Molecular Phylogeny, Radiation Patterns and Evolution of Life-History Traits in *Ursinia* (Anthemideae, Asteraceae)

by

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Declaration

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Abstract

Sequence data from the Internal Transcribed Spacer (ITS) of the nuclear ribosomal DNA were used to study the phylogenetic relationships in the genus *Ursinia* Gaertn. (Asteraceae, Anthemideae) in the southern African region. Closely related genera, i.e. *Cotula* L., *Osteospermum* L. and *Agoseris* Raf., were used as outgroups. The study also included maximum parsimony and principal component analyses.

The taxa within the genus *Ursinia* had previously been classified into two subgenera, *Ursinia* and *Sphenogyne* R.Br., mainly on the basis of distinct cypsela characters. The maximum parsimony, principal component and the phylogenetic analyses revealed two subgenera, corresponding to the existing subgeneric classification. Principal component analysis shows that the pappus, the number of pappus bristles and the colour of the cypsela are the most informative characters.

However, the low number of phylogenetically informative characters of the ITS sequences, the poor resolution in the consensus tree, and low branch support values indicate that the ITS data contain weak phylogenetic signals. The low bootstrap values for many nodes suggest that one should be cautious in using the ITS region alone to make final conclusions about the origin and evolution of taxa. In maximum parsimony analysis, the RI, CI and bootstrap values are low; principal component analysis values are also low. Furthermore, there is a lack of resolution in subgenus *Sphenogyne*. In the literature, *Ursinia* is divided into seven series but they were not retrieved as monophyletic in this study, probably because of short branch lengths in the phylogeny. Further molecular data are therefore required to be able to support or reject the present classification. Maximum parsimony, principal component and molecular analyses show that *U. trifida* f. *calva* Prassler and *U. trifida* (Thunb.) N.E.Br. f. *trifida* are not sister taxa, supporting the recognition of these two taxa as separate species.

The *Ursinia* taxa from the summer-rainfall region are not monophyletic and are sister to a clade of Cape species. This supports a hypothesis that *Ursinia* migrated from the Cape into the Drakensberg which has been shown for a number of other Cape groups that have Drakensberg relatives.

Opsoming

Sekwens data uit die Interne Transkripsie Spasieërder (ITS) van die nukluêre ribosoom-DNS is gebruik om die filogenetiese verwantskappe van die genus *Ursinia* Gaertn. (Asteraceae, Anthemideae) in die Suider-Afrikaanse streek te ondersoek. Naverwante genusse, d.i. *Cotula* L., *Osteospermum* L. en *Agoseris* Raf., is as buitegroepe gebruik. Die ondersoek het maksimum parsimonie en hoofkomponentanalise ingesluit.

Taxa binne die genus *Ursinia* is voorheen in twee subgenusse, *Ursinia* en *Sphenogyne*, ingedeel, hoofsaaklik op grond van opvallende vrugkenmerke. Die maksimum parsimonie, hoofkomponentanalise en die filogenetiese analise het dieselfde twee subgenusse opgelewer, in ooreenstemming met die voorgestelde subgeneriese klassifikasie. Die hoofkomponentanalise toon dat die pappus, die aantal pappusskubbe en die vrugkleur die meeste inligting bied.

In hierdie ondersoek impliseer die min filogeneties belangrike kenmerke van die ITS-ordenings, die swak skeiding in die konsensusboom, en die swak skeidingondersteuningswaardes dat die ITS-data swak filogenetiese seine bevat. Die lae *bootstrap*-waardes vir baie knope dui daarop dat 'n mens versigtig moet wees om slegs op grond van die ITS-ordenings finale afleidings oor die oorsprong en evolusie van taksons te maak. In maksimum parsimonie die RI, KI en die *bootstrap*-waardes laag; hoofkomponentanalise-waardes is ook laag. Verder is daar’n gebrek aan verwantskappe in subgenus *Sphenogyne*. In die literatuur word *Ursinia* in sewe series verdeel maar in hierdie studie het die series nie as monofileties na vore gekom nie, moontlik as gevolg van die kort lengtes van die vertakkings in die filogenie. Verdere molekulêre data word dus benodig om die klassifikasie in sewe series te ondersteun of te verwerp. Maksimum parsimonie, hoofkomponentanalise en molekulêre analise toon dat *U. Trifida* (Thunb) N.E.Br. f. *calva* Prassler en *U. trifida* (Thunb) N.E.Br. f. *trifida* nie sustertaksons is nie. Dit ondersteun die erkenning van hierdie twee taksons as afsonderlike spesies.

Die *Ursinia*-taksons van die somerreënvalgebiede is nie monofileties nie en is suster tot ’n klade van Kaapse spesies. Dit ondersteun ’n hipotese dat *Ursinia* van die Kaap na die Drakensberg migreer het; soortgelyk aan ’n aantal ander Kaapse groepe met Drakensberg-verwante taksa.

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Preface

The presented thesis, entitled ***"Taxomomic History and Current Status of the Genus Ursinia (Anthemideae, Asteraceae)"***, is composed of three chapters.

Chapter 1 features taxonomic history of the genus *Ursinia* (sensu lato) and sets the scene of the subsequent 2 chapters. The format of this chapter was adapted to meet the requirements for publication in the *South African Journal of Botany*.

Chapter 2 deals with the cypsela morphology and its importance for taxonomy of the subgeneric taxa within *Ursinia*. This chapter is formatted according to the requirements of the *Botanical Journal of the Linnaean Society*.

Chapter 3 features the molecular phylogeny of the genus *Ursinia* base don ITS marker. We plan to extend this chapter by additional research and prepare it for publication in an international journal to be chosen at later stage.

Chapter 1

Genus *Ursinia* (Anthemideae, Asteraceae): History and Current Taxonomic Account

1.1 Introduction

The southern and south-western regions of the former Cape [of Good Hope] Province of South Africa (now largely part of the Western Cape Province) possess a distinct flora often referred to as the "Cape Flora" (Linder 1990, 2003). Takhtajan (1986), in acknowledgement of its uniqueness and high endemism, recognised this region as the Cape Floristic Kingdom (known also as Capensis) comprising one phytochorion of lower rank—the Cape Floristic Region (CFR). This 90,000 km^2 large region, representing less that 0.5% of the area of Africa, is home to nearly 20% of the flora of the continent (Linder 2001). Two most striking features of this flora are the remarkable species richness and high level of endemism, which could be a result of geographical and ecological isolation (Linder 2003) and/or low extinction rates during the glacials (Jansson & Dynesius 2002). Almost 69% of the species and 16% of the genera of the South African flora are endemic to the CFR (Goldblatt & Manning 2000, Linder 2003). The high levels of endemism and remarkable diversity (especially beta-diversity; see Linder 2003) as well as presence of many species-rich genera (e.g. the genus *Erica* L. has as many as 750 species here; E.G.H. Oliver, personal communication) makes the CFR a prime address of studies on the evolution of species-rich floras.

One of the species-rich genera, having its centre of diversification in the CFR, is the broadly-conceived genus *Ursinia* of the tribe Anthemideae (Asteraceae). Species of this genus occurs in southern and eastern Africa, and show notable species concentration in the Western Cape (Fig. 1.). One of the species is found as far north as Ethiopia (*U. nana* according to the taxonomic concept of Prassler 1967). Within southern Africa, most of the species occur in the winter-rainfall area, such as endemic *U. quinquepartita* (DC.) N.E.Br. (Fig. 2), while some are limited to summer-rainfall regions of north-eastern South Africa, Lesotho and Swaziland (Fig. 3). Some widely distributed taxa such as *U. nana* DC., *U. chrysanthemoides* (Less.) Harv. and *U. paleacea* (L.) Moench, appear to be morphologically (and genetically) complex and therefore deserve further taxonomic attention (Fig. 4). So far all studied taxa in *Ursinia* were found to be diploid (mainly 2n =18; Haesler 1967).

Ursinia anthemoides (L.) Poir. and *U. speciosa* DC. were introduced to Western Australia and to New Zealand (Prassler 1967, Bremer & Humphries 1993, Bremer 1994, Barrett & Eng Pin Tay 2005). Here they successfully established and in places even became invaders.

1.2 Taxonomic History of the Genus *Ursinia*

Partial historical survey of the taxonomy surrounding the current genus *Ursinia* was published by Prassler (1967). She established that in the past, since Linnaeus (1760), Bergius (1767) and Burman (1768), the many current *Ursinia* taxa were first described as *Arctotis* (Arctotideae, Asteraceae). Linnaeus (1760) described *A. paradoxa* (later selected as the nomenclatural type of the genus *Ursinia* by Gaertner 1791) and in 1764 he listed three relevant *Arctotis* species (*A. paleacea, A. anthemoides and A. dentata*). Bergius (1767) described *A. crithmoides* and *A. pilifera*, Linnaeus (1771) added *A. tenuifolia* in his Mantissa, and his son (Linnaeus filius) described *A. serrata* in 1782. Later Aiton (1789) described *A. scariosa* and Jacquin (1797) added *A. foeniculacea*.

Of 42 *Arctotis* species listed by Thunberg (1800), 11 species are now classified as *Ursinia*. These include: *Arctotis serrata* L.f., *A pectinata* Thunb., *A. trifida* Thunb., *A. punctata* Thunb., *A. pinnata* Thunb., *A. nudicaulis* Thunb., *A. anthemoides* L., *A. paleacea* L., *A. dentata* L., *A. pilifera* Berg., and *A. sericea* Thunb. The classification of many current *Ursinia* species as *Arctotis* has been also followed by Willdenow (1803).

Gaertner (1791) in his protologue of the genus *Ursinia* diagnosed *Ursinia* on the basis of the pappus consisting of two rows (pappus arranged in an outer row of scales and an inner row of bristles). The type of his new genus is *U. paradoxa* Gaertn. (based on the *Arctotis paradoxa* Thunb.; synonymised by Prassler 1967 with *Ursinia chrysanthemoides* (Less.) Harv.). He listed three more species, such as *Arctotis pilifera* Berg., *A. anthemoides* Berg. and *A. paleacea* L., which he never saw, but on the basis of pappus arranged in double row, he also considered these as member of the genus *Ursinia*.

Poiret (1808) interpreted *Ursinia* in sense of Gaertner (1791), but he described only species with a single pappus, such as *U. paradoxa* Gaertn., *U. pilifera Berg., U. dentata L., U. anthemoides L., U. paleacea L., U. scariosa* Aiton, *U. crithmoides* Berg., *U. leucanthemifolia* Poir., *U. foeniculacea* Jacq., *U. serrata* L.f. and *U. tenuifolia L.*

Brown (1813, p. 142) published the first diagnosis of the genus *Sphenogyne* R.Br., which would include *S. anthemoides* (L.) R.Br., *S. crithmifolia* R.Br., *S. scariosa* (Aiton) R.Br., *S. abrotanifolia* R.Br., *S. dentata* (L.) R.Br. and *S. odorata* R.Br. He has not, however, addressed explicitly the relationship of *Sphenogyne* with *Ursinia* and chose not to mention the latter genus at all. Sprengel (1826) synonymised the two genera *Arctotis* and *Sphenogyne* under genus *Ursinia* and listed 11 species. Lessing (1832) subdivided *Sphenogyne* into three subgenera, such as *Anthemoides* Less., *Thelythamnos* Less. and *Xerolepis* Less., and described four new *Sphenogyne* species (*S. chrysanthemoides, S. anethifolia, S dentata* and *S. coronopifolia*).

The first revision of both *Ursinia* and *Sphenogyne* (further referred to as *Ursinia-Sphenogyne* complex) was presented by A.P. De Candolle (1836), who distinguished 49 species of *Sphenogyne* and 13 species of *Ursinia.* Many of the taxa described by De Candolle (l. c.) were later relegated into synonymy uncritically by Prassler (1967). Harvey (1865) presented a new, detailed taxonomic revision of the *Ursinia-Sphenogyne* complex as a part of the Flora Capensis. He reduced nine species to varieties, described four as new species, and further recognised 44 species and 15

varieties within *Sphenogyne* as well as 10 species and one variety within *Ursinia*. Brown (1887) reversed to the old concept of the genus *Ursinia* by including all *Sphenogyne* taxa into *Ursinia*.

Further additions to *Ursinia-Sphenogyne* complex were made by Schultz Bipontinus (1844, *S. natalensis*), Sonder (1850, *S. eckloniana*), Brown (1894, *U. saxatilis*), Brown (1901, *U. alpina*), Woods & Evans (1901, *U. brevicaulis*), Bolus (1906, *U. subintegrifolia*), Phillips (1917, *U. tysoniana*), Compton (1931, *U. argentea*), Dinter (1932, *U. frutescens*), Phillips (1951, *U. caledonica*), Bolus & Hall (1961, *U. gayeri*) and Prassler (1967, *U. merxmuelleri* and *U. trifida* forma *calva*).

Phillips (in Dyer 1951) noted that three of *Ursinia* species, namely *U. eckloniana* Sond., *U. quinquepartita* DC., and a new taxon described as *U. caledonica* E. Phillips, differed in general habit and floral morphology from other *Ursinia* species. In these three species, ray florets are female, and may be either fertile or sterile, whereas in the other *Ursinia-Sphenogyne* taxa, the ray-florets are neuter, with neither ovary nor style developed. These three taxa are restricted to the Caledon Mountains (incl. Kogelberg, Kleinrivierberge, Hottentots Holland). Based on their morphological uniqueness and concerted geographical distribution, Phillips (l.c.) placed them into a new genus called *Ursiniopsis*.

Prassler (1967) subjected the genus *Ursinia* (incl. the known taxa of the genus/subgenus *Sphenogyne* as well as the genus *Ursiniopsis*) to the third full taxonomic revision. Unfortunately, obviously her study was based on herbarium material only. It does not appear that Prassler (l. c.) has collected any material herself or used living (cultivated) material in her revision. She recognised only 37 species, nine subspecies and two formas, and classified them into two subgenera, *Ursinia* and *Sphenogyne.* The latter subgenus is further subdivided into five series: *Ursiniopsis*, *Pinnatae*, *Heterodontae*, *Thelythamnos* and *Xerolepis*, while the subgenus *Ursinia* is divided into two series: *Frutescentes* and *Nanae*.

1.3 Current Taxonomic Views

Beauverd (1915) was the first to place *Ursinia* in the tribe Anthemideae, a view supported by both Merxmüller (1954) and Prassler (1967) and upheld by later molecular evidence (Kim & Jansen 1995, Oberprieler & Vogt 2000, Watson *et al.* 2000, Francisco-Ortega *et al*. 2001, Oberprieler 2001, 2002, Watson *et al*. 2002, Oberprieler 2004a, b, Oberprieler *et al*. 2007, Himmelreich et al. 2008). The tribe Anthemideae of the subfamily Asteroideae is composed of about 110 genera with more than 1700 species. As many as 12 subtribes have to date been recognised within the tribe on morphological grounds (Bremer & Humphries 1993). The tribe has a worldwide distribution, with members occurring in Central Asia, the Mediterranean Region and South Africa; and less so in South America and Australia (Bremer 1994), hence the tribe has primarily of Old World and Temperate distribution (Heywood & Humphries 1977, Bremer & Humphries 1993).

Although the revision of the genus *Ursinia* by Prassler (1967) satisfactorily solved the problem of the relationship between the subgenera *Ursinia* and *Sphenogyne*, and has identified a number of characters which clearly distinguish these two taxa (Table 1),

the Prassler's (1967) identification key is poorly operational as it does not use well-defined characters, such as morphology of involucral bracts, flower size and coloration of petals and the growth habit as well. Some of these characters might disappear or become blurred in pressed herbarium material and therefore escape attention in taxonomic revisions. Limited scope of characters and inconsistent use of thereof lead to confusion, frequent misinterpretations and wrong plant identifications.

