Nuclear and chloroplast DNA-based phylogenies of *Chrysanthemoides* Tourn. ex Medik. (Calenduleae; Asteraceae) reveal extensive incongruence and generic paraphyly, but support the recognition of infraspecific taxa in *C. monilifera*

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**Abstract**

The small genus *Chrysanthemoides* comprises two species within which a number of infraspecific taxa have been recognized, some of which are invasive aliens in Australia and New Zealand. Here we investigate the relationships of the species and infraspecific taxa using both chloroplast and nuclear non-coding DNA sequence data. Results of the analyses of the plastid and nuclear data sets are incongruent, and neither *Chrysanthemoides* nor *Osteospermum* is resolved as monophyletic, although there is some support for the recognition of infraspecific taxa. Analyses of the separate and combined data sets resolve two clades within *Chrysanthemoides* (which include some species of *Osteospermum*), and these appear to have a geographic basis, one being restricted to the mainly winter rainfall region, the other the eastern bi-seasonal rainfall area. Our results suggest that there is evidence of past or ongoing hybridization within and possibly between these two lineages.

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1. **Introduction**

The tribe Calenduleae comprises some 120 species and 12 genera (Nordenstam, 2006, 2007; Nordenstam and Källersjö, 2009). Earlier assessments of the tribe recognized seven (Norlindh, 1977) or ten genera (Nordenstam, 1994). Since 1994 there have been several taxonomic re-arrangements. Nordenstam (1994, 1996) considered the previously recognized genus *Castalis* Cass. and sect. *Blaxium* (Cass.) T. Norl. of *Osteospermum* L. to belong to *Dimorphotheca* Vaill. and revived the genera *Oligocarpus* Less. and *Tripteris* Less., both of which had been included in *Osteospermum* by Norlindh (1943, 1977). Nordenstam (1996) also questioned the monophyly of the reduced *Osteospermum*, noting that despite these taxonomic changes, it was still heterogeneous. Further splits from *Osteospermum* were described as the new genera *Norlindhia* B. Nord. and *Monoculus* B. Nord. (Nordenstam, 2006), and a species of *Gibbaria* Cass. was recognized as a distinct new genus *Nepbrotheca* B. Nord. & Källersjö (Nordenstam et al., 2006). This move left *Gibbaria* as a monotypic genus until a second species was recently added by a transfer from *Osteospermum* (Nordenstam and Källersjö, 2009).

Norlindh (1943) established the genus *Chrysanthemoides* Tourn. ex Medik. for two species of *Osteospermum* that had fleshy drupe-like fruits. However, fleshy or semi-drupeaceous fruits have later also been reported in some species of *Osteospermum* such as *O. junceum* Berg., *O. asperulum* (DC.) T. Norl., *O. corymbosum* L. and *O. triquetrum* L. f. (Wood and Nordenstam, 2003). The evolution of drupes or fruits with a fleshy exocarp is extremely rare.
Table 1
Comparison of the species and infraspecific taxon names recognized by Norlindh (1943) and the unpublished entities recognized by Griffioen (1995), with key characteristics of each entity provided.

| Norlindh (1943) | Griffioen (1995) | Morphological characteristics | Ecology |
|-----------------|------------------|-----------------------------|---------|
| C. monilifera subsp. monilifera | “C. monilifera subsp. monilifera” | Shrub, 1.5 m in height×2.0 m in diameter, stems tanniferous, pubescence absent. | Elliptic or slightly obovate, 0.4–0.6 mm thick, 7–30 mm × 20–50 mm, glabrous, tannins absent, margins toothed, petioles 6–2 mm long. | Inner involucral scales lanceolate, outer involucral scales linear, slightly pubescent, 4–7 mm long. | Fleshy, orange-red when mature, globose to sub-globose, 5–8 mm in diameter, length: breadth ratio 1.1–1.2, Achenes not ridged. | Found in disturbed sites on margins of climax vegetation in a range of soils including TMS, limestone and loams in the SW Cape, on mountain slopes from Piketberg in the north to Hermanus in the east. |
| “C. monilifera subsp. floribunda” form 1 | “C. monilifera subsp. floribunda” form 1 | Small to large erect bush, 1–3.5 m in height×1–6 m in diameter, stems tanniferous, pubescence absent from young tissues. | Obovate (6–40 mm × 10–50 mm), margins pubescent or scapose, lamina leathery or fleshy. Young leaves and capitula clustered at branch terminals, covered with loose pubescence. | Inner and outer involucral scales narrowly lanceolate to ovate, glabrous of pubescent, 4–7 mm long. | Fleshy, purple-black when mature, ovoid, 4–6 mm long, achene ridged. | Coastal sand dunes, limestones and along roadsides from Langebaan to Knysna. |
| “C. monilifera subsp. floribunda” form 2 | “C. monilifera subsp. floribunda” form 2 | Small to large erect bush, 1–3.5 m in height×1–6 m in diameter. Stems tanniferous, pubescence absent from young tissues. | Broadly ovate, covered with loose pubescence. | — | — | — |
| C. monilifera subsp. pisifera | “C. monilifera subsp. pisifera” var. pisifera form 1 | Large bushes or small trees, 2 m in diameter, 2.5 m high. | Elliptic or slightly obovate, 0.4–0.6 mm thick, 7–30 mm × 20–50 mm, glabrous, tannins absent, margins toothed, petioles 6–2 mm long. | Inner involucral scales lanceolate, outer involucral scales linear, slightly pubescent, 4–7 mm long. | Fleshy, orange-red when mature, globose to sub-globose, 5–8 mm in diameter, length: breadth ratio 1.1–1.2, Achenes not ridged. | Found in disturbed sites on margins of climax vegetation in a range of soils including TMS, limestone and loams in the SW Cape, on mountain slopes from Piketberg in the north to Hermanus in the east. |
| “C. monilifera subsp. pisifera” var. pisifera form 2 | “C. monilifera subsp. pisifera” var. pisifera form 2 | Small shrub to 1.5 m high. | Variable, 5–30 mm × 8–45 mm, elliptic, glabrous, leathery, deeply dentate, 2–4 teeth per margin. | — | — | — |
| “C. monilifera subsp. pisifera” var. borealis | “C. monilifera subsp. pisifera” var. borealis | Stems without tannins, pubescence absent. | Somewhat leathery, narrowly elliptic, 10–20 mm × 25–45 mm, glabrous, margins spinosecent. | — | — | — |
| “C. monilifera subsp. pisifera” var. angustifolia | “C. monilifera subsp. pisifera” var. angustifolia | Erect bush, 1–2 m tall, 1–2 m diameter. | Narrowly elliptic, 10–25 mm × 35–60 mm, glabrous, margins scapose. | — | — | — |
| C. monilifera subsp. canescens | “C. monilifera subsp. canescens” | Stems pubescent, tannins absent. | Elliptic to broadly elliptic, 0.3–0.5 mm thick, 10–35 mm × 25–55 mm, pubescent, tannins absent, margins toothed, petiole 5–20 mm long. | Inner scales slightly ovate, outer scales lanceolate, pubescent, 5–7 mm long. | Obovoid, purple-black when mature, 2–6 mm long, achene not ridged. | Associated with Protea and grassland vegetation in the Drakensberg and Swartberg. |

