doppelter Wand und deutlichem Porus, relativ hell gelbbraun in KOH. Basidien: 4-sporig, 20–26 × 7–11 µm. Cheilozystiden (vom Fragment mit den größeren Sporen): lecythiform, 15–24 × 5–9 µm,

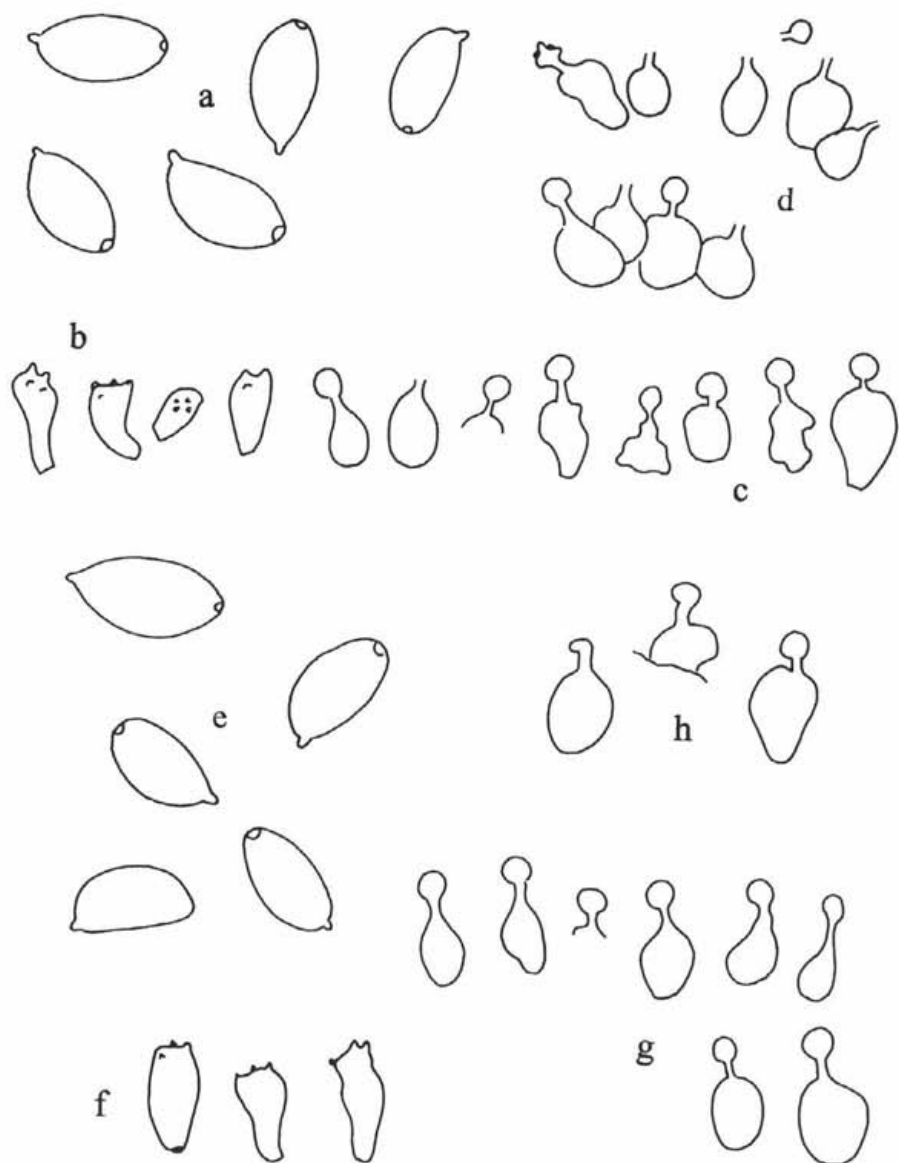


Abb. 1. a-d *Galera albipes* (PRM 153767, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Stielbekleidung,  $\times 800$ . Abb. 1. e-h *Galera brachypodii* (PRM 153778, Holotypus); e Sporen,  $\times 2000$ ; f Basidien,  $\times 800$ ; g Cheilozystiden,  $\times 800$ ; h Stielbekleidung,  $\times 800$ .



mit 3,7–4,5  $\mu\text{m}$  großen Köpfchen. Stielbekleidung: stark kollabiert, einige spindelige bis unregelmäßig länglich-rundliche Zellen vorhanden, keine lecythiformen Zystiden beobachtet. Huthaut: hymeniform aus rundlich-gestielten Elementen, 30–45  $\times$  15–24  $\mu\text{m}$ .

Der Typus könnte eine gemischte Sammlung aus zwei verschiedenen Arten beinhalten, leider ist nur mehr bei einem Fragment ein Stielstummel vorhanden. Singer (1951: 435) kombiniert die Art in *Conocybe bulbosa* (Velen.) Singer um, mit dem Hinweis auf eine mögliche Konspezifität zu *Conocybe mairei* Kühner und *Galera tenera* var. *hyalopoda* Bresadola. Diese vermutete Konspezifität mit *Pholiotina mairei* stimmt zumindest auf jenen Teil des Beleges nicht, bei dem kopfige Cheilozystiden gefunden wurden. In Anbetracht der Inhomogenität des Materials und auch seines schlechten Zustandes ist es wohl besser, *C. bulbosa* als zweifelhafte Art zu betrachten.

Eine weitere Kollektion Velenovskýs unter dem Namen *Galera bulbosa*, Mnichovice, Robinienwald, Juli 1940 (PRM 153781, Abb. 2 g-j), hat folgende Mikromerkmale: Sporen: 6,4–10  $\times$  4–5,2  $\mu\text{m}$ , im Mittel 8,0  $\times$  4,6  $\mu\text{m}$ , ellipsoidisch, dünnwandig mit deutlichem Porus (unter 1  $\mu\text{m}$  breit). Basidien: wahrscheinlich 4-sporig. Cheilozystiden: lecythiform, bis 20  $\times$  9,5  $\mu\text{m}$ , mit Köpfchen bis 5  $\mu\text{m}$ . Stiel: zwar oft kollabierte, aber deutlich lecythiforme Zystiden ähnlich den Cheilozystiden.

Diese Kollektion gehört einer ganz anderen Sektion an und hat mit Ausnahme der Sporengröße nichts mit dem Typusmaterial gemeinsam. Sie stimmt mikroskopisch exakt mit *Galera albipes* bzw. *G. brachypodii* überein.

**Galera conferta** Bolt. 1791, České houby 1921: 544, (mit SW-Abbildung).

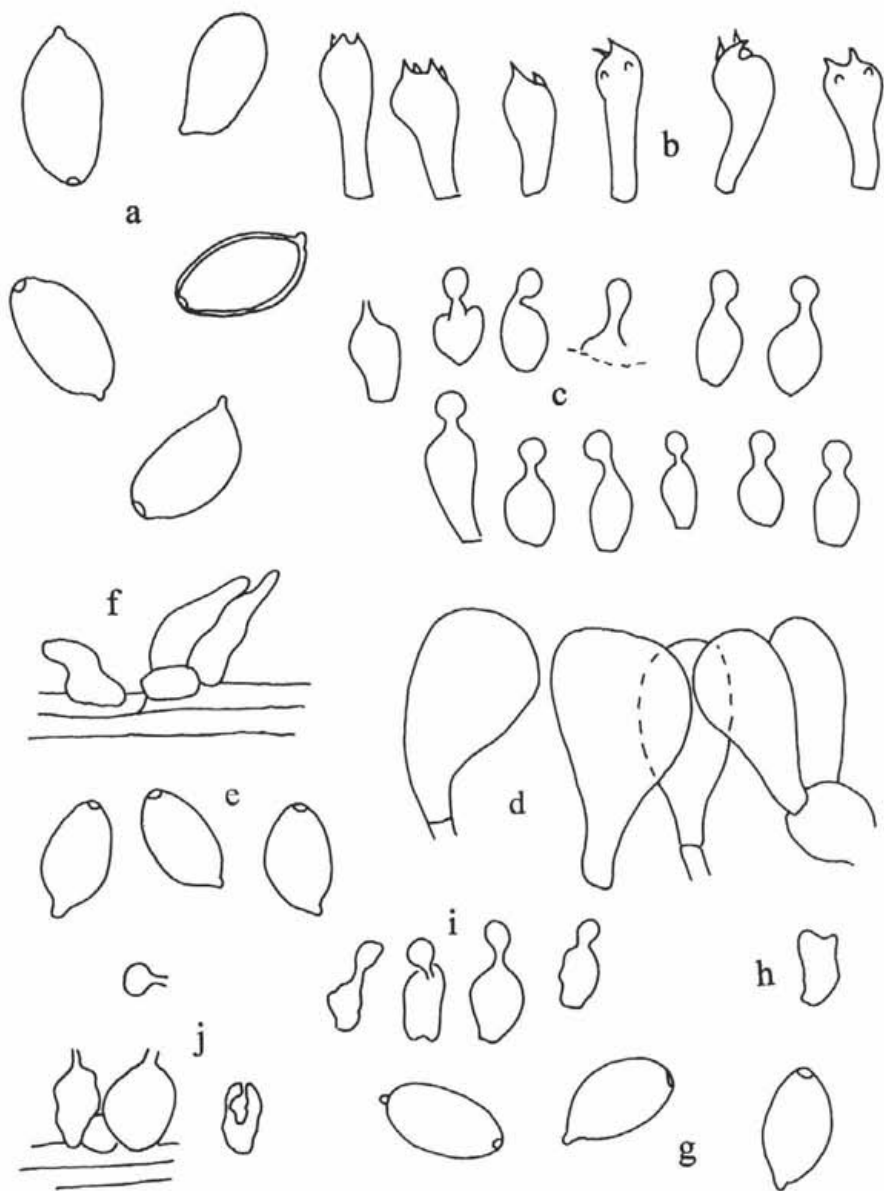
Abb. 3 a-e

Material: Böhmen, Prag, Botanischer Garten, November 1914 (PRC, Flasche 501).

Mikroskopische Eigenschaften: Sporen: 11–15,2  $\times$  7,6–9,8  $\mu\text{m}$ , im Mittel 13,7  $\times$  8,8  $\mu\text{m}$ , eiförmig-ellipsoidisch mit großem Apiculus, nicht linsenförmig breitgedrückt, extrem dickwandig (1,5–2  $\mu\text{m}$ ), mit großem, um 1  $\mu\text{m}$  breitem Porus, stumpf gelb- bis dunkelrotbraun in KOH, mit rötlicher Wand; keine Makro- und Mikrosporen untermischt. Basidien: 2-sporig, 20–26  $\times$  7–10  $\mu\text{m}$ . Cheilozystiden: lecythiform, 11–19  $\times$  4,5–8,5  $\mu\text{m}$ , mit 2,5–4,5  $\mu\text{m}$  großen Köpfchen. Stielbekleidung: nur aus lecythiformen Elementen bestehend, diese oft haarförmig dünn mit deutlichem Köpfchen, auch schlank lecythiform, 18–26  $\times$  2–9  $\mu\text{m}$ , Köpfchen mit 2–4  $\mu\text{m}$  Durchmesser. Huthaut: hymeniform, aus rundlich-gestielten Elementen (bis 32  $\times$  20  $\mu\text{m}$ ).

Diese Kollektion stellt eine eigenständige, bisher nicht beschriebene Sippe dar und wird nachfolgend als neue Art beschrieben:





**Abb. 2.** a-e *Galera bulbosa* (PRC, aus Flasche 440, Holotypus). a-d stielloses Hutfragment: a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Huthaut,  $\times 800$ . e-f Fragment mit Stielstummel: e Sporen,  $\times 2000$ ; f Stielbekleidung,  $\times 800$ . **Abb. 2.** g-j *Galera bulbosa* (PRM 153781): g Sporen,  $\times 2000$ ; h kollabierte Basidie,  $\times 800$ ; i Cheilozystiden,  $\times 800$ ; j Elemente der Stielbekleidung,  $\times 800$ .

*Conocybe pragensis* Hausknecht, spec. nova

= *Galera conferta* Bolt. ss. Velenovský 1921: 544

#### Diagnosis latina

Pileus 20–30 mm latus, campanulato-convexus, saepe flexuosus, distincte obtuse umbonatus, pallide ochraceus, non hygrophanus, non striatus. Superficies circum umbonem radiato-rugosa. Lamellae infirme adnatae, mox liberae, non latae, insigniter confertae, pallide ochraceae. Stipes (sine radice) ca. –35 mm longus, 3–5 mm latus, radice firmo, –55 mm longo, albus (radix pallide ochracea), superficies rimoso-striata pruinosa. Caro firma, pileo distincte crassa. Sporae 11–15,2 × 7,6–9,8 μm, ellipsoideae, guttiformes, non lentiformes, crassetunicatae, pariete 1,5–2 μm crasso, poro germinativo distincto (ca. 1 μm lato), ochraceo-ferrugineae usque rufobrunneae, pariete rubescente in KOH. Basidia bispora, 20–26 × 7–10 μm. Fibulae desunt. Cheilocystidia lecythiformia, 11–19 × 4,5–8,5 μm, capitulo 2,5–4,5 μm lato. Stipitipellis solum elementis lecythiformibus saepe haud ventricosis, sed anguste cylindricis distincte capitatis, 18–26 × 2–9 μm. Pileipellis hymeniformis, e cellulis sphaericis ad 32 × 20 μm latis. Ad stratum fimi, gregarius.

Typus: Bohemia, Prag, Hortus Botanicus, November 1914, J. Velenovský legit (PRC 501, holotypus).

Singer und Hausknecht (1988) glaubten zunächst, daß *Galera conferta* ss. Velenovský identisch mit *Conocybe neoantipus* (Atk.) Singer sei. Der Autor hat jedoch inzwischen nachgewiesen (Hausknecht 1996), daß der Typus von *C. neoantipus* aus wahrscheinlich drei verschiedenen Arten besteht und dieses Taxon deshalb nicht interpretierbar ist. Zusätzlich steht fest, daß die Kollektion aus Prag mit keiner der drei im Typusmaterial von *C. neoantipus* inkludierten Sippen konspezifisch sein kann.

Sie unterscheidet sich von der ebenfalls wurzelnden *Conocybe alboradicans* Arnolds und ihrer var. *carinthiaca* (Singer und Hauskn.) Hauskn. durch nicht hygrophanen, viel helleren, semmelfarbigen Hut, viel kräftigere Gestalt mit dickerem, länger wurzelndem Stiel ("Wurzel bis fünfmal länger") und als hart bezeichnetes Fleisch. Die Sporenmaße der beiden Arten sind annähernd gleich, die neue Art ist aber auf Grund der Sporen mit mehr als doppelt so dicken Wänden und viel gleichförmigerer Gestalt, Fehlen von Makrosporen und viel schlankeren und zarteren Stielzystiden mikroskopisch klar verschieden.

