Cryptogamie, Mycologie, 2003, 24 (4): 339-358

New and rare hyphomycetes from streams of northwest Portugal. Part I.

L. MARVANOVÁ [a], C. PASCOAL [b] & F. CÁSSIO [b]

[a] Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic

[b] Department of Biology, University of Minho, Campus de Gualtar 4710-057, Braga, Portugal

Abstract – *Collembolispora barbata* gen. et sp. nov. is described from a mountain freshwater stream in Portugal. Descriptions from pure cultures are provided for the following rare species: *Flagellospora curta, Geniculospora grandis, Heliscus submersus, Tetracladium palmatum, Tricladiopsis flagelliformis* and *Tricladium terrestre*. Conidia of *Flabellospora amphibia* and *Titaeella capnophila* are reported from stream foam.

Aquatic hyphomycetes / taxonomy / pure cultures / distribution / water pollution

INTRODUCTION

During studies on aquatic hyphomycetes (Marvanová *et al.,* 2002) of some streams in the vicinity of Braga and in the National Park Peneda-Gerês, a hitherto unknown spore form was isolated into pure culture. It is described below as a new taxon.

At the same occasion, a few uncommon species were also isolated. These belong either to rarely encountered and therefore not well known taxa, or with their main distribution being in the Tropics. Our pure cultures of these species from temperate climate support the reliability of published records of such species presently based on detached conidia from foam. Each new collection adds useful information to the characterisation of a taxon. This is very important for the recognition of phenotypic variation, especially when only description of the type species, sometimes based on a single isolate, is available.

In samples of foam, conidia of several very rare species were also observed. Unfortunately attempts to obtain pure cultures were unsuccessful. Their occurrence in Northwest Portugal waters is here documented by illustrations in order to encourage other mycologists for their search.

MATERIAL AND METHODS

Sampling sites

The study area is located within 40 km of Braga (Northwest Portugal, ca. 41°N) and includes part of the National Park Peneda-Gerês (NPPG). The

Table 1. Physical and chemical characteristics of stream water at the sampling sites

Parameters	*Sampling sites (sampling dates)*				
	S1 (20.3.2001)	*S2 (20.3.2001)*	*S3 (22.11.1999)*	*S4 (20.3.2001)*	*S7 (26.3.2001)*
Altitude (m)	400	140	220	150	1100
Temperature (°C)	10.6	10.2	12.1	12.0	10.2
pH	6.0	6.0	6.6	6.2	5.4
Conductivity (μS cm^{-1})	42	170	57	150	14
Total hardness (ppm)	12	42	42	32	10
Ammonium (mg L^{-1})	< 0.01	0.32	0.07	0.70	< 0.01
Nitrate (mg L^{-1})	2.2	22.0	5.2	17.0	0.4
Orthophosphate (mg L^{-1})	< 0.01	0.54	0.13	2.07	0.03

survey was carried out in March 2001 in several streams of the Ave River basin (S1, S2, S3 and S4). It was also extended to the Cávado River basin (S7), with stream water of different physical and chemical characteristics (Tab. 1). The sampling sites are briefly characterised below.

S1 is located at the spring of the Este River, 5 km above the town of Braga. This stream has soft water and a low concentration of nutrients, primarily ammonium and orthophosphate (Tab. 1). The riparian vegetation is mainly *Eucalyptus globulus*, *Pinus pinaster*, *Pteridium aquilinum* and *Juncus* sp. Leaves, twigs and other debris from these plants may be used as substrates by aquatic hyphomycetes. The stream bed consists of granite rocks, pebbles and gravels.

S2 is located 10 km downstream from S1 at the Industrial Park of Braga. Effluents from textile, metallurgic and food industries are discharged into the stream, and may account to the high values recorded for ammonium, nitrate, orthophosphate and conductivity in the stream water (Tab. 1). The riparian vegetation consists mainly of *Alnus glutinosa*, *Quercus robur*, *Populus tremula*, *Platanus hybrida* and *Salix* sp. The stream bed differs from S1 by the presence of mud.

S3 is located in the Ave River at Pontilhão de São Cláudio in an agricultural area. Nutrient concentrations in stream water proved to be lower than those of S2 and S4 (see Tab. 1.). *Alnus glutinosa* is the dominant watershed woody vegetation, but some exotic species like *Acacia* sp. may occur as well.

S4 is located 5 km below the town of Braga in the village of Lamas, in a small unnamed tributary of the Este River. The tributary flows through an agricultural area, is bordered by vineyards and its water exhibited high values for ammonium, nitrate and orthophosphate (Tab. 1).

S7 is located at São João do Campo in a stream on the border of the NPPG. At the sampling site the water flow is relatively slow and the bed is sandy. Physical and chemical parameters (Tab. 1) point to an oligotrophic stream with softwater. The riparian vegetation consists mainly of *Betula pendula*, *Quercus robur*, *Pinus pinaster* and *Salix* sp.

Field and laboratory techniques

Autumn shed *Alnus glutinosa* leaves were collected prior to abcission and air-dried. The leaves (4g) were placed into 0.5-mm mesh bags (20 × 20 cm) and submerged in the streams. Leaf bags were recovered after two weeks and transported in a cool box to the laboratory. The leaves were rinsed, cut into disks, placed in 100 ml Erlenmeyer flasks (15 disks per flask) containing 40 ml sterile distilled water and aerated for two days, at 18°C. Foam was collected into sterile glass bottles, whenever it was found at the sampling sites.

Conidia either present in foam or released from leaf disks in the Erlenmeyer flasks were used for isolations. A loopful of spore suspension was streaked on a thin layer of 1.5% water-agar in Petri dishes and incubated at 18°C for 24-36 hours. The agar surface was scanned under the microscope for germinating spores. The areas around the spores were marked with a fine needle, pieces of agar with single conidia were cut out and transferred onto 1% malt extract agar (MA, Difco) containing 0.5 g L^{-1} streptomycin (Sigma). The isolates are preserved at the Department of Biology, University of Minho, Portugal.

To obtain the characteristic colony appearance, the isolates were cultivated on 2% MA at 15°C in diffuse light. Sporulation was induced at 18°C (when not otherwise stated) by the following methods: (1) incubation of pieces of colony in a shallow layer of standing distilled water under either diffuse light or NUV radiation, and (2) aeration of colony pieces in Erlenmeyer flasks containing distilled water, using cultures from either 2% MA or LCA (Low Carbon Agar, Miura & Kudo, 1970). Colony colour descriptions were done according to Rayner (1970).

Nuclei were stained as follows: conidia were air-dried on slides for *ca.* 5 min, fixed with ethanol and hydrochloric acid (3:1 v/v) for 10 min, washed with distilled water, and stained with DAPI (4´,6-diamidino-2-phenylindole, 0.01 mg mL^{-1}). Slides were examined with epifluorescence microscope Leitz Laborlux S.

At the sampling sites, temperature, pH and conductivity of stream water were measured with field probes (Multiline F/set 3 n°400327, WTW). Water samples were collected to determine the concentration of ammonium, nitrate and orthophosphate (see methodology in Pascoal *et al.* 2001). A kit (4824 DR-LT, LaMotte) was used to quantify total hardness of stream water.

NEW TAXON

***Collembolispora* anam. gen. nov.**

Etym.: conidia resemble Collembola (springtails).

Fungi mitosporici, hyphomycetosi. Conidiophora simplicia vel ramificata. Cellulae conidiogenae holoblasticae, sympodialiter prolificantes. Conidia ramificata, axis ad apicem versus attenuatus, ramum unum retrorsum, lateralem, gerens. Apices conidiorum extensionibus simplicibus vel ramosis ornati.

Species typica:

Collembolispora barbata anam. sp. nov. Figs. 1 and 2

Coloniae in cultura artificiali griseae, medium celeriter crescentes, mycelium aerium lanosum, parietibus glabris vel asperatis, pars reversa atrogrisea. Fructificatio subaquatica. Conidiophora semimacronematosa, precipue terminalia,

