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This part of  
*Czech Mycology*  
is dedicated to Dr. Ludmila Marvanová, CSc.  
on the occasion of her 70th birthday.

# CZECH MYCOLOGY

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## Ludmila Marvanová – 70th birthday

JAN ŠPAČEK

In 1991, Olga Fassatiová published an article in *Česká mykologie*, Praha (45:123–127, 1991) on the occasion of Ludmila Marvanová's 60th birthday (born on February 22, 1931), including a list of Ludmila Marvanová's publications. Ten years after we commemorate her continuing activities.

Ludmila Marvanová as curator has been working in the Czech Collection of Microorganisms with unchanging intensity. Along with the development of electronic media the way of keeping data on collection cultures has changed, too, and the Czech Collection of Microorganisms joined the European project MINE (Microbial Network Europe). Ludmila Marvanová has had her share in refining definitions of database fields for fungi, and in implementing the project launched by the Ministry of the Environment (Building of National Node for the Database Researches) as part of the collection of fungi.

In the nineties various grant agencies and other institutions started to operate in the Czech Republic, opening competitions or entering into contracts for important projects. Ludmila Marvanová took part in several Czech and international projects, focussed mainly on aquatic hyphomycetes. At that time hydrobiologists started to be increasingly interested in these organisms, as they proved to play an important part in the food networks of aquatic ecosystems where decomposed leaf mass often was the only food source for benthic zoocenoses. Hand in hand went a growing interest in detailed study of the fungi, their life cycles and environmental requirements. Within the Czech Republic Ludmila Marvanová studied the fungi under the project Social and Natural Landscape Elements in the Extended Area



*L. Marvanová at a collecting trip to streams in Portugal.*

Photo Amaro Rodrigues,  
January 2002.



of the Dukovany-Dalešice Power System (1993–95), and between 1998–2000 as project researcher of the Taxonomy of Aquatic Hyphomycetes of Temperate Climate and their Species Diversity in Waters of the Czech Republic opened by the Grant Agency of the Czech Republic. Nowadays, many world research institutes study these fungi and their ecosystem functions. Ludmila Marvanová has been respected as one of the leading experts in this fungal ecologic group worldwide. She has often been called to participate in foreign research programmes, the solution of which requires taxonomy. In 2001 and 2002 Ludmila Marvanová won a scholarship under the INVOTAN programme for shared research of aquatic hyphomycetes at the University do Minho in Portugal. Several research institutes engaged in molecular methods in fungal taxonomy have shown their interest in co-operating with Ludmila Marvanová and utilize her unique collection of aquatic hyphomycetes (such as Technical University Berlin, New University of Lisbon, Mount Allison University in Sackville, University of Illinois, Urbana, Clark University in Massachusetts). In addition to that, Ludmila Marvanová travelled abroad several times with the purpose of preparing the manuscript of a monograph of aquatic fungi, together with other two foreign authors.

However, Ludmila Marvanová's professional activities were not concentrated on studies of aquatic fungi only. In the past ten years, she took part in the following projects: Analysis of Natural Conditions of the Peat Bog Locality Kaltwasser in the Flood Area of the Reservoir Turček (1992–1993), Basic Microbial Research in the Caves of the Moravian Karst (1994), or Biodiversity Protection Programme (1996–1997) in the region of lower River Dyje. Ludmila Marvanová published the results of her research activities both in scientific publications and also by way of presentation at congresses, conferences and symposiums held in the Czech Republic and abroad: (7<sup>th</sup> IUMS Congresses, Prague 1994, 5<sup>th</sup> International Mycological Association Congress, Vancouver 1994, 20<sup>th</sup> Czechoslovak Microbiological Society Congress, Ostrava 1995, 8<sup>th</sup> International Congress for Culture Collections, Veldhoven 1996, 2<sup>nd</sup> International Meeting on Plant Litter Processing in Freshwaters, Lunz 1999).

To get an idea of the volume of work carried out by Ludmila Marvanová we must not forget services related to her collection of fungi, mainly identification of microscopic fungi upon the requirements placed by various research institutes, industrial plants, private entrepreneurs, etc. These identifications, and in most cases also isolation of microscopic fungi from industrial products affected by biodeterioration, from food-processing plants, agricultural production, etc. have not been published.

The scope of activities rendered by experts of the Czech Collection of Micro-organisms does not include teaching primarily. Ludmila Marvanová, however, got involved in teaching in the last ten years, too. She was a consultant to several degree works and PhD theses, took part in determination courses held for graduate biolo-

gists in hygienic and water management practice, both here and abroad (such as in Riegersburg, Austria, for students of the University of Vienna, at a course focussed on identification, biodiversity, ecology and aquatic hyphomycetes preservation held in Coimbra, Portugal in 1998).

The description of Ludmila Marvanová's activities in the past ten years is supported by the bibliography thereof, which shows that the biggest part of her publications concentrated on taxonomic-morphologic aspects (description of new or little known taxons, new information on conidiogenesis, etc.). Another substantial part of Ludmila Marvanová's work mentioned in bibliography dealt with biodiversity of aquatic hyphomycetes in the Czech Republic and abroad, and also of micromycetes in caves and soil. Most of the publications are to be found in renowned international journals of a high quoting rate.

Ludmila Marvanová is a member of several mycological societies. The Czech Scientific Society awarded Ludmila Marvanová the title of Honorary Member for her credit of mycology development in 1996, on the occasion of the 50<sup>th</sup> anniversary of the Society. Her present commitment in the Society is that of a member of the editorial board of the journal *Czech Mycology*. She is also a member of the British Mycological Society, Mycological Society of Japan and since 1998 Honorary Member of the Mycological Society of America.

A general overview of Ludmila Marvanová's activities clearly shows that her achievements are based on a studied, systematic and dedicated work and working possibilities. She managed to combine scientific talent with publishing abilities, communication skills and readiness for action. Her work also proves that certain branches, tasks and problems have to be studied on a lifetime basis to obtain results from the research and routine for permanent service. Her knowledge and experience is based on a wide insight in the sciences and deep biological background extended earlier on by several years of agricultural research.

We express our congratulations to Ludmila, wishing her good health in the coming years to continue her work.

Ad multos annos!

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## On a new *Atractiella*

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Bandoni R. J. and Inderbitzin P. (2002): On a new *Atractiella*. – Czech Mycol. 53: 265–273

*Atractiella columbiana* Bandoni et Inderbitzin sp. nov. is described from felled poplar logs in southwestern British Columbia. The species is characterized by sporocarps typical of the genus, but these can bear either conidia or basidia, or both. The pileolus is globose to cupulate or discoid; it typically has a differentiated outer hyphal layer of parallel hyphae extending from the base of the stalk to the apex of the capitulum. Basidia are 4-celled; the sessile basidiospores being mostly unilateral and developing synchronously. Conidia develop sympodially on conidiogenous cells arising from assimilative hyphae, hyphae on sporocarp stalks, or in or on the capitulum. All such conidia are of similar appearance and dimensions.

**Key words:** Atractiellales, Hoehnelomycetaceae, *Atractiella*, *A. columbiana*, *Hoehnelomyces*.

Bandoni R. J. a Inderbitzin P. (2002): Nový druh rodu *Atractiella*. – Czech Mycol. 53: 265–273

Je popisován nový druh *Atractiella columbiana* Bandoni et Inderbitzin ze spadlých větví topolů v jihozápadní Britské Kolumbii. Tento druh je význačný plodnicemi typickými pro tento rod avšak mohou nést jak konidie tak i basidie. Klobouček je kulovitý, číškovitý nebo terčovitý a má typicky diferencovanou vnější hyfovou vrstvu složenou z paralelních hyf táhnoucích se od báze třeně až do vrcholku hlavičky. Basidie jsou čtyřbuněčné s jednostraně přisedlými basidiosporami, které se vyvíjejí současně. Konidie se vyvíjejí sympodiálně na konidiogenních buňkách, které vyrůstají z asimilativních hyf, z hyf na tření plodnice nebo na hlavičce. Všechny tyto konidie jsou podobné vzhledem i svými rozměry.

The genus *Atractiella*, based on *A. brunaudiana* Saccardo (Saccardo, 1886), included only three species in the survey of gasteroid auricularioid basidiomycetes by Oberwinkler and Bandoni (1982). A species of *Hoehnelomyces*, possibly a member of this group, was described and illustrated, but not named, by Boidin et al. (1979). During the past two years, several collections have been made of an *Atractiella* similar to *A. brunaudiana* on felled logs of *Populus trichocarpa* Torr. et Gray in a single locality in southwestern British Columbia. The taxon differs from the type species in dimensions of the basidia and spores, in having conidial synnemata

superficially identical to basidiomata, or in having such conidia formed externally on stalks of basidiomata, in the hymenium together with basidia, or on hyphae on the substrate. The species is described here as *A. columbiana* sp. nov. here.

#### MATERIALS AND METHODS

Material was mounted in 3% KOH-Phloxine and/or 3% KOH-Weak Congo Red for bright field microscopy. Spores and other structures were tested for amyloidity using Melzer's reagent, and for cyanophilly by staining with Lacto-phenol Cotton Blue (solutions as indicated in Hawksworth et al., 1995). Weak Congo red solution (Bandoni, unpublished) consists of: distilled water, 30 ml, glycerol, 3 ml, 1% aqueous Congo Red, 3 ml. A drop each of the stain solution and 3% KOH are mixed on the slide and the material is added; the preparation is then allowed to stand for 5–10 minutes for stain penetration. After covering, more stain is added at the edge of the coverslip as drying occurs or, alternatively, 10% glycerine in dist. water is added in this way to make the preparation semipermanent. The weak Congo Red preparation stains hyphal walls clearly, the intensity varying with the amount of stain added and with staining time. These preparations show less crystal formation than often occurs in mounts prepared with 1% Congo Red-KOH.

The collections examined, and the herbaria in which they are deposited, are listed under the specific description. Abbreviations for herbaria where specimens are preserved follow Holmgren et al. (1990).

#### TAXONOMY

*Atractiella columbiana* Bandoni et Inderbitzin, sp. nov. Figures 1–3.

Basidiomata stilboidea, 750–1100  $\mu\text{m}$  alta, hyalina vel lactea, sicca albolutescentia, caules 400–720  $\mu\text{m}$  longi, ad basim 98–230  $\mu\text{m}$  crassi, attenuati et 55–75  $\mu\text{m}$  ad apicem; tunica e hyphis 3.5–6.5  $\mu\text{m}$  in diam., efibulatis; hyphae internae 2.5–5  $\mu\text{m}$  in diam., efibulatae, compactae. Pilei globosi, 245–390  $\mu\text{m}$  lati, 320–430  $\mu\text{m}$  lati vel discoidei itaque, 120–160  $\mu\text{m}$  lati. Probasidia 50–90  $\times$  3.5–6  $\mu\text{m}$  demum anguste clavata vel cylindracea, 4-cellularia. Basidiosporae 17–22(–27.5)  $\times$  5–7  $\mu\text{m}$ , sessiles, subcylindraceae vel subfusiformes, anguste clavatae, rectae vel curvatae, per hypham germinantes. Conidiomata stilboidea, partim basidiomati-bus similia; cellulae conidiogenae plerumque 50–90  $\times$  4–8.5  $\mu\text{m}$ , ad apicem attenuatae sympodialiter proliferantes, conidia 16–25  $\times$  6–9  $\mu\text{m}$ , anguste clavata vel subcylindracea, ad basim truncata, per hypham vel per repetitionem germinantia.

Ad truncum *Populi trichocarpae* putrescentem, subcorticale vel in ligno nudo vicino. Delta (Ladner), British Columbia, Canada. A. & R. Bandoni 12992, HOLOTYPE (DAOM).

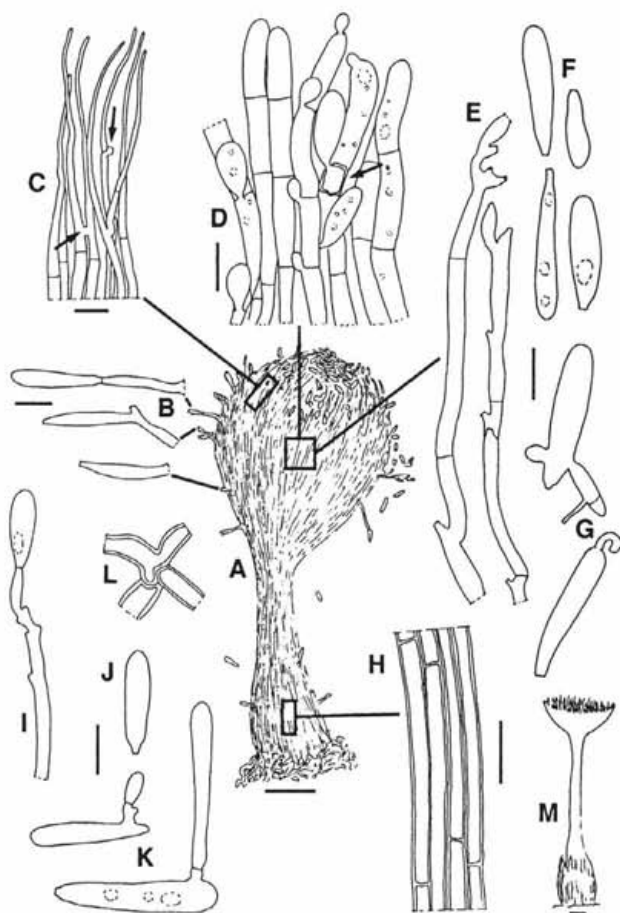
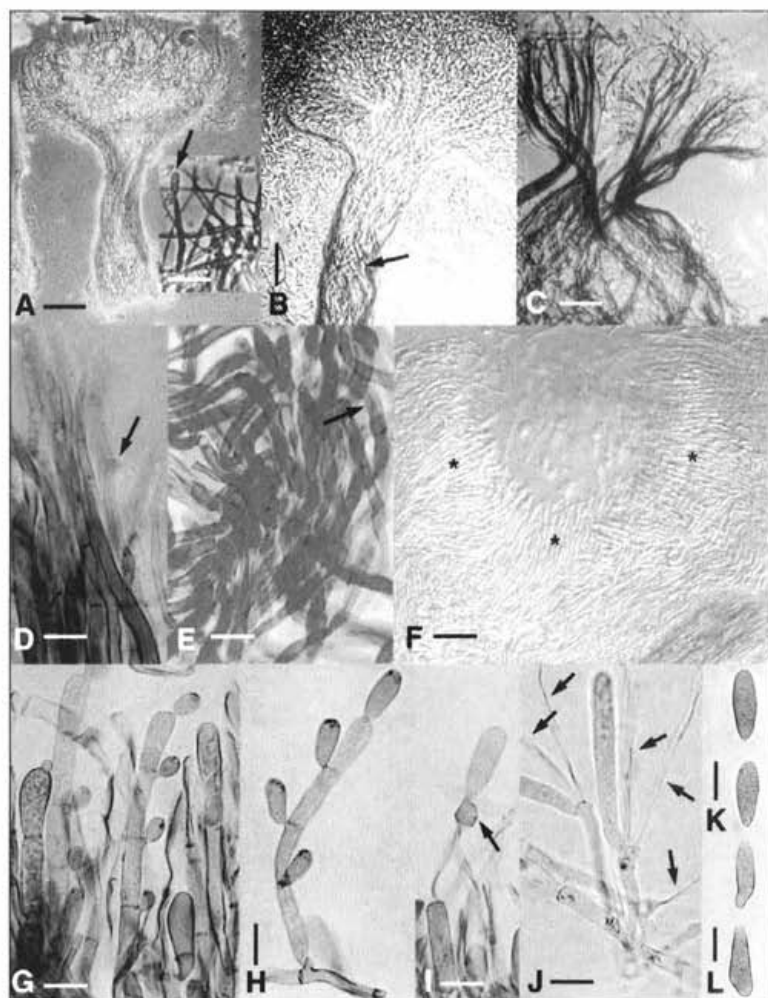


Fig. 1. *Atractiella columbiana* (A-C, H, M, from A. & R. B. 13992, Holotype; I - L from A. & R. B. 12993; E-G, from A. & R. B. 13008). A. Habit (drawn from photograph) of a complex sporocarp, the sheath filaments extending over the fertile portion. B.-L. Details (camera lucida drawings) of the sporocarp structure and types of reproductive structures in, on, or associated with such sporocarps. B. Conidia borne on conidiogenous cells protruding from sporocarp (lines). C. tapering apices of sheath hyphae; note clamp-like septum (arrow, above, right) and anastomosis (arrow, lower center). D. Hymenium, showing compact basidia of different stages in development. (Note central basidium showing typical unilateral and synchronous development of the 4 basidiospores; arrow, right, indicates chlamydospore-like structure from which a spore appears to be developing). E. Conidiogenous hyphae from hymenium of sporocarp, the successive conidiogenous levels suggesting extension to keep pace with extending hymenium. F. Conidia. G. Germinating conidia. H. Hyphae of the sheath showing slightly thickened walls and close, parallel arrangement. I. Conidiogenous cell and attached conidium, from hyphae on substratum adjacent to basidiome. J. Conidium, and K. germinating conidia, the lowermost germinating by a repetitive process. L. Clamp-like septum of hypha producing conidiogenous cells on substrate surface. M. Sporocarp with a discoid pileolus, that sketched had both basidia and conidiogenous cells in the hymenium. The volva-like basal portion probably is a rudimentary sheath. (Bars all equal 10  $\mu$ m)





**Fig. 2.** *Atractiella columbiana* (all figures from A. & R. B. 12993, Holotype). **A.** Sporocarp with discoid pileolus, lightly stained with KOH – Weak Congo Red. Enlarged portion, from margin of disk, the same attached conidium indicated by arrows in the habit and inset. Note the tapered apices of sheath hyphae in both insert and main print. **B.** Sporocarp stained as in A, the sheath hyphae of the stalk showing characteristic spiral arrangement found in most. **C.** Sheath, from crushed mount, separated from most of the stalk core and hymenium, showing continuity of sheath hyphae around stalk (below central constriction) and those extending around pileolus. **D.** Detail showing stained sheath hyphal apices from their widest points (ca. midpoint on capitulum) upward. Arrow indicates clamp like septum on one hypha. **E.** Thick-walled, irregularly arranged hyphae of emplacement, the lumen almost occluded in spots (arrow). **F.** Hymenium of a basidiome showing the compact, concave form in some basidiomata, the external form not discoid. **G.** Hymenium with developing basidia and basidiospores. **H.** Typical basidium with 4 unilateral, sessile, synchronously-formed basidiospores. **I.** Atypical basidium, a basidiospore apparently developing from a chlamydospore-like remnant of protoplasm in a partially emptied basidial cell (below arrow). **J.** Probasidium, upper center, and numerous collapsed basidia (arrows) showing proliferation pattern. **K.** Basidiospores of the most common form, showing inconspicuous attachment scars. **L.** Curved basidiospores. (Bars in A, left = 10  $\mu$ m; right bar = 30  $\mu$ m. All other bars = 10  $\mu$ m)

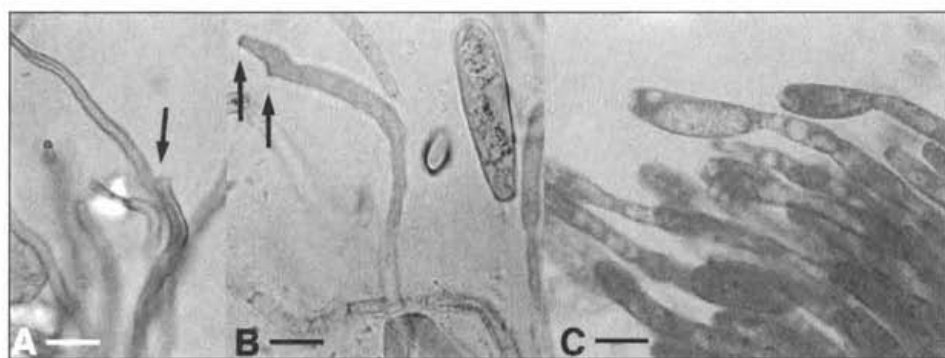


Fig. 3. *Atractiella columbiana* (from A. & R. B. 12992). A. Sheath hypha showing locus (arrow) where conidium developed during elongation and B. hymenial conidiogenous cell from basidiome, two sporogenous loci indicated by arrows. C. Hymenium of conidial sporocarp showing young conidiogenous cells and attached conidia. (Bars = 10  $\mu$ m).

Basidiomata scattered to gregarious, often intermixed with conidiomata of similar form or differing slightly, most 750–1100  $\mu$ m high, stipitate-pileolate (Fig. 1A, M; 2A–B); stalk 400–720  $\mu$ m long, 98–230  $\mu$ m wide basally, tapering to 55–75  $\mu$ m below the pileolus, the latter 245–390  $\mu$ m wide, ca. 120–160  $\mu$ m high centrally if discoid, 320–430  $\mu$ m high if globose or oval, hyaline to milky when fresh, the basal half sometimes appearing gelatinous, with a slime drop above surrounded by paraphysis-like tapered tips of the sheath hyphae (Fig. 2A), these enclosing numerous basidiospores and/or conidia, drying inconspicuous, whitish. Stalk arising from a pulvinate emplacement of irregularly arranged hyphae (Fig. 2E), 3.5 to 6.5  $\mu$ m in diam., the walls to 2  $\mu$ m thick. A sheath like layer commonly surrounds the stalk; it is composed of hyphae 2.5–5  $\mu$ m in diam., their walls thickened to ca. 1  $\mu$ m, these hyphae closely adherent laterally, typically arranged in a steep spiral around the stalk (Fig. 2B); they are septate, efibulate, branched, the main axis swollen immediately below the infrequent branch points, branches recurving and adhering to the parent; the sheath typically continuous upward around the capitulum, the hyphae branching more frequently and separating from one another there, swollen to 5–7  $\mu$ m at the level of the hymenium, then tapering upward, arching to form a loose enclosure over the fertile zone, the tips narrowed to 1–1.5  $\mu$ m in diam., the terminal cell slightly larger, rounded apically; some such hyphae conidiogenous, producing a conidium, then extending below its base, the locus visible after secession (Fig. 3A); sheath lacking entirely in some basidiomata and conidiomata, otherwise extending to varying distances up the stalk, to the base of the pileolus, or to the edge of the disk and appearing as a marginal fringe (Fig. 2A); central core hyphae of stalk 3.5–6  $\mu$ m in diam., thin walled, septate, some septa adventitious, without clamps, compactly arranged, parallel, strongly adherent, infrequently branched, continuing to the hymenium to

produce basidia and/or conidia. Hymenium of compactly arranged probasidia, and basidia, these developing continuously, older basidiomata with numerous collapsed basidia present (Fig. 1J); probasidia narrowly clavate (Fig. 1J), finally 4-celled,  $50-90 \times 3.5-6 \mu\text{m}$ , the 3 upper cells cylindrical, the basal cell longer and tapering to  $2.5-3.5 \mu\text{m}$ ; predominantly clearly simple septate basally (Fig. 2G, H, J), but infrequently appear to be clamped there; basidial walls thin, each cell producing a sessile basidiospore (Fig. 1D; Fig. 2G, H), then collapsing as the protoplast migrates into the spore. Basidiospores  $17-22(-27.5) \times 5-7 \mu\text{m}$ , subcylindrical to subfusiform, narrow clavate, straight or variously curved (Fig. 2K, L), some approaching allantoid or sigmoid; walls smooth, thin, inamyloid, not cyanescent; germination by germ tube. Conidia produced in or on some basidiomata (Fig. 1B, E-G, I-L; Fig. 3B-C) in purely conidial structures essentially like the basidiomata, on tapered apices of sheath hyphae (Fig. 3A), or sometimes along stalks of both synnemata and basidiomata; conidia also develop on hyphae in culture and on the substrate surface in nature (Fig. 1I-L); these can be born on single, separate conidiogenous cells, but rudimentary synnemata are more common on the natural substrate, often enclosing conidiophores of associated dematiaceous hyphomycetes; synnemata variable, many indistinguishable from the basidiomata superficially, but others often under  $500 \mu\text{m}$  high, the capitulum globose, oval, cupulate or discoid to irregularly clavate; stalk structure as with basidiomata, the sheath present or absent, if present, either extending to the margin of the discoid or cupulate portion of the synnema (as in Fig. 2A) and forming a marginal fringe, or otherwise ending below the capitulum, the hyphae and their arrangement as in basidiomata. Conidiogenous cells proliferating sympodially in all cases, many of those on the stalk and fertile portion of sporocarps  $50-90 \times 4-8.5 \mu\text{m}$ , tapered apically, producing a terminal conidium, then proliferating immediately below it (Fig. 1I; Fig. 3B), leaving a conspicuous conical locus and often bent at an angle below this; conidia  $16-25 \times 6-9 \mu\text{m}$ , thin-walled, smooth, narrowly clavate to subcylindrical (Fig. 1B, F, J; Fig. 3B), indistinguishable from basidiospores in general form, but most visibly truncate basally or with an abruptly broadened shoulder a short distance above the attachment point; germination by germ tube (Fig. 1G) or by a repetitive process (Fig. 1K).

**Habitat:** Growing on felled trunks of *Populus trichocarpa* (cut 2-3 years previously), beneath the loose bark, on the inner bark layer, or immediately adjacent to the bark on recently exposed wood.

**Collections examined:** All collections decaying logs of *Populus trichocarpa* (felled, on the ground for 2-3 years); on wood beneath loosened bark, on inner bark, or on exposed wood immediately adjacent to bark edges; all came from a single locality, as follows: Canada: British Columbia; Delta (Ladner), Lagoon Walk, off Ferry Rd. A. & R. Bandoni, 12992, 14 Oct. 2000, HOLOTYPE (DAOM); A. & R. Bandoni 12993, 19 Oct. 2000 (DAOM); 12997, 15 Oct. 2000 (DAOM);

13004, 25 Oct. 2000 DAOM); 13007, 2 Nov. 2000 (UBC); 13008B, 2 Nov. 2000 (UBC); 13028, 30 Nov. 2000 (UBC).

#### DISCUSSION

All previously described species of *Atractiella* are clampless, and almost all septa appear simple in *A. columbiana*. However, rare septa at the bases of basidia and elsewhere are possibly clamped (Figs. 1C, L; Fig. 2D). The basidia are similar to those in other species of the genus, typically producing passively released sessile basidiospores unilaterally and synchronously. Exceptions with respect to synchrony were observed several times; these might represent delayed development of a single spore on a basidium. In each such case, a small chlamydospore-like compartment within the basidium giving rise to a typical basidiospore (Fig. 1D, 2I, arrows)

The species is distinctive in having conidia often present superficially on the stalks of basidiomata and conidiomata, on the capitulum surface (Fig. 1B; Fig. 2A), mixed with basidia (Fig. 3B), free on the substratum or in synnematosus conidiomata. The surface sometimes arising from the growing sheath hyphae (Fig. 3A). Conidial synnemata can be stilbelloid, cupulate, or discoid, the conidiogenous cells often occurring in a hymenium-like layer (Fig. 3C) on the planate to convex or concave distal surface. Similar conidia are produced on the mycelium in nature (Fig. 1L) and in culture; sporocarps developed in culture were mostly anamorphic, but no attempt was made to determine conditions necessary for formation of basidiomata.