Es gibt in Europa bisher nur zwei Vertreter der Gattung *Conocybe*, die ähnlich langwurzelnde Stiele haben, nämlich *Conocybe watlingii* Hausknecht und *Conocybe fiorii* (Saccardo) Watling (von welcher 1997 ein Neufund gelang – siehe Hausknecht und al. 1998). Erstere wächst direkt auf Dung und hat noch größere, viel dünnwandigere Sporen, 4-sporige Basidien und eine andere

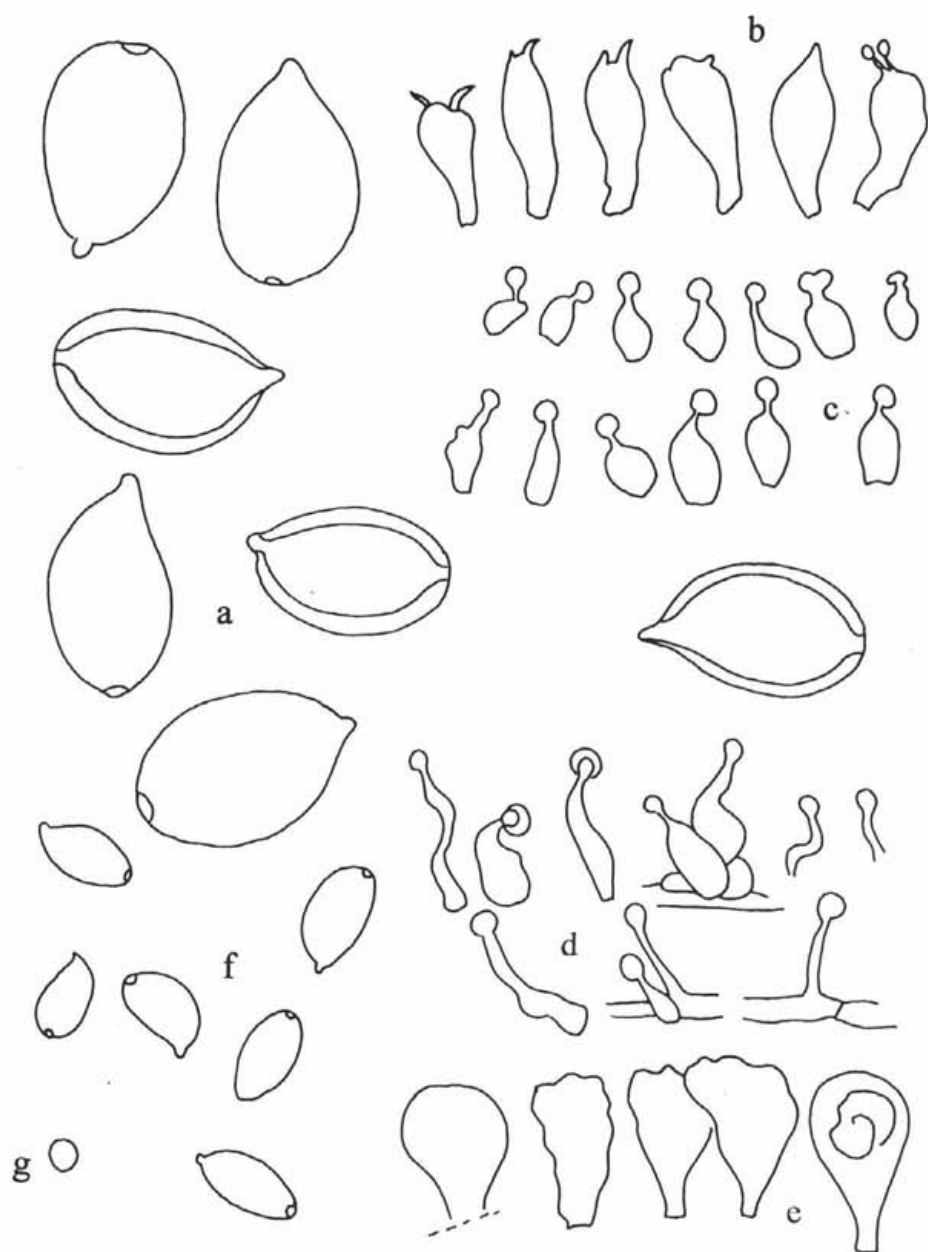


Abb. 3. a-e *Galera conferta* (PRC Flasche 501, Holotypus von *Conocybe pragensis*); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Stielbekleidung,  $\times 800$ ; e Elemente der Huthaut,  $\times 800$ . Abb. 3. f-g *Galera dumetorum* (PRC, aus Flasche 78 g, Lektotypus); f Sporen,  $\times 2000$ ; g Köpfchen einer Cheilozystide (?),  $\times 800$ .

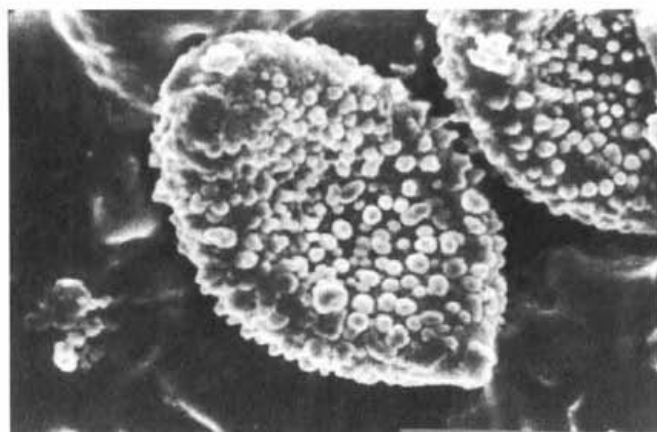


Abb. 3. h *Galera dumetorum* (PRC, aus Flasche 78 g, Lektotypus); Spore,  $\times 10000$ .

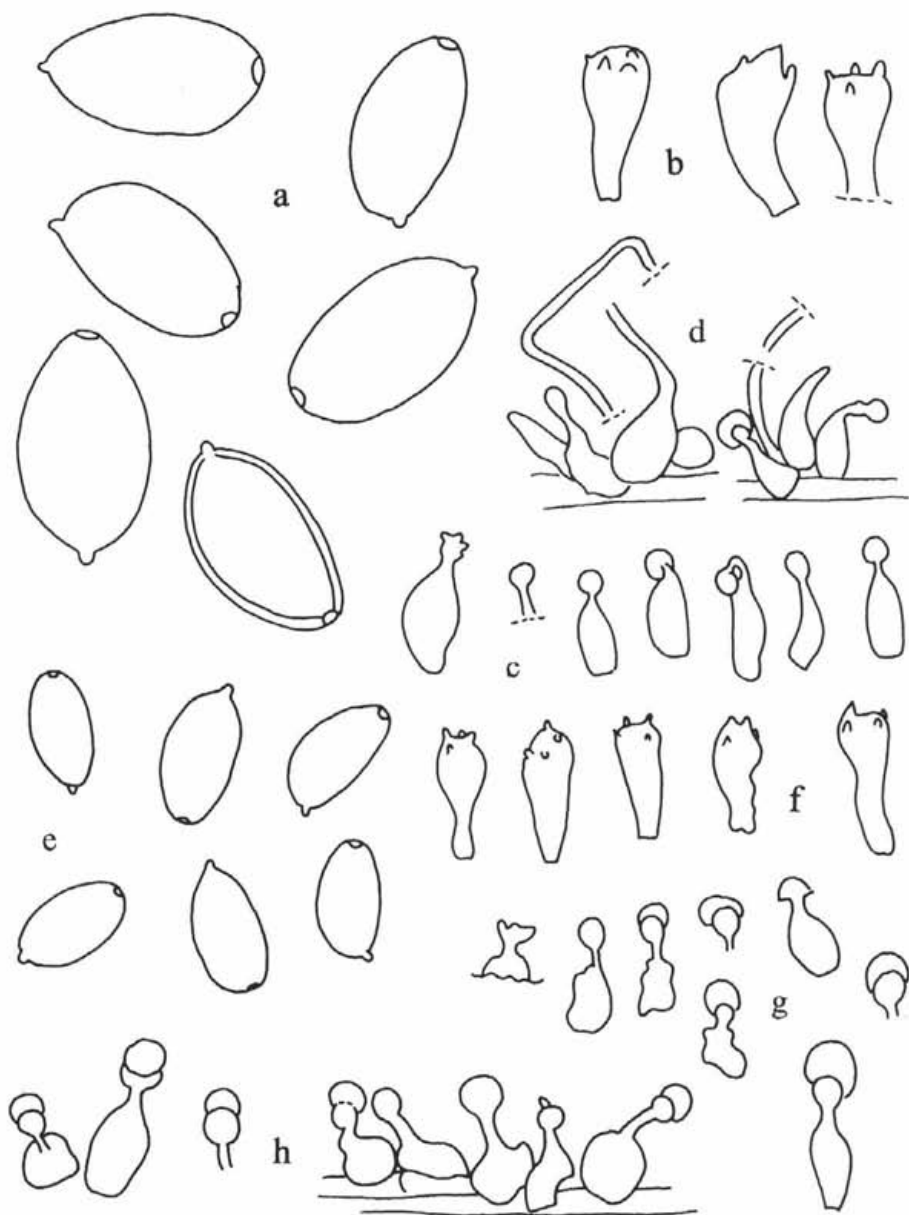
Stielbekleidung sowie eher nördliche Verbreitung (siehe dazu Hausknecht 1996: 193), die zweitgenannte Art ist bisher nur aus Italien bekannt, wächst direkt im Sand, ihre Sporen haben Wände von maximal  $0,5 \mu\text{m}$  Dicke und einen viel größeren Keimporus, weiters hat sie 2-4-sporige Basidien und überwiegend nicht-leycythiforme Zystiden am Stiel.

***Galera digitalina*** Velen., Novitates mycologicae novissimae 1947: 70. Abb. 4 a-d

Material: Böhmen, Mnichovice, bei Myšlín, bei *Epilobium angustifolium* (= *Chamerion angustifolium*), 9. 9. 1941 (PRM 153719, Holotypus).

Mikroskopische Eigenschaften: Sporen:  $12,8-15,2 \times 6,8-8,8 \mu\text{m}$ , im Mittel  $14,0 \times 7,9 \mu\text{m}$ , ellipsoidisch, nicht linsenförmig breitgedrückt, mit bis  $0,7 \mu\text{m}$  dicker Wand und  $1,2-1,5(-2,5) \mu\text{m}$  breitem Porus, in KOH rotbraun mit weinrötlicher Wand. Basidien: 4-sporig, bis  $27 \times 12,5 \mu\text{m}$ . Cheilozystiden: lecythiform,  $17-23 \times 5,5-9,5 \mu\text{m}$ , mit  $3-4,5 \mu\text{m}$  breitem Köpfchen. Stielbekleidung: vom Typ einer echten *Mixtae* mit annähernd gleichvielen lecythiformen und nicht-leycythiformen Elementen und langen Haaren. Huthaut: aus rundlich-gestielten Elementen.

Die Ergebnisse meiner Untersuchung decken sich voll mit jenen von Singer (1989), der die Art zu *Conocybe digitalina* (Velen.) Singer umkombiniert und *Conocybe subpubescens* Orton sowie *Conocybe cryptocystis* (Atk.) Singer ss. auct. als konspezifisch ansieht. Zwar werden in der Literatur für die beiden letzteren Taxa oft etwas kleinere Sporenmaße angegeben, meine zahlreichen österreichischen Kollektionen erreichen aber auch öfter die beim Typus vorgefundenen Maße.



**Abb. 4. a-d** *Galera digitalina* (PRM 153719, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Stielbekleidung,  $\times 800$ . **Abb. 4. e-h** *Galera echinata* (PRM 153744, Holotypus); e Sporen,  $\times 2000$ ; f Basidien,  $\times 800$ ; g Cheilozystiden,  $\times 800$ ; h Stielbekleidung,  $\times 800$ .

**Galera dumetorum** Velen., České houby 1921: 541. Tafel I, Abb. 3 f-h

Material: Böhmen, Mnichovice, 1918 (PRC, Lectotypus, ein ca.  $1 \times 1$  mm großes Hutfragment aus Flasche 78 g von mir aussortiert und zum Lectotypus bestimmt).

Mikroskopische Eigenschaften: Sporen  $5,7-7,5 \times 3,6-4,4 \mu\text{m}$ , im Mittel  $6,4 \times 4,0 \mu\text{m}$ , ellipsoidisch, nicht lentiform, im Lichtmikroskop mit  $\pm$  glatter Wand, im REM mit deutlicher, meist isoliert warziger Oberfläche; in KOH hell gelblicher mit dünner Wand und deutlichem, nicht truncatem Porus. Basidien: nicht gefunden. Cheilozystiden: kollabiert, ein Köpfchen mit einem Durchmesser von  $5 \mu\text{m}$  gesehen. Struktur der Stielbekleidung und Huthaut: unbekannt.

Der Typus von *Galera dumetorum* galt als verschollen. *G. dumetorum* sollte – jeweils zusammen mit einigen anderen Arten – in den Flaschen 78 und 381 aufbewahrt sein; in letzterer konnte ich nichts finden, was auf einen zarten, kleinsporigen Vertreter der Gattung *Conocybe* paßte. Nach intensiver Nachsuche in Flasche 78 g, die von E. Gerhardt nach Aufteilung des Inhalts der Flasche 78 in 6 Teile als "indet." beschriftet worden war, gelang es, ein Hutfragment zu finden, das auf Grund der Sporenform und -größe zu keiner anderen der ursprünglich in Flasche 78 aufbewahrten Arten, auch nicht zu *Galera velata* (Mikrodaten siehe dort), gehören konnte. Die an diesem Fragment festgestellten Sporen haben im REM eine typisch warzige Oberfläche (siehe Abb. 3h), wie sie unter allen Vertretern der Untergattung *Ochromarasmius* nur *C. dumetorum* in der Interpretation von Svrček (1956) und zuletzt auch Hausknecht (1995) hat (siehe dazu auch Hausknecht und Krisai-Greilhuber 1998). Somit dürfte auch der von Enderle (1996: 21) geäußerte Zweifel an der Interpretation von *C. dumetorum* ausgeräumt sein; wie aber Enderle richtig feststellt, entspricht die Sporenform in der Beschreibung von Velenovský – und auch bei Betrachtung im Lichtmikroskop – nicht der typischen *Conocybe laricina* (Kühner) Kühner; sie hat vielmehr große Ähnlichkeit mit *C. dumetorum* var. *austriaca* Hauskn., auch wenn sie um eine Spur breiter sind als beim Typus der var. *austriaca*. Im REM zeigt der Typus von *C. dumetorum* teilweise etwas spitzere, weniger abgerundete Warzen, stimmt aber insofern perfekt überein, als die Warzen fast bis zum Porus hin annähernd gleich hoch sind und nicht wie bei den beiden anderen Varietäten viel niedriger werden und in der Nähe des Porus gänzlich fehlen. Als Konsequenz ergibt sich, daß *C. dumetorum* var. *austriaca* ein Synonym von var. *dumetorum* wird und für die häufigste Varietät, bisher als var. *dumetorum* bezeichnet, folgende Neukombination zu machen ist:

*Conocybe dumetorum* (Velen.) Svrček var. *laricina* (Kühner) Hausknecht, comb. nova

Basionym: *Galera laricina* Kühner, Le Botaniste 17: 170, 1926

**Galera echinata** Velen., *Novitates mycologicae novissimae* 1947: 69. Abb. 4 e-h

Material: Böhmen, Mnichovice, Božkov, im Wald unter *Crataegus*, 21. 8. 1940 (PRM 153744, Holotypus).

Mikroskopische Eigenschaften: Sporen:  $7,2-9 \times 4-5,2 \mu\text{m}$ , im Mittel  $7,8 \times 4,5 \mu\text{m}$ , ellipsoidisch, nicht lentiform, mit dünner Wand und kleinem, aber deutlichem Porus ( $0,5-0,8 \mu\text{m}$  breit), in KOH hellbraun. Basidien: 4-sporig,  $19-24 \times 6,5-8 \mu\text{m}$ . Cheilozystiden: bis  $22 \times 8,5 \mu\text{m}$  groß, mit  $4-6 \mu\text{m}$  breiten Köpfchen (oft schwer zu messen, da alle Zystidenköpfchen Schleimkappen tragen). Stielbekleidung: fast nur aus lecythiformen Zystiden bestehend, diese  $19-25 \times 8-10 \mu\text{m}$ , mit Köpfchen  $3,5-7,5 \mu\text{m}$  breit. Huthaut: hymeniform, aus rundlichen Elementen mit langem Pedicel.

Singer (1989: 103) hat die Art in *Conocybe echinata* (Velen.) Singer umkombiniert und *Conocybe sordida* Kühn. et Watling in Synonymie gestellt. Die mikroskopischen Eigenschaften ähneln aber eher *Conocybe sordescens* Orton, mit Ausnahme der etwas zu kleinen Sporen. Bis zur Klärung aller offenen Fragen rund um den *Conocybe magnicapitata*-Komplex sehe ich aber davon ab, Synonyme zu benennen.

**Galera juniana** Velen., *Novitates mycologicae novissimae* 1947: 68. Abb. 5 a-c

Material: Böhmen, Mnichovice, Menčice, unter *Ligustrum*, 9. 6. 1941 (PRM 153717, Holotypus).

Mikroskopische Eigenschaften: Sporen:  $(8-10-11(-12) \times 5,2-6(-7) \mu\text{m}$ , im Mittel  $10,4 \times 5,7 \mu\text{m}$ , ellipsoidisch bis leicht zitronenförmig, nicht lentiform, mit doppelter Wand und  $1-1,5(-2,0) \mu\text{m}$  breitem Porus, in KOH rotbraun mit weinrötlicher Wand. Basidien: kollabiert, keine Sterigmen gefunden; Svrček konnte bei seiner Untersuchung eindeutig 2- und 4-sporige Basidien feststellen. Cheilozystiden: großteils kollabiert, insgesamt drei lecythiforme Zystiden bis  $17 \times 12 \mu\text{m}$ , mit  $3,5-6 \mu\text{m}$  breiten Köpfchen, gesehen (Svrček hat seinerzeit Cheilozystiden  $20-25 \times 8-12 \mu\text{m}$ , mit bis  $7 \mu\text{m}$  breitem Köpfchen, gemessen). NH<sub>3</sub>-Reaktion: auch nach 10 Stunden negativ. Stielbekleidung: nur aus lecythiformen Zystiden bestehend, diese  $25-35 \times 12-19 \mu\text{m}$ , mit  $6-11 \mu\text{m}$  großen Köpfchen. Huthaut: hymeniform aus rundlich-gestielten Elementen, dazwischen (laut Svrček) zahlreiche riesige, lecythiforme Pileozystiden (bis  $28 \times 16 \mu\text{m}$ , mit Köpfchen  $8-10 \mu\text{m}$ ).