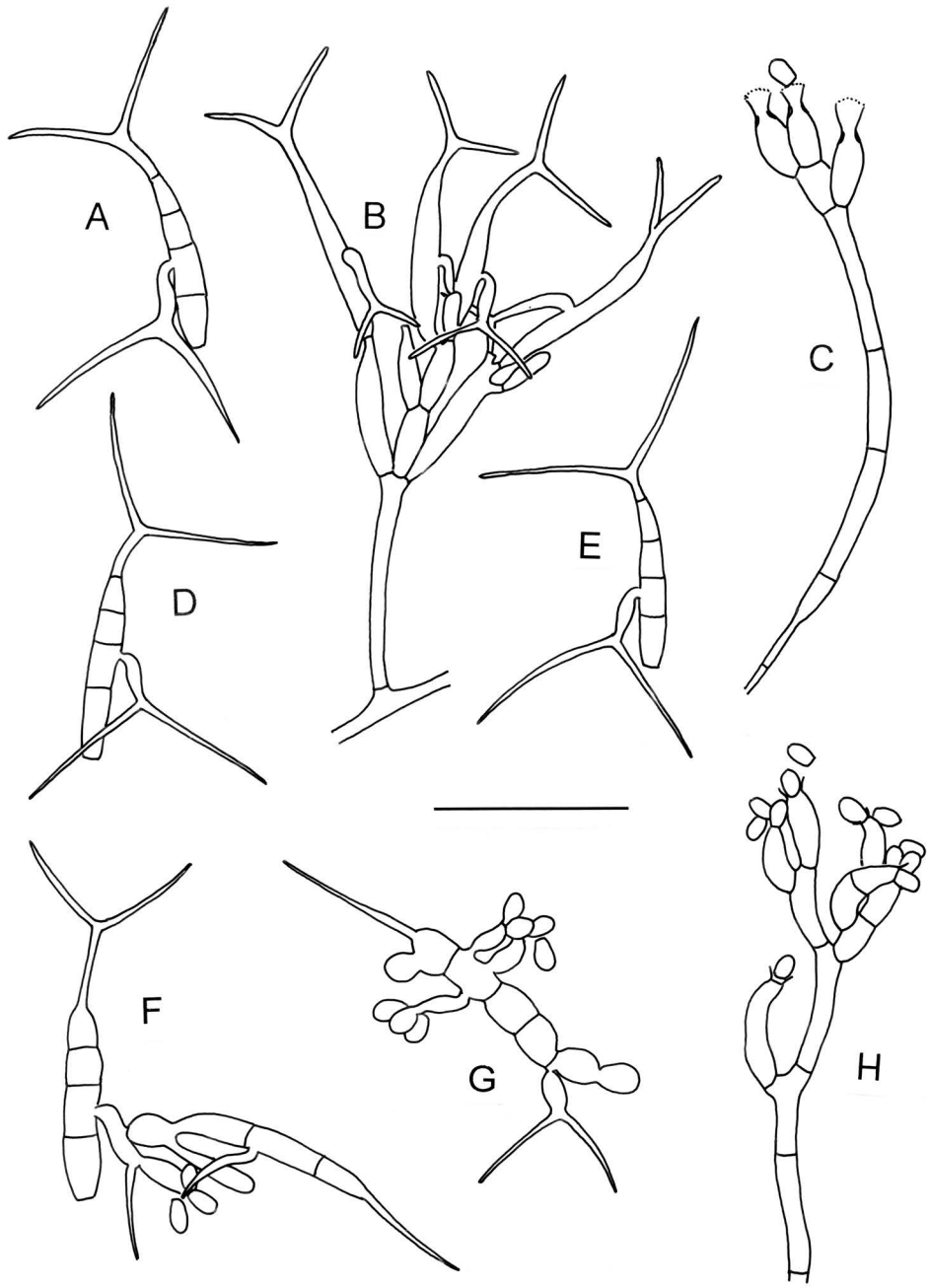

Fig. 1. *Collembolispora barbata* UMB-88.01: A,D,E. Detached conidia with forked extensions. B. lateral conidiophore with developing conidia. F. Conidium from water surface, producing on its branch secondary conidia and microconidia. G. Another conidium from the water surface, producing the microconidial state on considerably swollen cells. H. Terminal conidiophore of the microconidial state. UMB 48.01: C. Conidiophore with empty phialides, showing funnel-shaped collarettes and periclinal thickenings. Scale bar = 20 μm.

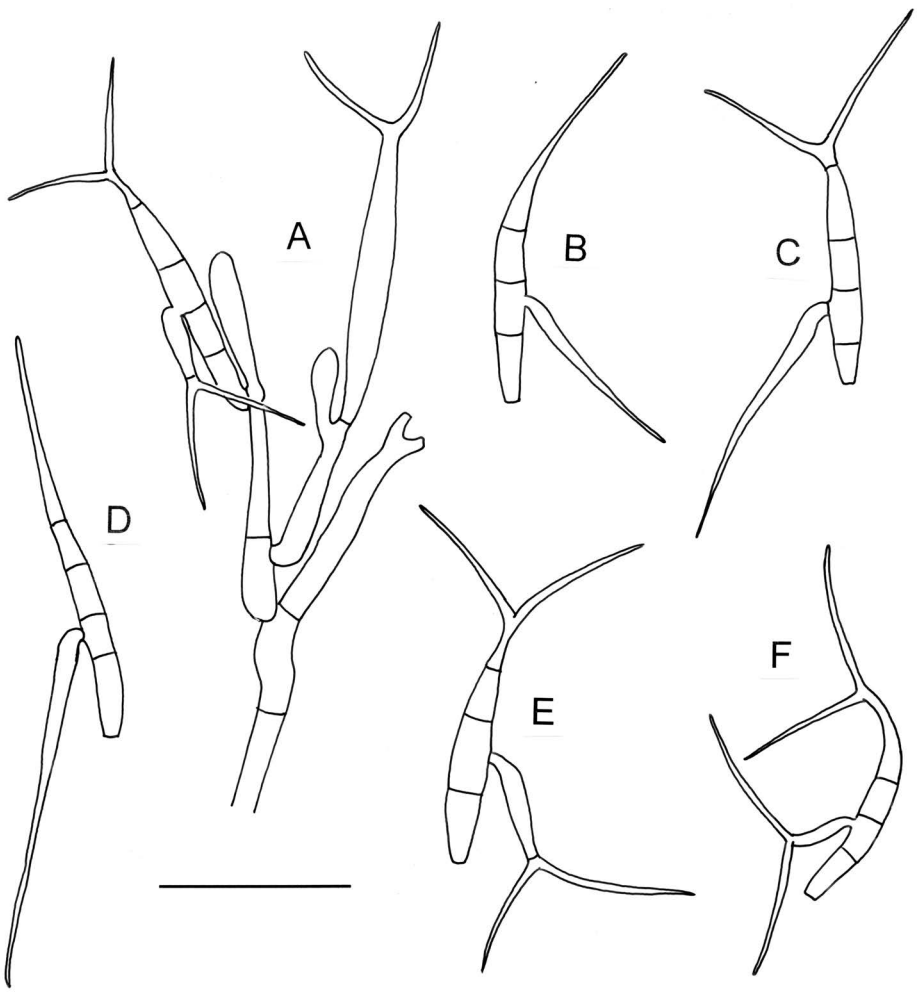

Fig. 2. *Collembolispora barbata* UMB-200.01: A. Conidiophore with developing conidia and one spent conidiogenous cell. B,D. Conidia with simple extensions. C. Conidium with single simple and forked extensions. E,F. Conidia with forked extensions. Scale bar = 20 µm.

raro lateralia, simplicia vel apice ramificata. Cellulae conidiogenae polyblasticae, 7-25 × 2.5-4 µm, elongationes sympodiales, breves. Conidia acrogena, axis rectus vel leniter curvatus, fusoideus, dorsiventralis, 3-4 septatus, 18-35 × 2-3 µm. Ramus singularis, ventralis, retrorsus, fusoideus vel sinuosus, 4-9(-12) × 1-2.5 µm. Apices conidiorum cum extensionibus setosis simplicibus vel 2-3 ramosis ornati.

Status microconidialis hyphomycetosus, ad hyphas vel ad cellulas macroconidiorum adest. Conidiophora semimacro- vel micronematosa, simplicia, terminalia vel lateralia. Cellulae conidiogenae phialidicae, dolii- vel lageniformes, 4-11 × 2-3.5 µm, collula usque ad 1.5 µm longa vel abest. Conidia aggregata, breviter ellipsoidea, basi truncata, unicellularia, 2-3 × 1.5-2 µm, glabra.

Holotypus: ***IMI*** *389661 (praeparatum e cultura UMB 88.01).*

Mitosporic fungi, hyphomycetes. Conidiophores simple or branched. Conidiogenous cells holoblastic, proliferations sympodial. Conidia branched, with axis tapering towards the apex, bearing single branch orientated downwards. Conidial apices with simple or branched extensions.

Colony growth moderately fast, reaching 20-25 mm diam. after 10 days at 15°C; aerial mycelium lanose, high, greyish, in one isolate blackish, margin paler, lacerate, reverse dark grey. Hyphae 1-3 µm wide, glabrous or with rough walls, inflated cells up to 8 µm wide. In older cultures prismatic crystals are produced. Sporulation under water within 2 days in aerated cultures and after 4-5 days also in standing water. Conidiophores typically terminal and then integrated with the supporting hypha or rarely lateral and then measuring up to 40 × 4 µm, simple or with acrotonous branches. Conidiogenous cells terminal or lateral, single or in groups of 2-3, lageniform, polyblastic, 7-25 × 2.5-4 µm, elongations few, sympodial, scars on denticles. Conidia in groups, acrogenous, closely sequential, branched. Conidial axis straight or slightly curved, fusoid or narrow obclavate, dorsiventral, 18-35 × 2-3 µm (length measured from the base to the point of branching of the extension; the entire length of conidia with unbranched apical extension being up to 52 µm), 3-4-septate, cells uninucleate, in older conidia often markedly inflated; conidial axis merging with mostly furcate, rarely three-pronged or simple extension, conidial base truncate, sometimes with a parabasal, simple or furcate, extension. Conidial branch single, ventral, strongly retrorse, cylindrical or sinuose, 4-9(-12) × 1-2.5 µm (with unbranched apical extension entirely up to 30 µm long), insertion often unilaterally more or less constricted, apex with a simple, furcate or three-pronged extension. All extensions are cellular, straight to slightly curved, setose, with broad insertion, in mature conidia often devoid of cytoplasm, 7-21 µm long when measured from the point of branching. Conidial secession schizolytic.