Intensive three-year field sampling in the *Ursinia-Sphenogyne* complex revealed an astonishing variability and existence of many very local, morphologically well-defined taxa representing good species pending formal description. Many of them became also supported or revealed by our molecular analyses. I believe that a revision of the identity of some De Candolle's (1836) and Harvey's (1865) taxa might result in resurrection of many species sunk by Prassler (1967) into synonymy. All these facts call for a new, comprehensive revision of the generic complex *Ursinia-Sphenogyne*, including many new field collections and implementing profound morphological and anatomical studies involving modern tools of plant genomics, such as chromosome banding, flow cytometry, construction of molecular phylogenies as well as phylogeographical studies zooming onto population-genetic level of variability. I expect that the understanding of the taxonomic and evolutionary patterns within *Ursinia-Sphenogyne* complex will enhance our knowledge of the evolutionary history and general pattern of radiations in the amazing Cape Flora.

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Table 1.1. Diagnostic characters of subgenera *Sphenogyne* and *Ursinia* as suggested by Prassler (1967).

Character	*Sphenogyne*	*Ursinia*
Palea	tip blunt or toothed	tip with round appendage
Pappus	in one series	in two series
Cypsela	straight or curved in lower part; usually tapering	bent almost at right angle much thicker in upper part (pipe-like)
Basal tuft of hairs	often present	absent

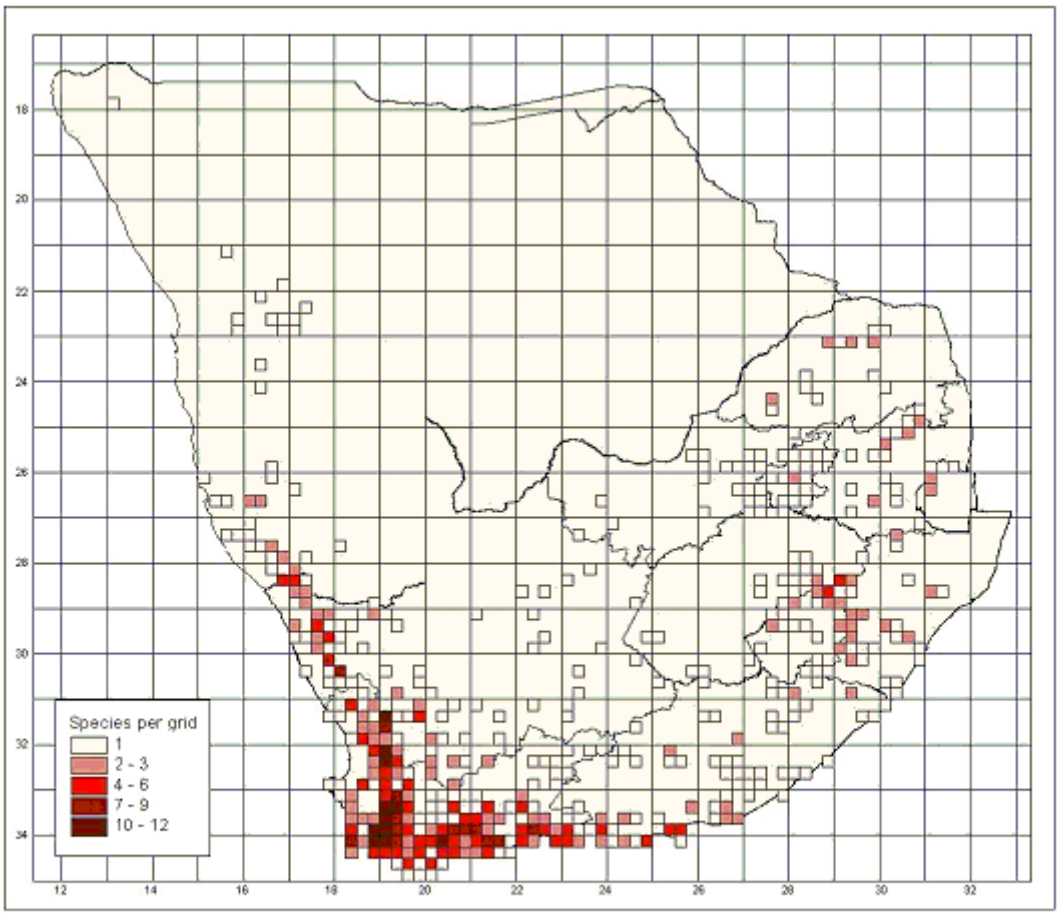

Fig. 1. Known distribution of Ursinia in southern Africa (with species count per quarter degree square).

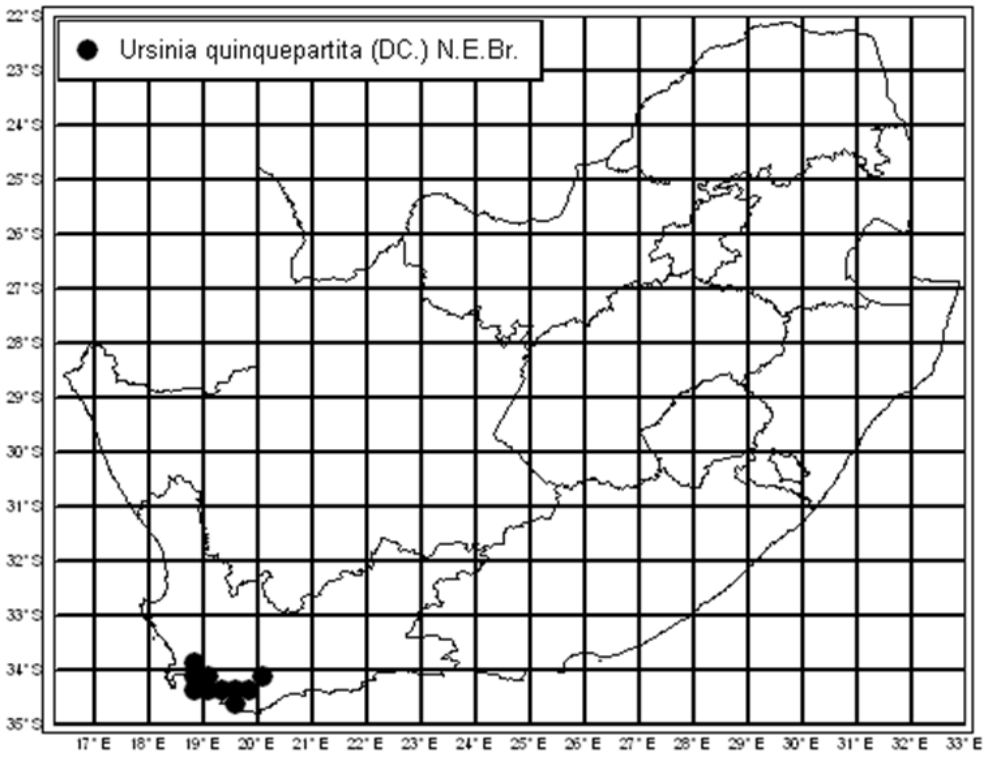

Fig. 2. Known distribution of U. quinquepartita - a Cape endemic taxon.

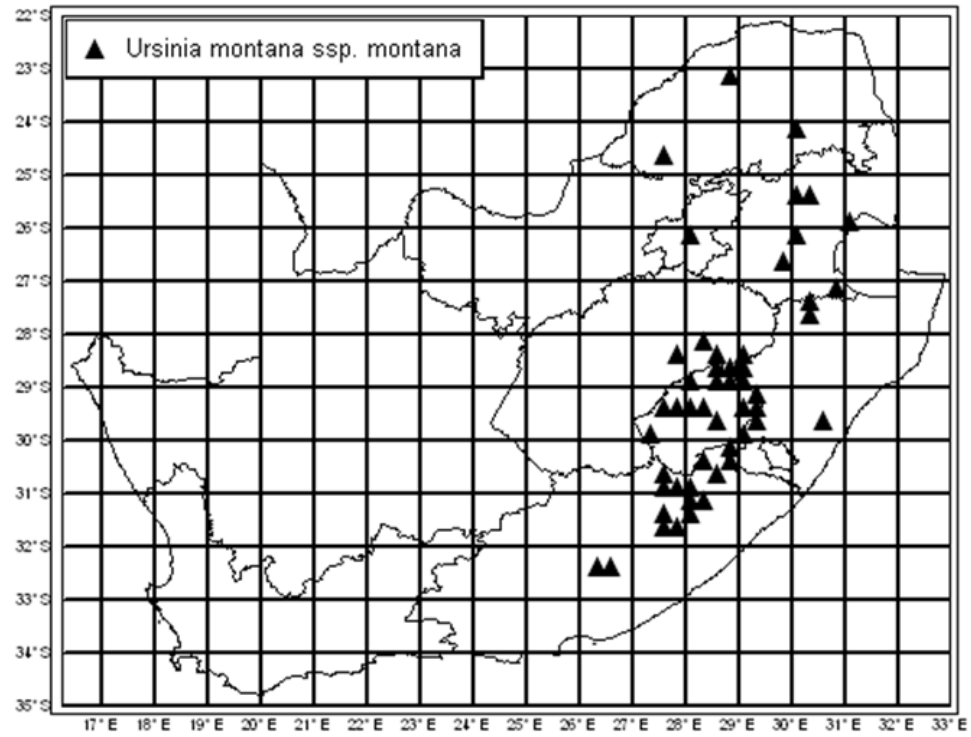

Fig. 3. Distribution of U. montana subsp. montana - a taxon limited to summer-rainfall regions of South Africa, Lesotho and Swaziland.

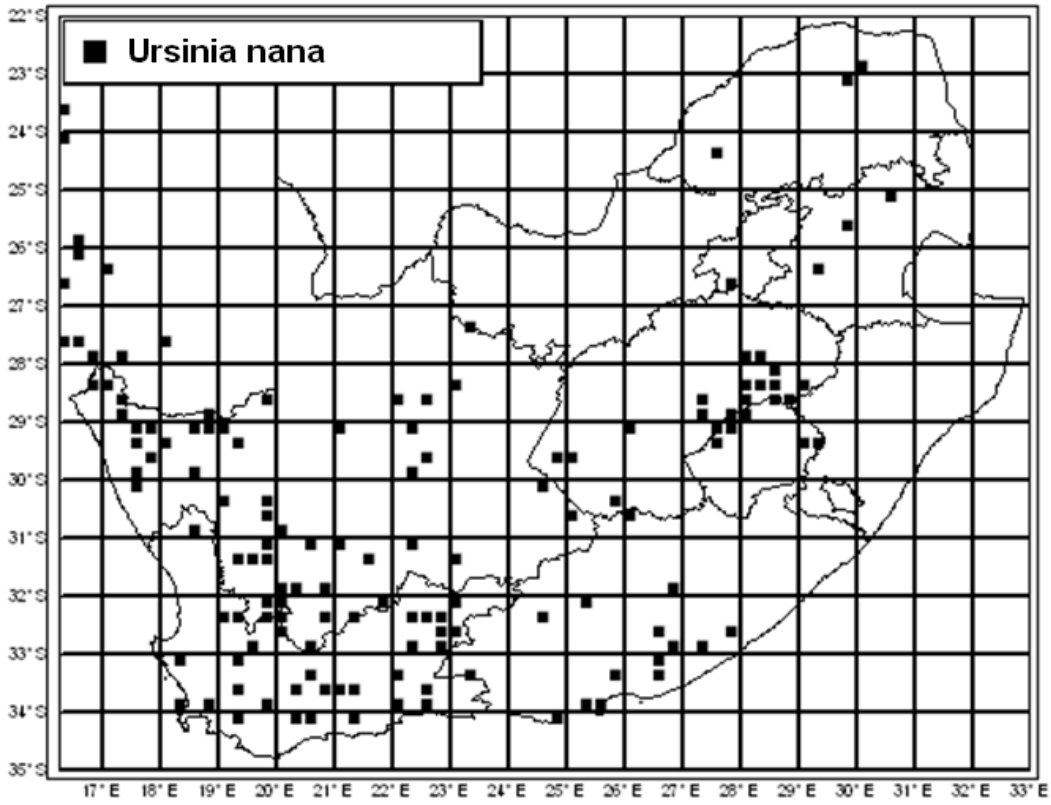

Fig. 4. Known distribution of *Ursinia nana* complex.

Chapter 2

Cypsela and pappus morphology of the southern African species of *Ursinia* (Anthemideae, Asteraceae)

2.1 Introduction

The Asteraceae is one of the largest plant families in the world, with about 1535 genera and 25 300 species arranged in three subfamilies: Asteroideae (11 tribes), Cichorioideae (6 tribes) and Barnadesioideae (1 tribe, exclusively South American) (Bremer 1994, Kim & Jansen 1995). The character that distinguishes this family from most other flowering plant families is the arrangement of the individual florets in a flower head or capitulum. The fruit type of most members of the family Asteraceae is a cypsela. These are indehiscent, one-seeded, and usually dry fruits with the testa adnate to the endocarp (Bremer 1994).

The tribe Anthemideae of the subfamily Asteroideae consists of c.111 genera and 1800 species worldwide. The most recent study by Oberprieler (2004) has shown that the evolutionary roots of the tribe are in southern Africa. One of the largest Anthemideae genera in the latter region is *Ursinia* Gaertn. Bentham (1873) placed the genus *Ursinia* in the tribe Arctoteae *sensu* Norlindh (1977), mainly because of its well-developed pappus scales. Cassini (1816), followed by Beauverd (1915) and Prassler (1967), considered it a member of the tribe Anthemideae. Robinson & Brettel (1973) argued that inclusion in the Anthemideae of the genus with its conspicuous pappus scales, widely ovate apical anther appendages and different pollen (exine without columnar structure) would destroy the workable tribal concept. Hence they proposed a monotypic new tribe Ursinieae. The large pappus scales and shape of the apical anther appendages may be plesiomorphic within Anthemideae, since similar characters also occur in outgroup. The pollen was investigated by Stix (1960) and she concluded that *Ursinia* belongs in the tribe Anthemideae. This is supported by the presence of furanosesquiterpenes (Greger 1977, Heywood & Humphries 1977).

Bremer & Humphries (1993), in their generic monograph of Anthemideae, placed *Ursinia* in the subtribe Ursiniinae. Because of micromorphological characters of the florets, anthers, tubular ray epidermal cells, polarized endothecial tissue, cypsela, pappus which are not easily observed and because of specialized floral and vegetative features ranging from annuals to desert shrubs. They regarded the Ursiniinae as one of the basal branches in the phylogeny of the tribe Anthemideae. However, their placement was unresolved or not supported. Anthemideae has its origin in the southern Hemisphere, with the genus *Ursinia* originating in southern Africa rather than in Eurasia (Watson *et al*. 2000, Francisco-Ortega *et al*. 2001).

Previously much emphasis was put on fruit morphology, hairs, involucral bracts and growth form for identification of Asteraceae taxa by Beauverd (1915), Hutchinson & Phillips (1917), Hilliard (1977), Källersjö (1991), Bremer & Humphries (1993), Bremer (1994), Watson *et al*. (2000) and Herman (2001).

The aim of this study was to investigate the taxonomic importance of fruit morphology, pappus and the basal tuft of hairs on cypsela in order (1) to ascertain the usability of the cypsela characters for the taxonomy of the species within *Ursinia*, (2) to define groups of the studied taxa based on the cypsela morphology, and to compare these groupings with Prassler's (1967) classification.