Diese Kollektion ist dem *Conocybe magnicapitata*-Komplex zuzuordnen. Das Typusmaterial besteht aus einem teilweise zerbrochenem Fruchtkörper in sehr schlechtem Zustand, weshalb ich eine Umkombination in *Conocybe* vorerst unterlasse. *C. magnicapitata* Orton weicht jedenfalls makroskopisch etwas ab, und auch mikroskopisch fehlen zu viele Informationen bzw. bestehen zu große Differenzen gegenüber *Galera juniana*, um Konspezifität zu behaupten. Sollte sich bei einer



genauen Durchleuchtung dieses Komplexes herausstellen, daß *C. magnicapitata* wesentlich weiter und variabler aufzufassen ist, als dies beim reinen Vergleich von *G. juniana* mit dem Typusmaterial von *C. magnicapitata* derzeit geschieht, müßte man wohl doch nomenklatorische Konsequenzen ziehen.

**Galera lateritia** Fr. 1821, České houby 1921: 539.

Material: existiert nicht.

Die makroskopische Beschreibung und das Vorkommen auf Kompost lassen darauf schließen, daß es sich um einen Vertreter der *Conocybe pubescens*-Gruppe handelt.

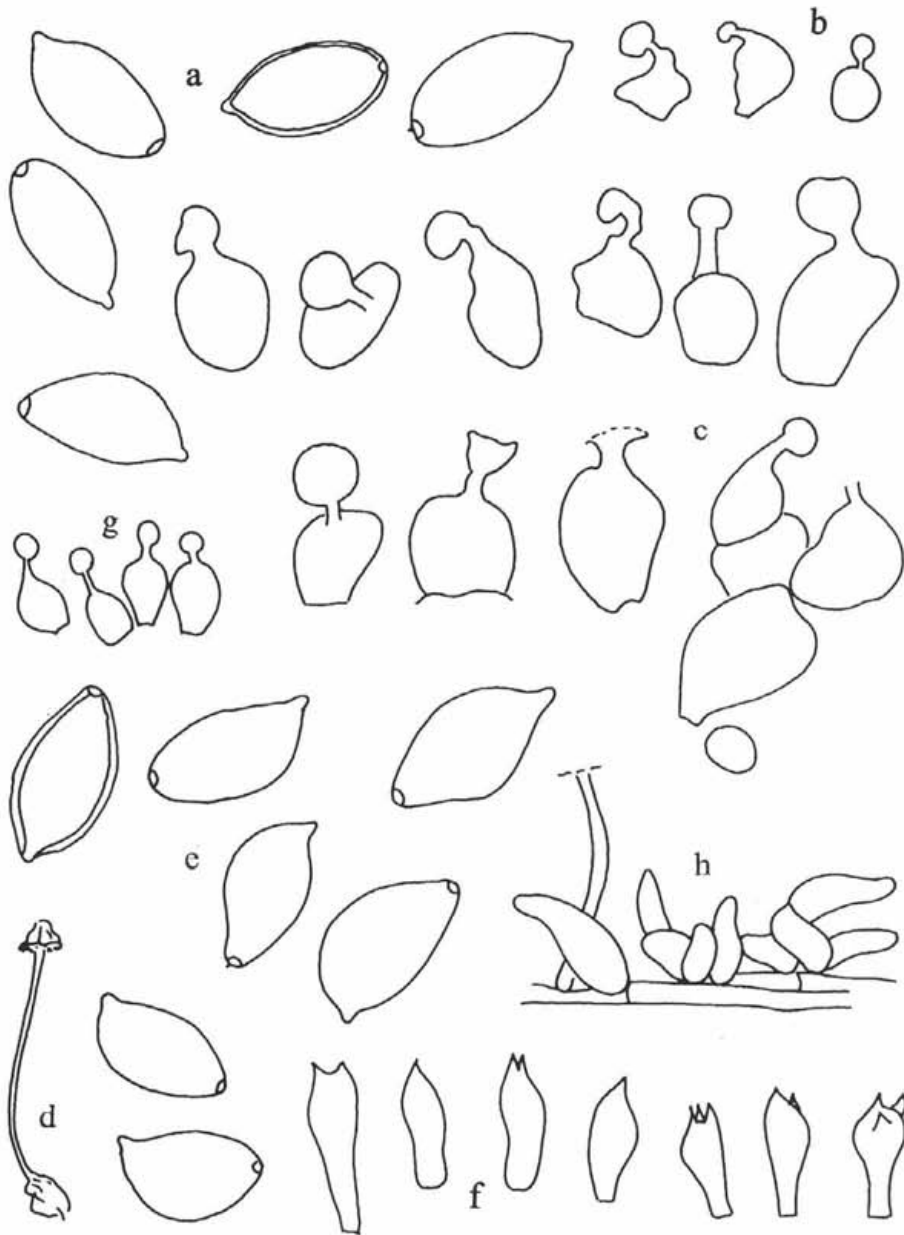
**Galera leporina** Velen., Novitates mycologicae novissimae 1947: 68. Abb. 5 d-h

Material: Böhmen, Mnichovice, auf Hasendung im Nadelwald, Juli 1940 (PRM 153750, Holotypus).

Zusätzlich untersuchtes Material: Österreich: Niederösterreich, Hainfeld, Michelbach Dorf (MTB 7960/2), 20. 5. 1984, leg. W. Klofac (WU 18015); – Hainfeld, Michelbach Markt (MTB 7860/4), 2. 5. 1988, leg. W. Klofac (WU 18016).

Mikroskopische Eigenschaften (nur vom Typus): Sporen: 9,2–12(–14,8) × 5–6,7 (–8,5) µm, im Mittel 11,8 × 6,1 µm, ellipsoidisch-zitronenförmig, nach beiden Polen zuspitzend, kaum lentiform, mit doppelter Wand und deutlichem, oft papilliertem, ca. 1 µm breitem Porus, kräftig gelbbraun in KOH. Basidien: (1–)2–(3–, 4–) sporig, davon 30–50 % 3– oder 4-sporig, 17–27 × 7–9 µm. Cheilozystiden: lecythiform, 15–18 × 6–8,5 µm, mit 3,5–4,5 µm breitem Köpfchen. Stielbekleidung: ziemlich kollabiert und von parasitischem Pilz befallen; einige haarförmige und rundliche bis länglich-spindelige Elemente (bis 24 × 8 µm) und an der obersten Stielspitze auch mehrere kopfige Zystiden gesehen. Huthaut: hymeniform aus rundlichen bis rundlich-gestielten Elementen.

Das Typusmaterial besteht aus einem ganzen Fruchtkörper, der offensichtlich im Substrat wurzelt. Im ersten Augenblick kam ich zu der Annahme, daß es sich auf Grund der variablen Sterigmenanzahl der Basidien und der dadurch bedingten sehr unterschiedlichen Sporengröße um ein etwas gestörtes Exemplar handeln könnte. Zwei Kollektionen aus Niederösterreich, beide aus sehr feuchtem Mischwald mit Fichte, Weide, Hainbuche und Erle, stimmen makro- und vor allem mikroskopisch (davon eine Aufsammlung allerdings mit überwiegend 4-sporigen Basidien) mit Velenovskýs Art so gut überein, daß ich nunmehr überzeugt bin, daß die Funde von offenen, wärmebegünstigten Stellen mit rein 2- oder 4-sporigen Basidien, viel breiteren oder kleineren, anders geformten Sporen und gänzlich fehlenden lecythiformen Zystiden am Stiel (auch nicht an der Spitze), also *Conocybe leporina* ss. Singer und Hausknecht 1988, eine selbständige Art darstellen.



**Abb. 5. a-c** *Galera juniana* (PRM 153717, Holotypus); a Sporen,  $\times 2000$ ; b Cheilozystiden,  $\times 800$ ; c Stielbekleidung,  $\times 800$ . **Abb. 5. d-h** *Galera leporina* (PRM 153750, Holotypus); d Fruchtkörper (Exsikkat),  $\times 0,8$ ; e Sporen,  $\times 2000$ ; f Basidien,  $\times 800$ ; g Cheilozystiden,  $\times 800$ ; h Stielbekleidung,  $\times 800$ .

*Galera leporina* wurde von Singer und Hausknecht (1988) gültig in *Conocybe leporina* (Velen.) Singer und Hausknecht umkombiniert, auch wenn die dort angefügte Beschreibung nunmehr einer anderen Sippe zugeordnet werden muß. Für die Funde aus Trockenrasen und warmen, offenen Stellen ist eine Neubeschreibung notwendig, welche ich nachfolgend gebe:

*Conocybe microrrhiza* Hausknecht, spec. nova  
(= *Conocybe leporina* ss. Singer et Hausknecht 1988)

Diagnosis latina

Pileus 6–22 mm latus, 6–15 mm altus, acute conicus, obtuse conicus ad late convexus, primum brunneus ad obscure brunneus rubrotinctus, interdum luteo-brunneus ad fulvobrunneus, hygrophanus, in statu humido striatus, superficies glabra. Lamellae anguste adnatae, confertae, ventricosae, luteobrunneae, deinde ferrugineae. Stipes 30–50 mm longus, 1–2 mm latus, radicans, primum albus, mox luteolus, demum melleus ad brunneus, superficies primum pruinosa, demum glabra. Caro stipitis firma, odor nullus. Sporae (9,0–)10,3–12,0 × 7,3–7,8 × 7,0–7,5 µm, late ellipsoideae, leviter guttiformes, saepe leviter lentiformes, pariete glabro crassiusculoque, poro germinativo 1 µm lato. Basidia 16–20 × 8–10 µm, bispora. Cheilocystidia lecythiformia, 18–25 × 8–10 µm, capitulo 2,7–4 µm lato. Reactio ammoniac nulla. Stipitipellis cystidiis non-lecythiformibus (10–25 × 6–9 µm) et pilis (30–80 × 2–3 µm) consistens, cystidia lecythiformia desunt. Pileipellis hymeniformis, cellulis sphaeropedunculatis (22,5–31–50 × 17–22 µm) consistens, parce cystidiis lecythiformibus cheilocystidiis similibus immixtis. In pratis siccis, locis apertis.

Typus: Austria: Vindobona, Lobau, Kreuzgrundtraverse (MTB 7865/1), 29. 9. 1984, A. Hausknecht legit (WU 5222, holotypus).

Weiters ergeben sich daraus folgenden Neukombinationen:

*Conocybe microrrhiza* var. *tetraspora* (Singer et Hausknecht) Hausknecht, comb. nova

Basionym: *Conocybe leporina* var. *tetraspora* Singer und Hausknecht, Pl. Syst. Evol. 159: 112, 1988

*Conocybe microrrhiza* var. *parvispora* (Hausknecht) Hausknecht, comb. nova

Basionym: *Conocybe leporina* var. *parvispora* Hausknecht, Österr. Z. Pilzk. 5: 190, 1996

**Galera major** Velen., České houby 1921: 540.

Material: existiert nicht.

Die Art wurde von Singer (1951: 439) als "species incertae sedis" eingestuft und in *Conocybe major* (Velen.) Singer umkombiniert. Die Beschreibung der Makro-

und Mikromerkmale von Velenovský (Hut bis 4 cm, Stiel weiß, bis 0,4 cm dick, Zystiden mit gestieltem Köpfchen, aber Sporen nur bis 10  $\mu\text{m}$ ), läßt m. E. keine eindeutige Identifizierung zu; *Conocybe major* ist demnach als nomen dubium anzusehen.

**Galera melea** Velen., České houby 1921: 541. Abb. 6 a-d

Material: Böhmen, Chuchle bei Prag, auf Kuh- und Pferdedung, Juli 1918 (PRC, Flasche 381/5, von E. Gerhardt aus Flasche 381 separiert). Durch die Aufbewahrung in Äthanol gänzlich entfärbt und in sehr schlechtem Zustand. Typusmaterial aus Mnichovice nicht mehr vorhanden.

Mikroskopische Eigenschaften: Sporen: 15–18  $\times$  9,6–11,2  $\times$  8,7–9,6  $\mu\text{m}$ , im Mittel 16,6  $\times$  10,4  $\times$  9,1  $\mu\text{m}$ , ellipsoidisch, linsenförmig plattgedrückt, dickwandig mit großem Porus, von Äthanol total entfärbt und die Außenwand teilweise aufgelöst. Basidien: kollabiert, die Anzahl der Sterigmen nicht feststellbar. Cheilozystiden: völlig kollabiert, nicht mehr gefunden. Stielbekleidung: einige rundliche, keulige bis haarförmige Elemente vorhanden, komplette lecythiforme Zystiden nicht gesehen. Huthaut: hymeniform aus rundlich-gestielten Elementen, ca. 25–33  $\times$  22–26  $\mu\text{m}$ , bestehend.

Singer (1951) hat die Art in *Conocybe melea* (Velen.) Singer umkombiniert. Der vorgefundene Beleg betrifft zweifellos einen Vertreter der *C. pubescens*-Gruppe, auf Grund der nicht mehr intakten Basidien und Stielbekleidung ist eine Zuordnung auf eine innerhalb dieses Komplexes derzeit unterschiedenen Arten nicht möglich und *C. melea* daher als zweifelhafte Art zu betrachten.

**Galera microcephala** Velen., České houby 1921: 543. Abb. 6 e-h

Material: Böhmen, Mnichovice, April 1918 (PRC, Lectotypus, separiert von mir in ein eigenes Gefäß aus Flasche 92, wo er zusammen mit anderen Pilzen aufbewahrt worden war). Die Art sollte auch in Flasche 352 vorhanden sein, ich konnte aber nichts finden, was der Beschreibung Velenovskýs entspricht.

Mikroskopische Eigenschaften: Sporen: 9,5–11,5  $\times$  5–6  $\mu\text{m}$ , im Mittel 10,3  $\times$  5,5  $\mu\text{m}$ , ellipsoidisch-zitronenförmig, nicht lentiform, mit leicht doppelter Wand und deutlichem Keimporus (ca. 1  $\mu\text{m}$ ), in KOH blaßgelb. Basidien: 4-sporig, 18–24  $\times$  8–11  $\mu\text{m}$ . Cheilozystiden: lecythiform, 15–20  $\times$  6–12  $\mu\text{m}$ . Stielbekleidung: ziemlich kollabiert, einige rundliche bis keulig-rundliche Elemente und eine lecythiforme Zystide unmittelbar unterhalb des Lamellenansatzes vorhanden. Huthaut: hymeniform aus rundlich-gestielten Elementen.

