

## Chapter 2

### Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.

#### ABSTRACT

Phylogenetic analyses of separate and combined plastid *trnL-trnF* and nuclear ITS DNA sequences were used to investigate (1) the position of *Cineraria* in the tribe Senecioneae (Asteraceae), (2) its delimitation with respect to five species previously included in *Cineraria*, and (3) its infrageneric structure. The results concur with previous reports that *Cineraria* is related to *Pericallis*, *Dendrosenecio* and *Euryops*, and our increased taxonomic sampling identifies *Bolandia* as the sister-genus to *Cineraria*. *Bolandia* comprises two South African species (*B. argillacea* and *B. pedunculosa*) previously included in *Cineraria*, and is characterised by pinnately veined, exauriculate leaves, solitary ecalyculate capitula on long peduncles, truncate style apices and heteromorphic fusiform cypselae with short, white mucilaginous hairs. Two other species previously placed in *Cineraria* are reinstated as *Senecio cordifolius* (syn: *C. mitellifolia*) or reassigned as *S. hederiformis* (syn: *C. hederifolia*) because of their close relationships to other *Senecio* species in a clade that also includes *Pericallis*. The fifth species is now treated as the monotypic *Oresbia heterocarpa* (syn: *C. tomentosa*) because of its unique morphological features and strongly incongruent placement with *Kleinia galpinii* versus *Dendrosenecio kilimanjari* in the *trnL-trnF* and ITS data-sets, respectively. *Cineraria* now has a coherent morphological concept as plants with palmately veined leaves, radiate, calyculate capitula, obtuse/penicillate style apices and obovate, compressed cypselae with two distinct margins or wings and a substantial carpopodium. The species-level comparisons within *Cineraria* show surprisingly limited amounts of DNA variation that nonetheless have striking points of incongruence between the *trnL-trnF* and ITS data-sets. *Cineraria* evidently experienced a rapid biogeographic and morphological diversification periodically punctuated by bouts of reticulate evolution.

#### INTRODUCTION

##### The genus *Cineraria*

*Cineraria* L. (Senecioneae, Asteraceae) mostly comprises perennial herbs and subshrubs with heterogamous, radiate capitula, all with yellow florets. As suggested by the name (from ‘*cinereus*’ meaning ash-coloured), many of the species have grey leaves and stems due to a tomentose or cobwebby indumentum. The genus is distinguished mainly by its compressed cypselae (Hilliard, 1977), palmately veined leaves (Bremer, 1994) and yellow, radiate capitula (Figure 1a–c).

*Cineraria* is essentially an African genus, ranging from the Cape Peninsula and mountains of South Africa, where it has its centre of diversity, along the eastern highlands of Africa to

Figure 1. Distinguishing features of *Cineraria*: (a) *C. ngwenyensis*, an herbaceous perennial. Scale bar = 10 mm. (b) Palmate venation in leaf of *C. austrotransvaalensis* [Cron 19 (J)]. Scale bar = 5 mm. (c) Laterally compressed cypsela of *C. vallis-pacis* with distinct wings [Balkwill *et al.* 11802 (J)]. Scale bar = 325  $\mu\text{m}$ . (d) Balusterform filament collars of *Cineraria canescens* [Pearson 5762 (BOL)]. Scale bar = 100  $\mu\text{m}$ . (e) Obtuse disc style apex with sweeping hairs centrally and fringing apex of *Cineraria aspera* [Cron & Goodman 531 (J)]. Scale bar = 50  $\mu\text{m}$ .

Ethiopia. In the west it is known from the mountainous areas of Namibia and southern Angola. One species, *C. abyssinica*, extends from Ethiopia into Yemen and Saudi Arabia and another, *C. anampoza*, is endemic to Madagascar. *Cineraria* is afroalpine (to afroalpine) in its affinity, occurring at altitudes of 1600–4300 m in the mountains of tropical East Africa, and between 1200–3300 m in the Drakensberg range in South Africa, but extends from mountain peaks (*C. aspera*, *C. erosa*) to sea-level (*C. geifolia*, *C. humifusa*) in the Western Cape. A list of all species included in this study (with author citations) is provided in Table 1 and a list of all species currently in *Cineraria* (with author citations and geographical distribution) is provided in Appendix 1.

### Placement in the Senecioneae

*Cineraria* has balusterform filament collars in its stamens (Figure 1d), discrete stigmatic areas and a chromosome number of  $x = 10$ , and is therefore a senecioid member in the subtribe Senecioninae (Nordenstam, 1978; Bremer, 1994). Traditionally, the Senecioneae have been loosely characterised by an epaleate receptacle and a pappus of capillary bristles. A narrower group is now circumscribed mainly by an involucre of uniseriate bracts (or sometimes biseriate), with or without an outer calyculus of smaller bracts (Bremer, 1987). Secondary compound chemistry (viz. the presence of pyrrolizidine alkaloids and sesquiterpenes of the furanoeromophilane type) is also useful in delimiting the tribe (Bremer, 1994). No pyrrolizidine alkaloids were found in a limited sample (eight species) of *Cineraria* (Cron, 1991), although terpenoids and polyacetylenes were detected (Gonser *et al.*, 1990; Cron, 1991).

Within the Senecioninae, *Pericallis* D.Don from the Canary Islands and *Dendrosenecio* (Hauman ex Hedb.) B.Nord. from the mountains of tropical East Africa have both been proposed as feasible close relatives to *Cineraria*. Nordenstam (1978) suggested a possible common ancestor for *Cineraria* and *Pericallis*, with *Pericallis* being more senecioid than cacalioid, despite its chromosome number ( $x = 30$ ) and non-yellow flower colour. It has dilated filament collars and its disc styles are truncate with sweeping hairs and discrete stigmatic areas (Nordenstam, 1978; Bremer, 1994). The style apices of *Cineraria* are obtuse (Figure 1e), but there are other distinct resemblances between the genera, with many species of *Pericallis* having been previously included in *Cineraria*. Both *Cineraria* and *Pericallis* have palmately veined leaves (Figures 1b, 2a), which are frequently auriculate. It was thought that the cypselae of both genera were compressed to some degree, a rare occurrence in the tribe (Nordenstam, 1978; Bremer, 1994), but careful examination of mature cypselae of *Pericallis* reveals that they are sub-cylindrical (Figure 2b).

Swenson & Manns (2003) found that *Cineraria* and *Pericallis* are more closely related to each other than either is to *Packera* in combined and separate analyses of ITS and morphological data. Combined plastid (the *trnT-trnL* intergenic spacer, the *trnL* intron and two parts of the

Figure 2. (a) *Pericallis steetzi*: habit, leaves and inflorescences. (b) Oblong, cylindrical disc cypsela of *P. tussilaginis* [Chaytor 75 (K)]. Scale bar = 240  $\mu\text{m}$ . (c) Habit of *Dendrosenecio battiscombei* near Mount Sattima in the Aberdares, Kenya. (d) Oblong, cylindrical cypsela of *D. kilimanjari* ssp. *cottonii* [Robertson 2 (K)]. Scale bar = 550  $\mu\text{m}$ .

Table 1. Vouchers and GenBank accession numbers of the species included in the study. An asterisk (\*) before the voucher specimen indicates that an herbarium specimen served as the source, rather than leaf material collected in the field and dried in silica gel.

Samples of <i>Cineraria</i> included in molecular study	Voucher Specimen	Locality (SA = South Africa)	Genbank Accessions	
			ITS	<i>trnL-trnF</i>
<i>Cineraria albicans</i> N.E.Br.	<i>Cron 482</i> (J)	Umtamvuna Nature Reserve, KwaZulu-Natal, SA.	AY275655	AY952894
<i>Cineraria alchemilloides</i> DC.	* <i>Winter 284</i> (J)	Nubib Mountain, Farm "Erfstuk", Namibia.	AY953913	AY952902
<i>Cineraria aspera</i> Thunb.	<i>Cron &amp; Goodman 549</i> (J)	Naude's Nek, Eastern Cape, S.A.	AY275656	AY952903
<i>Cineraria austrotransvaalensis</i> Cron	<i>Cron &amp; McCallum 341</i> (J)	Linksfield Ridge, Gauteng, S.A.	AY953920	AY952910
<i>Cineraria cyanomontana</i> Cron	<i>Cron, Knox &amp; Winter 348</i> (J)	Blouberg, Limpopo Province, S.A.	AY953909	AY952898
<i>Cineraria deltoidea</i> Sond. (Sn)	<i>Cron &amp; Balkwill 528</i> (J)	Cashel-Chimanimani road, Zimbabwe.	AY953907	AY952896
<i>C. deltoidea</i> Sond. (EA) (= <i>C. grandiflora</i> Vatke)	<i>Muasya, Cron &amp; Knox 9</i> (EA, J).	Aberdares, Kenya.	AY953905	AY952893
<i>Cineraria erodioides</i> DC.	<i>Cron &amp; Goodman 538</i> (J)	Joubert's Pass, Eastern Cape, S.A.	AY953914	AY952904
<i>Cineraria geraniifolia</i> DC. (dissected form)	<i>Cron &amp; Goodman 563</i> (J).	Hogsback, Eastern Cape, S.A.	AY953921	AY952911
<i>Cineraria glandulosa</i> Cron x <i>C. atriplicifolia</i> DC.	* <i>Cron &amp; Scott-Shaw 9</i> (J)	Richmond District, KwaZulu-Natal, S.A.	AY953906	AY952895
<i>Cineraria lobata</i> L'Hér. ssp. <i>lobata</i> (R, robust form)	<i>Hodgkiss C2</i> (J)	Cape Point, Western Cape, S.A.	AY953904	AY952892
<i>Cineraria lobata</i> L'Hér. ssp. <i>lobata</i> (UN; small-leaved, jointed form)	<i>Cron &amp; Goodman 569</i> (J)	Uniondale Division, near Joubertina, Western Cape, S.A.	AY953922	AY952912
<i>Cineraria lobata</i> L'Hér. ssp. <i>soutpansbergensis</i> Cron	<i>Cron, Knox &amp; Winter 344</i> (J)	Blouberg, Limpopo Province, S.A.	AY953912	AY952901

<i>Cineraria longipes</i> S.Moore	<i>Cron, Pfab &amp; Mills 485 (J)</i>	Suikerbosrand Nature Reserve, Gauteng. S.A.,	AY953917	AY952907
<i>Cineraria lyratiformis</i> Cron	<i>Cron &amp; Balkwill 430 (J)</i>	Krom Ellenboog Farm, KwaZulu-Natal, S.A.	AY953918	AY952908
<i>Cineraria mazoensis</i> S.Moore	<i>Cron &amp; Balkwill 486 (J)</i>	Hwedza Mountain, Zimbabwe.	AY953911	AY952900
<i>Cineraria mollis</i> E.Mey. ex DC.	<i>Cron &amp; Goodman 545 (J)</i>	Witteberg, Joubert's Pass, Eastern Cape, S.A.	AY953923	AY952913
<i>C. parvifolia</i> Burt Davy	<i>Cron &amp; Dallas 483 (J)</i>	Witpoortjie Falls, Gauteng, S.A.	AY953919	AY952909
<i>Cineraria pinnata</i> O.Hoffm.	<i>Kyle 2 (PRE)</i>	Kosi Bay, northern KwaZulu-Natal, S.A.	AY953908	AY952897
<i>Cineraria pulchra</i> Cron	<i>Cron &amp; Balkwill 500 (J)</i>	Mountt Nyangani, Nyanga, Zimbabwe.	AY953910	AY952899
<i>Cineraria saxifraga</i> DC.	<i>Hodgkiss C4 (J)</i>	Kirstenbosch Botanic Garden, Western Cape, S.A.	AY953916	AY952906
<i>Cineraria vallis-pacis</i> Dinter ex Merxm.	<i>Balkwill &amp; McDade 11802 (J)</i>	Olifantshoek, Northern Cape, S.A.	AY953915	AY952905
<b>Species previously in <i>Cineraria</i></b>				
<i>Bolandia argillacea</i> (Cron) Cron (= <i>Cineraria argillacea</i> Cron)	<i>*Esterhuysen 35117 (BOL)</i>	Worcester, Brandwag Peak, Western Cape, S.A.	AY953924	AY952914
<i>Bolandia pedunculosa</i> (DC.) Cron (= <i>Cineraria pedunculosa</i> DC.; <i>C. albomontana</i> Hilliard)	<i>Cron &amp; Goodman 544 (J)</i>	Witteberg, Joubert's Pass, Eastern Cape, S.A.,	AY953925	AY952915
<i>Oresbia heterocarpa</i> Cron & B.Nord. (= <i>Cineraria tomentosa</i> Less.; <i>Senecio lanatus</i> L.f.)	<i>*Cron, Hodgkiss, Stander &amp; Cocks 322 (J)</i>	Sneeuberg, Cederberg, Western Cape, S.A.	AY953935	AY952925