2.2 Material and Methods

2.2.1 The ingroup and outgroup taxa

Mature cypselae were obtained from herbarium specimens housed in PRE, NBG and from plants collected in the wild. For most species 25 cypselae (at least five from different collections) were studied for morphological, except for *U. merxmuelleri, U. alpina, U. tenuifolia* subsp. *ciliaris, U. dregeana*, and *U. hispida* due to lack of sufficient material. The length of the pappus, and the length and width of the cypselae were measured with the aid of a Bausch & Lomb StereoZoom® Series microscope. Eighteen characters with different states were recorded (Appendix 2.1). Two cypselae in each taxon, except in the case of the above mentioned taxa, were examined by means of the scanning electron microscope. All samples were dry and were not chemically treated before being sputter-coated with gold-palladium.

Morphology of the cypselae and pappus were also studied under a dissecting microscope and pictures were taken with a digital camera mounted on the dissecting microscope. A representative from *Cotula hispida* was included in the analysis as outgroup as it is closely related to *Ursinia*. This is in accordance with recommendation of Maddison & Maddison (1992) that taxa to be used as outgroup should not be too distantly related from those of the ingroup. A total of 43 *Ursinia* taxa were studied. The sources of the study material are listed in Appendix 2.2.

2.2.2 Phylogenetic analysis

Thirteen encodable discrete and meristic characters were identified for all ingroup and outgroup taxa. Character-states were either assigned values ranging from 0–5, or coded as present or absent and were all treated as unordered. Outgroup was used to designate apomorphic and plesiomorphic character-states (Appendix 2.1). The characters 12, 13, 14, 15 & 16 were not included in the analysis).

Maximum parsimony analysis was performed on these morphological characters using PAUP* version 4.0b10 (Swofford 2002). All characters were considered as unordered and unweighted, and 100 trees were retained from the full heuristic search. The strict consensus tree was derived from the 100 retained trees. Level of homoplasy for individual traits and all characters combined were assessed using the consistency index and for comparison, the retention index. Reliability of individual nodes on the most-parsimonious trees was estimated by bootstrap re-sampling using the 'BOOTSTRAP' option in PAUP.

2.2.3 Principal Component Analysis

A data matrix describing 18 traits for 42 taxa was constructed using average values for quantitative traits and mean value for multistate traits. The data matrix was

submitted to standard Principal Component Analysis using STATISTICA, version 8.0.

2.3 Results

The cypselae in subgenus *Sphenogyne* are less uniform compared to those in subgenus *Ursinia*. The cypselae in subgenus *Sphenogyne* are narrowly obconical to obconical (Fig. 2.1a), obovate, terete, ± straight to slightly curved. The cypselae in subgenus *Ursinia* are curved and taper towards the base (Fig. 2.6f). The cypselae in subgenus *Sphenogyne* are creamy white, turning dark brown at maturity except in *U. trifida* forma *calva* where both the young and mature cypselae are dark brown. The cypselae in subgenus *Ursinia* are white. The cypselae surfaces in subgenus *Sphenogyne* varies from transversely rugose (Fig. 2.1b), muricate, rugulose to smooth. The cypselae surfaces in subgenus *Ursinia* are either rugulose to smooth, muricate (Fig. 2.6e) or papillate (Fig. 2.6b). In subgenus *Sphenogyne* cypselae are broadly ribbed (Fig. 2.3d) compared to cypsela in subgenus *Ursinia* (Fig. 2.6d). In subgenus *Sphenogyne* the cypsela have a tuft of hairs at the base of the cypsela (Fig. 2.3a). As seen under the SEM the tuft of hairs have spiral wall thickenings (Fig. 2.1f). A basal tuft of hairs are absent in subgenus *Ursinia*. In both subgenera when glands are present they are either scattered all over the cypsela (Fig. 2.2b) or distributed between the ribs (Fig. 2.3d & 2.3e).

In subgenus *Sphenogyne* the cypsela have a uniseriate pappus (Fig. 2.3a), whereas in subgenus *Ursinia* the cypsela have a biseriate pappus (Fig. 2.5d). In subgenus *Sphenogyne* the cypsela consist of 5 white pappus scales (Fig. 2.3b), that have a brown streaks towards the base of the pappus scale. In subgenus *Ursinia* the cypsela consist of 5 white pappus scales (no streaks), surrounding 5 inner linear white bristle-like scales (Fig. 2.5d). In subgenus *Sphenogyne* the pappus size is mostly half the cypsela size, except in *U. coronopifolia* and *U. punctata* where the pappus is a quarter the size of the cypsela. In subgenus *Ursinia* the pappus is almost equal to the cypsela in length. The pappus scales in subgenus *Sphenogyne* are elliptic or elliptic to round (Figs. 2.2c, d, & e), whereas in subgenus *Ursinia* they are obovate (Fig. 2.5f). When fruits are young, the pappus scales are spirally rolled (Fig. 2.5e), but as they mature they open up like an umbrella so that they can easily be dispersed by wind (Fig. 2.5f). Under SEM the surface of both pappus scales and bristle-like scales are striate (Fig. 2.2f).

Maximum parsimony analysis of thirteen cypsela morphological characters using PAUP generated a single tree, 98 steps in length, with a Consistency Index of 0.316 and a Retention Index of 0.553. Bootstrap values >50% are displayed below the branches (Fig. 2.7). The strict consensus tree retrieved a monophyletic genus *Ursinia* as it is presently circumscribed. However within genus *Ursinia* two strongly supported clades, corresponding to subgenus *Ursinia* (98% bootstrap) and subgenus *Sphenogyne* (87% bootstrap) were retrieved. Within subgenus *Ursinia* another well-resolved clade was retrieved while within subgenus *Sphenogyne* more clades were retrieved. Lack of resolution in subgenus *Sphenogyne* did not allow accurate establishment of the relationships within the clades, although a number of monophyletic groupings were retrieved. *U. trifida* forma *trifida* and *U. trifida* forma *calva* are not sister taxa, with *U. trifida* forma *calva* being unique to the rest of the group (Fig. 2.7).

Principal Component Analyses also showed species belonging to subgenus *Ursinia* and subgenus *Sphenogyne* group separately, with *U. trifida* forma *calva* forming its own group. The combination of the PCA Axes 1 and 2 accounted for respectable 56% of variability (Fig. 2.8). Uniseriate or biseriate pappus, cypsela colour and number of pappus bristles are the most informative characters in the whole analysis (Appendix 2.3).

When *U. trifida* forma *calva* was removed from the analysis, the combination of PCA Axes 1 and 2 explained 59% of the variation, which does not constitute remarkable improvement when compared to the analysis involving *U. trifida* forma *calva*. *Ursinia frutescens* (point 35) is shown as an outlier within the cluster of the subgenus *Ursinia* (Fig. 2.9).

2.4 Discussion

The taxa within *Ursinia* have previously been classified into two subgenera by Prassler (1967), mainly based on distinct cypsela characters. The maximum parsimony and PCA support Prassler's (1967) classification based *inter alia* on the basis of uniseriate vs. biseriate pappus, cypsela curvature, presence basal tuft of hairs, and number of pappus bristles. Still, the internal relationships within subgenus *Sphenogyne* remains unresolved.

Prassler (1967) divided subgenus *Ursinia* into two series, series Frutescentes and series *Nanae. U. frutescens* which belong to series Frutescentes is a sister to some species that belong to series Nanae. Taxa in series Nanae are not retrieved as monophyletic. Under SEM the *U. nana* complex, *U. montana* subsp. m*ontana, U. tenuiloba* and *U. montana* subsp. *apiculata* have glands while *U. nana* subsp. *leptophylla*, *U. cakilefolia*, *U. pygmae*, *U. chrysanthemoides* and *U. speciosa* are eglandular (Fig. 2.7). Presence or absence of glands is probably a homoplasious character. Maximum parsimony and PCA analyses also suggest that *U. trifida* forma *trifida* and *U. trifida* forma *calva* are not sister taxa. These analyses support the recognition of these two taxa as separate species.

Cypsela and pappus morphology is very important in Asteraceae classification. These two features, together with growth form, capitula size, florets, involucral bracts and leaf shapes were used by Bremer & Humphries (1993) in separating Anthemideae into 12 subtribes. The significance of cypselae in Asteraceae taxonomy is illustrated in naming of genera in tribe Calenduleae (e.g. *Dimorphotheca, Tripteris*, *Osteospermum*, *Gibbaria* and *Nephrotheca*).

Cypsela hairs have been shown to be important in taxonomic studies, for instance within tribe Mutisieae (Asteraceae) Jeffrey (1967) and Hansen (1990) use cypsela hairs to delimit genera and sections. Freire & Katinas (1995) also studied morphology and ontogeny of the cypsela hairs in subtribe Nassauviinae (Asteraceae, Mutisieae). They found eight morphological types of non-glandular hairs, including basic, asymmetric, divergent, radiate, crenate, single 3-celled, single 2-celled, branched as well as three types of glandular hairs, such as uniseriate, typically biseriate, and atypically biseriate. Prassler (1967) also relied on indumentums characters in her

classification. She used, *inter alia,* the presence or absence of the basal tuft of hairs to distinguish *Ursinia* into two subgenera.

In some species cypselae appear to change in colour as they mature. For example in *Athanasia* (Anthemideae) young cypselae are always brownish. As they mature they may remain brown or they may turn either creamy-white or very dark reddish brown (Källersjö 1991). In genus *Ursinia* subgenus *Sphenogyne* young cypselae are creamy white. As they mature they turn brown except, in *U. trifida* forma *calva* where both the young and mature cypselae are brown. The cypselae in subgenus *Ursinia* are white.

Ribs on cypselae are also reported in *Athanasia* (Anthemideae) species, where some taxa have five major ribs containing both veins and resin sacs, while most taxa have six–ten ribs. Ribs may be extended to wings as in *A. oocephala*. Occasionally lateral wings can be extended to wings, giving the fruit to give a flattened appearance (Källersjö 1991). All the cypselae in subgenus *Sphenogyne* are broadly ribbed. In subgenus *Ursinia* most of the cypselae are ribbed on the upper half, fading in the lower half except in *Ursinia frutescens* which have smooth cypselae.

The cypselae shape, the ribs, the colour, the presence or absence of basal tuft of hairs, pappus scales are important taxonomic characters. However, in *Ursinia* these characters need to be viewed in conjuction with characters such as involucral bracts, growth form and distribution. Potentially new taxa discovered within this study cannot be identified based on cypsela and pappus characters only. Involucral bracts, growth form, leaf division, size of capitula and distribution also need to be considered.

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Appendix 2.1. List of characters and the coding of their states used in the principal component and maximum parsimony analyses of the cypsela and pappus morphology of the studied *Ursinia* taxa.

1. Pappus:
   (0) absent, (1) present
2. Tuft of hairs on cypsela:
   (0) absent, (1) present
3. Pappus:
   (0) absent, (1) uniseriate, (2) biseriate
4. (Mature) Cypsela colour:
   (0) light brown, (1) dark brown, (2) white
5. Curvature of cypsela:
   (0) flat, (1) ± straight, (2) slightly curved, (3) curved and tapering towards the base
   (4) cypsela not so obviously curved
6. Cypsela shape:
   (0) ovate, (1) narrowly obconical, (2) broadly obconical, (3) obovate, (4) terete
7. Cypsela surface:
   (0) smooth, (1) transversely rugose, (2) muricate, (3) rugulose-smooth,
   (4) papillate, (5) undulating
8. Position of cypsela glands:
   (0) absent, (1) between ribs, (2) scattered on the cypsela
9. Number of pappus scales:
   (0) 0, (5)1, (6) 2, (10) 3
10. Shape of pappus:
    (0) absent, (1) elliptic-round, (2) elliptic, (3) obovate
11. Surface of pappus:
    (0) absent, (1) indistinctly striated, (2) densely striate
12. Length of cypsela (mm)
13. Width of cypsela (mm)

14. Length of pappus scales (mm)
15. Length of cypsela including pappus (mm)
16. Ratio of pappus length to cypsela length
17. Spot on pappus scales:
    (0) absent, (1) brown, (2) pink, (3) light brown
18. Number of pappus bristles
    (0) 0, (5) 1

Appendix 2.2. Sources of the fruit material used in morphological analyses. The taxa are arranged according to Prassler (1967); *Cotula hispida* was used as outgroup. The codes such as 3418BD designate the quarter-degree based on the 1:50,000 mapping sheets of South Africa. The abbreviation of the herbaria follows Holmgren *et al.* (1990).

***U. quinquepartita* (DC.) N.E.Br.**

Western Cape.—3418BD: Palmiet River, lower slopes, mountains near Palmiet River Mouth, 25 Jan 1947, *Esterhuysen 13698* (PRE); Palmiet River, 8 Nov 1942, *Compton 14111* (PRE); Kogelberg summit, 1 Mar 1947, *Compton 19391* (NBG). 3419AA: Elgin Mountains west of Palmiet River, Elgin, lower slopes, 25 Apr 1943, *Leighton Pre 44767* (PRE).

***U. caledonica* (E.Phillips) Prassler**

Western Cape.—3318DD: Jonkershoek Forest Station, Little Dwarsberg Peak, 6 Jan 1953, *Rycroft 1484* (PRE); Jonkershoek Forest Station, Dwarsberg, 13 Mar 1982, *Viviers 208* (PRE). 3319CC: Jonkershoek, Zigzag path, 1 Mar 1980, *Taylor 10113* (PRE). 3419AA: West of Landroskop Hut, 2 km from hut towards Sir Lowrie's Pass on east slope, edge of pass, 27 Feb 1984, *Fellingham 490* (PRE). 3418AA: Victoria Peak, 2 Jan 1944, *Esterhuysen 9749* (PRE).

***U. eckloniana* (Sond.) N.E.Br.**

Western Cape.—3418BD: Bettiesbaai, 19 Oct 1960, *Van Rensburg 2180* (PRE). 3419AD: Vogelgat, Vogelpool, 4 Oct 1979, *Williams 2861* (PRE); Klein River Mountains, *sine dato*, *Stokoe 44783* (PRE). 3419BD: Kogelberg, Platberg, 16 Sept 1967, *Taylor 7145* (PRE); Cape Hangklip, Whaling Station, mountain, 10 Oct 1923, *Stokoe 963a* (PRE).

***U. filipes* (E.Mey. ex DC.) N.E.Br.**

Western Cape.—3319CA: Bainskloof, at edge of streamlet, 13 Mar 1934, *Galpin 12873* (PRE); Bainskloof Mountains, 13 Nov 1896, *Schlechter 9156* (PRE); 3319CC: Franschhoek, French Hoek Pass, 2 Nov 1947, *Barker 4907* (NBG).

***U. merxmuelleri* Prassler**

Western Cape.—3319AD: Mosterts Hoek Twins, in steep rocky somewhat moist gulley, 24 Feb 1985, *Esterhuysen 36216* (PRE).

***U. pinnata* (Thunb.) Prassler**

Western Cape.—3319AA: Tulbagh, Great Winterhoek, slopes, 14 Jan 1923, *Andreae 909* (PRE). 3319AD: Ceres, Michell's Pass, 20 Nov 1994, *Watson & Panero 94-61* (NBG). 3319CA: Du Toit's Kloof, 15 Oct 1949, *Barker 5985* (NBG); Du Toits Kloof, valley floor at exit of Tierkloof, 30 Dec 1987, *Boucher 5331* (PRE). 3319CC: Franschhoek, on south slope of Hottentots Holland, 2 miles north west of town, 24 Mar 1926, *Smith 2659* (PRE).

