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Hyphomycetes from Canadian streams. VI. Rare species in pure cultures

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We describe and illustrate a series of rare or inadequately known species of mitosporic fungi (hyphomycetes), which were isolated from foam from Canadian streams: *Alatospora constricta*, *Arborispora dolichovirga*, *Calcarispora hiemalis*, *Cladoconidium articulatum*, *Lateriramulosa quadriradiata*, *Sympodiocladium frondosum*, *Tetrabrunneospora ellisii*, *Tricladium caudatum*, *Trifurcospora irregularis*, *Triglyphium alabamense*, *Varicosporium trimosum*. *Ypsilina graminea* is reported with microconidial state.

Key words: aquatic hyphomycetes, species descriptions, distribution in Canada.

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V článku popisujeme a zobrazujeme některé vzácné nebo málo známé druhy hyfomycetů, které byly izolovány z pěny na kanadských potocích a řekách: *Alatospora constricta*, *Arborispora dolichovirga*, *Calcarispora hiemalis*, *Cladoconidium articulatum*, *Lateriramulosa quadriradiata*, *Sympodiocladium frondosum*, *Tetrabrunneospora ellisii*, *Tricladium caudatum*, *Trifurcospora irregularis*, *Triglyphium alabamense*, *Varicosporium trimosum*. Pro druh *Ypsilina graminea* bylo zjištěno mikrokonidiové stádium.

INTRODUCTION

Freshwater leaf-inhabiting hyphomycetes occupy an essential trophic level between deciduous leaves and detritus-feeding invertebrates (shredders) in running waters (Bärlocher 1992). A recent estimate puts their annual production at a level comparable to that of bacteria and macroinvertebrates, two other major heterotrophic groups in streams (Suberkropp 1997). These fungi have also become popular objects to study fungal successions (Dix and Webster 1995)

or potential connections between biodiversity and ecological functions (Raviraja et al. 1998). Both objectives require accurate identification, based primarily on conidial morphology and development.

There are several freshwater hyphomycete species which have been isolated only very rarely or not at all since their description. Often the protologue is based either on a single isolate or on a few isolates from geographically close areas. This lowers the probability of appreciating the full variability within and potential overlaps between species. Reproductive structures of aquatic hyphomycetes are difficult to preserve in a good shape. Permanent preparations may dry out and attempts to restore them are not always successful. Moving the coverslip may distort the long arms of some teraradiate conidia, or even entire scolecoform conidia, and cause them to become entangled. Pure cultures often lose the ability to sporulate after a few transfers. It is therefore advisable to document new isolations of rare species with good drawings or photomicrographs, which may allow others to recognize the species even when misidentified by the author. Good illustrations may also to some extent serve as substitutes for voucher specimens if deposition of material is for some reason impossible.

The sporadic occurrence of some species at very distant localities raises the question of whether they have in fact a disjunct distribution or whether their rarity is simply due to a lack of sustained effort to find them. Some of them have distinctly shaped conidia (*Lateriramulosa quadriradiata*) or quite large (*Tetrabrunneospora ellisii*, *Varicosporium trimosum*). It seems unlikely that these would escape attention. We therefore have to assume, that they or their hypothetical teleomorphs may have special ecological requirements, which are rarely met, either on a temporal or spatial scale.

MATERIAL AND METHODS

Foam samples were collected into glass jars. Inoculations were performed in the field or the same day after return to the laboratory. Foam was smeared on thin layers of 0.1 % malt agar (MA, Difco), on object slides fixed to the bottom of Petri dishes. After inoculation they were kept in a cool box with ice cubes, or, on return to the laboratory in the fridge at c. 10 °C. Isolations were done within 24–48 hours. The agar layers were scanned under a low power compound microscope. Germinating conidia were cut out with a flamed needle together with a piece of agar and transferred to Petri dishes with 2 % MA. a disadvantage of this method is the impossibility of checking the density of conidial suspension in the field. If it is too high, conidia will be crowded, which makes their isolation uneasy or even impossible. On the other hand, it prevents losses of conidia during transport by sedimentation and adhesion to the walls of the sampling containers. Descriptions of colonies are based on pure cultures on 2 % MA incubated at 15 °C,

if not otherwise stated. Sporulation was obtained by submerging pieces of agar cultures into standing sterile distilled water in Petri dishes, incubated at 15–18 °C in diffuse daylight.

Terms:

“Phialidic conidiogenous cells” is used in the sense of Hawksworth et al. (1995), i.e. such, which give rise to several conidia at the same level in a basipetal sequence. “Primoculture” is the one, which is obtained from a conidium collected in nature.

DESCRIPTIONS OF ISOLATES

Alatospora constricta Dyko, Trans. Br. Mycol. Soc. 70: 409, 1978.

(Figs. 1 and 15 D, E)

Colony on MA compact, tough, cream-coloured, greyish (the primoculture chestnut brown) in the centre, slow-growing, reaching 14 mm diam. within 20 days at 15 °C, elevated, glabrous but funiculose in the centre. Globose inflated cells abundant, with thicker walls, in chains or in clumps. Sporulation after submergence, in standing distilled water. Conidiophores terminal or lateral, simple or with a single branch, thin, flexuous. Conidiogenous cells phialidic, single, sometimes proliferating and forming a series of 3–4, rarely in terminal pairs or threes, or intercalary with lateral conidiogenous locus (Fig. 1 F) Conidia tetra- or polyradiate, with axis and two opposite synchronous laterals, axis 42–58 × 2–2.7 μm, branches 21.5–41 × 1.7–2.5 μm, one usually shorter. Elements rather cylindrical, straight or less often curved, with more or less subulate ends. Conidial secession schizolytic.

Specimens examined: Pure culture: CCM F-23394, Canada, Nova Scotia, Cape Breton National Park, right tributary of the Cheticamp River, ca 8 km downstream from the First Salmon Pool, isolated from foam collected by L. Marvanová on 9. 10. 1994. Foam samples: conidia seen in foam in Arsenic Brook near Williamstown, in the Cobequid Hills, Nova Scotia, and in the Catamaran Brook near its confluence with the Southwest Branch of the Miramichi River, Miramichi Region, New Brunswick.

A chestnut brown colouration of the colony on Malt Yeast Soytone Agar was reported in the protologue (Dyko 1978). Our isolate showed such colour only in the primocultures on 2 % MA. Dark colour was observed also in primocultures of *A. flagellata* (J. Gönczöl) Marvanová (1977). The Canadian isolate differs in some details from the Dyko's culture (ATCC 32680), namely by having simpler, tiny conidiophores often with proliferating phialides and a single terminal phialide. However, the characteristic intercalary phialides with lateral necks seen in ATCC 32680 (cf. Marvanová and Descals 1985, Fig. 4A) were confirmed in our material.

In spite of c. 15 reports published from various countries this species has not been reisolated in pure culture since the original collections from the Appalachian Mountain streams, where it was reported as common in winter and spring months (Dyko 1978). Owing to the similarity of its conidia to those of *Alatospora acuminata* Ingold sensu lato (Marvanová and Descals 1985) and of *Stenocладиella neglecta* (Marvanová et Descals) Marvanová et Descals 1987, reported identifications might not always be correct.

Arborispora dolichovirga K. Ando, Trans. Mycol. Soc. Japan 27: 125, 1986.
(Fig. 2)

Colony compact, pale beige, very slow-growing, reaching 5 mm diam. after 10 weeks at 15 °C, granular, brain-like convoluted, aerial mycelium low, reverse pale brown. Conidiophores absent. Conidiogenous cells mostly intercalary with a short lateral stalk bearing conidia; proliferation not seen. Conidial initiation integrated with the conidiogenous cell. Conidia solitary, terminal, branched, more or less fan-shaped, conidial elements long fusoid, distal ends rounded to subulate. Axis straight, widest in the lower third, 36–51 × 2–3 µm, base truncate. Branches 2–8 (typically 4–5), lateral, sequential, primary and secondary, 9–38 × 2–3 µm, bases obconic, sometimes slightly pedicellate. Primary branches in 1–2(-3) verticils, the lower on the first or second proximal cell of the axis, the upper mostly on the neighbour distal cell. Secondary branches 0–2, more frequent in the lower verticil, typically inserting very near the base of the primary ones ("pedate"), but also remote (Fig. 2 E). Conidial secession schizolytic.

Pure culture examined: CCM F-40394, Canada, New Brunswick, Miramichi Region, near Renous, Catamaran Brook, isolated from foam collected on 4. 11. 1994 by L. Marvanová and H. Garnett

For more than ten years the first author tried to isolate this fungus from foam. Unfortunately, the conidia usually do not germinate or if they do, the growth is so slow that the colony is easily overgrown. The Canadian isolate is probably the first from outside Japan, where the type specimen originated as well as a recent isolate by Matsushima and Matsushima (1996), which, however, remained sterile on artificial media. On dead tree leaves the above authors observed up to 12 branches per conidium. Our isolate corresponds closely to the protologue, but there is a greater variability in the branch number; conidia with tertiary branches are also encountered.

The earlier name for this species is very probably *Magdalaena monogramma* Arnaud (1952), which, however, was published invalidly (without Latin diagnosis). The protologue (diagnosis generico-specifica) consists of a brief description of the conidia only, which, according to Arnaud, were the only material at his disposal. None of the two depicted conidia show the secondary branches inserting near