<i>Senecio cordifolius</i> L.f. (= <i>Cineraria mitellifolia</i> L'Hér.; <i>C. chamaedrifolia</i> Lam.)	<i>Cron &amp; Hodgkiss</i> 369 (J)	Table Mountain, Western Cape, S.A.	AY953926	AY952916
<i>Senecio hederiformis</i> (Cron) Cron (= <i>Cineraria</i> <i>hederifolia</i> Cron)	<i>Cron, Knox &amp;</i> <i>Winter 355</i> (J)	Blouberg, Limpopo Province, S.A.	AY953928	AY952918
<b>Other Senecioneae</b>				
<i>Dendrosenecio kilimanjari</i> (Mildbr.) E.B.Knox ssp. <i>cottonii</i> (Hutch & G.Taylor) E.B.Knox	<i>Knox 50</i> (MICH)	Mount Kilimanjaro, Tanzania.	AY953933	AY952923
<i>Emilia discifolia</i> (Oliv.) C. Jeffrey	<i>Cron &amp; Balkwill</i> 490 (J)	Hwedza Mountain, Zimbabwe.	AY953930	AY952920
<i>Euryops brownei</i> S.Moore	<i>Muasya, Cron &amp;</i> <i>Knox 10</i> (J)	Aberdares, Kenya.	AY953936	AY952926
<i>Kleinia galpinii</i> Hook.f.	<i>Cron 537</i> (J)	Hort. University of Witwatersrand, Johannesburg, S.A.	AY953934	AY952924
<i>Pericallis multiflora</i> (L'Hér.) B.Nord.	<i>Santos-Guerra</i> 97–9 (ORT)	Tenerife, Canary Islands.	AY953931	AY952921
<i>Pericallis murrayi</i> (Bornm.) B.Nord.	<i>Santos-Guerra</i> 97–10 (ORT)	El Hierro, Canary Islands.	AY953932	AY952922
<i>Senecio deltoideus</i> Less.	<i>Cron &amp; Balkwill</i> 373 (J)	Groenvlei, KwaZulu- Natal, S.A.	AY953927	AY952917
<i>Senecio achilleifolius</i> DC.	<i>Cron &amp; Goodman</i> 556 (J)	Naude's Nek, Eastern Cape, S.A.	AY953929	AY952919
<i>Tephroses atropurpurea</i> (Ledeb.) Holub	<i>Golden 318</i> (LEA)	Whitehorse, Yukon	AF345306 AF345314	
<i>Tephroses kirilowii</i> (DC.) Holub	No voucher listed	Locality unknown		AF468164

*trnK* intron) and ITS DNA data from a broader range of genera in the Senecioneae (Pelser *et al.*, 2002), also place *Cineraria* and *Pericallis* within a clade that contains *Senecio scandens*, *S. lineatus* and *Dendrosenecio*. However, the relationship between *Cineraria* and *Pericallis* is only weakly supported and the entire clade has <50% bootstrap support and does not appear in the consensus trees of the separate plastid and nuclear analyses (Pelser *et al.*, 2002).

In contrast, Panero *et al.* (1999) and Bain & Golden (2000) found the New World genus *Packera* to be sister to *Pericallis* in a phylogeny based on the ITS region and supported by palynological studies (Bain *et al.*, 1997). In this study *Cineraria* is placed sister to two New World genera, *Dorobaea* and *Pseudogynoxys*, which, in turn, are sister to the clade with *Packera* and *Pericallis* (Panero *et al.*, 1999).

A putative close relationship between *Dendrosenecio* and *Cineraria* was first discerned by Knox & Palmer (1995) in a phylogeny based on cpDNA restriction sites. Among the 37 taxa sampled, *Cineraria deltoidea* was found to be the closest relative to *Dendrosenecio* (Knox & Palmer, 1995). However, the large number of diagnostic restriction site mutations for *Dendrosenecio* indicated that closer relatives were likely to be found (Knox & Palmer, 1995; Knox 1996). *Dendrosenecio* differs from both *Cineraria* and *Senecio* in having a biseriate involucre and continuous stigmatic areas, in addition to the ‘megaphytic’ rosette-tree habit (Figure 2c) and the pyramidal-paniculate synflorescence (Nordenstam, 1978; Bremer, 1994). It was reported to show some tendency towards cypselae compression (Nordenstam, 1978), one of the key features in *Cineraria*, but this is probably due to immaturity and/or lack of fertilization (*pers. obs.*) as mature fertilised cypselae are clearly oblong and cylindrical (Figure 2d).

### **Generic circumscription and taxonomic history of *Cineraria***

*Cineraria* as circumscribed in recent times (Dyer, 1975; Hilliard, 1977; Cron, 1991; Lisowski, 1991; Bremer, 1994) consisted of a slightly heterogeneous group due to the inclusion of some anomalous species amongst the 46 comprising the genus. It included herbs and subshrubs with alternate (rarely radical) leaves that are usually dentate and lobed or pinnatifid, and mostly auriculate. Its capitula were described as generally small and arranged in a lax corymb, rarely solitary, radiate with ligulate rays and tubular, 5-lobed, hermaphrodite disc florets, but one species was discoid. The uniseriate, campanulate involucre was usually subtended by a few calyculus bracts, but a few species were ecalyculate. The anthers were characterised by obtuse appendages and minutely sagittate bases. The style branches are mainly obtuse and penicillate, with sweeping hairs, but a few were truncate or rounded. An important diagnostic feature was the laterally compressed, obovate cypselae with distinct margins or wings, but even here, a few species deviated. The pappus was noted to consist of delicate scabrid bristles, soon caducous, and the receptacle is flat and epaleate.

*Cineraria* was first treated in *Species Plantarum II* (Linnaeus, 1763) and was not formally diagnosed, although *C. geifolia* (*Hortus Cliffortus Solidago* 7) is recognised as the type for the genus (Wijnands, 1983; Jarvis *et al.*, 1993). De Candolle (1838) described the cypselae as being “usually compressed”, often with winged margins (at least the outermost ones). He also noted that the involucral bracts have scarious margins, the broadness and spacing thereof (in our experience) certainly assists in distinguishing *Cineraria* from *Senecio* L. Harvey (1865)

distinguished *Cineraria* from *Senecio* by its "cone-tipped style and the usually flattened or many-angled achenes".

The name *Cineraria* as first applied by Linnaeus in 1763 referred to 13 species, of which *C. geifolia* is the only one currently remaining in the genus. This very heterogeneous assemblage included species later transferred to *Senecio*, *Ligularia*, *Othonna*, *Felicia* and *Tephrosia*. Cassini (1817) distinguishes *Cineraria* from *Jacobaea* Gaertner by its involucre bracts and calyculus. He notes that *Cineraria* includes sixteen species but names only two of them, *C. campestris* Retz. and *C. cruenta* L'Hér. He does not mention any of Linnaeus's original species but neither does he exclude them. Therefore his concept is *not* considered to be the creation of a new genus, as suggested by Holub (1973). Lessing (1832) later restricted *Cineraria* to a group of three South African species (*C. aspera*, *C. geifolia*, *C. tomentosa*), with the only Linnaean species included being *C. geifolia*. Cassini (1829) did however propose a new genus *Xenocarpus* Cass. 1829, which is synonymous with *Cineraria* L. *sensu* Less. 1832 (Holub, 1973).

Over the past two to three centuries, more than 400 species have been placed in *Cineraria*, the majority of these having been subsequently removed to other genera in the Senecioneae (Index Kewensis, 1997), leaving only African and Madagascan species in the genus. Apart from De Candolle (1838) in his *Prodromus systematis naturalis regni vegetabilis* (vol. 6), subsequent treatments of *Cineraria* have all been regional: Harvey (1865) in *Flora Capensis*; Oliver & Hiern (1877) in *Flora of Tropical Africa*; Hilliard (1977) in *Compositae in Natal*; Lisowski (1991) in *Les Asteraceae dans la flore d'Afrique Centrale*; Goldblatt & Manning (2000) in *Cape Plants*. Prior to this current revision, about 46 species were recognised in *Cineraria* (Cufodontis, 1967; Lebrun & Stork, 1997; Herman, 2003), although this has long been thought to exceed the true number (Jeffrey, 1986; Bremer, 1994).

Subgeneric treatment of *Cineraria* has been limited. In his *Prodromus*, De Candolle (1838) recognised four divisions in *Cineraria*: *Hebecarpae*, *Blepharicarpae*, *Leiocarpae* and *Polypterae*. Most of the species (16) were placed in the *Blepharicarpae* because of their compressed fruits and ciliate, winged margins, including *C. exilis*, unusual because of its discoid capitula. Three species, *C. geraniifolia*, *C. atriplicifolia* and *C. lyratiformis* (previously *C. lyrata* DC. - illegitimate due to prior use by Ledebour, 1818) were described in the *Leiocarpae*, characterised by glabrous cypselae, compressed and winged ray cypselae and radiate capitula. The divisions *Hebecarpae* and *Polypterae* were established to accommodate three species, two of which De Candolle described at the time. He noted however, that both divisions might better be considered as separate, related genera, as they lacked truly compressed cypselae (De Candolle, 1838). The *Hebecarpae* included two species: *C. pedunculosa* and *C. dregeana*, which have either fusiform cypselae (*C. pedunculosa*; Figure 3b – c) or subcylindrical cypselae (*C. dregeana*). Based on separate and combined molecular and morphological cladistic analyses (described here and Chapter 3), these species have been removed from *Cineraria*, the former

Figure 3. *Bolandia*: (a–c) *Bolandia pedunculosa*: (a) Habit [*Compton 2722* (K)]. Scale bar = 5.2 mm. (b) SEM glabrous inner surface and distinct median rib of ray cypsela. Scale bar = 1 mm. (c) SEM of ray cypsela with convex outer surface covered with short white hairs. Scale bar = 1 mm. (d) Truncate style apex with central tuft and fringe of sweeping hairs [*Barker 5398* (NBG)]. Scale bar = 117  $\mu$ m. (e–f) *Bolandia argillacea*: (e) Habit [*Esterhuysen 35117* (BOL)]. Scale bar = 4.1 mm. (f) Ray cypsela [*Esterhuysen 35117* (BOL)]. Scale bar = 600  $\mu$ m. (g) Truncate style apex with sweeping hairs [*Esterhuysen 36192* (K)]. Scale bar = 50  $\mu$ m.

constituting one of two species in a new genus, *Bolandia* (Cron *et al.*, submitted a) and the latter placed in *Senecio sensu lato* (Chapter 6).

De Candolle's *Polypterae*, characterised by glabrous, four-winged ray cypselae and radiate capitula (Figure 4a, b), comprised only *Cineraria tomentosa*, previously *Senecio lanatus* L.f. (*C. lanata* L. being pre-occupied). Based on the current work, a new monotypic genus has been erected to accommodate this unusual species, *Oresbia heterocarpa* (Cron & Nordenstam, submitted), the name *C. tomentosa* being illegitimate due to prior use by Miller (1768). As the specific epithet suggests, the disc cypselae of *O. heterocarpa* are quite different from the ray cypselae, being covered with mucilaginous hairs and without wings or even margins (Figure 4c). This species is nevertheless similar to *Cineraria* in having an obtuse style with sweeping hairs (Figure 4d).

