Fungal species richness in Western Ghat streams (southern India): is it related to pH, temperature or altitude?

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Foam and leaf samples from ten streams in the Western Ghat region (south-west India) were examined for aquatic hyphomycetes. A total of 47 conidial forms were distinguished, 15 of them unknown. Cluster and principal component analysis suggested that stream category (defined primarily by distance from source) was the primary factor connected with the composition of fungal communities. Data from the current and earlier studies in the same region showed a negative correlation between species number and pH of streams ($r = 0.3$, $p = 0.18$). The correlation was stronger ($r = 0.4$, $p = 0.051$), when one stream with an unusually high number of species was excluded. A resampling test suggested that fungal species richness differed among stream categories, with the highest numbers occurring in mid-altitude streams.

Introduction

Aquatic hyphomycetes are important intermediaries between vascular plant remains and stream detritivores (Bärlocher, 1992). Their diversity has been shown to fluctuate seasonally (Iqbal and Webster, 1973; Webster, Moran and Davey, 1976; Suberkropp, 1984), and to be correlated with water chemistry, altitude and quality of riparian vegetation (Bärlocher and Rosset, 1981; Wood-Eggenschwiler and Bärlocher, 1983; Shearer and Webster, 1985a, b; Bärlocher, 1987; Gönczöl, 1989). In an early review, Wood-Eggenschwiler and Bärlocher (1985) concluded that on a worldwide scale, temperature, together with its influence on vegetation in different climatic regions, is the main factor determining fungal distribution patterns, whereas, within smaller areas, the chemistry of stream water appeared to dominate. More recent, detailed studies in south-western France suggested that altitude (or factors associated with it) is of primary importance in structuring aquatic hyphomycete communities (Chauvet, 1991; Fabre, 1996). As in the vast majority of previous investigations, these conclusions are based on temperate conditions. By comparison, detailed information on fungal communities in tropical streams is scarce. Our goal was to remedy this situation by investigating ten locations in the Western Ghat area of southern India, where previous studies provide considerable background information on fungal species occurrence and distribution (Sridhar, Chandrashekhar and Kaveriappa, 1992). Our major objective was to determine whether pH, temperature or altitude was most closely correlated with fungal species richness.

Materials and methods

All examined streams originate at peaks of the Western Ghat mountains and are tributaries of 5 major rivers, Gurpur, Kollur, Nethravathi, Payaswini and Sita, which empty into the Arabian Sea. The 10 sampling locations are located in the state of Karnataka, in the area enclosed between 12° 20' to 14° 50' N and 74° 45' to 75 ° 45' E. Information on the 10 sites is summarized in Table 1. All samples were taken between October 13 and 25, 1995, between 9 and 11 a.m. A number of earlier studies had consistently shown that fungal communities peak at this time (number and types of conidia in transport, or produced from randomly collected leaves; Sridhar *et al.*, 1992). The altitude of the location was determined with an altimeter (BARIGO, Germany). Water temperature and pH were recorded in the field with a Systronic instrument. Generally, five measurements were taken during a 2 h period. During this time, fluctuations within instrumental limits were negligible. In some cases, temperature and pH values were measured during an entire 24 h cycle. Daily temperature fluctuations varied between 1 and 3 C, and pH by up to 0.2 units.

The fungal communities were characterized by collecting leaf litter and foam along a 40-50 m long stream section. Randomly collected leaves were brought to the laboratory in polythene bags, and rinsed in distilled water to remove attached debris. Disks (10 mm diam.) were prepared from 4-5 different types of leaves, and aerated in 250 ml sterile distilled water in a 500 ml Erlenmeyer flask (generally 3 disks per leaf type, 48 h, 27 ± 2 C). Five replicate flasks were prepared from each location. After aeration, the water was filtered through 5 μm membrane filters. The filters were immediately stained with a 0.05 % solution of aniline blue in lactophenol, and scanned for conidia of aquatic hyphomycetes. To determine percentage distribution of the various species, at least 500 conidia were identified. Leaf disks were collected, dried at 100 C for 24 h, and their mass determined. This allowed us to estimate the number of conidia produced per unit leaf mass.