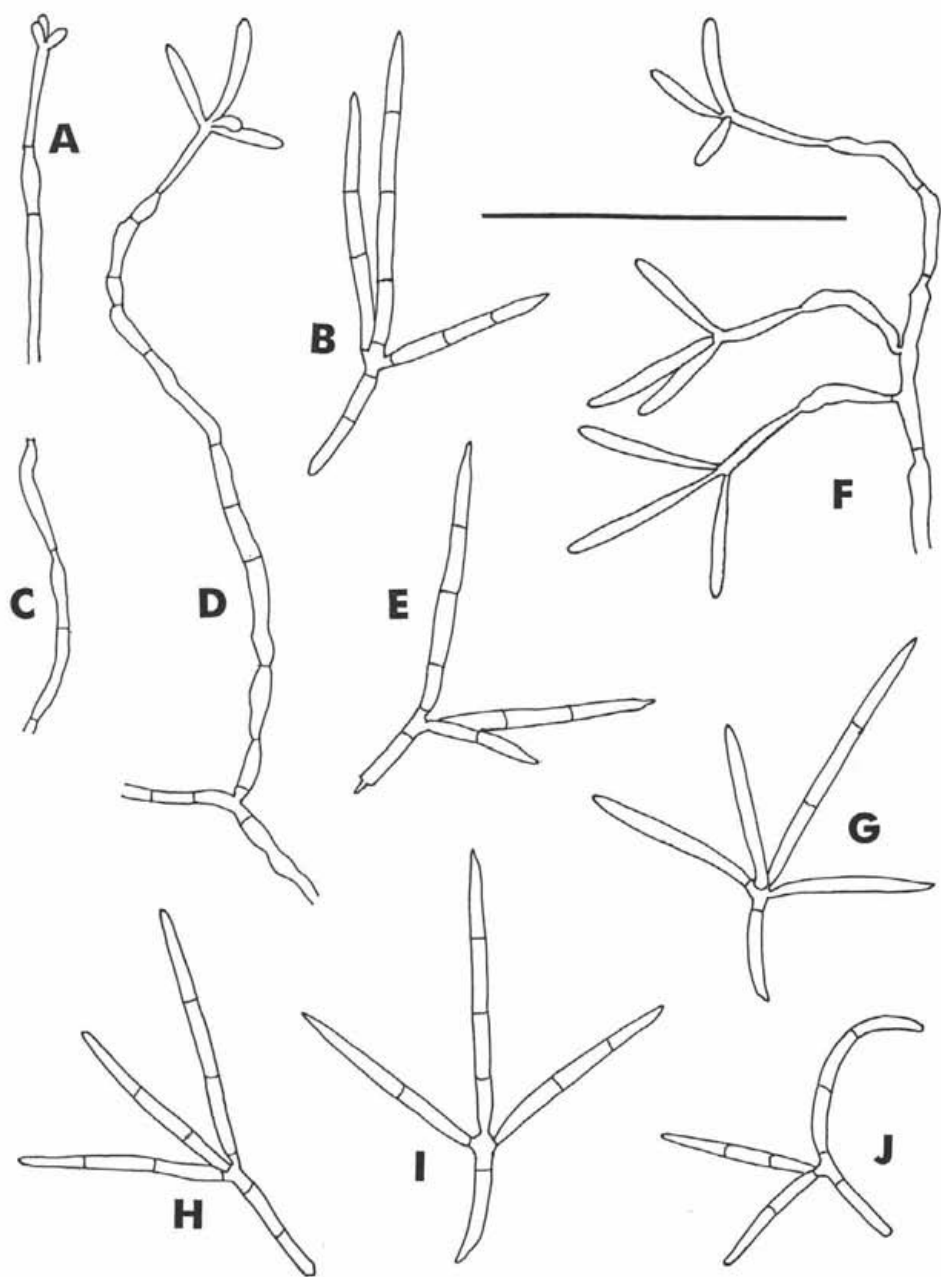


Fig. 1. *Alatospora constricta*, CCM F-23394. A,D,F, conidial development. Note lateral phialides in F. C, spent phialide with indistinct collarette. B,E,G-J, detached conidia. Scale bar = 50 μ m.

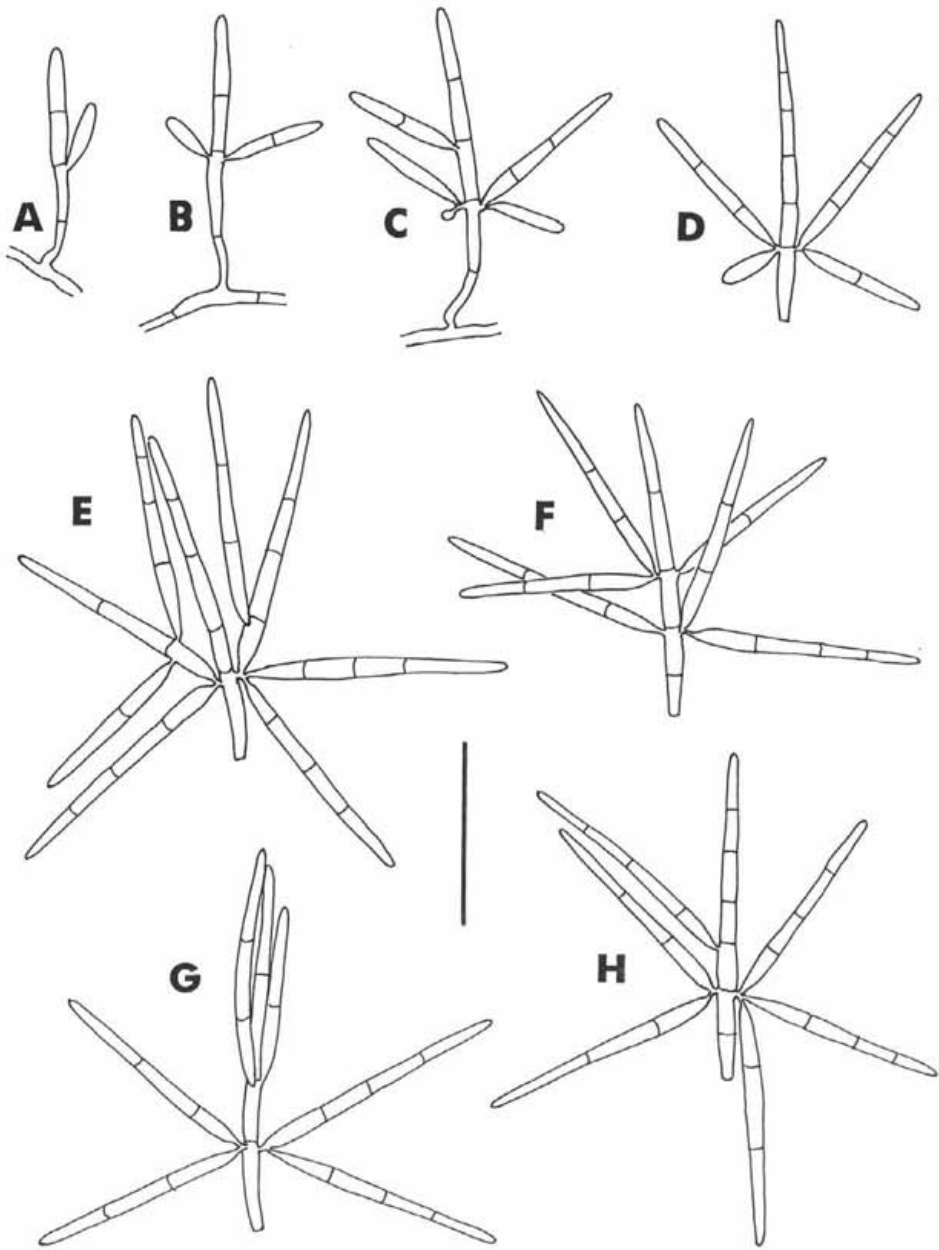


Fig. 2. *Arborispora dolichovirga*, CCM F-40394. A-C, conidial development. E-H, detached conidia. Scale bar = 25 μ m.

the base of the primary ones, which was claimed an important generic feature in *Arborispora*; on the contrary, secondary branches are shown as arising at a distance from the base of the primary. However, among the morphological variation in conidial shapes drawn by Ando (Fig. 3, the right bottom conidium) such branch arrangement is illustrated. Because the genus *Arborispora* is a well established taxon documented by type material and other specimens in contrast to the poor information given by Arnaud, we prefer not to validate Arnaud's name.

Ecologically *A. dolichovirga* is probably more close to aquatic hyphomycetes than the other *Arborispora* species; it sporulates profusely under water and its conidia are relatively often being collected in foam on streams. Ando and Kawamoto (1986) collected *A. dolichovirga* on leaf litter. Conidia from plant debris in water or from foam are mostly reported under the name *Magdalaenaea monogramma* (e.g. Gönczöl and Révay 1983, Marvanová 1984, Bärlocher 1987).

Calcarispora hiemalis Marvanová et Marvan, Acta Mus. Silesiae (Opava), Ser. A, 12: 109, 1963. (Figs. 3, 4)

Colonies pale beige to drab, slow-growing, reaching 8–15 mm diam. after 20 days at 15 °C, with low aerial mycelium, coarsely funiculose in the centre. Reverse isabelline, paling towards margin. Numerous globose thicker-walled hyaline inflated cells appear in chains or clusters in older cultures. Conidiophores single, simple or sparsely branched, flexuous, terminal or lateral. Conidiogenous cells discrete, acrogenous, 1–3 per conidiophore apex, mostly widening distally, often irregularly inflated, 11–21 × 2–5 µm, proliferation not seen. Conidial initiation discrete ("budding"). Conidia single, terminal or subterminal, sigmoid or arcuate, (72) 96–219 × 2.5–3.5 µm (basal extension included), parallelwalled, multiseptate, apex subulate, base truncate, basal extension excentric, (7-)18–51 µm long, appearing before secession. Conidial secession schizolytic. Microcycle conidiation with sessile conidiogenous cells producing secondary conidia seen in vigorously sporulating culture in standing distilled water.

Pure cultures examined: CCM F- 23594 Canada, Nova Scotia, Cape Breton National Park, Right tributary of the Cheticamp River, near the First Salmon Pool, isolated from foam collected on 9. 10. 1994 by L. Marvanová CCM F-35994, CCM F-36394, CCM F-40994, Canada, New Brunswick, Miramichi Region, near Renous, Catamaran Brook, isolated from foam collected on 4. 11. 1994 by L. Marvanová and H. Garnett.

Beside two subsequent isolations from the region nearby the type locality in the Czech Republic (Marvanová 1972 and unpubl.) this species is unlikely to have been isolated elsewhere. There are repeated records by Dudka (1973, 1974) from the Ukrainian Carpathians from *Fagus* leaves and from foam. Other reports are

from submerged twigs from Pakistani mountain streams (Iqbal et al. 1979) and from a river in Poland (Czeczuga et al. 1989–1990)

The conidiogenesis was described as phialidic in the conventional sense, i.e. the conidium initiation is discrete and the conidiogenous cells produce more than one conidium at the same level and do not change the shape or size after prolonged conidiation. However, no periclinal thickening has been seen on the phialide apex. The shape of some conidiogenous cells of the Canadian isolates (Fig. 3 C) suggests the possibility that polyblastic or polyphialidic conidiogenesis may be involved. However, with the light microscope and with the absence of periclinal thickening it is impossible to distinguish between polyblastic and polyphialidic nature of the conidiogenous cell and only single conidia have been seen at any given time on one conidiogenous cell. Wolfe (1977) who has seen authentic material (permanent preparation in lactophenol) of *C. hiemalis* doubted the phialidic conidiogenesis as published in the protologue and considered the conidia "aleuriospores". This would imply integrated conidium initiation and production of only one conidium at all from the same conidiogenous locus. However, the initiation of a conidial primordium in *C. hiemalis* is clearly discrete.

The Canadian isolates differ from the Czech ones by the temperature optimum for sporulation, which was 5 °C for the Czech, but around 15 °C for the Canadian.

Cladoconidium articulatum Bandoni et Tubaki, Trans. Mycol. Soc. Japan 26: 426, 1985. (Fig. 5)

Colonies very-slow growing, dark olive, aerial mycelium sparse, thinwalled, substrate mycelium thickwalled, reverse black. Characteristic hyphopodia present in older cultures. Conidiogenous structures fuscous. Conidiophores absent or semimacronematous, sometimes bearing a sympodial conidiogenous cell with several denticles (Fig. 5 B). Conidia fan-shaped or cheiroid, axis branched up to 4 levels, branches up to fifth order, sometimes more abundant on one side of the axis, apices rounded or broadly subulate, bases sometimes slightly constricted. Conidial span 20–36 μm . Conidial secession schizolytic.

Pure culture examined: CCM F-33094, Canada, Nova Scotia, Colchester County, Cobequid Mountains, Black Brook, isolated from foam collected on 22. 10. 1994 by L. Marvanová.

In their branching pattern, the conidia resemble those of *Tetracladium palmatum* A. Roldán. However, the latter are hyaline and possess digitiform as well as filiform and narrowly obclavate elements.