De Candolle (1838) also listed eleven species as “*non satis notae*”, only one of which (*C. erosa*) is currently recognised as a valid member of the genus. In addition, he excluded 176 species from the genus, placing most of them in *Senecio*, but also in *Ligularia*, *Othonna*, *Steirodiscus*, *Brachyrinchus*, and *Agathaea*, amongst others.

In *Flora Capensis*, Harvey (1865) listed 22 species in *Cineraria* and two doubtful species (*C. arctotidea* and *C. humifusa*) which he indicated might both be forms of *C. mollis*. He recognised only three sections in *Cineraria*: (I) *Senecioides*, (II) *Eu-Cineraria* and (III) *Othonnoides*. He included the species then known as *C. pedunculosa* and *C. dregeana* in Section *Eu-Cineraria* (along with 18 other species), thereby expanding the concept of the “true” genus to include species with entire, exauriculate, pinnately veined leaves and ecalyculate capitula. He placed the unusual *C. tomentosa* (now *Oresbia heterocarpa*) in Section *Senecioides*, and another anomalous species “*C.? othonnoides*” (previously *Othonna pinnatifida* Thunb.) in Section *Othonnoides*. *Cineraria othonnoides* has sessile, pinnati-partite leaves, lacks auricles and is pinnately veined. The involucre is ecalyculate and has minute tooth-like ridges at the base of the involucre bracts, tooth apices alternating with the bracts. The cypselae are four-angled, covered in long mucilaginous brown hairs, with an obscure carpopodium. This species is obviously misplaced in *Cineraria* and its true affinity requires further investigation.

### **Problematic species in *Cineraria***

As indicated by the outline above, several species from southern Africa not conforming to the key vegetative and reproductive features of *Cineraria* have remained part of the genus. These include the four dubiously placed species noted above (*C. pedunculosa*, *C. dregeana*, *C. tomentosa* and *C. othonnoides*), as well as *C. mitellifolia*, *C. argillacea*, *C. hederifolia*, *C. exilis* and *C. microglossa*. Two other species which De Candolle listed as doubtful (*C. purpurata* L. and *C. viscosa* L'Hér.) have never officially been excluded from *Cineraria* or placed elsewhere.

Figure 4. *Oresbia heterocarpa* [Cron *et al.* 322 (J)]: (a) Habit. Scale bar = 19 mm. (b) Four-winged ray cypsela. Scale bar = 340  $\mu\text{m}$ . (c) Disc cypsela. Scale bar = 405  $\mu\text{m}$ . (d) Obtuse style apex with sweeping hairs. Scale bar = 50  $\mu\text{m}$ .

Six of these dubiously placed species are known only from type collections: *C. microglossa*, *C. exilis*, *C. dregeana*, *C. othonnoides*, *C. purpurata* and *C. viscosa*.

Material for molecular analysis was obtained for five of these dubiously placed species and on the basis of the results presented below, they have been reinstated or reassigned (Cron *et al.*, submitted a; Cron & Nordenstam, submitted; Cron, 2005: Chapter 6) as follows: *Bolandia argillacea* (syn: *C. argillacea*), *B. pedunculosa* (syn: *C. pedunculosa*), *Senecio cordifolius* (syn: *C. mitellifolia*) and *S. hederiformis* (syn: *C. hederifolia*) and *Oresbia heterocarpa* (syn: *C. tomentosa*). The status of the remaining species is addressed in a morphological cladistic study (Chapter 3).

*Bolandia argillacea*, a narrow endemic from Brandwacht Peak near Worcester in the Western Cape (Cron & Balkwill, 1997), matches *B. pedunculosa* (also mainly from the Western Cape) in having pinnately veined, exauriculate leaves and ecalyculate solitary capitula on long peduncles (Figure 3a, e). *Bolandia argillacea* has pinnatifid leaves, while those of *B. pedunculosa* are lyrate-pinnatifid (to ovate/obovate). Although the cypselae of both species appear compressed when young, the mature ray cypselae are 3-angled with a prominent median rib on the inner surface and the outer surface slightly convex (Figure 3b, c, f); the overall shape being more fusiform than obovate. The mature disc cypselae are sub-cylindrical with four ribs and are oblong to fusiform in shape, with no wings or margins present. Both species have a truncate style apex (Figure 3d, g) as opposed to the obtuse one in *Cineraria* (Figure 1e).

*Senecio cordifolius*, from the mountains of the Western Cape, has a sparsely sub-calyculate involucre, but its ovate to elliptic leaves are exauriculate with pinnate venation (Figure 5a, c) and its leaves are slightly succulent, which is unusual for *Cineraria* (seen only in *C. saxifraga*). It thus lacks the palmate venation so typical of *Cineraria* as well as the auricles. Its cypselae are narrowly oblong, are not compressed when mature and lack distinct margins (Figure 5b). They are certainly not “extremely flattened” as noted by Moore (1903) when he transferred *S. cordifolius* to *Cineraria*, although they might appear so when immature.

*Senecio hederiformis* has exauriculate leaves, although the venation of its lobed leaves could be interpreted as palmate or pinnate (Figure 5d, e). The leaf margins differ from *Cineraria* in being entire rather than dentate, although the apices of the lobes are acute. Its cypselae are also oblong and neither compressed nor margined when mature (Figure 5f). It has a disjunct distribution in South Africa, known only from Blouberg Mountain in north-west Limpopo Province, and the Graskop region in Mpumalanga Province.

*Oresbia heterocarpa* has sessile, pinnately veined, narrowly obovate to elliptic or ovate leaves and few capitula on ebracteolate peduncles (Figure 4a). As noted previously, its cypselae are very different from the obovate, laterally compressed cypselae in *Cineraria*: four-winged ray

Figure 5. (a–c) *Senecio cordifolius* (= *Cineraria mitellifolia*) [Cron & Hodgkiss 369 (J)]: (a) Habit. Scale bar = 13·5 mm. (b) Disc floret with cypsela. Scale bar = 1·1 mm. (c) Leaf with pinnate venation. Scale bar = 5 mm. (d–f) *Senecio hederiformis* (d) Exauriculate leaves [Codd 6747 (K)]. Scale bar = 9 mm. (e) Habit: growing in crevices of rocks near Graskop, Mpumalanga. Scale bar = 9·3 mm. (f) Mature cypsela [Cron *et al.* 247 (J)]. Scale bar = 400  $\mu$ m.

cypselsae (Figure 4b) and multi-ribbed disc cypselsae (Figure 4c), both oblong/cylindrical with an obscure carpodium.

### **Purpose of the study**

The main aims of this study are therefore to use molecular data in a phylogenetic study to (1) investigate the position of *Cineraria* in the Senecioneae, testing the hypotheses of the putative close relationships of *Pericallis* and *Dendrosenecio* to *Cineraria*, (2) more clearly delimit the genus *Cineraria* as a monophyletic homogenous group characterised by a set of well-defined diagnostic features, and (3) provide a useful framework for elucidating infrageneric relationships in *Cineraria*, a morphologically very difficult group to investigate. Morphological features are to be used to more fully interpret the molecular phylogenies. Diverse representatives of the tribe are included to assist with the placement of problematic species currently included in *Cineraria*. This study therefore also aims to add to the existing and growing molecular and morphological evidence concerning relationships of genera and species in the Senecioneae.

## **MATERIALS AND METHODS**

### **Taxon sampling**

Twenty-two samples of *Cineraria*, including 18 species, two subspecies of *C. lobata*, two “forms” of ssp. *lobata*, two samples of *C. deltoidea* from different geographic regions and a putative hybrid between *C. glandulosa* and *C. atriplicifolia* were analysed. The geographic sampling includes Kenya, Zimbabwe, Namibia and areas throughout South Africa. Five of the species previously placed in *Cineraria* (*Bolandia argillacea*, *B. pedunculosa*, *Senecio cordifolius*, *S. hederiformis*, and *Oresbia heterocarpa*) were analysed to determine their position relative to *Cineraria* and other Senecioneae. Voucher information and GenBank accession numbers are provided for the samples included in this study (Table 1).

### *Outgroup selection*

The tussilaginoïd genus, *Tephrosieris* Reichenb., was included to provide a definitive outgroup. *Tephrosieris* is a Eurasian genus, with one species occurring in North America (Bremer, 1994). The group comprises mainly perennial herbs with florets varying in colour from yellow to orange and purplish-red, and possessing oblong cypselsae. The sequences for the *trnL-trnF* and ITS1 and ITS2 regions for *Tephrosieris* were obtained from GenBank. As it was not possible to obtain sequences for both chloroplast and nuclear regions for the same species, sequence data for *T. kirilowii* (Liu, unpublished) and *T. atropurpurea* (Golden *et al.*, 2001), respectively, were used (Table 1). Data were not available from *Tephrosieris* for the flanking portion of the *trnF* exon or the 5.8S and small subunit genes, and these regions were coded as missing data.

A number of potential sister taxa and related genera in the Senecioneae (*Dendrosenecio kilimanjari* ssp. *cottonii*, *Emilia discifolia*, *Senecio deltoideus*, *S. achilleifolius*, *Euryops*

*brownei*, *Kleinia galpinii*, *Pericallis multiflora* and *P. murrayi*) were included in the analysis (Table 1), based on relationships suggested in previous molecular studies (Knox & Palmer, 1995; Kadereit & Jeffrey, 1996; Panero *et al.*, 1999). *Kleinia galpinii* was initially hypothesised as a potential outgroup for this study as the clade containing four species of *Kleinia* (*K. neriifolia*, *K. fulgens*, *K. pendula* and *K. stapeliiformis*) and 17 species of *Senecio* (as well as four other genera: *Gynura aurantiaca*, *Delairea odorata*, *Solanecio gigas*, *S. manni* and *Packera aurea*) was found to form a clade sister to the clade comprising *Cineraria*, *Dendrosenecio* and *Euryops* in the cpDNA restriction site study of Knox & Palmer (1995). However, the position of *Kleinia galpinii* proved unstable in cladograms resulting from our preliminary analyses and therefore could not be used to root them. *Kleinia* Mill. itself is not necessarily monophyletic (Jeffrey, 1986) and *K. galpinii* may not be part of the same group as the species used by Knox & Palmer (1995).

## **Molecular analyses**

### *Choice of regions*

Chloroplast DNA seems best suited for higher taxonomic levels in the Asteraceae, especially among genera, where there is sufficient variation and the incidence of homoplasy is low, resulting in few equally parsimonious trees (Jansen *et al.*, 1992). Non-coding regions of the chloroplast, such as the *trnL* intron and *trnL-trnF* spacer region, have been demonstrated to evolve more rapidly than genes such as *rbcL* in some genera and therefore are appropriate for use at taxonomic levels lower than families and orders (Gielly & Taberlet, 1994). Sequence data from the *trnL* intron and the *trnL-trnF* intergenic spacer regions have been used extensively to investigate the phylogenetic relationships at the intrafamilial or tribal levels (Bayer & Starr, 1998) and at intergeneric levels, such as within the Gnaphalieae (Bayer *et al.*, 2000), the Apocynaceae (Potgieter & Albert, 2001) and the Crassulaceae (Ham *et al.*, 1994).

ITS sequences are valuable for elucidating relationships within and among closely related genera (Baldwin, 1991). Sequences from ITS1 and ITS2 were recently successfully used to elucidate species relationships within two senecionoid genera, *Pericallis* (Panero *et al.*, 1999; Swenson & Manns, 2003) and to a lesser extent in *Packera* (Bain & Golden, 2000), as well as in other groups of Asteraceae (Kim & Jansen, 1992; Baldwin, 1993a, b). DNA sequence data from the nuclear ribosomal internal transcribed spacers (ITS) and 5.8S ribosomal RNA and from the chloroplast *trnL-trnF* region were therefore obtained for use in this study.