U. trifida* (Thunb.) N.E.Br. forma *trifida

Western Cape.—3321CC: Riversdale, Garcia Pass, 28 Sept 1976, *Hugo 587* (PRE). 3420AB: Swellendam, slopes below 12 O'clock Peak, 11 May 1963, *Taylor 4749* (PRE). 3421: Langeberg, slopes near Riversdale, 20 Nov 1892, *Schlechter 1837* (PRE). 3421AA: Corente River Farm, Nov 1908, *Muir 251* (PRE). 3422BB: Plattekloof, Jun 1909, *Muir 399* (PRE).

***U. trifida* (Thunb.) N.E.Br. forma *calva* Prassler**

Western Cape.—3320DC: Tradouw Pass, 24 Sept 1949, *Acocks 15450* (PRE); Tradouw Pass, 26 Oct 1986, *Stirton & Žantovská 11271* (PRE); Barrydale, Tradouw Pass, south of town at end of pass, 22 Oct 1989, *Greuter 22119* (PRE). 3320DD: Grootvadersbosch, Feb 1904, *Marloth 3496b* (PRE); Grootvadersbosch State Forest, Langeberg, along path between Bobbejaankloof and Vaalrivierkloof, SE facing slopes, 27 Nov 1987, *McDonald 1509* (PRE); Grootvadersbosch, east borderline, fringe of forest, steep south facing slope, 21 May 1983, *Van Wyk 1231* (PRE); Grootvadersbosch State Forest, Boosmansbos Wilderness Area, near Helderfontein, south-facing slope, 6 Dec 1985, *McDonald & Morley 1088* (PRE). 3420BB: Langeberg, Strawberry Hill, Dec 1954, *Stokoe PRE 44862* (PRE).

***U. heterodonta* (DC.) N.E.Br.**

Western Cape.—3320DB: Touwsberg, lower slope on the north side above Mistkraal, 19 Oct 1990, *Kurzweil 1785* (PRE). 3421AD: Korente River Dam, slope above second arm, 30 Mar 1979, *Bohnen 5301* (PRE). 3422AA: 9 miles west of Mossel Bay on hillock, 7 Sept 1947, *Story 3098* (PRE); Moordkuilrivier Valley, on road from Kleinbrakrivier to Gannakraal, along valley on hillside, 19 Oct 1990, *Joffe 862* (PRE).

***U. discolor* (Less.) N.E.Br.**

Western Cape.—3418BB: Between Gordon's Bay and Van der Stel, below railway line, 31 Oct 1927, *Smith 4706a* (PRE). 3419AB: Caledon, Zwartberg, vicinity of the Baths, Nov 1830, *Zeyher 2795b* (PRE). 3420AB: Bredasdorp, Bontebok National Park, experimental plots, Mar 1963, *Liebenberg 7159* (PRE); Bredasdorp, Bontebok National Park, Sept 1962, *Liebenberg 6383* (PRE). 3420BA: Bredasdorp, Buffeljagts 6 km on road to Witsand 28 Sept 1976, *Hugo 585* (PRE).

***U. dentata* (L.) Poir**

Western Cape.—3318CD: Table Mountain, Platteklip Gorge, 18 Nov 1897, *Galpin 4246* (PRE); Kirstenbosch, 24 Jan 1929, *Gillet 3327* (NBG). 3418BD Hottentots Holland Mountains, Kogelberg State Forest, ca. 1 km from 2nd dwelling, along N road at 1st bridge near water under bridge, 3 Feb 1992, *Kruger 371* (PRE).

***U. rigidula* (DC.) N.E.Br.**

Western Cape.—3218: Piquetberg, 3 Nov 1951, *Barker 7557* (NBG). 3319: Worcester, Brandvlei Lake, against hill, 4 Nov 1961, *Van Breda 1576* (PRE).

***U. nudicaulis* (Thunb.) N.E.Br.**

Western Cape.—3319CA: Wellington, Bainskloof, 21 Oct 1946, *Compton 18642* (NBG). 3419BB: Nuweberg State Forest, near Stonehenge, 18 Nov 1976, *Haynes 1216* (PRE).

***U. saxatilis* N.E.Br.**

Mpumalanga.—2531CC: Barberton, Jan 1907, Thorncroft TRV 2830 (PRE). KwaZulu-***Natal.—2831CA: Nkandla Forest, 29 Mar 1939,* Gerstner 3953 *(PRE).***

***U. alpina* N.E.Br.**

KwaZulu-Natal.—2828DB: Bergville, Royal Natal National Park, Mont Aux Sources, Mar 1964, *Trauseld 243* (PRE).

U. tenuifolia* (L.) Poir. subsp. *tenuifolia

Western Cape.— 3418AB: Cape Peninsula, near Klaasjagers, 20 Sept 1949, *Barker 5907* (NBG); Cape Peninsula, Schustuskraal, 10 Oct 1945, *Compton 17469* (NBG); Witsand, 30 Oct 1927, *Smuts PRE 44844* (PRE).

***U. tenuifolia* (L.) Poir. subsp. *ciliaris* Prassler**

Western Cape.—3421AD: Riversdale, Milkwoodfontein, 7 Oct 1897, *Galpin 4242* (PRE).

***U. paleacea* (L.) Moench**

Western Cape.—3318DD: Jonkershoek, Swartboskloof Nature Reserve, 7 Nov 1970, *Werger 1211* (PRE); Paarl, Haelhoek Spitskop, 2 Jan 1947, *Esterhuysen 13531* (PRE). 3418AB: Cape Peninsula, Froggy Farm, mountain slope, 20 Nov 1964, *Taylor 6055* (PRE); 3418BB: Gordon's Bay, mountains south of Gordon's Bay, Nov 1919, *Marloth 10122* (PRE).

***U. oreogena* Schltr. ex Prassler**

Western Cape.—3319DC: Riviersonderend Moutains, Jonaskop, Oct 1977, *Hugo 954* (PRE).

***U. macropoda* (DC.) N.E.Br.**

Western Cape.—3219AC: Cederberg, Heuningvlei, 16 Jan 1933, *Esterhuysen 21137* (PRE); North Cederberg, Groot Koupoort Nek, 28 Dec 1983, *Taylor 10864* (PRE); Cederberg State Forest, on west-facing slopes, 14 Nov 1983, *Richardson 37* (PRE). 3219AA: Clanwilliam, Pakhuisberg, Dec 1940, *Leipoldt 3632* (PRE). 3219CC: Olifants River Mountains, 27 Dec 1946, *Esterhuysen 13480* (PRE); 3219CD: Wupperthal, Kleinveld Farm, north of Rosendal Farm, 3 Feb 1980, *Hugo 2257* (PRE).

***U. sericea* (Thunb.) N.E.Br.**

Western Cape.—3320DA: Anysberg, 28 Jan 1987, *De Lange 3* (PRE). 3319: Hexberg, old Elandskloof, 24 Mar 1951, *Johnson 28* (NBG).

***U. dregeana* (DC.) N.E.Br.**

Western Cape.—3118DB: Vanrhynsdorp, Matsikammaberg, north of Die Vlei Farm, 11 Nov 1985, *Snijman 958* (NBG).

***U. abrotanifolia* (R.Br.) Spreng.**

Western Cape.—3319CA: Wellington, Bainskloof, 13 Mar 1934, *Galpin 12657* (PRE); 4 Feb 1947, *Rehm s.n.* (NBG).

***U. hispida* (DC.) N.E.Br.**

Western Cape.—3421BA: Langeberg, Riversdale, Welgevonden, Nov 1912, *Muir 1419* (PRE).

***U. coronopifolia* (Less.) N.E.Br.**

Western Cape.—3319AA: Tulbagh, Winterhoek, Jan 1887, *Marloth 1712* (PRE); Tulbagh, in a marsh in valley between the Klein Winterhoek and Klein Poortberg, 13 Mar 1983, *Esterhuysen 35893* (PRE).

***U. punctata* (Thunb.) N.E.Br.**

Western Cape.—3218BB: Clanwilliam, Kleinkliphuis, 8 miles north of town, Dec 1940, *Leipoldt 3635* (PRE). 3219AA: Clanwilliam, Pakhuis Pass, top of Pakhuis Pass, 6 Dec 1981, *Stirton 10170* (PRE). 3219AC: Cedarberg State Forest, Groot Koupoort, 5 Feb 1976 *Haynes 1212* (PRE); Cedarberg, Wolfberg slopes, 26 Dec 1953, *Esterhuysen 22427* (PRE). 3219CD: Citrusdal, Latjieskloof Height, 24 Oct 1977, *Emdon 94* (NBG).

U. anthemoides* (L.) Poir. subsp. *anthemoides

Northern Cape.— 3017BB: Namaqualand, Kamieskroon, at road to Skilpad, 4 Sept 2005, *Mucina & Swelankomo 040905/09* (STEU). 3119AC: Vanrhynsdorp, below Vanrhynspass, pull-in, 2 Sept 2005, *Mucina 020905/13* (STEU). 3119BD: Calvinia, 1936, *Schmidt 345* (PRE). Western Cape.— 3118DC: Klawer, on N7 near turnoff to the town, 11 Sept 2005, *Mucina 110905/05* (STEU). 3318AD: Darling, Mamre Road, *Wasserfall 1005* (PRE).

***U. anthemoides* (L.) Poir. subsp. *versicolor* (DC.) Prassler**

Northern Cape.—3018AC: Namaqualand, Garies, Studer's Pass, 6 Sept 2005, *Mucina 060905/18* (STEU). 3119CA: Nieuwoudtville, Lokenburg, 30 Aug 1953, *Acocks 17087* (PRE). Western Cape.— 3218BD: Olifants River Valley, 23 km north of Citrusdal, 7 Sept 1976, *Hugo 424* (PRE). 3318DA: Durbanville, west end of excavation at Klipheuwel Farm north of Durbanville, 15 Sept 1982, *Van Zyl 3169* (PRE). 3318DD: Stellenbosch, Marais Park, 18 Oct 1960, *Van Rensburg 2140* (PRE).

***U. calenduliflora* (DC.) N.E.Br.**

Northern Cape.—2817AC: Namaqualand, Richtersveld, Sept 1925, *Marloth 12267b* (PRE). 2817CA: Richtersveld, between Kawarass and Lekkersing, 4 Sept 1925, *Marloth 12438* (PRE). 2917BB: 12 km from Steinkopf on road N7 to Namibia, 23 Aug 1995, *Rodriguez-Oubiña & Cruces 2042* (PRE). 2917DB: Namaqualand, Springbok, 1970, *Small, Olivier & Robbertse 72* (PRE); Springbok, Goegab Nature Reserve, Aug 1994, *Rösch 24* (PRE); Springbok, Goegab Nature Reserve, Aug 1989, *Van Rooyen 2059* (PRE).

***U. serrata* (L.f.) Poir.**

Western Cape.—3320CD: Swellendam, Langeberg, south slopes of 12 O'clock Peak, 6 Nov 1962, *Taylor 4245* (PRE).

U. scariosa* (Aiton) Poir. subsp. *scariosa

Western Cape.—3422AB: Plettenberg Bay, Nov 1921, *Rogers 26779* (PRE). 3422BB: Belvedere near Knysna, 7 Nov 1928, *Hutchinson 1315* (PRE). 3423AA: Knysna, Springfield Plantation, Sept 1918, *Keet 18* (PRE). Eastern Cape.—3423BB: Tsitsikamma, Storms River, 15 Sept 1897, *Galpin 4239* (PRE).

***U. scariosa* (Aiton) Poir. subsp. *subhirsuta* (DC.) Prassler**

Western Cape.—3322CD: George, Montagu Pass, 28 Dec 1949, *Martin 79* (NBG). 3420AB: Swellendam, Langeberg, *sine dato*, *Kennedy 1707* (PRE). 3420: Langeberg, 22 Oct 1894, *Schlechter 5661* (PRE).

***U. pilifera* (P.J. Bergius) Poir.**

Northern Cape.—3119AD: Zoetwater, 21 km west of Calvinia, 24 Sept 1952, *Maguire 1918* (NBG). 3119BD: Calvinia, Calvinia Nature Reserve, 3 Oct 1986, *Strydom 23* (PRE); Western Cape.—3221AD: Aarfontein Farm between Sutherland and Fraserburg, below koppie just west of farmhouse, 21 Sept 1985, *Moffett 3735*

(PRE). 3320BC: Witteberge, Bantam Crossing at foot of mountain, on a flat area, 29 Sept 1983, *Van Zyl 3554* (PRE).

***U. frutescens* Dinter**

NAMIBIA.—2715B?: Lüderitz-Süd, Klinghardtberge, northern part in the mountains near Sargdeckel, 18 Sept 1977, *Merxmueller & Giess 32152* (PRE). 2716CA: Sperrgebiet South, 74 km east of Kakaoberg Escarpment, 8 Oct 1988, *Jürgens 28177* (PRE).

***U. nana* complex**

Northern Cape.—2917BA: Steinkopf; Anenous Pass, 23 Aug 1983, *Van Wyk 6182* (PRE). 2917BC: 20 km from Steinkopf on road to Port Nolloth, 22 Aug 1995, *Rodriguez-Oubiña & Cruces 2010*, (PRE). 2917BB: 12 km from Steinkopf on road N7 to Namibia, 23 Aug 1995, *Rodriguez-Oubiña & Cruces 2041* (PRE). 2917DB: Springbok, Hester Malan Nature Reserve (today Goegap N.R.), along the Pofadder road, 16 Apr 1974, *Rösch & Le Roux 344* (PRE). 2918DC: Between Gamoep and Aggeneys, SE of Springbok, 19 Sept 2002, *Koekemoer 2507* (PRE).

***U. nana* (DC.) subsp. *leptophylla* Prassler**

Gauteng.—2528CA: Magaliesberg, Wonderboom, Mar 1930, *Obermeyer 395* (PRE); Magaliesberg, Wonderboom Research Station, upper slopes of mountain, north and south sides, 20 Jan 1944, *Repton 1585* (PRE).

U. montana* DC. subsp. *montana

Mpumalanga.—2629DB: Amsterdam, Athol Pasture Research Station, 14 Nov 1935, *Norval 28* (PRE). Free State.—2828BC: Clarens, Golden Gate Highlands National Park, 18 Jan 1966, *Liebenberg 7448* (PRE). KwaZulu-Natal.—2929CC: Bergville, Cathedral Peak Forest Research, 20 Dec 1985, *Everson 280* (PRE). 3028BD: Matatiele, flat rocks on hillsides, 7 Mar 1936, *Galpin 14081* (PRE). Eastern Cape.—3226AD: Katberg, Tarkastad road, 18 km from Fairview, N side of Groot Winterberg, 28 Apr 1995, *Victor 1315* (PRE).

***U. tenuiloba* DC.**

Swaziland.—2631AA: Malolotja Nature Reserve, Ngwenya viewpoint road after Logwaja turn off, at base of Ntabamhlophe hill, 25 Sept 1992, *Braun 1344* (PRE). KwaZulu-Natal.—2829AD: Harrismith, Van Reenen's Pass, 17 Nov 1892 *Medley-Wood 4664* (PRE). 2930AC: Nottingham Road, 26 Oct 1897, *Medley-Wood 6542* (PRE). Eastern Cape.—3225BC: Farm Karreebosch, 20 km north of Somerset East, top of Blomfonteinberge, 28 Apr 1995, *Victor 1310* (PRE).

