Chapter 5
Multivariate analysis of morphological variation in *Cineraria deltoidea* (Senecioneae, Asteraceae)

Abstract

Cluster Analysis and Principal Coordinates Analysis are used to investigate phenetic variation in *Cineraria deltoidea*, a species that ranges from near sea level in KwaZulu-Natal, South Africa, to 4300 m on the mountains of East Africa and Ethiopia. Earlier taxonomic revisions reduced nine previously recognized species to synonyms of *C. deltoidea*. Two closely related species, *C. decipiens* and *C. atriplicifolia*, were also included in the analyses. Thirty-six morphological characters were examined on 111 specimens. Phenograms and scattergrams show partial clusters of specimens of *C. deltoidea* from individual mountains or geographic regions, but no groups are sufficiently distinct to warrant formal recognition at any rank. The East African specimens from 3000 m and higher tend to cluster together. Growth at high altitude in East Africa is correlated with fewer, larger capitula on longer peduncles, and an absence of a cobwebby indumentum comprising long, narrow-based trichomes. *C. deltoidea* is thus a highly variable species with geographic and clinal variation evident throughout its range. *C. atriplicifolia* and *C. decipiens* are maintained as distinct species, distinguished from *C. deltoidea* by their growth form, life span and auricle shape.

Introduction

Cineraria L. is essentially an afromontane genus that extends from the Western Cape, South Africa through the mountains of the Northern Cape to Namibia and southern Angola and along the eastern mountain ranges of South Africa, Zimbabwe and East Africa to Ethiopia. One species also occurs in Yemen and Saudi Arabia and another species is endemic to Madagascar. The genus mainly comprises perennial herbs and subshrubs, with a few annuals or short-lived perennials at lower altitude in South Africa. *Cineraria* is distinguished by its palmately-veined, 5–7-lobed leaves (usually auriculate) and radiate, yellow capitula. A substantial carpopodium and laterally compressed, obovate cypselae with distinct margins or wings are also diagnostic of the genus. As a senecioid member of the Senecioneae, Asteraceae, it has balusterform filament collars and a chromosome number of x = 10 (Nordenstam, 1978).

The genus *Cineraria* comprises 35 species, 27 of which occur in South Africa. The most wide-ranging species is *C. deltoidea* Sond., which occurs from South Africa to Ethiopia (Figure 1). In South Africa, *C. deltoidea* occurs at forest margins in the midlands of KwaZulu-Natal and the Transkei region of the Eastern Cape, as well as in forests near Magoesbaskloof and the Soutpansberg in Limpopo Province. From there it extends along

the ‘spine of Africa’ on peaks in Zimbabwe (Chimanimani, Vumba and Inyanga), Mozambique (Gorongoza), and Malawi (Mulanje, Zomba and Nyika) before reaching the numerous peaks of East Africa and Ethiopia, including the Imatong Mountains in southern Sudan. Its altitudinal range extends from 200–1700 m in KwaZulu-Natal to 1600–4300 m in East Africa, with the highest specimen recorded from Mount Elgon on the border of Uganda and Kenya. Its growth form shows altitudinal variation. In the alpine zone, *C. deltoidea* forms a compact herb or small shrub (Figure 2a), but at lower altitudes throughout Africa it typically grows as a straggling herb up to 1·5 m in forest margins or undergrowth (Figure 2b). It also grows as a pioneer herb on newly deposited lava at high altitude on the Virunga Mountains (Figure 2c), but with a much smaller form than in the forests below (Figure 2d).

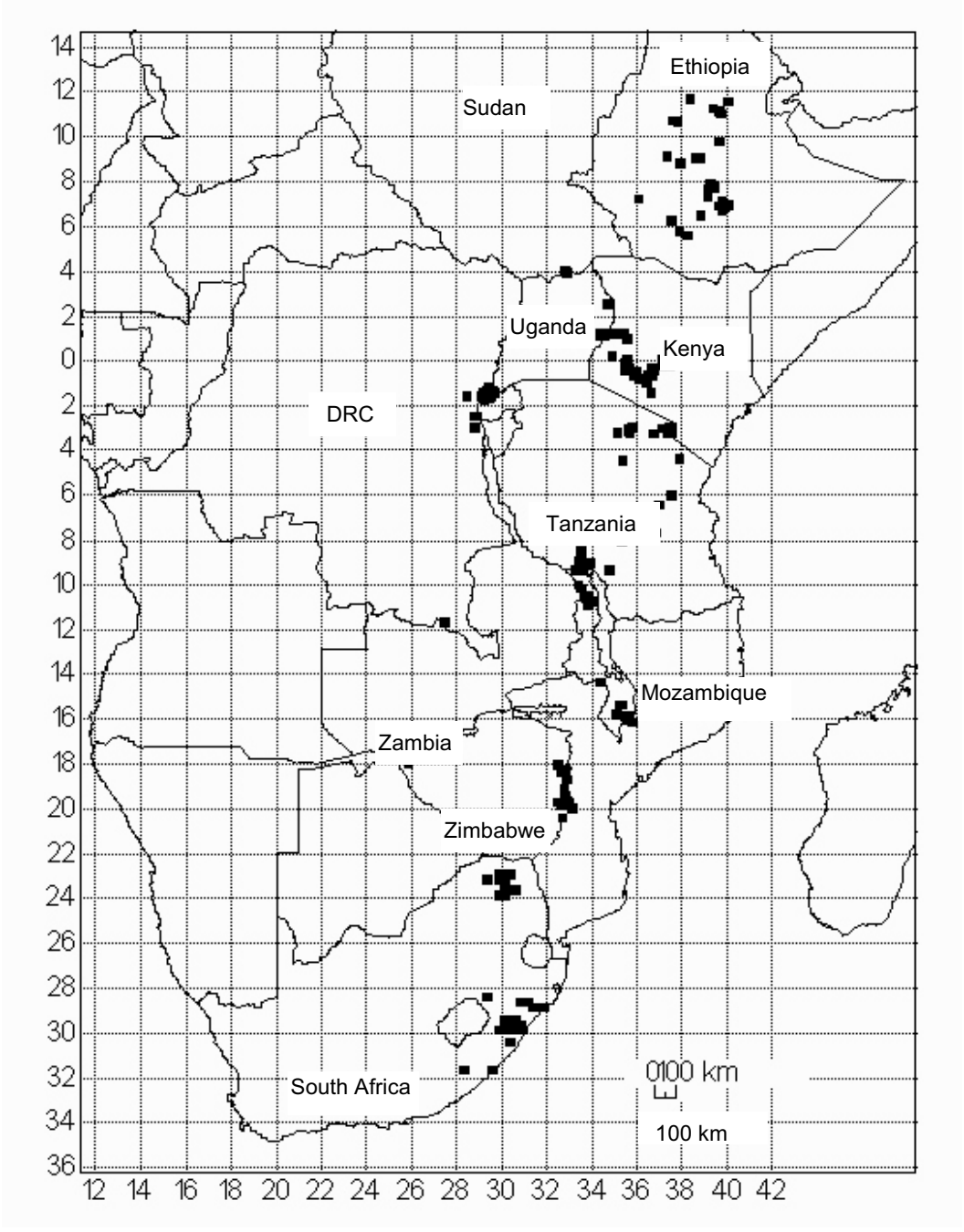


Figure 1. Distribution of *Cineraria deltoidea.*

The plasticity and phenotypic variation of *Cineraria deltoidea*, combined with the nature of colonial exploration in east Africa, resulted in a proliferation of species descriptions and names that were applied to plants from individual mountains or a particular altitudinal range. Hedberg (1957) placed six species (Table 1) into synonymy with *C. grandiflora* from Ethiopia, noting no consistent discontinuity in the characters supposedly distinguishing them. In particular, Hedberg (1957) stated that there is continuous variation in the indumentum of the cypsela and the size and number of capitula and that leaf shape is highly variable. Leaves from the upper part of the stem are frequently lyrately or pinnately divided, while those from the lower part of the stem are normally undivided and have smaller petiolar auricles. Combinations of all these characters were used to distinguish these previously described species (Table 1).

Table 1. Types and synonyms of *Cineraria grandiflora* according to Hedberg (1957).

Species	Type and type locality	Distinguishing features
C. grandiflora Vatke	*Schimper 1517* (B†, holo.; BM, K, S, iso.) Ethiopia, Dshan Mèda.	lyrate-pinnatifid leaves, 6, 7 or 8 rays, cypselae glabrous.
C. bequaertii De Wild.	*Bequaert 5863* (BR, holo.) Democratic Republic of Congo (DRC), lava plains between Tongo and Mukule.	lyrate-pinnati-partite leaves, cypselae ciliate on margins.
C. bracteosa O.Hoffm. ex Engl.	*Götzen 64* & *106* (B†, syn.) DRC, Ninagongo, 2500 and 3000 m.	many bracts on peduncles, 8 involucral bracts, cypselae hairy.
C. densiflora R.E.Fr.	*Fries & Fries 1555* (UPS, holo.; S, iso.) Kenya, Mount Kenya.	numerous, small capitula with 6 to 8 rays, cypselae glabrous.
C. kilimandscharica Engl.	*Johnson 4, 120, 129* (K, lecto.) Tanzania, Mount Kilimanjaro, 1300–2300 m.	leaves not lyratiform, with 100 km ler petioles, large capitula with long rays and 12 or 13 involucral bracts, cypselae hairy or ciliate.
C. laxiflora R.E.Fr.	*Fries & Fries 2651* (UPS, holo.; S, iso.) Kenya, Aberdares, Sattima, 3000 m.	coarsely-toothed, rotund-ovate leaves, few-headed inflorescences, long peduncles, 12 rays, cypselae glabrous and winged.
C. prittwitzii O.Hoffm.	*Götzen 29* (B†, holo.) DRC, plains at Ninagongo, 2000 m.	8 involucral bracts, cypselae hairy and/or ciliate.

Hedberg (1957) noted that much of the variation in the *Cineraria grandiflora* complex is environmentally conditioned (e.g., the size of the capitula positively correlates with altitude). He also observed that the southern African species *C. deltoidea*, *C. monticola* and *C. buchanani* did not appear to be convincingly different from the East African *C.*

Figure 2. Variation in growth form of *Cineraria deltoidea* in East Africa: (a) a large, sprawling subshrub at 3000 m at Chania Waterfall, Aberdares, Kenya. Scale bar = 5 cm; (b) a dwarf suffrutex (with larger capitula) at 3800 m in the Aberdares, Kenya. Scale bar = 15 cm; (c) A pioneer herb at 3700 m, Volcan Mikeno, Kivu District, DRC [*Humbert 8090* (BR)]. Scale bar = 34 mm; (d) scrambling herb/suffrutex on Volcan Bishoke near Susa, DRC, at 2400 m *De Witte 2222* (BR). Scale bar = 34 mm.

grandiflora, but refrained from placing them in synonymy because of a lack of exposure to them in the field. He called for a monographic study of the whole genus so that the taxonomy of *C. deltoidea s.l.* could be established.

Although much of the variation in the East African populations is environmentally conditioned, with altitude affecting growth form, size and number of capitula and density of indumentum, some of the variation does seem to reflect regional patterns. The specimens from the Ngong Hills south of Nairobi, Kenya are distinguishable by their grey, cobwebby leaves, small capitula with short ray florets (4.8–6.0 mm long) and many dentitions on their leaves [(6–) 9–10 per cm]. The specimens from Ethiopia and Mount Kenya tend to have larger capitula with longer (and more) ray florets and many involucral bracts, glabrous cypselae or glabrous faces and ciliate margins. Collections from the Virunga Mountains tend to have many more and smaller capitula (5–6 rays, 20 or fewer disc florets) than those from the other East African mountains, and distinctly winged cypselae. Specimens from southern Tanzania have fine trichomes creating a cobwebby indumentum on the leaf surfaces and/or petioles, and commonly have many capitula with few rays (5, rarely 8), on short peduncles.

Hilliard (1977) also noted that *Cineraria deltoidea* from KwaZulu-Natal is either closely allied to or is conspecific with *C. grandiflora*. Jeffrey (1986) observed that *Cineraria* was in need of critical revision, but placed *C. grandiflora* and the southern African species *C. monticola* and *C. buchanani* into synonymy with *C. deltoidea* (Table 2), simply stating that he could see no distinction between them.

Table 2. Types and synonyms of *Cineraria deltoidea* according to Jeffrey (1986; including *C. grandiflora*, Table 1).

