

Chapter 3

Phylogenetic analysis of *Cineraria* (Senecioneae, Asteraceae) based on morphological and molecular data.

ABSTRACT

A narrower, more homogenous generic concept for *Cineraria* (Senecioneae, Asteraceae) is supported by separate and combined morphological and molecular (*trnL-trnF* and ITS regions) cladistic analyses and a hypothesis of species relationships within *Cineraria* is presented. Four species previously misplaced in *Cineraria* (viz. *C. exilis*, *C. microglossa*, *C. othonnoides*, *C. dregeana*) are shown to be outside of its generic limits. *Bolandia* is placed sister to *Cineraria* amongst the genera included in the study, according to combined morphological and molecular analyses, whereas the morphological data alone placed *Pericallis* sister to *Cineraria* due to numerous convergent morphological features. Morphological data alone provide insufficient information to unambiguously resolve species relationships within *Cineraria*. Combined molecular and morphological data provide the most resolved hypotheses of species relationships in the genus, but not all species are included in the molecular analyses and neither the *trnL-trnF* nor the ITS regions appear to have sufficient variation to fully resolve relationships amongst all species in *Cineraria*. Separate morphological and combined morphological and molecular cladistic analyses with *Bolandia* as the outgroup indicate that the ancestral condition of *Cineraria* was herbaceous with long peduncles and large capitula, and the woody subshrub developed later with a number of reversals back to the herbaceous condition. Deltoid-reniform upper leaves and reniform lower leaves with a cobwebby to tomentose indumentum appear to be the plesiomorphic states. Leaf shape and trichome complement are important characters in elucidating species relationships in the genus, while a subisodiametric cell on the cypsela epicarp is an additional feature distinguishing *Cineraria* from most other senecioid genera.

INTRODUCTION

The genus *Cineraria* L. (Senecioneae, Asteraceae) as currently circumscribed (Chapter 6) includes herbs and subshrubs, with heterogamous, radiate capitula with yellow florets. Distinguishing characteristics include palmately-veined, mostly auriculate leaves and obovate, compressed cypselae with distinct margins or wings and a well developed carpodium (Hilliard, 1977; Bremer, 1994; Cron, 1991). The leaves are characteristically dentate, 5–7-lobed and/or occasionally pinnatifid. The capitula are generally small with ligulate rays and tubular, 5-lobed, hermaphroditic disc florets and are arranged in a lax corymb, rarely solitary. The uniseriate involucre is campanulate, with few calyculus bracts. The anthers have obtuse appendages and minutely sagittate bases and the style apices are obtuse and penicillate, with sweeping hairs. The pappus consists of delicate scabrid bristles, soon caducous, and the

receptacle is flat and epaleate. *Cineraria* is a senecioid member of the tribe Senecioneae, having balusterform filament collars in its stamens, discrete stigmatic areas and a chromosome number of 10 (Nordenstam, 1978; Bremer, 1994). Several of these distinguishing features are illustrated in Figure 1.

Molecular evidence (Chapter 2) has provided a narrowly circumscribed concept of the genus *Cineraria*. Currently 35 species are included in *Cineraria*, with the majority of these occurring in South Africa. *Cineraria* is afromontane (to afroalpine) in its affinity and ranges from the Cape Peninsula and eastern and western mountain ranges of South Africa, along the eastern highlands of Africa to Ethiopia. It is also known from the mountainous areas of Namibia and south-western Angola. One species, *C. abyssinica*, extends from Ethiopia into Yemen and Saudi Arabia and another, *C. anampoza*, is endemic to Madagascar. (A complete list of species with author citations and their distribution is provided for *Cineraria* and other genera included in the study in Table 1.)

The name *Cineraria* was first used by Linnaeus in 1763 and included 13 species, of which *C. geifolia* was listed first and subsequently used to lectotypify the genus (Jarvis *et al.*, 1993). This was a very heterogeneous assemblage and included species later transferred to *Senecio*, *Ligularia*, *Othonna*, *Felicia* and *Tephrosieris*. Lessing (1832) restricted *Cineraria* to a group of three South African species, with *C. geifolia* the only Linnaean species included.

Two treatments of *Cineraria* [De Candolle's (1838) *Prodromus systematis naturalis regni vegetabilis* and Harvey's (1865) *Flora Capensis*] formed the basis of the generic concept of *Cineraria* until this current revision. De Candolle included 22 species in *Cineraria* and listed eleven as "insufficiently known", of which only *C. erosa* is currently an accepted species in the genus. His infrageneric classification was based mainly on fruit characteristics: (i) *Hebecarpae* (cypselae hairy, not truly compressed): two species; (ii) *Blepharicarpae* (compressed cypselae with ciliate, winged margins): 16 species; (iii) *Leiocarpae* (glabrous, compressed cypselae with winged margins, radiate cypselae): three species; and (iv) *Polypterae* (glabrous cypselae, 3–4-winged in rays, radiate capitula): one species (*C. tomentosa*).

Harvey (1865) merged three of De Candolle's divisions (*Hebecarpae*, *Blepharicarpae* and *Leiocarpae*) into *Eu-Cineraria*, thereby combining both species with compressed and non-compressed cypselae, and radiate and discoid capitula. He retained *Cineraria tomentosa* on its own in *Senecioides*, and created a new division, *Othonnoides*, for *C. othonnoides* (previously *Othonna pinnatifida*). Harvey's list of species in *Cineraria* also comprised 22 species, and two doubtful ones (*C. arctotidea* and *C. humifusa*). Since then, many species have been described and recently numbered 46 (Cufodontis, 1967; Lebrun & Stork, 1997; Herman, 2003) with a number of manuscript names in circulation. Clearly a monographic treatment/revision was required (Hilliard, 1977; Jeffrey, 1986; Cron, 1991; Bremer, 1994).

Table 1. List of taxa investigated (Asteraceae, Senecioneae) and their ITS and trnL-trnF accession numbers with DNA voucher specimens where applicable, distribution. The abbreviation S.A. = South Africa. * indicates those species excluded from *Cineraria* as a result of this work.

Species	Voucher specimen and locality for species used in molecular analyses	Genbank Accessions		Distribution of Species
		ITS 1 & 2	trnL-trnF	
<i>Bolandia argillacea</i> Cron (= <i>Cineraria argillacea</i> Cron)	S.A., Western Cape, Worcester, Brandwag Peak, <i>Esterhuysen 35117</i> (BOL).	AY953924	AY952914	S.A.: Western Cape
<i>Bolandia pedunculosa</i> Cron (= <i>Cineraria pedunculosa</i> DC.; <i>Cineraria albomontana</i> Hilliard)	S.A., Eastern Cape, Witteberg, Joubert's Pass, <i>Cron & Goodman 544</i> (J).	AY953925	AY952915	Lesotho; S.A.: Western Cape, Eastern Cape.
<i>Cineraria abyssinica</i> Sch.Bip. ex A. Rich.				Ethiopia, Yemen, Saudi Arabia, Somali
<i>Cineraria albicans</i> N.E.Br.	S.A., KwaZulu-Natal, Umtamvuna Nature Reserve, <i>Cron 482</i> (J).	AY275655	AY952894	S.A.: Eastern Cape, KwaZulu-Natal
<i>Cineraria alchemillioides</i> DC.	Namibia, Nubib Mountain, Farm "Erfstuk", <i>Winter 284</i> (J).	AY953913	AY952902	Namibia; S.A.: Western Cape
<i>Cineraria anampoza</i> (Baker) Baker				Madagascar
<i>Cineraria aspera</i> Thunb.	S.A., Eastern Cape, Naude's Nek, <i>Cron & Goodman 549</i> (J).	AY275656	AY952903	S.A.: Eastern Cape, Gauteng, KwaZulu-Natal, Mpumalanga, Northern Cape, North-West, Western Cape
<i>Cineraria atriplicifolia</i> DC.				S.A.: KwaZulu-Natal
<i>Cineraria austrotransvaalensis</i> Cron	S.A., Gauteng, Linksfield Ridge, <i>Cron & McCallum 341</i> (J).	AY953920	AY952910	S.A.: Gauteng, Mpumalanga, North-West
<i>Cineraria canescens</i> Wendl. ex Link				Namibia; S.A.: Western Cape, Northern Cape
<i>Cineraria cyanomontana</i> Cron	S.A., Limpopo Province, Blouberg, <i>Cron, Knox & Winter 348</i> (J).	AY953909	AY952898	S.A.: Limpopo Province

Species	Voucher specimen and locality for species used in molecular analyses	Genbank Accessions		Distribution of Species
		ITS 1 & 2	<i>trnL-trnF</i>	
<i>Cineraria decipiens</i> Harv.				S.A.: KwaZulu-Natal
<i>Cineraria deltoidea</i> Sond. (Sn); (= <i>C. monticola</i> Hutch.)	Zimbabwe, Cashel-Chimanimani road, <i>Cron & Balkwill 528</i> (J).	AY953907	AY952896	Zimbabwe, Zambia, Malawi, S.A.: Kwazulu-Natal, Eastern Cape
<i>C. deltoidea</i> Sond. (EA); (= <i>C. grandiflora</i> Vatke)	Kenya, Aberdares, <i>Muasya, Cron & Knox 9</i> (EA, J).	AY953905	AY952893	Ethiopia, Sudan, Kenya, Tanzania, Rwanda, DRC, Uganda,
* <i>Cineraria dregeana</i> DC.				S.A.: Northern Cape
<i>Cineraria dryogeton</i> Cron				S.A.: KwaZulu-Natal
<i>Cineraria 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.)	S.A., Eastern Cape, Joubert's Pass, <i>Cron & Goodman 538</i> (J).	AY953914	AY952904	Lesotho; S.A.: Eastern Cape, Free State, KwaZulu-Natal, Mpumalanga, Limpopo Province
<i>Cineraria erosa</i> (Thunb.) Willd.				S.A.: Western Cape, Northern Cape
* <i>Cineraria exilis</i> DC.				S.A.: Northern Cape
<i>Cineraria foliosa</i> O.Hoffm.				Tanzania, Kipengere Range
<i>Cineraria geifolia</i> (L.) L.				S.A.: Western Cape
<i>Cineraria geraniifolia</i> DC.	S.A., Eastern Cape, Hogsback, <i>Cron & Goodman 563</i> (J).	AY953921	AY952911	S.A.: Eastern Cape, Kwazulu-Natal
<i>Cineraria glandulosa</i> Cron				S.A.: KwaZulu-Natal
<i>Cineraria grandibracteata</i> Hilliard				S.A.: Kwazulu-Natal
<i>Cineraria huilensis</i> Cron				Huila Province, Angola

Species	Voucher specimen and locality for species used in molecular analyses	Genbank Accessions		Distribution of Species
		ITS 1 & 2	<i>trnL-trnF</i>	
<i>Cineraria humifusa</i> L'Hér. (= <i>C. angulosa</i> Lam.)				S.A.: Western Cape
<i>Cineraria lobata</i> L'Hér. ssp. <i>lobata</i> (R; robust form)	S.A., Western Cape, Cape Point, <i>Hodgkiss C2</i> (J).	AY953904	AY952892	S.A.: Eastern Cape, Western Cape
<i>C. lobata</i> ssp. <i>lobata</i> (UN; small-leaved, jointed stem form)	S.A., Uniondale Division, near Joubertina, <i>Cron & Goodman 569</i> (J)	AY953922	AY952912	S.A.: Eastern Cape, Western Cape
<i>C. lobata</i> L'Hér. ssp. <i>soutpansbergensis</i> Cron	S.A., Limpopo Province, Blouberg, <i>Cron, Knox & Winter 344</i> (J).	AY953912	AY952901	S.A.: Limpopo Province
<i>Cineraria longipes</i> S.Moore	S.A., Gauteng, Suikerbosrand Nature Reserve, <i>Cron et al. 485</i> (J).	AY953917	AY952907	S.A.: Gauteng
<i>Cineraria lyratiformis</i> Cron	S.A., KwaZulu-Natal, Krom Ellenboog Farm, <i>Cron & Balkwill 430</i> (J).	AY953918	AY952908	S.A.: Eastern Cape, Free State, Gauteng, Northern Cape, North-West, Lesotho
<i>Cineraria magnicephala</i> Cron				Malawi: Northern Province, Mzimba District
<i>Cineraria mazoensis</i> S.Moore	Zimbabwe, Mount Hwedza, <i>Cron & Balkwill 486</i> (J).	AY953911	AY952900	Malawi, Zimbabwe, Zambia
* <i>Cineraria microglossa</i> DC. = <i>Mesogramma apiifolium</i> DC.				S.A.: Northern Cape
<i>Cineraria mollis</i> E. Mey. ex DC.	S.A., Eastern Cape, Witteberg, Joubert's Pass, <i>Cron & Goodman 545</i> (J).	AY953923	AY952913	S.A.: Eastern Cape, KwaZulu-Natal, Western Cape, Lesotho
<i>Cineraria ngwenyensis</i> Cron				Swaziland
* <i>C?. othonnoides</i> Harv. [= <i>Othonna pinnatifida</i> Thunb.]				S.A.: "Cape of Good Hope"

Species	Voucher specimen and locality for species used in molecular analyses	GenBank Accessions		Distribution of Species
		ITS 1 & 2	<i>trnL-trnF</i>	
<i>Cineraria parvifolia</i> Burt Davy	S.A., Gauteng, Witpoortjie Falls, <i>Cron & Dallas 483</i> (J).	AY953919	AY952909	S.A.: Gauteng, Limpopo Province (Wolkberg), Mpumalanga, North-West Province
<i>Cineraria pinnata</i> O.Hoffm.	S.A., northern KwaZulu-Natal, Kosi Bay, <i>Kyle 2</i> (PRE).	AY953908	AY952897	KwaZulu-Natal, Mozambique
<i>Cineraria platycarpa</i> DC.				S.A.: Northern Cape, Eastern Cape, Western Cape
<i>Cineraria pulchra</i> Cron	Zimbabwe, Nyanga, Mt. Nyangani, <i>Cron & Balkwill 500</i> (J).	AY953910	AY952899	Zimbabwe, Mozambique
<i>Cineraria saxifraga</i> DC.	S.A., Western Cape, Kirstenbosch Botanic Garden, <i>Hodgkiss C4</i> (J).	AY953916	AY952906	S.A.: Eastern Cape (Cultivated widely, including in St. Helena)
<i>Cineraria vagans</i> Hilliard				S.A.: Eastern Cape
<i>Cineraria vallis-pacis</i> Dinter ex Merxm.	S.A., Northern Cape, Olifantshoek, <i>Balkwill & McDade 11802</i> (J).	AY953915	AY952905	Botswana, Namibia, S.A.: Northern Cape, (North West?)
<i>Dendrosenecio battiscombei</i> (R.E.Fr. & T.C.E.Fr.) E.B. Knox				Kenya
<i>Dendrosenecio kilimanjari</i> (Mildbr.) E.B. Knox subsp. <i>cottonii</i> (Hutch. & Taylor) E.B.Knox	Tanzania, Mount Kilimanjaro, <i>Knox 50</i> (MICH).	AY953933	AY952923	Tanzania
<i>Emilia discifolia</i> (Oliv.) C.Jeffrey	Zimbabwe, Hwedza Mountain, <i>Cron & Balkwill 490</i> (J).	AY953930	AY952920	Zimbabwe
<i>Euryops brownei</i> S.Moore	Kenya, Aberdares, <i>Muasya, Cron & Knox 10</i> (J).	AY953936	AY952926	Kenya, Uganda
<i>Kleinia galpinii</i> Hookf.	S.A., Johannesburg, Hort. University of Witwatersrand, <i>Cron 537</i> (J).	AY953934	AY952924	South Africa, Mpumalanga

Species	Voucher specimen and locality for species used in molecular analyses	GenBank Accessions		Distribution of Species
		ITS 1 & 2	<i>trnL-trnF</i>	
<i>Oresbia heterocarpa</i> Cron & B. Nord. [= <i>C. tomentosa</i> Less.; <i>Senecio lanatus</i> L.f.]	S.A., Western Cape, Cederberg, Sneeuberg, <i>Cron et al.</i> 322 (J).	AY953935	AY952925	S.A.: Western Cape
<i>Othonna natalensis</i> Schultz. Bip.				S.A.: Eastern Cape, Gauteng, KwaZulu-Natal, Mpumalanga, Swaziland, Zimbabwe
<i>Pericallis appendiculata</i> (L.f.) B.Nord.				Canary Islands
<i>Pericallis aurita</i> (L'Hér.) B.Nord.				Madeira
<i>Pericallis multiflora</i> (L'Hér.) B.Nord.	Canary Islands, Tenerife, <i>Santos-Guerra</i> 97–9 (ORT).	AY953931	AY952921	Tenerife
<i>Pericallis murrayi</i> (Bornm.) B.Nord.	Canary Islands, El Hierro, <i>Santos-Guerra</i> 97–10 (ORT).	AY953932	AY952922	Hierro
<i>Pericallis tussilaginis</i> (L'Hér.) D.Don				Gran Canaria, Tenerife
<i>Senecio achilleifolius</i> DC.	S.A., Eastern Cape, Naude's Nek, <i>Cron & Goodman</i> 556 (J).	AY953929	AY952919	S.A.: Eastern Cape
<i>Senecio cordifolius</i> L.f. [= <i>Cineraria mitellifolia</i> L'Hér.]	S.A., Western Cape, Table Mountain, <i>Cron & Hodgkiss</i> 369 (J).	AY953926	AY952916	S.A.: Western Cape
<i>Senecio deltoideus</i> Less.	S.A., KwaZulu-Natal, Groenvlei, <i>Cron & Balkwill</i> 373 (J).	AY953927	AY952917	Malawi; Mozambique; Swaziland; Zimbabwe; S.A.: Eastern Cape, KwaZulu-Natal, Limpopo Province, Mpumalanga, Western Cape
<i>Senecio hederiformis</i> (Cron) Cron ined. [= <i>Cineraria hederifolia</i> Cron]	S.A., Limpopo Province, Blouberg, <i>Cron, Knox & Winter</i> 355 (J).	AY953928	AY952918	S.A.: Limpopo Province, Mpumalanga
<i>Tephroseris atropurpurea</i> (Ledeb.) Holub	USA, Yukon, Whitehorse, <i>Golden</i> 318 (LEA).	AF345306 AF345314		Arctic Europe, Arctic and Eastern Siberia, Bering Strait and Arctic America, Far East
<i>Tephroseris kirilowii</i> (DC.) Holub	No voucher or locality provided.		AF468164	China

Figure 1. Distinguishing features of *Cineraria*: (a) *Cineraria austrotransvaalensis*, suffrutescent habit with many radiate capitula in a lax corymb. (b) *C. longipes*, perennial herb with few capitula on long peduncles. Scale bar = 15 mm. (c) *C. deltoidea* with deltoid, palmately-veined leaves. Scale bar = 5 mm. (d) Obtuse, penicillate style apex of *C. aspera* [Cron & Goodman 551 (J)]. Scale bar = 50 μm . (e) Balusterform filament collar of *C. canescens* [Leipoldt 3274 (BOL)]. Scale bar = 100 μm . (f) *C. erodioides* var. *tomentosa* with conspicuous auricles at base of reniform leaf. Scale bar = 15.5 mm. (g) Ray cypsela of *C. vallis-pacis* [Seydel 4403 (WAG)]: obovate with distinct wing, mucilaginous trichomes have been triggered by wetting. Scale bar = 275 μm . (h) Carpopodium of *C. austrotransvaalensis* [Louw 1083 (PRE)]. Scale bar = 10 μm .

Formerly misplaced species in *Cineraria*

Both De Candolle (1838) and Harvey (1865) included five species in *Cineraria* that have recently been shown by molecular cladistic analyses and corroborated by key morphological features to belong outside of the genus (Chapter 2). These five species previously considered to be within *Cineraria* have therefore been removed and placed in neighbouring genera: *Senecio cordifolius* (previously *C. mitellifolia*), *S. hederiformis* (*C. hederifolia*), *Oresbia heterocarpa* (*C. tomentosa*), *Bolandia pedunculosa* (*C. pedunculosa*) and *B. argillacea* (*C. argillacea*). These species all possess combinations of morphological features that do not conform with distinguishing features of *Cineraria*: pinnate venation, entire/unlobed leaves, ecalyculate capitula, truncate or rounded style apices, and obscure or small carpopodia; all lack the laterally compressed, obovate cypselae so diagnostic of *Cineraria*. The exclusion of these formerly misplaced species results in a more homogenous genus according to features well correlated with the monophyletic delimitation provided by the molecular analyses.

Six other species not included in the molecular analyses also do not conform to the narrower circumscription of *Cineraria*. All of these species are known only from type collections with very vague localities: *C. exilis*, *C. microglossa*, *C. dregeana*, *C. othonnoides*, *C. purpurata* L. and *C. viscosa* L'Hér. Four of these species are included in the morphological cladistic analysis presented here; *C. purpurata* and *C. viscosa* are considered to be too far outside the senecioid group of genera included in this study, and possibly not even members of the Senecioneae.

Cineraria exilis and *C. microglossa* are both from the Gariep region of the arid Northern Cape in South Africa. *Cineraria exilis* is a perennial herb and is discoid, which was unique in *Cineraria* as previously delimited. Its leaves are pinnately veined, exauriculate, and vary in shape from oblanceolate to elliptic, occasionally with lateral pinnae near the base of the petiole (Figure 2a). *Cineraria microglossa*, a small annual herb, also has pinnately veined, exauriculate leaves, obovate to lanceolate, occasionally with lateral pinnae (Figure 2c). Although the capitula of *C. exilis* and *C. microglossa* are sparsely calyculate, the large number of involucre bracts (17–20 in *C. exilis* and about 15 in *C. microglossa*) is uncharacteristic for *Cineraria* and the bracts are much narrower. The disc cypselae of both *C. exilis* and *C. microglossa* appear to be three- (outer) to four-angled (inner), not distinctly compressed nor margined or winged, and more oblong to elliptic than obovate, as is typical of *Cineraria*.

Cineraria dregeana also has pinnately veined leaves, which are sessile and amplexicaul, not auriculate (Figure 2b). Although *C. dregeana* was originally reported to be discoid (De Candolle, 1838; Harvey, 1865), examination of the type revealed reduced, not absent, ray florets. Its cypselae are elliptic to fusiform in shape and appear four-sided, lacking a clear

carpopodium. Thus all three species, *C. exilis*, *C. microglossa* and *C. dregeana*, lack various diagnostic features of *Cineraria* and need to be reassigned elsewhere in the Senecioneae¹.

The three other dubious species are even more clearly misplaced in *Cineraria*. “*C. othonnoides*” has “rufo-pubescent” cypselae (Harvey, 1865), ecalycylate capitula, sessile pinnati-partite leaves and ebracteolate peduncles (Figure 2d). Neither does it belong in *Othonna*, its previous designation (*O. pinnatifida*), as its involucre bracts are not connate and its leaves are not succulent. The expanded apex of the peduncle is “minutely dentate, the teeth alternating with the involucre scales” (Harvey, 1865), an unusual feature in the Senecioneae.