Singer (1951) nahm die Umkombination zu *Conocybe microcephala* (Velen.) Singer vor. Die Art gehört zweifelsohne in den bisher von mir noch nicht kritisch durchleuchteten Komplex um *C. sienophylla* (Berk. et Br.) Singer, auch eine

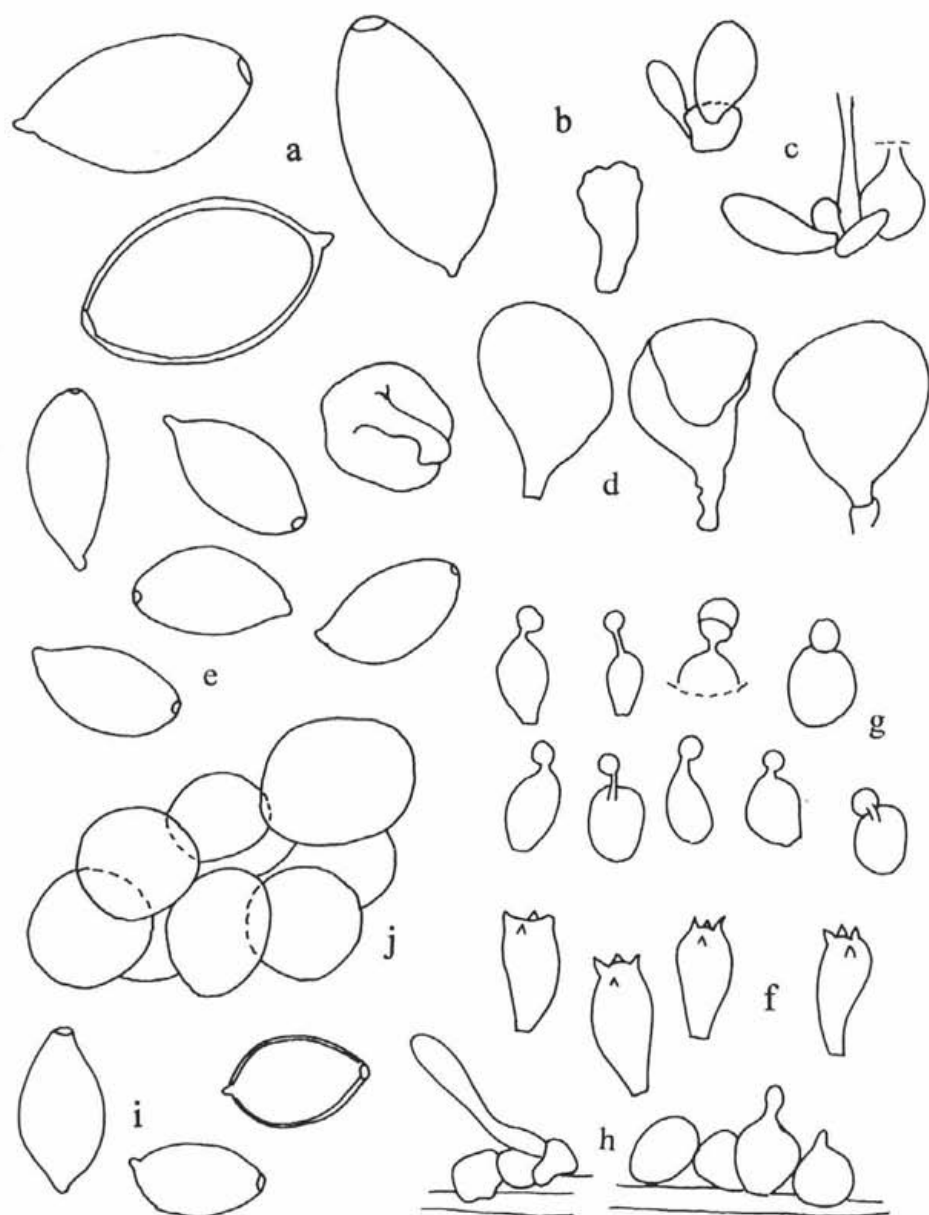


Abb. 6. a-d *Galera melea* (PRC Flasche 381/5); a Sporen,  $\times 2000$ ; b kollabierte Basidie,  $\times 800$ ; c Elemente der Stielbekleidung,  $\times 800$ ; d Elemente der Huthaut,  $\times 800$ . Abb. 6. e-h *Galera microcephala* (PRC Flasche 92, Lektotypus); e Sporen,  $\times 2000$ ; f Basidien,  $\times 800$ ; g Cheilozystiden,  $\times 800$ ; h Stielbekleidung,  $\times 800$ . Abb. 6. i-j *Galera sordida* (PRC Flasche 381/6); i Sporen,  $\times 2000$ ; j Huthaut (Aufsicht),  $\times 800$ .

Verwandtschaft zu *Conocybe moseri* Watling (Hut: "vertice atrofusco") ist nicht auszuschließen. Der schlechte Zustand des Beleges, vor allem der Stieloberfläche, erlaubt es aber nicht, eine Synonymie zu letzterem Taxon zu vermuten, sodaß ich *C. microcephala* zu den zweifelhaften Arten zähle.

**Galera microspora** Velen., České houby 1921: 543.

Material: Böhmen, Radotín, 1918 (PRC, laut Etikett in Flasche 400). Die Flasche ist ausgetrocknet und trotz intensiver Suche konnte nichts gefunden werden, was auf eine *Conocybe* mit kleinen Sporen hindeutet.

Svrček (1983) hat fast an derselben Stelle wie Velenovský im Jahr 1956 eine *Conocybe* gefunden, die ausgezeichnet auf die Originalbeschreibung paßt. Da der Typus in PRC nicht mehr vorhanden ist, schlage ich die Kollektion von Svrček, 17. 6. 1956, Prag-Radotín (PRM), als Neotypus vor.

Laut Singer und Hausknecht (1992) wurde *G. microspora* von Dennis (1953) korrekt zu *Conocybe microspora* (Velen.) Dennis umkombiniert, obwohl das zugrundeliegende Material eine andere Art darstellte, nämlich *Conocybe microsperma* Singer 1992. Die von Svrček (1983) vorgenommene Neukombination war daher überflüssig.

**Galera pulchella** Velen., České houby 1921: 543. Abb. 7 a-d

Material: Böhmen, Mnichovice, im Rasen, Juli 1919 (PRM 678157, Holotypus).

Mikroskopische Eigenschaften: Sporen: (12-)12,7-15,8 × 7,6-9,3(-10) µm (Riesensporen bis 19 µm lang), im Mittel 14,6 × 8,6 µm, ellipsoidisch, leicht linsenförmig, mit doppelter, dicker Wand und großem (1,5-2,5 µm breitem) Keimporus, in KOH kräftig rotbraun mit weinroter Wand. Basidien: 4-sporig, 22-30 × 12-17 µm. Cheilocystiden: lecythiform, 17-22 × 6-9 µm, mit 3,5-5 µm großem Köpfchen. Stielbekleidung: einer echten *Mixtae*, aus lecythiformen Zystiden (bis 37 × 13 µm), nicht-lecythiformen Elementen und Haaren zusammengesetzt. Huthaut: hymeniform aus rundlich-gestielten Elementen.

Das gut erhaltene Typusmaterial und die makroskopische Beschreibung erlauben eine eindeutige Bestimmung als das, was bisher in der Literatur *Conocybe pseudopilosella* Kühner et Watling bezeichnet worden ist. Da Velenovskýs Art Priorität hat, ist folgende Neukombination notwendig:

*Conocybe pulchella* (Velen.) Hausknecht et Svrček, comb. nova

Basionym: *Galera pulchella* Velen., České houby 1921: 543

Synonyme: *Conocybe pubescens* var. *pseudopilosella* Kühner 1935, ungültig publiziert

*Conocybe pseudopilosella* Kühner et Watling 1980.

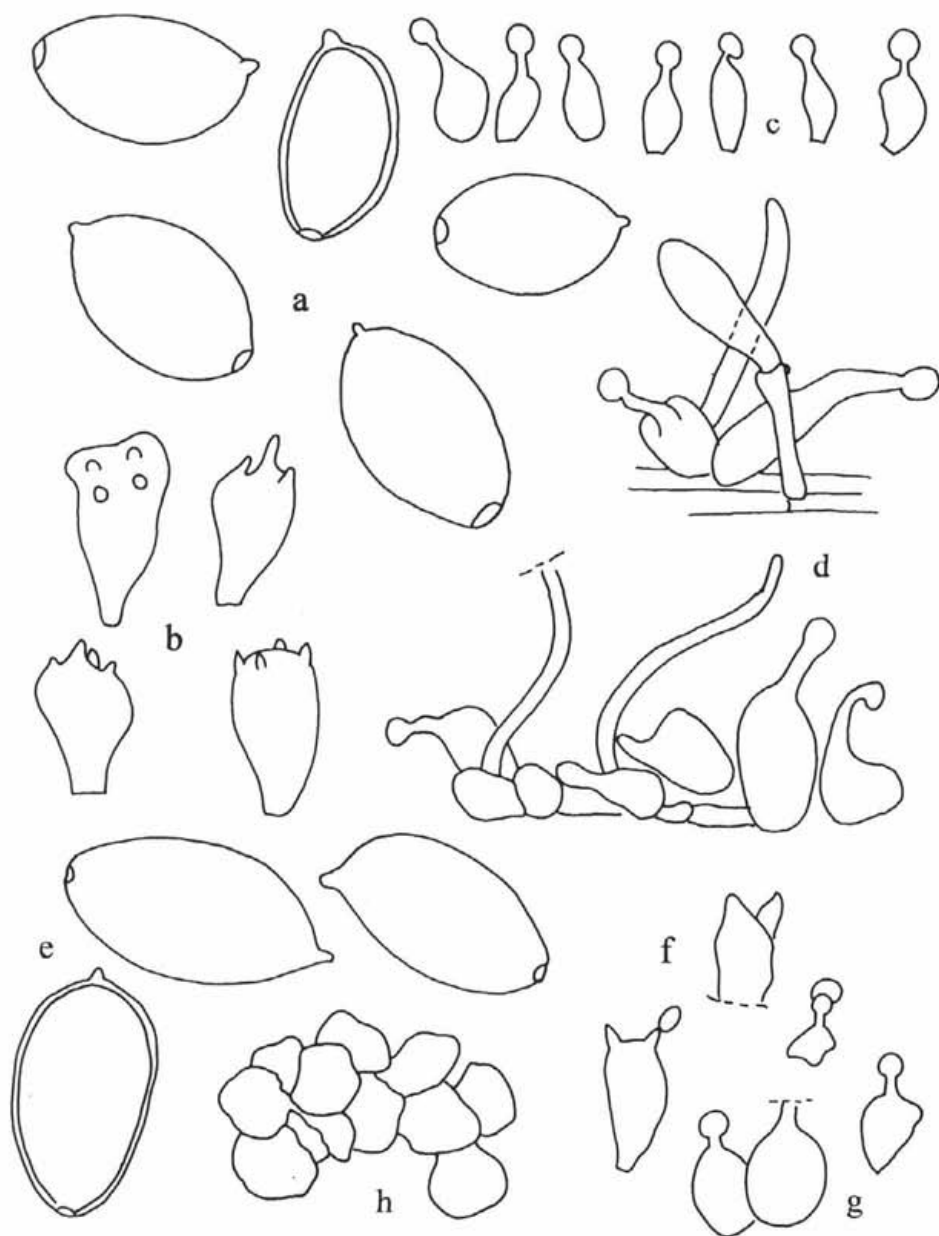


Abb. 7. a-d *Galera pulchella* (PRM 678157, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Stielbekleidung,  $\times 800$ . Abb. 7. e-i *Galera reticulata* (PRC, aus Flasche 435, Holotypus); e Sporen,  $\times 2000$ ; f Basidien,  $\times 800$ ; g Cheilozystiden,  $\times 800$ ; h Huthaut (Aufsicht, kollabiert),  $\times 800$ .



**Galera pygmaeoaffinis** Fr. 1863, České houby 1921: 542.

Material: nicht vorhanden.

Die Erwähnung von Zystiden mit langgestielten Köpfen von 3  $\mu\text{m}$  und satt gelben, großen Sporen in Verbindung mit dem Habitat (Pferde- und Rinderexkrementen) lassen fast nur die Möglichkeit einer *Conocybe* zu. Mit einiger Wahrscheinlichkeit handelt es sich um *Conocybe rickenii* (Schaeff.) Kühner mit etwas zu groß angegebenen Sporen – Fruchtkörpergröße, Hutfarbe und fehlende Riefung würden passen.

**Galera reticulata** Velen., České houby 1921: 542. Abb. 7 e-i

Material: Böhmen, Mnichovice, im Gras, Juli 1918 (PRC, Holotypus, von mir ausgeschieden aus Flasche 435 und in ein separates Gefäß überführt). Die Flasche war ausgetrocknet, das Material (aus verschiedenen Pilzen bestehend) zusammengeklebt und völlig hart und spröde.

Mikroskopische Eigenschaften: Sporen: 14,5–18,5  $\times$  7,9–9,6  $\mu\text{m}$ , im Mittel 16,1  $\times$  8,8  $\mu\text{m}$ , ellipsoidisch, teilweise leicht eckig, nicht lentiform, mit über 0,5  $\mu\text{m}$  dicker, doppelter Wand und 1–1,3  $\mu\text{m}$  großem Porus, in KOH olivlich gelbbraun (verfärbt?). Basidien: 2-sporig, ca. 22  $\times$  10  $\mu\text{m}$ , mit breiten, fast dreieckigen Sterigmen. Cheilozystiden: lecythiform, bis 25  $\times$  13,5  $\mu\text{m}$ , mit 3,5–5  $\mu\text{m}$  großen Köpfchen. Stielbekleidung: nicht feststellbar, das Fragment enthielt nur einen völlig kahlen Stielstummel. Huthaut: hymeniform aus rundlichen, stark geschrumpften Zellen.

Kühner (1935: 123) zitiert *G. reticulata* im Zusammenhang mit *Conocybe lateritia* (= *C. lactea* = *C. albipes*) und spricht davon, daß sich erstere nur durch den stark netzig gezeichneten Hut unterscheidet; in seinem provisorischen Schlüssel stellt Singer (Singer, ined.) *Galera reticulata* mit Fragezeichen ebenfalls zu *Conocybe lactea*. Die Sporen der ersteren Art sind aber wesentlich größer, dunkler gefärbt und auch etwas dickwandiger, sodaß ich eine nahe Verwandtschaft der beiden Sippen nicht für möglich halte. Ich kenne allerdings Aufsammlungen einer *C. albipes* mit ausgebreitetem, grubig-netzig gezeichnetem Hut aus Deutschland und den Niederlanden (var. *rugata* Hauskn., siehe Hausknecht 1998), die recht gut zur makroskopischen Beschreibung Velenovskýs passen würden, nur sind deren Sporen sogar noch etwas kleiner als bei *C. albipes*, und eine Konspezifität ist daher auszuschließen; *Galera reticulata* ist deshalb für mich auch eine zweifelhafte Art.

**Galera rimosa** Velen., Novitates mycologicae 1940: 130. Abb. 8 a-d

Material: Böhmen, Mnichovice, Jidášky, im Gras, September 1934 (PRM 153772, Holotypus).

Mikroskopische Eigenschaften: Sporen:  $7,2-9,1 \times 4,4-5,6 \mu\text{m}$ , im Mittel  $8,4 \times 5,2 \mu\text{m}$ , ellipsoidisch mit dünner, einfacher Wand und deutlichem Keimporus ( $1-1,2 \mu\text{m}$  breit), hell gelbbraun in KOH. Basidien: 4-sporig,  $16-20 \times 6-8 \mu\text{m}$ . Cheilozystiden:  $29-55 \times 6,5-9,5 \mu\text{m}$ , unregelmäßig zylindrisch, gegen die Basis zu bauchig verdickt, oft auch unregelmäßig wellig und mit leicht kopfig angeschwollener Spitze. Huthaut: aus rundlich-gestielten Elementen ( $35-50 \times 17-25 \mu\text{m}$ ).

Diese Kollektion stellt eine makroskopisch etwas untypische ("centro depresso") *Pholiotina sulcatipes*/*P. aberrans* dar, da ja die Frage, ob man in Europa wirklich zwei Arten unterscheiden kann, noch immer nicht geklärt ist (siehe zuletzt Krisai-Greilhuber und al. 1997: 176, Enderle 1997). Für den Fall, daß es in Europa nur eine Art, *Pholiotina aberrans* (Kühner) Singer, gibt und diese nicht konspezifisch mit der nordamerikanischen Sippe ist, hätte Velenovskýs Art Priorität. Ich schlage daher folgende Neukombination vor:

*Pholiotina rimosa* (Velen.) Hausknecht et Svrček, comb. nova

Basionym: *Galera rimosa* Velen., Novitates mycologicae 1940: 130

**Galera rostellata** Velen., Novitates mycologicae 1940: 129. Abb. 9 a-e

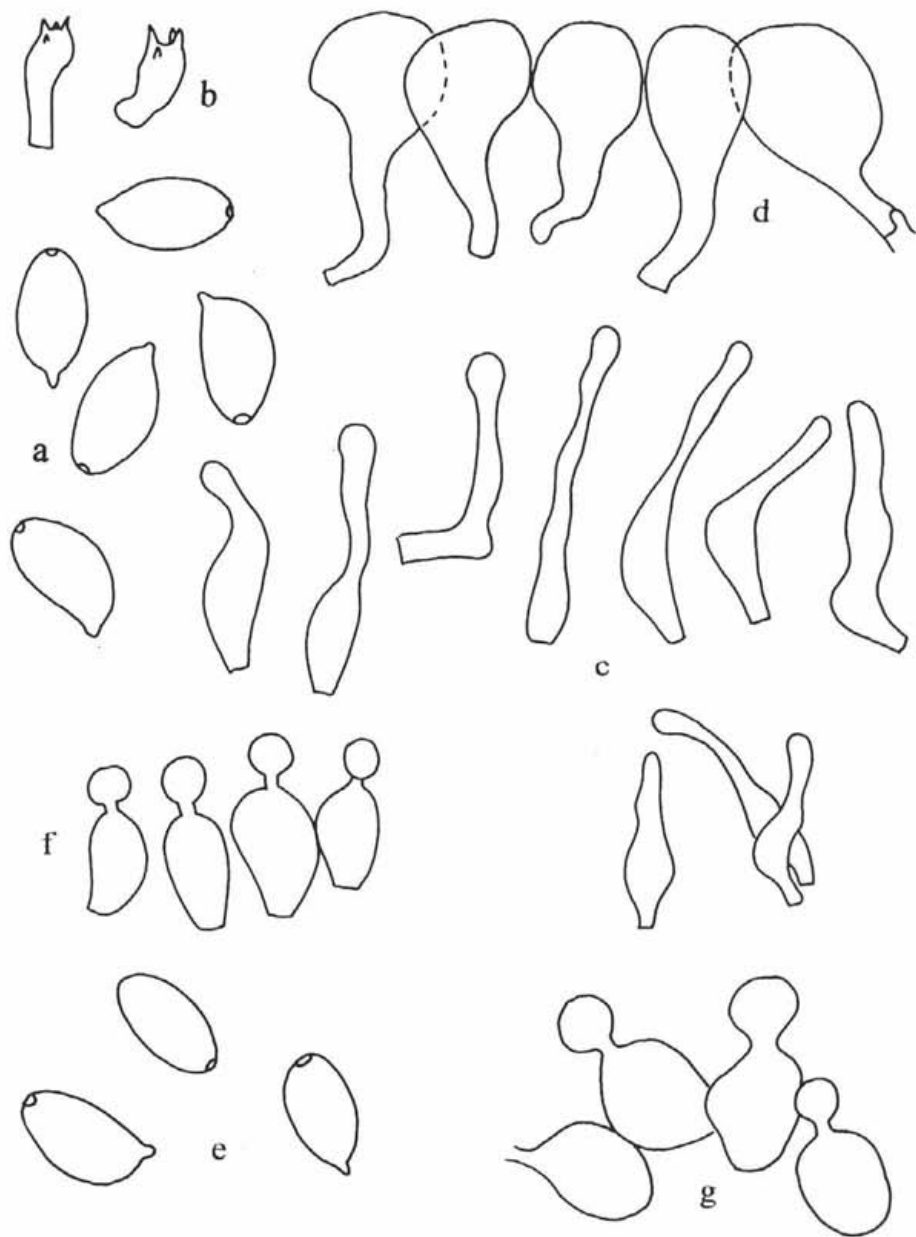
Material: Böhmen, Mnichovice, im Gras, 5. 10. 1935 (PRM 153691, Holotypus)

