Julia Watson

Botany Honours

2004

Supervisor: Prof. Terry Hedderson

Morphometric and molecular analysis of two distinct forms of *Pseudocrossidium crinitum.*

Abstract

Two distinct forms of *Pseudocrossidium crinitum* (Bryophyta) exist – a form with a white hair point on the leaf, and one with a yellow hair point on the leaf. The white and yellow forms exist in similar areas, but the yellow hair point form is found on its own in arid areas. In this paper we analyse 16 morphometric variables and chloroplast and nuclear DNA molecular information to determine whether *Pseudocrossidium crinitum* should be separated into two species based on morphological differences. We use ANOVA, Principal components analysis and cluster analysis to analyse the morphometric data. Four significantly different morphological differences were found between the two forms. Five haplotypes were revealed from 8 samples, with only one haplotype shared amongst them. Two samples from the Cedarburg exhibit highly different DNA to the rest of the samples. Although genetic sampling was not large enough on which to base significant conclusions, we find that the two forms are morphologically differentiated enough to separate *Pseudocrossidium crinitum* into two species.

Introduction

Pseudocrossidium crinitum (first described as Barbula crinita by Schultz in 1823, also known as Tortula pilifera) was subsequently described as P*seudocrossidium crinitum* by R.H Zander in 1993.

The genus has been identified and reported in North America (mainly the western United States and Mexico), South America, and Sub-Saharan Africa. We are primarily concerned with the populations sampled in the Northern and Western Cape regions of South Africa. They are distributed widely throughout South Africa, although comprehensive sampling of the country is not complete. In the selected regions, two morphs (or forms) have been identified. However, the existence of these two forms is not treated in any existing publication to date. The two distinct morphs are distinguishable by the colour of the hair-point of each leaf. The white form can be found growing alone, whereas the yellow form is commonly seen growing amidst the white plants. The yellow form is observed more commonly in drier regions than the white form. Studies of this type have been done before (e.g. Buck et al 2000, Cao et al 2003, Gerdol1987, Shaw and Rooks 1994, Vanderpoorten et al 2003).

Hypothesis

We hypothesise that *Pseudocrossidium crinitum* can be divided into two separate groups based on the colour of the hair point. This project attempts to test this hypothesis and to determine any factors that may explain (ecologically and

historically) why *Pseudocrossidium crinitum* has two distinct forms. This hypothesis was formed for two reasons. Firstly, *Pseudocrossidium crinitum* appears to be distinct in its growth form in the field. i.e. the white and yellow hair point forms are easily distinguishable in the field and they grow in separate patches, although no other features are recognisably different under field observation. Secondly, when the species is regarded at closer range, the hair point colour is distinctly different and it is a delimiting character within the species that can not readily be dismissed.

Statistical analyses

The statistical methods used in this paper can be grouped into two kinds: descriptive statistics (means, medians, standard deviations, Principal components and cluster analysis) and inferential statistics (Analysis of variance and regression analysis). The factor that discriminates between these two kinds is that descriptive statistics do not test hypotheses, but inferential statistics do test hypotheses. Discriminant analysis is used to classify information into two or more naturally defined groups (see also Gerdol, 1987). An effective discriminant analysis will have a high percentage of correctly classified cases. Discriminant analysis also establishes the percent of variance in the dependent variable that is explained by the independent variables, and to eliminate variables which are little related to group discriminations. The qualities of discriminant analysis therefore allow one to use variables to classify cases into groups.

Analysis of variance (ANOVA)

ANOVA functions in a similar way to discriminant analysis. With ANOVA, one can determine whether two or more groups (more specifically, their means) are significantly different from each other. If the means are significantly different from each other, then this variable distinguishes between the two groups. The null hypothesis in this paper is that the means of the two forms (white and yellow) do not differ. The alternative hypothesis therefore is that the means of the morphological variables that were measured do differ, and that the white and yellow forms are distinct in certain morphological aspects.

Principal components analysis (PCA)

A PCA is useful in this type of study, as it extracts factors, which account for less and less of the variation that is viewed in the data. The factor with the highest Eigenvalue accounts for the most variation. PCA is also useful because it takes the group of data points and plots them so that the highest amount of variation is visible. This allows one to identify the most important factors in one's data. PCA also allows one to use variables, which are not measured in the same units. The covariance matrix measures how each variable contributes information to the data set (Raychaudhuri et al 2004). One problem with PCA, is that there is no *a priori* valuation that the PCA will yield axes that correspond to species boundaries. One can therefore use hypothesis testing (such as can be used for ANOVA) to determine if the means differ in multivariate space.

Cluster analysis

This method groups cases of a similar kind into categories. Cluster analysis is useful because it adds structure to a group, and any relationship between cases is emphasised. It is similar to PCA because it reduces the dimensionality of the data (Raychaudhuri et al 2004). Cluster analysis arranges the cases (defined as each sample in this paper) within each cluster so that they are more similar to the cases in the cluster than to cases in other clusters.

Therefore, the type of analysis can be split into two distinct groups. Firstly, discriminant analysis and ANOVA distinguish between groups. Secondly, PCA and cluster analysis assemble data into similar groups. Both of these forms of analysis have one factor in common – they attempt to portray a body of data in meaningful and easy-to-visualise components.

Materials and Methods

Morphology

I used 16 variables (table 1, fig. 2) including leaf shape and cellular level details in the morphometric analysis. I measured leaf characters using a light microscope at 10 times magnification, and cell and costa characters at 400 times magnification. The data matrix comprised observations of 58 specimens, all from the Western and Northern Cape, South Africa (fig. 1). Specimens from other provinces in South Africa were not available, and so the results reflect patterns only in the two provinces from which the specimens were used (fig. 1).

Molecular

DNA extraction

Material for extraction was selected by breaking off one shoot of the *Pseudocrossidium crinitum* plant per sample for five white and five yellow samples. The white samples were labeled w1-w5, and the yellow y1-y5. These labels will be used throughout this paper. These were placed in 1.5ml micro-centrifuge tubes. Samples w2 and y3 did not yield good quality DNA and so were not included in further analysis due to time constraints.

DNA was extracted by grinding the sample with several grains of sand in a pestle and mortar. Each sample was then incubated at 65 degrees C for approximately one hour in 700ul of preheated hexadectyltrimethylammonium bromide (CTAB) and B-mercaptoethanol. 600ul of chloroform-isoamyl alcohol (24:1) was added and mixed by inversion. Samples were spun for five minutes and the aqueous phase was transferred into a clean microcentrifuge tube. An equal volume of ice-cold isopropanol was added and tubes were stored in a –10C freezer overnight to precipitate the DNA. The samples were spun and washed with 75% ethanol. The samples were left to dry overnight and then resuspended with 50ul of TE (10mM Tris-Cl ph 7.4, 1mM EDTA pH 8.0). Primers trnC and trnF, psbA and trnH were used to isolate the chloroplast region. The nuclear ITS 1 region was amplified with 18KRC and ITS1 primers. Primer sequences are shown below. Target DNA regions were amplified by Polymerase Chain Reaction (PCR) using

0.75 units of BIOTAQ™ DNA polymerase (Bioline) in 30ul volumes also containing1 X NH4 buffer and 5mM MgCl2, 0.1mM of each dNTP and 0.3uM of each primer, with 3ul of unquantified diluted DNA template. Thermo-cycling was carried out set to the following thermal conditions: initial denaturation at 94®C for two minutes, 30 cycles of 94®C for 1 minute, 52®C for one minute, 72®C for two minutes and a final polymerization stage at 72®C for seven minutes.

trn c CGAAATCGGTAGACGCTACG Taberlet et al. 1991

trn f ATTTGAACTGGTGACACGAG Taberlet et al. 1991

18KRC GCACGCGCGCTACACTGA Hamby et al. 1988

ITS 1 TCCGTAGGTGAACCTGCGG White et al 1990

The amplified DNA was cleaned using GFX™ PCR DNA and Gel Band Purification Kits (Amersham Biosciences). Cycle sequencing was carried out by PCR in 10ul volumes containing the following 2ul of BigDye R Terminator v. 1.15 X Sequencing Buffer (Applied Biosystems), 1ul of BigDye R Terminator v 3.1 Cycle Sequencing RR-100 (Applied Biosystems), 0.16ul primer, 2ul DNA template, and the remaining volume of PCR water. The University of Stellenbosch resolved cycle sequencing products.

