A TAXONOMIC STUDY OF *SENECIO SPECIOSUS, SENECIO MACROCEPHALUS* AND POSSIBLE HYBRID POPULATIONS USING MORPHOLOGICAL DATA, TOXICTY TESTS AND CHROMATOGRAPHY.

THESIS

Submitted in fulfilment of the requirements for the Degree of MASTER OF SCIENCE of Rhodes University

by
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ABSTRACT

The variation between populations of *Senecio speciosus Willd., Senecio macrocephalus DC.* and intermediate plants was investigated in a comparative study of morphological characters, toxicity of plant extracts to brine shrimps (*Artemia salina*) and chromatography of plant extracts.

Specimens were collected at 18 localities in the Eastern Cape Province. All these specimens were examined morphologically and chemical extracts were tested for toxicity and by comparative chromatography. The collection of *Senecio speciosus* and *Senecio macrocephalus* specimens in the Selmar Schonland Herbarium (GRA) was also examined morphologically. Six geographical areas were represented in the combined collections.

Analysis of morphological data separated typical *Senecio speciosus* and *Senecio macrocephalus* at either end of hybrid index histograms and principal components analysis diagrams. The intermediate populations displayed morphological characters of both *Senecio speciosus* and *Senecio macrocephalus*. Some specimens were intermediate between these two species, falling within the range of variation of these species while others fell outside this range.

The Brine Shrimp Assay was used to test for toxicity and to investigate the possibility of using toxicity data as a genetic marker in taxonomic studies. As *Senecio speciosus* extracts were less than 1% toxic and *Senecio macrocephalus* extracts were at least 95% toxic to the brine shrimps it is suggested that in this case toxicity can be used as a genetic marker. Toxicity can even be described as a good taxonomic character as discontinuity is very sudden and complete. The intermediate plants in the Grahamstown area were at least 92% toxic to the brine shrimps linking them to *Senecio macrocephalus*.

Thin layer and paper chromatography were used as comparative techniques to study the chemical profiles of the specimens. Alkaloids, terpenoids and flavonoids were studied.

Thin layer chromatography to separate the alkaloid components of the plant extracts showed *Senecio speciosus* and *Senecio macrocephalus* to have distinct chemical profiles suggesting that they are separate species. The intermediate plants were found to contain chemical compounds matching either or both *Senecio speciosus* and *Senecio macrocephalus* suggesting that they may have arisen by hybridisation.

In a preliminary investigation *Senecio speciosus* extracts showed a complete lack of terpenoid compounds whereas extracts from *Senecio macrocephalus* and the intermediate specimens tested gave a terpenoid colour reaction in the basal spot only. This links the intermediate populations with *Senecio macrocephalus.*

Paper chromatography to separate the flavonoid constituents of the plant extracts also showed typical *Senecio speciosus* and *Senecio macrocephalus* to be distinct. The intermediate populations contained flavonoid compounds from one or both of these species.

The populations in the Grahamstown area show morphological features close to and in some cases indistinguishable from *Senecio speciosus.* Chemically these specimens show some similarities with *Senecio macrocephalus.* In the East London area specimens show a similar mixture of characters but appear morphologically to be closer to *Senecio macrocephalus.* However, in the Amatole Mountains, despite both species being present in the same locality it appears that no hybridisation has occurred.

It is therefore suggested that at some of the localities where the geographical ranges of *Senecio speciosus* and *Senecio macrocephalus* overlap in the Eastern Cape Province hybridisation between these two species occurs.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The purple flowered *Senecio speciosus* Willd. and *Senecio macrocephalus* DC. are herbaceous perennials found in the wetter areas of southern Africa.

According to Hilliard (1977), *Senecio speciosus* ranges from the Cape Peninsula eastwards through the Cape Coastal districts to the Transkei, Natal and the Eastern Transvaal, Swaziland and Mozambique. This species is typically coastal in distribution and in Natal and Transkei it is not found above c.750 metres. *Senecio macrocephalus* ranges from about Alexandria and Grahamstown in the Eastern Cape to Natal and the Eastern Transvaal. In Natal this species is found between 600 and 2500 metres above sea level. Hilliard (1977) compares these species as follows:

> "In the South West Cape leaves of *Senecio speciosus* are thinner and may be more deeply pinnately dissected than in specimens from the Eastern Cape and beyond. Here distribution overlaps with *Senecio macrocephalus* which is absent from the South West Cape. *Senecio speciosus* is characterised by its leaves, mostly with long broad flat petioles and deeply toothed or lobed margins and smaller heads (involucral bracts mostly 10mm long) corymbosely arranged. *Senecio macrocephalus* has leaves narrowed to the base but scarcely petioled, margins entire, repand or shallowly toothed, involucral bracts mostly 12mm long, the heads tending to be racemosely arranged. Involucral bracts and inflorescence branches are often more or less invested with long jointed hairs. Although some specimens are difficult to place, both names are upheld here as the taxonomy of these concolorous purple senecios is far from understood".

Nomenclatural information for these taxa from Hilliard (1977) is given below.
Senecio speciosus Willd, Sp. Pl. 3: 1991 (1804); Ker in Bot. Reg. t. 41 (1815); Loddiges, Bot. Cabinet 12, t. 1113 (1826); DC, Prodr. 6: 407 (1838); Wood, Natal Plants 6 (2) t. 550 (1910); Hilliard and Burtt in Notes Roy. Bot. Gard. Edinb. 34: 98 (1975). Type: Andrews Bot. Rep. 5, t. 291 (1803).

Senecio macrocephalus DC, Prodr. 6: 407 (1838); Harv. in Fl. Cap. 3: 362 (1865); Hilliard and Burtt in Notes Roy. Bot. Gard. Edinb. 34:92 (1975).
Lectotype: Cape, Kat River Mountains, Drège 5891 (G - DC). Noted by Hilliard and Burtt (1975).

There is long standing confusion over the correct application of the name *Senecio concolor DC.* (Hilliard 1977). This name was used by Harvey and Sonder in Flora Capensis 3 (1865) and by other authors. Specimens originally described as *Senecio concolor* and varieties of *Senecio concolor* were treated by Hilliard (1977) as either *Senecio speciosus* or *Senecio polyodon DC. Senecio concolor DC.* var. *subglaber* was regarded as a variety of *Senecio polyodon,* and *Senecio concolor, Senecio concolor* var. *hispido-scaber DC.* and *Senecio concolor* var. *hispidus* as synonyms of *Senecio speciosus.* In this way, Hilliard appears to have satisfactorily solved this particular problem.

Senecio sp. aff. *S. speciosus* is an unnamed mountain species ranging along the Natal-Cape Drakensberg from about Cathkin to Naudés Nek (between Maclear and Rhodes) between 1600 and 2800 metres above sea level. It is also found in the mountains of East Griqualand, the Transkei and the Amatole Mountains (Hilliard 1977). Since the situation in the Eastern Cape was not fully understood Hilliard (1977) declined to formally describe this species. However, as there is an area of overlap at least with *Senecio macrocephalus*, *Senecio* sp. aff. *S. speciosus* has been included in this project.

Arnold and De Wet (1993) in a checklist giving names and distributions of southern African plant species give the distribution of *Senecio speciosus* as Transvaal, Orange Free State, Natal, Cape Province, Swaziland and Lesotho. *Senecio macrocephalus* is given as Transvaal, Orange Free State, Natal, Cape Province and Lesotho. Jacot Guillarmod (1971) refers to both these species as being found in the Eastern Cape.

Pictorial studies also contain references to these species but sometimes the identification is unclear. Batten and Bokelmann (1966) depict a plant which appears like *Senecio speciosus*, call it *Senecio macrocephalus* and give its description as coastal in the Eastern Cape. Gibson (1975) gives *S. speciosus* as coastal and *S. macrocephalus* as inland in

Natal and Gledhill (1981) describes a typical plant from the Grahamstown area under the name *S. speciosus*.

Given such a wide distribution range for both *Senecio speciosus* and *Senecio macrocephalus* it is not difficult to envisage the occurrence of both ecotypic and hybrid variants particularly in the area of overlapping distribution of the two species in the Eastern Cape. Ecotypes may develop in response to their habitat and the microclimates prevailing in that habitat. Genecotypes and phenecotypes of various plant species have been recognised, genecotypes being a result of evolution within the species and phenecotypes being a result of phenotypic variation within the environment (Stebbins, 1963; Barbour *et al.* 1980; Briggs & Walters, 1986; Jones and Luchsinger, 1986).

Hybrid swarms of *Senecio* species are reported from Natal (Hilliard, 1977). In a marshy area of the Garden Castle Nature Reserve, the yellow flowered *Senecio parentalis* which is a rayed species and the discoid *Senecio submontanus* appear to form a hybrid swarm and in the Estcourt District the purple flowered discoid *Senecio polyodon* and the purple flowered discoid *Senecio cathcartensis* also appear to have formed a hybrid swarm.

When hybridisation occurs in nature it frequently gives taxonomists some difficulty (Jeffrey 1982). Hybridisation is defined by Stebbins (1959) as the crossing between individuals belonging to separate populations which have different adaptive norms and (quoting Wagner 1868) which would be separated in ordinary taxonomic practice as readily defined phenetic species. While Mayr (1940), cited by Briggs and Walters (1986), defines biological species as groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups, Solbrig (1970) states that hybridisation can occur whenever two species that possess no genetic sterility barrier are within pollination range of each other.

Hybrid plants are often sterile but may, by apomixis, produce a large population of intermediate plants (Jeffrey 1982). The term, vegetative apomixis, is used when plants grow out radially by means of rhizomes, stolons, runners, or bulbils. Agamospermy is the

term used when plants set normal seed without the occurrence of sexual fusion of gametes (Briggs & Walters 1986).

Fertile hybrids may be able to cross among themselves or backcross with the parent species giving populations of very diverse intermediates known as hybrid swarms. Repeated backcrossing is known as introgressive hybridisation and is common where both parent species and hybrids grow in close proximity. This results in only small amounts of germplasm appearing to be transferred from one species to the other (Jeffrey 1982, Briggs and Walters 1986; Jones and Luchsinger 1986).

Hybrid plants may acquire fertility through the doubling of chromosomes and may contain 2, 4, 6 or 8 times the number of chromosomes of the parent species. These polyploid individuals are usually intermediate between the parent species, but in some cases have been interpreted by taxonomists as a single widely variable species. To quote Jeffrey (1982) "Hybridisation combined with polyploidy is a common cause of taxonomic confusion".

When hybridisation is suspected a number of experimental techniques can be used to assess this possibility. According to Stace (1989) hybrids may be recognised by the following 5 criteria.

1. Phenetic intermediacy between putative parents.
   This is generally shown by analysis of morphological and anatomical characters. The resulting data is displayed by constructing hybrid index histograms, scatter diagrams, polygonal graphs or by principal components analysis (Radford *et al.* 1974; Briggs and Walters 1986; Jones and Luchsinger 1986).

2. Reduced fertility.
   Interspecific hybrids range from completely sterile to being as fertile as the parent species. Reduction in fertility or absolute sterility is usually noticeable in hybrid populations.

3. F_2 Segregation

   F_1 generations are known to be generally uniform whereas F_2 generations may be very variable. If seed from suspected F_1 hybrids is grown and a wide range of variation in characters is observed in the F_2 generation it is reasonable to suggest that the parents were F_1 hybrids.

4. Distributional evidence.

   The study of the distribution of the likely parents and possible hybrids is important. Putative parents found near to a hybrid population are more likely to be the parents of this population than other species located at a distance.

5. Artificial resynthesis.

   Breeding experiments may be undertaken between possible parent species in an attempt to resynthesise the hybrid population. Although these experiments do provide useful information much time and space is needed and plants may need to be moved from their natural habitats and subjected to unnatural environmental conditions to ensure that flowering takes place at the required time (Jones & Luchsinger 1986).

Two further established methods of testing for hybrids can be added to Stace's list, namely cytological studies and chemotaxonomic studies.

Cytological studies to test for polyploidy are a useful guide to detecting possible hybrids. Squashes of bud material are made and the chromosome numbers in the pollen mother cells and chromosome morphology are determined (Jones & Luchsinger 1986).

Chemotaxonomic studies are frequently used to correlate chemical constituents with morphological and cytological evidence. Alkaloid, flavonoid and terpenoid chemical profiles are compared as these compounds are generally found to be additive in hybrids (Smith 1976; Harborne 1984).

Payne (1976) and Payne *et al.* (1973) are cited by Kubitzki (1984) as having shown that the "non-specific hypothesis" of Sokal and Sneath (1963) does not always hold true. This hypothesis states that a classification based on one set of characters will necessarily be compatible with that based on another set of characters. Examples are quoted in which the morphological and chemical traits of populations are not correlated, showing that different sets of characters may be subjected to different selection pressures. This is important to remember when dealing with possible hybrid populations.

Generally, however, interspecific hybrids are intermediate between the evolutionary extremes achieved by their parent species (Jones & Luchsinger 1986).

The taxonomy of the genus *Senecio* is the subject of a recent study. Jeffrey *et al.* (1977, 1979) and Jeffrey (1986, 1992) have attempted to define generic and sectional limits in the genus *Senecio* as part of a wider study of the tribe *Senecioneae* particularly in Southern America, Asia and East Tropical Africa. Using a study of certain characteristics, they group the *Senecio* species into 3 series (A, B, C), 16 groups (I - XVI) and 62 clusters (1 - 62). They point out however that intermediate states are always found between various character states of the characters being considered.

A suggestion is made that each of groups I-VIII and X-XVI should become separate genera and only group IX, into which *Senecio vulgaris* (the type species of the genus) falls, should remain as the genus *Senecio*. The three series (A,B,C) may then eventually be recognised at sub-tribe level. Jeffrey (1986) states, "*Senecio* is a paraphyletic group. Attempts have been made to sub-divide (it) into what may reasonably be considered to be monophyletic sub groups but the true relationships of many species are still to be elucidated."

South African *Senecio* species are mainly found in Series C, the *Senecionoids* and in group IX, the *Eusenecionoids*. This group as stated above contains the European type species *Senecio vulgaris*. The South African species are spread through clusters 18, 19, 21, 33, 37, 38, 39, 40, 41 and 43. Exactly where *Senecio speciosus* and *Senecio macrocephalus* fit in is not stated by Jeffrey. However, the purple flowered, discoid

species, *Senecio erubescens* which is also found in the Eastern Cape, and is, according to Hilliard (1977), very like *S. speciosus*, is definitely placed in cluster 39. It seems most likely then that *S. speciosus* and *S. macrocephalus* should also be placed in cluster 39.

1.2 Morphological Characters and Taxonomic Data

Jones and Luchsinger (1986) suggest two possible approaches in the analysis of local population samples. Firstly, an intensive study can be made of one or two characters, especially those that show geographical or ecological variation, using a large number of population samples. Alternatively, following Stebbins (1963), an analysis may be performed on 8 - 20 characters on fewer population samples. Where there is little variation a sample of 25 individuals is adequate but if two species are hybridising 50 -75 individuals are necessary.

1.3 Taxonomic Studies of *Senecio* species in the Eastern Cape

McCartan (1991), in a two-part survey, studied *Senecio* sp. aff. *S. speciosus* from the Menziesberg area of the Amatole Mountains and a variety of plants from the Grahamstown, East London and East Cape Drakensberg areas using taxonomic indices such as leaf shape, leaf length : width, numbers of rays and involucral bracts and length of involucral bracts. She concluded that *Senecio* sp. aff. *S. speciosus* should be regarded as a distinct species but her results from the Grahamstown and East London areas were inconclusive. The use of polygonal graphs brought out certain taxonomic features in definitely identifiable species but only succeeded in showing the vast array of variability in the Grahamstown and East London specimens.

1.4 Chemical Studies on *Senecio* species with special reference to those from the Eastern Cape

Alkaloids, flavonoids and terpenoids are the chemical compounds most frequently studied for comparative taxonomic data (Hegenaur 1966; Smith 1976).

Hegenaur (1963) defined "alkaloidal" plants as plants which accumulate large amounts of alkaloids, up to 0.01% of the plant body. Many species of the family Asteraceae are considered alkaloidal, particularly those of the genus *Senecio.*

The quantity and proportions of alkaloids in alkaloidal plants are to some extent under genetic control but they are also greatly affected by fluctuations in the environment (Smith 1976). Quantitative and proportional differences in alkaloid content are therefore unlikely to be reliable taxonomic characters. The possibility that genetic control of alkaloid synthesis is disturbed in hybrids was also noted by Nowacki (1963). However, there are many examples of agreement between the presence or absence of alkaloids and taxonomy (Hegenaur 1963 and 1966; Manske 1944; Mears and Mabry 1971).

Grue (1991) extracted pyrrolizidine alkaloids from *Senecio speciosus* and *Senecio macrocephalus* plants collected on the Menziesberg area of the Amatole Mountains and from populations of the *"Senecio speciosus/macrocephalus* complex" around Grahamstown. Pyrrolizidine alkaloids have been used as taxonomic markers in various studies (Borstel *et al.* 1989; Van Wyk *et al. 1989).* Apparent alkaloid fractions obtained by Grue were first subjected to Thin Layer Chromatography and visualised using ultra violet light and Dragendorff reagent prepared according to the variation by Munier and Machenboef (1951). Spots visible under ultra violet light did not always respond to Dragendorff reagent or only showed a faint response. However, these fractions were subjected to GC-MS and tentatively identified as pyrrolizidine alkaloids.

Several pyrrolizidine alkaloids were definitely identified in Grue's study using high field NMR techniques. *Senecio speciosus* was found to contain two new alkaloids, 7-senecioyl-9-sarracinylheliotridine and 7-isosarracinyl-9-sarracinyl-heliotrinidine. The Grahamstown populations were found to be variable. Three of the four populations studied contained 7-

senecioyl-9-sarracinylheliotridine and 7-angelyl-9-sarracinylheliotridine indicating a close relationship with *S. speciosus*, the fourth population was found to contain the known alkaloid retrorsine and a new alkaloid 2-hydroxyl-1, 2-dihydrosenkirkine. *Senecio macrocephalus* was found to contain a very small amount of total alkaloid. A number of other pyrrolizidine alkaloids were tentatively identified using GC-MS in all three taxa. Those found in the Grahamstown populations could have been genetically-derived from either *S. speciosus* or *S. macrocephalus.*

The pyrrolizidine alkaloids identified by Grue in *Senecio speciosus* are mainly acyclic diesters which are less toxic to stock than the pyrrolizidine alkaloids tentatively identified in *Senecio macrocephalus* which are macrocyclic diesters (Mattocks, 1989). Retrorsine, the known pyrrolizidine alkaloid isolated from Grue's fourth Grahamstown population is also a macrocylic diester.

The production of retrorsine in one of the populations is thought to be due to grazing pressure as these plants were collected from a grassy slope near Jameson Dam, Grahamstown, know to be subject to grazing by both sheep and cattle. The production of toxic alkaloids by plants as a defense mechanism against grazing has been noted by Stebbins (1963), Levin (1976), Jeffrey (1979) and Meinwald (1990). The other specimens studied by Grue came from populations not subjected to grazing.

Flavonoids or other phenolic markers have been used in 2 dimensional paper chromatography to establish that the flavonoid pattern of hybrid plants is intermediate between the parent patterns (Smith 1976). This technique can also be used to demonstrate the inheritance of phenolic characters (Alston and Turner 1959).

McCartan (1991) investigated the use of flavonoids as taxonomic markers in a study of *Senecio* species from the Hogsback, East London and Grahamstown areas. Flavonoids can be used as taxonomic markers as they are chemically stable and are under genetic control (Crawford, 1978). Results from this survey showed variability both between and within the population studied with 0-4 flavonoid spots showing up on paper chromatograms.

The third group of chemical compounds which can be used as taxonomic markers are terpenoids such as sesquiterpene lactones and furanosesquiterpenes. Jeffrey (1979), using phytochemical evidence from Bohlmann *et al.* (1979) and Robins (1977, 1978) states that although the occurence of low molecular weight secondary metabolites may appear to be independent of morphological characters there does in some cases appear to be an association between chemical constituents and life form. The presence of the sesquiterpene lactones, furanoeremophilanes, together with pyrrolizidine alkaloids is basic in the tribe *Senecioneae*. The coarse herbaceous perennial *Senecio* species thought to be the least specialised members and possibly the ancestral life form of the tribe are most highly vulnerable to grazing and browsing by insects and mammals. So early development of furanoeremophilanes and pyrrolizidine alkaloids as chemical defense mechanisms is not surprising.

However, chemical evolution in the tribe *Senecioneae* has proceeded in two directions, firstly elaboration by means of extension of synthetic ability and secondly, simplification by loss of synthetic ability. Loss of furanoeremophilanes may be accompanied by high development of alkaloids and tough leaves by perennial species, or by the development of life forms such as annuals, climbers and succulents and plants occupying ecological niches where grazing and browsing do not occur. In the latter case loss of pyrrolizidine alkaloids may also occur (Jeffrey, 1979).

Regarding South African and particularly East Cape species, Seaman (1982) cites Bohlmann *et al.* (1979) who found a total lack of furanoeremophilanes in *Senecio speciosus, Senecio polyodon* and *Senecio purpureus*. These three species are perennial herbs, the leaves of which are not particularly coarse and which tend to occupy ecological niches not necessarily subjected to grazing pressure. It can be postulated that these South African species have become simplified chemically by loss of furanoeremophilanes and a reduced complement of macrocyclic diester pyrrolizidine alkaloids but when grazing pressure is applied the plants have the ability to produce at least the macrocyclic diester retrorsine.

1.5 The Brine Shrimp Assay

Brine Shrimp (*Artemia salina*) have been used over the past 25 years in a bioassay to test for toxicity in many diverse systems, (See Appendix 3). Until the present study, this assay had not been used as a tool in the chemotaxonomy of plants. This assay is simple, fast and inexpensive, the apparatus needed should be found in any biological laboratory.

In a study to show the toxicity to brine shrimps of plant extracts from different families known to contain different chemical constituents three species of *Senecio* were tested (Lewis in press). The yellow flowered *Senecio pterophorus*, known to contain toxic pyrrolizidine alkaloids (De Waal 1941; Watt and Breyer Brandwijk 1962; Rose 1972 and Smith and Culvenor 1981), was tested together with *Senecio speciosus* from Kasouga and plants from the intermediate *S. speciosus/macrocephalus* complex from the Grahamstown area.

This study showed definite differences in toxicity to brine shrimps in the three *Senecio* extracts tested. It was decided therefore to use toxicity tests in this project to assess whether toxicity data could be used as an extra character in taxonomic studies.

1.6 Aims and Objectives of this Study

The aim of this study is to be able to answer the following questions:

1. Are *Senecio speciosus* and *Senecio macrocephalus* taxonomically distinct?
2. Is there hybridisation between these two species where their ranges overlap in the Eastern Cape Province?
3. If this is so, how can such hybrids be recognised?
4. Do environmental factors have an effect on the morphology and distribution of *S. speciosus, S. macrocephalus* and possible hybrids between the two?
5. Can toxicity data, for example from the Brine Shrimp Assay be used as a character in taxonomic studies?
6. How useful is chromatography in such taxonomic studies?

In the course of this project the variation between the populations of *Senecio speciosus* and *Senecio macrocephalus* in the Eastern Cape Province will be studied.

Three of the criteria discussed by Stace (1989) will be used to investigate the possibility of populations in the area of overlap of the two species being hybrid populations. Phenetic intermediacy between putative parents will be studied using morphological characters given by Hilliard (1977) as being distinguishing characters of the two species. Techniques used to display these results will be hybrid index histograms, scatter diagrams and principal components analysis.

Distributional evidence of the two species over the area studied will be mapped to show the distribution pattern of putative parent species and intermediate populations. Data from the hybrid index histograms will be mapped to show parent species, intermediate populations and possible migration routes.

Instead of using the methods of F_2 segregation and artificial resynthesis to complete the project two newer experimental techniques will be used to complement the morphological and geographical analysis.

Brine shrimp toxicity tests will be carried out using extracts from all the specimens collected. These results will be used in hybrid index histograms as an extra character over and above the morphological data.

Methanol extracts of all the specimens collected will be chromatographed to examine comparatively the presence or absence of alkaloids, flavonoids and terpenoids in the populations. This comparative data will be used to attempt to recognise both the intermediate populations and the parent species.

Therefore, using morphological and geographical data, toxicity and chromatography, the hypothesis to be tested is that where the coastal *Senecio speciosus* overlaps with the more inland *Senecio macrocephalus*, there is hybridisation between the two species.

CHAPTER 2: MATERIALS AND METHODS

2.1 Plant Materials and Geographical Distribution

Specimens of *Senecio speciosus* and *Senecio macrocephalus* were collected at fifteen different localities in the Eastern Cape Province. Five closely related species, *Senecio erubescens*, *Senecio barbatus*, *Senecio polyodon*, the undescribed speeies *Senecio* sp. aff. *S. speciosus* and a specimen unidentified using Hilliard (1977) were also collected from these and three additional localities within the Eastern Cape. Three more distantly related species, *Senecio elegans* and *Senecio radicans* and the yellow-flowered *Senecio inaequidens* were collected to be used for comparative purposes in the study. It was expected that their chemical constituents would differ more markedly from those of *S. speciosus* and *S. macrocephalus* than would the chemistry of the possible hybrid variants.

The eighteen localities collected are listed below.

1. Kasouga, between Kenton-on-Sea and Port Alfred, open grassland above the river.
2. Port Alfred beach, *Senecio elegans* growing in sand, apparently planted as a stabiliser.
3. 10km from Port Alfred on the East London road, at the roadside.
4. Fish River Mouth, grassland.
5. Potter's Pass Nature Reserve, East London, grassland beside the beach.
6. Gonubie Nature Reserve, East London.
7. Amatole Mountains, Menziesberg area, open grassland.
8. Amatole Mountains, above Kettlespout Waterfall, beside the footpath.
9. Amatole Mountains, near Plaatjies Kraal, sheltered grassland between trees.
10. Bokspruit, North Eastern Cape Province, on the farm Birnam, wet patches beside a mountain stream.
11. Naudés Nek, North Eastern Cape Province, wet area beside a stream just below the summit of the pass.
12. Howison's Poort, Brackenhill Farm, grassland among *Protea* bushes.

13. Stoneshill, Firdene, bank beside the farm drive.
14. 1820 Settler's Monument, rough ground surrounding the Monument and the frequently mown lawn in front of the Monument.
15. Beggarsbush Outspan, bushy area beside the gate, open grassland beside the path and the side of a kranz.
16. Mountain Drive, grassland in an exposed situation.
17. Coldsprings, Faraway, grassland among *Protea* bushes, more exposed than at Brackenhill.
18. Alicedale, on top of a kranz near the village.

All specimens collected at each locality, with Grid References cited according to Raper (1989) Dictionary of South African Place Names, are given in Appendix 1.

2.1.1 Mapping the Collections

Both the collections of fresh material and the collections of *Senecio speciosus, Senecio macrocephalus*, intermediate specimens and *Senecio* sp. aff. *S. speciosus* in the Selmar Schonland Herbarium were mapped to show the overall distribution of the species in the Eastern Cape Province and the areas of overlap and possible hybridisation between *Senecio speciosus* and *Senecio macrocephalus* in this area. The localities of collection of fresh material for this study are shown on Figure 1, the Grahamstown localities are shown on Figure 2 and the localities of collection of the specimens in the Selmar Schonland Herbarium are shown on Figure 3.

Figure 1.
Localities of collection of fresh material for the present project. (Lewis Collection) Other *Senecio* species are: *Senecio* sp. aff. *S. speciosus*, *Senecio polyodon*, *Senecio erubescens*, *Senecio elegans*, *Senecio radicans* and *Senecio inaequidens*.

Figure 2.
Localities of collection for the present project in the Grahamstown area.

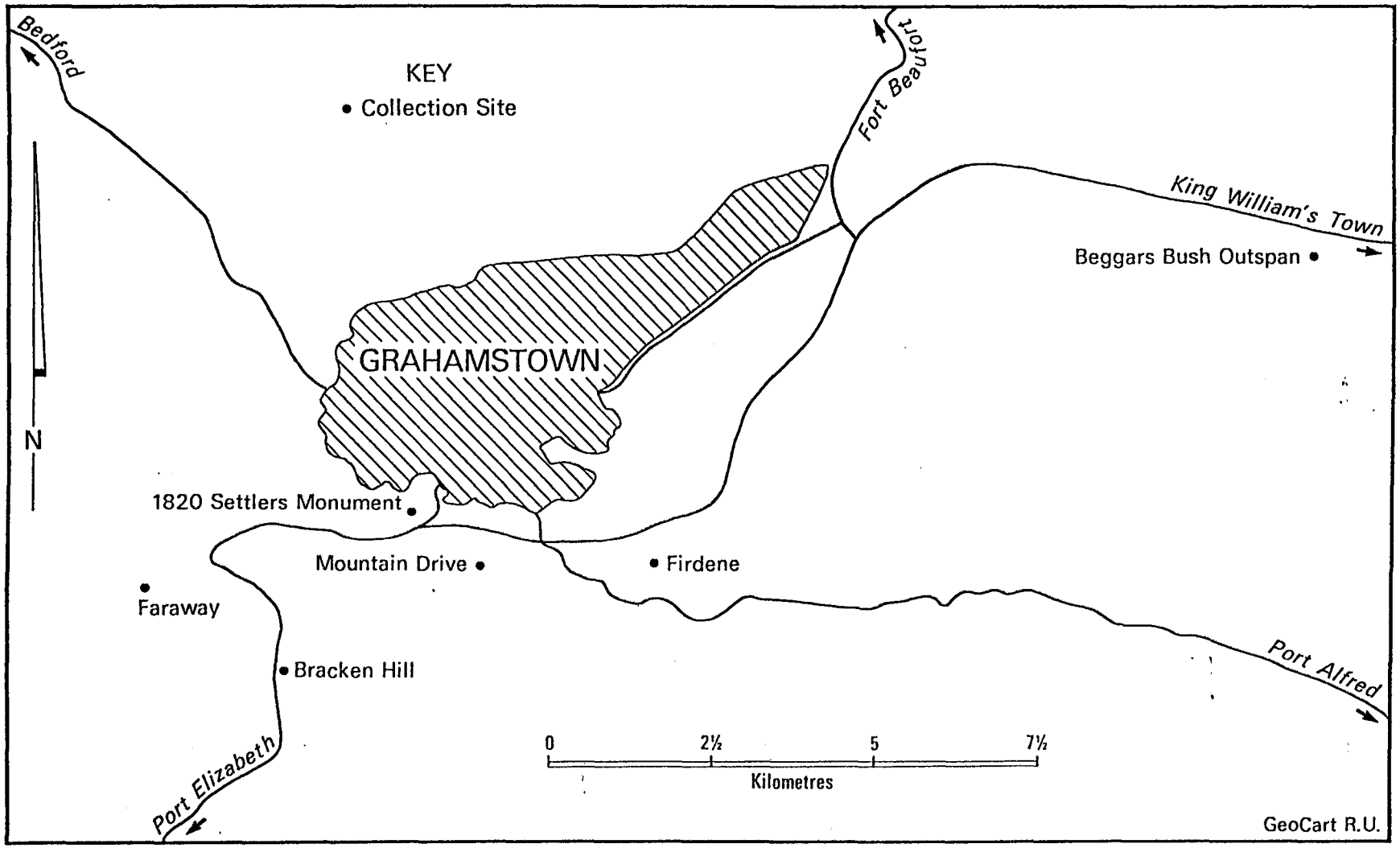
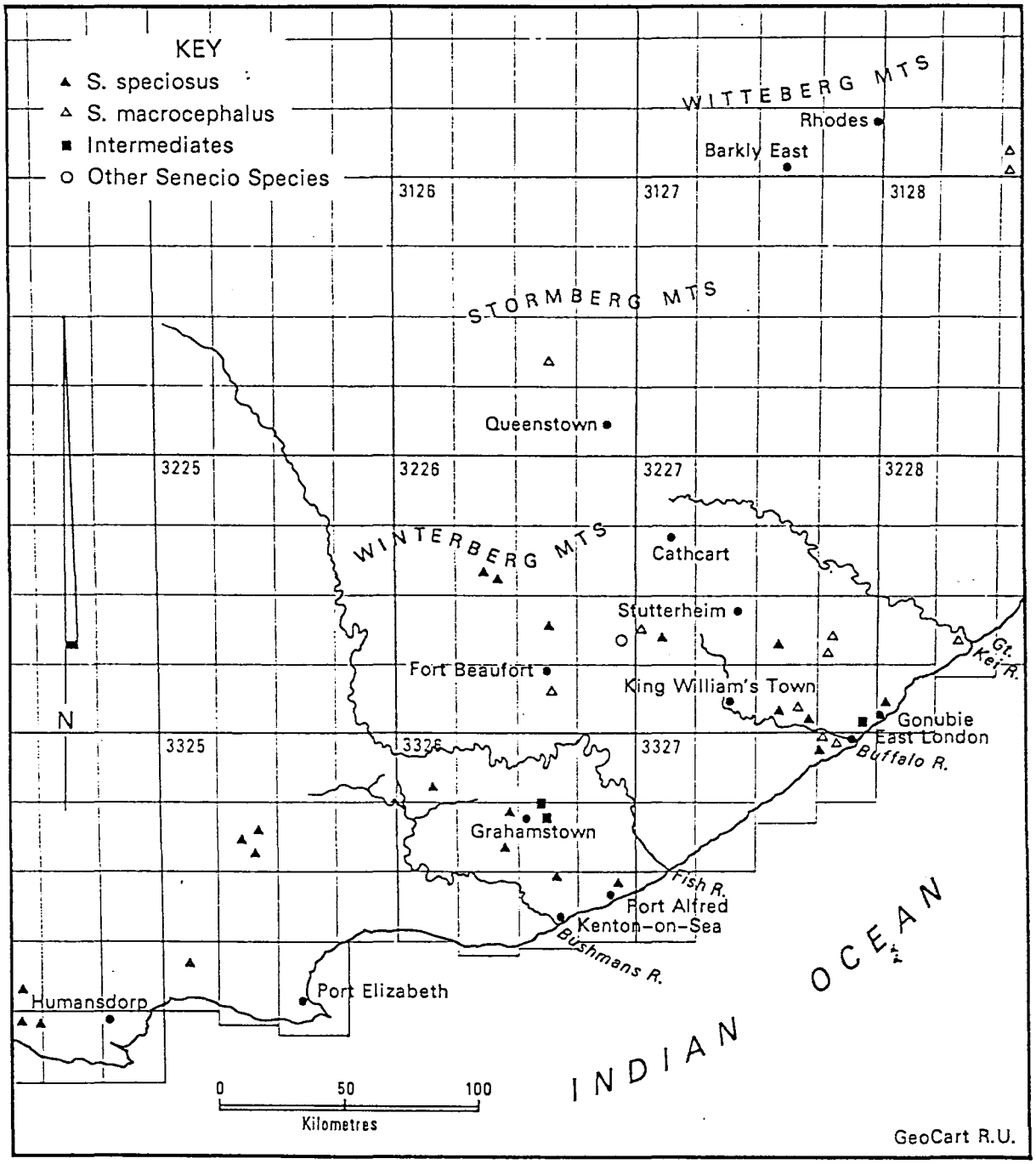


Figure 3.
Localities of collection of the specimens in the Selmar Schonland Herbarium. The other *Senecio* species is *Senecio* sp. aff. *S. speciosus*.



