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MULTIVARIATE ANALYSIS OF THE SCUTELLINIA UMBRORUM COMPLEX (PEZIZALES, ASCOMYCETES) FROM FIVE ECOTOPES IN THE NETHERLANDS

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The multivariate structure of the *Scutellinia umbrorum* complex (*Pezizales, Ascomycetes*), based on the morphometrical parameters of 81 specimens from five ecotopes in the Netherlands, was analysed. According to conventional expert estimation, five putative taxa resp. species were established: *S. patagonica* (Rehm) Gamundi, *S.* aff. *subhirtella* Svrček, *S. umbrorum* (Fr.) Lambotte, *S. parvispora* J. Moravec and *S. subhirtella* s. Kullman. These taxa form a taxonomic continuum hardly separable by traditional taxonomy. Five clusters obtained by UPGMA (with the generalized J-distance for mixed data as a measure of resemblance) are more distinct and in good accordance with ecological factors; some of them, however, are statistically not well separated. The revision of the clusters' structure by k-means approach yields highly discontinuous clusters. The morphometric characters of specimens differ when going from open habitats to the forest. Differences are also revealed in phenology: the growing season starts in the forest later than in open habitats. The data are divided into two subsets according to spore ornamentation and spore width, the withingroup variation of either subset is caused mainly by the length of marginal hairs. On the basis of several statistical methods a supposition was introduced that the *S. umbrorum* complex probably consists of two polymorphic species, *S. umbrorum* (Fr.) Lambotte and *S. subhirtella* Svrček s.l., with the mean value of marginal hairs longer than 450 µm and shorter than 450 µm, respectively. The UPGMA clusters can be interpreted as ecodemes of respective species.

INTRODUCTION

The systematics of the order *Pezizales* (*Ascomycetes*) is disputable and rather unstable (Kimbrough & Gibson, 1989; van Brummelen, 1994; Bunyard et al., 1995). The separation of some species is based on the morphological description of one or two specimens only. The result is a multitude of putative taxa. When studying more abundant material of closely related species in *Pezizales*, an extensive variation of morphological characters becomes evident.

Taking into account that the *Pezizales* have "a virtually invariant haplophase with predominantly homomictic mating" (Burnett, 1987; Weber, 1992) it may be considered that these fungi exist in natural conditions as lineages, i.e. as groups of clones with the same DNA haplotype. In the genus *Scutellinia* (Cooke) Lambotte emend. Le Gal there are homothallic species, e.g. *Scutellinia scutellata* (Fr.) Lambotte (Gwynne-Vaughan & Williamson, 1933).

The evolutionary pattern of *Scutellinia* follows continuous divergence, the substrate being the most important natural selection factor for these saprotrophic fungi (Kullman,

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1979, 1982, 1986; Kullman & Rahi, 1988a, 1988b, 1989, 1990; Schumacher, 1990). Sometimes response to the substrate and other environmental conditions is expressed also by local ecotypical variability which is revealed in pronounced morphological difference (Stebbins, 1950; Kullman, 1977, 1995).

The present paper aims to (i) analyse the structure of a comparatively large data set of the *Scutellinia umbrorum* complex, hardly distinguishable morphologically, (ii) test the statistical significance of clusters obtained by different phenetic approaches of classification of these data, and (iii) analyse the dependence of data structure on substrate and habitat conditions.

MATERIAL AND METHODS

Study sites

The material was collected in the Netherlands during 1979–89. Five localities, constituting a trophic gradient from ecotopes poor in nutrients to richer ones were studied:

(i) Best-Son (denoted as B). Former loam-pit of brick-work. The habitat represents a deposition of loam or loamy sand being permanently wet or drying slowly after the removal of loam. Only pioneer vegetation has formed, dominated by *Equisetum* spp., *Salix* spp., *Alnus glutinosa*, mosses, etc. 16 specimens collected.

(ii) Nuenen (N). Abandoned loam-pit. Habitat and vegetation quite similar to that of Best Son. 16 specimens collected.

(iii) Eindhoven, Urkhoven (E). Paludified meadow with a sparse field layer on sandy-loamy soil. Traversed by car-tracks. Transition zone between wet heathland and reed swamp. The main vascular species are: *Pedicularis* spp., *Gentiana pneumonanthe*, *Dactylorhiza maculata*, *Platanthera bifolia*, *Carex* spp., *Phragmites australis*, *Sphagnum* spp. 7 specimens collected.

(iv) Helmond, De Schouw (H). Rather open moderately eutrophic moist meadow (hayland) on sandy-loamy soil. Traversed by car-tracks. Dominant vasculars are: *Carex* spp., *Equisetum* spp., and *Dactylorhiza majalis*. 25 specimens collected.

(v) Elsloo-Geulle, Bunderbos (L). Eutrophic forest slope (with calcareous springs) on slowly drying black mud along a regularly overflowing brooklet. Abundant vasculars are: *Equisetum telmateia*, *Impatiens noli-tangere*, *Chrysosplenium* sp., and *Allium ursinum*. 16 specimens collected.

Measurements

The characteristics of fungal fruit-bodies were measured with the microscope 'Olympus BH-2', magnification up to 1000 ×. Tap water was used as an observation medium. For all 81 specimens, 4 morphological parameters were measured: length of marginal hairs on the apothecium (PILL), width of marginal hairs on the apothecium (PILW), spore length (SPOL), spore width (SPOW). For every specimen all morphological parameters were measured in not less than ten replications. The mean values were used as initial data for multivariate analysis. A subtracted parameter, the ratio of spore length to spore width (Q), was also included in the data matrix. Additionally, the type of spore ornamentation (OR) was identified as (i) tuberculate, (ii) verrucose, and treated as a binary variable. The type of ornamentation was studied by staining the spore wall in cotton blue solution in lactophenol as well as from photographs of the scanning electron microscope (Figs. 1 and 2).