Statistical analyses

Univariate

I performed ANOVA on the 16 variables for all of the specimens measured using Statistica v. 6. The variables were log transformed to the requirement criteria of equal variances and normal distribution for these statistical tests.

Multivariate

I performed principal components analysis, cluster analysis and discriminant analysis on the data obtained from 56 of the 58 specimens measured using Statistica v. 6.

Molecular

Chloroplast and nuclear data were analysed separately. Sequences were assembled on SeqMan (LaserGene System Software, DNAStar, Inc.) and aligned manually using MegAlign (LaserGene System Software, DNAStar, Inc.). Alignment ends were trimmed to exclude missing data.

Haplotype distributions were calculated (with combined trnF and psbA data) using Arlequin version 2.0. TCS version 1.13 was used to construct a statistical parsimony network that was arranged into a nested cladogram.

Results

Morphological

Univariate

Analysis using ANOVA identified four significantly different variables (table 1). The average length of upper cells in white forms is significantly greater than that of yellow forms. On average, yellow forms have more guide cells than white forms, and papilla height is smaller in yellow forms than in white forms. The ratio of the length of the hair point: width of the leaf at the widest point is greater in the yellow than in the white forms, as is the ratio of the height of the papilla: hair point length. The ratio of the height of the papilla: thickness of the costa is greater in white forms than in yellow forms.

Table. 1. Mean ± std deviation for morphological data for all white (W, n = 30) and yellow (Y, n = 28) forms of *Pseudocrossidium crinitum*

(ANOVA Significance level: * $p < 0.05$; *** $p < 0.005$)

	Mean W	**Mean Y**	R^2
Width leaf at base	47.1 ± 12.0	52.8 ± 13.1	0.10
Width leaf widest point	67.7 ± 21.2	75.1 ± 13.5	0.46
Distance to widest	52.0 ± 14.4	54.1 ± 15.1	0.32
Length widest to tip	145.9 ± 34.4	151.7 ± 37.1	0.19
Width of shoulder	19.9 ± 4.5	21.8 ± 4.3	0.03
Ave. length basal cells	18.3 ± 5.2	17.3 ± 3.7	0.21
Ave. width basal cells	4.6 ± 0.8	4.3 ± 0.7	0.04
Ave. length upper cells *	**3.1± 0.9**	**2.8 ± 0.4**	**0.05**
Ave. width upper cells	2.83 ± 0.6	2.7 ± 0.5	0.02
Thickness costa	26.8 ± 5.2	28.1 ± 4.9	0.04
Width costa	45.2 ± 8.5	47.8 ± 8.9	0.03
No. of guide cells *	5.6 ± 0.7	6.0 ± 0.8	0.32
Ave width guide cells	7.2 ± 1.5	7.4 ± 1.2	0.27
Ave height papillae ***	**2.5 ± 0.6**	**2.2 ± 0.5**	**0.34**
Thickness stereid band	10.3 ± 2.1	9.9 ± 1.6	0.16
Hair point length	86.4 ± 30.8	99.5 ± 31.8	0.24
width widest :dist to widest	1.3 + 0.33	1.5 + 0.37	0.08
width widest : length leaf	0.3 + 0.07	0.4 + 0.06	0.24
length hair pt : width leaf @ widest pt ***	1.3 + 0.43	2.0 + 0.86	0.29

width leaf at base : length leaf	0.2 + 0.05	0.3 + 0.06	0.29
thickness stereid : no guide cells	1.9 + 0.4	1.7 + 0.36	0.21
width guide : no guide cells	1.3 + 0.3	1.3 + 0.26	0.28
height papillae : thick costa ***	**0.10 + 0.02**	**0.08 + 0.02**	**0.15**
length basal cell : no guide cells	3.3 + 0.9	2.9 + 0.76	0.22
width costa : thick costa	1.7 + 0.28	1.7 + 0.32	0.02
height papilla : hair pt length *	**0.04 + 0.02**	**0.1 + 0.02**	**0.37**
width shoulder : leaf length	0.1 + 0.03	0.1 + 0.02	0.01

Multivariate analyses

PCA

Of the 16 variables that were measured for 56 specimens, factor one explained 33.66% of the variance, and factor two 16.48% of the variance (Table 2). The length of the hair point contributed the highest of all the variables. The projection of the variables on the factor plane (fig. 3 and 4) presents these findings graphically. The values for factor one are all positive, indicating that this factor describes size variation. Factor two describes the axis on which the length of the hair point and with width of the costa are contrasted to most other measurements. Factor 3 (fig. 4) also describes the axis on which the length of the hair point and with width of the costa are contrasted, but it the average length of the basal cells, average length of the upper cells , average width of the upper cells and the average height of the papilla are also contrasted highly to the other variables.

Table 2. Results of PCA on the measured morphometric variables. The values in the columns are co-efficients of the principal components. Principal component 1 and 2 contain 48% of the variance of the data, while the first six components in total account for 79% of the variance.

Variables	Principal Components					
	1	2	3	4	5	6
Width leaf at base	0.3424	-0.2272	-0.3188	-0.0310	0.2624	0.0483
Width leaf widest point	0.4145	-0.1652	-0.2320	0.0867	0.1243	0.1101
Distance to widest point	0.3993	-0.2572	0.1094	-0.0529	-0.5436	0.0700
Length: widest to tip	0.3219	-0.2166	0.0207	-0.1962	0.1522	0.3527
Width of shoulder	0.2494	-0.1564	-0.2009	-0.0233	0.0585	-0.2320
Ave. length basal cells	0.1826	-0.2409	0.3779	0.2042	-0.4339	-0.3896
Ave. width basal cells	0.0744	-0.1908	-0.1058	-0.1178	0.1581	-0.2464
Ave. length upper cells	0.0575	-0.1122	0.4697	-0.3655	0.3163	-0.1218
Ave. width upper cells	0.0596	0.0122	0.3883	-0.4072	0.2495	-0.1744
Thickness costa	0.1166	0.0375	0.0406	0.4248	0.3421	-0.1366
Width costa	0.1958	0.3137	0.0572	0.1817	0.1038	-0.3085
No. of guide cells	0.0033	0.0797	-0.0300	-0.0461	0.0569	-0.1540
Ave. width guide cells	0.2039	-0.0384	0.1006	0.1236	0.1585	-0.2516
Ave. height papillae	0.0490	-0.1058	0.4891	0.3741	0.1557	0.5561
Thickness stereid band	0.0643	0.0633	0.0892	0.4471	0.1452	-0.1198
Hair point length	0.4899	0.7440	0.0567	-0.1546	-0.1290	0.1422
Eigenvalue	0.056	0.029	0.019	0.014	0.012	0.009
% variance	31.7	16.5	10.5	8.2	6.9	5.3

Figures 5 and 6 do not show any strong groupings, but there is a trend towards the y forms assembling in the top right quadrant in figure 5. This therefore separates the two forms according to the length of the hair point and the width of the costa.