In addition, fresh (i.e. white) foam was collected at each site and placed in a measuring cylinder. The volume of the foam was estimated after allowing it to settle for 20-30 min. It was then fixed with an equal volume of FAA (10 ml of 40 % formaldehyde + 5 ml of glacial acetic acid + 85 ml of 70 % ethanol) and transferred to air-tight plastic containers. In the laboratory, 5 ml of the foam/FAA mixture was filtered through a 5 μm membrane filter, which was again stained and scanned under the microscope for conidia of aquatic hyphomycetes.

All analyses were done with SYSTAT (Wilkinson *et al.*, 1996) or Resampling Stats (Simon, 1992). For some analyses, sampling sites were placed in one of the following five categories: (i) Mountain locations: headwater streams, altitude 900-1325 m, sparse vegetation, few riparian trees; water temperature 17-22 C; (ii) Mid-altitude: 500-1000 m below peaks, rapid water flow, species-rich riparian forests, 19-23 C; (iii) Foot-hill locations: altitude < 350 m, moderate water flow, rich forests, 26-30 C; (iv) Coastal locations: altitude < 100 m, close to Arabian Sea, slow water flow, natural vegetation scarce, many plantations; 26-30 C; (v) Plains: altitude 600-900 m, streams and rivers flowing east from Western Ghat peaks, moderately dense forests.

Results

Study sites

Altitude, water temperature and pH of the 10 locations are summarized in Table 1. The first six streams were classified as mid-altitude streams, and the last four as foot-hill streams.

Table 1. Description of sampling locations. The first six streams are 10-12 m and the last four, 15-20 m wide. All run through dense, species-rich forests.

Location	River system	Altitude (m)	Temperature (C)	pH
Kudremukh (KU)	Gurpur	1350	22	6.4
Kotachadri (KO)	Kollur	1100	19	5.9
Samse (SS)	Nethravathi	1100	23	6.6
Marcara (MC)	Payaswini	900	22	7.0
Sampaje (SJ)	Payaswini	500	23	7.1
Agumbe (AB)	Sita	700	22	6.7
Venur (VR)	Gurpur	100	28	6.8
Kollur (KR)	Kollur	350	30	7.1
Navuru (NR)	Nethravathi	250	26	6.5
Someshwara (SR)	Sita	350	26	6.9

Fungal species

A total of 47 conidial forms were distinguished, 32 of which could be identified. Another 15 could not be linked to described species; we are currently trying to isolate these forms in pure culture for identification and description.

The occurrence of conidial forms in the 10 streams is listed in Table 2. All statistical analyses were done with and without unidentified forms; since this did not significantly change the results, only those including all forms are reported here.

The numbers of conidia produced per mg leaf mass are reported in Table 3. They varied between 243 (KR) and 2610 (VR). Overall, the two most productive species were *Lunulospora curvula* and *L. cymbiformis*.

Statistical analysis

Species numbers were negatively correlated with temperature and pH, and positively correlated with altitude; however, all regressions were non-significant (temperature: $F = 0.776$, $r = -0.19$, $p = 0.39$; pH: $F = 1.9$, $r = 0.3$, $p = 0.18$; altitude: $F = 0.062$, $r = 0.04$, $p = 0.84$). Temperature significantly decreased with altitude ($F = 25.3$, $r = -0.74$, $p < 0.001$). Multiple regression with two independent factors again failed to reveal a significant correlation (species vs. pH and temperature: p for pH = 0.271, p for temperature = 0.635; species vs. pH and altitude: p for pH = 0.10; p for altitude = 0.31). Because of collinearity between temperature and altitude, no regression with all three variables was performed.

