The reclassification of the genus *Ceratocaryum* Nees (Restionaceae) and describing the variation of the species *C. argenteum* Nees ex Kunth.

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Abstract

An African Restionaceae, *Ceratocaryum*, displays a wide range in variation. It consists of 6 species, which occur strictly in the Cape Floristic Region. Inspection of herbarium material suggests that the species *C. argenteum* Nees ex Kunth presently contains a species complex that can be differentiated into three segregates. The possibility of distinguishing the segregates from each other was explored, thereby testing the hypothesis that the segregates fulfill species status requirements. Phenetic analysis was used to derive the extent of the variation and to decide whether the phenetic data could be used to distinguish between the segregates. Cladistic analysis was also used to derive the phylogeny of the genus to discern the relation between the segregates to the rest of the genus. The cluster analysis reflected three distinct groups based on bract length and nut width. The most parsimonious tree described segregates 1 and 2 as sister taxa and segregate 3 as being a sister group to *C. pulchrum*. They do not form a monophyletic group, which places more confidence on the hypothesis. Segregates 1, 2 and 3 can be distinguished form each other and contain diagnosably distinct features which implies that these taxa are diagnosable species. A description of each species follows.

Contents

Introduction

The Cape Floristic Region, situated in the south-western Cape, is renown for it high floral biodiversity. The region is characterised by a Mediterranean-type climate (Taylor, 1978) characterised by winter rainfall. Bedrock types range from Table Mountain sandstones to Cape granites to Malmesbury shales, giving the region a disrupted, patchy environment. Fynbos, which is the word used when referring to the Cape flora, is said to be defined by either a lack of species dominance, or the conspicuous presence of members of Restionaceae (Taylor, 1978). Due to the complexity and large variation presented by the Flora, taxonomic classification has been problematic. It is for this reason that previously classified taxa are being re-classified in the attempt to achieve a more accurate account of the diversity.

The genus *Ceratocaryum* (family Restionaceae), consisted of 5 species (Linder, 1985) up until 1995. Linder's reclassification involved the addition of a species, *C. pulchrum,* which was previously miss-identified as *C. argenteum.*

Kunth first described *C. argenteus* from the specimen, *Willdenow herbarium,* in 1841. In 1928, Pillans grouped *Ceratocaryum* together with *Willdenowia.* It was in 1930 that Gilg-Benedict (also previously by Masters, 1897) (cited in Linder, 1984) recognized the groups as distinct and separate genera.

The *Willdenowia-Ceratocaryum* clade is recognized by the presence of two styles and silica bodies in the outer sclerenchyma and they also shed their spathes at anthesis (Linder 1990). *Ceratocaryum* is distinguished by its large woody nuts that are usually sessile. Sclerenchyma ridges found in the culm are absent in all the *Ceratocaryum.*

Ceratocaryum is currently a genus consisting of 6 species (Linder, 1995) that occur strictly in the South West cape region of South Africa, namely; *C. fistulosum, C. xerophylum, C. argenteum, C. fimbriatum, C. decipens* and lastly *C. pulchrum*. This last species is the 6th species added by Linder in 1995. The genus *Ceratocaryum* is not only limited to southern Africa, but is endemic to the Cape Floristic Region.

Upon closer inspection of the herbarium specimens it is evident that the species *C. argenteum* represent a species complex with extensive variation. The aim of this study therefore, is to explore the possibility of quantifying the variation and thereby testing the hypothesis that the group consists of 3 distinct species. Morphological features are commonly used amongst descriptive taxonomists when classifying a specimen, for reasons that will be discussed at a later stage. One such morphological feature represented by the species complex is the variation within the nutlet. The nutlets possess different ornamentation and vary in size.

Field observations revealed that the segregates in the species complex not only differed in nutlet morphology, but also growth form of the rhizome. However this was not explored for the simple reason that this variation would be difficult to quantify and for all intents and purposes the nutlet analysis contained ample evidence.

To describe a new species it is advisable to decide what defines a species, and what would make one species different from another. There are numerous species concepts, each with their own parameters, which one can consult. The different species concepts define their species according to different criteria. The reasons and theory behind the different concepts, especially with reference to the biological and the phylogenetic,

will be briefly discussed. There is always the fundamental use of phenetic data as these infer similarities or dissimilarities amongst the taxa, but do not necessarily allocate taxonomic rank Essentially, the determinant of what species concept should be used lies in the nature of the data.

Methods and Material

Data was collected from both field and herbarium material. The herbarium specimens were divided into three segregates according to nut morphology.

Field data

The fresh data was collected towards the end of July from two populations: one on top of Sir Lowry's Pass and the other at the Palmiet River catchment near Kleinmond. The populations represented segregate 1 and segregate 2 respectively.

Segregate 2, was sampled using 20 culms from a single individual to measure the variation within an individual of the population. Thirty culms were sampled from randomly chosen individuals so that the variation within the whole population could be estimated. Only culms from female plants were sampled, as no distinguishing features on the male spikelet could be used to distinguish between the groups.

Segregate 1 was also sampled using only the female culms. The number of individuals that were sampled are however not consistent with those of Seg. 2. Female plants were few and far between and were observed to occur in a large colony-like patch as opposed to the small tussocks found in segregate 2. Only four female plants were found to sample. Five culms were sampled from the one plant and 19 culms in all

from the other three plants. This inconsistency by no means jeopardizes the comparability of the data. The difference in growth form that was observed is probably due to a difference in rhizome structure. Seg1 has a large rhizome producing few, but extensive colonies of a single sex. Seg2 produces many small tufts.

Herbarium data

All herbarium material used were specimens from the Bolus Herbarium, University of Cape Town. The details of each are given in appendix 1.

Data

Once all the material was gathered, the nutlet width and length was recorded from both field and herbarium material. All the viable nutlets that could be found in the herbarium material were measured. 13, 23, and 19 nutlets were measured for segregate 1,2 and 3 respectively. The field data, consisting of only segregate 1 and 2, reflected measurements of variation with in the individual and within the population. From segregate 1, four plants were sampled from which 14, 2,3 and 5 nuts were measured. Segregate 2 produced more nuts per spikelet and per individual (observation) and as a consequence, 27 nuts were measured from a single plant and 31 nutlets from the population.

Data from the herbarium specimens included culm diameter, bract length, spathe length, spathe width, and nut width and nut length (see figure 1 below). The values were averaged for each specimen and produced a matrix (table 1) which was used for Cluster analysis and ordination.

