

## Chapter 8

### General Discussion and Conclusions

The aims of this project were outlined in the introduction (Chapter 1) and comprised investigating the phylogeny, generic circumscription, species limits, biogeography and conservation and the revision of the genus *Cineraria*. An overview of the results and conclusions for each of the objectives of the thesis is provided below, as well as recommendations for future studies.

**Objective 1:** *To use the phylogenetic approach to delimit the genus Cineraria as an homologous entity, unified by diagnostic apomorphic characters, and to investigate possible infrageneric level classification.*

Firstly, a narrower, more homogenous generic concept for *Cineraria* (Senecioneae, Asteraceae) is described, supported by molecular and morphological cladistic analyses. The genus is now clearly monophyletic, with a large number of diagnostic characters, including the obovate, compressed cypselae with distinct margins or wings, subisodiametric epicarp cells and a well developed carpodium. Vegetative features characterising *Cineraria* are its palmately-veined, 5–7 lobed leaves that are usually auriculate. Other reproductive features are the radiate, heterogamous capitula with yellow ray and disc florets, obtuse or penicillate apices of the disc styles, surrounded and often tipped by sweeping hairs. It thus fulfils the requirements for good genera as specified by Funk (1985) and Schrire & Lewis (1996), a coherent phylogenetic unit with predictive and communicative value.

Eleven species not conforming to key morphological features for *Cineraria* have been removed from the genus, leading to the establishment of two new genera: *Bolandia* Cron and *Oresbia* Cron & B.Nord. to accommodate three of these species. One species has been reinstated in and two others moved to *Senecio sensu lato*. The affinities of four/five species remain unresolved or unclear, viz. species previously known as *C. exilis*, *C. microglossa*<sup>1</sup>, *C. othonnoides*, *C. purpurata*, *C. spathulata*. (For authorities of species, see Chapter 6.)

No infrageneric classification has been proposed for *Cineraria*, as a robust phylogeny for the whole genus is yet to be hypothesised. Plastid and nuclear regions reflect different evolutionary histories, with strong evidence for reticulate evolution in the nuclear ITS data. This is not unusual, especially in the Asteraceae (Funk *et al.*, 2004; Guo *et al.*, 2004), but also in other families such as the Apiaceae (Yoo *et al.*, 2002). Additional regions from the chloroplast genome (possibly *ndhF* or *matK*) are needed to more fully reveal and resolve evolutionary relationships as recorded by the maternal pattern of inheritance in *Cineraria*. An alternative or additional site for nuclear DNA is also needed to assist with unravelling the

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<sup>1</sup>*Cineraria microglossa* DC. has since been identified as *Mesogramma apiifolium* DC. (Nordenstam & Pelsner, 2005).

complex phylogeny apparent in the ITS data/analyses. Differing phylogenetic signals between plastid and nuclear DNA sequences may provide useful evidence for past reticulation events (e.g. Guo *et al.*, 2004), and that is certainly the case in *Cineraria*.

It is clear that morphology provides too few unambiguous characters to convincingly resolve species level relationships in *Cineraria*. Many of the vegetative characters so important in distinguishing the various species are nonetheless variable and difficult to accurately characterise. However, morphological data are very useful at the generic level, where they unequivocally support a monophyletic group of species in *Cineraria* and distinguish it from other genera in the Senecioneae. Thus, although morphological characters are not easy to use, I would not support their exclusion from further cladistic analyses, as proposed by Scotland *et al.* (2003), but rather their judicious use in combination with molecular data and careful analysis of the nature and distribution of the homoplasy (Donoghue & Sanderson, 1992). Morphological features have been used to effectively investigate phylogenies at the generic and infrageneric level in the Asteraceae e.g. *Naussauvia* Comm. ex Juss. (Freire *et al.*, 1993) and *Pericallis* D.Don (Swenson & Manns, 2003), although studies at higher levels are more common, e.g. relationships in the Asteroideae (Karis, 1993), the Cichorioideae - Mutiseae (Karis *et al.*, 1992) and the Australian Gnaphalieae (Breitweiser & Ward, 2003).