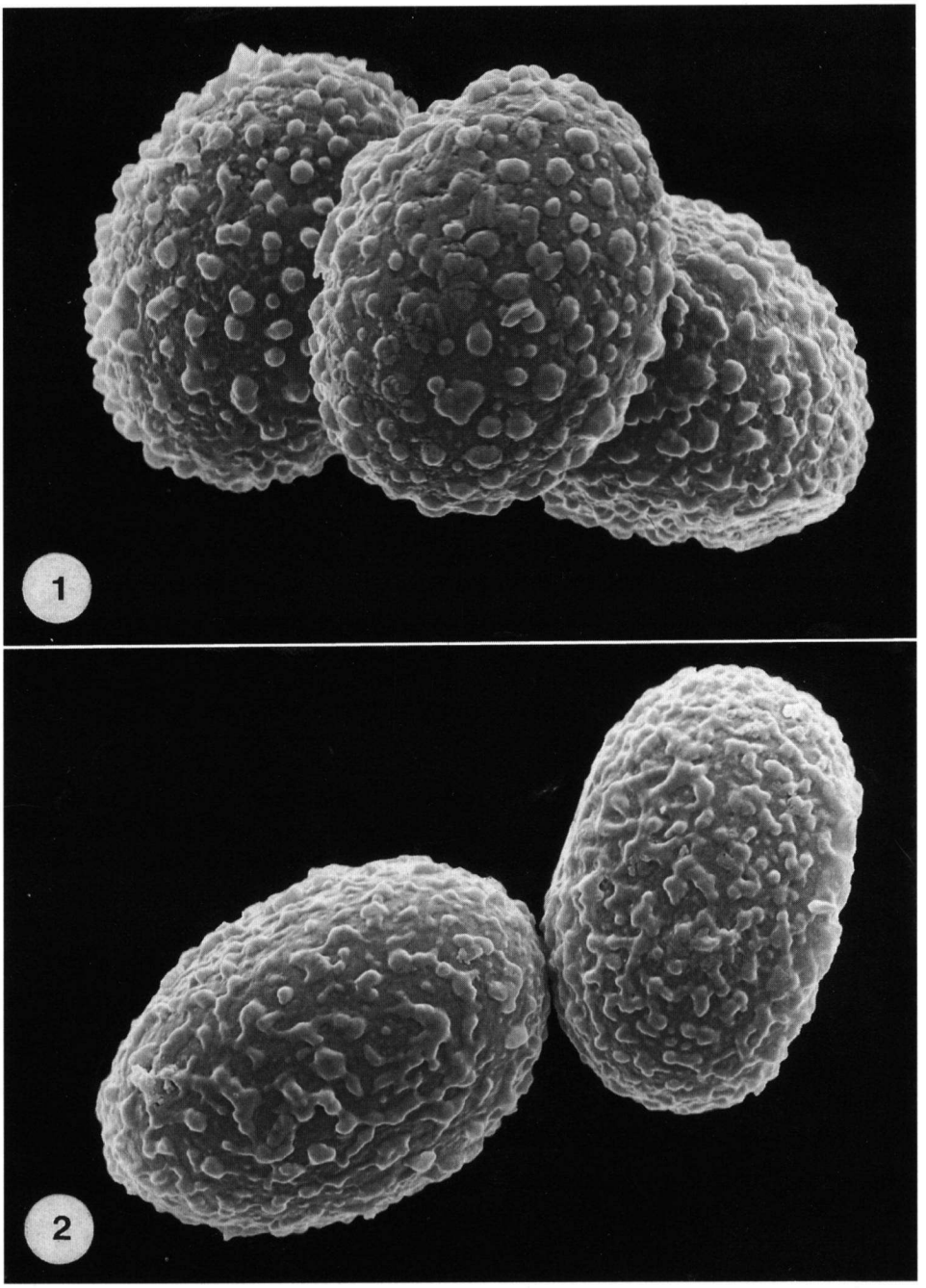


Fig. 1. Spores with a tuberculate type of ornamentation. Specimen of *S. parvispora* according to the expert estimation. Magnification 3000 × (15 mm = 5 μm). — Fig. 2. Spores with a verrucose type of ornamentation. Specimen of *S. subhirtella* s. Kullman according to the expert estimation. Magnification 3000 × (15 mm = 5 μm).

Two nominal characteristics were used as environmental parameters. One describes the type of the ecotope according to its trophicity and openness from the loam pit to the eutrophic forest slope (see study sites: B, N, E, H, and L). The other corresponds to the substrate: S = mineral soil, O = humous soil or decaying wood, W = wood.

Data

At first the specimens (OTUs) were classified traditionally (see diagnoses below). The results of this were further taken as a basis for comparison and search for an optimal solution by numerical methods.

1. *Scutellinia patagonica* (Rehm) Gamundi (denoted as Pt)

Diagnosis: Apothecia 3–12(–20) mm. Marginal hairs 200–1160 × 18–40(–45) µm, sample means between 535–695 × 25–32 µm, average 600 × 28.8 µm, 3–28 septate; walls (2–)3–6 µm thick; base with 1–3 roots, the longest hairs often more complex. Ascospores (17.5–)18–22 × 15–18.5 µm, sample means between 19.4–20.7 × 15.7–17.5 µm, average 20.1 × 16.7 µm; Q = 1.1–1.3, means between 1.14–1.27, average 1.20; ornamentation tuberculate, warts of different size rarely confluent 0.5–1.2(–1.5) µm high and 0.3–1.7(–2.5) µm wide. Habitat H.

These characteristics are in good agreement with *S. patagonica* as described by Gamundi (1975) and Schumacher (1990), but also correspond with *S. arenosa* (Velen.) Le Gal (Le Gal, 1966b and Moravec, 1974).

2. *Scutellinia* aff. *subhirtella* Svrček (denoted as Sb)

Diagnosis: Apothecia 1.5–10(–15) mm. Marginal hairs 110–650(–720) × 12–35 µm, sample means between 255–465 × 19–27.4 µm, average 360 × 23.6 µm, (0–)2–14 septate; walls 1–5 µm thick; base usually with 1–3 roots, exceptionally more complex. Ascospores (17–)18–23(–24) × (13–)14–16.5(–17.5) µm, sample means between 18.9–22.5 × 14.3–16.3 µm, average 20.5 × 15.3 µm; Q = 1.2–1.5(–1.65), means between 1.26–1.46, average 1.34; ornamentation heterogeneous tuberculate(-verrucose), variable warts of different size and shape 0.3–1.0 µm high and 0.3–2.0 µm wide, often isolated but sometimes confluent, forming short ridges and irregular complexes 1–1.5 µm high and 2–3(–4) µm wide. Habitats B and N.

The marginal hairs fit well Schumacher's (1990) description of *S. subhirtella* Svrček, but spore shape and ornamentation deviate too much and look more like his *S. umbrorum*. Because of this resemblance such collections have certainly been conceived as *S. umbrorum* (cf. Le Gal, 1947; Maas Geesteranus, 1969; they might even represent the real *Peziza umbrorum* Fries, 1823).

3. *Scutellinia umbrorum* (Fr.) Lambotte (denoted as Um)

Diagnosis: Apothecia 3–16(–20) mm. Marginal hairs 200–1120 × 16–43 µm, sample means between 430–745 × 23–30.4 µm, average 568 × 25.9 µm, 2–29 septate; walls 2–7 µm thick; base with 1–3 conspicuous roots but longest hairs also multifurcate. Ascospores 18.5–24 × (13–)13.5–17.5 µm, sample means between 20.4–22.5 × 14.1–16.4 µm, average 21.3 × 15.5 µm; Q = 1.25–1.55(–1.7), means between 1.30–1.47, average 1.38; ornamentation tuberculate, mostly isolated semiglobose or slightly angular warts of different size 0.5–1.5(–2.5) µm high and 0.5–2(–3) µm wide, sometimes crateriform.

Habitats H and E. In H often growing together with *S. patagonica* which starts fruiting one or two weeks earlier and differs mainly in the lower Q-value for the ascospores. During the research period no mixing of the two species could be observed.

These characteristics correspond well with the descriptions of *S. umbrorum* by Denison (1959) and Kullman (1982), which are based on the neotypification of this species by the material of 'Ellis & Everhart's North American Fungi' No. 2911 in CUP. According to both authors the ascospores of the neotype clearly show a tuberculate ornamentation. Le Gal (1966a), however, found the ornamentation to be more confluent warty, like in *Scutellinia ampullacea* (Limm. ex Cooke) O. Kuntze and therefore rejected Denison's neotype. The diagnosis above does also correspond with the descriptions by Le Gal (1966a) and Schumacher (1990), which are based on another neotypification of *S. umbrorum* by Le Gal (1966a) with the material of Boudier's Icones mycologicae 2: plate 369, 1906 (leg. Boudier, Montmorency, 1883, "No. 369", in Herb. Boudier, PC). Unfortunately this shows different or poorly developed marginal hairs.

4. *Scutellinia parvispora* J. Moravec (denoted as Pr)

Diagnosis: Apothecia 3–13 mm. Marginal hairs 200–950 × 15–38 µm, sample means between 385–665 × 21.3–30.6 µm, average 510 × 26.3 µm, (0–)2–22 septate; walls (1–)2–7 µm thick; base with 1–3 roots sometimes more complex. Ascospores 17–22 × 13.3–16.6 µm, sample means between 18.1–20.6 × 14.1–15.5 µm, average 19.9 × 14.6 µm; Q = 1.25–1.50, means between 1.27–1.40, average 1.36; ornamentation rather homogeneous tuberculate, mostly isolated semiglobose warts 0.3–1.2 µm high and 0.5–1.5(–2) µm wide. Habitat L.

Except for the habitat and the somewhat smaller spores, the differences with *S. umbrorum* are marginal.

5. *Scutellinia subhirtella* Svrček s. Kullman (denoted as Sk)

Diagnosis of the first morph: Apothecia (2–)4–17 mm. Marginal hairs 150–1000 × 11–33 µm, sample means between 340–650 × 18.3–25.6 µm, average 505 × 22.8 µm, 3–31 septate; walls (1.5–)2–6 µm thick; base with 1–3 roots, the longest hairs more complex. Ascospores 17.5–23.5 × 12.5–16 µm, sample means between 19.4–21.8 × 13.7–15.1 µm, average 20.4 × 14.2 µm; Q = 1.3–1.6(–1.7), means between 1.38–1.50, average 1.43; ornamentation confluent verrucose, like *S. scutellata* up to 1.0(–1.5) µm high. Habitat mostly wet woodland with *Alnus* and *Salix* surrounding B, H and E, but also in H.

Diagnosis of the second morph: Apothecia 2–6(–8) mm. Marginal hairs 100–465 × 14–28 µm, sample means between 245–365 × 17.5–22 µm, average 295 × 20.6 µm, 2–10 septate; walls 1.5–5 µm thick; base with 1–3 roots very rarely more complex. Ascospores 19–21.8 × 13.4–15 µm, sample means between 20.0–20.7 × 13.9–14.3 µm, average 20.3 × 14.1 µm; Q = 1.3–1.6, means between 1.40–1.47, average 1.44; ornamentation confluent verrucose up to 0.5(–1) µm high. Habitat L on drier mossy wood of *Fraxinus*(?).