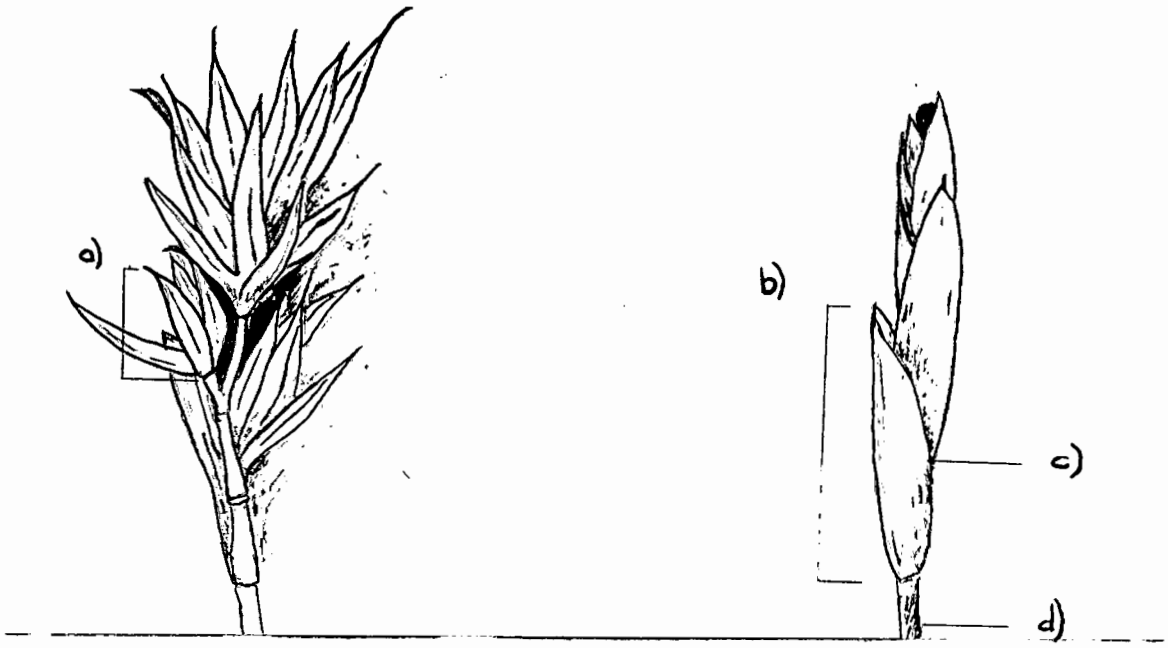


Figure 1 a) The length of the basal bract is measured from the base of the spikelet to the tip; b) the length of the basal spathe is measured from the node to the tip; the width c) was measured at half the length, where it is broadest; d) apical culm diameter was measured a few millimeters below the basal spathe.

Statistical Analysis

Phenetic analysis

Univariate analysis

Measurements of bract length, spathe length, spathe width and culm diameter, recorded from the herbarium specimens were plotted as Box-and-whisker plots. The aim thereof was to observe any significant difference in ranges of the variables within the species complex, which could correlate to the difference in nut morphology. The plots were performed in STATISTICAvs5.0. The reason herbarium specimens were used, and not fresh material, is that the fresh material only represented two populations and the herbarium material was a better representative of the species complex as a whole.

Bivariate analysis

Excel was used to produce a scatterplot of the nut width and nut length for all three groups within the species complex. Both fresh and herbarium material was used, giving a good indication of how well the sampled population represents the species, i.e. if the population data is nested in with in the data collected from herbarium specimens, the population can be regarded as a good representative of the species. The scatterplot is also used to determine the extent of overlap in the data.

Multivariate analysis

Phenetic analysis was applied using NTSYS 2.02i (Rohlf, 1998). The operational taxonomic unit (OTU) was represented by averaged values of each herbarium collection that was measured. The data matrix used consisted of continuous variables (see table 1) and was standardised for all the calculations so that large ranges in the data weren't weighted. The analysis was used to retrieve a dendrogram that produces clusters of organisms more similar to each other than any other such cluster. The cluster analysis is based on a similarity matrix. The similarity matrix was calculated using the Distance coefficient, which, according to Sneath and Sokal (1973) allows considerable violation of the assumptions of multivariate normality. The data used in the analysis is continuous, a requirement to calculate the distance coefficient. Clusters of specimens were grouped using UPGMA (unweighted pair-group method using arithmetic averages) to avoid chaining of the dendrogram. The method involves equal weighting of OTU's and examines the dissimilarity matrix for most mutually similar pairs (Sneath and Sokal, 1973). The dendrogram reflects the maximum phenetic differentiation, if any, (as computed by the Distance coefficient) between taxa. The

cluster analysis also implies to what degree a group of taxa are phenetically different by the distance values calculated on the dendrogram.

All the specimens that were measured were coded. S1 represents the herbarium specimens divided into segregate 1, s2 codes for all the specimens separated into segregate 2 and like wise for s3. The letters a, b, etc simply code for the different specimens used within each segregate. Therefore 5, 7 and 6 specimens were used for segregate 1, 2 and 3 respectively.

Table 1. The data matrix used for phenetic analysis, prior to standardization.

	Nut width (mm)	Nut length (mm)	Culm diameter (mm)	Bract length (mm)	Spathe length (mm)	Spathe width (mm)
s1a	7.32	8.5	3.67	18.5	34	20
s1b	7.1	9.35	3.2	16.5	28.5	16
s1c	8	10.4	3.2	16	28	16.5
s1d	7.4	9.7	4.2	24	32	16.3
s1e	7.9	9.9	4.2	15.5	46.5	14
s2a	4.9	8.2	3.8	16	39.6	16
s2b	5.2	7.8	3.65	18	41.5	17
s2c	4.9	8.2	3.8	16	38.3	15
s2d	5.5	8.45	3.8	19	37	13.5
s2e	5.2	7.9	4.1	18	47.3	19
s2f	5.6	10	4	21	46	16
s2g	9	5.1	4	25.5	54.5	19.5
s3a	9.7	11.5	2.7	27.5	49.5	16
s3b	9.3	11.3	3.4	38.5	70	23
s3c	11.2	12.5	3.2	32	55	18.5
s3d	8.5	9.9	3.4	29	55.5	22
s3e	9.1	10.6	3.8	25	31	17
s3f	11.4	12.5	3	24.5	48.5	13.5

Principle Components Analysis (or PCA) is used to determine which one of the characters is predominantly responsible for the grouping of the clusters. The results are reflected in the projected eigenvalues and eigenvectors (vectors also known as components). Eigenvectors are important as they describe the relationships between the OTU's and the data. A large proportion of the variation in the data is accounted for in a relatively low framework of dimensionality (Sneath and Sokal, 1973) i.e., the first few components can account for most of the variation seen in the data. These are called Principle Components. Both the vectors and values are used to produce the components and therefore, the eigenvalues for the first three components provide information on which variable is accountable for the variation observed within the component.

A co-phenetic analysis was also performed to assess how well the data was reflected in the cluster analysis, therefore determining how significant the results are and with how much confidence they can be represented. The values range from 0 to 1, one being the perfect fit.

Cladistic analysis

A cladistic analysis was run for the whole genus to determine its phylogeny. The coded data matrix used to run the analysis is a modification of the matrix used by Linder (1995). The matrix was modified, as it did not satisfy the variation observed in the *C. argenteum* complex (Table 2). In the modified matrix, *C. argenteum* was represented as segregates 1, 2 and 3. Character 4 was changed to describe the variation of tuberculation observed on the nutlet, and character 5 was added to account for the differentiation of the nutlet cap from the rest of the nut, as seen in Seg 1 and Seg 2.

Character 12 was also added to account for the large difference in nutlet size between Seg3 and *C. pulchrum* and the rest of the genus. The character of rhizome length was also changed for Seg1, which was found to have an extensive, robust rhizome.

Table 2. The data matrix used to determine the phylogeny of *Ceratocaryum*.

	1 5 10
Can	1000000100100----
Hypo	1000000100100----
Willdenowia	1000000100100----
Seg 1	0000110011000----
Seg 2	1000110011000----
Seg 3	1000201011001----
C. pulchrum	1001201010001----
C. fistulosum	1100201011000----
C. decipens	0010000000110----
C. fibriatum	0010100100100----
C. xerophilum	0000000000100----

Character coding used for the construction of the matrix above.

1. Rhizomes: long (0); short (1)
2. Culms: not fistular (0); fistular (1)
3. Spathes: acute, pale (0); long-acuminate, dark (1)
4. Spikelets: numerous (0); solitary (1)
5. Tuberculation: smooth (0); moderately tuberculate (1); extremely tuberculate (2)
6. Ornamentation: no cap differentiation (0); cap differentiated from body of nut (1)
7. Nut outline: barrel (0); round (1)
8. Female tepals: broadly oblate, overlapping (0); oblong, not overlapping (1)
9. Female tepals: apiculate (0); long apiculate (1)

10. Culm diameter: 2 – 4 mm (0); 6 – 8 mm (1)

11. Elaiosome: absent (0); present (1)

12. Elaiosome: short<ca 1/10 of nut> (0); long< ca 1/3 of nut> (1)

13. Nut size: small (width<10mm, length<11mm) (0); large (width>10, length>11) (1)

The program, Hennig 86 (Farris, 1988), was used to produce a phylogeny that would reflect the most parsimonious tree. Hennig 86 was chosen as the data matrix that was used was relatively small and, according to Platnick (1989), Hennig 86 provides better results over other programs and is both effective and efficient compared to other available programs. It also makes use of Heuristic methods to find the shortest possible tree, i.e. the minimal amount of steps. One of the ways in which this was done was by using the bb* function in Hennig 86 to include branch swapping. Branch swapping rearranges the tree in an attempt to decrease its length (Kitching, 1992). The trees that are produced in Hennig86 can be saved using the tsave function, which can then be recalled in an editing program, CLADOS, which runs in conjunction with Hennig 86. CLADOS was used to plot the characters and the phylogenetic state each character represented.

