Relationships and evolution in the Drakensberg near-endemic genus, Craterocapsa (Campanulaceae)

E. Uys *, G.V. Cron

School of Animal, Plant & Environmental Sciences, University of the Witwatersrand, Private Bag 3, WITS 2050, South Africa

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The Drakensberg near-endemic genus Craterocapsa of the Campanulaceae comprises four species as circumscribed by Hilliard & Burtt with a fifth, Craterocapsa alfredica, recently distinguished from Craterocapsa insizwa based on dubious and variable morphological characters. A phylogenetic study of the relationships and evolutionary trends in Craterocapsa was undertaken based on morphological and DNA sequence data (the nuclear ribosomal ITS and the plastid trnL–trnF regions). An additional aim was to investigate the status of the various species in the genus. The generic circumscription of Craterocapsa was supported, however there is a lack of morphological evidence for the recognition of C. alfredica as a distinct taxon, and topologies of the molecular phylogenies suggest hybridisation and/or introgression as likely processes for its origin. C. alfredica is therefore here excluded from the genus. The morphological and molecular phylogenies are highly incongruent, suggesting convergence in features such as growth form and leaf shape in response to environmental factors, most notably altitude. The widespread Craterocapsa tarsodes is the first species to diverge, with dispersal northwards to Mount Inyangani a likely explanation for its highly disjunct distribution. C. insizwa and C. tarsodes are hypothesised to have speciated sympatrically in the Kokstad–Weza region, a ‘hotspot’ for the genus, whereas the sister species Craterocapsa montana and Craterocapsa congesta appear to have diverged in allopatry with ecological speciation playing a role.

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1. Introduction

Craterocapsa Hilliard & B.L. Burtt is a genus of perennial herbs in the sub-family Campanuloideae of the ‘bellflower family’, Campanulaceae. The Campanulaceae, one of five (Lammers, 2007) or four sub-families (Eddie et al., 2010) currently recognised in the family, occurs primarily in the temperate zones of the Old World, unlike the Lobelioideae, which inhabit mainly tropical and subtropical regions. Many of the Campanulaceae live in montane habitats, and the Campanuloideae tend to occur in fairly open habitats (Lammers, 2007), as does Craterocapsa, which grows in mats or colonies in stony, montane grassland or on rocky outcrops (Pooley, 2003; pers. obs.).

Craterocapsa occurs in southern Africa, a centre of diversity for the Campanulaceae (Lammers, 2007), where it is a near-endemic of the Drakensberg mountain range, occurring across the Eastern Mountain Region (EMR) in South Africa and northwards to Zimbabwe (Phillips, 1917; Hilliard and Burtt, 1973, 1987; Carbutt and Edwards, 2001). It is one of four genera near-endemic to the Drakensberg region and one of seven wahlenbergioid genera endemic to southern Africa and Zimbabwe (Hilliard and Burtt, 1973; Goldblatt, 1978). Most of these wahlenbergioid genera occur within the Greater Cape Floristic Region (Goldblatt and Manning, 2002), and include Theilea Phillips, Prismatocarpus L’Hér., Microcodon A.DC., and Roella L., which extends northwards into the Eastern Cape and southern KwaZulu-Natal.

Craterocapsa was described by Hilliard and Burtt (1973) and distinguished by its cup-shaped, usually trilocular ovary, which may rarely be bilocular. The capsule (Fig. 1B) forms a deep calyx chalice crowned by the persistent calyx tube (Hilliard and Burtt, 1973). Dehiscence of the capsule is apical (i.e. above the calyx lobes) by means of an operculum formed by the enlarged style base (or disc) and apex of the ovary (Hilliard and Burtt, 1973; Lammers, 2007). The lid is lifted out cleanly to release the seeds. The actinomorphic, pale blue or white flowers are usually solitary and terminal (rarely a few in leaf axils), sessile or on pedicels 1–20 mm long (Fig. 1). Another characteristic feature of the genus is that the leaves have a tendency to form rosettes.