(continued on next page)
Table 1 (continued)

| Author          | Morphological characteristics                                                                 |
|-----------------|------------------------------------------------------------------------------------------------|
| **C. monilifera** |                                                                                             |
| subsp.          |                                                                                             |
| septentrionalis | Erect shrub or bush, stems without tannins, pubescent.                                       |
| **C. incana**   |                                                                                             |
| var. gracilis   | Low, spreading bush, stems slender, pubescent.                                               |
| var. hirsuta    | Prostrate, spinescent bush, 0.5 m high, stems pubescent.                                     |
| var. microphylla| Prostrate, spinescent bush or small shrubs up to 1 m tall, 1–4 m in diameter, stems           |
| var. rangei     | Prostrate shrub 0.5 m high, stems pubescent, bearing leaves at tips.                          |
| var. rotundata  | Scrambling shrub or small tree, stems tanniferous, glabrous.                                 |
| var. subcanescens| Bushes up to 2.5 m high, spreading, young tissue pubescent, stems up to 2 m long, glabrous. |
| var. septentrionalis | Inner scales lanceolate or narrowly ovate, outer scales lanceolate, slightly pubescent or glabrous, 2.5–5 mm long. |
| var. subcanescens| Narrowly elliptic, 3–10 mm × 10–32 mm, margins minutely spinescent or entire, petiole 2–10 mm long. |
| var. var. gracilis | Pubescent.                                                                                 |
| var. hirsuta    | Pubescent, 3–5 mm long.                                                                      |
| var. microphylla| Pubescent, 5–7 mm long, achenes not ridged.                                                  |
| var. rangei     | Pubescent.                                                                                  |

Norlindh (1943) recognized two species in *Chrysanthemoides*, viz. *C. incana* (Burm. f.) T. Norl. and *C. monilifera* (L.) T. Norl., and further divided the latter into five subspecies, some of which were noted to be highly variable. In an unpublished thesis, Griffioen (1995) used morphological data supplemented with isozyme and ecological data to re-assess the infraspecific taxonomy of both species in the genus. A phenetic analysis of morphological characters indicated that *C. incana* could be distinguished from *C. monilifera* on the basis of spinescence, prostrate growth form, and the distribution of pubescence on the stems, leaves and receptacles in the former species (Griffioen, 1995). These differences are accompanied by various geographic and ecological attributes. Furthermore, isozyme electrophoresis indicated that the two species are distinct, and do not hybridize when co-occurring. Within *C. incana*, Griffioen (1995) recognized six infraspecific taxa,
and within *C. monilifera* 10 taxa were recognized (at subspecies, variety and form rank; Table 1).

Norlindh (1943) noted that the drupes of *Chrysanthemoides* are edible and most likely bird dispersed, and he ascribed the presence of the species on St. Helena since before 1839 to bird dispersal. *Chrysanthemoides* fruits are eaten by a variety of birds in South Africa (Rowan, 1967; Keath et al., 1992; Joffe, 2001). In St. Helena dispersal by the introduced Indian Myna has been reported (Ashmole and Ashmole, 2000). In Australia, where *Chrysanthemoides* is an invasive weed, various mammals have been recorded as dispersers as well as emus and flying frugivorous birds (Weiss, 1986; Meek, 1998). Man has thus also acted as a dispersal agent, and both *C. monilifera* subsp. *rotundata* (DC.) T. Norl. and *C. monilifera* subsp. *monilifera* are legislated as “weeds of national significance” in Australia (http://www.weeds.org.au/WoNS/bitoubush/), and there has been over two decades of biocontrol research in Australia on *C. monilifera* (Downie et al., 2007). This species is also a problem in New Zealand (Roy et al., 2004). The study of these taxa in their native region is thus of vital importance if the spread of these species as weeds is to be controlled (Scott, 1996). However, the fact that these taxa are able to establish easily has resulted in their use in rehabilitation efforts following mining activities (Hälbich, 2003) and to stabilize coastal dunes in urban areas of South Africa (Nichols, 1996).

*Chrysanthemoides* is distributed across a number of biomes and vegetation types in southern Africa, ranging from the Fynbos of the South Western Cape, to the montane Grassland and vegetation types in southern Africa, ranging from the Transvaal, and the Eastern Cape mountains and the Drakensberg into northern Namibia (Angra Pequena) and along the southern coast to about flying frugivorous birds (Weiss, 1986; Meek, 1998). Man has also acted as a dispersal agent, and both *C. monilifera* and *C. monilifera* subsp. *monilifera* are legislated as “weeds of national significance” in Australia (http://www.weeds.org.au/WoNS/bitoubush/), and there has been over two decades of biocontrol research in Australia on *C. monilifera* (Downie et al., 2007). This species is also a problem in New Zealand (Roy et al., 2004). The study of these taxa in their native region is thus of vital importance if the spread of these species as weeds is to be controlled (Scott, 1996). However, the fact that these taxa are able to establish easily has resulted in their use in rehabilitation efforts following mining activities (Hälbich, 2003) and to stabilize coastal dunes in urban areas of South Africa (Nichols, 1996).