Species	Type and type locality	Distinguishing features
C. deltoidea Sond.	*Gueinzius 343* (S, holo.; MEL, P, W, iso.) South Africa, KwaZulu-Natal.	deltoid leaves, large auricles, 5 rays, cypselae glabrous.
C. buchanani S.Moore	*Buchanan 10* (BM, holo.; GRA, PRE, SAM, iso.) Malawi.	very broad leaves with long petioles, many-headed cymes, small heads with 8 involucral bracts.
C. monticola Hutch.	*Hutchinson & Gillett 3201* (K, holo.) South Africa, Limpopo Province, Soutpansberg.	leaves lobed and "repando"-denticulate, ventral surface of leaves lanate, glabrescent, cypselae sparsely hairy.

As noted in Table 2, plants previously treated as *Cineraria monticola* were distinguished from *C. deltoidea* by the lobing and indumentum of their leaves, having more distinct

lobing and a grey tomentum on the young leaves and the lower surface of older leaves. They also have a more shrubby growth form, as opposed to the trailing nature of *C. deltoidea* in the forest margins of KwaZulu-Natal and Eastern Cape. Specimens from the Chimanimani mountains and near Inyanga in Zimbabwe also match *C. monticola*.

Very few specimens match the type of *C. buchanani*, which is distinctive due to the size of its leaves (especially petiole length) and the mucronate teeth on the leaf margins. The leaves are also membranous, and the stems are unusually pale (a light straw colour) and are covered with white hairs that are especially thick on the young parts.

Other species with deltoid leaves apparently closely allied to *Cineraria deltoidea* are *C. atriplicifolia* DC. and *C. decipiens* Harv., both of which are endemic or near-endemic to KwaZulu-Natal, with *C. decipiens* extending into Swaziland. Sonder (1850) noted that *Cineraria deltoidea* differs from *C. atriplicifolia* in having weaker stems and leaves not deeply incised but with larger auricles. Harvey (1865) distinguished *C. decipiens* on the basis of the lobing of its leaves and by its ciliate/hairy cypselae. Hilliard (1977) noted that both *C. atriplicifolia* and *C. decipiens* are probably annual herbs; our observation is that they may be short-lived perennial herbs. They do not develop the trailing habit nor reach the size of *C. deltoidea*. Hilliard (1977) drew attention to the large apical lobe of *C. atriplicifolia*, which distinguishes it from both *C. decipiens* and *C. deltoidea* (Figure 3).

Cineraria atriplicifolia and *C. decipiens* are both diploid (n =10; Cron, 1991; Cron *et al.,* 1994), which may correspond with their shorter life span and less spreading habit (Stebbins, 1950; Solbrig, 1977). In contrast, *C. deltoidea* from KwaZulu-Natal is tetraploid (n = 20; Cron, 1991), a number also reported for *C. deltoidea* from Kenya and Tanzania (Turner & Lewis, 1965) and from Mount Kenya and Mount Kilimanjaro (2n = 40; Hedberg & Hedberg, 1977a). Diploid populations of *C. deltoidea* have not been reported.

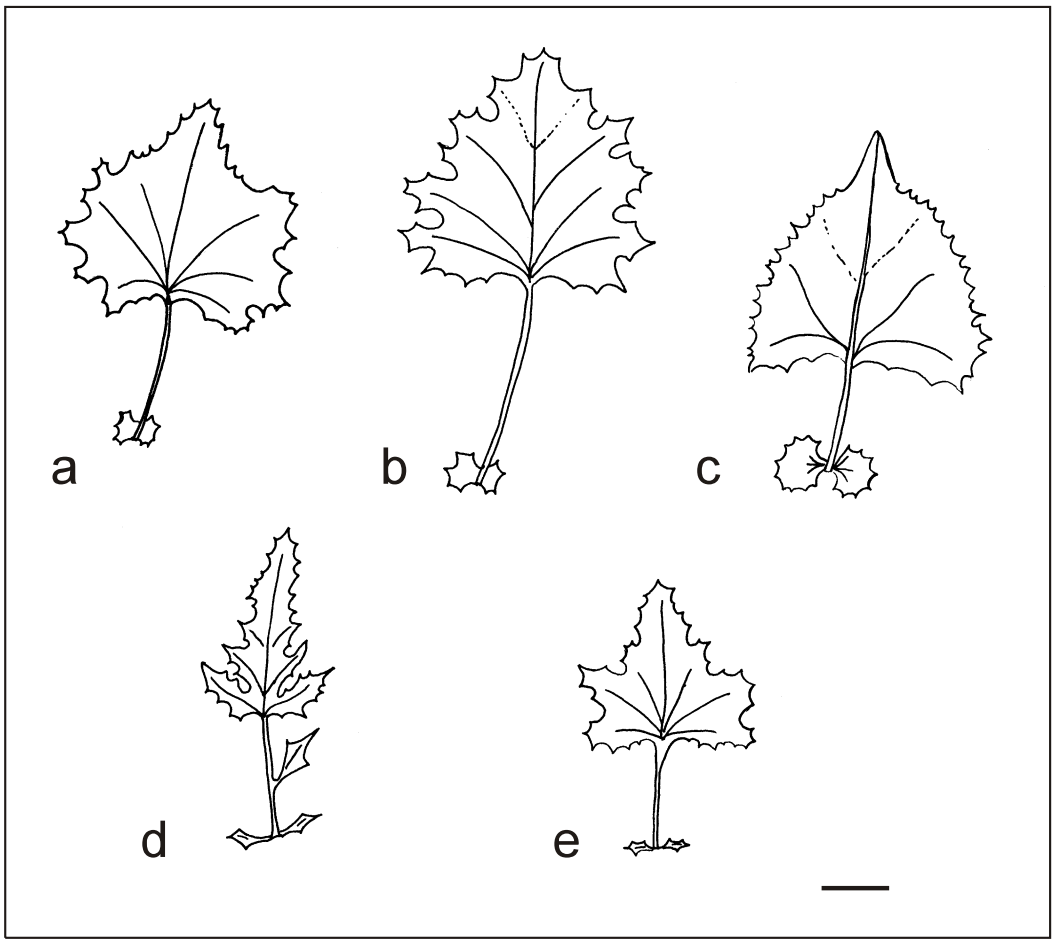


Figure 3. Upper leaves of *Cineraria*: (a) *C. deltoidea*, *Hilliard & Burtt 10168* (MO), Peak of Byrne, KwaZulu-Natal, South Africa; (b) *C. deltoidea*, *de Wilde 5939* (WAG), 5 km north of Addis Ababa, Ethiopia; (c) *C. deltoidea*, *Chase 8302* (BR), Vumba mountains, Mutari district, Zimbabwe; (d) *C. atriplicifolia*, *Cron 7* (J): Montesseel, Camperdown District, KwaZulu-Natal, South Africa; (e) *C. decipiens*, *Galpin 14778* (PRE), Tugela Ferry, Msinga District, KwaZulu-Natal, South Africa. Scale bar = 9 mm.

Multivariate techniques

Two main approaches have been used in numerical taxonomy based on morphological data, ordination and cluster analysis. Ordination techniques summarise large amounts of information in only a few dimensions and have been much used in taxonomic studies (Pimentel, 1981; Chandler & Crisp, 1998). Cluster analysis is useful in separating organisms into groups that may be used in a classification and has been widely used to examine geographical patterns of variation (Thorpe, 1983). It imposes a hierarchical structure on the data and a disadvantage of this is that the analysis may show distinct clusters even if the variation is clinal, as may be seen using ordination techniques (Thorpe, 1983). Therefore both Cluster Analysis and Principal Coordinates Analysis (PCO) were used in this study.

PCO is recommended for data sets combining quantitative and qualitative characters (Legendre & Legendre, 2003). The method has been used to good effect in several taxonomic studies (e.g., Brysting & Elven, 2000; Olvera, 2003). Michener (1970) and Dunn & Everitt (1982) conclude that numerical taxonomic methods are best seen as tools for data exploration, rather than for the production of a formal classification. It is in this sense that we use these approaches here.

We use a phenetic species concept essentially equivalent to the traditional concept based on morphological similarity among organisms. Although the phylogenetic species concept of Nelsen & Platnick (1981) and the composite species concept of Kornet & McAllister (1993) are more satisfactory and intuitive, with species being mutually exclusive groups of organisms, morphologically distinguishable by combinations of diagnostic characters and (in the latter concept) existing over time, the method for applying these concepts to highly variable and wide-ranging species or species complexes is unclear. Certain taxonomists have admitted to adopting a pluralistic approach (e.g., Sidwell, 1999), making use of phenetic practice to investigate variation in widespread and variable species, while adhering to a phylogenetic concept for other species in a group. Other taxonomists probably do the same, however many do not clearly state the species concepts being applied (McDade, 1995).

Aim of study

This study investigates the morphological variation in *Cineraria deltoidea s.l.* (Jeffrey, 1986) to determine whether plants previously described as *C. grandiflora*, *C. monticola* or *C. buchanani* are sufficiently different from *C. deltoidea s.s* to warrant formal recognition. Some analyses include *C. decipiens* and *C. atriplicifolia* from KwaZulu-Natal as points of comparison.

Materials and Methods

Specimens and characters

One hundred specimens that span the geographic and altitudinal range of *Cineraria deltoidea* were examined, along with six specimens of *C. decipiens* and five specimens of *C. atriplicifolia* (Appendix 1). Six type specimens were included (Appendix 1). In 38 of these 111 specimens, multiple herbarium sheets were available for comparison. Each specimen was named according to its geographic location. Species from southern Africa were named either according to their original specific epithet (when certain) or by geographic locality or mountain range (when uncertain).

Analyses were performed for three partitions of the data: (i) southern Africa (including specimens from South Africa, Zimbabwe and Malawi; (ii) East Africa (including specimens from Ethiopia, Kenya, Tanzania, DRC, Uganda, Sudan, Rwanda and Nyika Plateau in northern Malawi; and (iii) a complete set of all specimens of *C. deltoidea* (i.e. excluding *C. atriplicifolia* and *C. decipiens*). The specimens from Nyika Plateau in northern Malawi

were included in both data sets to allow some overlap between the southern tropical region and the East African Highlands. Reasons for separating the specimens geographically were twofold: firstly, to assist in ease of interpretation of any patterns in the data, especially in regard to altitudinal influences (which could be complicated by increase in latitude) and secondly, to facilitate reading of the ordination plots. The southern data set was also analyzed excluding *C. decipiens* and *C. atriplicifolia* to examine the influence of characters on the associations and distribution of C. *deltoidea* specimens.

The characters in this study include those used to distinguish the previously described species now included in *Cineraria deltoidea*, as well as characters known to be generally useful within *Cineraria* (Cron, 1991). Forty-three characters were investigated and 35 characters were used in the final analyses: 14 qualitative (11 binary and three multistate ordered) and 21 quantitative characters, including both vegetative and floral characters (Table 3). Sixteen characters, including three multistate unordered characters, were used in a separate analysis of the qualitative data only (Table 3). Invariant and nearly invariant characters were excluded from the analysis. Pearson's product-moment correlation coefficient was used to calculate correlations between characters and highly correlated (> 0.80) characters were excluded. Although numbers of ray florets, disc florets and involucral bracts were fairly highly correlated, this was logical as an increase in capitula size could be reflected in all these characters, but does not necessarily imply a genetic link. Sneath and Sokal (1973) recommend the use of partially correlated characters, rather than exclusion.

Inclusion of multistate ordered characters in the data set is based on the statement by Sneath and Sokal (1973) that multistate ordered characters may be treated like continuous quantitative characters. Ratios were used to provide indicators of leaf shape rather than size (Table 3). In addition to leaf length:leaf width ratios, leaf width was retained in the data set as it resulted in phenograms with a better cophenetic correlation coefficient than with leaf length and the length:width ratio. The use of ratios has been shown to be valuable in several studies (Hill, 1980; Estabrook & Gates, 1984; West & Noble, 1984; Frampton & Ward, 1990), even though their use is controversial as they are non-normal and have been shown to increase spurious correlations between variables (Atchley *et al.,* 1976; Atchley & Anderson, 1978).

