Aquatic hyphomycete diversity in streams of Northwest Portugal

Cláudia Pascoal[1*], Ludmila Marvanová[2] and Fernanda Cássio[1]

[1]Department of Biology, University of Minho, Campus de Gualtar 4710-057, Braga, Portugal
[2]Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Tvrdého 14, 602 00 Brno, Czech Republic

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This work examines the distribution of aquatic hyphomycetes in streams of the Northwest Portugal with different environmental conditions. Five sites are in the Ave River basin in an area with high population density, intensive agriculture and industrial activities. Other three sites are in the Cávado River basin and belong to the Peneda-Gerês National Park. Despite this survey has been conducted within a small area and during a short period of time, high aquatic hyphomycete species diversity was found. A total of 113 fungal taxa were identified at least at the generic level, of which *ca*. 90% were classified as aquatic hyphomycetes in the traditional sense. Several rare aquatic hyphomycete species were found, of these five are reported here for the first time in the Iberian Peninsula and seven are new records in Portugal. The highest species richness was found at the spring of the Este River - Ave River basin (77 taxa) and in streams at the National Park (40-58 taxa). A decline in the richness of aquatic hyphomycete species was found at polluted sites of the Ave River basin (23-29 taxa). The distribution of aquatic hyphomycete species by cluster analysis and CA ordination opposed polluted and non-polluted sites, suggesting that water chemistry was the main factor regulating the structure of aquatic hyphomycete communities.

Key words: aquatic hyphomycetes, freshwater pollution, fungal diversity, Northwest Portugal.

Introduction

Aquatic hyphomycetes are a phylogenetically heterogeneous group of fungi occurring mainly in lotic waters. They are known to release a great amount of asexual (mitotic, anamorphic) spores (conidia) underwater with characteristic stauroform or scolecoform shapes, which facilitate the identification of species. However, in some cases the conidiogenesis must be observed to ensure unequivocal classification. Few aquatic hyphomycete taxa are known to have sexual (meiotic, teleomorphic) states, and the hitherto discovered anamorph/teleomorph connections show links mainly to

*Corresponding author: Cláudia Pascoal; e-mail: cpascoal@bio.uminho.pt

Ascomycota, lesser to *Basidiomycota* (Marvanová, 1997, 2002). This has been supported by recent studies of phylogenetic relationships based on homologies in rDNA sequences (e.g. Campbell *et al*., 2002; Nikolcheva and Bärlocher, 2002; Baschien, 2003; Bauer *et al*., 2003).

Aquatic hyphomycetes are the major fungal decomposers of leaf litter entering into streams and are an important trophic link between decaying leaves and stream invertebrates (Bärlocher, 1992). Numerous studies have demonstrated that the distribution of aquatic hyphomycetes is affected by physical and chemical characteristics of the stream water and riparian vegetation. On a worldwide scale, temperature together with its influence on vegetation in different climatic regions is the main factor determining aquatic hyphomycete distribution, whereas within smaller areas the chemistry of stream water appears to dominate (Wood-Eggenschwiler and Bärlocher, 1983). Altitude is also an important factor in structuring aquatic hyphomycete communities within a stream (Chauvet, 1991; Raviraja *et al.*, 1998a). Several studies have reported that softwater streams contain higher richness in aquatic hyphomycete species than hardwater streams (Wood-Eggenschwiler and Bärlocher, 1983; Harrington, 1997; Raviraja *et al*., 1998a; Gönczöl and Révay, 2003), although this is not always the case (Gönczöl *et al*., 2003). The increase of nitrogen and/or phosphorus concentration in oligotrophic freshwaters has been reported to raise species richness (Gulis and Suberkropp, 2003, 2004).

Human enterprises such as agriculture, urbanization and industry greatly affect rivers and streams, and pristine ecosystems are becoming scarce. Aquatic hyphomycetes are generally associated with clean and well-aerated freshwaters and are believed to be sensitive to pollution (Bärlocher, 1992). A decline in aquatic hyphomycete diversity has been found in streams affected by organic pollution (Raviraja *et al*., 1998b) or heavy metals (Bermingham *et al*., 1996; Niyogi *et al*., 2002). However, the occurrence of several aquatic hyphomycete species in heavily polluted streams has been reported (Sridhar *et al*., 2000; Krauss *et al*., 2001; Luo *et al*., 2004; Pascoal *et al*., 2003, 2005).

This work examines the distribution of aquatic hyphomycetes in streams of the Northwest Portugal and correlates its diversity with different environmental conditions. Conidia collected from foam in the field and/or released in laboratory from leaves, previously immersed in streams, were examined at eight sites with different water chemistry, altitude and riparian vegetation. Five sites are in the Ave River basin in an area with high population density, intensive agriculture and industrial activities. The other three sites are in the Cávado River basin and belong to the Peneda-Gerês National Park.

Material and methods

Sampling sites

Field studies were conducted in the Northwest Portugal at eight sites in streams of the Ave River and Cávado River basins (Fig. 1), where granitic rock dominate geological substratum. Three sampling sites (S5, S6 and S7) belong to the Cávado River basin and are included in the Peneda-Gerês National Park. The other five sampling sites (S1, S2, S4, S8 and S9) are located in the Ave River basin in an area with high population density, intensive agriculture and industrial activities. S5 is placed in the Ribeira da Laja and it is bordered by *Arbutus unedo, Quercus pyrenaica* and *Quercus robur*. S6 is located in the Maceira River and its riparian vegetation differs from S5 by the absence of *A. unedo* and the presence of *Chamaecyparis* sp. and *Ilex aquifolium*. S7 is located at São João do Campo and it is bordered by *Betula pendula*, *Pinus pinaster*, *Q. robur* and *Salix* sp. S1 is at the spring of the Este River, and S2 is 10 km downstream near the industrial park of the city of Braga, where contamination by heavy metals has been reported in both stream water (Gonçalves, 2001) and sediments (Soares *et al*., 1999). The dominant riparian vegetation at S1 is *Eucalyptus globulus, Juncus* sp. (also in the stream bed), *P. pinaster* and *Pteridium aquilinum*, while that at S2 is *Alnus glutinosa*, *Platanus hybrida*, *Populus tremula*, *Q. robur* and *Salix* sp. S4 is located in a tributary of the Este River bordered by *Vitis vinifera* at the village of Lamas, where agriculture is the main human activity. S8 is surrounded by *A. glutinosa*, *Populus alba*, *P. tremula*, *Rubus* sp. and *Salix* sp., and S9 is bordered by *Salix* sp. and *V. vinifera*; both sites are located in the Pelhe River in an agricultural area with some industrial activity. Further information about the characteristics of S1, S2, S4 and S7 is given in Marvanová *et al*. (2003).