Mikroskopische Eigenschaften: Sporen:  $6,4-8,7(-9,5) \times 4,0-4,8 \mu\text{m}$ , im Mittel  $7,6 \times 4,3 \mu\text{m}$ , ellipsoidisch-eiförmig, nicht lentiform, nicht bohnenförmig, mit einfacher, dünner Wand und deutlichem, bis  $1 \mu\text{m}$  breitem Keimporus, in KOH roströtlich. Basidien: 4-sporig,  $13-20 \times 6-8 \mu\text{m}$ . Cheilozystiden: lecythiform,  $14-20 \times 6-10,5 \mu\text{m}$ , mit  $4-5,5 \mu\text{m}$  breitem Köpfchen. Stielbekleidung: in sehr schlechtem Zustand, trotz intensiver Suche konnten einige kollabierte haarförmige Elemente sowie rundliche bis rundlich-längliche Zellen entdeckt werden, das Vorhandensein von überwiegend lecythiformen Zystiden ist jedenfalls auszuschließen. Huthaut: hymeniform aus rundlich-gestielten Elementen ( $30-45 \times 15-25 \mu\text{m}$ ), dazwischen vereinzelt lecythiforme Pileozystiden ähnlich den Cheilozystiden.

Diese Art ist ein kleinsporiges Mitglied des Artenkomplexes rund um *C. sienophylla*, sie ist aber von der echten *C. sienophylla* sicher verschieden; trotz des schlechten Zustandes des Beleges und einer nicht gerade typischen Hutform ("acute rostellato") spricht sehr vieles dafür, daß sie mit den vielen Funden aus verschiedenen Ländern Europas konspezifisch ist, die ich unter dem provisorischen Namen "forma paupera" führe. Für den Fall, daß sich diese Annahme bestätigen sollte und die Sippe Artrang verdient, ist folgende Neukombination vonnöten:

*Conocybe rostellata* (Velen.) Hausknecht et Svrček, comb. nova

Basionym: *Galera rostellata* Velen., Novitates mycologicae 1940: 129



**Abb. 8.** a-d *Galera rimosa* (PRM 153772, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Huthaut,  $\times 800$ . **Abb. 8.** e-g *Galera rostellata* (PRM 153718); e Sporen,  $\times 2000$ ; f Cheilozystiden,  $\times 800$ ; g Stielbekleidung,  $\times 800$ .

**Galera rostellata** Velen., *Novitates mycologicae novissimae* 1947: 68. Abb. 8 e-g

Material: Böhmen, Mnichovice, bei Mirošovice, auf einer Waldwiese, 25. 10. 1941 (PRM 153718)

Mikroskopische Eigenschaften: Sporen:  $7,5-9,5 \times 4-5,2 \mu\text{m}$ , im Mittel  $8,1 \times 4,5 \mu\text{m}$ , ellipsoidisch, dünnwandig mit deutlichem Porus, in KOH gelbbraun. Basidien: 4-sporig. Cheilozystiden: lecythiform,  $24-31 \times 10-16 \mu\text{m}$ , mit  $5,5-8,5 \mu\text{m}$  breiten Köpfen. Stielbekleidung: nur aus lecythiformen bestehend, diese bis  $33 \times 17 \mu\text{m}$  groß, mit bis  $12 \mu\text{m}$  großen Köpfchen.

Diese Kollektion ist etwas völlig anderes als der Typus. Die Farbangabe "species pulchella" zusammen mit den riesigen Cheilo- und Kaulozystiden und kleinen Sporen führen ganz klar zu *Conocybe rickeniana* Orton.

**Galera siliginea** Fr. 1824, *České houby* 1921: 540 (mit SW-Abbildung)

Material: existiert nicht.

Auf Grund der in Abbildung 5 gezeichneten Zystide handelt es sich zweifelsohne um eine *Conocybe*, die kleinen Sporen schließen *C. siliginea* (Fr.: Fr.) Kühner aus; es könnte sich aber auf Grund der Standortangabe "im Gras auf Viehweiden, im Sommer allgemein entlang von Wegen" und der als gelblich-bräunlich beschriebenen Farbe des Hutes um *C. fuscimarginata* (Murrill) Singer gehandelt haben.

**Galera sordida** Velen., *České houby* 1921: 541. Abb. 6 i-j

Material: Böhmen: Chuchle bei Prag, Juli 1918 (PRC, Flasche 381/6, von E. Gerhardt aus Flasche 381 separiert und durch die Aufbewahrung in Äthanol in extrem schlechtem Zustand). Typusmaterial nicht vorhanden.

Mikroskopische Eigenschaften: Sporen  $8,7-10 \times 5,2-6,2 \mu\text{m}$ , im Mittel  $9,5 \times 5,7 \mu\text{m}$ , ellipsoidisch, leicht lentiform, glatt, mit deutlichem Keimporus, vom Äthanol total entfärbt, die Außenwand teilweise aufgelöst. Basidien und Cheilozystiden: nicht gefunden. Huthaut in Aufsicht deutlich zellig-hymeniform. Stielbekleidung: nur Hyphen, keine Haare oder Zystiden gefunden.

Eine Interpretation der echten *Galera sordida* ist auf Grund des Fehlens des Originalbeleges nicht möglich und die Art als zweifelhaft zu betrachten.

**Galera spartea** Fr., *Novitates mycologicae* 1940: 129.

Material: Böhmen, Mnichovice, Kunice, 26. 7. 1938 (PRM 153774, ursprünglich als *G. carbonaria*)

Mikroskopische Eigenschaften: Sporen:  $7-8 \times 3,8-4,6 \mu\text{m}$ , im Mittel  $7,5 \times 4,3 \mu\text{m}$ , ellipsoidisch-äpfelkernförmig, nicht lentiform, mit dünner Wand und

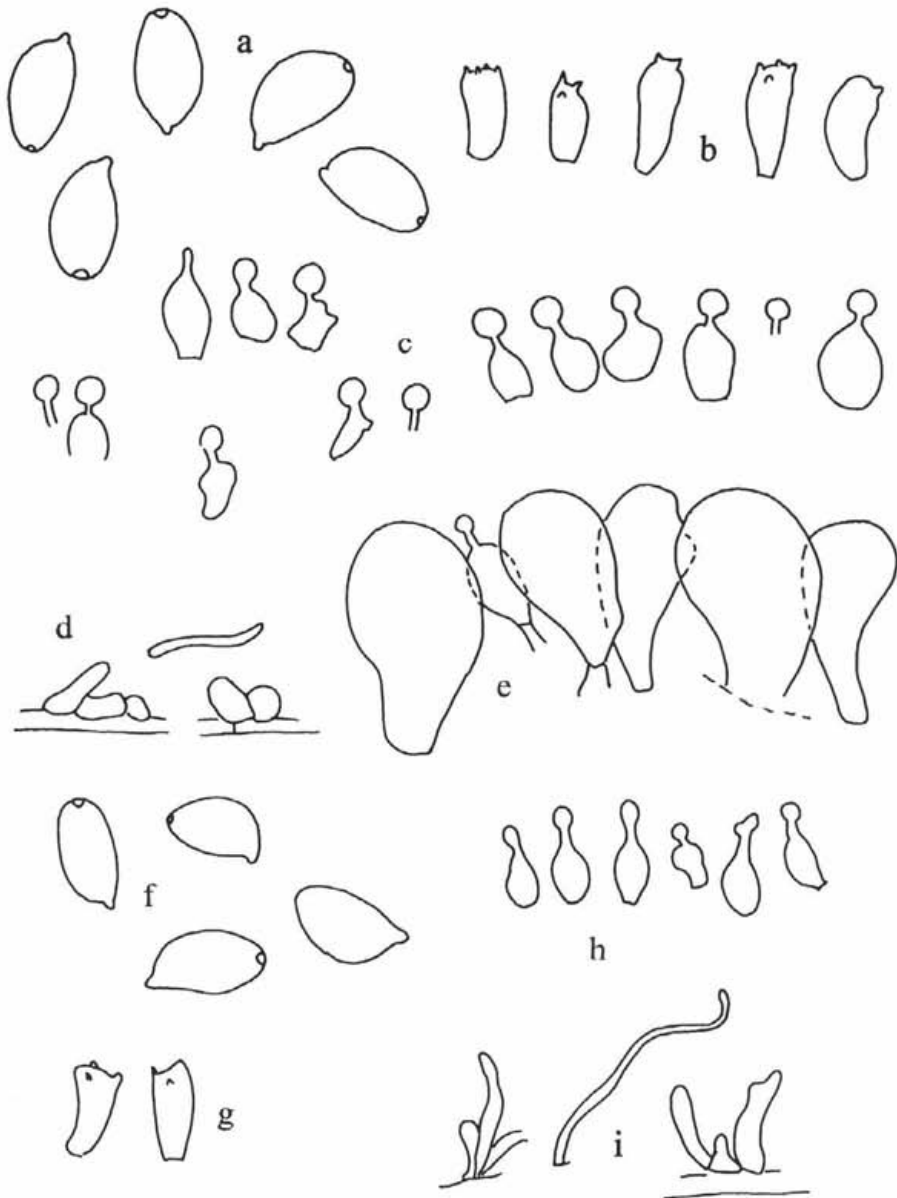


Abb. 9. a-e *Galera rostellata* (PRM 153691, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Elemente der Stielbekleidung,  $\times 800$ ; e Huthaut,  $\times 800$ . Abb. 9. f-i *Galera spartea* ss. Velen. (PRM 153774); f Sporen,  $\times 2000$ ; g Basidien,  $\times 800$ ; h Cheilozystiden,  $\times 800$ ; i Elemente der Stielbekleidung,  $\times 800$ .

teilweise undeutlichem Porus, sehr hell gelblich in KOH. Basidien: 4-sporig. Cheilozystiden: lecythiform,  $11-17 \times 5-6,5 \mu\text{m}$ , mit  $2,5-4 \mu\text{m}$  großem Köpfchen. Stielbekleidung: nur aus Haaren und nicht-lecythiformen, meist zylindrischen Elementen zusammengesetzt.

Auf Grund der hellen, schwach gelblichen, kleinen Sporen und der Stielbekleidung stellt diese Kollektion *Conocybe pallidospora* Kühn. et Watling dar.

**Galera spicula** Lasch 1828, České houby 1921: 543.

Material: in PRC nicht mehr vorhanden.

Die makroskopische Beschreibung mit dem Hinweis auf eine Ähnlichkeit mit *Galera hypnorum* und die Angabe von Zystiden mit besonders großen Köpfen und großen Sporen führen zu *Conocybe magnicapitata* Orton. Singer (1951: 440) hat diesen Fund *Conocybe velenovskyi* Singer, nomen nudum, genannt. Laut Watling und Gregory (1981) ist dieses Taxon als 2-sporige Form von *C. magnicapitata* aufzufassen.

**Galera tenera** Schaeff. 1762, České houby 1921: 540 (mit SW-Abbildung)

Material: in PRC nicht vorhanden.

Die Abbildung stellt einen zarten, gerieften Pilz dar, die gezeichnete Zystide paßt zur Gattung *Conocybe*. Mehr kann dazu nicht gesagt werden.

**Galera togularis** Bull. 1792, České houby 1921: 552 und Novitates mycologicae novissimae 1947: 74

Material: im PRC nicht vorhanden.

Die makroskopische Beschreibung und die Angaben, die über Sporen und Zystiden ("lang, scharf zuspitzend") gemacht werden, weisen darauf hin, daß es sich um *Pholiotina arrhenii* (Fr.) Singer handeln müßte.

**Galera urticae** Velen., Novitates mycologicae 1940: 131. Abb. 10 a-c

Material: Böhmen, Mnichovice, Plecháč, unter Brennessel, Oktober 1939 (PRM 153688, Holotypus).

Mikroskopische Eigenschaften: Sporen:  $6,4-7,9 \times 3,8-4,6 \mu\text{m}$ , im Mittel  $7,3 \times 4,2 \mu\text{m}$ , ellipsoidisch, nicht linsenförmig breitgedrückt, mit dünner, einfacher Wand und teilweise undeutlichem Porus, hell gelblich in KOH. Basidien: 4-sporig, z. B.  $15 \times 7 \mu\text{m}$ . Cheilozystiden: einige stark kollabierte, zylindrisch-spindelige Elemente gesehen; die genaue Form der Zystiden läßt sich nicht mehr feststellen. Lamellen-Trama von *Pholiotina*-Typ. Huthaut: aus rundlich-gestielten Elementen zusammengesetzt.

Singer (1986) nahm eine Umkombination in *Conocybe urticae* (Velen.) Singer vor und stellt sie in die Sektion *Mixtae*. Das Material ist in schlechtem Zustand und von einem Parasiten befallen, sodaß sich die Struktur der Lamellenschneide nicht mehr exakt feststellen läßt. Bei den von mir untersuchten Exemplaren handelt es sich aber zweifelsohne um eine *Pholiotina*, auch Svrček kam laut beiliegendem Begleitzettel zum selben Schluß. *C. urticae* kann demnach nur als fragliche Art aufgefaßt werden.

**Galera velata** Velen., České houby 1921: 547. Abb. 10 d-g

Material: Böhmen, Mnichovice, im Garten, 1918 (PRC, Holotypus, von mir selektiert aus Flasche 78 g und in ein eigenes Gefäß überführt).

Mikroskopische Eigenschaften: Sporen: 6-7,9 × 4-5 µm, im Mittel 6,9 × 4,2 µm, ellipsoidisch, nicht breitgedrückt, dünnwandig mit deutlichem Porus, hell gelbbraun in KOH. Basidien: 4-sporig, 17-22 × 5-9 µm. Cheilozystiden: 21-37 × 5-10 µm, zylindrisch-spindelrig, seltener leicht flaschenförmig mit kopfig erweiterter Spitze. Schnallen vorhanden. Huthaut: hymeniform aus rundlich-gestielten Elementen, bis 43 × 25 µm.

Dies ist unzweifelhaft *Conocybe appendiculata* Lange et Kühner 1935, ungültig publiziert (= *C. appendiculata* Watling 1971). Das ausgewählte Material besteht zwar nur aus zwei Hutfragmenten, ist aber gut erhalten, man kann alle wichtigen mikroskopischen Eigenschaften nachvollziehen. Die nachfolgende Neukombination ist notwendig:

*Pholiotina velata* (Velen.) Hausknecht, comb. nova

Basionym: *Galera velata* Velen., České houby 1921: 547.

Synonyme: *Conocybe appendiculata* Lange et Kühner 1935, ungültig publiziert  
*Conocybe appendiculata* Watling 1971

*Pholiotina appendiculata* (Watling) Courtecuisse

**Galera velutipes** Velen., Novitates mycologicae 1940: 128. Abb. 11 a-d

Material: Böhmen, Mnichovice, September 1939 (PRM 153695, Holotypus).