2.1.2 Collection of Voucher Specimens

Voucher specimens were made of each population studied and these are lodged in the Selmar Schonland Herbarium, Grahamstown (GRA). Voucher numbers are recorded in Appendix 1. All references to specimens in the text are according to voucher numbers. The specimens referred to as the Lewis collection consist of fresh material collected for this project and given Lewis collection numbers, fresh material collected on a joint expedition to the Amatole Mountains and given Phillipson collection numbers and two unwanted herbarium specimens collected by McCartan and kindly donated by the Selmar Schonland Herbarium (GRA).

2.2 Morphology

2.2.1 Description of Fresh Material

In her descriptions of *Senecio speciosus* and *Senecio macrocephalus*, Hilliard (1977) gives fifteen morphological characters which can be used to distinguish between the two species. Of these fifteen morphological characters, five were either discarded as being too variable or not always available or were incorporated together with other characters, leaving ten characters to be examined and analysed.

These ten characters are:

1. Arrangement of leaves at flowering.
2. Leaf shape (basal and cauline leaves).
3. Leaf margin.
4. Inflorescence type.
5. Diameter of heads.
6. Number of rays (if present).
7. Number of involucral bracts.
8. Length of involucral bracts.
9. Indumentum on - involucral bracts
   flowering stems
   leaves.
10. Achenes (Cypselae).

These ten characters give information on both conservative and plastic features and sufficient data was obtained for the construction of hybrid index histograms, distribution maps and for principal components analysis. The amount and variety of this information is within the parameters suggested by Jones & Luchsinger (1989).

This information is recorded in Appendix 1.

2.2.2 Description of Herbarium Material

The specimens of *Senecio speciosus* and *Senecio macrocephalus* in the Selmar Schonland Herbarium (GRA) were also examined and the ten morphological characters recorded in the same way as for the fresh material.

This information is recorded in Appendix 2.

2.2.3 Illustration of Plant Material

Plates 1-4 show potted plants several weeks after collection, demonstrating variations in leaf shape. Plates 5-8 show plants *in situ* and localities of collection. Figure 4 shows comparative leaf shapes and Figure 5 shows comparative inflorescence types demonstrated by *Senecio macrocephalus, Senecio speciosus* and some of the intermediate specimens collected.

2.2.4 Hybrid Index Histograms

Hybrid Index Histograms were constructed from the plant descriptions given in Appendix 1 and 2 after the method given by Radford *et al.* (1974). Characters of *Senecio speciosus* according to the descriptions given by Hilliard (1977) were given the score of 1. Characters of *Senecio macrocephalus* were given the score of 5. Intermediate characters were scored 2, 3 or 4. Of the ten characters included, seven characters were recognised as being diagnostic between these two species only. For the fresh material, toxicity data from the brine shrimp assay was scored as character 8. Other characters given earlier either refer to *Senecio* sp. aff. *S. speciosus* or are too variable to use.

Characters, character states and points scored are given in Table 1.

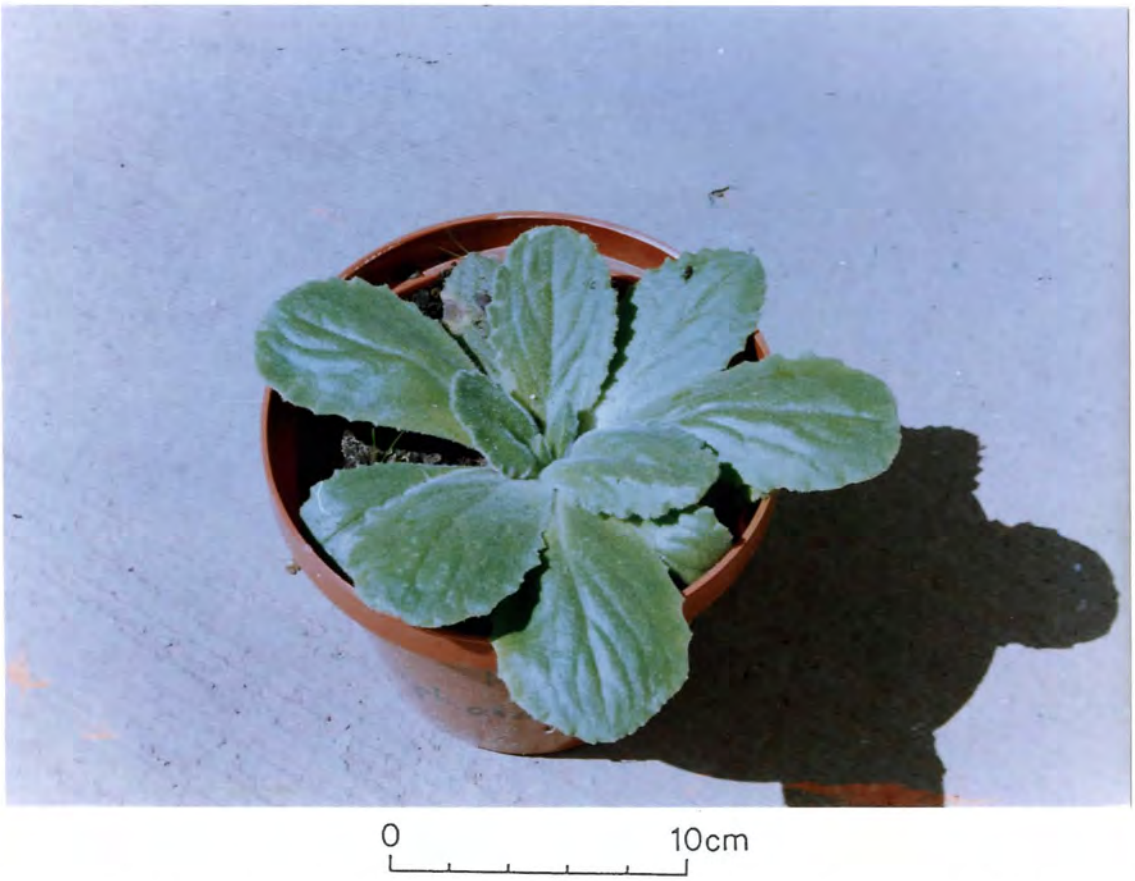


Plate 1 - *Senecio macrocephalus* specimen from the Menziesberg area of the Amatole Mountains.

Plate 2 - *Senecio speciosus* specimen from the Menziesberg area of the Amatole Mountains.

Plate 3 - *Senecio erubescens* specimen from the Menziesberg area of the Amatole Mountains. This specimen has purple discoid heads.

Plate 4 - Unidentified *Senecio* specimen, Voucher number Phillipson 3822, from the Menziesberg area of the Amatole Mountains. This plant has leaves which are deeply lobed towards the base and has deep red discoid heads. The plant is sticky to the touch.

Plate 5 - The collection locality at Brackenhill, Howison's Poort, near Grahamstown. This is a sheltered area between *Protea* bushes. Scattered basal rosettes of *Senecio speciosus/macrocephalus* intermediate specimens are seen.

Plate 6 - A *Senecio speciosus/macrocephalus* intermediate specimen *in situ* at Brackenhill, Howison's Poort, near Grahamstown. The leaves are elongated and spathulate and the inflorescence is corymbose.

Plate 7 - The collection locality at Naudés Nek, North Eastern Cape which is very exposed.

Plate 8 - The unidentified *Senecio* specimen, Voucher number Lewis 112, collected from Naudés Nek *in situ*, showing the large head and small leaved basal rosette.

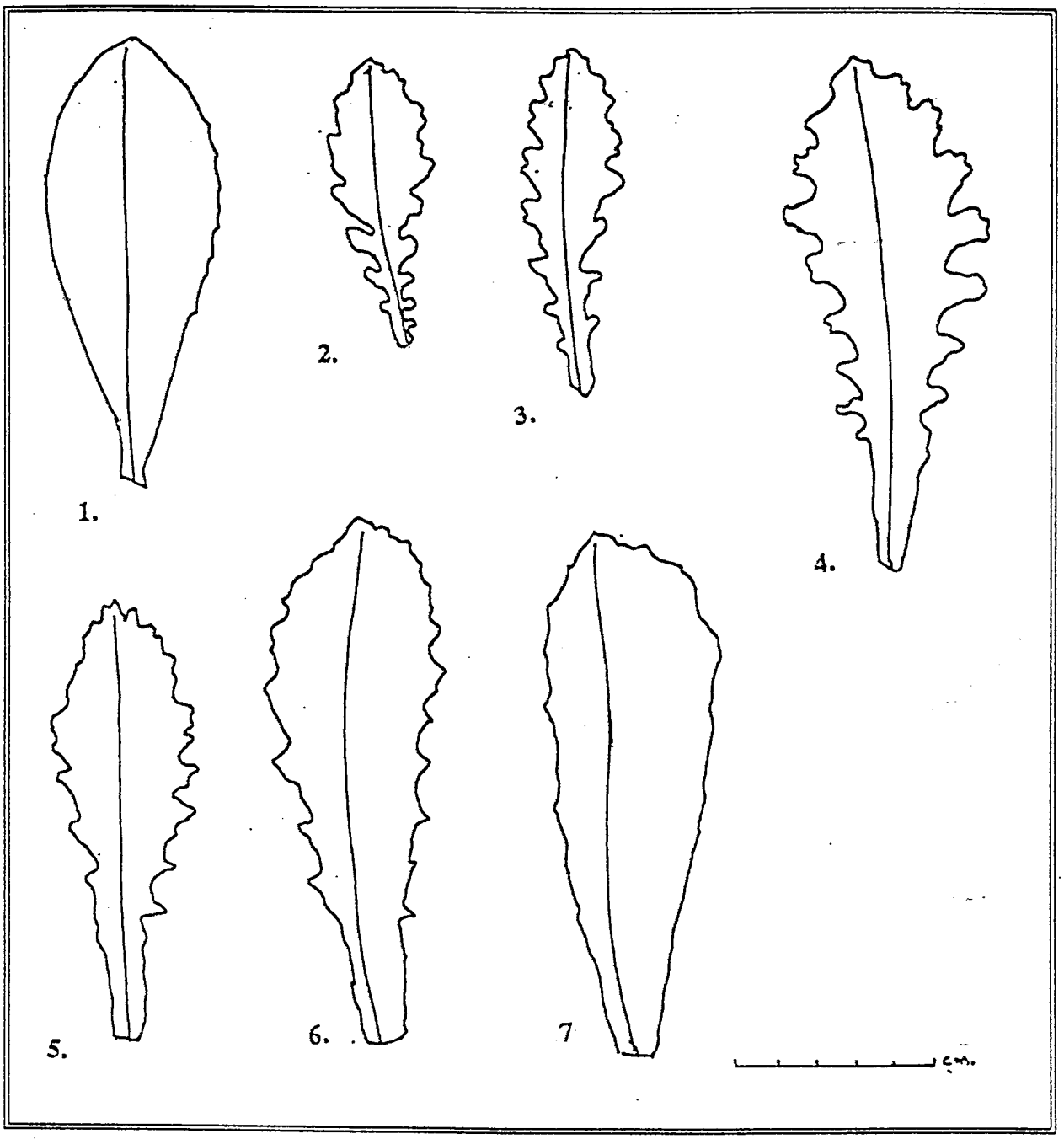


Figure 4 - Comparative leaf shapes.

1. Lewis 82. *Senecio macrocephalus* specimen collected on the Menziesberg area of the Amatole Mountains.

2-3. Phillipson 3823. *Senecio speciosus* specimen collected on the Menziesberg area of the Amatole Mountains.

4, 5, 6, 7. Lewis s.n. a variety of leaf shapes collected from the intermediate populations in the Grahamstown area.

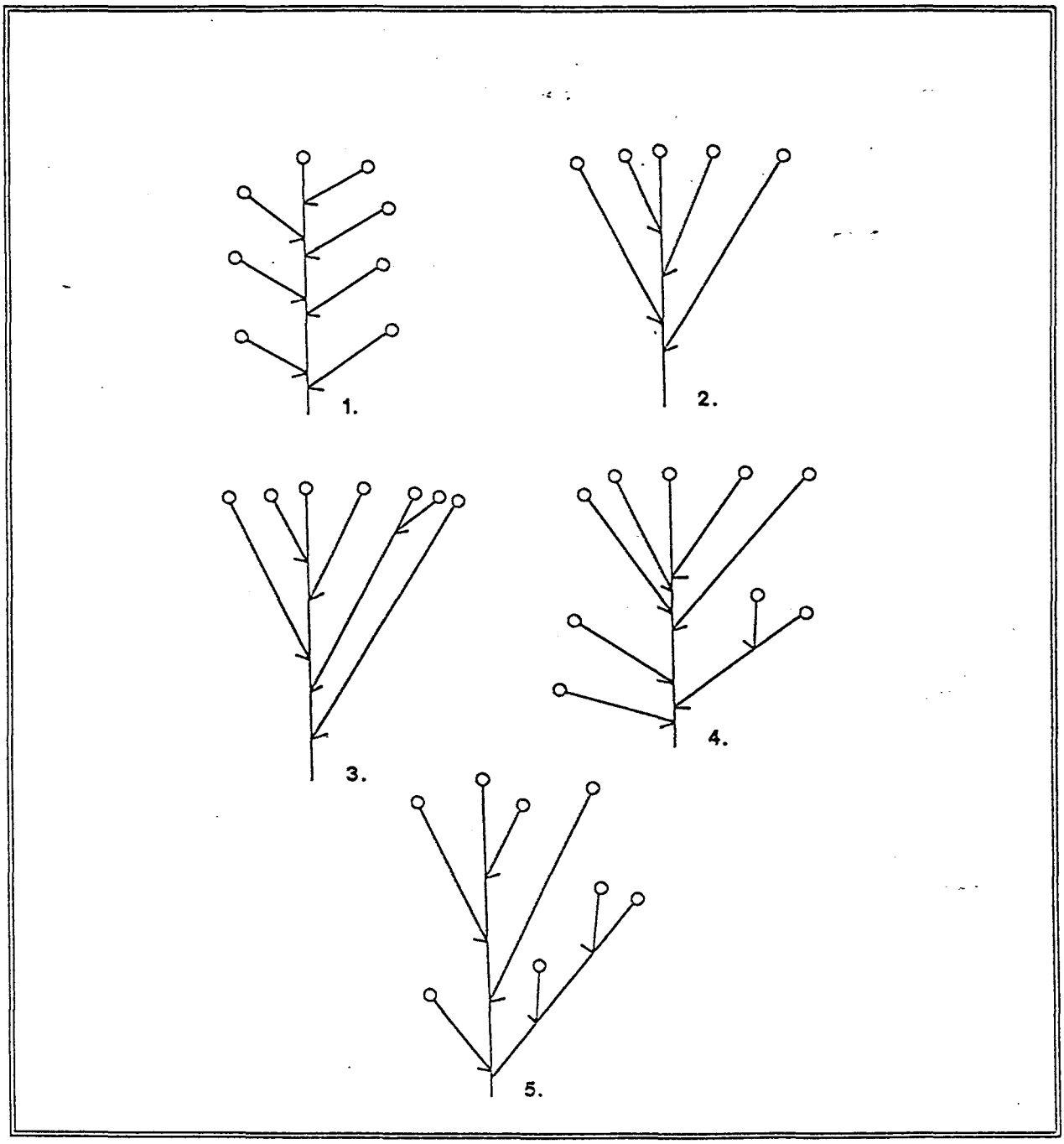


Figure 5.

Comparative inflorescence types. Diagrams showing branching patterns and relative peduncle lengths of the three classes of inflorescence, corymbose (simple or compound), Intermediate and racemose.

1. Racemose inflorescence, Rattray 98. *Senecio macrocephalus.*
2. Corymbose inflorescence, Lewis 65A, *Senecio speciosus.*
3. Corymbose inflorescence, Lewis 65, *Senecio speciosus.*
4. An intermediate inflorescence, Lewis 57 which is racemose below tending to corymbose above.
5. An intermediate inflorescence, Lewis 71, where mixed racemose and corymbose features are shown.

Table 1 **Hybrid Index Histogram Analysis - Characters, Character States and Points Scored**

Character	Character State	Points Scored
Leaf shape	Spathulate, petiole-like base	1
	Spathulate to elliptical	2
	Elliptical, broader base	3
	Elliptical to oval	4
	Oval or oblong scarcely petioled base	5
Leaf margin	Lobed and deeply cut	1
	Lobed, not deeply cut	2
	Lobed and toothed	3
	Nearly entire, toothed	4
	Entire, toothed	5
Inflorescence type	Corymbose	1
	Intermediate	3
	Racemose	5

Note: Inflorescence types are corymbose, intermediate or racemose (See Figure 5). No score of 2 or 4 is possible for this character.

Diameter of heads	10 - 15mm	1
	15 - 20mm	2
	20 -25mm	3
	25 - 30mm	4
	> 30mm	5

Length of involucral bracts	10mm	1
	10.5mm	2
	11mm	3
	11.5mm	4
	12mm+	5
Indumentum (of leaves, flowering stems and achenes)	Sparsely hairy, very short hairs < 0.5mm	1
	Densely hairy, short hairs < 0.5mm	2
	Medium length 0.5 - 1mm or mixed (long, medium, short) hairs	3
	Sparsely hairy, long hairs > 1mm	4
	Many long hairs > 1mm	5
Achenes (Cypselae)	Cylindrical, ribbed, short hairs between ribs	1
	Cylindrical, ribbed, medium hairs between ribs	2
	Cylindrical, ribbed, long hairs between ribs	3
	Densely hairy (short hairs)	4
	Densely hairy (long hairs)	5
Toxicity	Below 20%	1
	20 - 40%	2
	40 - 60%	3
	60 - 80%	4
	80 - 100%	5

Specimens giving a complete score were used to construct the hybrid index histograms. Where achenes were not available from the herbarium specimens, a score commensurate with that expected in relation to the rest of the characters of the specimens lacking achenes was given.

2.2.5 Principal Components Analysis

The hybrid index histogram data was used as a matrix in principal components analysis. The programme used was CANOCO (C.J.F. Ter Braak, Agricultural Mathematics Group, Wageningen, The Netherlands). CANOCO is an acronym for Canonical Community Ordination and was originally designed for data analysis in community ecology. The programme was run using the Cornell condensed format and default settings throughout.

2.2.6 Scatter Diagram

A scatter diagram was constructed using information given in Appendix 1 and Appendix 2. The only two characters which could be used as axes on this type of diagram were the diameter of heads and the number of ray florets. All the other characters did not give enough numerical range. It was decided to plot these species as they are identified in Appendix 1 and Appendix 2 although some of the original identifications are doubtful in the light of the work in this project.

2.3 Preparation of Plant Material

2.3.1 Plant Material for Cultivation

At least one plant from each population collected was cultivated in potting soil in the Department of Botany, Rhodes University. These plants were harvested six months later and analysed in an attempt to show whether soil conditions affect toxicity or chemical composition.

2.3.2 Plant Materials for Analysis

Whole plants, that is inflorescences, leaf material and root material were collected wherever possible. For all samples, leaf material and root material were tested separately for toxicity and chemical constituents, however root material was not always available. Inflorescences were not used. The bulk of the plant material collected was dried immediately at 30-40 °C in drying ovens. It was found that these plants would not air dry successfully and at temperatures below 25 °C they became black and slimy. When completely dry the plant material was finely ground and stored in airtight jars.

2.3.3 Chemical Extraction

2.5 g of dried, ground plant material were shaken with 100 ml de-ionised water for 24 hours. The extracts were filtered and used immediately for the Brine Shrimp Assay. If not used immediately a precipitate or growth tended to appear, the shrimps became entangled and the results were therefore suspect. Professor P. Rose of the Microbiology Department at Rhodes University could not identify the growth as being specifically bacterial or fungal. This only occurred in *Senecio* extracts and not in any other types of plant material used when checking the Brine Shrimp Assay (see Appendix 3).

2.5 g of dried, ground plant material were shaken with 100 ml of Methanol for 24 hours. These extracts were filtered and could be stored for several weeks before being used for the Brine Shrimp Assay and Thin Layer Chromatography.

2.4 The Brine Shrimp Assay

The assay using brine shrimp (*Artemia salina*) was carried out as described by Meyer *et al.* (1962) and modified by Lewis (in press, see Appendix 3).

2.4.1 Hatching the Shrimps

The brine shrimps were hatched in a shallow polythene box consisting of 2 compartments with holes bored through the dividing walls. The box was filled with fresh or filtered and autoclaved seawater. Freshly collected seawater can be used within two or three days, thereafter it should be filtered and autoclaved to avoid bacterial growth. Shrimp eggs, obtained from a pet food store, were sprinkled on one side only. This side was covered and a lamp was trained onto the open side. After 12-24 hours the eggs hatched and the larvae swam through the holes in the dividing wall towards the light. This ensured firstly that the shrimp culture was free of eggs and other debris and secondly that the culture was concentrated enough to be picked up easily, in a minimum of water, by a simple pipette.

2.4.2 Preparation of Water Extracts

Testing was carried out in Kimble vials of 25-30 ml capacity. 0.05 ml, 0.5 ml and 1 ml of each water extract were pipetted into the Kimble vials and these were made up immediately

to 5 ml with fresh or filtered, autoclaved seawater. Controls were set up using 5 ml seawater only.

2.4.3 Preparation of Methanol Extracts

0.05 ml, 0.5 ml and 1 ml of extract were pipetted into the Kimble vials. These extracts were then left for the methanol to evaporate either overnight or in the drying oven at 30-40 °C. This is necessary as a concentration of more than 10% methanol is toxic to the shrimps. Controls were set up using 1 ml pure methanol and also left to evaporate to dryness. When only dry plant extract remained in the vials these were made up to 5 ml with fresh or filtered, autoclaved seawater and shaken well.

2.4.4 Testing the Toxicity of Plant Extracts

10-20 brine shrimp larvae, at least 24 hours after hatching, that is second or third stage nauplii, were pipetted into each prepared vial. These were kept at 25 °C for 24 hours. After 24 hours the numbers of dead and living shrimps were counted and the percentage deaths calculated. Five replicates were used per dilution and five replicates as controls for each set of experiments.

It was found that feeding the larvae was not necessary for such a short experimental time as all the controls stayed alive for the duration of the experiment. Shrimp deaths could then be directly attributed to the toxic effects of the plant material. Mean percentage deaths were calculated over the five replicates for each dilution of plant extracts.

2.5 Thin Layer Chromatography

2.5.1 Preparation of Methanol Extracts for Chromatography

The crude methanol extracts were chromatographed directly. Further purification of the extracts was attempted in order to separate the flavonoid, terpenoid and alkaloid fractions with a view to identifying the toxic constituents. Purified extracts and controls, which consisted of pure methanol subjected to the same purification process, were tested in a trial against the brine shrimps. In both the extracts and controls a 100% death rate of the brine shrimps was recorded. It was therefore concluded that, however carefully the extraction was carried out, some of the chemicals used in the purification procedure, for example hydrochloric acid (HCl)

and ammonia (NH_4OH), remain in the samples and are toxic to the shrimps. In this case the toxic fractions could not be identified.

2.5.2 Chromatography Plates and Reagents

The methanol extracts were chromatographed on Merck ready prepared silica gel plastic sheets reference number 6OF 254. The solvent used was 85 chloroform: 14 methanol:1 ammonia. Other solvents were investigated but this gave the best spread of spots. Pure retrorsine donated by Dr J.R. Liddell of the Chemistry Department, Rhodes University was used as a marker. All extracts were chromatographed several times.

Spots were visualised using ultra violet light and Dragendorff reagent modified according to Munier and Machenboef (1951) for alkaloids and other nitrogen containing compounds. Many of the spots visible under ultra violet light did not react with the Dragendorff reagent or reacted only slightly. This was also reported by Grue (1991).

Other spray reagents were used to check for the presence of flavonoids or terpenoids. A 5% solution of ferric chloride ($FeCl_3$) in 0.5N hydrochloric acid and a 3% solution of aluminium chloride in methanol were used to test for flavonoids (Rhodes University, Botany Department, Practical Schedule). Both these reagents showed that flavonoids had not separated out and were still located in the basal spot.

Bohlmann *et al.* (1979) reported that the terpenoid compounds, sesquiterpene lactones and furanosesquiterpenes are absent in *Senecio speciosus* and *Senecio polyodon* but he did not examine *Senecio macrocephalus*. Plates were therefore sprayed with a solution of 1 part anisaldehyde to 100 parts glacial acetic acid to 2 parts concentrated sulphuric acid and heated at 100 °C to test for terpenoid compounds (pers. comm. Dr J.R. Liddell, Department of Chemistry, Rhodes University). Brown colouration only occurred in extracts of certain specimens in the basal spot, showing that terpenoids had not separated out in this method.

It was therefore assumed at this stage that the spots visible under ultra violet light were all alkaloids. This was also assumed by Grue (1991) in her work on *Senecio* alkaloids.

2.6 Paper Chromatography

Paper chromatography is the more usual technique for separating flavonoids. The crude methanol extracts were chromatographed in 1 dimension on Whatman No.1 paper using the pure flavonol glycoside Rutin as a marker. The solvent used was TBA (Tertiary Butyl Alcohol) and the system was run for at least 16 hours.

Some spots were visible to the naked eye, others were visualised under ultra violet light after spraying with a 3% solution of aluminium chloride in methanol. The papers were then fumed with ammonia and checked again for any colour change. Colour changes indicate different flavonoids (Harborne, 1984).

CHAPTER 3: RESULTS

3.1 Morphological and Geographical Data

3.1.1 Hybrid Index Histograms

The hybrid index scores for all the specimens in the Lewis Collection and the herbarium specimens are given in Table 2. Total A is the score for characters 1-7 in the Lewis collection. Total B includes character 8, the toxicity of plant extracts to brine shrimps. Separate histograms were constructed with and without the toxicity scores. Histograms for the herbarium specimens were constructed using the morphological characters (1 - 7) alone as sufficient material to perform toxicity tests was not available.

Table 2. HYBRID INDEX SCORES

Table 2A Hybrid Index Scores: Lewis Collection

Total A is the score for characters 1-7 (excluding toxicity data)

Total B is the score for characters 1-8 inclusive.

Voucher Specimens	Character								Totals	
	1	2	3	4	5	6	7	8	A	B
Lewis 51	2	4	3	3	5	4	5	5	26	31
Lewis 53	3	3	3	5	5	5	3	5	27	32
Lewis 55	3	1	3	5	5	5	1	5	23	28
Lewis 57	3	3	3	5	5	3	1	5	23	28
Lewis 65	1	1	1	1	1	5	1	1	11	12
Lewis 65A	1	1	1	1	1	1	1	1	7	8
Lewis 66	2	2	3	3	3	2	5	3	20	23
Lewis 71	3	2	3	3	3	3	3	5	20	25
Lewis 79	3	3	3	5	5	3	2	5	24	29
Lewis 88	2	3	3	1	3	2	4	5	18	23
Lewis 90	2	3	3	5	5	5	1	5	24	29
Lewis 91	3	3	3	5	1	3	1	5	19	24
Lewis 96	2	1	5	1	1	1	1	1	12	13

Lewis 98	3	3	1	5	3	4	4	5	23	28
Lewis 100	3	3	1	1	5	3	3	5	19	24
Lewis 101	3	3	1	5	5	1	1	5	19	24
Lewis 102	2	1	1	1	1	1	1	1	8	9
Lewis 103	5	5	3	5	5	5	1	5	29	34
Lewis 104	3	3	3	5	5	5	1	5	25	30
Lewis 105	3	3	3	5	5	5	1	5	25	30
Lewis 106	2	1	3	5	5	5	1	5	22	27
Lewis 112	2	3	1	5	5	1	1	2	18	20
Lewis 115	3	3	3	1	1	3	1	5	15	20
Lewis 116	3	3	1	1	1	1	1	5	11	16
Lewis 117	1	5	1	3	5	5	1	5	21	26
Lewis 118	3	3	1	1	1	4	1	5	14	19
Lewis 119	5	5	5	5	5	5	5	5	35	40
Phillipson 3820	1	3	5	1	1	2	1	1	14	15
Phillipson 3822	1	1	1	1	1	2	1	1	8	9
Phillipson 3823	1	1	1	1	1	1	1	1	7	8
McCartan 3	3	1	3	5	5	3	1	5	21	26
McCartan 13	3	1	1	5	3	1	1	5	15	20

Table 2B Hybrid Index Scores: Selmar Schonland Herbarium Collection

Voucher Specimens	**Character**							**Total**
	1	2	3	4	5	6	7	
Archibald 4934	1	1	1	1	1	1	1	7
Bandert 31	5	5	5	3	5	3	5	31
Bandert 141	5	5	5	5	5	4	5	34
Bradley 9	2	1	1	3	3	4	1	15
Comins 1785	5	5	5	5	5	5	5	35
Cummings s.n.	2	4	1	3	3	1	1	15

Dahlstrand 285	4	4	5	5	5	5	5	33
Daly 841	2	1	1	3	3	1	1	12
Dickson 42	1	1	1	1	3	1	1	9
Dix 142	1	2	3	1	1	1	1	10
Dornbell 39	2	3	1	1	5	1	3	16
Dyer 767	1	1	1	1	1	1	1	7
Dyer 1087	3	1	3	5	3	3	5	23
Dyer 2052	1	2	1	1	1	5	1	12
Flanagan 1797	4	5	1	3	5	5	5	28
Flanagan 1049	4	5	1	5	5	5	5	30
Flanagan 768	4	5	1	5	3	3	3	24
Fourcade 867	1	1	1	3	1	1	1	9
Fourcade 2556	1	1	1	1	1	1	1	7
Francis 66	2	1	1	1	5	5	3	18
Galpin 2178	4	5	5	5	5	3	5	32
Galpin 2666	1	1	1	1	1	1	1	7
Galpin 2665	3	1	1	1	1	1	1	9
Garrard 26	1	1	5	5	5	5	3	25
Gordon Gray 554	1	1	1	5	5	4	3	20
Heeg 212	2	3	3	1	3	1	3	16
Hobson (CD) 32	3	2	1	3	1	5	3	18
Hobson (S) 1296B	1	3	1	1	1	1	1	9
Hutton 1027	1	3	1	1	1	1	1	9
Lawrence 40	5	5	5	5	3	1	1	25
Long 23	1	1	1	1	1	1	1	7
Macowan	1	1	1	1	1	1	1	7
McCartan 29	5	5	1	3	1	1	1	17
McCartan 28	5	5	1	1	1	3	1	17
McCartan 6	4	5	1	5	5	5	1	26
McCartan 25	1	3	1	1	1	1	1	9
McCartan 19	2	1	1	3	3	1	1	12
McCartan 23	1	3	1	1	1	1	1	9

McCartan 8	2	1	1	5	5	4	1	19
McCartan 18	2	1	1	3	5	5	3	20
McCartan 9	1	3	1	5	5	5	1	21
McCartan 2	5	1	1	5	5	1	1	19
Noel 943	3	1	3	3	3	2	3	18
Olivieri 31	1	1	3	3	1	3	1	13
Rattray 99	1	1	1	3	1	4	1	12
Rattray 98	5	4	5	5	5	5	5	34
Rogers 3160	1	1	1	3	1	1	1	9
Rogers 903	2	1	1	1	1	3	1	10
Schonland 4089	2	1	1	3	3	2	1	13
Schonland 3322	3	1	3	3	1	3	1	15
Schonland 3196	1	1	1	3	1	1	1	9
Schonland 4248	1	1	1	1	1	1	1	7
Schlechter 2425	1	3	1	1	1	1	1	9
Schlechter 6537	1	1	1	3	5	1	1	13
Thomas Baines NR	3	4	1	5	3	5	1	22
Van Heeren 8	1	1	3	3	3	2	3	16
Whitty 19	1	1	1	5	5	5	3	21
Woods Davies 96	1	1	1	3	3	4	3	16
Woods Davies 57	3	1	1	1	1	1	1	9
Wormald 28	3	3	1	3	1	5	1	17

Figure 6 shows the hybrid index histogram of the combined collection (herbarium and Lewis collection). This shows a definite peak between 7 and 10 which corresponds to the total of the scores given for *Senecio speciosus* and a small peak at 33 - 35 which corresponds to the total of the scores given for *Senecio macrocephalus*. Most of the plants in the collection fall between these two values suggesting a large degree of hybridisation in these populations. There is a pronounced peak between 15 and 20 which indicates the majority of the intermediate specimens and this is skewed towards *S. speciosus*.