Stream water analysis

Physical, chemical and microbial analyses of the stream water were performed in March 2001 and in January 2002. Water temperature, pH and conductivity were measured *in situ* with field probes (Multiline F, WTW). Stream water samples were collected in sterile glass bottles transported on ice and analysed within 24h. Total hardness was quantified using a kit (4824 DR-LT, LaMotte). A HACH DR/2000 photometer (Hach Company, Loveland, CO, USA) was used to measure chemical oxygen demand (COD) by dichromate digestion, nitrate concentration by cadmium reduction, ammonium concentration by the Nessler method and orthophosphate concentration by the

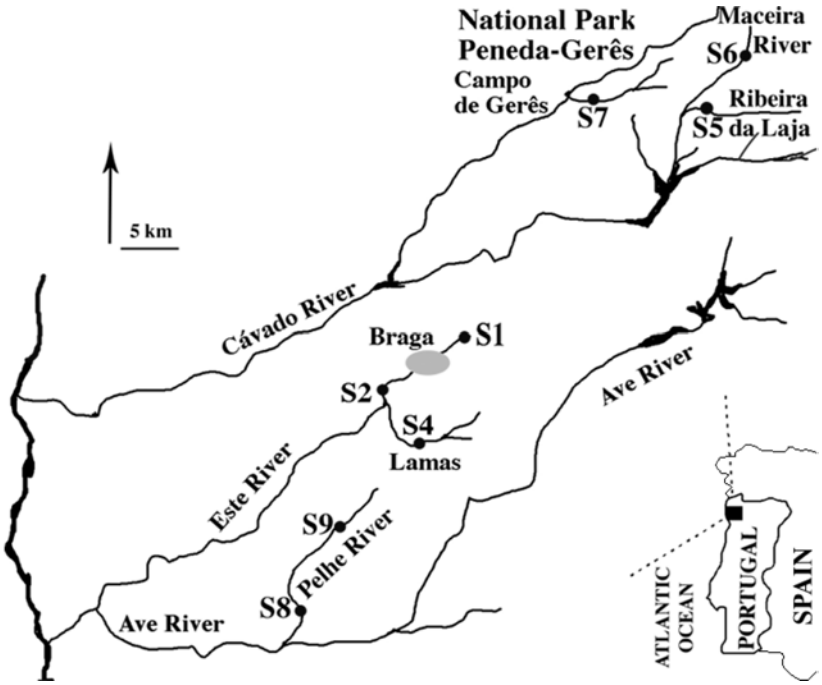

Fig. 1. Location of the sampling sites in the Ave River (S1, S2, S4, S8 and S9) and Cávado River (S5, S6 and S7) basins in the Northwest Portugal.

molybdate reagent, according to HACH manual. The colony-forming units (CFU) of total heterotrophs, total and fecal coliforms were quantified according to standard methods (APHA, 1998).

Aquatic hyphomycete sampling

In March 2001 and in January 2002, air-dried autumn shed leaves of *Alnus glutinosa* (alder) were weighed into 4 g-groups, placed in bags (16 × 20 cm, 0.5 mm mesh size) and submerged at each sampling site. After 15 days, three leaf bags were retrieved from each site, except at S4 due to bag losses. In the laboratory, the leaves were rinsed with deionised water to remove debris and cut into discs (12 mm diameter). To induce sporulation, 15 leaf discs were placed under aeration in Erlenmeyer flasks containing 40 mL of sterile deionised water for 48 ± 4 h at 18 ºC.

Fresh foam was collected at each site (see dates in Table 2) with a spoon and transferred to sterile glass bottles. Subsamples of foam were preserved with 70% ethanol, iced acetic acid and 37% formalin (16:3:1, v/v/v) for further examination. At sites where foam was not available (S2, S8 and S9), stream water was collected and foam formation was induced in the laboratory by

Table 1. Physical, chemical and microbial characteristics of sampling sites in the Ave River (S1, S2, S4, S8 and S9) and Cávado River (S5, S6 and S7) basins. Mean values of, at least, two independent measurements.

Parameters	**Ave River basin**					**Cávado River basin**		
	S1	**S2**	**S4**	**S8**	**S9**	**S5**	**S6**	**S7**
Altitude (m)	400	140	150	105	160	1050	1150	1100
Temperature (°C)	10.6	10.2	12.0	11.1	12.0	7.9	8.3	10.2
pH	6.0	6.0	6.2	6.2	6.1	6.6	6.2	5.4
Conductivity ($\mu S\ cm^{-1}$)	42	170	150	179	158	21	9	14
Total hardness ($mg\ L^{-1}$)	12	42	32	30	36	10	10	10
$N\text{-}NH_4^+$ ($\mu g\ L^{-1}$)	<8	249	544	117	334	<8	<8	<8
$N\text{-}NO_3^-$ ($\mu g\ L^{-1}$)	497	4968	3839	5194	5194	99	99	99
$P\text{-}PO_4^{3-}$ ($\mu g\ L^{-1}$)	<3	176	675	538	261	10	10	10
COD ($mg\ O_2\ L^{-1}$)	16	39	77	43	52	<1	<1	<1
Total heterotrophs ($CFU\ mL^{-1}$)	50	$1.4x10^5$	$8.2x10^5$	$4.3x10^5$	$1.1x10^5$	2	7	1
Total coliforms ($CFU\ mL^{-1}$)	60	$3.2x10^3$	$1.5x10^3$	$3.7x10^3$	$4.2x10^3$	<1	<1	<1
Fecal coliforms ($CFU\ mL^{-1}$)	<1	815	330	185	420	<1	<1	<1

CFU, colony-forming units.

adding 100 µL of 0.5% tween 80 per 1 L of stream water, in order to facilitate isolation of conidia.

Conidia present in fresh and induced foam, as well as released from leaf discs were inoculated onto thin layers of 1.5% water-agar and incubated at 18º C. The agar surface was scanned under the compound microscope for germinating spores and pieces of agar with a single conidium were cut out and transferred onto 1% malt extract agar (Difco), containing 0.5 $g\ L^{-1}$ streptomycin (Sigma). Samples of conidium suspensions from preserved foam and aerated leaves were mounted on microscope slides for identification of conidia. Some detached conidia could be identified only tentatively; these names are cited as "cf." Pieces of *ca*. 2-week-old pure agar cultures were submerged in either standing deionised water in Petri dishes or aerated deionised water in Erlenmeyer flasks, and then species were identified based on fertile structures.

Data analysis

Ordination of the sampling sites according to the stream water variables was done by Principal Component Analysis (PCA) after standardisation of the variables (Legendre and Legendre, 1998). Ordination of the sites based on the aquatic hyphomycete assemblages was done by Correspondence Analysis (CA, Legendre and Legendre, 1998). The similarity among sites based on aquatic hyphomycete assemblages was quantified by Jaccard coefficient and the resulting matrix was subjected to cluster analysis by Unweighed Pairgroup

Method Average (UPGMA, Legendre and Legendre, 1998). Both CA and UPGMA cluster analyses were based on species presence/absence. Multivariate statistical analyses were performed with ADE-4 for Macintosh (Thioulouse *et al.*, 1997).

The relationship between stream water variables and richness in aquatic hyphomycete species was examined by Spearman rank correlation, using Statview 5.0 for Macintosh (SAS Institute Inc., North Carolina).

Results

Physical, chemical and microbial characteristics of stream water at the sampling sites are in Table 1. Mean values of temperature varied from 7.9-12ºC; the lowest ones being measured at sites with high altitude (S5 and S6). Data from pH ($5.4 < pH < 6.6$) and total hardness (10 to 42 mg L^{-1}) indicated moderately acidic softwaters at all sampling sites. Apart from S1, sites in the Ave River basin had higher values of conductivity, COD, concentration of ammonium, nitrate and phosphate, and density of culturable microorganisms than sites of the Cávado River basin. S1 had intermediate nitrate concentration, although 10-times lower than that at other sites of the same basin.

PCA ordination of the sampling sites according to the stream water variables (Fig. 2) showed that axes 1 and 2 explained 98% of the total variance and grouped S1 with sites of the Cávado River basin (S5, S6 and S7), which were clearly separated from other sites of the Ave River basin (S2, S4, S8 and S9).