The leaves were divided into upper leaves (UL) and 'middle to lower leaves' (LL), as these differ somewhat in shape and degree of dissection as well as length of petiole. As far as possible, specimens with a reasonable length of stem and number of leaves were selected for the analysis; in a few specimens (notably from Mount Mulanje), lower leaves were not available for measurement, resulting in some missing data. Mature capitula were used for the floral measurements and measurements for disc floret length and cypsela length were taken from different capitula. A range of peduncle lengths were included (i.e. longest to shortest). A minimum of three measurements was taken for each character, more (5–7) when characters appeared highly variable. Cypsela length was excluded to eliminate considerable missing data due to the rarity of mature fruits on many specimens. (Data matrix available on website: www.wits.ac.za/apes/ggoodman/cineraria.html)

Table 3. Characters used in the NTSYS analysis of *Cineraria deltoidea*

1. Growth form: (1) annual/short-lived perennial; (2) long-lived perennial.
2. Shape of lower leaves (LL): (1) deltoid to deltoid-reniform; (2) reniform.
3. Upper leaf (UL) base: (0) truncate (to cuneate); (1) truncate to subcordate; (2) subcordate; (3) subcordate to cordate; (4) distinctly cordate; (Figure 4).
4. Lower leaf (LL) base: (0) truncate (to cuneate); (1) truncate to subcordate; (2) subcordate; (3) subcordate to cordate; (4) distinctly cordate; (Figure 4).
5. Lateral pinnae below main lamina: (0) Absent; (1) Present on at least one leaf (excluding uppermost bract-like leaves).
6. UL width (average of 3 measurements; Figure 4).
7. LL width (average of 3 measurements; Figure 4).
8. UL length to width ratio (average of 3 measurements; Figure 4).
9. LL length to width ratio (average of 3 measurements; Figure 4).
10. UL lobe depth (length) to width ratio (average of 3 measurements of second lobe from the base; Figure 4).
11. LL lobe (depth) length to width ratio (average of 3 measurements of second lobe from the base; Figure 4).
12. Apical lobe length to leaf length ratio (UL) (average of 3 measurements; Figure 4).
13. Apical lobe length to leaf length ratio (LL) (average of 3 measurements; Figure 4).
14. Number of teeth per cm (UL), measured from apex of leaf (mean for 3 leaves).
15. Number of teeth per cm (LL), measured from apex of leaf (mean for 3 leaves).
*16. Indumentum of leaf: (1) glabrous above and below; (2) glabrous above, hairy below; (3) hairy above and below; (4) cobwebby above and below/below only.
17. Trichome 1: Tapering multi-celled base (granular or agranular) with long appendage (Figures 5a, 6a, b, c): (0) absent (1) present.
18. Trichome 2: Eglandular, long but no wisp (Figures 5b, 6d): (0) absent; (1) present.
19. Trichome 3: Glandular in lobes of leaves / surface (Figures 5c, 6f, g): (0) absent; (1) present.
20. Trichome 4: Fine, cobwebby type (2–6 narrow basal cells; Figures 5d, 6h): (0) absent; (1) present.
*21. Petiole indumentum: (1) glabrous; (2) hairy; (3) cobwebby.
22. Petiole length (UL) (average of 3).
23. Petiole length (LL) (average of 3).
24. Shape of auricles (present and either persistent/caducous): (1) lanceolate; (2) auriculate.
*25. Stem indumentum: (1) glabrous; (2) hairy; (3) cobwebby.
26. Indumentum of peduncles: (1) glabrous; (2) cobwebby.
27. Length of peduncle nearest capitulum (average of 3–5 measurements).
28. Number of bracts on peduncle (average of 3–4 measurements).
29. Number of capitula per stem branch (average of 3).
30. Number of involucral bracts (average of 3 measurements).
31. Length of involucral bracts (mm) (average of 3 measurements)
*32. Indumentum of involucral bracts: (1) glabrous; (2) cobwebby.
33. Number of ray florets (average of 3 measurements).
34. Length of ray florets (mm) (maximum total length, i.e. limb + tube).
35. Number of veins on ray florets: (1) four veins only; (2) more than four veins on one or more ray florets.
36. Number of disc florets (average of 3 measurements).
37. Length of mature disc floret corolla (average of 3 measurements).
38. Lateral extension of cypsela (when mature): (1) margined; (2) winged.
39. Cypsela indumentum: (1) glabrous; (2) glabrous faces, ciliate margins; (3) hairy faces and margins/ (1) glabrous; (2) ciliate and/or hairy (used for southern data set).

(* = not included in final data set; used in qualitative analysis only.)

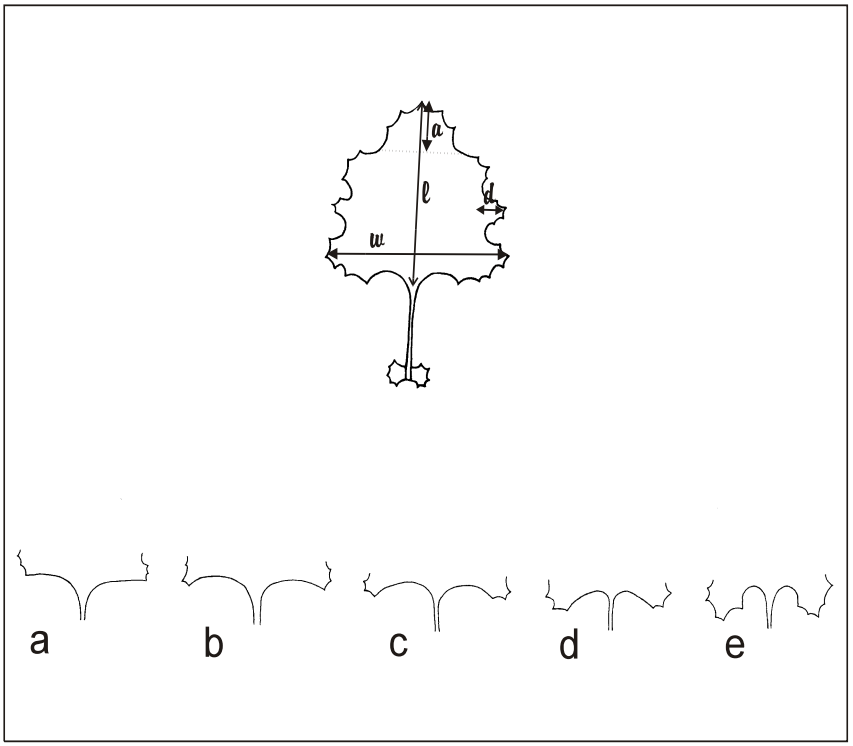


Figure 4. Diagrams showing: (i) dimensions of leaf measurements taken: *a* = apical lobe, *l* = lamina length, *w* = lamina width, *d* = lobe depth; and (ii) leaf bases: (a) truncate (to cuneate); (b) truncate to subcordate; (c) subcordate; (d) subcordate to cordate; (e) distinctly cordate.

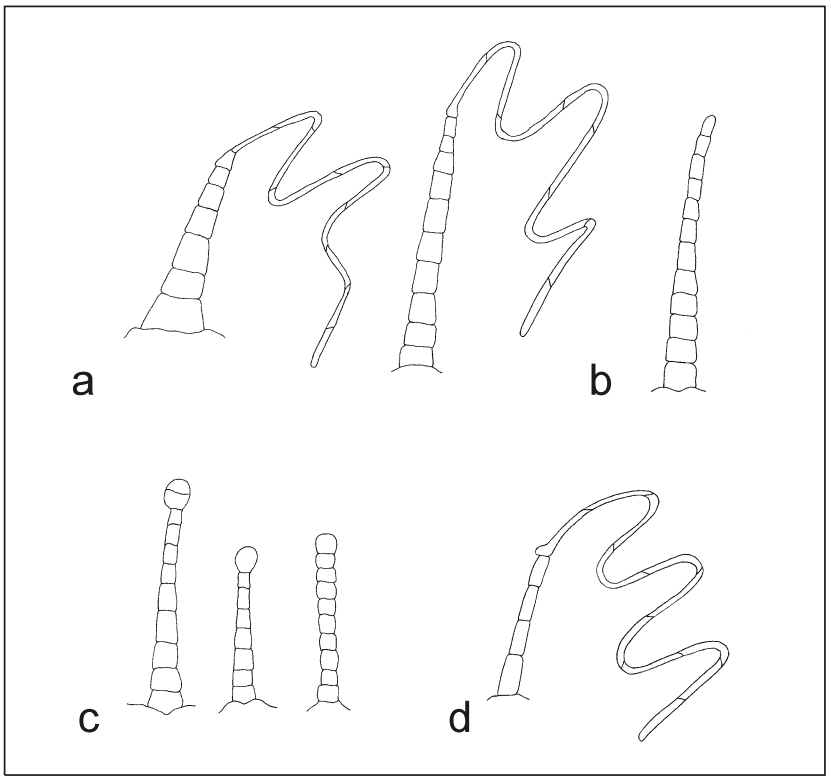


Figure 5. Diagrams showing types of trichomes: (a) Trichome 1: tapering basal cells (4–8) with long multi-celled apical appendage; (b) Trichome 2: eglandular, 8–16 cells; (c) Trichome 3: glandular, present in angles of lobes; (d) Trichome 4: 2–6 narrow basal cells with long multi-celled apical appendage.

Figure 6. (Opposite) SEM of trichomes: (a) Trichome 1: *Hilliard & Burtt 10168* (PRE), KwaZulu-Natal, South Africa; (b) Trichome 1, *Hedberg 1293* (K), Mount Kilimanjaro; (c) Trichomes 1 and 4 (arrowed), *Cron et al. 292* (J), Soutpansberg, South Africa; inset shows slightly granular base of Trichome 1; (d) Trichome 1, *Nappier 536* (K), Ngong Hills, Kenya, inset shows very granular base; (e) Trichome 2, *Bally 6414* (K), Mount Kenya; (f) Trichome 3 (glandular) in angles of lobes of leaves, *Greenway 8404* (K), Rungwe District, Tanzania; (g) Trichome 3 (glandular) in angles of lobes, *Hedberg 1293* (K), Mount Kilimanjaro, Tanzania; (h) Trichome 4: *Nappier 536* (K), Ngong Hills, Kenya. Scale bars: a, b, c, d inset, e, f, h and h inset: 10 µm; d, g: 100 µm.

All measurements were made on dried herbarium specimens housed at J and EA or borrowed from the following herbaria: BOL, BR, COI, GRA, K, MO, NU, PRE, TCD, WAG. Leaf trichomes were examined at 400X magnification on a dissecting microscope, and representative specimens from each location were also examined using a compound microscope and/or the scanning electron microscope. Leaves examined under the compound microscope were cleared and autoclaved according to the procedure of O'Brien & von Teichman (1974). Scanning electron microscopy (SEM) preparation of leaves involved dehydration in an alcohol series, critical point drying and coating with a mixture of gold palladium and carbon. Specimens were viewed under a JEOL JSM 800 scanning electron microscope. Cypsela surfaces from representative specimens were also examined using the SEM. The outline of the epicarp cells and their surface patterns were noted and photographed. As it was not practical to examine cypselae of all specimens with the SEM, these features were not included in the matrix, but are referred to in the results and discussion.

All analyses were performed using NTSYS-PC version 2.0 (Rohlf, 1998). In the Cluster Analysis, the characters were standardised by dividing the difference between the mean and the actual measurement by the standard deviation (default standardization option in NTSYS), then a dissimilarity matrix based on average taxonomic distance was calculated and the UPGMA clustering method used to hierarchically cluster the specimens. The data set comprising only the 16 qualitative characters was compared using the simple matching coefficient, then clustered using the UPGMA algorithm. The cophenetic correlation coefficient (r) for each resultant tree and the distance matrix were used as a measure of goodness of fit. PCO was also performed on the data set comprising 33 (for East Africa) or 34 (for southern Africa) qualitative and quantitative characters, by standardising the matrix by variables and computing and double-centring a matrix of distances between the specimens, factoring the double-centred matrix and plotting the results in 2-dimensional and 3-dimensional space (Rohlf, 1993; Rohlf, 1998).

Results

East Africa

Two characters (life span and auricle shape) were invariant for this data set and were excluded, resulting in a matrix of 69 specimens and 33 characters: 21 quantitative, 9 binary and three multistate ordered (UL base, LL base and cypsela indumentum).