The specimens in the Selmar Schonland Herbarium collected over large areas of the Eastern Cape between 1890 and 1970 are shown on Figure 7. This shows a comparatively large peak for *S. speciosus*, between 7 and 10, a small peak for *S. macrocephalus,* between 33 and 35, and a peak between 15 and 20 corresponding to possible hybrids. Figure 8 shows the specimens from the Selmar Schonland Herbarium from 1970 to the present. These plants were mostly collected by students in the Grahamstown and East London areas and tend to show a collecting bias towards intermediate populations.

The Lewis collection, excluding the toxicity data (Figure 9), shows *S. speciosus* and *S. macrocephalus* at either end of the graph and an intermediate peak from 19 to 24. This is somewhat more central than in Figures 18, 19 and 20 although there are still numerically more specimens towards *S. speciosus*. However when the toxicity data is added (Figure 10) a definite skew towards *S. macrocephalus* is apparent.

Figure 6.
Hybrid Index Histogram of the combined collections (Selmar Schonland Herbarium specimens and the Lewis collection).

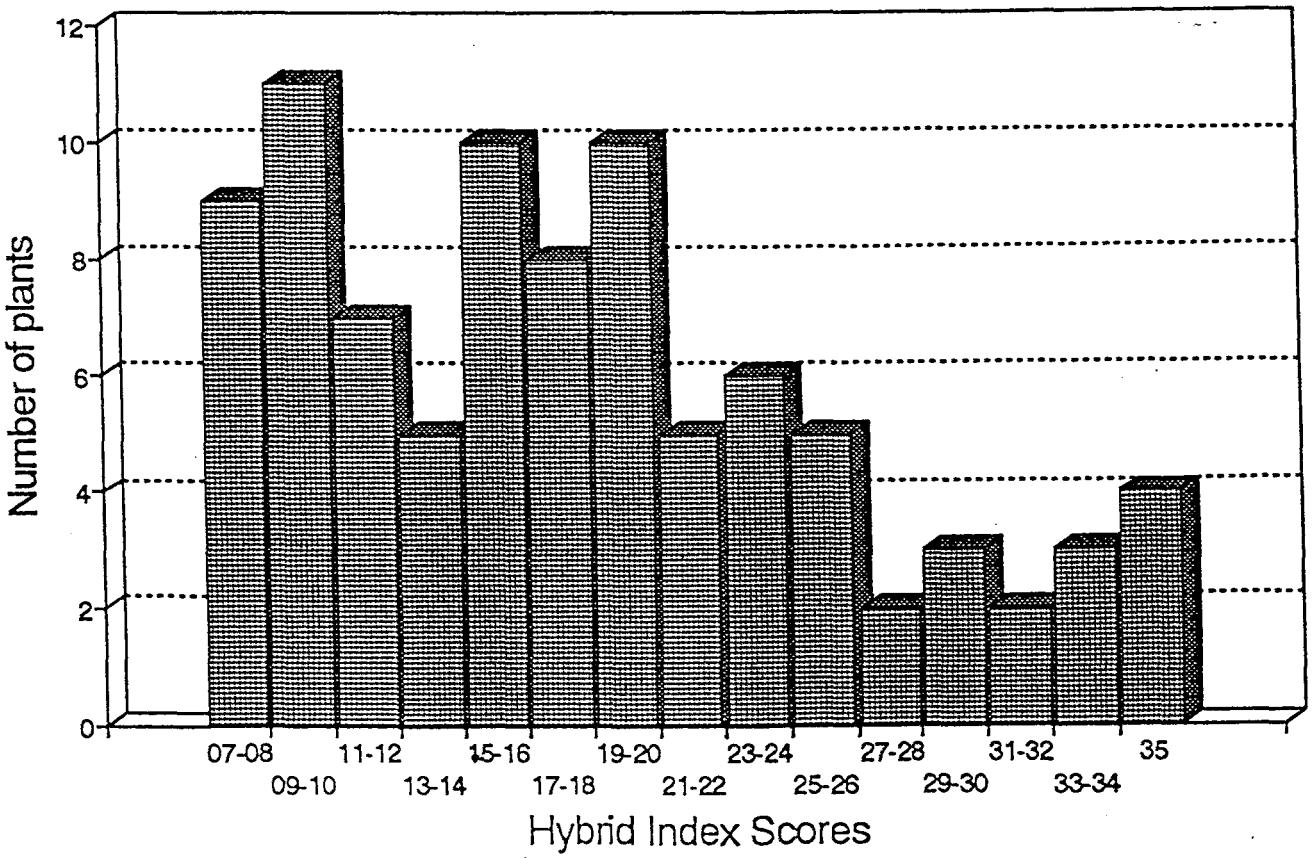


Figure 7.
Hybrid Index Histogram of the specimens in the Selmar Schonland Herbarium collected between 1890 and 1970.

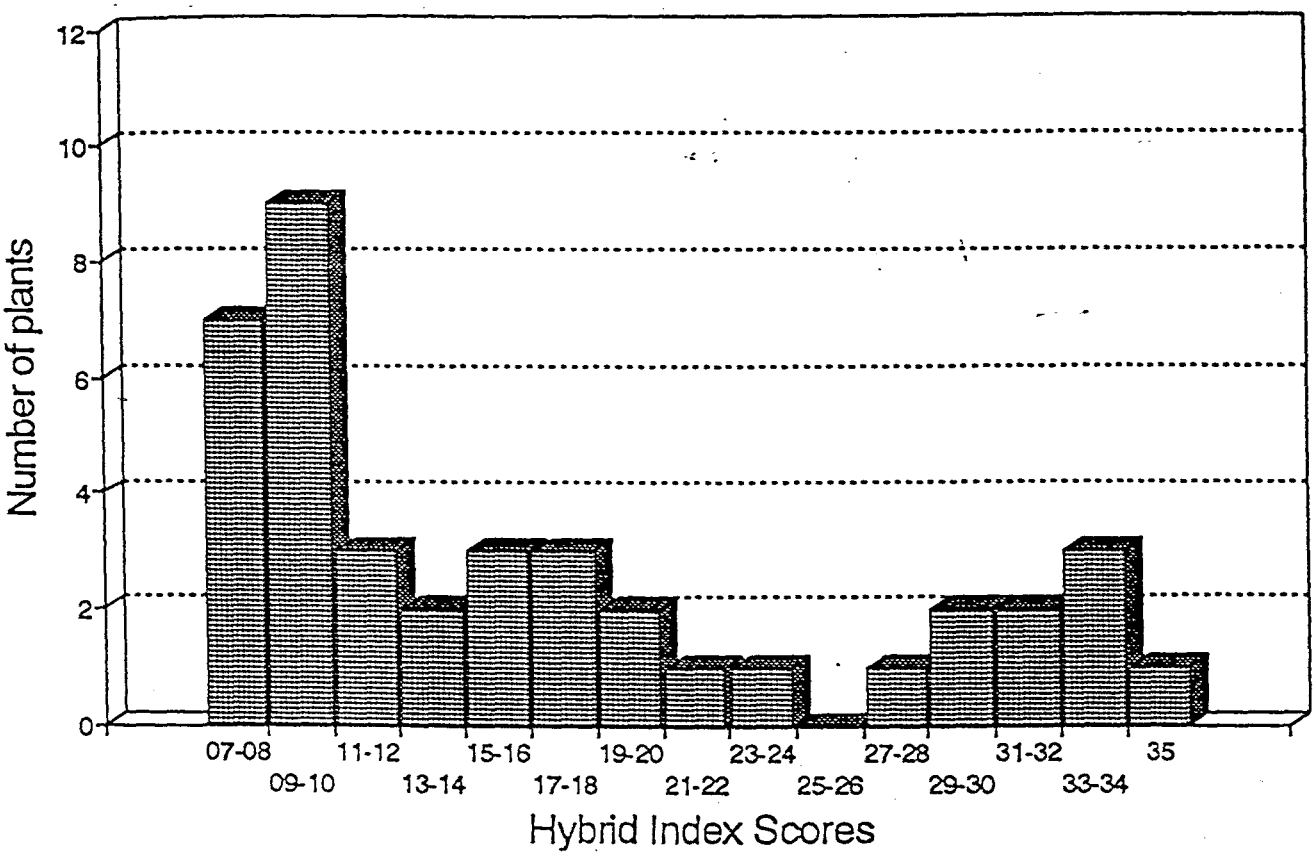


Figure 8.
Hybrid Index Histogram of the specimens in the Selmar Schonland Herbarium collected between 1970 and 1992.

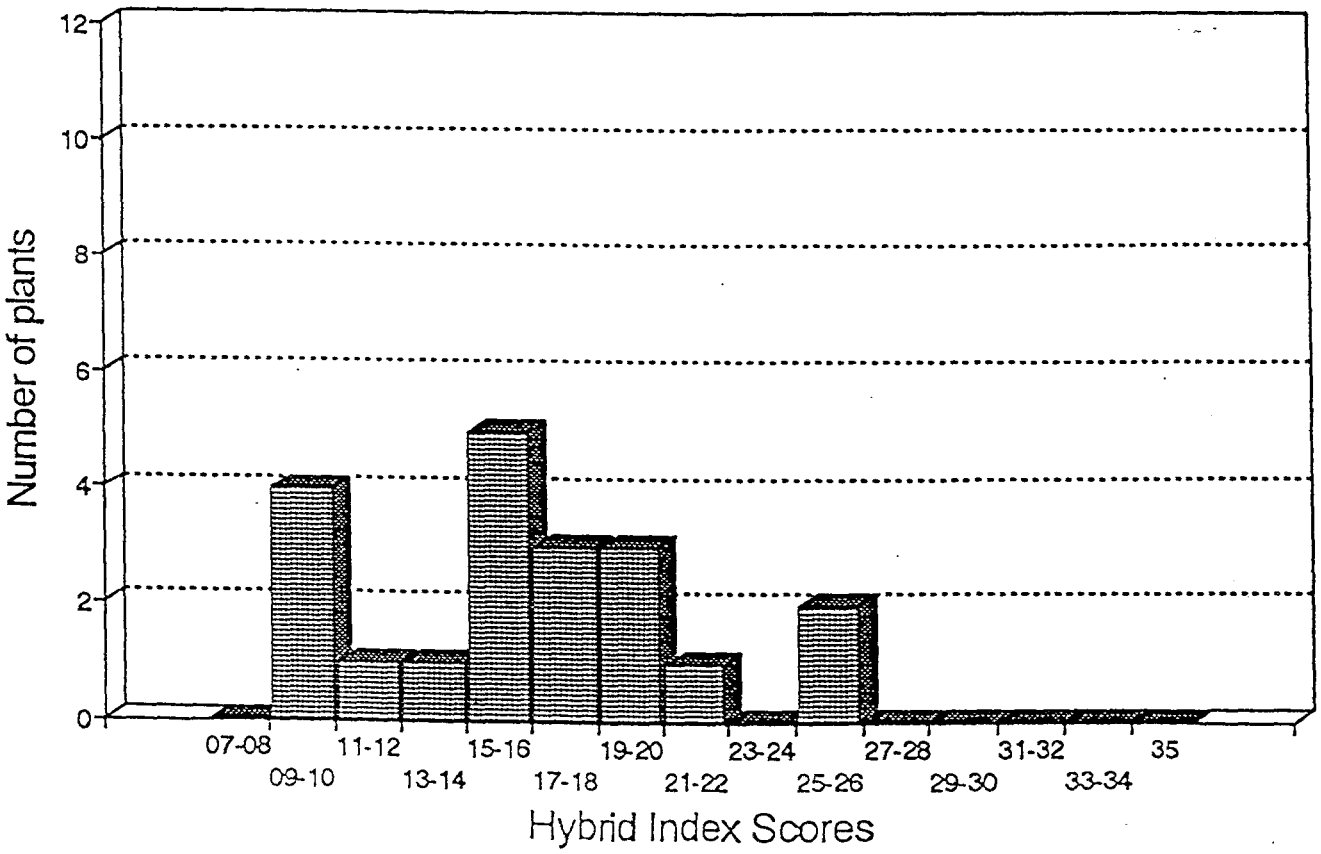


Figure 9.
Hybrid Index Histogram of the Lewis collection (morphology only).

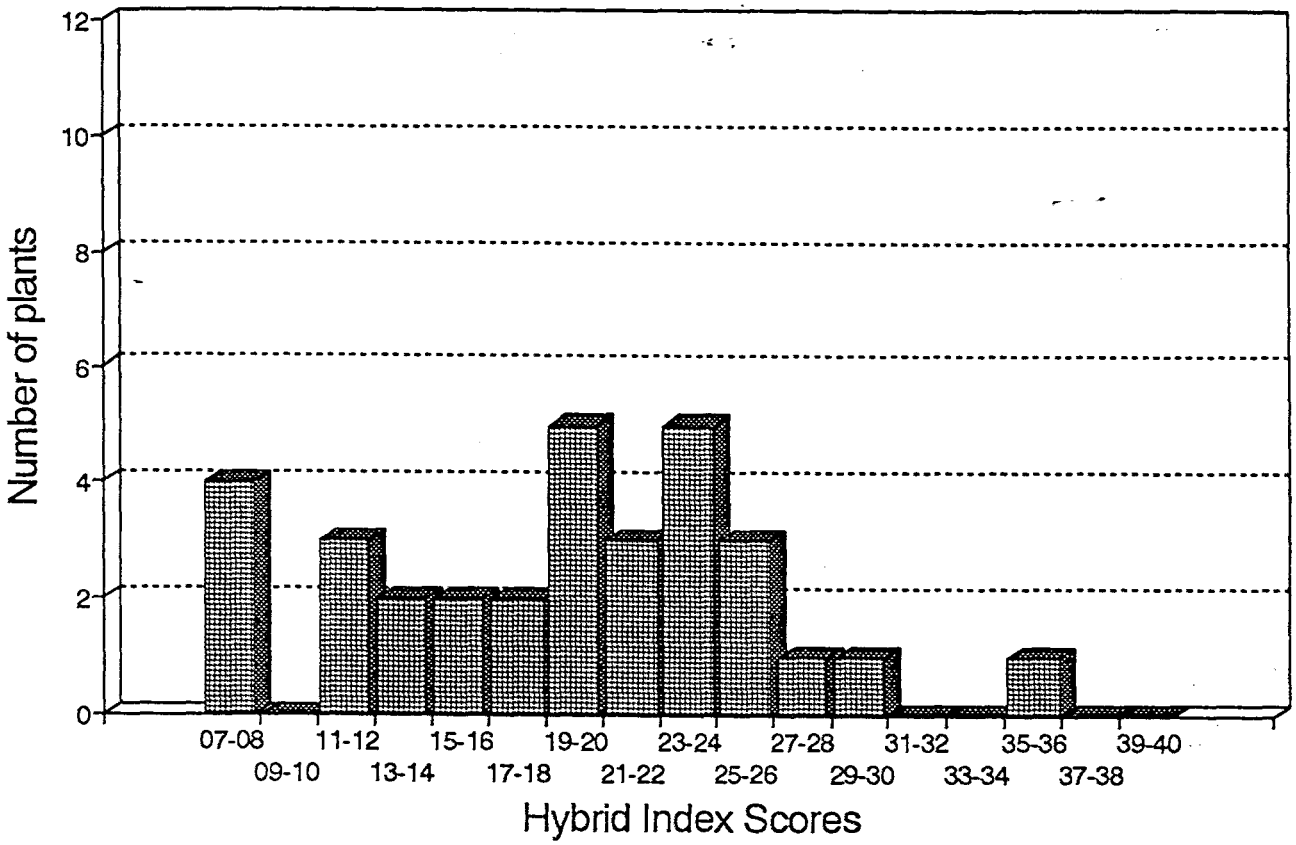
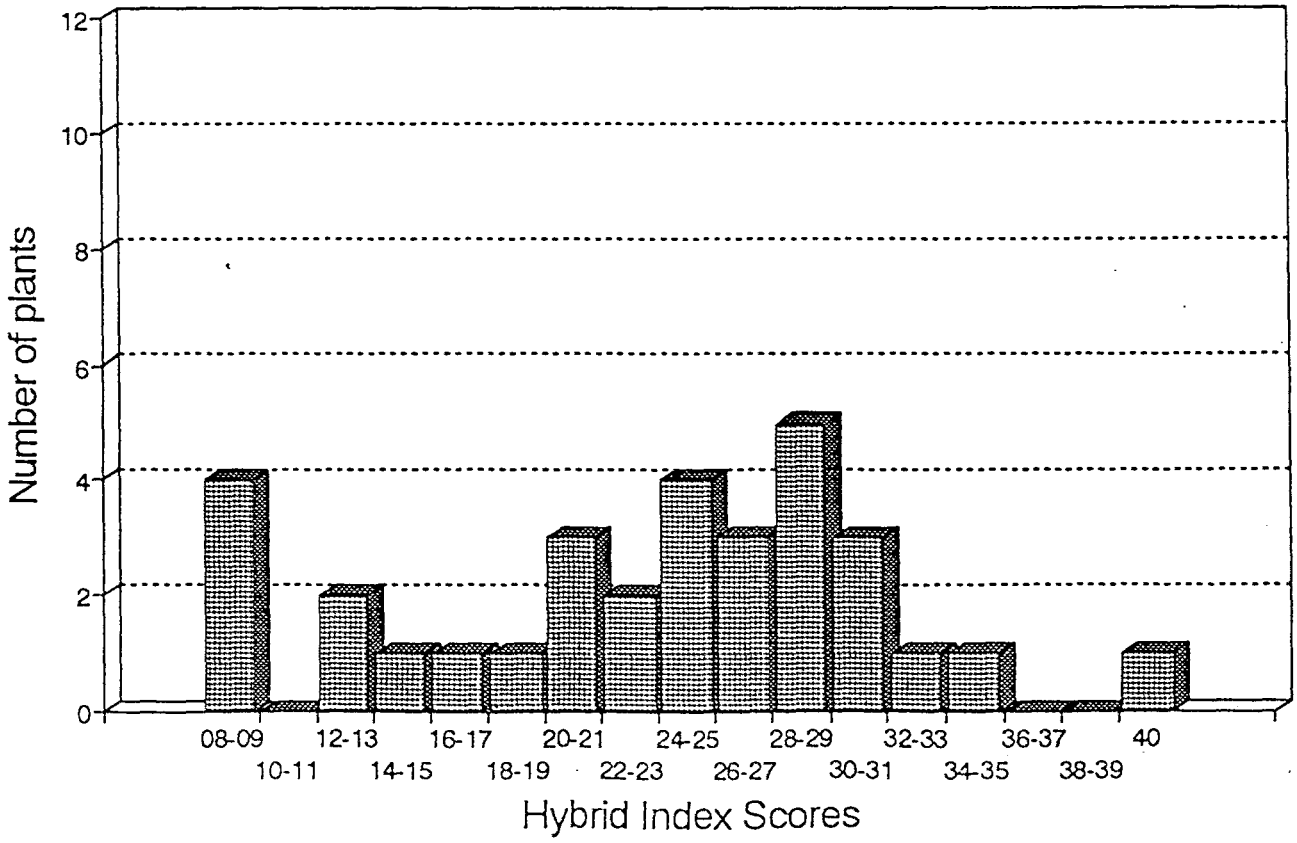


Figure 10.
Hybrid Index Histogram of the Lewis collection (morphology and toxicity).



3.1.2 Geographical Distribution

The distribution of all the specimens included in this study is shown in Figures 1, 2 and 3 on pages 15-17. *Senecio speciosus* is located to the south and west, from Tsitsikamma to Port Alfred, and inland in the Zuurberg, Winterberg and Amatole Mountains. *Senecio macrocephalus* is found from East London inland along the Buffalo River to the Katberg and Amatole Mountains to the west and also in the Transkei to the north east. Around Grahamstown intermediate types are recorded.

On Figure 11 the hybrid index histogram scores (excluding Total B, Table 2A) are marked directly onto a map of part of the Eastern Cape. The map is then divided into areas taking into account geographical features and the distribution of hybrid index scores. The six areas delimited are described below:

Area 1 is the south and west coastal belt from Tsitsikamma to Port Elizabeth and includes the Zuurberg. This is an area of Devonian and pre-Devonian rocks with a coastal lowland succeeded inland by rugged mountain ranges trending parallel to the coast. In this area only *Senecio speciosus* specimens are found.

Area 2 is the coastal belt from Port Elizabeth to the Fish River including Sidbury, Salem and Southwell. this is an area of dissected topography below an altitude of c.450m corresponding essentially with the region submerged during the Pliocene marine transgression (Rust *et al.* 1990; Lewis 1995). *Senecio speciosus* specimens and low-scoring intermediate plants are found in this area.

Area 3 is the Grahamstown area which is essentially a dissected peneplain at altitudes of 630-736m developed on rocks of the Karoo sequence and the Cape Supergroup (Mountain 1980; Lewis 1995). This is an area of intermediate populations with lower scoring specimens to the west, towards the range of typical *Senecio speciosus* and higher scoring intermediates to the east towards the range of typical *Senecio macrocephalus* .

Area 4 contains East London and the border corridor to Komgha and King Williams Town. This is a marine erosion surface of Cretaceous age dissected by the south-easterly flowing Buffalo River (Maud and Partridge 1990). Mainly high-scoring (*macrocephalus* type) intermediates are found in the East London area. Towards King Williams Town *Senecio speciosus* and *Senecio macrocephalus* specimens have been collected.

Area 5 encompasses the Katberg, Winterberg and Amatole Mountains which form part of the Great Escarpment and rise to altitudes of nearly 2000m where dolorite sills have been dissected to form prominent ridges (Phillipson 1987). Specimens collected in this area are *Senecio speciosus*, *Senecio macrocephalus* and *Senecio* sp. aff. *S. speciosus*. No intermediate plants were collected here.

Area 6 is the north-eastern Cape and Transkei. This forms the highest and most dissected topography in the whole study area due to fluvial erosion consequent upon Pliocene uplift (Moon and Dardis 1988). The highest point, Ben MacDhui, reaches 3001m. *Senecio polyodon* and a specimen (Lewis 112) with a large capitulum, raggedly lobed leaves and a low toxicity to brine shrimps were collected in these mountains.

The Grahamstown area is shown separately on Figure 12.

Figure 13 is a hybrid index histogram constructed using the combined collections (excluding toxicity data) divided into the areas shown on Figure 11. *Senecio speciosus* is found in all areas, *Senecio macrocephalus* however is only found in areas 4,5 and 6, in the north and east of the study area. Intermediate populations are mainly in areas 2, 3 and 4 with the lower scoring intermediates (closer to *S. speciosus*) in areas 2 and 3 and the higher scoring intermediates (closer to *S.macrocephalus*) in areas 3 and 4.

Figure 11.
Hybrid Index Scores of the combined collections plotted onto the East Cape base map and divided into areas. The division into areas is explained in the text

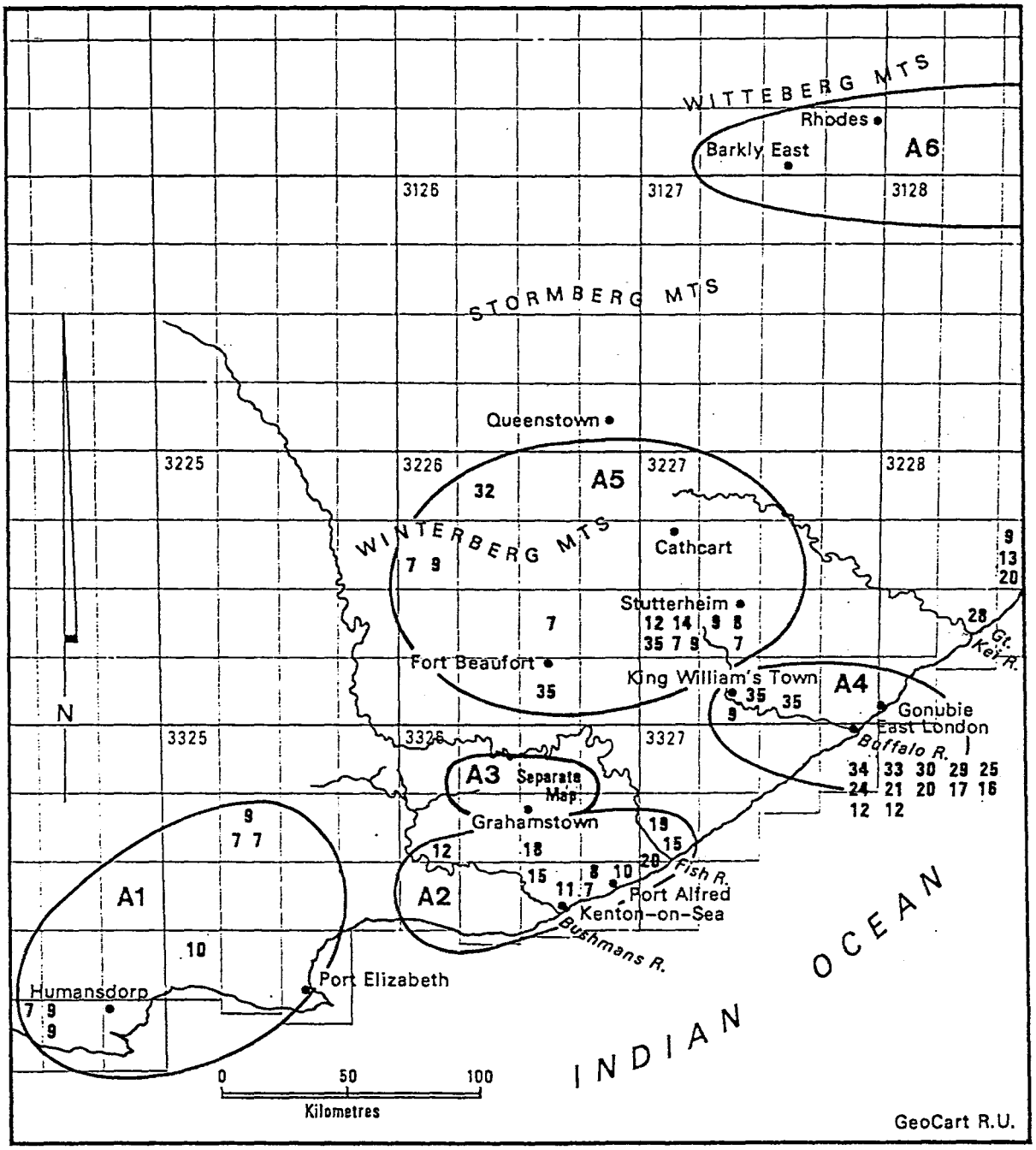


Figure 12.
Hybrid Index Scores of specimens in the combined collections from the Grahamstown area plotted onto the Grahamstown base map.