Very little is known about the ecological characteristics of this species, described from *Scirpus microcarpus* Presl litter (Bandoni et Tubaki 1985). Other collections of the well recognizable conidia of this species are known from softwater streams

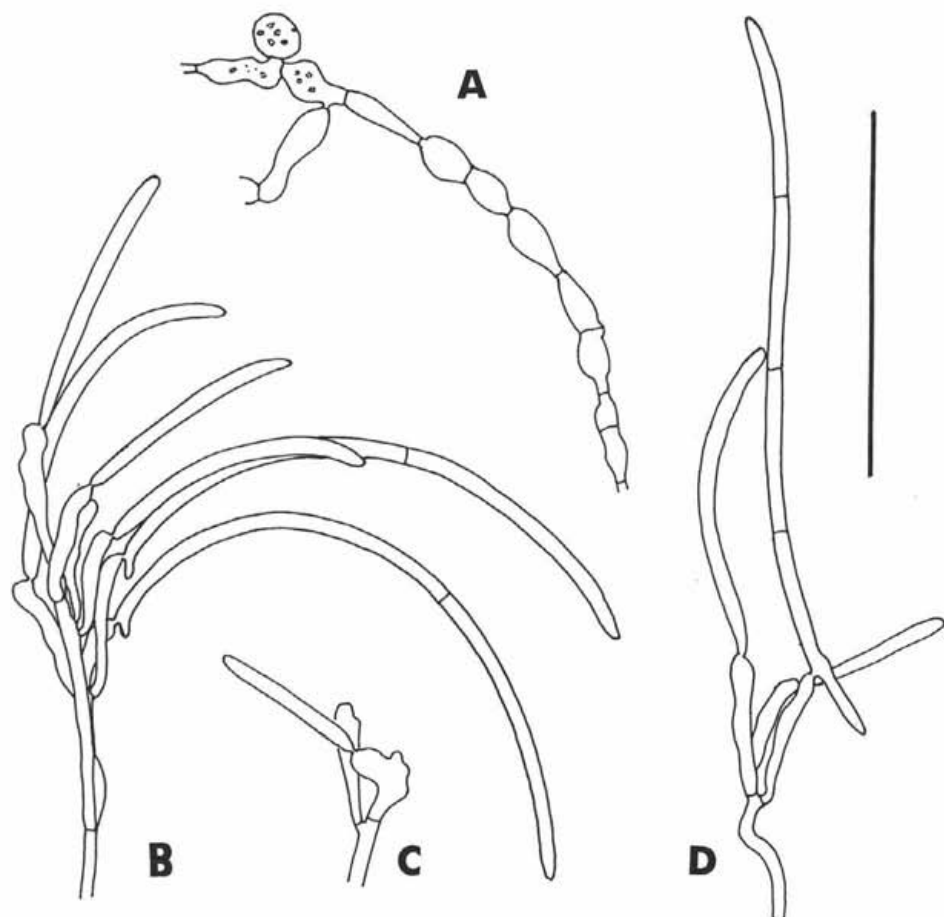


Fig. 3. *Calcarispora hiemalis*, CCM F-23594. A, chain of inflated cells. B-D, conidial development. Note the discrete initiation of the conidial primordium and formation of parabasal extension *in situ* in B and irregularly inflated conidiogenous cell in C. Scale bar = 50 μ m.

in Austria (Voglmayr 1996, Fig. 1 e, as ? *Tetracladium* sp.), and in the Czech Republic (Marvanová, unpublished).

Lateriramulosa quadriradiata K. Miura et Okano, J. Jap. Bot. 54: 209, 1979. (Figs. 6 and 14 E, F)

Colony slow-growing, chalky white, with cottony aerial mycelium, reverse pale. Sporulation underwater. Conidiophores typically terminal, single, simple. Conidio-

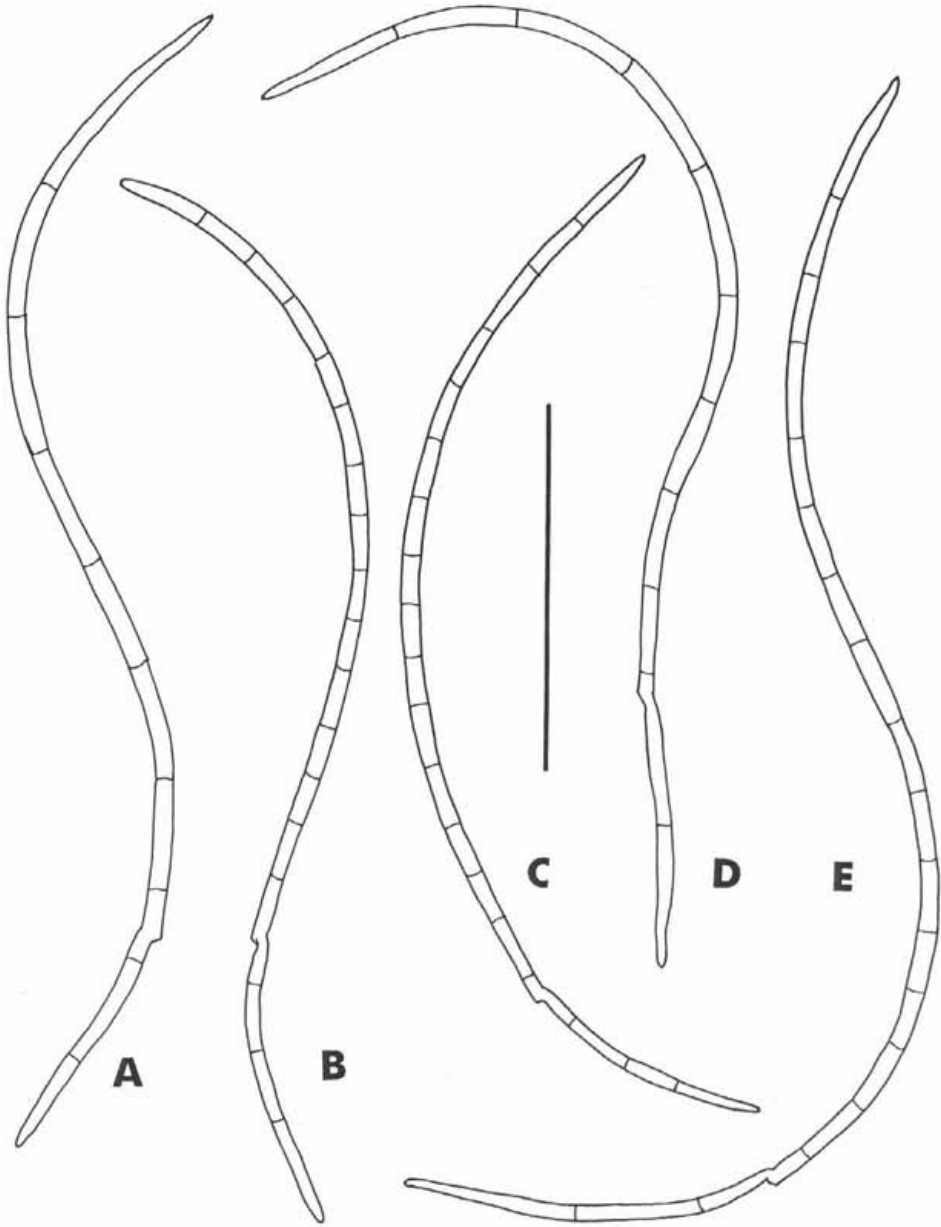


Fig. 4. *Calcarispora hiemalis*, A-E, detached conidia (A,D = CCM F-23594; B,C,E = CCM F-35994). Scale bar = 50 μ m.

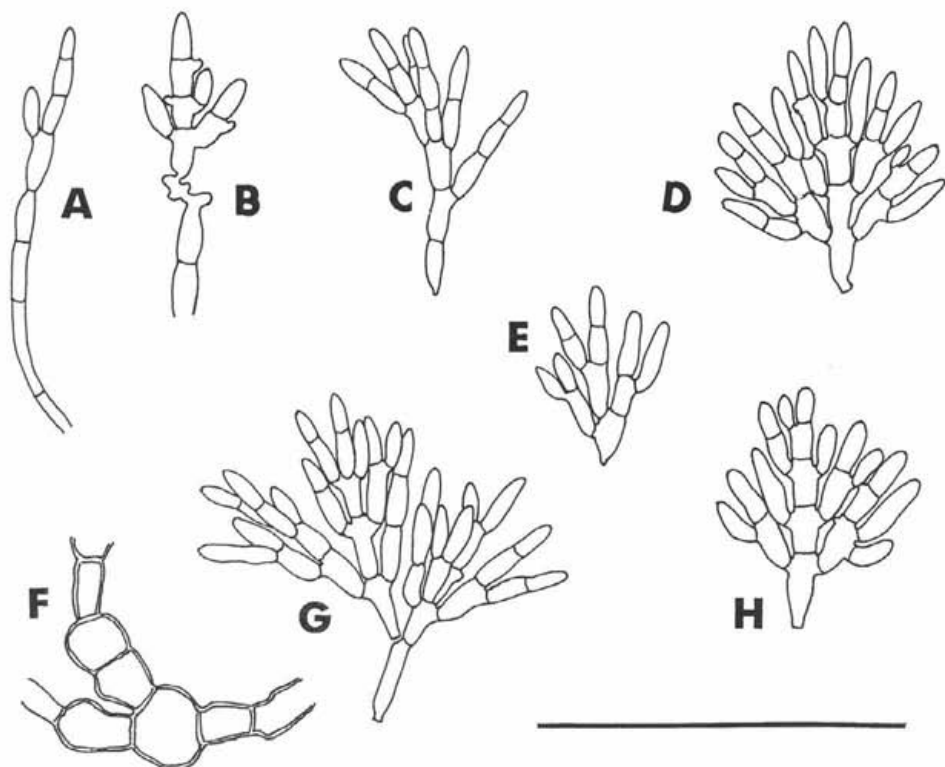


Fig. 5. *Cladoconidium articulatum*, CCM F-33094. A-C, conidial development. Note the symphydial proliferation of conidiogenous cell in B. D, E, H, detached conidia. G, disarticulation of detached conidium into part-conidia. F, thickwalled inflated cells. Scale bar = 50 μm .

genous cells integrated, with distinct denticles, proliferation symphydial. Conidia acrogenous, stauroform, central body short-clavate, aseptate, $5-7 \times 2.5-3.5 \mu\text{m}$. Branches three, primary, lateral, closely sequential, two on one side of the central body, one on the opposite side. The paired branches straight, inflated or bulbous (sometimes inequilaterally) at the base. The distal one $14-34 \times 1.5-2.5 \mu\text{m}$ in the inflated part, the proximal $15-34 \times 1 \mu\text{m}$, the bulbous base $2-3 \mu\text{m}$ wide. The opposite branch gently curved, $21-49 \mu\text{m}$ long, attached near the median, slightly inflated part. Conidial secession schizolytic.

Pure cultures examined: CCM F-28394, CCM F-29194. CCM F-29394, Canada, Nova Scotia, Cape Breton National Park, side ditch near the Cheticamp River, near the First Salmon Pool, isolated from foam collected on 9. 10. 1994 by L. Marvanová; CCM F-31194, Canada, Nova Scotia, Cumberland County, Cobequid Mountains, Arsenic Brook, isolated from foam collected on 17. 10. 1994 by L. Marvanová.