Cluster analysis

The cluster analysis performed on the discriminant analysis canonical scores for the six most influential factors (i.e. the first six factors) did not group the two forms into separate clusters (fig. 7). The samples on which molecular data was gathered in addition to the morphological data are highlighted along the x-axis. W1, y1 and w3 were clustered together which is similar to the haplotype analysis

(fig. 6). W4 and w5 were in separate clusters, as were y2 and y5 – although they were more closely grouped. The histogram (fig. 8) shows that the two forms do overlap in multivariate morphological space, but also that there is a definite degree of separation based on the morphological differences between the two forms.

Discriminant analysis

Of the 56 samples, 80.4% (45 out of 56) were correctly classified into their correct groups according to the squared Mahalanobis distances. Of the 11 incorrect classifications, four were borderline (i.e. close to 50%). Therefore, only samples w2, 19, 23 and 28, and Y4, 9 and 12 were significantly incorrectly classified.

Molecular

Chloroplast regions

With the psbA and trnlF regions, we constructed a haplotype diagram (fig. 9)and a cladogram (fig. 10). The haplotype diagram reveals six haplotypes from eight samples. W4 and w5 are very different from all the other samples, and from each other. W1, y1 and y4 were grouped in one haplotype, with the haplotype of w3 closely related to this haplotype. In fig. 9, the dashed line indicates that the connection between the w4 and w5 haplotypes and the other six haplotypes is dubious as it fails the 95% connection limit of the TCS. The two main patterns seen in the cluster analysis were also seen in the haplotype diagram of psbA and trnlF molecular data, although other relationships seen in the haplotype diagram were not mirrored in the cluster analysis. Of the six haplotypes, only one haplotype was shared among the species. Therefore, most of the yellow forms

were different from the white forms. The cladogram of the same gene regions depicts these relationships. (The main difference between the cladogram and the haplotype diagram is that the haplotype diagram is not constrained to be dichotomously branched).

DNA region

The ITS data was not sufficiently clear to manipulate in a significant manner because the sequences obtained were not clean enough (i.e. they contained a lot of 'noise' from other factors such as dye in the sample). Sequence alignments for samples w4 and w5 were very different from those of the other five samples. The 18S region was identical for all sequences, but significant (approximately 19%) differences were noticed in the ITS regions of w4 and w5. Samples w4 and w5 were most similar to each other.

Discussion

Morphology

The ANOVA data suggests that the white and yellow forms of *Pseudocrossidium crinitum* exhibit significant morphological variation. These differences relate to leaf shape characters and also to cellular details. To hypothesise as to the relevance of these morphological differences is difficult and is beyond the scope of this paper. It would also require further, more anatomically detailed research. The fact that the yellow forms exist in more arid areas (T Hedderson, pers.comm.) is an interesting phenomenon. The morphological differences that

are apparent between the two forms may be present for different reasons – both historical (genetic) and ecological. Differences in climate in an area where *Pseudocrossidium crinitum* were found may have lead to arid adaptations (e.g. the yellow form.

Molecular

Samples w4 and w5 are from similar areas in the Cedarburg, and yet they are so genetically different from each other (and the other samples), as seen from the haplotype diagram and cladogram. The yellow and the white forms differ genetically, with only one haplotype shared between them. This information confirms one part of the hypothesis – that the white and the yellow forms should possibly be designated as two different species. Even though one haplotype is shared among the two forms, the proportion that is shared differs. The two forms (as seen in from the molecular data) are not reciprocally monophyletic, (some yellow forms are more related to some white forms than the white forms are related to one another), but the large differentiation between the haplotypes (15 steps between samples w4 and w5, and almost no relation to the other samples) anticipates that genetic differentiation is occurring. The sample size is not adequate from which to infer any real conclusions regarding relationships, but as a preliminary survey, the results are intriguing and warrant further research into whether there are large genetic differences between species of *Pseudocrossidium crinitum*.

Species concepts

Species concepts have been debated for several decades. There are many different views on what defines a species and on taxonomic delineations (see Wheeler and Meier, 2000). In this project, I define a species according to the morphological species concept. The phylogenetic species concept as defined by Mishler and Theriot (Mishler and Theriot 2000) is becoming more widely accepted with the increase in molecular analyses, but for the purpose of this project, I will utilise the morphological species concept. The reason that I do not use the phylogenetic species concept is that the data I gathered focuses on morphological differences, and in order to apply the phylogenetic species concept, I would need to have performed a different array of sampling, including other *Pseudocrossidium* species and data to determine mono/paraphyly of the species (Mishler and Churchill, 2004). The basic tenet of the morphological species concept is that two very similar organisms are more likely conspecifics than two less similar organisms. Although this concept has been much criticised, (e.g. Wheeler and Meier, 2000), it is still widely used and applicable, especially to plants where relationships are occasionally analysed without judgment on speciation and associated reproductive isolation. Therefore, based on the morphological data and the analyses above, I propose that *Pseudocrossidium crinitum* be separated into two species, based primarily on the colour of the hair point. Other characters (supporting the morphological discontinuity) include the average length of the upper cells, average height of the papillae, the ratio of the height of the papilla to the hair point length. *Pseudocrossidium crinitum* may

therefore be separated into the white form and the yellow form, although further research is necessary in order to make this distinction with absolute confidence.

Future considerations

Studies have shown that substantial molecular differentiation can occur in bryophytes without much morphological differentiation (Cronberg 1997, Appelgren and Cronberg 1999, Shaw 2001). *Pseudocrossidium crinitum* appears to exhibit this pattern, with morphological differences outweighed by large genetic differences. Genetic diversity within species is also found to be very high in bryophytes (Itouga et al 2002), and further work on *Pseudocrossidium crinitum* would useful to yield a more rigorous idea of genetic diversity within the species.

The two very different forms (w4 and w5) are found in the same geographical area (in the Cedarburg). This warrants further investigation. There is not much evidence that the two forms share haplotypes, due to a small sample size. Future combined molecular and morphological studies require a secure morphological background (Hedenas 2004) and this study would be highly suitable for additional investigation.

References:

1. Appelgren L and Cronberg N. 1999. Genetic and morphological variation in the rare epiphytic moss *Neckera pennata* Hedw. Journal of Bryology. 21: 97-107.

2. Buck W, Goffinet B, Shaw A. 2000. Testing morphological concepts of orders of pleurocarpous mosses (bryophyte) using phylogenetic reconstructions basted on trnL-trnF and rps4 sequences. *Molecular Phylogenetics and Evolution.* 16 (2) 180-198.

3. Cao T, Guo S, Yu J and Song G. 2003. Taxonomic assessment of *Ptychomitrium lepidomitrium* and *P. tortula* (Bryopsida, Ptychomitriaceae). *Journal of Bryology*. 25: 263-270.

4. Compton, J.A. & Hedderson, T.A J. 1997. A morphometric analysis of the *Cimicifuga foetida* L. complex *(Ranunculaceae). Botanical Journal of the. Linnean. Society.* 123, 1-23

5. Cronberg, N. 1997. Differentiation of *Sphagnum rubellum* and *S. capillifolium. Journal of Bryology*19:715-729.

6. Donoghue, M. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *Bryologist* 88: 172-181.

7. Gerdol R. Discrimination of Sphagnum sect. Acutifolia based on a quantitative expression of stemleaf forms. *Lindbergia* 13: 150-154. 1987.

8. Hamby, R. K., Sims, L., Issel, L. and Zimmer, E. 1988. Direct ribosomal RNA sequencing: Optimization of extraction and sequencing methods for work with higher plants. *Plant. Molecular. Biology. Rep.* 47: 175-92.