These morphs correspond well with Kullman's (1982) interpretation of *S. subhirtella* Svrček. According to Schumacher (1990) who studied the type, the spore ornamentation is tuberculate instead of confluent verrucose. In his monograph no description could be found which fully fits the above mentioned characteristics. Perhaps it has been included in his conception of *S. olivascens* (Cooke) O. Kuntze (= *S. ampullacea* (Limm. ex Cooke)

O. Kuntze) which shows broader hairs and larger spores. Recent type studies of both *S. olivascens* and *S. ampullacea* by Yao & Spooner (1996) also demonstrated somewhat larger ascospores. The larger apothecia of the Dutch specimens with hairs up to 1000 µm look very much like *S. umbrorum*, but differ in the slightly more slender marginal hairs and spore ornamentation.

DATA PROCESSING

Cluster analysis

For classification the unweighted average linkage method (UPGMA) was used, where the process of specimens fusion is based on the minimum average distance between specimens and clusters. By this method cluster sizes are considered while recalculating values in the distance matrix. If cluster i has 1, cluster j has 4 and cluster h has 2 specimens, then for calculating the distance between newly formed cluster ij and cluster h, d_{jh} must be considered twice as much as d_{ij} (Podany, 1994).

For optimization of UPGMA-clusters as well as, for testing the stability or invariantness of classification, the k-means clustering (MacQueen, 1967) was used. This method minimizes the within-cluster variation (sum of squares). The process is iterative: as initial centers (means) of clusters the centroids of UPGMA-clusters were exploited. Then, in each step of the procedure it is examined if the relocation of any specimen from one cluster to another provides decrease of the sum of squares. The object for which maximum decrease may be achieved is moved to the new group. The iterations stop if no further reduction is possible.

To measure the fit of hierarchical UPGMA dendrogram to the originating distance matrix **D**, the matrix of ultrametric distances **C** was computed. The last matrix consists of elements c_{ij}, which are defined to be the first level in the dendrogram at which specimens i and j occur in the same cluster (Everitt, 1993). Then, the product moment correlation or the cophenetic correlation (Sokal & Rohlf, 1962) between these two matrices was calculated.

Both classifications, as well as estimation of cophenetic correlation were realized by SYN-TAX 5.0 program package (Podany, 1993).

Ordination

To visualize graphically the specimens' mutual relationship in multidimensional character space, principal components analysis (PCA) of $\ln (1 + x_{ij})$ transformed data was exploited. By means of PCA new 'artificial' variables (scores) are computed on the bases of the original data in attempt to achieve a more efficient representation of data in few dimensions (principal components, shown in figures as axes of the ordination). For ordination CANOCO package, version 3.1 (ter Braak, 1988, 1990), and CANODRAW package, version 3.0 (Smilauer, 1992) were used.

Estimation of adjacency

When focusing on taxonomic continuum we usually do not mean all possible transitions between clusters but only relations between clusters which are most similar, or adjacent in the character space. Thus, the number of clusters to which a specimen or an operational taxonomic unit (OTU) with intermediate characteristics can belong is always smaller than the total number of clusters. Numerical analysis requires a formal criterion for deciding whether the clusters should be regarded as adjacent.

One can postulate: the j-th cluster is treated as adjacent to the i-th cluster if the distance between at least one of the OTUs of the i-th cluster and the centroid of the j-th cluster is smaller than the distance to the centroids of all other clusters (Paal & Kolodyazhnyi, 1983; Paal, 1994). This definition of adjacency is non-symmetric: if the j-th cluster is adjacent to the i-th cluster, the latter need not necessarily be adjacent to the OTUs belonging to cluster j.

According to such a criterion the distance of all OTUs from all centroids (except the cluster to which the OTU belongs) can be calculated and the adjacent clusters estimated. The results are presented in the form of the adjacency matrix.

Testing of clusters' distinctness

In order to measure the degree of distinctness the α-criterion (Duda & Hart, 1976) was used. To acquire a better interpretation of the estimates, it is more convenient to apply the corresponding probabilities as coefficients of indistinctness (CI) instead of direct values (Paal, 1987, 1994):

$$CI = 100 / \sqrt{2 \pi \int \exp(-x^2/2) dx}. \quad (1)$$

To visualize the distribution of OTUs located between the centroids of two adjacent clusters in the character space, the split window method (Parzen, 1962) appears appropriate. The density of the OTUs projection probability distribution on a straight line passing through the centroids of both clusters can be calculated as

$$p(x) = 1/n \sum_{i=1}^{n} (1/h) \Phi [(x-x_i) / h], \quad (2)$$

where p(x) is the distribution density at point x, Φ – the window function, h – the smoothing parameter or window breadth, n – the number of OTUs in the cluster, x_i – the projection of the i-th OTU on the line. The density of the normal distribution was regarded as the window function.

The smoothing parameter h was determined according to the formula:

$$h = 2s (0.05 + 1 / \sqrt{n}), \quad (3)$$

where s is the standard error of the projections. The density of projection probability for the OTUs of either cluster was calculated for the line segment ± 3s for every 0.1 unit of the standard error. Normalization to the standard error makes it possible to estimate the expression of distinctness independently of the number of OTUs in the cluster.

The transition zone between centroids, denoted by two dotted lines perpendicular to the line connecting the centroids in figures is estimated so that its length is exactly half the distance between the centroids but, in depending on the within-group dispersion rate, the transition zone shifts toward one or the other centroid (Paal & Kolodyazhnyi, 1983; Paal, 1987).

RESULTS

Expert classification

Adjacent taxa for *S. patagonica* (cluster 1) are *S. umbrorum* (cluster 3) and *S. parvispora* (cluster 4) (Table I). All these three taxa are mutually insufficiently separated (Table II). The distribution of OTUs projection probability on a line joining the centroids of these clusters is multimodal and largely overlapping. An example is shown in Fig. 3.

Table I. Adjacency matrices of clusters obtained by different classification procedures. Figures in the matrix indicate the percentage of OTUs of the analysed cluster for which the centroid of the compared cluster is the nearest in the character space.

Cluster analysed	Cluster compared				
	1	2	3	4	5
Expert classification					
1	–	–	66.7	33.3	–
2	–	–	–	–	96.2
3	56.3	31.3	–	12.5	–
4	12.5	25.0	37.5	25.0	–
5	–	40.9	22.7	31.8	–
UPGMA classification					
1	–	–	100	–	–
2	–	–	–	76.8	21.4
3	50.0	–	–	9.1	36.4
4	–	100	–	–	–
5	7.7	7.7	84.6	–	–
k-means classification					
1	–	–	100	–	–
2	–	–	–	75.9	24.1
3	52.4	–	–	9.5	38.1
4	–	100	–	–	–
5	7.7	7.7	84.6	–	–

Table II. Coefficients of indistinctness between the taxa of *S. umbrorum* complex. Below the diagonal: evaluation of clusters estimated by the expert classification, above the diagonal: evaluation of clusters obtained by UPGMA.

Cluster	1	2	3	4	5
1	X	0.0	60.7	0.0	0.7
2	0.0	X	0.0	10.2	0.0
3	63.5	0.0	X	0.0	76.0
4	10.9	0.0	48.0	X	0.0
5	0.2	9.8	2.1	48.7	X

For specimens of the *S.* aff. *subhirtella* Svrček (cluster 2), a single adjacent cluster is 5, *S. subhirtella* s. Kullman (Table I), from which it is separated non-significantly (Table II).

The main neighbour in the character space for cluster 3 is cluster 1; adjacency is seen also with *S.* aff. *subhirtella* Svrček, and with *S. parvispora,* i.e. with clusters 2 and 4 (Table I).

Cluster 4, *S. parvispora,* is the most diffuse, varying in different directions and being adjacent to all other clusters to an almost equal degree (Table I). It is distinctly separated only from cluster 2, *S.* aff. *subhirtella* Svrček (Table II).