The phenogram (Figure 7) resulting from the UPGMA analysis of the East African specimens reveals as association among some specimens from a given geographic region (e.g. southern Tanzania and Nyika, Ngong, the Aberdares, Ethiopia), but no unambiguous geographic clusters are evident. Overlying the weak regional patterns, some influence of altitude on morphology is evident, with a number of specimens from higher altitudes (>3000 m) tending to cluster together. The cophenetic correlation coefficient (r) is 0.68, indicating a poor match of pair distances to the tree.

The influence of altitude on the distribution of specimens is more clearly seen in the scattergram based on the first two axes of the PCO (Figure 8), where specimens from 3000 m and higher are distributed towards the left of the phenetic space and those from lower altitudes towards the right. There is strong correlation between altitude and the possession of fewer, larger capitula, as evidenced by a Pearson's product moment correlation of altitude with the data set and also by the eigen vector values (Table 4). Specimens with many, smaller capitula, mainly from Malawi and southern Tanzania, but also from lower altitudes in other regions and mountains, are distributed towards the right of the scattergram (Figure 8). Characters reflecting size of capitula (in order of importance in determining distribution along the first axis) are length and number of involucral bracts, number and length of ray florets and number and length of disc florets. A larger number of capitula per stem is correlated with shorter average peduncle length, also important in influencing distribution of specimens along the first axis. A fine, cobwebby indumentum on the leaves due to Trichome 4 (Figures 5d, 6h) is more common in those specimens towards the right of the first axis (Figure 8; Table 4) and is more prevalent in specimens growing at lower altitudes. As shown in Table 4, the first three coordinates only account for 38.5 % of the variation in the data set.

Important characters influencing distribution of specimens on the second axis are shape of leaf bases and leaf widths (strongly positive), presence or absence of lateral pinnae, peduncle indumentum (glabrous/cobwebby) and the ratio of depth of lobe to leaf width on lower leaves (in the negative direction). On the third axis, shape and dentition of lower leaves are important influences in the positive direction, while lower leaf length:width ratio and presence/absence of glandular trichomes in angles of lobes (Trichome 3, Figures 5c, 6f, g) are important in the negative direction.

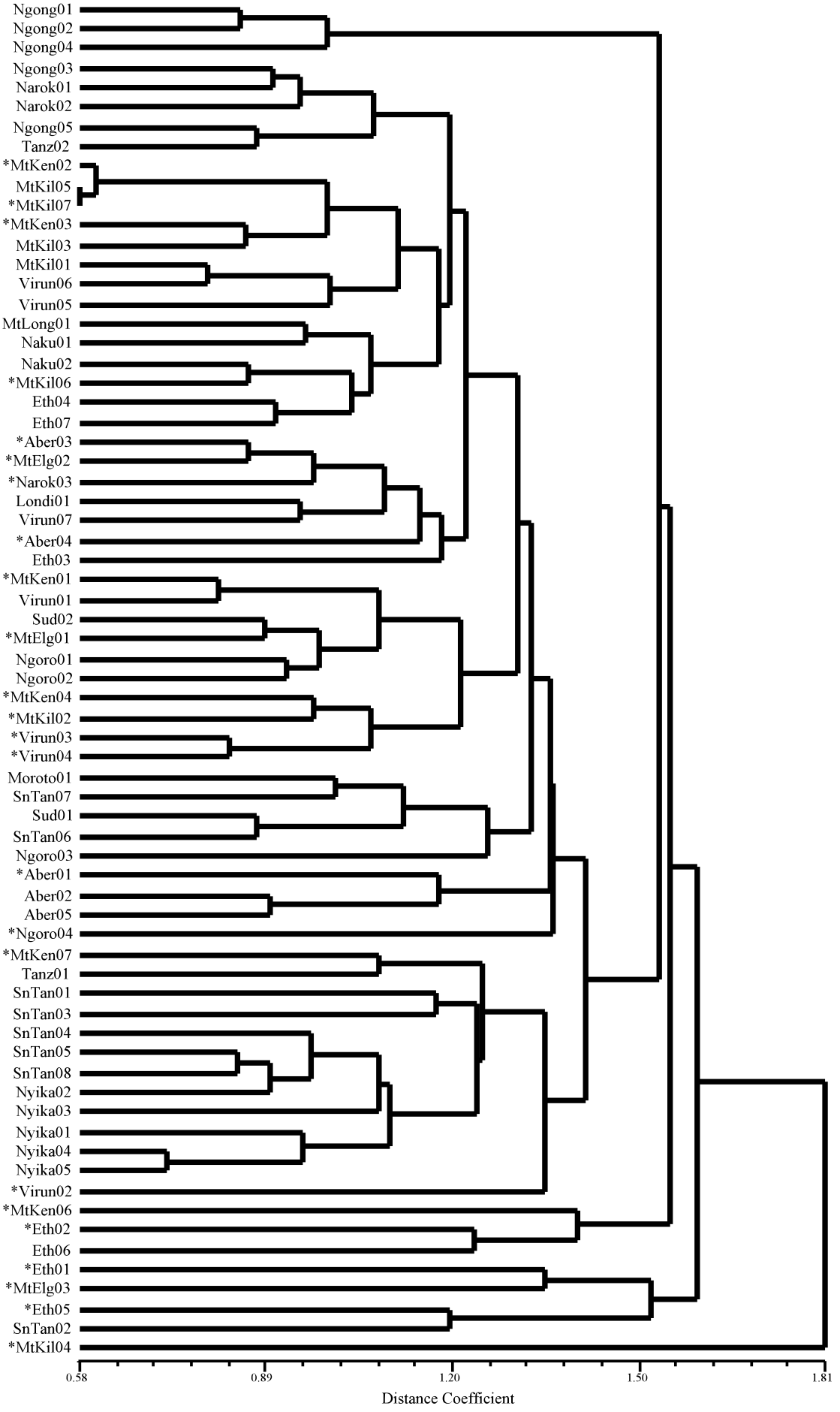


Figure 7. Phenogram resulting from the UPGMA cluster analysis of average taxonomic distance of *Cineraria deltoidea* in East Africa based on 33 morphological characters, r = 0.67. (Abbreviations identifying specimens as in Appendix 1; * specimens growing at 3000 m or more.)

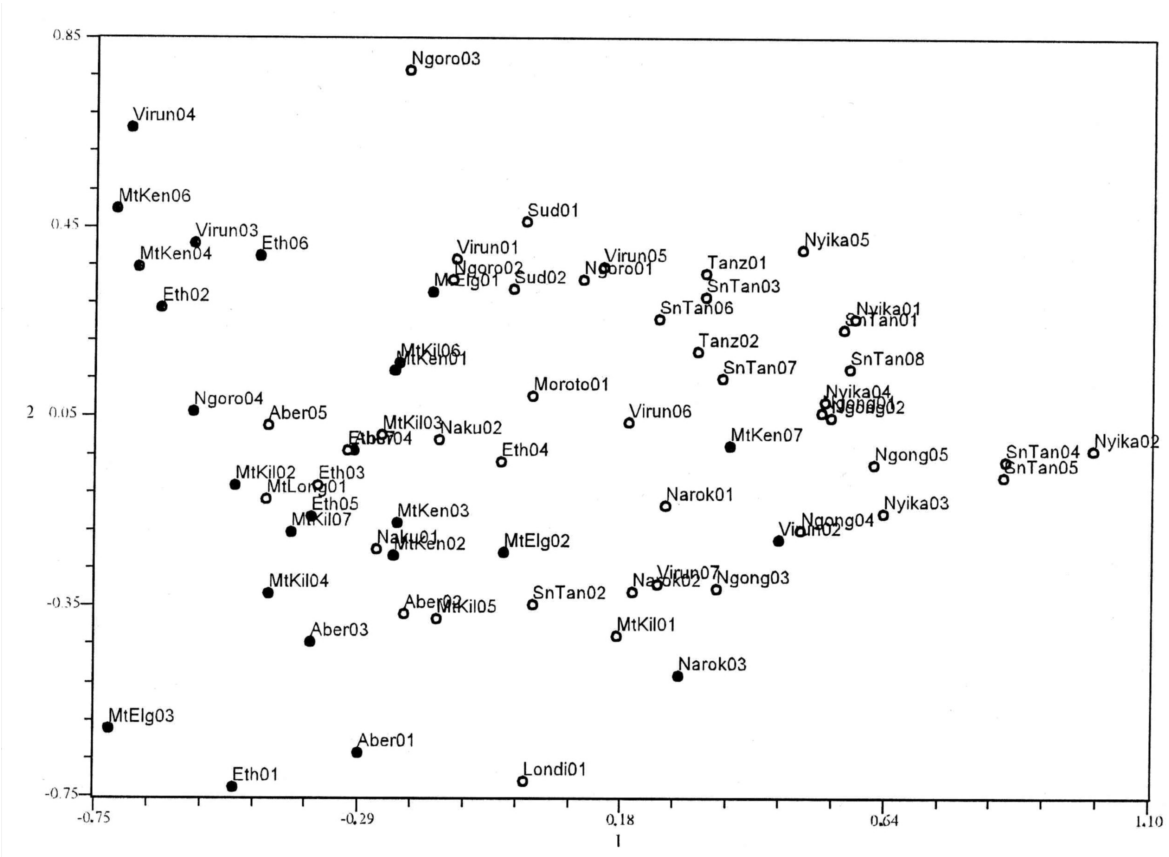


Figure 8. Scattergram of the first two principal coordinates of *Cineraria deltoidea* in East Africa based on 33 morphological characters.(●filled circles indicate plants collected at an altitude of 3000 m or more). (Abbreviations identifying specimens as in Appendix 1.)

Table 4. Eigen vector coefficients for Principal Coordinates Analysis of *Cineraria deltoidea* in East Africa. Character loading values are for the first three Principal Coordinate (PC) axes. The percent variation explained by each axis is presented. The correlation of each character with altitude is also presented.

Character	PC1 (18.4 %)	PC2 (10.5 %)	PC3 (9.6 %)	Correlation with altitude
Lower leaf shape	0.156	0.047	0.662	0.122
UL base	0.15	0.552	0.272	–0.098
LL base	0.23	0.696	0.258	–0.126
Lateral pinnae	0.061	–0.483	0.014	–0.063
UL width	–0.247	0.55	–0.110	–0.066
LL width	–0.131	0.537	–0.173	–0.119
UL length:width	0.422	–0.346	–0.333	–0.276
LL length:width	0.216	–0.240	–0.545	–0.161
UL lobe depth:leaf width	0.239	–0.371	–0.324	0.115
LL lobe depth:leaf width	0.375	–0.412	–0.410	–0.041
UL apical lobe:leaf length	0.486	0.286	–0.312	–0.293
LL apical lobe:leaf length	0.388	0.491	–0.076	–0.231
UL # teeth	0.518	–0.072	0.346	–0.085

LL # teeth	0.484	–0.192	0.519	–0.450
Trichome 1	–0.073	–0.004	0.362	0.266
Trichome 2	–0.332	0.046	–0.219	0.373
Trichome 3	0.05	–0.031	–0.429	0.171
Trichome 4	0.686	0.062	0.084	–0.447
UL petiole length	–0.336	0.335	0.118	0.329
LL petiole length	–0.405	0.364	0.086	0.235
Peduncle indumentum	0.183	–0.433	0.262	–0.031
Peduncle length	–0.569	0.043	–0.157	0.347
# Bracts	0.24	–0.277	0.483	0.088
# Capitula	0.804	0.107	–0.317	–0.524
# Involucral bracts	–0.690	–0.240	0.177	0.562
Involucral bract length	–0.697	–0.167	–0.046	0.681
# Ray florets	–0.555	–0.386	0.171	0.584
Ray floret length	–0.544	–0.036	–0.282	0.478
# Veins on ray florets	–0.183	–0.143	–0.249	0.418
# Disc florets	–0.471	–0.337	0.415	0.436
Disc floret length	–0.438	0.22	–0.284	0.445
Cypsela extension	–0.286	0.028	–0.047	0.145
Cypsela indumentum	0.268	–0.191	0.086	–0.074

Cluster analysis of only the qualitative characters based on a similarity matrix calculated using the simple matching coefficient resulted in a phenogram (r = 0.65; not shown) with two distinct clusters of exclusively Ngong specimens and Nyika specimens, but these clusters do not include all the specimens from these regions as one specimen is missing in each case. Analysis of the quantitative data only produces a similar result for the Ngong specimens (r = 0.68).