### *Procedure*

DNA was extracted for most samples from leaf material dried in silica gel using the modified CTAB method of Doyle & Doyle (1987). For a few species, leaf material from fairly recently collected herbarium specimens or fresh leaf material was extracted using the Qiagen DNeasy Minikit.

For the ITS sequences, a segment comprising ITS1, the 5.8S gene and ITS2 (Baldwin, 1992; Baldwin *et al.*, 1995) was amplified using the 'universal' primers ITS 4 and ITS 5 of White *et al.* (1990). In some taxa, specially designed internal primers anchored in the 5.8S gene [ITSint1F: 5'-CGGCACGGCATGTGCCAAGGA-3'; ITSint2R: 3'-GCTTCGGGCGCAACT TGCGTTC-5'] were used to confirm the ITS sequence. PCR amplification of this region using a Hybaid PCR Sprint involved a 2 minute premelt at 97°C, 28 cycles of 1 minute of denaturation at 94°C, 1 minute annealing at 54°C and a 1 minute extension at 72°C, followed by a final extension for 5 minutes at 72°C with a hold at 4°C.

The *trnL-trnF* segment comprised the sequences for the *trnL* 3' intron, the 3' *trnL* exon and the intergenic spacer between this exon and the *trnF* gene. This segment was amplified using the 'c' and 'f' primers designed by Taberlet *et al.* (1991). The PCR procedure used here was identical to that described above for ITS, except that an annealing temperature of 40°C was used because of the low melting temperature of the primers. The 'd' and 'e' primers of Taberlet *et al.* (1991) were used to confirm the *trnL-trnF* sequences where required.

A total volume of 100 µl was used in the PCR amplification process. 5 units of Taq polymerase prepared following the protocol described by Desai and Pfaffle (1995) was used, as well as 0.25 µl of each primer (20 µM), 12 µl of MgCl<sub>2</sub> (25mM), 2 µl of dNTPs (10mM) and 10 µl PCR buffer (10X) for Taq Polymerase A.

PCR products were purified using the Qiagen PCR Purification kit. Sequences were generated on ABI Prism 310 and 3100 automated sequencing facilities at the University of the Witwatersrand and the University of Stellenbosch (respectively) using the same primers as in amplification. Electropherograms of all sequences were assembled into contigs and proof-read manually. Consensus sequences were aligned using Sequencher 4.1.2 (Genecodes Corp) and all apomorphies were confirmed by reference back to electropherograms.

After initial alignment using Sequencher 4.1.2, the alignment of the matrix was refined manually and then moved into PAUP version 4.0b10 (Swofford, 2001). Alignment was done following the convention of moving nucleotides to the left if alternative alignments were possible and taking possible tandem repeats and length mutations due to slipped strand mispairing into account (Wolfson *et al.*, 1991; Kelchner, 2000). Most gap alignment was unambiguous because of adjacent sequence conservation. In a few cases (all involving mononucleotide strings), positional homology was evaluated against phylogenetic estimates derived from point mutations only. Non-homologous insertions were aligned and coded separately. Non-homologous deletions were aligned together (with no consequences in the phylogenetic analysis), but coded separately. All insertion/deletion events were treated as missing data and an extension to the data matrix coded each indel for presence or absence.

Clear polymorphisms in the ITS regions, identified by equivalent signals present in both forward and reverse readings, were coded in the data matrix. Polymorphisms in the ITS region were recorded for 11 samples of *Cineraria* and three of the other Senecioneae. Dubious polymorphisms (a signal 30% or less of the dominant signal) were investigated for possible synapomorphic information, but none was found, and they were considered to be background noise and were excluded.

Data matrices were analysed separately using PAUP\* 4.0b10. Fitch parsimony analyses were conducted using rigorous heuristic searches with 100 random addition sequences and TBR swapping; ACCTRAN and MULPARS options in operation. Multiple most parsimonious trees were combined as strict consensus trees. All characters were weighted equally.

The strength of support for individual branches was estimated using bootstrap support (BS) values (Felsenstein, 1985), reported from 100 replicates and 10 random addition sequences, using the same settings as for the general heuristic search analyses, and were based on matrices containing point mutations only (i.e. indels not coded). Standard measures of fit of characters: consistency index (CI) excluding uninformative characters, retention index (RI) and tree lengths of the most parsimonious trees are reported. The minimum number of character changes present at a node or branch is also indicated.

It should be noted that bootstrapping is considered an inappropriate measure of branch support for sequence data due to the violation of its core assumptions by the following aspects of evolution of a non-coding region of the genome: (1) nucleotides do not uniformly evolve independently in non-coding regions of the chloroplast; and (2) mutations are not distributed randomly throughout chloroplast non-coding regions (Kelchner, 2000; Bain & Golden, 2000). Therefore Bremer support may be used as an additional measure of branch support (Bremer 1988; Donoghue *et al.*, 1992). Bootstrapping can nevertheless still be considered a useful ‘index of support’ rather than as a statistical statement (Swofford & Olsen, 1990).

The partition homogeneity test as implemented by PAUP (1000 replications; Farris *et al.*, 1995) was used to test for congruence between the phylogenetic signal of the *trnL-trnF* and ITS data sets (excluding the hybrid *Cineraria glandulosa* X *atriplicifolia*). The two data sets were subsequently combined and points of conflict and consensus between the various data sets are noted and discussed. The putative hybrid between *Cineraria glandulosa* and *C. atriplicifolia* was included in the analyses of the individual data sets, but not in the combined analysis as hybridization is “inconsistent with a method designed to depict hierarchies” (Funk, 1985a: 681).

## RESULTS

The *trnL-trnF* region is more highly conserved and therefore less informative than the ITS region. Only six point mutations (and no indels) distinguish the tussilaginoïd outgroup *Tephrosieris* from the senecionoid genera for *trnL* intron, 3' exon and *trnL-trnF* intergenic spacer. Only 40 of 107 variable characters (out of a total of 892 characters) in the *trnL-trnF* region are parsimony informative (as well as seven of the 26 indels coded). In contrast, the ITS region includes 283 variable characters, 132 of which are parsimony informative, with 38 indels, eight of which are parsimony informative.

Although there is little variation in the *trnL-trnF* region, there is little homoplasy (homoplasy index (HI) = 0.07, Figure 6), and the phylogenetic signal is therefore fairly consistent. Analysis of the character distribution in the cladogram generated by the ITS data set reveals a fair amount of homoplasy (HI = 0.27; Figure 7). This is consistent with the results of other studies in the Asteraceae using *trnL-trnF* and/or ITS (e.g. Baldwin, 1992; Kim & Jansen, 1994; Panero *et al.*, 1999; Francisco-Ortega *et al.*, 2001; Pelsner *et al.*, 2001). The greater homoplasy in the ITS region has commonly been attributed to a higher substitution rate in nuclear DNA compared to chloroplast DNA and evolutionary constraints that result in more frequent parallelisms and reversals. Homoplasy may also arise from intragenic recombination (via concerted evolution) of divergent alleles following evolutionary reticulation events.

### Sequence characteristics

#### *trnL-trnF* region

The entire *trnL-trnF* region varies in length from 812 to 849 nucleotides, with the *trnL* 3' intron ranging from 423 to 448 nucleotides and the intergenic spacer from 339 to 351 nucleotides. The *trnL* exon is an invariant 50 bases long. The aligned lengths are 471 bases for the *trnL* 3' intron and 356 for the intergenic spacer, resulting in a total aligned length of 892 bases (including the first 15 bases in the *trnF* gene before the primer). The intergenic spacer has twice the amount of variation due to point mutations (11.6 %) compared to the *trnL* intron (6.2 %). (The aligned data set will be available on the website:

<http://www.wits.ac.za/apes/ggoodman/cineraria.html> as from November 2005.)

Among the 35 senecionoid samples (i.e. excluding *Tephrosieris*), seven of 26 indels occurring in the *trnL-trnF* region are parsimony informative; four in the *trnL* intron and three in the *trnL-trnF* spacer region. Four of these informative indels, varying in length from one to 11 base pairs (bp), are unique to *Bolandia*. *Kleinia galpinii*, *Oresbia heterocarpa* and *Euryops brownei* share a 6-bp deletion in the *trnL* intron. The insertion of a single bp in the *trnL* intron distinguishes the 22 samples of *Cineraria* from all the other Senecioneae included in the study. In addition, a number of unique indels are present in the *trnL* intron from certain samples: *Dendrosenecio kilimanjari* (5-bp insertion), *Euryops brownei* (two 5-bp duplications and a 4-bp insertion),

Strict

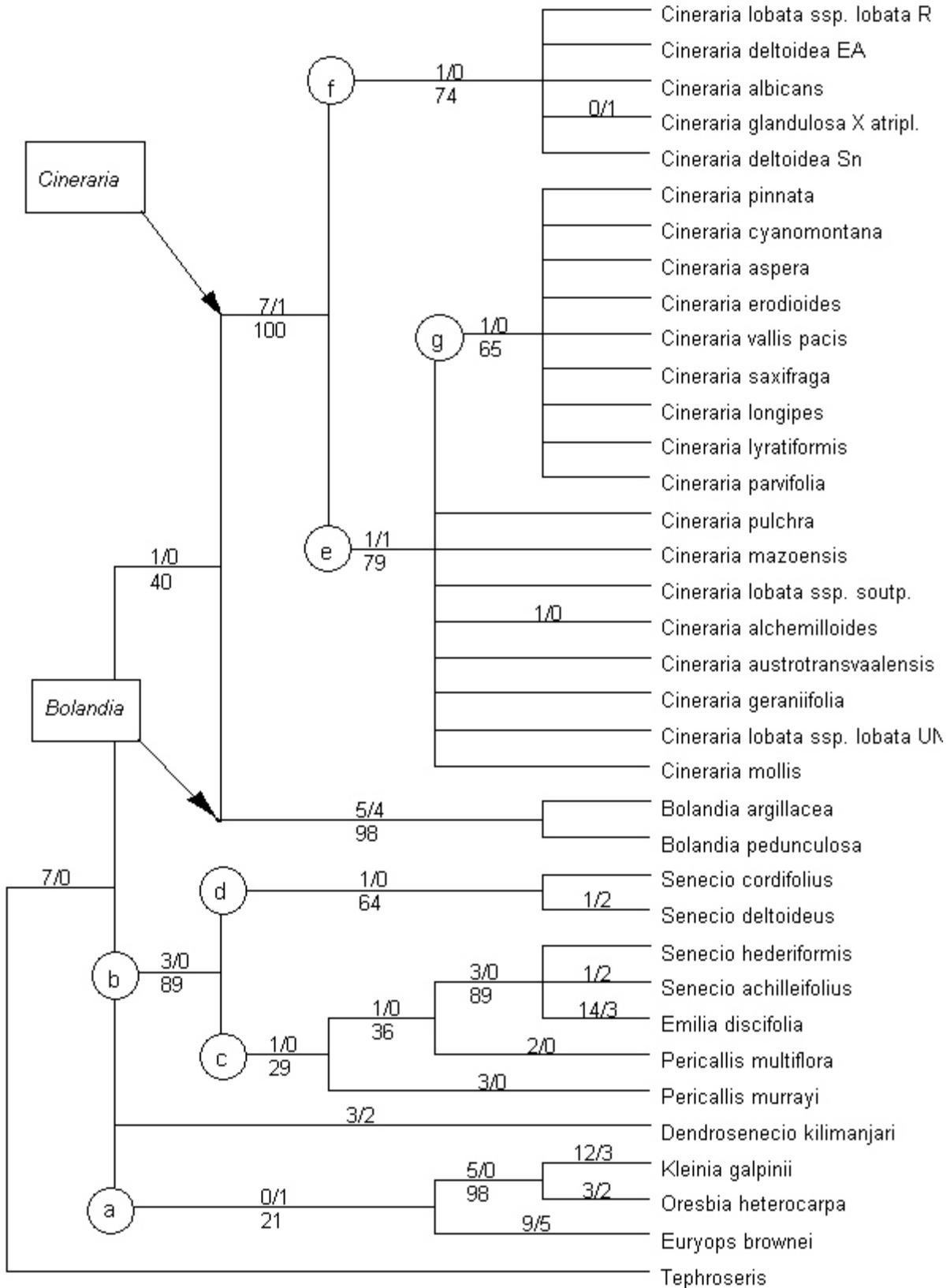


Figure 6. Strict consensus of two most parsimonious trees (with *Bolandia* sister to *Cineraria*; see text) from unweighted Fitch parsimony analysis of 36 *trnL-trnF* sequences of the Senecioneae (116 steps, CI = 0.86, RI = 0.97). The minimum number of steps (point mutations/indels) are indicated above the branches and Bootstrap values (calculated for point mutations only) are indicated below the branches.