The species designated as *Cineraria purpurata* (Figure 3a) has purple ray florets, a bi/triseriate involucre, an atypical, very bristly pappus, pinnately-veined, exauriculate leaves and clearly does not belong within *Cineraria*. A handwritten note on the specimen suggests it may be in the Anthemidae. As *C. purpurata* is possibly not even a member of the Senecioneae, it is excluded from this study.

Cineraria viscosa, the other dubious inclusion in *Cineraria*, has solitary, ecalyculate capitula (Figure 3b) and although its cypselae appear to be compressed, this is very likely a function of their immaturity. Its cypselae are also not distinctly margined, nor are they obovate - very diagnostic characters for *Cineraria*. The disc style apex is truncate to slightly rounded (Figure 3c), as opposed to the penicillate/obtuse apex in *Cineraria*. Examination of its stamens revealed that the filament collars are not at all balusterform (dilated) and it is therefore possibly not even a senecioid member of the Senecioneae (Figure 3d). As its subtribal affinities are unclear, *C. viscosa* is also excluded from this study.

Previous cladistic studies

Morphological cladistic studies within the Asteraceae have mainly been at the tribal and generic levels (e.g. Bremer, 1987; Karis *et al.*, 1992; Karis, 1993; Bremer, 1994; Urtubey & Stuessy, 2001; Breitweisser & Ward, 2003). Fewer studies have investigated species relationships within a genus or genera using morphological and/or anatomical characters (e.g. Funk, 1982; Keeley & Turner, 1990; Freire *et al.*, 1993; Katinas & Crisci, 2000; Swenson & Manns, 2003) with varying degrees of success. Molecular data have given additional, potentially more reliable means for investigating species relationships in genera (examples in the Asteraceae include Funk, 1982; Bain & Jansen, 1995; Torrell *et al.*, 1999; Bain & Golden, 2000; Pelser *et al.*, 2002). There appear to be few combined morphological and molecular cladistic studies at the species and/or genus level in the Asteraceae (e.g. Esselman & Crawford, 1997; Swenson & Manns, 2003).

¹*Cineraria microglossa* has since been identified as the monotypic *Mesogramma apiifolium* DC., a genus resurrected by Nordenstam and Pelser (2005).

Figure 2. Type specimens of species not conforming to generic characters of *Cineraria* and erroneously placed in *Cineraria*: (a) *C. exilis* [Burchell 2274 (G-DC)]. Scale bar = 12.3 mm (b) *C. dregeana* [Drége 2717 (G-DC)]. Scale bars = 11 mm; inset: 9 mm. (c) *C. microglossa* [Drége 5926 (iso, K)] Scale bar = 11 mm. (d) *C. othonnoides* [Thunberg 29801 (UPS-THUNB)]. Scale bar = 15.2 mm.

Figure 3. (Overpage) (a) *Cineraria purpurata*, type from Linnaean Herbarium in London. Scale bar = 11.2 mm. (b–d) *C. viscosa*: (b) Type specimen Hort Kew 1778 (BM). Scale bar = 13 mm. (c) Truncate style apex. (d) Narrow, undilated filament collar. Scale bars b, c: 100 µm. (*C. viscosa* photographed and images used with permission, Natural History Museum, London.)

A preliminary morphological cladistic analysis of *Cineraria* (Cron, 1991) using only 18 characters and including 24 South African species with *Pericallis* as the outgroup proved inconclusive in resolving species relationships. The study was also not ideal in that data for some species were obtained from the literature and photographs/microfiche illustrations of type specimens. Nevertheless, several characters were revealed as being useful, namely, leaf shape, presence/absence of certain trichomes and the size and number of capitula and length of peduncles.

Separate and combined cladistic analyses using DNA sequence data for plastid (*trnL-trnF*) and nuclear (ITS) regions clearly indicated the monophyletic nature of 22 samples of *Cineraria*, well correlated with the suite of diagnostic morphological features outlined above (Chapter 2). It was not possible to include all species then considered to be within *Cineraria* in the analyses, nor did the amount of variation in the sequenced regions warrant further investigation. The molecular data nevertheless provide a framework for elucidating species relationships using the morphological data and identified *Bolandia* as the sister group to *Cineraria*, within the limited sampling of Senecioneae in the study.

Combining multiple data sets

As the practice of applying multiple data sets to a common group of taxa has become increasingly widespread, it has become evident that phylogenetic incongruence is quite common (Wendel & Doyle, 1998). Phylogenetic incongruence, if significant, may inform us of various evolutionary processes in the history of the organisms/genes. On the other hand, incongruence may be simply due to “technical causes” such as insufficient data, gene choice, sequencing error and taxon sampling (Wendel & Doyle, 1998). “Soft incongruence” or insignificant incongruence (Seelanan *et al.*, 1997) may be attributed to weak support of certain clades and/or to alternative topologies being only slightly less parsimonious OR an absence of sufficient signal, rather than fundamentally different evolutionary histories. In contrast, “hard incongruence” (statistically supported) may be due to many and varied evolutionary processes (Seelanan *et al.*, 1997).

Different character sets may have different underlying evolutionary histories (Wendel & Doyle, 1998) and there has been considerable debate regarding the optimal modes of analysis of data over the last few decades with no clear consensus on the issue (Miyamoto, 1985; Hillis, 1987; Kluge, 1989; Barret *et al.*, 1991; Doyle, 1992, Bull *et al.*, 1993; de Queiroz, 1993; de Queiroz *et al.*, 1995, Miyamoto and Fitch, 1995). Two general approaches have been advocated for gaining an overall estimate of phylogeny based on two or more data sets, viz. the consensus approach and the combined approach, both with their limitations and advantages (de Queiroz, 1993). The combined approach is used here, but separate morphological analyses and all possible combinations of the molecular and morphological data are investigated. Possible causes of incongruence and areas of consensus are discussed.

Weighting of characters in cladistic analyses has also been a controversial subject /procedure, with *a priori* weighting being judged as either totally subjective and biased (e.g. Mayr, 1969; Sneath and Sokal, 1973) or as the only rational and noncircular approach to weighting (Neff, 1986). *A posteriori* weighting of characters is generally accepted as the more acceptable form of weighting if preliminary cladistic analyses are unresolved due to character conflict or homoplasy (Neff, 1986). The successive weighting method of Farris (1969) is commonly used in morphological analyses (e.g. Breitweiser & Ward, 2003) and is based on successive weighting of those characters found to be consistent with the shortest trees found in each successive analysis.

Aims of the study

The aims of this study were therefore to use morphological cladistic analyses separately and combined with molecular data to: (1) Confirm the monophyly of the genus *Cineraria* as it is now more narrowly conceived; (2) Assist in the placement of those species not conforming to the narrower generic circumscription of *Cineraria*; (3) Investigate the putative sister relationship of *Bolandia*; (4) Investigate species relationships and character evolution within *Cineraria*; and (5) Explore the usefulness of various morphological characters in cladistic analyses at generic and specific levels within the Senecioneae.

MATERIALS AND METHODS

Taxa

All 35 species of *Cineraria* (Chapter 6) were included in the morphological aspect of this study, plus four of the dubiously placed species: *C. dregeana*, *C. exilis*, *C. microglossa* and *C. othonnoides*. Two subspecies (including two forms) of *C. lobata* (a very variable species) and two samples of *C. deltoidea* (from populations in East Africa and southern Africa) were included in combined morphological and molecular data sets to assist in interpreting their status and relationships.

Selected members of related African senecioid genera were included to help place those species that fall outside a revised delimitation of *Cineraria*. The same senecioid genera and species used in the molecular analyses (Chapter 2) were included here to facilitate combination of the molecular and morphological data sets, as well as the five species excluded from *Cineraria* by the molecular studies, viz. *Bolandia pedunculosa*, *B. argillacea*, *Oresbia heterocarpa*, *Senecio cordifolius* and *S. hederiformis* (Table 1). Two species of *Dendrosenecio* (Hauman ex Hedb.) B.Nord. and five of *Pericallis* D.Don were included as previous authors/studies have suggested a close relationship between these two genera and *Cineraria* (Nordenstam, 1978; Knox & Palmer, 1995; Panero *et al.*, 1999). In addition, *Othonna natalensis* was included in the morphological study. *Tephroseris* Reichenb., a cacalioid genus, was used as the designated outgroup. *Tephroseris* was first published as a

section of *Cineraria* in Reichenbach's '*Flora Germania Excursoria*' in 1831. The genus has a number of cacalioid features, including cylindrical filament collars, a continuous stigmatic area, ecalyculate involucre and a base chromosome number of $x = 24$, probably derived from $x = 30$, in contrast to the base chromosome number of 10 found in the senecioid genera (Nordenstam, 1978). *Tephroseris* is therefore placed in the Tussilagininae (Jeffrey & Chen, 1984; Bremer, 1994) or less formally in the tussilaginoid group (Jeffrey 1992).

Data Sampling

Field observations and collections of 27 of the 35 species of *Cineraria* were made, as well as for four of the five species now excluded from the genus and all of the senecioid taxa. Types of all species of *Cineraria* (and the erroneously placed ones) were examined and a large range of specimens were borrowed from the following herbaria: BM, BOL, BR, E, GRA, K, LISC, MO, NBG, NH, NU, PRE, SAM, TCD and WAG. Types and other collections were examined on site at BM, G-DC, K, LINN, P, S and UPS. Measurements of macromorphological features (habit, stems, leaves, capitula, florets and cypselae) and notes were made from as wide a geographic range as possible for each species, covering the spectrum of variation for the variable, widespread species and for all specimens in species of limited distribution. (Selected voucher specimens are listed in Appendix 1. A more complete list of specimens examined is provided as species citations in Chapter 6.)

Micromorphological features (stamens and styles, trichomes on florets and floret surfaces) were examined and measured for at least three ray and three disc florets per specimen and at least three specimens per species (except in cases where only the type species is known or was available, and in some outgroup species). Florets were softened in warm water with a wetting agent (e.g. Windowlene) and dissected under a Nikon SMZ1500 dissecting microscope. Dissected florets were mounted in Hoyer's solution (Anderson, 1954) and viewed under an Olympus BH2 compound microscope. They were digitally photographed and further analyses and measurements were executed on enlarged computer generated images.

To facilitate microscopic examination of trichomes, leaves were autoclaved in 80% ethanol for 15 minutes at 104 kPa and cleared in 1% KOH (for a further 15 minutes at 104 kPa) and bleached for approximately 5 minutes in household bleach according to the technique described by O'Brien and von Teichman (1974). Leaves or portions of leaves were mounted in 1% glycerol and coverslips sealed with lacquer.

Scanning electron microscopy (SEM) was used to examine certain leaf trichomes in detail, especially those fine trichomes comprising cobwebby and/or woolly indumenta, and also to examine mature ray and disc cypselae for surface features such as epicarp cell shape, surface ornamentation and size of carpopodium. Fresh leaves were fixed for four hours in 1% glyceraldehyde in 0.1 M Na-cacodylate buffer at pH 7.2, washed in three changes of buffer for

90 minutes and dehydrated in a graded alcohol series. Leaves from pressed specimens and cypselae (if necessary) were simply dehydrated in the graded alcohol series. All leaves and cypselae were then critical point dried and coated with carbon and gold palladium and viewed and photographed on a JEOL JSM 800 SEM. (Voucher specimens for micromorphological features, for light microscopic and SEM examination of leaf trichomes and for cypselae examined under the SEM are included in Appendix 1.)

For related senecioid genera, characters were scored for the species sampled for the molecular data (Chapter 2), viz., *Dendrosenecio battiscombei*, *D. kilimanjari* spp. *cottonii*, *Emilia discifolia*, *Euryops brownei*, *Kleinia galpinii*, *Pericallis murrayi*, *P. multiflora*, *Senecio achilleifolius* and *S. deltoideus*. In addition, *Pericallis appendiculata*, *P. aurita*, *P. tussilaginis* and *Othonna natalensis* were sampled and scored. The scoring for the outgroup *Tephroseris atropurpurea* was based on descriptions (Ledebour, 1814; Schischkin & Bobrov, 2000) and an illustration (Schischkin & Bobrov, 2000, p. 720, Plate 35), although herbarium specimens of other species of *Tephroseris* were examined for micromorphological features. Scanned images of specimens of *Pericallis murrayi* and *P. multiflora* were examined to supplement information obtained from the literature (L'Héritier, 1788; Willdenow, 1803; Swenson & Manns, 2003).

Character selection and coding

Fifty-four morphological characters were included in the analysis and the majority (35/54 or 65%) were coded as multistate. A list of the morphological characters and their states is provided in Table 2. All characters were coded as unordered. Variation within a species was captured by coding the species as polymorphic where necessary (i.e. where two or more states commonly occur), although PAUP does not really support a polymorphic population genetics concept. It assumes that polymorphisms are independently derived, i.e. that the ancestral lineages are all monomorphic (Swofford, 2001).

Characters were selected on the basis of their usefulness in distinguishing the various genera included in the study, as well as species of *Cineraria*. Many of these characters are those referred to in brief descriptions of the senecioid genera (e.g. in Bremer, 1994) and in keys (e.g. in Hilliard, 1977). Jeffrey's (1987) list of characters also provided a useful foundation for the development of the set of characters. These were modified according to observations of the taxa included in this study.

'Natural' disjunctions observed in the characters were used as far as possible, but to some extent traditional groupings (e.g. 5–8 ray florets vs. 8–13 ray florets in *Cineraria*) were applied. In a few cases, perceived differences were abandoned because of too much overlap across the ranges in certain species (e.g. lengths of appendages and filament collars in *C. geraniifolia* and *C. erodioides*). Character numbers are given in brackets in the following descriptions.

Table 2. Morphological Characters for cladistic analysis.

Habit and phyllotaxy:

1. Habit: Annual or short-lived perennial herb (0); Long-lived perennial herb (1); Woody perennial shrublet (2); Pachycaul tree (3).
2. Phyllotaxy: Leaves more or less evenly distributed along stem (0); Crowded at base/rosulate (1); Crowded at tips of stem (2).

Leaves:

3. Leaf venation: Pinnate (0); Palmate (1).
4. Leaf attachment: Petiolate (0); Pseudopetiolate/with decurrent base (1); Sessile (2).
5. Leaves: Exauriculate (0); Amplexicaul base (1); Auriculate (2); Petiole widens at base (3).
6. Upper leaf shape: Reniform (0); Lyratiform (1); Deltoid (2); Deltoid-reniform (3); Linear (4); Ovate to lanceolate (5); Obovate to oblanceolate (6); Elliptic (7).
7. Middle to lower leaf shape (outline): Reniform (0); Lyratiform (1); Deltoid (2); Deltoid-reniform (3); Linear (4); Ovate to lanceolate (5); Obovate to oblanceolate (6); Elliptic (7).
8. Leaf dissection: Entire, not lobed (0); 5–7-lobed (1); Pinnatifid (2).
9. Dissection of apical leaf lobes: Not dissected (0); Dissected from the apex (1).
10. Leaf margin: Entire (0); Dentate (1); Denticulate (2); Serrate (3); Crenate (4).
11. Upper leaf base: Cuneate to attenuate (0); Cuneate to truncate (1); Truncate to subcordate (2); Subcordate to cordate (3); Distinctly cordate (4); Rounded (5).
12. Lower leaf base: Cuneate to attenuate (0); Cuneate to truncate (1); Truncate to subcordate (2); Subcordate to cordate (3); Distinctly cordate (4); Rounded (5).
13. **Glandular** leaf trichomes: Glandular with a uniseriate stalk and single-celled gland (0); Glandular with a uniseriate stalk (6–10 cells long) and multi-celled gland (walls horizontal) (1); Glandular with a uniseriate stalk (12–16 cells long) and multi-celled gland (walls horizontal and vertical) (2); Glandular with a multiseriate stalk (3); Glandular hairs absent (4).
14. **Eglandular** leaf trichomes (short), without distinct basal cells and without elongated apical cells forming a long wisp: Hair cells uniform, short, not tapering much towards the apex, apex rounded, (6–7 cells long) (0); Hairs cells uniform, short, tapering towards the apex, apical cell rounded (6–8 cells long) (1); Hair cells more or less uniform, tapering towards the apex, 12–16 cells long (cells sometimes collapsing when dry) (2); Eglandular hairs of this type absent (3).
15. **Eglandular** leaf trichomes (long), with **broad, tapering basal cells**, and with elongated cells forming an apical wisp: Hair tapering towards the apex, 4–6 granular basal cells, tipped by elongated cells forming a long apical wisp (0); Hair tapering gradually towards the apex, ca. 14 cells, tipped by 4–6 (–many) elongated cells forming an apical wisp (1); Hair tapering towards the apex, 4–6 agranular basal cells, tipped by many elongated cells forming an apical wisp (2); Trichomes of this type absent (3).
16. Woolly leaf trichomes, with 2–4 **distinct narrow agranular basal** cells and a long unicellular or multicellular appendage, **perpendicularly attached**: Appendage multicellular, attached end cell rounded (0); Appendage unicellular or multicellular, end cell rounded (1); Appendage multicellular, apical cells stiff, end cell sharply pointed (2); Trichomes of this type absent (3).
17. Woolly leaf trichomes, with 2–4 **distinct narrow agranular basal** cells and a long unicellular or multicellular appendage, **obliquely attached**: Appendage multicellular, end cell rounded (0); Trichomes of this type absent (1).

18. Woolly leaf trichomes with narrow **granular** basal cells, with multicellular appendage of agranular cells, transversely attached: Granular basal cells (3–4) tapering towards the apex, first basal cell appressed to epidermis (0); Two to three basal cells of decreasing diameter, first cell not appressed to epidermis (1); Trichomes of this type absent (2).

19. Capitulescence: Solitary (0); Few capitula: 2–4, rarely 6 (1); Multi-headed cymose or corymbose panicle [> 8 heads] (2); Pyramidal paniculate synflorescence (3).

20. Peduncles: Bracteolate (0); Ebracteolate (1).

21. Peduncles (length): Short: < 70 mm (0); Long: 80–300 mm (1).

Capitula

22. Capitula: Radiate (0); Discoid (1).

23. Capitula: Homogamous (0); Heterogamous, ray or marginal florets female, disc florets bisexual (1); Heterogamous, outer florets female, inner male (2).

24. Corolla colour: Yellow (0); Orange (1); White, pink, and/or purple (2).

25. Number of rays: 5–8 rays (0); 8–13 rays (1); > 13 rays (2).

26. Ray tube: limb ratio: Tube $>$ limb (0); Tube equal to limb (1); Tube $<$ limb (2).

27. Glandular hairs on ray corolla tube: Absent (0); Present (1).

28. Eglandular hairs on ray corolla tube: Absent (0); Present (1).

29. Ray limb upper epidermis: Smooth (0); Papillose (1).

30. Number of Disc florets per capitulum: Few: < 20 (0); 21–45 (1); 50–100; > 100 (3).

31. Involucre: Calyculate (0); Ecalyculate (1).

32. Involucral bracts: Free (0); Connate to a third or half the length (1).

33. Series of involucral bracts: Uniseriate (0); Biseriate (1).

34. Involucral bract indumentum: Glabrous (0); Glabrous, but cobwebby amongst calyculus bracts (1); Cobwebby to tomentose, glabrescent or persistent (2); Multicellular eglandular tapering hairs (no long wisp) (3); Uniseriate glandular hairs (4); Multiseriate glandular hairs (5); Multicellular jointed hairs, eglandular, with long wisp (6).

35. Number of involucral bracts: 6–8 (0); 8–13 (1); 12–13 but never as few as 8 (2); 14–20 (rarely more) (3).

Pappus:

36. Presence and duration: Present on all florets, persistent (0); Present on all florets, caducous (1); Absent from ray florets, caducous (2).

37. Series of rows of cells: Biseriate (0); Pluriseriate (1).

Stamens:

38. Anther bases: Rounded (0); Minutely sagittate [≤ 75 μ m long] (1); Shortly tailed [≥ 100 μ m long] (2).

39. Filament collar: Cylindrical or very slightly widening towards the base (0); Balusterform - distinctly enlarging towards the base (1).

Styles:

40. Style branches (of perfect florets): Truncate (0); Rounded (1); Obtuse to cone tipped (2); Acute to acuminate (3).

41. Stigmatic areas on style branches (of perfect florets): Continuous (0); Two discrete regions (1).

Cypselae:

42. Ray Cypselae: Not compressed (0); Slightly compressed laterally (1); Distinctly compressed laterally (2).

43. Ray Cypselae Rib Number: 1–4 (0); 5 or 6 [–8] (1); [8–] 10 or more (2); Ribs absent (3).
44. Ray Cypselae extensions: Not margined or winged (0); Two distinct margins or narrow wings (1); Two broad wings (2); Four wings (3).
45. Cypselae trichomes: None (0); Duplex (1).
46. Disc Cypselae: Not compressed (0); Slightly compressed laterally (1); Distinctly compressed laterally (2).
47. Disc Cypselae Rib Number: 1–4 ribs (0); 5 or 6 ribs (1); [8 –] 10 ribs (2).
48. Disc Cypselae extensions: Not margined or winged (0); Two distinct margins/ wings (1); Two broad wings (2).
49. Cypsela shape (outline): Oblong to elliptic/fusiform (0); Obovate (1).
50. Ray cypsela epicarp shape: Rectangular (long with straight ends) (0); Oblong-elongate (rounded ends) (1); Square or subisodiametric to shortly rectangular (2).
51. Disc cypsela epicarp shape: Rectangular (long with straight ends) (0); Oblong-elongate (rounded ends) (1); Square or subisodiametric to shortly rectangular (2).
52. Cypsela surface ornamentation: Smooth (including papillate or sunken and ridged) (0); Striate (1).
53. Carpopodium: Absent or obscure/small (2/3 rows of cells or less) (0); Medium: 4–6 rows of cells (1); Large: 7–10 (–14) rows of cells (2).

Receptacle:

54. Receptacle shape: Flat to slightly rounded (0); Convex to conical (1).
-

Habit (1) and phyllotaxy (2)

The growth form in *Cineraria* typically a perennial herb or subshrub, with only a few species being clearly annual or short-lived perennials. Long-lived perennial herbs have some wood development at the base of the stem, but it is not as extensive as in a subshrub, which is also more branched than the herb. For example, *C. deltoidea* was classified as a perennial herb as it has slender stems and a climbing/straggling habit, even though it may become slightly woody towards the base.

Leaves (3–18)

Leaves offer a large number of characters useful for distinguishing both senecioid genera included in the study and species within *Cineraria*. Leaf shape (6, 7) is particularly useful in distinguishing species in *Cineraria* and is often used in keys (Hilliard, 1977; Cron, 1991; Cron 2005: Chapter 6). Two shapes and variations of these shapes predominate in *Cineraria*, deltoid and reniform. Leaf shape may vary according to the position on the stem, hence the distinction between the upper leaves (6) and middle to lower leaves (7). Leaf shape also varies to some degree within a population and species were coded as polymorphic where two or more states commonly occur (as noted above).