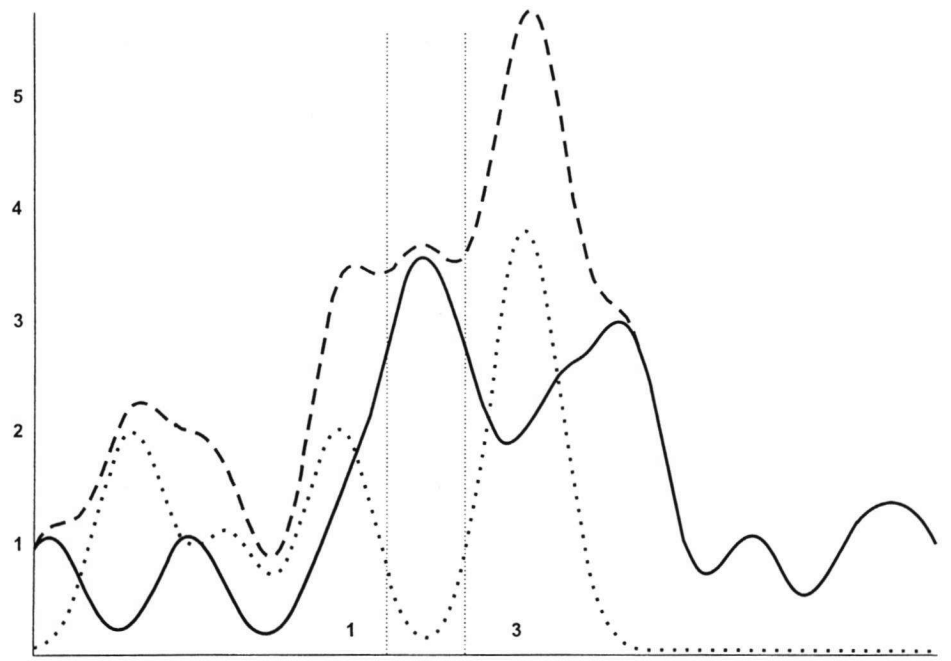


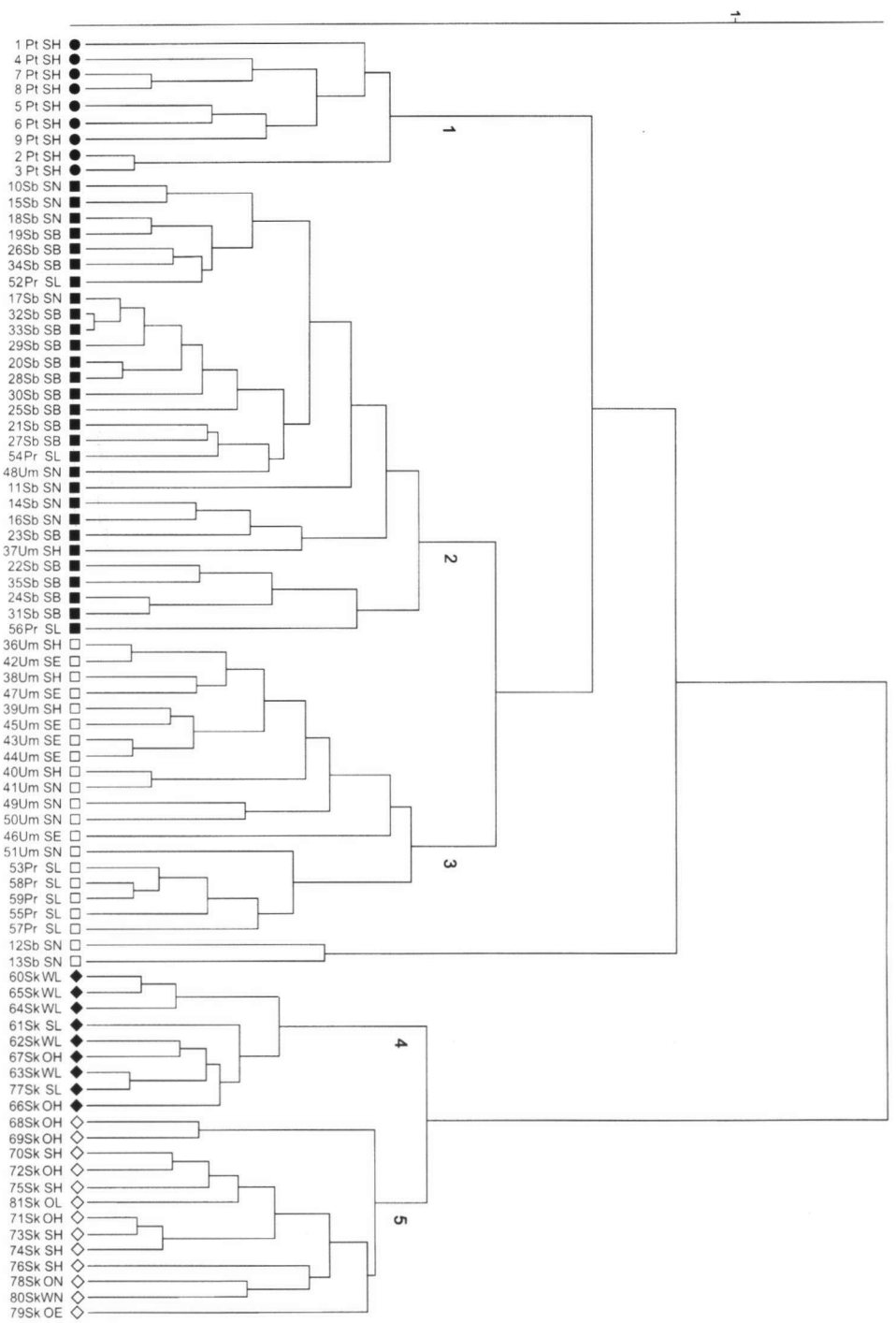
Fig. 3. OTUs projections probability distribution according to the split window method. The curve marked with dots portrays the left-hand cluster, the continuous curve corresponds to the right-hand cluster. The curve with short lines above them represents the OTUs projection probability distribution of the joint cluster. Clusters 1 and 3 of the expert classification.

For the OTUs of cluster 5, *S. subhirtella* s. Kullman, cluster 2, *S.* aff. *subhirtella* Svrček, cluster 3, *S. umbrorum*, and cluster 4, *S. parvispora* are adjacent (Table I). For cluster 5 indistinctness appears with respect to cluster 2 and cluster 4 (Table II).

These results allow to conclude that the adjacency matrix generally conforms with coefficients of indistinctness. On the basis of the adjacency matrix asymmetric relationship between clusters becomes apparent. Thus, cluster 5 is adjacent for 96% of the specimens of cluster 2, whereas cluster 2 is adjacent only for 41% of the specimens of cluster 5. It appears, too, that the taxa estimated according to the expert classification are rather insufficiently separated. Only *S.* aff. *subhirtella* Svrcek has no more than one indistinct relation with other taxa, whereas the other taxa have two, or even three, indistinct relationships like cluster 4, *S. parvispora.*

UPGMA *classification*

The dendrogram received by UPGMA is split into two large subsets at very high level of dissimilarity (Fig. 4). Further, the first of them is divided into three clusters, two OTUs (12 and 13) providing an additional separate branch. (Development of these apotheciums