Strict

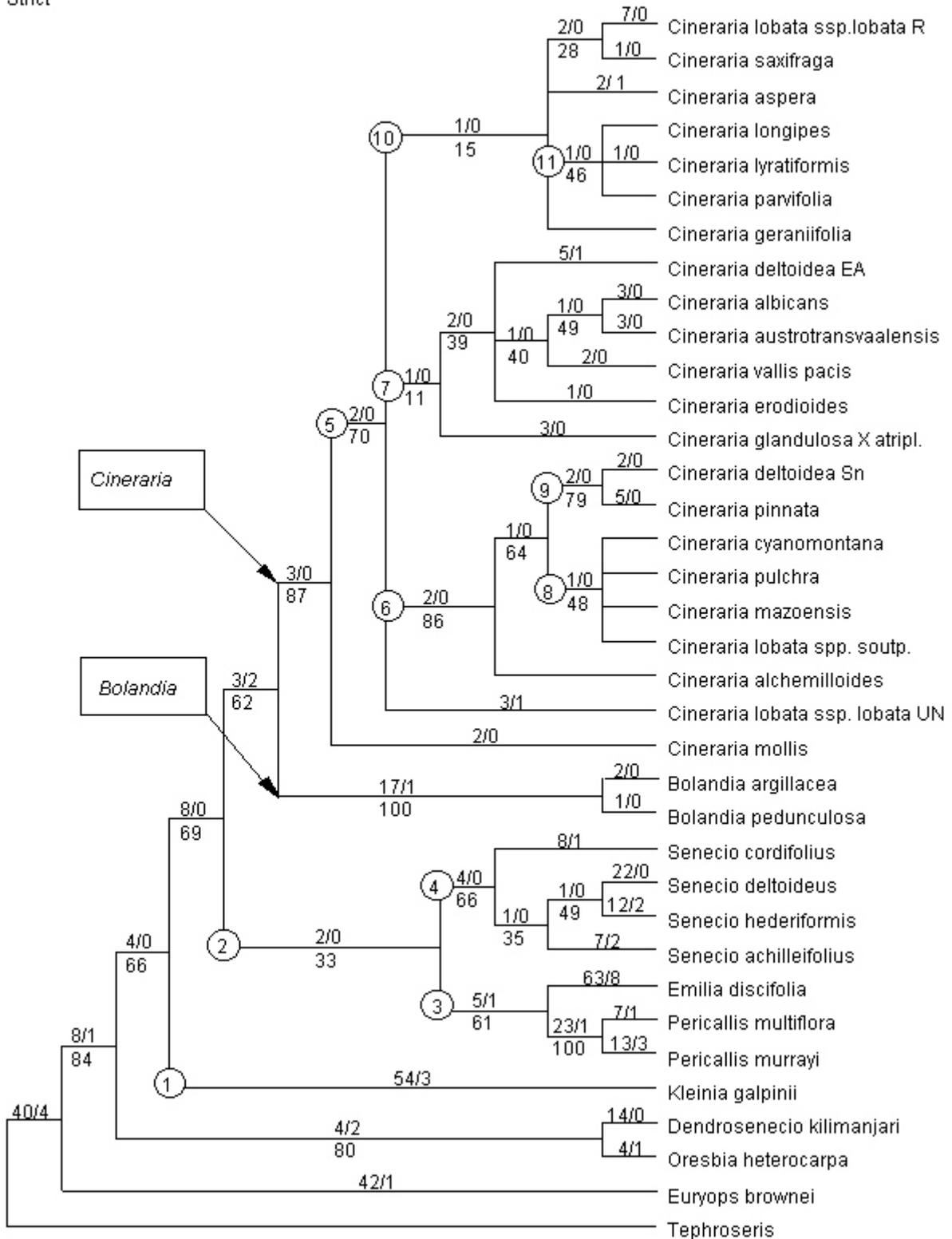


Figure 7. One of two most parsimonious trees from unweighted Fitch parsimony analysis of 36 ITS sequences of the Senecioneae using point mutations only (504 steps, CI = 0.59, RI = 0.67), and the strict consensus of these two trees, as well as of two most parsimonious trees from the analysis including indels (541 steps, CI = 60, RI = 0.69). The minimum number of steps (point mutations/indels) are indicated above the branches and Bootstrap values (calculated for point mutations only) are indicated below the branches.

*Senecio achilleifolius* (4-bp duplication) and *Emilia discifolia* (1-bp deletion, 2-bp and 3-bp insertions). Only three indels occur in the *trnL-trnF* region amongst the 22 *Cineraria* samples: a unique 6-bp duplication in *C. alchemilloides*, a 1-bp duplication in *C. glandulosa x atriplicifolia* and a 4-bp deletion shared by seventeen samples (Figure 6, Clade e).

### *ITS Region*

The data matrix includes 105 nucleotides of the small subunit (SSU) gene, ITS1 (254–262 bases), 164 nucleotides of the 5.8S gene, ITS 2 (201–226 bases) and 54 nucleotides at the beginning of the large subunit (LSU) gene. The total aligned length for the region is 833 nucleotides.

Three autapomorphic point mutations are present in certain members of the Senecioneae (but not in *Cineraria*) in the portion of the SSU gene included in the data matrix, as well as a single synapomorphy shared by *Emilia discifolia* and the two samples of *Pericallis*. There are nine autapomorphic and six synapomorphic point mutations in the 5.8S gene, one of the latter occurring amongst four samples of *Cineraria* (Figure 7, Clade 8) and *C. alchemilloides* is polymorphic for the site. The 54 bases of the beginning of the LSU gene incorporated at the end of the data matrix are invariant for all 36 samples in this study.

In contrast to this limited variation in the genic portions of the data set, there is considerable variation in the internal transcribed spacers, with the ITS2 region having more variable sites (46%) than the ITS1 region (37%). The majority of indels in ITS1 are 1-bp long, with the longer ones being a unique 5-bp insertion due to a direct duplication in *Emilia discifolia* and a 2-bp insertion in *Senecio hederiformis*. In contrast, eight of the 19 indels in ITS2 are more than 1-bp long, the longest being a 19-bp deletion in *C. lobata* ssp. *soutpansbergensis*. There is a 9-bp insertion in *Kleinia galpinii* and a 4-bp deletion in *Senecio achilleifolius*. *Pericallis murrayi* and *P. multiflora* share a 3-bp deletion and *P. murrayi* has a unique insertions of 1-bp and 2-bp in ITS2. *Dendrosenecio* and *Oresbia heterocarpa* share a 1-bp deletion, while *Emilia discifolia* has three unique deletions (two 1-bp and 3-bp) in this region. *Bolandia argillacea* and *B. pedunculosa* also share a 1-bp deletion.

Homology assessment of the indels resulted in only a single ambiguous case (the presence of three versus two adenines in ITS1 in *C. lobata* ssp. *lobata* (robust form, R), *C. saxifraga* and *C. austrotransvaalensis*) which is referred to as a putatively synapomorphic indel (PSI). PSI has three plausible alignments and codings. PSI-1: Independent mutations in all three samples; PSI-2: Synapomorphic for *Cineraria lobata* ssp. *lobata* (R) and *C. saxifraga*, but independent for *C. austrotransvaalensis*; PSI-3: Synapomorphic for all three samples. Because homology in these mononucleotide strings cannot be unambiguously determined and the choice of coding scheme strongly affects the phylogenetic results in some analyses, this case is not included among the coded indels, and the effect of the alternative coding schemes is investigated. The

choice of alignment has no effect on the phylogenetic analysis of the point mutations only and the additional nucleotide in all three samples is aligned together.

### ***trnL-trnF* cladograms**

Ten equally most parsimonious (EMP) trees of 90 steps (CI = 0.83, RI = 0.97, not shown here) result from the analysis of only the point mutations of the 36 sequences for the *trnL-trnF* region with *Tephrosieris* as the outgroup. Inclusion of the indels in the analysis reduces to five the number of EMP trees (116 steps, CI = 0.86, RI = 0.97) because a unique 5-bp deletion affirms the relationship of *Euryops brownei* with *Kleinia galpinii* and *Oresbia heterocarpa* (Figure 6, Clade a), which is otherwise unresolved. Of these five trees, one is an invalid (or false) topology because a single character-state transition cannot simultaneously support two different nodes (Knox & Palmer, 1998), two support the sister-group relationship of *Cineraria* and *Bolandia*, and two trees place *Bolandia* in a clade with the samples of *Senecio*, *Emilia*, *Pericallis* and *Dendrosenecio*. However, one of these latter trees has two nodes that are supported by a single character and only when optimized using ACCTRAN, making this result conditional and unlikely. Since the ITS data also support the sister status of *Cineraria* and *Bolandia* (see below), we present the consensus of only these two trees (Figure 6); the consensus of all five trees is as shown (Figure 6) except that the lineage leading to *Bolandia* also arises directly from the basal polytomy. The instability in the position of *Bolandia* is due to conflict between two synapomorphic point mutations, one of which is shared with *Cineraria*, the other shared with Clade b and *Dendrosenecio*.

The 22 samples of *Cineraria* are strongly supported (100% BS) as a monophyletic group, as is the *Bolandia* clade (98% BS; Figure 6). Within *Cineraria*, there is little resolution: two clades emerge basally, each supported by a minimum of a single point mutation (Figure 6). Clade f comprises five samples and Clade e comprises seventeen samples and is also supported by a 4-bp deletion. Nested within Clade e is a third clade (Clade g), with nine samples united by a single point mutation (Figure 6). The three/four point mutations within *Cineraria* are fully compatible and there is no internal homoplasy in *Cineraria*.

Of the five species previously placed in *Cineraria*, two of them: *Senecio cordifolius* and *S. hederiformis* are consistently placed in Clade b, containing *S. deltoideus*, *S. achilleifolius*, *Emilia discifolia*, *Pericallis* and other species of *Senecio*. *Senecio cordifolius* groups with *S. deltoideus*, whereas *S. hederiformis* groups with *S. achilleifolius*. However, little can be construed from this because of the limited sampling within *Senecio*, itself a large paraphyletic or polyphyletic genus.

### **ITS cladograms**

Two EMP trees of 504 steps (CI = 0.59; RI = 0.67) result from analysis of 36 sequences for the ITS region with *Tephrosieris* as the outgroup. The consensus tree (Figure 7) is the same as the better of the two trees, the alternative tree being due to interpretation of a polymorphism in ITS

2 in *Cineraria erodioides* as either a C or a G. Including the coded indels results in the same two trees, with slightly improved tree statistics (541 steps, CI = 0.60, RI = 0.69). Including PSI according to the alternative coding schemes outlined above has the following effects: PSI-1 (all autapomorphic) adds no cladogenic information; PSI-2 (synapomorphic for *C. lobata* ssp. *lobata* (R) and *C. saxifraga*) is compatible with the other ITS mutations and the same two trees result as before (543 steps, CI = 0.60, RI = 0.69); PSI-3 (synapomorphic for all three samples) results in six EMP trees, the consensus of which has greatly reduced resolution in *Cineraria*: Clade 5 loses all resolution except for Clades 6 and 10 (Figure 7). The reason for this effect is that two point mutations also link these three samples and the coordinated conflict of these characters provides clear evidence of a reticulate evolutionary pattern within the ITS data set.