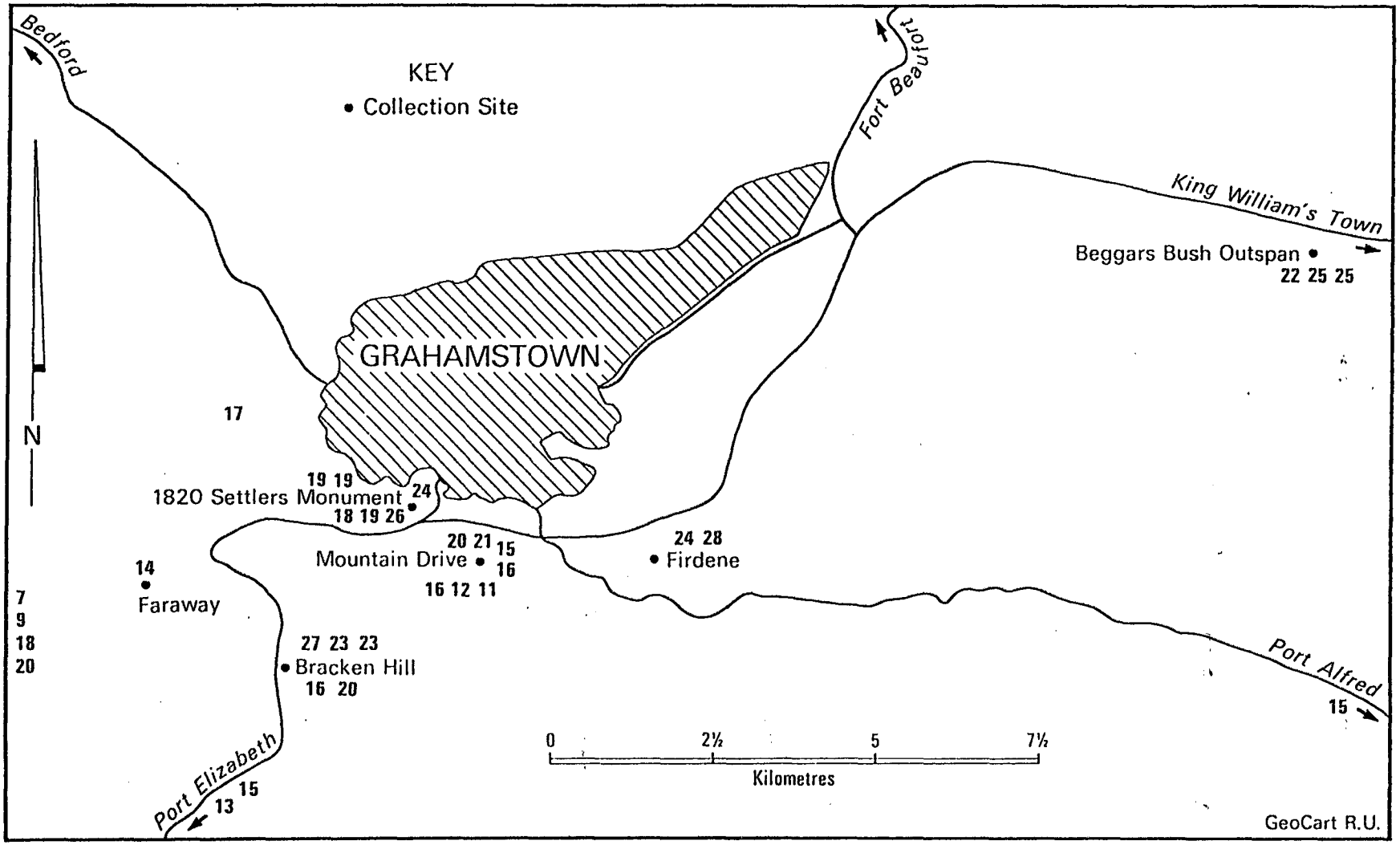
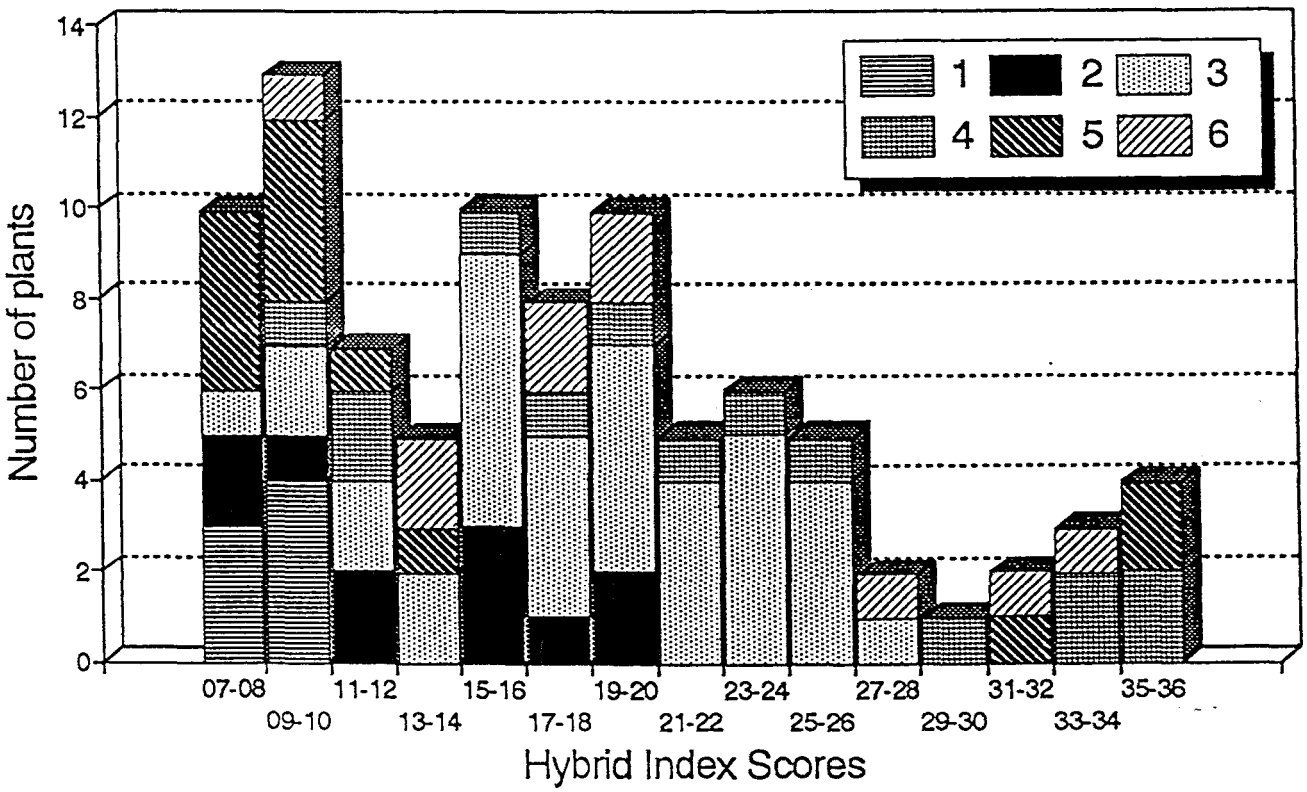


Figure 13.
The Hybrid Index Histogram of the combined collections (Figure 4) divided into the areas given on Figure 11.



Key To Areas

1. The south and west coastal belt from Tsitsikamma to Port Elizabeth and the Zuurberg.
2. The coastal belt from Port Elizabeth to the Fish River, including the Sidbury, Salem and Southwell areas.
3. The Grahamstown area.
4. East London and the border corridor to Komgha and King Williams Town.
5. The Katberg, Winterberg and Hogsback Mountains.
6. The North Eastern Cape and the Transkei

3.1.3 Principal Components Analysis

Three graphs were constructed using the data produced by the programme CANOCO. These are shown as Figures 14-16. On all three graphs *Senecio speciosus* and *Senecio macrocephalus* are located at either end of the X axis and there is a wide spread of points between the two indicating the presence of intermediates. The dots in the small circle at the left hand end of the X axis are the nine specimens which scored 7 points on the hybrid index histogram scale and which are regarded as typical *Senecio speciosus*. The points in the small circle at the right hand end of the X axis are the two specimens which scored 35 points on the hybrid index scale and which are regarded as typical *Senecio macrocephalus*. The wider circle at the left hand end of the X axis includes 15 specimens which scored 8, 9 or 10 points and the wider circle at the right hand end of the X axis includes 5 specimens which scored 31 - 34 points. These represent specimens which differ only slightly from *Senecio speciosus* and *Senecio macrocephalus* respectively.

Closer scrutiny of the pattern of dots showed that intermediate specimens giving lower hybrid index scores are located on the left hand side of the Y axis, either below or above the X axis and specimens giving higher hybrid index scores are located on the right hand side of the Y axis either below or above the X axis. There appears to be no correlation of single characters with the position of the dots either below or above the X axis. It also appears that the intermediate specimens from the different populations and areas are scattered at random on the four quarters of the graph.

Figure 14.
Principal Components Analysis 1
Axis 1 x Axis 2

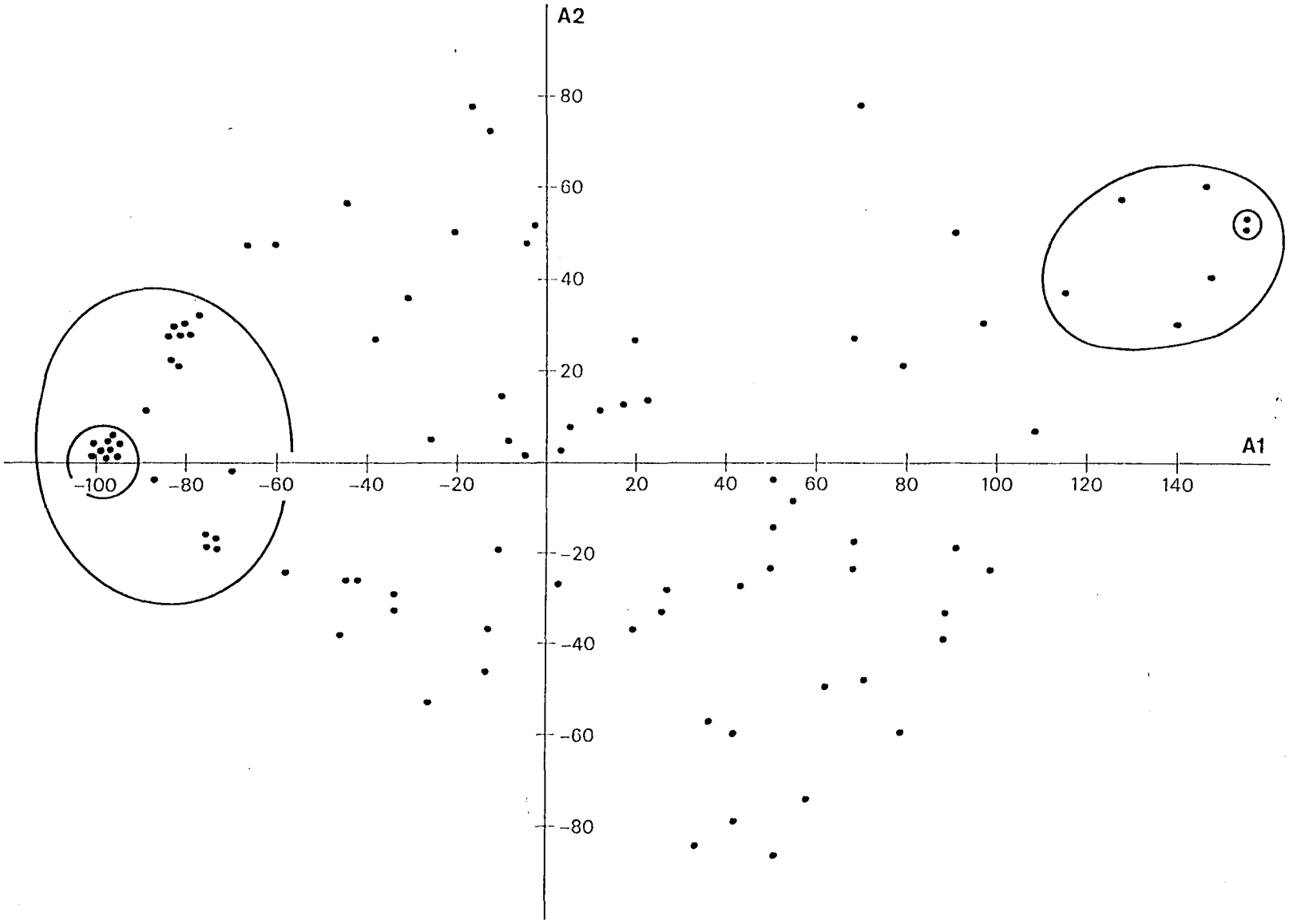


Figure 15.
Principal Components Analysis 2
Axis 1 x Axis 3

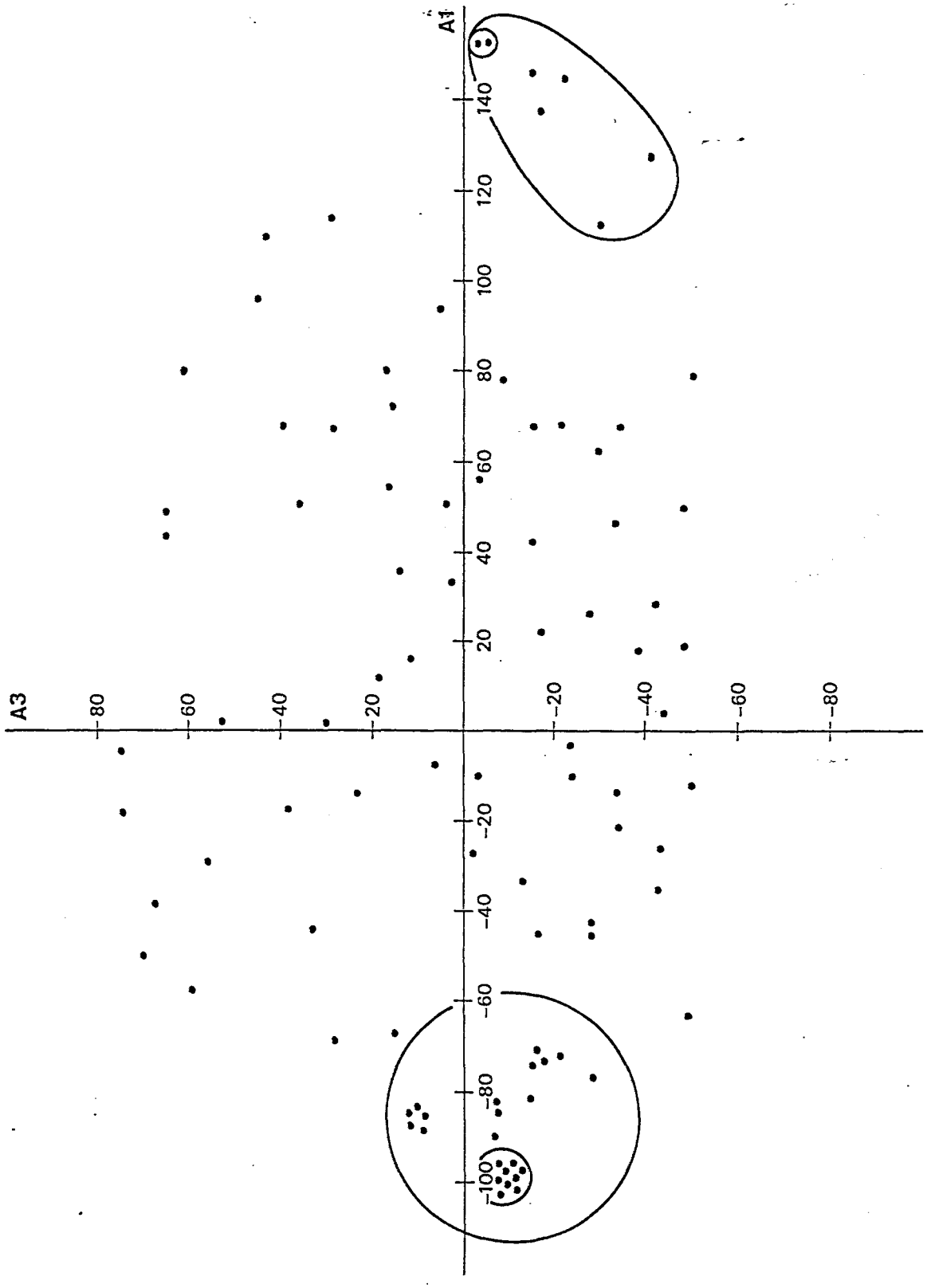
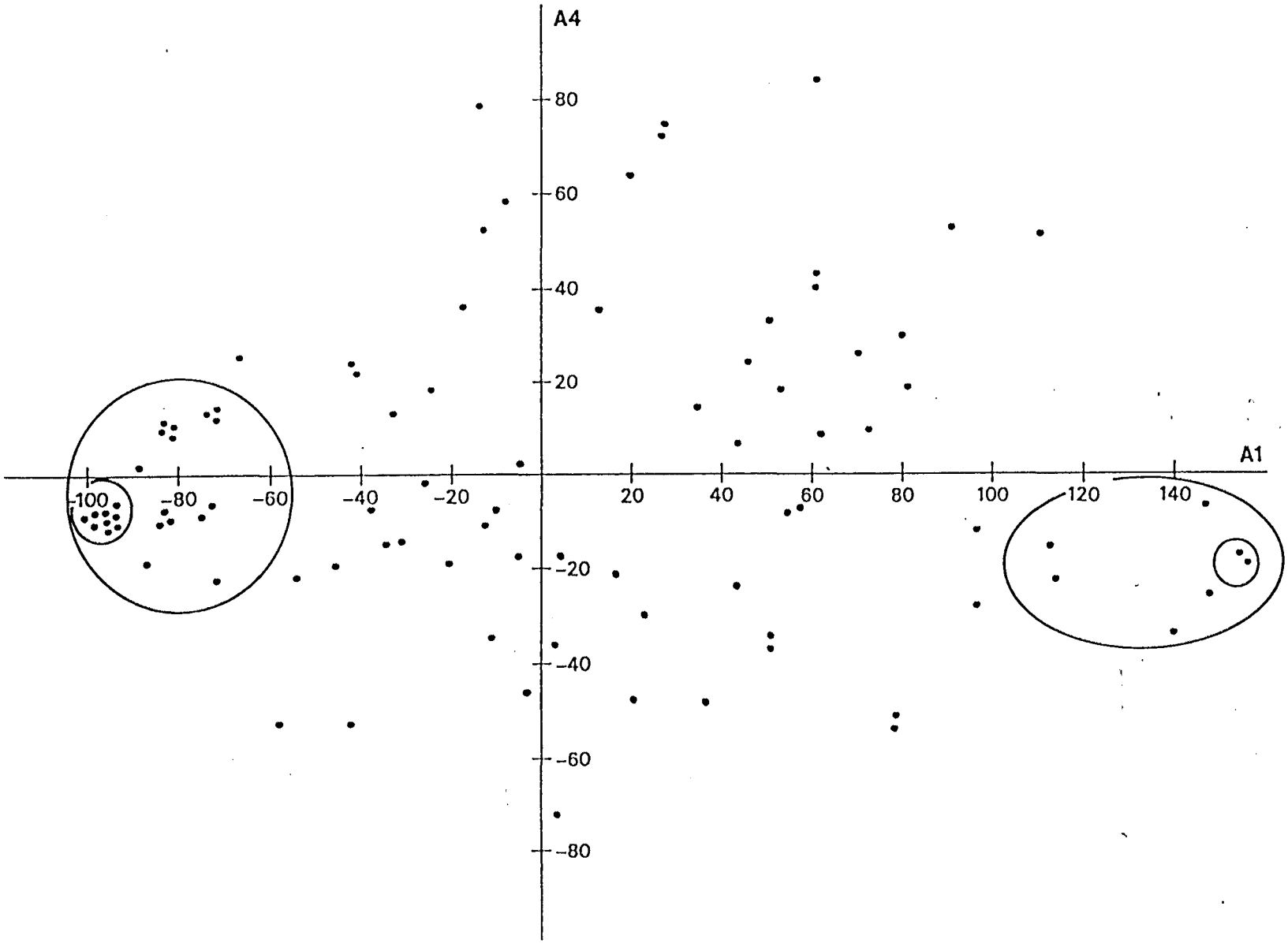


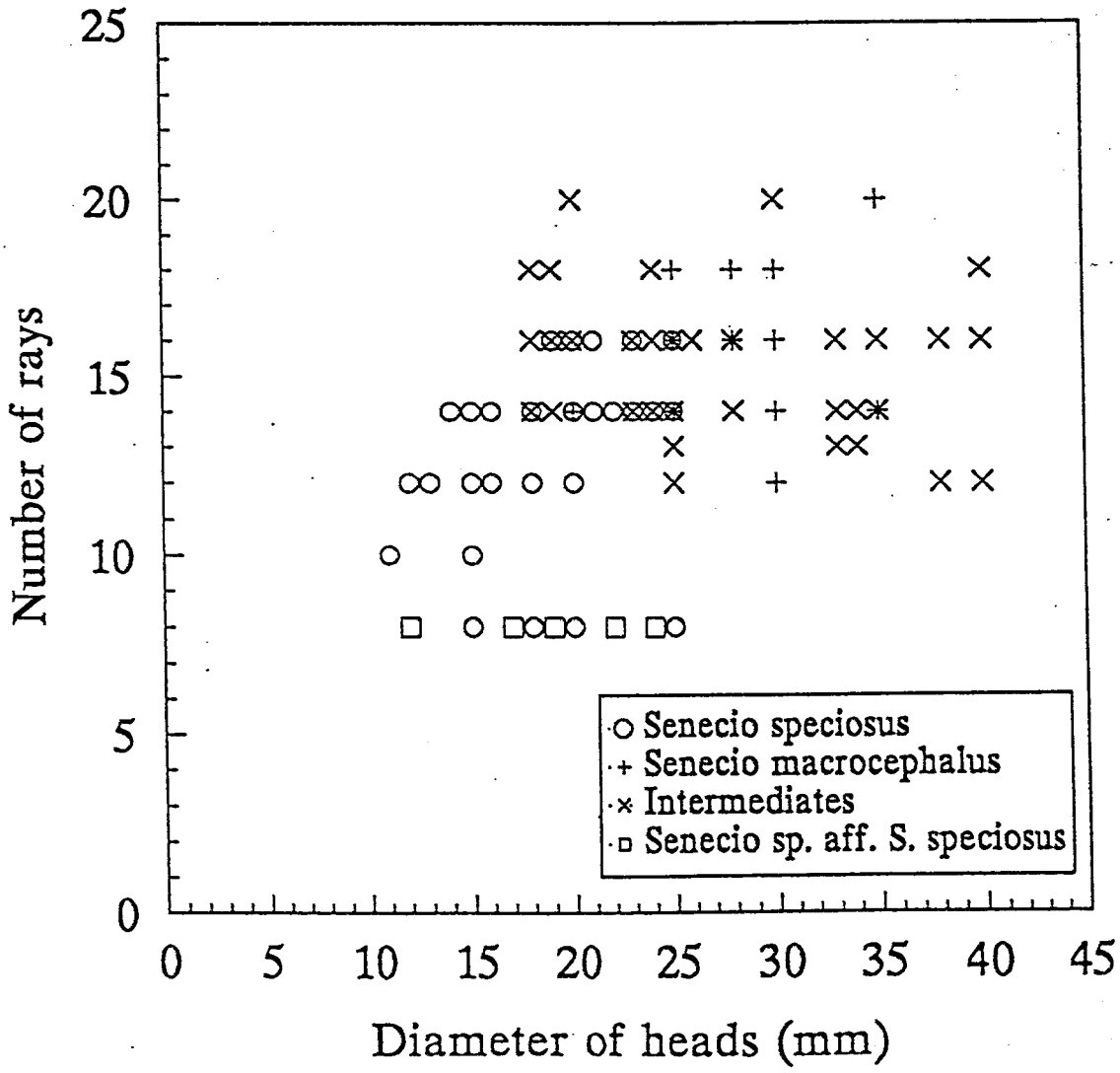
Figure 16.
Principal Components Analysis 3
Axis 1 x Axis 4



3.1.4 **Scatter Diagram**

A scatter diagram was produced based on information listed in Appendices 1 and 2, using diameter of heads as the X axis and number of rays as the Y axis. *Senecio* sp. aff. *S. speciosus* is located towards the bottom of the graph. Specimens identified as *Senecio speciosus* in the appendices occur on the left hand side with some specimens close to or in the *Senecio* sp. aff. *S. speciosus* group. This is to be expected as some specimens of *Senecio speciosus* have only 8 ray florets. Specimens identified as *Senecio macrocephalus* in the appendices occur towards the right hand side but somewhat scattered. Intermediate specimens occur in the centre of the graph but intermingling with both *Senecio speciosus* and *Senecio macrocephalus* and occurring further towards the right and top of the graph than some of the *Senecio macrocephalus* specimens.

Figure 17.
Scatter Diagram using diameter of heads x number of ray florets



3.2 The Toxicity of *Senecio* species to Brine Shrimps (*Artemia salina*)

The following tables (3 - 14) give the percentage deaths of brine shrimps (2nd and 3rd stage nauplii of *Artemia salina*) at 24 hours when tested with three dilutions of the plant extracts, 0.05 ml, 0.5 ml and 1 ml of plant extract each made up to 5 mls with seawater.

Five replicates per plant extract were used and five controls per batch of samples were set up using only seawater. If widely varying results were obtained between replicates in any sample this was deemed to be due to contamination and the experiment was repeated. If any controls died the experiment was repeated.

Results from both methanol and water extracts are given. Results from the water extracts are very variable due to a contamination factor which was difficult to remove, so the discussion and conclusions are based on the results from the methanol extracts only.

Toxicity results from the yellow flowered species *Senecio inaequidens* have been included for comparative purposes.

TABLE 3

Percentage Deaths of Brine Shrimps after 24 hours in water extracts, (mean of 5 replicates) for *Senecio speciosus*

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 65	3	6	7
Lewis 95	0	0	0
Lewis 96	0	0	0
Lewis 102	0	0	0
Phillipson 3823	0	0	0
Controls	0	0	0

TABLE 4

Percentage Deaths of Brine Shrimps after 24 hours in water extracts (mean of 5 replicates) for *Senecio macrocephalus*

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 82	0	15	37
Controls	0	0	0

TABLE 5

Percentage Death of Brine Shrimps after 24 hours in water extracts (mean of 5 replicates) for *Senecio speciosus/macrocephalus* intermediate specimens from the Grahamstown Area

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 51	0	22	95
Lewis 51 (Roots)	55	90	100
Lewis 55	0	100	100
Lewis 57	0	100	100
Lewis 57 (Roots)	100	100	100
Lewis 71	0	0	70
Lewis 72	0	0	50
Lewis 79	0	0	15
Lewis 87	0	90	100
Lewis 88	0	50	50
Lewis 90	0	60	85
Lewis 91	30	98	100
Lewis 98	0	0	60
Lewis 100	0	5	10

Lewis 101	0	12	50
Lewis 104	7	44	89
Lewis 104 (Roots)	5	33	45
Lewis 105	0	0	47
Lewis 107	10	15	20
Lewis 107 (Roots)	7	17	87
Lewis 108	10	10	35
Lewis 108 (Roots)	7	50	90
Lewis 115	0	50	60
Lewis 116	23	54	97
Lewis 118	0	66	100
Controls	0	0	0

TABLE 6

Percentage Deaths of Brine Shrimps after 24 hours in water extracts (mean of 5 replicates) for *Senecio speciosus/macrocephalus* intermediate specimens from Fish River Mouth to East London

Voucher Specimen	**Dilution**		
	0.05ml	**0.5ml**	**1.0ml**
Lewis 66	2	6	5
Lewis 66	0	0	0
Lewis 103	3	0	3
Lewis 117	3	7	35
Controls	0	0	0

TABLE 7

Percentage Deaths of Brine Shrimps after 24 hours in water extracts (mean of 5 replicates) for other *Senecio* species.

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 64 (*Senecio elegans*)	5	10	20
Lewis 67 (*Senecio inaequidens*)	7	7	10
Lewis 94 (*Senecio radicans*)	0	0	0
Lewis 111 (*Senecio polyodon*)	10	40	92
Lewis 112 (*Senecio sp.*)	5	7	8
Phillipson 3820 (*Senecio erubescens*)	0	0	0
Phillipson 3822 (*Senecio sp.*)	0	0	20
Controls	0	0	0

TABLE 8

Percentage Deaths of Brine Shrimps after 24 hours in water extracts (mean of 5 replicates) for *Senecio* specimens grown in potting soil for 6 months

Voucher specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 82A (*S. macrocephalus*)	0	80	100
Lewis 82A (*S. macrocephalus*)(Roots)	0	4	20
Lewis 86A (*S. barbatus*)	0	0	12
Lewis 86A (*S. barbatus*)(Roots)	25	36	60
Phillipson 3822A (*Senecio sp.*)	22	15	73
Phillipson 3822A (*Senecio sp.*)(Roots)	33	66	90
Phillipson 3823A (*S. speciosus*)	0	4	20
Phillipson 3823A (*S. speciosus*)(Roots)	0	0	0
Controls	0	0	0

TABLE 9

Percentage Deaths of Brine Shrimps after 24 hours in methanol extracts (mean of 5 replicates) for *Senecio speciosus*

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 65	0	5	9
Lewis 95	0	0	0
Lewis 96	0	0	0
Lewis 102	0	0	0
Phillipson 3823	0	0	0
Controls	0	0	0

Mean Death Rate: 0.77%

TABLE 10

Percentage Deaths of Brine Shrimps after 24 hours in methanol extracts (mean of 5 replicates) for *Senecio macrocephalus*

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 82	86	100	100
Controls	0	0	0

Mean Death Rate = 95%

TABLE 11
Percentage Deaths of Brine Shrimps after 24 hours in methanol extracts (mean of 5 replicates) for *Senecio speciosus/macrocephalus* intermediate specimens from the Grahamstown area

Voucher Specimen	Dilution 0.05ml	0.5ml	1.0ml
Lewis 51	80	100	100
Lewis 51(Roots)	90	100	100
Lewis 55	95	100	100
Lewis 57	95	100	100
Lewis 57(Roots)	90	100	100
Lewis 71	30	86	92
Lewis 72	81	100	100
Lewis 79	40	86	99
Lewis 79(Roots)	95	95	99
Lewis 87	70	100	100
Lewis 88	16	100	100
Lewis 90	100	100	100
Lewis 91	100	100	100
Lewis 98	98	100	100
Lewis 100	56	100	100
Lewis 101	56	98	100
Lewis 104	92	100	100
Lewis 104(Roots)	100	100	100
Lewis 105	40	100	100
Lewis 105(Roots)	100	100	100
Lewis 106	58	100	100
Lewis 107(Roots)	100	100	100
Lewis 108(Roots)	100	100	100
Lewis 115	68	99	100
Lewis 116	84	100	100
Lewis 118	96	100	100
Controls	0	0	0

Mean Death Rate = 92%

TABLE 12

Percentage Deaths of Brine Shrimps after 24 hours in methanol extracts (mean of 5 replicates) for *Senecio speciosus/macrocephalus* intermediate specimens from Port Alfred to East London

Voucher Specimen	Dilution		
	0.05ml	0.5ml	1.0ml
Lewis 66	0	38	45
Lewis 103	58	90	91
Lewis 117	50	95	98
Lewis 117	75	100	100
McCartan 3	60	98	100
McCartan 13	17	82	100
Controls	0	0	0

Mean Death Rate = 80.9%

TABLE 13

Percentage Deaths of Brine Shrimps after 24 hours in methanol extracts (mean of 5 replicates) for Specimens of other *Senecio* species.

Voucher Specimen	Dilution			Mean Death
	0.05ml	0.5ml	1.0ml	Rate %
Lewis 64 (*Senecio elegans*)	0	5	6	3.6%
Lewis 67 (*Senecio inaquidens*)	0	3	3	2.0%
Lewis 86 (*Senecio barbatus*)	5	5	25	11.6%
Lewis 94 (*Senecio radicans*)	0	0	0	0
Lewis 111 (*Senecio polyodon*)	100	100	100	100.0%
Lewis 112 (*Senecio sp.*)	2	19	14	11.6%
Phillipson 3820 (*S. erubescens*)	5	5	5	5.0%
Phillipson 3822 (*Senecio sp.*)	0	10	15	8.3%
Controls	0	0	0	

TABLE 14

Percentage Deaths of Brine Shrimps after 24 hours in methanol extracts (mean of 5 replicates) for *Senecio* specimens grown in potting soil for 6 months

Voucher Specimen	Dilution			Mean Death
	0.05ml	0.5ml	1.0ml	Rate %
Lewis 82A (*S. macrocephalus*)	96	100	100	98.6%
Lewis 82A (*S. macrocephalus*)(R)	54	100	100	84.6%
Lewis 86A (*S. barbatus*)	0	0	9	3.0%
Lewis 86A (*S. barbatus*)(R)	2	54	76	44.0%
Phillipson 3820A (*S. erubescens*)	0	0	16	5.3%
Phillipson 3822A (*Senecio sp.*)	2	20	34	18.6%
Phillipson 3822A (*Senecio sp.*)(R)	36	98	100	78.0%
Phillipson 3823A (*S. speciosus*)	0	0	0	0
Phillipson 3823A (*S. speciosus*)(R)	0	4	20	8.0%
Controls	0	0	0	

R = Root Material

3.3 Thin Layer Chromatography

A total of eleven spots visible under ultra-violet light were counted on the thin layer chromatography plates. R_f values for these spots, which are given in Table 15, were calculated using the formula: distance travelled by the trace divided by distance travelled by the solvent. The R_f value for the retrorsine marker was calculated on all plates as 0.33 and this spot always gave an orange colour reaction when sprayed with Dragendorff reagent.

TABLE 15

R_f values for the 11 spots counted on Thin Layer Chromatography Plates

Spot No.	1	2	3	4	5	6	7	8	9	10	11	Ret. Mark.
R_f Value	0.13	0.16	0.19	0.27	0.32	0.36	0.51	0.62	0.70	0.79	0.85	0.33

It can be seen that the spot number 5 is very close to the R_f value of the retrorsine marker, but since no colour reaction was seen in spot number 5, unlike retrorsine, it is assumed to be a different alkaloid or retrorsine in low concentration.

The presence of each of the eleven spots was recorded for each chromatography plate. Results from the replicates were rationalised to give the presence or absence of each spot for each extract. The spots detected for each extract, together with their colour reaction, are shown on Tables 16 - 19. Spots, shown as +, were only visible under ultra violet light and were assumed to be alkaloids in low concentration (Grue 1991). Spots which gave a positive (orange) alkaloid colour reaction when sprayed with Dragendorff reagent are shown as ++ on the tables and are assumed to be alkaloids in higher concentrations.

TABLE 16 Thin Layer Chromatography.

Presence of alkaloid spots. Plant extracts according to species.

+ low concentration ++ high concentration

Voucher Specimen	Spot no. 1	2	3	4	5	6	7	8	9	10	11
Senecio speciosus											
Lewis 65	+		+			+	+		+		+
Lewis 102	+		+	+	+	+	+		+	+	+
Phillipson 3823	+		+			+	+			+	
Phillipson 3823A	+		+	+	+	+				+	+
Phillipson 3823A (Roots)	+			+						+	
Senecio* sp. aff. *S. speciosus											
Lewis 96			+	+	+				+	++	+
Senecio macrocephalus											
Lewis 82	+		+	+	+	+		+		+	
Lewis 82A	+		+	+	+			+		++	+
Lewis 82A(Roots)	+		+	+		+		+		++	

TABLE 17

Thin Layer Chromatography.

Presence of alkaloid spots. Plant extracts from the Grahamstown Populations.