1
1 Pt SH
4 Pt SH
7 Pt SH
8 Pt SH
5 Pt SH
6 Pt SH
9 Pt SH
2 Pt SH
3 Pt SH
10Sb SN
15Sb SN
18Sb SN
19Sb SB
26Sb SB
34Sb SB
52Pr SL
17Sb SN
32Sb SB
33Sb SB
29Sb SB
20Sb SB
28Sb SB
30Sb SB
25Sb SB
21Sb SB
27Sb SB
54Pr SL
48Um SN
11Sb SN
14Sb SN
16Sb SN
23Sb SB
37Um SH
22Sb SB
35Sb SB
24Sb SB
31Sb SB
56Pr SL
36Um SH
42Um SE
38Um SH
47Um SE
39Um SH
45Um SE
43Um SE
44Um SE
40Um SH
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61Sk SL
62Sk WL
67Sk OH
63Sk WL
77Sk SL
66Sk OH
68Sk OH
69Sk OH
70Sk SH
72Sk OH
75Sk SH
81Sk OL
71Sk OH
73Sk SH
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76Sk SH
78Sk ON
80Sk WN
79Sk OE
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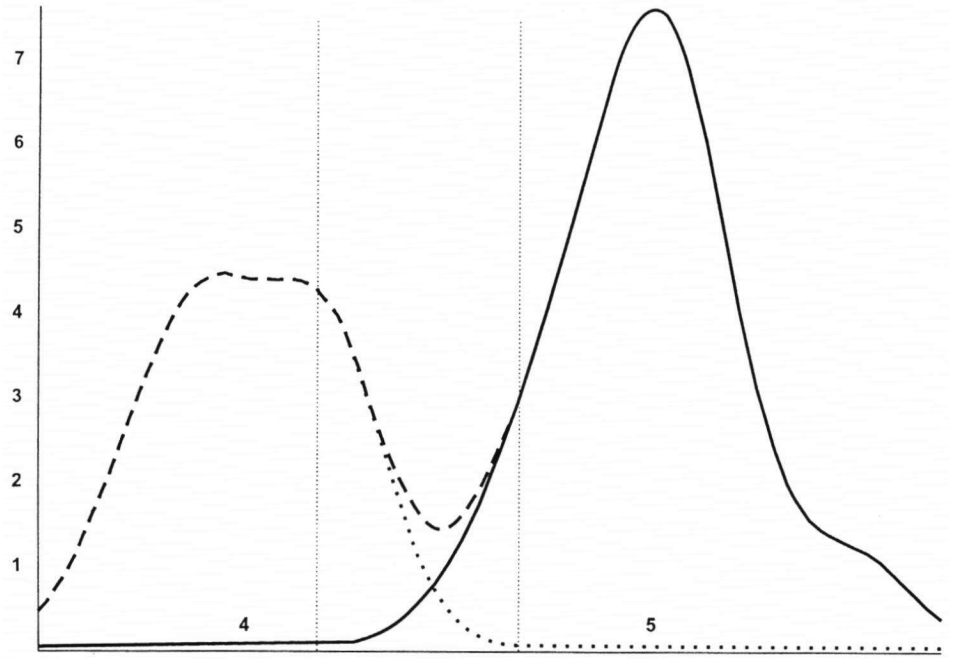

Fig. 5. OTUs projection probability distribution according to the split window method. Clusters 4 and 5 of the UPGMA classification.

is somewhat disturbed, many asci contain only 2–6 mature spores.) The second subset comprises two clusters. Cluster 1 incorporates all specimens of the preliminarily estimated *S. patagonica* but no specimens of the other putative taxa. Cluster 2 includes mainly specimens of *S.* aff. *subhirtella* Svrček of the expert identification, cluster 3 (plus OTUs 12 and 13) merges specimens of two previously recognized taxa, *S. umbrorum* and *S. parvispora*. The specimens assumed to belong to *S. subhirtella* s. Kullman are divided into clusters 4 and 5.

Changes in cluster structure are reflected plainly in the adjacency matrix (Table I). For the objects of cluster 1 (*S. patagonica*) the only neighbour is now cluster 3 (*S. umbrorum* + *S. parvispora*). On the other hand, cluster 1 is also the main neighbour for the specimens of cluster 3, but only for 50% of its OTUs. These clusters are separated non-significantly ($CI_{1,3} = 60.7$, Table II).

For the objects of cluster 2 (*S.* aff. *subhirtella* Svrček) cluster 4 becomes the most adjacent. For the OTUs of cluster 4 only cluster 2 is adjacent, but not cluster 5 as we could

Fig. 4. Dendrogram of UPGMA clustering based on the generalized J-distance for mixed data. The specimen's label includes its number in the sample, abbreviation of empirically estimated taxon name, denotation of substrate, and denotation of habitat. The cluster numbers are marked beside the branches of the dendrogram, the specimens of different clusters are denoted with various symbols.

Table III. Clusters obtained by k–means classification procedure. For the specimen's label cf. Fig. 4.

Cluster	Specimen				
1	1Pt SH	4 Pt SH	7 Pt SH	8 Pt SH	46Um SE
	49Um SN	50Um SN	51Um SN	57Pr SL	81Sk HL
2	14Sb SN	16Sb SN	17Sb SN	20Sb SB	21Sb SB
	22Sb SB	23Sb SB	25Sb SB	26Sb SB	28Sb SB
	29Sb SB	32Sb SB	33Sb SB	35Sb SB	52Pr SL
	56Pr SL	63Sk WL	66Sk OH	67Sk OH	77Sk SL
3	2Pt SH	3Pt SH	5Pt SH	6Pt SH	9Pt SH
	36Um SH	38Um SH	39Um SH	40Um SH	41Um SN
	42Um SE	43Um SE	44Um SE	47Um SE	53Pr SL
	58Pr SL	59Pr SL	68Sk OH	70Sk SH	71Sk OH
	72Sk OH	73Sk SH	75Sk SH	76Sk SH	78Sk ON
4	10Sb SN	11Sb SN	12Sb SN	13Sb SN	15Sb SN
	18Sb SN	19Sb SB	34Sb SB	60Sk WL	61Sk SL
	62Sk WL	64Sk WL	65Sk WL		
5	24Sb SB	27Sb SB	30Sb SB	31Sb SB	37Um SH
	45Um SE	48Um SN	54Pr SL	55Pr SL	69Sk OH
	74Sk SH	79Sr OE	80Sk WN		

expect, because both clusters (4 and 5) were referred to in the expert classification as belonging to *S. subhirtella* s. Kullman. Clusters 2 and 4 represent another pair of indistinct clusters ($CI_{2,4}$ = 10.2); at the same time, clusters 4 and 5 are separated even more significantly ($CI_{4,5}$ = 0.0, Table II) than several of the putative species. The probability of OTUs projection distribution of both clusters is almost normal, and the overlapping of the corresponding curves quite limited (Fig. 5). According to the adjacency matrix (Table I), cluster 5 is now closely related to cluster 3, both constituting the third indistinct pair of clusters ($CI_{3,5}$ = 76.0, Table II).

If the OTUs 12 and 13 are excluded from cluster 3 as outliers, the classification structure will not be considerably more distinct. The same cluster pairs will remain indistinct, only for clusters 3 and 5 the coefficient of indistinctness will be lower ($CI_{3,5}$ = 29.4, Table II).

Taking into account that "hierarchical clustering techniques impose a hierarchical structure on data and it is usually necessary to consider whether this is merited or whether it introduces unacceptable distortions of the original relationships amongst the individuals, as implied by their observed proximities" (Everitt, 1993: 72), the cophenetic correlation coefficient was calculated. In the present case the coefficient is 0.90. This value is close to the upper bound of the range (0.74–0.90) of most frequently occurring cophenetic correlations (Sneath & Sokal, 1973). Rohlf & Fisher (1968) stated that values above 0.8 are sufficient to reject the null hypothesis that "The specimens represent a random sample from a single multivariate normal distribution." In that way, we can conclude that the obtained UPGMA dendrogram is in good accordance with the real structure of data, and the loss of information due to arranging the specimens into a hierarchical classification system is rather limited.

K-means classification

A further attempt to optimize the classification by the k-means procedure, using the previous result as the initial group membership vector, enables to get clusters which are all well distinct, with the coefficient of indistinctness close to zero. However, now correspondence with the empirical classification is much weaker than in the case of UPGMA results (Table III). Still, adjacency relations for clusters obtained on the basis of the k-means algorithm will remain rather similar to the relations established for UPGMA clusters (Table I).