Microconidial synanamorph (andromorph ?) hyphomycetous, on hyphae, on macroconidiophores or on macroconidia. Conidiophores semimacronematous, simple, terminal or lateral, up to 48 mm long, sometimes micronematous. Conidiogenous cells phialidic, acrogenous or lateral, single or grouped, dolii- or lageniform, 4-11 × 2-3.5 µm, periclinal thickenings present, collarette cup-shaped, up to 1.5 µm long, often hardly discernible on sporulating phialides but distinct on spent ones, rarely absent. Conidia grouped at the phialide apex, short ellipsoidal with truncate base, unicellular, glabrous, 2-3 × 1.5-2 µm. Germination not seen.

Pure cultures: UMB-48.01, UMB-88.01 (both with microconidial state); UMB-200.01, UMB-206.01. All are monoconidial isolates from foam at S7.

The microconidial state appears several days after the onset of conidiation. Macroconidia tend to swell considerably when floating on water surface in standing water and then they usually produce the microconidial state from intercalary cells of the conidial axis.

The branching of setose conidial extensions varies. Most often they are forked, less frequently unbranched and exceptionally three-pronged. Four combinations of the forked and unbranched extensions may be possible: (1) both axis and branch extensions forked, (2) both axis and branch extensions simple, (3) axis extension simple, branch extension forked, (4) axis extension forked, branch extension simple. The isolates may differ by the proportion of conidia with each combination (100 randomly chosen conidia per isolate counted). Three isolates have 90-95 percent of conidia with the combination 1. Markedly different is UMB-200.01, which has *ca.* 55 percent of conidia with the combination 1, *ca.* 30 percent with the combination 2, and the remaining conidia with combinations 3 or 4.

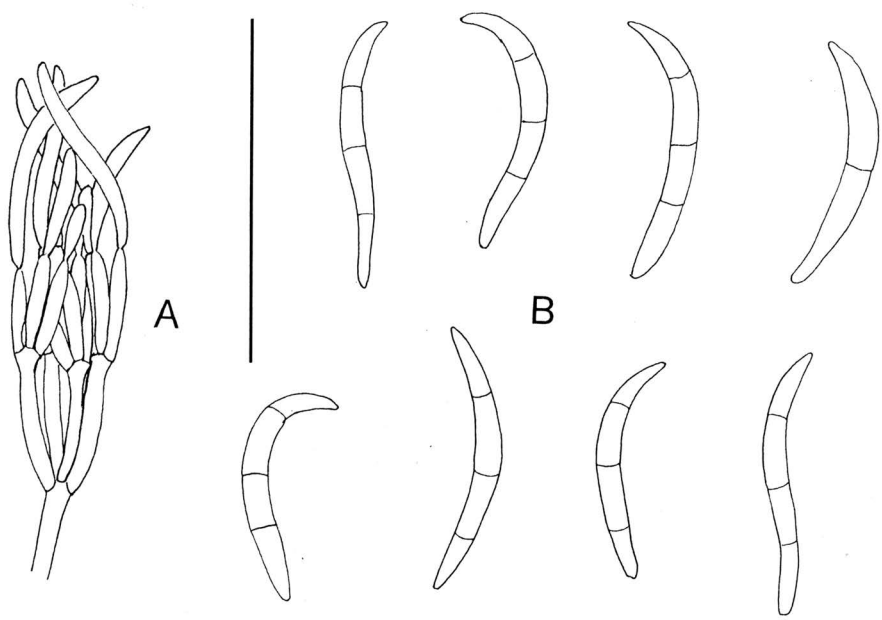

Fig. 3. *Flagellospora curta* UMB-39.01: A. Conidiophore with conidia. B. Detached conidia. Scale bar = 50 µm.

As far as we know, this taxon has neither been described nor reported as unknown conidia, but a similar fungus with unbranched extensions was isolated in the Czech Republic (Marvanová, unpubl.). Its conidia bear some resemblance to those of *Ramulispora bromi* (R. Sprague) R. Sprague, but the latter is a pathogen on grasses, its conidia are mostly unbranched and lack extensions.

RARE SPECIES IN PURE CULTURES

Flagellospora curta J. Webster, *Nova Hedwigia* 56: 456, 1993. Fig. 3

Colony cinnamon brown, aerial mycelium abundant, funiculose, exudate honey to cinnamon. Reverse sepia, reddish brown extracellular pigment produced in agar. Hyphae hyaline or brown, glabrous, elongate inflated cells in chains scarcely seen. Sporulation very profuse in aerated distilled water within 4 days at 20 ± 2°C. Conidiophores penicillate, stipes up to 260 × 3-7 µm, well developed penicilli 85-100 µm long, branches cylindrical, 12-25 × 2-2.5 µm, conidiogenous cells phialidic, lageniform, typically in groups, 12-16 × 1.8-2 µm, collarette indistinct, up to 1 µm long. Conidia long fusoid, apically often somewhat rostrate, curved or sigmoid, 21-40 × 2-4 µm, with 1-3 septa, base truncate or rounded.

Pure culture: UMB-39.01, from leaf baits at S2.

Other cultures examined: HME 4390 (ex-type) and HME 4374, both isolated by J. Webster.

This fungus is not easy to recognise even in pure culture. There are two similar species described: *Flagellospora penicillioides* Ingold and *F. minuta* S.H. Iqbal & Bhatty. The conidial length, seen in the ex-type culture of *F. curta*, i.e. 14-45 × 3-4 µm, overlaps with that of *Flagellospora penicillioides* but the conidia of the latter are only 2-2.5 µm wide, typically with a single septum. *F. minuta* has conidia similar to those of *F. penicillioides*, but with up to three septa. The conidiophores are similar in all three species, ranging from short and branched near the base to robust with distinct stipes over 200 µm long and up to 5 µm wide. The ex-type culture of *F. curta* differs from our isolate by its more curved, slightly broader conidia with rounded apices. Conidial apices in our isolate are rather subulate and conidia seem to be morphologically intermediate between those of *F. penicillioides* and *F. curta*, but in our opinion more similar to the latter. In the absence of teleomorph (*Nectria curta* J. Webster) or molecular taxonomic studies it is difficult to make an unequivocal identification. *F. minuta* is not well known and the type specimen is not available for study. As far as we know, *F. curta* has not been reported since its description.

Our culture was derived from leaf baits in a heavily polluted stream, whereas the type specimen developed on corticated *Fraxinus* twigs found floating in an unpolluted river in England.

Geniculospora grandis (Greath.) Nolan, *Mycologia* 64: 1173, 1992 Fig. 4

Colonies pale olivaceous grey, aerial mycelium abundant, lanose, high, reverse olivaceous black. Hyphopodia rare. Sporulation on culture pieces aerated for 4 days at 18°C and then incubated in standing distilled water, under or at water level. Conidiophores simple, mostly terminal and then integrated, up to 4 µm wide. Conidiogenous cells integrated, proliferations percurrent. Conidia single, terminal, branched (tetraradiate), conidial elements cylindrical, 4-5 µm wide, multiseptate, cells sometimes slightly inflated, stalk straight, 31-46 µm long, branches 3 (4), terminal, sequential, diverging, straight or gently curved, 48-98 µm long, insertion strongly constricted.

Pure cultures: UMB-176.01 from S4 and UMB-198.01 from S1, both from foam.

This species was for more than 20 years known only from the African continent: Greathead (1961), South Africa, without pure culture, Descals *et al.* (1984), isolated from apothecia of *Hymenoscyphus africanus* Descals, P.J. Fisher & J. Webster developed in the laboratory on leaf detritus collected in Malawi, and Webster *et al.* (1994), South Africa, as conidia in foam. In 1989, conidia were isolated from stream foam in Thailand (Marvanová & Hywel-Jones 2000). The Thai isolate has much longer conidial elements than that from Malawi. Conidia assignable to *G. grandis* were also reported from Zimbabwe (Ingold 1960, Fig. 1D, as unknown), and from Swaziland (Ingold 1973, Fig. 1C, as *Articulospora* sp.).

Our present isolates from Portugal are the first records from a temperate climate, confirmed in pure cultures. Very recently, conidia of this species in stream foam in central Spain were reported by Descals & Rodríguez Pérez (2002). The preceding reports by Mil´ko 1965, Ukraine, Czeczuga *et al.*. 1990, Poland, and Sridhar & Bärlocher 1993, Canada (for details see Marvanová & Hywel-Jones 2000). were not documented by voucher specimens. They were suggested doubtful. The confirmation of this species in Northwest Portugal enhances the credibility of previous reports from cooler climates.