Dissection of the leaf (8) into 5–7 lobes is a common feature in *Cineraria*, and upper leaves tend to have one or two lateral pinnae, but these leaves were not considered pinnatifid unless the lamina itself was further divided. Certain species exhibit further dissection of the apex of the main lobes of the leaves (9), creating a characteristic ‘frilly’ appearance (Figure 4a). In addition,

the margins (10) of all *Cineraria* leaves are dentate to some degree. Leaf base (11, 12) was coded for upper and lower leaves separately because of the variation in leaf shape along the length of the stem in most species of *Cineraria*. The five states used were based on observations of the leaves and application of traditional terminology to these shapes/bases (illustrated in Figure 4b).

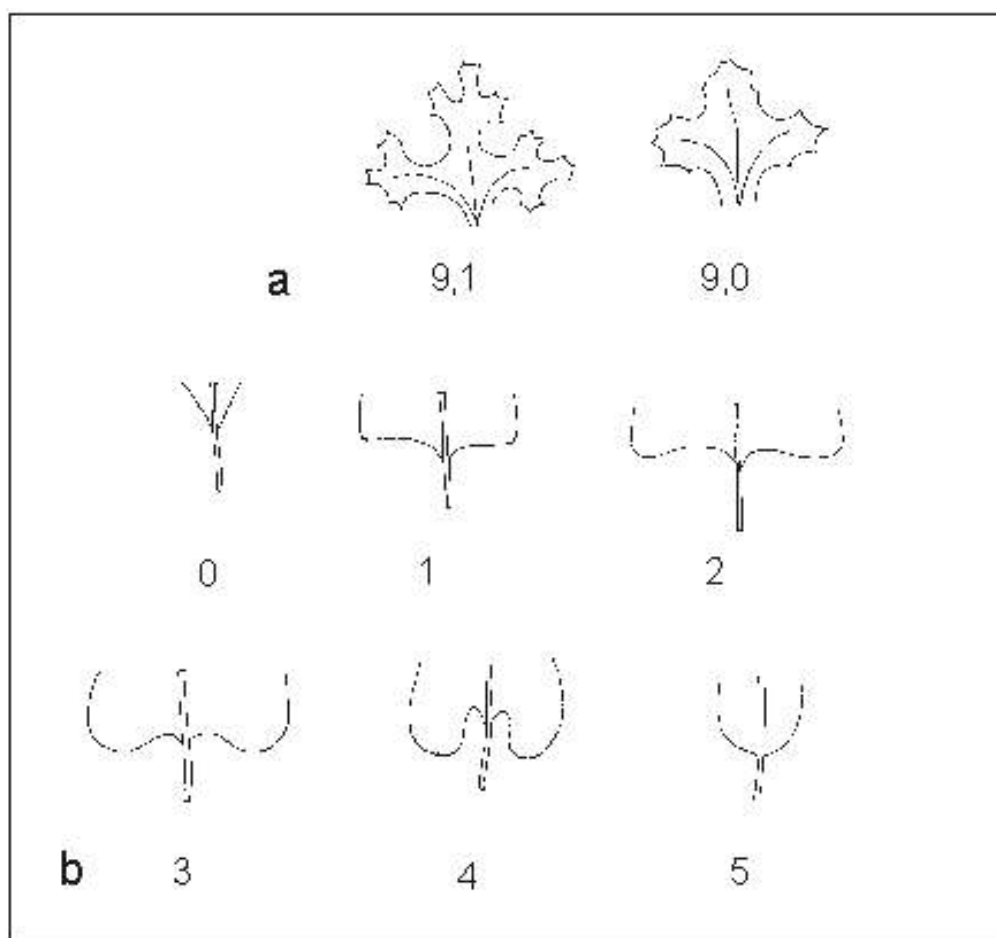


Figure 4. (a) Dissection of leaf lobes (Character 9): (9,1) Lobes dissected; (9,0) Lobes not further dissected. (b) Leaf bases (Characters 11 and 12): (0) Cuneate to attenuate; (1) Cuneate to truncate; (2) Truncate to subcordate; (3) Subcordate to cordate; (4) Distinctly cordate; (5) Rounded.

Six categories/types of leaf trichome were identified for use in the cladistic analyses: glandular (13), relatively short eglandular hairs (14), and four types of long eglandular hairs (15–18) creating a cobwebby or woolly appearance (illustrated in Figures 5–7). Some of these trichomes corresponded to those described by Drury & Watson (D&W; 1965): short eglandular (D&W7 = 14,1), long tapering (D&W5 = 15,2; D&W8 = 15,0) and long with narrow basal cells (D&W1 = 16,0; D&W3 = 17,0). The size and shape of the basal cells provided the basis for distinction between the types of trichomes, as well as their granular/agranular surfaces. Although persistence and extent of indumentum vary considerably within a species in *Cineraria*, even sometimes within a single population (Cron, 1991), the hair types are fairly fixed and this is an extremely useful character in distinguishing species. In a few large variable species (e.g. *C. erodioides*, *C. lobata* and *C. deltoidea*), trichomes vary according to geographical regions and the species were coded as polymorphic for these characters. *Senecio achilleifolius* was the only species to possess a branched trichome, so this seventh type of trichome was excluded from the data set (and is not included in the character list).

Figure 5. (Opposite) Trichomes types in *Cineraria* and other Senecioneae included in this study:

(a) **Glandular trichomes** (Character 13): (0) Uniseriate with undivided apical gland cell; (1) Uniseriate with multicellular gland (horizontal walls); (2) Uniseriate with glandular region divided horizontally and vertically; (3) Multiseriate stalk to gland.

(b) **Short eglandular trichomes** (Character 14): (0) 6–8 cells, not tapering, with rounded apical cell, (14,0); (1) 6–8 cells, tapering; (2) 12–16 cells, broadly tapering, cells may collapse in alternate directions when dry.

(c) **Long woolly trichomes** with multi-celled tapering bases and multi-celled apical wisps (Character 15): (0) 4–6 basal cells, granular; (1) Multicelled, gradually tapering, agranular; (2) 4–6 basal cells, agranular.

(d–f) **Long woolly trichomes with narrow basal cells and long multi-celled apical wisp:**

(d) Apical wisp joined perpendicularly to agranular basal cells (Character 16): (0) 2–4 basal cells, “soft” long wisp, rounded apex; (1) 4 basal cells, apical cells stiff/firm, apex sharply acute.

(e) Apical cells attached obliquely to agranular basal cells (Character 17): (0) 2–4 agranular basal cells.

(f) Apical cells joined perpendicularly to granular basal cells (Character 18): (0) basal cell appressed to leaf surface of similar size; (1) 2–3 granular basal cells, decreasing in size towards apex.

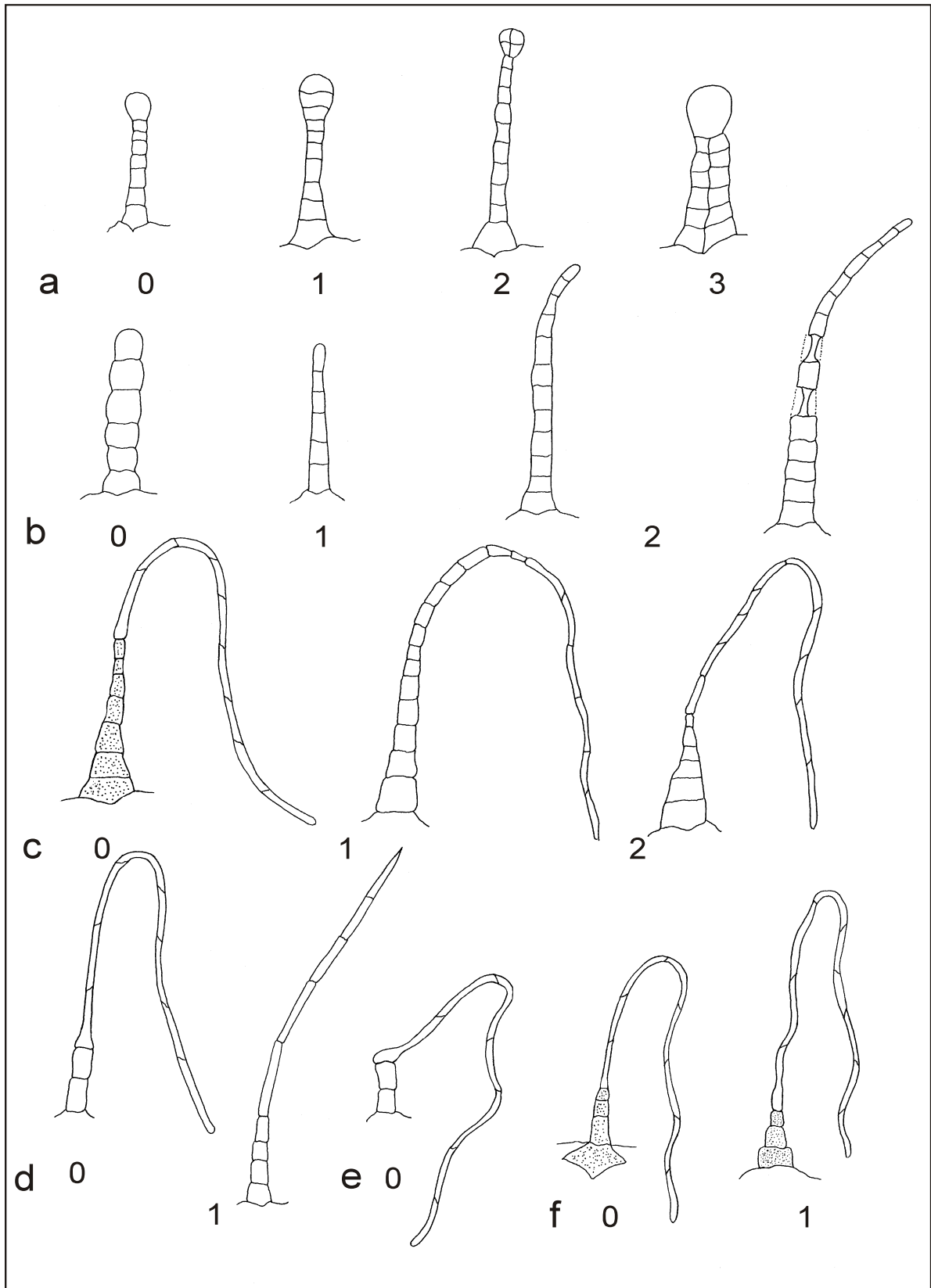


Figure 6. Glandular and short (non-woolly) eglandular trichomes (characters 13 and 14) in *Cineraria*: (a) Uniseriate glandular trichome (13,1) on leaf margin of putative hybrid *C. glandulosa* X *C. atriplicifolia* [Cron & Scott Shaw 9 (J)], LM. Scale bar = 15 µm. (b) Uniseriate glandular trichome (13,1) on ventral surface of leaf of *C. erodioides* [Cron & Wilson 217 (J)], SEM. Scale bar = 10 µm. (c) Eglandular trichome (14,1) on ventral surface of leaf of *C. ngwenyensis* [Cron et al. 308 (J)], SEM. Scale bar = 15 µm. (d) Eglandular trichome (14,1) on ventral surface of leaf of *C. geraniifolia* [Cron & Ching 2 (J)], LM. Scale bar = 10 µm. (e) Eglandular trichome (14,2) on ventral surface of *C. geraniifolia* [Cron & Ching 2 (J)], LM. Scale bar = 16 µm. (f) Eglandular trichome (14,2) on ventral surface of leaf of *C. geifolia* [Cron 314 (J)], SEM. Scale bar = 100 µm.

Figure 7. (Overpage) Scanning electron micrographs of eglandular long woolly trichomes (Characters 15, 16, 17 and 18) in *Cineraria*: (a) Tapering basal cells, granular, long multicelled apex (15,0) on dorsal surface of leaf of *C. austrotransvaalensis* [Cron 20 (J)]. (b) Tapering, multicelled base, agranular, with long multi-celled apex (15,1) on ventral surface of *C. deltoidea*, Hedberg 1496 (K). (c) Tapering basal cells, agranular, with long multi-celled apex (15,2) on dorsal surface of leaf of *C. mazoensis* [Bayliss 10147 (MO)]. (d) Narrow 2–4-celled base, with long multi-celled apex (16,0) on ventral surface of leaf of *C. pulchra* [Cron & Balkwill 459 (J)]. (e, f) Narrow 2–4-celled base, with obliquely attached multi-celled long apex (17,0) on ventral surface of *C. albicans* [Cron 4 (J)]. (g) Granular base, first cell appressed to leaf surface, with long multi-celled apex (18,0) on ventral surface of *C. austrotransvaalensis* [Cron 20 (J)]. (h) Granular basal cells with long multi-celled apex (18,1) on ventral surface of *C. alchemilloides* [Mauve & Hugo 36 (PRE)]. Scale bars: a, c, e, f: 10 µm; b, d: 100 µm.

Capitula (19–24)

The number of capitula (19) is generally a useful character in distinguishing species of *Cineraria*. Long peduncles (21) are commonly associated with solitary capitula, but are also sometimes present on those species of *Cineraria* with few capitula. Only *Emilia discifolia* and *Euryops brownei* have ebracteolate peduncles (20). All ‘true’ *Cineraria* species have radiate capitula (22) and are heterogamous (23) with female ray florets and bisexual disc florets. Amongst the genera included in this study, only *Pericallis* has the cacalioid feature of non-yellow flower colour (24).

Florets (25–31)

Traditionally, species of *Cineraria* have been grouped or keyed out as those with 5–8 ray florets and those with 8–13 rays or more (Hilliard, 1977). Although there is some overlap in these numbers, they form useful categories as those with 5–8 rays never have more than 8, and those with 8–13 rays never have fewer than 8. All species of *Cineraria* possess pluriseriate glandular hairs (27) on the tube of the ray floret, near the base of the limb. A few species also sometimes have eglandular hairs (28). The papillate upper epidermis of the ray limb (29) is a unique apomorphy for *Pericallis*. In contrast, the apices of the disc corolla lobes were observed to be papillate in all species examined and therefore did not provide a useful cladistic character.

The number of disc florets (30) reflects the size of the capitula and numbers are based on perception of small, medium, large and very large capitula. The degree of constriction of the disc floret tube was investigated as a potentially useful character, but was found to vary considerably within a species of *Cineraria* and also within a capitulum depending on degree of maturity. Hilliard (1989) noted the presence of dark reddish-brown median veins in the disc floret corolla lobes to be a useful character in delimiting species of *Cineraria* and Jeffrey (1987) includes it in his proposed list of useful characters for Senecioneae. However we found it to be an unreliable feature among species of *Cineraria*.

Involucre (31–35)

The involucre bracts of core/true species of *Cineraria* are free (32) and are subtended by a calyculus (31). *Dendrosenecio* has a biseriate involucre, while that of *Euryops brownei* is “subuniseriate to subbiseriate” (Nordenstam, 1968). The presence and type of indumentum on the involucre (34) is a useful feature within *Cineraria*, although the persistence of the cobwebby or tomentose indumentum is not. The number of involucre bracts (35) is another feature reflecting the size of the capitulum. Although it is well correlated with the number of rays, it is not consistently correlated (i.e. is independent) and was found to be of some use in resolving the species relationships in *Cineraria*.

Pappus (36–37)

Only *Kleinia galpinii* and *Othonna natalensis* possess a persistent, pluriseriate pappus amongst the genera included in this study. The rest of the genera, including *Cineraria*, have a caducous pappus, although it is absent from the ray florets of some species of *Pericallis* in the study.

Stamens (38–39)

Anthers of all species of *Cineraria* possess minutely sagittate bases (Figures 1e; 8a), but some of the senecioid genera/species included in the study have shortly tailed bases. Anther bases (38) were considered to be shortly tailed if the ‘tails’ exceeded 100 μm , e.g. *Oresbia heterocarpa* (Figure 8b). Anther appendages are ovate in *Cineraria* (Figure 8c) and all the other senecioid genera included in the study, so this was not a useful character. Differences in terms of anther appendage length and their length to width ratios were investigated for potential use in the cladistic analysis, however there were no clear disjunctions in the measurements obtained and the character was not included in the final analyses. An exception was *Kleinia galpinii*, which had unusually long anther appendages (0.75–1 mm long; Figure 8d).

Endothelial thickening of the anther lobes is polarised in the tussilagionoid outgroup *Tephroseris* and radial in the senecioid genera, although a transitional state has been reported for *Dendrosenecio* and *Pericallis* (Nordenstam, 1978). However, it was observed that the endothelial thickening became more polarised towards the edges of the lobes of many of the species and genera examined and the transitional state was difficult to distinguish. The character was also uninformative in preliminary parsimony analyses and was excluded.

Filament collars (39) are reportedly balusterform (dilated) for all the senecioid genera included in this study, however, they are barely dilated in *Euryops brownei* and *Othonna natalensis* and not at all in *Kleinia galpinii* (Figure 8e–g). They are markedly dilated in all other genera studied here, including *Cineraria* (Figure 8a) and *Bolandia*.

Styles of disc florets (40–41)

The shape of the disc style apex (40) appears to be very useful at a generic level and certainly serves to delimit *Cineraria* from most other senecioid genera as it has an obtuse/cone-tipped style apex with sweeping hairs surrounding it (Figures 1d, 9a–j). Both *Kleinia galpinii* and *Othonna natalensis* have appendaged style branch apices, however, it is the style of the disc floret of *Kleinia* (Figure 9h, i) that is appendaged and the style of the ray floret in *Othonna* (Figure 9j). They therefore cannot be considered homologous features and were not included as they would simply serve as autapomorphies for the two genera/species concerned. All genera, barring *Tephroseris* and *Dendrosenecio*, have discrete stigmatic areas (41).

Cypselsae (42–54)

Cypselsae provide an extremely useful array of characters that clearly distinguish *Cineraria* from the other genera in this study (Figure 10). *Cineraria* has characteristically compressed cypselsae, with two to four ribs (Cron, 1991, Figure 3.27). The lateral compression of the cypselsae results in two distinct margins, which are extended into a narrow or broad wing in certain species (Figure 10a). Compression of the cypselsae (42, 46), the number of ribs (43, 47), and number and type/degree of extensions (44, 48) and were coded separately for ray and disc cypselsae as they differed in species of some genera. Degree of extension also differed between ray and disc cypselsae for some species of *Cineraria*. [Note: It is debatable whether the four wings on the ray cypselsae of *Oresbia heterocarpa* (Figure 10g) have evolved from and are homologous to ribs. They were coded assuming non-homology.]

Cypsela shape (49) was coded as a single character and only two states were recognised (Table 2), as oblong and ellipsoid shapes were difficult to accurately characterise for some species (e.g. *Kleinia galpinii* Figure 10k, l), as they varied according to the maturity of the cypselsae, the position in the capitulum and whether or not they had been fertilised. This range of shapes was therefore coded as a single state, with obovate as the alternate state.

Only *Oresbia heterocarpa* has distinctly heteromorphic cypselsae: large, glabrous, winged ray cypselsae and smaller, ciliate, ribbed disc cypselsae (Figure 10g, h). *Othonna natalensis* has large, ellipsoid, fertile ray cypselsae and smaller, oblong, sterile disc ovaries that do not develop into cypselsae (Figure 10m). The heteromorphism of the cypselsae *Othonna natalensis* and cypselsae/sterile ovaries of *Oresbia heterocarpa* is therefore not homologous and the character is treated as autapomorphic for these taxa. Certain species within *Cineraria* exhibit minor differences in the breadth of the wing on the ray and disc cypselsae margins. However these differences in cypsela features are already captured in the coding for other characters and no single character for presence/absence of heteromorphic cypselsae was included.

The majority of species in this study with duplex hairs on their cypselsae have mucilaginous trichomes that secrete a long spiral thread that splits into two in response to wetting (Figure 11a–d). In *Cineraria*, the possession of these mucilaginous trichomes is well correlated with arid habitat, being prominent in species that occur in the dry western and northern regions of southern Africa. However, the trichomes on cypselsae of some *Cineraria* species appear to have lost the ability to secrete these long threads and did not respond at all to wetting, or only emitted shorter, thicker threads (Figure 11e, f). These two responses were initially distinguished as different states, but this distinction was later abandoned as it was found to be variable and insufficient sampling was done to be sure of the response. Cypsela indumentum (45) was excluded from the final analyses due to its very homoplasious nature.

Figure 8. (Opposite) Light micrographs of filament collars of stamens: (a) Balusterform filament collars of *Cineraria canescens* [Pearson 5762 (BOL)]. (b) Filament collars of *Oresbia heterocarpa* [Cron et al. 322 (J)]. (c) Anther appendage of *C. erodioides* [Hilliard & Burt 12343 (NU)]. (d) Anther appendage of *Kleinia galpinii* [Cron 537 (J)]. (e) Filament collar of *Euryops brownei* [Muasya, Cron & Knox 10 (J)]. (f, g) Base of anthers and filament collars of *K. galpinii* [Cron 537 (J)]. Scale bars = 100 μm .

Figure 9. (Overpage) Style apices of senecioid genera: (a) Truncate disc style apex with central tuft of sweeping hairs of *Bolandia pedunculosa* [Barker 5398 (NBG)]. Scale bar = 50 μm . (b) Truncate style apex of *Senecio cordifolius* [Cron et al. 369 (J)]. Scale bar = 50 μm . (c) Rounded style apex with sweeping hairs of *S. achilleifolius*. Scale bar = 75 μm . (d) Rounded style apex of *S. deltoideus* [Burrows 4693 (J)]. Scale bar = 50 μm . (e) Truncate style apex of *Pericallis appendiculata* [R.P.M. (K)]. Scale bar = 50 μm . (f) Obtuse style apex of *Oresbia heterocarpa* [Cron et al. 322 (J)]. Scale bar = 50 μm . (g) Obtuse disc style apex of *Euryops brownei* [Muasya, Cron & Knox 10 (J)]. Scale bar = 100 μm . (h, i) Disc style apex of *Kleinia galpinii* [Cron 537 (J)], with appendage. Scale bars (h) 200 μm ; (i) 100 μm . (j) Ray style apex of *Othonna natalensis* [Burrows 4719 (J)]. Scale bar = 100 μm .

Figure 10. (Overpage) Range of shapes of cypselae in the senecioid genera in study: (a) Obovate, broadly winged ray cypselae of *Cineraria lyratiformis* [Balkwill et al. 11804 (J)]. Scale bar = 4.4 mm. (b) Fusiform/elliptic cypselae of *Bolandia argillacea* [Esterhuysen 36192 (K)]. Scale bar = 500 μm . (c) *Emilia discifolia* [Cron & Balkwill 490 (J)]. Scale bar = 385 μm . (d) *Senecio achilleifolius* [Cron & Goodman 556 (J)]. Scale bar = 575 μm . (e) *Senecio deltoideus* [Burrows 4693 (J)]. Scale bar = 340 μm . (f) *S. hederiformis* [M. Van Wyk 5024 (J)]. Scale bar = 325 μm . (g, h) *Oresbia heterocarpa* [Cron et al. 322 (J)]. Scale bar = 580 μm . (i) *Pericallis tussilaginis* [Chaytor 75 (K)]. Scale bar = 350 μm . (j) *Dendrosenecio kilimanjari* ssp. *cottonii* [Robertson 2 (K)]. Scale bar = 1.1 mm. (k, l) *Kleinia galpinii* (k) [McCallum 636 (J)]. Scale bar = 1.7 mm. (l) [Cron 537 (J)]. Scale bar = 0.95 mm. (m) *Othonna natalensis* [Strey 3417 (J)]. Scale bar = 2.3 mm.