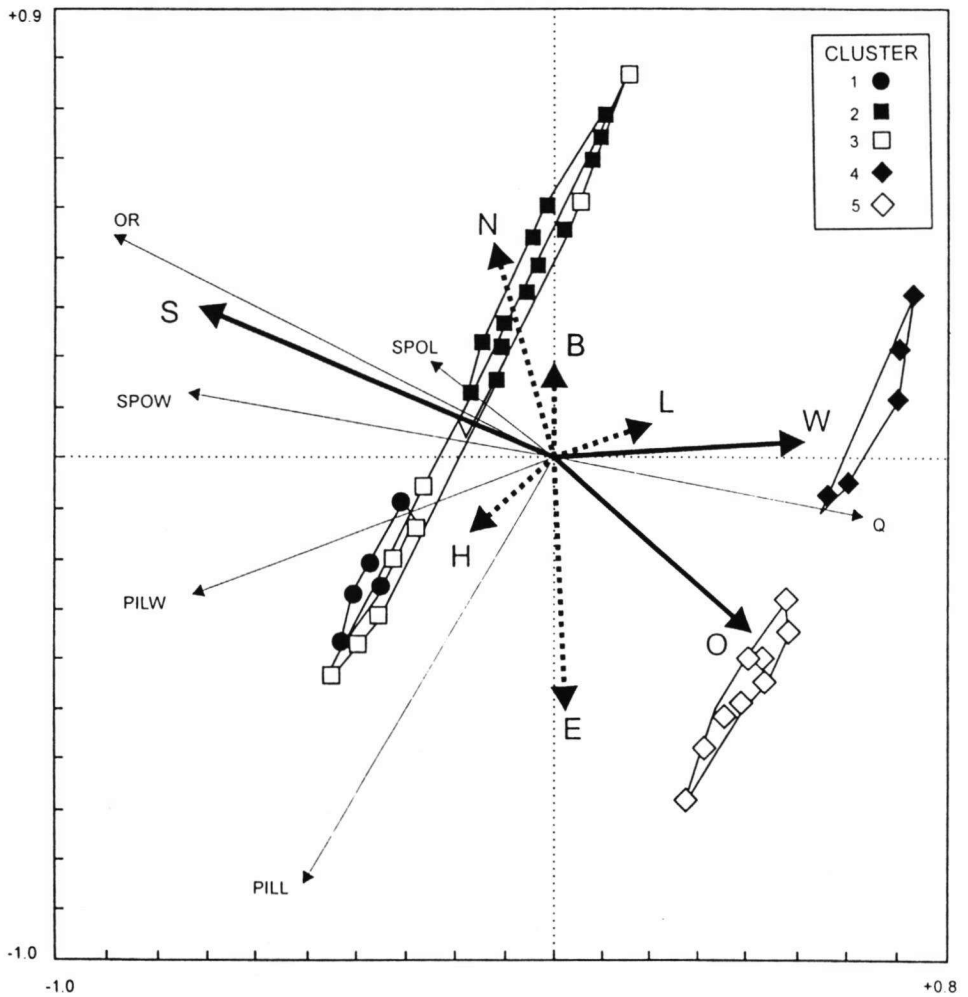

Fig. 6. Classification polygons superimposed onto a PCA ordination and specimen-character-environmental variables triplot. Clusters obtained by UPGMA. The first component (abscissa) explains 55.0% of the total variation and the second component (ordinate) 39.7%.

Ordination analysis

The PCA demonstrates that the whole sample consists of two obviously separated subsets, both having parallel variation (Fig. 6). The two-dimensional solution of PCA is well acceptable, as the first axis accounts for 55.0% of total variance, and the second axis for 39.7%. Amount of variation connected with the next axes is much smaller: the third axis explains 3.3% of variation and the fourth 1.2%. Therefore, these axes are not considered further. The subsequent superposition of clusters onto the ordination plot (Figs. 6 & 7) enables to visualize the issue of clusters estimated by different methods.

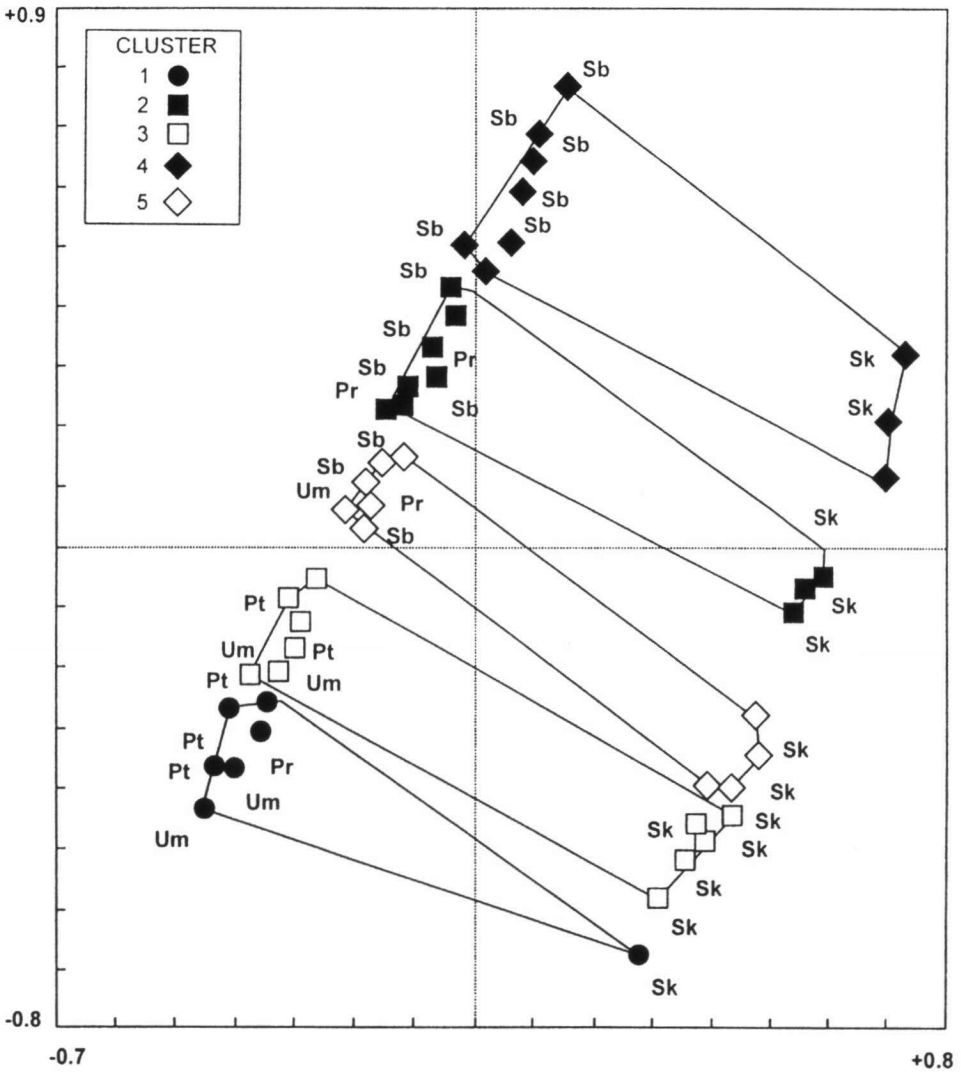

Fig. 7. Classification polygons superimposed onto a PCA ordination. Clusters obtained by the k-means algorithm.

The principal similarity between the classification structure achieved empirically and the one achieved by UPGMA is conspicuous. In both cases the specimens of the bigger subset are separated into clusters parallel to the axis of within-group variation, while the OTUs of several putative taxa are to some extent intermixed (Fig. 6). The OTUs of the smaller subset remain all in a separate cluster or clusters. Parallel variation explains also the large overlapping of the OTUs projection probability distribution (cf. Fig. 3).

K-means clustering provides spherical clusters; in this case we cannot expect that revision of the cluster structure obtained by the hierarchical UPGMA algorithm will produce the same solution as the sum of squares criterion followed by k-means clustering (Podany, 1994). According to the k-means solution, the elongated subsets of OTUs are divided perpendicularly to the direction of their main variation, and there is no overlapping of classification polygons (Fig. 7).

On the basis of the triplot method in which the distribution of the specimens and the loading of the characters for the principal axes are combined in the same figure (Fig. 6), we can get an understanding of the most important characteristics determining the data structure, and how they are related to environmental factors (Jongman et al., 1987; ter Braak, 1990). The specimens are divided clearly into two subsets according to the type of spore ornamentation (OR) and spore width (SPOW) (Figs. 6 & 8). The larger subset of