***U. montana* DC. subsp. *apiculata* (DC.) Prassler**

Eastern Cape.—3126AC: Molteno, Henning Station, 8.5 miles SSW of station, 29 Jun 1950, *Acocks 15905* (PRE). 3224AC: Aberdeen, Palmietfontein, 21 Jun 1961, *Acocks 21776* (PRE). 3225AB: Cradock, Mountain Zebra National Park, on Bankberg, 5 May

1963, *Liebenberg 7213* (PRE); 3225AD: Zwagershoek Pass, top of Zwagershoek Pass, 18 Mar 1950, *Acocks 15698* (PRE).

***U. cakilefolia* DC.**

Northern Cape.—2917BA: Namaqualand, Klipfontein, in mountains, Aug 1883, *Bolus 396* (PRE).—3119CA: Nieuwoudtville, Lokenburg, *sine dato*, *Dyer 5428* (PRE); Lokenburg, ridges north of dam, 18 Sept 1955, *Leistner 349* (PRE); Lokenburg, valley, 26 Sept 1953, *Acocks 17226* (PRE). Western Cape. —3218BD: Clanwilliam, hills 2 miles up Hex River Valley, 14 Oct 1939, *Pillans 9059* (PRE).

***U. pygmaea* DC.**

Western Cape.—3118AB: Vanrhynsdorp, Kareeberg, 17 July 1896, *Schlechter 8180* (PRE); Bitterfontein, in hills, 1 Sep 1897, *Schlechter 11022* (PRE).

***U. chrysanthemoides* sensu Prassler (1967)**

Northern Cape.—3017BB: Namaqualand, Kamieskroon, Grootvlei, Skilpad Nature Reserve, 1 Sept 1956, *Lewis 5000* (PRE). 3018AC: Namaqualand, ca. 10 km off Leliefontein near Kamieskroon, 25 Aug 1983, *Van Wyk 6383* (PRE).

***U. speciosa* DC.**

Western Cape.—3117BD: Vredendal, Brandsebaai, proposed mining area, 29 Sept 1992, *Van Rooyen 2125* (PRE). 3118DB: Tigerberg, near foot of berg, 15 miles east of Vanrhynsdorp, 4 Sept 1955, *Lewis SAM 68801* (PRE). 3118DC: Klawer, Nov 1917, *Roberts & Adendorf TRV 17680* (PRE).

***Cotula hispida* (DC.) Harv.**

Mpumalanga.—2530CB: Machadodorp, Slaaihoek, 26 Feb 1989, *Burgoyne 855B* (PRE). KwaZulu-Natal.—2929BA: Estcourt, Ntabamhlophe Mountain, 14 Mar 1937, *West 60* (PRE). Lesotho.—3028: Hillslope above Orange River at Rapari, near Qachasnek, 11 Mar 1936, *Galpin 14076* (PRE).

Appendix 2.3. Eigenvectors (factor loadings) of 18 pappus and cypsela characters traits used in principal components analysis (PCA Axes 1 & II).

Trait	I	II
1. Pappus (absent or present)	0.06	0.76
2. Tuft of hairs on cypsela	0.48	0.72
3. Pappus (uniseriate or biseriate)	0.92	0.92
4. Cypsela colour	0.90	0.96
5. Curvature of cypsela	0.72	0.72
6. Cypsela shape	0.24	0.35
7. Cypsela surface	0.12	0.13
8. Position of glands	0.02	0.08
9. Number of pappus scales	0.00	0.11
10. Shape of pappus	0.18	0.57
11. Surface of pappus	0.00	0.32
12. Length of cypsela (mm)	0.06	0.19
13. Width of cypsela (mm)	0.77	0.82
14. Length of pappus scales (mm)	0.59	0.74
15. Length of cypsela, including pappus (mm)	0.32	0.50
16. Ratio of pappus length to cypsela length	0.57	0.62
17. Spot on pappus scales	0.45	0.65
18. Number of pappus bristles	0.91	0.96

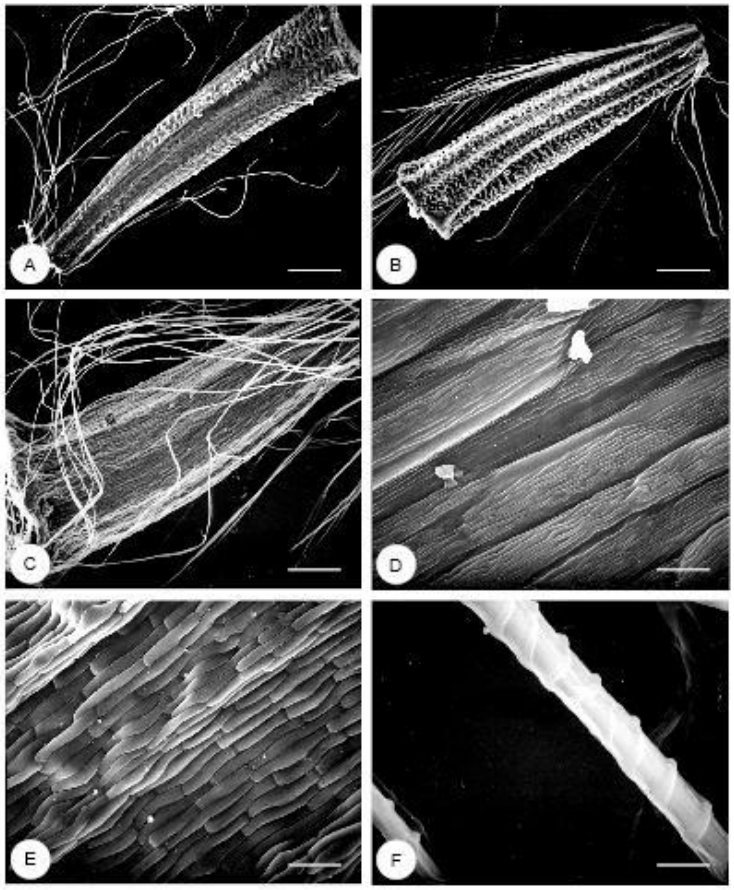

Fig. 2.1. A. U. scariosa subsp. scariosa, Keet 18, cypsela shape. Scale bar = 395.940 μm.—B. U. dregeana, Snijman 958, cypsela shape. Scale bar = 407.253 μm.—C. U. alpina, Trauseld 243, cypsela shape. Scale bar = 254.532 μm.—D. U. alpina, Trauseld 243, cypsela surface. Scale bar = 14.590 μm.—E. U. scariosa subsp. scariosa, Keet 18, cypsela surface. Scale bar = 39.594 μm.—F.U. paleacea, Esterhuysen 13531, tuft of hairs at base of cypsela. Scale bar = 4.658 μm. All SEM micrographs.

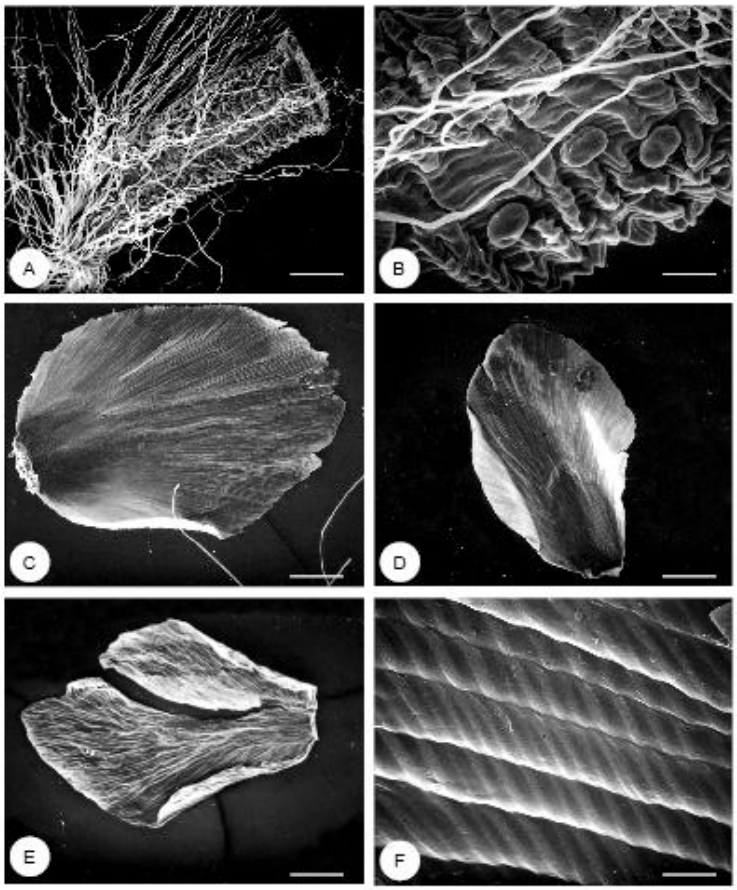

Fig. 2.2. A. U. punctata, Haynes 1212, cypsela. Scale bar = 419.231 μm.—B. U. punctata, Haynes 1212, cypsela surface with glands. Scale bar = 83.846 μm.—C. U. heterodonta, Kurzweill 1785, pappus shape. Scale bar = 250.067 μm.—D. U. paleacea, Werger 1211, pappus shape. Scale bar = 395.940 μm.—E. U dregeana, Snijman 958, pappus shape. Scale bar = 395.940 μm.—F. U. macropoda, Esterhuysen 21137, pappus surface. Scale bar = 7.272 μm. All SEM micrographs.

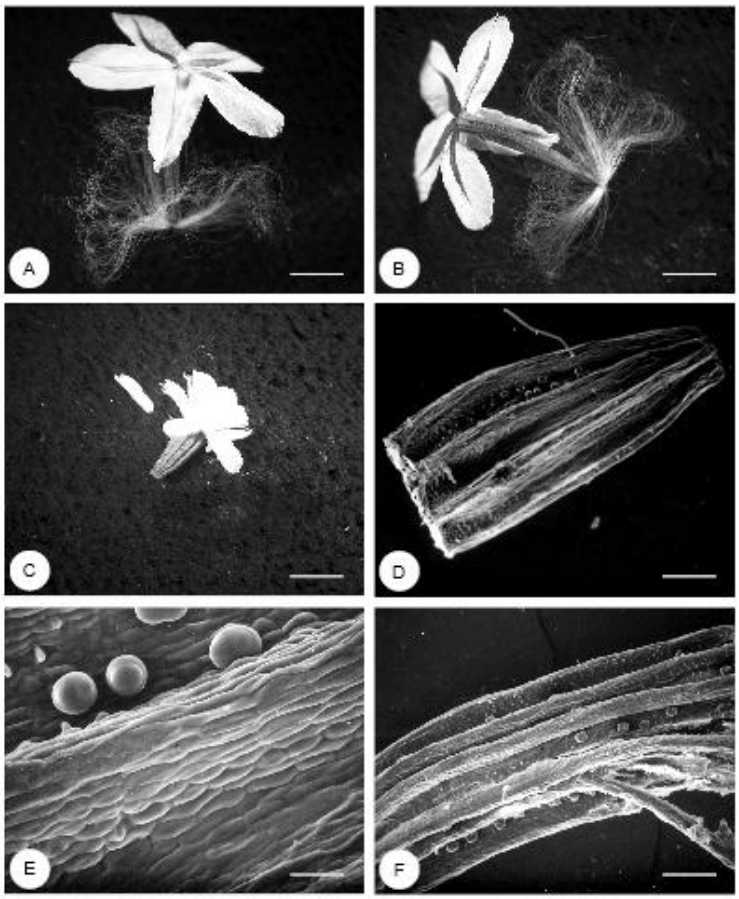

Fig. 2.3. A & B. U. anthemoides subsp. anthemoides, Mucina 110905/5 cypselae, pappus scale & basal tuft of hairs in different positions. Scale bar = 5 mm.—C. U. quinquepartita, Compton 14111, cypsela. Scale bar = 5 mm.—D. U. quinquepartita, Compton 14111, cypsela shape. Scale bar = 419.231 μm.—E. U. quinquepartita, Compton 14111, cypsela surface with glands. Scale bar = 63.919 μm.—F. U. quinquepartita, Esterhuysen 13698, cypsela surface with glands between ribs. Scale bar = 263.960 μm. A–C: digital camera photo mounted on dissecting microscope; D–F: SEM micrographs.

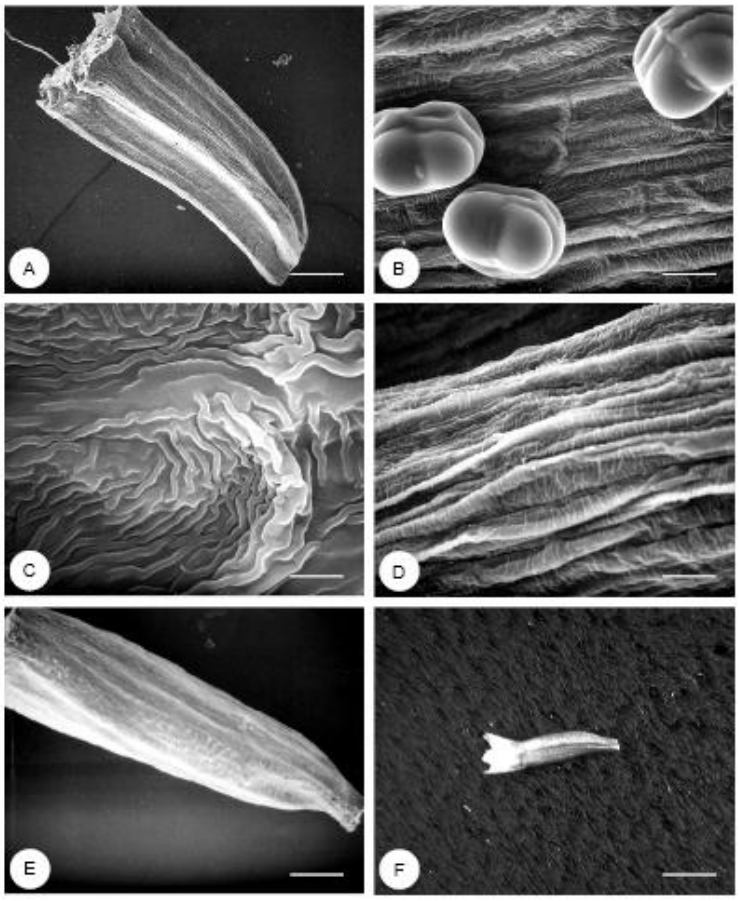

Fig. 2.4. A. U. caledonica, Viviers 208, cypsela shape. Scale bar = 431.935 μm.—B. U. caledonica, Rycroft 1484, cypsela surface with glands. Scale bar = 254.533 μm.—C. U. caledonica, Rycroft 1484, cypsela surface showing undulating margins. Scale bar = 5.221 μm.—D. U. caledonica, Rycroft 1484, ribbed cypsela. Scale bar = 25.453 μm.—E. U. coronopifolia, Marloth 1712, cypsela shape. Scale bar = 356.346 μm.—F. U. coronopifolia, Marloth 1712, cypsela, pappus scale. Scale bar = 5 mm. A–E: SEM micrographs; F–: digital camera photo mounted on dissecting microscope.