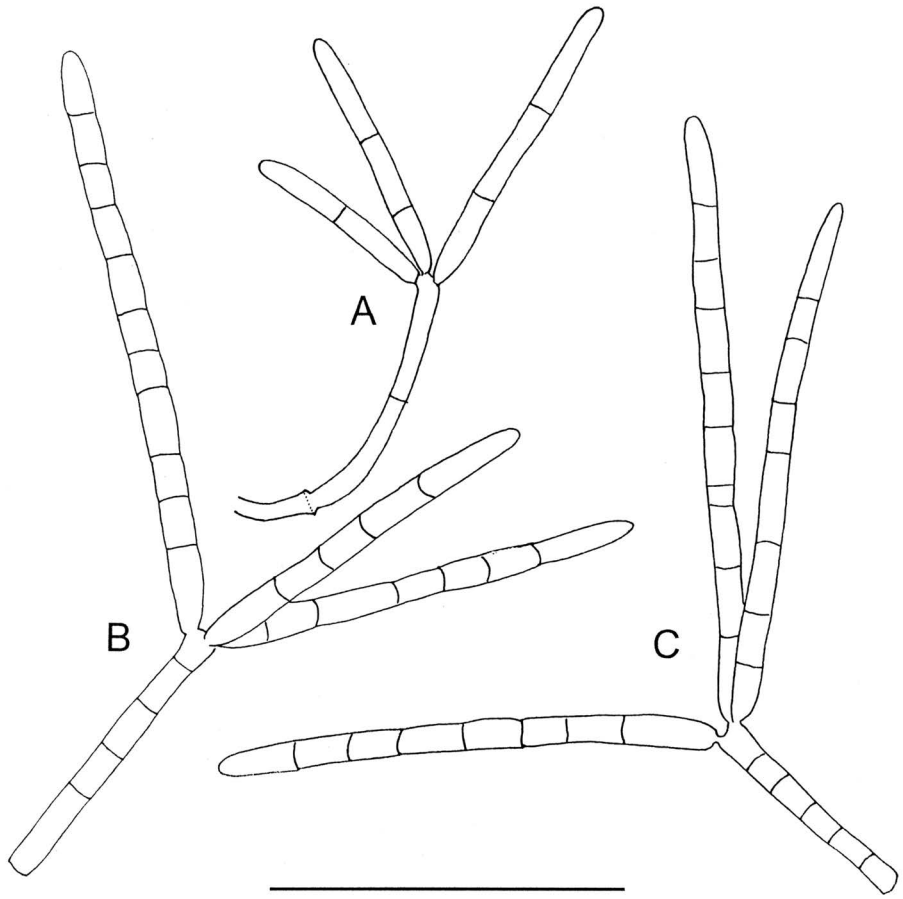

Fig. 4. *Geniculospora grandis* UMB-198.01: A. Developing conidium. Note the percurrent proliferation of the conidiogenous cell. B,C. Detached conidia. Scale bar = 50 μm.

Heliscus submersus H.J. Huds., *Trans. Br. Mycol. Soc.* 44: 91, 1961. Fig. 5

Colony growing fast, pale or cinnamon, exsudate honey to cinnamon. Reverse sepia or pale; reddish brown extracellular pigment is produced in agar in one isolate. Aerial mycelium abundant, funiculose, consisting of hyaline and/or brown hyphae, which may be up to 15 μm wide, smooth, with slightly thickened walls. Chlamydospores single, in chains or clusters, more or less globose or irregular, 15-35 × 14-22 μm, with slightly thickened smooth walls. Sporulation profuse in aerated distilled water at 20 ± 2°C after 3 days. Conidiophores penicillate, stipes 50-320 × 2-4 μm, penicillus consisting of 1-3 verticils of primary branches 13-16 × 2.8-3.5 μm, which may produce secondary branches. Each branch bears 2-4 phialidic lageniform conidiogenous cells 11-15 × 1.5-2 μm, without collarettes but with distinct periclinal thickenings. Conidia straight or slightly curved, long-clavate, furcate at the apex 19-40 × 2.5-4 μm below the fork, with 1 distinct and 2-3 indistinct

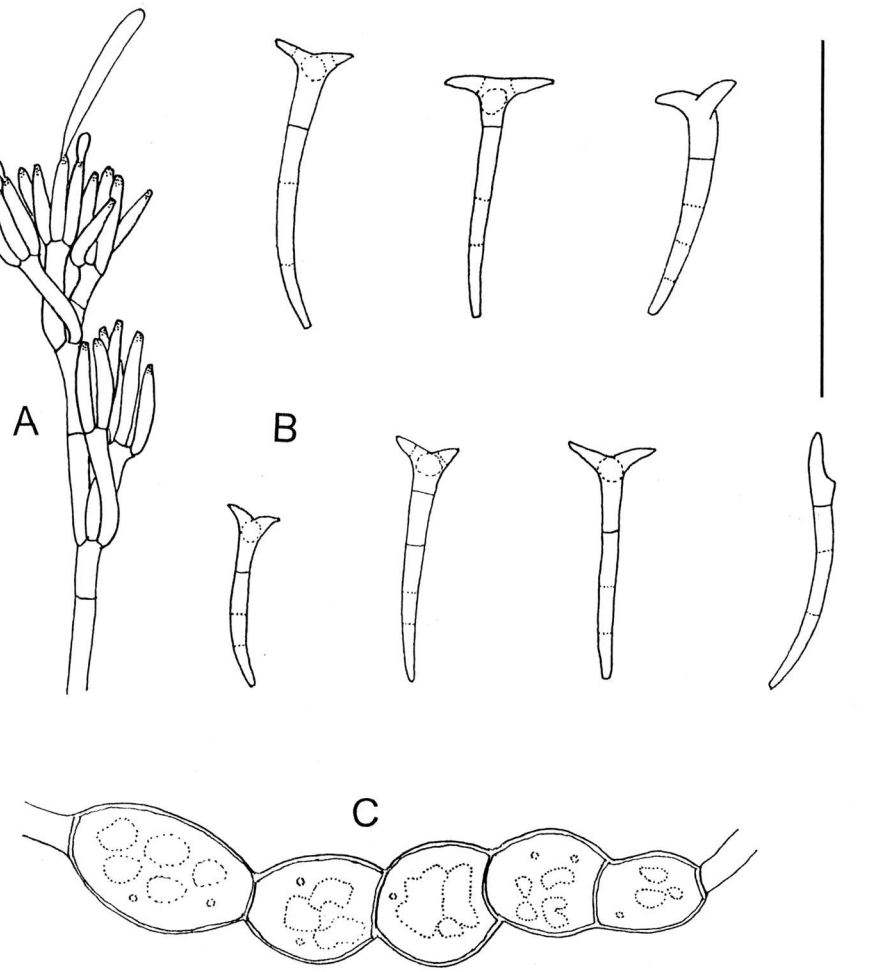

Fig. 5. *Heliscus submersus* UMB-135.01: A. Conidiophore with spent phialides and one developing conidium. B. Detached conidia. C. Chain of inflated cells. Scale bar = 50 μm.

septa. Span of the fork is 7-15 μm, the conoid outgrowths are 3-8 μm long. Usually there is a vacuole in the apical part of the conidium.

Pure cultures: UMB-1.99 from S3 and UMB-135.01 from S2, both from leaf baits.

Our isolates differ slightly from the type, isolated in Jamaica, by the conidial size as well as the shape: the conidial length in the type is up to 51 μm, the apical outgrowths are slightly longer and a single septum was reported below the fork.

This species appears on lists of fungi from streams of tropical and subtropical countries in both Hemispheres. Our isolates give more credibility to previous reports of conidia or isolates from European countries, which are not

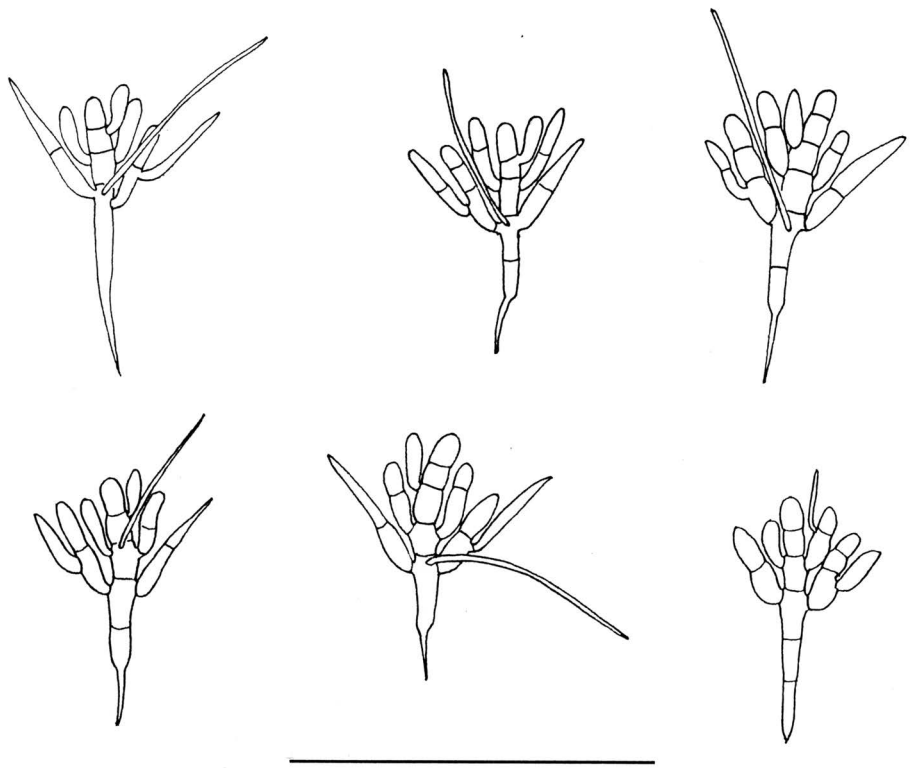

Fig. 6. *Tetracladium palmatum* UMB-180: Detached conidia. Scale bar = 50 µm.

documented by voucher specimens or at least descriptions (e.g. England: Dalton *et al.* 1970; Austria: Messner & Oberzill 1974; Ukraine: Dudka 1973). Arnold (1968a, 1968b) reported this species from Germany as conidia from foam or growing on submerged leaves, respectively. The first record is accompanied by a drawing (Arnold 1968a, Fig. III,8), which shows conidia smaller, but in shape true to type.