Since conidia and basidiospores are of similar form, overlap in dimensions, and have the same wall characteristics, they can easily be confused with one another. Generally, however, the conidia are symmetrical and are relatively conspicuously truncate basally. The basidiospores are attached asymmetrically, and the scar left after secession is inconspicuous.

Known species of *Atractiella* fall into two groups: In *A. brunaudiana* Saccardo (type species) and *A. columbiana*, hyphae of the sheath terminate in narrowed hyphidium-like filaments apically. These species lack hyphidia in the hymenium. The sheath sometimes is absent or is only partially developed, but it generally is present and surrounds the stalk, the same hyphae extending upward around the capitulum. The sheath hyphae of the stalk and basal portion of the capitulum are closely parallel and are more strongly adherent to one another than to the inner core of cells. Pressure on the coverslip of a whole mount often results in separation of the sheath from the central core, as seen in Figure 2C. The apices of individual sheath hyphae are free (Figs. 1C; 2D), and they sometimes appear to be present in the hymenium when whole mounts of single basidiomes are flattened. However, in *A. columbiana*, they are restricted to the area around the hymenial margin.



*A. brunaudiana* appears to have a sheath like that in *A. columbiana*, but true hyphidia are present in hymenia of other species in the genus. In *A. columbiana*, the sheath hyphae can be conidiogenous (Fig. 3A). In *A. solani* (Cohn et Schroet.) Oberw. et Bandoni and *A. delectans* (Moeller) Oberw. et Bandoni, a sheath is present around the stalk, where it is at least superficially similar to that described above, but, the tapered extensions over the fertile area are absent. Sporocarps of these species do have hyphidia that develop from the fertile hyphae (Oberwinkler et Bandoni, 1982). It is not clear at this time whether the two groups are closely related (i.e., actually congeneric) as the study of most of these species is based upon limited herbarium material, some (e.g., *A. brunaudiana*) from a single poorly preserved collection. Occasional stalked conidiomata of *A. columbiana* lack both a sheath and a capitulum; here the stalk terminates in a ring of conidiogenous cells through which the central core of cells sometimes protrudes. In typical sporocarps, the central core of the stipe extends to the hymenial area, where they produce the fertile hyphae bearing basidia or conidia.

Conidial production has been reported only on germ tubes arising from basidiospores in *A. delectans* and *A. solani*; they occur on conidiophores in a *Hoehnellomyces* sp. reported on olives from Italy (Boidin et al., 1979). The description and figures of the last named fungus appear to be *Atractiella* - like. Although the dimensions of basidia and spores given for this species are within those noted here for *A. columbiana*, the conidia are much smaller and the conidiophores and conidiogenous cells also differ sharply. Thus far, no other atractyelloid taxa have been found which produce synnematus anamorphs that closely resemble their basidiocarps. Nor are conidium-bearing cells known on the stipes, on the tapered hyphae of the sheath, and on conidiogenous cells in the hymenium in species other than *A. columbiana*.

In conclusion, the unusual habitat in which much of the *A. columbiana* material grew, (i. e. beneath the slightly loosened bark of relatively sound trunks of felled or fallen trees) is rich in arthropods, worms, bacteria, and fungi. The presence of arthropods, and the sticky spore drops characteristic of *Atractiellas* suggest transport by such animals. Although we contemplated erecting a new genus for the species described here, the similarities to *A. brunaudiana* dictate against such a course at this time.

#### ACKNOWLEDGMENTS

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

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Gulis V. and Suberkropp K. (2002): The effect of excluding plant litter on the aquatic hyphomycete conidia in a headwater stream. – *Czech Mycol.* 53: 275–284

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

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

Gulis V. a Suberkropp K. (2002): Důsledek odstranění rostlinného opadu na konidie vodních hyfomycetů v horním toku. – *Czech Mycol.* 53: 275–284

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

### INTRODUCTION

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

Most aquatic hyphomycetes form tetra- or polyradiate (predominant), branched or filiform conidia which are adapted for dispersal in flowing water (Webster & Descals 1981). Conventionally shaped conidia are less frequent. Tetra- or polyradiate conidia can

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

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

#### METHODS

The study was conducted at two headwater streams draining catchments 53 (C 53) and 55 (C 55) in the Coweeta Hydrologic Laboratory, Macon county, North Carolina, USA. These streams are small (avg. discharge about  $2 \text{ L s}^{-1}$ ), circumneutral, softwater and contain low nutrient concentrations ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N} < 7 \mu\text{g L}^{-1}$ , soluble reactive phosphorus  $< 3 \mu\text{g L}^{-1}$ ). They drain mixed deciduous forest with a dense understory of rhododendron (*Rhododendron maximum* L.) in the southern Appalachian Mountains at an elevation of ca. 800 m a.s.l. Physical and hydrochemical characteristics of the two streams are very similar (Wallace *et al.* 1999). Water temperature during the study period was monitored with StowAway temperature probes (Onset Computer Corp.) that recorded temperature every 30 min.

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



declined even more than it was in 1997. As a result, organic substrates available for fungal colonization in C 55 included very limited amounts of leaf litter (e.g. some needles of *Pinus strobus* L.), woody debris, dead and living roots of riparian trees, mosses, and plant parts (e.g. stipules, bud scales) that were smaller than the mesh. C 53 served as the reference stream and contained a variety of types of leaf litter and woody substrates for colonization.

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

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

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

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

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

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

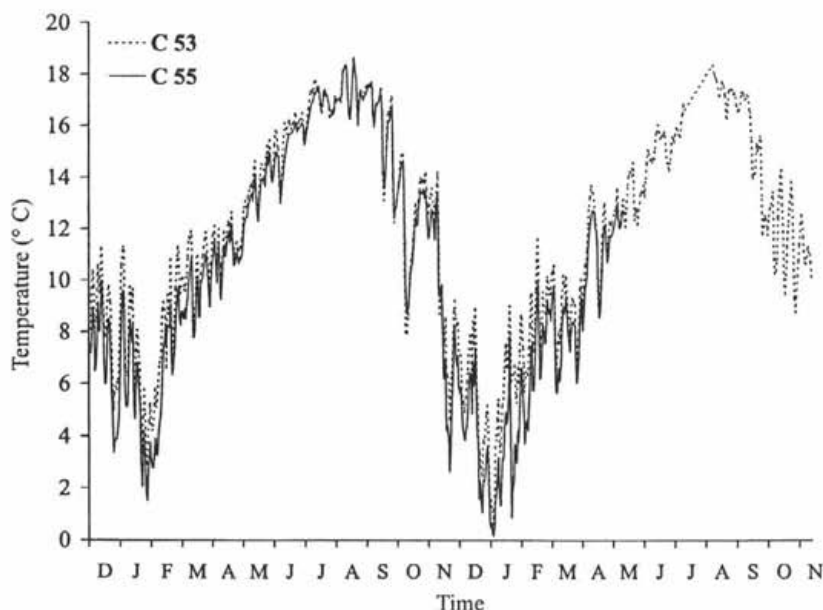


Fig. 1. Average daily water temperatures of C 53 and C 55 during the study period.

## RESULTS

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

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

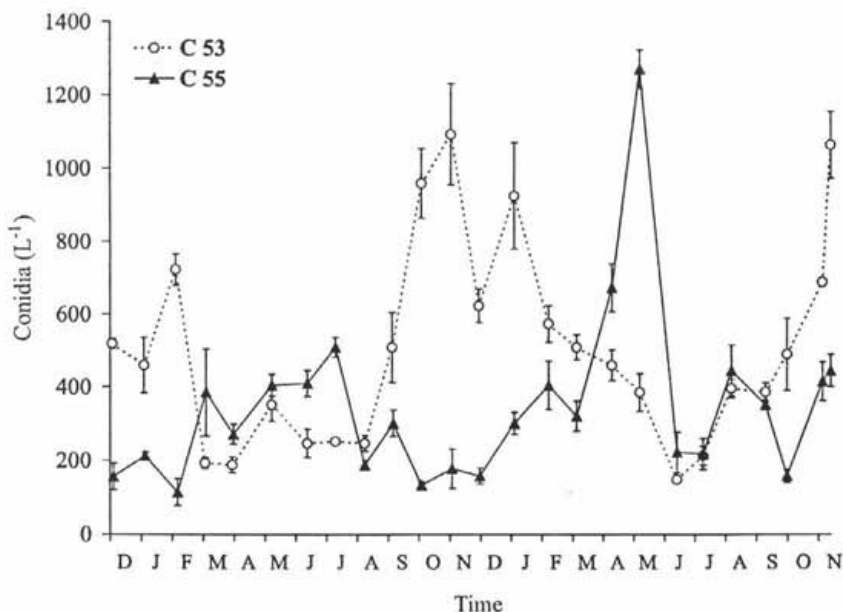


Fig. 2. Conidia concentration of aquatic hyphomycetes in the water of C 53 and C 55. Symbols indicate mean  $\pm$  SE.

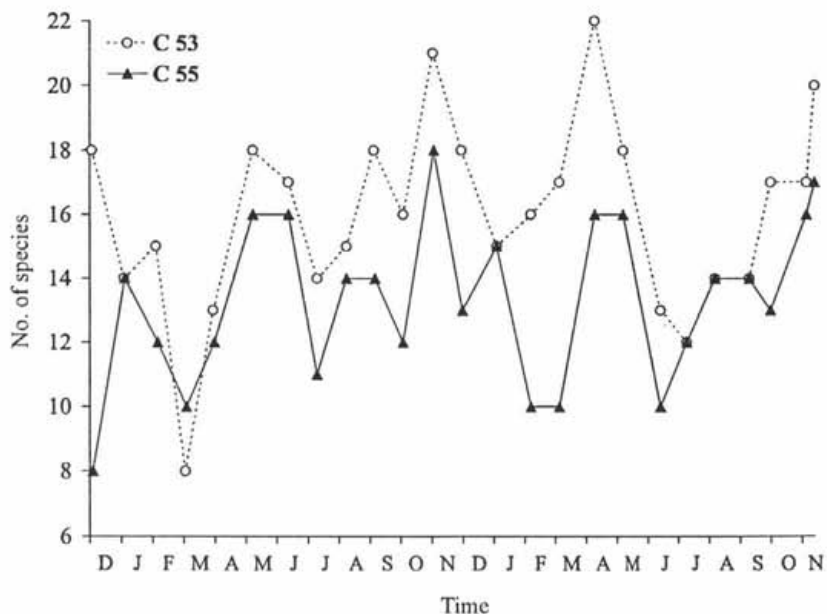
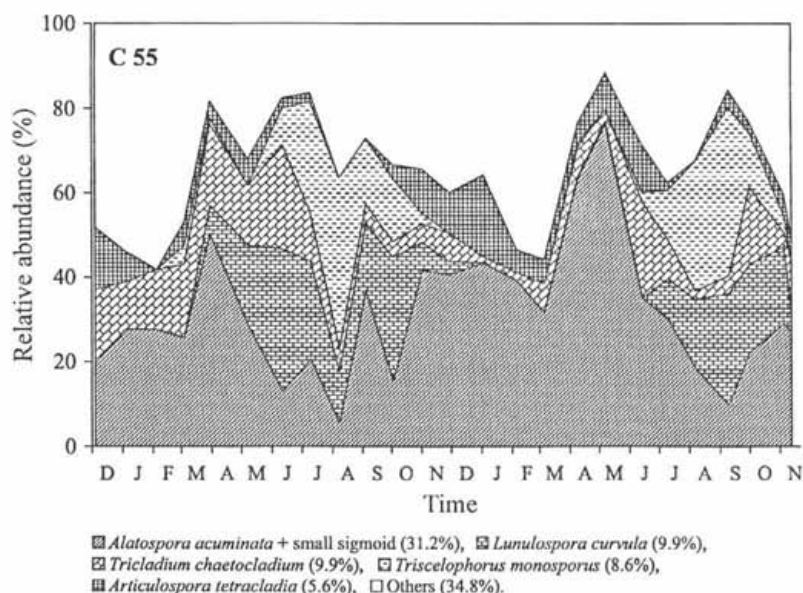
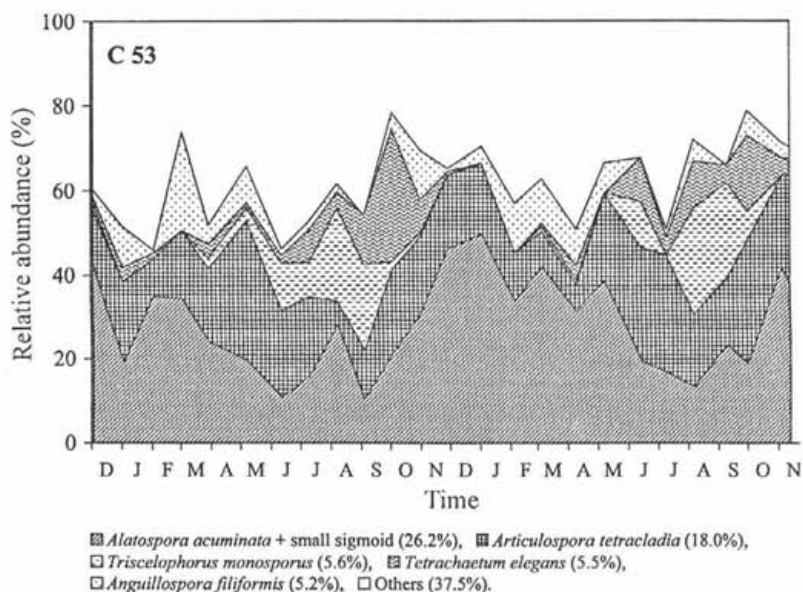


Fig. 3. Species richness of aquatic hyphomycete conidia in transport in C 53 and C 55 during the study period.



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

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

### DISCUSSION

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

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

Maximum conidia concentrations observed in C 53 and C 55 are relatively low in comparison to some published values (up to 22000 (Shearer & Webster 1985a), 18000 (Suberkropp 1997) or 11000 conidia L<sup>-1</sup> (Gönczöl & Révay 1999))

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

The unexpected difference we observed was that conidia concentrations exhibited maxima at different times in the two streams. The reference stream, C 53 exhibited a pattern that is typical for temperate forested streams with maximum conidia concentrations in autumn and winter. Such a response to seasonal substrate input is well documented (e.g. Iqbal & Webster 1973, Bärlocher & Rosset 1981, Shearer & Webster 1985b, Suberkropp 1997, Gönczöl & Révay 1999, Bärlocher 2000). In contrast, conidia concentrations in the litter-exclusion stream exhibited maxima in the spring-summer (Fig. 2). We speculate that in the absence of the influence of seasonal litter inputs, activity of aquatic hyphomycetes in C 55 was controlled more by temperature (cf. Figs. 1 and 2).

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

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



changes in community structure took place in response to litter exclusion. Higher species richness and less even distribution of conidia between taxa in 1999–2001 in both streams (Table 2) appear to be due to the higher number of relatively rare species found over two years (1999–2001) in contrast to the one-year period (1988–1989).

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

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## Phylogeny of *Tetracladium* based on 18S rDNA

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Complete sequences of 18S rDNA of seven strains of *Tetracladium* were determined. The following species were included: *T. apiense*, *T. furcatum*, *T. maxilliforme*, *T. setigerum* (one strain each) and *T. marchalianum* (3 strains). Sequence homology among the 7 strains was  $\geq 98\%$ . The closest published match (NCBI database) to the *Tetracladium* sequences is one by *Bulgaria inquinans* (homology 95–96%). Phylogenetic analysis placed the *Tetracladium* complex in the vicinity of the Ascomycete orders Onygenales, Erysiphales and Leotiales.

**Key words:** *Tetracladium*, 18S rDNA, Leotiales, Erysiphales, Onygenales

Nikolcheva, L. a Bärlocher, F. (2002). Fylogeneze rodu *Tetracladium* založená na 18S rDNA. – Czech Mycol. 53: 285–295

Byly stanoveny kompletní sekvence 18S rDNA sedmi kmenů rodu *Tetracladium*. Následující druhy *T. apiense*, *T. furcatum*, *T. maxilliforme*, *T. setigerum* (od každého druhu jeden kmen) a *T. marchalianum* (3 kmeny). Homologie sekvencí mezi sedmi kmeny byla  $\geq 98\%$ . Nejbližší obdoba (NCBI databáze) k sekvencím rodu *Tetracladium* je sekvence druhu *Bulgaria inquinans* (homologie 95–96%). Fylogenetická analýza umístila komplex druhů rodu *Tetracladium* do blízkosti řádů vřeckatých hub a to Onygenales, Erysiphales a Leotiales.

### INTRODUCTION

Aquatic hyphomycetes are the primary agents of leaf litter decay in streams and rivers (Bärlocher 1992). They 'condition' the substrate for consumption by detritus-feeding invertebrates and thus form an important trophic link in the food web (Bärlocher 1985, Suberkropp 1992). Aquatic hyphomycetes (also known as freshwater hyphomycetes, amphibious hyphomycetes, Ingoldian fungi) are not monophyletic, but are grouped together on the basis of morphological and ecological similarities (Bärlocher 1992). Their taxonomy is based on anamorph-genera (asexual or mitosporic states), which include species with conidia of similar development and morphology. These spores are typically tetra- or sigmoidal (Ingold 1975, Webster and Descals 1981). Since these shapes facilitate attachment to the substrate and provide a stable base for rapid germination (Read et al. 1992), they are believed to have evolved repeatedly and independently in fungi living in a similar habitat. This suggests that spore morphology provides little information on phylogenetic relationships (Seifert 1993, Dix and Webster 1995, Alexopoulos

et al. 1996). Studies connecting anamorphs to teleomorphs have shown that the majority of aquatic hyphomycetes belong to the Ascomycetes and only a few to the Basidiomycetes. They also revealed that several of the anamorph-genera are heterogeneous, i.e. include taxa of diverse relationship (Webster and Descals 1981). These findings support the assumption of convergent evolution and suggest that the current classification does not reliably reflect evolutionary relationships. An alternative, preferred approach is based on molecular data such as nucleotide sequences of selected genes (Hillis 1987, Olsen 1988, Smith, 1989). DNA sequences allow comparing groups at all taxonomic levels and are independent of an organism's stage in the life cycle or its reproductive phase. In particular, ribosomal genes have been an important source of phylogenetic information for many taxa, including fungi (Bruns et al. 1991, Hibett 1992). They are present in all organisms, highly conserved in form and function (White et al. 1990, Holst-Jensen et al. 1997, van de Peer and De Wachter 1997,) and are homologous (Brus et al. 1991). In eukaryotic organisms, the emphasis has been on 18S rDNA (small subunit nuclear rDNA; Berbee and Taylor 1993, Van de Peer and De Wachter 1997).

*Tetracladium* was one of the three genera of aquatic hyphomycetes with tetradiate conidia that were discovered by de Wildeman (1893, 1894, 1895). He noticed the similarity between the newly described *Tetracladium marchalianum* and some old, doubtful or poorly defined genera of algae such as *Asterothrix* and *Cerasteria*. To complicate matters further, *T. marchalianum* was often confused with *T. setigerum* (first described as *Tridentaria setigera*; Grove 1912) or *T. maxilliforme* (described as *Titaca maxilliformis* by Rostrup 1894). The confusion dates back to de Wildeman (1893), whose drawings seem to include *T. marchalianum* and *T. setigerum*. A fourth species, *T. apiense*, was described by Sinclair and Eicker (1981), and *T. furcatum* was added two years later (Descals and Webster 1983). Based on pure culture studies, two more species, *T. breve* and *T. palmatum*, were defined by Roldán et al. (1989). Members of this genus have been reported from a wide range of geographic locations; however, conidial shapes of the various species show considerable overlap and identification from single conidia is problematic (Roldán et al. 1989). To date, no sexual state has been reported from any species.

The primary objective of this study was to determine 18S rDNA sequences of selected representatives of the genus *Tetracladium* and use them to establish its position within the Fungi. In addition, our goal was to determine if the molecular data confirm the monophyletic status of *Tetracladium*, as suggested by traditional, morphology-based taxonomy.

## MATERIALS AND METHODS

## Isolates examined in study

Pure cultures of 7 strains belonging to 5 species were obtained from the Czech Collection of Microorganisms (CCM). They are listed in Table 1.

**Table 1.** Fungal strains examined in this study. All were obtained from the Czech Collection of Microorganisms (CCM). Sequences were deposited in the GenBank database (NCBI).

Taxon	CCM Number	Origin	GenBank Accession Number
<i>Tetracladium apiense</i>	F-23199	Czech Republic	AF388575
<i>T. furcatum</i>	F-11883	Czech Republic	AF388578
<i>T. marchallanum</i>	F-11391	Czech Republic	AF388579
<i>T. marchallanum</i>	LH-89	Czech Republic	AF388580
<i>T. marchallanum</i>	F-312	Slovak Republic	AF388576
<i>T. maxilliforme</i>	F-14286	Czech Republic	AF388577
<i>T. setigerum</i>	F-20987	Canada, New Brunswick	AF388574

## DNA extraction

Fungal mycelia were grown in 1% malt broth at 20 °C for 14–21 days. Mycelia were harvested on filters, freeze-dried overnight and ground in liquid nitrogen for up to 1 min. The crushed mycelia (150 mg) were placed in an Eppendorf tube together with 300 µl of lysis buffer (50 mM Tris-HCl, 50mM EDTA, 3% SDS, 1% 2-mercaptoethanol) and the sample was incubated at 75 °C for 10 min. Standard chloroform: phenol (1:1) extraction was performed with isopropanol precipitation of DNA. The supernatant was drained, the pellet was washed with ethanol, dried and resuspended in 100 µl of ddH<sub>2</sub>O.

## Amplification and purification of DNA

Partial nuclear SSU rDNA regions were amplified using combinations of primer pairs of NS1 to NS8 (White et al. 1990). Amplification was performed in 50 µl volumes containing 1 ng µl<sup>-1</sup> template DNA, 2 U Taq polymerase (Pharmacia Biotech), 1X Taq polymerase buffer, 250 µM of each dNTP (Pharmacia Biotech), and 4 mM MgCl<sub>2</sub>. The PCR was performed in a T-gradient Biometra thermocycler (Whatman). The program started with initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30s, annealing at 55 °C for 30s and extension at 72 °C for 2 min. The final extension was done at 72 °C for 5 min. The PCR product was purified from solution with a GFX DNA purification kit (Amersham Pharmacia Biotech). The product was eluted with 40 µl of ddH<sub>2</sub>O. The

DNA was sequenced with the PCR amplification primers and a dideoxy terminator sequencing kit (as instructed by ABI Prism) at the Molecular Supercentre at the University of Guelph.

### Sequence alignment and phylogeny construction

The 18S rDNA gene sequences of all seven *Tetracladium* isolates were submitted to the BLAST Search engine at NCBI. The sequences of the first hits together with other selected sequences were used for phylogeny construction. Altogether, 18S rDNA gene sequences of 42 fungal species were retrieved from GenBank for use in phylogenetic analyses. The selected species followed by accession number are: *Anamylospora pulcherrima* (AF119501), *Arthrocladiella mougeotii* (AB033477), *Arthroderma ciferrii* (AB015678), *Aspergillus sparsus* (AB008408), *Aureobasidium pullulans* (M55639), *Beauveria bassiana* (AB027336), *Blumeria graminis* (AB033476), *Bothryosphaeria ribis* (U42477), *Bulgaria inquinans* (AJ224362), *Byssosascus straitosporus* (AB015776), *Coccodinium bartschii* (U77668), *Comminutispora* sp. (Y18699), *Cryphonectria havanensis* (L42440), *Cyttaria darwinii* (U53369), *Erysiphe orontii* (AB133483), *Graphium silanum* (AB007661), *Helvella terrestris* (AF006306), *Lecidea fuscoatra* (AF088239), *Lepo-lichen coccophorus* (AF274110), *Leveillula taurica* (AB033479), *Monilinia laxa* (Y14210), *Microsphaera friestii* (AB033478), *Myxotrichum deflexum* (AB015777), *Nectria aureofulva* (AB013010), *Oidiodendron tenuissimum* (AB015787), *Ophiostoma piliferum* (AJ243295), *Peziza echinospora* (AF006309), *Phyllactinia guttata* (AF021796), *Pseudogymnoascus roseus* (AB015778), *Psora decipiens* (AF184759), *Rhizina undulata* (U42664), *Rhizomucor miehei* (AF192506), *Sarcoscypha austriaca* (AF006318), *Selenaspora guernisacii* (AF104667), *Sordaria fimicola* (X69851), *Spathularia flavida* (Z30239), *Sphaerotheca cucurbitae* (AB033477), *Stereocaulon paschale* (AF140236), *Taplrina maculans* (AB000953), *Tuber excavatum* (X98089), *Uncinula mori* (AB033484).

All sequences were aligned manually with Se-Al (Rambaut 1995). Regions with ambiguous alignment were excluded from the data set in order to increase bootstrap support for the branches. Phylogenetic trees were generated with PHYLIP (Felsenstein 1995) using the parsimony (DNAPARS) and neighbour-joining distance methods (DNADIST/NEIGHBOR) with equal weighing of the 1695 most conserved alignment positions. All trees were calculated with a random addition of taxa. Branching order stability was estimated by bootstrap analysis of 100 replicates. *Rhizomucor miehei* was used as an outgroup in all phylogenetic trees. For the Neighbour-Joining tree, dissimilarity values based on pairwise comparisons of sequences were transformed into distances according to the Kimura two-parameter correction and using a transition to transversion ratio of 2.



## RESULTS

## Sequence alignment and BLAST search results

The sequences of the nuclear rDNA of the 7 *Tetracladium* strains were approximately 1780 bp long (all sequences were deposited in the GenBank database; accession numbers are listed in Table 1). Their alignment revealed very little variation in the 18S region: sequence homology was  $\geq 98\%$ ; rare events often consisted of insertion/deletion or substitution of a single nucleotide.