The species diversity based on conidia found in natural and induced foam, released from submerged alder leaves and obtained from pure cultures is shown in Table 2. A total of 113 fungal taxa were identified at least at the generic level; *ca.* 90% of them were classified as aquatic hyphomycetes as traditionally understood. The rest are miscellaneous propagules of terrestrial (nine taxa) and aeroaquatic hyphomycetes (two taxa) often encountered in water. From the samples, 72 strains (41 fungal species) were obtained in pure culture, increasing substantially the credibility of their identification.

The highest species richness was found at S1 (77 species) and in streams at the National Park (40-58 species). Richness in aquatic hyphomycete species was negatively correlated with concentration of phosphate ($r=-0.92$, $P=0.0014$), ammonium ($r=-0.84$, $P=0.006$) and nitrate ($r=-0.75$, $P=0.028$), hardness ($r=-0.83$, $P=0.008$) and density of culturable microorganisms (heterotrophs, $r=-0.88$, $P=0.0038$; total coliforms, $r=-0.88$, $P=0.0039$; and fecal coliforms, $r=-0.87$, $P=0.0031$). No other stream water variables were significantly correlated with species richness.

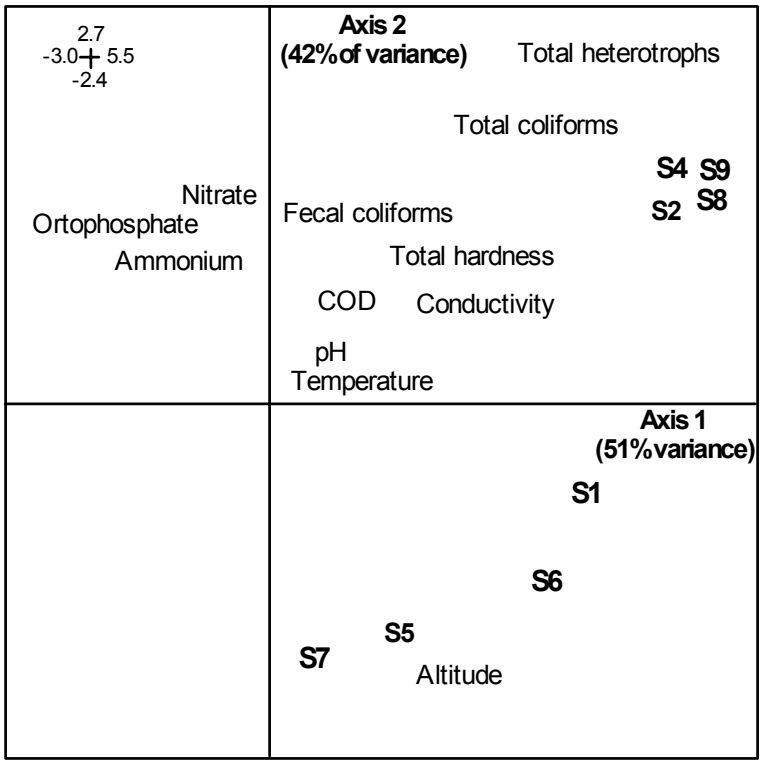

Fig. 2. Principal component analysis of physical, chemical and microbial characteristics of the sampling sites in the Ave River (S1, S2, S4, S8 and S9) and Cávado River (S5, S6 and S7) basins.

CA ordination of the sampling sites based on aquatic hyphomycete assemblages showed that axes 1 and 2 explained 46% of the total variance and separated three groups (Fig. 3A): (i) S1 and sites at the National Park (S5, S6 and S7); (ii) S2, S8 and S9; and (iii) S4. The analysis of relative and absolute contributions of CA ordination (not shown) suggested that *Fontanospora fusiramosa*, *Gyoerffyella rotula*, *Naiadella fluitans*, *Pleuropedium multiseptatum*, *Tetracladium breve*, *T. furcatum*, *Tricladium attenuatum* and *T. biappendiculatum* were strongly related to the first group, while *Flabellospora acuminata*, *F. amphibia*, *Isthmolongispora lanceata*, *Tetracladium palmatum* and *Tricellula inaequalis* were clearly associated with S4. Taxa as *Dendrospora erecta*, *D. tenella*, cf. *Digitodochium* sp., *Taeniospora gracilis* var. *enecta* and *T. gracilis* var. *gracilis* also exhibited high relative and absolute contributions (not shown), and were mainly associated with sites at the National Park. Several taxa were widespread and did not contribute to the ordination of the sampling sites. That was the case of *Articulospora tetracladia* and *Varicosporium*

Table 2. Aquatic hyphomycete taxa in foam (F), induced foam (IF) and on leaves (L) in streams from the Ave and the Cávado River basins. Sampling sites (dates): S1, S2, S5, S6 and S7 (26 Mar. 2001, 20 and 27 Jan. 2002) S4, S8 and S9 (20 Mar. 2001).

Taxon	Ave River Basin					Cávado River Basin		
	S8	S9	S4	S2	S1	S5	S6	S7
Alatospora acuminata s.s. Ingold	IF L	IF L	F		F*L	F	F*L	F*L
Alatospora acuminata s.l. Ingold		L						
Alatospora pulchella Marvanová	L	L		L	L	F	F	L
Anguillospora crassa Ingold			F			F*		
Anguillospora filiformis Greath.	L*	L		L*	L*			L
Anguillospora longissima (Sacc. & Syd.) Ingold	L			L	L			L
Anguillospora rosea J. Webster & Descals					F L	F	F	
Arborispora dolichovirga K. Ando					F	F		F
Arborispora palma K. Ando					F			
Articulospora tetracladia Ingold	IF L	IF L	F	IF*L	F*L*	F* L	F*L	F*L
Candelabrum spinulosum Beverw.					F*	F		
Clathrosphaerina zalewskii Beverw.					F			
Clavariopsis aquatica De Wild.	L*	L		L*		F		
Clavatospora longibrachiata (Ingold) Marvanová & Sv. Nilsson	L	L		L	L	F		F
Collembolispora barbata Marvanová, Pascoal & Cássio								F*
cf. *Cristula integra* Chenant.					F	F	F	
Culicidospora aquatica R.H. Petersen					L			F*
Cylindrocarpon spp.	L	L		L	L	F	L	L
Dactylellina appendiculata (Anastasiou) M. Scholler, Hagedorn & A. Rubner					F			
Dendrospora erecta Ingold						F	F	F
Dendrospora fusca Descals & J. Webster						F		
Dendrospora cf. *juncicola* S.H. Iqbal					F			
Dendrospora tenella Descals & J. Webster						F	F*	F*
Dendrosporium cf. *lobatum* J.L. Crane					F*			
cf. *Descalsia cruciata* A. Roldán & Honrubia					F			
cf. *Digitodochium* sp.						F	F	F
Dimorphospora foliicola Tubaki	L	L		L*	L			
Enantioptera tetraalata Descals					F			F
Flabellospora acuminata Descals			F					
Flabellospora amphibia (I.P. Price & P.H.B. Talbot) Descals			F					
Flabellospora cf. *verticillata* Alas.			F		F			
Flabellospora sp.					F L			
Flagellospora curta J. Webster	L	L		L*				
Flagellospora curvula Ingold					F L	F	L	F
Flagellospora sp.		L		L	F L			F
Fontanospora cf. *alternibrachiata* Dyko								F
Fontanospora eccentrica (R.H. Petersen) Dyko					F		F L	F L
Fontanospora fusiramosa Marvanová, P.J. Fisher & Descals					F*L	F	F*L	F*L

Table 2 continued. Aquatic hyphomycete taxa from the Ave and the Cávado River basins.