Both of our isolates were obtained from polluted waters (in one case strongly polluted), whereas the collections from the Tropics and Subtropics are mostly from presumably clean mountain streams; an exception is the record from the Nile River, whose water has higher content of organic matter as well as of soluble salts (Khalil *et al.* 1993). There are strikingly few drawings of *H. submersus* conidia from its main distribution area, i.e. tropical and subtropical regions.

Tetracladium palmatum A. Roldán, Descals & Honrubia, *Mycol. Res.* 93: 460, 1989. Fig. 6

Colony whitish, adpressed, reaching 23 mm diam. after 15 days at 15°C, reverse colourless. After several days of submergence in diffuse day light the colony becomes pale saffron. Sporulation abundant in standing distilled water after 2-3 days. Conidiophores simple or sparsely branched, up to 27 µm long, coni-

diogenous cells integrated, polyblastic, sympodial, with scars on denticles, conidia grouped, acrogenous, axis subclavate, bearing primary branches at three levels, broader elements in one plane, filiform branch diverging, usually at the lowermost level of branching, sometimes at the second level or occasionally as a secondary branch. Conidial span is 17-38 (-45) × 17-32 µm.

Pure culture: UMB-180.01, from foam at S4.

This species was reported as unknown from a stream in Spain by Roldán *et al.* (1987a, Figs. 2 L-N and 3 D) and Roldán *et al.* (1987b, Fig. b), and then described, also from Spain, by Roldán *et al.* (1989). Since then conidia were recorded scarcely in stream foam in Austria (Voglmayr 1996, Marvanová & Gulis 2000), in the Czech Republic (Marvanová 2002), although relatively frequently from Spain (Descals & Rodriguez Pérez 2002). The water chemistry on the Spanish localities was not given, but the other published collections are from softwater streams with oligotrophic water.

Tricladiopsis flagelliformis Descals, *Trans. Br. Mycol. Soc*. 78: 418, 1982. Fig. 7

Colony dark grey, aerial mycelium in the centre cottony, isabelline, margin submerged, fimbriate. Substrate mycelium fuscous to dark brown, reverse black with fuscous margin. Dark thick-walled "hyphopodia" (or chlamydospores ?) form sporadically. Sporulation under aeration after 5 days, in standing water tardy, after 4 weeks, at water level. Conidiophores short, lateral, simple, 24-31 × 3-3.5 µm, conidiogenous cells sympodial, scars flat, on denticles. Conidia solitary or in small groups of 2-3, densely septate. Axis straight or gently curved, narrow-obclavate, 110-190 × 3-3.5 µm, distal part attenuate, base truncate. Branches 0-2 and then alternate, orientated antrorsely, retrorsely or perpendicular to the axis, broadly diverging, 12-67 × 1.5-2 µm, cylindrical, insertion more or less constricted. Distance between 2 branches 31-38 µm. In standing water most of the conidia develop as branchless.

Pure culture: UMB-210.01, from foam at S4.

Our isolate differs from the type by the absence of stromata, reported in the ex-type culture. Conidia of the Portuguese isolate exceed in their maximum length those described in the protologue by nearly 1.5 times.

This is a rare species, the description being based on an isolate from ascospores of *Coccomyces* sp. Another specimen (isolate ?) from foam (as "isotypus", but from another locality) is mentioned in the protologue (Descals & Webster 1982a). As far as we know, since then no further pure cultures of this species have been reported. There are several collections of detached conidia from Spain (Descals & Mánjón 1989, Descals & Rodríguez Pérez 2002) and two records from Portugal: streams in Serra de São Macário and in Mata da Margaraça (Descals & Rodríguez Pérez 2002). Another collection was published from the Indian Himalaya (Sati *et al.* 2002 Fig. 13 and Pl.1 Fig. 6).

Tricladium terrestre D. Park, *Trans. Br. Mycol. Soc*. 63: 180, 1974. Figs. 8,9

Colonies dark grey, cottony to softly floccose, aerial mycelium abundant, grey, with ropes in the elevated paler centre, indistinctly zonate in the flat part of the colony, margin fimbriate, darker grey, reverse black. Both substrate and aerial hyphae fuscous, those in ropes brown with slightly thickened, glabrous or rarely roughened walls. Hyphal coils present on aerial mycelium. Sporulation after 2 days under aeration or after 6 days in standing distilled water, at the water level. Conidiophores terminal or more often lateral, simple or sparsely branched, 2-5 µm wide. Conidiogenous cells terminal or lateral, single or in groups of 2-3, irregularly

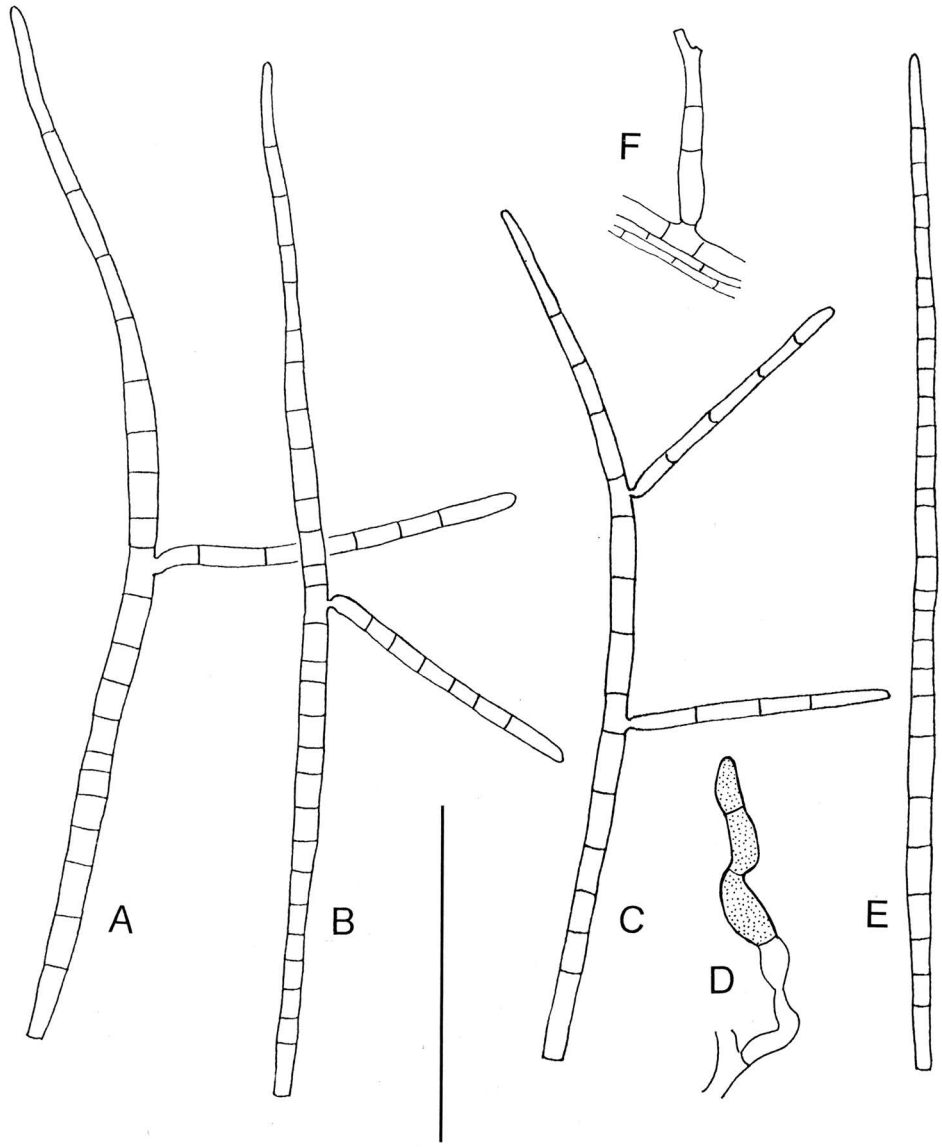

Fig. 7. *Tricladiopsis flagelliformis* UMB-210.01: A,B,C,E. Detached conidia. Note the varying number of branches. D. Dark hyphopodium-like lateral outgrowth on hypha. F. Short lateral conidiophore with terminal conidiogenous cell proliferating sympodially. Scale bar = 50 μm.