**Objective 2:** *To further elucidate Cineraria's position within the Senecioninae with regard to sister groups.*

Amongst the senecioid genera included in this study, *Bolandia* is established as the sister genus to *Cineraria*. *Cineraria mollis* is placed sister to the rest of the genus in the ITS cladistic analysis, as well as combined molecular and morphological analyses. Based on this evolutionary premise, the plesiomorphic condition for *Cineraria* is herbaceous with a cobwebby to tomentose indumentum on its deltoid-reniform to reniform leaves, long peduncles with relatively large, solitary or few capitula.

**Objective 3:** *To appraise the specific limits in Cineraria by applying the phylogenetic species concept (the practicable version of the composite species concept) and assess its applicability.*

Despite their variability within certain species, a number of important characters delimiting species and elucidating species relationships in *Cineraria* are recognised. These include leaf shape, auricle shape, number and size of capitula (as expressed by the number of ray florets, disc florets, and/or involucre bracts), maximum length of peduncles and, to some extent, indumentum of the involucre bracts. Trichome complement is also extremely useful in distinguishing species, subspecies and varieties in *Cineraria*.

In this study, morphology is used to construe common ancestry, consistent with the phylogenetic species concept of Nixon & Wheeler (1990). Suites of diagnostic characters are used to delimit the species in *Cineraria*, allowing for variation in a species across geographic range. The criterion of majority fixation of characters was used, for example, with trichome types. However, until a robust phylogeny of species has been hypothesised, it is not possible to evaluate which of the characters currently used to diagnose species are apomorphic and which are plesiomorphic.

**Objective 4:** *To produce a monograph of the genus Cineraria.*

Thirty five species of *Cineraria* have been recognised here, with eight species having been placed in synonymy (*C. angulosa*, *C. britteniae*, *C. burkei*, *C. dieterlenii*, *C. gracilis*, *C. hamiltoni*, *C. polycephala*, *C. sebaldei*), as well as *C. chamedraefolia* being placed in synonymy with *Senecio cordifolius* (previously *C. mitellifolia*). Five new species have been described in the monograph (Chapter 6) or in its preparation (Appendices 3 & 4): *C. dryogeton*, *C. ngwenyensis*, *C. pulchra*, *C. magnicephala*, *C. huilensis*, as well as four new subspecies (three for *C. lobata* and one for *C. alchemilloides*) and two new varieties (*C. erodioides* var. *tomentosa* and *C. mazoensis* var. *graniticola*).

**Objective 5:** *To investigate variation within a number of polymorphic species or species complexes in Cineraria using the phenetic approach.*

Two widespread, highly variable species were investigated phenetically using multivariate and clustering techniques: *Cineraria lobata* and *C. deltoidea*, resulting in clarification of the variation and in *C. lobata*, formalization of the variation at the infraspecific level. *Cineraria lobata* was found to be a very variable species with geographic patterns of character distribution. The placing of eight species in synonymy with *C. deltoidea* by Hedberg (1957) and Jeffrey (1986) was confirmed by these phenetic analyses. Evidence for reticulate evolution occurring in both *C. lobata* and *C. deltoidea* is provided by the ITS cladistic analyses. Morphological variation in *C. aspera* and *C. erodioides* was also investigated and found to reflect geographic distribution and altitudinal differences amongst populations.

**Objective 6:** *To investigate distribution patterns within Cineraria and to hypothesise a reconstruction of its biogeographic history.*

Southern Africa is the centre of diversity for *Cineraria*, particularly the KwaZulu-Natal Midlands and the Eastern Cape. *Cineraria* is an afromontane genus, occurring in the

archipelago of mountains throughout the Rift Valley of Africa, but also in the mountains of the Western and Northern Cape, Namibia and southern Angola. It is likely that dispersal has played a major role in the current biogeographic pattern of *Cineraria*, as seen in *C. anampoza*, endemic to Madagascar and *C. abyssinica* in the highlands of Ethiopia and south-east Asia. Vicariance has also played a role, as *Cineraria* was probably present in the alpine vegetation that spread inland from the eastern escarpment across the high plateau of South Africa in the cooler, wetter glacial periods during the Quaternary (Van Zinderen Bakker, 1978; Werger, 1983), leaving remnants of this vegetation in the high-lying areas. Phylogenetic analyses based on nuclear ITS regions and morphology (separate and combined) hypothesise an origin for *Cineraria* in the Western and/or Eastern Cape, based on current distributions of *C. mollis* and the sister group *Bolandia*.