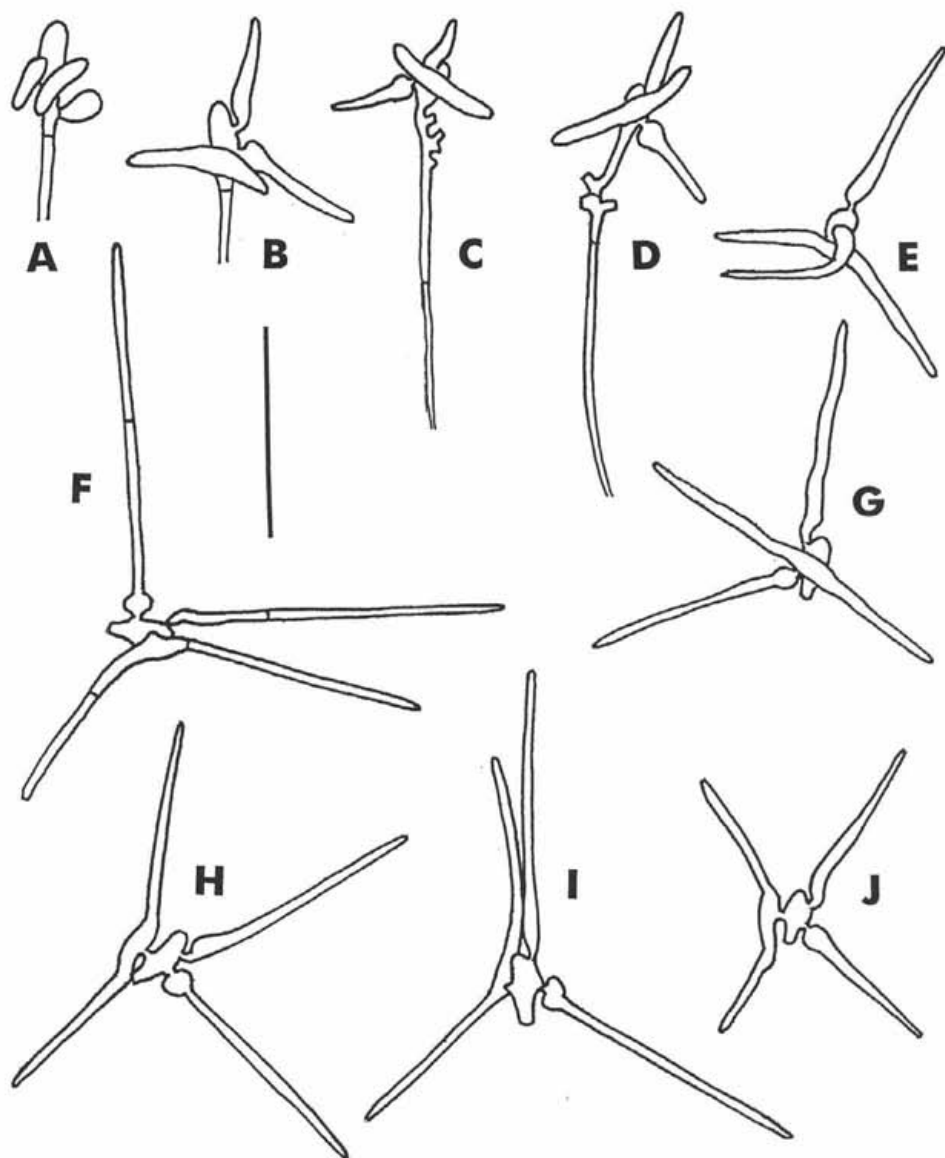


Fig. 6. *Lateriramulosa quadriradiata*. A-D, conidial development. Note the denticles in C and D, left behind after secession of conidia. E-J, detached conidia. (A-D,F,H,I = CCM F-29194; E,G,J = CCM F-28394). Scale bar = 20 μ m.

This species has not been isolated after its description and as far as we know, its conidia have not been reported from anywhere else. Our observations are in accordance with the protologue. In spite of the common site of origin of the first three Canadian isolates, the CCM F-28394 differs by having conidial arms roughly one third shorter than those in CCM F-29194 and CCM F-29394.

Sympodiocladium frondosum Descals, Trans. Br. Mycol. Soc. 78: 429, 1982. (Fig. 7)

Colony compact, dirty white or rosy, later becoming dark purple, very slow-growing, reaching 3 mm after 28 days at 15 °C, remaining restricted, elevated and brain-like convoluted, aerial mycelium sparse, granular, reverse very dark blue, blue diffusing pigment in agar appears in one month old cultures. Conidiophores micronematous. Conidiogenous cells integrated with the conidiophore, apex rounded. Conidia acrogenous, attached to the conidiogenous cells by abrupt narrow isthmus. Conidial elements long fusoid, septate, ends subulate. Axis bent at branch insertion, 42–58 × 2–2.7 µm, primary branch single, median, 21.5–41 × 1.7–2.5 µm, bearing one (rarely two) somewhat shorter secondary branch(es) at its lowermost cell. Conidial secession schizolytic.

Pure cultures examined: CCM F-17294, Canada, Nova Scotia, Chignecto Bay Coast, Shulie River, isolated from foam collected on 1. 10. 1994 by L. Marvanová, CCM F-33594, CCM F-33694, Canada, Nova Scotia, Colchester County, Cobequid Mountains, Murphy Brook, isolated from foam collected on 22. 10. 1994 by L. Marvanová.

This is the first published isolation into pure culture after the protologue. Our isolates match fully the original description. Conidia of this species are rarely reported from streams. They were encountered from alkaline stream water (e.g. Roldán et al. 1987, South Spain) or from softwater streams (e.g. Descals 1998, Cantabria, Spain; Marvanová, the Czech Republic, unpubl.).

Tetrabrunneospora ellisii Dyko, Trans. Br. Mycol. Soc. 70: 414, 1978. (Figs. 8 and 14 D, G-I)

Colonies isabelline with a yellow tinge, restricted, reaching c. 10 mm after 8 weeks at 18 °C with dense cottony aerial mycelium, reverse dark brown. Substrate mycelium hyaline or brownish, with numerous globose or irregular swellings on hyphae, aerial mycelium brown. Sporulation on a submerged piece of colony, but above the water level. Conidiogenous structures with brown thick walls. Conidiophores lateral, inconspicuous to macronematous and then erect, simple, septate. Conidiogenous cells single, terminal, integrated with the conidiophores; proliferation or regeneration not seen. Conidial primordia integrated with the

conidiogenous cells, forming a rod-like structure; branches lateral, arising more or less simultaneously from a slightly swollen cell near the middle of the primordium; at this time the upper part of the conidial axis bends slightly backwards. Mature conidium is tetra- or poly-radiate, septate, distal ends paler, rounded to subulate; axis 92–175 × 10–12.5 µm, proximal part shorter than the distal part, detachment scar mostly truncate, rarely with a short, percurrent, sometimes furcate, basal extension. Branches diverging, 69–132 × 10–12.5 µm, branch insertion slightly constricted. Conidial secession tardy, schizolytic.

Specimens examined: Pure culture: CCM F-28294 Canada, Nova Scotia, Cape Breton National Park, Right tributary of the Cheticamp River, near the First Salmon Pool, isolated from foam collected on 9. 10. 1994 by L. Marvanová. Herbarium material: DAOM 164967 (holotype.).

Due to the failure of conidia to germinate, the original description of this species was not based on a pure culture (Dyko 1978). There is a confusing information as regard the holotype specimen: on p. 415, following the Latin diagnosis, Dyko (l.c.) gives the holotype as "B. J. Dyko no. 579, IMI 222995 holotypus" (from N. C.), but on p. 416, in the paragraph Specimens examined she cites... "BJD 579, DAOM 164967, holotype...". The collection deposited as IMI 222995 is here claimed equal to BJD 578, which is a collection from N. Y. The specimen No. 164967 sent to the senior author from DAOM was labelled "holotype" and bears Dyko's collection No. 579, written in her handwriting. The holotype material was accompanied by copies of two letters from Dyko to DAOM, expressing her intention to deposit the holotype of *T. ellisii* in DAOM.

The holotype consisted of two slide preparations with the objects mounted in lactofuchsin (?). The one contained a piece of angiosperm leaf (probably *Betula* sp.) with a few scattered conidia and conidiophores (?). Conidial ontogeny or mature conidia attached to conidiophores were not seen. The conidia were not well discernible because of the thickness of the preparation. The second slide contains 4 mostly partly damaged detached conidia of *Tetrabrunneospora ellisii*.

There are small differences between our isolate and Dyko's material: in Dyko's specimen the walls of the end cells gradually become thinner and paler towards the apex and the edge of the brown cell wall layer is visible as an indistinct line under light microscope (Fig. 8 F,G, arrows). In our isolate there is a thin hyaline septum separating the paler end cells. The different thickness of the entire conidial cell wall (compare Fig. 8 D, E with F, G) of both specimens may be caused by differences in the conidial maturation *in vivo* vs *in vitro*. Slight differences in conidial arm length and width may be due to strain variation or again to *in vivo* vs. *in vitro* effects. It is known, that some conidia become 'fatter' when obtained in culture on artificial media. Dyko gives the maximum width of the axis in her material as 7–8.5 µm, but our measurements in her holotype extended up to 9.5 µm.

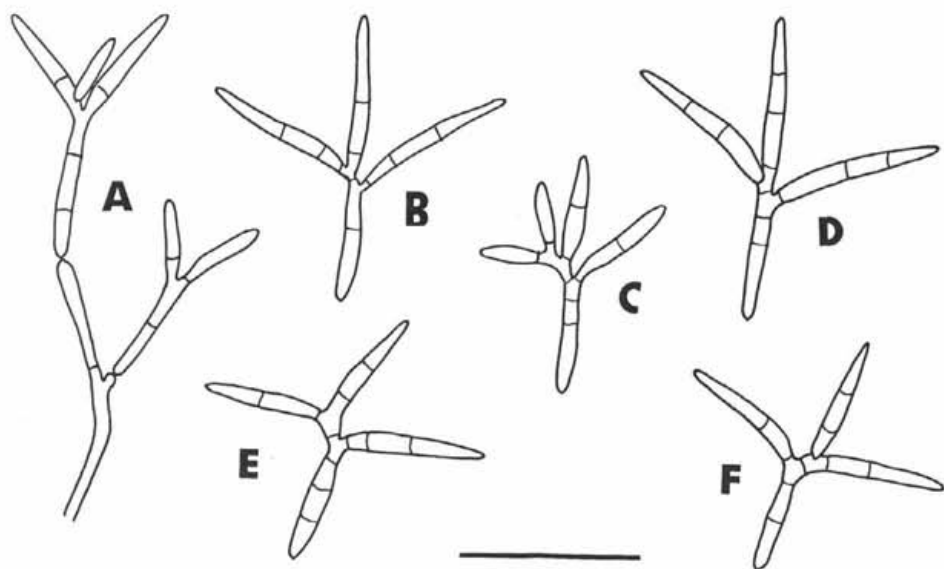


Fig. 7. *Sympodiocladium frondosum*. A, conidial development. B-F, detached conidia. C, aberrant conidium with two secondary branches. (A = CCM F-33594; B-F = CCM F- 17294). Scale bar = 25 μ m.

In our collection the conidia germinated tardily, grew slowly and became easily overgrown by more competitive species. Originally this fungus was collected in small streams in U.S.A. (N.C., N.Y. and Va). Conidia were later reported from streams in U.S.A., Va. (Suberkropp and Wallace 1992).

Tricladium caudatum Kuzuha 1973, J. Jap. Bot. 48: 222.