geniculate, elongations sympodial, scars sometimes indistinct. Conidia acrogenous, single or in groups of up to 4, elements slightly tapering distally, densely septate, with smooth or barely sinuous outline, sometimes fuscous with age, cells often sub-inflated. Conidial axis gently curved or slightly geniculate at branch insertion or

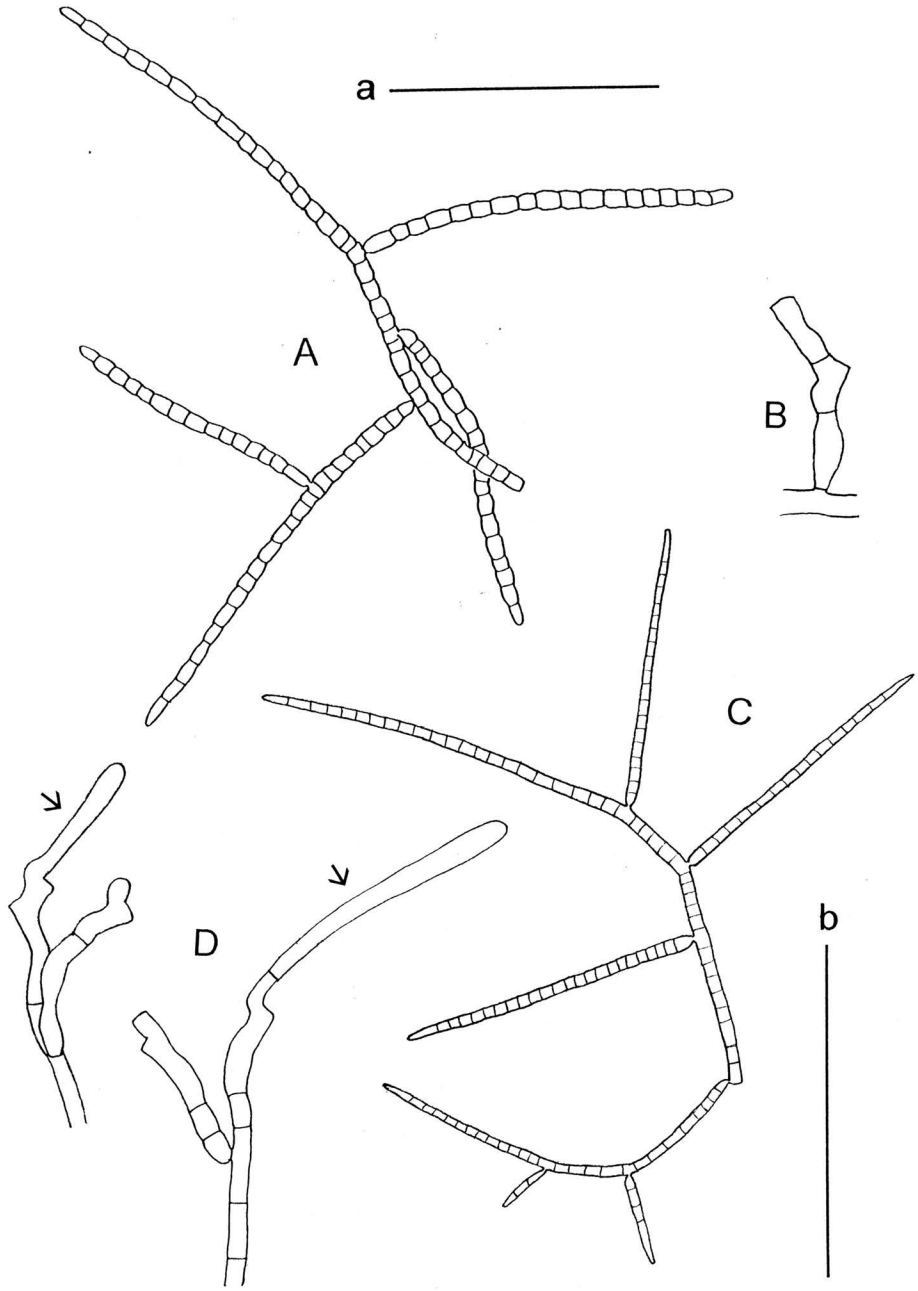

Fig. 8. *Tricladium terrestre* A. Detached conidium with slightly inflated cells. Note the secondary branch. B. Short lateral conidiophore. C. Detached primary conidium with secondary conidium on the basal cell of the axis. D. Sparsely branched conidiophores with conidial initials (arrows). A = UMB-181.01: B-D = UMB-89.01. Scale bar a = 100 μm (Figs. A,C); scale bar b = 50 μm (Figs. B,D).

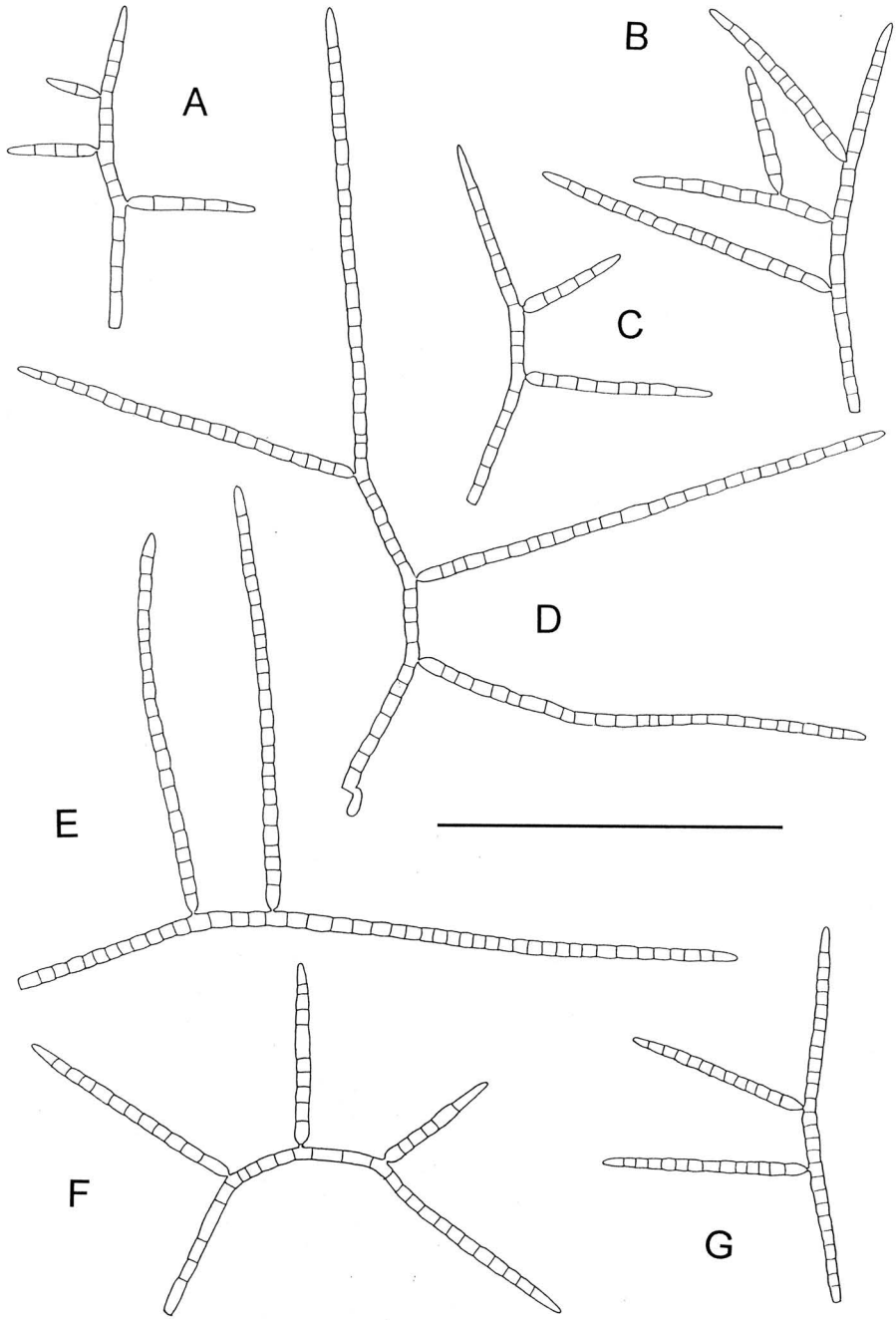

Fig. 9. *Tricladium terrestre.* Detached conidia. A,C. From culture aerated for 3 days. The rest from standing water. A,C = UMB-181.01; B = UMB-179.01; D,E,G = UMB-83.01; F = UMB-89.01. Scale bar = 100 μm.

rarely straight, (67)118-283 × 3-4.5 (up to 5.5 µm when cells are inflated), base truncate, occasionally with short eccentric extension, distal end subulate, primary branches usually unilateral, alternate, (0)1-4, broadly divergent, 14-33 µm apart, appearing in basifugal sequence, (15)26-180 × 3-4.5 µm, the distal ones shorter, secondary branches rare, typically on the proximal primary arm, 15-103 × 3-4.5 µm, branch bases short obconic or more or less rounded when swollen, ends subulate or (in inflated conidia) rounded. Secondary conidia occasional, near the basal scar of the primary ones.