The potential correlation between numbers of fungal species in streams and their pH values was extended by including results from earlier studies. The

Table 2. Fungal conidia from 10 streams in the Western Ghat area. Symbols as in Table 1.

	KU	KO	SS	MC	SJ	AB	VR	KR	NR	SR
Actinospora megalospora Ingold			F		F					
Anguillospora crassa Ingold	F	F			F	F		F	F	
A. longissima (Sacc. and Syd.) Ingold	L	L	L		FL	FL	F	L	FL	L
Articulospora inflata Ingold					F	F				
Brachiosphaera tropicalis Nawawi	F									
Campylospora chaetocladia Ranzoni		F	F	F	F			F		
C. filicladia Nawawi			F	F	F					
C. parvula Kuzuha	F		F		F					
Clavariopsis aquatica de Wild.				F	F					
Condylospora spumigena Nawawi						F				F
Dendrospora erecta Ingold									F	

Table 2. (continued).

	KU	KO	SS	MC	SJ	AB	VR	KR	NR	SR
D. juncicola Iqbal			F							
Dwayaangam cornuta Descals			F							
Flabellocladia tetracladia (Nawawi) Nawawi	F	F	F	F	F	F			F	F
Flabellospora crassa Alasoadura		F	F		F					
F. multiradiata Nawawi	F	F							F	
F. verticillata Alasoadura	F	F	F		F		F		F	
Flagellospora curvula Ingold				F						
F. penicillioides Ingold	F		F							
Hydrometrospora sp.	F									
Ingoldiella hamata Shaw	F	F								F
Isthmotricladia gombakiensis Nawawi		F		F	F	F			L	
Lunulospora curvula Ingold	L	L	L	FL	FL	FL	L	L	L	FL
L. cymbiformis Miura	L	L	L	FL	FL	FL		L	L	L
Mycocentrospora acerina (Hartig) Deighton	L	L	FL	L	L	L			L	L
Phalangispora constricta Singh		F	F							
Pyramidospora fluminea Miura and Kudo						F				
Speiropsis pedatospora Tubaki						F		F		
Tricladium sp.									F	F
Triscelophorus acuminatus Nawawi	F	F	F		F	F	FL	F	F	F
T. konajensis Sridhar and Kaveriappa	F		F				L		L	
T. monosporus Ingold	F	L	FL	F	F	F	L			
Unknown 1										F
Unknown 2	F									F
Unknown 3			F						F	
Unknown 4						F				F
Unknown 5	F	F	F		F	F	F		F	F
Unknown 6	F									
Unknown 7										F
Unknown 8									F	
Unknown 9	F									
Unknown 10	F		F							
Unknown 11	F									
Unknown 12										F
Unknown 13		F				F				F
Unknown 14										F
Unknown 15										F
Total Species	22	17	21	10	18	16	7	7	16	18
Unidentified	6	2	3	0	1	3	1	0	3	9
Exclusive species	5	0	2	1	0	1	0	0	2	5

F = foam samples. L = leaf samples.

Table 3. Conidia released per mg dry mass of leaf litter from 10 streams in the Western Ghat area. Symbols as in Table 1. Averages of 5 samples, with SEM in parentheses.

	KU	KO	SS	MC	SJ	AB	VR	KR	NR	SR
A. longissima (Sacc. and Syd.) Ingold	<1	68	<1		<1	676		25	63	60
Isthmotricladia gombakiensis Nawawi									<1	
Lunulospora curvula Ingold	129	116	220	737	99	1	2610	179	1214	593
L. cymbiformis Miura	286	292	238	475	429	935		39	614	925
Mycocentrospora acerina (Hartig) Deighton	68	68	118	213	207	171			<1	110
Triscelophorus acuminatus Nawawi							<1			
T. konajensis Sridhar and Kaveriappa							<1		<1	
T. monosporus Ingold		<1	<1				<1			
Total	483 (56)	549 (43)	576 (63)	1425 (319)	735 (125)	1783 (74)	2610 (633)	243 (29)	1891 (263)	1688 (250)