Mikroskopische Eigenschaften: Sporen: 10-13,5 × 7,2-9 × 6,4-7,2 µm, im Mittel 11,1 × 7,8 × 6,8 µm, ellipsoidisch, deutlich linsenförmig breitgedrückt, mit doppelter Wand und großem, deutlichem Keimporus, in KOH rötlichbraun mit weinrötlich gefärbter Wand. Basidien: meist kollabiert, 4-sporig, 17-20 × 8,5-10 µm. Cheilozystiden: lecythiform, 12-22 × 6-9 µm, mit 3,5-5 µm breiten Köpfchen. Stielbekleidung: nur aus nicht-lecythiformen, zylindrischen, spindeligen bis keuligen Elementen (bis 47 × 8,5 µm) und Haaren bestehend. Huthaut: hymeniform aus rundlich-gestielten Elementen.

Obwohl das Exsikkat nur aus einem Fruchtkörper in mäßig gutem Zustand besteht, kann kein Zweifel darüber herrschen, daß dies *Conocybe siliginea* var.



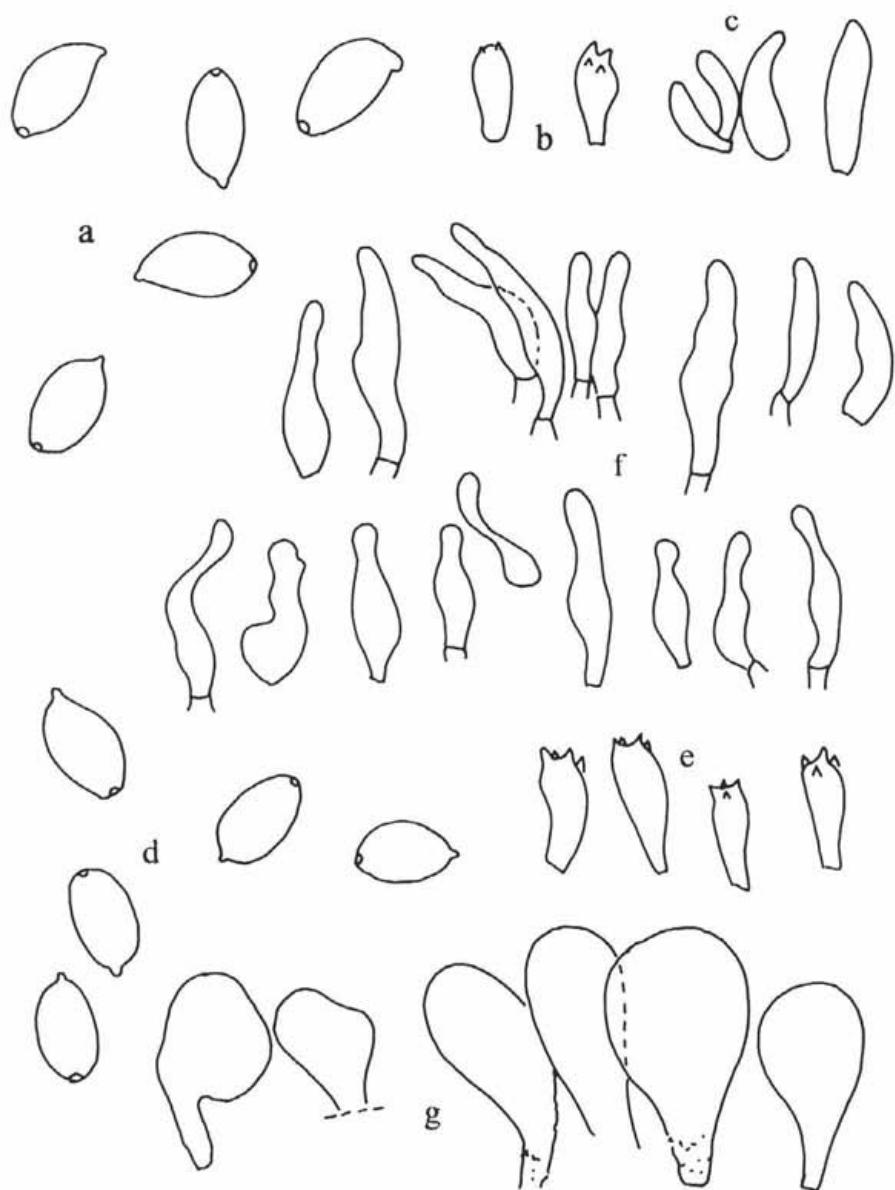


Abb. 10. a-c *Galera urticae* (PRM 153688, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Elemente der Stielbekleidung,  $\times 800$ . Abb. 10. d-g *Galera velata* (PRC, aus Flasche 78 g, Holotypus); d Sporen,  $\times 2000$ ; e Basidien,  $\times 800$ ; f Cheilozystiden,  $\times 800$ ; g Elemente der Huthaut,  $\times 800$ .

*ochracea*, forme macrospore (Kühner 1935) ist, die von Singer (1969) gültig in *C. kuehneriana* Singer umbenannt wurde. Da Velenovskýs Art Priorität hat, wird folgende Neukombination vorgeschlagen:

*Conocybe velutipes* (Velen.) Hausknecht und Svrček, comb. nov.

Basionym: *Galera velutipes* Velen., Novitates mycologicae 1940: 128

Synonyme: *Conocybe siliginea* var. *ochracea*, forme macrospore (Kühner 1935)

*Conocybe kuehneriana* Singer 1969

**Galera vesicaria** Velen., Novitates mycologicae novissimae 1947: 69. Abb. 11 e-h

Material: Böhmen, Mnichovice, Myšlín, auf Brandstelle, 2. 8. 1940 (PRM 153732, Holotypus).

Mikroskopische Eigenschaften: Sporen: 14,3–16,6 × 8,5–10,3 μm, im Mittel 15,3 × 9,0 μm, ellipsoidisch, nicht lentiform, mit dicker, doppelter Wand und 1,2–1,8 μm breitem Porus, in KOH rötlich gelbbraun mit weinrötlicher Wand. Basidien: 2-sporig, 17–21 × 10–12 μm, mit dicken, fast dreieckigen Basidien. Cheilozystiden: lecythiform, 14–25 × 5,5–9,5 μm, mit 4–5,5 μm breiten Köpfchen. Stielbekleidung: überwiegend aus Haaren und nicht-lecythiformen Zystiden bestehend, dazwischen nicht selten kopfige Zystiden ähnlich den Cheilozystiden, aber schlanker. Huthaut: hymeniform aus rundlich-gestielten Elementen.

Dies ist *Conocybe siliginea* (Fr.: Fr.) Kühner. Dafür sprechen der frisch tonfarbene, trocken weiße Hut, die zarte Statur, das Habitat sowie natürlich die Mikromerkmale (siehe dazu auch Hausknecht und Passauer 1997).

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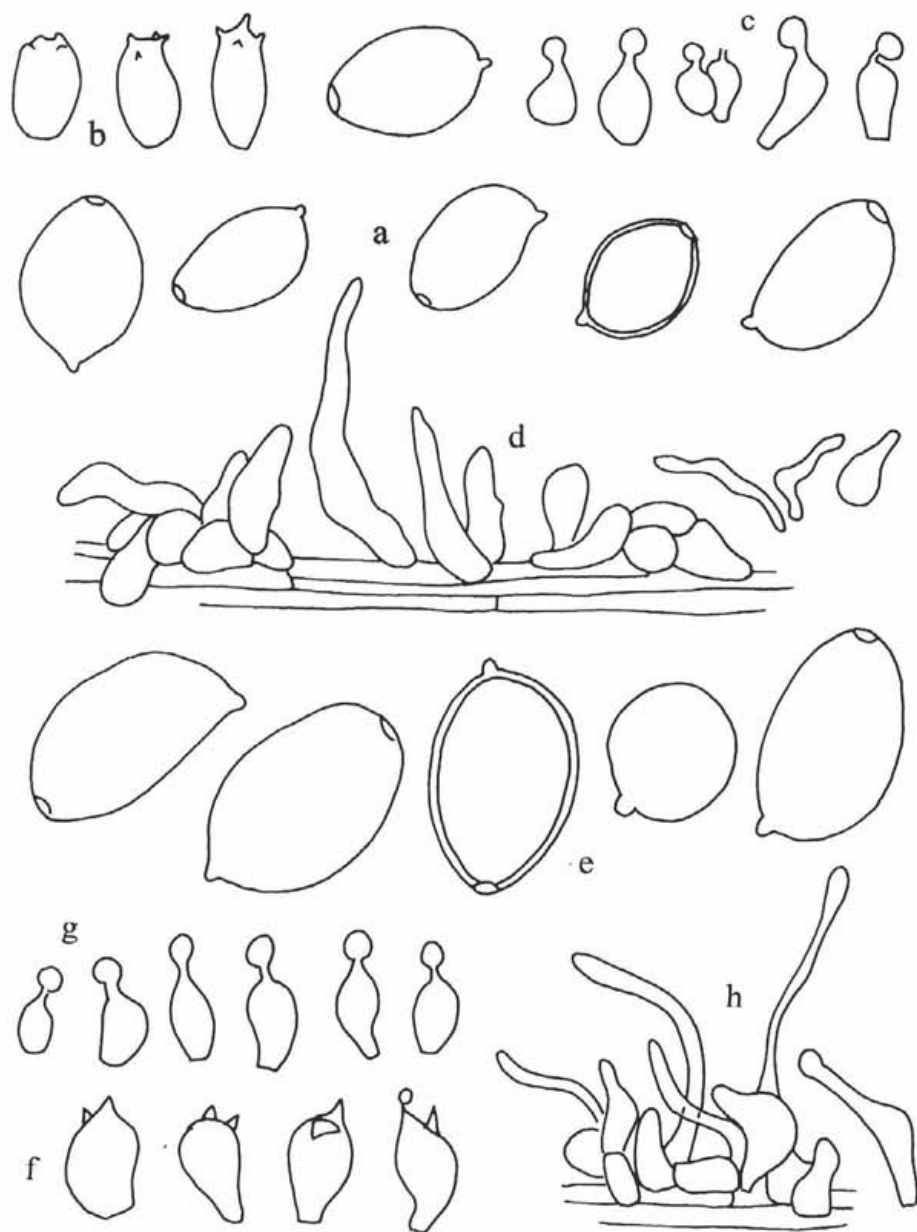


Abb. 11. a-d *Galera velutipes* (PRM 153695, Holotypus); a Sporen,  $\times 2000$ ; b Basidien,  $\times 800$ ; c Cheilozystiden,  $\times 800$ ; d Stielbekleidung,  $\times 800$ . Abb. 11. e-h *Galera vesicaria* (PRM 153732, Holotypus); e Sporen,  $\times 2000$ ; f Basidien,  $\times 800$ , g Cheilozystiden,  $\times 800$ ; h Stielbekleidung,  $\times 800$ .

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## Induction of extracellular glycosidases in filamentous fungi and their potential use in chemotaxonomy

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Huňková Z., Kubátová A., Weignerová L. and Křen V. (1999): Induction of extracellular glycosidases in filamentous fungi and their potential use in chemotaxonomy – Czech Mycol. 51: 71–87

Data on the occurrence and inducibility of extracellular  $\beta$ -*N*-acetylhexosaminidase,  $\alpha$ -galactosidase,  $\alpha$ - and  $\beta$ -mannosidase and  $\alpha$ -L-fucosidase, including inductors, are given for selected *Aspergillus*, *Penicillium* and *Fusarium* strains. These data represent additional information on the strains in the Culture Collection of Fungi, Department of Botany, Charles University, Prague, and in the Culture Collection of the Institute of Microbiology, Prague, Czech Republic, thus extending their usability in biochemistry and biotechnology. With respect to these biochemical data a taxonomic evaluation of the examined strains is presented. Several strains were re-identified after biochemical and morphological comparisons with the type strains. The strains of *A. niveus* CCF 544, *A. terreus* CCF 76, CCF 869, and CCIM USA were re-identified as *A. flavipes*, the strain *A. oryzae* CCF 1301 as *A. wentii*.

**Key words:** glycosidases, induction, *Aspergillus*, *Penicillium*, *Fusarium*

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Pro vybrané kmeny rodů *Aspergillus*, *Penicillium* a *Fusarium* jsou uvedeny údaje o výskytu a indukovatelnosti  $\beta$ -*N*-acetylhexosaminidasy,  $\alpha$ -galaktosidasy,  $\alpha$ - a  $\beta$ -manosidasy a  $\alpha$ -L-fukosidasy. Tyto údaje představují další informaci o kmenech ve Sběrce kultur hub při katedře botaniky Přírodovědecké fakulty Karlovy university a ve sbírce mikroorganismů Mikrobiologického ústavu AV ČR, což zvyšuje jejich využitelnost v biochemii a biotechnologii. S ohledem na získaná biochemická data bylo provedeno taxonomické vyhodnocení zkoumaných kmenů. Po biochemickém a morfologickém porovnání s typovými kulturami byly některé zkoumané kmeny přeřuceny. Kmeny *A. niveus* CCF 544, *A. terreus* CCF 76, CCF 869 a CCIM USA byly přeřuceny jako *A. flavipes*, kmen *A. oryzae* CCF 1301 byl přeřucen na *A. wentii*.

### INTRODUCTION

Glycosidases from fungi have proved to be very useful in the preparation of many glycosidic structures, such as  $\beta$ -galactosides,  $\beta$ -glucosides,  $\beta$ -mannosides,  $\beta$ -*N*-acetylglucosaminides and  $\beta$ -*N*-acetylgalactosaminides, by transglycosylation or a reversed glycosylation (Křen et al. 1994, Taubken et al. 1993, Crout et al. 1992). Despite of the copious methods developed for the chemical synthesis

of glycosides, the use of enzymes as catalysts is an attractive alternative, since the sugar coupling steps can be performed with high stereoselectivity and certain regioselectivity (Křen and Thiem 1997). There is a continuous effort to identify and to characterise novel glycosidases applicable in biochemistry (Koga et al. 1991, Holazo et al. 1992, Yamamoto et al. 1986a).

Extensive screening to obtain the desired glycosidases from selected fungal strains originating mostly from the Culture Collection of Fungi, Department of Botany, Charles University, Prague was started. Our attention was concentrated mostly on the fungal genera already known for their glycosidase production. Induction by specific inductors (oligosaccharides, glycomimetics) enabled us to obtain the desired glycosidases in sufficient quality and quantity (Huňková et al. 1996a,b, Huňková et al. 1997).

In addition, biochemical characteristics, i.e. production of particular glycosidases, and their inducibility can reveal new chemotaxonomic features for better characterisation and eventual taxonomic re-identification of the strains. So far, profiles of secondary metabolites and isoenzymes were used as an aid in the identification of many fungal genera, e.g. *Penicillium* (Cruickshank and Pitt 1987, Frisvad and Filtenborg 1989, Paterson et al. 1989), *Aspergillus* (Zohri and Ismail 1994, Bridge and Hawksworth 1984), *Fusarium* (Wasfy et al. 1987), *Phoma* (Monte et al. 1991), *Monascus* (Bridge and Hawksworth 1985), and *Beauveria* (Mugnai et al. 1989). Glycosidase activities were also used in some of these studies but only in combination with other features (Bridge and Hawksworth 1984, Bridge and Hawksworth 1985, Bridge et al. 1989). Besides their production, enzyme inductibility and catabolic repression can provide an additional set of data reflecting regulatory systems and physiological typology of the strains studied. Biochemical and physiological differences can, therefore, help to identify species which can be hardly distinguished morphologically. Thus, we have also focused on strains requiring further taxonomic characterization. For comparison we examined in parallel strains derived from the type specimen (ex-type cultures).

Here, we summarize the data on the occurrence of the most important extracellular glycosidases and their inducibility. These results represent further biochemical information on the strains in the Collections and they can also serve as the basis for their chemotaxonomy.

Some glycosidases from our screening were already successfully employed for glycoside synthesis (Weignerová et al. 1996, Rajnochová et al. 1997, Huňková et al. 1997, Křen et al. 1998, Weignerová et al. 1998).

## MATERIAL AND METHODS

## Strains and cultivation conditions

The strains used originated from the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, from the Culture Collection of the Institute of Microbiology (CCIM), Prague, Czech Republic, from the American Type Culture Collection (ATCC), Rockville, Maryland, U. S. A., and from the International Mycological Institute (IMI), Egham, U. K.

Before the glycosidase assay, the cultures were maintained on the slants [g/l]: agar-agar, 20; bacto-peptone, 5; malt extract, 35. Conical flasks (500 ml) with 100 ml of medium were inoculated with a suspension of spores in 0.1% Tween 80 solution. The flasks were cultivated on a rotary shaker at 28 C. Media used: Sabouraud's medium [g/l]: mycological peptone, 10; glucose, 40; pH 5.6. Casamino-acid medium [g/l]: yeast extract, 0.5; mycological peptone, 5;  $\text{KH}_2\text{PO}_4$ , 3;  $\text{NH}_4\text{H}_2\text{PO}_4$ , 5; casein hydrolysate, 7.5; pH 6.0. After sterilization each flask was supplemented with 0.5 ml of 10 %  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . The inductor supplemented medium (production medium) was the same as the casamino-acid medium, the casamino-acids were replaced by respective amounts of inductor. Concentrations of inductors are given in the respective cases – see Tab. 1–3.