When the 18S rDNA sequences of the seven isolates were aligned and submitted to the BLAST search engine on NCBI, the closest match was with *Bulgaria inquinans* (placed in Leotiales by Ainsworth et al. 1995, Alexopoulos et al. 1996). Sequence identity with *B. inquinans* varied between 95% (*T. apiense*) and 96% (*T. marchalianum* LH89). Other close matches were with species of the genera *Erysiphe*, *Blumeria* (Erysiphales); *Myxotrichum*, *Oidiodendron* (Onygenales); *Rhizina* (Pezizales).

Phylogeny of *Tetracladium* and its position in the fungal kingdom

A phylogeny of the aquatic hyphomycete genus *Tetracladium* inferred from the Neighbour-Joining distance method is presented in Fig. 1. Bootstrap values greater than 50% supporting the recovered branches in either the distance or the parsimony tree are placed at the internal nodes of the tree.

Parsimony analysis of the data set yielded 16 equally parsimonious trees, one of which is shown (Fig. 2). There were no major differences between the Parsimony tree and the Neighbour-Joining tree. *Tetracladium* species grouped together in 100% of the bootstrapped samples, suggesting that the genus is monophyletic. Within the *Tetracladium* genus there was no significant grouping. *T. setigerum* and *T. marchalianum* F-11391 grouped together in both trees but the branch was weakly supported. All members of the genus *Tetracladium* were closely related to species from the Ascomycete orders Onygenales, Erysiphales and Leotiales. In the Neighbour-Joining tree *Tetracladium* branched together with Onygenales and was more distantly related to Erysiphales. In the Parsimony tree this order was reversed. As seen in previous studies (Doering 1998), the 18S rDNA data were unable to give good resolution and bootstrap support for the relationship between many of the lineages within the Leotiales, Onygenales and Erysiphales. *Tetracladium* grouped significantly apart from Sordariales, Clavicipetales, Hypocreales, Diaporthales, Ophiostomatales and Sphaeriales. *Rhizomucor* was used as an outgroup and was significantly different from the other species in 100% of the analyses.

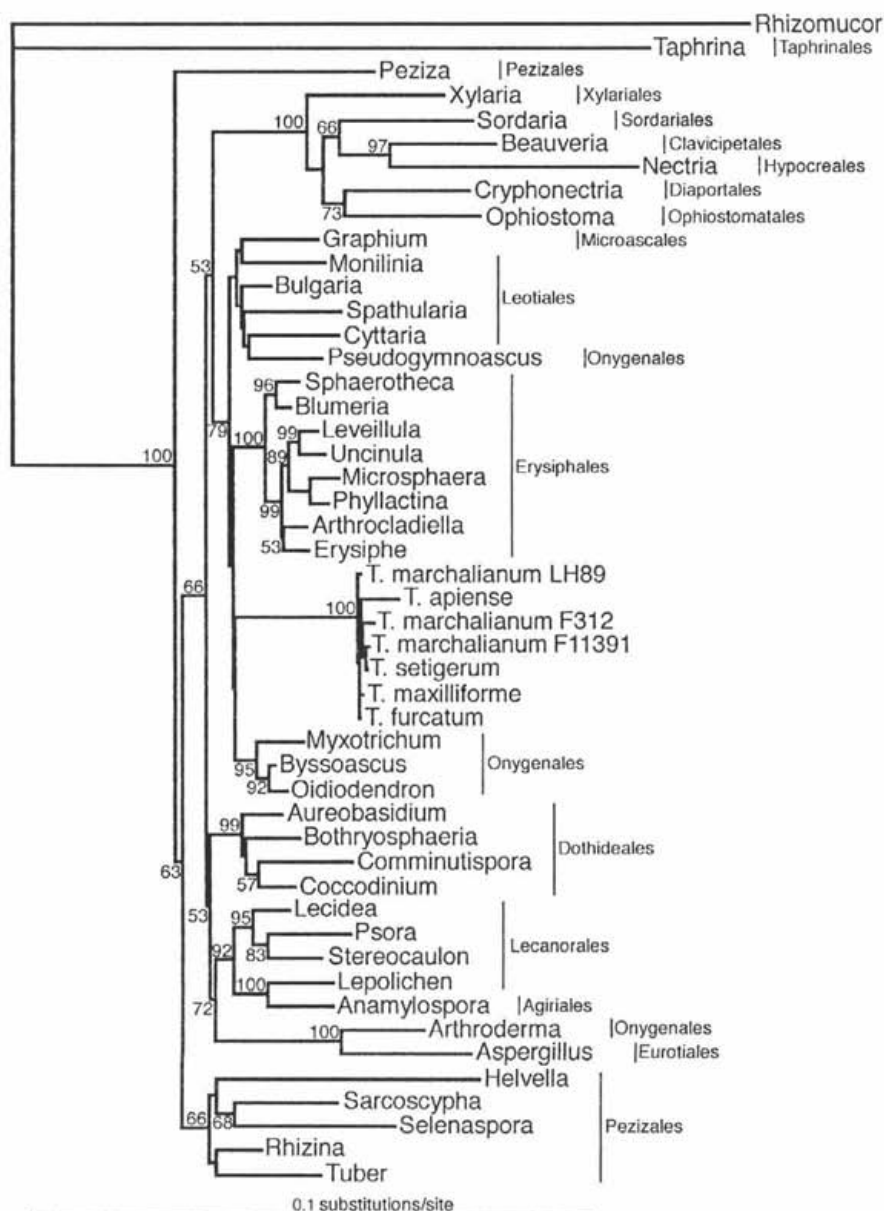
## DISCUSSION

Sequence analysis of 18S genes clearly indicates that the *Tetracladium* strains available for this study are part of a monophyletic group, supporting traditional, morphology-based taxonomy. The seven species of the genus have been reported from many different geographic locations (Roldán et al. 1989). It is unlikely that this wide distribution could be based on long-range transport of the relatively fragile aquatic conidia (Bärlocher 1992). Instead, the original dispersal of the various species, or their persistence in widely separated areas, are more likely due to sexually produced spores. In aquatic hyphomycetes, such spores generally occur on moist substrates that are no longer covered in water; dispersal is through airborne propagules (Webster 1992). The evidence presented here clearly shows that this presumed teleomorph (or teleomorphs) belongs to the Ascomycota. The closest hit in the GenBank database is *Bulgaria inquinans*, placed in the Leotiaceae (Leotiales according to Ainsworth et al. 1995; Helotiales according to Alexopoulos et al. 1996). This species decomposes dead wood in terrestrial habitats (Alexopoulos et al. 1996). Other members of the same family are found on stems of annual plants, on cones and fruits as well as on living plants (Alexopoulos et al. 1996). Interestingly, roughly 50% of all established meiosporic states associated with aquatic hyphomycetes are members of the Leotiales (Webster 1992, Webster and Descals 1979).

However, it would be premature to accept members of the Leotiaceae as the closest relatives of *Tetracladium*. When other hits are included, and depending on the details of the analysis, the *Tetracladium* complex can be closest to the Leotiales, Onygenales or Erysiphales, without any clear pattern emerging. Although the order Onygenales contains many human pathogens, members of the family Myxotrichaceae (e.g., *Byssosascus*, *Oidiodendron*, *Myxotrichum*) are saprobic on cellulosic substrates and are typically psychrophilic (Alexopoulos et al. 1996). Both properties may be useful preadaptations to colonize leaves in streams (Bärlocher 1992). However, terrestrial conidia of the Onygenales secede rhexolytically (a trait rarely found outside this order); the aquatic conidia of *Tetracladium* are holoblastic, and conidiogenous cells are polyblastic with sympodial proliferation (Roldán et al. 1987, 1989).

Members of the Erysiphales are obligate biotrophs that cause plant diseases known as powdery mildews (Alexopoulos et al. 1996). It is unlikely that *Tetracladium* would have evolved from an obligate fungal pathogen. But Erysiphales and *Tetracladium* might have a common, relatively recent ancestor.

Onygenales, Erysiphales and Helotiales (or Leotiales) generally cluster closely together. In the NCBI database (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>) they are all placed within the Pezizomycotina; Onygenales are one of two defined orders in the Euratiomycetes, while Erysiphales



**Fig. 1.** Neighbour-joining tree based on 18S rDNA sequences *Tetracladium* and other selected fungal species. Distances were calculated using the aligned sequences and the PHYLIP program DNADIST with Kimura two-parameters method and random addition of taxa. *Rhizomucor* was used as an outgroup. The bar indicates a distance of 0.1 (10 bases change per 100 nucleotide positions). Bootstrap support of 50% or greater from 100 bootstrap replicates is shown in the internal nodes of the branches.

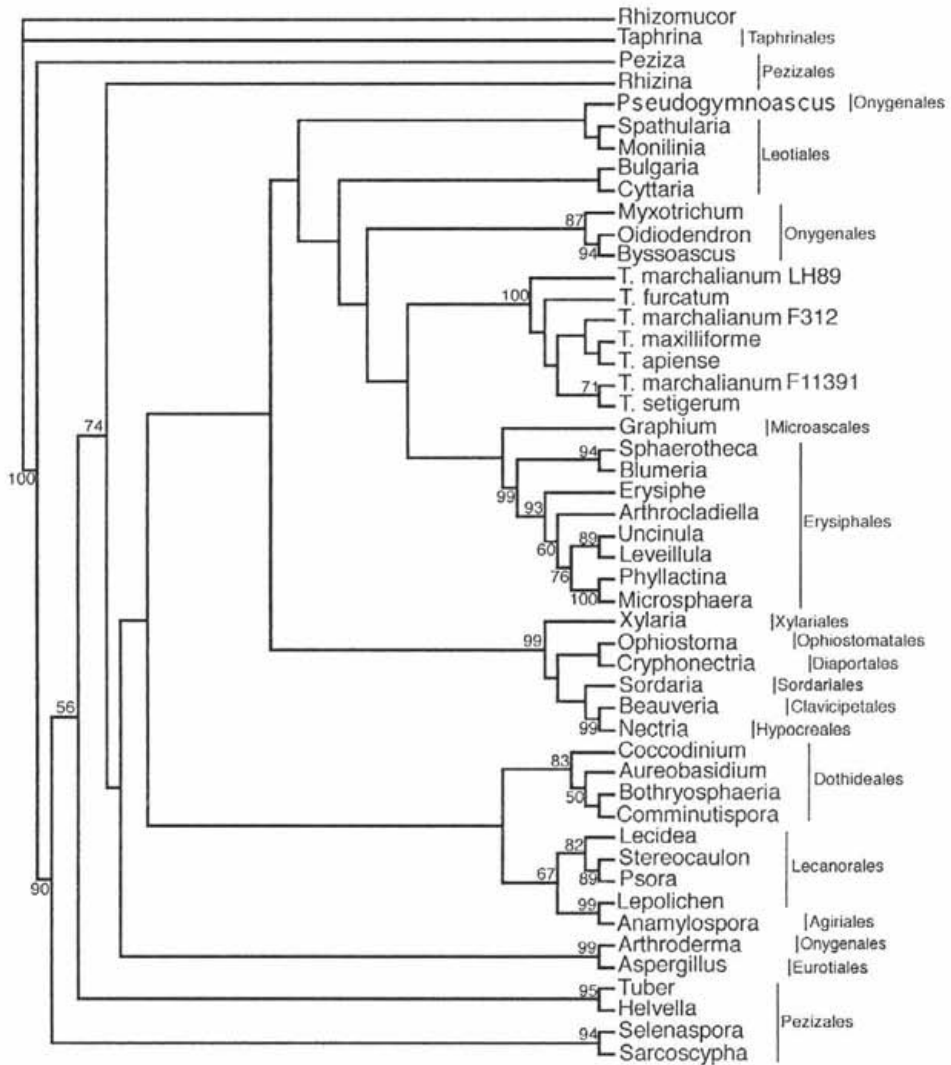


Fig. 2. One of 16 most parsimonious trees based on 18S rDNA sequences of *Tetracladium* and other selected fungal species. Branch topology was calculated using the aligned sequences and the PHYLIP program DNAPARS with random addition of taxa. *Rhizomucor* was used as an outgroup. Bootstrap support of 50% or greater from 100 bootstrap replicates is shown in the internal nodes of the branches.

and Helotiales represent two of the four defined orders in the Leotiomycetes. Our data suggest that the most common recent ancestors connects *Tetracladium* with one of these orders; without additional data, we cannot narrow it down any further. The major difficulty with establishing the phylogenetic relationship of fungal

species or genera is the presence of the large gaps in published databases. While rDNA sequences of pathogenic taxa are common, saprobic species are very much underrepresented (Cannon 1999). To our knowledge, our data on *Tetracladium* represent the first complete 18S rDNA sequences of any aquatic hyphomycete.

The evolutionary relationships among the members of the genus *Tetracladium* could not be further elucidated from our sequences. Although some strains, such as *T. setigerum* and *T. marchalianum* F-11391 consistently grouped together, the branching events were not significant. Also, there was considerable disparity among the results obtained when different criteria were used for the analysis. This was not surprising, since the sequences of the 18S rRNA gene evolve slowly and typically are not suited to comparisons between closely related taxa (Smith 1989). To elucidate such relationships, and to determine whether subdivision of *Tetracladium* into the seven described species (Roldán et al. 89) is justified, requires analysis of more variable regions of the fungal genome. Internal transcribed spacers (ITS) between the coding regions for the ribosomal subunits are generally more informative. In addition, a convincing analysis would require many more isolates of the various species, preferably from various geographic locations. Unfortunately, there are few strains of aquatic hyphomycetes available from culture collections. We were unable to obtain living cultures of *T. breve* or *T. palmatum*.

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**Arthroxyllaria elegans, a new coprophilous anamorphic fungus allied with the Xylariaceae, with notes on the genus Bisporostilbella<sup>\*</sup>**

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Seifert K. A., Gams W. and Louis-Seize G. (2002): *Arthroxyllaria elegans*, a new coprophilous anamorphic fungus allied with the Xylariaceae, with notes on the genus *Bisporostilbella*. – Czech Mycol. 53: 297–307

The new genus and species *Arthroxyllaria elegans* is described for a synnematosus hyphomycete isolated from pack rat dung. The fungus is characterized by the production of tall, lightly pigmented, indeterminate synnemata covered with a layer of unbranched or sparingly branched chains of 0–1-septate meristem arthroconidia. A synanamorph with sympodially-proliferating conidiogenous cells, producing minute aseptate conidia, is also produced. Phylogenetic analyses of partial small subunit ribosomal DNA sequences suggest that the fungus is related to the Xylariaceae, Xylariales, and analysis of internal transcribed spacer sequences places the fungus in *Xylaria*. The new species is compared with other anamorphs of the Xylariaceae, and a number of similar synnematosus and mononematosus hyphomycetes, including the poorly understood *Bisporostilbella fusca*, which is illustrated based on holotype material.

**Key words:** anamorph taxonomy, coprophilous fungi, hyphomycetes, biodiversity

Seifert K. A., Gams W. a Louis-Seize G. (2002): *Arthroxyllaria elegans* nová koprofilní anamorfní houba příbuzná čeledi Xylariaceae, s poznámkami o rodu *Bisporostilbella*. – Czech Mycol. 53: 297–307

Je popisován nový druh synematičtých hyfomycetů *Arthroxyllaria elegans* izolovaný z krysích exkrementů. Houba je význačná vytvářením vysokých světle pigmentovaných, indeterminovaných synemat pokrytých vrstvou nevětvených nebo řídce rozvětvených řetězků neseptovaných nebo septovaných meristemových arthrokonidií. Tvoří se též synanamorfa se sympodiálně proliferujícími konidiogenními buňkami, které vytvářejí drobné, neseptované konidie. Fylogenetická analýza částečné podjednotky sekvencí ribosomální DNA naznačuje že houba je příbuzná čeledi Xylariaceae z řádu Xylariales. Analýza vnitřní prostorové transkribované sekvence zařazuje tuto houbu do rodu *Xylaria*. Nový druh je srovnáván a ostatními anamorfami čeledi Xylariaceae a s četnými podobnými synematičtými a mononematičtými hyfomycety včetně málo známé *Bisporostilbella fusca*, která je vyobrazena na základě holotypu.

<sup>\*</sup>) We are pleased to dedicate this contribution to our friend and colleague Ludmila Marvanová on the occasion of her 70th birthday. We remember with fondness Ludmila's visits to CBS in Baarn and pleasant evenings of conversation in the Gams' home. We have admired her observant eye and patient coaxing of many aquatic hyphomycetes into pure culture for the first time, from both European and Canadian streams.

## INTRODUCTION

In 1977, Dr. R. K. Benjamin isolated a synnematosus hyphomycete from the dung of pack rats (*Neotoma* sp.) in California, USA. The fungus produced spectacular synnemata that would grow as much as 6 cm tall if the lid of the Petri dish was removed to allow continued growth. The culture was circulated to several hyphomycete specialists, who were unable to assign the fungus to an appropriate anamorph genus. We began studying the fungus in 1981, including it in an as yet unpublished study of some nondescript, mononematous hyphomycetes with similar conidiogenesis. Our recent molecular results have convinced us that this fungus is unrelated to the mononematous species under consideration, and have stimulated the description of a new hyphomycete genus and species here.

## MATERIALS AND METHODS

**Cultures.** For morphological studies, the culture was grown on oatmeal agar (OA, Gams et al. 1998) and 2% Malt Extract Agar (MEA, using brewer's malt available at CBS) at room temperature under ambient light or in the dark at 25 °C for 14 days. For DNA extraction, cultures were grown on OA in the dark at 25 °C for 10 days.

**DNA Sequencing.** DNA was isolated using a FastDNA™ Kit and the Fast-Prep™ FP120 (BIO 101 Inc.) using synnemata removed from OA cultures. PCR and cycle sequencing reactions were performed on a Techne Genius thermocycler (Techne Cambridge Ltd.). PCR reactions were performed using Ready-To-Go™ Beads (Amersham Canada Ltd.) in 25 µL volumes, each containing 20–100 ng of genomic DNA, 1.5 units Taq DNA Polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 0.4 µM of each primer, and stabilizers including BSA. The reaction profile included an initial denaturation for 3 min at 94 °C, followed by 30 cycles of 1 min denaturation at 94 °C, 1.5 min annealing at 56 °C, 2 min extension at 72 °C, with a final extension of 10 min at 72 °C. Amplicons were purified using UltraClean™ PCR Clean-up™ DNA Purification Kit (MO BIO Inc.) following the manufacturer's directions. Amplification products were sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (ABI Prism/Applied Biosystems) system, and the ABI PRISM™ 310 DNA Sequencer (Applied Biosystems, Foster, CA) following the manufacturer's directions. A portion of the small ribosomal subunit (18S) DNA was amplified and sequenced using primers NS1 and NS4. The complete internal transcribed spacers and 5.8S rDNA were amplified using primers ITS1 and ITS4, and cycle-sequenced using primers ITS1, ITS2, ITS3 and ITS4 (primers from White et al. 1990).

**Phylogenetic analysis.** The partial small subunit sequence of *A. elegans* (GenBank AF432180) was subjected to a BLAST search on GenBank

(<http://www.ncbi.nlm.nih.gov/BLAST/>), and then aligned with the closest matches. The ITS sequence of *A. elegans* (GenBank AF432179) was aligned with selected sequences for *Xylaria* and similar genera reported by Lee et al. 2000. GenBank accession numbers for these sequences are included on Fig. 3. Alignments were calculated using the pileup option of GCG 10.1 (Canadian Bioinformatics Resource <http://www.cbr.nrc.ca/>) with a gap weight of 5 and a gap length penalty of 1. Parsimony analysis of alignments were performed with PAUP \*4.0b8 (Swofford 1999) using heuristic searches with uninformative characters removed, and further evaluated using bootstrap analysis with 1000 replications.

## RESULTS

**Phylogenetic analysis.** Parsimony analysis of the partial nuclear small subunit sequence placed *A. elegans* clearly in the *Xylariaceae*, sister to *Xylaria carpophila* Z49785, and also closely related to *Poronia punctata* AF064052, *Xylaria curta* U32417 and *X. hypoxylon* U20378 with weak bootstrap support for the topology, which was otherwise robust based on the strict consensus (results not shown).

The complete ITS alignment was 586 characters. To create a data set that was comparable for all sequences, 28 bp at the beginning and 56 bp at the end of the alignment were omitted. Removal of uninformative positions left a remainder of 167 characters. Heuristic searches resulted in four equally parsimonious trees of 464 steps (CI 0.641, RI 0.586, RC 0.381 and HI 0.349). The ITS-based phylogenetic hypothesis placed *A. elegans* in the *Xylariaceae* with strong bootstrap support. The topology of the tree presented here (Fig. 3) compares well with the more detailed analysis of Lee et al. (2000), placing *A. elegans* basal to Groups A and B of *Xylaria*.

## TAXONOMIC PART

*Arthroxyllaria elegans* Seifert et W. Gams, gen. et sp. nov.

Figs. 1, 2

Synnemata indeterminata, ad 6 cm alta, albida vel cremea in parte sterili basilari, in parte fertili olivaceo-viridia et 300–1000  $\mu\text{m}$  lata, apicem versus modice rosea, filiformia, sinuosa, rotunda in sectione, gregaria vel caespitosa, simplicia vel in quavis parte ramis aequalibus dichotoma vel trichotoma, in parte fertili tomentosa vel lanosa. Hyphae stipitis 1.5–3  $\mu\text{m}$  latae, hyalinae, tenuitunicatae, septis simplicibus divisae. Arthroconidia catenis 50–280  $\mu\text{m}$  longis adhaerentia, (0–)1(–3)-septata, 3.5–8  $\mu\text{m}$  lata, conidia 0-septata 7.5–13  $\mu\text{m}$  longa, 1-septata 12.5–21  $\mu\text{m}$ , 3-septata 28–38  $\mu\text{m}$ , quorum cellulae globosae, subglobosae vel oblongae ellipsoideae, aequales vel inaequales, ad septum constrictae an

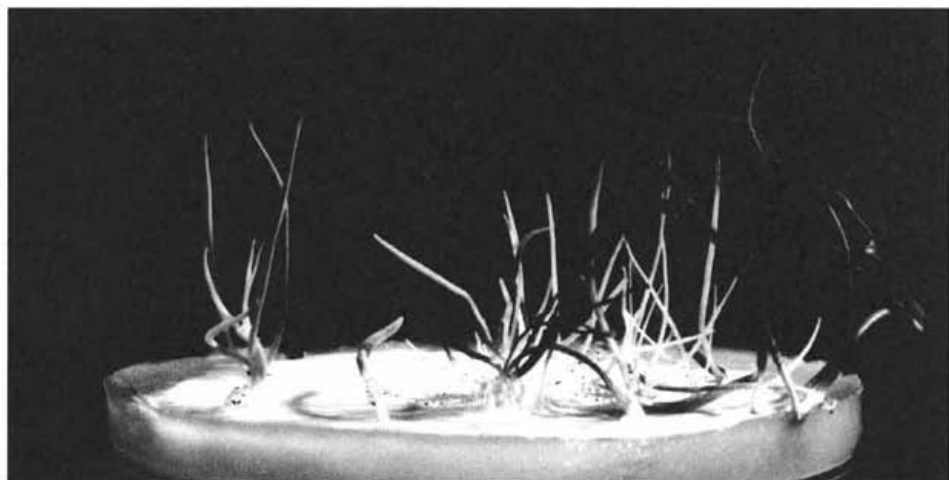


Fig. 1. *Arthrozyllaria elegans*, ex-type culture on OA. Habit of synnemata growing on deep oatmeal agar with the lid replaced by a beaker to allow indeterminate growth.

non, tenuitunicatae vel modice crassitunicatae, nonnumquam asperulatae; conidia utrinque applanata et magis tenuitunicata, eximie cicatricata. Synanamorphosis polyblastica e catenis arthroconidiorum oriunda, cellulis conidiogenis 30–70  $\mu\text{m}$  longis, hyalinis, denticulatis, conidiis 2–4.5  $\times$  1.5–2.5  $\mu\text{m}$ , ovoideis vel ellipsoideis. Chlamydosporae submersae, intercalares, hyalinae, 6.5–10  $\mu\text{m}$  diam.

Holotypus: U.S.A., California, Cottonwood Springs, May 15, 1977, leg. R. K. Benjamin, dried culture of CBS 537.79 (Herb. CBS)

Synnemata indeterminate, up to 6 cm tall, white to cream-coloured in the basal sterile 1–2 mm, olive-green and 300–1000  $\mu\text{m}$  wide in the sporulating area, slightly pink at the growing tip, filiform, sinuous, terete, gregarious or caespitose, unbranched or with dichotomous or trichotomous equivalent branches anywhere along the stipe, tomentose to lanose in the fertile zone. Hyphae of stipe 1.5–3  $\mu\text{m}$  wide, hyaline, with smooth, thin walls and simple septa. Arthroconidia forming chains 50–280  $\mu\text{m}$  long, (0-)1(-3)-septate, 0-septate conidia 7.5–13  $\mu\text{m}$  long, 1-septate conidia 12.5–21  $\mu\text{m}$  long, 3-septate conidia 28–38  $\mu\text{m}$  long, all conidia 3.5–8  $\mu\text{m}$  wide, individual cells globose, subglobose to oblong-ellipsoidal, with cells of equal size or one smaller, constricted or not at the septum, walls thin to slightly thickened, sometimes slightly rough, resulting in flattened poles with slightly thinner walls and a minute frill.