*Chrysanthemoides* is distributed across a number of biomes and vegetation types in southern Africa, ranging from the Fynbos of the South Western Cape, to the montane Grassland of the Drakensberg, Chimanimani and mountains of eastern Africa (Griffioen, 1995). Of the two recognized species, *C. incana* is mostly restricted to the South Western Cape but extends mainly along the coast northwards to Namaqualand and southern Namibia (Angra Pequena) and along the southern coast to about Cape Aguilhas. *C. monilifera* is more widespread, with *C. monilifera* subsp. *canescens* (DC.) T. Norl. extending from the Eastern Cape mountains and the Drakensberg into northern Transvaal, and *C. monilifera* subsp. *septentrionalis* T. Norl. distributed in the montane regions of Tropical East Africa from Zimbabwe north to Tanzania (Norlindh, 1943).

Thus, while *Chrysanthemoides* in current taxonomy comprises only two species, there is ample evidence (published and unpublished) of considerable variation within each species. Here we use both nuclear and chloroplast DNA sequence data to undertake a phylogenetic analysis of the genus to test not only the monophyly of the genus, but also to assess if there is any genetic evidence to support the recognition of the infraspecific taxa as recognized by either or both Norlindh (1943) and Griffioen (1995), especially within *C. monilifera*. We emphasise that while we are using the taxa as recognized by Griffioen, they are not validly published, and hence the names in Table 1 and the text that follows appear in quotation marks to indicate this status.

In order to test taxon monophyly, we adopt a multiple exemplar approach, including two or more specimens representative of each taxonomic entity. This approach is important, as some molecular studies have indicated that species non-monophyly (as determined by molecular data) can be quite common (e.g. Crisp and Chandler, 1996; Ohsako and Ohnishi, 2000; Syring et al., 2007; Howis et al., in press; Ramdhani et al., in press), and when noted requires careful analysis and explanation. Monophyly at infraspecific ranks is likely to be compromised by hybridization and incomplete lineage sorting. However, as noted by Hølser et al. (2001), distinguishing between these two processes is difficult, but a phylogeographic approach may enable us to identify monophyletic infraspecific taxa. If so, then we suggest that this be viewed as evidence favouring their recognition as valid infraspecific taxa, and certainly as “Evolutionary Significant Units” (ESUs sensu Ryder, 1986; cf. Fraser and Bernatchez, 2001).

Two widely utilised chloroplast spacer regions were selected for this investigation: the *psbA-trn*H spacer, and the *trn*L intron in conjunction with the associated *trn*-L*F* spacer (hereafter termed the *trn*L*F* region). The *psbA-trn*H region is being increasingly used in phylogenetic studies at the intrageneric level (Gielly et al., 1996; Sang et al., 1997; Kim et al., 1999; Chandler et al., 2001; Pels et al., 2003; McKenzie et al., 2006; McKenzie and Barker, 2008) as well as the intraspecific level (Storchová and Olson, 2004; Yamashiro et al., 2004). The *trnL*-trnF intergenic spacer region is one of the most commonly used non-coding regions of cpDNA in phylogenetic studies at the intrageneric and species level, and has occasionally been found to be sufficiently variable for use below the species level (Barker et al., 2005).

While the use of the Internal Transcribed Spacer (ITS) region for phylogenetic purposes is considered controversial by some (see for example Alvarez and Wendel, 2003; Bailey et al., 2003; Small et al., 2004 for critiques), it is still the most tractable nuclear region for molecular systematics at the species and genus level (e.g. Feliner and Rosselló, 2007; Mort et al., 2007), and has been widely used in phylogenetics of many groups of Asteraceae. Baldwin (1993) was probably the first to notice intraspecific variability in ITS sequences in the Asteraceae, and this region has been used in phylogeographic studies in the Asteraceae (for example Comes and Abbott 2001; Simurda et al., 2005; Zachariades et al., 2004; Pelser et al., 2007). However, at least some of these studies use ITS data in conjunction with additional data such as AFLP’s or plastid DNA data so as to address the potentially problematic issues or paralogy and lineage sorting.

2. Methods

2.1. Sampling, DNA extraction, amplification and sequencing

Multiple samples from each of the species of *Chrysanthemoides* were obtained, and in particular we focused on ensuring coverage of the various subspecific entities within *C. monilifera* as recognized by Norlindh (1943) and Griffioen (1995). Where possible, we collected material at sites that had been sampled for Griffioen’s phenetic analysis (Griffioen, 1995). All samples were identified to subspecies, variety, and in some cases form, using Griffioen’s (1995) key. A total of 35 *Chrysanthemoides* samples were used (Table 2). Additional sequence data for several species of *Osteospermum* (sections *Homocarpa* T. Norl. and *Coriacea* T. Norl.) were selected in order to test generic monophyly. Only
species from these sections were considered, as other studies have indicated a close relationship between Chrysanthemoides and these sections of Osteospermum (Wood and Nordenstam, 2003; Nordenstam and Källersjö, 2009). It should be noted that O. subulatum DC. (of sect. Trialata in Norlindh, 1943) and O. triquetrum (unassigned to section in Norlindh, 1943) were transferred to sect. Homocarpa by Wood and Nordenstam (2003). The more distantly related Tristeris microcarpa Harv. was used as outgroup.

Leaf samples were collected into silica gel according to the method of Chase and Hills (1991), and the DNA was extracted using a modified CTAB DNA extraction protocol (Doyle and Doyle, 1987). The ITS region was amplified using “ITS-5” (White et al., 1990) and a modified “ITS-4” primer (“ITS-
Chrys-4”; 5′-TCCTCGGCTATGGATATGC-3′). The psbA-trnH spacer was amplified using the primers “psbA” and “trnH” (Sang et al., 1997), and the trnL-F region amplified using the primers “c” and “f” (Taberlet et al., 1991).