Both the consistency index and the retention index values were calculated to indicate how well the data fits the cladogram. The consistency index (ci) expresses the amount of homoplasy present in the tree and the retention index (ri) expresses the amount of synapomorphy present in the tree (Siebert, 1992).

Three outgroups were used for the analysis, namely *Cannomois parviflora* (Thunb.) Pillans, *Hypodiscus aristatus* (Thunb.) Krauss and *Willdenowia glomerata* (Thunb.) (Also used by Linder 1995).

The purpose of and criteria for choosing outgroups has, according to Nixon and Carpenter (1993), been confused in the literature. Outgroups are said to polarise the character states, or, in other words to determine the direction of evolution by implying an ancestral and derivative state. Numerous authors dispute this assumption, stating that all the characters should be assessed equally and arguing that the phylogeny could determine ancestral and derived states. Nixon and Carpenter (1993) concluded by stating that; outgroups do not have to be, or include the sister group (a common misconception), although cladistic conclusions may be better founded if they are. Also, that outgroups need not be monophyletic and lastly, that it is not necessary to have more than one outgroup, although more taxa might produce a more stable cladistic inference. Outgroups are based on inclusive synapomorphies in both the in- and outgroup. If this is not possible outgroups can be base on previous classifications (Nixon and Carpenter, 1993).

In this study, three outgroups were chosen. They did not form a monophyletic group, but are closely related to *Ceratocaryum* based on the phylogeny described by Linder (1991).

The three outgroups share the same character states in the data matrix as shown in table 2 and therefore the states are decisively the maximally parsimonious reconstruction (Kitching in Forey et al, 1992).

Bootstrapping and Bremer support

Bootstrapping and the Decay index (or branch/Bremer support) were both calculated in PAUP vs. 3.11 (Swofford, 1993) using the data matrix according to the characters and coding in Table 2. Both methods calculate the branch strength. This is necessary to establish how strongly the nodes and branches are supported by the data, which in turn provides either a stronger or weaker argument for certain tree morphologies.

Bootstrapping

Bootstrap values are calculated by randomly re-sampling characters to approximate the distribution (Siebert, 1992), (each random sample consisting of same number of characters as presented in the matrix) and running a most parsimonious analysis on the data. This means that a character could be chosen more than once, adding weight to the character. This is normally done for 100 runs or more. Larger data matrices would need to have more than 100 runs to accurately represent the distribution. The value given on each branch, is the percentage of occurrences of a particular branch (Siebert, 1992).

Bootstrapping procedure, although popular, has been criticized. Bremer (1994) argues that the statistical methods are erroneous in that the re-sampling technique which produces a new matrix, applies zero weighting to those characters that have not been sampled and over weights the characters that have been sampled more than once. Bootstrapping is best applied to molecular data, as the strength of bootstrap results is dependent on the number of replicates of samples it can produce and morphological data is often not extensive enough. Although bootstrapping may not be accurate, Sanderson (1989) believes that it provides, along with other analyses, a more complete picture of the confidence of tree morphology.

An alternative would be to use a Decay index or Bremer support to determine the strength and confidence on the nodes. The Decay index relaxes the tree parsimony in single steps and notes which nodes collapse and which remain, until the whole tree collapses.

Results

Phenetic

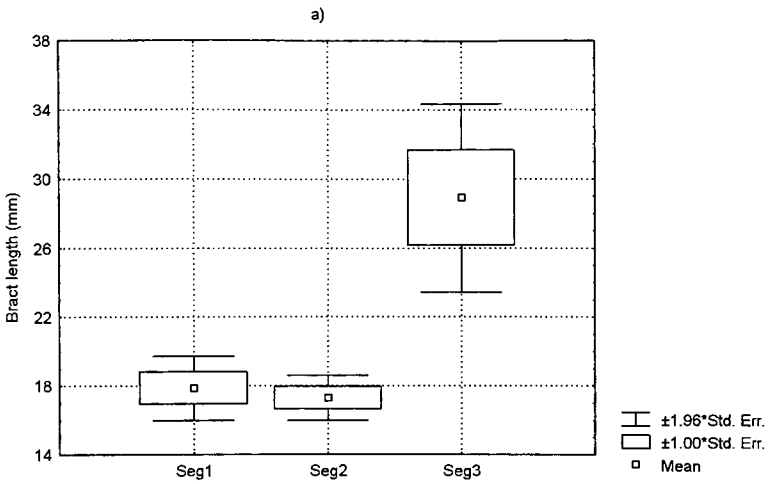
Univariate analysis

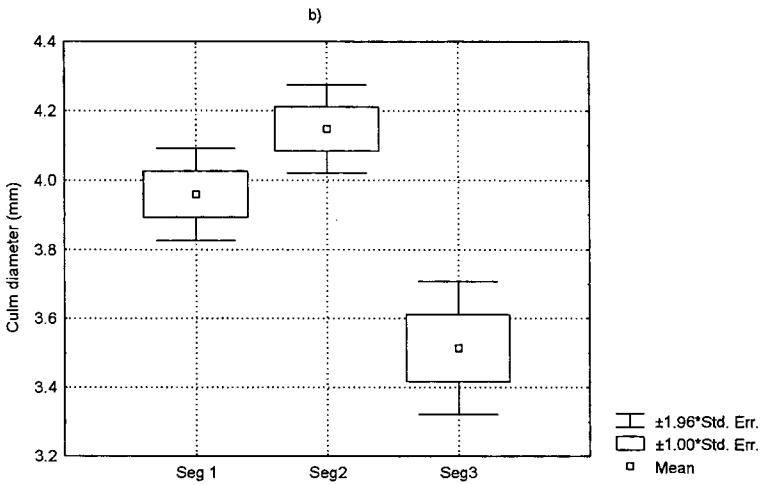
The variation between the morphological features was presented as box and whisker plots (Figure 2a, b and c). Figure 2a show that Seg1 and Seg2 share relatively smaller bract lengths (approximately 18mm) as opposed to Seg3 at a higher average of 29mm. They also share a higher average culm diameter (4-4.2 mm) than Seg3 (average of 3.5m).

Bivariate analysis

There is no overlap in the variation of nutlet width between species Seg1 and Seg2 (figure 3). A dividing line could be drawn at a width of 6mm where Seg2 would be smaller and Seg1 would be wider than. Seg3 represented on figure 3 seems to be well nested in Seg1 when comparing the nutlet dimensions, but these two complexes can easily be distinguished from each other by comparing the nut ornamentation.

The difference in shape can be seen from the drawings on figure 4a and 4b. Seg2 is oblong in profile and Seg1 is barrel-shaped.