(Figs. 9 and 15 B,C)

Colony compact, greyish in the centre, with black margin, slow-growing, reaching 20 mm diam. after 20 days at 15 °C; aerial mycelium abundant, funiculose, reverse black. Sporulation after submergence in a few days, under water, abundant. Conidiophores terminal, single, simple, hyaline, flexuous. Conidiogenous cells terminal, integrated, sometimes caducous and then remaining attached to the conidia (Fig. 9 F), proliferation percurrent (Fig. 9 A,C, arrows). Conidia branched, axis twice bent, attenuate at each point of flexion, 55-85 \times 3-4.5 μ m, apex rounded, base slightly convex, basal extension excentric, appearing before secession (Fig. 9 D), up to 17 μ m long, very rarely lacking in some conidia; branches typically two, alternate, remote, slightly constricted at the insertion, the proximal one 29-34 \times 3-5 μ m, the distal one 14-27 \times 2-3.5 μ m, ends rounded. Conidial secession schizolytic.

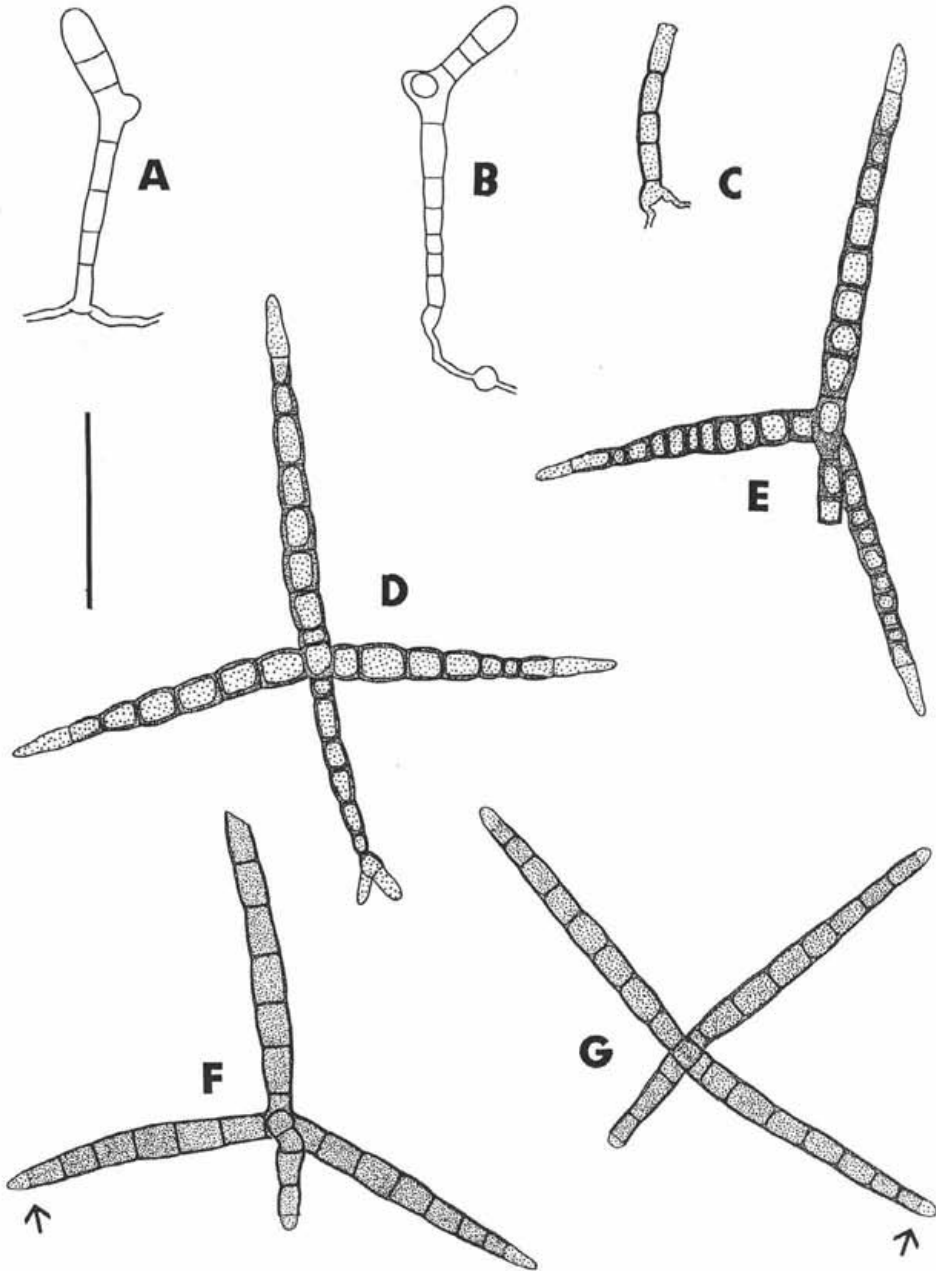


Fig. 8. *Tetrabrunneospora ellisii*. A,B, conidial development. C, spent conidiophore. D-G, detached conidia. (A-E = CCM F-28294; F,G = DAOM 164967). Scale bar = 50 μ m.

Pure cultures examined: CCM F-39594, Canada, New Brunswick, Miramichi Region, near Renous, Otter Brook, isolated from foam collected on 4. 11. 1994 by L. Marvanová and H. Garnett. CCM F-37794, 42194, dtto; CCM F-23787, Canada, Nova Scotia, Lime Kiln Brook, isolated from foam collected on 29. 4. 1987 by L. Marvanová and F. Bärlocher.

Our description of the pure culture is the first one published. Kuzuha (1973) isolated conidia into pure culture from stream foam in Japan, but did not give the colony characters. Her original collection was the only one for a long time. The first illustrated published record of conidia of this fungus from outside Japan was probably from Austria (Regelsberger et al. 1987). We isolated the species in Canada in 1987. The conidiation was very sparse and with that isolate we have not succeeded in observing conidiogenesis. Since then, records of conidia of this species from foam accompanied with drawings were published from Spain (Descals 1987), France (Descals and Chauvet 1992) and Austria (Voglmayr 1996, Marvanová and Gulis 2000). Conidia in stream foam were also encountered by Marvanová (unpubl.) in the Czech Republic. The above collections differ from the conidia seen in the type specimen by more curved (especially the "internodal" part of the axis between the two branches), sometimes variously inflated elements and a smaller span. We do not consider these differences distinct enough to justify separation at the species or subspecies level.

The Canadian as well as the European collections are mostly from softwater streams.

Trifurcospora irregularis (Matsush.) K. Ando et Tubaki, Trans. Mycol. Soc. Japan 28: 471, 1987. Figs. 10 and 14 A, B

≡ *Flabellospora irregularis* Matsush., Mats. Mycol. Mem. No. 2, p. 9, 1981.

Colony dirty white, cottony, slow-growing, reaching 12 mm diam. in 14 days at 15 °C; aerial mycelium abundant, delicate, funiculose, reverse pale beige with orange tinge. Sporulation in submerged cultures, moderate, under water and at the water level. Conidiophores mostly lateral, single, simple, short, monilioid; conidiogenous cells single or rarely in pairs, inflated, similar to the conidiophore cells, with one or two monoblastic conidiogenous loci on denticles. Conidia acrogenous, mostly triradiate, but also with 1, 2 or 4 arms. Central body globose, 3.5–5 µm wide. Arms narrowly obclavate, slightly constricted at septa, 24–76(-89) × 3.5–5 µm, often gently curved, sometimes with very thin long ends.

Pure culture examined: CCM F-37494, Canada, New Brunswick, Miramichi Region, near Renous, Catamaran Brook, isolated from foam collected on 4. 11. 1994 by L. Marvanová and H. Garnett.

Originally described from U.S.A., Alabama, as *Flabellospora irregularis* (Matsushima 1981). The variation of the conidial arm length is great: the upper limits

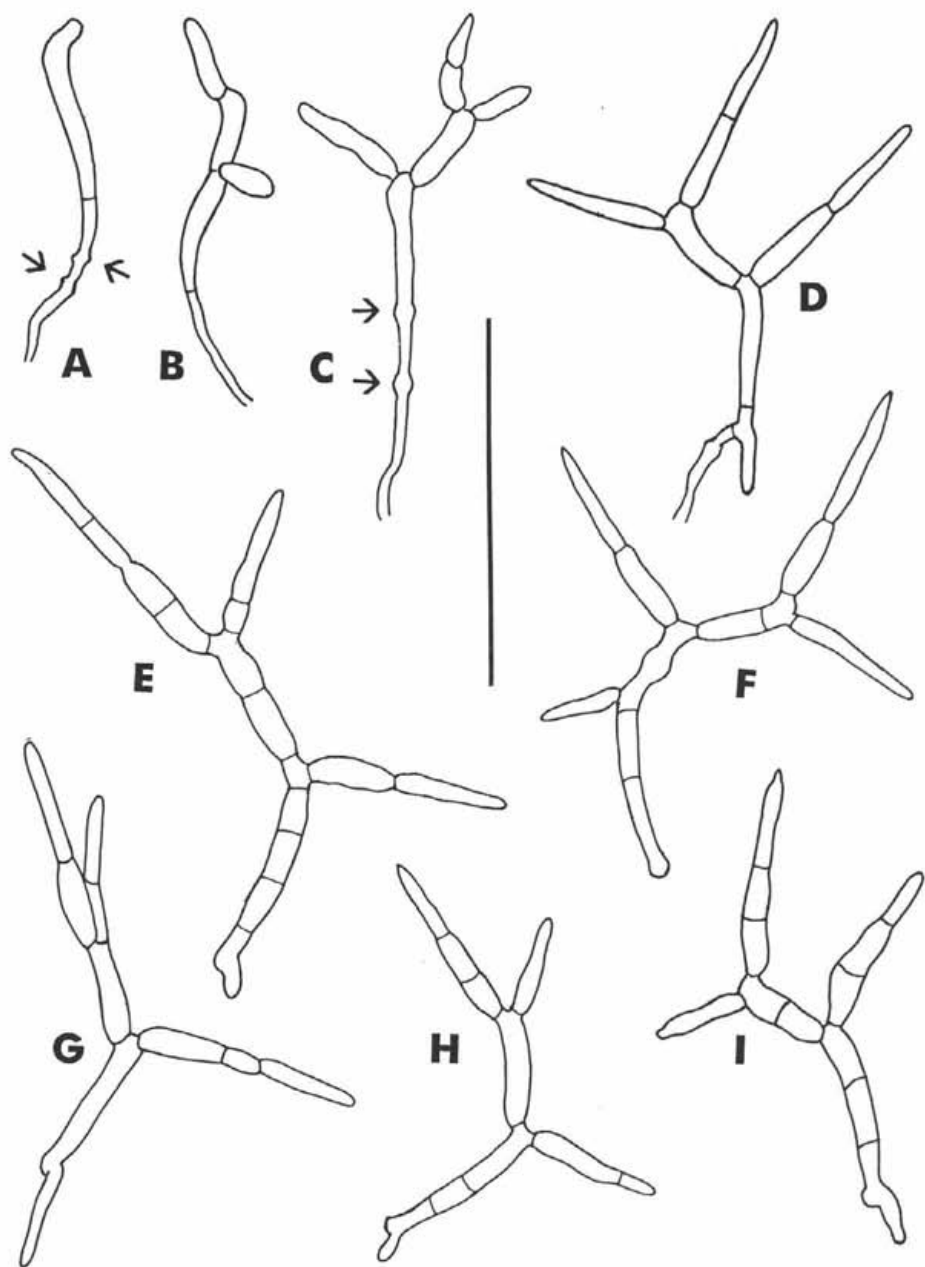


Fig. 9. *Tricladium caudatum*, CCM F-39594. A-D, conidial development. Arrows point out the percurrent proliferation of conidiogenous cells. Note the *in situ* development of the basal extension in D. E-I, detached conidia. F, detached conidium bearing caducous conidiogenous cells attached to the base. Scale bar = 50 μ m.