PCR amplifications were carried out using either a Thermo-Hybird PCR Sprint Temperature Cycling System or a Corbett Research PC-960 G Microplate Gradient Thermal Cycler, with 35-40 cycles of amplification. Successful PCR amplification was confirmed by electrophoresing the PCR products on a 1% agarose gel. PCR products were cleaned using either the QIAquick PCR purification kit or the PROMEGA Wizard SV Gel and PCR purification kit and resuspended in 30 µl of dH2O before being sequenced using the ABI prism BigDye Terminator v3.0 or v3.1 Ready Reaction Cycle sequencing kit (Applied Biosystems) according to the manufacturer’s instructions.

The ITS PCR product was sequenced in both directions with the primers “ITS-1” (White et al., 1990), “ITS-Chrys-4”, “Chromosoma 5.8-R” (5′-GATTCTGGAATTCAACC-3′; Barker et al., 2005), and the novel internal primer “Chrysanthemoides 5.8-F” (5′-GACTCTCGGCAACGGATATC-3′). The psbA-trnH spacer was sequenced using the same primers as used in the PCR process, and the trnL-F region was sequenced using the primers “c”, “d”, “e” and “f” (Taberlet et al., 1991).

Sequence data was checked and edited using SEQUENCHER (Version 3.1.1; Gene Code Corporation). Assembled sequences were imported into MACLADE (Version 4.06; Maddison and Maddison, 2000) and aligned manually.

2.2. Phylogenetic analyses

As the psbA-trnH and trnL-F are both found on the chloroplast genome which is inherited as a single unit, these data sets were combined to form what we term the cpDNA data set. Prior to analysis, the incomplete 5′ and 3′ ends of the psbA-trnH and trnL-F regions were excluded. The ITS nrDNA data set was analysed separately. Data sets were analysed by means of Bayesian inference (BI) and maximum parsimony (MP). As Bayesian analysis is based on explicit models of DNA evolution, the program MrModelTest (Nylander, 2004) was used to select the model of DNA substitution that best fit the data. The Bayesian analysis was run using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001) as follows: four Markov chains, three heated and one cold, were run simultaneously for 2,000,000 generations and trees were saved every 100 generations. The starting tree was random, the branch lengths were saved and the first 4000 trees were discarded as burn-in following a visual inspection of a plot of the likelihood values to ensure stationarity had been reached well within this limit. The remaining trees were combined and used to generate a 50% majority rule consensus tree and to determine the PP for each node.

Parsimony analyses were conducted on the two data sets as follows: Using PAUP version 4.0 b10, one hundred random input analyses were conducted using the parsimony-informative characters, keeping one tree (TKEEP=1) from each of the analyses. A heuristic search was then conducted on all the shortest trees in memory (with MAXTREES set 20,000) using the TBR branch swapping algorithm. A strict consensus tree was then calculated from the set of equally parsimonious trees. A FULL HEURISTIC Bootstrap analysis was conducted on each data set using 1000 replicates, with MAXTREES set to 2000.

3. Results

MrModelTest identified the best DNA substitution model for the ITS data as the HKY + G (Hasegawa et al., 1985) model with variable sites assumed to follow a discrete gamma distribution. For the cpDNA data, the best DNA substitution model was identified as GTR+G. The Bayesian Inference consensus topologies for the ITS and cpDNA data sets are presented in Fig. 1, with the parsimony bootstrap values also indicated. The parsimony analyses produce poorly resolved consensus trees, and the nodes that are retained in common with the BI trees are indicated on Fig. 1. Table 3 lists the details of the parsimony analysis of each data set. Support for the various nodes in both trees is generally lacking, if one is to accept that any Bayesian posterior probability (PP) value < 0.95 and bootstrap percentages < 70% is weak (Alfaro and Holder, 2006).

The ITS region is twice as variable and contains approximately three times as many parsimony-informative sites as the cpDNA regions (Table 3). Despite this lower variability, the psbA-trnH region included three synapomorphic insertion–deletion events (indels) that, when mapped on the plastid tree, provide additional support for three of the main nodes retrieved (Fig. 1; note the exception of a subsequent loss of one of these insertions in specimen SH100 – “C. monilfera var. pisifera form 2” – within Clade 1). However, indel data were not coded and included in phylogenetic analyses.

It must be noted that all three samples of C. incana possessed multiple ITS paralogues of different lengths, which meant that not all the data from this region could be used, as the trace files became unreadable at the point where the paralogues diverged. This problem was particularly severe in the Wood 20 specimen, where 361 aligned sites were affected, and coded as unknown. In the remaining two C. incana samples, this problem affected less than 10 sites. Reasons for the presence of multiple paralogues in these samples include hybridization followed by incomplete lineage sorting, or the presence of pseudogenes. However, as the 5.8S regions of these sequences are highly conserved, it seems unlikely that the sequences used here are pseudogenes (Razafimandimbison et al., 2004). The obvious solution to this problem would be to clone the PCR product and sequence the different paralogues. While this has been done for a larger study based on ITS data only (Howis et al., in prep.), we used the data obtained from directly sequenced PCR product in this study. This finding is nonetheless interesting, and this species needs detailed molecular and morphological investigation.

3.1. Topological comparisons and incongruence

It is immediately apparent that the ITS and cpDNA topologies are not congruent, and in some instances this incongruence is well supported. The genus Chrysanthemoides is indicated by both
datasets to be non-monophyletic, as in each tree there are various species of *Osteospermum* embedded within the *Chrysanthemoides* clade. Furthermore, the two additional lineages, representing the Drakensberg and East African taxa ("C. monilifera subsp. canescens" and "C. monilifera subsp. septentrionalis"), are placed in a more basal position, among species of *Osteospermum* in the ITS analysis. Both data sets resolve "C. incana subsp. *incana*" as a monophyletic species which is embedded within *C. monilifera*.