In the strict consensus (Figure 7), the 22 samples of *Cineraria* form a well supported clade, sister to *Bolandia*. Within *Cineraria*, *C. mollis* is sister to the remaining samples. Among the remaining 21 samples, four lineages emerge basally, but two of the three clades are very weakly supported (Figure 7). There is little correspondence between the twelve clades in *Cineraria* in this analysis versus those in the *trnL-trnF* analysis (Figure 6). Five samples in Clade 10 (Figure 7) occur together in Clade g (Figure 6) and three samples (*C. albicans*, *C. deltoidea* EA and the hybrid *C. glandulosa* X *C. atriplicifolia*) occur together in Clade 7 (Figure 7) and Clade f (Figure 6). *Cineraria erodioides*, *C. vallis-pacis* and *C. austrotransvaalensis* in Clade 7 (Figure 7) share a polymorphic for a point mutation in ITS2, with *C. vallis-pacis* and *C. austrotransvaalensis* also similarly polymorphic at a second point. *Cineraria pulchra* and *C. mazoensis* in Clade 8 (Figure 7) share a polymorphism, which PAUP does not treat as a shared feature.

Clade 2 (Figure 7) comprises the same samples as the *trnL-trnF* Clade b (Figure 6), although the topologies differ somewhat. *Pericallis* is monophyletic, and is weakly supported as sister to *Emilia discifolia*. *Oresbia heterocarpa* groups with *Dendrosenecio* (Clade 1, Figure 7), as opposed to *Kleinia galpinii* and *Euryops brownei* in the *trnL-trnF* analysis (Figure 6). This conflicting placement is individually well supported in both cases.

### **Combined analysis of *trnL-trnF* and ITS data sets**

In the combined analysis of the *trnL-trnF* and the ITS data sets, 142 trees (614 steps, CI = 0.58, RI = 0.73, not shown here) result using point mutations only, but with limited resolution in *Cineraria*: Clades 5 and 6 of the ITS analysis (Figure 7) occur and three samples unite in a clade (as in Figure 6, Clade f, excluding *C. deltoidea* Sn). Including the coded indels and excluding PSI, or using either coding PSI-I or PSI-2, results in 79 trees (680 steps, CI = 0.59, RI = 0.57), but with very little resolution in *Cineraria* in the consensus tree (not shown here). This is due to the 4-bp deletion in the *trnL-trnF* region of 17 samples (Figure 6, Clade e) counterbalancing portions of the ITS data. Including the coded indels and with PSI-3 coding in operation, three

trees (682 steps, CI = 0.59, RI = 0.75) result and a very well-resolved consensus, but with an overlapping pattern of coordinated reversals and parallelisms (Figure 8).

These results are indicative of reticulate evolution occurring in *Cineraria*, notably amongst *C. lobata* ssp. *lobata* (R), *C. saxifraga*, *C. austrotransvaalensis* and *C. erodioides*. The partition homogeneity (PH) test indicated that the two data sets are on the cusp of congruence/incongruence ( $P = 0.01$ ) and the test is thus inconclusive. However, inspection of the data clearly indicates that points of conflict exist between the topologies supported by the plastid and nuclear data and the possible effects of reticulate evolution in ITS data need to be identified.

*Cineraria* is once again clearly monophyletic in the consensus of the combined analysis (Figure 8), with *Bolandia* the sister-genus, consistent with the ITS analysis and two of four *trnL-trnF* valid EMP trees. *Senecio cordifolius* and *S. hederiformis* are grouped in a clade (Clade 4) with *S. deltoideus* and *S. achilleifolius* respectively, similar to the ITS analysis (Figure 7), but the clades are not identical in topology. The placement of the remaining senecioid genera/species (including *Oresbia heterocarpa*) is also consistent with the phylogeny produced by analysis of the ITS region (Figure 7), but differs from that assembled according to the phylogenetic estimation from the *trnL-trnF* data (Figure 6). This is due to the larger number of ITS mutations dominating over the fewer, and in some cases markedly incongruent, mutations in the cpDNA.

Within *Cineraria*, very few of the clades are strongly supported (>50% BS), reflecting the conflict between the cpDNA and the nuclear ITS data. *Cineraria mollis* is placed sister to the rest of the samples included in the study (Figure 8, Clade 5), consistent with the ITS phylogeny. Within Clade 5 (Figure 8), there is further subdivision into three lineages: *C. lobata* ssp. *lobata* (small-leaved form indicated 'UN'), Clade 6 with seven samples reflecting the dominant phylogenetic signal from the ITS data, and Clade 7 with 12 samples. Within Clade 7, only subclade 10 (with three samples) reflects the phylogenetic signal from the *trnL-trnF* data, although the position of *C. austrotransvaalensis* is also influenced by the interaction between mutations in the cpDNA and PSI when treated as a synapomorphy (PSI-3).

## DISCUSSION

### Comparison of the *trnL-trnF* and ITS regions

The *trnL* intron and the *trnL-trnF* intergenic spacer evolve at similar rates (Gielly & Taberlet, 1994). Our results show more point mutations in the spacer region, but fewer indels. As expected, the ITS region has more variation than the *trnL-trnF* region. Most of the ITS variation comprises point mutations rather than indels, as previously observed (Baldwin *et al.*, 1995; Panero *et al.*, 1999). Both data sets support the monophyly of *Cineraria*, its sister-group relationship with *Bolandia* and exclusion of the other three samples previously included in *Cineraria*. Some fairly well supported clades in *Cineraria* emerge in cladograms resulting from

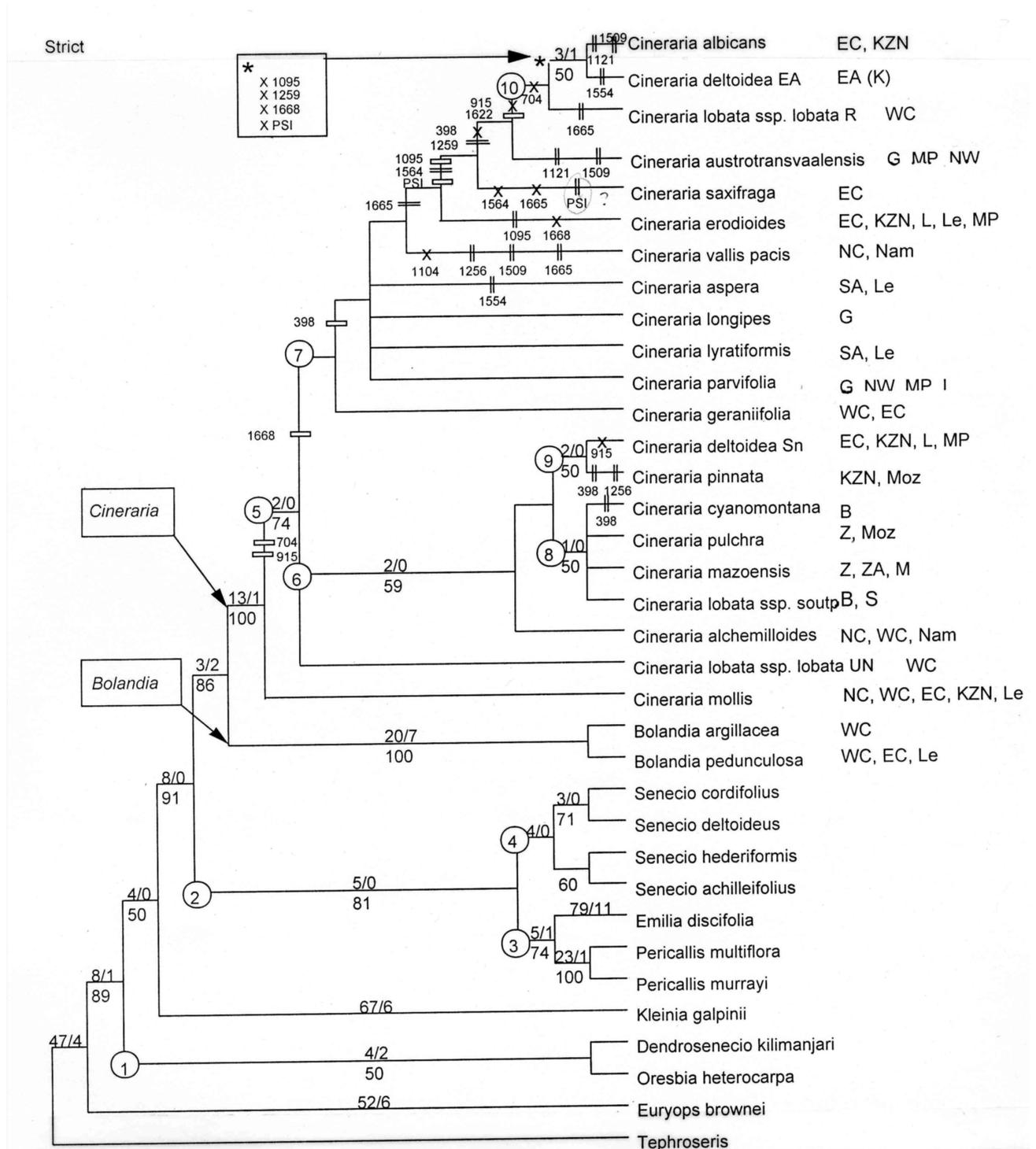


Figure 8. Strict consensus of three most parsimonious trees from unweighted Fitch parsimony analysis of the combined plastid (*trnL-trnF*) and nuclear ITS data sets for 35 samples in the Senecioneae including point mutations, coded indels and PSI-3 (682 steps, CI = 0.59, RI = 0.75). The minimum number of steps (point mutations/indels) are indicated above the branches and Bootstrap values of  $\geq 50\%$  (calculated for point mutations only) are indicated below the branches. Conflicting homoplasious characters are mapped on the branches for *Cineraria*: □ homoplasious characters, = parallelism, X reversal.

Geographic regions indicated as follows: B Blouberg, Bo Botswana, EA (K) East Africa (Kenya), EC Eastern Cape, FS Free State, G Gauteng, KZN KwaZulu-Natal, L Limpopo, Le Lesotho, M Malawi, Moz Mozambique, Nam Namibia, NC Northern Cape, NW North West, S Soutpansberg, SA South Africa (widespread), Sw Swaziland, WC Western Cape, Z Zimbabwe, Za Zambia.

analysis of both data sets (Figures 6 and 7), but due to conflicting phylogenetic signals between the maternally inherited plastid region and the biparentally inherited ITS regions, the support for clades retained in the combined analysis is considerably weaker (Figure 8). The conflict in the information in each region very likely reflects different evolutionary histories in the nuclear (biparental) and plastid (maternal) DNA in the genus *Cineraria*.

Support for reticulate evolution in *Cineraria* is provided by observations of putative hybrids in the field; viz. *C. deltoidea* x *C. pulchra* in the Chimanimani region of Zimbabwe (Cron *et al.*, submitted b) and *C. glandulosa* x *C. atriplicifolia* in the Midlands of KwaZulu-Natal (Cron *et al.*, in press). In addition, including the putative hybrid *C. glandulosa* x *atriplicifolia* in the combined data set results in all resolution within *Cineraria* (except for the sister status of *C. mollis*) collapsing due to additional conflict between the nuclear and plastid DNA data.