Hilliard and Burtt (1973) included four species in Craterocapsa:

- Craterocapsa montana, Craterocapsa congesta, Craterocapsa tarsodes and Craterocapsa insizwa (Fig. 1C–G). All but one (C. congesta) of these four species were previously placed either in Wahlenbergia or in Roella due to the similarity of the capsule. In both Craterocapsa and most species of Roella, the capsule dehisces apically via a pore or a lid that splits open or pops off in some manner to release the seeds (Adamson, 1951; Hilliard and Burtt, 1973). However, in Roella glomerata the capsule dehisces by five vertical sutures (Bentham, 1876; Hilliard and Burtt, 1973). In Wahlenbergia on the other hand, the capsule dehisces by apical valves, which project to varying degrees above the base of the calyx (Hilliard and Burtt, 1973; Lammers, 2007), with the exception of Wahlenbergia acaulis E. Mey.
and *Wahlenbergia suffruticosa* Cupido (Cupido et al., 2011). *Roella* differs from *Craterocapsa* in that its ovaries are always bilocular (not trilocular) and the fused hypanthium and ovary are narrowly cylindrical, with two thirds of the species having few or many bracts arising from the fruit wall. In contrast, in *Craterocapsa* the fruit is cup-shaped and lacks leafy bracts (Adamson, 1951; Hilliard and Burtt, 1973). *Roella* also has decurrent leaves or leaves in clusters in axils, features not seen in *Craterocapsa*.

Three molecular phylogenetic studies of the Campanulaceae (Eddie et al., 2003; Haberle et al., 2009; Eddie et al., 2010) include some southern African genera. In Eddie et al.’s (2003) study based on ITS nuclear ribosomal DNA, *Craterocapsa congesta* and *Roella ciliata* are sister to each other, however they were the only ‘wahlenbergioid’ taxa included in the study (i.e. in the tribe Wahlenbergieae of Yeo (1993), resembling *Wahlenbergia* s.l. of Eddie et al. (2010)).

In the phylogeny produced by Haberle et al. (2009) based on three chloroplast regions (*atpB*, *matK* and *rbcL*), *Craterocapsa* and *Theileera* form a well supported clade sister to *Microcodon*. This clade with these three genera is sister to a clade with two branches: the first comprises *Wahlenbergia gloriosa* and *Wahlenbergia angustifolia*; the second clade comprises *R. ciliata*, *Prismatocarpus* and *Merciera*. Both *Wahlenbergia* and *Prismatocarpus* are shown to be polyphylectic, and *Roella* is nested in *Prismatocarpus*. The status of these genera in southern Africa is currently under review (Cupido pers. comm.). Prior to this work, no phylogenetic study of *Craterocapsa* has been done.

Subsequent to Hilliard and Burtt’s (1973) delimitation of *Craterocapsa*, a fifth species was described, *C. alfredica* D.Y. Hong (Hong, 2002). The type of this new species was previously identified as *C. insizwae*, a species noted to include plants with a bilocular or trilocular ovary and a bifid or trifid style respectively (Hilliard and Burtt, 1973). Hong (2002) described *C. alfredica* as having a tri-lobed style, but a bilocular ovary, and ovate to ovate-lanceolate or cuneate leaf bases as opposed to the rounded, truncate or cordate leaf base of *C. insizwae*, which he denoted as possessing a bifid style. [It should be noted that this difference was present in two of the four collections cited by Hilliard and Burtt (1973) in their description of *C. insizwae*.] However, such variation in stigmatic lobe number has been reported elsewhere in the Campanulaceae; for example in *Campanula* the stigmatic lobe number varies irrespective of the number of locules (Eddie and Ingrouille, 1999). Calyx lobes were also noted to differ in shape and reflexion with linear-lanceolate and
unflexed calyx lobes in *C. alfredica* versus the ovate-orbicular, reflexed calyx lobes of *C. insizwae* (Hong, 2002). The new species *C. alfredica* was based only on the type specimen [holotype: South Africa, Natal, Alfred District, Main Cape road near Weza turnoff, 14 Nov 1973, Hilliard and Burtt 7227 (PRE)]. This fifth species has not been widely recognised (Manning, 2009), although Lammers (2007) listed five species of *Craterocapsa*. The status of *C. alfredica* as a distinct species thus needed to be investigated using both morphological and molecular data.