The two main clades of samples on the plastid phylogeny receive at best moderate support (pp = 0.93 and BS = 84% for Clade 2). The ITS analysis shows higher levels of support (pp = 0.99, BS = 84% for Clade 1 and pp = 0.92 for Clade 2, which has no BS support). Furthermore, each of these clades corresponds reasonably well with the Griffioen’s taxonomy. Within Clade 1, the samples of "C. incana subsp. *incana*" are sister to the clade containing the remaining samples of "C. monilifera subsp. pisifera var. pisifera form 1" and "C. monilifera subsp. *rotundata*". Clade 2 comprises samples of "C. monilifera subsp. floribunda form 1", "C. monilifera subsp. *monilifera*", "C. monilifera subsp. pisifera var. pisifera form 2" and "C. monilifera subsp. *pisifera var. angustifolia". In addition, the cpDNA places the two montane subspecies ("C. monilifera subsp. canescens" and "C. monilifera subsp. septentrionalis") in this clade as well. However, the ITS data places these taxa in a more basal position, among species of *Osteospermum*, but with no support.

In the cpDNA phylogeny, none of the infraspecific taxa apart from "C. monilifera subsp. canescens" are resolved as monophyletic, the two-sample clade of which receives pp = 1.0 and BS = 88. In contrast, the ITS data resolves samples of four of the taxa as recognized by Griffioen (1995) as monophyletic, but (with one exception) with insignificant support. These are "C. monilifera subsp. pisifera var. pisifera form 1" (Clade 1), "C. monilifera subsp. floribunda form 2" (Clade 1), "C. monilifera subsp. *monilifera*" (Clade 2, with good PP and BS support) and "C. monilifera subsp. pisifera var. angustifolia" (Clade 2). With one exception, samples of "C. monilifera subsp. *rotundata*" (Clade 1) are also monophyletic. Other taxa such as "C. monilifera subsp. pisifera var. pisifera form 2" are paraphyletic.

These results indicate some (but not complete) agreement in terms of membership of each of the two clades. This incomplete agreement is further exacerbated by five samples of *C. monilifera* that swap clades between the two phylogenies. Three samples in Clade 1 of the cpDNA phylogeny are placed in Clade 2 of the ITS phylogeny (indicated by solid dots in Fig. 1), and two samples placed in Clade 2 of the cpDNA phylogeny are in Clade 1 of the ITS phylogeny (indicated by squares in Fig. 1).

*Osteospermum ciliatum* Berg. and *O. aciphyllum* are placed near the base of the tree in both phylogenies, while *O. subulatum* is in an unsupported clade of four *Osteospermum* taxa collectively sister to Clade 1 in the cpDNA phylogeny, but placed within Clade 1 of the ITS topology, with moderate to good support (Fig. 1). *O. junceum* and *O. asperulum* are sister taxa in the ITS topology, and are in turn sister to the "C. monilifera subsp. *canescens*" clade, a relationship that lacks support. However, in the cpDNA topology, *O. junceum* is sister to part of Clade 1 that comprises samples of *C. monilifera* (with moderate) and *O. asperulum* is sister to the rest of Clade 2 (with moderate support at best).

*O. pyrifolium* T. Norl. and *O. triquetrum* are members of Clade 2 of the cpDNA phylogeny, and placed in a well supported clade (pp = 0.97) with "C. monilifera subsp. *canescens*" samples. However, in the ITS phylogeny they placed as members of separate clades (*O. pyrifolium* is part of Clade 1 and *O. triquetrum* is sister to Clade 2).

### 3.2. Combined data set

Wiens (1998) argues that conflicting data sets can be combined, and that the accuracy of the recovered topology as being a reflection of the true phylogeny is enhanced. We thus combined the data in an attempt to enhance the phylogenetic signal in the data. Parsimony analysis of the combined data resulted in a poorly resolved tree (nodes retained in the strict consensus tree are indicated in Fig. 3), a result typical of instances where hybridization has occurred and reduced resolution in parsimony analyses (McDade, 1992). However, the results of the BI analysis are encouraging, in that most samples of *C. monilifera* formed monophyletic lineages corresponding to their taxonomic identity (Fig. 3), although BI posterior probability support for most nodes was still lacking.

### 4. Discussion

#### 4.1. Support for infraspecific taxa within *C. monilifera*

While our sample size here is limited, the ITS sequence data does provide some evidence to support the recognition of the
multitude of infraspecific taxa recognized by Griffioen (1995), although few nodes receive good support. It is unfortunate that the plastid data is too conservative to provide independent support for these lineages. It should also be borne in mind that gene trees do not equate to species trees (Doyle, 1992), and that infraspecific relationships may be reticulate rather than hierarchical in nature. If so, then our results represent efforts to place square pegs (reticulating taxonomic entities) into round holes (a hierarchical representation of relationships).

Our results (especially the combined analysis; Fig. 3) do however indicate some genetic support for the infraspecific entities recognized by Griffioen (1995) on the basis of morphology (which is a phenotypic representation of the nuclear genotype). This lends weight to their validity, and we thus feel that it is important to encourage their use by the end-users of taxonomies. We thus present the key features of these entities in Table 1. This result highlights the value of careful phenetic and ecological studies in variable species (Griffioen, 1995), which should accompany molecular phylogeographic studies, as all sources of data need to be considered in assessing species limits and the recognition of ESUs within species. The fact that other of Griffioen’s (1995) taxonomic entities are not monophyletic should not be viewed negatively — it is entirely possible that paraphyletic taxa (such as “C. monilifera subsp. rotundata”, “C. monilifera subsp. pisifera” form 2” and “C. monilifera subsp. floribunda” form 1”) represent surviving ancestral lineages, out of which some of the mono- phylectic lineages have recently evolved, and or lineage sorting has not yet reached completion in these taxa. Another possibility is that this is a side effect of relatively rare hybridization. This should, in particular, affect the plastid phylogenies, as they tend to give more categorical results, and so can be expected to be categorically wrong.