Secondary conidiophores sometimes developing from the apex of arthroconidial chains, hyaline, unbranched or with 1–2 lateral branches, up to 100  $\mu\text{m}$  long, 1–2  $\mu\text{m}$  wide. Secondary conidiogenous cells 30–70  $\mu\text{m}$  long, hyaline, proliferating sympodially, with 1–15 proliferations forming a terminal conidiogenous zone

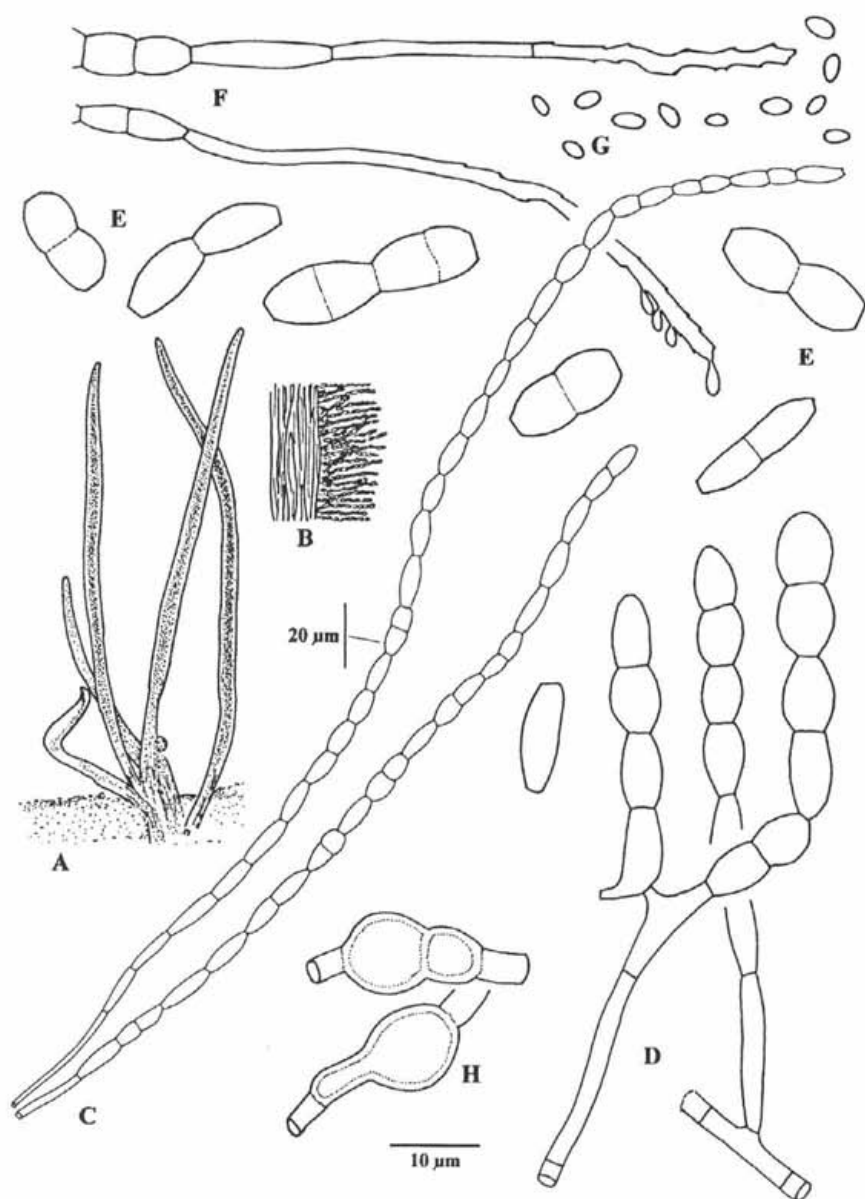


Fig. 2. *Arthroxyllaria elegans*, ex-type culture on OA, habit sketches and camera lucida drawings. A. Habit. B. Diagrammatic section of synnema showing arrangement of conidial chains. C. Complete conidial chains. D. Partial conidial chains. E. Seceded conidia. F. Sympodially-proliferating conidiogenous cells of the synanamorph. G. Conidia of the synanamorph. H. Chlamydospores imbedded in agar. Scale bar at bottom for all figures except A-C. A, B not to scale.



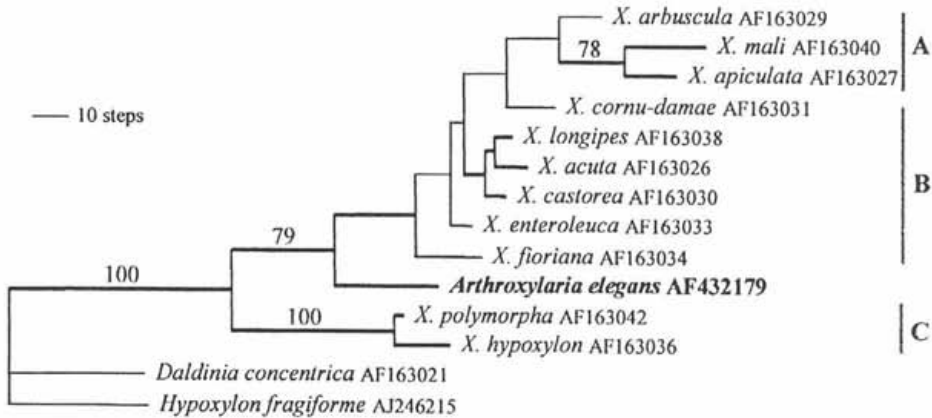


Fig. 3. One of four equally parsimonious phylograms showing postulated relationships between *Xylaria* species and *Arthroxyllaria elegans* based on heuristic analysis of internal transcribed spacer (ITS) sequences. The groups A-C identified within *Xylaria* by Lee et al. (2000) are shown on the right of the figure. Branches in bold occur in all equally parsimonious trees. Bootstrap values above 50% are based on 1000 replicates. See Results for further details.

up to 30  $\mu\text{m}$  long, 1–2  $\mu\text{m}$  wide, proliferations visible as minute denticles or notches, sometimes with single denticles up to 1.5  $\mu\text{m}$  on intercalary cells of the conidiophore. Secondary conidia 2–4.5  $\times$  1.5–2.5  $\mu\text{m}$ , ovoid to ellipsoidal.

Colonies on OA at 25 °C under ambient light 19–24 mm diam after 7 days, 32–37 mm diam after 14 days, covered with cottony to lanose white aerial mycelium about 1 mm deep, with vague concentric zonation, margin gnawed, reverse white to cream, mottled, with brown spots. Hyphae 2–3.5  $\mu\text{m}$  wide, with swollen cells up to 9  $\mu\text{m}$  wide, hyaline, with thin, smooth walls. Chlamydospore-like cells intercalary in submerged hyphae, hyaline, 6.5–10  $\mu\text{m}$  diam, walls smooth, about 0.5  $\mu\text{m}$  thick. Synnemata developing near the inoculum after 1 week. Colonies on MEA at 25 °C under ambient light 18–23 mm diam after 7 days, 34–37 mm diam after 14 days, with cottony to felty white aerial mycelium up to 2 mm deep near the inoculum and in patches elsewhere, surface light brown, wrinkled, margin gnawed, reverse cream to light orange-brown, wrinkled, with concentric zonation. Synnemata scattered over the colonies on OA and MEA, starting to develop within 2 weeks; if the Petri dish lid was left in place, the synnemata grew into it and spread radially to form an arachnoid aerial growth.

Material examined: CBS 537.79 (= UAMH 4811 = IMI 286722 = DAOM 226684), ex-type culture, USA, California, San Bernadino Co., Cottonwood Springs, N. side of Mid Hills, New York Mountains, isol. from pack rat dung by R. K. Benjamin, # RSA 2455, 15 May 1977.

## DISCUSSION

*Arthroxyllaria elegans* is a coprophilous hyphomycete distinguished by the production of tall, lightly pigmented, indeterminate synnemata covered with long, dry chains of usually 0–1-septate conidia that sometimes terminate in a sympodially proliferating conidiogenous cell bearing minute, aseptate microconidia. Conidium ontogeny on the primary conidiophores of this fungus follow the meristem arthroconidium pattern (Hughes 1953). Contorted hyphae grow laterally and terminally from the growing synnema. These hyphae become septate as they grow, and the cells then swell in a retrogressive sequence, resulting in constrictions at the septa. Mostly 2-celled conidia mature while in the chain, giving the now fertile hypha a monilioid appearance. Conidia maturing near the base of the chain tend to be less swollen, cylindrical to clavate, and aseptate. Secession is schizolytic.

Parsimony analyses of partial small subunit and internal transcribed spacer ribosomal DNA sequences clearly place this fungus in the *Xylariales*, allied with species of *Xylaria*. The production of stipitate, anamorphic stromata is a common feature in this order, as is the production of a sympodially proliferating microconidial synanamorph (often referred to as selenosporella-like). Although synnematos anamorphs are relatively common in the *Xylariales* (classified in genera such as *Acanthodochium* Samuels, Rogers et Nagasawa, *Dematophora* R. Hartig, *Moelleroclavus* P. Hennings, *Nodulisporium* Preuss and *Xylocoremium* J. D. Rogers), these anamorphs all have sympodially-proliferating conidiogenous cells, each aperture producing a single, blastic conidium with a truncate base. The known anamorphs of *Xylaria* tend to be produced directly on the immature teleomorph stroma, or are known only from culture (eg. Callan & Rogers 1990, van der Gucht 1996, Ju & Rogers 1999). Although anamorphs are considered an important taxonomic character in *Xylaria* (Rogers 1985), most lack anamorph-generic names (Whalley 1996). Exceptions include the synnematos *Moelleroclavus* anamorph reported for *X. moelleroclavus* Rogers et al. (1997). *Xylocoremium flabelliforme* (Schw.) J. D. Rogers is the anamorph of *Xylaria cubensis* (Mont.) Fries (Rogers 1984), a species that is separate from the main groups of *Xylaria* according to the ITS analysis of Lee et al. (2000).

Apart from *A. elegans*, meristem arthroconidium production is unknown in the *Xylariales*. There are a few aberrant anamorphs in this order that do not always conform to the typical pattern, however. The *Lindquistia* anamorphs of *Poronia* species produce typically xylariaceous conidiogenous cells on a capillitium-like mass of interwoven conidiophores at the apex of synnematos conidiomata. The cells of the conidiophores sometimes disarticulate, resulting in hyphal fragments capable of germinating and acting as propagules (Rogers & Læssøe 1992).

There are a few synnematos hyphomycete genera with arthroconidia that warrant comparison with *Arthroxyllaria*. None of these have known teleomorph

connections or available DNA sequence data, so their phylogenetic relationships remain unknown.

*Briosia ampelophaga* Cavares is most similar. This species causes lesions on leaves of *Vitis* vines, produces short (<500  $\mu\text{m}$  tall) unpigmented synnemata terminating in branched chains of golden-brown, aseptate conidia. The conidia are meristem arthroconidia that mature in basipetal sequence and are cuboid when in the chain, but become globose after schizolytic secession (Sutton 1973).

*Arthrographis cuboidea* (Sacc. et Ellis) Sigler produces minute synnema-like conidiomata on wood, with dry, terminal heads of schizolytically-seceding, fission arthroconidia (Sigler & Carmichael 1983).

*Coremiella cubispora* (Berk. et M. A. Curtis) M. B. Ellis produces small (<1 mm tall) determinate synnemata on dying or dead plants, with dry, terminal capitula comprising divergent chains of alternate, cuboid arthroconidia (Ellis 1971). Separating cells in the conidial chains lose their cytoplasm and break, a type of rhexolytic conidial secession. The conidia have characteristic, doliipore-like occlusions at both ends, visible with light microscopy (Cole & Samson 1979).

The poorly understood soil hyphomycete *Bisporostilbella fusca* Brandsberg et E. F. Morris (1971) also warrants comparison with *Arthrozyllaria*. Our examination of the dried agar cultures that comprise the holotype of *B. fusca* (WSP 58777) revealed radiating, feathery to funiculose dark olivaceous-brown colonies (Fig. 4). The feathery or funiculose bundles of hyphae, which are several mm long (Fig. 4a), are not synnemata in the usual sense of the term (Seifert & Okada 1990). Marginal hyphae of the bundles may be sterile and bear contorted short branches (Fig. 4e), or integrated, lateral or discrete, terminal conidiogenous cells may be produced (Fig. 4e). The bundles terminate in divergent, branching, dematiaceous hyphae that become frequently septate, and bear terminal or lateral conidiogenous cells (Fig. 4b, c). Conidiogenous cells are 3–7.5  $\mu\text{m}$  long and 2–4.5  $\mu\text{m}$  wide. The base of the conidium is truncate, lying flat against the conidiogenous aperture, but we saw no evidence of the percurrent proliferations of the conidiogenous cells described in the protologue, although percurrent regeneration of the conidiophore was observed (arrowhead in Fig. 4c). Indeed, the mechanism for the production of conidial chains could not be deduced from the specimen, and it was unclear whether conidium ontogeny is blastic or thallic. Conidial secession is clearly schizolytic. Chains of 2–4 conidia were noted. Each conidium is 5–6  $\times$  3–4.5  $\mu\text{m}$ , olivaceous-brown, ellipsoidal to somewhat doliiform, with a single thick, darkened septum, sometimes constricted at that septum, with lateral walls that are conspicuously thicker than the polar walls (Fig. 4d). Unfortunately, a culture derived from the holotype of *B. fusca* (CBS 253.72) is now a *Cladosporium* sp. and no longer represents the original fungus. Thus *B. fusca* remains an enigma, but our impression of the holotype is of a fungal culture expressing aberrant morphology that may be unrepresentative of the wild type of the fungus.

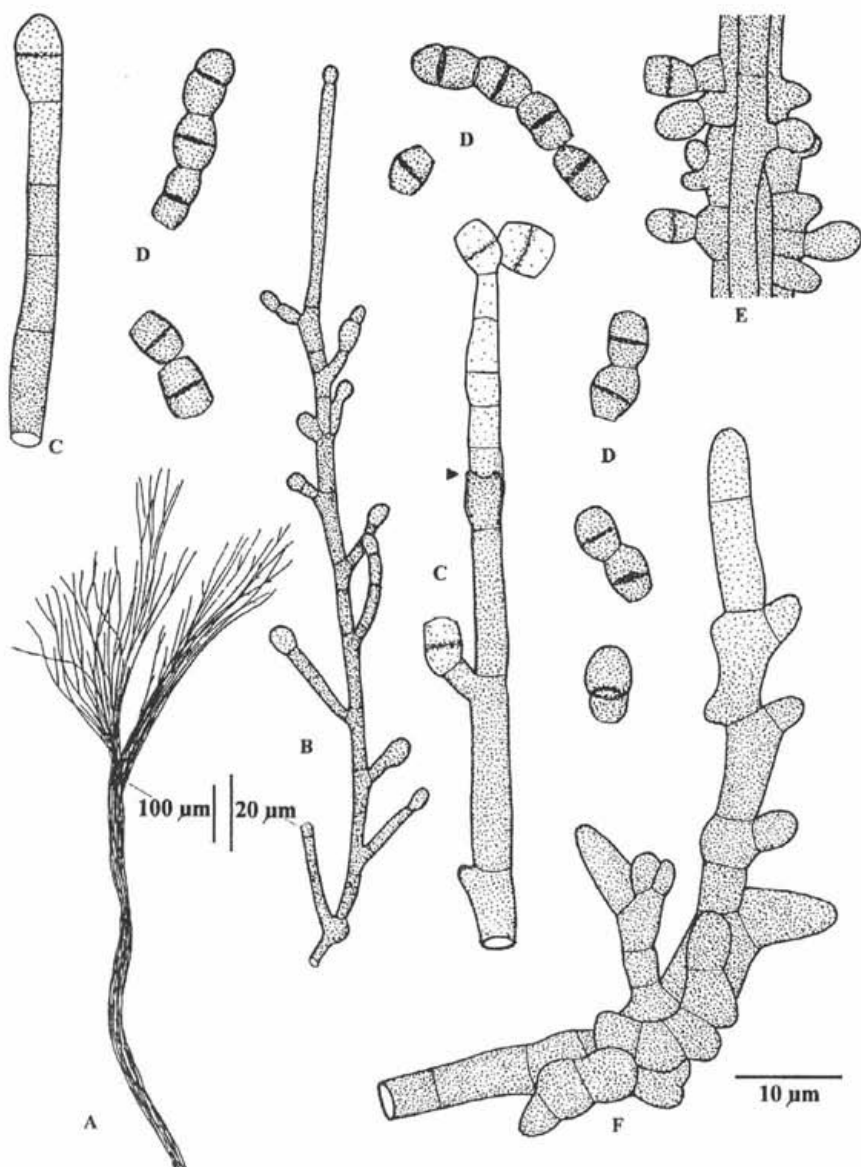


Fig. 4. *Bisporostilbella fusca*, holotype, camera lucida drawings, showing feathery hyphal aggregations, conidial chains and seceded conidia. A. Synnema-like bundles. B, C. Terminal hyphae bearing conidia; the arrow in C indicates percurrent regeneration of the conidiogenous hypha. D. Conidia. E. Part of hyphal bundle with lateral conidiogenous cells. F. Contorted, branched hypha from side of hyphae bundle. Scale in lower right for all figures except A, B.

If the synnematos nature of the conidiomata of *Arthroxyllaria elegans* is discounted, there are some mononematous hyphomycetes producing similar chains of conidia of mixed arthric and blastic conidiogenesis. *Basipetospora chlamydosporis* Matsushima (1975), for example, produces erect, branching aerial hyphae that are converted into chains of 4–10 conidia, with the longest conidia generally near the middle of the chain. Like *A. elegans*, *B. chlamydosporis* also produces chlamydospores. Phylogenetic analysis of partial small subunit ribosomal DNA sequences of three cultures identical with or similar to this species [listed as *Monilia pruinosa sensu* Gilman CBS 249.68 (AF437893), CBS 217.74 (AF437894) and *Basipetospora variabilis* Mats. 995.87 (AF437892)] suggests that it is unrelated to the *Xylariales* and hence *Arthroxyllaria*. It instead has affinities with the *Microascales* (Seifert & Louis-Seize, unpublished), and is consequently also unrelated to *Basipetospora rubra* Cole & Kendrick, the anamorph of *Monascus ruber* van Tiegham (*Eurotiales*). Similar undescribed anamorphs are found in the *Sporormiaceae* and *Onygenales*.

*Arthroxyllaria elegans* illustrates the taxonomic and nomenclatural dilemma presently posed by anamorphic fungi (Seifert & Samuels 2000). No teleomorph is known but the fungus is clearly related to *Xylaria* based on parsimony analysis of rDNA sequences. Morphologically, the conidiogenesis of the fungus is dissimilar from other known anamorphs in this group, although the synnema may be a homologous structure to the erect, multiloculate perithecial stroma that characterizes *Xylaria*. Does this fungus really require the formal description of a genus? There are no provisions in the International Code of Botanical Nomenclature to allow the naming of an anamorphic species in a teleomorphic genus in the absence of the morphological manifestation of a sexual apparatus. We argue that a well-defined descriptor is necessary for such a distinctive anamorph, to facilitate its identification by morphological means when the teleomorph (if extant) is not seen.

#### ACKNOWLEDGEMENTS

We are grateful to S. Berch and R. K. Benjamin for sending us this fungus and L. Sigler, who first noticed the synanamorph. Portions of this work were completed while K. Seifert was a student at CBS, and he is grateful for the support received at that time. Presubmission reviews of the manuscript were kindly provided by J. D. Rogers, S. Hambleton and J. Bissett.

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## Book Review

B. A. SUMMERELL, J. F. LESLIE, D. BACKHOUSE, W. L. BRYDEN AND L. W. BURGESS  
(EDS.)

**Fusarium: Paul E. Nelson Memorial Symposium**

APS Press, St. Paul Minnesota, USA, 2001, 392 p.

ISBN 0-89054-268-6. Price 59 USD

(The book is deposited in the library of the Society)

*Fusarium* has mostly been studied in the context of its ability to cause diseases of many economically important crop plants. Besides its considerable economic significance, *Fusarium* has become a valuable experimental pathogen for the study of genetic, molecular and biochemical aspects of plant-fungus associations.

This book includes either revised versions of papers presented at the P. E. Nelson Memorial Symposium held at Pennsylvania State University, State College, Pennsylvania, in November 1997 or additional contributions written for this book.

The preface of this book is dedicated to P. E. Nelson, to his research on the *Fusarium* genus and his achievements in this field of interest.

The contributions were summarized in 25 articles (chapters) in five sections and are supplemented with tables, black and white illustrations, photos and references.

The first section ("Taxonomy") contains three contributions. The very interesting chapter "Developments in the taxonomy of *Fusarium* species based on secondary metabolites" is supplemented with a table of *Fusarium* metabolites and a survey of analytical techniques for their detection. The author emphasises the use of secondary metabolite profiles in connection with morphological, physiological and genetical characters as a powerful tool for classification of *Fusarium* species. The next chapters deal with the study of perithecial species of *Fusarium* and anamorph generic concepts of *Fusarium*.

The second section ("Genetics") includes 5 chapters, which present results of research on genetics of *Fusarium* toxins, evolution of host specificity in *Fusarium oxysporum* and vegetative compatibility group diversity in *Fusarium*.

The third section ("Ecology") contains four chapters. These contributions include the results of studies on *Fusarium* species from the view of their biogeography and influence of various conditions.

The section "Pathology" with nine contributions is the most voluminous. The first chapter ("Molecular assays as aids in the detection, diagnosis and quantification of *Fusarium* species in plants") is crucial; it deals with modern methods of study of the complex host-fungus. Immunoassay and DNA-based assays developed toward *Fusarium* species are valuable tools in epidemiological studies, providing a rapid reliable means of detecting and quantifying pathogens in host tissues throughout the growing season. The other contributions of this section deal with *Fusarium* spp. as very significant pathogens of a wide range of important crop plants.

The last part ("Mycotoxicology") covers four contributions. They confirm the significance of mycotoxin production by agriculturally important *Fusarium* spp. Developments in mycotoxicology have progressed rapidly over the last four decades.

This book gives new information and strengthens the knowledge of specialists in this field of study and it will in particular initiate further research concerning the *Fusarium* genus.

Michaela Zemánková

## *Anguillospora mediocris* sp. nov. from streams in Hungary

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Gönczöl J. and Marvanová L. (2002): *Anguillospora mediocris* sp. nov. from streams in Hungary. – *Czech Mycol.* 53: 309–317

*Anguillospora mediocris* sp. nov. is described from the Morgó stream system in Hungary. It produces relatively short, falcate or sigmoid conidia on percurrent conidiogenous cells. A pycnidial microconidial synanamorph was observed in pure culture. The natural substrate is leaves, mainly of alder (*Alnus glutinosa*). The fungus seems to prefer hard waters. Conidia in nature, even when abundant in stream, occur only sporadically in foam.

**Key words:** aquatic hyphomycetes, taxonomy, ecology.

Gönczöl J. a Marvanová L. (2002): *Anguillospora mediocris* sp. nov. z potoků v Maďarsku. – *Czech Mycol.* 53: 309–317

V článku je popsán druh *Anguillospora mediocris* sp. nov. z vodních toků v Maďarsku. Je charakterizován tvorbou srpovitých nebo sigmoidních konidií na krátkých nevětvených konidioforech. V čistých kulturách bylo pozorováno také mikrokonidiové stadium vytvářející pyknidy. V přírodě se tento druh vyskytuje na ponořených listech, zejména olšových (*Alnus glutinosa*). Dosavadní nálezy jsou převážně z tvrdých vod. Konidie se jen sporadicky najdou ve vzorcích pěny a to i v případech, kdy jsou ve vodním toku hojné.

### INTRODUCTION

Stream water filtration and identification of detached conidia on filters is a common method used in ecological and biodiversity studies of aquatic hyphomycetes. Whereas identification is relatively safe in the case of stauroconidia, it often fails when applied to scolecoconidia, not only because few scolecoconidia have diagnostic characters distinct enough, but also because of the great variation in size, especially in length, which causes frequent overlap and uncertainty in identification unless conidiogenesis is seen.

Abundant scolecoform conidia of undoubtedly the same species were repeatedly encountered in the Morgó stream system since the first analyses of the stream water with membrane filtration in 1996. The conidia have been referred to as *Filosporella* sp. 1 in Gönczöl et al. (1999) and Gönczöl and Révay (1999a), and *Filosporella* sp. in Gönczöl and Révay (1999b). After several attempts the first author succeeded in isolating the fungus into pure culture. It appears that it fits relatively well into the genus *Anguillospora* and it is described here as a new species.

## MATERIAL AND METHODS

Decaying leaves of *Alnus glutinosa* (L.) Gärtn. were collected from the Nacsagrom stream in the Börzsöny Mountains, NE Hungary, in November 2000. Leaves were placed into Petri dishes with distilled water and incubated for several days at 10 °C. Some of the incubation water was then poured onto a plate with 0.1 % malt agar (MA), germinating conidia were located with the aid of a New England Finder, and with a sterile needle transferred to 2% MA. Subcultures were made on 3% MA. Pieces of 15–60 day old colonies from 3% MA were submerged in standing or aerated sterile distilled water and incubated at 12 °C. Aeration had negative effect (no conidia detected), whereas in standing water a weak to abundant production of conidia took place after 5–7 days.

## DESCRIPTION

*Anguillospora mediocris* Gönczöl et Marvanová, sp. nov. (Figs. 1, 2)

Etym. *mediocris* (Lat.) = medium, not large, size of the conidia in comparison with other species of the genus.

Coloniae in agar maltoso moderate crescentes, griseae vel atrogriseae, margine lobatae. Mycelium in substrato crustosum, e cellulis plus-minusve isodiametricis seu breviter elongatis, 7.5–15 µm in diametro, cum parietibus tenuibus, atris, compositum. Pars reversa nigra. Conidiophora singularia vel aggregata, brevia, non ramificata, 10–60 × 2–3 µm. Cellulae conidiogenae incorporatae, percurrenter vel raro sympodialiter proliferantes. Conidia scolecoformia, septata, falcata vel raro sigmoidea rectaque, attenuata in parte distali, 62–104 × 3.3–4.8 µm, apex subulatus, basis truncata vel cum extensione brevi. Dehiscencia conidiorum schizolytica.

Status microconidialis (andromorphosis ?) coelomycetosus, in cultura agarosa. Pycnidia inter mycelium immersa, globosa, brunnea, 50–175 µm in diametro, parietibus tenuibus, e cellulis isodiametricis compositis. Conidiophora cellulae conidiogenaeque non visae. Conidia hyalina, ellipsoidea vel irregulariter elongata, 1.5–4 × 1.5–1.8 µm.

Holotypus: BP-95701, praeparatum e cultura monoconidiali JG 2000–5 e folio putrido *Alni glutinosae* isolata, in rivulo Nacsagrom-patak pr.pag. Szokolya, montibus Börzsöny-hegység, Hungaria, 14 Nov. 2000., J. Gönczöl.