9. Hedenas L. 2004. Morphological and anatomical evidence suggest that 'Hylocomiaceae' taxa belong to at least two clades. *Journal of Bryology.* 26 125-135.

10. Itouga M, Yamaguchi T, Deguchi H. 2002. Allozyme variability within and among populations in the Asian liverwort *Asterella liukiuensis. Journal of Bryology.* 24: 267-276

11. Mishler, BD and Churchill SP. 1984. A cladistic approach to the phylogeny of the "bryophytes". *Brittonia.* 36 (4) 406-424.

12. Mishler, BD 1985. The morphological, developmental and phylogenetic basis of species concepts in bryophytes. *Bryologist* 88: 207-214

13. Raychaudhuri S, Stuart JM, Altman RB. 2000. Principal components analysis to summarize microarray experiments: application to sporulation time series. *Pacific Symposium on Biocomputing.*:455-66.

14. Shaw J and Rooks P. 1994. Systematics of Mielichhoferia (bryaceae:Mucsi) I. Morphological and genetic analyses of M. elongata and M. mielichhoferiana. *The Bryologist.* 97(1) 1-12.

15. Shaw J. 2001.Biogeographic patterns and cryptic speciation in bryophytes. *Journal of Biogeography*, 28 (2): 253

16. Taberlet P, Gielly L., Patou G., Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular. Biology*. 17:1105-1109

17. Vanderpoorten A., Hedenäs L. & Jacquemart A. 2003. Differentiation in DNA fingerprinting and morphology among species of the pleurocarpous moss genus, *Rhytidiadelphus*. *Taxon* 52: 229-236.

18. Wheeler Q and Meier R (editors). 2001. *Species Concepts and Phylogenetic Theory A Debate*. pp256

19. White T, Innis M, Gelfand H and Sninsky J (editors). 1990. PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., New York.

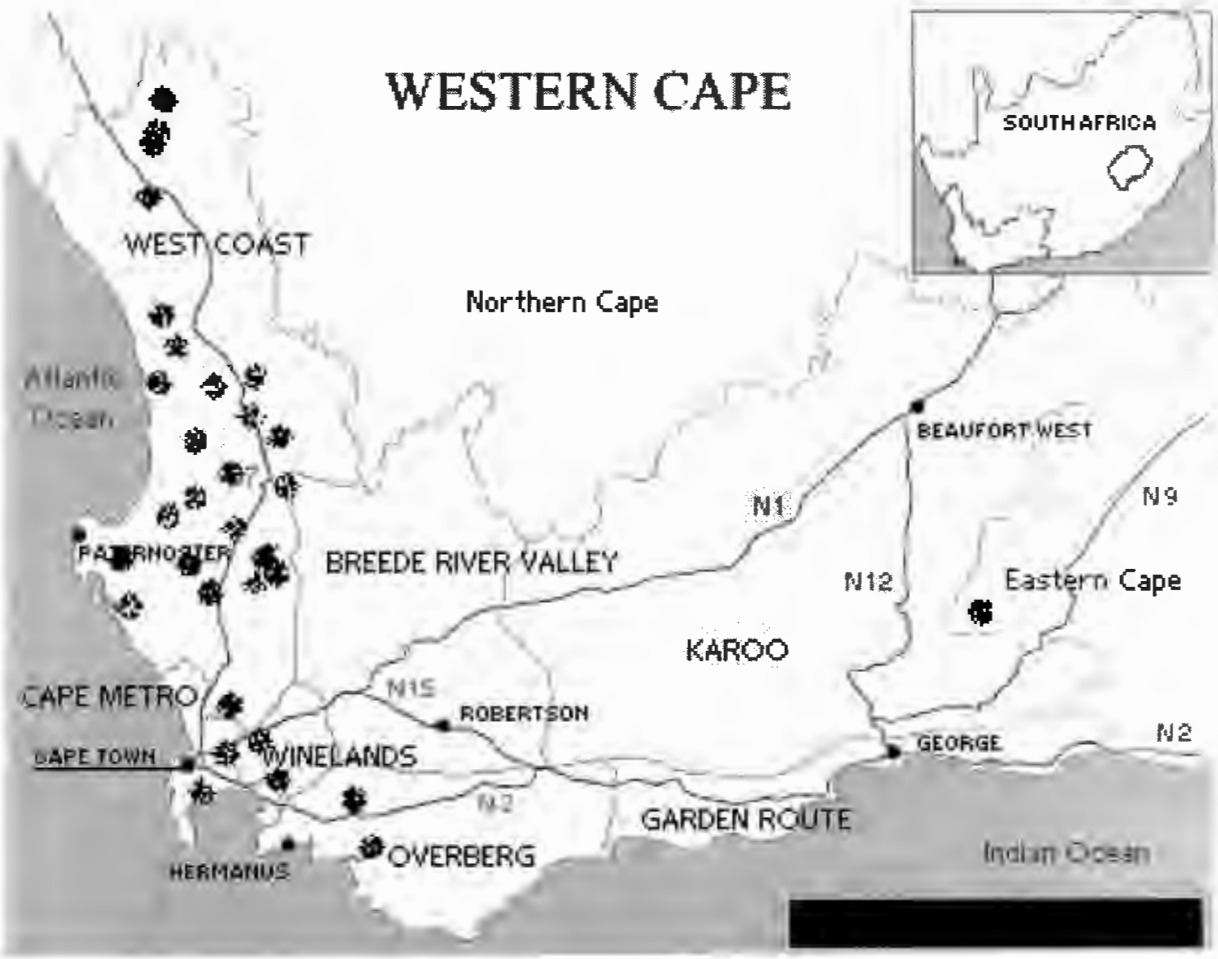

Fig. 1. Map of the Western and Eastern Cape, South Africa. Samples indicated with black dots.