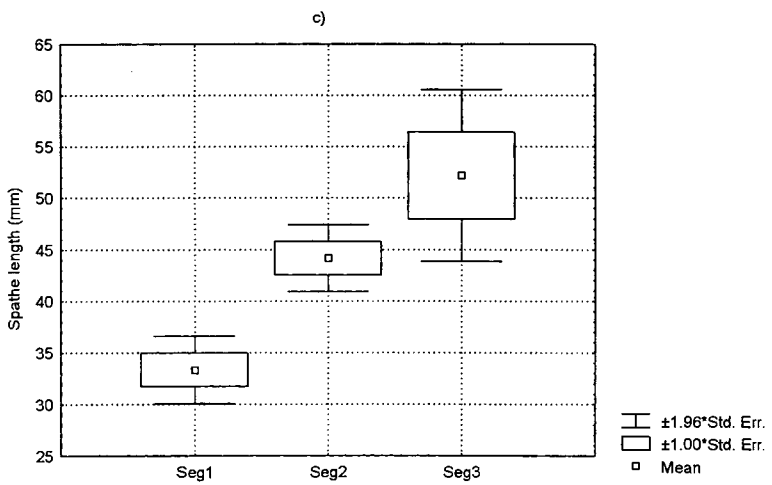


Figure 2a, b and c represent bract length, culm diameter and spathe length respectfully.

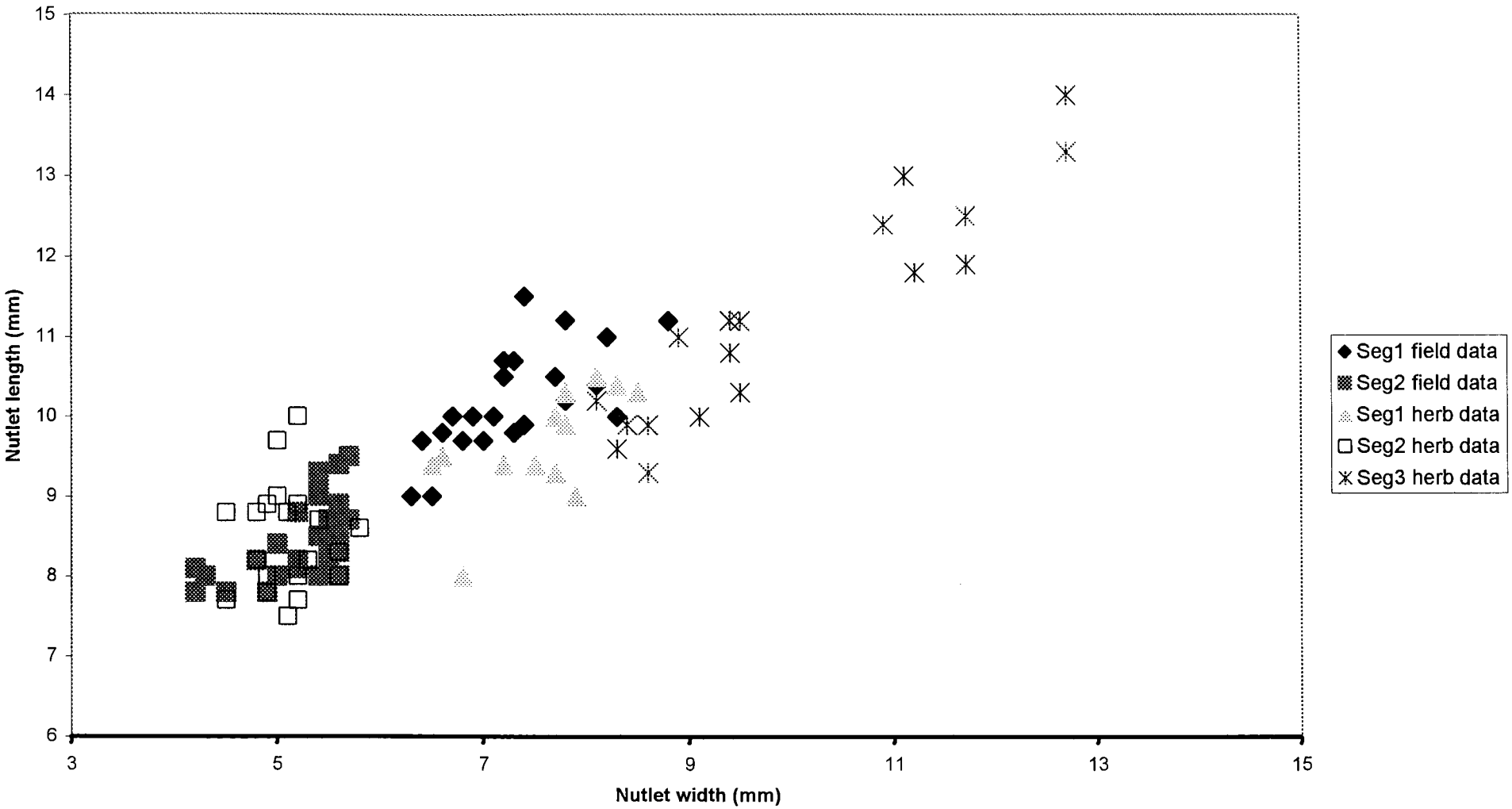


Figure 3. Nutlet width against nutlet length. The field data from seg1 and seg2 are nested well within the herbarium data (Seg1, 2a nd 3 herb data)

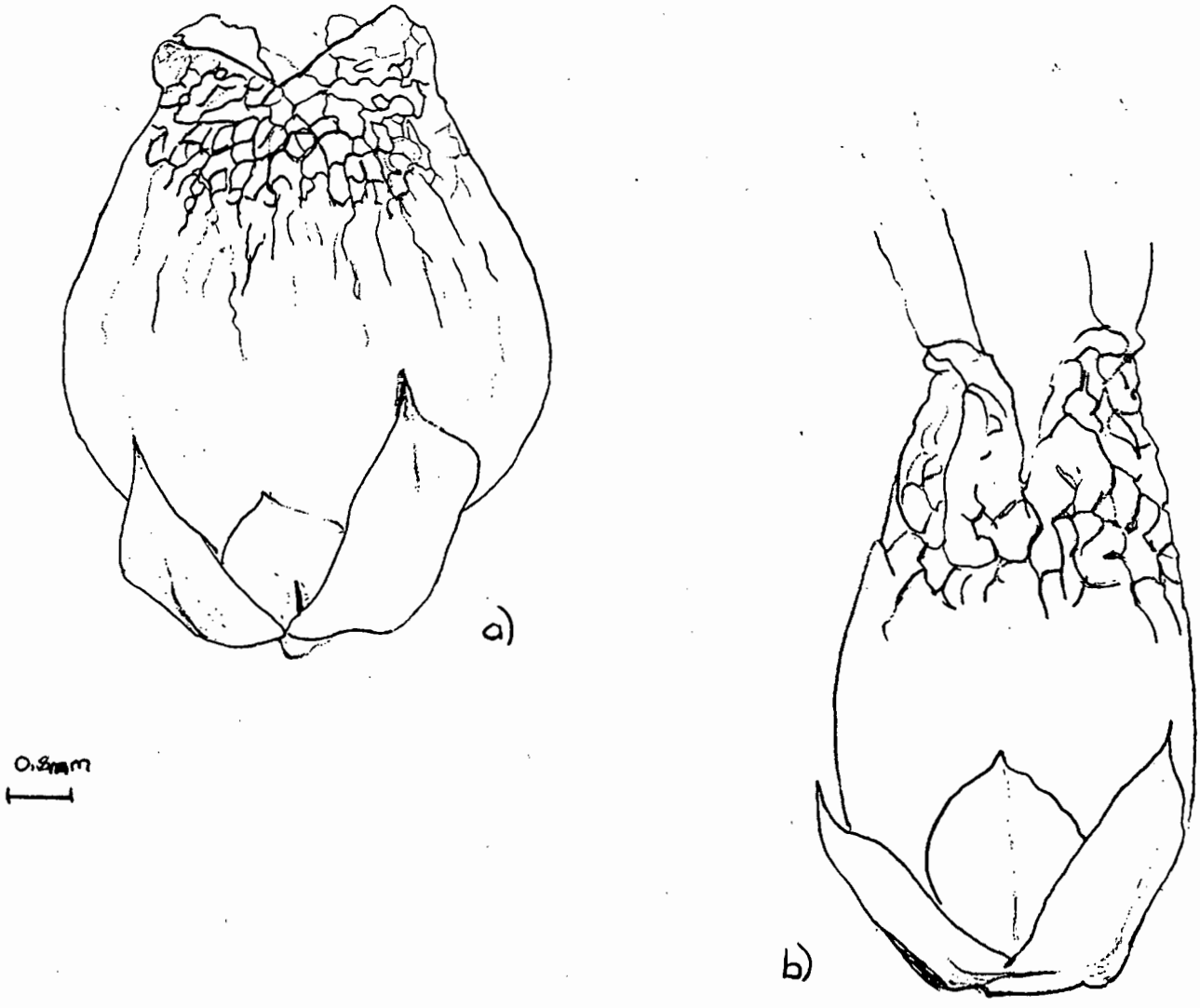


Figure 4. Nutlets representative from a) Segregate 1 and b) Segregate 2, showing detailed nutlet tuberculation and differentiation of the cap.