Figure 11. (Overpage) Mucilaginous trichomes on cypselae of *Cineraria*: **a–d** emitting **long** mucilaginous threads on wetting; **e, f** - emitting **short** mucilaginous threads on wetting (a) Cypselae of *Cineraria aspera* after wetting [Cron & Goodman 551 (J)]. Scale bar = 260 μm . (b) Trichomes on margin of cypselae of *C. alchemilloides* [Merxmüller & Giess 3558 (BR)]. (c) Single duplex hair from cypselae of *C. alchemilloides* [Merxmüller & Giess 3558 (BR)] with mucilaginous threads released. (d) Margin of cypselae of *C. humifusa* [Pillans 6971 (BOL)]. (e) Cypselae margin of *C. austrotransvaalensis* [Cron & McCallum 341 (J)]. (f) Duplex trichome on cypselae of *C. deltoidea* [Schlieben 4470 (BR)]. Scale bars b–e: 100 μm ; f: 50 μm .

The shape of the epicarp cells was found to be a useful character at the generic level, being subisodiametric in *Cineraria* (Figure 12a–c) and elongate in the other genera in the study (Figure 12d–f). The shape of the epicarp cells of the ray and disc cypselae differed in *Othonna natalensis* and therefore two characters (50 & 51) were needed to code for this variation. Cypselae surface ornamentation (52) was useful to some degree, though it was difficult to distinguish smooth vs. sculptured surfaces (Figure 12b, c) as dehydration treatment affected the surface and caused it to wrinkle. Smooth and sculptured were therefore ultimately treated as one state, but the alternative state of a striate surface was usually very distinctive (Figure 12a, d–f).

The size of the carpopodium (53) expressed as the number of rows of cells it comprised was based on Jeffrey's (1987) states. The number of rows of cells comprising the carpopodium varied between the middle versus the sides of the cypselae and the largest number was consistently used.

Receptacle shape (54)

Variation in the shape of the receptacle (54) was found to be limited, with only *Othonna natalensis* and *Euryops brownei* having a convex (to conical) receptacle, and all other genera/species having a flat to slightly rounded receptacle.

Cladistic Analyses

The morphological data matrix (Table 3) was analysed using heuristic parsimony algorithms of the software package PAUP Version 4.010b (Swofford, 2002) for Macintosh, together with MacClade Version 4.0 PPC (Maddison & Maddison, 2000) for analysis of character evolution. Heuristic searches were undertaken under the unordered and equal weighting criteria of non-additive Fitch parsimony with 10 replicates of random addition sequences and tree bisection-reconnection (TBR) branch swapping, retaining 20 trees at each step with the ACCTRAN option in operation. This initial analysis was followed by successive character weighting using the rescaled consistency index and the maximum value (best fit) was applied to develop better-resolved evolutionary hypotheses (Farris, 1969, 1989). Reweighting continued until the weighting values, tree topology and tree length exactly repeated on two consecutive analyses. Strict consensus trees were obtained for both unweighted and weighted analyses. Simple bootstrap analysis (Felsenstein, 1985) was performed with 100 repetitions and maximum trees set at 5000 (7000 for the complete unweighted morphological analysis). Minimum branch lengths of internal nodes are also presented.

In addition, a cladistic analysis of a reduced data set comprising all species in *Cineraria* with *Bolandia* set as the outgroup (as indicated by the molecular analyses) was performed applying the same procedures and criteria as for the complete data set.

A second reduced version of the morphological data set comprising only those taxa for which molecular data had been obtained, viz. 22 samples of *Cineraria* and 17 senecioid species in eight genera, was combined with two molecular data sets: plastid *trnL-trnF* data and nuclear Internal Transcribed Spacer (ITS) regions separately and in a combined analysis of the three sets of data (total analysis). *Cypsela indumentum* (45) was also excluded from these analyses. The option *mstaxa* = polymorphic was used for the combined morphology and ITS and the total data analyses. Consistency index (CI) values are given excluding uninformative characters for all analyses. Partition homogeneity tests (Farris *et al.*, 1994; Farris *et al.*, 1995) were conducted to test for congruence of the various data sets (100 reps, random heuristic analyses, 10 replicates, with maximum trees set at 5 000).

Figure 12. (Opposite) Epicarp shape and patterning on cypselae (a–c) Subisodiametric epicarp cells of *Cineraria* (a) with striate pattern of *Cineraria foliosa* [Goetze 973 (BR)]. (b) Sunken and smooth to sculptured, *Cineraria erodioides* [Olivier 1985 (WAG)]. (c) Bulging (papillose), smooth epicarp of cypsela of *Cineraria lobata* [Compton 1158 (NBG)]. (d) Elongate epicarp cells with striate surface of *Senecio cordifolius* [Taylor 10094 (K)]. (e) Elongate cells with rounded ends, striate surface of cypselae of *Senecio deltoideus* [Leifeldt *s.n.* sub J32124]. (f) Elongate, raised rounded cells, striate surface of disc cypsela of *Pericallis tussilaginis* [Chaytor 75 (K)]. Scale bars a, b, d, e, f: 10 µm; c: 100 µm.

Table 3: Morphological data set : Character-state values for species used in the cladistic analyses. Unknown character-states are marked as ‘?’; inapplicable states as ‘-’. Polymorphic states are coded as follows: a = 0,1; b = 0,2; c = 0,3; d = 0,4; e = 0,5; f = 1,2; g = 1,3; h = 1,4; i = 1,6; j = 2,3; k = 5,7; m = 6,7; n = 0,1,3; p = 3,4.

Character:	00000	00001	11111	11112	22222	22223	33333	33334	44444	44445	55555
	12345	67890	12345	67890	12345	67890	12345	67890	12345	67890	12345
Tephrosieris (atropurpurea)	110a0	55000	00?23	31200	10102	2000j	10063	10001	00f00	0f000	0?10
Bolandia pedunculosa	11010	i i b0h	12433	01200	10101	21001	1002f	10110	11001	00001	1110
Bolandia argillacea	00010	km201	01433	01200	10101	21001	10021	10110	11001	00001	1110
*C.? dregeana	10021	66001	44433	31220	00100	0??01	?0011	10???	1010?	0100?	??00
*C.? exilis	000f0	66b01	00433	312a0	1100-	- - - -1	00003	1011?	1- - - -	0?00?	??10
*C.? microglossa	00010	55201	00433	31210	00101	2??01	00003	1011?	1??01	0?00?	??10
*C.? othonnoides	10010	67201	11413	31200	10101	2??02	10001	1???a	10101	0100?	??00
Dendrosenecio battiscomb.	32010	77001	00433	21230	00101	21002	00111	10210	00200	02000	0010
D. kilimanjari ssp. cottonii	32013	55001	ee433	21230	00102	20103	00103	10210	00200	02000	0010
Emilia discifolia	10010	66001	00431	31201	00101	00002	10001	10110	10101	01001	1110
Euryops brownei	20020	44003	00433	31201	00102	20102	11003	10201	10100	01100	0011
Kleinia galpinii	11010	56000	00433	31210	1101-	- - - -2	00001	01103	1- - -0	02000	0bf0
Oresbia heterocarpa	11011	76003	0e433	01210	00101	10001	00023	102f1	10330	01001	1aa0
Othonna natalensis	11010	55000	00433	312a0	10201	21002	11001	01101	1020a	02002	00f1
Pericallis aurita	20102	30002	33433	01220	00121	20011	10002	10110	1020a	02001	1100
Pericallis appendiculata	2010b	55102	43433	01220	00121	20011	00002	20110	1020a	02001	1100
Pericallis multiflora	20100	55002	42433	01220	00121	20011	10002	10110	10201	02001	1?00
Pericallis murrayi	11112	3c102	23431	01220	00121	20012	10032	20110	10200	02001	1?00
Pericallis tussilaginis	11102	37a02	34431	01210	a0121	20013	10002	20110	10201	02001	1100
Senecio achilleifolius	20012	57200	11333	31210	00101	21001	00051	10211	10100	01001	1120
Senecio cordifolius	10000	55001	23413	01200	10101	20101	00002	10210	10100	01001	1120
Senecio deltoideus	10002	22001	22413	31220	0a100	20100	00010	10211	10101	01001	1120
Senecio hederiformis	10000	55101	33433	30210	00101	20001	00022	10111	10100	01001	1000
Cineraria abyssinica	20102	10f11	23433	00220	00100	01001	00021	10112	12010	20112	2120
Cineraria albicans	20102	30101	34433	00210	00101	21001	00021	10112	12011	20112	2020
Cineraria alchemilloides	20102	20101	j3433	00f20	00101	21000	000ba	10112	12011	20112	2020
Cineraria anampoza	10102	3c101	2p113	31220	00100	b1001	00002	10112	12011	20112	2120
Cineraria aspera	20102	55211	23430	c1220	00100	21001	00011	10112	12011	20112	2020
Cineraria atriplicifolia	00102	2j101	f2113	31220	00100	21001	00001	10112	12010	20112	2120
Cineraria austrotransvaal.	20102	30112	23430	31020	00101	21001	00021	10112	12011	20112	2020
Cineraria canescens	20102	00112	23433	0a120	00100	21000	000f1	10112	12011	20112	2020
Cineraria cyanomontana	20102	55201	22433	01220	00100	21001	00001	10112	12011	20112	2020
Cineraria decipiens	00102	23101	23113	31220	00100	21001	00001	10112	12011	20112	2020
Cineraria deltoidea	10102	2c101	jphgn	c1220	001a0	2100f	000a1	10112	1201a	20112	2020
Cineraria dryogeton	00102	00101	34423	31210	00100	21000	00000	10112	12010	20112	2120
Cineraria erodioides	20102	c0101	234jn	c1220	0010a	21a01	00011	10112	120fa	20112	2020
Cineraria erosa	20102	00112	33430	31220	00100	21000	00020	10112	12011	20112	2020
Cineraria foliosa	10102	33111	34433	01220	00100	21001	00011	10112	12011	20112	2120
Cineraria geifolia	10102	00101	34423	31210	00100	21a01	000c1	10112	12011	20112	2020
Cineraria geraniifolia	10102	00101	3p223	31210	10101	21001	00001	10112	12010	20112	2020
Cineraria glandulosa	10102	3c101	33133	31220	00100	21001	00041	10112	12010	20112	2020

<i>Cineraria grandibracteata</i>	10100	30101	23432	31200	10101	21002	00022	10112	12010	20112	2020
<i>Cineraria huilensis</i>	10103	j3101	23433	01220	0010a	21001	000b1	10112	12011	20112	2020
<i>Cineraria humifusa</i>	10102	00101	33003	31210	a0101	21001	00032	10112	12011	20112	2120
<i>Cineraria lobata</i>	20102	c0101	2j43g	312f0	00100	21001	00001	10112	12011	20112	2020
<i>Cineraria longipes</i>	10102	30101	12433	31210	10101	21001	00002	10112	12010	20112	2020
<i>Cineraria lyratiformis</i>	00102	112a1	22h30	01220	00100	21001	000b2	10112	12020	20212	2020
<i>Cineraria magnicephala</i>	20102	j3101	23433	012f0	00102	21002	00022	10112	12010	20112	2?20
<i>Cineraria mazoensis</i>	10102	30111	2343b	00220	00100	21a01	00021	10112	12011	20112	2020
<i>Cineraria mollis</i>	10103	00101	34433	00200	10101	21002	00021	10112	12011	20112	2020
<i>Cineraria ngwenyensis</i>	10102	30101	23423	31210	0010a	21001	00001	10112	12010	20112	2a20
<i>Cineraria parvifolia</i>	00102	3cf01	23133	31220	00100	21001	00001	10112	12011	20112	2020
<i>Cineraria pinnata</i>	00102	j3f01	13133	31220	00100	21001	00001	10112	12011	20112	2120
<i>Cineraria platycarpa</i>	00102	a0101	23133	31220	00100	2100a	00001	10112	120f1	20112	2020
<i>Cineraria pulchra</i>	20102	30112	23433	00220	00101	21001	00021	10112	12011	20112	2010
<i>Cineraria saxifraga</i>	10100	00101	1f113	31220	00100	21000	00001	10112	12011	20112	2020
<i>Cineraria vagans</i>	10103	00101	34223	31200	10100	21001	00002	10112	12010	20112	2120
<i>Cineraria vallis-pacis</i>	20102	30111	23433	01220	00100	21001	00011	10112	12021	20112	2120

Coding for regional populations/forms used in combined morphological and molecular analyses:

<i>Cineraria deltoidea</i> EA	10102	2c101	23hgn	c1220	0010a	2100f	00001	10112	1201a	20112	2a20
<i>Cineraria deltoidea</i> Sn	10102	23101	33110	31220	00100	21001	00001	10112	12011	20112	2020
<i>Cineraria lobata</i> ssp lobR	20102	c0101	23431	31220	00100	21001	00001	10112	12011	20112	2020
<i>Cineraria lobata</i> ssp lobUN	20102	00101	22433	31210	00100	21001	00001	10112	12011	20112	2020
<i>Cineraria lobata</i> ssp soutp	20102	c0101	22433	01220	00100	21001	000a1	10112	12011	20112	2020

RESULTS

Morphological analyses

Complete data set with Tephroseris as outgroup

1450 equally most parsimonious (EMP) trees of 316 steps resulted from the unweighted analysis of the morphological data set with *Tephroseris* as outgroup (Figure 13, CI = 0.37, RI = 0.71). Successive weighting of the data resulted in three EMP trees with improved resolution amongst species of *Cineraria* in the consensus tree (Figure 14).

Cineraria sensu stricto (as currently conceived) is strongly supported as a monophyletic group in both unweighted and weighted analyses of the morphological data with *Tephroseris* as outgroup and *Pericallis* is placed sister to *Cineraria* (Figures 13 and 14). In the strict consensus tree resulting from the unweighted analysis (Figure 13), *Bolandia* is grouped in a clade (Clade E) with *Emilia discifolia*, and four of the species previously misplaced in *Cineraria*: *C. dregeana*, *C. othonnoides*, *C. exilis* and *C. microglossa*. However Clade E is only weakly supported and represents an anomalous grouping of species with truncate style apices and solitary or few capitula on long peduncles. In contrast, in the strict consensus tree of the weighted analysis (Figure 14), *Bolandia* (Clade F) is placed sister to the clade

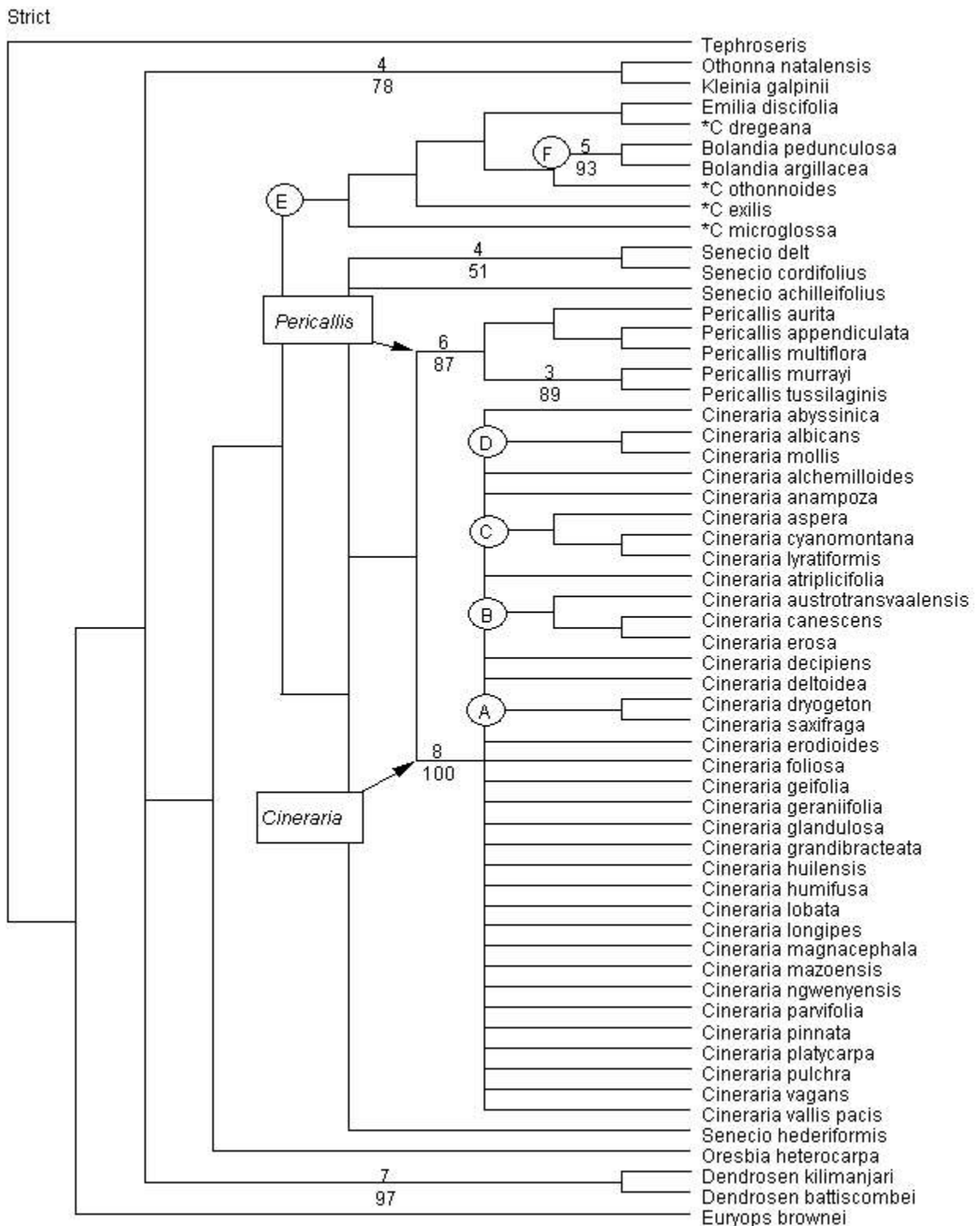


Figure 13. Strict consensus tree of 1450 most parsimonious trees of 316 steps (CI = 0.37, RI = 0.71) in an unweighted parsimony analysis of 35 species of *Cineraria* and 23 Senecioneae based on the morphological data set with *Tephroseris* as outgroup. Minimum branch length is shown above the branches and bootstrap values are indicated below the branches and for those branches with >50% bootstrap support. (*) indicates species previously misplaced in *Cineraria*.)

Strict

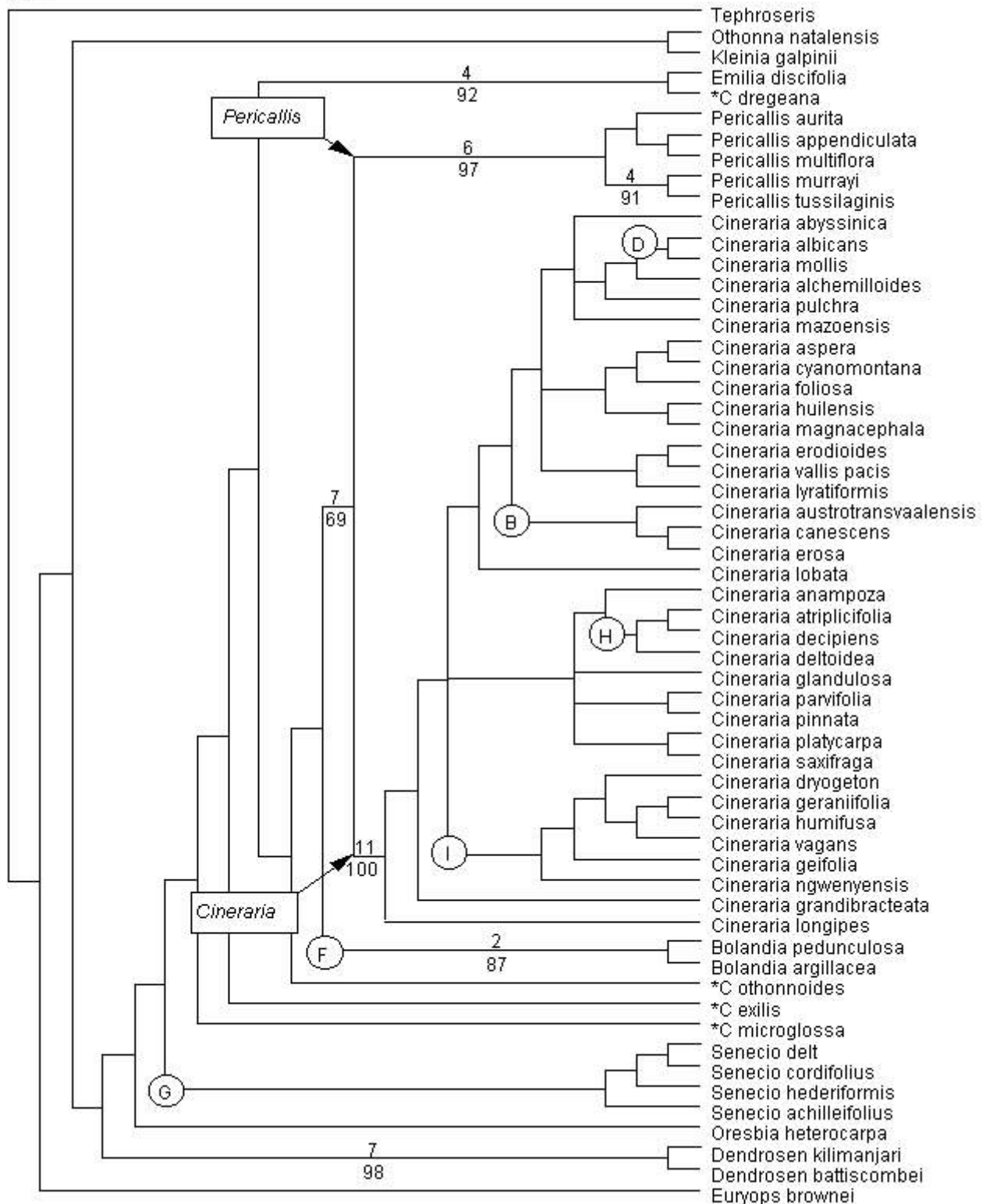


Figure 14. Strict consensus tree of 3 most parsimonious trees after successive weighting of the morphological characters for 35 species of *Cineraria* and 23 Senecioneae with *Tephroseris* as outgroup. Minimum branch length is shown above the branches and bootstrap values are indicated below the branches and for those branches with >50% bootstrap support. (* indicates species previously misplaced in *Cineraria*.)

comprising *Pericallis* and *Cineraria*, with *C. othonnoides* sister to the clade containing *Bolandia*, *Pericallis* and *Cineraria*. Further back along the inferred ancestral lineage are the branch points for lineages leading to *Emilia* and *C. dregeana*, *C. exilis* and *C. microglossa* and Clade G with the four species of *Senecio*.