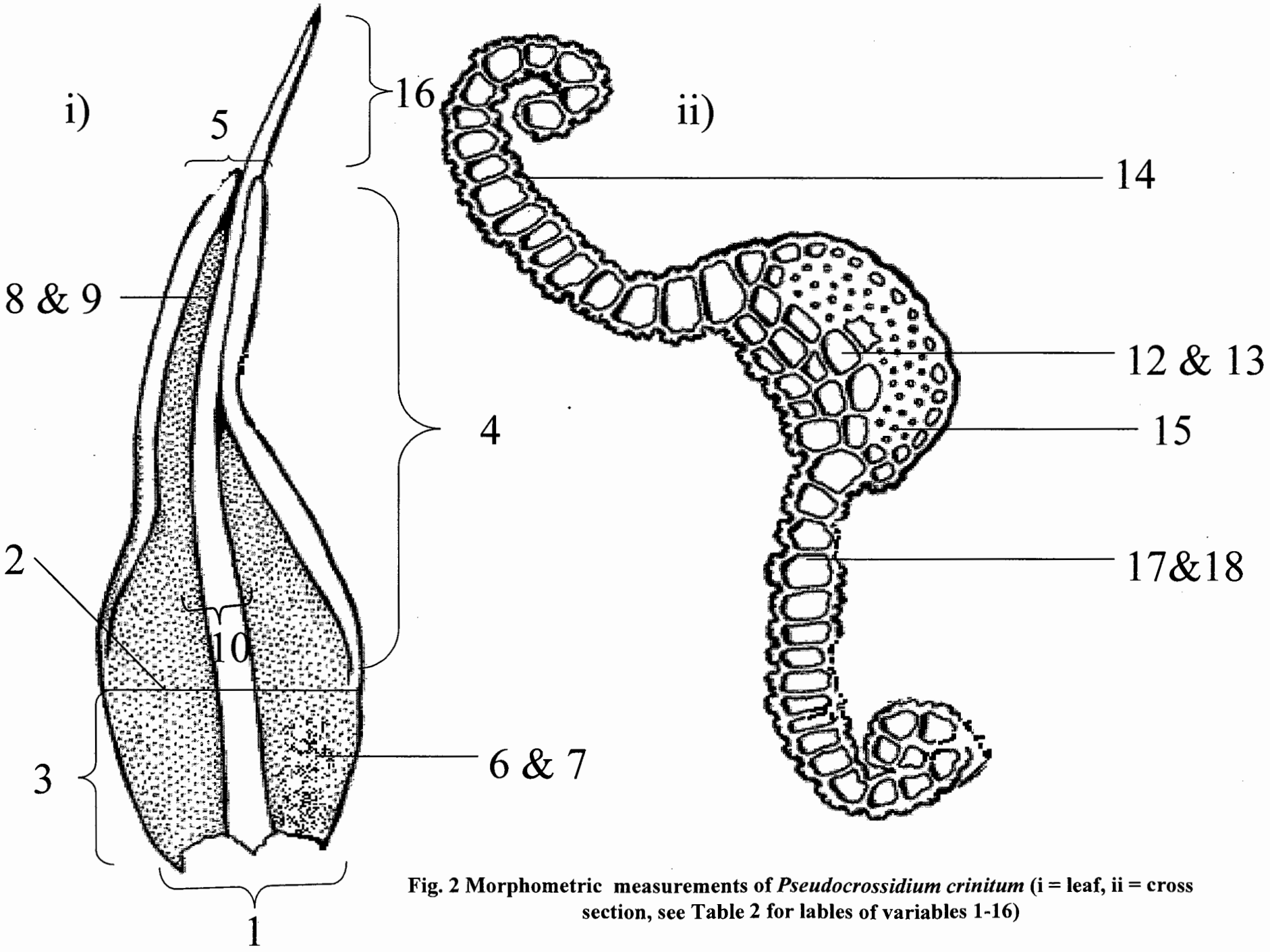

Fig. 2 Morphometric measurements of *Pseudocrossidium crinitum* (i = leaf, ii = cross section, see Table 2 for lables of variables 1-16)

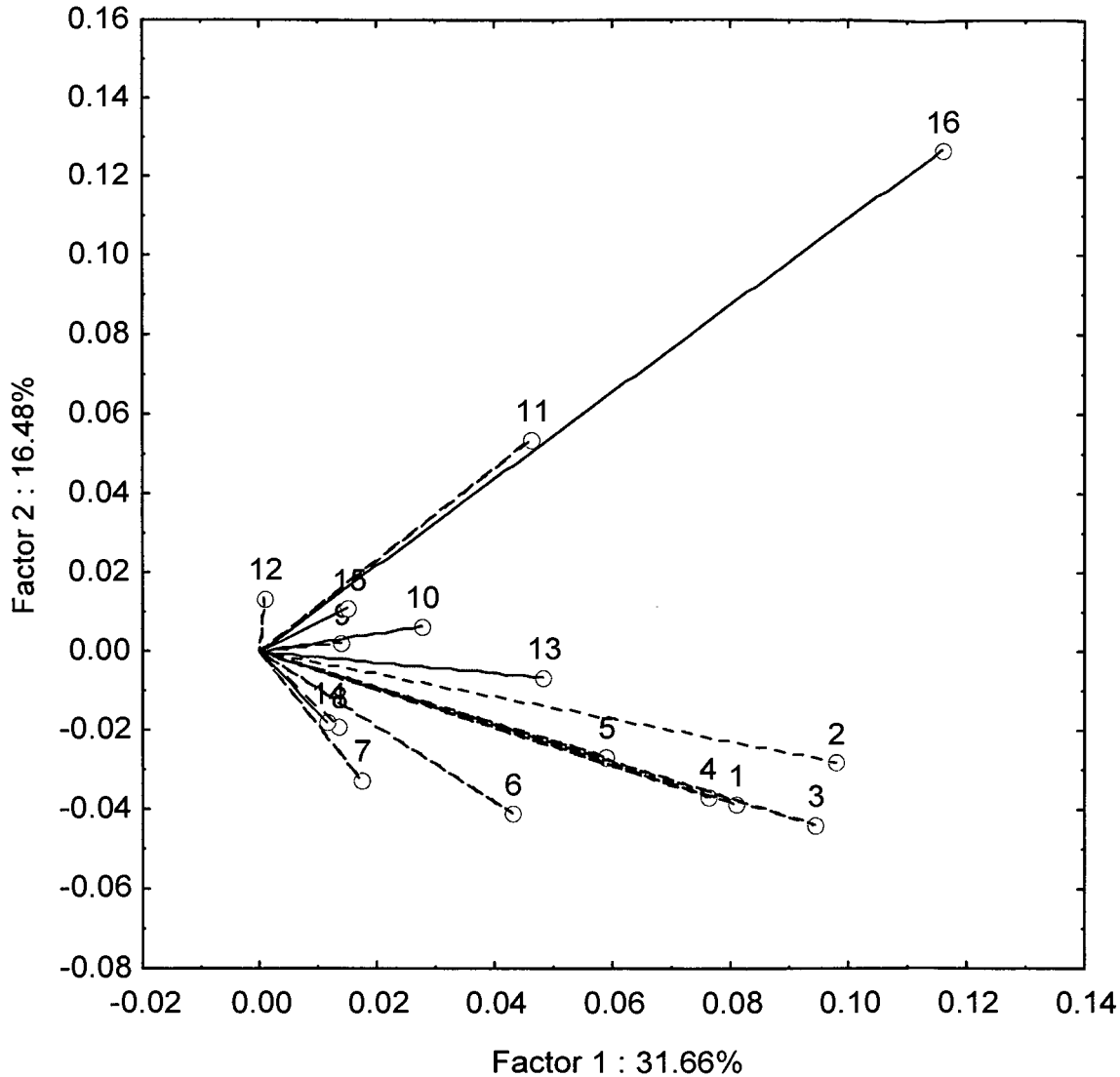

Fig. 3. Projection of the morphological variables (1-16) on the factor plane. Factors 1 and 2 contain 48% of the variance (eigenvalues from PCA).