Multivariate analysis

Cluster analysis

The cluster diagram is based on a distance similarity matrix that projects clusters of specimens that are more similar to each other that any other specimen. Figure 5 separates the specimens into the three clusters that we had previously hypothesised, i.e. the three groups corresponded to the three that were separated from the herbarium specimens (with the exception of s3c and s1d) therefore confirming a clear distinction between the segregates.

Principle Component Analysis

The exceptions of s3c and s1d can be explained by possible overlaps in variation amongst the variables that were measured. The graph (Figure 6) of the first two components eliminate any ambiguity of the placing of s3c and s1d by showing that the first two (and therefore the more influential factors determining the position of the specimens) components group the s1, s2 and s3 together with no potential overlap. The associated eigenvalues for the first three components are represented in table 3. The first three components account for most of the variation (Sneath and Sokal, 1973). The first component showing that the strongest influencing factor is bract length valued at 0.9116 and nut width at 0.8432, the closer the value is to one the stronger. The second component is strongly influenced by spathe width with a value of –0.72.

The cophenetic value was calculated to be 0.875, which means that clusters correspond and reflect the data fairly accurately.

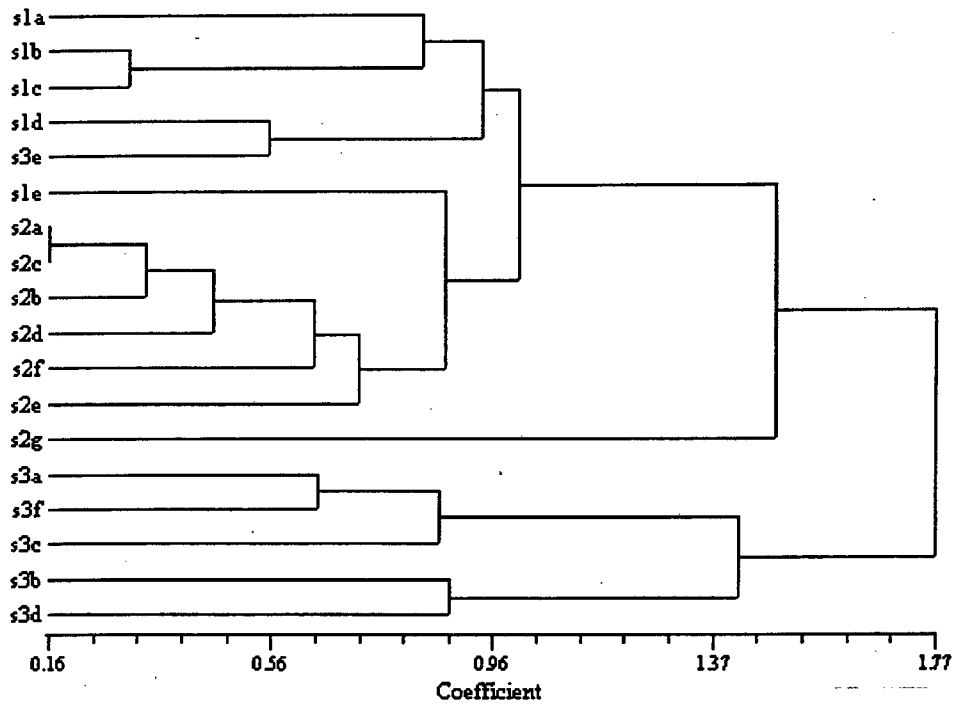


Figure 5. Cluster diagram using UPGMA and Distance coefficient. Seg 1, 2 and 3 form seperate clusters.

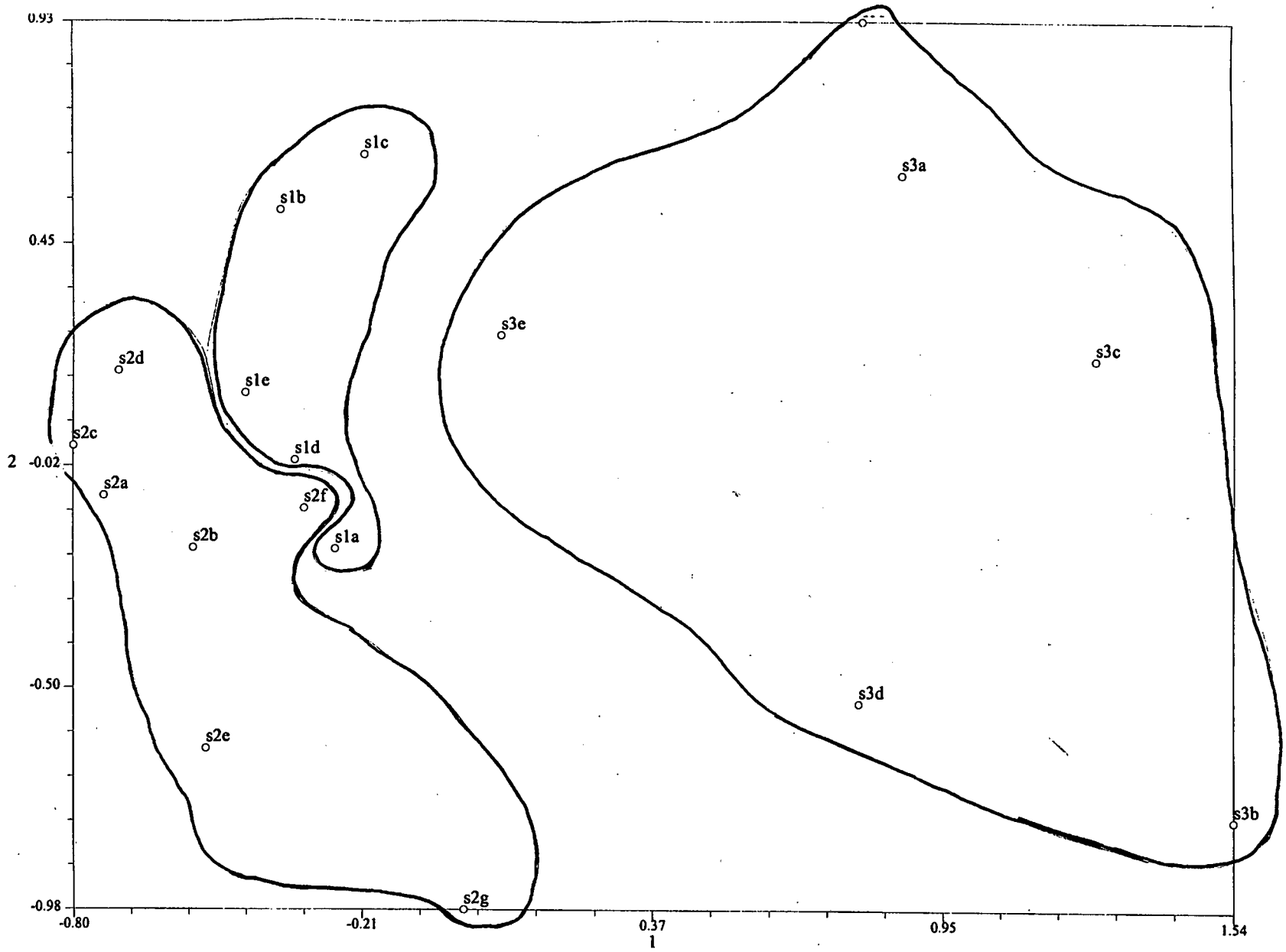


Figure 6. The axes represent the first two Principle components. The specimens are plotted according to the extent of similarity as reflected by the components. The positions can be used to group more similar specimens.