following locations, with their categories, were used: Kempu Hole (mid-altitude) and Kumaradhara (foot-hill), both from Chandrashekar, Sridhar and Kaveriappa (1990); three tributaries of the Cauvery River (plains streams, Rajashekhar and Kaveriappa, 1993); two coastal streams, a foot-hill and a mid-altitude stream (Sridhar and Kaveriappa, 1989a, b), and four mountain streams (Sridhar and Kaveriappa, 1989c). In all cases, only the values from one sample taken in the post-monsoon season were used for analysis. The data are summarized in Fig. 1, which indicates that species number declined with pH, but the regression was not significant at the 0.05 level ($F = 1.9$, $r = 0.3$, $p = 0.18$). In order to identify potential outliers, the analysis was repeated with one data pair deleted at a time. The highest correlation coefficient was found when the values for the Sampaje stream were deleted (from Sridhar and Kaveriappa 1989b; $F = 4.316$, $r = 0.4$; $p = 0.051$). Finally, we tested the possibility that species number differs among the five stream categories. As test statistic, we used the variance attributable to treatment (= stream category). The data were randomly shuffled 50,000 times, and each time the test statistic was determined. To estimate a p value, we determined how often the test statistic of randomly shuffled data reached or exceeded the value given by the original data (Simon, 1992). With all 23 streams, this proportion was 0.077. When only streams flowing east from the Western Ghat peaks were used, the proportion was 0.054.

Presence/absence data of conidial forms in the 10 streams were examined with cluster analysis (Wilkinson *et al.*, 1996). Regardless of linkage method or whether or not all forms or only identified species were used, the first clusters always appeared within flood-plain or within mid-altitude streams. One example is shown in Fig. 2. Similarities (1-Distance) between streams or clusters of streams varied between 53.3 and 33.6 %.

Principal Component Analysis of the 10 communities is shown in Fig. 3. The first three factors accounted for 22.5, 17.2, and 14.4 % of the total variance. The first axis appears to separate the four foot-hill streams from the mid-altitude streams, but there is considerable overlap among the two categories. The second axis further subdivides the mid-altitude streams.

Discussion

Temperate regions

Bärlocher and Rosset (1981) found more aquatic hyphomycete species in two softwater than in two hardwater streams. In a more extended study with 16 streams, species richness was negatively correlated with pH or associated factors, such as alkalinity or Ca content (Wood-Eggenschwiler and Bärlocher, 1983). There was no significant correlation with the diversity of riparian vegetation. Similarly, Marvanová (1984) stated that in Czechoslovakia the highest number of fungal species generally occur in small streams passing through beech forests on acid substrata. On the other hand, studies on the Morgó stream system in Hungary showed that alkalinity clearly influenced community composition, but had no obvious effect on overall species numbers (Gönczöl, 1987; Gönczöl and Révay, 1992). In a comparison of Canadian and European streams, Bärlocher (1987) showed a slow, possibly negligible decrease in species numbers in circumneutral waters (pH 5.7-7.2) with a more rapid decline in alkaline waters (pH > 7.2). There are many potentially confounding factors. For example, fungal communities change as one follows a stream from its source to lower reaches. The River Teign in Devon, England, originates in moorland where the water is very acidic (pH 5.4-6), and riparian trees, and therefore the type of substrates favoured by aquatic hyphomycetes, are rare. Not surprisingly, conidial numbers and diversity are low in this region, and increase in lower reaches where the pH rises to 7-7.2 and riparian trees become abundant (Shearer and Webster, 1985a, b). Pronounced longitudinal changes were also documented by Gönczöl (1987, 1989) and Gönczöl and Révay (1992). As one goes downstream, riparian vegetation, water flow, chemistry and temperature all change, and human influence on most of these becomes more pervasive (Bärlocher, 1992; Allan, 1995; Dix and Webster, 1995). Under these conditions, it becomes difficult to isolate dominant factors. Chauvet (1991) concluded that altitude was more important than pH, temperature or season in determining the species composition of aquatic hyphomycetes in 27 streams in south-western France. In a study of three streams in the French Pyrenees, with 40 collecting sites at various distances from the source, the major conclusion emerging was that fungi and riparian tree communities were simultaneously but independently structured by environmental conditions, which were summarized by elevation (Fabre, 1996). Elevation was used as a "synthetic variable" controlling most other physical and biological characteristics of running waters.