Voucher Specimen	Spot no. 1	2	3	4	5	6	7	8	9	10	11
Brackenhill, Howisonspoort											
Lewis 71				+	+	+	+	+		+	
Lewis 71(Roots)	+		+		+		+	++		++	
Lewis 87	+		+	+				+		++	+
Lewis 98	+		+					+		+	+
1820 Settlers Monument											
Lewis 51	+			+	+	+				+	
Lewis 51(Roots)		++			+		+	+			
Lewis 88	+		+	+				+		++	+
Lewis 90	+		+	+	+	+		+		+	+
Lewis 91	+		+	+			+	+		+	+
Lewis 100	+			+	+		+	+		+	+
Lewis 101	+				+			+		+	+
Lewis 107(Roots)	+		+	+		+	+	+		+	
Lewis 108(Roots)	+		+	+		+	+	+		++	
Beggarsbush Outspan											
Lewis 104	+		+					+		+	+
Lewis 104(Roots)	+		+	+		+		+		++	
Lewis 105			+	+		+		+		+	+
Lewis 105(Roots)	+			+				+		++	
Lewis 106			+	+		+		+			+
Mountain Drive											
Lewis 115	+		+	+		+		+		+	+
Lewis 116			+		+	+		+		+	+
Faraway, Coldsprings											
Lewis 118			+		+	+	+	+		+	+
Firdene, Stoneshill											
Lewis 57			+	+	+	+	+	+			
Lewis 57(Roots)	+		+	+	+		+	++		++	
Lewis 79	+		+	+	+			+		+	+
Lewis 79(Roots)	+				+			++		++	

TABLE 18

Thin Layer Chromatography.

Presence of alkaloid spots. Extracts from specimens collected at the coast.

Voucher Specimen	Spot no. 1	2	3	4	5	6	7	8	9	10	11
Lewis 64			+				+				
Lewis 66			+	+	+				+		
Lewis 103	+		+	+	+		+	+		+	+
Lewis 117	+		+	+	+			+		+	+
McCartan 3	+				+	+	+	+	+	+	+
McCartan 13			+		+	+	+				+

TABLE 19

Thin Layer Chromatography

Presence of alkaloid spots. Extracts from specimens collected in the mountains.

Voucher Specimen	Spot No. 1	2	3	4	5	6	7	8	9	10	11
Phillipson 3820	+		+	+	+		+			+	+
Phillipson 3820A	+		+	+	+		+			++	+
Phillipson 3822	+		+	+			+	++		+	
Phillipson 3822A			+	+	+	+	+	++	++	+	+
Phillipson 3822A(Roots)			+	+			+	+		++	
Lewis 86	+	++		+		+	+			+	
Lewis 86A	+	++		+	+					+	+
Lewis 86A(Roots)	+	++								+	
Lewis 111	+		+	+	+			+		+	+
Lewis 112	+		+			+	+	+	+	+	+
Lewis 94	+		+	+					++	++	+

Table 16 shows spots given by extracts from *Senecio speciosus*, *Senecio* sp. aff. *S. speciosus* and *Senecio macrocephalus*. Spots 1 - 6 are variable but it is important to note the absence of Spot 8 in *S. speciosus* and *Senecio* sp. aff. *S. speciosus* and the absence of spots 7 and 9 in *S. macrocephalus*. Spot 8 is present in *S. macrocephalus*. Table 17 shows the results from the specimens collected in the Grahamstown area. Spot 9 is absent as in *Senecio macrocephalus* and Spot 8 is present also as in *S. macrocephalus*. Spot 7 is

occasionally present. However, spot 7 was not detected in extracts from the specimens from Mountain Drive and Beggarsbush Outspan.

Table 18 shows spots given by extracts from other *Senecio* specimens collected at the coast. These results are very variable, the specimens from East London definitely showing spot 8 but spots 7 and 9 seeming to appear at random.

Table 19 shows the results given by other specimens collected on the Menziesberg area of the Amatole Mountains and the North Eastern Cape Drakensberg. These all have their own distinct distribution of spots as they are all separate species. These will be discussed later.

When thin layer chromatography plates were used to test for the presence of terpenoid compounds only the basal spot on the plate gave a colour reaction with certain plant extracts.

The basal spot from an extract of Lewis 82 (*Senecio macrocephalus*) gave a dark brown colour. Three of the intermediate specimens were tested (Lewis 57 root material, Lewis 79 and Lewis 101) and these also gave a brown colour reaction in the basal spot. Extracts from specimens of *Senecio speciosus* (Lewis 65 and Phillipson 3823) tested gave no colour reaction at all.

3.4 Paper Chromatography

Single dimension paper chromatography was used to separate the flavonoid constituents of the methanolic plant extracts. Eight spots were visible on the chromatograms after spraying with 3% aluminium chloride in methanol, fuming with ammonia and visualising under ultra violet light. R_f values were calculated for these spots using the formula: distance travelled by the trace (measured to the centre) divided by the distance travelled by the solvent. These are shown on Table 20. The pure flavonol glycoside, Rutin, was used as a marker on all chromatograms and the R_f value of this was calculated to be 0.38.

TABLE 20

R_f Values for the 8 Spots counted on Paper Chromatograms

Spot No.	1	2	3	4	5	6	7	8	Rutin Mark.
R_f Value	0.25	0.30	0.38	0.40	0.47	0.50	0.55	0.64	0.38

Spot 2 is a brown or grey-brown colour after spraying and fuming which possibly indicates the presence of a flavone (Harborne 1984). Spots 3 and 4 are close in R_f value and colour to the marker Rutin and may be this compound. The other 5 spots are wide enough apart to be distinct. The presence of each spot for each plant extract tested is shown on Table 21.

TABLE 21

Presence of flavonoid spots.

Voucher Specimen No.	Spot Number							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Senecio speciosus								
Lewis 65	+			+				
Lewis 102		+		+				
Phillipson 3823		+		+				
Senecio macrocephalus								
Lewis 82		+						
Firdene, Stoneshill								
Lewis 79		+		+				
Brackenhill, Howison's Poort								
Lewis 98		+		+				
1820 Settlers Monument								
Lewis 101		+		+				
Lewis 107 (Roots)		+						

Beggarsbush Outspan								
Lewis 104		+	+					
Lewis 106		+						
Mountain Drive								
Lewis 115		+		+				
Lewis 116		+						
Faraway, Coldsprings								
Lewis 118		+						
Other *Senecio* specimens collected at the coast								
Lewis 66	+			+		+		
Lewis 103		+		+		+		
Other *Senecio* specimens collected in the mountains								
Lewis 86	+			+				
Lewis 111				+				
Lewis 112		+		+				+
Phillipson 3820	+			+	+		+	
Phillipson 3822	+				+			+

Table 21 shows that no plant extract gave more than four spots. The extracts from *Senecio macrocephalus* only showed spot 2, and *Senecio speciosus* extracts showed spot 4 and either spots 1 or 2. The extracts of specimens from the Grahamstown area showed either spots 2 and 4 or spot 2 only. Other specimens tested gave their own distinctive spot arrangements.

CHAPTER 4 - DISCUSSION

4.1 Morphological and Geographical Data

This section of the project aims to show, using morphological analysis together with geographical distribution, whether *Senecio speciosus* and *Senecio macrocephalus* are taxonomically distinct species and, if this is so, that there is hybridisation between the two species where their ranges overlap in the Eastern Cape Province. There are five possibilities to be considered.

Firstly, as suggested by Hilliard (1977), that *Senecio speciosus* is a distinct species ranging from the South West Cape along the coast at least to the Fish River and possibly through the Transkei and into Natal. *Senecio macrocephalus* is also a distinct species ranging inland through the mountains from Natal to the Winterberg, Katberg and Amatole mountains and down to the King William's Town and East London areas. Where these two ranges overlap there is a complex of intermediate plants. These are considered to be hybrids.

The second possibility is that the whole complex, from the South West Cape to Natal and the Transvaal is a very variable single species showing a great deal of phenotypic plasticity. Solbrig (1970) states that the characters of the phenotype are a reflection of the interaction of the internal genetic factors of the plant with the environment. Phenotypic plasticity is defined as being the response of a population of plants to the environment. Over the wide range occupied by these populations there are many different environmental areas and conditions possibly leading to a great deal of phenotypic plasticity.

Thirdly that *Senecio speciosus* and *Senecio macrocephalus* both show a great deal of phenotypic plasticity which tends to blur the distinction.

Fourthly that in the Eastern Cape Province there is more than one point of contact between the ranges of these two species and at each point of contact different types of populations result. Some of these populations are considered to be hybrids.

The fifth possibility is that the whole complex is a single species with numerous genetically distinct races.

The description of *Senecio macrocephalus* by Harvey and Sonder (1865) together with the description of *S. speciosus* and *S. macrocephalus* by Hilliard (1977) define these as separate species. These two species can be identified from my present collection and from the Selmar Schonland Herbarium specimens (Appendix 1 and 2, Figures 4 and 5). These two species however only occur in certain areas and in other areas many intermediate forms are found which cannot be equated with Hilliard's concept of each species. Where the areas of distribution of the two species overlap the boundaries between the species tend to blur and populations showing characters of both species or intermediate characters occur. The hybrid index histograms, principal components analysis and scatter diagrams show this variation together with the two species (*S. speciosus* and *S. macrocephalus*) at either end of the graphs.

To consider the first possibility in more detail. The hybrid index histogram of the combined collections (Figure 6) shows a definite hybrid peak between 15 and 20 points and also a wide spread of specimens between the parents and the hybrid peak. This wide spread of plants indicates the presence of a hybrid swarm or large hybrid complex.

The hybrid peak on Figures 6-9 is skewed towards *Senecio speciosus*. As *S. speciosus* is more plentiful in the area and *S. macrocephalus* is near the end of its range this is not unexpected. On Figure 7 the large peak for *Senecio speciosus* and the definite skew towards this species indicates the possibility of introgressive hybridisation taking place. Figure 8 shows a collecting bias towards the intermediate populations as these specimens were collected mainly in the Grahamstown and East London areas. No typical *Senecio macrocephalus* plants were collected in these areas. The plants with the hybrid index scores of 9 were two *Senecio speciosus* specimens collected on the Menziesberg area of the Amatole Mountains and two specimens collected south west of Grahamstown towards the range of typical *S. speciosus*. It is suggested that only intermediate plants are present in these areas.

Figure 9 which is my own collection plotted using morphological data only gives a definite hybrid peak between 19 and 24 points with a certain amount of backcrossing indicated

particularly with the *Senecio speciosus* parent. When toxicity data is added to the morphological data and Figure 10 is constructed a definite skew towards *Senecio macrocephalus* is apparent with the hybrid peak at 24 - 29 points. Introgressive hybridisation towards *S. macrocephalus* may be indicated. The addition of toxicity data to Figure 9 to give Figure 10 shows that although morphological data alone places the intermediate populations closer to *Senecio speciosus* the addition of chemical evidence may prove this to be different.

Further evidence for this first possibility is shown on Figures 11-13. These maps are divided into areas A1 - A6 taking into account hybrid index scores, and geographical features. Only *S. speciosus* is found in Area 1. It is assumed that *S. macrocephalus* localities are too far away for hybridisation to take place. Area 2 contains *S. speciosus* and low scoring intermediates close to *S. speciosus*. In the Grahamstown area (Area 3) there are only intermediates present. Low scoring intermediates are found west of Grahamstown and southwards towards the coast. Higher scoring intermediates are found at Stoneshill and Beggarsbush Outspan on the King William's Town road. High scoring intermediates together with *S. macrocephalus* specimens are found in Area 4.

Since only apparent hybrids are present in the Grahamstown area it is important to dicuss how *S. speciosus* and *S. macrocephalus* genetic material came into the area.

It is recognised that plants and animals migrate along well defined corridors. In Britain recently the Tyne Tees Planning Authority has recognised definite plant and animal corridors within its jurisdiction and has banned further development in these areas (Professor C. Lewis, Dept of Geography, Rhodes University, pers. comm).

Migration routes and corridors are environmentally determined. Coastal lowlands and river catchment areas between coastal lowlands and wetter uplands are potential migration routes. There appears to be a corridor, a two-way migration route, along the Buffalo River catchment as demonstrated by the specimens collected in this area (Figure 11). *Senecio speciosus* and *Senecio macrocephalus* are usually found on grassy ridges above the river valleys and within the river catchment areas. There are other such river catchments in this area which are not so well collected. These could also be migration routes. There is also the possibility of a

coastal migration along the Southern Cape Coast, into the Eastern Cape coastal regions and on into the Transkei and Natal. Suggestions for potential migration routes are shown on Figures 18 and 19.

Figure 18.
Map showing possible migration routes in the Eastern Cape, tentatively suggested according to the morphological similarities of the specimens indicated by the hybrid index scores and environmental factors as discussed in the text.

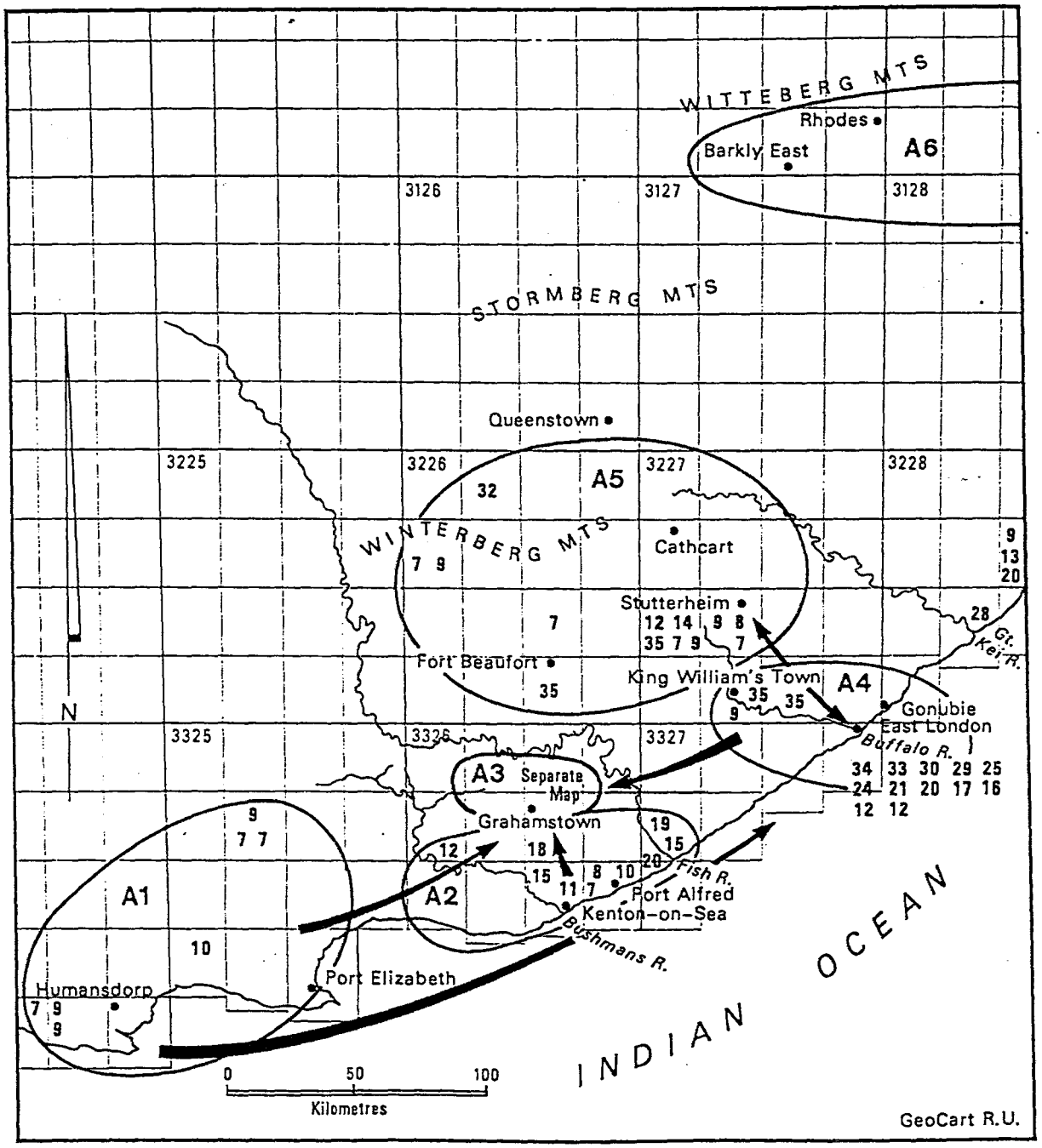


Figure 19.
Map showing possible migration of *Senecio speciosus* and *Senecio macrocephalus* towards Grahamstown tentatively suggested according to the spread of Hybrid Index Scores.

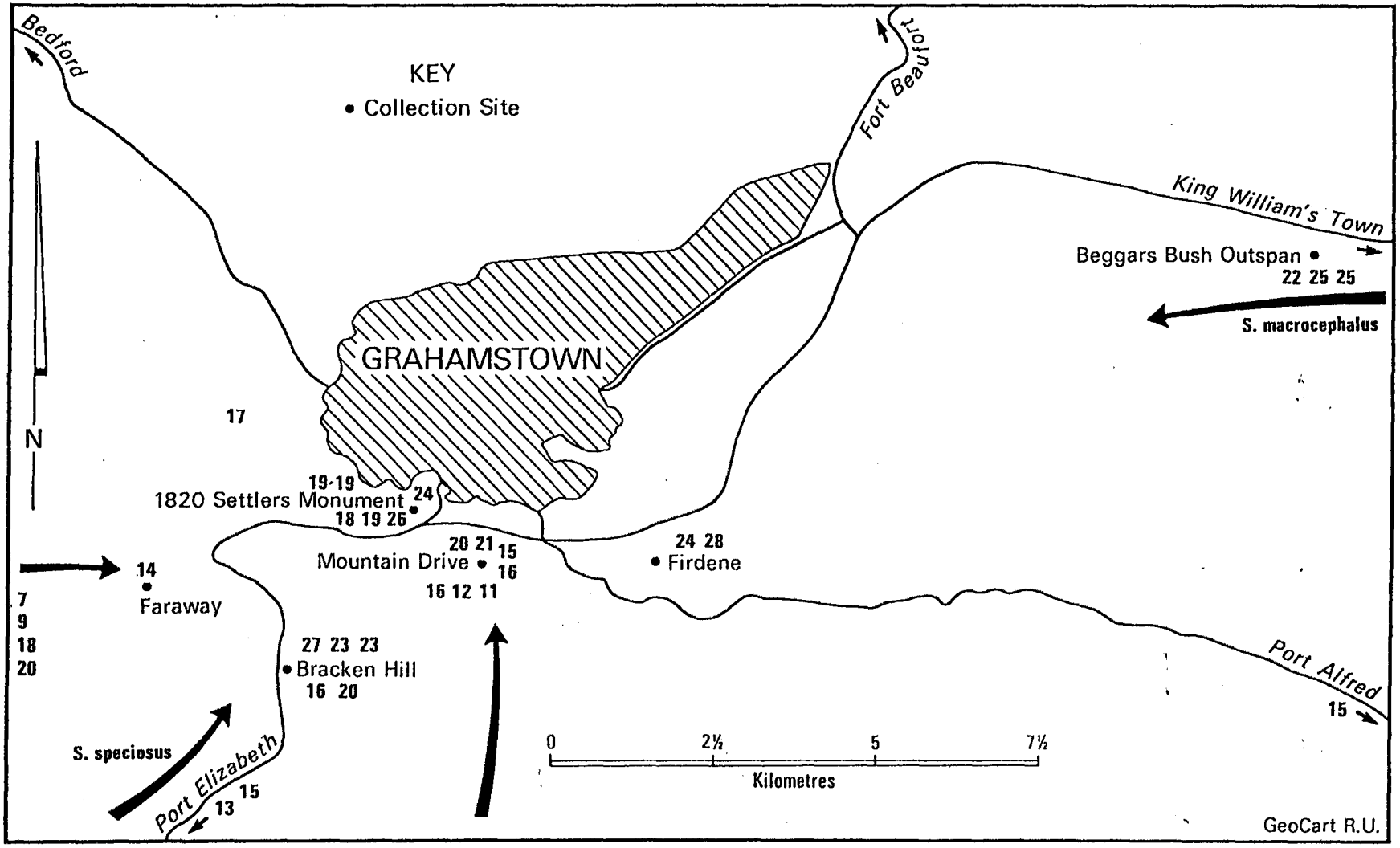


Figure 18 shows the Buffalo River corridor and the suggested coastal migration route. There could have been migration of genetic material into the Grahamstown area via the Bushmans River Valley, the Kariega River Valley between Kenton-on-Sea and the Grahamstown area, and the Kowie River Valley between Port Alfred and Grahamstown at a time when the climate was wetter than at present. The arrows between areas 1, 2 and 3 and areas 4 and 3 link morphological similarities between plants in these areas. *Senecio speciosus* and low scoring intermediates are located in areas 1 and 2 and on the west side of area 3. High scoring intermediates are located on the East side of Grahamstown (Area 3) and *Senecio macrocephalus* is found in the King William's Town area. Possible migration routes towards Grahamstown are shown on Figure 19.

The intermediate populations in the East London area (Area 4) also appear to be hybrids with some specimens being close to *S. macrocephalus*. If there is, as suggested, a migration corridor along the Buffalo River then the *S. macrocephalus* parent material may have come from the King William's Town area. *Senecio speciosus* genetic material may have come eastwards along the coast.

The second possibility, that all the populations studied are one very variable species showing a great deal of phenotypic plasticity does not seem likely. The morphological features of the populations in the Grahamstown area are similar to those of the coastal populations but the plants are larger. This could appear to be phenotypic plasticity in response to environmental changes from a coastal, more exposed, habitat to the inland, less exposed areas. However the sudden appearance in the East London area of specimens with oblong to oval leaves (*S. macrocephalus* type) poses a problem. This could be construed as phenotypic plasticity in a population of plants originating further eastwards in the Transkei but then it is apparent that there are two distinct populations or species of *Senecio* involved. This is the third possibility, where two species of *Senecio* are recognised but the intermediate populations of plants are thought to be due to phenotypic plasticity. This cannot be proved or disproved on the morphological evidence alone.

The fourth possibility also cannot be proved on morphological evidence alone but this is postulated to be the most likely. The intermediate populations around Grahamstown show

distinct characteristics particularly regarding leaf shape and leaf margin. Leaves are elongated and spathulate with deeply lobed margins, sometimes coarsely toothed. These specimens appear to be derived from *Senecio speciosus* and a somewhat more distant *S. macrocephalus* parent. The intermediate populations in the East London area also have distinctive leaf shapes and margins. Leaf shape is oblong to elliptical, sometimes almost rounded and the margins are entire and shallowly toothed. These plants appear to be derived from a closer *S. macrocephalus* parent and a more distant *S. speciosus* parent. It is postulated that the populations in the Grahamstown area arose from *S. speciosus* parents migrating inland from the coast and *S. macrocephalus* parents migrating westwards from the King Williams Town area, and the population in the East London area arose separately from *S. speciosus* parents migrating eastwards along the coast and *S. macrocephalus* parents migrating along the Buffalo River Corridor from King Williams Town.

The third point of contact known at present is the Menziesberg area of the Amatole Mountains. Here both species are present, together with *Senecio* sp. aff. *S. speciosus* but no hybridisation appears to take place. Species in this area seem to have a reproductive isolating mechanism and do not appear to be able to interbreed. These species are growing sympatrically, that is, growing together without losing their identity by hybridisation. Plants collected on the Menziesberg area of the Amatole Mountains, and cultivated in potting soil for two years did not flower in Grahamstown although they grew well and reproduced vegetatively. There are, therefore, three different possible points of contact between these two species and three different resulting populations.

Phenotypic plasticity, however, does occur within the intermediate populations. According to Jones and Luchsinger (1989) local populations of species of flowering plants may be very variable. Plants occupying territories that are broken into mosaics of sharply distinct ecological conditions tend to have mosaic variation. Mosaic pattens are often related to soil, moisture, aspect and other environmental factors. The specimens in the Grahamstown area occupy several different ecological niches, from the exposed hilltop of Mountain Drive where the plants are small, very similar to *S. speciosus* and can only be distinguished by chemical means, to the sheltered environment at Howison's Poort where the plants are very large possibly due to environmental conditions or to hybrid vigour. And from the 1820 Settlers

Monument where the plants are short and stumpy, possibly due to frequent mowing, to Beggarsbush Outspan where the specimens are tall and spreading, growing in long grass or straggling along the edge of the kranz.

Principal components analysis and the scatter diagram are a further basis for discussion. All three principal components analysis diagrams show definite groups of dots at either end of the X axis corresponding to the specimens identified as *Senecio speciosus* on the left hand side and *Senecio macrocephalus* on the right hand side. There is a wide spread of dots arranged round the X and Y axes suggesting intermediate plants showing a great degree of variation. Principal components analysis is used to transform a set of highly correlated characters to a new set of uncorrelated characters. The principal components are simply the new uncorrelated characters (Radford *et al.* 1974). This type of multivariate analysis yields patterns of dots arranged round axes of a highly derived kind (Briggs and Walters 1986). These diagrams may be over complex and unsatisfactory when dealing with a wide range of variation, however certain trends are apparent from the present analysis. The definite groupings of dots at either end of the X axis suggest that *Senecio speciosus* and *Senecio macrocephalus* are distinct and separate species. From these groups of dots there is a gradation in morphological characters from left to right across the diagram. Attempts to correlate any particular character with any particular position on the diagram did not succeed. Two possible explanations for the gradation of characters across the principal components analysis diagrams are offered. Firstly, as the variation is numerically from the left hand side (*S. speciosus*) increasing towards the centre and from the right hand side (*S. macrocephalus*) decreasing towards the centre this could suggest phenotypic plasticity in the two distinct species with specimens spreading into an area between the ranges of these two species. This is the third possibility discussed earlier.

Secondly, as in the first possibility discussed, the intermediate populations could be hybrid populations between the two distinct species forming a large hybrid complex. The true F_1 hybrids would be located closest to the Y axis and hybrid index scores would decrease towards the left hand side of the diagram and increase towards the right hand side as backcrossing towards the parent species occurred. This could be postulated to be the case with these diagrams.

The scatter diagram (Figure 17) plotting diameter of heads against number of rays brings out a distinct grouping of *Senecio* sp. aff. *S. speciosus* specimens. It is suggested that this should be regarded as a separate species. *Senecio speciosus* specimens are generally grouped on the left of the diagram and *Senecio macrocephalus* specimens are scattered towards the right hand side. The intermediate specimens are centrally situated but also spread and intermingle between the *S. speciosus* and *S. macrocephalus* specimens. Some of the intermediate specimens have a greater head diameter and more ray florets than typical *Senecio macrocephalus* and appear at the top right of the diagram. These specimens are those collected at the Fish River Mouth and in the East London area. Specimens from the Grahamstown populations intermingle mainly with *S. speciosus* towards the left hand side of the diagram.

Phenotypic plasticity cannot definitely be ruled out at this stage but it is suggested that this diagram demonstrates the fourth possibility discussed, that *S. speciosus* and *S. macrocephalus* come into contact at more than one point in the Eastern Cape and at each point of contact hybrids showing different characteristics arise. The Grahamstown intermediate populations are shown to be morphologically closer to *Senecio speciosus* intermingling with that species and indicating the possibility of introgressive hybridisation in this area. The East London intermediate populations are closer to and sometimes have heads larger than *S. macrocephalus*. This suggests the phenomenon of hybrid vigour or heterosis in these populations. According to Briggs and Walters (1986), such hybrid plants are characteristically of great vegetative vigour and high fertility.

It is possible therefore using morphological and geographical data to suggest various relationships between *S. speciosus, S. macrocephalus* and the intermediate populations. No definite proof can be offered at this stage as to the hybrid status of the intermediates.

4.2 Toxicity Tests

The aim of this section of the project is to ascertain whether toxicity data from assays such as the brine shrimp test can be used as genetic markers in taxonomic studies. If this is so, do the results of the brine shrimp test conducted using extracts from *Senecio speciosus*, *Senecio macrocephalus* and their intermediate populations provide any information on the taxonomic relationships of these species? Table 9 shows the percentage deaths of brine shrimps after 24 hours using methanol extracts of *Senecio speciosus* specimens. A mean death rate of 0.77% was calculated, most of the specimens giving no deaths at all. Table 10 shows percentage deaths of brine shrimps over the same time and dilutions using extracts from *Senecio macrocephalus* specimens. Here a 95% overall death rate was recorded with the more concentrated solutions giving 100% mortality. Table 11 gives death rates when the specimens collected in the Grahamstown area were tested. Here the mean death rate over all the specimens tested was 92% again with the more concentrated solutions giving 100% mortality.

Kubitzki (1984) cites Levin (1976) as stating that closely related plant species tend to deploy the same biogenetic group of metabolites as key chemical barriers, that is, toxic chemicals, in their defense systems. It appears here that a metabolite or group of metabolites, highly toxic to brine shrimps is deployed by *S. macrocephalus* and that these metabolites are not found in *S. speciosus*. These metabolites appear to be present in all the extracts from specimens collected in the Grahamstown area.

It is suggested therefore that since secondary metabolites are said to be under genetic control (Hegenaur 1963, 1966; Alston and Turner 1959; Nowacki 1963), toxicity to brine shrimps can be used as a genetic marker in this study. It appears that the toxicity of plant extracts to brine shrimps can even be regarded as a "good" character in taxonomic terms. Briggs and Walters (1986) state that characters which show phenotypic plasticity are regarded by taxonomists as "bad" characters. "Good" characters are those that are least phenotypically variable and show discontinuity in variation. Methanol extracts of *Senecio speciosus* and *Senecio macrocephalus* plants cultivated in potting soil for six months showed the same toxicity to brine shrimps as extracts from freshly collected material (Tables 9, 10 and 14).

Also extracts of intermediate specimens collected in six different localities in the Grahamstown area and from a range of habitats (See Chapter 4.1) all showed similar levels of toxicity to the brine shrimps (Table 11). These two observations suggests that toxicity of *S. speciosus* and *S. macrocephalus* extracts is not under phenotypic control.

Percentage death rates of brine shrimps after 24 hours using extracts of specimens from the East London Area are shown on Table 12. Here again the more concentrated solutions give nearly 100% mortality, the mean death rate being 80.9%. Lewis 66 is not included in this calculation as it only showed 45% toxicity to the brine shrimps at the highest concentration. This specimen was collected at the edge of the road near Port Alfred and was the only *Senecio* plant in sight. It is suggested that this is a spurious plant, possibly brought into the area as seed in road making material. All results from this plant will be treated with caution.

Table 13 shows the percentage deaths of brine shrimps after 24 hours when tested with extracts from other *Senecio* species. Toxicity levels here differ from those displayed by *Senecio speciosus*, *Senecio macrocephalus* and the intermediate populations. *Senecio inaequidens* and *Senecio elegans* showed 2.0% and 3.6% toxicity respectively, Phillipson 3822, a plant with red discoid heads and sticky leaves which could not be identified as any known species using Hilliard (1977) or matched with any other species in the Selmar Schonland Herbarium shows 8.3% toxicity. *Senecio barbatus* and Lewis 112, a specimen from Naude's Nek each show 11.6% toxicity. *Senecio polyodon* show 100% toxicity throughout and *Senecio radicans* 0% toxicity. Levin (1976) cited by Kubitzki (1984) suggested that distantly related species deploy different chemical compounds in their defense systems. It is possible that this is the case with these species of *Senecio* since there is a wide range of toxicity levels to the brine shrimps.

The plants which had been cultivated in potting soil for six months were harvested and methanol extracts were also tested on the brine shrimps (Table 14). The results from leaf extracts were very similar to those given by freshly collected plant material. However, some specimens gave higher death rates than expected. Extracts from root material of Lewis 86A and Phillipson 3823 and extracts from both leaf and root material of Phillipson 3822 gave high death rates. The rise in toxicity of extracts from these particular specimens could be due

to several factors, environmental, stress or genetically related. Lewis 86A (*Senecio barbatus*) appeared to be under stress when cultivated in Grahamstown as after 9 months all the plants died. It is suggested that this could be due to environmental influences such as a difference in relative humidity between the Menziesberg and Grahamstown. The increase in toxicity could be due to an increase in secondary metabolites produced as a mechanism to combat stress. Phillipson 3822 is a plant so far unidentified and possibly an undescribed species. Chemical results in this study point to this possibility and this will be discussed further in Chapter 4.3.

When toxicity was used as a genetic marker in this study certain new relationships between *Senecio speciosus, Senecio macrocephalus* and the intermediate populations become apparent.