Southern Africa

UPGMA analysis of the southern African data set using 34 characters (lower leaf shape is invariant) yields a phenogram (Figure 9) with r = 0.78, which indicates a good fit of the data to the tree. *Cineraria atriplicifolia* and *C. decipiens* are distinct from *C. deltoidea s.l.* and from each other. *C. deltoidea s.s.* forms a distinct cluster, but is nested within other clusters. Apart from the Vumba specimens clustering together, there is no other grouping which exclusively reflects the geographic region or mountain from which the specimens originate. The four specimens of *C. monticola* from the Soutpansberg clustered together, but this cluster excludes the fifth specimen (Magoebaskloof) and includes one specimen from Zimbabwe (Chimanimani).

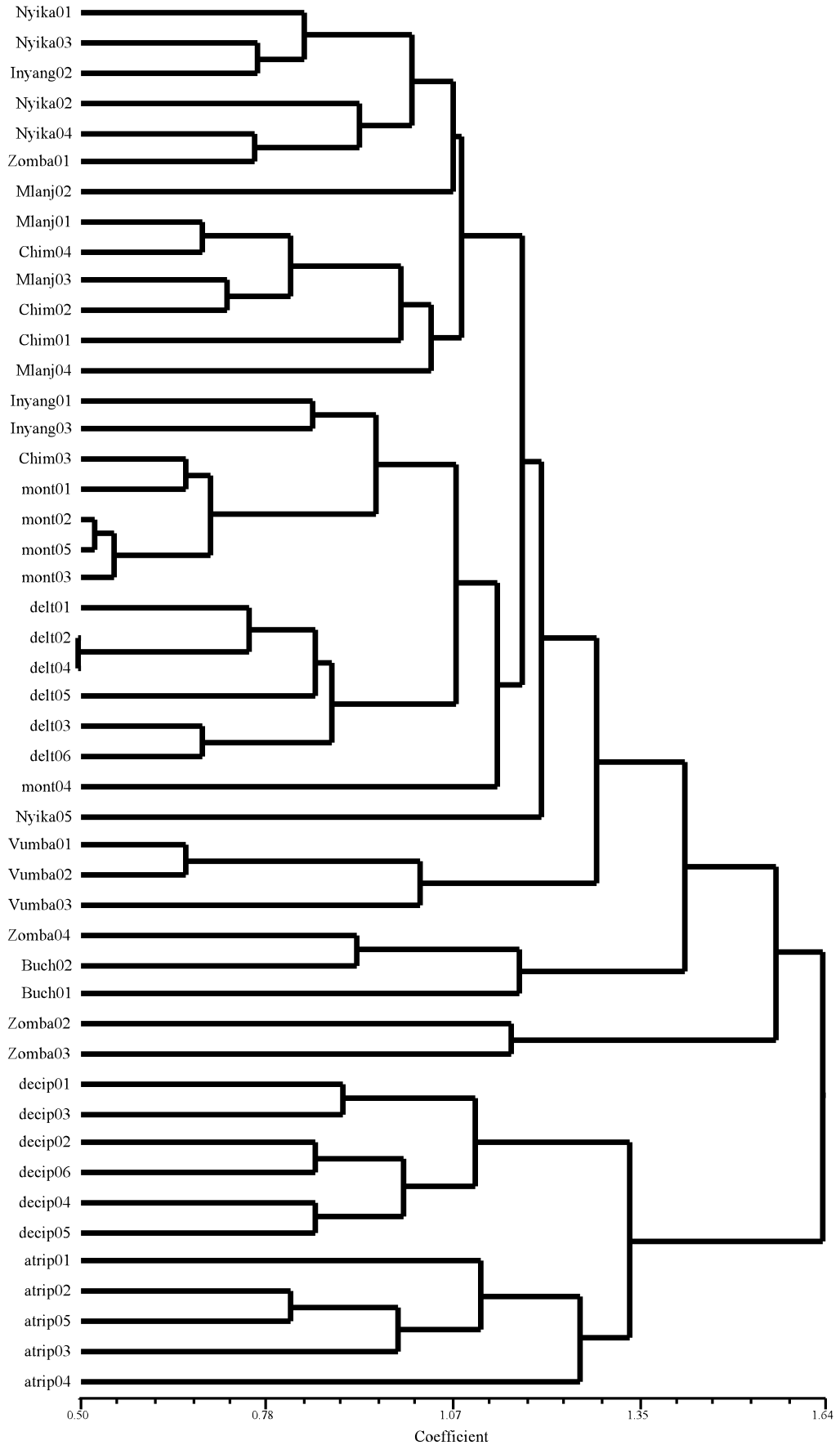
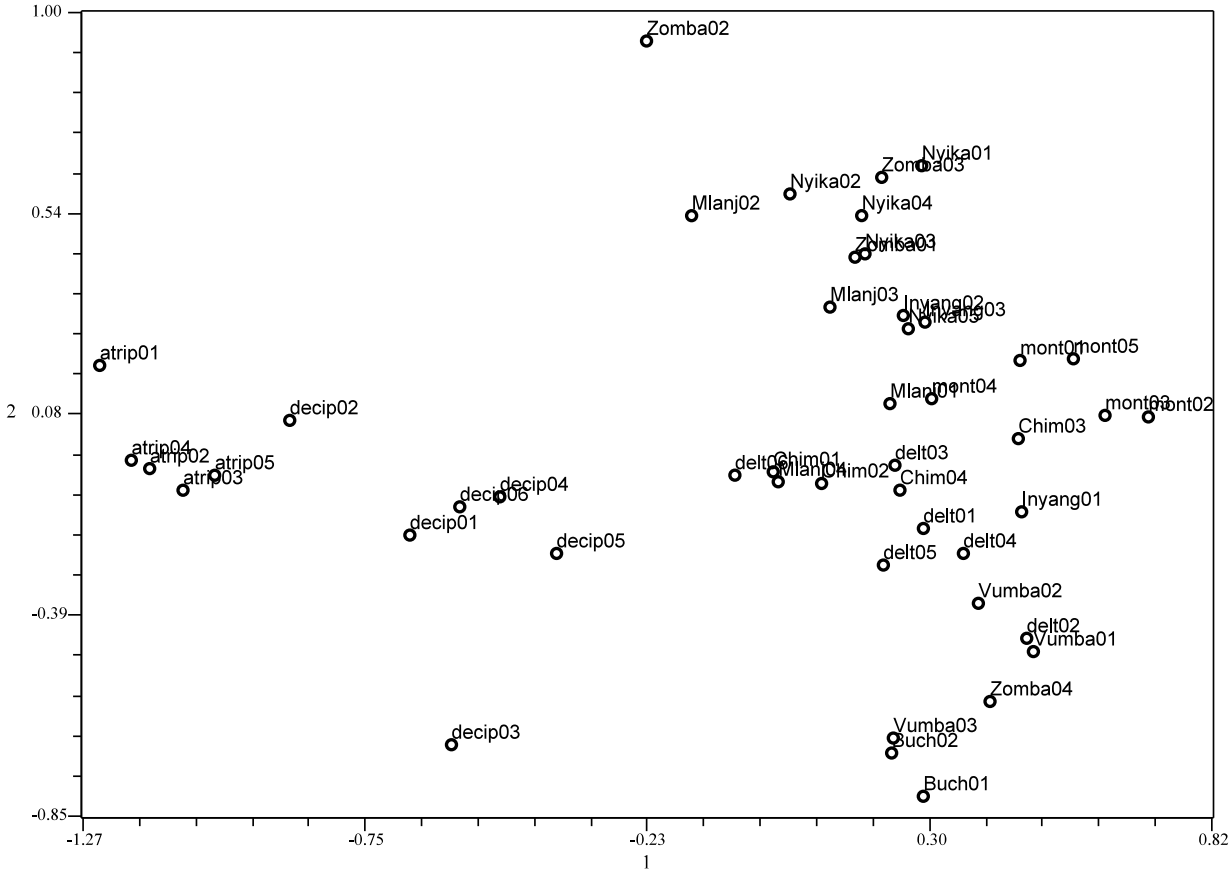


Figure 9. Phenogram resulting from the UPGMA cluster analysis of average taxonomic distance of *Cineraria deltoidea*, *C. atriplicifolia* and *C. decipiens* in southern Africa based

on 34 morphological characters, r = 0.78. (Abbreviations identifying specimens as in Appendix 1.)

A similar picture emerges from the scattergram (Figure 10) produced by PCO analysis of the same data set. The two annual or short-lived perennial species, *Cineraria atriplicifolia* and *C. decipiens*, are set apart from *C. deltoidea*, with *C. decipiens* more scattered than *C. atriplicifolia*. The separation of these two species from *C. deltoidea* is based on their lifespan, auricle shape and degree of lobing of the leaves, as seen by the eigen values of the principal coordinates (Table 5). Other characters contributing significantly to the distribution of specimens along this axis are the presence or absence of Trichome 1 (Figures 5a, 6a, c) and Trichome 3 (Figures 5c, 6f, g) in the angles of lobes. Although there is a tendency for specimens of *C. deltoidea* from the same geographic region to be nearer to one another in the two-dimensional space of the scattergram, no distinct geographic groupings emerge within the complex. Leaf width and dentition influence distribution of specimens along the second coordinate, while peduncle length, involucral bract length and number of capitula are of importance along the third coordinate (Table 5). However, only 42.4 % of the variation in the data is accounted for by the first two axes in Figure 10.

Figure 10. Scattergram of the first two principal coordinates of *Cineraria deltoidea*, *C.*



atriplicifolia and *C. decipiens* in southern Africa based on 34 morphological characters. (Abbreviations identifying specimens as in Appendix 1.)

Table 5. Eigen vector coefficients for Principal Coordinates Analysis of *Cineraria deltoidea* in southern Africa. Character loading values are for the first three Principal Coordinate (PC) axes. The percent variation explained by each axis is also presented.

Character	PC1 (27.5 %)	PC2 (14.9 %)	PC3 (9.1 %)
Life span	0.847	0.182	0.149
Upper leaf (UL) base	0.71	–0.004	–0.039
Lower leaf (LL) base	0.771	–0.004	–0.265
Lateral pinnae	–0.497	–0.173	0.284
UL width	0.381	–0.771	0.157
LL width	0.53	–0.716	0.035
UL length:width	–0.530	0.059	0.203
LL length:width	–0.615	0.152	0.224
UL lobe depth: leaf width	–0.856	0.084	0.132
LL lobe depth: leaf width	–0.902	–0.020	0.043
UL apical lobe: leaf length	–0.882	0.187	0.033
LL apical lobe: leaf length	–0.732	–0.093	0.091
UL # teeth	0.266	0.767	0.184
LL # teeth	0.216	0.788	0.256
Trichome 1	0.606	0.335	–0.147
Trichome 2	0.044	–0.647	–0.026
Trichome 3	–0.809	–0.225	–0.418
Trichome 4	0.378	0.525	0.089
UL petiole length	0.06	–0.637	0.401
LL petiole length	0.307	–0.624	0.347
Auricle shape	0.847	0.182	0.149
Peduncle indumentum	0.357	0.088	–0.038
Peduncle length	–0.469	–0.187	–0.723
# Bracts	–0.151	0.149	–0.399
# Capitula	–0.167	–0.164	0.547
# Involucral bracts	–0.300	0.332	0.015
Involucral bract length	0.079	–0.323	–0.656
# Ray florets	–0.138	0.472	–0.025
Ray floret length	0.45	–0.200	–0.345
# Veins on ray florets	–0.078	0.286	–0.060
# Disc florets	0.1583	0.4403	–0.135
Disc floret length	0.477	0.0985	–0.449
Cypsela extension	0.0856	–0.2914	–0.023
Cypsela indumentum	–0.0797	–0.0480	0.472

Excluding *Cineraria decipiens* and *C. atriplicifolia* from the cluster analysis of the southern data set results in a phenogram (not shown) with r = 0.71. Some clustering of specimens mirrors original names and localities, notably *C. buchanani*; Zomba and *C. monticola*. *C. deltoidea s.s.* does not form a distinct hierarchical cluster, but in the scattergram produced by PCO analysis of this reduced data set (Figure 11), they are in close proximity to one another. Leaf width, the presence or absence of the eglandular Trichome 2 (Figures 5b, 6e), the apical lobe to leaf length ratio for the upper leaves and degree of dentition of the leaf margin are important in influencing the distribution of specimens along the first principal coordinate (Figure 11). Disc floret length, petiole length and cypsela indumentum are important in determining distribution of specimens along the second principal coordinate and the leaf length to width ratio and shape of leaf base are important in determining distribution along the third principal coordinate, also influenced by number of disc florets and presence of a cobwebby indumentum.