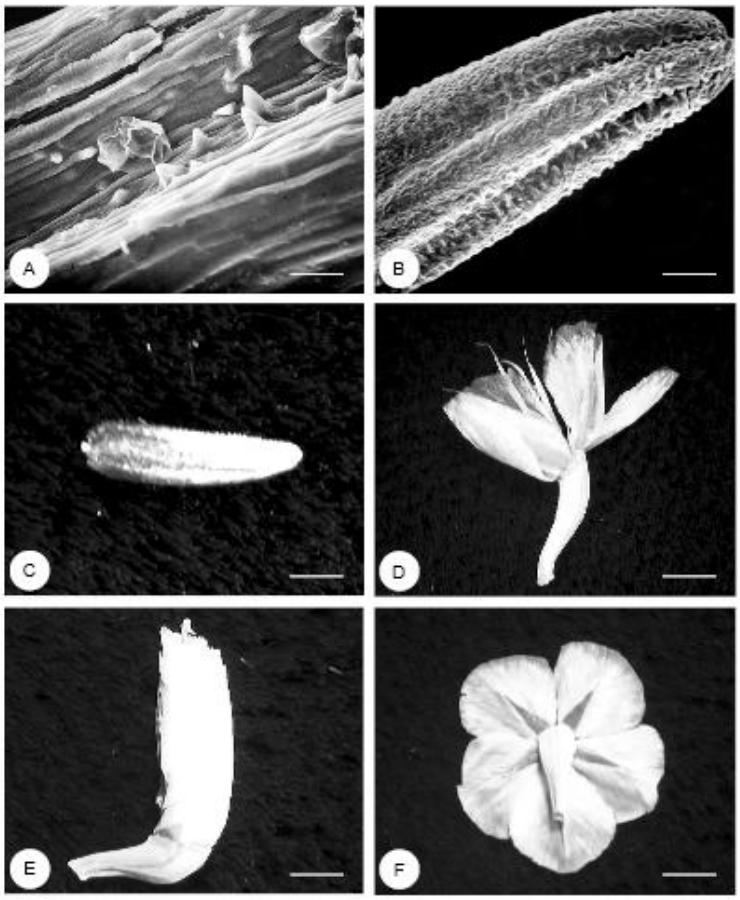

Fig. 2.5. A. U. hispida, Muir 1419, cypsela surface with glands. Scale bar = 34.765 μm.—B. U. trifida forma calva, Acocks 15450, cypsela surface. Scale bar = 259.160 μm.—C. U. trifida forma calva, Acocks 15450, cypsela. Scale bar = 5 mm.—D. U. frutescens, Jürgens 28177, cypsela, pappus scale & inner bristles. Scale bar = 5 mm—E. U. nana complex, Koekemoer 2507, cypsela & pappus scale. Scale bar = 5 mm.—F. U nana complex, Van Wyk 6182, cypsela & pappus scale at different position. Scale bar = 5 mm. A&B: SEM micrographs; C–F: digital camera photo mounted on dissecting microscope.

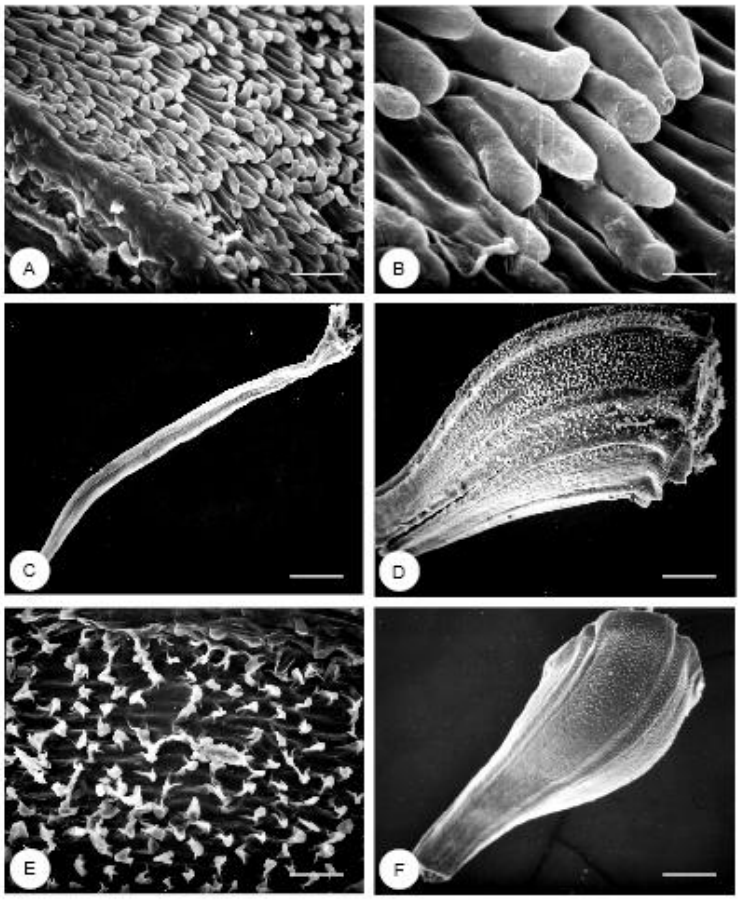

Fig. 2.6. A. U. nana subsp. leptophylla, Obermeyer 395, cypsela surface. Scale bar = 71.989 μm.—B. U. nana subsp. leptophylla, Obermeyer 395, cypsela surface at higher magnification. Scale bar = 15.327 μm.—C. U. nana subsp. leptophylla, Repton 1585, pappus bristle. Scale bar = 445.433 μm.—D. U. tenuiloba, Braun 1344,cypsela shape. Scale bar = 375.101 μm—E. U. tenuiloba, Braun 1344, cypsela surface. Scale bar = 59.639 μm.—F. U. cakilefolia, Leistner 349, cypsela shape. Scale bar = 395.940 μm. A–F: SEM micrographs.

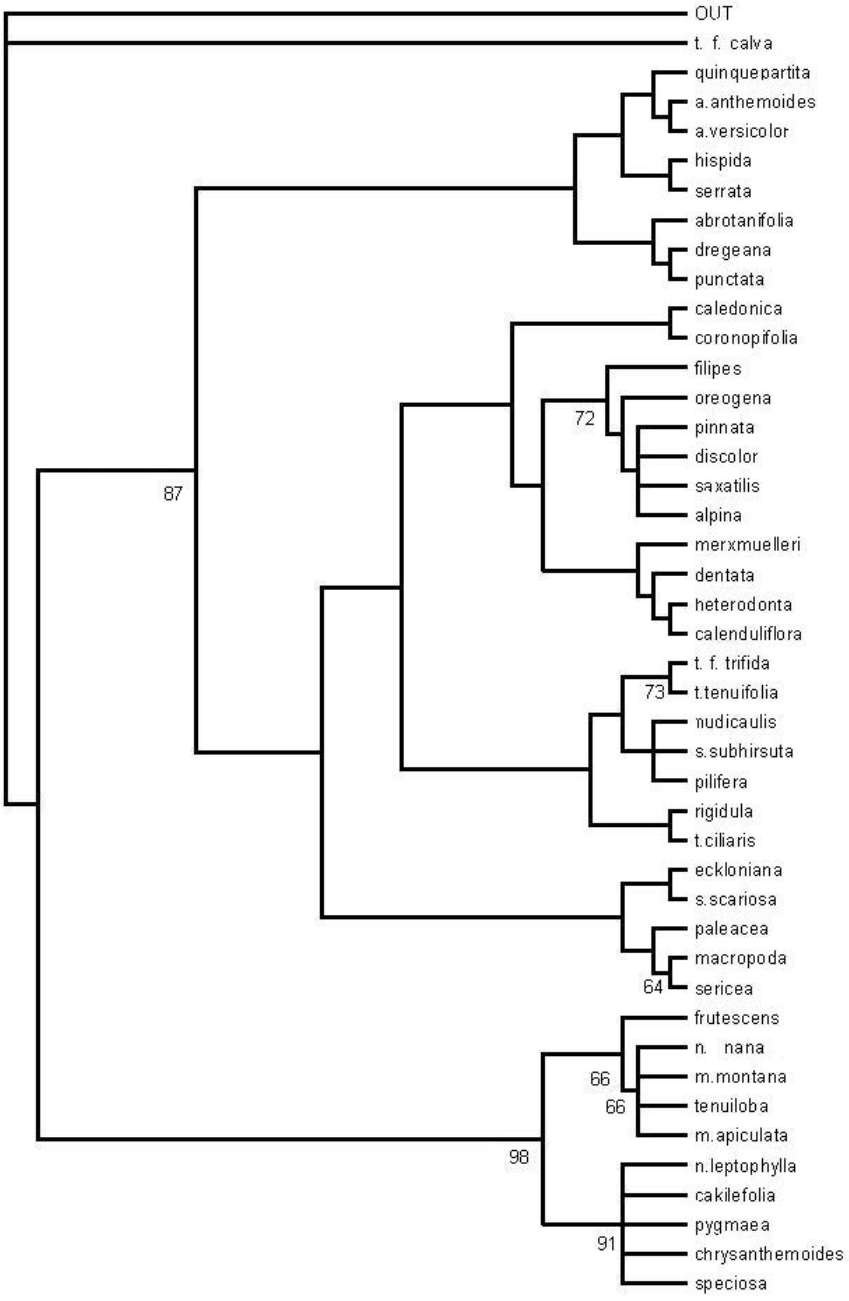

Fig. 2.7. Maximum parsimony tree, 98 steps in length derived by PAUP for Ursinia and outgroup based on 13 pappus and cypsela characters. The values below the branches indicate bootstrap support (not indicated if less than 50%).

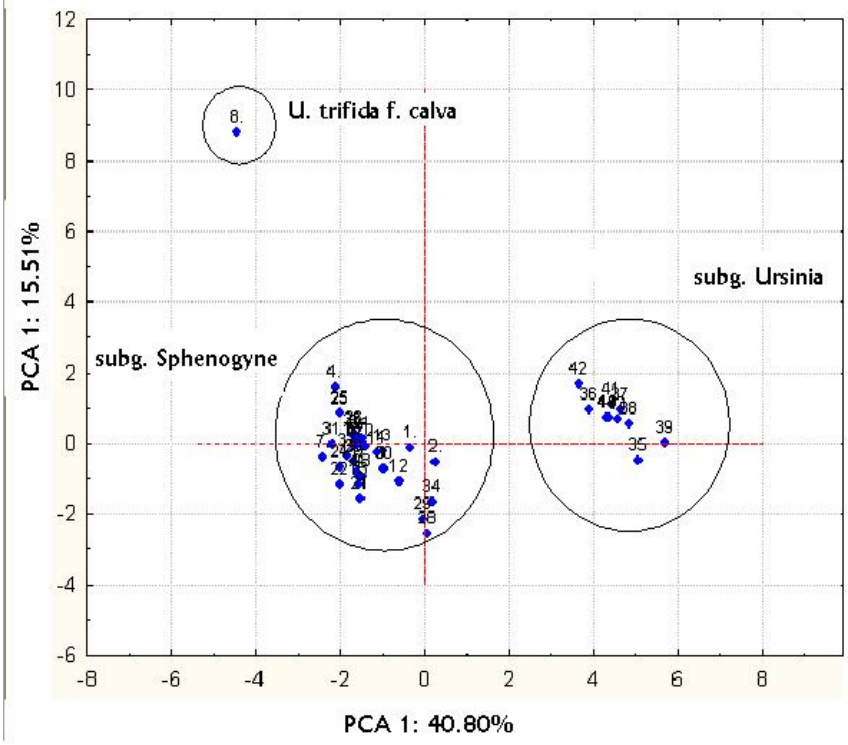

Fig. 2.8. Plot of the first two principal components (PC 1 and PC 2) obtained from a principal components analysis based on all cypsela and pappus characters listed in Appendix 2.1. This analysis shows the split of genus Ursinia into subgenus Sphenogyne and subgenus Ursinia, with U. trifida forma calva being unique to the rest of the group.

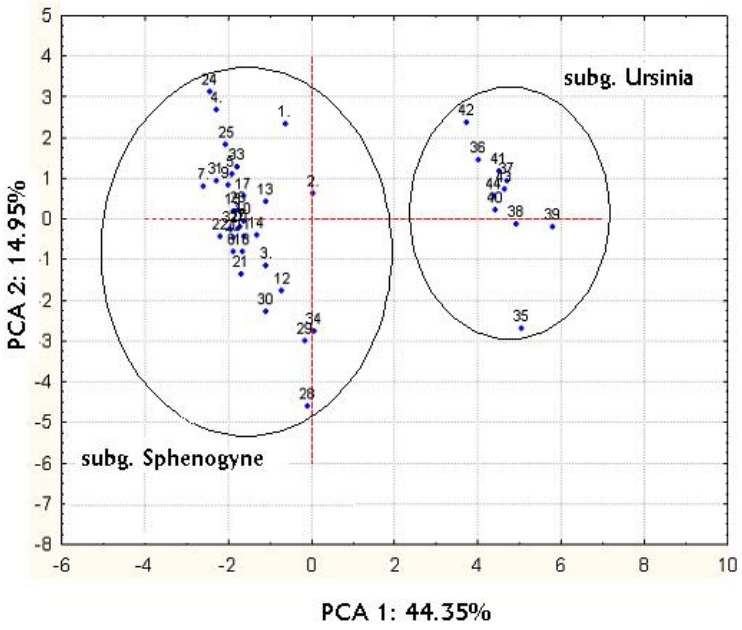

Fig. 2.9. Plot of the first two principal components (PC 1 and PC 2) obtained from a principal components analysis based on all cypsela and pappus characters listed in Appendix 2.1. when U. trifida forma calva was removed from the analysis.

Chapter 3

A Molecular Phylogeny of the Genus *Ursinia* (Anthemideae, Asteraceae) Based on ITS Marker

3.1 Introduction

Within the tribe Anthemideae molecular phylogenetic techniques, in particular sequencing of traditional nuclear and chloroplast markers (such as nrITS and cpDNA), has been applied successfully by in solving relationship within the southern African tribe Antemideae. In particular, sequencing was very instrumental in elucidating position of southern African Anthemideae in bigger picture of the taxonomy of the Asteraceae (Oberprieler et al. 2007, Himmelreich et al. 2008).

The genus *Ursinia* (Anthemideae, Asteraceae) is one of the species-rich Cape genera, having a basal position within the tribe Anthemideae (Himmelreich et al. 2008). The taxonomic history of the genus *Ursinia* (sensu lato) have revealed controversial issues surrounding the identity of the subgenus *Sphenogyne* (Brown 1813) and *Ursiniopsis* (Phillips in Dyer 1951), in relation to the genus *Ursinia*. The revision of *Ursinia* (incl. both *Sphenogyne* and *Ursiniopsis*) did not solve this controversy in satisfactory manner (see Chapter 1 in this thesis). Besides, the intensive field collecting of the past 3-4 years has revealed existence of numerous (to date around 30) morphologically well-defined taxonomic units (still pending formal description or deserving nomenclature resurrection), placing the most recent revision of the genus (Prassler 1967) under light of serious doubt.

In this study, I applied ITS marker to explore the following questions regarding the taxonomy of the broadly conceived genus *Ursinia*:

(1) Does nuclear *ITS* marker lend support for monophyletic nature of the subgenera *Ursinia* Gaertn. and *Sphenogyne* R.Br.?

(2) Do molecular data support the concepts of the series within *Ursinia* as defined by Prassler (1967)?

(3) Is there molecular support for separating the genus *Ursiniopsis* (as suggested by Phillips in Dyer 1951) as a genus in its own right?

(4) Is the ITS based molecular phylogeny corroborating the species concepts within *Ursinia*?

(5) Can the forms within *Ursinia trifida* (forma *trifida* and forma *calva*), described by Prassler (1967), be considered sister taxa lending support to their monophyletic origin?

(6) Do the taxa of *Ursinia* from the summer-rainfall regions of southern Africa (incl. Drakensberg, KwaZulu-Natal Midlands, Mpumalanga Escarpment and Soutpansberg) form a monophyletic clade?