4.2. Correlation to distribution

When the geographic distribution of the Chrysanthemoides samples in each clade identified in Fig. 1 is mapped, it becomes apparent that Clades 1 and 2 are correlated to geographic distribution: Clade 1 comprises samples from the eastern portion of the distribution range, and Clade 2 the western (Fig. 2). The western clade (Clade 2) comprises specimens from the predominantly winter rainfall region, whereas Clade 1 covers the bi-seasonal (but predominantly summer) rainfall region. Interestingly, the five samples that exchange clade membership between the ITS and cpDNA phylogenies are all from the geographic region intermediate between the main distribution areas of the two main clades; the Tsitsikamma – Nature’s Valley – Oudtshoorn area, suggesting that hybridization between the two main clades in this region cannot be ruled out. It is thus possible that gene flow between these lineages has taken place, and that the different positions of these five samples in the ITS phylogeny reflects the fixation of one particular set of parental paralogues via concerted evolution — most likely those from the paternal source which are incongruent with the maternal cpDNA topology.

In the analysis of the combined data, three of the five samples that swap clade membership (those indicated in Fig. 1 by circles) are placed within a clade that comprises most samples from Clade 1 with good support (pp=0.95), reflecting the results of cpDNA analysis. The other two samples are placed basal to a clade that comprises [Clade 1; O. subulatum], a position that approximates the ITS result. The geographic patterns based on the analysis of the combined data are thus even more striking, with the enlarged Clade 1 (which includes O. pyrifolium and O. subulatum and having a pp=0.98) now assuming a distinct Eastern distribution, while the remaining samples form a Western lineage (Figs. 2 and 3).

4.3. Relationship of Chrysanthemoides to Osteospermum

On the basis of both ITS and cpDNA gene trees as well as the combined analysis, both Osteospermum and Chrysanthemoides are paraphyletic, bearing in mind that many placements are not well supported. If these gene trees are taken to be straightforward reflections of evolutionary history, the simplest nomenclatural and taxonomic solution to this problem is to subsume all Chrysanthemoides taxa as well as Osteospermum sect. Coriacea (O. junceum) into Osteospermum sect. Homocarpa, which would then be monophyletic. This would mean that the defining morphological characteristic of Chrysanthemoides (drupaceous fruit) would then have to be interpreted as having originated several times, followed by losses/reversals in those species of Osteospermum shown to be embedded within the lineages of Chrysanthemoides. The number of gains and losses depends on the phylogeny used: cpDNA or nrDNA. However, it would be most profitable to re-examine the fruit morphology and its ontogeny for all species in Osteospermum section Homocarpa, as there is recent evidence (Wood and Nordenstam, 2003) that fruits with a fleshy exocarp are not restricted to Chrysanthemoides as claimed by Norlindh (1943) and that exocarp features (including colour) may have evolved independently and repeatedly as adaptations to different dispersal strategies (bird vs. ant dispersal; cf. Wood and Nordenstam, 2003).

4.4. The role of hybridization in Osteospermum and Chrysanthemoides

Irrespective of proposed taxonomic and nomenclatural changes required to preserve generic monophyly, the issue of conflicting species-level relationships within this assemblage remains. This could be caused by several processes. Generally, such conflict is attributed to hybridization and/or incomplete lineage sorting as well as the questionable utility of ITS as a suitable nuclear marker (see for example Vriesendorp and Bakker, 2005; Mort et al., 2008). Given the numerous instances of incongruence in this group, we would have to invoke multiple instances of hybridization (and possibly subsequent introgression), such that the general “Chrysanthemoides” form or morphology is retained throughout in disparate lineages, such as “C. monilifera subsp. canescens” and “C. monilifera subsp. rotundata”. Certainly the intermediate geographic distribution of samples that swap clade membership may be considered as evidence for past or ongoing hybridization. The report of a natural hybrid between Osteospermum potbergense A. R. Wood & B. Nord. and Chrysanthemoides monilifera is noteworthy in this
Fig. 2. Map showing sample sites for South African specimens of Chrysanthemoides. Key to shapes: squares indicate localities of C. monilifera samples in Clade 1; circles indicate localities of C. monilifera samples in Clade 2; stars indicate localities of C. monilifera samples which swap clade membership between cpDNA and ITS data sets; triangles indicate localities of C. monilifera subsp. canescens; diamonds indicate localities of samples of C. incana. The grey outline indicates samples that fall into the Eastern clade in the analysis of the combined data set. Numbers within symbols indicate samples as listed in Table 1.
context (Wood and Nordenstam, 2003). Alternatively, the entire Osteospermum section Homocarpa is of very recent origin, and the results here represent incomplete lineage sorting, a scenario that cannot be examined fully until a more complete phylogeny is obtained and subjected to some form of dating analysis.

5. Conclusion

Our results indicate the existence of substantial intraspecific variation within C. monilifera, corresponding in part to the various morphotypes recognized by Griffioen (1995) at different
taxonomic ranks. Unfortunately, the taxonomic recognition and disentangling of these entities, along with the relationships of these to each other and other *Osteospermum* species is bedeviled by incongruence between nuclear and plastid markers, suggesting a history of hybridization events and reticulation or incomplete lineage sorting further confused by the distinct possibility of multiple paralogues of the ITS region. Despite the slight improvement in the phylogeny obtained when the data are combined, we refrain from nomenclatural validation of Griffioen’s unpublished infraspecific taxa.

5.1. Significance to users of taxonomies

Our results are of considerable significance for biocontrol scientists working to control *Chrysanthemoides* in regions where it is invasive. As the current taxonomy does not adequately address the genetic diversity within species of *Chrysanthemoides*, it is essential for biocontrol scientists to ascertain which genetic lineage and geographic area the invasive plants are from e.g. Zachariades et al. (2004) and Paterson et al. (2009). Once this is known, it may be possible to obtain more effective biocontrol organisms from natural populations of that specific genotype, as for example shown for the control of the invasive fern *Lygodium microphyllum* by phytophagous mites (Goolsby et al., 2006).

Our findings are also relevant to the horticultural and landscaping industry, as *C. monilifera* is used for a range of horticultural practices (including landscaping and restoration, noted above). As we show that genetic diversity is correlated with geographic origin, it is clearly important to ensure that only locally adapted lineages are used for these activities, otherwise it is possible that foreign genotypes will be imported and may interbreed with local genotypes. This can have disastrous genetic consequences such as outbreeding depression and genetic swamping (Hufford and Mazer, 2003).