2. Distribution — a Drakensberg near endemic

*Craterocapsa* is a near-endemic of the Drakensberg and outlying mountains (Fig. 2), with its known distribution extending into the Eastern Highlands of Zimbabwe (not shown). The alpine and subalpine zones of the Drakensberg (1800–3500 m) have been found to constitute a centre of endemism, the Drakensberg Alpine Centre (DAC) (Killick, 1994; Carbutt and Edwards, 2001; Van Wyk and Smith, 2001). Floristically the DAC includes the KwaZulu-Natal Drakensberg summit and its outliers Mahwaqa and Ngeli Mountains, the Lesotho Malotis, the northern and southern KwaZulu-Natal Drakensberg scarp face, the Eastern Cape Drakensberg and Witteberg, Sehlabathebe and East Griqualand (Hilliard and Burtt, 1987; Carbutt and Edwards, 2006; Mucina and Rutherford, 2006). The DAC constitutes the fourth richest regional flora in southern Africa; regrettably the endemic plants and their conservation status are poorly known, with only about 5.5% of the DAC currently conserved (Carbutt and Edwards, 2006). *Craterocapsa* is found on all of the mountains comprising the DAC, extending southwards into the Sneeuwberg and Winterberg in the Eastern Cape and northwards to the mountains of Mpumalanga and Swaziland, with a single population of *C. tarsodes* known from Mount Inyangani in Zimbabwe [Wild 4906 (K; SRGH)]. The genus occurs at altitudes ranging from 1200 to 3550 m and is thus present in alpine, subalpine and montane altitudinal bands (Hilliard and Burtt, 1973).

*C. tarsodes* has the most widespread distribution, extending from KwaZulu-Natal to Mpumalanga and Swaziland to a highly disjunct population on Mount Inyangani in Zimbabwe, and occurs over a wide altitudinal range in both montane and sub-alpine bands: 1200–2500 m. *C. congesta* is centred in the Maloti mountains of Lesotho and the KwaZulu-Natal Drakensberg escarpment with isolated populations on the nearby mountains Ngeli and Insizwa in the Eastern Cape (Fig. 2). The majority of collections of *C. congesta* occur between 2400 and 3350 m in the Drakensberg Alpine Centre and at 1250–1600 m on the outlying mountains (e.g. Mount Ngeli). In contrast, *C. montana* has a disjunct distribution both geographically and altitudinally, occurring below 1900 m on the north eastern border of Lesotho and in the eastern Free State and around 2000 m on the Eastern Cape mountains, the Boschberg, Sneeuwberg and Great Winterberg, and more recently discovered, on the Katberg Pass near Hogshack (T. Dold pers. comm.). *C. insizwae* is limited to the Drakensberg outliers (e.g. Mt. Ngeli, Mt. Insizwa) near Kokstad, while *C. alfredica* is known only from the type locality near Weza State Forest (Fig. 2). Both species occur between 1250 and 1350 m. The Weza–Kokstad region (including Mt. Insizwa and Mt. Ngeli) is thus a centre of diversity as four of the five species occur here: *C. congesta, C. insizwae, C. alfredica* and *C. tarsodes*.

As *Craterocapsa* is a near endemic of the Drakensberg mountain range (Hilliard and Burtt, 1973), the topography, habitat ranges and development of the Drakensberg may have played an important role in the speciation and distribution patterns of *Craterocapsa*. The Drakensberg mountains are included in the Afromontane Archipelago which extends beyond the eastern highlands of Zimbabwe into the mountains of Malawi and East Africa to Ethiopia (Hedberg, 1965; Van Wyk and Smith, 2001). They comprise the southernmost regional mountain system of the Afromontane Archipelago (White, 1983), the most south-eastern range of which includes the Winterberg of the Eastern Cape (Meadows and Linder, 1993).

Many species occurring in the Drakensberg have Cape relatives (Hilliard and Burtt, 1973, 1978; Carbutt and Edwards, 2001; Galley...
and Linder, 2006). Some of the Cape flora are hypothesised to have colonised the Drakensberg region, for example Protea, Disa and Oxalis, and some species nested within Cape genera have geographical ranges extending into tropical Africa, e.g. Cliftorria, Disa and Pentaschistis (White, 1983; Carbutt and Edwards, 2001; Galley and Linder, 2006; Galley et al., 2007). As the Cape Fold Belt arose long before the Drakensberg mountains, around 330 mya when a subduction zone developed on the southern margin of Gondwana causing a compression within the interior (McCarthy and Rubidge, 2005 Fig. 7.7 p. 193), an established montane flora from the Cape would have had the opportunity to colonise the Drakensberg.