Taxon	Ave River Basin					Cávado River Basin		
	S8	S9	S4	S2	S1	S5	S6	S7
Fusarium aquaeductuum var. *medium* Wollenw.				L*				
Fusarium sp.	L			L				
Geniculospora grandis (Greath.) Nolan			F*		F*L			F
Geniculospora inflata (Ingold) Marvanová & Sv. Nilsson							F	
Goniopila monticola (Dyko) Marvanová & Descals						F L		
Gyoerffyella gemellipara Marvanová								F*
Gyoerffyella rotula (Höhn.) Marvanová					F	F	F	F
Heliscella stellata (Ingold & V.J. Cox) Marvanová	L				F L	F	F*	F*
Heliscina campanulata Marvanová			F				L	F
Heliscus lugdunensis Sacc. & Thérry	L	L		L*	L	L	F*L	F*L
Heliscus submersus H.J. Huds	L	L		L*				
Infundibura adhaerens Nag Raj & W.B. Kendr.			F		F	F	F	
Isthmolongispora lanceata de Hoog & Hennebert			F					
Isthmotricladia britannica Descals					F L			F
Isthmotricladia cf. *gombakiensis* Nawawi					F			
Isthmotricladia cf. *laeensis* Matsush.					F			
Lateriramulosa ainflata Matsush.					F			
Lateriramulosa biinflata Matsush.					F		F	
Lateriramulosa minitriangularia Matsush.					F			
Lateriramulosa uniinflata Matsush.			F		F		F	F
Lemonniera cf. *alabamensis* R.C. Sinclair & Morgan-Jones	L	L					L	
Lemonniera aquatica De Wild.	IF L	L		L*	L			L
Lemonniera filiformis Dyko				IF				
Lemonniera terrestris Tubaki	L							
Lunulospora curvula Ingold	L*	L		L*	L			
Margaritispora aquatica Ingold					F			
Mycocentrospora acerina (R. Hartig) Deighton			F	L	L	F	F	
Naiadella fluitans Marvanová & Bandoni					F	F	F	F
Pestalotia sp.						F	F	F
Pleuropedium multiseptatum Marvanová & Descals					L	F	F L	F*
Pleuropedium tricladioides Marvanová & S.H. Iqbal					F			
Pleuropedium sp.					F			
Pseudoanguillospora stricta S.H. Iqbal			F		L	F		F*
cf. *Radiatispora* sp.								F
Retiarius sp.							F	
cf. *Rhynchosporium* sp.					F	F		F
Spermospora sp.					F			F
Stenocladiella neglecta (Marvanová & Descals) Marvanová & Descals					F	F		
Sympodiocladium frondosum Descals					F	F	F	
Taeniospora descalsii Marvanová & Stalpers			F	IF*L	L			F L
Taeniospora gracilis var. *enecta* Marvanová & Stalpers						F	F	F

Table 2 continued. Aquatic hyphomycete taxa from the Ave and the Cávado River basins.

Taxon	Ave River Basin					Cávado River Basin		
	S8	S9	S4	S2	S1	S5	S6	S7
Taeniospora gracilis Marvanová var. *gracilis*						F	F	F
Tetrachaetum elegans Ingold	L	L		L*	L	L*		L
Tetracladium cf. *apiense* R.C. Sinclair & Eicker			F				F	F
Tetracladium breve A. Roldán					L	F	F	F*
Tetracladium furcatum Descals					L	F	F	F
Tetracladium marchalianum De Wild.	L*	L	F	L*	F*L	F		F
Tetracladium maxilliforme (Rostr.) Ingold					F			
Tetracladium palmatum A. Roldán			F*					
Tetracladium setigerum (Grove) Ingold	L	L	F	IF L	F	F		F
Titaeella capnophila K. Ando & Tubaki					F			
Tricellula aquatica J. Webster					F			F
Tricellula aurantiaca (Haskins) Arx				L	F*L			L
Tricellula inaequalis Beverw.			F*					
Tricellula sp.			F					
Tricladiopsis flagelliformis Descals			F*		F L	F		
Tricladium attenuatum S.H. Iqbal					F*L	F	L	F L
Tricladium biappendiculatum (G.R.W. Arnold) Marvanová & Descals					F*	F	F*L	F*
Tricladium castaneicola B. Sutton			F		F			
Tricladium chaetocladium Ingold	L*	L		L*	F L	F	L	L
Tricladium cf. *indicum* Sati & N. Tiwari			F		F*			
Tricladium minutum (S.H. Iqbal) Marvanová & Descals					F			F
Tricladium patulum Marvanová			F					F
Tricladium splendens Ingold	L	L	F	L*	F*L	F		F*
Tricladium terrestre D. Park			F*		F*L			
Tridentaria sp.					F		F	F
Tripospermum camelopardus Ingold, Dann & P.J. McDougall								L
Tripospermum myrti (Lind) S. Hughes		L				F		
Triscelophorus cf. *acuminatus* Nawawi			F	L	F L	F	L	F
Triscelophorus cf. *monosporus* Ingold					F			
Varicosporium delicatum S.H. Iqbal					F*			F*L
Varicosporium elodeae W. Kegel	L	L	F	L	F*L	F	F*L	F*L
Varicosporium tricladiiforme A. Roldán & Marvanová					F*		L	
Varicosporium cf. *trimosum* Wolfe							F	
Ypsilina graminea (Ingold, P.J. Mc Dougall & Dann) Descals, J. Webster & Marvanová			F	IF*L	F L			F*L
Species richness	24	23	29	28	77	45	40	58

* isolated into pure culture.