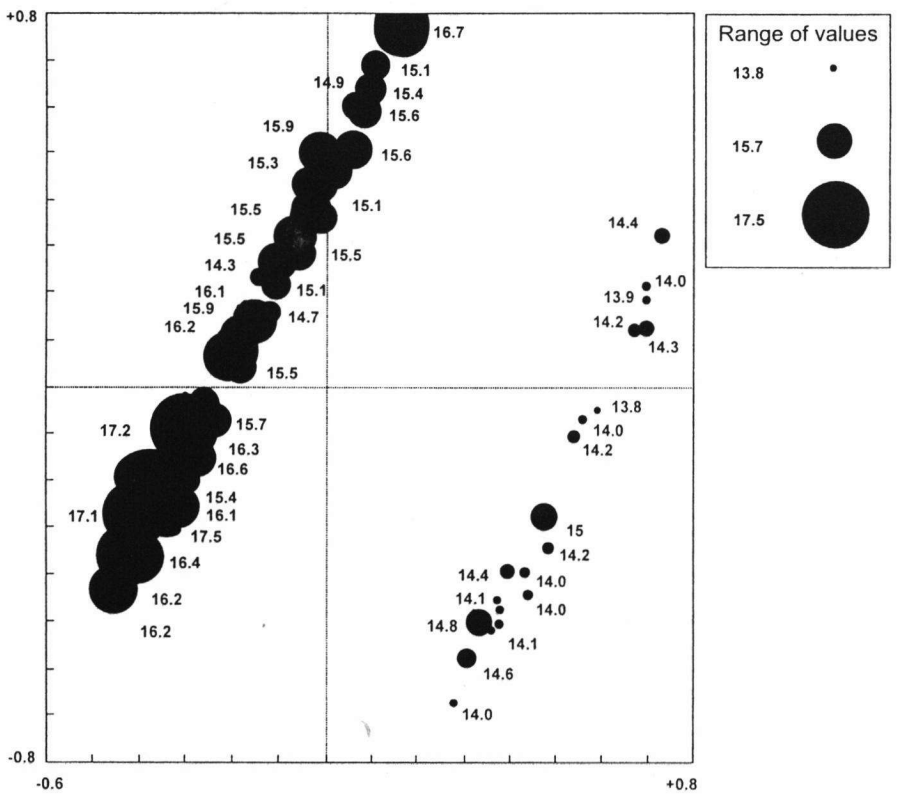

Fig. 8. Distribution of spore width values.

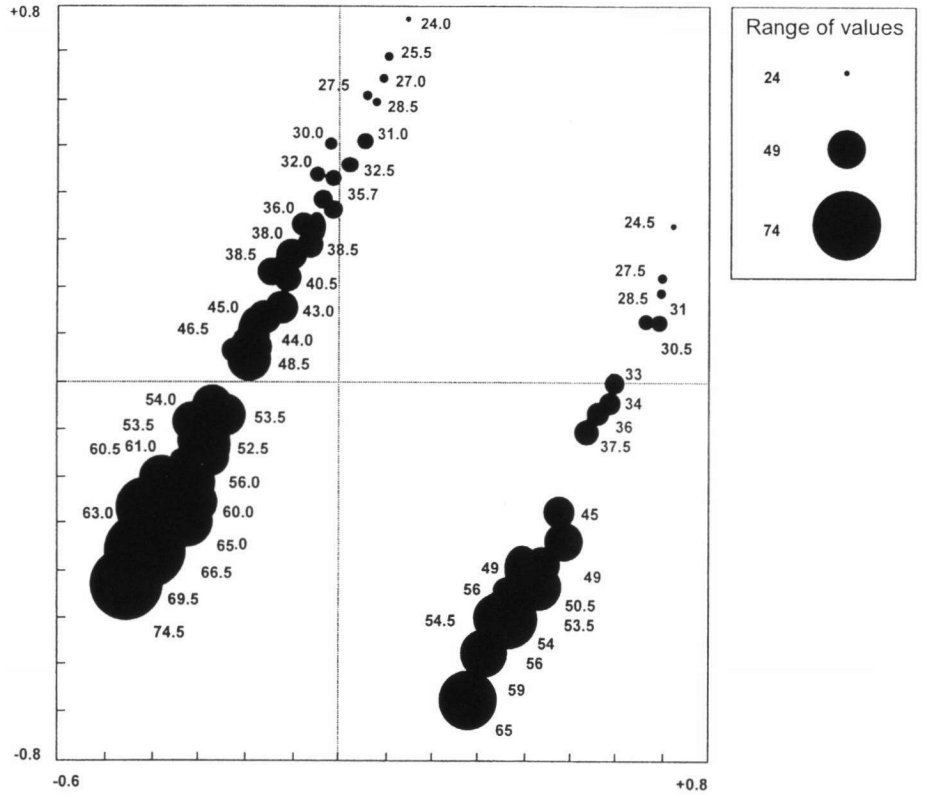

Fig. 9. Distribution of the values of length of marginal hairs. Values in the figure are 10 times lower than real values.

specimens includes only specimens with tuberculate ornamentation and wide spores; to the smaller subset belong specimens with verrucose ornamentation and much narrower spores. These variables are opposed by the ratio of spore length to spore width (Q), but the values of this substracted parameter do not have such a clear distribution pattern. The within-group variation of either subset is caused mainly by the length of marginal hairs (PILL, Figs. 6 & 9).

These results indicate that in this case UPGMA clusters are in a much better accordance with the character of the parameters' variation than the clusters obtained by k-means procedure. Now, the separation of the empirically estimated *S. subhirtella* s. Kullman into two distinct clusters also finds an explanation: it is based mainly on the length of marginal hairs. Cluster 4 includes specimens with short hairs (245–375 µm), while specimens with longer hairs (450–650 µm, Fig. 9) belong to cluster 5. In this way, these clusters correspond well to two morphs of *S. subhirtella* s. Kullman distinguished on the basis of marginal hairs length (Kullman, 1982). Besides, specimens of these clusters are evidently connected with different substrata: specimens with short hairs grow on wood under drier

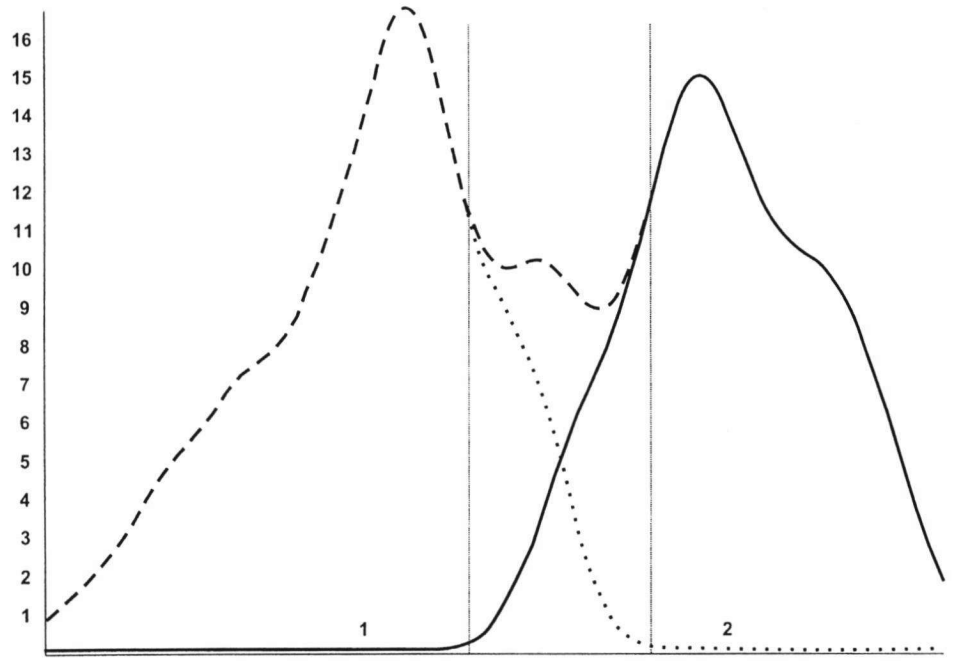

Fig. 9. Distribution of the values of length of marginal hairs. Values in the figure are 10 times lower than real values.

conditions, specimens with long hairs are related to humous soil saturated with water or wet decayed wood (Fig. 6). The specimens of three other UPGMA-clusters all grow on mineral soil with various content of humus.

The data structure is not so clearly associated with general habitat features. Still, the specimens of UPGMA-clusters 1, 3, and 5 (*S. patagonica, S. umbrorum*, and the *S. subhirtella* s. Kullman morph with long hairs) grow mainly on moist meadows (Fig. 6, H & E). Specimens of cluster 2 (*S.* aff. *subhirtella* Svrcek) are associated with loam-pits (N & B). With forest habitat (L) above all the specimens of cluster 4 (*S. subhirtella* s. Kullman morph with short hairs) are related.

According to field records, there is also a remarkable divergence in the phenology of the studied fungi, depending on the habitat type. The growth period in open habitats lasts from May till July, in the forest from June till October.