The wahlenbergioid genera are similarly a group that are predominantly found in the Western and Eastern Cape (Welman and Cupido, 2003) and appear to have followed a similar path northwards. Furthermore very little is known about the modes of speciation, or patterns and events pertaining to speciation in this Eastern Mountain Region (Hilliard and Burtt, 1973, 1978, 1987; Carbutt and Edwards, 2001, 2006).

The main aims of this study were to clarify the species relationships in Craterocapsa and elucidate evolutionary trends, and to evaluate the taxonomic status of the more recently described species C. alfredica and its relationship to C. insizwa. We also hypothesise speciation modes based on the phylogeny and species’ distribution patterns of this near-endemic Drakensberg genus. The utility of morphological and molecular data to hypothesise relationships in Craterocapsa are also compared here. The nuclear ribosomal internal transcribed spacer (ITS) region, comprising ITS1, the 5.8S gene and ITS2, was selected to represent the bi-parental history, whilst the trnL–trnF plastid region (viz. the trnL 3′ intron, 3′ trnL exon and intergenic spacer between the exon and the trnF gene) was used for the maternal lineage. The ITS region has proven to be very useful at species-level and/or for depicting intra-generic relationships (Baldwin, 1992), although it proved too variable in a study of the genus as a whole (Antonelli, 2009).

3. Materials and methods

3.1. Taxon sampling

Roella and Wahlenbergia serve as outgroups for this study based on relationships in the phylogeny of the Campanulaceae hypothesised by Eddie et al. (2003). Roella is represented by R. glomerata, a species belonging to the putatively more primitive Spicatae (Adamson, 1951) and the only one that extends from the Eastern Cape into KwaZulu-Natal (Goldblatt and Manning, 2002; Welman and Cupido, 2003). Wahlenbergia, represented by the species Wahlenbergia krebssii, was used to root the molecular phylogenies, and compared to topologies when rooted with R. glomerata. Morphological characters and subsequent phylogenetic analyses used Roella as the outgroup, in order to simplify interpretation of homologous features. The ingroup comprises all five species of Craterocapsa, with multiple exemplars where possible: C. tarsodes (four), C. montana (three), C. congesta (four), C. insizwa (two) and C. alfredica (one). Species were identified by comparison with types (C. alfredica and C. congesta) and/or specimens determined by O.M. Hilliard & B.L. Burtt, and descriptions provided in Hilliard and Burtt (1973).

Our phylogenetic investigation of Craterocapsa was thus based on three sources of phylogenetic evidence: morphology, plastid and nuclear DNA sequence data, viz. the trnL–trnF and the ITS regions respectively.

3.2. Morphological component

A list of potentially phylogenetically informative morphological characters and states was drawn up based on examination of herbarium specimens and field observations and collections (Appendix A) and also guided by literature, notably Hilliard and Burtt (1973). A minimum of 15 specimens was examined for each species, using specimens from BOL, E, GRA, MO, NH, NU and PRE (abbreviations as per Holmgren et al., 1990). However the maximum numbers of specimens available for C. insizwa and C. alfredica were 11 and three (including duplicates) respectively. A final list of 27 characters with character states is provided in Table 1 and the data matrix in Table 2.

3.3. Molecular component

DNA was extracted using the Qiagen DNeasy Minikit from leaf material collected during fieldwork and dried in silica gel, except for C. alfredica where a leaf from the isotype [Hilliard & Burtt 7227 (NU)] was used (with permission) as this is the only collection cited by Hong (2002) and the population appears to no longer occur at the type locality. Vouchers are housed at J, PRE and NH (Appendix A).