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References

Alfaro, M.E., Holder, M.T., 2006. The posterior and the prior in Bayesian phylogenetics. Annual Review of Ecology and Systematics 37, 19–42.
Alvarez, J. A., Wendel, J. F., 2003. Ribosomal ITS sequences and plant phylogenetic inference. Molecular Phylogenetics and Evolution 29, 417–434.
Ashmole, P., Ashmole, M., 2000. St. Helena and Ascension Island: A Natural History. Anthony Nelson, Oswestry.
Bailey, C.D., Carr, T.G., Harris, S.A., Hughes, C.E., 2003. Characterization of angiosperm nrDNA polymorphism, paralogy and pseudogenes. Molecular Phylogenetics and Evolution 29, 435–455.
Baldwin, B.G., 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA. American Journal of Botany 80, 222–238.
Barker, N.P., Von Senger, I., Howis, S., Zachariades, C., Risley, B.S., 2005. Plant phylogeography based on rDNA ITS sequence data; two examples from the Asteraceae. In: Bakker, F.T., Chatrou, L.W., Gravendeel, B., Pelser, P.B. (Eds.), Plant Species-level Systematics: New Perspectives on Patterns and Process. Gantner Verlag, Ruggell, pp. 217–244.
Chandler, G.T., Bayer, R.J., Crisp, M.D., 2001. A molecular phylogeny of the endemic Australian genus *Gastrolobium* (Fabaceae: Mirbeliaceae) and allied genera using chloroplast and nuclear markers. American Journal of Botany 88, 1675–1687.
Chase, M.W., Hills, H.H., 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. Taxon 40, 215–220.
Connes, H.P., Abbott, R.J., 2001. Molecular phylogeography, reticulation, and lineage sorting in Mediterranean Senecio sect. Senecio (Asteraceae). Evolution 55, 1943–1962
Crisp, M.D., Chandler, G.T., 1996. Paraphyletic species. Telopea 6, 813–844.
Downie, P.O., Holtkamp, R.H., Ireson, J.E., Kwong, R.M., Swirepek, A.E., 2007. A review of the *Chrysanthemoides monilifera* biological control program in Australia: 1987–2005. Plant Protection Quarterly 22, 24–32.
Doyle, J.J., 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Systematic Botany 17, 144–163.
Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochemical Bulletin 19, 11–15.
Feliner, G.N., Rosselló, J.A., 2007. Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. Molecular Phylogenetics and Evolution 44, 911–919.
Fraser, D.J., Bernatchez, L., 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology 10, 2741–2752.
Gibbs, L., Yuan, Y-M., Kupper, P., Taberlet, P., 1996. Phylogenetic use of noncoding regions in the genus *Gentiana* L.: chloroplast trnL(UAA) intron versus nuclear ribosomal internal transcribed spacer sequences. Molecular Phylogenetics and Evolution 5, 460–466.
Goolsby, J.A., De Barro, P.J., Makinson, J.R., Pemberton, R.W., Hartley, D.M., Frohlich, D.R., 2006. Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control programme. Molecular Ecology 15, 287–297.
Griffioen, R.C., 1995. A taxonomic study of *Chrysanthemoides* Tourn. ex Medik. (Compositae). MSc Thesis, University of Cape Town.
Häßl, T.J.R., 2003. Mine rehabilitation in the arid Succulent Karoo vegetation zone of the South African west coast, Namakwa Sands — case study. Heavy Minerals 2003, Johannesnums, South African Institute of Mining and Metallurgy.
Hasegawa, M., Kishino, H., Yano, T., 1985. Dating the human–ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22, 160–174.
Holder, M.T., Anderson, J.A., Holloway, A.K., 2001. Difficulties in detecting hybridization. Systematic Biology 50, 978–982.
Howis, S., Barker, N.P., Macina, L., (in press). Globally grown, but poorly known: species delimitation, relationships and biogeography in *Gazania* Gaertn. (Asteraceae). Taxon. (http://caliban.ingentaselect.co.uk/fstemp/a45e2b2eb892d0d6a7b77bf0922c3f593.pdf).
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hufford, K.M., Mazer, S.J., 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology and Evolution 18, 147–155.
Joffe, P., 2001. Creative Gardening with Indigenous Plants: A South African Guide. Briza, Pretoria.
Keating, A., Urban, E.K., Fry, C.H., 1992. The Birds of Africa, vol. 4. Academic Press, London.
Kim, S.C.-H., Crawford, D.J., Jansen, R.K., Arnold, S.-G., 1999. The use of a non-coding region of chloroplast DNA in phylogenetic studies of the subtribe Sonchinae (Asteraceae: Lactuceae). Plant Systematics and Evolution 215, 85–99.
Maddison, W.P., Maddison, D.R., 2000. MacClade 4: Analysis of Phylogeny and Character Evolution. Sinauer Associates, Sunderland.
McDade, L.A., 1992. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. Evolution 46, 1329–1346.
Mckenzie, R.J., Barker, N.P., 2008. Radiation of southern African daisies: biogeographic inferences for subtribe Arctotideae (Asteraceae, Arctotideae). Molecular Phylogenetics and Evolution 49, 1–16.

McKenzie, R.J., Barker, N.P., Muller, E.M., Karis, P.O., 2006. Phylogenetic relationships and generic delimitation in subtribe Arctotideae (Asteraceae: Arctotideae) inferred by DNA sequence data from ITS and five chloroplast regions. American Journal of Botany 93, 1222–1235.

Meek, P.D., 1998. ‘Weed seeds and whoopsie daisies’: viability of bitou bush Chrysanthemoides monilifera seeds in fox (Vulpes vulpes) scats. Plant Protection Quarterly 13, 21–26.

Mort, M.E., Archibald, J.K., Randle, C.P., Levens, N.D., O’Leary, T.R., Topalov, K., Wiegand, C.M., Crawford, D.J., 2007. Inferring phylogeny at low taxonomic levels: utility of rapidly evolving cpDNA and nuclear ITS loci. American Journal of Botany 94, 173–183.

Mort, M.E., Randle, C.P., Kimball, R.T., Mesfin Tadesse, Crawford, D.J., 2008. Molecular data indicates the origin of weed populations of Chrysanthemoides monilifera in South Africa and its relevance to biological control. Biological Control 48, 84–91.