Since *Senecio speciosus* is non toxic and the intermediate populations are nearly 100% toxic the intermediate populations cannot be phenotypic variants of *Senecio speciosus*, the discontinuity in the toxicity character is too sudden and complete. Neither can the intermediate populations, at least those in the Grahamstown area, be *Senecio macrocephalus*. Even though the toxic compounds are present in both *Senecio macrocephalus* and the intermediate populations the morphological characters, particularly those of leaf shape and margin are very different.

In the Grahamstown area therefore the intermediate plants appear to be a hybrid population between *Senecio speciosus* and *Senecio macrocephalus* having morphological characters similar to *S. speciosus* and the toxicity character of *S. macrocephalus*. This is not enough evidence however to provide proof of hybridisation. A comparative chromatographic study of the plant extracts is needed to provide further evidence and to show the position of the East London populations.

4.3 Thin Layer Chromatography

4.3.1 Comparative Alkaloid Content

This section of the project is designed to demonstrate the use of thin layer chromatography in taxonomic studies by showing similarities and differences between extracts of *Senecio*

speciosus, *Senecio macrocephalus* and the intermediate populations. No attempt was made to identify the alkaloids present, chromatography was used purely as a comparative technique. Harborne (1984) states that the value of chemistry in solving problems of hybrid identification lies in the likelihood that the various putative parents have different chemical profiles so that it may be obvious from a chemical analysis that compound characteristics of two particular parental species both appear on the chromatogram of the hybrid.

The results shown on Tables 16-19 for the 11 recorded spots show variation between species and also within species. However all plant extracts were chromatographed several times and results for each plant extract are constant. Variability in alkaloid content of related plant species is discussed by Jeffrey *et al.*(1979) citing Stebbins (1963) and Swain (1963) who state that difference in chemical constituents of plants, particularly differences in alkaloid content, may be due to environmental influences on the plant. Environmental influences can differ from place to place and also from time to time.

Specimens Lewis 65 and Lewis 102 are both *Senecio speciosus* collected from the same area at Kasouga but 12 months apart. Table 16 shows that extracts from Lewis 65 gave three fewer alkaloid spots than extracts from Lewis 102 although they both have six spots in common. (spots 1, 3, 6, 7, 9 and 11). Phillipson 3823 is *Senecio speciosus* collected from the Menziesberg area of the Amatole Mountains and extracts from this plant show spots number 1, 3, 6, 7 and 10. The common feature in all these specimens is the absence of spot number 8.

After cultivating in potting soil for six months the extract from the *Senecio speciosus* from the Amatole Mountains (Phillipson 3823) showed spots 1, 3, 4, 5, 6, 10 and 11. Lewis 96, thought to be *Senecio* sp. aff *S. speciosus* showed spots 3, 4, 5, 9, 10 and 11. Again spot 8 is not present but there is a definite orange (alkaloid) reaction at spot 10 when sprayed with Dragendorff reagent. Extracts from *Senecio macrocephalus* (Lewis 82) showed spots 1, 3, 4, 5, 6, 8 and 10 and after cultivating in potting soil for six months (Lewis 82A) a similar spread of spots is seen (spots 1, 3, 4, 5, 8, 10 and 11). Extracts of root material of Lewis 82A gave spots 1, 3, 4, 6, 8 and 10. These extracts all show the presence of spot 8, absent in *Senecio speciosus* and the absence of spots 7 and 9 generally present in *S. speciosus*. It

is already apparent therefore that, using thin layer chromatography certain differences between these two species can be demonstrated.

Extracts from the intermediate specimens collected in the Grahamstown area (Table 17) show a variable spread of spots. Some show spot 7, absent in *S. macrocephalus* but present in freshly collected *S. speciosus*. All chromatograms show spot 8, absent in *S. speciosus* and present in *S. macrocephalus*. Spot 9 is absent in all these chromatograms as in *S. macrocephalus*. Extracts of specimens from Beggarsbush Outspan and Mountain Drive all lack Spot 7 and appear chemically to be very similar to *S. macrocephalus*, although their morphological characters place them closer to *Senecio speciosus*.

The intermediate populations in the Grahamstown area therefore appear to be hybrids as they contain chemical constituents from either or both parents but generally more from *Senecio macrocephalus* and their morphological characters are generally closer to *Senecio speciosus*. The amount of variation within specimens tested, both chemically and morphologically seems to demonstrate that continous crossing has taken place both within the hybrid population and with the parent species.

Extracts from the roots of Lewis 51 collected from the frequently mown lawn area in front of the 1820 Settlers Monument in Grahamstown show a definite alkaloid colour reaction (orange) at R_f value 0,16 (Spot 2). This is thought to be due to the plant's defense mechanism. A specific alkaloid may be synthesised by the plant as a defense against damage, in this case by the lawnmower. R_f value 0,16 is at the base of the retrorsine marker spot on these plants. Grue (1991) found plants containing retrorsine on a heavily grazed area at Slykraal near Grahamstown and Jeffrey *et al.*(1979) stated that plants can synthesise alkaloids as a protection against grazing. The lawnmower, it appears, is just another type of grazer!

Extracts from the specimens collected between Port Alfred and East London show certain trends (Table 18). Lewis 103, Lewis 117 and McCartan 3 all collected at East London nature reserves have spot 8 on their chromatograms, linking them with *Senecio macrocephalus*. In Lewis 103 and McCartan 3, spot 7 is also present linking these specimens also to *S. speciosus*. These East London nature reserve specimens therefore also appear to be hybrids.

Lewis 64 (*Senecio elegans*) has its own completely different chemical profile and the extract from McCartan 13 shows spot 7 linking this with *S. speciosus*. This is a plant collected at the Fish River Mouth which shows high toxicity to brine shrimps, linking it to *S. macrocephalus*. It is suggested therefore that this specimen is also a hybrid.

Details of chromatograms of extracts from other mountain species are given on Table 19. Phillipson 3820 gives a constant chemical profile both when freshly collected and after cultivation in potting soil for 6 months. This specimen has been identified as *Senecio erubescens*. Extracts of Phillipson 3822 which appears to be a separate and undescribed species also show a unique chemical profile having spots at 7 and 8 or 7, 8 and 9 with an alkaloid colour reaction at spots 8 and 9.

Lewis 86 is *Senecio barbatus* collected from the Menziesberg area of the Amatole Mountains but some distance from the other specimens and on a mountain top. Extracts from these plants give a definite orange colour reaction at R_f value 0.16 and no spot 8 or 9. This colour reaction is in the same position as that given by the root material of Lewis 51. It has already been suggested that these *Senecio barbatus* plants were under stress while being cultivated in Grahamstown as extracts showed high toxicity to brine shrimps and all the plants died after 9 months. It is suggested that the production of alkaloid giving a colour reaction at spot 2 may have been the result of stress.

Lewis 94 is *Senecio radicans* which also shows its own unique chemical profile. This species is not closely related to the others under study and belongs to a group of species that have now been excluded by some taxonomists from *Senecio* and placed in the genus *Kleinia* (Jeffrey 1986).

Lewis 111 is *Senecio polyodon*. Extracts of this specimen shows a similar arrangement of spots to *Senecio macrocephalus*. Although extracts from both species are highly toxic to brine shrimps the plants show some clear morphological difference and *Senecio polyodon* has been confused with *S. speciosus* (Hilliard 1977).

Extracts from Lewis 112 shows spots 7, 8 and 9 making this appear as an intermediate specimen as indeed do its morphological characteristics. However the brine shrimp tests only show a low toxicity level. Naude's Nek is a high mountain pass between the North East Cape Drakensberg, the Transkei and Lesotho. More specimens need to be collected from this area and analysed to provide information on the relationships of this plant.

It is noted throughout these results that extracts from root material generally give fewer spots than extracts from leaf material. It may be that roots being subjected to less stress than the aerial parts of the plant need to deploy fewer alkaloids.

To draw together the threads followed in this discussion it is necessary to look at tables 16 - 19 where similarities and differences in the plant extracts can be clearly seen. Spots 1 - 6 are seen to vary within and between species as would be expected in alkaloid producing plants. However spot 8 is always absent in *Senecio speciosus* and is always present in *Senecio macrocephalus*. Spots 7 and 9 are always absent in *S. macrocephalus*. Extracts from the intermediate populations in both the Grahamstown and East London areas show spot 8 and sometimes spots 7 and 9. Since these populations contain chemical constituents from both putative parents it is suggested that they are hybrids. Other species studied are seen to have their own distinct chemical profiles.

4.3.2 Comparative Terpenoid Content

Colour reaction due to terpenoids on these chromatography plates only occurred in the basal spots showing that terpenoid compounds had not separated out. Other solvents were used and no movement of terpenoid compounds was shown. It was decided therefore to list the specimens which had shown a brown colour reaction in the basal spot and those which had not.

The specimens which gave positive results were *Senecio macrocephalus* ((Lewis 82) and the three specimens from the intermediate populations that were tested (Lewis 57 root material, Lewis 79 and Lewis 101). The intermediate specimens however did not give such a strong

colour reaction as *Senecio macrocephalus*. *Senecio speciosus* specimens tested (Lewis 65 and Phillipson 3823) gave negative reactions in line with Bohlmann's results (1979) showing no sesquiterpene compounds in *S. speciosus*.

If there are, as seems likely from these preliminary tests, terpenoid compounds in *Senecio macrocephalus* and also in the intermediate populations this is another chemical character linking the intermediate populations to *Senecio macrocephalus*.

4.4 Paper Chromatography

The eight spots visualised when paper chromatography was used to separate the flavonoid constituents of the methanolic plant extracts are shown on Table 21. No extract gave more than four spots. Extracts from *Senecio macrocephalus* only showed spot 2 and extracts from *Senecio speciosus* showed spots 1 or 2, and 4. This again distinguishes between the two species.

Extracts from the specimens collected in the Grahamstown area gave sometimes spots 2 and 4 or sometimes spot 2 only, thus linking these plants with both *S. speciosus* and *S. macrocephalus*.

The extract from Lewis 66 showed spots 1, 4 and 6 and the extract from Lewis 103 showed spots 2, 4 and 6. These are specimens collected on the coast, at Port Alfred and East London. Spot 6 is not obtained from any other extract and this appears to show that the intermediate populations in the East London area are different from the intermediate populations in the Grahamstown area although both may be hybrids between *S. speciosus* and *S. macrocephalus*.

Specimens of other *Senecio* species collected in the Menziesberg area of the Amatole Mountains (*S. erubescens* and *S. barbatus*) and in the north-eastern Cape Drakensberg (*S. polyodon*) each have their own specific chemical profile again showing each to be a distinct species.

This simple separation of flavonoids has distinguished between *Senecio speciosus* and *Senecio macrocephalus* and linked the Grahamstown populations to both these species. It has also shown that other species of *Senecio* tested have their own specific chemical profiles for flavonoids as for alkaloids. Since the intermediate populations in the Grahamstown area contain chemical constituents from either or both *Senecio speciosus* and *Senecio macrocephalus* it is suggested that this is yet another piece of evidence pointing to their hybrid status.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

This project has aimed to study the variation in the populations of *Senecio speciosus*, *Senecio macrocephalus* and intermediate populations in the Eastern Cape region and to correlate morphological and geographical data with toxicity tests and chromatography carried out on extracts from these species.

Kubitzki (1984) states that where chemosystematics complements plant population studies, deep insights into the nature and dynamics of the population are possible that cannot be attained on the basis of morphological analysis alone.

In this study morphological analysis has demonstrated the separation of *Senecio speciosus* and *Senecio macrocephalus* as distinct species at either end of the hybrid index histograms and principal components analysis diagrams. These two species are also located, geographically, at either end of the study area apart from the populations on the Menziesberg area of the Amatole Mountains. Here three species, *Senecio speciosus, Senecio macrocephalus* and *Senecio* sp. aff. *S. speciosus* grow sympatrically.

Maps and diagrams show intermediate populations of plants displaying a great amount of morphological variability. Hybrid index histograms show a definite hybrid peak not quite centrally situated between *Senecio speciosus* and *Senecio macrocephalus* with morphologically variable specimens spreading between the hybrid peak and the two species. In all cases where morphological data alone is plotted the hybrid peak is skewed towards *Senecio speciosus*. It is postulated that *Senecio speciosus* and *Senecio macrocephalus* are distinct parent species and that the peak shows the intermediate plants of hybrid origin. These are not necessarily the F_1 generation, and it is suggested that hybridisation, including backcrossing to the parent species, has been occurring for many generations. The skew on the hybrid peak suggests stronger selection for *S. speciosus* morphological characteristics, and it is worth noting that the current geographical range of this species is closer to the Grahamstown area than that of *S. macrocephalus*. The range of variation between the hybrid peak and the parent species shows that genetic recombination has taken place. It is also possible that backcrossing with the parent species has occurred.

Briggs and Walters (1986) suggest that there is selection pressure in hybrid populations and cite observations by Stebbins and Daly (1961) on a hybrid population of *Helianthus*. Here the population changed remarkably, as shown by hybrid index histograms, over the 8 years of the study, both morphologically towards one or other of the parents and in terms of increasing fertiity over the years. The intermediate populations in the Grahamstown area appear to be highly fertile. It has been noted that all the populations are increasing. That this is not due to vegetative apomixis is shown by the observation of seedlings arising in new locations including one specimen on the garage roof at Brackenhill.

From the survey of herbarium specimens it is apparent that intermediate specimens have been collected in the Grahamstown area since 1928, for example, Dyer 1807 (1928); Lawrence 40 (1947); Noel 943 (1953); Heeg 212 (1962); Francis 66 (1963) and Whitty 19 (1965). It is therefore suggested that the populations of intermediate plants in this area are a hybrid complex where crossing has been occurring over a long period of time giving the great degree of variation that is seen, the selection pressure towards *Senecio speciosus* and the high fertility observed. A similar scenario is seen in the East London populations however there the selection pressure appears to be towards *S. macrocephalus*.

Hybridisation is not established on such morphological analyses alone. A method of establishing the presence of hybrids is by crossing possible parent species to produce an F_1 generation and then allowing these offspring to interbreed giving an F_2 generation with a high degree of variability. This method was not used in this project for several reasons. Firstly, there is a time constraint on this type of project. The chemical analysis and toxicity tests around which this project was conceived are time consuming as are breeding experiments. Furthermore these populations of intermediate plants are believed to have been in this area for nearly 70 years. The possibility of locating the parent stock of these populations and producing similar offspring was minimal in the time available. However *Senecio speciosus* plants from Kasouga and *Senecio macrocephalus* plants from the Menziesberg area of the Amatole Mountains were cultivated in Grahamstown with this possibility in mind. The *Senecio speciosus* from Kasouga flowered readily but the *Senecio macrocephalus* would not flower. When placed in controlled environment rooms in an attempt to initiate flowering the plants died. It was therefore decided to abandon any idea of breeding experiments and to

concentrate on chemical analyses in order to determine the status of the intermediate populations.

Chemical evidence, as suggested by Kubitzki (1984) gives a clearer picture of the relationships between these species than using morphological evidence alone. Toxicity to brine shrimps has in this project been identified as a characteristic of *Senecio macrocephalus* and the intermediate populations but not of *Senecio speciosus*. Other species of *Senecio* used as controls in the toxicity and chromatography studies have different levels of toxicity to the brine shrimps indicating that their chemical profiles are different from those of *S. speciosus*, *S. macrocephalus* and the intermediate populations. Figure 8, a hybrid index histogram using toxicity to brine shrimps as the 8th character scored, shows the hybrid peak to be skewed towards *Senecio macrocephalus*. It is suggested that this demonstrates the hybrid nature of the intermediate populations as morphologically they appear to be most similar to *Senecio speciosus*, but when chemical evidence is added they appear also to have an affinity with *Senecio macrocephalus*.

Evidence from the chromatographic analysis also suggests a hybrid nature for these plants. Thin layer chromatography for comparative alkaloid contents shows a variety of combinations of spots 1-6. Grue (1991) also showed a variety of alkaloids in these populations. Kubitzki (1984) states that much of the immense diversity of secondary metabolites appears as a result of reciprocal evolution between plant populations and their herbivores or pathogens. It is suggested that this is shown in the production of strong alkaloid reactions in some of the specimens tested. Different populations growing in different areas are reacting to environmental stimuli and producing different combinations of alkaloids. However there are definite chemical markers in *Senecio speciosus* and *Senecio macrocephalus*. *S. macrocephalus* extracts always show spot 8. *Senecio speciosus* extracts never show spot 8 but always show spots 7 and/or 9. *Senecio macrocephalus* extracts do not show spots 7 or 9. It is suggested that the chemical compounds visualised at spots 7, 8 and 9 on these chromatograms can also be used as genetic markers to distinguish between *Senecio speciosus, Senecio macrocephalus* and the intermediate populations. These spots clearly distinguish between *S. speciosus* and *S. macrocephalus* and place the intermediate populations as hybrids containing chemical markers from both the parent species.

The variety in chemical constituents of extracts from the specimens collected on the Menziesberg area of the Amatole Mountains has been noted. Kubitzki (1984) cites Waterman *et al.* (1978) who, in a chemical analysis of West African Rutaceae found that species with similar alkaloid and triterpene chemistry have different ecological ranges while the species co-existing with each other are chemically diverse. There appears to be much pressure for each species to be different from other species growing in the same area in the chemical weapons they deploy. The species found growing sympatrically on the Menziesberg do indeed have distinct chemical profiles.

Paper chromatography to demonstrate the flavonoid constituents of these plant extracts also distinguishes between *Senecio speciosus* and *Senecio macrocephalus* and indicates the hybrid status of the intermediate populations. These populations contain flavonoids shown by either or both of the parent species. Again the control species have their own distinct flavonoid profiles. Therefore using morphological and geographical data, toxicity studies and chromatography the aims and objective of this study have been fulfilled.

Senecio speciosus and *Senecio macrocephalus* can be regarded as distinct species on both morphological and chemical evidence. Morphological analysis places these species at either end of hybrid index histograms and principal components analysis diagrams. Chemical analysis for alkaloids, flavonoids and terpenoids gives each species a distinct chemical profile and toxicity tests using brine shrimps show that *Senecio macrocephalus* extracts contain chemical compounds which are 100% toxic to the shrimps but *Senecio speciosus* extracts do not contain these compounds and are non toxic to the shrimps.

Where the ranges of these species overlap in the Eastern Cape Province it can be concluded that hybridisation has occurred. Morphological evidence shows intermediate plant populations to have hybrid characteristics, that is characters of both parent species or intermediate characters. Chemical data shows the intermediate populations to contain chemical compounds from both parent species or, in the case of terpenoid content and toxicity to brine shrimps, to have morphological characters from one parent and chemical characters from the other. The hybrid populations may be recognised generally by their morphological characters, however where the plants are morphologically indistinguishable from *Senecio speciosus*, as

are some specimens from Mountain Drive and Faraway near Grahamstown, brine shrimp toxicity tests and chromatography can be used to ascertain their hybrid status.

It is further suggested that if these chemical techniques could be used on some of the early herbarium specimens collected in the Grahamstown area and identified as *Senecio speciosus* they too would be found to be hybrids.

Phenotypic plasticity displayed by the individual intermediate populations in response to microclimates in their particular habitats shows that environmental factors have an effect on the morphology of these species. Distribution of these species at present and in the past may be affected by changing climatic conditions. Wetter conditions than at present may be necessary for river valleys to act as migration routes. The width of meander belts and other geomorphological features in the Eastern Cape indicates that much wetter conditions prevailed during interstadials within the last Glacial stage (Lewis 1994). Considerable variations in precipitation are known to have occurred in the Eastern Cape during the Holocene, from c.10,000BP to the present (Coetzee 1967).

The brine shrimp toxicity data and chromatography profiles are crucial to this study as morphological evidence alone does not provide strong enough evidence of hybridisation.

When all the evidence is presented it is seen that these intermediate populations cannot be phenotypic variants of one or even two *Senecio* species. Both their morphological and chemical profiles strongly suggest that these populations are hybrids between *Senecio speciosus* and *Senecio macrocephalus*.

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Note: Further references to the Brine Shrimp Assay are in Appendix 3.

APPENDIX 1

Morphological Descriptions

The ten characters which were used in the morphological analysis of all the specimens collected are listed below. Hilliard's descriptions (1977) for *Senecio speciosus, Senecio macrocephalus* and *Senecio* sp. aff. *S. speciosus* are given first followed by the present collection in order of Voucher number. The specimens with Voucher numbers Phillipson 3820, 3821, 3822 and 3823 were collected on a joint expedition to the Menziesberg area of the Amatole Mountains. The specimens with Voucher numbers McCartan 3 and 13 are unwanted duplicate herbarium specimens kindly donated by the Selmar Schonland Herbarium.

Details of each specimen are set out as follows:

Voucher number. Locality. Grid Reference. Date collected.
Morphological characters 1 - 10.

Senecio speciosus (Hilliard, 1977)

1. Arrangement of leaves at flowering - mainly rosetted, few cauline.
2. Leaf shape - elliptical to spathulate.
3. Leaf margin - lobed and toothed, base narrow, petioled.
4. Inflorescence type - corymbose.
5. Diameter of heads - 12-15mm.
6. Number of rays - 8-12-14(-20).
7. Number of involucral bracts - 16-20.
8. Length of involucral bracts - 9-12mm (10).
9. Indumentum - short, glandular, hairs.
10. Achenes - cylindrical, pubescent between ribs.

Senecio macrocephalus (Hilliard, 1977)

1. Arrangement of leaves at flowering - mainly rosetted, few cauline.
2. Leaf shape - oblong to elliptical or spathulate, tapering to a broad, flat base, but scarcely petioled.
3. Leaf margin - entire, shallowly toothed.
4. Inflorescence type - racemose.
5. Diameter of heads - 15-20mm.
6. Number of rays - 12-18.
7. Number of involucral bracts - 16-20.
8. Length of involucral bracts - 10-15mm (12).
9. Indumentum - long, jointed glandular hairs.
10. Achenes - cylindrical with long white hairs.

Senecio sp. aff. *S. speciosus* (Hilliard, 1977)

1. Arrangement of leaves at flowering - mostly cauline.
2. Leaf shape - elliptical to spathulate.
3. Leaf margin - denticulate or toothed and denticulate, sometimes pinnately cut.
4. Inflorescence type - corymbose.
5. Diameter of heads - 10mm (may be radiate or discoid).
6. Number of rays - 8 (when present).
7. Number of involucral bracts - 12-14.
8. Length of involucral bracts - 7-9mm.
9. Indumentum - glandular, hairy.
10. Achenes - hairy.

Lewis 51. 1820 Settlers Monument, Grahamstown. 3326BC. October 1991.

1. Arrangement of leaves at flowering - mostly basal, rosetted, few cauline.
2. Leaf shape - spathulate, to elliptical, petiolate.
3. Leaf margin - toothed.
4. Inflorescence type - racemose to corymbose.
5. Diameter of heads - 20-25mm.
6. Number of rays - 12-14.
7. Number of involucral bracts - 18-20.
8. Length of involucral bracts - 12-15mm.
9. Indumentum -
   involucral bracts - medium length, jointed glandular hairs
   flowering stem - as above
   leaves - more or less glabrous.
10. Achenes - cylindrical, ribbed, very hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 53. Brackenhill, Howison's Poort, Grahamstown. 3326BC. November 1990.

1. Arrangement of leaves at flowering - large basal rosette, small, cauline leaves.
2. Leaf shape - spathulate, very long tapering to scarcely petioled base.
3. Leaf margin - slightly lobed and coarsely toothed.
4. Inflorescence type - immature, possibly will be corymbose.
5. Diameter of heads - 25-30mm.
6. Number of rays - 18-20.
7. Number of involucral bracts - 18-20.
8. Length of involucral bracts - 10-12mm.
9. Indumentum -
   Involucral bracts - long jointed glandular hairs.
   Flowering stems - long jointed glandular hairs.
   Leaves - few small glandular hairs.
10. Achenes - hairy (between ribs?)

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 54. Port Alfred Beach. 3326DB. October 1991.

1. Arrangement of leaves at flowering - no basal rosette, numerous small stem leaves.
2. Leaf shape - round to elliptical with long petioled base.
3. Leaf margin - coarsely lobed and toothed.
4. Inflorescence type - corymbose.
5. Diameter of heads - 20-25mm.
6. Number of rays - 12 (yellow disc).
7. Number of involucral bracts - 12.
8. Length of involucral bracts - 8mm.
9. Indumentum -
   involucral bracts - small-medium hairs
   stem - small-medium hairs
   leaves - more or less glabrous.
10. Achenes - many short hairs.

Identification - *Senecio elegans.*

Lewis 55. Brackenhill, Howison's Poort near Grahamstown. 3326BC. October 1991.

1. Arrangement of leaves at flowering - basal rosette, stem leaves and cauline leaves.
2. Leaf shape - Elliptical tapering to scarcely petioled base.
3. Leaf margin - lobed.
4. Inflorescence type - more or less racemose.
5. Diameter of heads - 25-30mm.
6. Number of rays - 14-16.
7. Number of involucral bracts - 16-18.
8. Length of involucral bracts - 12mm.
9. Indumentum -
   involucral bracts - medium and long hairs
   stem - short and medium hairs
   leaves - few hairs on margin only.
10. Achenes - hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 57. Firdene, Stoneshill near Grahamstown. 3326BC. October 1991.

1. Arrangement of leaves at flowering - basal rosette, few stem and cauline leaves.
2. Leaf shape - elliptical to spathulate with scarcely petioled base.
3. Leaf margin - coarsely toothed.
4. Inflorescence type - racemose below, corymbose above.
5. Diameter of heads - 30-40mm.
6. Number of rays - 12.
7. Number of involucral bracts - 18.
8. Length of involucral bracts - 12mm.
9. Indumentum
   - involucral bracts - short to medium length hairs
   - stem - short to medium length hairs
   - leaves - short hairs on midribs and margin.
10. Achenes - cylindrical, ribbed, hairy between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 64. Port Alfred Beach. 3326DB. October 1991.

1. Arrangement of leaves at flowering - no basal rosette, numerous small stem leaves.
2. Leaf shape - round to elliptical with a long petiole.
3. Leaf margin - coarsely lobed and toothed.
4. Inflorescence type - more or less corymbose.
5. Diameter of heads - 20-25mm.
6. Number of rays - 12 (NB. disc is yellow).
7. Number of involucral bracts - 12.
8. Length of involucral bracts - 8mm.
9. Indumentum -
   involucral bracts - numerous small/medium hairs
   stem - numerous small/medium hairs
   leaves - more or less glabrous.
10. Achenes - covered with numerous short hairs.

Identification - *Senecio elegans.*

Lewis 65. Kasouga. 3326DA. November 1991.

1. Arrangement of leaves at flowering - basal, rosette, few small cauline leaves.
2. Leaf shape - spathulate to elliptical with short petioled base.
3. Leaf margin - lobed and toothed.
4. Inflorescence type - corymbose - sometimes compound
5. Diameter of head - 15mm
6. Number of rays - 12
7. Number of involucral bracts - 16
8. Length of involucral bracts - 10mm
9. Indumentum -
   - involucral bracts - med - long jointed glandular hairs
   - flowering stems - med-long jointed glandular hairs
   - leaves - small glandular hairs
10. Achenes - cylindrical, ribbed, short hairs between ribs.

Identification - *Senecio speciosus.*

Lewis No. 65a. Kasouga. 3326DA. November, 1991.

1. Arrangement of leaves at flowering - basal rosette, few small cauline leaves.
2. Leaf shape - elliptical tapering to petioled base.
3. Leaf margin - finely lobed.
4. Inflorescence type - corymbose
5. Diameter of heads - 20mm
6. Number of rays - 12-14 (white)
7. Number of involucral bracts - 18
8. Length of involucral bracts - 10mm
9. Indumentum -
   - involucral bracts - few short glandular hairs
   - flowering stems - few short glandular hairs
   - leaves - some hairy, some nearly glabrous
10. Achenes - hairy between ribs.

Identification - *Senecio speciosus.*

Lewis 66. 10km from Port Alfred on the East London road. 3326DB. November 1991.

1. Arrangement of leaves at flowering - basal and cauline leaves present.
2. Leaf shape - spathulate, petiolate base.
3. Leaf margin - lobed and toothed.
4. Inflorescence type - paniculate or compound, racemose tending to corymbose at top.
5. Diameter of heads - 20-25mm.
6. Number of rays - -16
7. Number of involucral bracts - 18
8. Length of involucral bracts - 10-12mm
9. Indumentum -
   - Involucral bracts - numerous short - medium jointed glandular hairs.
   - Flowering stems - as above
   - Leaves - as above.
10. Achenes - cylindrical, very hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 71. Brackenhill, Howison's Poort, Grahamstown. 3326BC. November 1991.

1. Arrangement of leaves at flowering - basal rosette, stem and cauline leaves.
2. Leaf shape - elongated, spathulate with scarcely petioled base.
3. Leaf margin - lobed and toothed.
4. Inflorescence type - racemose/corymbose.
5. Diameter of heads - 20-25mm.
6. Number of rays - 14.
7. Number of involucral bracts - 18-20.
8. Length of involucral bracts - 10-12mm.
9. Indumentum -
   involucral bracts - long-medium jointed glandular hairs.
   stem - medium-short jointed glandular hairs.
   leaves - few medium-short hairs.
10. Achenes - hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 79. Firdene, Stoneshill near Grahamstown. 3326BC. November 1991.

1. Arrangement of leaves at flowering - basal rosette, stem and cauline leaves.
2. Leaf shape - elliptical to spathulate tapering to base.
3. Leaf margin - coarsely lobed and toothed.
4. Inflorescence type - corymbose.
5. Diameter of heads - 30-40mm.
6. Number of rays - 12.
7. Number of involucral bracts - 18.
8. Length of involucral bracts - 12mm.
9. Indumentum -
   - involucral bracts - short and medium hairs
   - stem - short and medium hairs
   - leaves - short hairs mainly on veins and margins.
10. Achenes - cylindrical, ribbed, long hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 82. Menziesberg area of The Amatole Mountains. 3226DB. December 1991.

1. Arrangement of leaves at flowering - basal rosette only available.
2. Leaf shape - oblong to elliptical, broad scarcely petioled base.
3. Leaf margin - shallowly toothed.
4. Inflorescence type - not available
5. Diameter of heads - not available
6. Number of rays - not available
7. Number of involucral bracts - not available
8. Length of involucral bracts - not available
9. Indumentum -not available
   - Involucral bracts - not available
   - Flowering stems - not available
   - Leaves -long hairs, cobwebbed below at first becoming less so later.

Identification - *Senecio macrocephalus.*

Lewis 86. Menziesberg area of The Amatole Mountains. 3226DB. December 1991.

1. Arrangement of leaves at flowering - basal rosette only available.
2. Leaf shape - oval to elliptical.
3. Leaf margin - toothed.
4. Inflorescence type - not available
5. Diameter of heads - not available
6. Number of rays - not available
7. Number of involucral bracts - not available
8. Length of involucral bracts - not available
9. Indumentum -not available
   - Involucral bracts - not available
   - Flowering stems - not available
   - Leaves - not available

Identification - *Senecio barbatus.* (Voucher mislaid)

Lewis 88. 1820 Settlers Monument Grahamstown. 3326BC. April 1992.

1. Arrangement of leaves at flowering - mainly basal, rosetted, few cauline.
2. Leaf shape - basal rosette spathulate to elliptical, long petioled base.
3. Leaf margin - lobed and toothed
4. Type of inflorescence - racemose.
5. Diameter of heads - 20mm
6. Number of rays -18-20
7. Number of involucral bracts - 20-22
8. Length of involucral bracts - 10-12mm
9. Indumendum -
   - involucral bracts - numerous short to medium jointed glandular hairs
   - flowering stems - as above
   - leaves - occasional short hairs
10. Achenes - cylindrical, ribbed hairy

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 90. 1820 Settlers Monument Grahamstown. 3326BC. October 1992.

1. Arrangement of leaves at flowering - basal rosette, few cauline leaves.
2. Leaf shape - spathulate tapering to long petiole like base.
3. Leaf margin - lobed and shallowly toothed.
4. Type of inflorescence - compound raceme or panicle tending at top towards corymbose arrangement
5. Diameter of heads - 25mm
6. Number of rays - 12
7. Number of involucral bracts - 18
8. Length of involucral bracts - 12mm
9. Indumentum -
   - involucral bracts - numerous long jointed glandular hairs
   - flowering stems - as above
   - leaves - glabrous
10. Achenes - cylindrical, ribbed, hairy between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 91. 1820 Settlers Monument, Grahamstown. 3326BC. October 1992.