Suboptimal solution

Considerably different results obtained by two cluster analyses used as well as indistinctness between several pairs of UPGMA-clusters indicate a need to search for a suboptimal solution at a more generalized level. For this purpose we merged mutually continuous UPGMA clusters 1, 3 and 5 into one cluster, and clusters 2 and 4 into another. Testing of the reliability of these joint clusters confirms the significance of their distinctness ($CI_{1,2} = 0.0$). Reorganization of these clusters by k-means procedure did not cause such a remarkable discrepancy as it did at the five-cluster level. Now only three

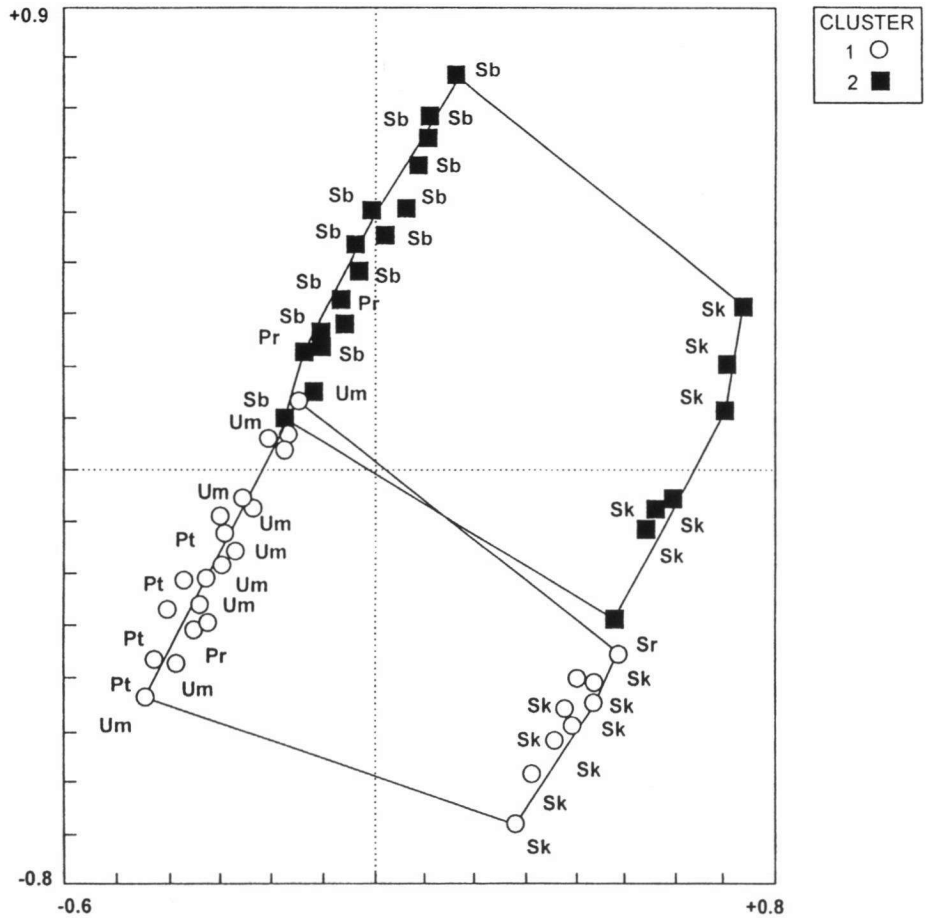

Fig. 11. Classification polygons superimposed onto a PCA ordination. Clusters obtained by the k-means algorithm at the two-cluster level.

OTUs from either cluster are shifted to another one. The coefficient of indistinctness for k-means clusters is very close to zero; the good separation of clusters is revealed by the OTUs probability distribution curves (Fig. 10) as well as by the ordination plot (Fig. 11). Remarkable similarity of the results of two classifications, obtained by rather different clustering procedures, gives a good reason to assume that now we have caught some essential or invariant features in the data structure.

DISCUSSION

The statistical indistinctness of empirically estimated taxa demonstrates the dubiousness of the conventional taxonomy of the *S. umbrorum* complex. At the same time, the present analyses show the sophisticated structure of the data even in case they are collect-

ed from a rather limited area. Several methods of clustering and comparative analysis of their results were needed to disentangle the structure of the data. Only after superimposing clusters onto the ordination plot we were able to grasp mutual relationships between them.

It is not simple to say which classification is the most appropriate. The use of cluster analysis does not simply involve the application of one particular technique, but rather necessitates a series of steps each of which may be dependent on the results of the preceding one. The final judgement of the quality of a particular result depends very much on the fact how informative it is, and what kind of new information it will give (Everitt, 1993).

It became evident that at the five-cluster level k-means clustering best provides significantly discontinuous clusters. However, it should be considered that clusters obtained by k-means procedure are approximately isodiametric, and thus we lose information about the directional variation of subsets in the character space, which is another important feature of data structure On the other hand, if the 'chain'-like configuration of subsets, related to their variation, is taken into account, not all clusters are distinctly separated. Figs. 6 and 7 illustrate convincingly these statements.

Despite the lack of discontinuity at the five-cluster level, UPGMA-clusters are ecologically better justified than k-means clusters. Also, they are in good accordance with the main features of the data structure by reflecting their division into two subsets according to spore ornamentation and width, as well as taxonomic continuum caused by the length of marginal hairs.

Still, the decision about the effectiveness of the obtained classification depends most of all at which taxonomic level we attempt to interpret the results and what the aim of a given classification is. Contradictory results obtained by UPGMA and by k-means procedure at the five-cluster level indicate difficulties in regarding either cluster as species. If the result at the two-cluster level is considered as species, the taxonomy of the *Scutellinia umbrorum* complex should be revised. Then, the first UPGMA-cluster compiling the specimens of the putative species *S.* aff. *subhirtella* Svrček and *S. subhirtella* s. Kullman morph with short marginal hairs can be called *S. subhirtella* Svrček s.l. The other cluster then joins the specimens of the putative species *S. umbrorum, S. parvispora* and *S. subhirtella* s. Kullman morph with long hairs on the apothecium, and it corresponds to *S. umbrorum* s.l. (Fr.) Lambotte. Taking into account the good relation of UPGMA-clusters obtained at the five-cluster level with habitat conditions, these clusters can be interpreted as ecodemes of respective species.

Hence, the type of spore ornamentation should not be considered the most important character for identifying species within the *Scutellinia umbrorum* complex, as it is traditionally done (Svrček, 1971; Kullman, 1982; Schumacher, 1990); instead the length of marginal hairs comes on the first plane. Thus, to *S. subhirtella* s.l. belong specimens with hairs shorter than 450 µm, to *S. umbrorum* s.l. specimens with hairs longer than 450 µm. The limit of 450 µm should certainly be taken with precaution, only as a pilot point established on the basis of data of a recently studied sample.

Unambiguous estimation of the type of spore ornamentation is quite often questionable because this character does not have clearly fixed states but is varying continuously like almost all quantitative morphological parameters. Van Brummelen (1993) has shown that the formation of spores ornamentation varies only slightly between *Scutellinia* species. Moreover, as it was proved earlier (Kullman, 1982), one ascus can contain spores with

different types of ornamentation. Restriction of the estimation of the ornamentation type to only two classes is a rather rough simplification which would overemphasize this parameter in comparison with other parameters in classification. Therefore, OTUs on the dendrogram (Fig. 4) as well as on the ordination plot are divided into two subsets namely according to their spore ornamentation type. If we established at least one more ornamentation type, the result would certainly be different. It is remarkable that the taxonomic continuum of morphs established by UPGMA appears between the very clusters which merge specimens with different types of spore ornamentation (Table II). The result suggests also that two well separated subsets of OTUs on the ordination plot are not located too far from each other in the character space. Due to the reduction of dimensionality all ordination plots represent, to some extent, a simplification of the existing relations between objects and contain an error due to which larger distances become more distorted (Paal et al., 1989). Dubiousness of the spore ornamentation type as one basic parameter in the *Scutellinia umbrorum* complex systematics is confirmed by the results of k-means clustering at the five-cluster level, where all clusters include specimens with both types of spore ornamentation.

On the basis of the current analysis we can suppose that the number of species we can distinguish in the *Scutellinia umbrorum* complex is not as large as it was so far expected. At the same time, the morphological plasticity of the species is rather great. The appearance of different morphs, usually interpreted as sister species, seems to be determined by ecological conditions in the habitat.

The present study was not intended to revise the systematics of the *Scutellinia umbrorum* complex, but to elucidate, by means of different multivariate methods, the complicated structure of the data and their dependence on ecological factors. It is obvious that in order to establish more or less invariate systematics of *Scutellinia*, we still are in need of sufficient data representing the variation of taxa in different ecotopes and geographical regions. Furthermore, more variables should be taken into account. To establish the variation amplitude of variables by the uniparental species of *Scutellinia*, cultivation under controlled conditions is necessary.

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