Nordenstam, B., 2007. Tribe Calenduleae Cass. In: Kadereit, J.W., Jeffrey, C. (Eds.), Asterales. Vol. 8 of Kubitzki, K. (Ed.), The Families and Genera of Compositae. Timber Press, Portland, Oregon, USA, pp. 365–388.

Nordenstam, B., 1996. Recent revision of Senecioneae and Calenduleae (Compositae, vol. 2). Academic Press, London, pp. 961–1033.

Nordenstam, B., Källersjö, M., 2009. Calenduleae. In: Funk, V.A., Susanna, A., Nordenstam, B., 2007. Tribe Calenduleae Cass. In: Kadereit, J.W., Jeffrey, C. (Eds.), Asterales. Vol. 8 of Kubitzki, K. (Ed.), The Families and Genera of Compositae. IAPT, Vienna, pp. 527–538.

Nordenstam, B., Bourke, G.H., 2006. Nephrotheca, a new monotypic genus of the Compositae-Calenduleae from the southwestern Cape Province. Compositae Newsletter 44, 32–37.

Norlindh, T., 1934. Studies in the Calenduleae I. Monograph of the genera Diaphorotheca, Castalis, Osteospermum, Gibbaria and Chrysanthemoides. Lund. Carl Blom Boktryckeri, CWK Gleerup, p. 432.

Norlindh, T., 1977. Calenduleae — systematic review. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The Biology and Chemistry of the Compositae, vol. 2. Academic Press, London, pp. 961–988.

Nylander, J.A.A., 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University. Program distributed by the author.

Ohsako, T., Okumura, M., 2003. Intra- and interspecific phylogeny of wild Fago- pyrum (Polygonaceae) species based on nucleotide sequences of noncoding regions in chloroplast DNA. American Journal of Botany 87, 573–582.

Paterson, J.M., Downie, D.A., Hill, M.P., 2009. Using molecular methods to determine the origin of weed populations of Paspalum aculeata in South Africa and its relevance to biological control. Biological Control 48, 84–91.

Pelser, P.B., Graavesen, B., Van der Meijden, R., 2003. Phylogeny reconstruction in the gap between too little and too much divergence: the closest relatives of Senecio jacobaea (Asteraceae) according to DNA sequences and AFLPs. Molecular Phylogenetics and Evolution 29, 613–628.

Pelser, P.B., Nordenstam, B., Kadereit, J.W., Watson, L.E., 2007. An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of Senecio L. Taxon 56, 1077–1104.

Ramdhani, S., Barker, N.P., Bijnath, H., (in press). Molecular data indicates rampant non-monophyly of species in Kniphofia Moench (Asphodelaceae): a consequence of a recent Afromontane radiation? Taxon.

Razafimandimbison, S.G., Kellogg, E.A., Bremer, B., 2004. Recent origin and phylogenetic utility of divergent ITS putative pseudogenes: a case study from Naucleeae (Rubieae). Systematic Biology 53, 177–192.

Rowan, M.K., 1967. A study of the colles of southern Africa. The Ostrich 38, 63–115.

Roy, B., Popay, I., Champion, P., James, T., Rahman, A., 2004. An illustrated guide to the common weeds of New Zealand. New Zealand Plant Protection Society. Manaaki-Whenua Press.

Ryder, O.A., 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution 1, 9–10.

Sang, T., Crawford, D.J., Stuessy, T.F., 1997. Chloroplast DNA phylogeny, reticulate evolution and biogeography of Paeonia (Paeoniaceae). American Journal of Botany 84, 1120–1136.

Scott, J.K., 1996. Population ecology of Chrysanthemoides monilifera in South Africa: implications for its control in Australia. Journal of Applied Ecology 33, 1496–1508.

Simurda, M.C., Marshall, D.C., Knox, J.S., 2005. Phylogeography of the narrow endemic, Heliumium virginicum (Asteraceae), based upon ITS sequence comparisons. Systematic Botany 30, 887–898.

Small, R.L., Cronn, R.C., Wendel, J.F., 2004. Use of nuclear genes for phylogeny reconstruction in plants. Australian Systematic Botany 17, 145–170.

Štorková, H., Olson, M.S., 2004. Comparison between mitochondrial and chloroplast DNA variation in the native range of Silene vulgaris. Molecular Ecology 13, 2909–2919.

Syring, J., Farrell, K., Businský, R., Cronn, R., Liston, A., 2007. Widespread genealogical nonmonophyly in species of Pinus subgenus Strobus. Systematic Biology 56, 163–181.

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17, 1105–1109.

Vriesendorp, B., Bakker, F.T., 2005. Reconstructing patterns of reticulate evolution in angiosperms: what can we do? Taxon 54, 593–604.

Weiss, P.W., 1986. The biology of Australian weeds 14. University of California Press, Berkeley, 384 p.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of three non-coding regions of chloroplast DNA. PCR Protocols: A Guide to Amplification and Sequencing Protocols. Academic Press, San Diego, California, pp. 315–324.

Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Systematic Biology 47, 568–581.

Wood, A.R., Nordenstam, B., 2003. An interesting new species of Osteospernum (Asteraceae-Calenduleae) from the Western Cape Province, South Africa, providing a link to the genus Chrysanthemoides. South African Journal of Botany 69, 572–578.

Yamashita, T., Fukuda, T., Yokoyama, I., Maki, M., 2004. Molecular phylogeny of Vincetoxicum (Apocynaceae-Asclepiadoideae) based on the nucleotide sequences of cpDNA and nrDNA. Molecular Phylogenetics and Evolution 31, 689–700.

Zachariades, C., Von Senger, I., Barker, N.P., 2004. Evidence for a northern Caribbean origin for the southern African biotype of Chromolaena odorata. In: Day, M.D., McFadyen, R.E. (Eds.), Chromolaena in the Asia-Pacific region. Proceedings of the 6th International Workshop on Biological Control and Management of Chromolaena, held in Cairns, Australia, May 6–9, 2003, pp. 25–27.