For morphological examination of the *Penicillia* and *Aspergilli* three agar media were used: Czapek yeast-extract agar (CYA) and malt-extract agar (MEA) according to Pitt (1980), and wort-beer agar. *Fusarium* strains were cultivated on potato-glucose agar and on synthetic nutrient agar according to Nirenberg (1976).

## Enzyme activity assay

Glycosidases were assayed using corresponding *p*-nitrophenyl glycosides as substrates according to Mega et al. (1970). Proteins were determined according to Bradford (1976) using the kit from Bio-Rad (München, Germany) with bovine serum albumin as a standard.

## Morphological examination

The identity of the *Penicillia* and *Aspergilli* strains was checked according to Raper and Thom (1949), Raper and Fennell (1965), Pitt (1980), Klich and Pitt (1988), Tzean et al. (1990), and Samson and Pitt (1990). *Fusarium* strains were identified according to Burgess et al. (1988).

## Preparation of crude chitin hydrolysate

Crude chitoooligomers were prepared by the acid hydrolysis of chitine according to Rupley (1964).

Preparation of  $\alpha$ -mannooligomers

Mannooligomers were prepared by the condensation of mannose catalysed by  $\alpha$ -mannosidase from *Canavalia ensiformis* (jack bean) under conditions as



published (Ajisaka et al. 1995) and separated by gel filtration on BioGel P2 (BioRad, U. S. A.).

## RESULTS AND DISCUSSION

### Constitutive production of glycosidases

Many fungal strains have low basal levels of glycosidases. For their purification and characterisation constitutively produced glycosidases were used, e.g.  $\beta$ -*N*-acetylhexosaminidase from *Trichoderma harzianum* (Koga et al. 1991) and *Penicillium oxalicum* (Yamamoto et al. 1985),  $\alpha$ -galactosidase from *Trichoderma reesei* (Savel'ev et al. 1996) and *Mortierella vinacea* (Shibuya et al. 1997),  $\alpha$ -galactosidase and  $\beta$ -*N*-acetylglucosaminidase from *Aspergillus niger* (Bahl and Agrawal 1969). *A. niger* is frequently used as a source microorganism for purification and characterisation of numerous glycosidases or for enzymatic syntheses (e.g., Jones and Kosman 1980, Bahl 1970, Ajisaka and Skirakabe 1992, Itoh and Kamiyama 1995).

We have found low constitutive production of extracellular glycosidases in many fungal strains, e.g.  $\beta$ -*N*-acetylhexosaminidase in *A. oryzae* strains 0.1–1.3 U/mg prot. and in *A. terreus* strains 0.5–2.2 U/mg prot. (Huňková et al. 1996a), in *P. vinaceum* CCF 2384 2.2 U/mg prot., and in *P. oxalicum* strains 0.22–6.3 U/mg prot.;  $\alpha$ -galactosidase in *A. terreus* strains 0.25–7.0 U/mg prot.;  $\alpha$ -fucosidase in *Fusarium oxysporum* strains 0.25–0.44 U/mg prot. and in *A. flavipes* IMI 171885 0.10–0.15 U/mg protein. Low constitutive production of  $\alpha$ -mannosidase was found in many strains and slight constitutive production of  $\beta$ -mannosidase was observed in several strains (see Table 1, 2). Such activities are, however, rather low for practical use. Higher constitutive production of  $\beta$ -*N*-acetylhexosaminidase was found in *P. chrysogenum* CCF 1269 (9.2 U/mg prot.). High constitutive production of  $\beta$ -*N*-acetylhexosaminidase (11.0–24.6 U/mg prot.) and  $\alpha$ -galactosidase (7.3–32.9 U/mg prot.), and slight production (under 0.5 U/mg prot.) of  $\alpha$ - and  $\beta$ -mannosidase and  $\alpha$ -L-fucosidase was found in culture filtrates of *A. niger* CCIM K1 and CCIM K2, *A. awamori* CCF 763 and *A. phoenicis* CCF 61. However, contamination of the main activity by other glycosidases complicates their purification and use in bioorganic chemistry.

### Induction and inductors

Some glycosidases in fungi are known to be inducible, e.g.  $\alpha$ -galactosidase in *Penicillium ochrochloron* was induced by galactomannan from guar (*Cyamopsis tetragonobola*) gum (Dey et al. 1993),  $\alpha$ -fucosidase in *Fusarium oxysporum* was substantially induced by L-fucose and slightly by D-arabinose (Yamamoto et al. 1986b),  $\beta$ -*N*-acetylhexosaminidase in *Aspergillus oryzae* was strongly induced

Table 1 Glycosidases in *Aspergillus*

STRAIN (original name)	$\beta$ -HexNAc		$\alpha$ -Gal	$\alpha$ -Man	$\beta$ -Man	$\alpha$ -Fuc
	$\beta$ -GlcNAc	$\beta$ -GalNAc/ $\beta$ -GlcNAc rate				
<i>A. oryzae</i> CCF 147	I+++ cho	0.26	I++ raf	I mo, MeMan C ca	0	0
<i>A. oryzae</i> CCF 172	I+++ cho	0.34	I+ raf	I mo	ND	ND
<i>A. oryzae</i> CCF 1063	I+++ cho	0.27	ND	ND	ND	ND
<i>A. oryzae</i> CCF 1065	I+++ cho	0.29	ND	ND	ND	ND
<i>A. oryzae</i> CCF 1066	I+++ cho I+++ GlcNAc I+ chi I GlcN	0.39	I++ raf	I MeMan, mo C ca	0	0
<i>A. oryzae</i> CCIM NZS	I+++ cho	ND	ND	ND	ND	ND
<i>A. oryzae</i> CCIM NZZ	I+++ cho	ND	ND	ND	ND	ND
<i>A. oryzae</i> CCF 1301*	C Glc	ND	0	0	0	ND
<i>A. oryzae</i> CCF 1602	I+ cho	0.26	I++ raf	I MeMan C ca	0	0
<i>A. oryzae</i> T IMI 16266ii	I++ cho	0.43	I raf	I MeMan C ca	0	0
<i>A. flavus</i> CCF 146	I cho	0.23	I++ raf	C ca, GlcN	0	0
<i>A. flavus</i> CCF 642	C ca, GlcN NI cho	0.54	I+++ raf	C ca	0	0
<i>A. flavus</i> CCF 814	I+ cho	0.60	I++ raf	I MeMan C ca	0	0
<i>A. flavus</i> CCF 1129	I++ cho I GlcN	0.66	I++ raf I GlcN	C ca	0	ND
<i>A. flavus</i> CCF 1624	C ca, GlcN NI cho	ND	I+ raf	C ca	0	0
<i>A. flavus</i> T IMI 124930	I++ cho I GlcN	0.57	I+++ raf I+ GlcN	C ca	0	0
<i>A. parasiticus</i> CCF 141	I cho	0.25	I+ raf I dGlc	C ca	0	0
<i>A. parasiticus</i> CCF 1298	I+++ cho I GlcN	0.59	I+++ raf I GlcN	I MeMan, mo	0	ND
<i>A. parasiticus</i> T IMI 15957ix	I+ cho	0.32	I++ raf I+ dGlc, GlcN	I MeMan, mo	0	0
<i>A. sojae</i> T IMI 191300	I cho	0.51	I++++ raf I dGlc, GlcN	C Glc, ca	0	0
<i>A. flavofurcatis</i> CCF 107	I+ cho	0.22	I+ raf	C ca	C- ca	0

Table 1 — Continued

STRAIN (original name)	$\beta$ -HexNAc		$\alpha$ -Gal	$\alpha$ -Man	$\beta$ -Man	$\alpha$ -Fuc
	$\beta$ -GlcNAc	$\beta$ -GalNAc/ $\beta$ -GlcNAc rate				
<i>A. flavofurcatis</i> T IMI 124938	I+ cho I dGlc, GlcN	0.35	I++ raf I+ GlcN I dGlc	C ca	0	0
<i>A. tamarii</i> CCF 1665	I+ cho I dGlc, GlcN	0.30	I+++ raf	I MeMan C ca	0	0
<i>A. tamarii</i> CCF 2492	I++ cho	ND	I+ raf	ND	ND	ND
<i>A. terreus</i> CCF 55	I+ cho	0.50	I++ raf I++ mel I Gal	ND	C- Glc	ND
<i>A. terreus</i> CCF 57	NI cho	1.00	I raf	ND	ND	ND
<i>A. terreus</i> CCF 58	I cho	ND	I++ mel I++ raf	ND	ND	ND
<i>A. terreus</i> ATCC 20542	ND	ND	I+ raf, mel I Gal	ND	ND	ND
<i>A. terreus</i> T IMI 17294	I cho	0.46	I+ raf I dGlc, GlcN	0	0	0
<i>A. terreus</i> CCF 2539	I+ cho	1.01	I+ raf, mel I+ Gal	ND	C- Glc	ND
<i>A. terreus</i> CCF 76*	I++++ cho	0.54	C ca NI raf, dGlc	ND	C- ca	ND
<i>A. terreus</i> CCF 869*	I++++ cho I+++ GlcNAc	0.56	C ca NI raf, dGlc	ND	C- ca	ND
<i>A. terreus</i> CCIM LM	I cho	ND	I dGlc NI raf	ND	ND	ND
<i>A. terreus</i> CCIM USA*	I++ cho	0.68	I dGlc NI raf	ND	ND	ND
<i>A. flavipes</i> CCF 1895	I++ cho NI dGlc, GlcN	0.70	I dGlc NI raf	I MeMan C ca	0	0
<i>A. flavipes</i> CCF 2026	I+++ cho NI dGlc, GlcN	1.00	I++ dGlc NI raf	0	0	0
<i>A. flavipes</i> T IMI 171885	I+++ cho NI dGlc, GlcN	0.90	NI raf, dGlc (C- Glc)	C ca	C Glc	C Glc
<i>A. niveus</i> CCF 544	I++++ cho	0.75	I dGlc NI raf	I MeMan C ca	0	0
<i>A. niveus</i> T IMI 171878	I cho C+ Glc	0.15	NI raf, dGlc C Glc	C- ca	C- Glc	0
<i>A. niger</i> CCIM K1	C++ Glc	0.40	C++ ca	C- ca	C ca	ND

Table 1 — Continued

STRAIN (original name)	$\beta$ -HexNAc			$\alpha$ -Gal	$\alpha$ -Man	$\beta$ -Man	$\alpha$ -Fuc			
	$\beta$ -GlcNAc	$\beta$ -GalNAc/ $\beta$ -GlcNAc rate								
<i>A. niger</i> CCIM K2	C++	Glc	0.42	C++	ca	C	ca	C	ca	ND
<i>A. awamori</i> CCF 763	C++	ca	ND	C+++	ca	C-	ca	ND		ND
<i>A. phoenicis</i> CCF 61	C++	ca	0.49	C++	ca	C	Glc	C ca, GlcN		C- Glc
<i>A. versicolor</i> CCF 2491	I++ I+	cho GlcN	0.09	I	raf, GlcN	0		0		0

**Abbreviations:**

- T type strain  
 \* re-identified strain, see chapter Taxonomic evaluation  
 ND not determined

**Constitutive production** (basal levels are not given in case of unquestionable induction)

- C- specific activity below 0.02 U/mg prot.  
 C spec. act. 0.02–2 U/mg prot.  
 C+ spec. act. 2–10 U/mg prot.  
 C++ spec. act. 10–20 U/mg prot.  
 C+++ spec. act. more than 20 U/mg prot.

**Inductive production** (compared with basal level)

- I induction less than 5×  
 I+ 5–10×  
 I++ 10–20×  
 I+++ 20× — 50×  
 I++++ more than 50×  
 NI no induction  
 $\beta$ -HexNAc  $\beta$ -N-acetylhexosaminidase  
 $\beta$ -GlcNAc  $\beta$ -N-acetylglucosaminidase  
 $\beta$ -GalNAc  $\beta$ -N-acetylgalactosaminidase  
 $\alpha$ -Gal  $\alpha$ -galactosidase  
 $\alpha$ -Ma  $\alpha$ -mannosidase  
 $\beta$ -Man  $\beta$ -mannosidase  
 $\alpha$ -Fuc  $\alpha$ -L-fucosidase

**Inductors and substrates used (concentrations)**

- Ara D-arabinose (0.1 %)  
 Gal galactose (1 %)  
 Glc glucose (4 %; Sabouraud medium)  
 GlcNAc N-acetylglucosamine (0.5 %)  
 dGlc 6-deoxyglucose (0.1 %)  
 GlcN glucosamine (0.1 %)  
 Fuc L-fucose (0.1 %)  
 MeMan  $\alpha$ -methylmannoside (0.1 %)  
 cho chitooligomers (0.2 %; crude chitin hydrolysate)  
 chi chitosan (MW 70000, 0.25 %)  
 raf raffinose (0.2 %; raffinose pentahydrate)  
 mel melibiose (0.2 %; melibiose monohydrate)  
 mo  $\alpha$ -mannooligomers (0.1%; crude  $\alpha$ -mannooligomers)  
 ca casamino acids (0.75 %; casein hydrolysate)

Table 2 Glycosidases in *Penicillium* (For legend and abbreviations see Table 1)

STRAIN (original name)	$\beta$ -HexNAc		$\alpha$ -Gal	$\alpha$ -Man	$\beta$ -Man	$\alpha$ -Fuc
	$\beta$ -GlcNAc	$\beta$ -GalNAc/ $\beta$ -GlcNAc rate				
<i>P. spinulosum</i> CCF 2159	I cho	0.92	I raf, dGlc I GlcN	ND	0	0
<i>P. vinaceum</i> CCF 2384	C+ Glc	ND	I+++ raf	I MeMan C ca	C Glc	0
<i>P. daleae</i> CCF 2365	I+ cho I dGlc, GlcN	ND	I+ raf I dGlc, GlcN	0	0	0
<i>P. melinii</i> CCF 2440	I+++ cho I GlcN	0.12	I+ raf	C- ca	0	0
<i>P. brasilianum</i> CCF 2155	I cho	0.75	I raf	I MeMan, mo	0	0
<i>P. brasilianum</i> CCF 2171	I++++ cho I++ dGlc	ND	I raf, dGlc	0	0	0
<i>P. ochrochloron</i> CCF 2379	C Glc	ND	I raf, mel	0	ND	ND
<i>P. commune</i> CCF 2962	0	ND	I+ mel	C- ca	ND	ND
<i>P. chrysogenum</i> CCF 1269	C++ Glc	ND	I+++ mel	C ca	ND	ND
<i>P. funiculosum</i> CCF 1994	I++ cho I dGlc, GlcN	1.20	I raf I+ dGlc	I MeMan, mo	0	I dGlc
<i>P. funiculosum</i> CCF 2325	I+ cho I dGlc, GlcN	0.76	I raf I+ dGlc	C- ca	0	I dGlc
<i>P. purpurogenum</i> var. <i>rubrisclerotium</i> CCF 2984	I+ cho	1.22	I raf I++ dGlc	0	C- Glc	I dGlc
<i>P. purpurogenum</i> var. <i>rubrisclerotium</i> CCF 2985	I cho	1.15	I raf I+ dGlc	I MeMan, GlcN	C- Glc	I dGlc
<i>P. pitii</i> CCF 2277	I cho	0.63	C+ ca	0	0	0
<i>Talaromyces flavus</i> CCF 2324	C Glc, ca	ND	I raf I++ dGlc	0	C Glc	0
<i>P. oxalicum</i> CCF 1659	I cho	2.8	ND	ND	ND	ND
<i>P. oxalicum</i> CCF 1667	I++ cho	2.0	ND	ND	ND	ND
<i>P. oxalicum</i> CCF 1959	I+ cho I dGlc	1.6	ND	I MeMan	ND	ND

Table 2 — Continued

STRAIN (original name)	$\beta$ -HexNAc		$\alpha$ -Gal	$\alpha$ -Man	$\beta$ -Man	$\alpha$ -Fuc
	$\beta$ -GlcNAc	$\beta$ -GalNAc/ $\beta$ -GlcNAc rate				
<i>P. oxalicum</i> CCF 2315	l+++ cho	1.4	ND	ND	ND	ND
<i>P. oxalicum</i> CCF 2430	l+++ cho	2.3	ND	ND	ND	ND
<i>P. oxalicum</i> CCF 3009	l+ cho	1.4	ND	ND	ND	ND
<i>P. asturianum</i> CCF 2062*	l cho	1.5	ND	ND	ND	ND

Table 3 Glycosidases in *Fusarium oxysporum* (for legend and abbreviations see Table 1)

STRAIN (original name)	$\beta$ -HexNAc		$\alpha$ -GlcNAc	$\alpha$ -Gal	$\alpha$ -Man	$\beta$ -Man	$\alpha$ -Fuc
	$\beta$ -GlcNAc	$\beta$ -GalNAc/ $\beta$ -GlcNAc rate					
<i>F. oxysporum</i> CCF 377	l cho, Fuc, GlcN	0.18	ND	l+ raf l Fuc l dGlc, GlcN	0	0	l+++ Fuc l Ara
<i>F. oxysporum</i> CCF 483	l Fuc	ND	ND	ND	ND	ND	l++ Fuc
<i>F. oxysporum</i> CCF 906	l Fuc	ND	ND	ND	ND	ND	NI Fuc C ca
<i>F. oxysporum</i> CCF 1389	l Fuc	ND	ND	ND	ND	ND	NI Fuc C ca
<i>F. oxysporum</i> CCF 1414	l Fuc	ND	ND	ND	ND	ND	l++ Fuc

by aminosugar derivatives (Huňková et al. 1996a). For obtaining the glycosidases in higher amount, we tested glycosidase induction susceptibility of all examined strains. Extracellular production of certain glycosidases can be remarkably increased by induction (see Tables 1–3). The data in the tables represent induction in comparison with the basal level (casamino-acids medium without any supplement).