Pure cultures: UMB-83.01, UMB-89.01, UMB-181.01 from S4 and UMB-146.01, UMB-179.01 from S1, all from foam.

Other material examined: Type of *Tricladium marylandicum* (**NY**).

Tricladium terrestre was originally described in North Ireland from terrestrial leaf litter of *Quercus* and *Prunus* submerged in the laboratory (Park 1974). Pure culture was established from the type material (Park, 1974), and later by Descals & Webster (1982b) from conidia collected from foam in Scotland. The latter authors pointed out the similarity and overlap with conidia of *T. castaneicola* B. Sutton in pure culture. They also discovered a phialidic microconidial state in their isolate of *T. terrestre,* which was not reported in the protologue and has not been seen by us even in aged cultures. The conidial axis in our isolate was longer by one third than reported by Descals & Webster (1982b, up to 220 µm), but approximated the length observed by Park (1974, up to 280 µm). The width of conidial axis given by Park is 4-5 µm, but according to Descals & Webster (l.c.) in their isolates it is 4-6 µm (on their Fig. 11 according to the scale only 4.5 µm). The conidial branch bases in our material vary between short-obconic to more or less rounded (rounder then drawn in Park or by Descals & Webster) but their appearance in phase contrast microscopy is very similar to that shown by the latter authors (Descals & Webster 1982b, Fig. 18). The conidial cells often swell soon after conidium release (after 3 day aeration), which was not mentioned by Descals & Webster or Park, but illustrated by the latter (Park 1974, Fig 2 A).

The conidial size in our isolates differed depending on conditions for sporulation: conidia in aerated cultures were in general smaller and had shorter branches, e.g. the isolate UMB 181.01 aerated for 3 days had the axis 72-125 µm long, the branches 17-55 µm long, the distance between branches was 14-24 µm, whereas the same culture when left for other 2 days in standing water produced conidia with axis up to 242 µm long, branches up to 146 µm and the distance between branches was 24-33 µm. The difference in branch distance cannot be explained by post-secessional apical growth of conidial ends known to change considerably the conidial size in some species (Marvanová 1997). It can be speculated that at least in this case standing water (less mechanical disturbance during the conidiogenesis resulting in larger conidia ?) may act as a morphogenic factor.

There are three other species in some way resembling our isolates: *Tricladium indicum* Sati & N. Tiwari, *T. marylandicum* J.L. Crane, and the anamorph of *Hymenoscyphus varicosporoides* Tubaki. All produce dark colonies and have large conidia with constricted branch insertion and rounded branch bases. However, conidia of *T. indicum* and of *Hymenoscyphus varicosporoides* have much longer elements, with cells 10-12 and 7-7.5 µm wide, respectively (Tubaki 1966, Sati & Tiwari 1992). According to the protologue, *Tricladium marylandicum* approximates our isolates in size of conidia (axis 135-265 × 3.9-6.5 µm), but the type material contains conidia with axis over 300 µm long and the conidial elements are parallel-walled. Details of conidiogenesis are not given, the illustration (Crane 1968, Fig. 3 A,B) shows a probably terminal conidiophore with integrated

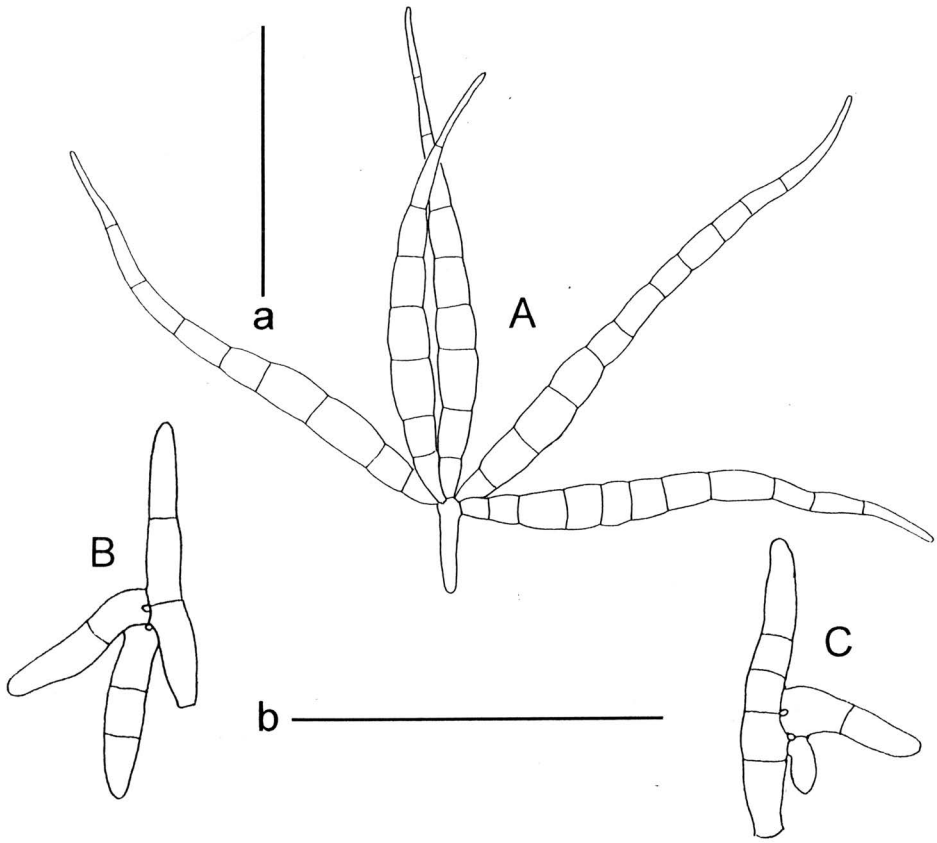

Fig. 10. Conidia from foam. A. *Flabellospora amphibia*. B,C. *Titaeella capnophila.* Note the primary and secondary branches each arising from clamp on the parent element. Scale bar a = 50 µm (Fig. A); scale bar b = 50 µm (Figs. B,C).

conidiogenous cell bearing an integrated conidial primordium. In the type material no conidiogenous cells could be found.

On the basis of the above considerations we conclude that our isolates are assignable to *Tricladium terrestre* as defined by Park 1974 and Descals & Webster (1982b).

There are four records of conidia of *T. terrestre* from Portugal (Descals & Rodríguez Pérez (2002): from streams in Serra de São Macário, Serra do Caramulo, Serra da Estrela and Mata da Margaraça, unfortunately not accompanied with illustrations and therefore no comparison with our specimens is possible.

No information on pure cultures appears in the following reports of *T. terrestre*: Chamier *et al.* (1984), U.K., revealed from minute leaf square platings from leaf packs of *Alnus* exposed in a stream; Swart (1986), Australia (substrate not given); Metwalli & Shearer (1989), Illinois, USA, on *Quercus* leaf packs submerged in a medium hard to hard stream water; Thomas *et al.* (1992), Australia,

on naturally colonized *Acacia* bark from a stream; Voronin (1993) Russia (Karelian Republic), on dead *Nuphar luteus* plants in a lake; Tóth (1994), Hungary (substrate not given); Garnett *et al.* (2000), New Brunswick, Canada, on *Acer* leaf packs submerged in a softwater stream; Descals & Rodríguez Pérez (2002), Spain, stream foam.

DETACHED CONIDIA IN FOAM

Flabellospora amphibia (I.P. Price & P.H.B. Talbot) Descals, *Trans. Br. Mycol. Soc*. 78: 414, 1982. Fig. 10 A

This species was described from South Australia as *Tetracrium amphibium* I.P. Price & P.H.B. Talbot. Originally it was collected on bark and wood of *Eucalyptus* sp. in terrestrial conditions, but the authors also saw the conidia in streams (Price & Talbot 1966). A second record is from dead insects in a pond in Uzbekistan (Sagdullaeva *et al.* 1989). As far as we know, it has not been obtained in pure culture. We found a single conidium in a foam sample at S4.

Titaeella capnophila K. Ando & Tubaki, *Trans. Mycol. Soc. Japan* 26: 155, 1985. Fig. 10 B,C

Originally this species was invalidly published by Arnaud (1951) in France, in association with sooty moulds. The name was validated by Ando & Tubaki (1985). Pure cultures were established by these authors (l.c.) from conidia collected in rainwater dripping off *Pinus densiflora* in Japan and later by Descals (1997) from foam in a Spanish mountain stream with broad-leaved trees and shrubs and conifers on the banks. Our collection is from foam at S1 with *Pinus pinaster* at close vicinity.

Acknowledgements. This work was supported by a fellowship from NATO Science Programme (L. Marvanová) and by the Portuguese project grant POCTI/34024/BSE/2000. The authors thank Dr. F. Baltazar and one of the reviewers for language corrections. The loan of type material of *T. marylandicum* from **NY** and the deposition of *F. curta* isolates by J. Webster in the Czech Collection of Microorganisms are acknowledged. We appreciate the numerous critical comments and suggestions of one of the reviewers; however, in some points we preferred our own view.

REFERENCES

ANDO K. & TUBAKI K., 1985 — Three new hyphomycetes from Japan: *Anthopsis microspora, Scutisporus brunneus* and *Titaeella capnophila*. *Transactions of the Mycological Society of Japan* 26: 151-160.