Table 3. The eigenvalues of the first three components

	1	2	3
Nut width	0.843	0.267	0.09
Nut length	0.673	0.593	0.191
Culm diameter	-0.63	-0.537	0.476
Bract length	0.911	-0.269	0.106
Spathe length	0.704	-0.506	0.274
Spathe width	0.501	-0.72	-0.395

Cladistic analysis

Only one most parsimonious tree was derived and calculated to be 18 steps long (Figure 7). The associated characters have been placed at each corresponding step on the branches. The ci and ri values at 77 and 85 respectively, reflect a good fit of data to both the basal and terminal nodes. The most parsimonious tree demonstrates that *C. pulchrum* and Seg3 are more closely related than either is to Seg1 or Seg2, and that Seg2 and Seg1 are sister species. The previous monophyletic grouping of *C. fimbriatum*, *C. decipens* and *C. xerophilum* has re-established as a monophyly, basal to the genus.

The Bootstrapping analysis (figure8) calculated a very strong node grouping of Seg1, Seg2, Seg3 and *C. pulchrum* and *C. fistulosum* with a value of 93. The grouping of *C. argenteum, C. pulchrum and C. fistulosum* is also strong, which means that the phylogeny represented by these nodes is backed up with a fair degree of confidence.

Bremer support showed that by relaxing the parsimony by 1 from tree length of 18 to 19, the node supporting the Seg1 and Seg2 group and the node Seg3 and *C. pulchrum* collapses. The others, *C. xerophilum*, *C. decipens* and *C. fimbriatum* also collapse right down to the bottom of the tree. By relaxing the tree by yet another step, collapses

the *C. argenteum, C. pulchrum* and *C. fistulosum*. At a tree length of 21 and less the tree morphology remains the same as before (tree length of 20 and less). Lastly, when the parsimony is relaxed a fourth step (trees 22 steps and less) the whole tree collapses The Bremer support values are shown on figure 8.

The bootstrap and Bremer support values seem to reflect similar branch support. The combination of the two analyses and their agreement produce strong evidence for the strength of the nodes.

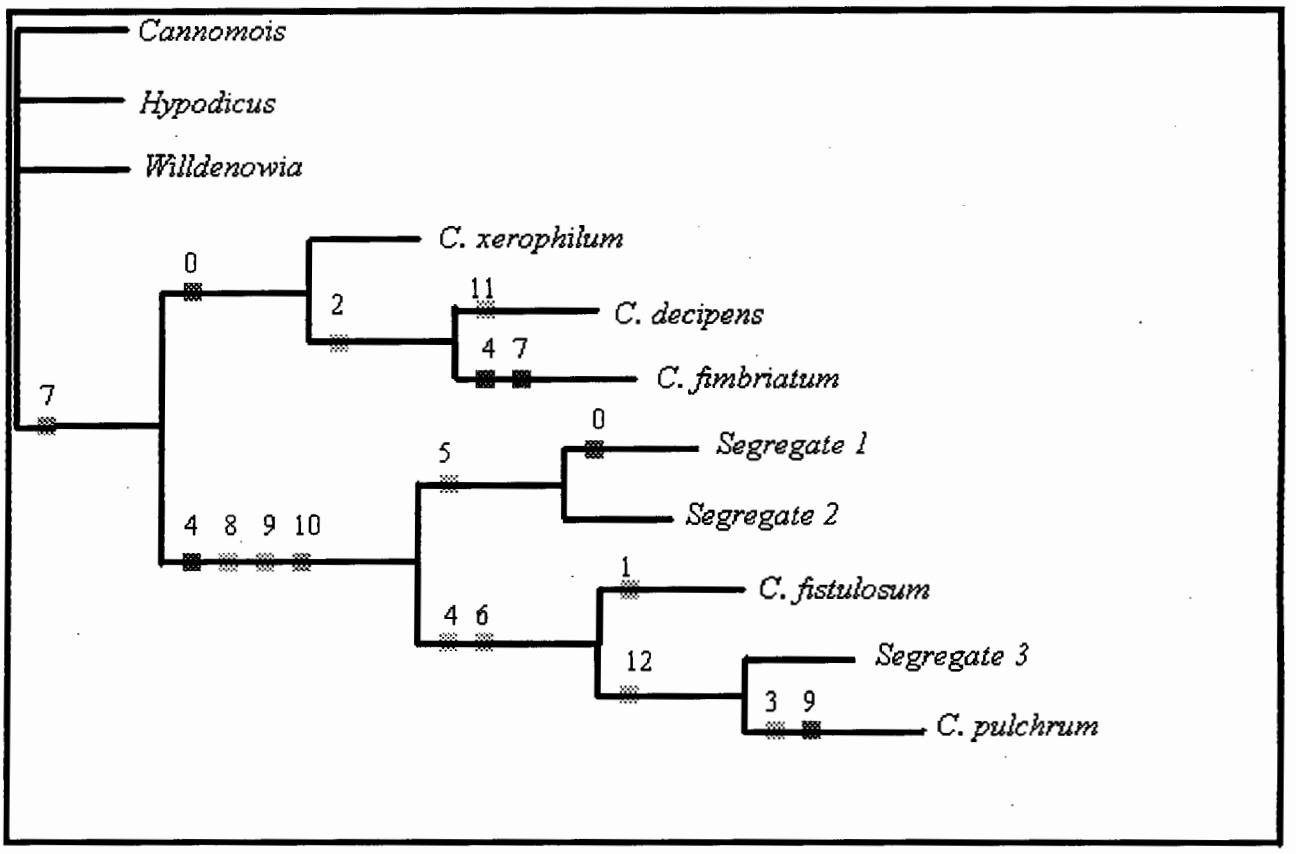


Figure 7. The most parsimonious tree calculated in Hennig 86, with the shortest tree length of 18, ri = 85 and ci = 77

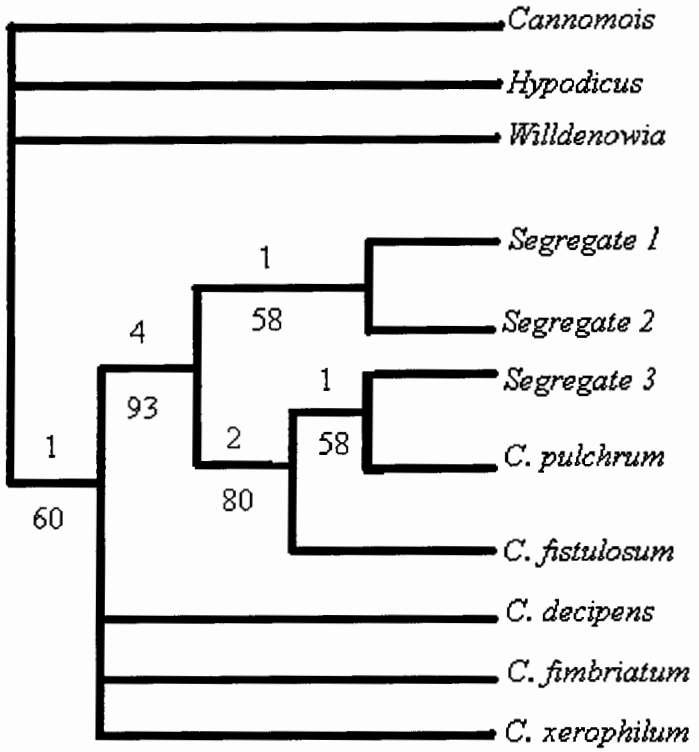


Figure 8. Bootstrap values below and Decay index values below.