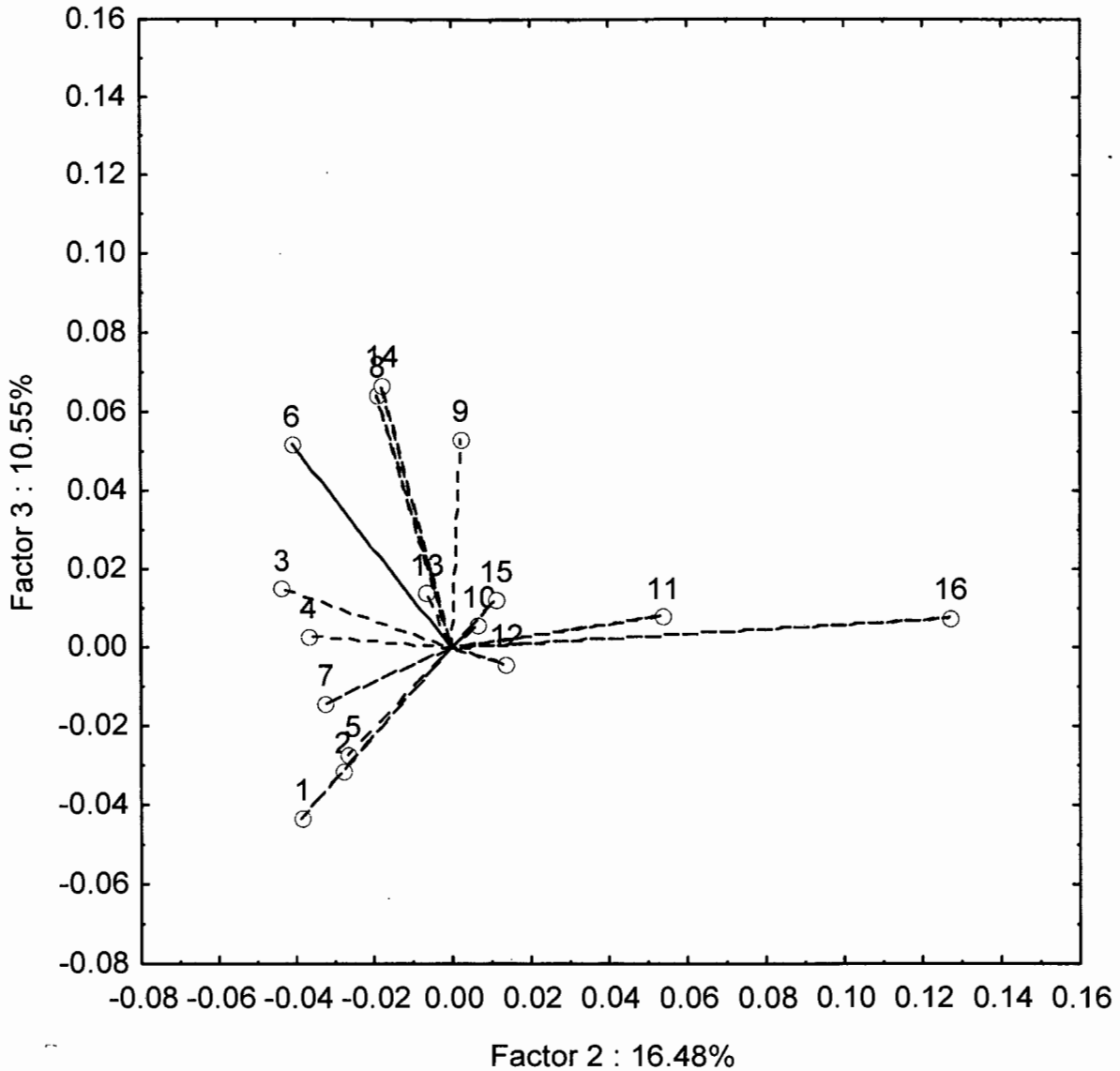

Fig. 4. Projection of the morpholgical variables (1-16) on the factor plane. (eigenvalues from PCA).

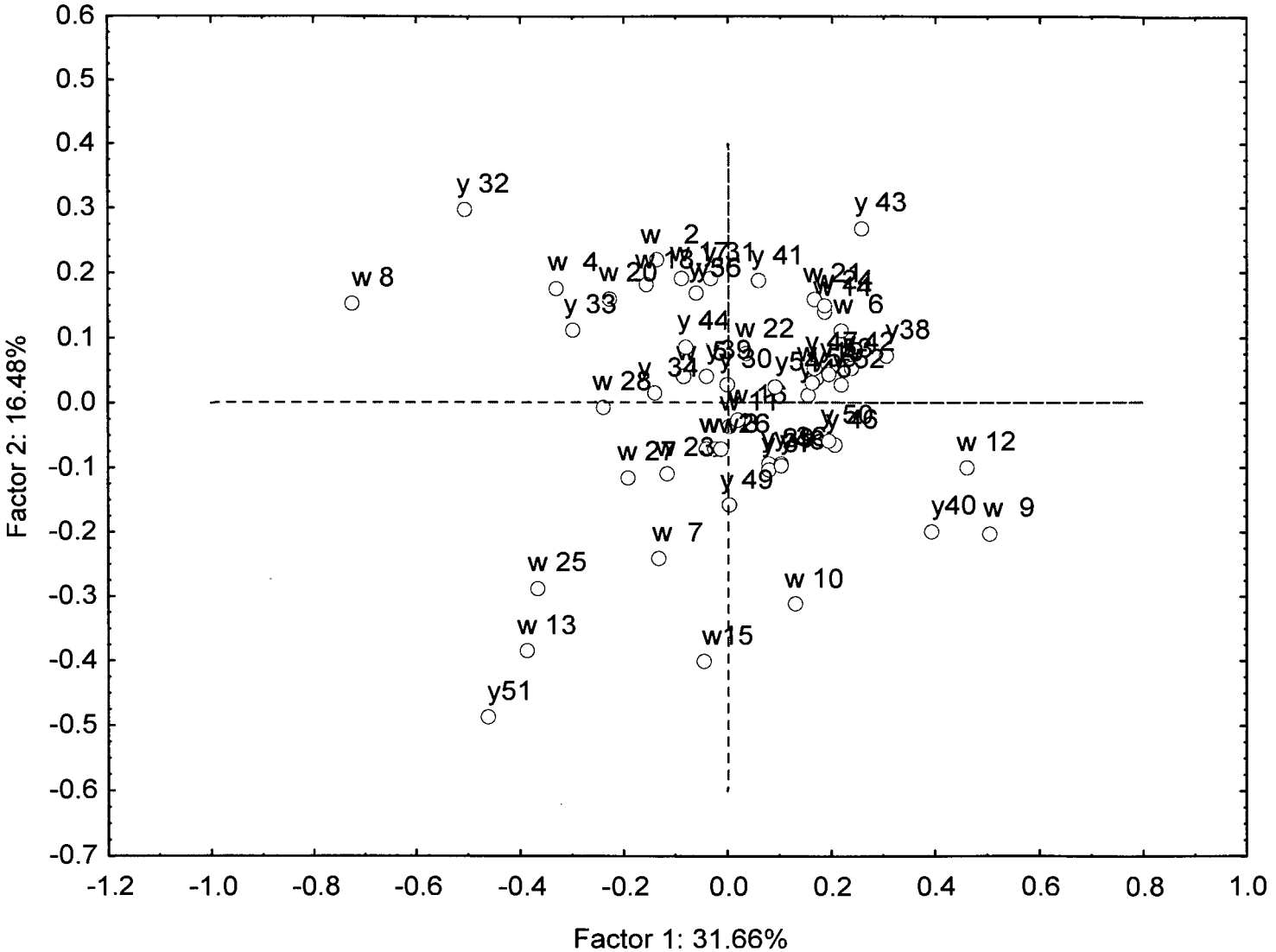

Fig. 5. Plot of each specimen of white and yellow Pseudocrossidium crinitum forms on the factor plane (based on morphological data).