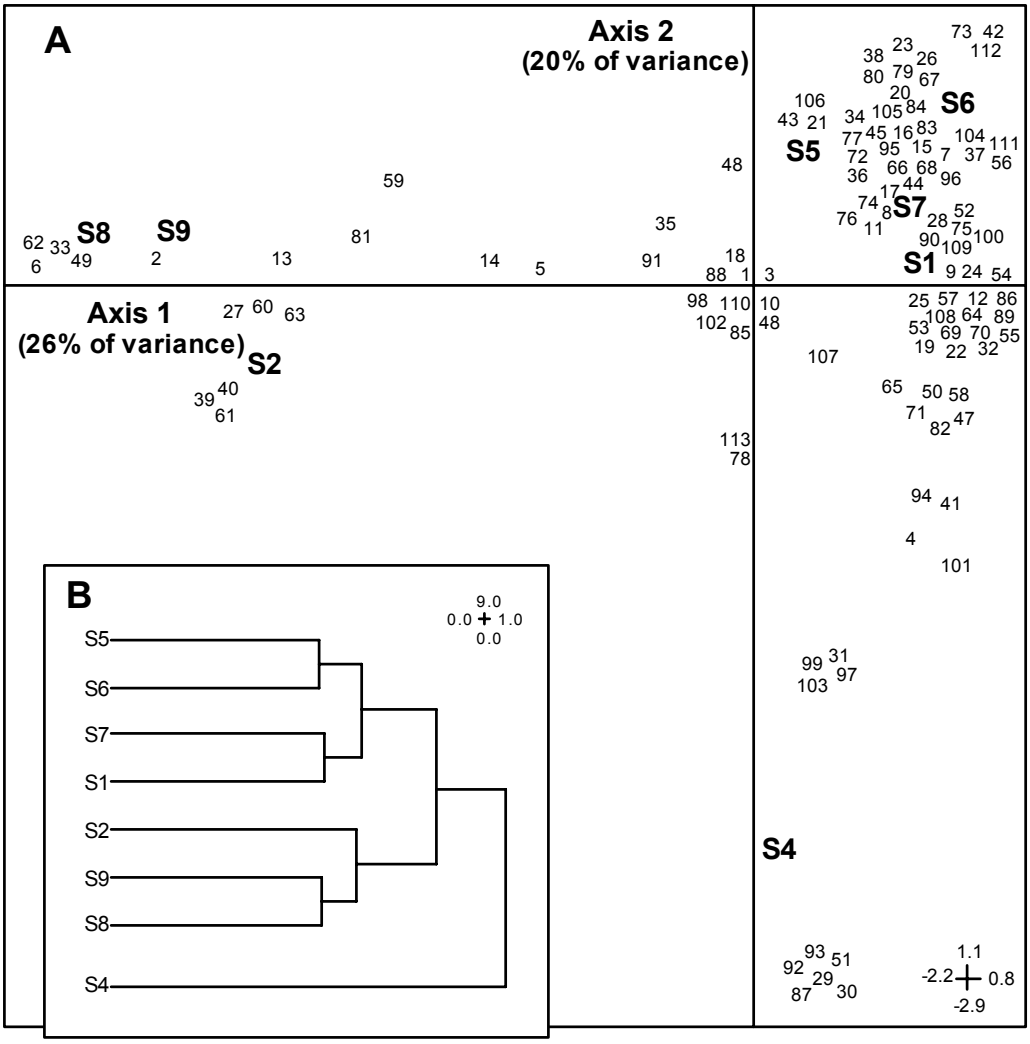

Fig. 3A. Correspondence analysis, and **B.** UPGMA cluster analysis based on the Jaccard coefficient of aquatic hyphomycete assemblages at the sampling sites of the Ave River (S1, S2, S4, S8 and S9) and Cávado River (S5, S6 and S7) basins. Numbers represent species as indicated in Table 2.

elodeae that were found at all sites, and *Alatospora acuminata*, *A. pulchella*, *Heliscus lugdunensis*, *Tetracladium marchalianum*, *T. setigerum*, *Tricladium chaetocladium* and *T. splendens*, which were present at seven sampling sites.

UPGMA cluster analysis, based on the similarity in aquatic hyphomycete assemblages among sites, showed two major hierarchical divisions: S4 and all the other sites (Fig. 3B). Among the latter, S2, S8 and S9 diverged from S1 and

from sites at the National Park (S5, S6 and S7). In addition, greater similarity was found between S8 and S9, S5 and S6, and S1 and S7.

Discussion

In this study about 12% of taxa are represented by conidia where our attempts to obtain them in pure culture failed. These could be only identified tentatively because (i) small morphological aberrations distinguish them from the respective protologues, (ii) the comparable species are not well known, and/or (iii) identification based on detached conidia was unreliable: cf. *Cristula integra*, *Dendrospora* cf. *juncicola*, *Dendrosporium* cf. *lobatum,* cf. *Descalsia cruciata*, cf. *Digitodochium* sp., *Fontanospora* cf. *alternibrachiata*, *Isthmotricladia* cf. *gombakiensis, I.* cf. *laeensis, Lemonniera* cf. *alabamensis*, cf. *Radiatispora* sp., cf. *Rhynchosporium* sp., *Tetracladium* cf. *apiense*, *Triscelophorus* cf. *acuminatus, T.* cf. *monosporus* and *Varicosporium* cf. *trimosum*.

Seventy percent of the species recorded by us are known from freshwaters of the Iberian Peninsula (Descals and Rodríguez-Pérez, 2002). Besides several ubiquitous species from temperate climates, less widespread species such as *Alatospora pulchella*, *Clavatospora longibrachiata, Mycocentrospora acerina* and *Triscelophorus* cf. *acuminatus* were also found at the majority of the sampling sites, and therefore they could not be assigned to any particular stream variable considered in this study. A recently described species *Collembolispora barbata* and a considerable number of rare species, namely *Flabellospora amphibia*, *Flagellospora curta*, *Geniculospora grandis*, *Heliscus submersus*, *Tetracladium palmatum*, *Titaeella capnophila*, *Tricladiopsis flagelliformis* and *Tricladium terrestre* were reported previously in Portugal (Marvanová *et al.*, 2002, 2003). Other less common species, whose occurrence in Iberian Peninsula is reported here for the first time, are *Arborispora dolichovirga*, *A. palma*, *Goniopila monticola*, *Isthmolongispora lanceata* and *Pseudoanguillospora stricta* (cf. Descals and Rodríguez-Pérez, 2002). Furthermore, some species documented from Spain (Descals and Rodríguez-Pérez, 2002) had not previously been reported in Portugal (*Dactylellina appendiculata*, *Dendrospora fusca*, *Flabellospora acuminata*, *Fontanospora fusiramosa*, *Lateriramulosa ainflata*, *L. minitriangularia,* and *Varicosporium tricladiiforme*).

Arborispora dolichovirga is probably conspecific with *Magdalaenaea monogramma* Arnaud, a not well-known taxon described from France and reported from Spain by Descals and Rodríguez-Pérez (2002). *Goniopila monticola* is difficult to distinguish from *Margaritispora aquatica* when only

detached isodiametrical conidia are seen (Marvanová and Descals, 1985). This is probably the reason why this fungus does rarely appear in studies where identification of taxa is based on water filtration or foam samples. *Pseudoanguillospora stricta* is also rarely recorded from streams, although it was documented in pure cultures from 11 out of 35 softwater streams in the Šumava National Park (Bohemian Forest, Czech Republic) by Marvanová (2001). On the basis of detached conidia it is hardly distinguishable from *Sigmoidea praelonga* Marvanová, which also has long, straight or little curved conidia. *Dactylellina appendiculata* originally described as *Dactylella* (Anastasiou, 1964), and later recombined in *Laridospora* (Nawawi, 1976), is a nematode capturing hyphomycete (Rubner, 1996, as *Monacrosporium tentaculatum* A. Rubner and W. Gams), which occurs scarcely on plant debris in either semiaquatic habitats or streams from temperate to tropical climates. *Dendrospora fusca* has been collected from leaf baits in European softwater streams (e.g. Bärlocher and Schweizer, 1983; Gessner *et al*., 1993) and from stream foam in Spain (Descals *et al*., 1995; Descals and Moya, 1996). *Fontanospora fusiramosa* was not for long time distinguished from *F. eccentrica*, and so its natural habitat was not known. It was described on the basis of three specimens (Marvanová *et al*., 1997) occurring in UK and Canada. The relative abundance of its conidia in streams of the Peneda-Gerês National Park and at the spring of the Este River indicates preference to unpolluted softwater in warmer climates. The two species of *Lateriramulosa* (*L. ainflata* and *L. minitriangularia*) were rarely seen in foam samples of the Iberian Peninsula (Descals *et al*., 1995; Descals and Moya, 1996; Descals and Rodríguez-Pérez, 2002). Conidia of *Varicosporium tricladiiforme* are very variable in shape as well as in size. Some strains have conidia very similar to larger ones of *Tricladium angulatum* and these might have been reported under the latter name when seen in foam samples. Besides the type locality, which is in Spain (Roldán *et al*., 1992), only a single record of *V. tricladiiforme* conidium from foam is cited from that country (Descals and Rodríguez-Pérez, 2002). This species seems to prefer streams flowing through grasslands (e.g. Marvanová, 2001), which is reinforced by Gulis (2001) reporting its high affinity to grass blades in Belarussian watercourses.