Although the resolution within *Cineraria* is substantially improved in the weighted analysis (Figure 14), none of the clades has bootstrap support of more than 50%. Only two of the clades within *Cineraria* (B and D) in the consensus tree of the unweighted analysis (Figure 13) are retrieved in the weighted analysis. The base of *Cineraria* remains unresolved in the strict consensus tree of the unweighted analysis (Figure 13), whereas *C. longipes* and *C. grandibracteata* (perennial herbs with long peduncles and solitary or few capitula) are placed sister to the rest of the species in *Cineraria* in the consensus tree of the weighted analysis (Figure 14). However, as noted, none of the clades in *Cineraria* is very robust and little can be concluded from these analyses.

Reduced data set with Bolandia as outgroup

Unweighted analysis of a reduced data set comprising all 35 species of *Cineraria* with *Bolandia* as the designated outgroup, resulted in 178 EMP trees of 140 steps (CI = 0.43, RI = 0.61). Of the 53 characters included in the analysis, 17 are constant and 2 variable characters are uninformative, resulting in 33 parsimony informative characters. There is, however, limited resolution within *Cineraria* in the strict consensus tree resulting from this analysis (Figure 15). Successive weighting of the characters in the reduced data set (five times to get unchanging values) resulted in 35 EMP trees and a much more resolved consensus tree (Figure 16), although none of the clades in *Cineraria* is very robust. Three of these clades (Figure 16, Clades V, VI and IX) are consistent with those in the unweighted analysis of the same data set (Figure 15, Clades c, d and e). *Cineraria longipes* and *C. grandibracteata* are grouped in a clade sister to the rest of the species in both analyses of the reduced data set, with *C. mollis* sister to the remaining species in this clade (Figures 15 and 16).

Character performance/usefulness

Twenty-six (49%) of the 53 characters included in the cladistic analyses in this study vary at the species level within *Cineraria*, with eight of these 26 characters also useful at the generic level. The remainder of the characters essentially reflect features delimiting genera and are useful in that they confirm the monophyly of the core species in *Cineraria* and assist in placing those species not fitting well within the genus, as well as hypothesizing sister group relationships.

Table 4 summarises the performance of the parsimony informative characters in the unweighted and weighted analyses of the reduced data set with *Bolandia* set as outgroup. In this analysis, nine characters serve to distinguish the outgroup/sister group *Bolandia* from

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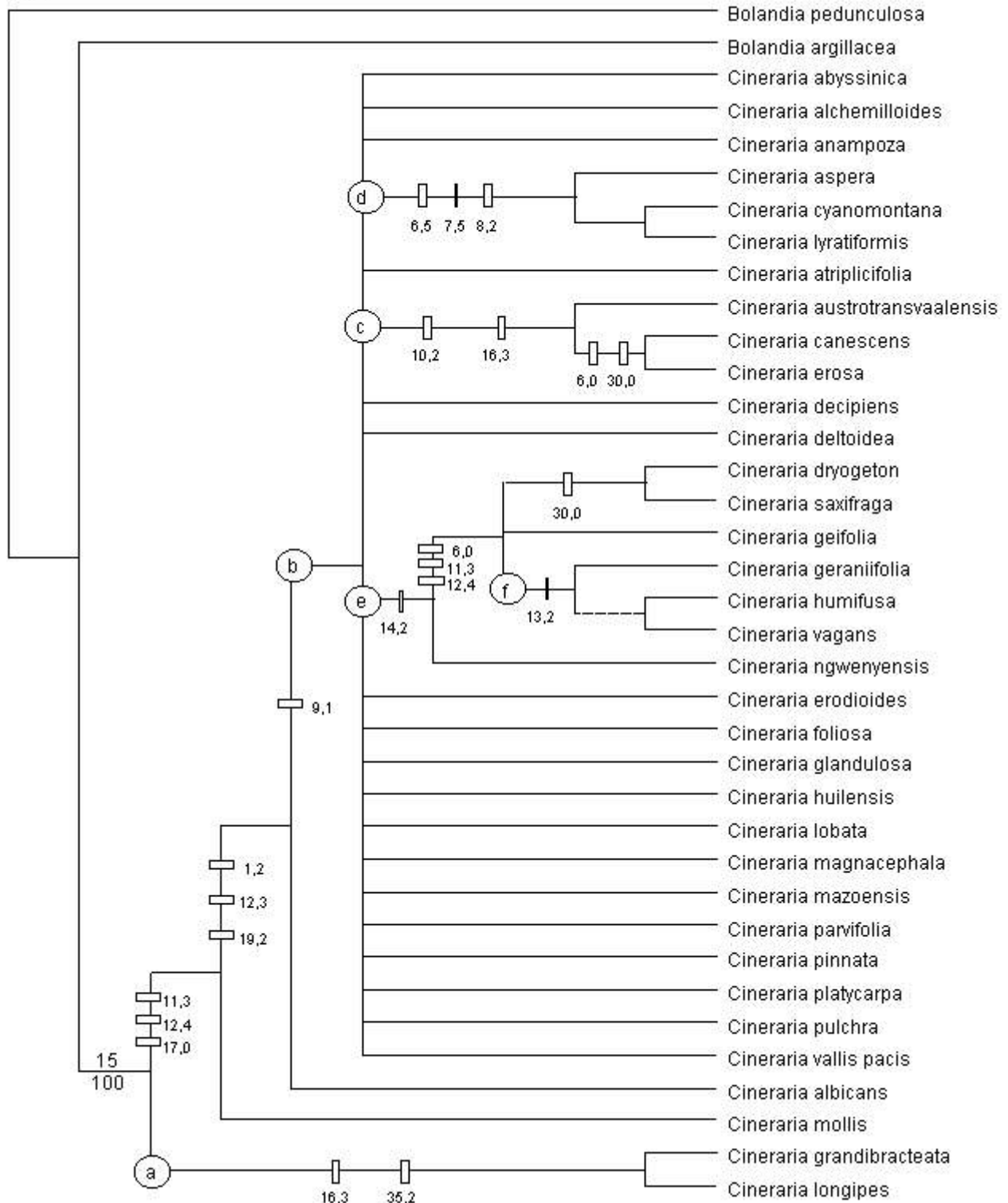


Figure 15. Strict consensus of 178 most parsimonious trees of 140 steps (CI = 0.43, RI = 0.61) resulting from parsimony analysis morphological data set of 35 species of *Cineraria* with *Bolandia* as outgroup, unweighted. Minimum branch length is shown above the branches and bootstrap values are indicated below the branches and for those branches with >50% bootstrap support. Selected characters for internal nodes shown: ■ (closed bar) apomorphies with no homoplasy; □ (open bar) homoplasious apomorphies and with subsequent reversals or parallelism.

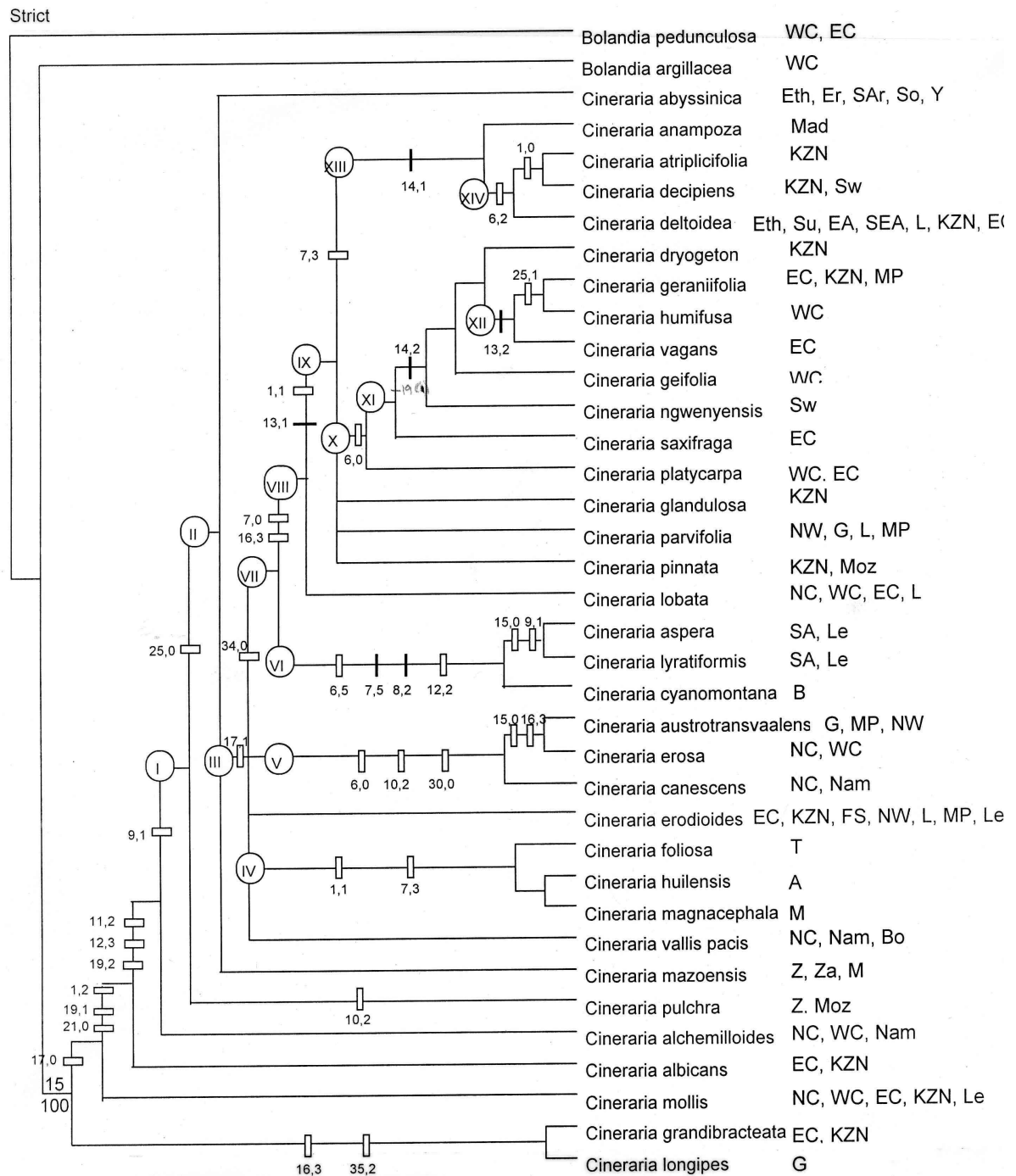


Figure 16. Strict consensus tree of 35 most parsimonious trees after successive weighting of morphological characters for 35 species of *Cineraria* with *Bolandia* as outgroup. Minimum branch length is shown above the branches and bootstrap values are indicated below the branches and for those branches with >50% bootstrap support. Selected characters for internal nodes shown: ■ apomorphies with no homoplasy; □ homoplasious apomorphies and with subsequent reversals or parallelism. Geographic regions indicated: A Angola, B Blouberg, Bo Botswana, EA East Africa, EC Eastern Cape, Er Eritrea, Eth Ethiopia, FS Free State, G Gauteng, KZN KwaZulu-Natal, L Limpopo, Le Lesotho, M Malawi, Moz Mozambique, Nam Namibia, NC Northern Cape, T Tanzania, S Soutpansberg, SA South Africa (widespread), SAr Saudi Arabia, SEA southern East Africa (Malawi, Mozambique, Zambia, Zimbabwe), So Somalia, Su Sudan, Sw Swaziland, WC Western Cape, Y Yemen, Z Zimbabwe.

Cineraria and thus have Consistency and Retention indices of 1.00: leaf venation (3), leaf attachment (4), presence or absence of a calyculus (31), shape of disc floret style apex (40), compression of ray cypselae (42) and disc cypselae (46), cypselae shape (49) and shape of the epicarp cells on the ray cypselae (50) and the disc cypselae (51). The remaining 25 parsimony informative characters are useful in elucidating species relationships. (Note: Character 18 is not informative, although it does vary amongst and within species of *Cineraria*.)

Table 4: Character diagnostics for the reduced data set (*Bolandia* set as outgroup), unweighted and weighted analyses. (Only variable, parsimony informative characters are presented.)

Character	Unweighted analysis			Weighted analysis		
	Tree steps	CI	RI	Tree steps	CI	RI
1. Habit	10	0.2	0.67	11	0.19	0.53
3. Leaf venation	1	1.00	1.00	1	1.00	1.00
4. Leaf attachment	1	1.00	1.00	1	1.00	1.00
5. Leaf auricles	6	0.33	0.2	6	0.33	0.2
6. Upper leaf shape	11	0.36	0.56	12	0.33	0.5
7. Lower leaf shape	7	0.57	0.57	5	0.8	0.86
8. Leaf dissection	2	0.5	0.75	2	0.5	0.75
9. Leaf dissection at apex	3	0.33	0.75	4	0.25	0.63
10. Leaf margin	2	0.5	0.67	2	0.5	0.67
11. Upper leaf base	10	0.3	0.42	10	0.3	0.42
12. Lower leaf base	10	0.3	0.3	11	0.27	0.2
13. Glandular trichome	4	0.75	0.88	4	0.75	0.88
14. Eglandular trichome (short)	3	1.00	1.00	3	1.00	1.00
15. Eglandular trichome (long)	5	0.4	0.25	4	0.5	0.5
16. Woolly trichome (narrow basal cells, perpendicular junction)	4	0.25	0.79	3	0.33	0.86
17. Woolly trichome (narrow basal cells, oblique junction)	3	0.33	0.6	2	0.5	0.8
19. Capitula arrangement	6	0.33	0.64	6	0.33	0.64
21. Peduncle length	2	0.5	0.83	2	0.5	0.83
25. Number of ray florets	5	0.4	0.7	4	0.5	0.8
30. Disc floret number	6	0.33	0.33	8	0.25	0.25
31. Involucre calyculus	1	1.00	1.00	1	1.00	1.00

34. Involucral indumentum	7	0.57	0.79	7	0.57	0.79
35. Involucral bract number	7	0.29	0.29	8	0.25	0.14
40. Style apices	1	1.00	1.00	1	1.00	1.00
42. Ray cypselae: Compression	1	1.00	1.00	1	1.00	1.00
44. Ray cypselae: Extensions	2	0.67	0.50	3	0.67	0.50
46. Disc cypselae: Compression	1	1.00	1.00	1	1.00	1.00
48. Disc cypselae: Extensions	2	1.00	1.00	2	1.00	1.00
49. Cypselae shape	1	1.00	1.00	1	1.00	1.00
50. Epicarp cell shape (ray cypselae)	1	1.00	1.00	1	1.00	1.00
51. Epicarp cell shape (disc cypselae)	1	1.00	1.00	1	1.00	1.00
52. Cypselae surface ornamentation	7	0.14	0.33	8	0.13	0.22
53. Carpodium	2	0.50	0.50	2	0.50	0.50

Combined morphological and molecular data analyses

Combination of the morphological and *trnL-trnF* data sets for those species of *Cineraria* and related Senecioneae for which the DNA sequences were available resulted in 521 EMP trees of 368 steps (CI = 0.50, RI = 0.76) with limited resolution within *Cineraria* (Figure 17). Analysis of the combined morphological and ITS data sets yielded 11 trees of 853 steps (CI = 0.56, RI = 0.65), with considerable resolution within *Cineraria* in the consensus tree (Figure 18).

The total combined data set generated a matrix of 1841 characters including 53 morphological, 918 from the *trnL-trnF* region and 870 from the ITS1, ITS2 and 5.8S regions, including 63 coded indels for the cpDNA and nuclear regions (26 and 37 respectively). Of these 1841 characters, 224 were parsimony informative, including 46 morphological characters, 163 point mutations and 15 indels. Only one tree (996 steps, CI = 0.56, RI = 0.70) resulted from the combined analysis of the morphological, nuclear and plastid molecular data sets, but with limited support for the clades within *Cineraria* (Figure 19).

In consensus trees of the combined analyses of the morphological and plastid or nuclear molecular data (Figures 17 & 18) and the tree generated by the total analysis (Figure 19), *Cineraria* is strongly supported as a monophyletic group with *Bolandia* as the sister group. In two of the three analyses (Figures 18 and 19), *C. mollis* is placed sister to the rest of the species of *Cineraria*, followed by *C. geraniifolia* and/or *C. longipes*. These three species are herbaceous and have either solitary or few capitula on long peduncles and medium to large capitula, features shared with the sister genus *Bolandia*. *Cineraria mollis* also lacks auricles, having only a dilated base to the petiole. In the consensus of the combined morphology and

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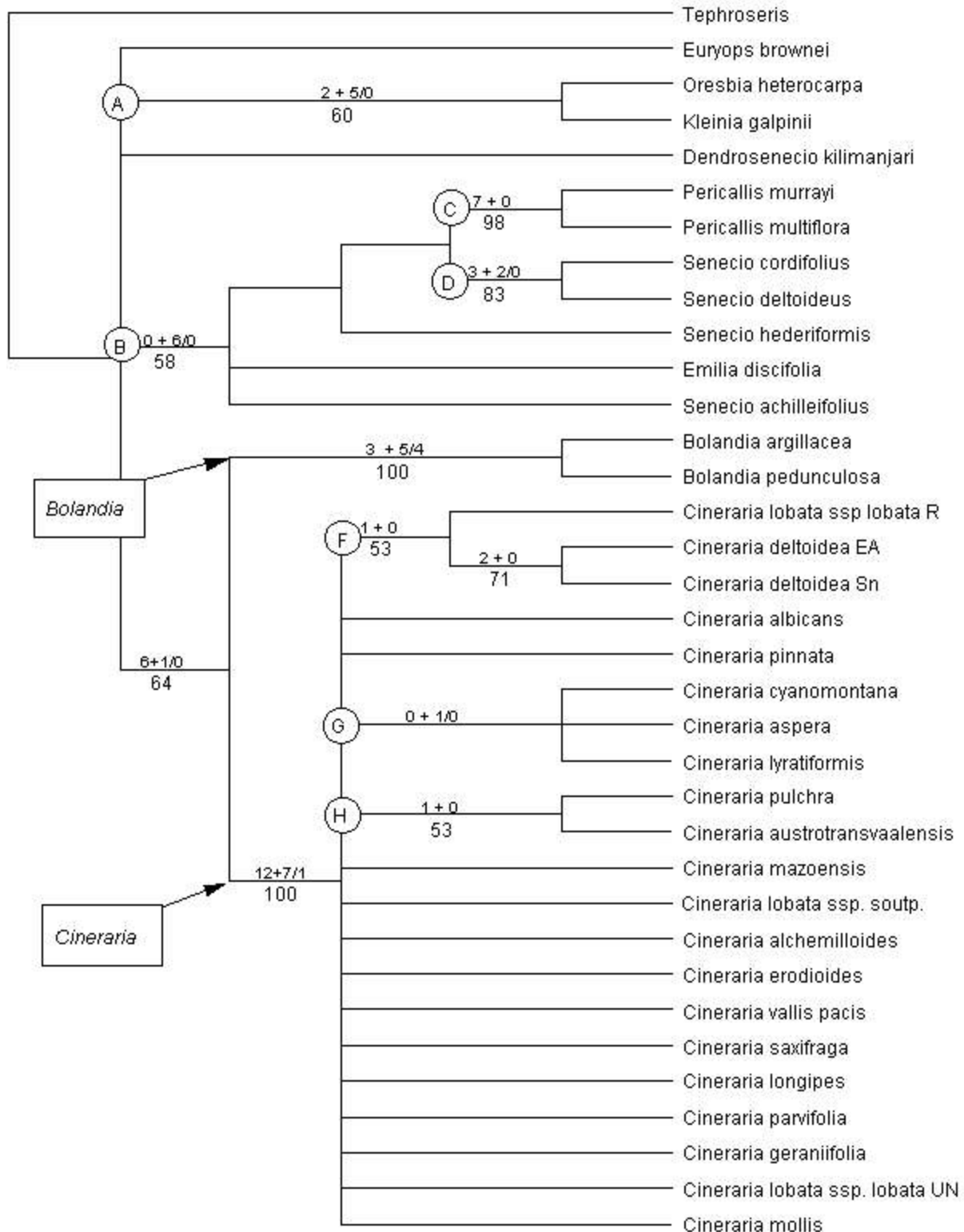


Figure 17. Strict Consensus tree of 521 equally most parsimonious trees ($l = 368$; $CI = 0.50$; $RI = 0.76$) based on parsimony analysis of combined morphological and *trnL-trnF* data sets for 21 samples (species and subspecies/forms) of *Cineraria* with *Bolandia* as outgroup. Minimum branch length (morphological + point mutations/indels) is shown above the branches and bootstrap values (calculated using point mutations only) are indicated below the branches and for those branches with $>50\%$ bootstrap support.