Conclusion

Based on the evidence provided by the cluster analysis and cophenetic values, as well as the cladogram phylogeny, three distinct groups are easily distinguished from each other. The three groups can be diagnosed by a combination of characters namely nut shape and nut ornamentation. According to Nixon and Wheeler (1990) the segregates can be defined as minimally diagnosable species as they posses a combination of diagnosable characters. Seg1 and 2 form a monophyletic group according to the phylogenetic analysis as they share nut ornamentation as a distinguishing feature that separates Seg1 from Seg3. The most parsimonious tree indicates that Seg3 is distinctively different from Seg1 and Seg2. Bremer support and bootstrap values support the argument b placing high values on the node supporting the monophyletic grouping of Seg3, *C. pulchrum* and *C. fistulosum*.

Species Concepts

There are numerous species concepts in the literature which one can utilize to define a species. This may lead to subjective decisions and possibly confusion amongst different classifications. For the purposes of this project I have chosen to use the Phylogenetic species concept as a guideline for defining a species.

The Morphological species concept has been used for centuries and as it implies, relies solely on morphology as a determinant for species rank. This concept is highly subjective as it basis species rank on the extent of similarity and dissimilarity of the specimens. The concept is also flawed in that it bears no information on the phylogenetic relationship between the taxa and is too simplistic.

The Biological species concept has been rejected in most fields of study as it also has an element of subjectivity. I have discarded the BSC because conceptually it does not address evolutionary processes and can therefore not be used consistently for systematic and cladistic analyses. The BSC places emphasis on reproductive power of populations, therefore species are defined as ecological, genetic and reproductive units (Mayr, 1991). The BSC relies solely on reproductive isolation as the central criterion for species status (Cracraft, 1989). The concept runs into problems when taxonomists work on species which readily hybridize, or when fossil records are involved. To add to the impracticality of the concept, Herbarium specimens cannot be tested for reproductive compatibility. The BSC does not explain or encompass evolutionary processes. It is causes inconsistencies in the literature as it relies on subjective decisions of species status based on varying levels of reproductive isolation.

The Phylogenetic species concept, although it uses phenetic data, emphasizes taxonomic differentiation, which may result in reproductive isolation and may not. It also emphasizes diagnostic variation for separating basal evolutionary taxa (Cracraft, 1989). The PSC defines species as an irreducible cluster of organisms, diagnosably distinct from other such clusters, which also reflect patterns of ancestry and descent (Cracraft, 1989). Phylogenetic species are basal taxonomic units, which place no limitation population size; therefore relatively small populations can acquire species status. The PSC has also been criticised by Mayr (1991) who claimed that the definition of a phylogenetic species is merely that of a phyletic lineage and that it lacked a satisfactory explanation of what mechanisms and processes result in speciation events. I believe these arguments are unfounded as the phylogeny of

species can describe in evolutionary terms which attributes have contributed to speciation. Also, a phylogenetic species is not merely an evolutionary unit, but must be defined by taxonomic differentiation. Therefore I have adopted the PSC and according to its principles and parameters, Segregate 1, 2 and 3 show evidence to be classified at species status.

Geographic distribution.

The three species seem to occur in separate micro regions. I call these micro regions as the ranges are comparably small and the species are recorded appear within the same vegetation regime in the southern Cape within the Cape Floristic Kingdom (Taylor, 1978). The range of all three species occurs from the Kogelberg mountains in the west to Albertinia and Riversdale in the east and from Landdrost Kop (in the Kogelberg range) in the north to Betty's Bay in the south west and Hermanus/Klein river mountains in the south east.

From figure 9 it is quite obvious that the three species do not overlap, but any comment on this matter would be purely speculative since no ecological information was recorded and no extensive geographical data has been collected. Possible speciation mechanisms associated with the different regions can only be hypothesised as either one of fire survival or a response to steep ecological gradients. A comparison of the two sampling sites did however show slightly different ecology. The site at Sir Lowry's pass seemed to be moister as water seeps were found everywhere. More information needs to be collected on this subject.

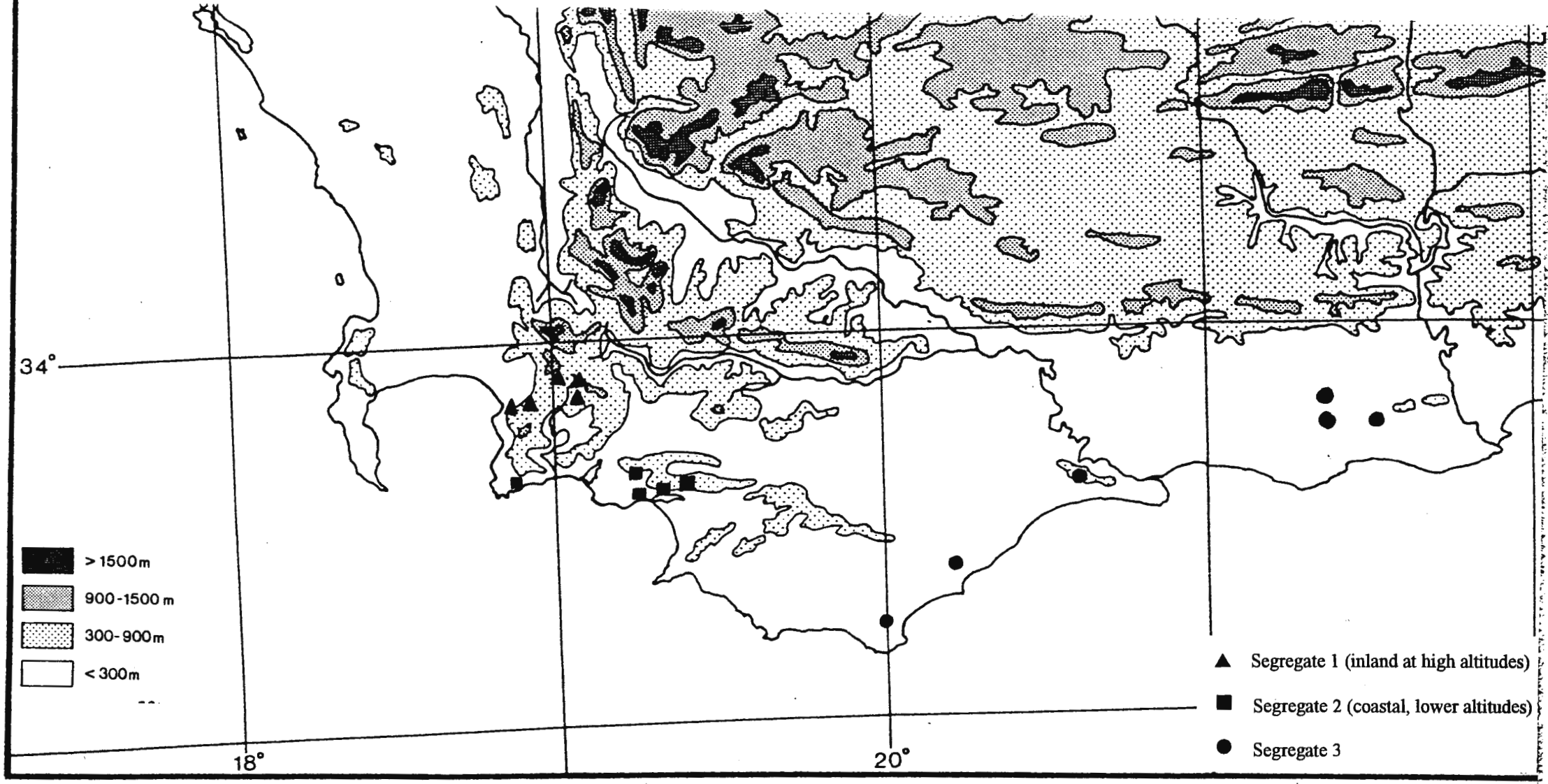


Figure 9. Geographic distribution of the three segregates as recorded by collections.