Although there is some variation in the ITS regions among the species of *Cineraria* sampled, much of it comprises autapomorphies and there are too few parsimony informative sites to robustly indicate relationships amongst all the species included in this study. In addition, evidence of reticulation complicates interpretation of the species relationships. At least four samples included in this study appear to have undergone reticulate evolution: *C. lobata* spp. *lobata* R., *C. saxifraga*, *C. austrotransvaalensis*, and *C. eroidoides*; possibly also *C. vallis-pacis*, *C. pinnata*, and *C. deltoidea* Sn.

## **Phylogenetic relationships**

### **Sister relationship of *Bolandia* and position of previously misplaced species**

A narrower circumscription of the genus *Cineraria* is supported by separate and combined analysis of sequence data from both the *trnL-trnF* and ITS regions of the chloroplast and nuclear genomes respectively. The diagnostic morphological characters corresponding to this monophyletic group are: palmately veined leaves, calyculate, (yellow) radiate capitula and laterally compressed, obovate cypselae with two distinct margins or wings. These features are those which have been noted by various authors (Hilliard, 1977; Cron, 1991; Bremer, 1994) to be of key importance in recognising species in this genus, although exceptions to the pattern had been traditionally included and were noted (Harvey, 1865; Cron, 1991).

The removal of five species previously included in *Cineraria* (*Bolandia pedunculosa*, *B. argillacea*, *Senecio cordifolius*, *S. hederiformis* and *Oresbia heterocarpa*) is supported by molecular evidence and corroborated by morphological features. *Bolandia* is placed sister to *Cineraria*, and is distinguished by its fusiform/elliptic 3-angled ray cypselae with a prominent median rib, four-angled disc cypselae, ecalyculate, solitary capitula on long, bracteate peduncles, as well as truncate style apices with a fringe and central tuft of sweeping hairs. The sister relationship of *Bolandia* to *Cineraria* is supported by the ITS analysis (Figure 7) and the combined data analysis (Figure 8), as well as in two of the four valid trees produced by analysis

of the *trnL-trnF* data (Figure 6). The monophyly of *Cineraria* could be extended back to below the node at which these taxa branch off, but that would considerably broaden the generic concept of *Cineraria* as *B. pedunculosa* and *B. argillacea* lack a number of features diagnostic for *Cineraria*.

If *Bolandia pedunculosa* and *B. argillacea* were nested within the *Cineraria* clade, they could be considered divergent species deviating from the major morphological patterns in the genus or exhibiting reversals in a number of features. However, as they are placed sister to the *Cineraria* clade, the question remains: Is it preferable to establish a more clear-cut morphological delimitation of *Cineraria* and treat *B. pedunculosa* and *B. argillacea* as a distinct sister genus OR to adopt the inclusive approach of Harvey and retain his expanded generic concept of *Cineraria*, reducing the need for taxonomic change?

From a purely cladistic standpoint, no change is necessary as either node could be the point at which a higher rank is accorded to the monophyletic group. However, if the category “genus” is a natural grouping of “species more closely related to one another than any are to other species” (Funk, 1985b), and not a purely artificial construct as purported by Bentham (1861) and Lamarck (according to Stevens, 2002), then a taxonomic criterion other than stability is necessary and appropriate.

*Cineraria sensu strictu* is clearly distinguished by a number of synapomorphic features (Figure 9), including an obtuse style apex with sweeping hairs, a feature commonly useful in distinguishing genera in the Senecioneae (Nordenstam, 1978; Bremer, 1994). The unique feature of obovate, compressed mature cypselae with distinct margins or wings makes this an easily recognisable group. *Bolandia* is similarly supported by morphological synapomorphies relating to the shape of its cypselae and style apices (Figure 9). The recognition of these two genera meets the recommendation that “the level of monophyly ultimately recognised as a genus should be from the node most strongly supported by diagnosing characters” (Schrire and Lewis, 1996, p. 362). It also meets conditions of ease of recognition and predictivity (Clayton, 1983; Schrire and Lewis, 1996) and reflects the pattern (the group of features) that has become “fixed during evolutionary history” (Jeffrey, 1987, p. 28).

To include *Bolandia pedunculosa* and *B. argillacea* within *Cineraria* would effectively dilute its generic concept and blur its homogeneity and coherence and minimise the informativeness and predictiveness of the group, important criteria for the validity and usefulness of the generic concept (Jeffrey, 1987). Moreover, *B. pedunculosa* and *B. argillacea* share very few synapomorphies with *Cineraria s.s.* and a large number of plesiomorphies with the rest of the Senecioneae (Figure 9).

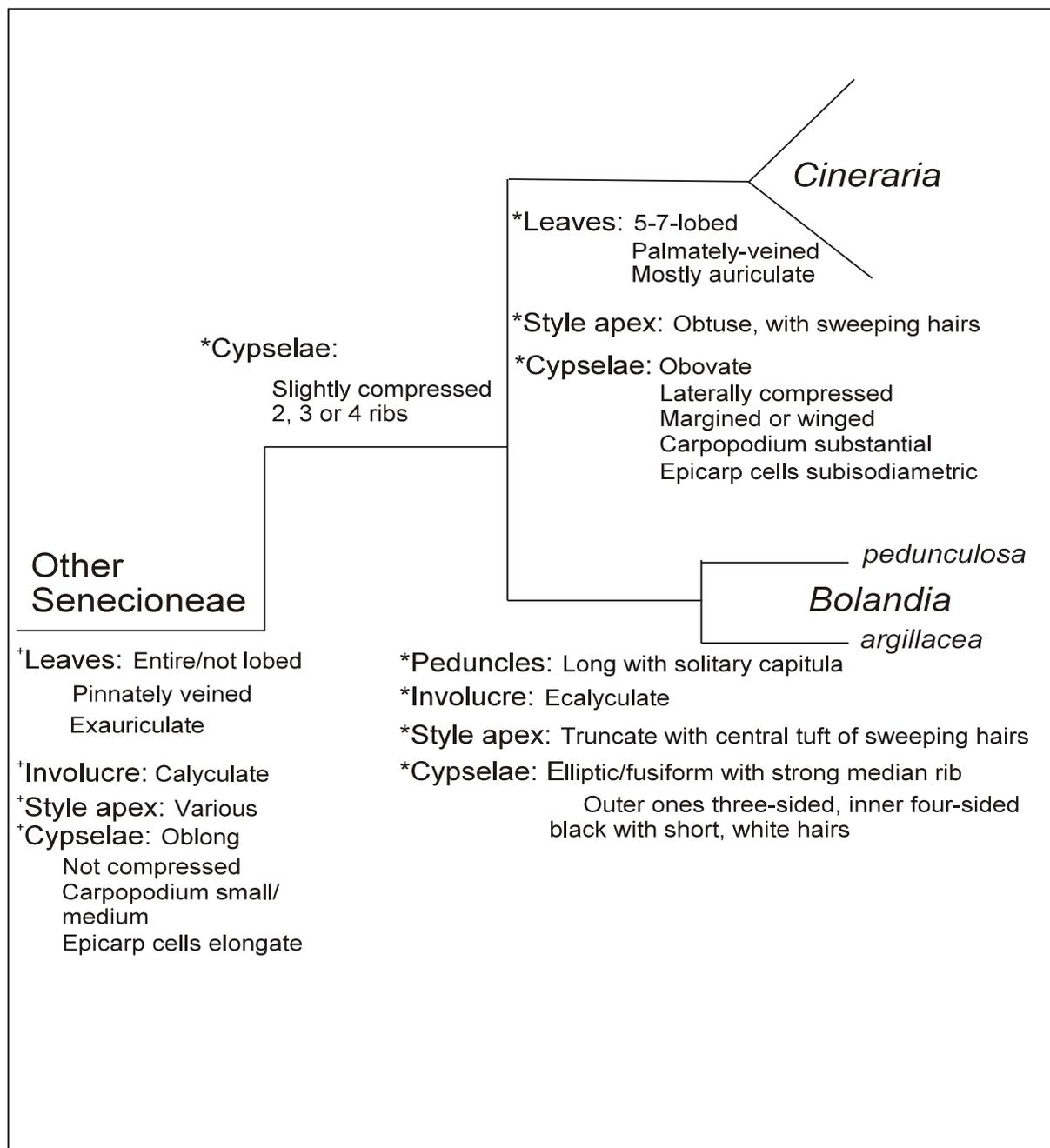


Figure 9. Summary cladogram of morphological synapomorphies (\*) for *Cineraria* and *Bolandia*; and plesiomorphies (+) shared by other members of the Senecioneae.

A narrower generic concept of *Cineraria* is thus supported by the molecular phylogenies shown here and by a consistent set of morphological characters as mapped onto these cladograms (Figure 9). Our conclusion therefore is that those species of *Cineraria* which have obovate, compressed cypselae with distinct margins, palmately veined, 5–7-lobed auriculate leaves and calyculate capitula form a natural group and should be considered a well defined genus. *Bolandia pedunculosa* and *B. argillacea* are therefore excluded from *Cineraria* and are recognised as a distinct genus, sister to *Cineraria*.

Considering the other problematic species previously included in *Cineraria*: *Senecio cordifolius* and *S. hederiformis* are both unequivocally placed with the two *Senecio* species included in this study by separate and combined analyses of the molecular data sets (Figures 6, 7 and 8). Morphologically, the oblong, cylindrical cypselae of *Senecio cordifolius* and *S. hederiformis* (Figures 5b and f) match those of the *Senecio* species included here. Both *S. cordifolius* and *S. hederiformis* have calyculate capitula and bracteate peduncles typical of *Cineraria*, but these features are also found in many species of *Senecio*. The style branch apices are truncate and fringed with sweeping hairs in *S. cordifolius* and rounded with short fringing sweeping hairs in *S. hederiformis*, both atypical of *Cineraria*. They are, however, similar to style apices seen in other African senecios (Vincent & Getliffe, 1992). These two species are therefore best placed in *Senecio sensu lato*.

*Oresbia heterocarpa* is a new monotypic genus, endemic to the Western Cape, with an affinity to *Dendrosenecio* according to the ITS analysis (Figure 7), although it is linked with *Kleinia* in the *trnL-trnF* analysis (Figure 6). In a more broad-based analysis of the Senecioneae using ITS data, *O. heterocarpa* is placed sister to a clade comprising *Phaneroglossa* and *Dendrosenecio* (P. Pelsner, pers. com.). *Phaneroglossa* is another monotypic genus from the Western Cape with heteromorphic cypselae with “5 or 6 obliquely-curved, wing-like ridges” (Nordenstam 1978, p. 66), with glabrous ray cypselae and the disc cypselae covered in mucilaginous hairs, but differs from *Oresbia* by virtue of its white-rayed, ecalyculate, solitary capitula. *Phaneroglossa bolusii* has a restricted range in the Hex River Mountains and the Koue Bokkeveld near Ceres in the Western Cape (Nordenstam, 1978), while *Oresbia* occurs mainly in the mountains around Worcester, Ceres and Clanwilliam in the Western Cape. The distributions of *Phaneroglossa* and *Oresbia* therefore overlap and it is quite feasible that they share a common ancestor.

### **Relationship with *Pericallis* and *Dendrosenecio***

Phylogenetic analysis of the ITS data (Figure 7) indicates that *Cineraria* is more closely related to *Pericallis* than to *Dendrosenecio*, but *Pericallis* is more closely related to *Emilia discifolia* and certain species of *Senecio* than to *Cineraria*. This basic conclusion is congruent with one of the four EMP trees obtained from the cpDNA. The close relationship between *Cineraria*, *Pericallis* and *Dendrosenecio* is thus confirmed by these analyses, but a more inclusive study of the Senecioneae is needed before exact relationships can be fully elucidated. This work nevertheless adds to the growing data set in GenBank, making such a task feasible in the future.

### **Species relationships within *Cineraria***

A limited framework for the resolution of species relationships within *Cineraria* is provided by both the *trnL-trnF* and the ITS sequence data. However, conflicting phylogenetic signals in the two data sets minimise the support for the clades obtained when they are combined. The sister relationship of *C. mollis* to the other samples in *Cineraria* evident in the ITS data is not

confirmed by the *trnL-trnF* data, where it shares a 4-bp deletion and a point mutation with sixteen other samples (Figure 6, Clade e).