The monoconidial primocultures on 2 % MA grew extremely slowly, reaching 5–8 mm diam. in one month at 20 °C. Black, hard, elevated colonies developed on this medium. On 3% MA, growth of cultures was moderately fast, reaching ca. 30 mm diam. in one month at 20 °C; colonies were mid to dark grey, with umbonate centre, zonate at the margins, lobed. Aerial hyphae brown, up to 5 µm wide,

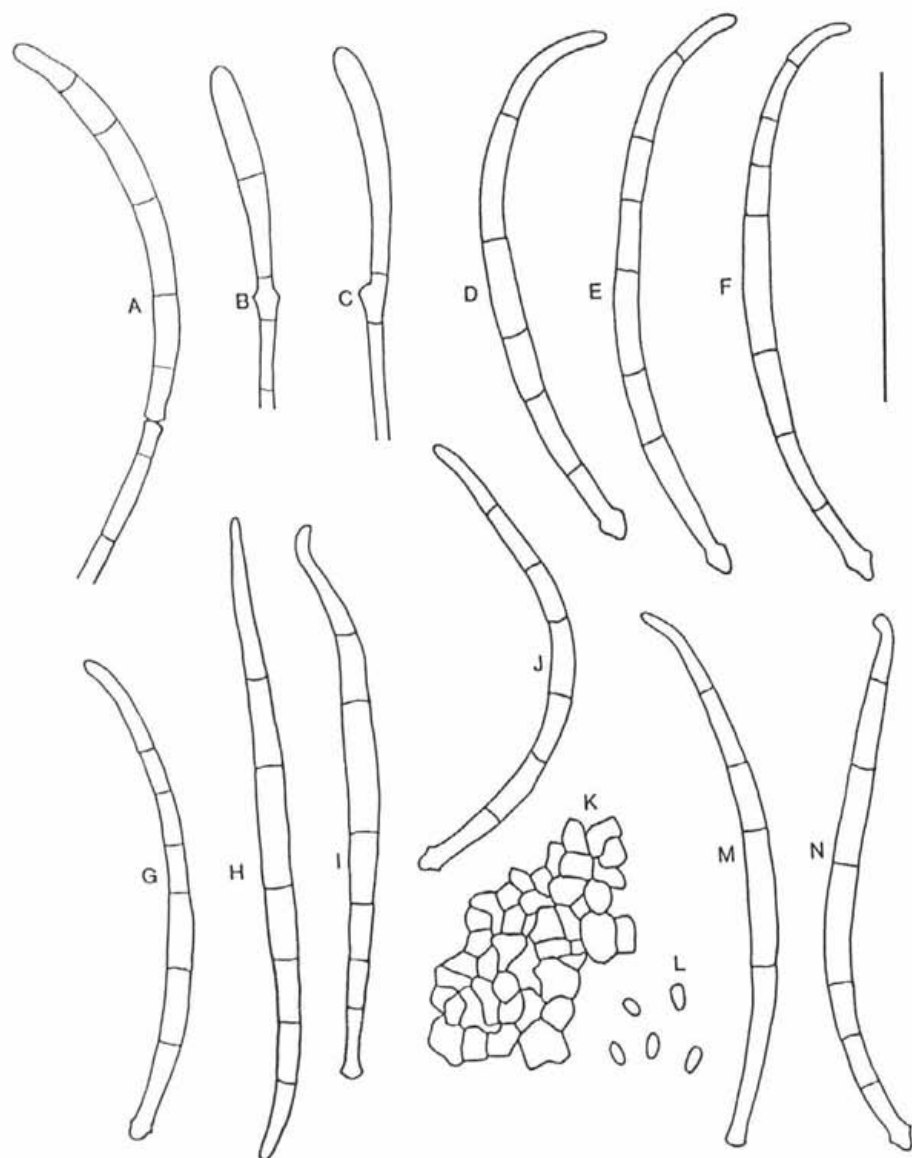


Fig. 1. A-N. *Anguillospora mediocris*. A. First-formed conidium detached in premature stage. B. Percurrent proliferation of a conidiogenous cell. C. Sympodial proliferation of a conidiogenous cell. D-F., G., J., M., N. Detached conidia from pure culture. H., I. Conidia from nature: H., from filter; I., from alder leaf. K. Surface structure of pycnidia of the microconidial state. L. Microconidia. Scale bar = 50  $\mu$ m.

walls slightly thickened; inflated globose cells 10–17  $\mu\text{m}$  wide present in chains or clusters. Substrate mycelium forming crusts of pseudoparenchymatous tissue composed of more or less isodiametric or shortly elongate cells 7.5–15  $\mu\text{m}$  diam. with thin dark walls. Some hyphae bear amorphous brown incrustations. Reverse black, marbled, with several slits. Sporulation after submergence in standing water, below water level, from substrate mycelium. Conidiophores single or grouped, short, unbranched, slightly widening towards the apex, 10–60  $\times$  2–3  $\mu\text{m}$ . Conidiogenous cells integrated, proliferations percurrent, rarely sympodial. Conidial initiation integrated. Conidia scolecoform, septate, typically falcate, resembling thin, long conidia of a *Fusarium*, sometimes more strongly curved and attenuate in the distal part, rarely sigmoid or straight; apex subulate, base slightly bulging, or snake-head-like (with a short percurrent extension) or with a pedicel. The conidial dimensions (50 conidia measured on filter from a filtration performed in November 2000 at the locus classicus) are 62–104  $\times$  3.3–4.8  $\mu\text{m}$ , with an average 81.8  $\times$  3.9  $\mu\text{m}$ . In pure culture (isolate J. Gönczöl 2000/5, submerged in standing distilled water) the conidia measure 70–92  $\times$  2.8–4.5  $\mu\text{m}$  with an average 80.5  $\times$  3.6  $\mu\text{m}$  (50 conidia measured). The length/width ratio is 20–22. In lactophenol with cotton blue the conidial dimensions are reduced roughly by a tenth. Freshly formed conidia are coated with a thin mucous layer, which disappears after some time. Conidial secession is schizolytic.

Microconidial state (presumed andromorph): coelomycetous, in agar cultures. Pycnidia embedded in the mycelial mat but easily separable, whitish in reflected, brownish in transient light, globose, 50–175  $\mu\text{m}$  across, wall thin, of more or less isodiametric cells in two (?) layers. Conidiophores or conidiogenous cells not seen. Conidia minute, ellipsoid, or irregularly elongate, 1.5–4  $\times$  1.5–1.8  $\mu\text{m}$ .

## DISCUSSION

The conidial shape and the percurrent proliferation of the conidiogenous cells point to the anamorphic genus *Anguillospora*. This is heterogeneous and polyphyletic. The teleomorphs, known in seven species, belong to four orders: Pleosporales (*A. longissima* (de Wild.) Ingold), Helotiales (*A. crassa* Ingold, *A. furtiva* J. Webster et Descals, *A. fustiformis* Marvanová and *Anguillospora* sp. anamorph of *Loramyces juncicola* W. Weston), Orbiliales (*A. rosea* J. Webster et Descals) and Lulworthiales (marine species *A. marina* Nakagiri et Tubaki). Classification above the genus level is according to Eriksson et al. (2001). Species without known teleomorph are: *A. filiformis* Greath. and *A. rubescens* Gulis et Marvanová. All the above species have distinctly longer and often broader or narrower conidia.

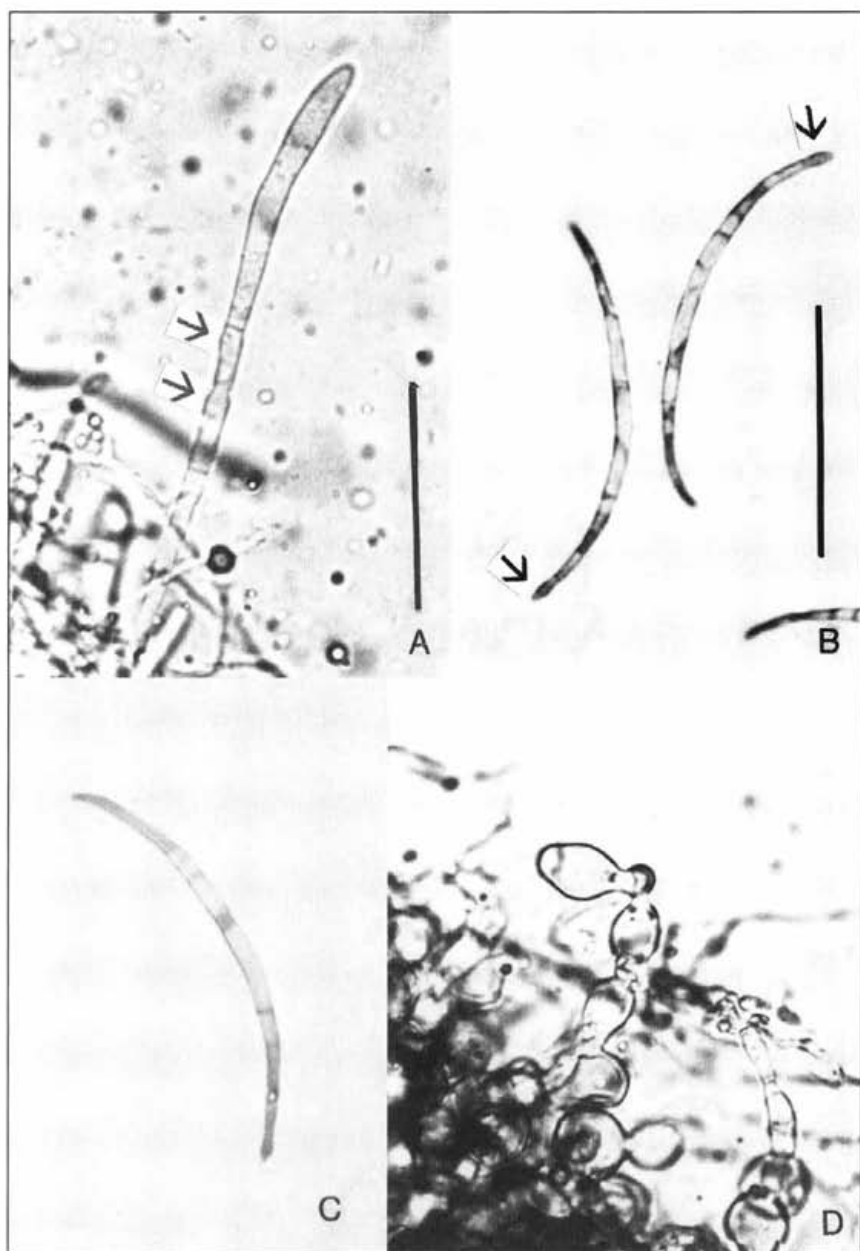


Fig. 2. A-D. *Anguillospora mediocris*. A. Developing conidium. Note the percurrent proliferation of one conidiogenous cells (arrows). B., C. Detached conidia from pure culture, the right one in B turned upside down. Note the snake-head-like bases. D. Crust-like substrate mycelium and inflated cells on the aerial mycelium. Scale bar for A,D = 30 mm, for B,C = 50 mm.



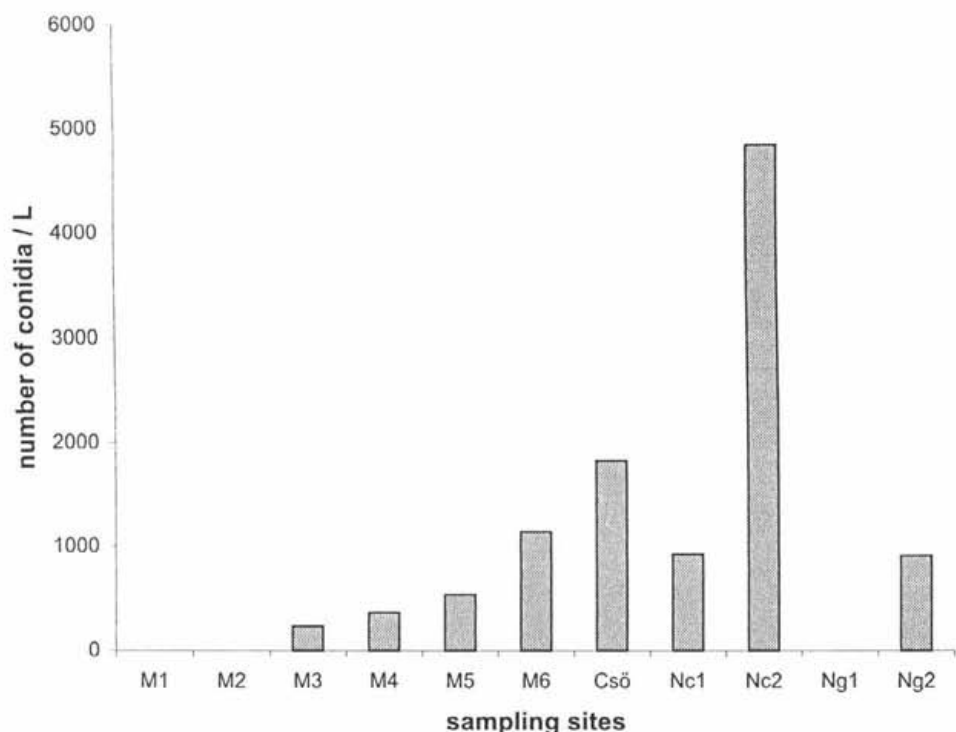


Fig. 3. Spatial dynamics in conidial concentration of *Anguillospora mediocris* in the October samples at the sampling sites of the four streams studied. M1 – M6 = sites downstream at the Morgó stream, Cső = Csömöle stream, Nc1 – Nc2 = Nacsagrom stream, Ng1 – Ng2 = Nagyvasfázék stream.

A coelomycetous microconidial state is known in *Anguillospora longissima*, but this species differs by longer macroconidia, rhexolytic conidial secession and profuse percurrent growth of basal extension in detached conidia.

There are three poorly known species of *Anguillospora*, presently considered doubtful: *A. curvula* S. H. Iqbal, *A. gigantea* Ranzoni and *A. pseudolongissima* Ranzoni. *A. gigantea* has sigmoidly curved conidia of length several times exceeding that of conidia of our fungus. *A. curvula* has conidia outside the range of dimensions known for our fungus. The colony was described as greyish brown, with age becoming reddish brown. The conidial dimensions of *A. pseudolongissima* (50–100 × 4.6–6 µm) almost match those of our fungus, but as noted by Marvanová et al. (1992) the latter is very probably a species of *Filosporella*. These authors support their opinion by the drawing in Ranzoni (1953, Fig. 1T) showing conidiophore with branching typical for *Filosporella* (see under *F. versimorpha*

Marvanová et al. below). Type material of *Anguillospora pseudolongissima* was not deposited.

*Anguillospora* sp. 1 reported by Descals (1997, Figs. 2,3) is described in terms which may characterize also our species: colony is dark grey, margin is lobed, conidiophores are short, unbranched, proliferation of conidiogenous cells is percurrent or sympodial. Conidia are long cylindrical or subfusiform, typically  $45\text{--}120 \times 3\text{--}4 \mu\text{m}$ . However, in *Anguillospora* sp. 1 the characteristic attenuation and curvature of the apex are lacking, the sites of percurrent proliferations are inflated, no pseudoparenchymatous crusts were reported in the colony. We do not think that these two taxa are conspecific.

There are few other taxa known from water, which approximate our fungus in their conidial dimensions: *Sigmoidea aurantiaca* Descals has scolecoform conidia  $25\text{--}90 \times 3.5\text{--}4.5 \mu\text{m}$ , but differs by typically sympodial rachis on conidiophore apex and by orange colonies. *Filosporella versimorpha* Marvanová et al. (1992) whose conidia are of similar length to our fungus, is out of consideration due to its long conidiophores with acrotonous branching. *Aquaphila albicans* Goh, Hyde et Ho (very similar to *Mirandina dactylelloides* Matsush.) has whitish colonies, conidia twice as broad and sympodial conidiophore proliferation.

Some fungi described from leaf litter or from moist conditions also have conidia similar to those of our fungus. However, most of these taxa have typically sympodial conidiophore proliferation:

*Mirandina flagelliformis* Matsush., anamorph of *Chaetonectrioides malaysiana* Matsush. (Matsushima 1996) has conidia very similar, but of smaller dimensions. It differs also in a denticulate conidiophore apex and pale colony.

*Paraarthrocladium amazonense* Matsush. has conidia almost matching those of our fungus in dimensions, but with slightly inflated cells. The conidiogenesis was reported as blastic, with up to two conidia appearing at the conidiogenous cell, which collapses after conidial secession (Matsushima 1993).

*Trichoconis antillana* Castañeda, Kendrick et Guarro (1997) has obclavate, rostrate conidia, overlapping considerably with those of *Anguillospora mediocris*, but formed on polyblastic denticulate conidiogenous cells.

#### ECOLOGICAL CHARACTERISTICS OF *A. MEDIOCRIS*

The importance of this fungus in the hyphomycete communities of the Morgó stream system has become evident since conidial populations in these streams have been studied by membrane filtration. Spatial and temporal distribution patterns of conidia of this species have been statistically analysed at 7 sites in the stream system in the subsequent years from 1996 to 1998. The fungus was reported as *Filosporella* sp. 1. (Gönczöl et al. 1999; Gönczöl and Révay 1999 a) and as *Filosporella* sp. (Gönczöl and Révay 1999 b). The current study on two

further tributaries confirmed our findings on the spatial distribution of this species (unpublished). Some characteristics of the stream habitats where *A. mediocris* has been detected by membrane filtration are given in Table 1. The spatial dynamics of the conidium concentration of *A. mediocris* in the stream water in October samples are shown in Fig. 3. The increasing conidial number in downstream direction in the main channel of the stream system suggests a longitudinal distribution pattern that may be structured by environmental heterogeneity. The stream sections of low altitudes seem to be the preferred habitats of this species. The high conidium concentration in one of the tributaries (Csömöle stream) and the extremely high conidial number in an other tributary (Nacsagrom stream) also suggests that *A. mediocris* may be a typical hardwater species. At higher altitude and/or in softwater stream portions only low conidial concentration or no conidia of this species could be detected.

**Table 1.** Geographical and physicochemical data of 11 sites in the Morgó stream system. Csöm = Csömöle stream, Nacs = Nacsagrom stream, Nagy = Nagyasfázék stream.

	Morgó-stream sites					
	1	2	3	4	5	6
altitude (m)	435	345	305	210	175	125
channel gradient %	12.66	5.66	3.07	2.37	1.09	1.51
stream order	second	second	third	fourth	fourth	fourth
temperature (°C)	2.2–15.5	1.2–17.1	0.2–15.1	0.2–17.2	0.8–19.1	0.1–19.5
pH	6.9–7.1	7.1–7.3	7.1–7.4	7.2–7.4	7.4–7.9	7.4–8.3
conductivity ( $\mu\text{S}/\text{cm}$ )	177–188	177–182	192–229	219–309	276–563	317–614
total hardness (°d)	2.3–4.8	3.3–5.9	4.2–5.9	4.7–7.0	5.6–12.7	7.0–22.0
	Tributary sites					
	Csöm	Nacs 1	Nacs 2	Nagy 1	Nagy 2	
altitude (m)	190	220	150	400	290	
channel gradient %	4.1	1.67	3.88	9.17	3.79	
stream order	second	second	second	second	second	
temperature (°C)	0–17	5.5–11.5	7.1–11.5	7.5–9	7.2–11.5	
pH	7.8–8.3	7.4–7.8	8–8.1	7.1–7.6	7.5–7.7	
conductivity ( $\mu\text{S}/\text{cm}$ )	400–720	630–770	750–970	175–200	220–290	
total hardness (°d)	13.5–17.5	15–19	19–22	5.0–7.0	6.0–10	

The conidia have been rarely seen in foam samples. It was pointed out that foam samples and membrane filtered water samples taken simultaneously at the same site differed dramatically in the conidium concentration of this species. While

a considerable proportion of foam samples contained only some or no conidia, the conidium concentration in filtered stream water ranged from some hundred to several thousands conidia/L (unpublished observation). The fungus, growing on submerged leaves, (mostly on alder) is also commonly found in the same distribution area (see above). Very probably the same species (conidial dimensions from nature were  $50\text{--}90 \times 4.5\text{--}6 \mu\text{m}$ ) was reported and illustrated from three hardwater streams in the Aggtelek National Park as *Anguillospora* sp. 1. (Gönczöl and Révay 1992, Fig. 2.).

Outside Hungary the fungus is probably rare. We found only one illustration, which may represent a conidium of *A. mediocris* (Descals 1998, Fig. 15 K, as unknown). Its dimensions correspond well to those of this species, and the depicted conidium has the characteristic short basal extension. The conidium was collected in the valley of Karrantza (Basque country, Spain), at ca 500 m alt. in foam in a stream flowing through mixed *Fagus* wood and meadows, with *Alnus*, *Corylus* and *Salix* on the banks. The water chemistry is not given.

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## Diseases of Cereal Grains.

APS Digital Image Collections, APS Press, The American Phytopathological Society, 2001, ISBN 0-89054-266-X.

(CD-ROM is deposited in the library of the Society)

The CD-ROM series of image collections edited by the American Phytopathological Society represents a useful compilation of diseases and injuries of different groups of cultivated plants.

The present title consists of 712 colour images illustrating the diseases of barley, corn, sorghum, rice and wheat. Consequently, the whole collection can be browsed through according to the host plant, e.g. images nos. 1-144 concerning the diseases of barley, nos. 145-327 of maize, nos. 328-466 of rice, nos. 467-595 of sorghum and nos. 596-712 of wheat. Each slide can be searched for in the checklist and zoomed in for a larger view. The image contains also the following information: source (author), name of the disease, full scientific name of the causal organism or specification of the abiotic cause and explaining caption. In addition, it is easy to search the images according to symptoms, common name of the disease or to causal organism.

The diseases are mostly shown as parts of the plants that were attacked. In some cases infected plants are compared with healthy ones. Furthermore, injuries of stored grains are included. In some cases, the characters of several pathogens are completed with micrographs. Only a few pictures show views of attacked fields and distribution maps of a certain disease.

The scope of biotic causes of the diseases includes viruses, phytoplasmas, bacteria, fungi, nematodes, insects and parasitic vascular plants. A large number of images show injuries caused by physiological, genetic and climatic factors.

In my opinion, pictures that show seedlings in nursery boxes did not have to be included. I wonder why mycorrhiza as shown in the slides No. 577 through 579 was included, as it is no disease. The sequence of slides sometimes appears a bit chaotic: I find for example that slides No. 700 through 705 (viruses) should come after slide No. 646. And there seem to be several other cases where another sequence would be more suitable. The entire slide No. 590 that shows a life cycle of *Claviceps africana* is illegible.

Despite these minor drawbacks this CD is going to be very useful for teachers and students of plant pathology. The CD contains a remarkable number of diseases, including tropical ones. Including with its installation, this CD is an easy to use tool to create the necessary visual impact in lectures and other presentations.

Jaroslava Marková



## Myxomycetes in Bohemian Karst and Hřebeny Mts.

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Dvořáková R. (2002): Myxomycetes in Bohemian Karst and Hřebeny Mts. – Czech Mycol. 53: 319–349

Data on species composition and ecology of Myxomycetes are presented for three National Reserves in the Bohemian Karst and the northern part of Hřebeny Mountains.

During a period of three years, all localities were investigated intensively. In addition to field collections, bark of living trees, twigs and dead leaves were cultivated in moist chambers. Specimens from the National Museum in Prague were revised. Ninety-five species of Myxomycetes belonging to 29 genera were registered with certainty, 17 of these new to the Czech Republic. Species descriptions are provided for newly recorded species including microhabitat preferences. Differences between species diversity in the Bohemian Karst and Hřebeny Mts. are discussed as well as the seasonal dynamics of Myxomycetes in Central European conditions.

**Key words:** Myxomycetes, Bohemian Karst, Hřebeny Mts., Species diversity, Moist chamber culture

Dvořáková R. (2002): Hlenky (Myxomycetes) v Českém krasu a na Hřebenech. – Czech Mycol. 53: 319–349

V letech 1996–1999 probíhal průzkum myxomycetů (hlenek) v NPR Karlštejn, Koda a Radotínské údolí a v severní části Hřebenů. Vedle sběru v terénu a revize herbářových položek v Národním muzeu v Praze byla využita metoda kultivace hlenek ve vlhkých komůrkách. Je uveden přehled 95 zastoupených druhů hlenek patřících do 29 rodů spolu s popisem a podrobnějšími údaji k 17 druhům novým pro ČR. Dále jsou diskutovány rozdíly v zastoupení druhů v Českém krasu a na Hřebenech a sezónní dynamika hlenek ve středoevropských podmínkách.

### INTRODUCTION

In the last 110 years little attention has been paid to Myxomycetes in the Czech Republic. Čelakovský's study on Czech Myxomycetes (Čelakovský 1890), focusing mainly on Western Bohemia, left a relatively large collection of Myxomycetes in the National Museum in Prague (PRM). This collection was further enlarged with material from Svrček (1959–1972) and Wichanský (1962–1968). Cejp (1962) reported 150 species of Myxomycetes from Western and Central Bohemia, leaving part of his material in the Herbarium of Charles University in Prague (PRC). Svrček (1972) also cultivated 12 species of Myxomycetes on bark of living trees in moist chambers. Vondrová (1991) reported 6 species from moist chamber cultures. Thus, until 1996, a total of 188 species of Myxomycetes were reported from the Czech Republic.

During the years 1996–1999 the author studied Myxomycetes in two relatively different localities near Prague (Hřebený Mts. and Bohemian Karst Protected Landscape Area). An intensive survey of all suitable microhabitats was carried out. The main tasks of the work were:

- revealing the species diversity
- contributing to the knowledge of Myxomycetes ecology in Central European conditions.
- comparing the spectrum of Myxomycetes in two areas with different geological structures (especially whether the limestone base in the Bohemian Karst influences the presence of the order *Physarales*)

#### STUDY AREA

Bohemian Karst Protected Landscape Area is a hilly area situated in central Bohemia to the south-west of Prague, touching the valley of the Berounka River between the capital and the town of Beroun. Elevation above sea level: 208–499 m. It is characterised by a moderately warm and dry climate with a low level of precipitation (500–550 mm). The mean annual temperature varies between 8 and 9 °C.

A limestone base, forming a considerable part of the geological structure, is broken by karst canyons and drilled through by caves. Due to extreme geomorphological conditions and the limestone base a wide variety of ecosystems occur (forest steppes with *Quercus pubescens*, calciphilous beechwoods, thermophilous mixed oak woods, slope forests etc.)

For this study, the three largest Nature Reserves were chosen – Karlštejn (49°57'N., 14°9'E.), Koda (49°56'N., 14°6'E.) and Radotínské valley (49°59'N., 14°18'E.) with a total area of c.21 km<sup>2</sup> (see Fig. 1).

Hřebený Mts. are the N-E part of the Brdy Mts., representing the largest complex of woodland in the neighbourhood of Prague. The maximum height above sea level reaches 600 m. It is also characterised by a moderately warm, quite dry climate with a low level of precipitation (550–610 mm). The mean annual temperature is 7–8 °C.

The northern part consists mainly of Ordovician quartzite. On the top parts and slopes the remnants of the original forest cover can still be found (woodrush and herb-rich beech forests, oak-hornbeam woods). Spruce forests or pinewood monocultures cover most of the area today, but large old oak or beech stumps are also present.

For the purpose of this study the northern part of Hřebený Mts. of a size comparable to the study area in the Bohemian Karst was chosen (49°54'N., 14°16'E.). The survey was concentrated especially on deep valleys and gorges of streams with plenty of decaying wood and high humidity. Material for moist chamber cultures was collected from the whole area studied (see Fig. 1).