Tropical regions

The tropics often pose substantial logistical problems for fieldwork. Of the 10 sites of the current study, several are difficult to sample during several months; for others, access remains somewhat hazardous throughout the year. Uninterrupted series of observations extending over several months or years are therefore often impossible. This inevitably weakens the validity of conclusions; it can also substantially lower the ability of statistical tests to detect existing trends or correlations. To a lesser extent this is also true for studies in temperate regions. Most of the published investigations are based on 4-12 samples taken

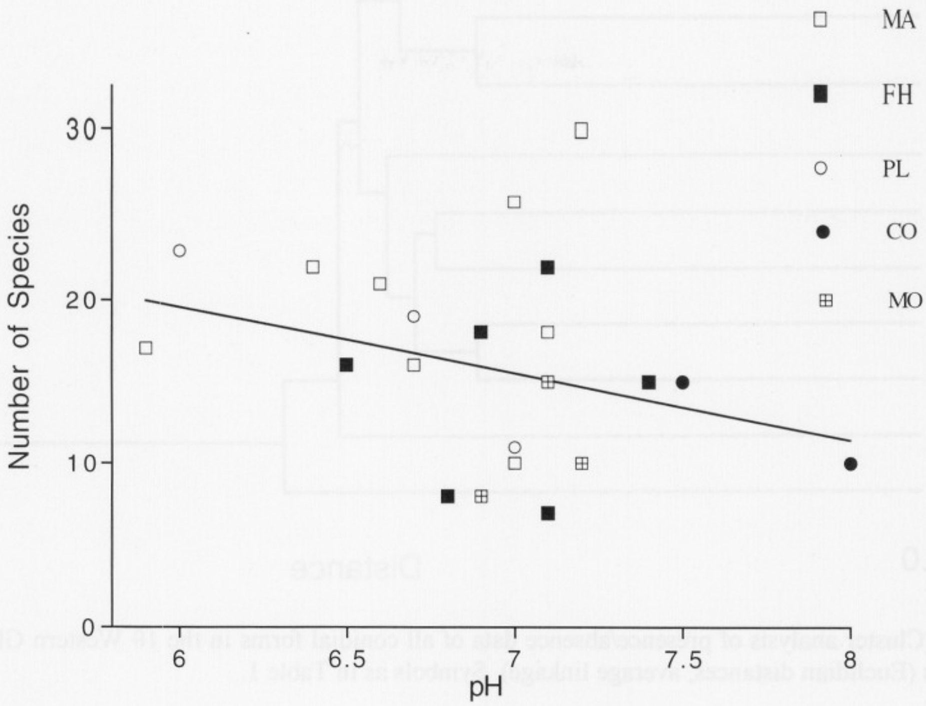

Fig. 1. Aquatic hyphomycete species vs. pH of stream. MA = mid-altitude streams; FH = foot-hill; PL = plains; CO = coastal; MO = mountain. Data from this study, and from Chandrashekhar *et al*. (1991), Rajashekhar and Kaveriappa (1993), Sridkar and Kaveriappa (1989 a,b,c).

during one year. This is insufficient to describe fungal stream communities: in the first 5 years of investigating the fungi in Catamaran Brook (New Brunswick, Canada), 40-50 species were identified per year. Over the entire period, a total of 75 species has been identified (FB, unpubl.). A preliminary conclusion is that the number of ecological roles remains fairly constant but are filled by different actors in different years. Thus, studies restricted to one year (or to the season of maximum diversity, which generally follows leaf-fall) may give a reasonable estimate of how many species can coexist in the short run. They are insufficient to make statements on the long-term stability of a given combination of species.