Examination of conidia released from leaves, previously immersed in a stream, can be useful for characterisation of the fungal community at a particular site. Nevertheless, this sampling technique may underestimate fungal diversity, because certain fungal assemblages show substrate preference (Gulis, 2001) and vary with time of leaf decomposition (Gessner *et al*., 1993). In this work, most of the species were detected in natural foam comparing to leaf samples (see Table 2). Some potential drawbacks to assess fungal communities

by foam examination have to be considered, such as the varying trapping efficiency of some conidial shapes (Webster, 1959; Gönczöl and Marvanová, 2002), the long-distance downstream transport of conidia, the difficulty to distinguish the origin of conidia (stream or terrestrial habitats) and to ascertain the ability of conidia to colonize substrates (Gessner *et al*., 2003). In addition, foam was not present at all sites and few conidia were found in induced foam (see Table 2). Further studies are needed to clarify if few conidia were actually present in stream water or low trapping efficiency of conidia by induced foam occurred.

Although this study has been conducted during a short period of time, and as such cannot give an exhaustive picture about the aquatic hyphomycete communities (Bärlocher, 2000), species diversity was high, particularly in streams of the Peneda-Gerês National Park and at the spring of the Este River (S1). At these sites, at least 60% of the total taxa were found at each sampling season (not shown). The highest richness in aquatic hyphomycete species found at S1 could be, at least, partially attributed to the moderate nitrate concentration in the stream water comparing with sites of the National Park. In oligotrophic softwaters the enrichment in inorganic nitrogen and phosphorus led to an increase in the number of both fungal species and conidia (Gulis and Suberkropp, 2003, 2004). Another factor that might have contributed to the unusual species composition is the near-water and streambed vegetation at S1 (*Juncus* sp. and *Pteridium aquilinum*). *Dendrospora juncicola* was reported as growing abundantly on *Juncus* culms in oligotrophic softwater streams in UK (Iqbal, 1972). Similarly, somewhat larger conidia of probably the same species were seen by us at S1. Some taxa collected from foam, which accounted for the high species diversity at S1 (*Arborispora dolichovirga, A. palma,* cf. *Cristula integra, Dactylellina appendiculata, Dendrosporium* cf. *lobatum, Isthmotricladia* cf. *gombakiensis*, *I.* cf. *laeensis, Lateriramulosa* spp., *Radiatispora* sp*., Retiarius* sp*., Tetracladium maxilliforme, Titaeella capnophila* and *Tricellula inaequalis*), are fungi occurring rather in humid terrestrial habitats than in water and their conidia may appear in streams as immigrants (Park, 1972), probably washed in with rain and persisting in foam for several weeks.

Apart from S1, sites of the Ave River basin presented much higher concentration of inorganic and organic nutrients than sites of the Cávado River basin. High levels of nutrients were probably related to agricultural exploitation, as well as inadequate functioning of sewage treatment plants in the highly populated area of the Ave River basin. Species diversity and/or conidium production of aquatic hyphomycetes are reported to decrease by organic pollution (Au *et al*., 1992; Raviraja *et al*., 1998b) or heavy metals (Bermingham

et al., 1996; Sridhar *et al*., 2001; Niyogi *et al*., 2002; Duarte *et al*., 2004). In our study, the richness in aquatic hyphomycete species was negatively correlated with hardness and concentration of phosphate, ammonium and nitrate. However, species richness (23-29 taxa) was considerable at polluted sites of the Ave River basin (S2, S4, S8 and S9), which agrees with other studies reporting the occurrence of several aquatic hyphomycete species at highly polluted sites (Sridhar *et al*., 2000; Krauss *et al*., 2001, 2003; Pascoal *et al*., 2003, 2005).

The distribution of the aquatic hyphomycete species by cluster analysis and CA ordination opposed polluted and non-polluted sites, since sites of the Cávado River basin together with S1 were clearly separated from those of the Ave River basin. This separation is in agreement with that of PCA ordination based on the stream variables, suggesting that water chemistry was the main factor regulating the structure of aquatic hyphomycete assemblages. *Flagellospora curta* and *Heliscus submersus* were exclusively associated with polluted sites, but were not found at S4. Both species are reported to be dominant on decomposing alder leaves in polluted streams of Northwest Portugal (Pascoal *et al*., 2003; Pascoal and Cássio, 2004). The distribution of *H. submersus* in Northwest Portugal is consistent with its record in organic enriched waters with soluble salts of the Nile River (Khalil *et al*., 1993). Despite of this, *H. submersus* has been mostly documented from presumably clean mountain streams from tropic and subtropic areas.

Other species, such as *Anguillospora filiformis, Clavariopsis aquatica, Dimorphospora foliicola, Lemonniera aquatica* and *Lunulospora curvula*, were often found at the polluted sites of the Ave River basin, but occurred at the unpolluted site S1 as well, and three of them were present in the National Park (S5 and S7). *Lunulospora curvula* is one of the most frequent species in the Iberian Peninsula (Descals and Rodríguez-Pérez, 2002) and was one of the dominant species (measured by conidial production) in streams of Central Portugal (Bärlocher *et al*., 1995; Bärlocher and Graça, 2002). *Anguillospora filiformis* and *D. foliicola* persisted as the dominant aquatic hyphomycetes on alder leaves at S2 during six weeks of exposure (Pascoal *et al*., 2005). This suggests possible existence of genetically different populations within these species, well adapted to pollution. Similarly, high resistance to heavy metal pollution of clean-water-preferring species was found in Canada (Miersch *et al.,* 1997) and in Germany (Krauss *et al*., 2001). The species spectrum found in Germany was different from that in Portugal. For example, *Tetracladium marchalianum* and *Heliscus lugdunensis* were consistently found among the ten top ranked species in severely polluted streams in Germany (Sridhar *et al*., 2000). It may be speculated that the tolerance to pollution evolves gradually in some individuals of the original community living in the stream, along with the

proceeding pollution. The combination of chemical factors and their possible interactions with the genetic potential of individual species make the resulting community pattern difficult to predict. Although no great genotype variation in *Tetrachaetum elegans* in relation to substrate preference was found by random amplified polymorphic DNA analysis (Charcosset and Gardes, 1999), it would be worth to apply this or similar techniques to isolates of the same species occurring in both polluted and unpolluted waters of the same geographic area.

The aquatic hyphomycete assemblage from S4, a lowland stream with high inorganic and organic load, was also discriminated from those of other sites, suggesting that other factor(s) may also be important in controlling the distribution of aquatic hyphomycete species. A possible explanation for the different fungal assemblage at S4 relies on the riparian vegetation, which, in contrast to other sites, is only constituted by vineyards. Because of differences in sampling techniques and frequency among sites, further studies should be done to clarify this point. Out of the conidia recorded only at S4, the two *Flabellospora* species are worth mentioning. *Flabellospora amphibia*, originally described from eucalypt bark and wood in Australia (Price and Talbot, 1966) may have been introduced with eucalypt plantation in Portugal in the 19th century. *Flabellospora acuminata* has worldwide distribution, but the records are rather scarce. An exception is from Spain, where it is reported from more than 10 localities (Descals and Rodríguez-Pérez, 2002). Chamier and Dixon (1982) collected *F. acuminata* conidia from foam in a moderately eutrophic river in the UK.