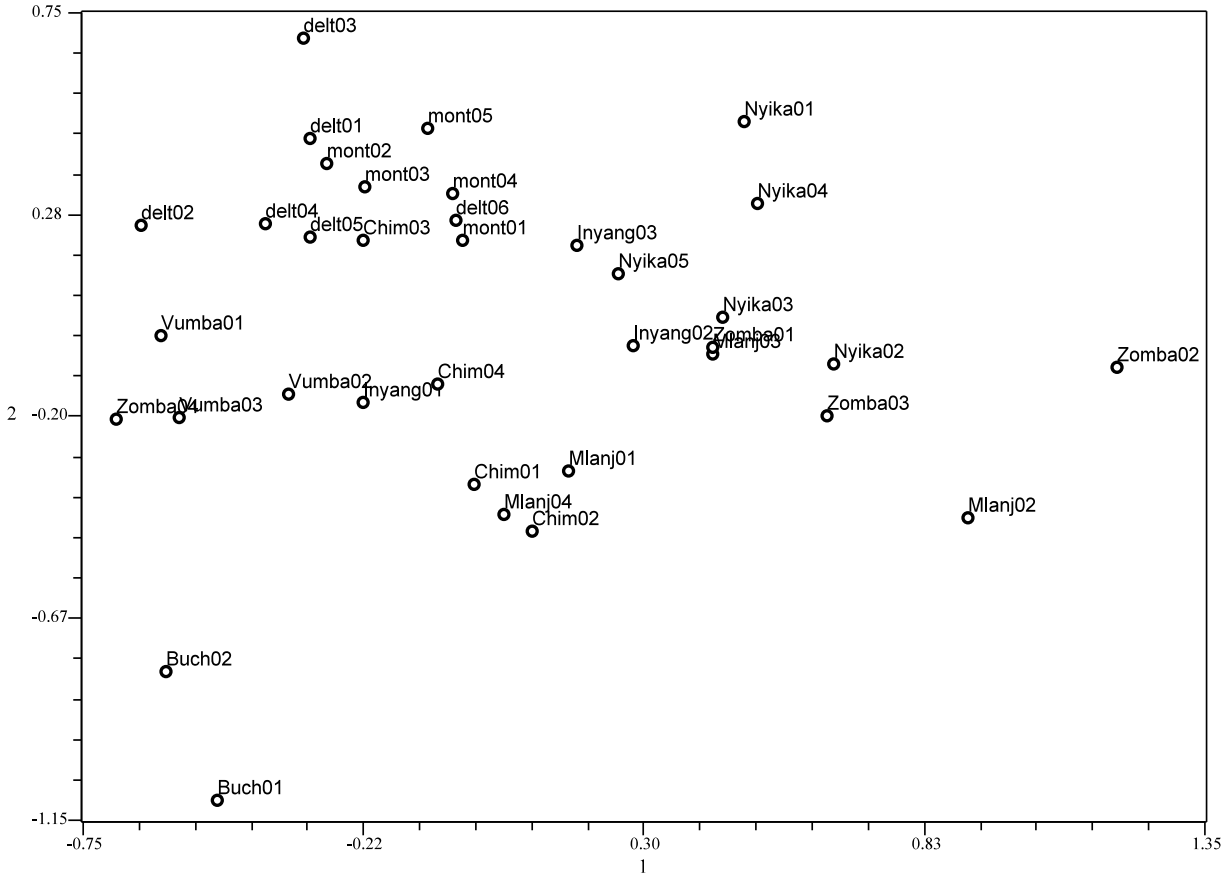


Figure 11: Scattergram of the first two principal coordinates of *Cineraria deltoidea* (excluding *C. atriplicifolia* and *C. decipiens*) in southern Africa based on 33 characters. (Abbreviations identifying specimens as in Appendix 1.)

Cluster analysis of only the quantitative characters results in a phenogram (not shown) without any distinct clusters corresponding to previously recognised groups; not even *Cineraria decipiens* and *C. atriplicifolia* are distinguishable. Although the cophenetic

correlation coefficient is relatively high (r = 0.80), quantitative characters alone are insufficient to distinguish clear groups.
In contrast, cluster analysis of only qualitative characters produces a phenogram (not shown; r = 0.84) in which *Cineraria decipiens* and *C. atriplicifolia* are distinct from *C. deltoidea*, but not from each other. *C. deltoidea s.s.* formed a cluster (except for delt06), but included Vumba03. No other clear groups emerged that correspond to geographic localities or previously recognised species.

Complete analysis
Cluster analysis of the complete data set resulted in a phenogram (r = 0.71; Figure 12) in which specimens of *C. deltoidea s.s.* are clustered together, as are most of the *C. monticola* and matching specimens, but they are nested within the larger group, as seen previously (Figure 9). Some grouping of specimens at higher altitude is evident, but this phenomenon is less apparent in the complete southern African and East African data set.

Principal Coordinates Analysis of the complete data set for only *C. deltoidea* (Figure 13) again shows an altitudinal influence, with high altitude specimens predominantly situated towards the left and those at lower altitudes towards the right. There is some overlap in the middle. The first two coordinates represent only 30.6 % of the variation in the data set. Characters strongly influencing the distribution of specimens on the first coordinate are those associated with size of capitula (number and length of involucral bracts, number of ray and disc florets), as well as peduncle length (in a positive direction) and upper and lower leaf width and number of capitula (in a negative direction). Upper and lower leaf width and petiole length, as well as length of ray florets and disc florets and presence/absence of the eglandular Trichome 2 (Figures 5b, 6h) influence the distribution along the second PC (in a negative direction), while a higher number of dentitions on the leaf margin and the presence of fine, cobwebby Trichome 4 cause the specimens to occupy the more positive end of the coordinate. Shape of the leaf base and leaf shape expressed as length to width ratio are important in determining distribution along the third component (not shown).

Epicarp surface patterning
Scanning electron microscopy of the cypselae of specimens from different mountains/regions reveals considerable variation in the epicarp surface sculpturing. Cypselae from Ethiopia and Mount Kenya have striate patterns on their surfaces (Figure 14a, b). Those from the Aberdares, Mount Elgon and Nakuru have ridged epicarp cell outlines with sculptured surfaces (Figure 14c, d), while those from the Virunga Mountains and Ngorogoro Crater are ridged and lined (Figure 14e, f), similar to cypselae of *C. deltoidea s.s.* from KwaZulu-Natal (Figure 14g). In contrast, Mount Kilimanjaro and the Soutpansberg have bulging, smooth epicarp cells (Figure 14h). There appears to be little relationship between geographic proximity and epicarp similarity, although not enough cypselae were examined to determine whether a consistent pattern is present on each mountain. There is also no apparent correlation of epicarp patterning with the types of trichome present on the leaves.

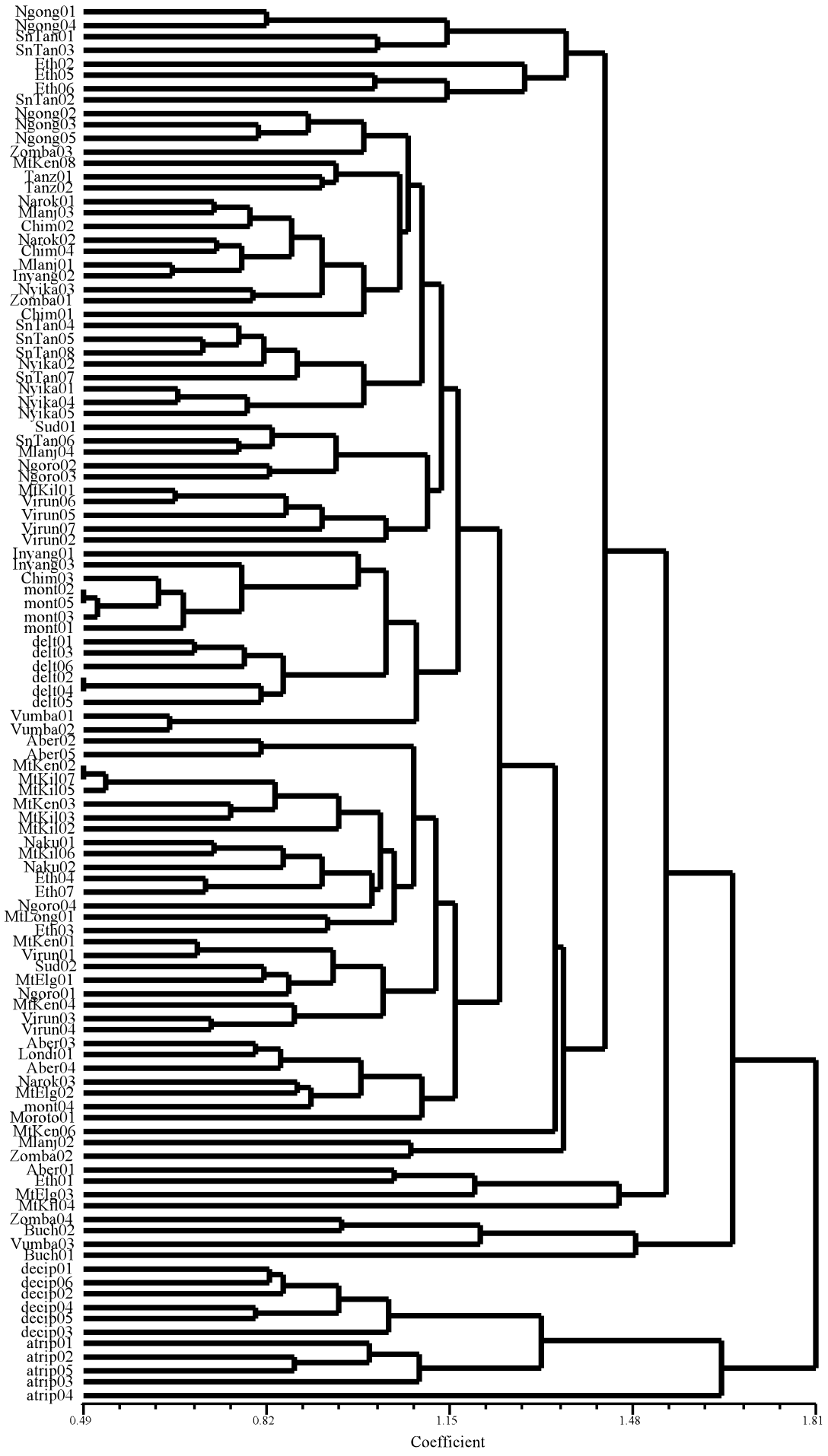


Figure 12. Phenogram resulting from the UPGMA cluster analysis of average taxonomic distance of the complete data set for *Cineraria deltoidea* based on 35 morphological characters, r = 0.71. (Abbreviations identifying specimens as in Appendix 1.)