#### $\beta$ -N-Acetylhexosaminidase

Overproduction of extracellular  $\beta$ -N-acetylhexosaminidase from *A. oryzae* by induction with aminosugar-containing inductors was already demonstrated by us

(Huňková et al. 1996a). Induction of this enzyme in *A. oryzae* strains was triggered by crude chitin hydrolysate (chitoooligomers containing 2–10 glycosyl units together with ca 75% NaCl produced by neutralization of HCl used for hydrolysis), by *N*-acetylglucosamine and by chitosan. Because the best results were obtained with the crude chitin hydrolysate, we have chosen this preparation as an inductor for all tested strains. Production of a large series of  $\beta$ -*N*-acetylhexosaminidases can be improved in this way (Tab. 1). Moreover, by induction we obtained  $\beta$ -*N*-acetylhexosaminidases in high specific activity without a significant amount of other glycosidases in some strains, e.g. *A. terreus* CCF 76\* and 869\*, *A. terreus* CCIM USA\*, *A. tamaritii* CCF 2492, *P. oxalicum* CCF 1959 and with all strains of *A. oryzae* (except the strain CCF 1301\*). Thus, culture filtrate containing  $\beta$ -*N*-acetylhexosaminidase could, after precipitation with for example ammonium sulphate, directly be used for many biochemical procedures (Rajnochová et al. 1997, Weignerová et al. 1996, Křen et al. 1998, Weignerová et al. 1997, Weignerová et al. 1998).

With some strains slight improvement of  $\beta$ -*N*-acetylhexosaminidase activity was also reached using 6-deoxyglucose, glucosamine or L-fucose.

#### *$\beta$ -N-acetylhexosaminidase with high $\beta$ -N-acetylgalactosaminidase activity*

As  $\beta$ -*N*-acetylhexosaminidase having high  $\beta$ -*N*-acetylgalactosaminidase activity and low  $\beta$ -*N*-acetylglucosaminidase activity was required, we have also determined the rate of these two activities. Most of  $\beta$ -*N*-acetylhexosaminidases from fungal sources have a low  $\beta$ -GalNAcase/ $\beta$ -GlcNAcase rate, being below 0.50 (Table 1–3). A higher  $\beta$ -GalNAcase/ $\beta$ -GlcNAcase rate was found only in several strains, e.g. *P. brasilianum* CCF 2155: 0.75, *P. funiculosum* CCF 2325: 0.76, *A. terreus* CCF 57: 1.00, *A. terreus* 2539: 1.01, *A. flavipes* CCF 2026: 1.00, *A. flavipes* IMI 171885: 0.90, *P. spinulosum* CCF 2159: 0.92, *P. funiculosum* CCF 1994: 1.20 and *P. purpurogenum* var. *rubrisclerotium* CCF 2985: 1.15 and CCF 2984: 1.22. Unique  $\beta$ -*N*-acetylhexosaminidase with the  $\beta$ -GalNAcase/ $\beta$ -GlcNAcase rate of about 1.50 was found only in culture filtrates of some strains of *P. oxalicum* (Huňková et al. 1997). These findings are in agreement with those of Yamamoto et al. (1985) obtained with the strain *P. oxalicum* IFO 5748 not exposed to induction. A high  $\beta$ -GalNAcase/ $\beta$ -GlcNAcase rate seems to be characteristic of the species *P. oxalicum*. Similar  $\beta$ -*N*-acetylhexosaminidase biochemical pattern was found only with *P. asturianum* CCF 2062<sup>1)</sup> (1.50). This species seems to be very close to *P. oxalicum*.

<sup>1)</sup> Strains with an asterisk (\*) were re-identified due to the results obtained – see the part “Taxonomic evaluation”.



*$\alpha$ -Galactosidase*

$\alpha$ -Galactosidase catalyzes hydrolysis of terminal  $\alpha$ -galactosidic linkages of glycosides (Savel'ev et al. 1996, Kaneko et al. 1990). Both raffinose and melibiose can serve as inducers of extracellular  $\alpha$ -galactosidase, giving similar induction rates (Huňková et al. 1996b). Cheaper raffinose was chosen for most of the experiments. After induction, extracellular enzyme was produced in several strains (e.g. *A. terreus* CCF 55, CCF 58, CCF 2539, ATCC 2052 and *P. commune* CCF 2962) in high specific activity and void of contaminating glycosidases (Huňková et al. 1996b). Thus, this enzyme could be directly precipitated by, e.g., ammonium sulphate and then directly used for further reactions (Weignerová et al. 1996, Weignerová et al. 1997, Weignerová et al. 1998).

Weak induction of  $\alpha$ -galactosidase was achieved using galactose. In some strains, a slight improvement of production was observed after adding of 6-deoxyglucose or glucosamine and in *F. oxysporum* also after adding of L-fucose.

In culture filtrates of the strains *Talaromyces flavus* CCF 2324, *P. funiculosum* CCF 2325, *P. purpurogenum* var. *rubrisclerotium* 2984 and CCF 2985, *A. fumigatus* CCF 1059, *A. flavipes* CCF 1895 and CCF 2026, *A. terreus* CCIM LM and CCIM USA\*, and *A. niveus* CCF 544\* induction of  $\alpha$ -galactosidase was triggered by 6-deoxyglucose whilst raffinose induced slightly or not at all. Induction of  $\alpha$ -galactosidase by 6-deoxyglucose in fungi is described here for the first time.

 *$\alpha$ -Mannosidase*

Although the occurrence of  $\alpha$ -mannosidase in different natural sources was described (Dey and Del Campillo 1984), no effective inducer for this enzyme was found yet. In several strains a slight improvement of extracellular  $\alpha$ -mannosidase was observed in the growth phase of cultivation when using  $\alpha$ -methylmannoside or  $\alpha$ -mannooligomers, but in the lytic phase constitutive production of this enzyme predominated (*A. oryzae* CCF 1066, CCF 147, CCF 172 and IMI 16266ii, *A. flavus* CCF 814, *A. tamarii* CCF 1665, *A. flavipes* CCF 1895, *A. niveus* CCF 544\*, *P. vinaceum* CCF 2384 – Table 1, 2). Slight induction both in the growth and lytic phases of cultivation was observed only in the strains *A. parasiticus* CCF 1298 and IMI 15957ix, *P. funiculosum* CCF 1994 and *P. purpurogenum* var. *rubrisclerotium* CCF 2985.

In some strains improvement of  $\alpha$ -galactosidase and  $\alpha$ -mannosidase was achieved using glucosamine. In our opinion, this improvement cannot be explained in terms of induction, similarly as the improvement of  $\beta$ -N-acetylhexosaminidase.

 *$\beta$ -Mannosidase*

No effective inducer for  $\beta$ -mannosidase was found so far. Brown copra meal (residual cake of coconut oil containing about 50%  $\beta$ -mannan) was used as a substrate in the screening test where *A. niger*, *A. awamori*, *A. sojae*, *P. wort-*

*manni* and *Emericella nidulans* were found to produce a detectable level of  $\beta$ -mannosidase (Holazo et al. 1992). For purification and characterization studies, the enzyme from *A. niger* produced constitutively was used (Monttreuil 1975, Elbein et al. 1977). In our study we observed slight constitutive production of extracellular  $\beta$ -mannosidase in several strains (*A. flavipes* IMI 171885, *A. niger* CCIM K1 and K2, *A. phoenicis* CCF 61, *P. vinaceum* CCF 2384, *P. purpurogenum* var. *rubrisclerotium* CCF 2984 and 2985 and *T. flavus* CCF 2324).

#### $\alpha$ -L-Fucosidase

Production of extracellular  $\alpha$ -L-fucosidase was slightly induced by 6-deoxyglucose in *P. funiculosum* CCF 1994 and CCF 2325 and *P. purpurogenum* var. *rubrisclerotium* CCF 2984 and CCF 2985. Effect of 6-deoxyglucose as inductor for  $\alpha$ -L-fucosidase was observed here for the first time.

On the contrary, in *F. oxysporum* extracellular  $\alpha$ -L-fucosidase was not induced by 6-deoxyglucose but its production was improved by L-fucose addition in some strains. The best results were reached with *F. oxysporum* CCF 377, good induction was observed in *F. oxysporum* CCF 483 and CCF 1414. No induction was observed in the strains CCF 906 and CCF 1389. Our results obtained with *F. oxysporum* CCF 377 are consistent with the results of Yamamoto et al. (1986b) obtained with *F. oxysporum*, strain S252. Just as these authors we also found that the amount of induced  $\alpha$ -L-fucosidase was increased proportionally with the concentration of L-fucose added (0.1–0.5%) and that the enzyme is slightly induced by D-arabinose.

#### Taxonomic evaluation

The data on the occurrence and inducibility of selected glycosidases represent additional information on the strains in the Collections. These data will appear in the addendum to the CCF Catalogue of filamentous fungi (Kubátová and Huňková 1998).

From the results given in the Tables 1–3 partial taxonomic conclusions can be deduced in such where more representatives of a particular species were examined, i.e. for *A. oryzae*, *A. flavus*, *A. terreus*, *P. oxalicum* and *F. oxysporum*.

The species *A. flavus*, *A. parasiticus*, *A. oryzae* and *A. tamarii* belong to economically important fungi, two first species producing aflatoxins, the other two species being used in food industry. All these species are included in the section *Flavi* of the genus *Aspergillus* and their morphological characters are very similar (Klich and Pitt 1988). The identification of these species based only on morphological features is difficult and quite often incorrect. Therefore, any aid in clarifying their taxonomy and their identification is very useful. Among our strains of *A. flavus*, *A. parasiticus* and *A. oryzae* large morphological variability was observed, especially in shape and surface texture of the conidias and in colony

habits. Some variability was found in the production of glycosidases as well. Contrary to *A. flavus*, the induction of  $\beta$ -*N*-acetylhexosaminidase in *A. oryzae* was higher than the induction of  $\alpha$ -galactosidase, only the strain *A. oryzae* CCF 1602 differed by a higher induction of  $\alpha$ -galactosidase. The morphological features of this strain were similar to the strains designated here as *A. tamarii*. Nevertheless, its correct placement in the species *A. oryzae* was confirmed by Dr. Z. Lawrence (IMI, Egham, U. K.). In *A. flavus* strains we found constitutive production of  $\alpha$ -mannosidase (except the strain CCF 814) whilst in *A. oryzae* slight induction was observed in the growth phase of cultivation and constitutive production prevailed in the lytic phase. However, the fact that no effective inductor exists for  $\alpha$ -mannosidase together with the fact that *A. flavus* CCF 814 exhibited typical morphological features makes definite conclusions impossible. In *A. oryzae* a slight induction of  $\alpha$ -mannosidase in the growth phase was observed, in *A. flavus* (except the strain CCF 814) constitutive production of this enzyme was found and in *A. parasiticus* CCF 1298 and IMI 15957ix induction of  $\alpha$ -mannosidase was observed both in the growth and lytic phase. Current results suggest that clarification of this situation requires detailed comparative study. Anyhow, one of the *A. oryzae* strains (CCF 1301) exhibited strong differences in the production of glycosidases manifested in the absence of induction of  $\beta$ -*N*-acetylhexosaminidase and  $\alpha$ -galactosidase and in the absence of constitutive  $\alpha$ -mannosidase production. After detailed morphological examination this strain was re-identified as *A. wentii*.

For the cultures of *A. terreus* (compared with the type strain IMI 17394) high induction of  $\alpha$ -galactosidase by raffinose or melibiose was typical. In the studied strains of *A. flavipes* (including the type strain IMI 171885) slight constitutive production of  $\alpha$ -galactosidase was observed, raffinose and melibiose being non-effective. Inducibility of  $\beta$ -*N*-acetylhexosaminidase in *A. flavipes* was very high but low or absent in *A. terreus*. In spite of the overall similarity of *A. terreus* and *A. flavipes*, these species have several distinct morphological features. *A. terreus* has remarkably shorter stipes, its phialides are more closely arranged and its colonies are growing faster. With respect to the differences in biochemical evaluations and distinct morphological features the strains *A. terreus* CCF 76, CCF 869 and CCIM USA were re-identified as to be *A. flavipes*.

Strain *A. niveus* CCF 544 was found to exhibit other biochemical characteristics than the type strain *A. niveus* IMI 171878 (see Table 1). Morphological examination revealed close resemblance of the strain CCF 544 to *A. flavipes* and the strain was therefore transferred to this species.

Black aspergilli, e.g. *A. niger*, *A. awamori* and *A. phoenicis*, are closely related to the section *Nigri* of the genus *Aspergillus*. Due to their morphological similarity, *A. awamori* and *A. phoenicis* were considered varieties of *A. niger* (Al-Musallam 1980, Klich and Pitt 1988, Kozakiewicz 1989). However, recent

molecular studies treated *A. awamori* and *A. phoenicis* as synonyms (Pařenicova et al 1997, Varga et al. 1997). Pařenicova et al. (1997) divided the studied strains of *A. awamori* and *A. phoenicis* into three groups: *A. niger*, *A. tubingensis* and *A. foetidus* varieties. Our results dealing with the glycosidase activity of four strains (constitutive production of all examined glycosidases) confirmed their close relationships. Nevertheless, correct placement of the four strains used in our study can be carried out only after a more detailed study involving comparative strains and molecular methods.

The slight induction of  $\alpha$ -L-fucosidase by 6-deoxyglucose in *P. purpurogenum* var. *rubrisclerotium* CCF 2984 and CCF 2985 as well as in *P. funiculosum* CCF 1994 and CCF 2325 together with other biochemical similarities correspond with the fact that *P. purpurogenum* var. *rubrisclerotium* is a species close to *P. funiculosum*. Both species belong to the series *Miniolutea* of the subgenus *Biverticillium*.

*P. oxalicum* strains represent a group which could be biochemically characterized by induction of unique  $\beta$ -N-acetylhexosaminidase with a very high  $\beta$ -N-galactosaminidase/ $\beta$ -N-glucosaminidase rate. *P. asturianum* CCF 2062, which possesses the same  $\beta$ -N-acetylhexosaminidase, has been considered as synonym of *P. oxalicum* since 1990 when Frisvad and Filtenborg (1990) and Frisvad et al. (1990) made a revision of *Penicillium* subgenus *Furcatum* based on secondary metabolites and conventional characters. Our results fully corroborate their conclusions.

*F. oxysporum* strains were preferentially examined for the production of  $\alpha$ -L-fucosidase and  $\beta$ -N-acetylhexosaminidase, and no other glycosidases were studied yet. For chemotaxonomic evaluation it would be necessary to examine all glycosidases, not only in *F. oxysporum* but also in closely related species. *F. oxysporum* is related to *F. solani*. Therefore, besides a morphological study a test of ammonium salts agar with sorbitol according to Brayford and Bridge (1989) was carried out. All *F. oxysporum* strains showed a red-vinaceous pigment on the colony reverse, one strain (CCF 1389) produced this pigment after a prolonged period of time (two weeks). Although the test confirmed the correct identification, the strains CCF 906 and CCF 1389 still differ from other strains by an absence of  $\alpha$ -L-fucosidase induction.

Differences in the production and inducibility of glycosidases certainly cannot serve as the only criterion for taxonomic determination of strains. Nevertheless, striking biochemical differences among strains of the same species drew our attention to a more detailed morphological examination of disputable strains and led us to eventual re-identification of some of them.

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