(7) Are the annual taxa within *Ursinia* of monophyletic origin?

3.2 Material and Methods

3.2.1 Sampling

Field collections were made from plants growing in their natural habitat for each of the species included in this study. Specimens are housed in STEU (Stellenbosch University Herbarium), South Africa. *Cotula coronopifolia* L., *Dimorphotheca fruticosa* (L.) Less, and *Agoseris grandiflora* (Nutt.) Greene have been included in the analysis as outgroups, as they are closely related to *Ursinia*. This is accordance with the recommendation of Maddison & Maddison (1992) that taxa to be used as outgroups should not be too distantly related from those of the ingroup. A total of 98 accessions of *Ursinia* were included as ingroup, some of which may well be new.

The source of the sequenced material (collections) are summarized in Appendix 3.1.

3.2.2 DNA Extraction

Total genomic DNA was extracted using a modified procedure of the CTAB method of Doyle & Doyle (1987). Fresh leaf material (0.8–1.0 g) was ground with the traditional mortar and pestle method in liquid nitrogen to snap freeze the tissue. Alternatively 0.2 g of silica dried tissue was ground up with the aid of the Qiagen tissue lyser in 500 µl of CTAB extraction buffer (+0.2% β-mercaptoethanol) and a spatula tip of Polyvinylpyrrolidone (PVP) (0.5 mg) was added.

The ground material was placed in a 1.5 ml Eppendorf tube and placed in a 60º C heating block for 45 min. An equal volume of chloroform: isoamylalcohol (24:1 v/v) was added and mixed thoroughly by repeated inversion for 10 min and then centrifuged at 7000 x g for 5 min. The supernatant was carefully decanted and transferred to a new tube, precipitated with an equal volume of cold isopropanol and gently mixed to produce fibrous DNA. This was incubated at -20° C for a minimum of 30 min. The samples were centrifuged at 3000 x g for 5 min. The pellet was washed with 500 µl of wash buffer, air dried and resuspended in 250 µl of TE buffer.

Subsequently, the DNA was precipitated for a second time. To this end, 100 µl of the TE buffer resuspended sample was mixed with 200 µl of water and 150 µl of 7.5 M of ammonium acetate, after which 2.25 ml of ice cold ethanol was added. The mixture was incubated at -20º C for 30 min, followed by centrifugation at 10 000 x g for 10 min. The pellet was air dried and resuspended in TE buffer. All the centrifugation steps were carried out at room temperature to avoid precipitation with CTAB, DNA degradation and to obtain good quality DNA.

3.2.3 PCR and DNA Sequencing

The nuclear ITS region was amplified (White et al. 1990). All PCR reactions were done using the Thermohybaid® PX2 thermal cycler and the Labnet International, Inc Multigene II PCR systems thermal cycler. A key to the shorthand for the PCR parameters used for amplification is as follows: initial denaturing step (temperature, time); number of repetitions of the amplification cycle X (denaturing temperature, time; primer annealing temperature, time; chain extension temperature, time)]; final extension step (temperature, time). All reactions were ended with a final 15° C hold step.

The nuclear ITS was first amplified using ITS 5P and ITS 4 (White et al. 1990) or AB 101 and AB 102 (Douzery et al. 1999). The combination of AB 101 and 8P (Baldwin 1992) and the combination of AB 101 and ITS 4 was used to amplify the region in instances where the above-mentioned primers failed to amplify the region. Parameters for PCR were 94º C for 1 min; 35 cycles (94º C, 1 min; 42º C, 1 min; 72º C, 1 min); 72º C, 6 min, hold at 15º C. Although these parameters were successful for many of the amplifications, some were unsuccessful resulting in the change of some of PCR parameters to 94º C for 30 sec; 35 cycles (94º C, 30 sec; 42º C, 1 min; 72º C, 1 min); 72º C, 6 min, hold at 15º C. These altered PCR parameters allowed amplification in all of the outstanding samples.

The amplification of all the regions was done in 100 µl PCR reactions with the following reaction components: 4 µl of template of total genomic DNA, 10 µl 10X buffer (JMR-Holdings, USA), 10 µl 2.5 mM of $MgCl_2$, (JMR-Holdings, USA), 4 µl (200 µM) dNTPs (BioLine), 0.2 µl (1 U) of *Taq* (Supertherm) (JMR-Holdings, USA), 1 µl (0.5 µM) of each primer, Bovine Serum Albumin with a final concentration of 0.2 mg/mL to improve amplification. The amplified PCR products were purified using Promega Wizard® SV gel and PCR cleaning system according to the manufacturer's protocol. The samples were then concentrated from 80 µl to 20 µl of the sample volume using a Savant® speed vacuum concentrator.

Cycle sequencing reactions were carried out in the forward and reverse direction in a Multigene II PCR system using 10 µl reactions with the following components: the sequencing buffer (BigDye®), Terminator mix (BigDye®) Terminator V3.1. Cycle sequencing Kit (Applied Biosystems), the designated sequencing primers, MQ and the purified DNA template strand according to the manufacturer's protocol. Each cycle consisted of 96º C denaturation for ten seconds, 52º C annealing for thirty seconds and 60º C extension for four minutes, amplified in 35 cycles followed by hold at 15º C. The products were purified and detected on an automated capillary sequencer (ABI 3100 Genetic Analyser) at the Central Analytical Facility, Stellenbosch University.

3.2.4 Sequence Assembly and Phylogenetic Analyses

Sequence assembly and editing was performed using Chromas version 2.23 (Technelysium) and the sequences were aligned using BioEdit Sequence Alignment Editor. All matrices were aligned using the ClustalW multiple alignment tool in BioEdit and further edited by eye. All the matrices that were produced were analysed using parsimony analyses in PAUP* version 4.01 b 10 (Swofford 2002).

3.2.5 Parsimony Analyses

In the parsimony analyses, the nucleotides were treated as unordered characters with equal weighting (Fitch parsimony; Fitch 1971). Heuristic searches used 1000 replicates of random taxon addition and tree bisection-reconnection (TBR) branch swapping with a limit of five trees saved during each replicate to reduce the time spent swapping on islands of equally parsimonious trees. The consistency (CI) and retention (RI) indices, which provide an indication of the measure of fit between the data and tree topologies, were calculated for each analysis. Clade support was assessed by means of nonparametric bootstrap analyses (Felsenstein 1985) with 1000 replicates (with one of the shortest trees saved per replicate), with simple taxon addition and TBR branch swapping. Bootstrap support ≥ 75% was considered as well supported.

3.2.6 Methodical Problems

Amplification of the ITS region of the DNA samples analyzed in the study could not be achieved with the primer sets routinely used in other Anthemideae (i.e. ITS 5P and ITS 4), as a result of which other primers had to be employed, often in different combinations. However, this allowed the successful amplification of the ITS region of all the samples that were collected. PCR amplification was also critically dependent on the quality of the isolated DNA and it was found that the second precipitation step in the DNA isolation protocol was essential. DNA concentrations in the samples were also very high, necessitating a suitable dilution prior to PCR amplification.

3.3 Results

The alignment of ITS sequence resulted in a data matrix which had a total of 318 constant characters, 94 parsimony uninformative characters and 192 parsimony informative characters. The heuristic search retrieved 5651 equally parsimonious trees, with a length of 656 steps, a Consistency Index of 0.637, and a Retention Index of 0.913. The strict consensus tree is shown in Fig. 3.1. One of the shortest trees of the parsimony analysis is shown in Fig. 3.2 to illustrate branch lengths in the different clades.

The strict consensus tree retrieved a monophyletic genus *Ursinia* (sensu lato) as it is presently circumscribed (see Chapter 2 of this thesis). Besides, within the broadly conceived genus *Ursinia,* two strongly supported clades corresponding to the subgenera *Ursinia* and subgenus *Sphenogyne* were also retrieved. Within the subgenus *Ursinia,* several resolved, but poorly supported clades were also retrieved. Within subgenus *Sphenogyne*, three strongly supported clades with some internal resolution and support were retrieved, but the relationships between these clades was not resolved. The sequences of all of the taxa in clade H2 (Fig. 3.1) were identical. This was confirmed by re-sequencing many of them. Branch lengths within these clades were short. Only *U. pilifera*, recognized by Prassler (1967) as member of the subgenus *Sphenogyne* has been shown to belong to subgenus *Ursinia* on the basis of the ITS marker.

Within subgenus *Ursinia, U. nana* (represented by the many samples) was not retrieved as monophyletic. Only two clades (B1 and B2; Fig. 3.1) were strongly supported. *U. pilifera*, which has been placed into subgenus *Sphenogyne* based on cypsela morphology (Prassler 1967), is retrieved in clade B3 within subgenus *Ursinia*.

The lack of resolution within the subgenus *Sphenogyne* clade did not allow an accurate establishment of the relationships between the clades with the subgenus. Still a number of monophyletic groupings were retrieved. The strict consensus tree (Fig. 3.1) and one of the shortest trees retrieved in the phylogenetic analysis (Fig. 3.2) revealed *U. montana* (incl. accessions representing two subspecies) to be as monophyletic species. Numerous species, amongst them many newly recognised taxa, appear as single units in the phylogeny, and because most of the branch lengths are short, the phylogeny does not support nor reject their taxonomic status. However, many of the presently recognised taxa, some of which have widespread distributions, were not retrieved as monophyletic groups. The subspecies within *U. scariosa* have not been retrieved as members of monophyletic clade and occur in two widely disparate clades G5 and H2. The same pattern was found also for two formas distinguished by Prassler (1967) within *U. trifida* (Fig. 3.1). *U. anthemoides* is apparently also polyphyletic since the used accessions are scattered among clades F and H2. *U. anthemoides* subsp. *anthemoides* is, however, a member of monophyletic clade (F; Fig. 3.1), shared further by several other, morphologically-clearly defined entities such as *U. bellstedtii, U. klipheuwelensis, U. microlutea* (all taxonomic entities pending formal description). *U. anthemoides* subsp. *versicolor* (placed in clade H2) appears to be a specific entity of its own right.

The field studies revealed existence of number of morphologically well-defined units considered here as new species pending formal description. When compared to known taxa, these new species find support of their unique genetic identity also by our molecular phylogenetic hypothesis (Fig. 3.2), both in subgenus *Ursinia* as well as in subgenus *Sphenogyne*.

U. quinquepartita and *U. caledonica* group together in a clade with strong support (Figs. 3.1 and 3.2) corresponding to the series *Ursiniopsis*. Prassler (1967) classified her series *Ursiniopsis* as part of the subgenus *Ursinia*, however, our study has shown that this clade appears well embedded within the subgenus *Sphenogyne.* In any case, the Phillip's (in Dyer 1951) *Ursiniopsis* has not been retrieved as a separate entity deserving status of genus.

The molecular phylogeny based on the ITS region did not recover the Prassler's (1967) classification of *Ursinia* into series, in particular within the subgenus *Sphenogyne* (Fig. 3).

The *ITS* analysis revealed that the taxa of *Ursinia* from the summer-rainfall regions do not form a monophyletic clade (Fig. 3.4).

The annual life-cycle apparently evolved within *Ursinia* (sensu lato) several times as the annual taxa do not form own monophyletic clade either within subgenus Ursinia or subgenus Sphenogyne (Fig. 3.4).

3.4 Discussion

The low number of phylogenetically informative characters of the ITS sequences, the poor resolution in the consensus tree as well as the low branch support values indicate that the ITS data contain weak phylogenetic signals. The low bootstrap values for many nodes suggest that we should be cautious in using the ITS region alone to make

strong conclusions about the origin and evolution of taxa. However, this does not mean that the ITS region is inappropriate for the analysis at the present taxonomic level. This is supported by the fact that this marker could be profitably deployed at higher taxonomic level when dealing with tribal inter-relationships in Anthemideae (Francisco-Ortega et al. 1997, 2001, Oberprieler & Vogt 2000).

3.4.1 Subgeneric Classification: Ursinia s.str. versus Sphenogyne

The taxa within genus *Ursinia* have previously been classified into two subgenera, *Ursinia* and *Sphenogyne* mainly on the basis of distinct cypsela characters (Stapf 1933, Prassler 1967, Bremer & Humphries 1993). The molecular analysis based on ITS sequences established in this study strongly supports this classification, and suggest that resurrection of the genus Sphenogyne should be considered. Preliminary molecular analysis of a chloroplast marker (G. Jakubowsky, unpublished data) so far also supported the monophyletic nature of the subgenera *Ursinia s.str.* and *Sphenogyne*.

3.4.2 Prassler's Series Within Ursinia

Prassler (1967) divided *Ursinia* into 7 series, but her series in subgenus *Sphenogyne* were not retrieved as monophyletic in this study (Fig. 3.3). She used different characters for different series. For example, series *Ursiniopsis* was based on leaf bases (on the stem), ray florets (female), cypsela (basal coma of hairs absent), whereas in series Pinnatae was based on flower heads (small), involucral bracts (linear, membrane edged) and distribution (more to the north of Calvinia, Hantam Plateau, Northern Cape). The reason why these groupings were possibly not retrieved may be due to the short branch lengths in the phylogeny. Further molecular and morphological data are, however, required to support or reject her classification.

3.4.3 Molecular Support for the Genus Ursiniopsis

Retrieval of Clade G2 as shown in Fig. 3.1 also provided answers to the position of some species which used to be problematic. These species include *U. quinqepartita* and *U. caledonica*, which formed a small clade with 84% bootstrap support in the present analysis. Retrieval of this clade ruled against Phillips' (in Dyer 1951) suggestion of recognising these taxa as a separate genus (*Ursiniopsis*) and supported Prassler's (1967) classification of placing these species into subgenus *Sphenogyne* of *Ursinia*. Phillips (l. c.) had separated a small group consisting of the new species *Ursiniopsis caledonica* with the otherwise very isolated *Ursinia quinquepartita*. In these species, cypselae are straight, more ribbed, the basal tuft of hairs absent, female ray florets either fertile or sterile and a habit which differentiates the species of *Ursiniopsis* from *Ursinia*. The group is now firmly embedded within the *Sphenogyne* in the present phylogeny, showing that these characters are not taxonomically informative.

3.4.4 Molecular Support for Monophyletic Origin of Widely-distributed Species

ITS sequence data give some support for the presently recognized taxomonic units in *Ursinia*, both in terms of species grouping together as monophyletic clades and in the isolated positions, sometimes on longer branches. However, many of the presently

recognized taxa were not retrieved as monophyletic units. It was also puzzling that taxa appeared in the U, the F or the G clades, as well as in the H2 clades, particularly as all the members of the H2 clade possessed identical sequences. The ITS region is known for reticulation (Alvarez & Wendel 2003), which may be the reason why taxa do not appear as monophyletic groups, but these patterns may also be indicative of possible ancient hybridization between different groups. Preliminary sequence data of chloroplast markers (G. Jakubowsky, personal communication) indeed indicate that hybridization has occurred in these groups. At this stage therefore, ITS sequence data cannot be used for taxonomic purposes in *Ursinia* in isolation. Before any major taxonomic changes can be made in the genus, these molecular patterns will have to analysed in greater detail using more informative markers. However, if these molecular data can be confirmed, it is also clear from this study that the taxonomy of *Ursinia* will have to be revised and expanded extensively.

3.4.5 Forms within Ursinia trifida

Our analysis showed that *U. trifida* forma *calva* and *U. trifida* forma *trifida* are not sister taxa, which is further supported by their very different fruit morphology (see Chapter 2 of this thesis). This analysis therefore gives support for the recognition of these two taxa as separate species.