In the current study, samples were taken once during the post-monsoon season. Earlier studies (Sridhar and Kaveriappa, 1989a, b, c; Chandrashekhar *et al*., 1990; Sridhar *et al*., 1992; Rajashekhar and Kaveriappa, 1993) suggested that typically 80-90 % of all species occur during this period (one notable exception is the Sampaje stream, where the post-monsoon sample accounts for

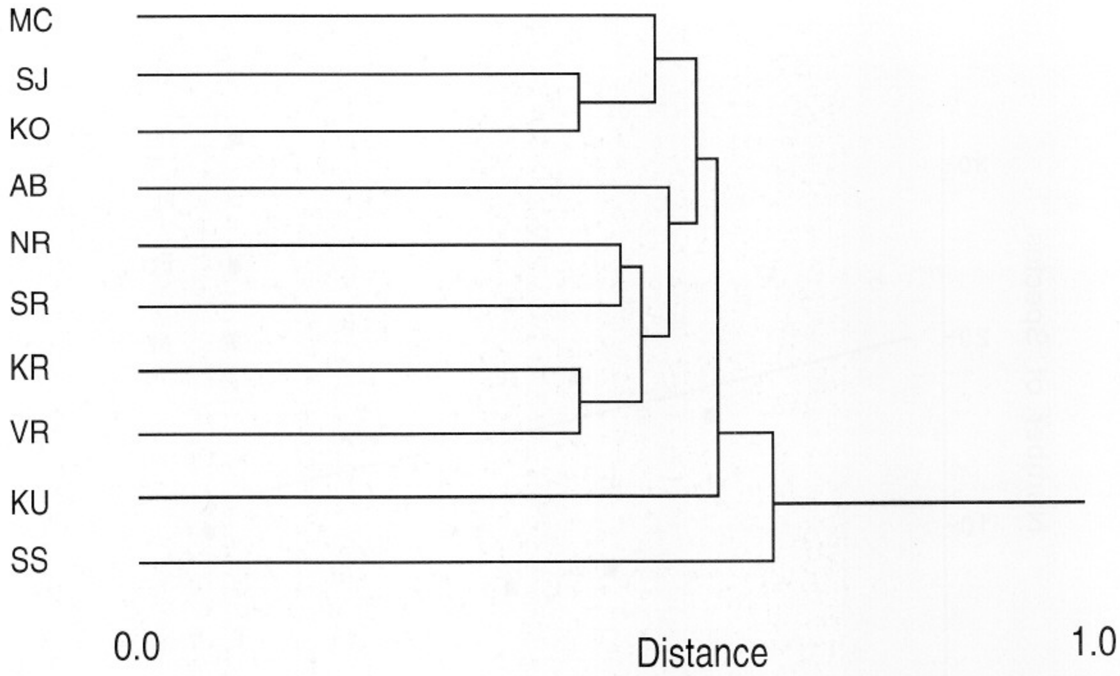

Fig. 2. Cluster analysis of presence/absence data of all conidial forms in the 10 Western Ghat streams (Euclidian distances, average linkage). Symbols as in Table 1.

only 63 % of the total). Despite these shortcomings, the results suggest that water chemistry influences fungal species numbers and composition in Western Ghat streams. In the combined data used in Fig. 1, the deletion of the Sampaje stream results in an almost significant correlation ($p = 0.051$) between pH and species number. Sampaje stream is unusually species-rich, and can reasonably be classified as an outlier: the total of aquatic hyphomycetes reported has reached 76 (Sridhar and Kaveriappa, 1992, 1993), by far the highest number in any stream of that area. It is also interesting to note that both in this and an earlier attempt (Bärlocher, 1987) to link pH and species numbers, the greatest variability seemed to occur between values of 6.8 and 7.2.