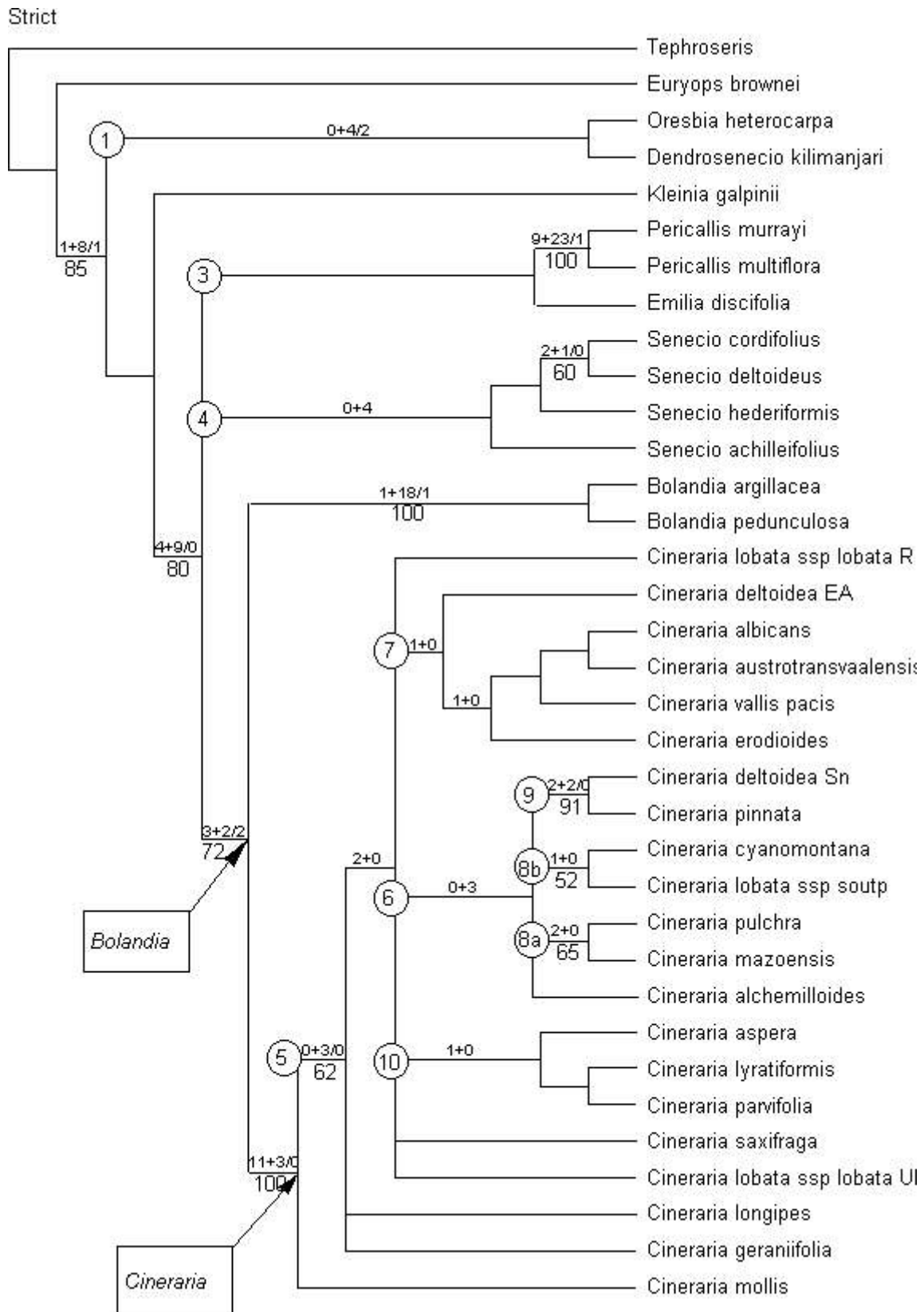


Figure 18. Strict consensus tree of 11 equally most parsimonious trees ($l = 853$, $CI = 0.50$, $RI = 0.65$) based on parsimony analysis of combined morphological and ITS data sets for 21 samples (species and subspecies/forms) of *Cineraria* with *Bolandia* as outgroup. Minimum branch length (morphological + point mutations/indels) is shown above the branches and bootstrap values (calculated using point mutations only) are indicated below the branches and for those branches with >50% bootstrap support.

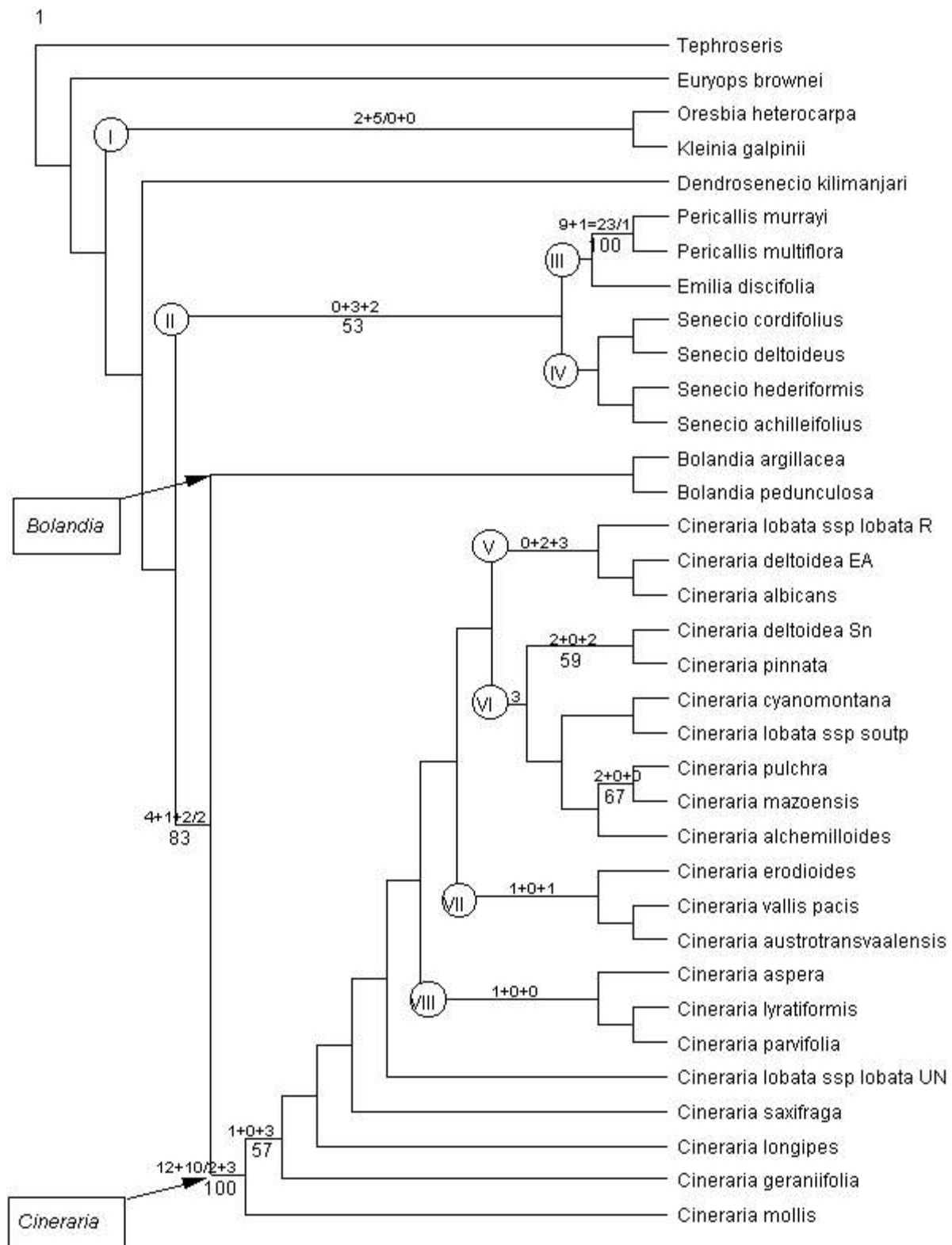


Figure 19. The single most parsimonious tree ($l = 996$ steps, $CI = 0.56$, $RI = 0.70$) resulting from parsimony analysis of combined morphological, *trnL-trnF* and ITS data sets for 21 samples (species and subspecies/forms) of *Cineraria* with *Bolandia* as outgroup. Minimum branch length (morphological + cpDNA + ITS point mutations / indels) is shown above the branches and bootstrap values (calculated using point mutations only) are indicated below the branches and for those branches with >50% bootstrap support and some of the internal nodes in *Cineraria*.

trnL-trnF analyses (Figure 17), however, the position of *C. mollis* is unresolved and it forms part of the basal polytomy.

Partition homogeneity tests resulted in a value of 0.01 for all three combinations of the morphological and molecular data. This is on the border of congruency/incongruency.

Evidence for conflict between the morphological data and the *trnL-trnF* data sets is seen within *Cineraria*, where of the three clades present in the consensus (Figure 17), only Clade F (Figure 17) is partially representative of Clade f in the consensus of the cpDNA data only (Chapter 2, Figure 6), as the position of *C. albicans* is unresolved. Clade e (Chapter 2, Figure 6), comprising seventeen species and defined by a unique 4-bp deletion and a point mutation, is not retained in the combined morphological and cpDNA analysis (Figure 17). Only three of the nine species previously united in Clade g (Chapter 2, Figure 6) remain united in the combined morphological and *trnL-trnF* analysis (Figure 17, Clade G).

In contrast, a considerable degree of the structure within *Cineraria* in the consensus of the combined morphological and ITS analysis (Figure 18) reflects the influence of the ITS data only (Chapter 2, Figure 7). Clades 6 and 7 (Figure 18) are very similar to the corresponding clades in the consensus of the ITS analysis (Chapter 2, Figure 7, Clades 6 & 7), and part of Clade 10 (Chapter 2, Figure 7) remains in the combination of *C. aspera*, *C. lyratiformis* and *C. parvifolia* (Figure 18, Clade 10). The weakly supported relationship between *C. lobata* ssp. *lobata* (R) and *C. saxifraga* evident in the ITS analysis (Chapter 2, Figure 7) is lost in the combined morphological and ITS analysis (Figure 18), and *C. geraniifolia* and *C. longipes* have also been removed from Clade 10 (Chapter 2, Figure 7), and placed sister to the remaining species due to morphological features shared with *C. mollis* (as noted above).

Most of the structure within *Cineraria* in the total analysis (Figure 19) is due to the influence of the ITS data and morphology. Only Clade V is representative of the signal from the *trnL-trnF* data. The basal nodes of the tree are more resolved, with *C. mollis* sister to *C. geraniifolia*, which is sister to *C. longipes*.

In the tree resulting from the total analysis (Figure 19) and the consensus of the combined morphological and *trnL-trnF* analysis (Figure 17), *Emilia discifolia*, *Pericallis* and the four *Senecio* species included in the study are placed in a clade (Clade B, Figure 17; Clade II, Figure 19) sister to *Bolandia*, although there is some variation within the clade. In the consensus for the combined morphological and ITS analysis (Figure 18), *Emilia discifolia* and *Pericallis* (Clade 3) are separated from the four species of *Senecio* (Clade 4).

There is a lack of congruence between the consensus of the combined morphological and *trnL-trnF* data and that of the combined morphological and ITS data in the placement of *Oresbia heterocarpa*, *Kleinia galpinii* and *Dendrosenecio*. *Oresbia* and *Kleinia galpinii* are grouped

together by four point mutations and a shared 6-bp deletion in the *trnL-trnF* data, and by a similarity in leaf phyllotaxy and shape. In the consensus of the combined morphological and ITS data, *Oresbia* and *Dendrosenecio* are grouped together by four point mutations and two indels (one bp insertion and one bp deletion). The morphological data tips the balance in the total analysis (Figure 19), where *Oresbia* and *Kleinia* form a clade (Clade 1), in contrast to the consensus of trees resulting from the combined molecular data only (Chapter 2, Figure 8). Additional sampling is needed to elucidate the extent of the evolutionary reticulations in the affected genera.

DISCUSSION

Generic concept of *Cineraria*

A narrower circumscription of *Cineraria* is strongly supported as a monophyletic group in all cladograms resulting from separate morphological and combined analyses. A minimum of eight characters unite species of *Cineraria* in the morphological analyses of the complete data set, where *Pericallis* is placed sister to *Cineraria* due to shared characters such as palmately-veined leaves, leaf shape and minutely sagittate anther bases. In the reduced data set, with *Bolandia* as the outgroup (Figure 15), a minimum of 15 synapomorphies are recognised for *Cineraria*, most of which distinguish the genus. These diagnostic features include the following: palmately veined, 5–7-lobed, mostly auriculate leaves; calyculate involucre; obtuse, penicillate style apices; obovate, distinctly compressed ray and disc cypselsae with 2–4 ribs and distinct lateral margins or wings; a substantial carpopodium. A useful diagnostic feature not noted previously is the subisodiametric (squarish) epicarp cells on the cypselsae (character 50), as opposed to the more elongated ones seen in the other senecioid genera and outgroup species (Figure 10).

Evidence for *Cineraria* as a well-defined, well supported genus is also provided by the strict consensus tree resulting from the “total evidence” analysis, 12 morphological synapomorphies constitute part of the minimum branch length for *Cineraria*, 13 point mutations (10 in the *trnL-trnF* region and three in the ITS regions) and one indel in the *trnL-trnF* region unite the species of *Cineraria* (Figure 19).

A corollary to verification of the narrower generic circumscription of *Cineraria*, was endorsement for the removal from the genus of the four species not conforming to key diagnostic characters for *Cineraria*. The dubious species *C. exilis*, *C. microglossa*, *C. othonnoides* and *C. dregeana* are all positioned outside the core group of species constituting the narrower generic concept of *Cineraria*, although their exact position varies between the two analyses (Figures 13 and 14).

Cineraria exilis, *C. microglossa*, *C. dregeana* and *C. othonnoides* are all grouped with *Bolandia* and *Emilia discifolia* in the unweighted analyses of the complete morphological data set in a weakly supported clade (Figure 13). Certain morphological features lend some credence to the possibility that *C. exilis* and/or *C. microglossa* are closely affiliated to *Bolandia*. They both have long peduncles, similar to *Bolandia*, although they bear paired capitula (to three's and four's in *C. microglossa*) and these are sparsely calyculate, whereas the capitula of *Bolandia* are borne singly and are ecalyculate. Examination with a light microscope reveals cypselae fairly closely matching those of *Bolandia* in shape and indumentum. There are some missing data for *C. othonnoides* and *C. dregeana* and their positioning is not well supported in either analysis. *Cineraria dregeana* is possibly a member of *Senecio s.l.* as its narrow involucral bracts resemble those of many *Senecio* species and its cypselae are also quite typical. Placement of *C. othonnoides*, however, requires much more investigation. Nevertheless, a clear outcome of this study is that none of these four species belongs in *Cineraria*.

Sister group to *Cineraria*

Consensus trees of all three combinations of morphological and molecular data, viz. *trnL-trnF*, ITS and combined plastid and nuclear data (Figures 17, 18 and 19), strongly support the placement of *Bolandia* as the sister group to *Cineraria*. This is due to the influence of the molecular data (both plastid and nuclear regions), which do not support the sister-group relationship of *Pericallis* with *Cineraria* seen in the morphological analyses (Figures 13 and 14). *Bolandia* and *Cineraria* are united by five molecular synapomorphies (three point mutations and two indels) and four morphological synapomorphies [1–4 ribs in the slightly compressed ray and disc cypselae, glandular hairs on the ray florets and the fine cobwebby trichome (16,0) - in certain species of *Cineraria* only].

Bolandia is distinguished from *Cineraria* by pinnately veined, exauriculate leaves, ecalyculate capitula, truncate style apices, fusiform-ellipsoid cypselae which lack extensions and have a medium sized carpodium (4–6 rows of cells) and only slight compression of the ray cypselae. A large number of molecular synapomorphies also characterise this genus (Figures 17–19).

As noted above, *Pericallis* is placed sister to *Cineraria* in the unweighted and weighted analyses of the morphological data set (Figures 13 and 14) as they are united by many morphological similarities, notably: palmately veined, petiolate leaves which are auriculate in some species of *Pericallis* and most species of *Cineraria*. Nordenstam (1978) first suggested that *Pericallis* and *Cineraria* share a common ancestor and subsequent molecular studies have corroborated a close relationship (Pelser *et al.*, 2002), but indicated that they are not sister taxa. From the results of the combined molecular and morphological analyses of this study,

it would appear that the strong morphological similarities (especially in the leaves) between the two genera are due to convergent evolution and not an immediate common ancestry.

Pericallis differs from *Cineraria* and the other senecioid genera included here by its non-yellow flowers and papillose ray corolla surface. It differs from *Cineraria* in that its cypselae are oblong to ellipsoid and not compressed when mature, lack wings/extensions and have 10 ribs, as opposed to the obovate compressed cypselae with margins or wings and 2 or 3 ribs of *Cineraria*. Epicarp cells of *Pericallis* are elongate with rounded ends, similar to those of *Emilia discifolia* and certain other Senecioneae (e.g. *Senecio deltoideus*), whereas in *Cineraria* they are subisodiametric. The carpopodium in *Pericallis* tends to be obscure or small, whereas in *Cineraria* it is medium to large, occasionally very large (with more than 10 rows of cells).

Previous analysis of cpDNA restriction-site variation among selected genera in the Senecioneae indicated that *Cineraria* is more closely related to *Dendrosenecio* than is *Euryops* (Knox & Palmer 1995). The morphological (Figures 13 and 14) and combined morphological and molecular analyses (Figures 17–19) all support this general conclusion, but the increased taxonomic sampling of this clade has identified many species that are more closely related to *Cineraria* than is *Dendrosenecio*. *Dendrosenecio* exhibits many unique synapomorphies: its unusual growth form (a pachycaul tree with leaves crowded towards the apex of the stem), the type of trichome on the leaves (eglandular, stiff, with pointed apical cell – which may possibly be present in other Senecioneae not included in this study), a pyramidal synflorescence and biseriate involucre bracts. *Dendrosenecio* is also unusual amongst the senecioid genera in that it has a continuous stigmatic area, a feature it shares with *Tephrosia*, although this is more likely to be a reversal in character evolution than an indication that it is primitive within the Senecioneae.

Lack of congruence between the *trnL-trnF* and ITS Data Sets

The three combinations of morphological and molecular data sets used here are on the border of incongruence at $P = 0.01$. This lack of clear congruence in the topologies of trees in *Cineraria* based on the different combinations of morphological and DNA data sets may be due to several reasons. It is possible that there is insufficient sampling of the taxa as only 18 of a possible 35 species in the genus (51%) are sampled for the molecular data sets (as well as two subspecies (and two forms) of *C. lobata* and two disjunct populations of *C. deltoidea*). The lack of variation in the *trnL-trnF* region is another possible reason for the lack of congruence (i.e. the region is evolving too slowly to reflect speciation in *Cineraria*). Most importantly though, the plastid and nuclear genomic regions are reflecting different evolutionary histories. Reticulate evolution would be reflected in them differently and there is reason to believe that it is common in *Cineraria* (Chapter 2) and in other Senecioneae (Pelser *et al.*, 2002). Hybridization has been proposed between a few species of *Cineraria*, e.g. *C. pulchra* and *C. deltoidea* in the Chimanimani region of Zimbabwe; *C. atriplicifolia*

and *C. glandulosa* from the KwaZulu-Natal Midlands (Cron *et al.*, submitted, Appendix 4; in press, Appendix 3).

Character performance/usefulness

Amongst the most useful and best performing characters in distinguishing species relationships in *Cineraria* are a number associated with the leaves. The presence or absence and type of glandular trichome on the leaves (or in the angles of the lobes of the leaves) (character 13) supports two clades (IX and XII) in the consensus tree of the weighted analysis (Figure 16) and one in the unweighted analysis (Figure 15, Clade f). Short (non-woolly) eglandular trichomes (character 14) also contributes considerably to the topology of the consensus trees (Figure 15, Clade e; Figure 16, Clades XI and XIII). Although the woolly trichomes (characters 15, 16 and 17) exhibit some homoplasy in the current hypotheses (Figures 15 and 16), they also play an important role in elucidating species relationships. The woolly trichome with a granular tapered base (character 15,0) unites *Cineraria canescens* and *C. erosa* in Clade V (Figure 16) and *C. aspera* and *C. lyratiformis* in Clade VI (Figure 16). The absence of a woolly or cobwebby indumentum due to fine cobwebby trichomes with an oblique base (character 17) unites all the species in Clade III (Figure 16).

Despite the variability in the shape of the leaves in *Cineraria*, lower leaf shape (7) performs well and is a synapomorphy for three clades in the weighted consensus tree (Figure 16, Clades IV, VI and VIII). Although upper leaf shape (6) is more homoplasious than lower leaf shape, it supports a substantial number of relationships in both unweighted and weighted analyses. Deltoid upper leaf shape is a synapomorphy for the species in Clade XIV (Figure 16) and a reniform upper leaf shape also unites the three species in Clade V and 11 of the 12 species in Clade X (Figure 16), as *C. ngwenyensis* reverts to a deltoid-reniform upper leaf shape. Pinnatifid dissection of the leaf (8,2) is instrumental in grouping *C. aspera*, *C. cyanomontana* and *C. lyratiformis* together (in Clade VI) and dissection of the leaf apex (9) is a key character in supporting the large unresolved clade (Clade b) in the unweighted analysis (Figure 15) and Clade I in the weighted analysis (Figure 16). A denticulate leaf margin (10,2) serves as a synapomorphy for *C. austrotransvaalensis*, *C. canescens*, *C. erosa* and *C. pulchra* (Clade V, Figure 16). Although both upper and lower leaf bases (characters 11 and 12) are homoplasious, they do contribute considerably to the topology of the consensus trees of both unweighted and weighted analyses (Figures 15 and 16).

Non-leaf characters useful in elucidating species relationships in both the unweighted and weighted analyses are peduncle length (21) and arrangement (number) of capitula (19), as well as size of capitula as indicated by ray floret number (25) and number of involucre bracts (35), although the latter is fairly homoplasious. Indumentum of the involucre bracts (34) also performs reasonably well. A small number (5–8) of ray florets (25) distinguishes a large group of species in Clade II (Figure 16), but reversals (i.e. increases and subsequent decreases) do

occur within these clades. Although the size of the carpopodium and the extensions of the disc cypselae appear to perform well, they are essentially generic characters, with variation in only one or two species of *Cineraria* (i.e. a carpopodium of only 6 rows in *C. pulchra* and disc cypselae with broad wings in *C. vallis-pacis* and *C. lyratiformis*).

Thus, both vegetative and reproductive characters serve to elucidate the pattern of evolution in *Cineraria*, with some being more stable than others. Both the stability and variability of the various characters are reflected in their usefulness in keys to the species. However, although the morphological features do provide useful information regarding species relationships in *Cineraria*, many of the characters are variable within a species and their usefulness is therefore limited due to their polymorphic nature. In addition, although a best attempt has been made to describe character states, it is possible that homology has been incorrectly interpreted or that differences are recognised where no substantial genetic changes exist. Furthermore, the very plastic nature of certain of the characters (e.g. change in leaf size according to habitat/environment) precludes them from being used in such an analysis.

Character evolution within *Cineraria*

The sister-group relationship of *Bolandia* and *Cineraria* provides a firm point of phylogenetic comparison for reconstructing certain aspects of morphological character evolution within *Cineraria*, despite the incomplete resolution and incongruence within and among data sets. The perennial herb with solitary, relatively large capitula on long peduncles is the plesiomorphic growth form/condition for *Cineraria* (Figures 15–20), with the woody subshrub developing slightly later in the evolution of the genus. However, there are several independent reversals to the herbaceous condition (Figures 16 and 17). Auricles at the base of the leaves also appear to have undergone independent losses (in *C. saxifraga* and *C. grandibracteata*) or reduction to a widening of the petiole (e.g. *C. huilensis* and *C. vagans*).

Along with the evolution of auricles and woody habit is an increase in the number of capitula and a decrease in the length of the peduncles and size of the capitula (Figures 16 and 17). However, there is a reversion to long peduncles with fewer (or solitary), larger capitula (in *C. geraniifolia*, *C. vagans* and *C. humifusa*) in the weighted analysis (Figure 16, Clade XII). The size of the capitula is indicated by both number of ray florets and disc florets and to some extent by the number of involucre bracts. It has been noted that an increase in altitude affects the size of the capitula in *Cineraria* (Hedberg, 1957), with capitula tending to become larger at higher altitude. As a consequence, the number of ray florets (25) and to a lesser extent number of disc florets (30) are polymorphic in certain species (notably *C. erodioides* and *C. deltoidea*). Reduction of ray limb length has only occurred twice in *Cineraria* - in *C. abyssinica* and also in *C. anampoza* subvar. *anampoza* from Madagascar.