Another species, isolated from S4 and S1, is what we call *Tricladium* cf. *indicum. Tricladium indicum* was described from submerged decaying needles of *Pinus roxburghii* from a high altitude stream in the Kumaun Himalaya, India (Sati and Tiwari, 1992). Other records are from Natal in South Africa (Webster *et al*., 1994, 1995). Our isolates exhibited greater variation in conidial morphology than described by the authors of the name (Sati and Tiwari, 1992). Some of our specimens had conidia similar to those reported by Webster *et al*. (1995) as *T. indicum* anamorph of *Cudoniella indica* J. Webster. Sivichai *et al*. (2003) drew attention to the similarity among conidia of *T. indicum, Varicosporium* anamorph of *Hymenoscyphus varicosporoides* Tubaki and *T. marylandicum* J.L. Crane, described from USA. More about our isolates will be published separately.

In the current study, none of the aquatic hypomycete species generally associated with tropical climate was found in high altitude streams in the Peneda-Gerês National Park. *Dendrospora erecta*, *D. tenella,* cf. *Digitodochium* sp*., Taeniospora gracilis* var. *enecta* and *T. gracilis* var. *gracilis* were mainly associated with oligotrophic fast flowing streams bordered by

mixed deciduous trees in the National Park. Apart from *Digitodochium*, a terrestrial sporodochial hyphomycete described from Japan (Tubaki and Kubono, 1989), the other species are mainly known from softwater mountain streams in Spain (e.g. Roldán *et al.*, 1988; Descals *et al*., 1995; Descals and Moya, 1996; Descals and Rodríguez-Pérez, 2002) and Central Portugal (Bärlocher *et al*., 1995, only *T. gracilis*; Bärlocher and Graça, 2002, *T. gracilis* and *D. erecta*).

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References

Anastasiou, C.J. (1964). Some aquatic Fungi Imperfecti from Hawaii. Pacific Science 18: 202-206.

APHA (1998). *Standard Methods for the Examination of Water and Wastewater*. 20th edn. American Public Health Association, Washington, DC.

Au, D.W.T., Hodgkiss, J.L. and Vrijmoed, L.L.P. (1992). Fungi and cellulolytic activity associated with decomposition of *Bauhinia purpurea* leaf litter in a polluted and unpolluted Hong Kong waterway. Canadian Journal of Botany 70: 1071-1079.

Bärlocher, F. (1992). *The Ecology of Aquatic Hyphomycetes*. Springer-Verlag, Berlin.

Bärlocher, F. (2000). Water-borne conidia of aquatic hyphomycetes: seasonal and yearly patterns in Catamaran Brook, New Brunswick, Canada. Canadian Journal of Botany 78: 157-167.

Bärlocher, F., Canhoto, C. and Graça, M.A.S. (1995). Fungal colonization of alder and eucalypt leaves in two streams in Central Portugal. Archiv für Hydrobiologie 133: 457-470.

Bärlocher, F. and Graça, M.A.S. (2002). Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. Freshwater Biology 48: 1123-1136.

Bärlocher, F. and Schweizer, M. (1983). Effects of leaf size and decay rate on colonization by aquatic hyphomycetes. Oikos 41: 205-210.

Baschien, C. (2003). *Development and Evaluation of rDNA Targeted in situ Probes and Phylogenetic Relationships of Freshwater Fungi*. Ph.D. thesis, Technical University, Berlin.

Bauer, R., Begerow, D., Oberwinkler, F. and Marvanová, L. (2003). *Classicula*: the teleomorph of *Naiadella fluitans*. Mycologia 95: 756-764.

Bermingham S., Maltby, L. and Cooke, R.C. (1996). Effects of a coal mine effluent on aquatic hyphomycetes. I. Field study. Journal of Applied Ecology 33: 1311-1321.

Campbell, J., Shearer, C.A. and Marvanová, L. (2002). Evolutionary relationships between aquatic anamorphs and teleomorphs: *Lemonniera*. 7th International Mycological Congress, 11-17 August 2002, Oslo: 193-194. (Abstract)

Chamier, A.-C. and Dixon, P.A. (1982). Pectinases in leaf degradation by aquatic hyphomycetes. I. The field study. The colonization pattern of aquatic hyphomycetes on leaf packs in a Surrey stream. Oecologia 52: 109-115.

Charcosset, J.-Y. and Gardes, M. (1999). Intraspecific genetic diversity and substrate preference in the aquatic hyphomycete *Tetrachaetum elegans*. Mycological Research 103: 736-742.
Chauvet, E. (1991). Aquatic hyphomycete distribution in South-Western France. Journal of Biogeography 18: 699-706.
Descals, E. and Moya, O. (1996). Fungal spora in streams of the Catalan Pyrenees. Boletín Sociedad Micológica de Madrid 21: 145-174.
Descals, E., Peláez, F. and López-Llorca, L.V. (1995). Fungal spora of stream foam from central Spain II. Chorology, spore frequency and unknown forms. Nova Hedwigia 60: 551-559.
Descals, E. and Rodríguez-Pérez, J. (2002). Cuadernos de Trabajo de Flora Micológica Ibérica 18. *Bases Corológicas de Flora Micológica Ibérica* (ed. F. Pando). CSIC, Madrid.
Duarte, S., Pascoal, C. and Cássio, F. (2004). Effects of zinc on leaf decomposition by fungi in streams: studies in microcosms. Microbial Ecology 48: 366-374.
Gessner, M.O., Bärlocher, F. and Chauvet, E. (2003). Qualitative and quantitative analyses of aquatic hyphomycetes in streams. Fungal Diversity Research Series 10: 127-157.
Gessner, M.O., Thomas, M., Jean-Louis, A.-M. and Chauvet, E. (1993). Stable successional patterns of aquatic hyphomycetes on leaves decaying in a summer cool stream. Mycological Research 97: 163-172.
Gonçalves, M.A.P. (2001). *Determinação de Metais Pesados em Águas Superficiais Recolhidas no Rio Este*. M.Sc. thesis, University of Minho, Braga, Portugal.
Gönczöl, J., Csontos, P. and Révay, Á. (2003). Catchment scale patterns of aquatic hyphomycetes. The role of physicochemical variables and substrate composition in structuring conidial communities. Archiv für Hydrobiologie 157: 249-266.
Gönczöl, J. and Marvanová, L. (2002). *Anguillospora mediocris* sp. nov. from streams in Hungary. Czech Mycology 53: 309-317.
Gönczöl, J. and Révay, Á. (2003). Aquatic hyphomycetes in the Morgó stream system, Hungary - tributary communities. Nova Hedwigia 76: 173-189.
Gulis, V. (2001). Are there any substrate preferences in aquatic hyphomycetes? Mycological Research 195: 1088-1093.
Gulis, V. and Suberkropp, K. (2003). Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48: 123-134.
Gulis, V. and Suberkropp, K. (2004). Effects of whole-stream nutrient enrichment on the concentration and abundance of aquatic hyphomycete conidia in transport. Mycologia 96: 57-65.
Harrington, T.J. (1997). Aquatic hyphomycetes of 21 rivers in southern Ireland. Biology and Environment: Proceedings of the Royal Irish Academy 97B: 139-148.
Iqbal, S.H. (1972). New aquatic hyphomycetes. Transactions of the British Mycological Society 59: 301-307.
Khalil, A.M., EL-Hissy, F.T. and Abdel-Raheem, A. (1993). Monthly variations of Oomycetes (zoosporic fungi) and aquatic hyphomycetes at Sohag (Upper Egypt). Acta Societatis Botanicae Poloniae 62: 67-73.
Krauss, G., Bärlocher, F., Schreck, P., Wennrich, R., Glässer, W. and Krauss, G.-J. (2001). Aquatic hyphomycetes occur in hyperpolluted waters in Central Germany. Nova Hedwigia 72: 419-428.
Krauss, G., Bärlocher, F. and Krauss, G.-J. (2003). Effects of pollution on aquatic hyphomycetes. In: *Freshwater Mycology* (eds. C.K.M. Tsui and K.D. Hyde). Fungal Diversity Press, Hong Kong: 211-230.