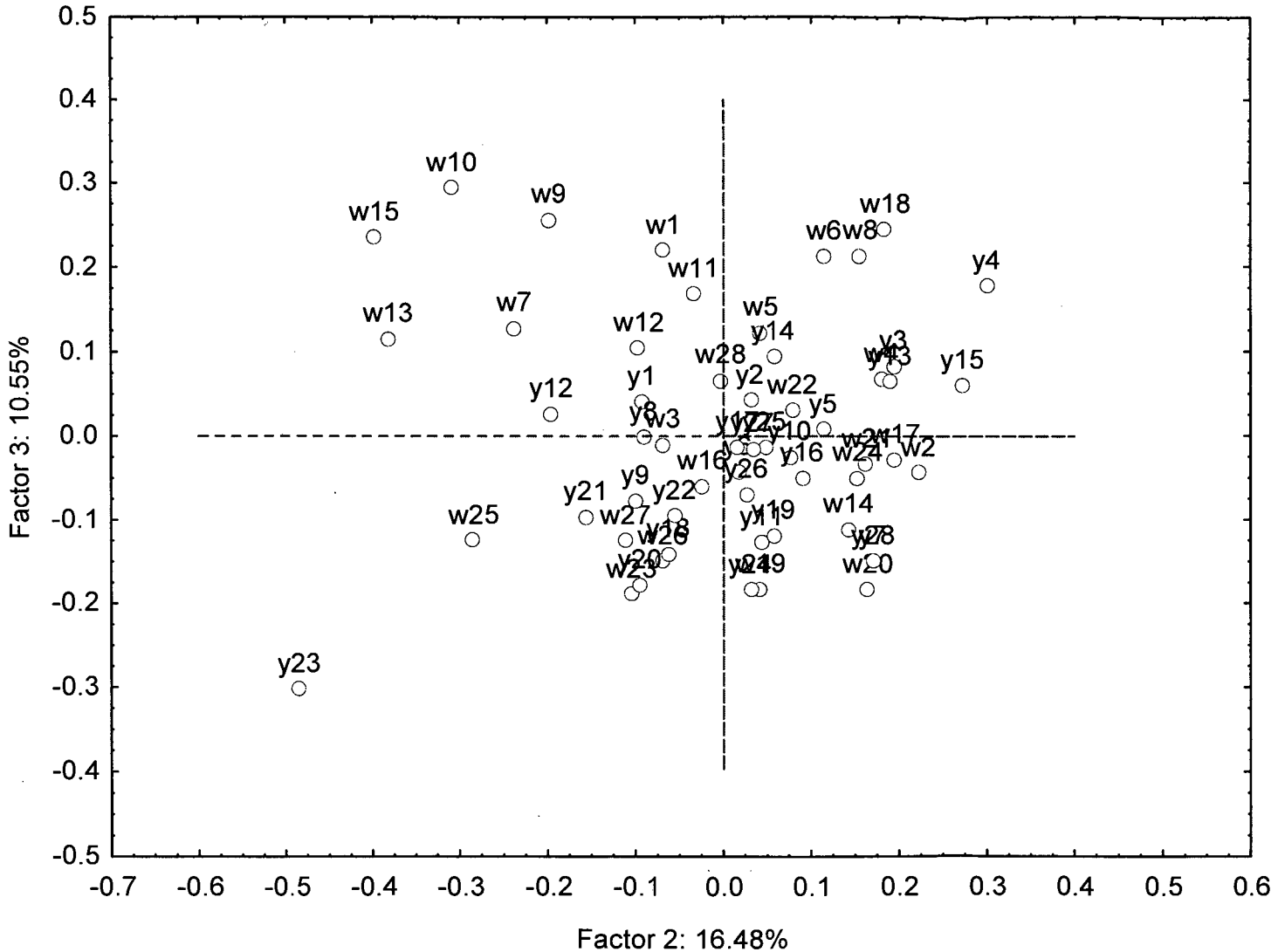

Fig.6. Plot of each specimen of white and yellow Pseudocrossidium crinitum forms on the factor plane (based on morphological cata.

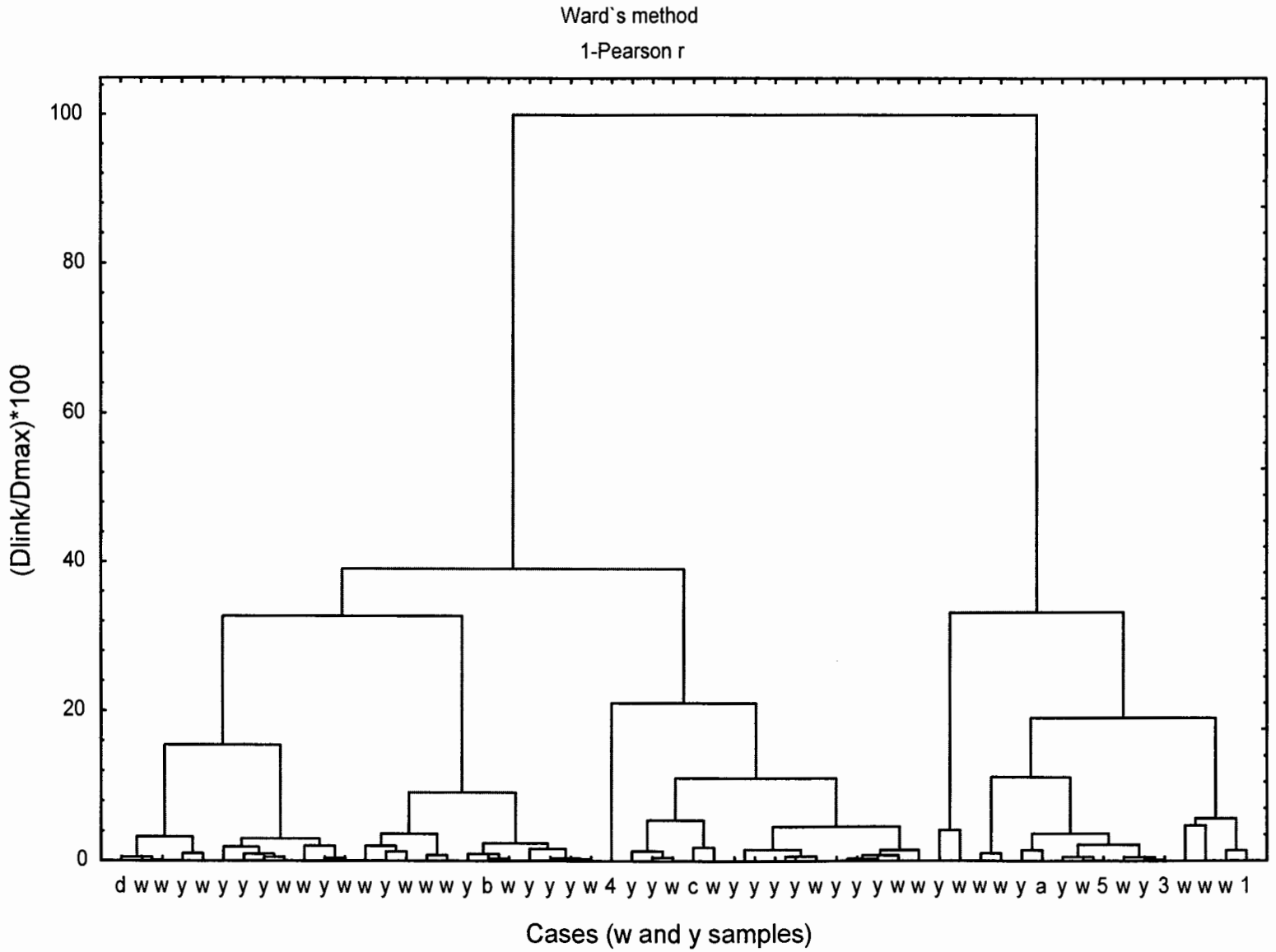

Fig. 7.Tree Diagram from the cluster analysis of the morphological data for 56 Cases. w and y samples that were analysed molecularly are indicated. (Numbers on the x-axis represent samples w1, w3, w4, w5 oand small letters a-d represent samples y1, y2, y4 and y5)