1. Arrangement of leaves at flowering - basal rosette, few stem and cauline leaves.
2. Leaf shape - spathulate tapering to scarcely petioled base.
3. Leaf margin - entire to sinuately lobed and toothed.
4. Inflorescence type - compound, probably raceme or panicle.
5. Diameter of heads - 25mm.
6. Number of rays - 14.
7. Number of involucral bracts - 18.
8. Length of involucral bracts - 10mm.
9. Indumentum -
   involucral bracts - short-medium jointed glandular hairs
   stem - short-medium jointed glandular hairs
   leaves - more or less glabrous, few hairs on midrib or margin.
10. Achenes - cylindrical, ribbed, hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 94. Near Alicedale. 3326AC. December 1991.

1. Arrangement of leaves at flowering - on prostrate stems forming mats.
2. Leaf shape - linear, short petiole like base, succulent.
3. Leaf margin - entire.

4 - 10 not available

Identification - *Senecio radicans*.

Lewis 95. Hogsback (Amatole Mountains) above Kettlespout Waterfall. 3226DB. December 1991.

1. Arrangement of leaves at flowering - cauline leaves, no basal rosette collected.
2. Leaf shape - elliptical to spathulate tapering to petiole like base.
3. Leaf margin - lobed and toothed.
4. Type of inflorescence - simple corymb.
5. Diameter of heads - 15mm
6. Number of rays - 10
7. Number of involucral bracts - 14
8. Length of involucral bracts - 8-10mm
9. Indumentum -
   - involucral bracts - short glandular hairs
   - flowering stems - short glandular hairs
   - leaves - few small hairs on veins only
10. Achenes - not available

Identification - *Senecio speciosus.*

Lewis 96. Amatole Mountains, near Plaatjies Kraal. 3226DB. December 1991.

1. Arrangement of leaves at flowering - no basal rosette. Stem and cauline leaves present.
2. Leaf shape - spathulate tapering to non-petioled base.
3. Leaf margin - deeply lobed.
4. Inflorescence type - immature, probably corymbose.
5. Diameter of heads - 20mm.
6. Number of rays - 8.
7. Number of involucral bracts - 14-16.
8. Length of involucral bracts - 8mm.
9. Indumentum -
   involucral bracts - few small hairs
   stem - few small hairs
   leaves - few small hairs.
10. Achenes - cylindrical, slightly hairy.

Identification - *Senecio* sp. aff. *Senecio speciosus.*

Lewis 98. Brackenhill, Howison's Poort, Grahamstown. 3326BC. November 1992.

1. Arrangement of leaves at flowering - basal rosette, few stem and cauline leaves.
2. Leaf shape - elongated, spathulate, tapering to non-petioled base.
3. Leaf margin - raggedly lobed and toothed.
4. Inflorescence type - corymbose.
5. Diameter of heads - 25-30mm.
6. Number of rays - 14.
7. Number of involucral bracts - 14-16.
8. Length of involucral bracts - 10-12mm.
9. Indumentum -
   involucral bracts - long jointed glandular hairs
   stem - long jointed glandular hairs
   leaves - more or less glabrous, few small hairs on margin only.
10. Achenes - densely hairy (short hair).

Identification - *Senecio speciosus/macrocephalus* intermediate.

Specimen No. 100. 1820 Settlers Monument, Grahamstown. 3326BC. November 1992.

1. Arrangement of leaves at flowering - basal rosette, few stem and cauline leaves.
2. Leaf shape - elliptical tapering to non-petioled base.
3. Leaf margin - coarsely lobed and toothed.
4. Inflorescence type - immature, probably corymbose.
5. Diameter of heads - 20mm.
6. Number of rays - 14.
7. Number of involucral bracts - 16-18.
8. Length of involucral bracts - 12-13mm.
9. Indumentum -
   involucral bracts - medium and long jointed glandular hairs
   stem - medium and long jointed glandular hairs
   leaves - few small hairs on midrib and margin.
10. Achenes - cylindrical, hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 101. 1820 Settlers Monument, Grahamstown. 3326BC. November 1992.

1. Arrangement of leaves at flowering -basal rosette, some stem and cauline leaves.
2. Leaf shape - spathulate tapering to broad base, not petiolate.
3. Leaf margin - coarsely lobed and toothed.
4. Inflorescence type - corymbose.
5. Diameter of heads - 35mm.
6. Number of rays - 14.
7. Number of involucral bracts - 18.
8. Length of involucral bracts - 12mm.
9. Indumentum -
   involucral bracts - short-medium glandular hairs
   stem - short glandular hairs
   leaves - short hairs mainly on midrib and margin.
10. Achenes - cylindrical, hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 102. Kasouga. 3326DA. November 1992.

1. Arrangement of leaves at flowering - mainly basal, rosetted, few cauline leaves
2. Leaf shape - elliptical to spathulate, petiole-like base.
3. Leaf margin - lobed and deeply cut.
4. Type of inflorescence - corymbose
5. Diameter of heads - 20mm
6. Number of rays - 12
7. Number of involucral bracts - 16
8. Length of involucral bracts - 10mm
9. Indumentum -
   - involucral bracts - few short jointed glandular hairs
   - flowering stems - many short jointed glandular hairs
   - leaves - as involucre
10. Achenes - cylindrical, short white hairs (between ribs?)

Identification - *Senecio speciosus.*

Lewis 103. Potters Pass Nature Reserve, East London. 3327BB. November 1992.

1. Arrangement of leaves at flowering - mainly basal, rosetted, some cauline leaves.
2. Leaf shape - oblong tapering to scarcely petioled base.
3. Leaf margin - shallowly toothed.
4. Type of inflorescence - corymbose or subracemose or paniculate
5. Diameter of heads - 25-35mm
6. Number of rays - 16
7. Number of involucral bracts - 18
8. Length of involucral bracts - 12-15mm
9. Indumentum -
   - involucral bracts - many long jointed glandular hairs
   - flowering stems - very occasional long jointed glandular hairs
   - leaves - short jointed glandular hairs
10. Achenes - cylindrical, ribbed, hairy between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 104. Beggarsbush Outspan near Grahamstown. 3326BC. December 1992. (on Kranz).

1. Arrangement of leaves at flowering - basal rosette, some stem and cauline leaves.
2. Leaf shape - spathulate with non-petioled base.
3. Leaf margin - from coarsely lobed and toothed to entire shallowly toothed.
4. Inflorescence type - tending to be corymbose.
5. Diameter of heads - 30 - 35mm.
6. Number of rays - 13 - 14.
7. Number of involucral bracts - 18 - 20.
8. Length of involucral bracts - 12 - 14 mm
9. Indumendum-
   Involucral bracts - long jointed glandular hairs
   Stem - long jointed glandular hairs
   leaves - glabrous, few medium length hairs on margin
10. Achenes - cylindrical, ribbed, short hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 105. Beggarsbush Outspan near Grahamstown. 3326BC. December 1992.

1. Arrangement of leaves at flowering - basal rosette, few small stem and cauline leaves.
2. Leaf shape - elliptical to spathulate, broad non-petioled base.
3. Leaf margin - coarsely lobed and toothed.
4. Inflorescence type - racemose to corymbose.
5. Diameter of heads - 30-35mm.
6. Number of rays - 12-13.
7. Number of involucral bracts - 16 - 18
8. Length of involucral bracts - 12mm+.
9. Indumentum -
   - involucral bracts - long jointed glandular hairs
   - flowering stems - medium to long jointed glandular hairs
   - leaves - few short hairs on midrib and margin.
10. Achenes - cylindrical, ribbed, short hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 106. Beggarsbush Outspan, near Grahamstown. 3326BC. December 1992.

1. Arrangement of leaves at flowering - mainly basal, rosetted, few cauline.
2. Leaf shape - elliptical to spathulate narrowing to scarcely petioled base.
3. Leaf margin - lobed.
4. Type of inflorescence - corymbose (compound) or racemose (simple) both occur together.
5. Diameter of heads - 30mm
6. Number of rays - 16
7. Number of involucral bracts - 18
8. Length of involucral bracts - 10-15mm
9. Indumentum -
   - involucral bracts - long jointed glandular hairs on corymbose inflorescence
   - flowering stems -as above
   - leaves - very sparsely glandular hairy
10. Achenes - cylindrical, ribbed, very hairy between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 111. Bokspruit, North Eastern Cape Province. 3027DD. December 1992.

1. Arrangement of leaves at flowering - basal rosette and cauline leaves
2. Leaf shape - spathulate, petiolate.
3. Leaf margin - toothed
4. Type of inflorescence - corymbose to paniculate
5. Diameter of heads - 10-15mm
6. Number of rays - 12 (disc florets - greyish)
7. Number of involucral bracts - 16-18
8. Length of involucral bracts - 6-8mm
9. Indumentum -
   - involucral bracts - short-medium-long jointed glandular hairs
   - flowering stems -as above
   - leaves - mainly medium length glandular hairs, jointed
10. Achenes - cylindrical, ribbed, no hairs visible.

Identification - *Senecio polyodon.*

Lewis 112. Naudés Nek. NE Cape Province. 3028CC. December 1992.

1. Arrangement of leaves at flowering - basal rosette and stem leaves present
2. Leaf shape - elliptical to spathulate with petiole like base.
3. Leaf margin - lobed and toothed
4. Type of inflorescence - immature but probably corymbose
5. Diameter of heads - 25mm
6. Number of rays - 18
7. Number of involucral bracts - 20
8. Length of involucral bracts - 12mm
9. Indumentum -
   - involucral bracts - few short jointed glandular hairs
   - flowering stems - as above
   - leaves - nearly glabrous, few hairs on veins
10. Achenes - cylindrcial slightly hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 115. Mountain Drive, near Grahamstown. 3326BC. October 1992.

1. Arrangement of leaves at flowering - basal rosette and stem leaves present.
2. Leaf shape - elliptical tapering to narrow scarcely petioled base.
3. Leaf margin - lobed and toothed (coarsely).
4. Inflorescence type - crowded at top of stem, probably corymbose.
5. Diameter of heads - 20mm.
6. Number of rays - 14-16.
7. Number of involucral bracts - 16.
8. Length of involucral bracts - 10mm.
9. Indumentum -
   involucral bracts - mixed (long, medium and short hairs)
   stem - short hairs
   leaves - short hairs mainly on margin.
10. Achenes - hairy.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 116. Mountain Drive, near Grahamstown. 3326BC. January 1993.

1. Arrangement of leaves at flowering - basal rosette and stem leaves
2. Leaf shape - basal leaves elliptical with petiole like base.
3. Leaf margin - toothed and lobed.
4. Type of inflorescence - corymbose
5. Diameter of heads - 15-20mm
6. Number of rays - 14-16
7. Number of involucral bracts - 18-20
8. Length of involucral bracts - 12mm
9. Indumentum -
   - involucral bracts - many medium jointed glandular hairs
   - flowering stems -as above
   - leaves - nearly glabrous, few hairs on veins only
10. Achenes - not available

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 117. Potters Pass Nature Reserve, East London. 3327BB. March 1993.

1. Arrangement of leaves at flowering - mostly basal rosetted, few cauline.
2. Leaf shape - elliptical long petiole like base,
3. Leaf margin - toothed
4. Type of inflorescence - corymbose
5. Diameter of heads - 20-25mm
6. Number of rays - 12
7. Number of involucral bracts - 16-18
8. Length of involucral bracts - 12-15mm
9. Indumentum -
   - involucral bracts - very few long jointed glandular hairs
   - flowering stems -very occasional long jointed glandular hairs
   - leaves - few glandular hairs on veins
10. Achenes - ribbed, hairy between ribs

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 118. Faraway, Coldsprings, near Grahamstown. 3326BC. September 1993.

1. Arrangement of leaves at flowering -basal rosette and few cauline leaves.
2. Leaf shape - spathulate with broad non-petioled base.
3. Leaf margin - raggedly lobed and toothed.
4. Inflorescence type - more or less corymbose.
5. Diameter of heads - 15-20mm.
6. Number of rays - 14.
7. Number of involucral bracts - 18.
8. Length of involucral bracts - 10mm.
9. Indumentum -
   involucral bracts - medium-long jointed glandular hairs
   stem - short-medium jointed glandular hairs
   leaves - short hairs at margin only
10. Achenes -. cylindrical, very hairy but mainly between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

Lewis 119. Menziesberg, Amatole Mountains. 3226DB. December 1993. (see also 82)

1. Arrangement of leaves at flowering - basal rosette, few stem and cauline leaves.
2. Leaf shape - oval with scarcely petioled base.
3. Leaf margin - entire shallowly toothed.
4. Inflorescence type - racemose.
5. Diameter of heads - 30 -35 mm.
6. Number of rays - 12 -14.
7. Number of involucral bracts - 16 -18.
8. Length of involucral bracts - 12 - 14 mm.
9. Indumentum -
   Involucral bracts - medium and long jointed glandular hairs
   Stems - medium and long jointed glandular hairs
   Leaves - lond jointed glandular hairs, cobwebbed below at first.
10. Achenes - hairy.

Identification - *Senecio macrocephalus.*

Phillipson 3820. Menziesberg, Amatole Mountains. 3226DB. January 1992.

1. Arrangement of leaves at flowering - basal rosette and cauline leaves.
2. Leaf shape - spathulate.
3. Leaf margin - toothed and lobed.
4. Inflorescence type - paniculate
5. Diameter of heads - 10-12mm.
6. Number of rays - absent
7. Number of involucral bracts - 12
8. Length of involucral bracts - 8-10mm
9. Indumentum -
   Involucral bracts - very occasional long jointed glandular hairs.
   flowering stems - glabrous
   leaves - very sparsely hairy
10. Achenes - cylindrical, ribbed no hairs visible.

Identification - *Senecio erubscens.*

Phillipson 3821. Menziesberg, Amatole Mountains. 3226DB. January 1992.

1. Arrangement of leaves at flowering - mostly cauline.
2. Leaf shape - lanceolate sessile becoming subauriculate
3. Leaf margin - coarsely denticulate
4. Inflorescence type - corymbose
5. Diameter of heads - 10-15mm
6. Number of rays - absent
7. Number of involucral bracts - 16
8. Length of involucral bracts - 8-10cms
9. Indumentum -
   involucral bracts - numerous short jointed glandular hairs
   flowering stems - as above
   leaves - short- long jointed glandular hairs
10. Achenes - cylindrical, hairy

Identification - *Senecio* sp. aff. *Senecio speciosus.*

Phillipson 3822. Menziesberg, Amatole Mountains. 3226DB. January 1992.

1. Arrangement of leaves at flowering - basal rosette and cauline leaves present.
2. Leaf shape - elliptical, petiolate.
3. Leaf margin - basal leaves often pinnately cut, lobed and toothed.
4. Type of inflorescence - simple corymbose.
5. Diameter of heads - 10-15mm disc florets red-purple.
6. Number of rays - absent.
7. Number of involucral bracts - 12.
8. Length of involucral bracts - 10mm.
9. Indumendum -
   - involucral bracts - short to medium jointed glandular hairs.
   - flowering stems - as above.
   - leaves - short glandular hairs (NB. the whole plant is very sticky)
10. Achenes - cylindrical, ribbed, hairy between ribs.

Identification - *Senecio sp.*

Phillipson 3823. Menziesberg, Amatole Mountains. 3226DB. January 1992.

1. Arrangement of leaves at flowering - basal rosette and stem leaves present.
2. Leaf shape - elliptical, petiolate.
3. Leaf margin - lobed and toothed.
4. Type of inflorescence - simple corymbose
5. Diameter of heads - 15-20mm
6. Number of rays -8
7. Number of involucral bracts - 12
8. Length of involucral bracts - 10mm
9. Indumendum -
   - involucral bracts - short jointed glandular hairs
   - flowering stems - as above
   - leaves - few small glandular hairs
10. Achenes - cylindrical, ribbed, few hairs on tips only.

Identification - *Senecio speciosus.*

McCartan 3. Gonubie Nature Reserve, East London. 3228CC. November 1991.

1. Arrangement of leaves at flowering - basal rosette, few stem leaves.
2. Leaf shape - elliptical to spathulate, broad base, stem leaves clasping.
3. Leaf margin - lobed.
4. Inflorescence type - bunched at top of stem, probably corymbose.
5. Diameter of heads - 35-40mm.
6. Number of rays - 14-16.
7. Number of involucral bracts - 18-20.
8. Length of involucral bracts - 12-15mm.
9. Indumentum -
   involucral bracts - few large stumpy hairs
   stem - few large stumpy hairs
   leaves - some small hairs.
10. Achenes - cylindrical, short hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

McCartan 13. Fish River Mouth Grassland. 3327AC. September 1991.

1. Arrangement of leaves at flowering - basal rosette, few cauline leaves.
2. Leaf shape - spathulate, scarcely petioled base.
3. Leaf margin - lobed and toothed.
4. Inflorescence type - corymbose.
5. Diameter of heads - 30-35mm.
6. Number of rays - 12-13.
7. Number of involucral bracts - 18.
8. Length of involucral bracts - 10-12mm.
9. Indumentum -
   involucral bracts - short hairs.
   stem - short hairs
   leaves - numerous wooly hairs.
10. Achenes - cylindrical, ribbed, hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

APPENDIX 2

Morphological features of the *Senecio speciosus* and *Senecios macrocephalus* specimens in the Selmar Schonland Herbarium (GRA)

This collection consists of two recently combined collections one from the Albany Museum Herbarium and one from the Rhodes University Herbarium. These two collections are described separately. Details of each specimen are set out as follows:

Voucher No. Locality (where stated) Grid Reference (where stated) Date Collected Morphological characters 1 - 10

The identifications given are those ascribed by the collector of each specimen.

1) Albany Museum Herbarium Specimens

Flanagan 768. Near Komgha. 3227DB. March 1890

1) Basal rosette and stem leaves.
2) Elliptical to oval.
3) Toothed.
4) Corymbose.
5) 25 - 30mm.
6) 12 +
7) 16 - 18.
8) 10 - 11mm.
9) Bracts - few medium to long hairs.
   Stem - short hairs.
   Leaves - numerous long hair on veins and margins.
10) Not available.

Identification: *Senecio macrocephalus*

Flanagan 1049. Near Komgha. 3227DB. 1891.

1) Only stem leaves on card.
2) Spathulate, clasping.
3) Toothed.
4) Compound corymb to panicle.
5) 20mm.
6) 12 - 14.
7) 18.
8) 12mm.
9) Bracts - long jointed glandular hair.
   Stem - long jointed glandular hair.
   Leaves - short hairs.
10) Not available.

Identification: *Senecio macrocephalus*

Flanagan 1797. Near Kei Mouth. 3228CB. July 1893.

1) Basal rosette, few cauline leaves.
2) Elliptical to oval tapering to narrow base but scarcely petioled.
3) Entire, shallowly toothed.
4) Corymbose.
5) 20mm.
6) 12 - 14.
7) 18.
8) 12mm.
9) Bracts - long jointed glandular hairs.
Stem - long jointed glandular hairs.
Leaves - ± glabrous.
10) Not available.

Identification: *Senecio macrocephalus*

Galpin 2178. Andriesberg. 3126DA. December 1901

1) Basal rosette, few cauline leaves.
2) Basal - elliptical to oval tapering to narrow base, scarcely petioled.
3) Entire very shallowly toothed.
4) Racemose.
5) 20 - 25mm.
6) 14.
7) 18 - 20.
8) 12 - 15mm.
9) Bracts - short to medium jointed glandular hairs.
Stem - short to medium jointed glandular hairs.
Leaves - short glandular hairs mainly on veins.
10) Not available.

Identification: *Senecio macrocephalus*

Rattray 98. East London. 3327BB. June 1907

1) Basal rosette, large stem leaves, small cauline leaves.
2) Oval, tapering to scarcely petioled base.
3) Toothed or lobed and toothed.
4) Racemose.
5) 25 - 30mm.
6) 18.
7) 18 - 20.
8) 15mm.
9) Bracts - long jointed glandular hairs.
Stem - long jointed glandular hairs.
Leaves - ± glabrous.
10) Not available.

Identification: *Senecio macrocephalus*

Bandert 31. Cedarville, Transkei. 3029CA. 1921.

1) Basal rosette and cauline leaves.
2) Oblong tapering to scarcely petioled base.
3) Entire.
4) Racemose.
5) 15 - 20mm.
6) 12 - 14.
7) 16 - 20.
8) 12mm.
9) Bracts - short to medium hairs.
   Stem - short to medium hairs.
   Leaves - hairy.
10) Cylindrical hairy.

Identification: *Senecio macrocephalus*

Bandert 141. Cedarville, Transkei. 3029CA. Feb 1921.

1) Basal rosette and cauline leaves.
2) Oval tapering to scarcely petioled base.
3) Shallowly toothed.
4) Racemose.
5) 20 - 25mm.
6) 18.
7) 18.
8) 12mm.
9) Bracts - numerous glandular hairs long\med\short.
   Stem - as above.
   Leaves - as above, mainly on veins.
10) Not available.

Identification: *Senecio macrocephalus*

Comins 1785. East London (Macleanstown Road). 3227DC. Dec 1957.

1) Basal rosette, some cauline leaves.
2) Oval tapering to scarcely petioled base.
3) Shallowly toothed.
4) Racemose.
5) 20 - 25mm.
6) 14 - 16.
7) 18 - 20.
8) 12 - 15mm.
9) Bracts - long jointed glandular hairs.
   Stem - long jointed glandular hairs.
   Leaves - ± glabrous.
10) Not available.

Identification: *Senecio macrocephalus*

Dahlstrand 285. Near East London. 3327BB. July 1964.

1) Basal rosette, some cauline leaves.
2) Elliptical tapering to narrow scarcely petioled base.
3) Coarsely toothed.
4) Racemose.
5) 25 - 30mm.
6) 14 - 16.
7) 16 - 18.
8) 12mm.
9) Bracts - numerous long jointed glandular hairs.
   Stem - numerous long jointed glandular hairs.
   Leaves - ± glabrous, few hairs on veins.
10) Long hairs.

Identification: *Senecio macrocephalus*

Schlechter 2425. Near Humansdorp. 3322CD. March 1893.

1) Basal rosette, few cauline leaves.
2) Elliptical to spathulate, long narrow petioled base.
3) Toothed and lobed.
4) Corymbose.
5) 15 - 20mm.
6) 10 - 12.
7) 16.
8) 10mm.
9) Bracts - few short hairs.
   Stem - few short hairs.
   Leaves - few short hairs.
10) Cylindrical, ribbed, pubescent between ribs.

Identification: *Senecio speciosus*

Hutton 1027. Kaboosie. 3227DA. 1895.

1) Basal rosette not on card. Stem + cauline leaves only.
2) Elliptical with long petiole.
3) Toothed.
4) Simple corymb.
5) 20mm.
6) 14 - 16.
7) 18.
8) 10mm.
9) Bracts - sparse short stubby hairs.
   Stem - sparse short stubby hairs.
   Leaves - numerous short hairs.
10) Not available.

Identification: *Senecio speciosus*

Schlechter 6527. 3029CD. 1898

1) No basal rosette, many stem leaves, few small cauline leaves.
2) Elliptical to spathulate with long petiole.
3) Deeply lobed, almost pinnately divided at base.
4) Corymbose.
5) 20 - 25mm.
6) 8.
7) 14.
8) 12mm.
9) Bracts - short jointed glandular hairs.
   Stem - short jointed glandular hairs.
   Leaves - numerous small hairs on veins and margins.
10) Not available.

Identification: *Senecio* sp. aff. *S. speciosus*

Galpin 2665. Great Winterberg. 3226AD. March 1900.

1) Large basal rosette, few cauline leaves.
2) Elliptical to spathulate base scarcely petioled.
3) Lobed.
4) Corymbose (few heads).
5) 15 - 20mm.
6) 14.
7) 18.
8) 10mm.
9) Bracts - short to medium length hairs.
   Stem - short to medium length hairs.
   Leaves - numerous short hairs.
10) cylindrical, ribbed, short hairs mainly between ribs.

Identification: *Senecio speciosus*

Galpin 2666. Great Winterberg. 3226AD. March 1900.

1) No basal rosette, many stem leaves, few cauline leaves.
2) Stem leaves lanceolate, auriculate.
3) Lobed.
4) Corymbose.
5) 10 - 15mm.
6) 8.
7) 16.
8) 10mm.
9) Bracts - short glandular hairs.
   Stem - short glandular hairs.
   Leaves - short glandular hairs.
10) Cylindrical, ribbed, short hair between ribs.

Identification: *Senecio* sp. aff. *S. speciosus*

Macowan s.n.. Grahamstown. 3326BC. 1904

1) Basal rosette, stem and cauline leaves.
2) Elliptical tapering to narrow petioled base.
3) Lobed, pinnately cut at base.
4) Corymbose.
5) 15 - 20mm.
6) Undistinguishable.
7) 18.
8) 10mm.
9) Bracts - short glandular hairs.
Stem - short glandular hairs.
Leaves - hairy.
10) Not available.

Identification: *Senecio speciosus*

Daly 841. Rockliffe near Sidbury. 3326AC. Nov 1904

1) Basal rosette, stem leaves and small cauline leaves.
2) Elliptical to spathulate tapering to nearly petioled base.
3) Lobed, but not coarsely.
4) Corymbose.
5) 20 - 25mm.
6) 16.
7) 18 - 20.
8) 10 - 11mm.
9) Bracts - short to medium length hairs.
Stem - short hairs.
Leaves - short hairs and cobwebbed.
10) Hairy mainly between ribs.

Identification: *Senecio speciosus*

Rogers 903. Port Alfred. 3326DB. Jan 1907.

1) Basal rosette, few small cauline leaves.
2) Spathulate tapering to nearly petioled base, cauline leaves lanceolate, clasping.
3) Lobed and toothed.
4) Corymbose.
5) 15 - 20mm.
6) Indistinguishable.
7) 18.
8) 10mm.
9) Bracts - medium to long hairs.
Stem - short hairs.
Leaves - numerous hairs mainly on veins.
10) Not available.

Identification: *Senecio speciosus*

Rattray 99. East London. 3327BB. June 1907.

1) Basal rosette, stem leaves and few cauline leaves.
2) Elliptical to spathulate with long petiole like base.
3) Coarsely lobed.
4) Corymbose.
5) 20 - 25mm.
6) 12 - 14.
7) 18.
8) 10mm.
9) Bracts - few long jointed glandular hairs.
   Stem - few medium length hairs.
   Leaves - numerous long to medium length hairs.
10) Cylindrical, ribbed, slightly hairy between ribs.

Identification: *Senecio speciosus*

Wormald 28. Cambridge E. London. 3227DD. Jan 1908.

1) Basal rosette, few stem and very small cauline leaves.
2) Elliptical to spathulate narrowing to scarcely petioled base.
3) Lobed and shallowly toothed.
4) Corymbose.
5) 20 - 25cm.
6) 14.
7) 16 - 18.
8) 10mm.
9) Bracts - numerous long hairs.
   Stem - medium to long hairs.
   Leaves - numerous long hairs.
10) Not available.

Identification: *Senecio speciosus*

Rogers 3160. Blaney Junction. 3227DC. Feb 1908.

1) Basal rosette, stem leaves and very small cauline leaves.
2) Spathulate, apex very acute, tapering to petiole like base.
3) Coarsely lobed.
4) Corymbose.
5) 20 - 25mm.
6) 12 - 14.
7) 18.
8) 10mm.
9) Bracts - some large stumpy hairs.
   Stem - small hairs.
   Leaves - small hairs.
10 Not available.

Identification: *Senecio speciosus*

Fourcade 2556. Tsitsikamma. Jan 1910.

1) No basal rosette on card, stem leaves present.
2) Stem leaves lanceolate, clasping.
3) Toothed.
4) Simple corymb.
5) Undistinguishable.
6) Undistinguishable.
7) 16 - 18.
8) 10mm.
9) Bracts - few short hairs.
Stem - very few short hairs.
leaves - short hairs.
10) Cylindrical, ribbed, very sparsely hairy between ribs.

Identification: Probably *Senecio speciosus*

S. Schonland 3196. Zuurberg near Sanatorium. 3325AD. April 1919.

1) Basal rosette, few stem leaves, tiny cauline leaves.
2) Elliptical, tapering to petiole like base.
3) Shallowly lobed.
4) Probably corymb (3 heads only).
5) 20 - 25mm.
6) 10 - 14.
7) 18.
8) 10mm.
9) Bracts - few short hairs.
Stem - few short hairs.
Leaves - many short hairs.
10) Cylindrical, ribbed, hairs between ribs.

Identification: *Senecio speciosus*

S. Schonland 3322. Southwell (during drought). 3326DA. December 1919.

1) Basal rosette few stem and cauline leaves.
2) Elliptical to spathulate tapering to scarcely petioled base.
3) Coarsely lobed.
4) Packed at top of stem, difficult to distinguish.
5) 20 - 25mm.
6) 14 - 16.
7) 18.
8) 10mm.
9) Bracts - few medium length hairs, more towards base.
Stem - numerous medium to long hairs.
Leaves - small hairs and cobwebbed.
10) Not available.

Identification: *Senecio speciosus*

S. Schonland 4089. Port St. Johns. 3129. January 1921.

1) Basal rosette, few stem leaves.
2) Spathulate tapering to narrow scarcely petioled base.
3) Coarsely lobed.
4) Crowded at top of stem, probably corymbose.
5) 20 - 25mm.
6) 14 - 16.
7) 18.
8) 10 - 11mm.
9) Bracts - few short hairs.
   Stem - numerous long hairs.
   Leaves - short and medium length and cobwebbed.
10) Hairy, mainly between ribs.

Identification: *Senecio speciosus.*

Fourcade 867. Ondebosch (Humansdorp District). Sept 1920.

1) Small basal rosette, stem and small cauline leaves.
2) Elliptical tapering to petiole like base.
3) Coarsely lobed.
4) Clumped at top of stem, may be corymbose.
5) 25mm.
6) 8.
7) 12.
8) 8 - 10mm.
9) Bracts - short stumpy hairs.
   Stem - short hairs
   Leaves - short stumpy hairs.
10) Not available.

Identification: *Senecio speciosus*

S. Schonland 4248. Katberg. 3226DA. January 1921.

1) Small basal rosette, few stem and cauline leaves.
2) Elliptical tapering to petiole like base.
3) Coarsely lobed.
4) Corymbose.
5) 10 - 15mm.
6) 8.
7) 12 - 14.
8) 8 - 10mm.
9) Bracts - small hairs.
   Stem - small hairs.
   Leaves - small hairs + cobwebbed
10) Cylindrical, ribbed, more or less glabrous

Identification: *Senecio speciosus*

Hilner 516. Qora River Mouth, Willowvale District. 3228CC. December 1921.

1) Only cauline leaves and inflorescence on card (2 Stems a and b).
2) Cauline leaves lanceolate, clasping.
3) Toothed.
4) Corymbose.
5) 30 + mm.
6) Undistinguishable.
7) 18.
8) 12 - 14mm.
9) Bracts - a) long hairs b) short hairs.
Stem - a) long hairs b) short hairs.
Leaves - few hairs at margin.
10) Cylindrical, hairy.

Identification: *S. speciosus*

Dyer 767. Amatole Mountains. 3227CS. November 1926.

1) Small basal rosette, very sparse stem and cauline leaves.
2) Elliptical tapering to petiole like base.
3) Lobed.
4) Simple corymb.
5) 10 - 12mm.
6) 12.
7) 16 - 18.
8) 5 - 6mm.
9) Bracts - short glandular hairs.
Stem - few short glandular hairs.
Leaves - very few short hairs.
10) Not available.

Identification: *Senecio speciosus*

Dyer 1807. Grahamstown. 3326BC. November 1928.

1) Large basal rosette few stem and cauline leaves.
2) Spathulate tapering to scarcely petioled base.
3) Coarsely lobed.
4) Corymbose tending to racemose.
5) 20 - 25mm.
6) 14.
7) 18.
8) 10 - 11mm.
9) Bracts - short to medium length hairs.
Stem - short hairs.
Leaves - numerous short hairs.
10) Cylindrical, ribbed, very hairy.

Identification: *Senecio speciosus.*

Dyer 2052. Gonubie. 3227DD. September 1929.

1) Basal rosette, large stem leaves, small cauline leaves.
2) Spathulate tapering to long petiole like base.
3) Lobed.
4) Corymbose.
5) 15 - 20mm.
6) 14 - 16.
7) 18.
8) 10mm.
9) Bracts - long jointed glandular hairs.
   Stem - long jointed glandular hairs.
   Leaves - long jointed glandular hairs.
10) Not available.

Identification: *Senecio speciosus.*

Long 23. Zuurberg Sanatorium. 3325AD. November 1930.

1) Small basal rosette, few stem leaves.
2) Spathulate tapering to petiole like base.
3) Lobed.
4) Corymbose.
5) 15mm.
6) 14.
7) 18.
8) 10mm.
9) Bracts - short glandular hairs.
   Stem - short glandular hairs.
   Leaves - short hairs on veins
10) Not available.