Fig. 1. Map of the area studied. Forested areas are marked with grey, localities of interest are striped and numbered: 1 = Karlštejn Nature Reserve, 2 = Koda Nature Reserve, 3 = Radotinské valley Nature Reserve, 4 = Northern part of Hřebeny Mts.

## MATERIAL AND METHODS

The fieldwork was carried out for 3 years, from July 1996 till May 1999, and included moist chamber experiments. All localities were visited regularly during the year, approximately once a month or more often in the season. Common and easily recognised myxomycete species were collected only occasionally, but rare species and species not easy to recognise in the field were always collected. Specimens collected by M. Svrček, E. Wichanský, J. Baier, V. Eckert, R. Fellner, B. Ježek, J. Nitka, A. Pilát, R. Škvrně, V. Vacek and S. Vondrová on the localities studied, deposited in the Mycological Herbarium of the National Museum in Prague (PRM), were also included in the study.

Samples for the moist chamber cultures were taken from bark of living and dead trees (up to 1.5 m high) and from litter (leaves, needles and twigs) of each tree species present. A total of 250 moist chamber cultures were cultivated. Cultures were prepared as described by Härkönen (1977, 1983), kept in a klimabox at a temperature of 19–22 °C and c.75 % humidity, illuminated artificially in a 12:12 light:dark cycle. The cultures were moistened with distilled water adjusted with KOH to pH 7. Moist chambers were first examined after 24 hours and then every second or third day under a dissecting microscope for about 5 weeks. Chambers with no or very few Myxomycetes developed were let to dry and after 6–8 weeks rewetted and cultivated once again.

From most of the collections, sporocarps were preserved as permanent slides in lactophenol or Hoyer medium (Nannenga-Bremekamp 1991). Specimens of Myxomycetes collected in the field or obtained from the moist chamber cultures were deposited in the herbarium at the Department of Botany, Faculty of Nature Science of Charles University in Prague (PRC), and in the personal collection of the author.

The abundance was estimated with a simple scale proposed by Stephenson et al. (1993), based on the proportion of records for one species on all fructifications recorded in the survey: R (rare <0.5%), O (occasional 0.5–1.5%), C (common 1.5–3%), A (abundant >3%). The geographical distribution of species is provided according to Nannenga-Bremekamp (1991) and Neubert et al. (1993, 1995).

## RESULTS

In total 813 specimens were studied. Out of these, 340 were collected in the field, 362 were harvested from moist chamber cultures and 99 were found in the Mycological Herbarium of the National Museum in Prague (PRM). Twelve specimens were kindly lent by M. Svrček from his personal herbarium. Thirty-two specimens from the moist chamber cultures were not mature or developed enough to be identified with certainty and were not included in further results.

The collections comprised 95 species, including 17 new to the Czech Republic<sup>1)</sup> and one seemingly undescribed (*Didymium* cf. *squamulosum*). The species were identified according to Martin and Alexopoulos (1969), Lado and Pando (1997), Nannenga-Bremekamp (1991), Neubert et al. (1993, 1995) and Ing (1999).

Table 1. shows all recorded species in alphabetical order, their occurrence on localities studied and estimated abundance.

**Table 1.** Occurrence and abundance estimations of all species and varieties of Myxomycetes recorded on localities studied. Those new to the Czech Republic are marked \*. (R = rare, O = occasional, C = common, A = abundant).

species	Hřebený Mts.	Bohemian Karst	abundance
<i>Arcyria affinis</i> Rostaf.	+	+	R
<i>Arcyria cinerea</i> (Bull.) Pers.	+	+	A
<i>Arcyria denudata</i> (L.) Wettst.	+	+	C
<i>Arcyria ferruginea</i> Sauter	+	+	R
<i>Arcyria incarnata</i> (Pers.) Pers.	+	+	C
<i>Arcyria insignis</i> Kalchbr. et Cooke	+	—	R
<i>Arcyria major</i> (G. Lister) Ing *	—	+	R
<i>Arcyria minuta</i> Buchet	+	+	R
<i>Arcyria obvelata</i> (Oeder) Onsberg	+	+	C
<i>Arcyria oerstedtii</i> Rostaf.	—	+	R
<i>Arcyria pomiformis</i> (Leers) Rostaf.	+	+	A
<i>Arcyria stipata</i> (Schw.) Lister	+	+	O
<i>Calomyxa metallica</i> (Berk.) Nieuwl. *	—	+	R
<i>Badhamia dubia</i> Nann.-Bremek. *	—	+	R
<i>Ceratiomyxa fruticulosa</i> (Müller) T. Macbr.	+	+	A
<i>Ceratiomyxa fruticulosa</i> var. <i>porioides</i> (Alb. et Schw.) A. Lister	+	+	C
<i>Comatricha elegans</i> (Racib.) G. Lister	—	+	R
<i>Comatricha nigra</i> (Pers.) Schroet.	+	+	C
<i>Comatricha pulchella</i> (C. Bab.) Rostaf.	+	+	R
<i>Craterium leucocephalum</i> (Pers.) Ditmar	+	+	C
<i>Craterium minutum</i> (Leers) Fr.	+	+	C
<i>Cribraria argillacea</i> (Pers.) Pers.	+	+	O
<i>Cribraria aurantiaca</i> Schrad.	+	+	R
<i>Cribraria cancellata</i> (Batsch) Nann.-Bremek.	+	+	A
<i>Cribraria macrocarpa</i> Schrad.	+	—	R
<i>Cribraria microcarpa</i> (Schrad.) Pers.	+	+	R

<sup>1)</sup> Nine species were previously published in Dvořáková (1999a): Myxomycetes developed in moist-chamber cultures. *Licea parasitica* was lately published by Kocourková (2000).

species	Hřebený Mts.	Bohemian Karst	abundance
<i>Cribraria persoonii</i> Nann.-Bremek. *	+	—	R
<i>Cribraria rufa</i> (Roth) Rostaf.	+	+	O
<i>Cribraria violacea</i> Rex	—	+	O
<i>Cribraria vulgaris</i> Schrad.	+	+	C
<i>Diachea leucopodia</i> (Bull.) Rostaf.	+	+	C
<i>Dictydiaethalium plumbeum</i> var. <i>ferrugineum</i> (Nann.-Bremek.) L. Krieglsteiner	+	—	R
<i>Diderma cingulatum</i> Nann.-Bremek. *	—	+	R
<i>Diderma crustaceum</i> Peck	—	+	R
<i>Diderma spumarioides</i> (Fr.) Fr.	—	+	O
<i>Diderma testaceum</i> (Schrad.) Pers.	+	+	A
<i>Didymium difforme</i> (Pers.) S. F. Gray	+	+	O
<i>Didymium melanospermum</i> (Pers.) T. Macbr.	+	+	O
<i>Didymium minus</i> (Lister) Morgan	+	+	C
<i>Didymium nigripes</i> (Link) Fr.	+	—	O
<i>Didymium serpula</i> Fr.	+	—	R
<i>Didymium squamulosum</i> (Alb. et Schw.) Fr.	—	+	C
<i>Didymium</i> sp. cf. <i>squamulosum</i> (Alb. et Schw.) Fr.	+	—	R
<i>Echinostelium minutum</i> de Bary	+	+	A
<i>Echinostelium apitectum</i> Whitney *	—	+	R
<i>Enerthenema papillatum</i> (Pers.) Rostaf.	+	+	A
<i>Enteridium lycoperdon</i> (Bull.) Farr	+	+	C
<i>Fuligo cinerea</i> (Schw.) Morgan	—	+	R
<i>Fuligo leviderma</i> Neubert, Nowotny et K. Baumann *	+	+	O
<i>Fuligo rufa</i> Pers.	—	+	R
<i>Fuligo septica</i> (L.) F. H. Wigg.	+	+	C
<i>Fuligo septica</i> var. <i>candida</i> (Pers.) Fr.	+	+	O
<i>Fuligo septica</i> var. <i>flava</i> (Pers.) Fr.	+	—	R
<i>Hemitrichia calyculata</i> (Speg.) Farr *	+	+	A
<i>Hemitrichia clavata</i> (Pers.) Pers.	+	+	C
<i>Hemitrichia serpula</i> (Scop.) Rostaf.	—	+	R
<i>Lamproderma arcyrioides</i> (Sommert.) Rost	—	+	R
<i>Lamproderma arcyronema</i> Rostaf.	+	+	O
<i>Lamproderma scintillans</i> (Berk. et Br.) Morgan *	+	+	O
<i>Leocarpus fragilis</i> (Dicks.) Rostaf.	+	+	O
<i>Licea biforis</i> Morgan *	+	+	O
<i>Licea kleistobolus</i> G. W. Martin *	+	+	A
<i>Licea minima</i> Fr. *	+	+	A



species	Hřebeny Mts.	Bohemian Karst	abundance
<i>Licea operculata</i> (Wingate) G. W. Martin *	—	+	R
<i>Licea parasitica</i> (Zukal) G. W. Martin *	+	+	O
<i>Licea variabilis</i> Schrad.	—	+	O
<i>Lycogala epidendrum</i> (L.) Fr.	+	+	A
<i>Lycogala flavo-fuscum</i> (Ehrenb.) Rostaf.	—	+	R
<i>Metatrichia floriformis</i> (Schw.) Nann.-Bremek.	+	—	O
<i>Metatrichia vesparium</i> (Batsch) Nann.-Bremek.	+	+	C
<i>Mucilago crustacea</i> F. H. Wigg.	—	+	C
<i>Paradiacheopsis fimbriata</i> (G. Lister et Cran) Hertel	+	+	A
<i>Paradiacheopsis solitaria</i> (Nann.-Bremek.) Nann.-Bremek. *	—	+	O
<i>Perichaena corticalis</i> (Batsch) Rostaf.	+	+	A
<i>Perichaena depressa</i> Libert	—	+	C
<i>Perichaena chrysosperma</i> (Currey) Lister	—	+	R
<i>Physarum bivalve</i> Pers.	+	—	O
<i>Physarum cinereum</i> (Batsch) Pers.	+	—	O
<i>Physarum decipiens</i> M. A. Curtis *	—	+	R
<i>Physarum leucophaeum</i> Fr.	—	+	O
<i>Physarum leucopus</i> Link	—	+	O
<i>Physarum nutans</i> Pers.	+	+	A
<i>Physarum rubiginosum</i> Fr.	—	+	R
<i>Physarum virescens</i> Ditmar	+	—	O
<i>Physarum viride</i> (Bull.) Pers.	+	+	R
<i>Physarum viride</i> (Bull.) Pers. var. <i>aurantiacum</i> (Pers.) Lister	+	+	O
<i>Stemonitis axifera</i> (Bull.) T. Macbr.	+	+	C
<i>Stemonitis fusca</i> Roth	+	+	O
<i>Stemonitopsis subcaespitosa</i> (Peck) Nann.-Bremek. *	—	+	R
<i>Stemonitopsis typhina</i> (F. H. Wigg.) Nann.-Bremek.	+	+	C
<i>Trichia botrytis</i> (J. F. Gmel.) Pers.	+	+	R
<i>Trichia contorta</i> (Ditmar) Rostaf.	+	+	R
<i>Trichia decipiens</i> (Pers.) T. Macbr.	+	+	A
<i>Trichia favoginea</i> (Batsch) Pers.	+	+	A
<i>Trichia munda</i> (Lister) Meylan *	+	+	O
<i>Trichia persimilis</i> Karst.	+	+	O
<i>Trichia scabra</i> Rostaf.	+	+	A
<i>Trichia varia</i> (Pers.) Pers.	+	+	A
<i>Tubifera ferruginosa</i> (Batsch) J. F. Gmel.	+	+	A

Brief descriptions of 17 species new to the Czech Republic are given, based on the Czech material. Notes on species ecology and geographical distribution are also added. (MCC = cultivated in moist chamber culture, RD = R. Dvořáková, PRC = Department of Botany Herbarium, Faculty of Nature Science, Charles University, Prague)

**Arcyria major** (G. Lister) Ing, Trans. Br. Myc. Soc. 50: 556. 1967.

Fig. 2, A-C

Specimens examined: Bohemian Karst: Srbsko, Karlštejn Nature Reserve, Bubovický potok valley; decaying log of *Carpinus betulus*, 5. XI. 1998, leg. RD, det. L. Kriegelsteiner (PRC).

Sporangia in small groups, shortly stalked, cylindrical, 2–3 mm tall, bright red-pink when fresh, later changing to orange-brown. Stalk short, yellow-brown, hypothallus extending under the whole group. Peridium fugacious except for the cup, which is shallow, pleated, translucent and iridescent; inner side of the cup covered with reticulum with fine ridges. Bottom of the cup and the stalk are filled with round cysts of the same size as spores or larger (up to 20  $\mu\text{m}$  toward the base of the stalk). Capillitium forming an elastic, expanding, large meshed net, easily blown out of the cup as a long plume, leaving remnants of the tubes attached to the cup; tubes yellow in transmitted light, 3–4.8  $\mu\text{m}$  in diam., branching and decorated with half-rings. Spores very pale yellow in transmitted light, almost smooth, with groups of small wartlets; 7.2–8.5  $\mu\text{m}$  in diameter.

Found only once on dead wood of *Carpinus*, specimen with c. 15 sporangia.

**Badhamia dubia** Nann.-Brem., Proc. K. Ned. Akad. Wet. Ser. C. 71: 49. 1968.

Fig. 3, A-C

Specimens examined: Bohemian Karst: Radotínské údolí Nature Reserve, east edge of the forest above Maškův mlýn; piece of bark of *Quercus sp.*, lying on a big pile of decaying herbaceous material, 11. VII. 1998, leg. et det. RD (PRC).

Sporangia crowded in small groups, sessile, almost spherical, about 1 mm in diam., blue-grey with metallic shine. Peridium thin, one-layered, translucent and shining, slightly covered with lime crystals, dehiscing irregularly. Capillitium consisting of a regular, wide net; tubes quite slender, filled with lime material. Spores black in mass, purple-brown in transmitted light, in clusters of 8–12 spores; individual spores spherical to ovoid, 9.6–12  $\mu\text{m}$  in diam., covered with large spines (forming a cup) on the outside of the cluster and scattered small warts on the other side.

Species described from the Netherlands in 1968, first record from the Czech Republic. This is the only *Badhamia* species found in the study area at all. Nine other *Badhamia* species have been reported from the Czech Republic, last record in Sept. 1961.

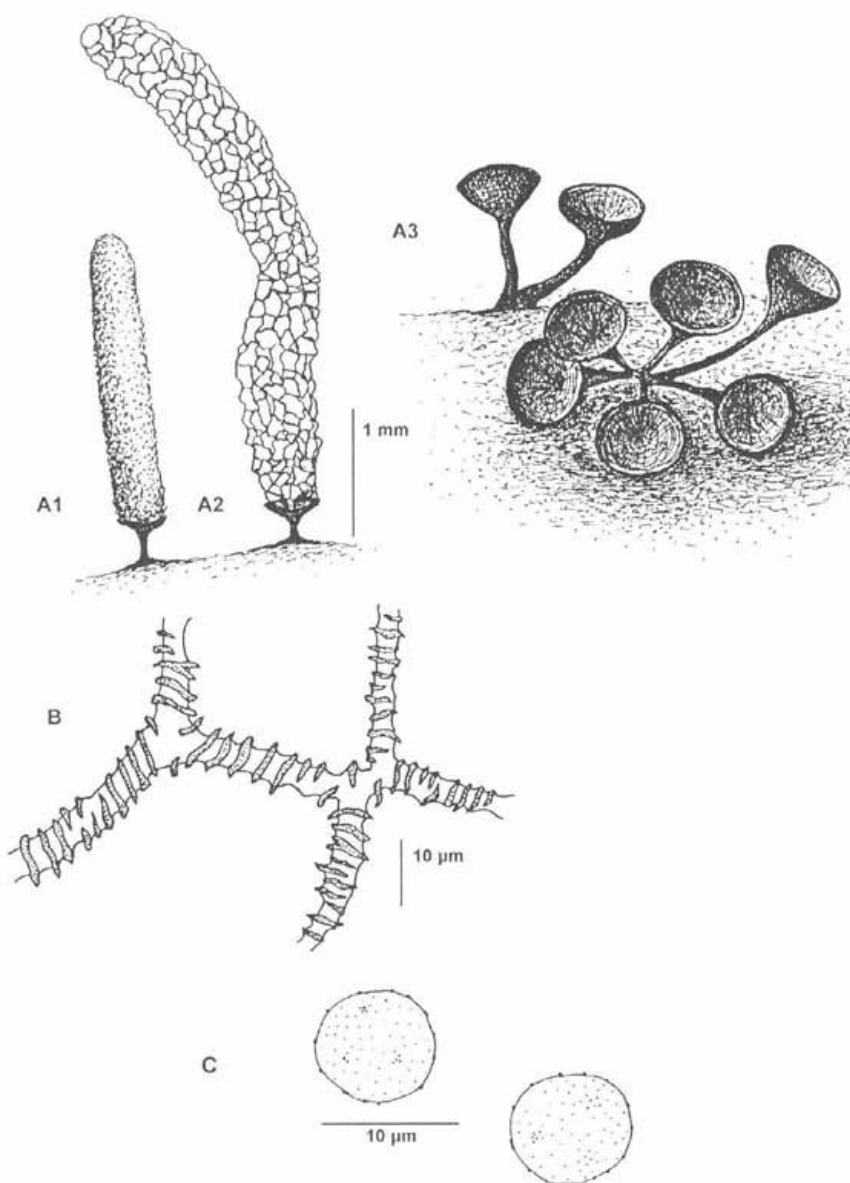


Fig. 2. *Arcyria major* (G. Lister) Ing. A1 - sporangium, A2 - sporangium with expanded capillitium, A3 - stalked cups, B - capillitium, C - spores

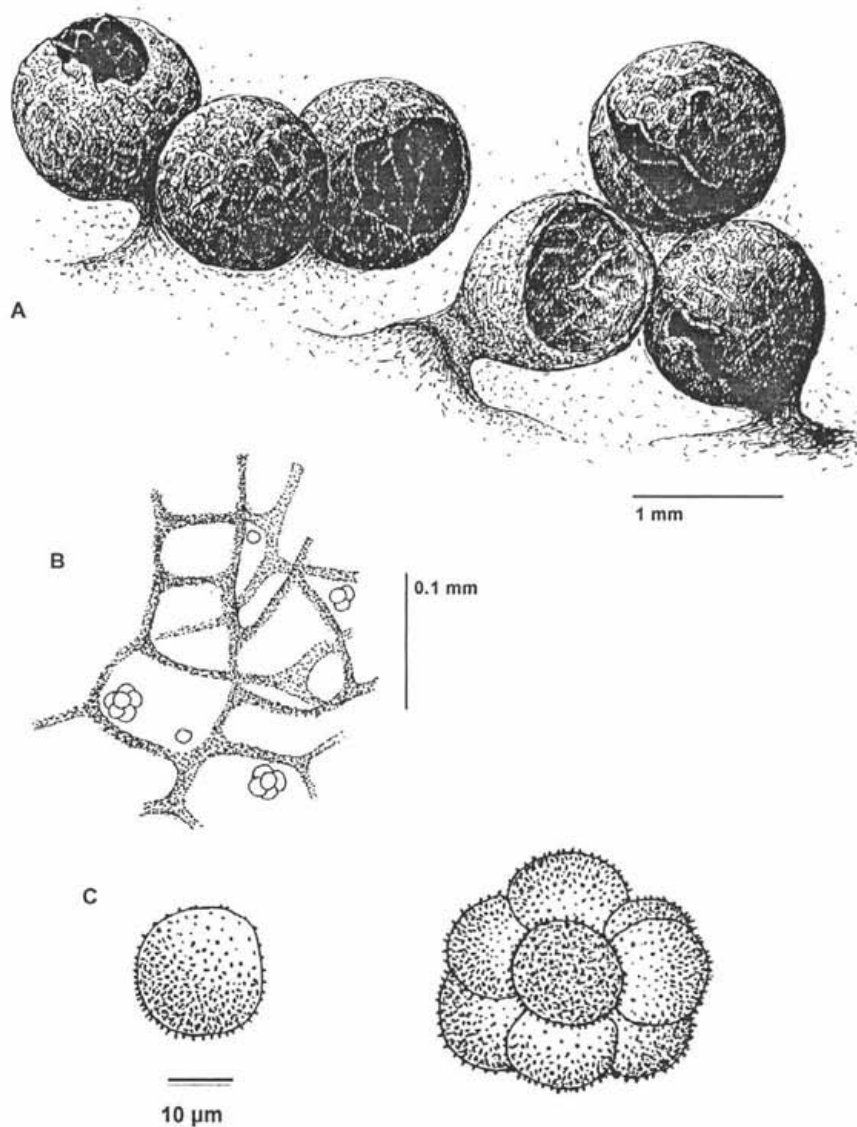


Fig. 3. *Badhamia dubia* Nann.-Bremek. A – sporangia, B – part of capillitium, C – spores

This material was found on a large pile of decaying herbaceous material together with extensive aethalia of *Fuligo cinerea*.

***Calomyxa metallica*** (Berk.) Nieuwl., Am. Mid. Nat. 4: 335. 1916.

Specimens examined: Bohemian Karst: Karlštejn Nature Reserve, east slope of Mt. Doutnáč; on the inner side of bark of *Quercus* sp., 5. XI.1998, leg. et det. RD (PRC). – Radotínské údolí Nature Reserve; on bark of living tree of *Tilia cordata* (MCC), 20. III.1998, leg. et det. RD (PRC).

Sporangia and/or short plasmodiocarps, sessile, spherical or pulvinate, 0.3–0.5 mm in diameter and up to 2 mm long, rosy when fresh, changing to golden iridescent. Peridium of one layer, thin and translucent, pale ochraceous in transmitted light. Threads of the capillitium very long, pale yellow, 0.5–1  $\mu\text{m}$  in diam., expanding elastically from the sporangia, tangled with many loops, covered with spirally arranged bands of spinules. Spores beige in mass, yellow in transmitted light, 9.7–10.7  $\mu\text{m}$  in diam., covered with distinct warts.

In moist chamber cultures developing together with *Trichia munda*.

Widely distributed (Europe, American continent, India, Japan, Jamaica) but not very common.

***Cribraria persoonii*** Nann.-Brem., Proc. K. Ned. Akad. Wet. Ser. C 74: 353. 1971. Fig. 4 A-C

Specimens examined: Hřebeny Mts.: Řevnice, valley of Moklický stream; dead coniferous wood, 14. VII.1998, leg. et det. RD (PRC).

Sporangia ochraceous-brown, stalked, subglobose, 1.5–2 mm in diameter. Stalk length 2 times the sporangium diameter, dark brown, plicate. Peridium persisting in the basal third as a cup, which is hazelnut brown and radially plicate, with thickened rim; there are short ribs or teeth regularly placed on the rim and connected by threads to the reticulum. Net nodes small and round, thickened with dark lime granules, almost without any free-ending threads. Spores almost colourless in transmitted light, with no oil inclusion, c. 7  $\mu\text{m}$  in diam. (6.5–7.5  $\mu\text{m}$  according to Nannenga-Bremekamp), covered with small warts.

Described by Nannenga-Bremekamp in 1971 from decayed pinewood only.

***Diderma cingulatum*** Nann.-Brem., Proc. K. Ned. Akad. Wet. C. 71: 91. 1968.

Specimens examined: Bohemian Karst: Radotínské údolí Nature Reserve; decayed leaves and branches of *Quercus* and *Carpinus* sp., several large colonies, 19. IX.1998, leg. et det. RD (PRC).

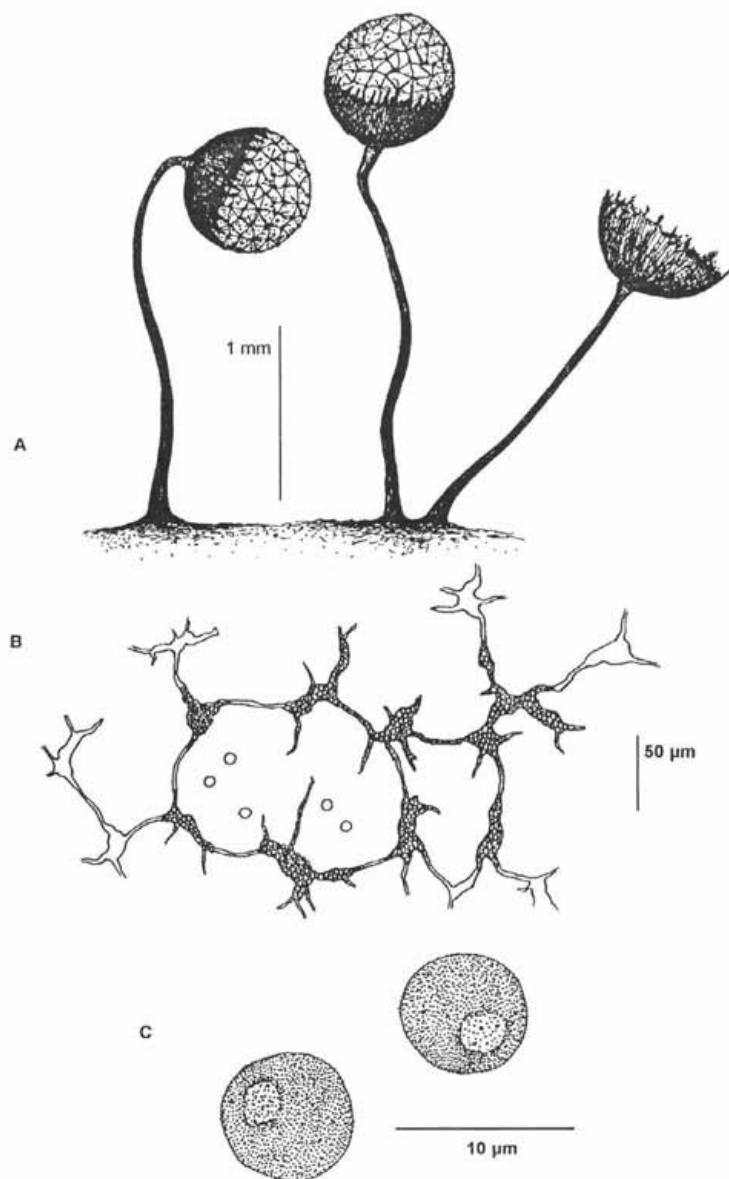


Fig. 4. *Cribraria persoonii* Nann.-Bremek. A – sporangia, B – peridial net, C – spores

Sporangia crowded in grape-like clusters, almost subglobose with a wide base, white, sessile. Limy hypothallus shared by the whole group. Peridium of two layers; the outer a thick white lime crust is brittle and crumbles easily, the inner one is membranous and colourless. White cylindrical columella slightly flattened and almost reaching the top of the sporangium. Capillitium consisting of dark thin tubules, pale on the top ends, sometimes dichotomously branched and only sparingly anastomosing, with few perforated swellings. Spores dark brown in mass, purple-brown in transmitted light, 9.6–12.5  $\mu\text{m}$  in diam., covered with fine warts and few fine ridges forming a lax reticulum; a pale narrow germination line is distinct.