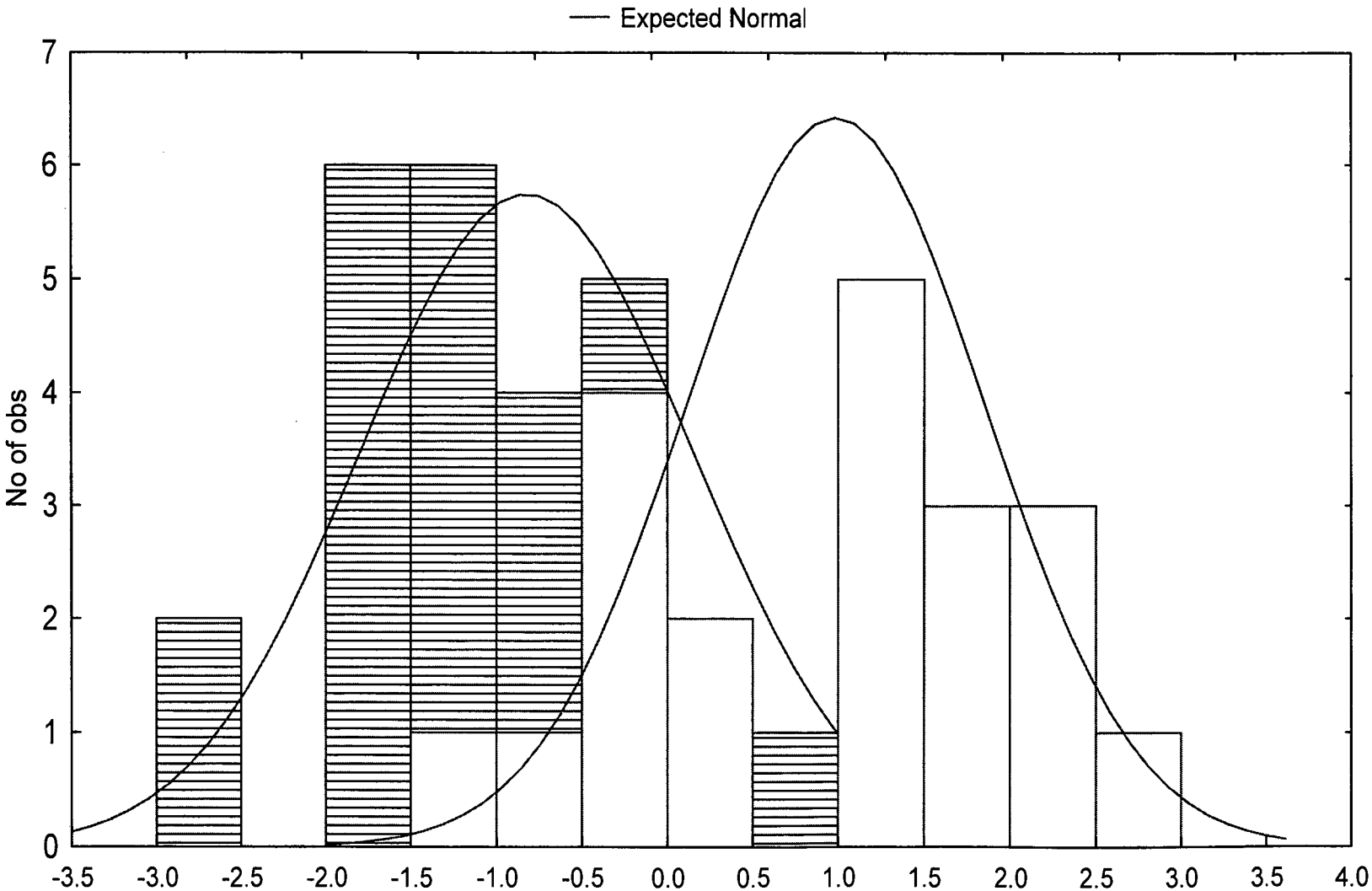

Fig.8. Histogram of canonical discriminant scores of w (hatched bars) and y forms (open bars) of *Pseudocrossidium crinitum*.

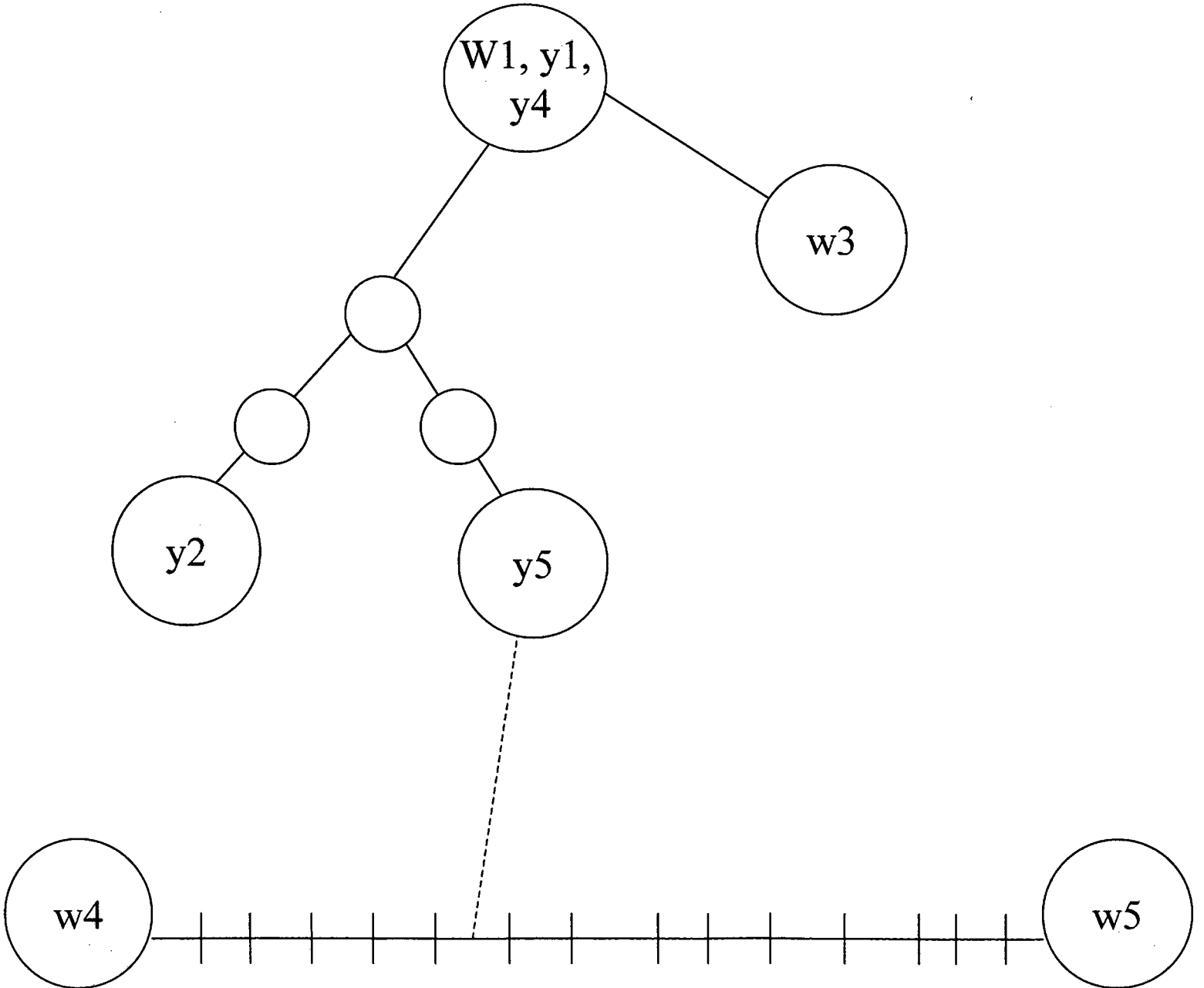

Figure 9. Haplotype network of combined psbA and trnlF data for w and y forms of Pseudocrossidium crinitum.