3.4.6 Summer-Rainfall Taxa in Ursinia

The taxa of *Ursinia* from the summer-rainfall region are not monophyletic (Fig. 3.4). For example *U. alpina* (a Drakensberg endemic) is sister to a clade of Cape species. *U. montana* subsp. *montana* (Northern Drakensberg) and *U. montana* subsp. *apiculata* (western continuation of Drakensberg) form a well-supported clade with 97% bootstrap support, and are also sister to a clade of Cape species. This supports a hypothesis that *Ursinia* repeatedly migrated from the Cape into the Drakensberg, which has been shown for a number of other Cape groups that have Drakensberg relatives (Galley et al. 2007). The latter study showed that the migration events from the Cape to the Drakensberg are more frequent than in the opposite direction, and these migration events are relatively frequent in other groups as well.

3.4.7 Origin of Annual Life-Form in Ursinia

An inspection of the ITS-based molecular phylogeny shows that annual life-cycle (short-lived habit) has evolved at least three times in subgenus *Ursinia*, and at least three times in subgenus *Sphenogyne* (Fig. 3.4). These taxa are found in habitats characterised by geology giving rise to nutrient-rich soils (granite, shale, dolerite). Some widely distributed taxa (e.g. *U. anthemoides subsp. versicolor* and other segregates currently considered as part of this taxon) are common in disturbed habitats such as roadsides. This may indicate that evolution of annual life history is dependant upon the availability of suitably fertile substrates, moisture availability and inherent capacity to grow and mature rapidly (Verboom et al. 2004).

3.5 Conclusion

The molecular analysis of the genus *Ursinia* based on ITS sequence data has revealed important insights that may have taxonomic implications. On the basis of this data

consideration could be given to the re-instatement of the genus *Sphenogyne*. However, the lack of resolution and the polyphyly of many taxa indicate that further study will be required to establish units of taxomony and to determine whether hybridization has played a role in the evolution of *Ursinia*. The numerous short terminal branch lengths that were retrieved in this analysis may indicate that the ITS region is evolving relatively slowly, and therefore the short branch lengths should rather be interpreted to indicate recent speciation and radiation in the genus *Ursinia*. This would be in agreement with the findings of many other studies that have indicated recent and rapid speciation in the Greater Cape Floristic Region (Linder 2003)**.**

3.6 References

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Appendix 3.1. Source of accessions used in molecular phylogenetic analysis (ITS) of *Ursinia*. Collections of *Agoseris*, *Dimorphotheca* and *Cotula* were used as outgroups. WC: Western Cape, EC: Eastern Cape, NC: Northern Cape, KZN: KwaZulu-Natal Provinces.

Taxa	Collection No.	Herb.	Locality
01. Agoseris grandiflora	AF386493		**GeneBank**
02. Dimorphotheca fruticosa	AF422131		**GeneBank**
03. Cotula coronopifolia	AF422118		**GeneBank**
04. Cotula turbinata	LM 250905/4	STEU	WC, Cape Peninsula, Kommetjie
05. U. arenaria sp. nov. *Piketberg*	LM 040806/32	STEU	WC, Piketberg, at road Piketberg–Velddrif, Banghoek
06. U. montana subsp. apiculata	Clark 458	STEU	EC, Sneeuberg near Graaff-Reinet
07. U. montana subsp. montana	Swelankomo 020406/30	STEU	KZN, Drakensberg, Sentinel, below the Chain Ladder
08. U. gariepensis sp. nov. *Koeboes*	LM 270806/11	STEU	NC, Richtersveld, Koeboes, SE of Sanddrif
09. U. quartzicola sp. nov. *Richtersveld*	LM 290806/4	STEU	NC, Richtersveld, E of Eksteenfontein
10. U. nana *Op-die-Berg*	LM 290805/9	STEU	WC, Koue Bokkeveld, Op-die-Berg, Sakrivier Farm
11. U. nana *Tanqua Karoo*	LM 010905/12	STEU	WC, Tanqua Karoo, N of Tankwarivier, Gebe
12. U. albicaulis sp. nov. *Kamiesberg*	LM 060905/1	STEU	NC, Namaqualand, Garies, Studers Pass
13. U. pilifera			origin unknown
14. U. pilifera	LM 280805/26	STEU	WC, Clanwilliam, Pakhuis Pass, below Klein Kliphuis
15. U. alba sp. nov. *Hondeklipbaai*	LM 040906/10	STEU	NC, Namaqualand, road Hondeklipbaai to Garies
16. U. alba sp. nov. *Hondeklipbaai*	Gwynne-Evans 181005/8	STEU	NC, Namaqualand, Honkeklipbaai, 15 km E of town
17. U. cakilefolia *Garies*	LM 040905/15	STEU	NC, Namaqualand, Garies
18. U. cakilefolia *Citrusdal*	LM 280805/15	STEU	WC, Olifantsrivier Valley, Citrusdal, east of the town
19. U. cakilefolia *Vanrhynsdorp*	LM 290706/15	STEU	WC, Namaqualand, Vanrhysdorp, on road to Gifberg
20. U. nana *Cederberg*	LM 270805/21	STEU	WC, Southern Cederberg, Farm Kunje, at road R303
21. U. kamiesbergensis sp. nov. *Kamiesberg*	LM 060905/2	STEU	NC, Namaqualand, Garies, Studers Pass
22. U. nana *Blouberg*	Winter 6889	PRE	Limpopo Prov., Blouberg
23. U. nana *Nieuwoudtville*	LM 020905/3	STEU	NC, Hantam, Nieuwoudtville, at Glacial Striations
24. U. picola sp. nov. *Riethuis*	LM 030906/25	STEU	WC, Namaqualand, Soebatsfontein, Riethuis
25. U. nana cf. subsp. leptophylla	AF046940		**GeneBank**
26. U. nana subsp. leptophylla	Burgoyne & Bhingwa 1	STEU	North-West Province
27. U. flava sp. nov. *Wilderness*	LM 050506/12	STEU	WC, Sedgefield, Wilderness National Park
28. U. nana *Worcester*	Koekemoer 3045	PRE	NC, Kakamas, Augrabies Falls National Park
29. U. nana *Steinkopf*	LM 100905/1	STEU	NC, Richtersveld, Steinkopf, Witputs NE of town
30. U. nana *Naip se Berg*	LM 080905/4	STEU	NC, northern Bushmanland, Aggeneys, Naip se Berg
31. U. nana *Kangas*	LM 060605/18	STEU	NC, Namaqualand, Aggeneys, Kangas Farm
32. U. nana *Vanrhynspass*	LM 020905/14	STEU	NC, Vanrhynsdorp, below Vanrhynspass
33. U. nana *Kommagas*	LM 050905/15	STEU	NC, Namaqualand, Kommaggas, at road to Springbok
34. U. nieuwoudtvillensis sp. nov. *Nieuwoudtville*	LM 290706/3	STEU	NC, Hantam, Nieuwoudtville Flower Reserve
35. U. alpina	Swelankomo 030406/1	STEU	KZN, Harrismith, Drakensberg, Oliviershoek Pass
36. U. bella sp. nov. *Swartruggens*	Bellstedt 100906/1	STEU	WC, Swartruggens, Kareefloof Private N.R.
37. U. bella sp. nov. *Bulletrap*	LM 310806/13	STEU	NC, Namaqualand, Bulletrap N of Springbok
38. U. bellstedtii sp. nov. *Karoopoort*	LM 210906/49	STEU	WC, Ceres, Karoopoort, Farm Vrede
39. U. bellstedtii sp. nov. *Swartruggens*	LM 290805/6	STEU	WC, Swartruggens, Dwarsbos turnoff
40. U. microlutea sp. nov. *Boschendal*	LM 180905/14	STEU	WC, Boland, Pniel, Boschendal, E foot of Simonsberg
41. U. klipheuvelensis sp. nov.	LM 130906/1	STEU	WC, Boland, Klipheuwel N of Stellenbosch
42. U. anthemoides s.str. *Pakhuis Pass*	LM 280805/34	STEU	WC, Clanwilliam, Pakhuis Pass
43. U. anthemoides subso. anthemoides *Boschendal*	LM 180905/10	STEU	WC, Pniel, Boschendal, E foot of Simonsberg
44. U. pinnata *Mitchells Pass*	LM 010905/1	STEU	WC, Ceres, Mitchells Pass
45. U. pinnata *Mosterts Twinpeaks*	LM 300805/6	STEU	WC, Ceres, Wolvekloof Resort
46. U. trifida f. calva	LM 310805/7	STEU	WC, Langeberg, top of Tradouw Pass
47. U. setigera	LM 130805/2	STEU	WC, Malgas, De Hoop N.R., near main entrance
48. U. discolor	LM 250904/18	STEU	WC, Cape Town, Helderberg, Gordon's Bay
49. U. outeniquensis sp. nov. *Outeniqua Pass*	LM 271105/7	STEU	WC, Outeniqua Mountains, George, top of Outeniqua Pass
50. U. outeniquensis sp. nov. *Yesternek*	LM 050506/1	STEU	WC, Outeniqua Mountains, Knysna, Ysternek N.R.
51. U. sericea	LM 180306/69	STEU	WC, Hexrivier Mountains, Erfdeel Farm, Spekrivier Valley
52. U. skurwebergensis sp. nov. *Skurweberg*	Gwynne-Evans 271005/34	STEU	WC, Ceres, Skurweberg
53. U. tsitsikammensis sp. nov. *Tsitsikamma*	LM 231105/4	STEU	WC, Tsitsikamma, Coldstream, E of the village
54. U. quinquepartita	Pienaar 62	STEU	WC, Betty's Bay, Kogelberg
55. U. caledonica	LM 250505/2	STEU	WC, Elgin, Hottentots Holland N. R., Landsroskop Hut
56. U. paleacea *Simonsberg*	LM 180905/9	STEU	WC, Pniel, Boschendal, E feet of Simonsberg
57. U. nudicaulis *Hottentots Holland*	LM 250505/4	STEU	WC, Elgin, Hottentots Holland N. R., Landsroskop
58. U. dentata *Kirstenbosch*	LM 161105/1	STEU	WC, Cape Town, Kirstenbosch Research Center
59. U. nudicaulis *Kleinmond*	LM 171205/12	STEU	WC, Overberg, Kleinmond, Kleinmond N.R.
60. U. punctata	LM 050405/3	STEU	WC, Wellington, Bainskloof, Twede Tol
61. U. hagelkraalensis sp. nov. *Klein Hagelkraal*	Gwynne-Evans 201105/43	STEU	WC, Overberg, Pearly Beach, Klein Hagelkraal
62. U. nigribracteata sp. nov. *De Hoop*	LM 030406/11	STEU	WC, Malgas, De Hoop N.R., Potberg section
63. U. paleacea *Silvermine*	LM 250905/10	STEU	WC, Cape Town, Fischhoek, Slivermine, Steenberg
64. U. paleacea *Du Toitskloof*	LM 031106/9	STEU	WC, Limietberg, old road to Du Toitskloof Pass
65. U. scariosa subsp. subhirsuta	LM 310805/3	STEU	WC, Langeberg, Barrydale, Tradouw Pass, Oskop
66. U. kammanassiensis sp. nov. *Uniondale*	LM 271105/17	STEU	WC, Uniondale, Kammanassie, Potjiespass
67. U. filipes	LM 050405/6	STEU	WC, Wellington, Bainskloof, E slope, near the Nek
68. U. subflosculosa	LM 290805/1	STEU	WC, Southern Cederberg, Kunje Farm, at bushman paintings
69. U. oreogena	LM 021106/3	STEU	WC, Limietberg, Paarl, old road to Du Toitskloof Pass
70. U. richtersveldensis sp. nov. *Jenkinskop*	LM 300806/15	STEU	NC, Richtersveld, Stinkfonteinberge, gorge SW of Jenkinskop
71. U. richtersveldensis sp. nov. *Noams*	LM 280806/5	STEU	NC, Richtersveld National Park, Noams
72. U. richtersveldensis sp. nov. *Ploegberg*	LM 290806/13	STEU	NC, Richtersvled, SW of Ploegberg, at road Koeboes–Lekkersing
73. U. mucronata sp. nov. *Stanford*	LM 100906/5	STEU	WC, Stanford, at road to De Kelders
74. U. clandestina sp. nov. *Bainskloof*	LM 270207/5	STEU	WC, Limietberg, Bainskloof, above Tweede Toll on road to The Nek
75. U. calenduliflora	Koekemoer 3004	PRE	NC, Namakwa National Park, near the Kanariesfontein turn-off
76. U. speciosa	LM 130906/2	STEU	WC, Stellenbosch, Klipheuwel N of Stellenbosch
77. U. speciosa	LM 270806/34	STEU	NC, Richtersveld, Steinkopf, Anenous Pass
78. U. trifida f. trifida	LM 271105/18	STEU	WC, Riversdale, top of Garcia Pass
79. U. maritima sp. nov. *De Hoop*	Radloff 7A	STEU	WC, Overberg, Malgas, De Hoop N.R.
80. U. anethoides s.str.	LM 261105/1	STEU	EC, Grahamstown, Dassie Krantz S of town
81. U. argentea	Jakubowsky 080207	STEU	WC, Koue Bokkeveld, Tafelberg
82. U. acocksii sp. nov. *De Hoop*	LM 211006/2	STEU	WC, Overberg, Malgas, N of De Hoop N.R., SW of Ouplaas
83. U. heterodonta *Barrydale*	LM 190206/23	STEU	WC, Barrydale, Op-die-Tradouw Pass
84. U. scariosa subsp. scariosa	Barker 1950	STEU	EC, Tsitsikamma
85. U. studentorum sp. nov. *Warme Bokkeveld*	LM 011106/3	STEU	WC, Warme Bokkeveld, Erfdeel Farm, road to the valley entrance
86. U. paleacea *Simonsberg*	LM 180905/3	STEU	WC, Pniel, Boschendal, E feet of Simonsberg
87. U. anthemoides subsp. versicolor	LM 040905/13	STEU	WC, Namaqualand, Garies
88. U. tenuifolia subsp. ciliaris	Radloff 5	STEU	WC, Overberg, Malgas, De Hoop N.R.
89. U. heterodonta *Rooiberg*	Gwynne-Evans 1005	STEU	WC, Rooiberg near Ladismith

90. U. pygmaea *Knersvlakte*	LM 050906/07	STEU	WC, Knersvlakte, at road between Vanrhynsdorp-Vredendal
91. U. rigidula	LM 170306/1	STEU	WC, Warme Bokkeveld, Farm Erfdeel, Spekrivier Valley
92. U. tenuiloba	DUB 011006/DB1	STEU	KZN, Midlands, Mt Gilboa
93. U. anethoides *Heroldsbaai*	LM 231105/1	STEU	WC, George, Heroldsbaai, at lookout at the coast
94. U. heterodonta *Ladysmith*	LM 190206/6	STEU	WC, Ladysmith, Hoeko Valley
95. U. ferricriticola sp. nov. *De Hoop*	LM 211006/10	STEU	WC, Overberg, Malgas, De Hoop N.R., Potberg section
96. U. soutpansbergensis sp. nov. *Soutpansberg*	DUB 1011	STEU	Limpopo, Soutpansberg, Farm Lajuma
97. U. hantamensis sp. nov. *Nieuwoudtville*	LM 030605/13	STEU	NC, Hantam, Nieuwoudtville, Nieuwoudtville Flower Reserve
98. U. abrotanifolia	LM 270207/3	STEU	WC, Wellington, Bainskloof, above Tweede Toll on road to The Nek