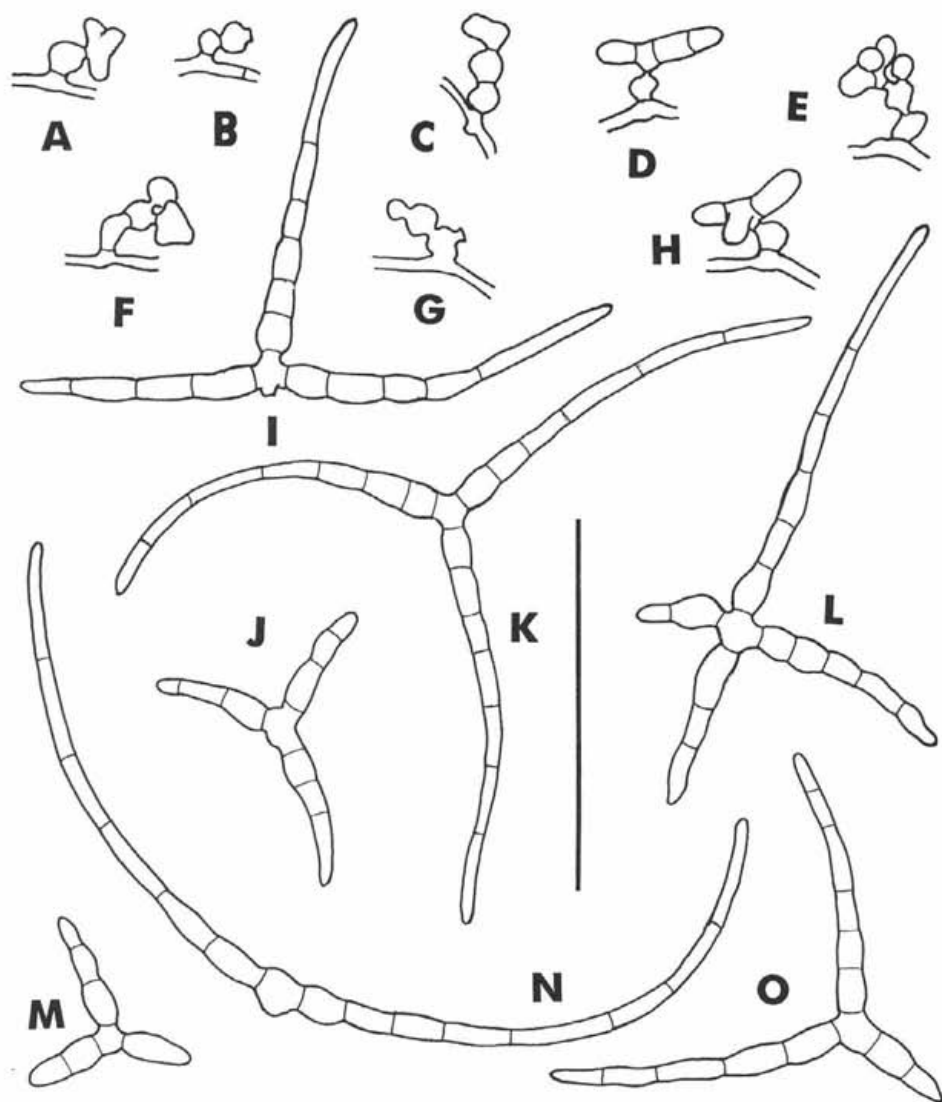


Fig. 10. *Trifurcospora irregularis*, CCM F-37494. A-H, conidial development. I-O, detached conidia. Note the abnormally elongate conidial ends in K and N, probably due to long persistence in foam. Scale bar = 50 μ m.

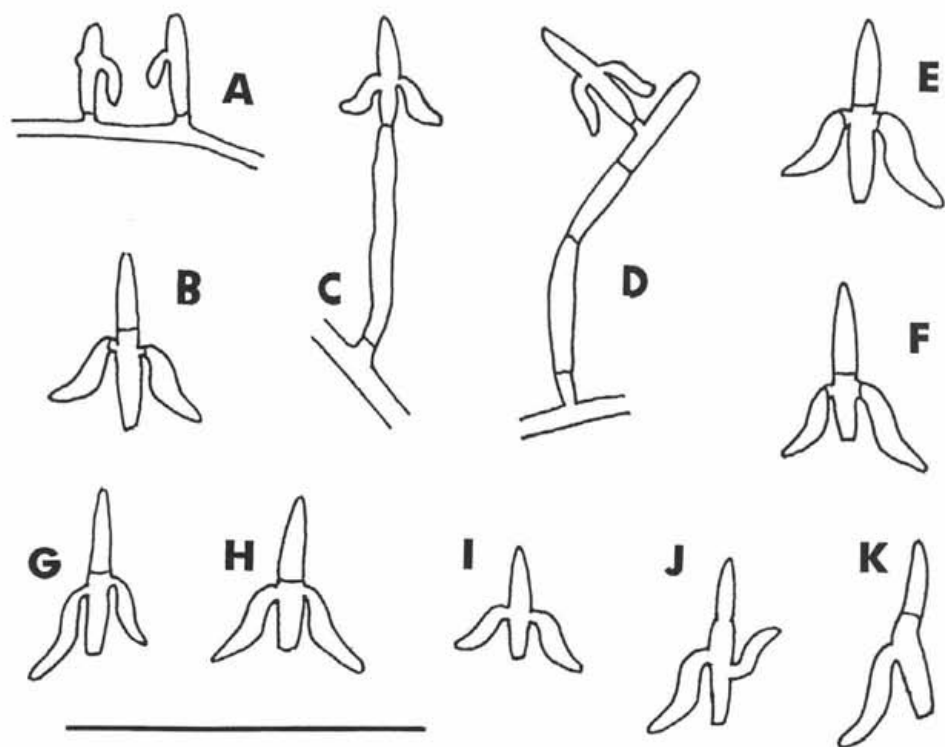


Fig. 11. *Triglyphium alabamense*, CCM F-30187. A,C,D, conidial development. B,E-K, detached conidia. Note the one antrorse branch in J and a single branch in K. Scale bar = 25 μ m.

are roughly three times greater than the lower limits (cf. also Matsushima 1981: 18–60 μ m, Ando et Tubaki 1987: 16–45 μ m and our values. The conidial arm length (27–75 μ m) in a new species *Trifurcospora subsessilis* K Matsush. et Matsush. (Matsushima 1995) approximates our measurements, but the conidiophores in *T. subsessilis* are cylindrical instead of monilioid (Matsushima 1995). *Quadricladium aquaticum* Nawawi et Kuthub. (Nawawi and Kuthubutheen 1989) should very probably be considered congeneric.

Triglyphium alabamense Matsush., Mats. Mycol. Mem. No. 2, p. 18, 1981. (Figs. 11 and 14 C).

Colony very slow-growing, pale yellow, tufted, with compact, elevated mycelial structures in the middle. Conidiophores absent or lateral, up to 30 μ m long. Conidiogenous cells intercalary, lateral or rarely terminal, integrated or discrete. Conidia terminal or lateral, stauroform. Axis navicular, typically two-celled,

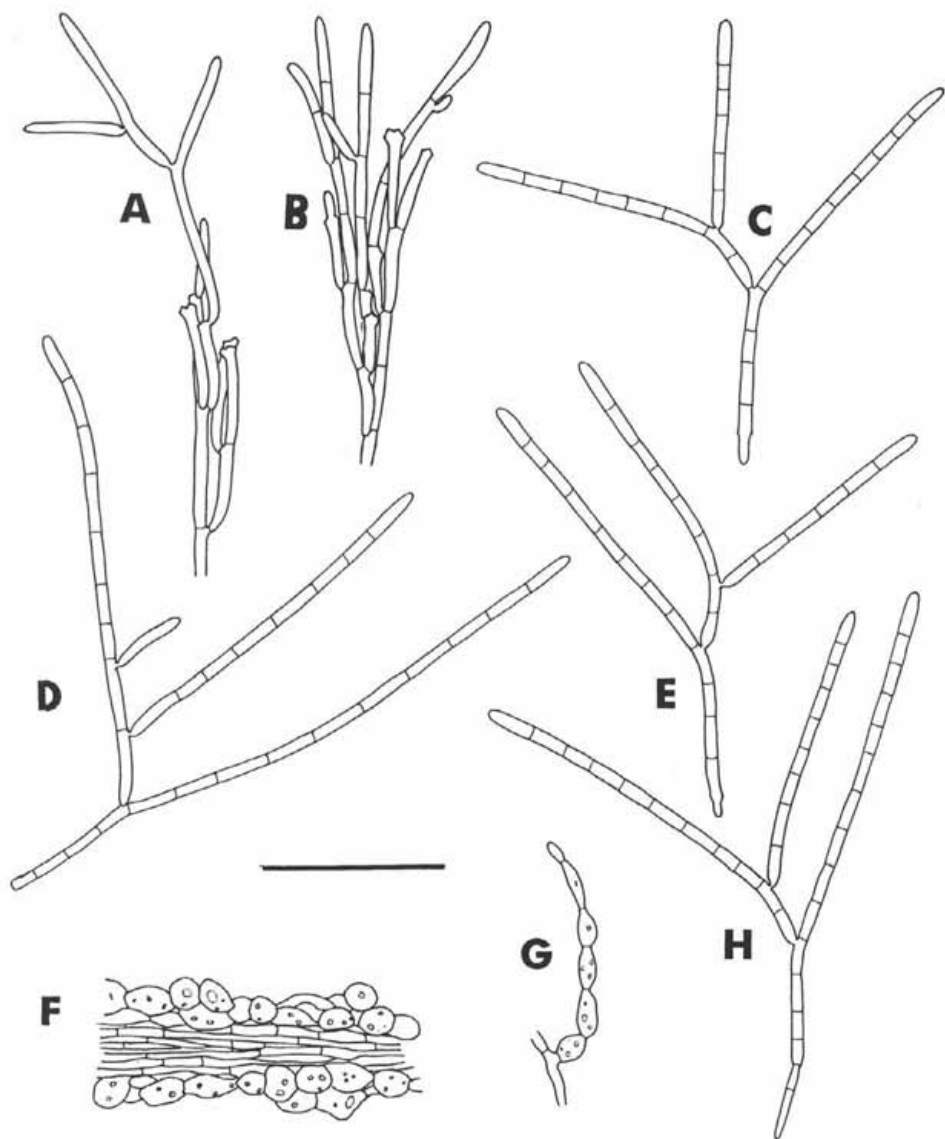


Fig. 12. *Varicosporium trimosum*, CCM F-32994. A,B, conidial development. C-E,H, detached conidia. D, aberrant conidium with two secondary branches. F, mycelial rope. G, inflated cells in chain. Scale bar = 50 μ m.

7–13 × 1.3–1.6 μm, apex subulate, base truncate; branches lateral, sequential, submedian, opposite or nearly so, retrorse, long fusoid, sigmoid or bent, tapering towards both ends; the first-formed 5–8 × 1–2 μm, the second 4–6 × 1–2 μm. Aberrant conidia with single branch or with one branch antrorse and one retrorse appear rarely. Conidial secession schizolytic.