Legendre, P. and Legendre, L. (1998). *Numerical Ecology*. Developments in Environmental Modelling 20. 2nd edn. Elsevier Science BV, Amsterdam.

Luo, J., Yin, J., Cai, L., Zhang, K. and Hyde, K.D. (2004). Freshwater fungi in Lake Dianchi, a heavily polluted lake in Yunnan, China. Fungal Diversity 16: 93-112.

Marvanová, L. (1997). Freshwater hyphomycetes: a survey with remarks on tropical taxa. In: *Tropical Mycology* (eds. K.K. Janardhanan, C. Rajendran, K. Natarajan and D.L. Hawksworth). Science Publishers, Inc., Enfield: 169-226.

Marvanová, L. (2001). Streamborne fungal spora in running waters of the Bohemian Forest. Silva Gabreta 7: 147-164.

Marvanová, L. (2002). Aquatic hyphomycetes - emerging outlines of their classification in the fungal system. 3rd International Meeting on "Plant Litter Processing in Freshwater", September 7-12, 2002, Szentendre, Hungary: 11. (Abstract)

Marvanová, L. and Descals, E. (1985). New and critical taxa of aquatic hyphomycetes. Botanical Journal of the Linnean Society 91: 1-23.

Marvanová, L., Fisher, P.J., Descals, E. and Bärlocher, F. (1997). *Fontanospora fusiramosa* sp. nov., a hyphomycete from live tree roots and from stream foam. Czech Mycology 50: 3-11.

Marvanová, L., Pascoal, C. and 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: 35. (Abstract)

Marvanová, L., Pascoal, C. and Cássio, F. (2003). New and rare hyphomycetes from streams of northwest Portugal. Part I. Cryptogamie, Mycologie 24: 339-358.

Miersch, J., Bärlocher, F., Bruns, I. and Krauss, G.-J. (1997). Effect of cadmium, copper and zinc on growth and thiol content of aquatic hyphomycetes. Hydrobiologia 346: 77-84.

Nawawi, A. (1976). A new genus of aquatic hyphomycetes. Transactions of the British Mycological Society 66: 344-347.

Nikolcheva, L.G. and Bärlocher, F. (2002). Phylogeny of *Tetracladium* based on 18S rDNA. Czech Mycology 53: 285-295.

Niyogi, D.K., McKnight, D.M., Lewis Jr., W.M. (2002). Fungal communities and biomass in mountain streams affected by mine drainage. Archiv für Hydrobiologie 155: 255-271.

Park, D. (1972). On the ecology of heterotrophic microorganisms in fresh water. Transactions of the British Mycological Society 58: 291-299.

Pascoal, C. and Cássio, F. (2004). Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Applied and Environmental Microbiology 70: 5266-5273.

Pascoal, C., Cássio, F. and Marvanová, L. (2005). Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low-order stream. Archiv für Hydrobiologie 162: 481-496.

Pascoal, C., Pinho, M., Cássio, F. and Gomes, P. (2003). Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. Freshwater Biology 48: 2033-2044.

Price, I.P. and Talbot, P.H.B. (1966). An aquatic hyphomycete in a lignicolous habitat. Australin Journal of Botany 14: 19-23.

Raviraja, N.S., Sridhar, K.R. and Bärlocher, F. (1998a). Fungal species richness in Western Ghat streams (southern India): is it related to pH, temperature or altitude? Fungal Diversity 1: 179-191.

Raviraja, N.S., Sridhar, K.R. and Bärlocher, F. (1998b). Breakdown of *Ficus* and *Eucalyptus* leaves in an organically polluted river in India: fungal diversity and ecological functions. Freshwater Biology 39: 537-545.

Roldán, A., Descals, E. and Honrubia, M. (1988). Hifomicetos acuáticos de Sierra Nevada y Sierra de los Filabres. Acta Botánica Malacitana 13: 77-90.

Roldán, A., Marvanová, L. and Descals, E. (1992). Concerning *Varicosporium tricladiiforme* anam. nov. Mycological Research 96: 935-938.

Rubner, A. (1996). Revision of predacious hyphomycetes in the *Dactylella-Monacrosporium* complex. Studies in Mycology 39: 134.

Sati, S.C. and Tiwari, N. (1992). A new species of *Tricladium* from Kumaun Himalaya, India. Mycological Research 96: 51-60.

Sivichai, S., Jones, E.B.G. and Hywel-Jones, N.L. (2003). Lignicolous freshwater Ascomycota from Thailand: *Hymenoscyphus varicosporoides* and its *Tricladium* anamorph. Mycologia 95: 340-346.

Soares, H.M.V.M., Boaventura, R.A.R., Machado, A.A.S.C., Esteves da Silva, J.C.G. (1999). Sediments as monitors of heavy metal contamination in the Ave river basin (Portugal): multivariate analysis of data. Environmental Pollution 105: 311-323.

Sridhar, K.R., Krauss, G., Bärlocher, F., Raviraja, N.S., Wennrich, R., Baumbach, R., Krauss, G.-J. (2001). Decomposition of alder leaves in two heavy metal-polluted streams in central Germany. Aquatic Microbial Ecology 26: 73-80.

Sridhar, K.R., Krauss, G., Bärlocher, F., Wennrich, R. and Krauss, G.-J. (2000). Fungal diversity in heavy metal polluted waters in central Germany. In: *Aquatic Mycology across the Millenium* (eds. K.D. Hyde, W.H. Ho and S.B. Pointing). Fungal Diversity 5: 119-129.

Thioulouse, J., Chessel, D., Dolédec, S. and Olivier, J.-M. (1997). ADE-4: a multivariate analysis and graphical display software. Statistics and Computing 7: 75-83

Tubaki, K. and Kubono, T. (1989). *Digitodochium*, a new staurosporous anamorph genus. Sydowia 41: 344-348.

Webster, J. (1959). Experiments with spores of aquatic hyphomycetes. I. Sedimentation and impaction on smooth surfaces. Annals of Botany (London), N.S. 23: 595-611.

Webster, J., Eicker, A. and Spooner, B.M. (1995). *Cudoniella indica* sp. nov. (Ascomycetes, Leotiales) the teleomorph of *Tricladium indicum*, an aquatic fungus isolated from a South African river. Nova Hedwigia 60: 493-498.

Webster, J., Marvanová, L. and Eicker, A. (1994). Spores from foam from South African rivers. Nova Hedwigia 57: 379-398.

Wood-Eggenschwiler, S. and Bärlocher, F. (1983). Aquatic hyphomycetes in sixteen streams in France, Germany and Switzerland. Transactions of the British Mycological Society 81: 371-379.

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