Morphologically, *Cineraria mollis* has a number of features in common with the sister genus *Bolandia* (especially *B. pedunculosa*), notably its caespitose habit and long peduncles with solitary capitula. However its capitula are calyculate and its obovate, compressed cypselae and obtuse style apex indisputably place it within *Cineraria*. As noted above, *C. mollis* is placed in Clade e in the consensus tree resulting from the *trnL-trnF* analysis (Figure 6), along with *C. geraniifolia*, which also has long peduncles and few capitula. It is possible that *C. mollis* has experienced a reticulation event that has resulted in its possession of a chloroplast genome that is not sister to the other species.

In the cpDNA-based phylogenetic estimate (Figure 6), Clade f comprises five samples, most of which have deltoid or deltoid-reniform leaves. Both the East African and southern African forms of *C. deltoidea* are positioned in this clade, supporting the synonymy of this very variable and widespread species (Jeffrey, 1986; Cron, 2005: Chapter 5). This is in contrast to the relationships hypothesised by analysis of the ITS data (Figure 7), where the more southerly sample/population of *C. deltoidea* is placed in a separate clade (Clade 7) with seven other species and sister to *C. pinnata* (Clade 9) from Maputaland (the coastal region of northern KwaZulu-Natal and southern Mozambique). This is indicative of possible reticulation events in the evolutionary history of this population of *C. deltoidea* from the Chimanimani region of Zimbabwe and *C. pinnata*, which also has deltoid leaves, although they tend to be very pinnatifid, especially in the upper leaves.

The putative hybrid between *Cineraria glandulosa* and *C. atriplicifolia* has a very restricted distribution in KwaZulu-Natal, South Africa, and is covered with glandular trichomes that distinguish it from *C. deltoidea* as well as from the other two species in Clade f (Figure 6). *Cineraria glandulosa* is considered to have a close affinity to *C. erodioides* due to its auricles and deltoid-reniform leaf shape, and the hybrid sample is associated with *C. erodioides* and *C. deltoidea* in Clade 7 in the ITS analysis (Figure 7). *Cineraria atriplicifolia* shows some affinity to *C. deltoidea* (Chapter 5) due to its deltoid leaf shape.

Of the four species in Clade f (Figure 6), only *C. albicans* has a fine grey tomentum on its leaves and stems. *Cineraria lobata* ssp. *lobata* and *C. albicans* are both somewhat woody and suffruticose in habit, whereas *C. deltoidea* and the putative hybrid are perennial herbs. The two “forms” of *C. lobata* ssp. *lobata* from the Western Cape are placed in two distinct clades (Figure 6), with the more robust form (with some similarity to *C. geifolia*) from the Cape Peninsula in Clade f and the small-leaved, jointed form from the Uniondale area in Clade g. This once again indicates that reticulate evolution is playing a role in the evolutionary history of *Cineraria*.

Clade f of the *trnL-trnF* consensus tree (Figure 6) contains mostly those species with a deltoid-reniform to reniform outline to the leaves. However, *Cineraria aspera* and *C. cyanomontana* have an elliptic outline and pinnatifid lobing and *C. lyratiformis* has a lyratiform leaf. Within Clade e (Figure 6) is a well supported clade of nine species (Clade g), three of which (*C. aspera*, *C. vallis-pacis*, and *C. cyanomontana*) have dissected leaf lobes and are grey in appearance due to a thick cobwebby indumentum. *Cineraria vallis-pacis* and *C. cyanomontana* possess the same type of trichome on their leaves (2–4 narrow, agranular basal cells with a multicelled apical extension); similarly *C. aspera* and *C. erodioides* have the identical leaf trichomes (4–6 granular, tapering basal cells and a long, multi-celled apical wisp). *Cineraria longipes*, *C. lyratiformis*, *C. parvifolia*, *C. pinnata* and *C. saxifraga* are green and mainly glabrous but commonly with glandular hairs in the angles of their leaves. As in Clade f, there is a mix of herbaceous and woody perennials in Clades e and g (Figure 6), with *C. pinnata* and *C. parvifolia* being the only annual herbs (both in Clade g).

*Cineraria pulchra*, *C. mazoensis*, *C. cyanomontana* and *C. lobata* ssp. *soutpansbergensis* form a weakly supported clade in the tree resulting from analysis of the ITS regions (Figure 7, Clade 8). All are tomentose-grey and from tropical Africa. The grouping of *C. cyanomontana*, a narrow endemic from Blouberg Mountain, within this clade may be due to reticulation as a hybrid origin from *C. aspera* and *C. lobata* ssp. *soutpansbergensis*, also occurring on Blouberg Mountain, is possible. *Cineraria cyanomontana* resembles *C. aspera* in having pinnatifid leaves with an ovate outline, and *C. lobata* ssp. *soutpansbergensis* in its lanceolate auricles.

Three herbaceous species of *Cineraria*, *C. longipes*, *C. parvifolia* and *C. lyratiformis*, are grouped together in a Clade 11 (Figure 7) in the strict consensus of trees resulting from the ITS analysis. This clade is present in only two of the three trees generated by the analysis based on the combined data set and therefore is not retained in the strict consensus tree (Figure 8). *Cineraria longipes* is endemic to Gauteng, and *C. parvifolia* also occurs there (as well as in the North-West, Mpumalanga and Limpopo Provinces), while *C. lyratiformis* is widespread and quite weedy.

Although only 51% of species in *Cineraria* are sampled here, the data and analyses provide a framework for further investigation into species relationships. Although no infrageneric classification can as yet be applied within *Cineraria*, it is evident that De Candolle's infrageneric categories (based on cypsela pubescence and extensions) are not upheld here as the two species with glabrous winged cypselae included in this study (*C. lyratiformis* and *C. geraniifolia*) do not group together in cladograms based on either the cpDNA or the nuclear ITS regions (Figures 6 and 7).

The ITS regions are clearly not evolving as rapidly in *Cineraria* as in *Pericallis* where species relationships are fairly well resolved by analyses using sequence data from these regions

(Panero *et al.*, 1999; Swenson & Manns, 2003). The restriction of most species in *Pericallis* each to a single Macaronesian island could also have served to promote this rapid rate of evolution and facilitate distinctive differences amongst species. *Cineraria* also occurs on the “island-like” archipelago of African mountains, but cycles of climatic change (notably moister and cooler periods in southern Africa) could have facilitated meeting of species and hybridization with subsequent isolation of new recombinant genotypes. *Cineraria* has apparently experienced rapid biogeographic and morphological diversification, with periodic points of reticulate evolution.

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Appendix 1. Complete list of species in *Cineraria* (with authorities and synonyms) and geographic distribution.

Species (and synonyms)	Distribution
<i>C. abyssinica</i> Sch.Bip. ex A.Rich (= <i>C. sebalzii</i> Cufod.)	Ethiopia, Eritrea, Yemen, Saudi Arabia
<i>C. albicans</i> N.E.Br.	South Africa: KwaZulu-Natal, Eastern Cape
<i>C. alchemilloides</i> DC.	South Africa: Western Cape, Northern Cape; Namibia
<i>C. anampoza</i> (Baker) Baker	Madagascar
<i>C. aspera</i> Thunb.	South Africa: Northern Cape, Western Cape, Eastern Cape, KwaZulu-Natal, Mpumalanga, Free State, Gauteng, North West; Lesotho
<i>C. atriplicifolia</i> DC.	South Africa: KwaZulu-Natal
<i>C. austrotransvaalensis</i> Cron	Gauteng, North West, Mpumalanga
<i>C. canescens</i> Wendl. ex Link (= <i>C. parviflora</i> Aiton; <i>C. aitoniana</i> Spreng.)	South Africa: Western Cape, Northern Cape
<i>C. cyanomontana</i> Cron	South Africa: Blouberg Mountain, Limpopo Province
<i>C. decipiens</i> Harv.	South Africa: KwaZulu-Natal, Mpumalanga; Swaziland
<i>C. deltoidea</i> Sond. (= <i>C. bequaertii</i> De Wild; <i>C. bracteosa</i> O.Hoffm. ex Engl.; <i>C. buchanani</i> S.Moore; <i>C. densiflora</i> R.E.Fr.; <i>C. gracilis</i> O.Hoffm.; <i>C. grandiflora</i> Vatke; <i>C. kilimandsharica</i> Engl.; <i>C. laxiflora</i> R.E.Fr.; <i>C. monticola</i> Hutch.; <i>C. prittwitzii</i> O.Hoffm.; <i>Senecio kirsteineanus</i> Muschl.; <i>Senecio schubotzianus</i> Muschl.)	Ethiopia, Sudan, Democratic Republic of Congo, Rwanda, Uganda, Kenya, Tanzania, Malawi, Zimbabwe, Zambia, South Africa.
<i>C. dryogeton</i> Cron	South Africa: Umtamvuna Forest, KwaZulu-Natal
<i>C. erodioides</i> DC. (= <i>C. britteniae</i> Hutch. & R.A.Dyer; <i>C. dieterlenii</i> E.Phill.; <i>C. polycephala</i> DC.; <i>C. tussilaginis</i> Thunb.)	South Africa: Eastern Cape, KwaZulu-Natal, Free State, Gauteng, Mpumalanga, Limpopo); Lesotho

<i>C. erosa</i> (Thunb.) Willd. (= <i>Doria erosa</i> Thunb.; <i>C. oxyodonta</i> DC.)	South Africa: Northern and Western Cape
<i>C. foliosa</i> O.Hoffm.	Kipengere Range, southern Tanzania,
<i>C. geifolia</i> (L.) L. (= <i>Othonna geifolia</i> L.)	South Africa: Western Cape
<i>C. geraniifolia</i> DC.	South Africa: Eastern Cape, KwaZulu-Natal, Mpumalanga
<i>C. glandulosa</i> Cron	South Africa: KwaZulu-Natal
<i>C. grandibracteata</i> Hilliard	South Africa: KwaZulu Natal and Eastern Cape
<i>C. huilensis</i> Cron	Angola, Huila Province, in the Serra da Chela mountains
<i>C. humifusa</i> L'Hér. = <i>C. angulosa</i> Lam.	South Africa: Western Cape
<i>C. lobata</i> L'Hér.	South Africa: W Cape, N Cape, E Cape, Limpopo
<i>C. longipes</i> S.Moore	South Africa: Gauteng
<i>C. lyratiformis</i> Cron (= <i>C. lyrata</i> DC.)	Western Cape, Northern Cape, Eastern Cape, KwaZulu-Natal, Free State, North West, Mpumalanga, Gauteng, Lesotho
<i>Cineraria magnicephala</i> Cron	Central Malawi
<i>C. mazoensis</i> S.Moore	Zimbabwe
<i>C. mollis</i> E.Mey. ex DC. (= <i>C. arctotidea</i> DC.; <i>C. polyglossa</i> DC.)	South Africa: Western Cape, Northern Cape, Free State, KwaZulu-Natal; Lesotho
<i>C. ngwenyensis</i> Cron	Swaziland
<i>C. parvifolia</i> Burt Davy	South Africa: Gauteng, Limpopo, Mpumalanga
<i>C. pinnata</i> O.Hoffm. ex Schinz	southern Mozambique, northern KwaZulu-Natal (South Africa)
<i>C. platycarpa</i> DC.	South Africa: Western Cape; Eastern Cape
<i>C. pulchra</i> Cron	Eastern highlands of Zimbabwe; Mozambique
<i>C. saxifraga</i> DC.	South Africa: Eastern Cape
<i>C. vagans</i> Hilliard	South Africa: Eastern Cape
<i>C. vallis-pacis</i> Dinter ex Merxm.	Botswana; Namibia; South Africa: Northern Cape