There is also evidence that altitude may influence species numbers (this comparison includes all the streams listed in Fig. 1): if only stream systems flowing west into the Arabian Sea are considered, the average number of species is 10.3 in the mountains (sparse riparian vegetation), and reaches a maximum of 20.1 at mid-altitude (rich, relatively undisturbed forests). It then declines to 14.2 (foot-hills) and 12.5 (coastal), as temperature rises, water flow becomes more sluggish and human influence stronger.

Community composition, as mentioned earlier, tends to be more variable than overall species numbers. There is nevertheless some evidence that the major factor separating fungal communities, as in reports from France (Chauvet, 1991;

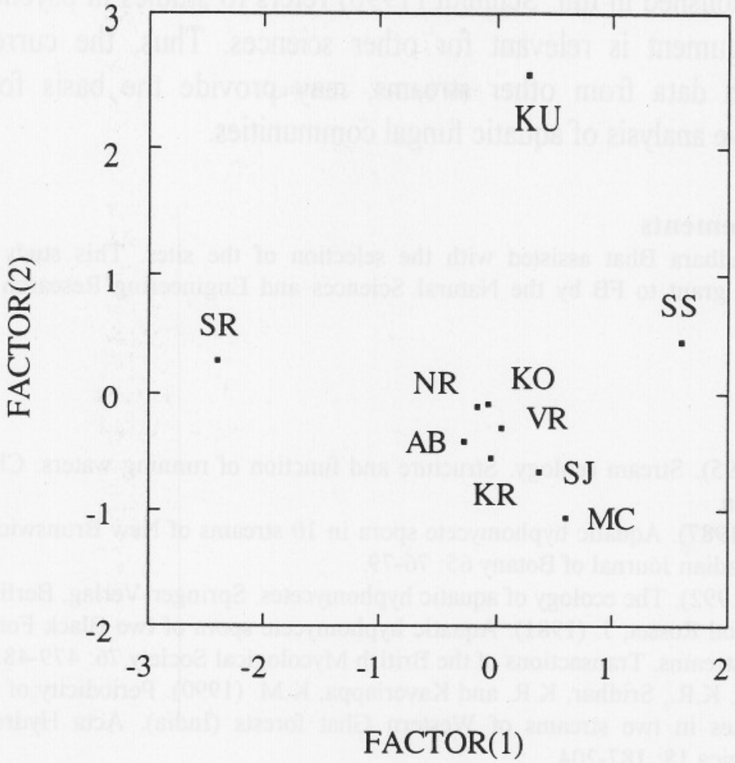

Fig. 3. Principal Component Analysis of fungal communities in 10 Western Ghat streams. Symbols as in Table 1.

Fabre 1996) is related to altitude (Figs. 2, 3). It is also obvious that the dominant species differ substantially between these two regions: in Indian streams, *Lunulospora curvula*, *L. cymbiformis*, *Flabellospora verticillata*, *Triscelophorus acuminatus* are common; if these occur at all in temperate streams, they are often restricted to summer months (Bärlocher, 1992; Dix and Webster, 1995). At least in some cases, this can be linked directly to temperature preferences (Webster *et al.*, 1976; Suberkropp, 1984).

Conclusions

Even though none of the correlations investigated here were significant at conventional p levels, it would be premature to conclude that no correlations exist. Negative results, i.e. the absence of significant correlations, are often due to insufficient power of the statistical test (power = ability of a test to correctly diagnose existing correlations; Zar, 1984). Schmidt (1996) therefore recommends that more emphasis be placed on meta-analysis, i.e. a systematic analysis of all relevant studies. This is only possible if negative and positive results are published in full. Schmidt (1996) refers to studies in psychology, but the same argument is relevant for other sciences. Thus, the current study, together with data from other streams, may provide the basis for a more comprehensive analysis of aquatic fungal communities.

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