ARNAUD G., 1951 — Les boucles mycéliennes des Eumycètes et la phylogenie des Uredinées. *Bulletin de la Société mycologique de France* 67: 196-198.

ARNOLD G.R.W., 1968a — Beiträge zur Kenntnis der Pilzflora Thüringens. I. Aquatische Hyphomyzeten. *Mykologisches Mitteilungsblatt* 13: 11-18.

ARNOLD G.R.W., 1968b — Sporen aquatischer Hyphomyzeten aus Gewässern im Bezirk Halle. *Wissenschaftliche Zeitschrift der Friedrich-Schiller-Univ. Jena, Mathematisch-Naturwissenschaftliche Reihe* 17: 369-373.

CHAMIER A.-C., DIXON P.A. & ARCHER S.A., 1984 — The spatial distribution of fungi on decomposing alder leaves in a freshwater stream. *Oecologia (Berlin)* 64: 92-103.

CRANE J.L., 1968 — Freshwater hyphomycetes of the Northern Appalachian Highland including New England, and three Coastal Plain states. *Americal Journal of Botany* 55: 996-1002.

CZECZUGA B., ORLOWSKA M. & WORONOWICZ L., 1990 — Studies of aquatic fungi. X. Some rare species Hyphomycetes in North-Eastern Poland. *Acta Mycologica* 25: 5-20.

DALTON S.A., HODKINSON M. & SMITH K.A., 1970 — Interactions between DDT and rare fungi I. Effects of p,p´-DDT on the growth of aquatic hyphomycetes. *Applied Microbiology* 20: 662-666.

DESCALS E., 1997 — Ingoldian fungi from the Catalan Pyrenees: pure culture studies. *Mycotaxon* 63: 431-466.

DESCALS E. & MÁNJÓN J.L., 1989 — Hongos acuáticos ingoldianos en la Sierra de Guadarrama. *Boletín de la Sociedad Micológica de Madrid* 13: 11-26.

DESCALS E. & RODRÍGUEZ PÉREZ J. 2002 — Bases corológicas de Flora Micológica Ibérica. Números 1933-2069. In: F. PANDO (ed.) Cuadernos de Trabajo de Flora Micológica Ibérica 18, 195 pp.

DESCALS E. & WEBSTER J., 1982a — Taxonomic studies on aquatic hyphomycetes III. Some new species and a new combination. *Transactions of the British mycological Society* 78: 405-437.

DESCALS E. & WEBSTER J., 1982b — Taxonomic studies on aquatic hyphomycetes IV. Pure culture and typification of various species. *Transactions of the British mycological Society* 79: 45-64.

DESCALS E., FISHER P.J. & WEBSTER J., 1984 — The *Hymenoscyphus* teleomorph of *Geniculospora grandis*. *Transactions of the British mycological Society* 83: 541-546.

DUDKA I.O., 1973 — Aqueous hyphomycetes from the Ukraine and new for mycoflora of the Soviet Union (in Ukrainian). *Ukrainskii Botanicheskii Zhurnal* 30: 449-456.

GARNETT H., BÄRLOCHER F. & GIBERSON D., 2000 — Aquatic hyphomycetes in Catamaran Brook: colonization dynamics, seasonal patterns, and logging effects. *Mycologia* 92: 29-41.

GREATHEAD S.K., 1961 — Some aquatic hyphomycetes in South Africa. *Journal of South African Botany* 27: 195-228.

INGOLD C.T., 1960 — Aquatic hyphomycetes in Southern Rhodesia. *Proceedings and Transactions of the Rhodesia Scientific Association* 48: 49-53.

INGOLD C.T., 1973 — Conidia of aquatic hyphomycetes from Swaziland. *Transactions of the British mycological Society* 61: 607-609.

KHALIL A.M., EL-HISSY F.T. & ABDEL-RAHEEM A., 1993 — Monthly variations of Oomycetes (zoosporic fungi) and aquatic hyphomycetes at Sohag (Upper Egypt). *Acta societatis botanicae Poloniae* 62: 67-73.

MARVANOVÁ L., 1997 — Freshwater hyphomycetes: a survey with remarks on tropical taxa. In: K.K. Janardhanan, C. Rajendran, K. Natarajan & D.L. Hawksworth (eds.) Tropical Mycology. Science Publishers, Inc, U.S.A., p. 169-226.

MARVANOVÁ L., 2002 — Streamborne fungal spora in running waters of the Bohemian Forest. *Silva Gabreta* 7: 147-164.

MARVANOVÁ L. & GULIS V.I., 2000 — Notes on aquatic hyphomycetes and streamborne spora from Austria. *Österreichische Zeitschrift für Pilzkunde* 9: 125-140.

MARVANOVÁ L. & HYWEL-JONES N.L., 2000 — *Sigmoidea contorta* and two rare hyphomycete species from streams in Thailand. *Cryptogamie, Mycologie* 21: 13-26.

MARVANOVÁ L., PASCOAL C. & CÁSSIO F., 2002. — New and rare hyphomycetes from streams of Northwest Portugal. *3rd International Meeting on "Plant Litter Processing in Freshwater", September 7-12, 2002, Szentendre, Hungary.* Abstract, p. 35.

MESSNER K. & OBERZILL W., 1974 - Aquatische Pilze in Fliessgewässern. *Zentralblatt für Bakteriologie 240, 1. Referate,* p. 403-406.

METWALLI A.A. & SHEARER C.A., 1989 — Aquatic hyphomycete communities in clear cut and wooded areas of an Illinois stream. *Transactions of the Illinois Academy of Science* 82: 5-16.

MIL´KO A.A., 1965 — Fungi isolated from water of the Soviet part of the Danube. *Mikrobiologicheskii Zhurnal* 27: 38-44.
MIURA K. & KUDO M.Y., 1970 — An agar medium for aquatic hyphomycetes (in Japanese). *Transactions of the Mycological Society of Japan* 11: 116-118.
PARK D., 1974 — *Tricladium terrestre* sp. nov. *Transactions of the British mycological Society* 63: 179-184.
PASCOAL C., CÁSSIO F. & GOMES P., 2001 — Leaf breakdown rates: a measure of water quality? *International Revue of Hydrobiology* 86: 407-416.
PRICE I.P. & TALBOT P.H.B., 1966 — An aquatic hyphomycete in a lignicolous habitat. *Australian Journal of Botany* 14: 19-23.
RAYNER R.W., 1970 — A Mycological Colour Chart. Commonwealth Mycological Institute Kew, 34 pp.
ROLDÁN A., DESCALS E. & HONRUBIA M., 1987a — Hifomicetos acuáticos en la Serranía de Cuenca. *Boletín de la Sociedad Micológica de Madrid* 11: 179-184.
ROLDÁN A., DESCALS E. & HONRUBIA M., 1987b — Notes on *Tetracladium apiense* Sinclair and Eicker. — *Cryptogamie, Mycologie* 8: 219-225.
ROLDÁN A., DESCALS E. & HONRUBIA M., 1989 — Pure culture studies on *Tetracladium. Mycological Research* 93: 452-465.
SAGDULLAEVA M.Sh., RAMAZANOVA S.S., KIRGHIZBAEVA Kh.M., GULIAMOVA M. & FAIZIEVA F.Kh., 1989 — Fungal Flora of Uzbekistan. Fan, Tashkent, 263 pp.
SATI S.C. & TIWARI A., 1992 — A new species of *Tricladium* from Kumaun Himalaya, India. *Mycological Research* 96: 229-232.
SATI S.C., TIWARI A. & BELWAL M., 2002 — Conidial aquatic fungi of Nainital, Kumaun Himalaya, India. *Mycotaxon* 81: 445-455.
SRIDHAR K.R. & BÄRLOCHER F., 1993 — Aquatic hyphomycetes on leaf litter in and near a stream in Nova Scotia, Canada. *Mycological Research* 97: 1530-1535.
SWART H.J., 1986 — A preliminary survey of aquatic hyphomycetes in Victoria, Australia. *Transactions of the British mycological Society* 86: 497-501.
THOMAS K., CHILVERS G.A. & NORRIS R.H., 1992 — Aquatic hyphomycetes from different substrates: substrate preference and seasonal occurrence. *Australian Journal of Marine and Freshwater Research* 43: 491-509.
TÓTH S., 1994 — Microscopic fungi of the Pilis and the Visegrád Mts, Hungary. *Studia botanica hungarica* 25: 21-57.
TUBAKI K., 1966 — An undescribed species of *Hymenoscyphus*, a perfect stage of *Varicosporium*. *Transactions of the British mycological Society* 49: 345-349.
VOGLMAYR H., 1996 — Spores of Ingoldian fungi in two Austrian rivulets. *Österreichische Zeitschrift für Pilzkunde* 5: 23-49.
VORONIN L., 1993 — Fungal complexes on dead plant substrata in small lakes of Karelia. (In Russian). *Proceedings of the Estonian Academy of Sciences, Biology* 42: 118-129.
WEBSTER J., MARVANOVÁ L. & EICKER A., 1994 — Spores from foam from South African rivers. *Nova Hedwigia* 57: 379-398.