Pure culture examined: CCM F-30187, Canada, New Brunswick, Sackville, near Ogden Mill Cross, isolated from foam collected in a stream below the Trans Canada Highway on 25. 4. 1987 by L. Marvanová.

We include this terrestrial taxon because the detached conidia may be found in streams and confused with those of *Tricellula aurantiaca* (Haskins) Arx. Conidia of *T. aurantiaca* differ by deep constrictions between all conidial elements. The tuberculariaceous genus *Triglyphium* Fres. is not well known, its type species, *Triglyphium album* Fres. is lacking. We follow here the concept of Matsushima (1981). *Triglyphium alabamense* was collected in Alabama, USA, on rotting leaves of *Quercus* on land. The authentic material sent by Matsushima to the first author contained dried leaves. They yielded a single detached conidium, which was in accordance with our material.

Varicosporium trimosum Wolfe, Aquatic Hyphomyc. of the Appal., p. 254, 1977. (Figs. 12 and 15 A)

Colony whitish with a rosy-beige hue or beige, slow-growing, reaching 20 mm diam. after 15 days at 15 °C, low, aerial mycelium finely funiculose, reverse pale brown. Globose or irregular inflated cells 5–12 μm in diam. in chains or clusters appear abundantly in culture. Mycelial ropes with parallel-running central hyphae surrounded by 1–2 layers of inflated cells (Fig. 12 F) were also observed. Conidiophores terminal, profusely branched, usually bearing two or three conidia per branch apex. Conidia branched, typically composed of axis and one primary and one secondary branches. Aberrant branching patterns with two primary, or two secondary branches or with a single primary branch may occur. Conidial elements cylindrical, septate, parent elements often slightly bent backwards at the point of daughter element insertion, ends sometimes slightly widening near the apex, apices rounded, branch bases constricted. Conidial axis 72–170(-192) × 2.5–5 μm, base usually with percurrent extension up to 21 μm long. Primary branch similar to the axis, 36–156(-168) × 2.5–3.5 μm. Secondary branch straight or gently curved, 17–77(-120) × 2.5–3.5 μm. Conidial secession schizolytic.

Pure cultures examined: CCM F-32994, Canada, Nova Scotia, Colchester County, Cobequid Mountains. Murphy Brook, isolated from foam collected on 22. 10. 1994 by L. Marvanová. CCM F-32694 dtto, Black Brook, isolated from foam collected on 22. 10. 1994 by L. Marvanová.

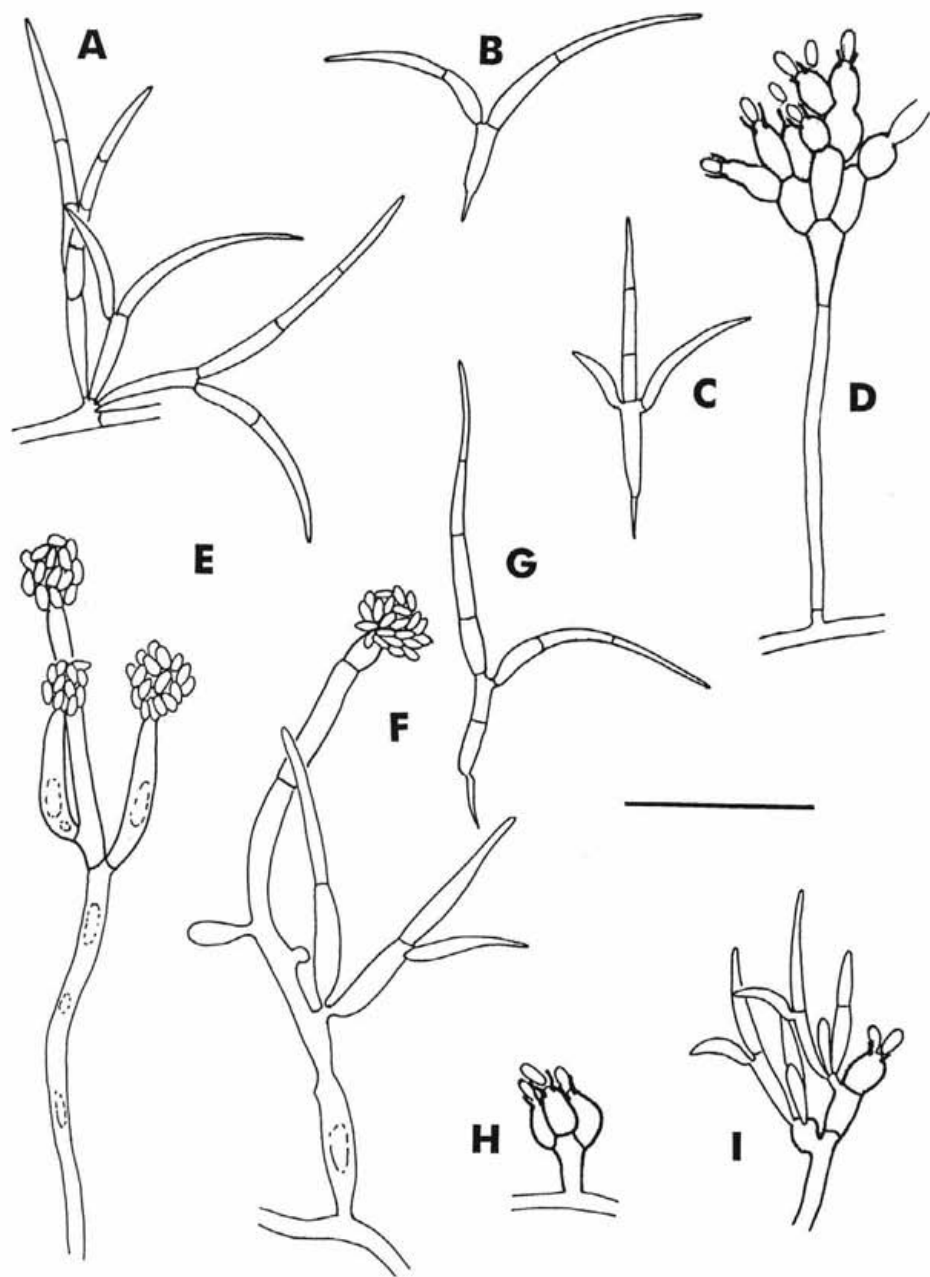


Fig. 13. *Ypsilina graminea*. A, macroconidial development. B, C, G, detached macroconidia. D-F, H, I, microconidial state. Note the occurrence of macro- and microconidial state on the same conidiophore in F and I. (A-D, G-I = CCM F-27687, E, F = unlabelled Czech isolate from 1964). Scale bar = 20 μ m.

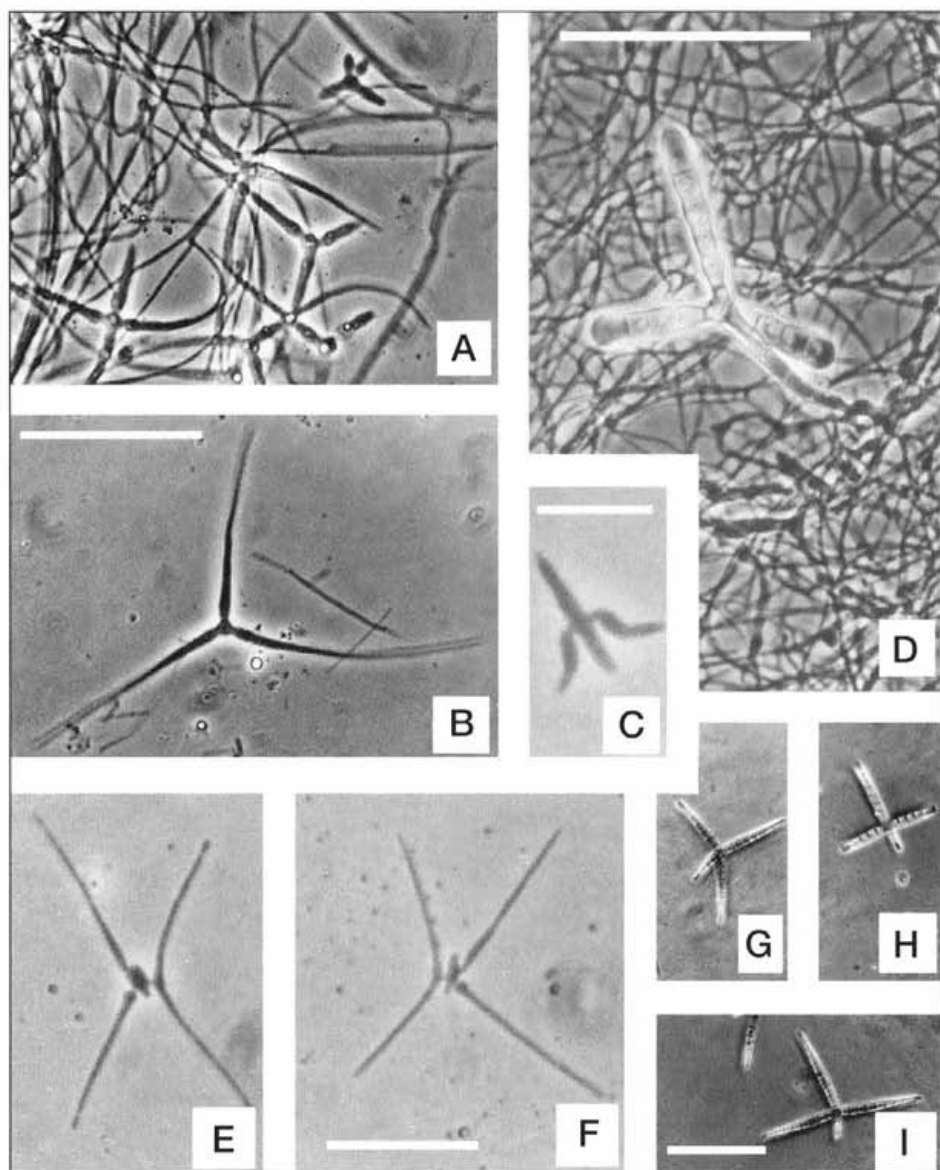


Fig. 14. A, B, *Trifurcospora irregularis*, CCM F-37494. A, developing conidia. B, detached conidium with extremely elongate branches. C, *Triglyphium alabamense*, CCM F-30187, detached conidium. D, G-I, *Tetrabrunneospora ellisii* CCM F-28294. D, developing conidium. G-I, detached conidia. E, F, *Lateriramulosa quadriradiata* CCM F-29194, detached conidia. Scale bar for A, B = 50 μ m, for C = 10 μ m, for D = 50 μ m, for E, F = 20 μ m, for G-I = 100 μ m.