*Cineraria* is present in various centres of diversity and/or endemism or hotspots, as described by Beentje *et al.* (2001), Van Wyk & Smith (2001) and Cowling & Hilton-Taylor (1994) respectively, with a large percentage/number of species (or subspecies) endemic or near-endemic to these centres or regions. In southern Africa, these regions include the Cape Floral Region, Albany Centre, Maputaland-Pondoland Centre, Little Karoo, Griqualand West Centre, Drakensberg Alpine Centre, Soutpansberg Centre, Wolkberg Centre, Barberton Centre, and the Chimanimani-Nyanga Centre. Northwards, *Cineraria* occurs on numerous mountains and plateau regions in Tanzania, Kenya, Sudan and Ethiopia that have also been recognised as important centres of diversity and/or endemism (see Chapter 7).

**Objective 7:** *To investigate levels of rarity and endemism in Cineraria, to identify some of the factors contributing to rarity in Cineraria and to highlight implications for conservation.*

Rarity in *Cineraria* is linked to edaphic specialization and altitudinal restriction; the latter being linked to a requirement for relatively moist and cool conditions. Sensitivity to fire has also resulted in a narrow ecological niche for many species: one in which protection from fire by rocks or cliffs is essential, and possibly also wind and frost. A few of the species of *Cineraria* are threatened to varying degrees, but at least seven are Data Deficient in their assessment and require urgent attention.

### ***Future studies***

Future studies on the genus *Cineraria* will involve searching for 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*. I suggest that both plastid and nuclear DNA regions are investigated to reveal the evolutionary history of *Cineraria*, as they appear to be fairly incongruent due to its reticulate nature.

The putative hybrids between *Cineraria deltoidea* and *C. pulchra* need to be tested using the molecular markers established in Chapter 2. The hypothesis that hybridization between *Cineraria lobata* and *C. geifolia* is occurring in the Cape Peninsula needs to be investigated making use of population-based DNA and/or isozyme studies. Further elucidation of the reticulation events between *C. lobata* ssp. *soutpansbergensis* and *C. cyanomontana* (as suggested by the analyses in Chapter 2) is also necessary.

The affinity of a few specimens tentatively placed in *Cineraria* cf. *erodioides* (Chapter 6) needs further investigation, as does a small number of specimens from the Eastern Cape that could equally well be placed in *C. geraniifolia* or *C. lobata*, depending on which characters are judged to be the determining ones. The plants have capitula on shorter peduncles (similar to *C. lobata*), but have leaves and glabrous cypselae like *C. geraniifolia*. Molecular analyses of the Eastern Cape populations of species of *Cineraria* might shed light on this morphologically highly complex group of plants. The Middleburg specimens designated *C. cf. lobata* (Chapter 4) should be included in such future analyses, as they also resemble *C. geraniifolia* to some extent and may even be the result of hybridization between it and *C. lobata*.

Fieldwork in southern Angola is needed to establish the identity/affinity of specimens named *C. paracanescens* Torre ined.; also to establish the variation in *C. huilensis*. Further collecting of *C. foliosa* and *C. magnacephala* from southern Tanzania and central Malawi is required, as is investigation into plants previously known as '*C. buchanani*' from Malawi (and possibly southern Zimbabwe), but now subsumed into *C. deltoidea*.

*Cineraria* has been a challenging genus with which to work due to its morphologically very variable species that are quite plastic in differing local environmental conditions. No monograph will ever be the final word on a genus, but hopefully this work has made clearer the species limits and affinities and highlighted problems requiring further investigation. The intricacies of unravelling evolution with only the current pieces of the puzzle are highlighted by O'Hara (1994):

*Because evolutionary history is something we are still in the midst of, it will not always be possible for us to determine which varieties - which distinctive populations in nature - are temporary and which are permanent, and so our counts of species across space and through time will also have some measure of ambiguity in them that we cannot escape. If there is any consolation in this, it must be that the very existence of this ambiguity - the very fact that some organisms in nature cannot easily be grouped into species - is itself, as Darwin recognised, one of the most important pieces of evidence for the historical process we call evolution.*

O Hara 1994: 20

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