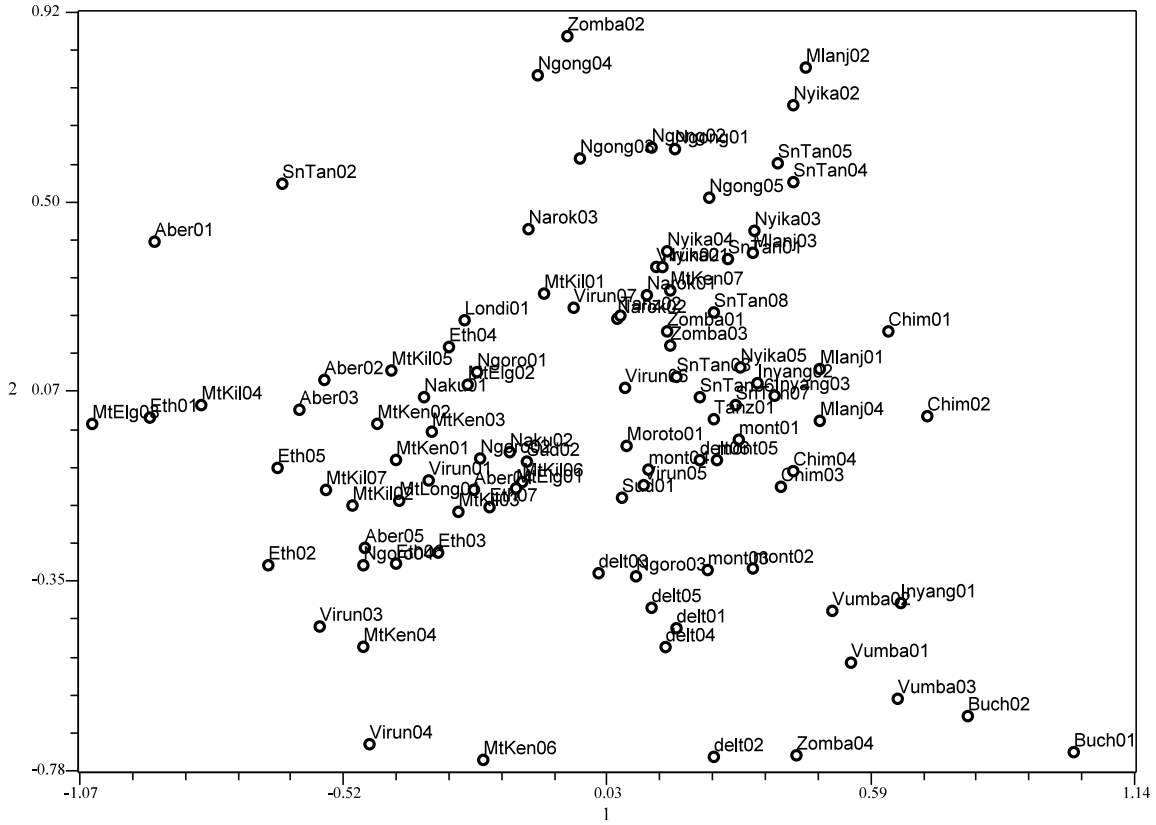


Figure 13. Scattergram of the first two principal coordinates of the complete data set for *Cineraria deltoidea* (excluding *C. atriplicifolia* and *C. decipiens*) based on 33 characters. (●filled circles indicate plants collected at an altitude of 3000 m or more). (Abbreviations identifying specimens as in Appendix 1.)

Figure 14. (Opposite) Scanning electron micrographs of epicarp surfaces of cypselae of *C. deltoidea*: (a) *Gillett 15002* (K), Ethiopia; (b) *Hedberg 1890,* (K), Mount Kenya; (c) *Polhill 2* (K), Kinangop, Aberdares; (d) *Lugard 30*1 (K), Mount Elgon; (e) *Mullenders 2522* (BR), Virunga National Park, DRC; (f) *Baynal 19141* (BR), Ngorogoro National Park; (g) *Hilliard & Burtt 10168* (PRE), KwaZulu-Natal, South Africa; (h) *Hutchinson & Gillett 4178* (PRE), Soutpansberg, South Africa. Scale bars = 10 μm.

Discussion

Multivariate analysis of the morphological variation in *Cineraria deltoidea s.l.* does not support the reinstatement of previously described species nor their reassignment at some infraspecific rank. These results support Hedberg's (1957) treatment of six East African species names as synonyms of *C. grandiflora* and Jeffrey's (1986) conclusion that *C. deltoidea s.s.*, *C. monticola* and *C. buchananii* form part of a single, widespread species. It seems clear that both regional and altitudinal factors affect the morphological features of various populations, especially those at high altitude in East Africa.

Although some geographic trends are evident in *C. deltoidea*, no population from a single mountain or geographic regions warrants taxonomic recognition. Hedberg (1957) reached similar conclusions in his study of variation in other species on the East African mountains. Instead, there is strong correlation between growth at high altitude and phenetic similarity amongst populations from the various tall mountains in East Africa. Characters strongly positively correlated with higher altitude are those indicative of larger (but fewer) capitula on longer peduncles. Characters strongly positively correlated with lower altitude and a more southerly distribution include more densely-packed, smaller capitula on shorter peduncles, as well as a cobwebby indumentum. However, the cobwebby indumentum is not present in populations in KwaZulu-Natal or the Eastern Cape of South Africa, where glandular trichomes in the angles of the leaves and glabrous cypselae are characteristic.

This high degree of infraspecific variation is also seen in other afroalpine/afromontane species, including species of *Bartsia*, *Dipsacus pinnatifidus* and *Swertia crassiuscula* (Hedberg & Hedberg, 1977b; Hedberg *et al.*, 1979; Lovett, 1993). The harsh environment of the afroalpine region, characterised by extremes of temperature and regular occurrence of frost, is thought to promote high rates of evolution in the afroalpine flora (Lovett, 1993). Natural selection drives morphological features towards the ability to survive in this harsh environment (Lovett, 1993). In addition, afroalpine species can be capable of long-distance dispersal, including inter-mountain and intercontinental dispersal (Hedberg, 1955), potentially resulting in gene flow between mountains.

Specimens of *Cineraria deltoidea* growing amongst rocks in the moorlands (and along tracks) at higher altitudes tend to have larger and fewer capitula, and their growth is more stunted. A reason for larger, fewer capitula on longer peduncles at higher altitude might be the occurrence of fewer potential pollinators at these altitudes. Plants therefore need to attract them more vigorously, or it may simply be a resource- or energy-saving feature. It was surprising to find that a fine cobwebby indumentum is not associated with higher altitude, as seen in other species in *Cineraria* in southern Africa (e.g., *C. erodioides* and *C. pulchra*), but Trichome 1 (Figures 5a, 6a, c, d), present in many of the specimens from high altitude, may perform the same function of protection against wind and ultra-violet radiation, as well as herbivores. Similar patterns in size and number of capitula have been observed in species occurring in southern Africa, viz. *C. erodioides* and *C. albicans*, where

specimens from higher altitudes have fewer, larger capitula, but indumentum here is usually more woolly due to fine trichomes.

This study is based entirely on morphological similarity only and may not reflect biogeographic relationships among populations. While it is hoped that morphological similarity is a good indicator of genetic relatedness, this is not necessarily the case. It is unlikely that populations on distant mountains or mountain ranges are more closely related than populations on the same mountain at different altitudes, although occasional long-distance dispersal of seeds between mountains is possible (Hedberg, 1969; Knox & Palmer, 1995). There is apparently gene interchange between species at different altitudinal ranges on a single mountain (Hedberg, 1969). The results of the cluster analysis and ordination here simply indicate the degree of phenetic similarity of specimens and show that in East Africa altitude is a strong force in natural selection of certain morphological features. Parallel adaptation of what is essentially a forest montane species to ecological conditions at higher altitudes appears to be occurring in *Cineraria deltoidea* on the high East African mountains. Interestingly, Agnew (1975) found the greatest variance in the high altitude populations of *C. deltoidea* on Mount Kenya, and lowest seed set at the lowest altitudes of his sampling. He ascribed this to *C. deltoidea* being an ecotonal species, best adapted to the forest edge in the afromontane forest zone. Possibly the sources of populations at higher altitudes vary more due to isolated dispersal events into the afroalpine zone and a lower rate of seedling recruitment than at lower altitude in the forest edges. There is also presumably less opportunity for gene interchange between these high altitude populations, as there are greater distances between the populations occurring in sheltered sites (e.g., at the base of cliff faces) in the moorlands than in the afromontane region where the forest margin is fairly continuous.

It is clear that vegetative features are more influential in the positioning of the more southerly populations of *Cineraria deltoidea* in the scattergrams (Figures 10 and 11), viz. type of trichome in the leaf indumentum, leaf size (width), leaf shape (lobing, bases) and dentition, although some floral features (cypsela indumentum, length and number of disc florets) do feature. Some of these characters (e.g. cypsela indumentum) are very variable in some species of *Cineraria*, while others are influenced by environmental factors. For example, leaf size has been observed to be influenced greatly by the amount of sunlight/shade received by the plant. Morphology is therefore probably not the best way of distinguishing degrees of relatedness in this species.

The recognition of *Cineraria decipiens* and *C. atriplicifolia* as species distinct from *C. deltoidea* is supported by these results. Distinguishing characters are growth form and ploidy (not included in this study), life span and the shape of their auricles. As noted by Hilliard (1977), the length of the apical lobe is characteristic of *C. atriplicifolia*, and serves, along with other characters such as indumentum of the cypselae, to distinguish it from *C. decipiens*.

Infraspecific ranks

The use of infraspecific ranks is important for recognising genetic variation that is significant for conservation. Variation that is not named tends not to be recognised (Snaydon, 1984). The ranks of subspecies and variety are most commonly used, although their use is not consistent (Hamilton & Reichard, 1992). They are most widely defined as being coherent evolutionary subsets of a species, with geographic, ecological and/or (often implied) phylogenetic integrity**.** Subspecies are usually defined as groups of individuals within a species that have some morphological distinctness as well as distributional or ecological integrity (Hamilton & Reichard, 1992). Pipoly (1987) emphasises the importance of phylogenetic integrity defining a subspecies as "groups of populations within a single lineage of ancestor-descendant populations ... correlated with biogeography and/or ecology."

Cineraria deltoidea s.s. from KwaZulu-Natal and the Eastern Cape is effectively geographically isolated from other populations in the mountain ranges northwards. The closest populations (previously recognised as *C. monticola*) are in forests near Tzaneen/Magoebaskloof and along the slopes of the Soutpansberg in Limpopo Province. It is unlikely that there is significant gene-flow between these populations which are 600–800 km apart. This isolation is reflected in the coherence of the morphological features characterising individuals of *C. deltoidea s.s.* from KwaZulu-Natal and the Eastern Cape region, viz. glabrous cypselae, 5 (–6) rays, 8 (–10) involucral bracts, glabrous peduncles and Trichome 1 (Figure 5a, 6a) present on leaves, as well as either Trichome 2 (short eglandular, Figure 5b) and/or Trichome 3 (glandular, Figure 5c) in the angles of the lobes of the leaves. There is an absence of fine cobwebby hairs compared to the cobwebby indumentum found in populations from Limpopo Province and the Chimanimani mountains in Zimbabwe. There is also an absence of lateral pinnae, common in many of the East African specimens.

However, *Cineraria deltoidea s.s.* has no unique features that separate it unequivocally from the remainder of the species. Although it is a morphologically coherent and geographically isolated group, it is best interpreted as part of the range of variation evident in the species as a whole. Similarly, plants previously named C. *monticola* from the Soutpansberg mountains are also part of the continuum of variation in *C. deltoidea s.l.* Future molecular studies might indicate whether genetic differences exist that are not apparent from the morphology.

The status of *Cineraria buchanani* also warrants further investigation. The two specimens included in these analyses are not sufficiently representative, and more fieldwork in central Malawi and southeast Zimbabwe is needed to resolve fully its status. Until such work has been done, the features used to describe *C. buchanani* are best considered part of the variation in *C. deltoidea s.l.*

Conclusion

Cineraria deltoidea is an extremely variable species that occupies the 'spine of Africa' from the Eastern Cape to Ethiopia. It varies in growth form, leaf size (and shape in lower leaves), the type and extent of indumentum, the number and size of capitula and the length of the peduncles. Much of this variation is regional, but altitude plays an important role in determining phenotype. No infraspecific recognition for local geographic variants or forms is warranted. *C. atriplicifolia* and *C. decipiens* are supported as distinct species, distinguished from *C. deltoidea* by their growth form, life span and auricle shape.

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Appendix 1. Specimens of *Cineraria deltoidea s.l.* included in phenetic study. Abbreviation/code used, geographic locality, altitude, month of flowering indicated.