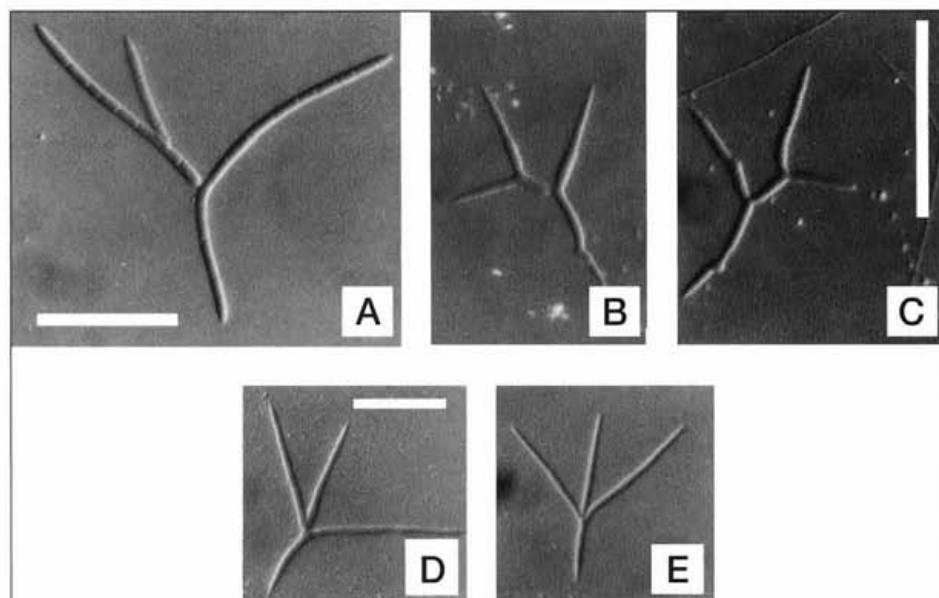


Fig. 15. Detached conidia. A, *Varicosporium trimosum*, CCM F-32994. B, C, *Tricladium caudatum*, CCM F-39594. D, E, *Alatospora constricta*, CCM F-23394. Scale bar for A = 50 μm , for B, C = 50 μm , for D, E = 20 μm .

This species has neither been isolated nor reported with certainty from nature since its description. The only record of conidia from foam (unillustrated) is from SW France (Descals and Chauvet 1992). Conidia of our isolates exceed the dimensions given by Wolfe; he gives the maximum length for the conidial axis, primary and secondary branch 121, 81 and 53.5 μm respectively, but in conidia with long arms such variation is not unusual. Conidia of *V. trimosum* are relatively large and therefore they are unlikely to be overlooked, but owing to their similarity to fragmented conidia of *Varicosporium elodeae* they may be misidentified. Conidial branching in *V. trimosum* is typically sympodial. This is in principle a pattern of conidial branching in *Pleuropedium*, which, however, has tapering elements usually with pointed ends.

This fungus was described from a mountain stream. Data on water chemistry were not given. According to our experience conidia of *V. trimosum* appear in low-nutrient waters on acidic bedrock. In such streams they were found in the Czech republic as well (Marvanová, unpubl.)

Ypsilina graminea (Ingold, P. J. McDougall et Dann) Descals, J. Webster et Marvanová, *Can. J. Bot.* 76: 1659, 1999. (Fig. 13)

≡ *Volucrispora graminea* Ingold, P. J. McDougall et Dann, *Trans. Br. Mycol. Soc.* 51: 326. 1968.

Colony 25 mm in diam after 43 days at 10 °C, white, cream-coloured, reverse isabelline, aerial mycelium cottony, slightly tufted in the centre. Macroconidial structures typical for this species, not described here.

Microconidial state hyphomycetous, presumably spermatial (andromorph): conidiophores lateral, rarely terminal, single, simple or with stipes and a penicillate head with cell walls somewhat thicker than that of the rest of structures; metulae up to five per conidiophore apex (or absent), crowded, inflated, $5-7 \times 3-4 \mu\text{m}$. Phialides lageniform, $5-7 \times 3-4 \mu\text{m}$, with distinct collarette up to $2 \mu\text{m}$ deep. Rarely the phialides with microconidia appear on the macroconidiophore along with macroconidia (Fig. 13 F,I). Conidia single, elliptical, smooth, $2-2.5 \times 1 \mu\text{m}$ (description based on CCM F-27687). The isolate from 1964 had no metulae, lower number of loosely arranged phialides per conidiophore apex, phialides cylindrical or long fusoid, collarette obscured among the slimy heads of conidia, conidia elliptical, smooth.

Pure cultures examined: CCM F-27687, Canada, Nova Scotia, Lime Kiln Brook, isolated from foam collected on 29. 4. 1987 by L. Marvanová and F. Bärlocher. Unlabelled culture, Czech Republic, isolated from leaves in 1964 by L. Marvanová, data on locality lost.

The microconidial state in pure culture has been seen for the first time by L. Marvanová in a Czech isolate from 1964, which is no more available. The presence of macro- and microconidia on one conidiophore confirm that both states belong to the same thallus (Fig. 13 F). At that time the fungus, later published as *Volucrispora graminea* was not yet formally described. The macroconidia and the conidiogenesis were very similar to the Canadian isolate, but the microconidial state differed in the phialide shape and length (cf. Fig. 13 E, F). The presence of microconidial state having the character of andromorph suggests existence of a teleomorph. The differences between the andromorphs of the two isolates rise a question, whether two morphological variants of spermatial state may indicate a possibility of *Ypsilina graminea* being a species aggregate. In fact the great variability of conidia seen in nature supports such considerations. The occurrence of andromorph in pure cultures of *Y. graminea* seems rare. The senior author has examined 12 isolates of *Y. graminea*, but only in the above two cases microconidial state developed.

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REFERENCES

- ANDO K. and KAWAMOTO (1986): *Arborispora*, a new genus of Hyphomycetes. – *Trans. Mycol. Soc. Japan* 27: 119–128.
- ARNAUD G. (1952): Mycologie concrete: Genera I. – *Bull. Soc. Mycol. France* 68: 181–223.
- BANDONI R. J. and TUBAKI K. (1985): *Cladoconidium*, new genus: a new hyphomycete anamorph-genus. – *Trans. Mycol. Soc. Japan* 26: 425–431.
- BÄRLOCHER F. (1987): Aquatic hyphomycete spora in 10 streams of New Brunswick and Nova Scotia. – *Can. J. Bot.* 65: 76–79.
- BÄRLOCHER F. (1992): Seasonal occurrence and movements of fungi in streams. – In: Khulbe R. D. (ed.), *Microbial Activity in the Himalaya*, p. 1–29. Books International, New York.
- CZECZUGA B., ORLOWSKA M. and WORONOWICZ L. (1989–1990): Studies of aquatic fungi X. Some rare species of Hyphomycetes in North-Eastern Poland. – *Acta Mycol.* 25(2): 5–20.
- DESCALS E. (1987): Muestro preliminar de hongos ingoldianos de Catalunya – *Rev. Iber. Micol.* 1987, 4: 17–32.
- DESCALS E. (1998): Streamborne fungi from Karrantza (Basque Country) and surroundings. – *Boll. Soc. Hist. Nat. Balears* 41: 191–219.
- DESCALS E. and CHAUVET E. (1992): Diversité des champignons Ingoldiens de quelques rivières du sud-ouest de la France. – *Nova Hedwigia* 54: 83–96.
- DIX N. J. and WEBSTER J. (1995): *Fungal Ecology*. – Chapman and Hall, London, 549 pp.
- DUDKA I. O. (1973): Aquatic hyphomycetes of Ukraine I. New species for mycoflora of the Soviet Union (in Ukrainian). – *Ukr. Bot. Zh.* 30: 449–456.
- DUDKA I. O. (1974): Fungal spores in foam and scum of running waters in the Ukrainian Carpathians (in Ukrainian). – *Ukr. Bot. Zh.* 31: 432–439.
- DYKO B. J. (1978): New aquatic and water-borne hyphomycetes from the Southern Appalachian Mountains of the United States. – *Trans. Br. Mycol. Soc.* 70: 409–416.
- GÖNCZÖL J. and RÉVAY Á. (1983): Observations on the hyphomycetes inhabiting forest litter of Hungary. – *Acta Bot. Hung.* 29: 107–125.
- HAWKSWORTH D. L., KIRK P. M., SUTTON B. C. and PEGLER D. N. (1995): *Ainsworth & Bisby's Dictionary of the Fungi*. 8th ed., – CAB International, Wallingford, 616 pp.
- IQBAL S. H., BHATTY S. F. and MALIK K. S. (1979): Freshwater hyphomycetes on decaying plant debris submerged in some streams in Pakistan. – *Trans. Mycol. Soc. Japan* 20: 51–61.
- KUZUHA S. (1973): Two new species of aquatic hyphomycetes. – *J. Jap. Bot.* 48: 219–224.
- MARVANOVÁ L. (1972): Concerning *Calcarispora hiemalis*. – *Čes. Mykol.* 26: 230–232.
- MARVANOVÁ L. (1977): Two new *Alatospora* species. – *Arch. Protistenk.* 119: 68–74.
- MARVANOVÁ L. (1984): Conidia in waters of the protected area Slovenský Raj. – *Biológia (Bratislava)* 39: 821–832.
- MARVANOVÁ L. and DESCALS E. (1985): New and critical taxa of aquatic hyphomycetes. – *Bot. J. Lin. Soc.* 91: 1–23.
- MARVANOVÁ L. and DESCALS E. (1987): New taxa and new combinations of aquatic hyphomycetes. – *Trans. Br. Mycol. Soc.* 89: 499–507.
- MARVANOVÁ L. and GULIS V. I. (2000): Notes on aquatic hyphomycetes and streamborne spores from Austria. – *Österr. Z. Pilzk.* 9: 125–140.
- MATSUSHIMA T. (1981): *Matsushima Mycological Memoirs No. 2*. – Matsushima, Kobe, 68 pp.
- MATSUSHIMA, T. (1995): *Matsushima Mycological Memoirs No. 8*. – Matsushima Fungus Collection, Kobe, 54 pp.

- MATSUSHIMA K. and MATSUSHIMA T. (1996): *Fragmenta Mycologica II*. – In: Matsushima T. (ed.), *Matsushima Mycological Memoirs No. 9*, p. 31–40. – *Matsushima Fungus Collection, Kobe*.
- NAWAWI A. and KUTHUBUTHEEN A. J. (1989): *Quadricladium aquaticum* gen. et sp. nov., an aquatic hyphomycete with tetra-radiate conidia. – *Mycotaxon* 34: 489–495.
- RAVIRAJA N. S., SRIDHAR K. R. and BÄRLOCHER F. (1998): Breakdown of *Ficus* and *Eucalyptus* leaves in two organically polluted streams in India: fungal diversity and ecological functions. – *Freshwat. Biol.* 39: 537–545.
- REGELSBERGER B., MESSNER K. and DESCALS E. (1987): Species diversity of aquatic hyphomycetes in four Austrian streams. – *Mycotaxon* 30: 439–454.
- ROLDÁN A., DESCALS E. and HONRUBIA M. (1987): Hifomicetos acuáticos en las cuencas altas de los Ríos Segura y Guadalquivir. – *Anal. Biol.* 13 (*Biología Vegetal* 3): 3–13.
- SUBERKROPP K. (1997): Annual production of leaf-decaying fungi in a woodland stream. – *Freshwat. Biol.* 38: 169–178.
- SUBERKROPP K. and WALLACE J. B. (1992): Aquatic hyphomycetes in insecticide treated and untreated streams. – *J. North. Am. Benthol. Soc.* 11: 165–171.
- VOGLMAYR H. (1996): Spores of Ingoldian fungi in two Austrian rivulets. – *Österr. Z. Pilzk.* 5: 23–49.
- WOLFE C. C. (1977): Aquatic hyphomycetes of the Southern Appalachians. – In: Parker B. C. and Roane M. K. (eds.), *The Distributional History of the Biota of the Southern Appalachians. Part IV, Algae and Fungi*, pp. 242–264. Virginia University Press.