Develops on dead wood, dead leaves and living herbs. Described from the Netherlands.

***Echinostelium apitectum* Whitney, Mycologia 72:954. 1980 Fig. 5**

Specimens examined: Bohemian Karst: Karlštejn Nature Reserve, west slope of Boubová Mt.; bark of living tree of *Quercus petraea* (MCC), 6. XI.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, valley of Loděnice River under the village of Hostim, bark of living tree of *Quercus petraea* (MCC), 7. XI.1998, leg. et det. RD.

Comparing to *Echinostelium minutum* the sporangia of this species were much smaller (70–150  $\mu\text{m}$ ), white, hyaline and very fragile. Stalk about 20  $\mu\text{m}$  in diam. at the base, tapering to the apex (up to 2  $\mu\text{m}$  in diam.), filled with granular material and hyaline in the upper part. Columella cylindrical to hemispherical, very small (c. 3  $\mu\text{m}$ ), with a globose or oval spore-like covering 7–12  $\mu\text{m}$  in diam. and a basal collar (remnant of peridium). Capillitium absent or present as one thread, arising from the columella (in one case the thread was dichotomously branched). Spores smooth, hyaline, 7–8  $\mu\text{m}$  in diam. (6–12  $\mu\text{m}$  in diam. according to Lado and Pando 1997).

Large colonies fructificated on bark of old trees of *Quercus petraea* together with *Arcyria cinerea*, *Licea kleistobolus* and *Paradiacheopsis fimbriata*. Incubation time 7 and 18 days respectively.

Reported from the USA (Whitney 1980), France and Spain (Lado and Pando 1997).

***Hemitrichia calyculata* (Speg.) Farr, Mycologia 66: 887, 1974.**

Specimens examined: Bohemian Karst: Karlštejn Nature Reserve, waterfalls on Bubovický stream; decayed mossy log, 18. IX.1997, leg. et det. RD (PRC). – Karlštejn Nature Reserve, Bubovický stream near Srbsko; on decayed deciduous wood, 5. VIII.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, Loděnice



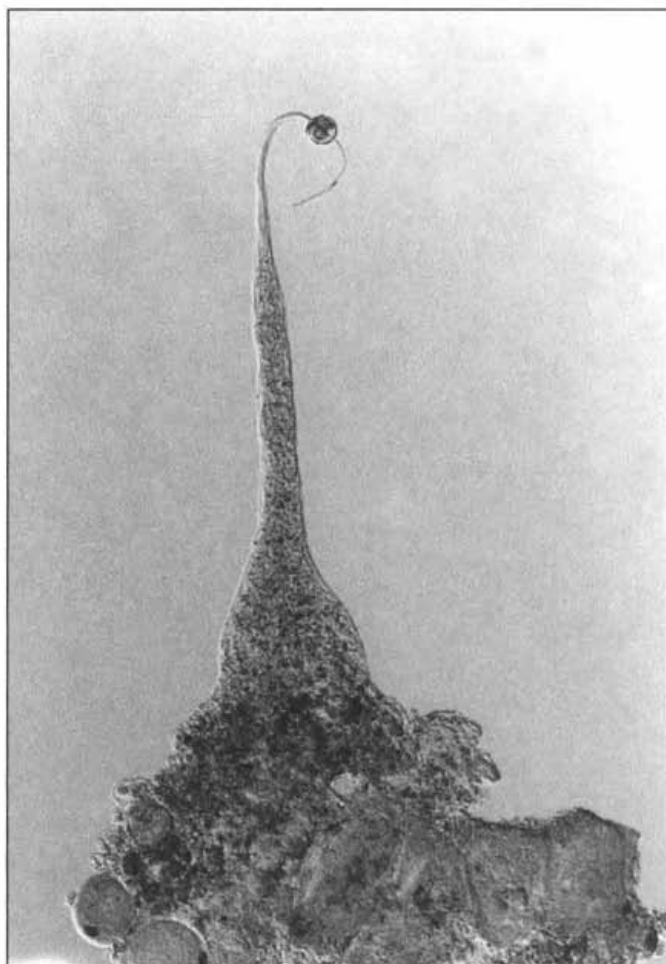


Fig. 5. *Echinostelium apitectum* Whitney (scale bar = 25  $\mu$ m)

stream; on decayed deciduous wood, 24. IX.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, Bubovický stream under Mt. Doutháč; on decaying log of *Quercus* sp., 5. XI.1998, leg. et det. RD (PRC). – Radotínské údolí Nature Reserve, Kalinův mlýn; on decayed log of *Carpinus betulus*, 8. XI.1998, leg. et det. RD (PRC). – Hřebeny Mts.: Moklický stream, on decayed log of *Fagus sylvatica*, 15. VII.1998, leg. et det. RD (PRC). – Kejná stream under Mt. Hvíždinec; on decayed log of *Fagus sylvatica*, 31. X.1998, leg. et det. RD (PRC).

Sporangia in groups, stalked, 2–5 mm high, yellow or ochraceous-yellow, shiny. Stalk dark brown, thin, abruptly tapering into the sporangium, 30–50 % of the

total sporangium high, on a red-brown membranous hypothallus. Peridium thin, yellow, translucent, dehiscing irregularly leaving a deep plicate cup with a revolute margin. Capillitium elastic, expanding and leaving the cup (not connected with the peridium or the wall of the stalk); long branching tubes are decorated with 4–5 spirals which are covered with fine spines (appearing velvety under oil immersion). Spores pale yellow, 7–8  $\mu\text{m}$  in diameter, covered with a delicate reticulum.

Typical of the autumn aspect, on strongly decayed wood of deciduous trees (esp. *Fagus sylvatica*), together with *Trichia favoginea*, *T. scabra*, *Metatrichia vesparium*, *M. floriformis* and *Arcyria denudata*. Large colonies found in autumn 1998. Known from Spain, France, Israel, Germany, Indonesia, etc.

**Lamproderma scintillans** (Berk. et Br.) Morgan, Jour. Cinc. Soc. Nat. Hist. 16:131. 1894.

Specimens examined: Bohemian Karst: Radotínské údolí Nature Reserve; decayed leaves of *Quercus robur*, 10. VII.1998, leg. et det. RD (PRC). – Hřebeny Mts.: Babský stream under Mt. Babka; decayed leaves of *Quercus* sp. and *Pinus sylvatica*, 15. VII.1998, leg. et det. RD (PRC).

Sporangia stalked, spherical, 0.5–1 mm tall; stalk slender, black, length 1.5–2 times the sporangium diameter. Peridium blue-purple iridescent in reflected light, falling off in large flakes and leaving only a red-brown collar around the stalk. Cylindrical columella with a blunt apex, up to about half of the sporangium height. Capillitium dark brown, colourless at the base around the columella, rather straight; threads emerging from the apex of the columella, radiating outwards, branching several times. Spores dark brown in mass, pale lilac-brown in transmitted light, 8–9.6  $\mu\text{m}$  in diam., with dark, dispersed warts.

Found on both localities in July on decayed leaves and needles. Material revised by L. Krieglsteiner.

**Licea biforis** Morgan, Jour. Cinc. Soc. Nat. Hist. 15: 131; 1893  
Fig. 6 A, B

Specimens studied: Bohemian Karst: Radotínské údolí Nature Reserve; inner side of bark of *Fraxinus excelsior*, 8. XI.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve; bark of living tree of *Sambucus nigra* (MCC), 3. X.1996, leg. et det. RD (PRC). – Hřebeny Mts.: Všenory railway station; bark of living tree of *Populus nigra* (MCC), 3. XII.1996, leg. et det. RD (PRC).

Sporangia in groups, sessile, laterally flattened, elongate, c. 240  $\mu\text{m}$  long, brown to yellow-brown. Peridium pale yellow-brown in transmitted light, covered with granulose inclusions which are absent from the pale longitudinal line of dehiscence.

Capillitium absent. Spores yellow in mass, very pale yellow in transmitted light, globose to oval, 8.4–10.8  $\mu\text{m}$  in diameter, minutely punctate.

The only field collection is from the inner side of bark of a decaying log, found together with *Trichia varia* and *Perichaena corticalis*. In moist chamber cultures it fructificated a few times on bark of *Populus nigra* and *Sambucus nigra*, together with *Echinostelium minutum*, *Enerthenema papillatum*, *Perichaena corticalis* and *Physarum nutans*. Development of sporangia in moist chambers completed in 21–30 days.

Note: This corticolous species seems to prefer tree species with higher bark pH (*Fraxinus excelsior*, *Populus nigra*, and *Sambucus nigra*).

**Licea kleistobolus** G. W. Martin, Mycologia 34: 702. 1942.

Fig. 7, A–C

Specimens examined: Bohemian Karst: Karlštejn Nature Reserve, Loděnice river; bark of living tree of *Quercus petraea* (MCC), 17. XI.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, Loděnice river; bark of living tree of *Alnus glutinosa* (MCC), 19. XI.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, Boubová Mt., bark of living tree of *Quercus petraea* (MCC), 19. XI.1998, leg. et det. RD (PRC). – Radotínské údolí Nature Reserve; bark of living tree of *Aesculus hippocastanum* (MCC), 16. I.1999, leg. et det. RD (PRC). – Radotínské údolí Nature Reserve; bark of living tree of *Pinus sylvestris* (MCC), 20. I.1999, leg. et det. RD (PRC). – Hřebený Mts.: under Mt. Hvízdinec; bark of living tree of *Aesculus hippocastanum* (MCC), 21. XI.1996, leg. et det. RD (PRC). – Kejná stream near the village of Řevnice; bark of living tree of *Quercus petraea* (MCC), 14. V.1998.

Black shiny sporangia in groups, sessile, very small (c. 0.1 mm in diam.), round, flattened, dehiscing along a sunken preformed lid with a distinct margin. Peridium yellow-brown in transmitted light, thin, the operculum on the inside covered with large hollow papillae, which get smaller towards the margin. Spores very pale in transmitted light, 9.6–12  $\mu\text{m}$  in diameter; the wall almost smooth with groups of tiny spinules, germination pore absent.

Corticolous species occurring on bark of *Quercus*, *Aesculus*, *Alnus*, *Pinus*. In moist chamber cultures developing together with *Paradiacheopsis fimbriata*, *Enerthenema papillatum*, *Arcyria pomiformis*, *Echinostelium minutum* (often) and *Licea parasitica* (occasionally), incubation time 7–28 days. Reported from Europe, Turkey, USA and Japan.

**Licea minima** Fr., Syst. Myc. 3: 199. 1829.

Fig. 8, A–C

Specimens examined: Bohemian Karst: Radotínské údolí Nature Reserve, Maškův mill; decaying barkless log, 10. VII.1998, leg. et det. RD (PRC). – Hřebený

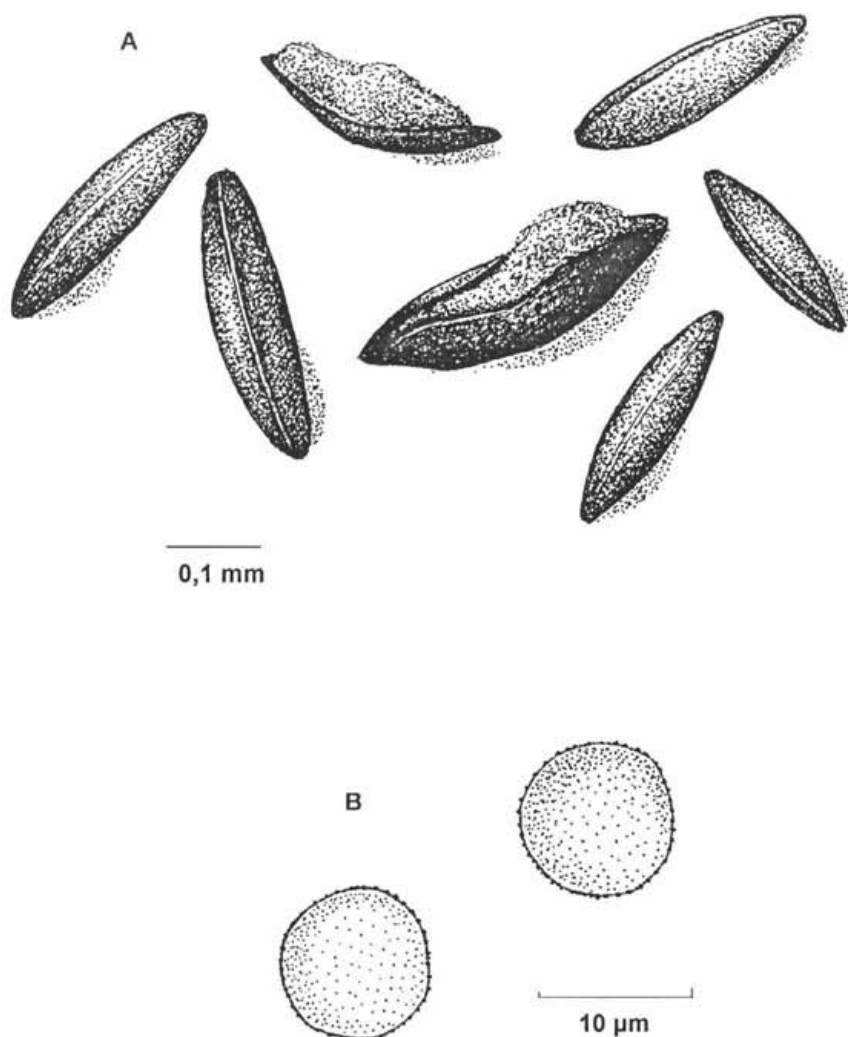


Fig. 6. *Licea biforis* Morgan. A – sporangia, B – spores

Mts.: Dobřichovice; bark of living tree of *Betula pendula* (MCC), 9. IX.1996, leg. et det. RD (PRC). - Dobřichovice; bark of living tree of *Pinus sylvestris* (MCC), 9. IX.1996, leg. et det. RD (PRC). - Mt. Chlum above Dobřichovice; bark of living tree of *Pinus sylvestris* (MCC), 12. II.1997, leg. et det. RD (PRC). - Dobřichovice;

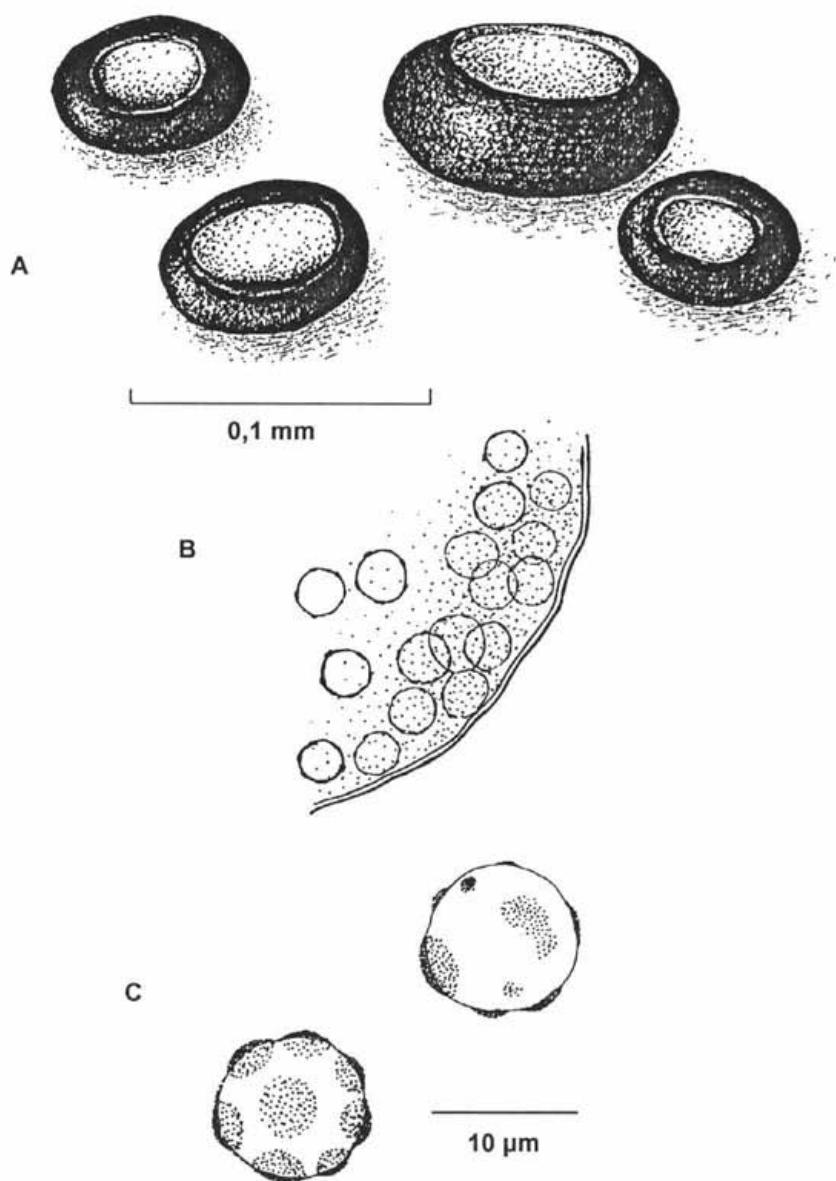


Fig. 7. *Licea kleistobolus* G. W. Martin. A – sporangia, B – part of the peridium, C – spores

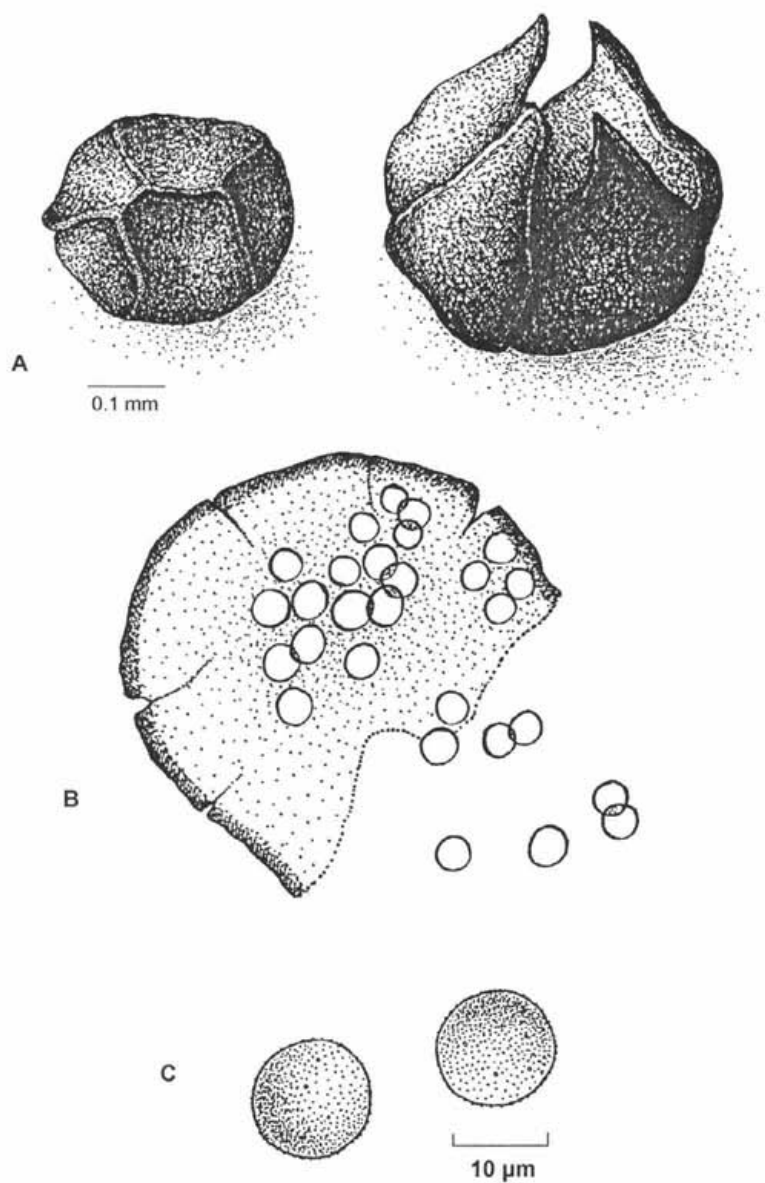


Fig. 8. *Licea minima* Fr. A – sporangia, B – part of peridium, C – spores

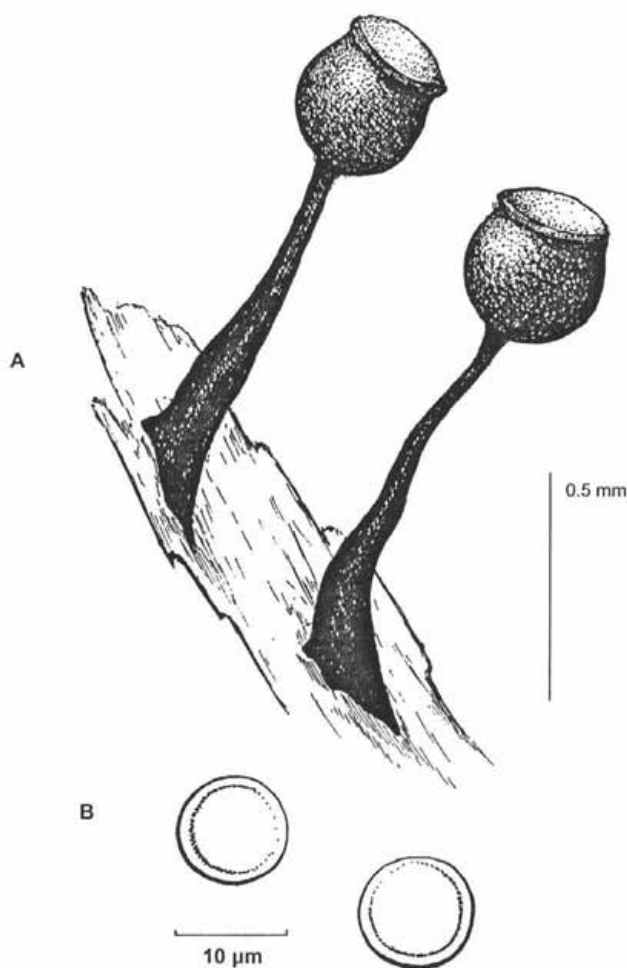


Fig. 9. *Licea operculata* (Wingate) G. W. Martin. A – sporangia, B – spores

bark of living tree of *Picea abies* (MCC), 22. IV.1997, leg. et det. RD (PRC). - Řevnice, valley of Kejná stream; bark of living tree of *Betula pendula* (MCC), leg. et det. RD (PRC). - Řevnice, valley of Kejná stream; bark of living tree of *Picea abies* (MCC), leg. et det. RD (PRC).

Sporangia sessile, small, 0.2-0.6 mm in diam., almost black, somewhat angular or spherical. Peridium thick, double-layered, splits along a network of prominent shining ridges; the outer layer covered with dark inclusions, the inner layer thin,



shiny, brown, translucent in transmitted light. Spores rust-red to brown in mass, pale ferruginous to smoke-brown in transmitted light, 9.6-13.2  $\mu\text{m}$  in diameter, minutely warted, the paler germination area sometimes visible.

Only one specimen collected in the field on decayed log of *Quercus* or *Carpinus*, together with *Arcyria pomiformis*. In moist chamber culture developing on bark of *Betula*, *Picea* and *Pinus* of samples from the Hřebený Mts. only. Occurring often together with *Echinostelium minutum*, *Paradiacheopsis fimbriata* and *Enerthenema papillatum*. Sometimes obtained after drying and rewetting, incubation time varying in 17-60 days.

Widely distributed in temperate and tropical regions throughout the world.

***Licea operculata*** (Wingate) G. W. Martin, Mycologia 34: 702. 1942.

Fig. 9, A-B

Specimens examined: Bohemian Karst, Karlštejn Nature Reserve, Mt. Boubová; bark of living tree of *Juniperus communis* (MCC), 18. XI.1998, leg. et det. RD (PRC).

Sporangia stalked, dark brown, urn-shaped with a lid, 0.5-1.2 mm high, 0.3 mm in diam. Lid flat, paler, with a golden shine. Stalk 2-3 times longer than the sporangium, black, cylindrical, wider towards the base and filled with granular inclusions. Spores beige in mass, almost colourless in transmitted light, 9.6-10.8  $\mu\text{m}$  in diam., spore wall smooth, paler on the large germination pore.

According to Nannenga-Bremekamp (1991) the sporangia of this species are 0.4-1.0 mm in diam. and the spore size is 10-13  $\mu\text{m}$ . Martin and Alexopoulos (1969), however, mention *L. operculata* with spore size 8-11  $\mu\text{m}$ , which fits to this material better.

Developed in moist chamber culture on bark of *Juniperus communis*, incubation time 20 days. The samples yielded only 8 fully mature sporangia. Developing together with *Enerthenema papillatum*.

Widely distributed in Europe, North America, West India and Japan, known from bark of various tree species. Not very common.

***Licea parasitica*** (Zukal) G. W. Martin, Mycologia 34: 702. 1942.

Fig. 10, A-C

Specimens examined: Bohemian Karst: Radotínské údolí Nature Reserve, Homolka; bark of living tree of *Sambucus nigra* (MCC), 4. III.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, alley near Hostim in the direction of Bubovice; bark of living tree of *Acer pseudoplatanus* (MCC), 16. VI.1998, leg. et det. RD (PRC). – Karlštejn Nature Reserve, Mt. Boubová; bark of living tree of *Acer campestre* (MCC), 16. VI.1998, leg. et det. RD (PRC). – Radotínské údolí

Nature Reserve; bark of living tree of *Aesculus hippocastanum* (MCC), 9. I.1999, leg. et det. RD (PRC). – Hřebený Mts.: Řevnice, valley of Kejná stream; bark of living tree of *Acer pseudoplatanus* (MCC), 30. III.1998, leg. et det. RD (PRC).

Sporangia sessile, black-brown, spherical when immature, later pulvinate, 0.02-0.5 mm in diam. Peridium thick, double-layered; granulose outer layer with dark deposits, the inner layer membranous and translucent. More or less distinct lid paler and sideways when immature. Spores dark brown in mass, pale olive-brown in transmitted light, often showing a few drops of pink inclusion, 12-13  $\mu\text{m}$  in diameter; the wall is smooth with a thinner, paler germination area.