Code	Specimen	Locality	Altitude (m)	Flowering
Ngong 01	*Nappier 536* (K)	Ngong Hills, Kenya		April
Ngong 02	*Beentje 1843* (EA, WAG)	Ngong Hills	2200–2350	Jan
Ngong 03	*Kayu 526* (BR, PRE)	Ngong Hills		March
Ngong 04	*Kibue K159* (BR, EA)	Ngong Hills	2500	Nov
Ngong 05	*Harmsen 6535* (WAG)	Ngong Hills, North_east facing slope	2300	July
Aber 01	*Muasya, Cron & Knox 24* (J)	Aberdares, road from Satima to Wanderi track	3250	July
Aber 02	*Polhill 2* (K)	Aberdares, Kinangop,	2600	March
Aber 03	*Coe 738* (BR, PRE)	Aberdares, Cave Waterfall Gorge	ca. 3000 m?	June
Aber 04	*Hedberg 1496* (K)	Aberdares, Nyeri Track	2950	July
Aber 05	*R.E. & Th.C.E Fries 1201* (BR)	Aberdares, lower Bamboo region	2500	April
Mt. Ken 01	*Bally 6414* (K)	Mount Kenya, Naro Moru	3200	Sept
Mt. Ken 02	*Townsend 2257* (K, EA)	Mount Kenya	3400	Jan
Mt. Ken 03	*Lewis 5921* (K)	Mount Kenya	3200	Sept
Mt. Ken 04	*Mearns 1352* (BR)	Mount Kenya, western slopes	3630	April
Mt. Ken 06	*Hedberg 1890* (EA)	Mount Kenya, western alpine zone	3900	Aug
Mt. Ken 07	R.E. & Th.C.E.Fries 1555 (S)(iso:C.densifl.)	Mount Kenya, northern side near Kongoni River		March
Mt. Long 01	*Gilbert & Hedberg 6297* (K)	Mount Longonot, Kenya	2500–2730	July
Londi 01	*Maas Geesteraanus 5503* (BR, K, PRE,	Kenya, Londioni district, Tinderet Forest Reserve	2800	July
Moroto 01	*Wilson 433* (K)	Karamoja district, Moroto Mountain, forest edge		April
Narok 01	*Greenway & Kanuri 13859* (PRE)	Narok district, Nasampulai Valley	2700	Nov
Narok 02	*Greenway & Kanuri 1504*1 (K, PRE)	Narok district, Nasampulai Valley	2650	Aug
Narok 03	*Glover, Gwynne & Samuel 1118* (PRE)	Narok district, 20 miles from Olokurtu on road to Kilburgon	3050	May
Naku 01	*Maas Geesteraanus 5931* (BR, K, PRE)	Nakuru district, Eastern Mau Forest Reserve	2750	Aug
Naku 02	*Maas Geesteraanus 6157* (BR, PRE)	Nakuru district, Eastern Mau Forest Reserve	2300	Sept
Sudan 01	*Johnston 1475* (BR, K)	Sudan, Imatong Mountains		Feb

Sudan 02	*A.S.T. Th1812* (K)	Sudan, Imatong Mountains, Kippia	2440	Dec
Eth01	*Gillett 15002* (K)	Ethiopia, Mount Delo, eastern slope	3110	Jan
Eth02	*Gillett & Jones 221* (K)	Ethiopia, Bale, 17 km SSW of Goba, Mount Dello	3800	April
Eth03	*de Wilde 5939* (BR, WAG)	Ethiopia, 5 km N of Addis Ababa, Mount Entotto,	2400	March
Eth04	*de Wilde 6278* (BR, K, WAG)	Ethiopia, Bonga region, Geetsha River, near Wush Wush	2200	Jan
Eth05	*Hedberg 4164* (K)	Ethiopia, Arussi, Chlialo Awraja, Galama Mountains	3750	Sept
Eth06	*Gilbert & Tewolde 3246* (K)	Ethiopia, Shoa Lake Wonchi, Montare Forest	2950	Feb (fruiting)
Eth07	*Schimper 1517* (E,K) (isotypes)	Ethiopia, Dschan Mèda	2600	Sept
Mt.Kil 01	*Rogers 131* (BR)	Mount Kilimanjaro, above Rongai	2250	Dec
Mt.Kil 02	*Schlieben 4470* (BR)	Mount Kilimanjaro, south_western side,	3000	Jan
Mt.Kil 03	*Richards 23 86*6 (K)	Arusha District, Meru Crater	2590	Jan
Mt.Kil 04	*Hedberg 1293* (K)	Mount Kilimanjaro, east of Peter's Hut	3700	June
Mt.Kil 05	*Greenway 3748* (BR)	Mount Kilimanjaro		
Mt.Kil 06	*Johnston 120* (K) (iso: *C. kilimansharica)*	Mount Kilimanjaro	3050	Feb
Mt.Kil 07	*Haarer 1126* (K)	Shira Mountains, west of Kilimanjaro	3660	Feb
Virun 01	*de Witte 2222* (K)	Virungas, Volcano Bishoke	2400	
Virun 02	*Burtt 3142* (K)	Virungas, Namlagira/Niamuragira	3000	Jan
Virun 03	*Louis 5324* (BR, K)	Kabara, northern side of Karasimbi	3000	Aug
Virun 04	*Lebrun 7370* (BR)	Kabara, between Karasimbi and Mikeno	3000–3100	Aug
Virun 05	*Lejoly 84/302* (BR)	Rwanda, Murura, Gishwati Forest	2370–2400	Aug
Virun 06	*Bamps 3087* (BR, K, WAG)	Rwanda, Ruhengeri_Gisenyi	2450	Feb
Virun07	*Bequaert 5863* (BR) (holo: *C. bequaerti*)	DRC, between Tongo and Mukule		Sept
Sn Tan 01	*Bredo 5727* (BR)	Mbeya District		Aug
Sn Tan 02	*Thulin & Mbhoro* (K)	Mbeya District, Poroto Mountains, Livingstone Forest Reserve	2600	Sept
Sn Tan 03	*Milne_Redhead & Taylor 11110* (K)	Tanzania, 19 km south of Njombe	1830	July
Sn Tan 04	*Proctor 1230* (K, PRE)	Mbeya District, Kawetire, North Usafwa Forest Reserve	2150	May
Sn Tan 05	*Greenway 8404* (K)	Rungwe District, Kiwara River banks	1850	Aug
Sn Tan 06	*Richards 6472* (K)	Rungwe District, Ngozi, Poroto Mountains	2100	Oct
Sn Tan 07	*Richards 16800* (BR, K)	Ufipa District, Sambawanga_Abercorn Road	1500	July

Sn Tan 08	*Bullock 3403* (BR, K)	Ufipa District, Mbisi	2150	Oct
Tanz 01	*Mabberley & Salehe 1502* (K)	Kilosa district, Ukaguru Mountaina, Mamiwa Forest Reserve	2302	Aug
Tanz 02	*Carmichael 1512* (K)	Shishiey area, Hanang, NE slope	2600	Oct
Mt Elg 01	*Dummer 3523* (K)	Mount Elgon, Uganda	3660	Jan
Mt Elg 02	*Lugard 301* (K)	Mount Elgon, Kenya	3100	Dec
Mt Elg 03	*Liebenberg 1586* (K)	Mount Elgon, Madangi to Jackson's Summit	3300	April
Ngoro 01	*Raynal 19141* (BR, K)	Enduleni_Ngorongoro, S slopes of Mt. Satiman	2650	Sept
Ngoro 02	*Raynal 19048* (BR, WAG)	Ngorongoro crater southern rim, outer slopes	2300	Sept
Ngoro 03	*Tanner 3271* (K)	Ngorongoro	1980	Nov
Ngoro 04	*Newbould 6227* (K)	Embagai, Crater Highlands	3050	July
Nyika 01	*Phillips 2318* (WAG)	Rumphi District, half a mile south of Chelinda	2280	May
Nyika 02	*Pawek 2170* (K)	Rumphi District, Nyika Plateau, Chelinda River Bridge	2260	April
Nyika 03	*Pawek 9314* (PRE, WAG)	Nyika Plateau, Chelinda Rock	2300	April
Nyika 04	*Salubeni 734* (K, WAG)	Nyika Plateau, in grassland along road to Chelinda Chalets	ca. 2300	May
Nyika 05	*Brass 17299* (K)	Nyika Plateau	2350	Aug
Mulanje 01	*Newman & Whitmore 447* (BR, WAG)	Mount Mulanje, Lake Ruo Plateau	1770	Aug
Mulanje 02	*Newman & Whitmore 662* (BR, COI, WAG)	Mount Mulanje, Chambe Plateau	1680	Sept
Mulanje 03	*Pawek 3774* (K, PRE)	Mount Mulanje, Lichenya Plateau	1980	Sept
Mulanje 04	*Chapman 5859* (K)	Mulanje Forest Reserve, Lichenya Plateau	1980	Aug
Zomba 01	*Salubeni & Tawakali 2599* (WAG)	Zomba Plateau, on road to Chingwe's Hole	ca. 1600	July
Zomba 02	*Salubeni & Balaka 3387* (K)	Zomba, slopes of Chiradzulu Peak	ca. 1700	Aug
Zomba 03	*LaCroix 3196* (PRE, WAG)	Zomba Plateau, near Chagwa dam	1650	Aug
Zomba 04	*Balake & Nachamba 508* (PRE)	Zomba, Chiradzulu Peak	ca. 1700	Aug
Buch 01	*Buchanan 10* (GRA, SAM) (isotypes)	Malawi		
Buch 02	*Brummit 11715* (K)	Malawi, Ndirande Mountain, SW side	1370–1530	June
Inyang01	*Rushworth 704* (K)	ca. 5 miles beyond Sanyatwe, along Rusape_Nyanga road	1935	April
Inyang02	*Wild 4591* (K, MO)	Nyanga: Mtenderere Source	2135	Sept
Inyang03	*Burrows 4591* (PRE)	Rhodes Nyanga Experimental Station		June
Vumba 01	*Chase 1740* (K)	Vumba Mountains, north slope	ca. 1600	Sept

Vumba 02	*Peter 30 597* (K)	Vumba Mountains, Bachschlucht	ca. 1600	Sept
Vumba 03	*Chase 7168* (K, PRE)	Imbeza Forest Estate, roadside to Ishitaka Forest	1220	Sept
Chiman 01	*Chase 8508* (COI, K, PRE)	Chimanimani District: Adam's Ridge, Lemon Kop	1525	July
Chiman 02	*Chase 8020* (K, PRE)	Chimanimani District: on road to Bridal Veil falls	ca.1500	May
Chiman 03	*Cron & Balkwill 515* (J, K, MO)	Cashel_Chimanimani road near Cashel	1150	May
Chiman 04	*Cron & Balkwill 523* (J, PRE)	Cashel_Chimanimani road near Tank Nek	1650	May
mont01	*Hutchinson & Gillett 4178* (K)	Soutpansberg, Klein Australe	1040	July
mont02	*Cron et al. 281* (J)	Soutpansberg, Wylies Poort	1300	May
mont03	*Cron et al. 292* (E, J, K, MO, PRE)	Soutpansberg, roadside on Farm Fife	1400	May
mont04	*Galpin 9389* (K)	Pietersburg District, Duiwelskloof, hillside	1400	July
mont05	*Hemm 125* (J)	Tato Vondo & Tshamnyatsha Forest Reserve	1219	June
delt01	*Hilliard & Burtt 10168* (MO, NU, PRE)	Richmond district, Peak of Byrne	1680	April
delt02	*Hilliard 5373* (MO, K) *5375* (NU)	Pinetown District, Everton, Molweni River	550	June
delt03	*Hilliard & Burtt 9037* (K, NU)	Ixopo District, 11 miles north of Ixopo on Donnybrook road	ca.1200	Feb
delt04	*Esterhuysen 20 292* (BOL, MO, PRE)	Pietermaritzburg District, Swartkops Hill, Cedara	ca.1100	July
delt05	*Hilliard 2664* (NU)	Nkandla District, Nkandla forest	1220	Feb
delt06	*Galpin 2890* (GRA)	West gate, Port St. John	230	April
decip01	*Gerrard & M'Ken 104*0 (K, TCD) (iso, holo)	Umvoti District, KwaZulu-Natal (KZN)	1200–1500	
decip02	*Galpin 14778* (PRE)	Tugela Ferry, river bank, KZN	500–600	Feb
decip03	*Ward 8858* (K. PRE)	Mtubatuba, KZN	ca. 200	May
decip04	*Huntley 891* (NU)	Ngoye, Umtunzini district, KZN	ca. 400	Feb
decip05	*Cron & Brummer 5a* (J)	Oribi Gorge Nature Reserve, KZN	ca. 500	May
decip06?	*Balkwill 9509a* (J)	Thankulu, Horseshoe Valley off Mzintlare	ca 700	Jan
atrip01	*Drége 5137* (MO, PRE) (isotypes)	Durban (Port Natal), KZN		
atrip02	*Cron 7* (J)	Montesseel, Pietermaritzburg district, KZN	800	May
atrip03	*Medley_Wood 515* (BOL, K)	Inanda, KZN, South Africa	ca. 150 m	
atrip04	*Hilliard & Burtt 10319* (MO, NU)	Ixopo District, valley of Umkomaas River, above Hella Hella	ca. 600 m	April
atrip05	*Hilliard 2830* (NU)	Pinetown District, Kloof, Forest Hills, KZN	650	April