Macro-morphology

The two species sampled also differed in growth form. Seg1 was found as large colonies. The species had a long well developed rhizome that possibly played a role as the primary mode of reproduction as cloning, rather than concentrate energies into producing seeds. This is evident in the number of viable seeds recorded per culm compared to Seg2. The role of fire also produces an argument for the chosen growth form. The species could resprout after fire due to the large well developed rhizome, and so seeding would be of secondary importance for reproduction.

Seg2 however has a comparatively small rhizome and can be found in small tufts composed of a single individual. The number of nutlets far exceeds that of Seg1, and possibly indicates that the plant is a reseeder and reinforcing a large seed bank is of primary importance.

This character unfortunately can not lend itself as a real valuable measurement as not enough is known about the structure. Infact it is the least studied structure. Apart from this unfortunate circumstance, herbarium specimens are rarely accompanied with rhizome samples.

Species description

Macro-morphology

Segregate 1

Stems erect, simple, terete and slightly fistular, minutely rugose, 3.8-4.2mm in diameter at the apex; sheaths tightly convolute, oblong-lanceolate, acute, mucronate, coriaceous, deeply membranous along upper margins, upper parts becoming chartaceous and disintegrating, 3 - 4.5cm long; male inflorescence not known; female

inflorescence spicate, oblong and narrow, containing 3 – 4 spikelets, 6 – 11 cm long; spathes disintegrating along margins, nerve marked and membranous, oblong-lanceolate, acute, smooth, dark brown in colour, 30 – 50mm long, loosely convolute; spikelets sessile, olblong, 1- or 2- flowered, 3-4 cm long; bracts loosely convolute, about 12 in number, lanceolate to linear lanceolate, 1- nerved, chartaceous, whitish-brown in colour, 1.5 – 3 cm long; perianth sessile, closely appressed to the ovary, 2- 5 mm long; segments broad-based, with margins overlapping, apiculate, suborbicular, cartilagenous, inner shorter than outer; ovary sessile, globose, moderately tubercled on upper part; styles 2, free erect-spreading, semi terete, acute, supported by channel up outer side, stigmatic surface feather-like extending over whole surface, 10 – 12mm long; fruit subglobose, depressed at apex, black and smooth on lower half of nutlet, reddish-brown and tuberculate upper half.

Segregate 2

Stem erect, unbranched, terete, slightly fistular, minutely rugose, 3.8 – 4.5 in diameter at apex; sheaths tightly convolute, oblong-lanceolate, acute, mucronate, coriaceous, deeply membranous along upper margins, upper parts becoming chartaceous and disintegrating, 3 – 5 cm long; male inflorescence erect, paniculate-cymose, dense, 6 – 9 cm long; spathes disintegrating along margins, nerve marked and membranous, oblong-lanceolate, acute, smooth, dark brown in colour, 35 – 40mm long, loosely convolute; rhachis, each node with callous-like thickening and bearing several racemes; recemes oblong, dense with many flowers1- 1.5 cm long; spathellae lanceolate, hyaline, 0.5 – 1.5 cm long; bracts expanded, linear or linear-lanceolate, long acuminate, membranous, silvery white; perianth shortly pedicellate, narrow-

oblong, about 5mm in length; segments not seen; female inflorescence spicate, oblong and narrow, containing 4 – 5 spikelets, 7 – 13 cm long; like that of the male inflorescence; spikelets sessile, oblong, 1- or 2- flowered, 2-3 cm long; bracts loosely convolute, about 12 in number, lanceolate to linear lanceolate, 1- nerved, chartaceous, whitish-brown in colour, 1.5 – 2.5 cm long; perianth sessile, closely appressed to the ovary, 2- 5 mm long; segments broad-based, with margins overlapping, apiculate, suborbicular, cartilagenous, inner shorter than outer; ovary sessile, globose, moderately tubercled on upper part; styles 2, free erect-spreading, semi terete, acute, supported by channel up outer side, stigmatic surface feather-like extending over whole surface, 10mm long; fruit oblong-ovate, depressed at apex between styles, black and smooth on lower half of nutlet, reddish-brown and tuberculate upper half.

Segregate 3

Stem erect, unbranched, terete, slightly fistular, minutely rugose, 3 – 4mm in diameter at apex; sheaths tightly convolute, oblong-lanceolate, acute, mucronate, coriaceous, deeply membranous along upper margins, upper parts becoming chartaceous and disintegrating, 4.5 – 7.5 cm long; male inflorescence erect, paniculate-cymose, dense, 6 – 12 cm long; spathes disintegrating along margins, nerve marked and membranous, oblong-lanceolate, acute, smooth, dark brown in colour, 50 – 70mm long, loosely convolute; rhachis, each node with callous-like thickening and bearing several racemes; recemes oblong, dense with many flowers1- 1.5 cm long; spathellae lanceolate, hyaline, 0.5 – 1.5 cm long; bracts expanded, linear or linear-lanceolate, long acuminate, membranous, silvery white, 4 – 5mm long; perianth shortly

pedicellate, narrow-oblong; segments not seen; female inflorescence spicate, oblong and narrow, containing 3 – 4 spikelets, 7 – 14 cm long; spathes like that of male inflorescence; spikelets sessile, oblong, 1- or 2- flowered, 2-3 cm long; bracts loosely convolute, about 12 in number, lanceolate to linear lanceolate, 1- nerved, chartaceous, whitish-brown in colour, 10 – 14 mm long; perianth sessile, closely appressed to the ovary, 2- 5 mm long; segments broad-based, with margins overlapping, apiculate, suborbicular, cartilagenous, inner shorter than outer; ovary sessile, globose, moderately tubercled on upper part; styles 2, free erect-spreading, semi terete, acute, supported by channel up outer side, stigmatic surface feather-like extending over whole surface, 10mm long; fruit subglobose, heavily tuberculate over entire nutlet wall, depressed at apex between styles, no differentiation between lower half and upper half, reddish-brown in colour.

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Appendix 1.

Specimens used to collect data for table1.

Species no.	Collector	Collector's no.	Herbarium	locality	Division
S1a	Esterhuysen	34943	BOL	Sir Lowry's pass (SLP)	Caledon
S1b	Esterhuysen	32764	BOL	Range between SLP and Gordon's bay	Caledon
S1c	Esterhuysen	3591	BOL	Landdrost kop	Caledon
S1d	Esterhuysen	31042	BOL	Nieweberg Forest	Caledon
S1e	Pillans	4812	BOL	Viljoen's Pass	Caledon
S2a	Esterhuysen	35303	BOL	Hermanus	Caledon
S2b	Levyns	10213	BOL	Betty's bay	Caledon
S2c	Esterhuysen	9991	BOL	Kogelberg SE slopes (stream side)	Caledon
S2d	Esterhuysen	31699	BOL	Klein river mnts (KRM)	Caledon
S2e	Esterhuysen	26957	BOL	KRM near Hermanus	Caledon
S2f	Esterhuysen	30971a	BOL	Kogelberg, SW slopes	Caledon

S2g	Esterhuysen	32321	BOL	Babylon's tower	Caledon
S3a	Linder	4197	BOL	Dekriet	Riversdale
S3b	Esterhuysen	33006	BOL	Rietfontein	Bredasdorp
S3c	Esterhuysen	272	BOL	2 miles from Albertinia	Albertinia
S3d	Esterhuysen	s.n.	BOL	Near Palmiet river	Bredasdorp
S3e	Esterhuysen	12557	BOL	2 miles from Pearly beach	Caledon
S3f	Esterhuysen	31793	BOL	?	Alberinia