Deltoid-reniform upper leaves (6,3) and reniform lower leaves (7,0) are the plesiomorphic leaf shapes in *Cineraria* (Figure 16), with two parallel changes towards deltoid upper leaves (6,2) and to deltoid-reniform lower leaves (7,3). Reniform upper leaves (6,0) apparently evolved twice and lyratiform upper leaves appear to have evolved independently in *C. abyssinica* and *C. lyratiformis*. Pinnatifid dissection of the leaves (8,2) is associated with an ovate to lanceolate outline/leaf shape that unites *C. aspera*, *C. lyratiformis* and *C. cyanomontana* (in Clade VI, Figure 16). Further dissection of the lobes of the leaves (9,1) occurs in eight species (*C. abyssinica*, *C. mazoensis*, *C. pulchra* and *C. austrotransvaalensis*, *C. canescens*, *C. erosa*, *C. foliosa* and *C. aspera*) which are hypothesised to arise independently in the large Clade I (Figure 16), although some of these species are grouped together in clades in the combined morphological and molecular analyses (Figures 17 and 19). Most notably, *C. mazoensis* and *C. pulchra* form a clade (Clade 8a, Figure 18; within Clade VI, Figure 19) supported mainly by morphological characters, as the polymorphic ITS characters that they share are not used by PAUP to evaluate alternative topologies (although they are identified with the *mstaxa* = polymorphic option in operation).

The tomentose/cobwebby indumentum present in many of the species of *Cineraria* is lost in Clade VIII (Figure 16) and replaced by glandular and or shorter eglandular trichomes. Different types of glandular trichomes on leaves (Character 13) appear to have evolved only once (Figures 16 and 17) and are associated with herbaceous life forms. A third type of trichome resulting in a tomentose indumentum (tapering basal cells and a long appendage: 15,0/2) apparently evolved independently a few times in *Cineraria* (with and without granular patterning on the surface).

A tomentose or cobwebby indumentum on the involucre bracts is plesiomorphic and occurs in many species of *Cineraria*. Total or partial loss of cobwebby or tomentose indumentum on the involucre bracts is fairly well correlated with loss of these trichomes on the leaves. A glabrous involucre unites species in Clade VII (Figure 16), although two species develop glandular trichomes and/or short eglandular trichomes (*C. glandulosa* and *C. humifusa*).

Broad wings on ray and disc cypselae have also evolved at least twice (*Cineraria vallis-pacis* and *C. lyratiformis*), and just on the ray cypselae in certain forms of both *C. platycarpa* and *C. erodioides*. Cypselae surface ornamentation (striate vs. smooth and sunken/papillose) is very homoplasious and therefore not useful in grouping species. It is also polymorphic for certain species.

Species relationships within *Cineraria*

In none of the morphological analyses do any of the clades within *Cineraria* have bootstrap support of over 50% in the strict consensus trees (Figures 13–16), although a few clades are consistently retrieved in all (or in three out of four) analyses and some are also reflected in the

clades from the species included in the combined morphological and molecular analyses (Figures 17–19).

No infrageneric classification is possible from these analyses, although some feasible relationships are suggested. For example, *Cineraria geraniifolia*, *C. dryogeton* and *C. vagans* are morphologically very similar and their distributions overlap in the Eastern Cape/KwaZulu-Natal; *C. geifolia* and *C. humifusa* also share a morphological resemblance, and *C. humifusa* has a restricted distribution in the Saldanah Bay region of the Western Cape, where *C. geifolia* also occurs. The placing of these five species with reniform leaves in a clade together with *C. ngwenyensis* from Swaziland (Clade I, Figure 14) and also with *C. saxifraga* from the Eastern Cape (Clades e and XI, Figures 15 and 16 respectively), requires further investigation using molecular markers.

Cineraria erosa and *C. canescens* are very likely related as they share many morphological features and both occur in the Western and Northern Cape. They are consistently placed together and with *C. austrotransvaalensis* in the morphological analyses (Clade B, Figures 13 and 14; Clade c, Figure 15 and Clade V, Figure 16). (Unfortunately molecular data were not available for *C. erosa* and *C. canescens* to include them in the combined analyses.) All three species have a denticulate leaf margin (10,2) and leaf lobes further divided (9,1), and *C. erosa* and *C. austrotransvaalensis* both possess the woolly trichome with the tapering granular basal cells (15,0).

It is also likely that *Cineraria aspera*, *C. cyanomontana* and *C. lyratiformis* share common ancestry as all have pinnatifid leaves and are shrubby with many capitula (Clade C, Figure 13; Clade d, Figure 15 and Clade VI, Figure 16). *Cineraria cyanomontana* is endemic to the Blouberg in Limpopo Province, South Africa, while the other two species are fairly widespread. The deltoid-leaved species are also probably closely related, viz. *C. atriplicifolia*, *C. decipiens*, and *C. deltoidea* (Clade H, Figure 14; Clade XIV, Figure 16), although the sister relationship of *C. anampoza* (from Madagascar) to these species needs to be tested using molecular markers. *Cineraria anampoza* has deltoid-reniform upper and lower leaves, and reportedly has a scrambling growth form, similar to *C. deltoidea*, which extends throughout the eastern highlands of Africa.

Other species relationships are not as clearly promoted by the various analyses and others are not tested as no molecular data were available for their inclusion in the combined analyses. For example, a relationship between *Cineraria austrotransvaalensis*, *C. canescens* and *C. erosa* suggested by the morphological analyses (Figures 13–16), but in the combined molecular and morphological analyses, *C. austrotransvaalensis* is variously associated with *C. albicans*, *C. erodioides*, *C. vallis-pacis* and/or *C. pulchra* (Figures 17–19). Analysis of the

ITS data (Chapter 2) suggests a reticulate history for *C. austrotransvaalensis* involving *C. lobata* and *C. saxifraga*, as well as for several other species.

Much of the structure of species relationships in *Cineraria* in the combined *trnL-trnF* and morphology consensus tree (Figure 17) is due to morphology; only Clade G is supported by a point mutation. In the consensus tree resulting from analysis of combined ITS and morphology data sets (Figure 18), a limited amount of molecular information supports the internal structure of *Cineraria*. As noted previously, only Clade V (Figure 19) shows congruence between the phylogenetic cpDNA and ITS data.

Intraspecific relationships in C. deltoidea and C. lobata

The two geographically disjunct populations of *Cineraria deltoidea* are placed in Clade F in the strict consensus tree of the combined *trnL-trnF* and morphology (Figure 17), which supports Jeffrey's (1986) decision to place *C. grandiflora* from East Africa and *C. monticola* from the Soutpansberg and southern Zimbabwe into synonymy in *C. deltoidea* (from KwaZulu-Natal and the Eastern Cape), although populations from the latter regions still need to be analysed. However, the combined morphological and ITS and total analyses (Figures 18 and 19) do not support any clear relationship between the two samples, indicating the different evolutionary histories which have occurred in these regions, resulting in a very variable, widespread species. As *C. deltoidea* EA is placed in Clade V of the tree resulting from total analysis (Figure 19), it is likely that this is the main 'organismal' lineage and that *C. deltoidea* Sn has gained its ITS Clade 9 haplotype through reticulate evolution.

Cineraria lobata is also a widespread, highly variable species occurring from the Western Cape to the Eastern Cape, with a disjunct subspecies in the Soutpansberg of South Africa, *C. lobata* ssp. *soutpansbergensis*. Two of the three infraspecific samples of *Cineraria lobata* that were included form part of the basal polytomy in the combined *trnL-trnF* and morphological analysis, viz. the small-leaved form of *C. lobata* ssp. *lobata* (from near Uniondale in eastern region of the Western Cape) and *C. lobata* ssp. *soutpansbergensis*. The robust form (R) of *C. lobata* ssp. *lobata* from the Cape Peninsula emerges in a clade separate from the other two forms of *C. lobata* in the combined morphological and *trnL-trnF* and total analyses (Figures 17 and 19 and its position is not fully resolved in the combined morphological and ITS analysis (Figure 18). It is suspected that this more robust, usually hairy form of *C. lobata* is hybridizing with *C. geifolia* in this region (Chapter 6). As noted previously, there is also evidence of reticulate evolution involving *C. lobata* ssp. *lobata* R, *C. saxifraga* and *C. austrotransvaalensis*. It is also possible that the high-altitude cobwebby form of *C. lobata* ssp. *soutpansbergensis* (sampled here) is hybridising with *C. cyanomontana* on the Blouberg, as suggested by their position in the separate ITS analyses (Chapter 2) and combined morphological and ITS and total analyses (Figures 18 and 19).

Future studies

Further work is needed to find more variable molecular regions than either *trnL-trnF* or ITS to provide a more fully resolved and thus more convincing hypothesis for speciation and species relationships within *Cineraria*. It is unlikely that analysis of morphological data alone will be sufficient to fully resolve species relationships in *Cineraria*. Difficulties inherent in the process of identifying homologous features (and determining states) and variability of certain characters (polymorphism) in some species confound the analysis. We suggest that both plastid and nuclear DNA regions are investigated to reveal the evolutionary history of *Cineraria*, as they appear to be not fully congruent due to the reticulate nature of this history. In addition, reticulate relationships amongst species in *Cineraria*, as indicated by the ITS sequence data, requires further attention.

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APPENDIX 1

Voucher specimens for micromorphological studies

Abbreviations for Use (Column 4): F = florets, T = trichomes cleared for (LM), Tr = trichomes (SEM), C = cypselae (SEM)

Genus/species	Specimen	Locality	Use
<i>Bolandia argillacea</i>	<i>Esterhuysen 36192</i> (K)	Western Cape, Worcester, Brandwacht Peak	F, C
	<i>Esterhuysen 35117</i> (BOL)	Western Cape, Worcester, Brandwacht Peak	F, Tr
<i>Bolandia pedunculosa</i>	<i>Jacot-Guillarmod 2325</i> (PRE)	Lesotho, Thaba Ntlenyana	F, T
	<i>Barker 5398</i> (NBG)	Western Cape, Swellendam District	F
	<i>Compton 2722</i> (BOL, NBG)	Western Cape, Laingsburg, Witteberg	C, Tr
	<i>Esterhuysen 7547</i> (BOL)	Western Cape, Cederberg	T
<i>Cineraria abyssinica</i>	<i>Thulin 1550</i> (K)	Ethiopia, Arussi Province, Chilalo Awraja	F, C, Tr
	<i>De Wilde & Gilbert 82</i> (WAG)	Ethiopia, Begemder Province, Semian Mts.	Tr
<i>C. albicans</i>	<i>Davidson 2012</i> (J)	KwaZulu-Natal, Port Shepstone District	F
	<i>Balkwill & Cron s.n. sub J 47735</i>	KwaZulu-Natal, Oribi Gorge	F, Tr
	<i>Hilliard 3138</i> (NU)	KwaZulu-Natal, Richmond District	F, Tr
	<i>Hilliard & Burt 16604</i> (PRE)	Eastern Cape, Matatiele District	F, C
	<i>Pellew 42</i> (NU)	KwaZulu-Natal, Port Shepstone District	F, C
	<i>Noberley 70</i> (NU)	Eastern Cape, Kokstad	F, C
<i>C. alchemilloides</i> ssp. <i>alchemilloides</i>	<i>Mauve & Hugo 36</i> (PRE)	Northern Cape, Porterville	F, Tr
	<i>Taylor 2813</i> (NBG)	Northern Cape, Calvinia, Ekerdam	F

	<i>Stephens 7005</i> (BOL)	Northern Cape, Koue Bokkeveld	F
<i>C. alchemilloides</i> <i>ssp. namibensis</i>	<i>Winter 284</i> (J)	Namibia, Nubib Mountain	F, Tr
	<i>Giess 13574</i> ()	Namibia, Nauchas District, Farm Göllschau,	F
	<i>Merxmüller & Giess 3558</i> (BR)	Namibia, Windhoek District, Erosgebirges	F
	<i>Schelppe 155</i> (K)	Namibia, Windhoek	F, C
<i>C. anampoza</i>	<i>Phillipson 1604</i> (WAG)	Madagascar, Antananarivo, E slope of Ankaratra	F, C
	<i>Baron 2438</i> (K)	Central Madagascar	T
	<i>Humbert 20835</i> (K)	Madagascar, Manjakatempo Forestry Station	T
	<i>Baron 2113</i> (K)	Madagascar, Imerina Province forests	T
<i>C. aspera</i>	<i>Cron & Goodman 551</i> (J)	Eastern Cape, Naude's Nek	F
	<i>Cron & Goodman 557</i> (J)	Eastern Cape Naude's Nek	F
	<i>Cron & Balkwill 257</i> (J)	Gauteng, Moffat Park, Johannesburg	F, T
	<i>Jacobsz 2535</i> (K)	Free State, Harrismith	T, C
	<i>Herman 473</i> (K)	Northern Cape, Colesburg	T
	<i>Bolus 589</i> (BOL)	Eastern Cape, Oudeberg near Graaff Reinett	Tr
	<i>Bolus 28</i> (BOL)	Eastern Cape, Elandshoek	Tr
	<i>Collett 484</i> (K)	Lesotho, Teyateyaneng District	C
	<i>Tait s.n. sub PRE 45366</i>	North-West, Potchefstroom	C
<i>C. atriplicifolia</i>	<i>J Medley Wood 515</i> (K)	KwaZulu-Natal, Inanda District	F
	<i>Medley Wood 1026</i> (K, Z)	KwaZulu-Natal, Durban District	F
	<i>Medley Wood 515</i> (BOL, K)	KwaZulu-Natal, Inanda District	F, C

	<i>Wood s.n.</i> (Z)	KwaZulu-Natal, Inanda District	F
	<i>Cron 7</i> (J)	KwaZulu-Natal, Camperdown District	F, C, T
<i>C. atriplicifolia</i> (cont.)	<i>Medley Wood 8295</i> (Z)	KwaZulu-Natal, Durban District	F
	<i>Medley Wood 640</i> (BOL, Z)	KwaZulu-Natal, Durban District	F
	<i>Hilliard 1506</i> (NU)	KwaZulu-Natal, Pinetown District	F
	<i>Wells 1322</i> (NU)	KwaZulu-Natal, Camperdown District	F, C
	<i>Hilliard & Burt 10319</i> (K, MO, NU, PRE)	KwaZulu-Natal, Ixopo District	F, C
	<i>Hilliard 1854</i> (NU)	KwaZulu-Natal, Pinetown District	F
	<i>Hilliard 2830</i> (K, NU)	KwaZulu-Natal, Pinetown District	F, C, T
	<i>Wylie Wood 8182</i> (K)	KwaZulu-Natal, Richmond District	F
	<i>Johnson 1499</i> (PRE)	KwaZulu-Natal, Camperdown District	F, C
	<i>Drege 5137</i> (MO, PRE)	KwaZulu-Natal, Stanger (Port Natal)	F
	<i>Munro P.S. 387</i> (PRE)	KwaZulu-Natal, Mtubatuba District	F
<i>Cineraria austrotransvaalensis</i>	<i>Cron & McCallum 341</i> (J)	Gauteng, Linksfield Ridge, Johannesburg	F, C
	<i>Burger 329</i> (PRE)	Gauteng, Linksfield Ridge	F
	<i>Rehmann 6825</i> (K)	Mpumalanga, Standerton District	F
	<i>Louw 1083</i> (PRE)	North-West, Potchefstroom District	F, C
	<i>Burger 329</i> (PRE)	Gauteng, Johannesburg District	F

	<i>Van Jaarsveld s.n.</i> (NBG 12390)	North-West, Potchefstroom District	F
	<i>Behr 486</i> (PRE)	North-West, Potchefstroom District	F
	<i>Young T.M. 2666584</i> (PRE)	North-West, Potchefstroom District	F
	<i>Bryant C42</i> (PRE)	Gauteng, Johannesburg District	F
<i>C. austrotransvaalensis</i> (cont.)	<i>Van de Westhuizen 789</i> (PRE)	North-West, Potchefstroom District	F
	<i>Sutton 589</i> (PRE)	North-West, Klerksdorp District	F
	<i>Mogg 21012</i> (PRE)	North-West, Potchefstroom District	F
	<i>Mogg 21975</i> (PRE)	Gauteng, Johannesburg District	F
	<i>Botha 2521</i> (PRE)	North-West, Potchefstroom District	F
	<i>Murray 563</i> (PRE)	Gauteng, Johannesburg District	F
	<i>Cron 19</i> (J)	Gauteng, Johannesburg District	F, Tr, T
	<i>Cron 20</i> (J)	Gauteng, Johannesburg District	F, Tr
<i>C. canescens</i> var. <i>canescens</i>	<i>Leipoldt 3274</i> (BOL)	Northern Cape, De Kom (now Farm Karas), near Leliefontein	F, Tr
	<i>Pearson 5762</i> (BOL)	Northern Cape, Great Namaqualand, Sneeuwkop	F
	<i>Compton 11560</i> (BOL)	Northern Cape, Namaqualand, Droedap	F
	<i>Hutchinson 859</i> (K)	Namaqualand, Khamiesberg, Sneeuwkop	F, C
<i>C. canescens</i> var. <i>flabellifolia</i>	<i>Rev. Whitehead</i> (TCD)	Northern Cape, Modderfontein	F
	<i>Salter 797</i> (K)	Northern Cape, Kamieskroon	C

	<i>Levyins 4044</i> (BOL)	Northern Cape, Leliefontein	C
<i>C. cyanomontana</i>	<i>Cron, Knox & Winter 350</i> (J)	Limpopo Province, Blouberg Mountain	F, C
	<i>Cron, Knox & Winter 355</i> (J)	Limpopo Province, Blouberg Mountain	F, C, Tr
	<i>Esterhuysen 21461</i> (BOL)	Limpopo Province, Blouberg Mountain	Tr
<i>C. decipiens</i>	<i>Huntley 891</i> (NU)	KwaZulu-Natal, Ngoye Forest, Mtunzini District	F
	<i>Gerrard & M'Ken 1040</i> (BM, K)	KwaZulu-Natal, Umvoti District	F
<i>C. decipiens</i> (cont.)	<i>Galpin 14778</i> (PRE)	KwaZulu-Natal, Msinga District	F, C
	<i>Ward 8858</i> (K)	KwaZulu-Natal, Mtubatuba	F
	<i>Hilliard 1916</i> (NU)	KwaZulu-Natal, Port Shepstone	F
	<i>Hilliard 2679</i> (NU)	KwaZulu-Natal, Mtunzini District	F
	<i>Huntley 891</i> (NU)	KwaZulu-Natal, Mtunzini District	F
	<i>Whellan 1096</i> (PRE)	KwaZulu-Natal, Port Shepstone District	F
	<i>Ward 8858</i> (K)	KwaZulu-Natal, Mtubatuba	F
	<i>Wood s.n.</i> sub NU 42042	KwaZulu-Natal, Mtunzini District	F
	<i>Cron & Brummer 5a</i> (J)	KwaZulu-Natal, Port Shepstone District	F, C, Tr
	<i>Lowrey & Van Wyk 1052</i> (J)	KwaZulu-Natal, Mtunzini District	F
<i>C. deltoidea</i>	<i>Hilliard 5055</i> (NU)	KwaZulu-Natal, Blinkwater Bush, Lion's River District	F, C
	<i>Compton 19753</i> (NBG)	KwaZulu-Natal, Qudeni Forest	F, C
	<i>Hilliard & Burt 10168</i> (MO, PRE)	KwaZulu-Natal, Richmond District, Byrne Peak	F, C, T
	<i>Acocks 12705</i> (PRE)	KwaZulu-Natal, Richmond District, Hella-Hella road	F, C

	<i>Esterhuysen 20292 (PRE)</i>	KwaZulu-Natal, Cedara	F, C
	<i>Edwards 3102 (NU)</i>	KwaZulu-Natal, Polela District	F, C
	<i>Hilliard 5373 (MO)</i>	KwaZulu-Natal, Everton, Pinetown district	T
	<i>Hilliard & Burt 9037 (K)</i>	KwaZulu-Natal, Donnybrook Farm, Richmond district	T
	<i>Cron et al. 281 (J)</i>	Limpopo Province, Soutpansberg, Road to Wyliespoort	F
	<i>Hutchinson & Gillett 4178 (K)</i>	Limpopo Province, Soutpansberg	T
<i>C. deltoidea (cont.)</i>	<i>Cron, Balkwill & Balkwill 289 (J)</i>	Limpopo Province, Soutpansberg	T
	<i>Cron, Balkwill, Balkwill & Otto 289 (J)</i>	Limpopo Province, Soutpansberg	T, Tr
	<i>Knox 4274 (J)</i>	Ethiopia	F
	<i>Ash 2326 (K)</i>	Ethiopia	T
	<i>Gillett 15002 (K)</i>	Ethiopia, Agheremarium	C, T, Tr
	<i>de Wilde 10057 (WAG)</i>	Ethiopia, Arussi Mountains	C, T
	<i>A.S.T. Th 1812 (K)</i>	Sudan, Kippia, Imatong Mountains	F, T
	<i>Johnston 1475 (BR)</i>	Sudan, Imatong Mountains	F
	<i>Nappier 536 (K)</i>	Kenya, Ngong Hills	C, T, Tr
	<i>Kibue K159 (BR)</i>	Kenya, Ngong Hills	C, T, Tr
	<i>Kayu 526 (PRE)</i>	Kenya, Ngong Hills	F
	<i>Muasya, Cron & Knox 24 (J)</i>	Kenya, Aberdares	F, T, Tr
	<i>Hedberg 1496 (K)</i>	Kenya, Aberdares	C, T, Tr
	<i>Fries & Fries 1201 (BR)</i>	Kenya, Aberdares	C, T, Tr
	<i>Bally 6414 (K)</i>	Kenya, Mount Kenya	C, T, Tr
	<i>Townsend 2257 (K)</i>	Kenya, Mount Kenya	C, T, Tr
	<i>Hedberg 1890 (K)</i>	Kenya, Mount Kenya	T