Identification: *Senecio speciosus*

Dix 142. Loerie Plantation. 3325CC. October 1934.

1) Basal rosette, stem leaves and very small cauline leaves.
2) Spathulate tapering to petiole like base.
3) Lobed.
4) Indistinct - raceme or corymb.
5) 10 - 15mm.
6) 8 - 10.
7) 16.
8) 10mm.
9) Bracts - short hairs.
   Stem - short hairs.
   Leaves - medium length hairs on veins.
10) Not available.

Identification: *Senecio speciosus*

Storey 2110. Fort Fordyce, Fort Beaufort. 3226DC. March 1947.

1) No basal rosette on card, large stem leaves present.
2) Stem leaves lanceolate, clasping.
3) Toothed.
4) Compound raceme.
5) 25 - 30mm.
6) 16 - 18.
7) 18 - 20.
8) 12 - 15mm.
9) Bracts - numerous long jointed glandular hairs.
   Stem - numerous long jointed glandular hairs.
   Leaves - medium length hair or medium and margin.
10) Not available.

Identification: *Senecio macrocephalus.*

Archibald 4934. Zuurberg, Alexandria District. 3325AD. Jan 1953.

1) Basal rosette, few stem and cauline leaves.
2) Elliptical tapering to narrow petioled base.
3) Shallowly lobed.
4) Corymbose.
5) 10 - 15mm.
6) 12 - 14.
7) 16 - 18.
8) 8mm.
9) Bracts - short to medium length hairs.
   Stem - short hairs.
   Leaves - numerous short hairs especially on midrib and margins.
10) Not available.

Identification: *Senecio speciosus.*

Commins s.n. Berlin, near King William's Town. 3227DC. October 1959.

1) Basal leaves not on card, stem and cauline leaves present.
2) Stem leaves oblong becoming lanceolate, clasping.
3) Toothed, cauline leaves ± entire.
4) Simple corymb.
5) 25 - 30mm.
6) 14.
7) 18 - 20.
8) 10 - 11mm.
9) Bracts - long stumpy hairs.
   Stem - medium to long hairs.
   Leaves - medium to long hairs.
10) Not available.

Identification: *Senecio macrocephalus?*

Gordon Gray 554. The Haven Bashee River Mouth. 3228BB. July 1966.

1) Basal rosette and stem leaves present.
2) Elliptical tapering to narrow petiole like base.
3) Lobed and toothed.
4) Probably corymbose.
5) 30 - 35mm.
6) 21.
7) 18 - 20.
8) 12mm.
9) Bracts - few long hairs.
   Stem - some medium to long hairs.
   Leaves - few short hairs.
10) Not available.

Identification: *Senecio macrocephalus?*

Dahlstrand 1806. Hogsback Forest Reserve. 3226DB. Nov 1969.

1) No basal rosette, many stem leaves, few cauline leaves.
2) Elliptical to spathulate tapering to scarcely petioled base.
3) Toothed.
4) Corymbose.
5) 20 - 25mm.
6) 8.
7) 12.
8) 8mm.
9) Bracts - few small hairs.
   Stem - few small hairs.
   Leaves - numerous small hairs.
10) Cylindrical, very sparsely hairy.

Identification: *Senecio* sp. aff. *S. speciosus.*

Dornbell 39. Howison's Poort, Grahamstown. 3326BC. 10/10/92.

1) Basal rosette, few stem leaves.
2) Spathulate tapering to base.
3) Shallowly lobed and toothed.
4) Corymbose.
5) 15 - 20mm.
6) 16.
7) 18 - 20.
8) 12 mm.
9) Bracts - short hairs.
   Stem - short hairs.
   Leaves - few short hairs.
10) Not available.

Identification: *Senecio speciosus.*

2) Senecio speciosus and Senecio macrocephalus specimens from Rhodes University Herbarium.

Specimen with no Voucher number. Grahamstown. 3326BC. July 1989.

1) No rosette on card but few stem leaves suggesting a basal rosette was present.
2) Stem leaves spathulate, petioled becoming lanceolate clasping.
3) Toothed.
4) Racemose - corymbose.
5) 30 - 35 mm.
6) 14 - 16.
7) 20.
8) 12mm.
9) Bracts - large stumpy hairs.
   Stem - medium length hairs.
   Leaves - few small hairs.
10) Not available.

Identification: *Senecio macrocephalus.*

Lawrence 40. Near Milner Dam, Grahamstown. 3326BC. 17/5/47.

1) Basal rosette, few cauline leaves.
2) Elliptical to oval, scarcely petioled base.
3) Toothed.
4) Racemose.
5) 25mm.
6) 14 - 16.
7) 20.
8) 10 - 12mm.
9) Bracts - short to medium length hairs.
   Stem - short to medium length hairs.
   Leaves - short hairs.
10) Cylindrical, few short hairs.

Identification: *Senecio macrocephalus*

Noel 943. Highlands near Grahamstown. 3326BC. 12/2/53.

1) Basal rosette, stem and cauline leaves.
2) Elongated, spathulate, scarcely petioled.
3) Lobed.
4) Racemose to corymbose.
5) 20 - 25mm.
6) 12 - 14.
7) 18.
8) 10 - 11mm.
9) Bracts - short to medium length hairs.
   Stem - short to medium length hairs.
   Leaves - short hairs mainly on veins and margins
10) Not available.

Identification: *Senecio speciosus.*

Heeg 212. Manley Flats road off National Road. 3326BC. 7/4/62.

1) Basal rosette, few stem leaves.
2) Spathulate with scarcely petioled base.
3) Toothed and lobed.
4) Racemose to corymbose.
5) 20mm.
6) 16 - 18.
7) 18 - 20.
8) 10 - 12mm.
9) Bracts - short stumpy and long glandular hairs.
   Stem - medium length hairs.
   Leaves - small hairs mainly on midrib and margin.
10) Not available.

Identification: *Senecio speciosus.*

Francis 66. Grahamstown (Old Brickfields). 3326BC. 12/3/63.

1) Basal rosette, stem and cauline leaves.
2) Elliptical to spathulate with scarcely petioled base.
3) Raggedly lobed.
4) Only 2 heads but at same level so probably corymbose.
5) 20mm.
6) 16 - 18.
7) 16.
8) 10 - 12mm.
9) Bracts - large stumpy hairs.
   Stem - large stumpy hairs.
   Leaves - small hairs.
10) Not available.

Identification: *Senecio speciosus.*

Whitty 19. Grahamstown (Mountain Drive) 3326BC. 18/4/65

1) Mainly basal rosette, few stem leaves.
2) Spathulate tapering to petiole like base.
3) Lobed.
4) Short, corymbose.
5) 20 - 30 mm.
6) 14 - 16.
7) 16.
8) 12mm.
9) Bracts - medium to long hairs.
   Stem - medium to long hairs.
   Leaves - few short hairs on margin.
10) Not available.

Identification: *Senecio speciosus.*

Dickinson 42. Port St. Johns (Near Umngazi River Mouth). 3129DA. 10/1/65

1) Basal rosette, few stem and cauline leaves.
2) Elliptical tapering to petiole like base.
3) Shallowly lobed.
4) Corymbose.
5) 20mm.
6) 14 - 16.
7) 18.
8) 10 - 11mm.
9) Bracts - sparse short hairs.
Stem - sparse short hairs.
leaves - sparse short hairs.
10) Not available.

Identification: *Senecio speciosus.*

Thomas Baines Nature Reserve s.n. 3326BC. 13/4/69.

1) Basal rosette, few stem and cauline leaves.
2) Round to elliptical with long petiole like base.
3) Toothed or shallowly lobed and toothed.
4) Corymbose.
5) 25mm.
6) 14 - 16.
7) 18 - 20.
8) 10 - 12mm.
9) Bracts - long jointed glandular hairs.
Stem - short hairs.
Leaves - long hairs.
10) Not available.

Identification: *Senecio speciosus.*

Olivieri 3. Grahamstown (Port Elizabeth Road). 3326BC. 25/4/71.

1) Basal rosette, few stem and cauline leaves.
2) Spathulate with petiole like base.
3) Shallowly lobed.
4) Corymbose.
5) 20 - 25mm.
6) 14 - 16.
7) 20.
8) 10 - 11mm.
9) Bracts - short hairs.
Stem - short hairs.
Leaves - short hairs.
10) Not available.

Identification: *Senecio speciosus.*

Bradley 9. Grahamstown (10 miles on the Port Elizabeth Road). 3326BC. 24/2/73

1) Basal rosette, few stem and cauline leaves.
2) Spathulate tapering to near petioled base.
3) Raggedly lobed.
4) Corymbose.
5) 20 - 25mm.
6) 12 - 14.
7) 18.
8) 10 - 12mm.
9) Bracts - mixed (long, medium and short hairs).
Stem - mixed (long, medium and short hairs).
Leaves - cobwebbed below.
10) Not available.

Identification: *Senecio speciosus.*

Cummings s.n. Grahamstown. 3326BC. 17/4/76.

1) Basal rosette, stem and cauline leaves.
2) Spathulate with near petioled base.
3) Mainly toothed, may be shallowly lobed.
4) Corymbose.
5) 20 - 25mm.
6) 14 - 16.
7) 16- 18.
8) 10 - 11mm.
9) Bracts - short to medium length hairs.
Stem - short to medium length hairs.
Leaves - sparse medium length hairs.
10) Cylindrical, ribbed, short hair mainly between ribs.

Identification: *Senecio speciosus.*

Hobson 32. Dassie Kranz near Salem. 3326AD. 10/4/83.

1) Basal rosette, stem leaves, very small cauline leaves.
2) Elliptical to spathulate, scarcely petioled.
3) Shallowly lobed.
4) Corymbose.
5) 25mm.
6) 14 - 16.
7) 18 - 20.
8) 10mm.
9) Bracts - large stumpy hairs.
Stem - large stumpy hairs.
Leaves - large stumpy hairs.
10) Not available.

Identification: *Senecio speciosus.*

Woods Davies 57. Highlands Rd. Grahamstown. 3326AD. 27/9/85

1) Basal rosette, few stem and cauline leaves.
2) Spathulate, scarcely petioled.
3) Shallowly lobed.
4) Corymbose.
5) 20mm.
6) 14 - 16.
7) 18 - 20.
8) 10mm.
9) Bracts - short to medium length hairs.
   Stem - short to medium length hairs.
   Leaves - more or less glabrous, a few short hairs.
10) Cylindrical, ribbed, short hairs between ribs.

Identification: *Senecio speciosus.*

Woods Davies 96. Gonubie, East London. 3328CC. May 1985.

1) Basal rosette, stem leaves few very small cauline leaves.
2) Elliptical few spathulate with petiole like base.
3) Lobed.
4) Corymbose.
5) 20 - 25mm.
6) 14 - 16.
7) 18 - 20.
8) 10 - 11mm.
9) Bracts - large stumpy hairs.
   Stem - medium length hairs.
   Leaves - large stumpy hairs.
10) Not available.

Identification: *Senecio speciosus.*

S. Hobson 1296B. Slaaikraal Dams Farm, Grahamstown. 3326BC. 1/12/86.

1) Basal rosette, stem leaves, few cauline leaves.
2) Elliptical to spathulate tapering to narrow petiole like base.
3) Raggedly lobed and toothed.
4) Corymbose.
5) 10 - 15mm.
6) 12 - 14.
7) 14 - 16.
8) 10mm.
9) Bracts - sparse short hairs.
   Stem - sparse short to medium length hairs.
   Leaves - large stumpy hairs on veins and margins.
10) Not available.

Identification: *Senecio speciosus.*

Garrard 26. Igoda, East London. 3227DB. 15/10/87.

1) Basal rosette, few stem leaves.
2) Round to elliptical tapering to petiole like base.
3) Shallowly lobed.
4) Racemose.
5) 25 - 30mm.
6) 14 - 16.
7) 18 - 20.
8) 12mm.
9) Bracts - few long jointed glandular hairs.
   Stem - very few short hairs.
   Leaves - medium to long hairs on veins and margin.
10) Not available.

Identification: *Senecio macrocephalus.*

Van Heeren 8. Grahamstown, Mountain Drive. 3326BC. August 1987.

1) Basal rosette, few stem and cauline leaves.
2) Elliptical to spathulate narrowing to base.
3) Lobed.
4) Short, racemose to corymbose.
5) 20 - 25mm.
6) 14.
7) 18 - 20.
8) 11mm.
9) Bracts - short to medium length hairs.
   Stem - short to medium length hairs.
   Leaves - short hairs mainly on veins and margins.
10) Not available.

Identification: *Senecio speciosus.*

McCartan 22. Menziesberg, Amatole Mountains. 3226DB. 9/3/91.

1) Stem leaves only.
2) Spathulate, clasping becoming lanceolate, auriculate.
3) Toothed.
4) Single head.
5) 20mm.
6) 8.
7) 14.
8) 8mm.
9) Bracts - few medium length hairs.
   Stem - few medium length hairs.
   Leaves - short hairs on midribs and margin.
10) Not available.

Identification: *Senecio* sp. aff. *S. speciosus.*

McCartan 23. Menziesberg, Amatole Mountains. 3226DB. 9/3/91.

1) Basal rosette, very few stem or cauline leaves.
2) Spathulate, tapering to petiole like base.
3) Toothed.
4) Only 2 heads.
5) 20mm.
6) 8.
7) 12.
8) 8 - 10mm.
9) Bracts - few short to medium length hairs.
   Stem - short to medium length hairs.
   Leaves - medium length hairs.
10) Not available.

Identification: *Senecio speciosus.*

McCartan 25. Menziesberg, Amatole Mountains. 3226DB. 9/3/91.

1) Basal rosette, stem and cauline leaves.
2) Spathulate tapering to petiole like base.
3) Toothed.
4) Corymbose.
5) 15 - 20mm.
6) 8.
7) 12.
8) 8mm.
9) Bracts - few short hairs.
   Stem - short hairs.
   Leaves - few short hairs.
10) Not available.

Identification: *Senecio speciosus.*

McCartan 28. Near Maclear. 3128AB. 8/4/91.

1) Basal rosette, stem and cauline leaves.
2) Oval, tapering to base, scarcely petioled.
3) Entire, shallowly toothed.
4) Corymbose.
5) 15 - 20 mm.
6) 10 - 12.
7) 18.
8) 9 - 10.
9) Bracts - medium length stumpy hairs.
   Stem - numerous short hairs.
   Leaves - few long hairs.
10) Cylindrical, ribbed, hairy between ribs.

Identification: *Senecio macrocephalus.*

McCartan 29. Near Maclear. 3128AB. 8/4/91.

1) Basal rosette, stem and cauline leaves.
2) Oval, tapering to base, scarcely petioled.
3) Entire, shallowly toothed.
4) Corymbose.
5) 15 - 20 mm.
6) 10 - 12.
7) 18.
8) 9 - 10.
9) Bracts - medium length stumpy hairs.
   Stem - numerous short hairs.
   Leaves - few long hairs.
10) Cylindrical, ribbed, hairy between ribs.

Identification: *Senecio macrocephalus.*

McCartan 8. East London (Gonubie Nature Reserve). 3228CC. 8/9/91.

1) Basal rosette, few stem leaves.
2) Elliptical to spathulate, with petiole like base.
3) Shallowly lobed.
4) Corymbose.
5) 40mm
6) 14 - 16
7) 20 - 22
8) 15mm
9) Bracts - few large stumpy hairs.
   Stem - few large stumpy hairs.
   Leaves - some small hairs.
10) Cylindrical, ribbed, short hairs between ribs.

Identification - *Senecio speciosus/macrocephalus* intermediate.

McCartan 18. Grahamstown (Mountain Drive). 3326BC. 8/9/91.

1) Base rosette, stem and cauline leaves.
2) Elliptical to spathulate tapering to base.
3) Lobed.
4) Corymbose.
5) 20 - 25mm.
6) 13.
7) 18.
8) 12mm.
9) Bracts - long, stumpy hairs.
   Stem - long, stumpy hairs.
   Leaves - medium to long hairs.
10) Not available.

Identification: *Senecio speciosus/macrocephalus* Intermediate.

McCartan 19. Grahamstown (Mountain Drive). 3326BC. 8/9/91.

1) Basalrosette, stem and cauline leaves.
2) Elliptical to spathulate tapering to base.
3) Lobed.
4) Corymbose.
5) 20 - 25mm
6) 13.
7) 18.
8) 12mm.
9) Bracts - short glandular hairs.
   Stem - short glandular hairs.
   Leaves - short glandular hairs.
10) Not available.

Identification: *Senecio speciosus/macrocephalus* intermediate.

McCartan 6. East London (Gonubie Nature Reserve). 3327BB. 8/9/91.

1) Basal rosette, stem and cauline leaves.
2) Oval to elliptical tapering to narrow base.
3) Finely toothed.
4) Corymbose.
5) 25mm.
6) 13 - 20.
7) 18 - 20.
8) 12 - 14mm.
9) Bracts - some long glandular hairs.
   Stem - few long glandular hairs.
   Leaves - hairs on veins and margins.
10) Cylindrical, ribbed, hairy between ribs.

Identification: *Senecio speciosus/macrocephalus* intermediate.

McCartan 9. East London (Gonubie Nature Reserve). 3327BB. 8/9/91.

1) Basal rosette, stem and cauline leaves.
2) Elliptical to spathulate tapering to base.
3) Shallowly toothed and lobed.
4) Short, probably corymbose.
5) 40mm.
6) 16 - 18.
7) 18 - 20.
8) 12 - 14mm.
9) Bracts - very long hairs.
   Stem - very long hairs.
   Leaves - long hairs on veins and margin.
10) Cylindrical, ribbed, hairy between ribs.

Identification: *Senecio speciosus/macrocephalus* intermediate.

McCartan 1. Fish River Grassland (Fish River Mouth). 3327AC. 11/9/91.

1) Basal rosette, few stem and cauline leaves.
2) Spathulate, scarcely petioled base.
3) Lobed and toothed.
4) Corymbose.
5) 30 - 35mm.
6) 12 - 13.
7) 18.
8) 10 - 12mm.
9) Bracts - short hairs.
   Stem - short hairs.
   Leaves - cobwebbed below.
10) Cylindrical, ribbed, short hairs between ribs.

Identification: *Senecio speciosus/macrocephalus* intermediate.

APPENDIX 3

TESTING THE TOXICITY OF SOME SOUTHERN AFRICAN PLANT EXTRACTS USING BRINE SHRIMP (*ARTEMIA SALINA*).

INTRODUCTION

In southern Africa herbal medicines are still widely used, especially in rural areas where herbal knowledge is passed down from generation to generation. However with the rapid urbanisation of the African communities, and their increased exposure to Western medicines and medical practices, a lot of this knowledge could disappear. Accordingly, a project in Zululand has resulted in a list of more than 1000 plants used by the Zulus for a variety of purposes ranging from love charm emetics to cancer cures. (pers. comm. Mrs. A. Hutchings). Although the chemical constituents of some of these plants have been identified very little is known about their pharmacological activity. In the Eastern Cape such a comprehensive list of plants does not as yet exist but it can be assumed that a large number of the Zulu plant species are also found and used medicinally in this area. Add to these other plants which are used only by the Xhosa and another long list ensues.

Therefore a simple, rapid and economical bioassay is required to screen the plethora of Southern African medicinal plants for general biological activity as a first step in the evaluation of their pharmaceutical potential. A bioassay that meets these criteria is the brine shrimp toxicity test.[1] Over the last decade the brine shrimp (*Artemia salina*) has been used in various bioassay systems to monitor the toxicity of mycotoxins,[2] wastewater and marine pollutants,[3/4] detergents and surfactants,[5] petroleum products,[6] food dyes,[7] antifouling paints for ships[8] and even sensitivity to cosmic rays.[9] A wide variety of bioactive chemical compounds are toxic to brine shrimps and the death of this organism when exposed to varying concentrations of these compounds forms the basis of the brine shrimp toxicity test.[1] Bioactive compounds are nearly always toxic in high concentrations and as toxicology can be described as pharmacology at higher doses this premise has been applied to the the screening of medicinal plant extracts in the brine shrimp toxicity test.[10] Although this test often detects a vast array of bioactive chemical compounds in a plant extract it provides a good initial screen for substances with possible pharmaceutical applications.

In this paper the application of the brine shrimp toxicity test to the screening of several Southern African plant extracts is reported. Modifications to the original method described by Meyer *et al.* are also discussed.

MATERIALS AND METHODS

Brine shrimp eggs can be obtained from pet shops where they are sold as fish food. It is possible to hatch the brine shrimp by placing the eggs in saline media, for example, filtered, autoclaved sea water or an artificial brine solution comprising sodium chloride (150g), magnesium sulphate (25g), sodium bicarbonate (0.1g) and water (2 litres). The relative suitability of these two media was compared. Comparable results were obtained with filtered, autoclaved sea water and aerated artificial brine solution. Few shrimps hatched or survived in unaerated artificial brine solution.

The newly hatched brine shrimp nauplii can survive for up to 48 hours without food. At this stage in their life cycle the nauplii have reached their second or third instar and exhibit their greatest sensitivity to test compounds.[11] Therefore to ensure that mortality observed in the bioassay could be attributed to bioactive compounds and not starvation two different foods were compared, a dried yeast solution and a marine unicellular alga *Tetraselmis seuccia* (cultured in filtered, autoclaved sea water with added nutrient medium). A disadvantage of the yeast solution is that if an excess is added to the media containing the brine shrimp nauplii the media becomes foul and the shrimps die. No such effects were observed with *Tetraselmis seuccia* and the shrimps thrived on this food source, living for up to seven weeks. Therefore filtered, autoclaved sea water and *Tetraselmis seuccia* were used in the bioassay.

BIOASSAY

A natural product lethal to brine shrimp[1], strychnine sulphate, was used as a control in dilutions of 10,100,1000 and 2000 ppm in autoclaved sea water (5ml) in kimble vials. Five replicates of each concentration were prepared and ten 48 hr old shrimps were pipetted into each vial with two drops of the *Tetraselmis* culture. After 24 hours the numbers of survivors were counted and percentage deaths calculated. From these percentages the LC50 in mg/ml was calculated[12] and the results are shown in Table 1. These results compare favourably with those of Meyer et al.

PREPARATION AND TESTING OF PLANT MATERIAL

Plant material to be tested was first air dried at 30°C and then finely ground. The dried plant material (5g) was extracted at room temperature, with water (200 ml) and shaken for 24 hours. A similar extraction was also carried out with methanol. The extracts were filtered and preliminary tests were carried out using 0.05ml, 0.5ml and 1.0ml of the extract made up to 5ml with seawater and tested with the brine shrimps as above. Methanol is toxic to the shrimps and the aliquots of the methanol extracts were allowed to evaporate to dryness (overnight) before seawater (5ml) and the shrimps were added. Controls were set up using sea water (5ml) for the water extracts and 1ml of methanol evaporated to dryness overnight and made up to 5 ml with sea water for the methanol extracts. The preliminary test results are given in Table 2.

If the extracts showed any activity in the preliminary tests the water extracts were freeze dried. The methanol extracts can be evaporated on a rotary evaporator and then freeze dried but this has not yet been tried. The resulting solid crude extracts were then used to make up solutions of 100, 1000 and 2000 ppm before testing in the bioassay as outlined earlier.

Extracts were made of a variety of plant species, some with known pharmacological activity and chemical composition and some with various medicinal reputations in the local African community. In some cases various parts of the plants were used as traditional medicine does not always use the whole plant in a cure.

The plants tested together with information on secondary metabolites and medicinal uses are shown in Table 3.

RESULTS

Results and calculated LC50's are shown in Table 4.

DISCUSSION AND CONCLUSION

Extracts of the families Liliaceae and Amaryllidaceae which are known to contain many toxic compounds including alkaloids, steroidal saponins and cardiac glycosides,[13] and in the case

of *Tulbaghia* species sulphur compounds, are very toxic to the brine shrimps. Plants containing saponins for example *Chrysanthemoides monilifera* from the Asteraceae, used by the early colonists for making soap,[14] also proved convincingly toxic. *Vinca major* (Apocynaceae) said to contain both alkaloids and triterpenoids and used as a diabetes remedy[15] proved toxic while only the methanol extract of *Lantana camara* (Verbenaceae) which contains both alkaloids and lantadenes (pentacyclic triterpenes)[15] was toxic. Surprisingly some plants containing poisonous alkaloids do not give such clear results, for example aqueous and methanolic extracts of *Datura stramonium,* which contain the alkaloids atropine, hyoscine and hyoscyamine,[15] had little effect on the shrimps although some toxicity was noted with aqueous extracts at higher concentrations. Also aqueous and methanolic extracts of *Senecio pterophorus* (known to contain toxic pyrrolizidine alkaloids)[15] were non-toxic to the shrimps. *Erythrophleum lasianthum,* one of the Zulu ordeal trees and containing the alkaloid erythrophleine[15] also showed very little activity although the aqueous extract of the pods and seeds which frothed slightly (indicating saponins?) was slightly toxic.

The inability of the brine shrimp test to detect these bioactive, toxic alkaloids poses a problem which could be due to either the method of extraction or the type of alkaloid present. *Datura* leaves are reported to be poisonous and to have caused deaths when they have been chewed.[15] The leaves are also smoked to produce a narcotic effect,[15] the active principle must therefore literally go up in smoke making it difficult to test this type of compound on brine shrimps. Pyrrolizidine alkaloids exert their toxic effects on the liver cells of humans and animals[15] and it is possible that no such adverse reaction occurs in the cells of the brine shrimp. Therefore these problems must be noted before the brine shrimp method is used as a general bioassay technique to test higher plants for biological activity. However the results as shown on Tables 1 - 4 indicate that the bioassay could be a very useful method of preliminary screening to identify plants which are of possible pharmaceutical importance.

TABLE 1:
BRINE SHRIMP ASSAY: PERCENTAGE DEATHS: 24 HOURS:

STRYCHNINE SULPHATE:

ppm	10	100	1000	2000	LC50
%	10	60	84	100	74.9

TABLE 2: RESULTS FROM PRELIMINARY TESTS:

Percentage deaths of brine shrimps at 24 hrs.

***Tulbaghia violacea*, leaves.**

	Water extract			**Methanol extract**		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	100	100	0	100	100

***Leucojum aestivum*, bulb.**

	Water extract			**Methanol extract**		
	0.05ml	0.5	1.0	0.05	0.5	1.0
%	50	100	100	0	100	100

***Vinca major*, whole plant.**

	Water extract			**Methanol extract**		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	56	96	98	100	100	100

Chrysanthemoides monilifera subsp rotundata.

	Water extract			**Methanol extract**		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	86	100	100	74	96	99

Chrysanthemoides monilifera subsp pisifera.

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	82	100	46	98	98

Nicotiana sp, **leaf.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	2	36	50	9	28	56

Lantana camara, **whole plant.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	0	0	60	84	94

Chelidonium majus, **whole plant.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	20	40	94	100	100

Datura stramonium, **fresh young plant 20gm/100ml.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	40	60	0	0	0

Erythrophleum lasianthum, **pods with seeds.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	0	63	0	0	0

***Senecio longifolius*, whole plant.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	55	94	15	30	50

***Senecio pterophorus*, whole plant.**

	Water extract			Methanol extract		
	0.05 ml	0.5	1.0	0.05	0.5	1.0
%	0	0	0	0	0	0

TABLE 3:

PLANTS EXAMINED ACCORDING TO FAMILIES AND ALLEGED MEDICINAL USE.[15]

FAMILY: LILIACEAE:

CHEMICAL CONTENTS: Alkaloids, sterols, cardiac glycosides, steroidal saponins, sulphur compounds.

PLANT EXAMINED:

Tulbaghia violaceae, used as an enema for stomach troubles (this usage has resulted in death from internal bleeding in children in the Eastern Cape, pers. comm. Livingstone Hospital, Port Elizabeth), for pulmonary tuberculosis and as an anthelmintic. The leaves are rubbed on the head for sinus headache and the plant is grown to keep snakes away.

FAMILY: AMARYLLIDACEAE:

CHEMICAL CONTENTS: Alkaloids, possibly saponins.

PLANT EXAMINED:

Leucojum aestivum, no specific medicinal use, poisonous.

FAMILY: PAPAVERACEAE:

CHEMICAL CONTENTS: Alkaloids, fixed oils.

PLANT EXAMINED:

Chelidonium majus is not native to Southern Africa but is grown widely as a garden plant, no specific medicinal use is known although the similar sap of other Papaveraceae is used as a narcotic, a purgative and to destroy warts.

FAMILY: FABACEAE:

CHEMICAL CONTENTS: Alkaloids, cyanogenic glycosides, saponins tannins.

PLANTS EXAMINED:

Erythrina caffra seeds contain alkaloids with a curare like action which can be used as a muscle relaxant in treatment of nervous disease.

Boiled bark and roots are used for earache toothache and as an eyewash. Paste made from leaves is also used medicinally.

Erythrophleum lasianthum bark is a powerful purgative and poison. Powdered, it is snuffed for headaches or colds. Seeds are also used but are much stronger. The leaf is a snake bite remedy in Tanzania. Erythrophleum alkaloids have a cardiac action and the seeds also contain haemolytic saponins.

FAMILY: APOCYNACEAE:

CHEMICAL CONTENTS: Alkaloids, cardiac glycosides, saponins, tannins.

PLANT EXAMINED:

Vinca major has been used as a diabetes remedy and the sap is used for insect bites and warts. It is thought to contain indole alkaloids and tannins and to have possible antimalarial and anti viral uses. The plant is astringent, used in menorrhagia and as an abortifacient.

FAMILY: VERBENACEAE:

CHEMICAL CONTENTS: Alkaloids, lantadenes (pentacyclic triterpenes)

PLANT EXAMINED:
Lantana camara has been used as a herb bath, a charm, for coughs, colds, jaundice, chest diseases and as a bath for rheumatism. Lantadene A is an icterogenic principle which causes photosensitisation in sheep.

FAMILY: SOLANACEAE:

CHEMICAL CONTENTS: Alkaloids.

PLANTS EXAMINED:

Datura stramonium is smoked for the relief of headaches and asthma and the leaves are used as a poultice. Poisonings have been reported after ingesting the seed. The plant is said to be narcotic. Atropine and hyoscine are mainly found in the young leaves and atropine and hyoscyamine in adult leaves.

Nicotiana sp. the tobacco plants are smoked, chewed and snuffed as a social habit. The alkaloids present are nicotine and anabasine together with some phenolic substances. The plant is somewhat insecticidal, being toxic to ticks.

FAMILY: ASTERACEAE:

CHEMICAL CONTENTS: Alkaloids, saponins, sesquiterpenes, volatile oils.

PLANTS EXAMINED:

Chrysanthemoides monilifera gives extracts which froth readily indicating the presence of saponins. Placing a burning branch of this bush in the hut of a madman is said to cure him.

Senecio pterophorus contains pyrrolizidine alkaloids particularily retrorsine and is toxic to stock.

TABLE 4:

PERCENTAGE DEATHS AND LC50's OF BRINE SHRIMPS AT 24 HOURS, USING FREEZE DRIED, AQUEOUS EXTRACTS OF PLANT MATERIAL:

***Tulbaghia violaceae*, leaves:**

ppm	100	200	300	400	500	1000	LC50
%	22	93	100	100	100	100	164.1

***Tulbaghia violaceae*, roots:**

ppm	100	200	300	400	500	1000	LC50
%	4	32	98	100	100	100	237.1

***Leucojum aestivum*, leaves:**

ppm	100	1000	2000	LC50
%	20	99	100	251.2

***Leucojum aestivum*, bulb:**

ppm	100	1000	2000	LC50
%	52	100	100	89.1

***Erythrina caffra*, seeds:**

ppm	100	1000	2000	LC50
%	0	66	100	631.0

***Erythrophleum lasianthum*, bark:**

ppm	100	1000	2000	LC50
%	7	10	15	2000

***Erythrophleum lasianthum*, leaves:**

ppm	100	1000	2000	LC50
%	0	20	30	2000

***Erythrophleum lasianthum*, pods with seeds:**

ppm	100	1000	2000	LC50
%	0	25	50	2000

***Vinca major*, whole plant:**

ppm	100	1000	2000	LC50
%	0	42	64	1334

***Datura stramonium*, young leaf:**

ppm	100	1000	2000	LC50
%	10	15	30	2000

***Nicotiana sp*, leaves:**

ppm	100	1000	2000	LC50
%	2	42	44	2000

***Chrysanthemoides monilifera subsp rotundata*:**

ppm	100	1000	2000	LC50
%	64	78	94	34.6

***Chrysanthemoides monilifera subsp pisifera*:**

ppm	100	1000	2000	LC50
%	18	70	87	398.1

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