Fructifications developed in moist chamber cultures on bark of *Aesculus*, *Acer* and *Sambucus*, together with *Arcyria cinerea*, *A. incarnata*, *Echinostelium minutum*, *Liccia kleistobolus* and *Physarum nutans*. Incubation time 12-31 days.

In the Czech Republic also collected by Kocourková as a lichenicolous species (Kocourková 2000). Common in moist-chamber cultures, reported from Germany, Austria, Corsica, Luxembourg, USA and Japan.

***Paradiacheopsis solitaria*** (Nann.-Brem.) Nann.-Brem., Proc. K. Ned. Akad. Wet. Ser. C. 70:209. 1967. Fig. 11

Specimens examined: Bohemian Karst: Karlštejn Nature Reserve, Svatý Jan pod Skalou; bark of living tree of *Acer pseudoplatanus* (MCC), 3. III.1998, leg. et det. RD (PRC). – Radotínské údolí Nature Reserve; bark of living tree of *Acer campestre* (MCC), 2. I.1999, leg. et det. RD (PRC). – Hřebený Mts.: Všenory, railway station; bark of living tree of *Populus nigra* (MCC), 15. XI.1996, leg. et det. RD (PRC).

Sporangia stalked, 0.5-0.8 mm high, spherical, dark brown, solitary or in small groups. Stalk 1-2 times longer than the sporangium, black, widened and fibrous at the base. Peridium fugacious. Columella at the centre of the sporangium divided into 2-3 main capillitial branches. Capillitium dark brown, rigid, a few times dichotomously branched; does not form a net. Free ends are not swollen or club-shaped at the tips. Spores smoke brown in transmitted light, 12-14.5  $\mu\text{m}$  in diameter, covered with fine warts which are less dense and more distinct than those of *Paradiacheopsis fimbriata*.

Material from Radotínské valley is somewhat different – the stalk is more reddish-brown, longer (2.5-3 times longer than the sporangium) and less fibrous at the base. The spores are smaller (9.6-12  $\mu\text{m}$  in diameter), but show the same colour and ornamentation. Perhaps this could be the small-spored variety with the relatively longer stalk mentioned by Nannenga-Bremekamp (1991, p. 365).

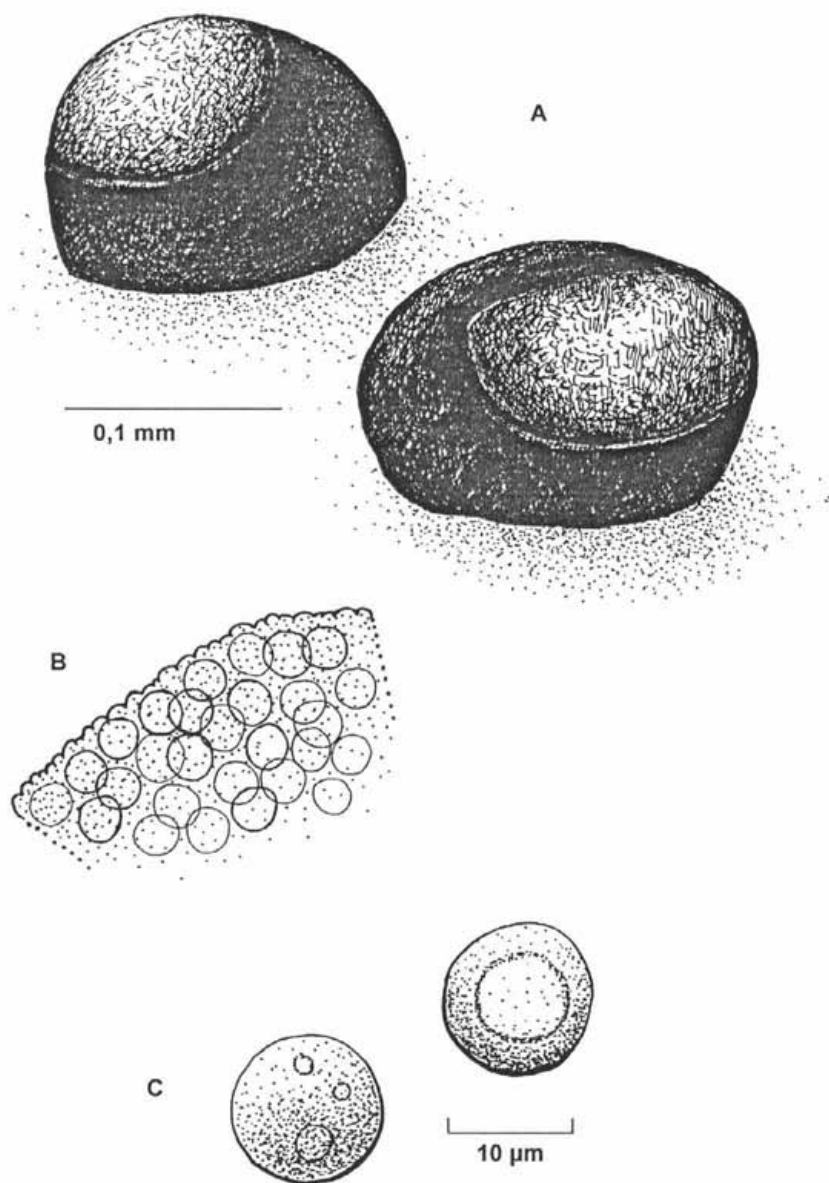
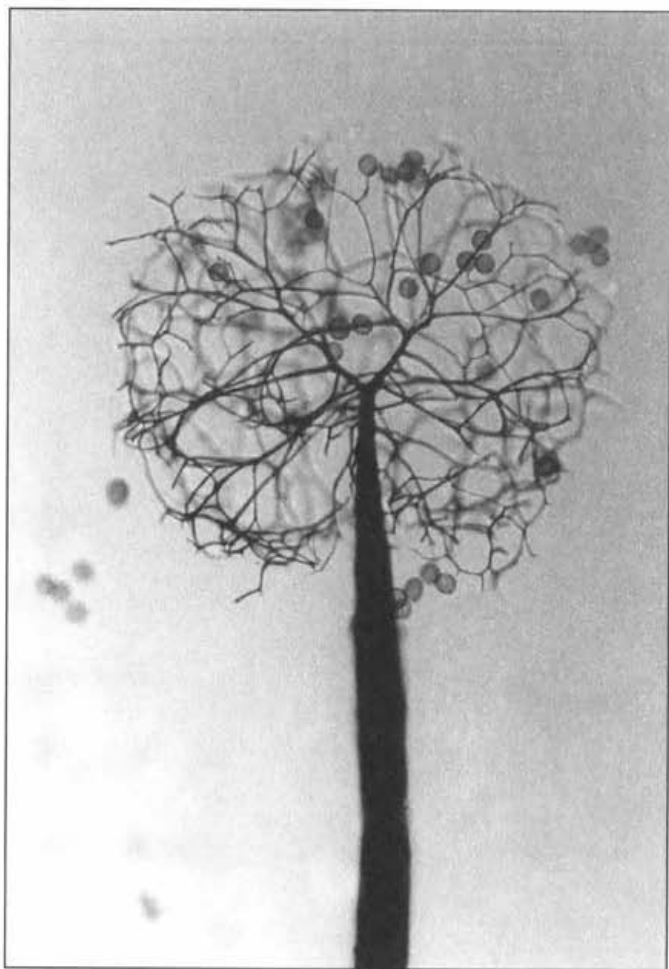


Fig. 10. *Licca parasitica* (Zukal) G. W. Martin



**Fig. 11.** *Paradiacheopsis solitaria* (Nann.-Bremek.) Nann.-Bremek., as seen under the microscope with Nomarski differential interference contrast. (Scale bar = 100  $\mu$ m)

Occurring in moist chamber cultures from Bohemian Karst only, incubation time 3-9 days, together with *Arcyria pomiformis*, *A. incarnata*, *Perichaena corticalis*, *P. chrysosperma* and *P. depressa*. The very small sporangia of *Paradiacheopsis solitaria* have so far been obtained only in moist chambers on bark of living deciduous trees. Described from the Netherlands in 1962.

**Physarum decipiens** M. A. Curtis, Am. Jour. Sci. II, 6: 352; 1848

Specimens examined: Bohemian Karst, Karlštejn Nature Reserve, Srbsko; bark of old living tree of *Crataegus* sp. (MCC), 16. VI.1998, leg. et det. RD (PRC).

Sessile sporangia and/or short plasmodiocarps, 0.2-0.8 mm in diameter and up to 2 mm long, on a colourless hypothallus. Peridium single-layered, light yellow, encrusted with yellow to orange-yellow lime scales (sometimes forming short veins). Columella absent. Capillitium consisting of a net with large flattened lime nodes, connected by colourless tubules; the lime nodes angular and irregularly shaped, filled with bright yellow lime inclusions. Spores black in mass, dark purple brown in transmitted light, 10-14 (15)  $\mu\text{m}$  in diameter, spore wall densely warted with a paler germination pore.

2 plasmodiocarps with one sessile sporangium developed in moist chamber culture together with *Arcyria cinerea* and *Perichaena corticalis*, incubation time 1 month.

Reported from Greece, Germany, the Netherlands and other countries of western Europe, USA, Canada, South America, Asia and Australia; not very common.

**Stemonitopsis subcaespitosa** (Peck) Nann.-Brem., De Nederlandse Myxomyceten, 1974

Specimens examined: Bohemian Karst, Radotínské údolí Nature Reserve; bark of living tree of *Malus domestica* (MCC), 10. III.1998, leg. et det. RD (PRC).

Sporangia stalked, cylindrical, c.2 mm tall, dark brown. Stalk black, shiny, 1/3-1/4 of the total height, fibrous at the base. Peridium fugacious. Columella reaching almost the apex of the sporangium and there merging into the capillitium. Capillitium lilac-brown, the inner net with large expansions, irregular surface net showing only fragments. Spores dark lilac-brown in transmitted light, covered with fine warts or spinules, 8.4-9.6  $\mu\text{m}$  in diameter. (According to Martin and Alexopoulos (1969) the spore size is (7)8-9(10)  $\mu\text{m}$ ).

Developed in moist chamber culture on bark of old *Malus domestica* with no other species, incubation time 29 days.

**Trichia munda** (Lister) Meylan, Bull. Soc. Vaud. Sci. Nat. 56: 327. 1925

Fig. 12, A-C

Specimens examined: Bohemian Karst, Radotínské údolí Nature Reserve; bark of living tree of *Tilia cordata* (MCC), 20. III.1997, leg. et det. RD (PRC). – Koda Nature Reserve, bark of living tree of *Crataegus* sp. (MCC), 21. III.1997, leg. et det. RD (PRC). – Hřebeny Mts.: Dobřichovice under Mt.

Hvízdinec; dead leaves of *Aesculus hippocastanum* (MCC), 21. III.1997, leg. et det. RD (PRC).

Sporangia stalked, small (0.8-1.2 mm high), almost globose, brown with pale yellow bands forming a large-meshed net. Stalk dark brown, thick, 2/3 of the total height, on a small dark brown hypothallus. Peridium consisting of two adherent layers; the outer layer thickened and encrusted with dark granulose material (except for the pale bands with the spore mass visible through), the inner layer thin and translucent; dehiscing along the bands. Elaters not branched, relatively short (140-200  $\mu\text{m}$ ), ornamented with three smooth spirals, tapering into 30-40  $\mu\text{m}$  long points with blunt apex. Spores and capillitium olive-yellow in mass, spores minutely warted, 9.6-12  $\mu\text{m}$  in diam.

Cultivated in moist chamber culture on bark of *Tilia* and *Crataegus* and on dead leaves of *Aesculus hippocastanum*, together with *Arcyria cinerea*, *A. pomiformis*, *Calomyxa metallica*, *Enerthenema papillatum* and *Physarum nutans*. Sporangia developing solitary, in small numbers (up to 10 sporangia per one chamber), incubation time 24-31 days. The specimen from dead leaves developed after drying and rewetting of the substrate (total time 4 months).

Usually obtained in moist chamber cultures only, recorded from Germany, the Netherlands, Switzerland, Portugal, Iceland and the USA.

#### DISCUSSION

Species diversity. – From the whole Czech Republic, 204 Myxomycete species have been reported (Dvořáková 1999b). The 95 species found in the area studied (about 40 sq km) indicate a rich offer of substrates for Myxomycetes present here as well as low research intensity in the rest of the country. Also the average number of species per genus (3.27) shows a relatively high species diversity. The degree of species classified as rare (collected 1-2 times) is relatively high – 40%, which corresponds with similar studies (Schnittler et Novozhilov 1996, Schnittler et Stephenson 2000) and shows the importance of long-term studies in Myxomycetes research.

Comparing the spectrum of Myxomycetes in the localities studied (as shown in Table 1), only 55 species (58 %) were found in both localities. There is a higher number of species recorded in the Bohemian Karst (84 species) than in the Hřebený Mts. (68 species). This is mainly due to a different and richer vegetation composition in Nature Reserves caused by the limestone base in the Bohemian Karst.

There were 12 species of *Physarales* collected in Bohemian Karst only (e.g. *Badhamia dubia*, *Diderma cingulatum*, *D. crustaceum*, *D. spumarioides*, *Fuligo cinerea*, *F. rufa*, *Mucilago crustacea*, *Physarum decipiens*, *P. leucophaeum*, *P. leucopus*, see Table 1.). Another 5 species belonging to *Physarales* were collected

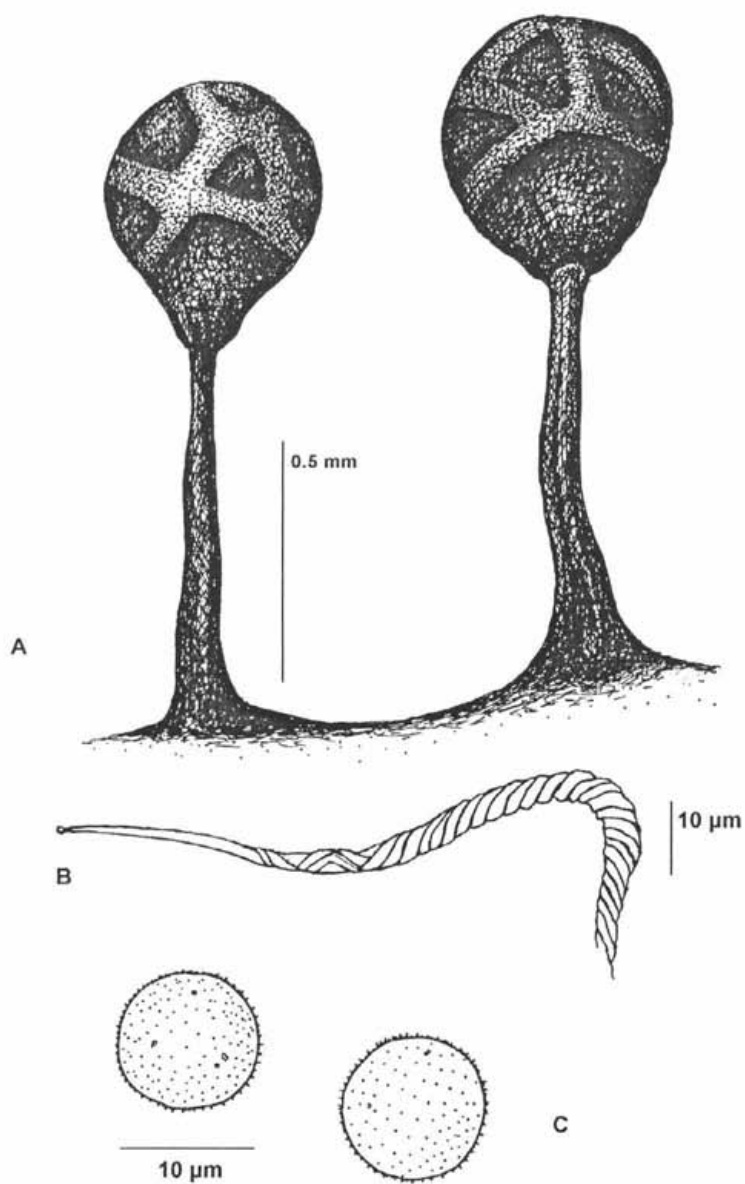


Fig. 12. *Trichia munda* (Lister) Meylan. A - sporangia, B - part of capillitium, C - spores



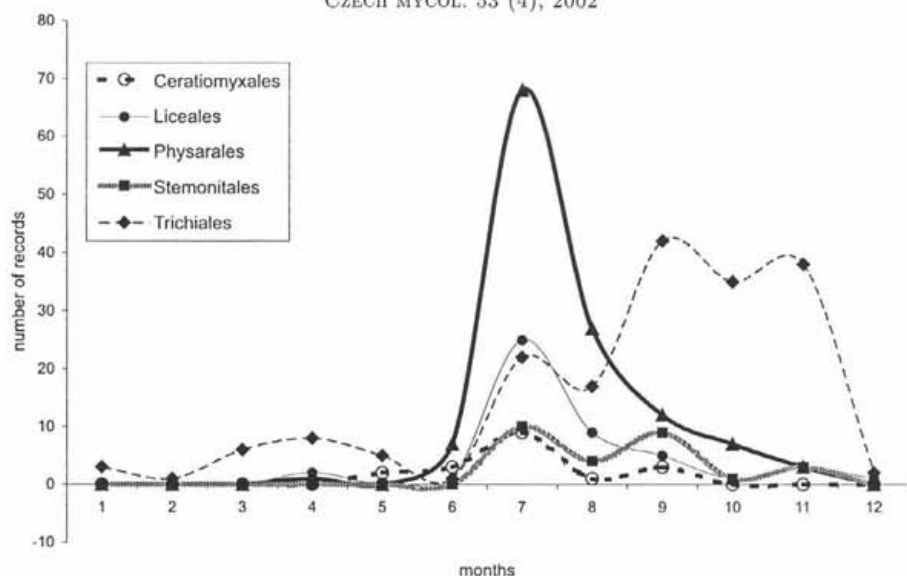


Fig. 13. Seasonal dynamics of orders of Myxomycetes in localities studied (based on data from Bohemian Karst and Hřebený Mts. from 1935–1999)

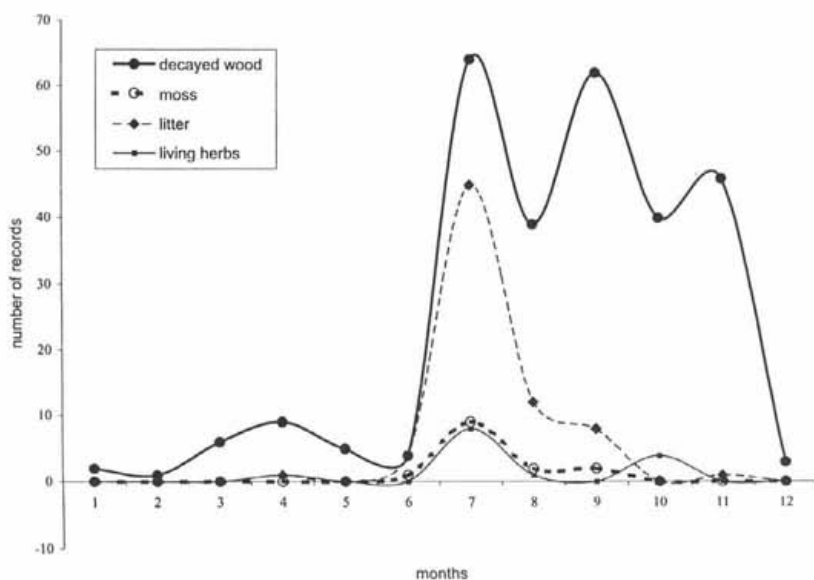


Fig. 14. Seasonal appearance of Myxomycetes on microhabitats (based on data from Bohemian Karst and Hřebený Mts. from 1935–1999)

only in the Hřebený Mts. (*Didymium nigripes*, *D. serpula*, *Physarum bivalve*, *P. cinereum* and *P. virescens* – mostly small folicolous species), indicating there was a conspicuous difference in both quality and quantity of Physarales present in the localities studied. The question whether this difference is directly due to the lime presented in Bohemian Karst or to other factors will remain an object of further long-term research and discussion.

Seasonal dynamics. – After putting together all data available for seasonal analysis (field collections only), a simple graph on seasonal dynamics came out (Fig. 13). It is only of informative value as the amount of fieldwork differed per every month of the year. It naturally reflects the Central-European climate with usually two main rain seasons suitable for Myxomycetes in July and September–November. As seen from the graph and well-known from field experience, the occurrence of the Myxomycete orders of *Physarales* and *Liceales* is more or less concentrated to one relatively short period of time during the summer season (the highest peak in July). Species of *Trichiales* (mostly xylophilous) are typical of the autumn aspect and otherwise fructified in a small quantity during the whole year. A similar situation is seen in Fig. 14, which reflects the preference of microhabitats in Myxomycete orders. The maximum of litter-inhabiting species in July is determined by the order *Physarales*.

Moist chamber cultures. – 250 chambers, prepared with samples of bark and litter, yielded 33 species representing 19 genera of Myxomycetes. Only 73 % of the moist chambers yielded Myxomycete fructifications. Species of the genus *Diderma* and *Didymium* fructified only after the cultivation was repeated. Among the most favourable substrates were *Acer* spp., *Aesculus hippocastanum*, *Betula pendula*, *Crataegus* sp. and *Quercus* spp. (see Table 2.). A quite specific Myxomycete flora occurred on *Juniperus communis*, *Malus domestica* and *Sambucus nigra*.

Spare material from moist chamber cultures (2–3 fructifications per moist chamber) was successfully multiplied by repeated cultivation several times. For example, a moist chamber with 2 sporangia of *Physarum viride* var. *aurantiacum* left on the substrate was rewetted and cultivated again. Finally 3 large colonies of this species were harvested. The same occurred with the species *Arcyria cinerea* and *Paradiacheopsis fimbriata*. If successful with other species of Myxomycetes as well, this could be a possible way of solving problems with lack of material from moist chambers.

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**Table 2.** List of substrates cultivated in moist chamber cultures with numbers of Myxomycete species and specimens developed (both on bark and litter).

substrate	moist chambers	species	specimens
<i>Acer campestre</i>	11	10	21
<i>Acer platanoides</i>	18	9	28
<i>Acer pseudoplatanus</i>	13	12	23
<i>Aesculus hippocastanum</i>	11	11	25
<i>Alnus glutinosa</i>	8	8	27
<i>Betula pendula</i>	11	10	26
<i>Crataegus</i> sp.	6	10	17
<i>Fagus sylvatica</i>	22	9	24
<i>Juniperus communis</i>	3	5	6
<i>Larix decidua</i>	13	3	17
<i>Malus domestica</i>	6	6	23
<i>Picea abies</i>	14	5	24
<i>Pinus nigra</i>	5	2	4
<i>Pinus sylvestris</i>	18	6	31
<i>Populus alba</i>	1	5	5
<i>Populus nigra</i>	4	5	7
<i>Pseudotsuga menziesii</i>	2	3	4
<i>Pyrus communis</i>	6	5	15
<i>Quercus petraea</i>	16	8	38
<i>Quercus robur</i>	15	10	39
<i>Robinia pseudacacia</i>	7	6	12
<i>Salix fragilis</i>	5	7	19
<i>Sambucus nigra</i>	13	7	23
<i>Sorbus aucuparia</i>	4	4	8
<i>Sorbus torminalis</i>	3	5	7
<i>Tilia cordata</i>	4	6	10

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In memoriam Prof. Zdeněk Černohorský (1910–2001)

JIRÍ LIŠKA

Zdeněk Černohorský, Professor Emeritus at Charles University, Prague, died in Prague on September 5, 2001, eight months after his 90<sup>th</sup> birthday. Zdeněk Černohorský's life was always associated with botany, and his activities in various branches of the discipline as well as his important personal image influenced the life of our botanical society.

Zdeněk Černohorský was born on December 27, 1910 in Chroustovice near Chrudim (East Bohemia). He graduated from Charles University, Prague, in 1933 with a specialization in lichenology, especially lichen sociology. However, during the next decade he studied also anatomy and morphology of seed plants. After university studies, he was a teacher at various schools (primary school in Chroustovice and later on secondary schools in Český Krumlov, Mělník and Prague). He started his professional career after World War II at the University of Agriculture and Forestry, from where he moved to the Paedagogical Faculty and after 1959 to the Faculty of Sciences, Charles University, after which he retired in 1977.

In lichenology, Professor Černohorský was known as co-author of a key to Czechoslovak macrolichens (Černohorský, Nádvorník and Servít 1956), as well as for introducing fluorescence analysis in lichen identification, and for a series of taxonomical and chorological studies in yellow *Rhizocarpon* species.

However, Zdeněk Černohorský devoted his interest, activities and work to all branches of botany, especially anatomy (a monograph on seeds of *Cruciferae*), morphology (a textbook with eight editions!) and education. Last but not least, he was active in organizing and managing science, namely in the Czechoslovak Botanical Society (he served as the Society Chairman for 12 years and Editor-in-Chief of *Preslia*, the journal of the Society, for 27 years) and in academic functions (Vice-Chancellor, Dean). He was elected an Honorary Member of the Czechoslovak Botanical Society, the Slovak Botanical Society and the Czech Scientific Society for Mycology.

Professor Černohorský was an excellent teacher. Education was another important field where he had impact on several generations of teachers and students. He loved to read lectures and to be in contact with students. His mind open to new trends and methods kept him young. He frequently published information on novelties in science in *Vesmír*, a Czech journal focused on popularization of science.

He loved discussions and regularly, every week, attended meetings of the Czech Botanical Society with lectures, as well as seminars for students at the university. He always knew how to ask a reasonable question, be it on any topic.

He always encouraged talented young people and followed them with fatherly love. His language and rhetorical abilities as well as conviviality facilitated good relationships with many colleagues abroad. Unfortunately, he spent most of his life in a period when travelling abroad was very difficult; so he was unable to establish as many contacts as he might have liked. Nevertheless, he was able to make use of all of the few journeys he made abroad both for educational and personal contacts. He had close links with many old friends in other countries (e.g. Á. Löve, J. Poelt and G. Clauzade).

Zdeněk Černohorský was a person of high personal integrity. His life was not easy, but he lived it both with honour and humility. He will be greatly missed by all generations of Czech and Slovak botanists. "Never dies who is still living in our minds."

Bibliographies of Z. Černohorský have been published in *Preslia* 42: 386–389 (1970), *Preslia* 52: 358–360 (1980) and in Buchberger J., ed., *Devadesáté narozeniny prof. RNDr. Zdeňka Černohorského*, TIS – nezávislé sdružení přátel přírody, Praha, 2001.

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