	<i>Greenway & Kanuri 13859</i> (PRE)	Kenya, Narok	C, T, Tr
	<i>Maas & Geesteranus 6157</i> (PRE)	Kenya, Nakuru	C, T
	<i>Hedberg 1293</i> (K)	Tanzania, Mount Kilimanjaro	C, T, Tr
	<i>Schlieben 4470</i> (BR)	Tanzania, Mount Kilimanjaro	F
	<i>Proctor 1250</i> (PRE)	Tanzania, Mbeya District	C, T
	<i>Greenway 8404</i> (K)	Tanzania, Rungwe District	C, T, Tr
	<i>Richards 16800</i> (BR)	Tanzania, Ufipa District	C, T, Tr
	<i>Bullock 3403</i> (BR)	Tanzania, Ufipa District, Bullock	C, T
	<i>Thulin & Mboro 1241</i> (K)	Tanzania, Mbeya District, Poroto Mountains	F
<i>C. deltoidea</i> (cont.)	<i>Mabberley & Salehe 1502</i> (K)	Tanzania, Kilosa District, Ukaguru Mts	C, T
	<i>Louis 5324</i> (BR)	DRC, Kabare, Karisimbi Volcano	C, T
	<i>Lebrun 7370</i> (BR)	DRC, Kabare	C, T
	<i>Bamps 3087</i> (BR)	Rwanda, Ruhengiri-Gisenye	C, Tr
	<i>Burt 3142</i> (K)	Rwanda, Namlagira	F
	<i>Lugard 301</i> (K)	Uganda, Mount Elgon	C, T
	<i>Dummer 3523</i> (K)	Uganda, Mount Elgon	C, T
<i>C. dryogeton</i>	<i>Abbott 7809</i> (J)	KwaZulu-Natal, Umtamvuna Nature Reserve	F, C, T
	<i>Abbott 1885</i> (NH)	KwaZulu-Natal, Umtamvuna Nature Reserve	F, T
<i>C. erodioides</i> var. <i>erodioides</i>	<i>Hilliard & Burt 12343</i> (NU)	Eastern Cape, Elliott District 3127 BB	F, C
	<i>Hilliard & Burt 12322</i> (NU)	Eastern Cape, Elliott District	F, C
	<i>Phillipson 1092</i> (MO, PRE)	Eastern Cape, Amatole Mountains	F
	<i>Bayliss 7511</i> (MO)	Eastern Cape, Old Quarry, Grahamstown	F

	<i>Evans 1357</i> (PRE)	KwaZulu-Natal, Mont Aux Sources	F, C, T
	<i>Jacot-Guillarmod 9336</i> (GRA)	Eastern Cape, Coldsprings, Grahamstown	F
	<i>Hilliard & Burt 10820</i> (NU)	Eastern Cape, Grahamstown Nature Reserve	F
	<i>Jacot-Guillarmod 9504</i> (GRA)	Eastern Cape, Coldsprings, near Grahamstown	T, C
	<i>Hilliard & Burt 11059</i> (K)	Eastern Cape, Stutterheim Division, Gubu Dam	F, T
	<i>Cron, Scott-Shaw & Ching 1</i> (J)	KwaZulu-Natal, Gladstone's Nose	F, T
	<i>Dieterlen 576</i> (NBG)	Lesotho, Leribe	T
	<i>Phillipson 1405</i> (K)	Lesotho, Leribe	T
	<i>Hilliard & Burt 9664</i> (K)	Lesotho, Sani Pass	C
<i>C. erodioides</i> var. <i>erodioides</i> (cont.)	<i>Hilliard & Burt 6727</i> (NU)	Eastern Cape, Naude's Nek	F, T
	<i>Bayliss 1606</i> (Z)	Eastern Cape, Kommadagga	T
	<i>Fourcade 3069</i> (K)	Eastern Cape	T
	<i>Cron & Wilson 271</i> (J)	Mpumalanga, Mariepskop	T, Tr
	<i>Merxmüller 583</i> (K)	Mpumalanga, Mariepskop	C
<i>C. erodioides</i> var. <i>tomentosa</i>	<i>Cron & Goodman 579</i> (J)	Limpopo, Venda, Gogogo	F, Tr
<i>C. erosa</i>	<i>Compton 18358</i> (NBG)	Western Cape, Baden Kloof, Montagu	F, C
	<i>Compton 22866</i> (NBG)	Western Cape, Laingsburg Div., Witteberg, Tweedside	F, Tr
	<i>Pillans 7970</i> (K)	Western Cape, Piketberg	C
	<i>Hanekom 1308</i> (K)	Western Cape, Ceres, Koue Bokkeveld	C
	<i>Cron & Perrett 329</i> (J)	Western Cape, Paarl Mountain	C (LM), Tr
<i>C. erosa/aspera</i> hybrid?	<i>Stokoe 60471</i> (SAM)	Western Cape, Prince Albert Division, Swartberg Pass	F

	<i>Wells 3740 (K)</i>	Western Cape, Schoemanspoort, Prince Albert	T
<i>C. foliosa</i>	<i>Goetze 973 (E)</i>	Tanzania, Kipengere Range	F, C
<i>C. geifolia</i>	<i>Robertson 462 (WAG)</i>	Western Cape, Hermanus, Fernkloof Nature Reserve	F, C
	<i>Smuts s.n. sub PRE 44090</i>	Western Cape, Stellenbosch District, Vlaggenberg Hills	F, C
	<i>Cron 314 (J)</i>	Western Cape, Davidskraal	Tr
	<i>Talbot 1840 (K)</i>	Western Cape, Caledon	T
<i>C. geraniifolia</i>	<i>Hilliard & Burt 9739 (MO)</i>	KwaZulu-Natal, Underberg District, Sani Pass	F, C
	<i>Cron & Ching 2 (J)</i>	KwaZulu-Natal, Mooi River District	F, T
	<i>Phillipson 1276 (MO)</i>	Eastern Cape, Hogsback	Tr
	<i>Wright 204 (NU)</i>	KwaZulu-Natal, Mpendhle District	F
<i>C. geraniifolia (cont.)</i>	<i>Wright 512 (NU)</i>	KwaZulu-Natal, Underberg District	F
	<i>Hilliard & Burt 12393 (K, NU)</i>	Eastern Cape, Fort Beaufort District	F
	<i>Hilliard & Burt 17609 (NU)</i>	KwaZulu-Natal, Mpendhle District	F
	<i>Balkwill et al. 1323 (NU)</i>	KwaZulu-Natal, Mooi River District	F
	<i>Hilliard 8230 (K, NU)</i>	KwaZulu-Natal, Mpendhle District	F
	<i>Hilliard & Burt 13228 (K, NU)</i>	Eastern Cape, Somerset East District	F
	<i>Hilliard & Burt 17345 (NU)</i>	KwaZulu-Natal, Underberg District	F
	<i>Thode A331 (PRE 17028)</i>	KwaZulu-Natal, Utrecht District	F
	<i>Hilliard & Burt 12618 (NU)</i>	KwaZulu-Natal, Underberg District	F

	<i>Grice s.n.</i> (NU)	KwaZulu-Natal, Underberg District	F, C
	<i>Killick 1427</i> (PRE)	KwaZulu-Natal, Bergville District	F
	<i>Compton 17794</i> (NBG)	Eastern Cape, East London District	F, C,
	<i>Flanagan 692</i> (BOL, NU)	Eastern Cape, Stutterheim District	F, C
	<i>Devenish 1917</i> (NU)	KwaZulu-Natal, Utrecht District	F
	<i>Rennie 1132</i> (NU)	KwaZulu-Natal, Polela District	F
	<i>Gibbs-Russell 3107</i> (MO, PRE)	Eastern Cape, Fort Beaufort District	F
	<i>Esterhuysen 27109</i> (PRE)	Eastern Cape, Uitenhage District,	F
	<i>Giffen 188</i> (PRE)	Eastern Cape, Fort Beaufort District	F
	<i>Rehmann s.n.</i> (Z)	Mpumalanga, Volksrust District	F
<i>C. geraniifolia</i> (cont.)	<i>J.M. Wood 4307</i> (K,Z)	KwaZulu-Natal, Pietermaritzburg District	F
	<i>Burger & Quicke 8</i> (PFV)	KwaZulu-Natal, Umvoti District	F
	<i>Rennie 351</i> (NU)	KwaZulu-Natal, Polela District	F
	<i>N.E. Shirley s.n.</i> (NU)	KwaZulu-Natal, Dundee District	F
	<i>Galpin 14071</i> (PRE)	Eastern Cape, Matatiele District	F
	<i>Tyson 1483</i> (PRE)	KwaZulu-Natal, Kokstad	F
	<i>Bokelmann 1 - PL10</i> (NBG)	Eastern Cape, East London District	F
	<i>Thode A2683</i> (PRE)	Eastern Cape, Port Elizabeth District	F

	<i>Johnson 1261 (PRE)</i>	Eastern Cape, Stutterheim District	F
<i>C. glandulosa</i>	<i>Huntley 154 (NU)</i>	KwaZulu-Natal, Mphendhle	F
<i>C. glandulosa</i> X <i>atriplicifolia</i>	<i>Cron & Scott-Shaw 9 (J)</i>	KwaZulu-Natal, Richmond District, Farm Wingfield	F, T, C
	<i>Moll 3037 (NU)</i>	KwaZulu-Natal, Richmond District, Tala Farm	F, T, C
<i>C. grandibracteata</i>	<i>Scott-Shaw 935 (J)</i>	KwaZulu-Natal, Umvoti District, Karkloof Nature Reserve	F
	<i>Hilliard & Burtt 10327 (PRE)</i>	KwaZulu-Natal, Lion's River District.	F, C
	<i>Tyson 1283 (BOL, NBG)</i>	KwaZulu-Natal, Kokstad	F
	<i>Galpin 11987 (PRE, BOL)</i>	KwaZulu-Natal, Richmond District	F
	<i>Van Jaarsveld & Tarr 5057 (NBG)</i>	KwaZulu-Natal, Richmond District	F, C
	<i>Hilliard 8095 (MO)</i>	KwaZulu-Natal, Richmond District	F, C, Tr, T
	<i>Cron & Scott-Shaw 10 (J)</i>	KwaZulu-Natal, Umvoti District	F, C, Tr, T
	<i>Fourcade s.n. sub BOL 50369</i>	KwaZulu-Natal, Underberg District	F
<i>C. grandibracteata</i> (cont.)	<i>Coleman s.n. (NU)</i>	KwaZulu-Natal, Alfred District	F
<i>C. huilensis</i>	<i>Borges 347 (K)</i>	Angola, Huila, Humpata, encosta da Leba	F, C, Tr
	<i>Borges 89 (LISC)</i>	Angola, Huila, Lubango	Tr
<i>C. humifusa</i>	<i>Pillans 6971 ()</i>	Western Cape, Malmesbury District, west of Langebaan	F
<i>C. lobata</i> ssp. <i>lobata</i>	<i>Cron & Goodman 569 (J)</i>	Western Cape, Uniondale, near Joubertina,	F, C
	<i>Cron & Hodgkiss 317 (J)</i>	Western Cape, Cape Point Nature Reserve	F
	<i>Page 47 (PRE)</i>	Western Cape Montagu Baths	F

	<i>Compton 23880</i> (NBG)	Western Cape, Uniondale Division	F
	<i>Boucher 4005</i> (PRE)	Western Cape, Yzerfontein	F
<i>C. lobata</i> ssp. <i>platyptera</i>	<i>Dahlstrand 3151</i> (K)	Eastern Cape, Port Elizabeth District	T
	<i>Cook s.n.</i> (NU)	Sapkamma Station	T
<i>C. lobata</i> ssp. <i>soutpansbergensis</i>	<i>Cron, Knox & Winter 344</i> (J)	Limpopo Province, Blouberg Mountain	F, Tr
	<i>Cron, Knox & Winter 363</i> (J)	Limpopo Province, Soutpansberg, Letjuma	F
	<i>Compton 18049</i> (NBG)	Limpopo Province, Soutpansberg	F
	<i>Cron, Knox & Winter 373</i> (J)	Limpopo Province, Soutpansberg, Letjuma	Tr
	<i>Cron, Balkwill & Balkwill 287</i> (J)	Limpopo Province, Soutpansberg	T
<i>C. longipes</i>	<i>Cron, Pfab & Mills 485</i> (J)	Gauteng, Suikerbosrand Nature Reserve	F
	<i>Cron & Balkwill 306</i> (J)	Gauteng, Mondeor	C
	<i>C.E. Moss 17834</i> (J)	Gauteng, Johannesburg, Thorntree Kloof	T
	<i>Bayer D94</i> (J)	Gauteng, Turffontein	T
<i>C. lyratiformis</i>	<i>Balkwill, McDade & Lundberg 11804</i> (J)	Postmasberg district, Northern Cape, S.A.	F
	<i>Killick 472</i> (J)	KwaZulu-Natal, Cathedral Peak Forestry Station	F
<i>C. lyratiformis</i> (cont.)	<i>Hoener 2217</i> (WAG)	Lesotho, Sehlabathebe National Park	F
	<i>Cron & Balkwill 459</i> (J)	KwaZulu-Natal, Utrecht	Tr
	<i>Phillips 1853</i> (J)	Mpumalanga, Standerton	T, C
	<i>West 378</i> (PRE)	KwaZulu-Natal, Estcourt	T
	<i>Leistner & Joynt 2732</i> (K)	Northern Cape, Postmasberg	T
<i>C. magnicephala</i>	<i>Pawek 9875</i> (WAG)	Malawi, Lwanjati Hill	F, T

<i>C. mazoensis</i> var. <i>mazoensis</i>	<i>Wild 6504</i> (K, BR)	Zimbabwe, Wedza Mountain	F, C, Tr
	<i>Cron & Balkwill 486</i> (J)	Zimbabwe, Wedza Mountain	F, C, Tr
	<i>Eyles 345</i> (BOL)	Zimbabwe, Mazowe	Tr
	<i>Baylis 10147</i> (MO)	Zimbabwe, Harare	Tr
	<i>Wild 6528</i> (K)	Zimbabwe, Mt Darwin	Tr
<i>C. mazoensis</i> var. <i>graniticola</i>	<i>Biegel, Pope & Simon</i> (K)	Zimbabwe, Mt Buhwa	Tr
	<i>Mahohoma 31</i> (K)	Zimbabwe, Mt. Buhwa	Tr
	<i>Biegel 3005</i> (K)	Zimbabwe, Bikita District	Tr
	<i>Chase 223</i> (K)	Zimbabwe, Mutare	Tr
	<i>Chase 802</i> (K)	Zimbabwe, Mutare, Dora Farm	Tr
	<i>Cron & Balkwill 532</i> (J)	Zimbabwe, Lake Mutirikwe	F, C, Tr
	<i>Miller 2850</i> (K)	Zimbabwe, Besna Kobila, Matobo District	F, C, Tr
	<i>Miller 5222</i> (K)	Zimbabwe, Matobo District, Besna Kobila	Tr
<i>C. mollis</i>	<i>Hilliard 5185</i> (K)	Eastern Cape, Naude's Nek, Barkley East District,	F, C, Tr
	<i>Hilliard & Burt 10362</i> (NU)	Eastern Cape, Renosterberg	F, C
	<i>Bayliss 012</i> (MO)	Lesotho, Sehlabathebe Reserve	C
<i>C. ngwenyensis</i>	<i>Cron et al. 308</i> (J)	Swaziland, Ngwenya Hills, Malolotja Nature Reserve	F, C, Tr
	<i>Maguire 7626/80</i> (J)	Swaziland, Ngwenya Hills	F
<i>C. parvifolia</i>	<i>Cron & Dallas 483</i> (J)	Gauteng, Witwatersrand Botanic Garden, Witpoortjie Falls	F, C
	<i>C.E. Moss 11365</i> (J)	Gauteng, Hartebeespoort	F, T
	<i>Codd 4175</i> (PRE)	Gauteng, Trichardtspoort, NE of Bronkhorstspuit	F, T, C
<i>C. pinnata</i>	<i>Junod 295</i> (LISC)	Mozambique, Maracuene, Ricatla	F

	<i>M. Moss s.n.</i> sub J277397	Mozambique, Inhaca Island	F, T
	<i>Moura et al.</i> 361 (WAG)	Mozambique, Zavala	F, C
<i>C. platycarpa</i> (broad-winged)	<i>Laidler</i> 57 (PRE)	Northern Cape, Little Karoo, Noukloof Nature Reserve	F, T
	<i>Retief & Reid</i> 314 (K)	Western Cape, Beaufort West District, Farm Kalkdam	F, T
	<i>Gillett</i> 1245 (BOL)	Western Cape, Klein Brak River	F, C
<i>C. pulchra</i>	<i>Cron & Balkwill</i> 508 (J)	Zimbabwe, Vumba Mountains	F, C
	<i>Cron & Balkwill</i> 499 (J)	Zimbabwe, Nyangani	F, Tr
	<i>Chase</i> 6127 (BR)	Zimbabwe, Vumba, Chikwera Peak	F
<i>C. saxifraga</i>	<i>Cron & Hodgkiss</i> 370 (J)	Western Cape, Kirstenbosch Botanic Garden	F
	<i>Bayliss BS</i> 4772 (MO)	Eastern Cape, Cradock Common, Albany	F, C
	<i>Taylor & Edwards</i> 8845 (WAG)	Eastern Cape, Botha's Hill	F, C
	<i>Bayliss BS</i> 724 (WAG)	Eastern Cape, Albany	C
	<i>Holland</i> 3658 (K)	Eastern Cape, Bulk River, Port Elizabeth	T
	<i>Phillipson</i> 1507 (K)	Eastern Cape, Grahamstown	F, T
	<i>Schlechter</i> 2646 (Z)	Eastern Cape, Albany District	T
<i>C. vagans</i>	<i>Hilliard & Burt</i> 12393 (K)	Eastern Cape, Stockenstroom District, Katberg Pass	F, C
	<i>Hilliard & Burt</i> 10961 (NU)	Eastern Cape, Katberg Pass	F, T
	<i>Hilliard & Burt</i> 18873 (NU)	Eastern Cape, Amatole mountains	T
<i>C. vallis-pacis</i>	<i>Balkwill, McDade & Lundberg</i> 11802 (J)	Northern Cape, Postmasberg District	F
	<i>Seydel</i> 4403 (WAG)	Namibia, Windhoek, Farm Finkenstein	F, T
	<i>Seydel</i> 3533 (K)	Namibia, Windhoek, Bergland	F, T, C
	<i>Wilman s.n.</i> (SAM 51167)	Northern Cape, Boetsap	Tr, C

<i>Dendrosenecio battiscombei</i>	<i>G. Allt 99 (K)</i>	Kenya, Mount Kenya, George's Valley	F, C, T
<i>Dendrosenecio kilimanjari</i> ssp. <i>kilimanjari</i>	<i>Hedberg 1332 (K)</i>	Tanzania, Mt. Kilimanjaro, above Marangu in the Philippia region (below Peter's hut)	F, C, T
<i>Dendrosenecio kilimanjari</i> ssp. <i>cottonii</i>	<i>Robertson 2 (K)</i>	Tanzania, Mt Kilimanjaro	F, C, T
<i>Emilia discifolia</i>	<i>Cron & Balkwill 490 (J)</i>	Zimbabwe, Wedza Mt	F, C, T
<i>Euryops brownei</i>	<i>Muasya, Cron & Knox 10 (J)</i>	Kenya, Aberdares	F, C
<i>Kleinia galpinii</i>	<i>McCallum 636 (J)</i>	Swaziland, Malolotja Nature Reserve	F, C
	<i>Cron 537 (J)</i>	Gauteng, ex hort Johannesburg	F, C
<i>Othonna natalensis</i>	<i>Balkwill 11288 (J)</i>	Mpumalanga, Middleburg District	F, C
	<i>Burrows 4719 (J)</i>	Mpumalanga, Lydenburg District, Buffelskloof Nature Reserve	F, C
	<i>Balkwill & Balkwill 4633 (J)</i>	KwaZulu-Natal, New Hanover District, Doornkop.	F, C
	<i>Strey 3417 (J)</i>	Mpumalanga, Balfast District, Stoffberg.	F, C
<i>Pericallis appendiculata</i>	<i>R.P.M. s.n. (K)</i> June 13 1894. (Ex Herb. Prior)	Canary Islands, Tenerife, Nanja Hills.	F, C, Tr
	<i>E. Beltrán (K)</i>	Canary Islands, Tenerife, Las Mercedes, Liano de los Viejos.	F, C
<i>Pericallis aurita</i>	<i>Hooker s.n. (K)</i> , 4–5 June, 1828; ex Herb. J. Gay	Madeira.	F, C, T, Tr
<i>Pericallis multiflora</i>	<i>Sventenius 88 (S)</i>	Canary Islands, Navaria, Valle Orotava.	Image viewed
<i>Pericallis murrayi</i>	<i>Swenson 574 (S)</i>	Canary Islands, Hierro, along road between Los Llanillos and Sabinosa	Image viewed
<i>Pericallis tussilaginis</i>	<i>Chaytor 75 (K)</i>	Canary Islands, Tenerife, Santa Ursula	F, C, T

	<i>Trethewy 137 (K)</i>	Canary Islands, Tenerife	F, T
<i>Senecio achilleifolius</i>	<i>Cron & Goodman 556 (J)</i>	Eastern Cape, Naude's Nek	F, C, T
<i>Senecio deltoideus</i>	<i>Burrows 4693 (J)</i>	Mpumalanga, Lydenburg District, Buffelspoort Nature Reserve	F, T
	<i>Maguire 22043 (J)</i>	Limpopo Province, Tzaneen	F, C
	<i>Major Leifeldt s.n. sub J32124</i>	Limpopo Province, Makhado (Louis Trichardt)	F, C
	<i>Hymans 12 (J)</i>	Mpumalanga, Songimvelo Nature Reserve	F, T
<i>Senecio cordifolius</i> (= <i>Cineraria mitellifolia</i>)	<i>Cron & Hodgkiss 369 (J)</i>	Western Cape, Table Mountain	F
	<i>Taylor 10094 (K)</i>	Western Cape, Stellenbosch, Jonkershoek	F, C
	<i>Taylor 10184 (K)</i>	Western Cape, Stellenbosch, Jonkershoek	F, T
	<i>Drège 10139 (K)</i>	Western Cape, Du Toit's Kloof	T
<i>Senecio hederiformis</i> (= <i>Cineraria hederifolia</i>)	<i>M. Van Wyk 5024 (J)</i>	Limpopo Province, Blouberg Mountain	F, C
	<i>Cron, Balkwill & Balkwill 247 (J)</i>	Mpumalanga, near Graskop	F
	<i>Cron, Balkwill & Balkwill 245 (J)</i>	Mpumalanga, near Graskop	F, Tr, C
<i>Oresbia heterocarpa</i> (= <i>Cineraria tomentosa</i> , <i>Senecio lanatus</i>)	<i>Cron et al. 322 (J)</i>	Western Cape, Cederberg, Sneeuwberg	F, C, Tr
	<i>Bodkin 7550 (K)</i>	Western Cape, Ceres, Skurfteberg	C, Tr
<i>Tephroseris papposa</i> (Reichenb.) Schur. [= <i>Senecio papposus</i> (Reichenb.) Less.]	<i>Dr. A. de Degen s.n. sub PRE 14579</i>	Hungary	F, C, T
<i>Tephroseris rivularis</i> [= <i>Senecio rivularis</i> (Waldst. & Kit.) DC.]	<i>Oberneder 5519 (PRE)</i>		F, C, T

<i>Tephroseris congesta</i> [<i>Cineraria palustris</i> L.]	<i>Kohlrausch</i> s.n. sub <i>SAM</i> 22476	Germany	F, C
<i>Tephroseris pratensis</i> Grieseb. & Schenk [= <i>Senecio pratensis</i> Hoppe]	<i>Eysn</i> 420 (PRE)		F, C