The ITS region was amplified using the primers AB101 (5′-AAG AAT TCA TGG TCC GGT GAA GTG TTC G-3′) and AB102 (5′-TAG AAT TCC CCG GTT CCG TCC CGG TTA C-3′) of Sun et al. (1994) for Craterocapsa, and AB101 and ITS4 (5′-TCC TCCCCGATTTGATAG 3′; White et al., 1990) for the outgroups Roella glomerata and Wahlenbergia krebssii. The PCR protocol, using Truestart Taq (Fermentas®), involved a 2 min pre melt at 95 °C, 35 cycles of 50 s of denaturation at 95 °C, annealing for 45 s at 54 °C and extension of 1 min 30 s at 72 °C, with a final extension at 72 °C for 7 min. Amplification of the trnL–trnF region was conducted using the ‘c’ (CGA AAT CGG TAG ACC CTA CG) and ‘p’ (ATT TGA ACT GGT GAC AG) primers designed by Taberlet et al. (1991) using the Pyrostart Fast PCR Master Mix (Fermentas®). Amplification involved a 1 min pre melt at 95 °C, 30 cycles of 1 s of denaturation at 95 °C, 5 s annealing at 40 °C, an extension of 90 s at 72 °C, followed by a final extension of 25 s at 72 °C.

The PCR products were purified using a Zymo DNA Clean and Concentrate Kit (Zymo Research®) and sequenced using the same primers as in the PCR protocols at the Central Analytical Facility, Stellenbosch University. Forward and reverse sequences were edited by checking

Table 1

<table>
<thead>
<tr>
<th>Character and states used in the morphological phylogenetic analysis of Craterocapsa.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fleshy roostock: absent (0), present (1).</td>
</tr>
<tr>
<td>Growth habit: erect, occasionally decumbent (0), tufted (1), prostrate (2).</td>
</tr>
<tr>
<td>Stems: woody (0), herbaceous (1).</td>
</tr>
<tr>
<td>Branch indumentum: hairy (0), not hairy (1).</td>
</tr>
<tr>
<td>Branches range of hairiness: &gt;0.5 mm (0), &gt;0.5 mm/leaf cells [1–3] (1), &gt;2 mm/many celled (2).</td>
</tr>
<tr>
<td>Lateral branches: absent (0), present and hairy (1).</td>
</tr>
<tr>
<td>Leaf clustering: axillary clustering (0), terminal clustering (1), no clustering (2).</td>
</tr>
<tr>
<td>Leaf rosettes: absent (0), present (1).</td>
</tr>
<tr>
<td>Leaf length: width ratio: 5 &lt; x &lt; 15 (0); 2.5 &lt; x &lt; 5 (1); &lt;2.5 (2).</td>
</tr>
<tr>
<td>Leaf shape: linear-lanceolate (0), narrowly-elliptic (1), ovate, to ovate-elliptic, to broadly elliptic (2).</td>
</tr>
<tr>
<td>Leaf indumenta adaxially: glabrous (0), thinly hairy (1).</td>
</tr>
<tr>
<td>Leaf indumenta abaxially: glabrous (0), thinly hairy (1), hairy (2).</td>
</tr>
<tr>
<td>Leaf margin: large teeth (0), minute teeth (1).</td>
</tr>
<tr>
<td>Floral stems: branched (0), unbranched (1).</td>
</tr>
<tr>
<td>Floral attachment: sessile (0), on pedicels (1).</td>
</tr>
<tr>
<td>Floral grouping: solitary (0), sparsely/few (1), dense heads (2).</td>
</tr>
<tr>
<td>Floral bracts: present (0), absent (1).</td>
</tr>
<tr>
<td>Capsule shape: cylindrical (0), cup-shaped (1).</td>
</tr>
<tr>
<td>Capsule top: plum (0), disc (1).</td>
</tr>
<tr>
<td>Capsule crown shape: flat lid (0), concave (1), concave with central depression (2).</td>
</tr>
<tr>
<td>Capsule dehiscence: vertical sutures (0), removal of cap (1).</td>
</tr>
<tr>
<td>Spike: bifid (0), trifid (1).</td>
</tr>
<tr>
<td>Style base broadening: present (0), absent (1).</td>
</tr>
<tr>
<td>Number of locules: bilocular (0), trilocular (1).</td>
</tr>
<tr>
<td>Hypanthium indumentum: glabrous (0), hairy (1).</td>
</tr>
<tr>
<td>Seed shape: elongated, ellipsoid (0), short, broad (1).</td>
</tr>
</tbody>
</table>

Calyx lobe indumentum: glabrous (0), ciliate on margins only (1), hairy (2).
against electropherograms and consensus sequences were created. The consensus sequences were then aligned using Sequencer 4.1.2 (Gene Code Corporation®) and alignment was refined manually and apomorphies confirmed by reference to electropherograms. Non-homologous insertions were aligned and coded as separate events, whilst non-homologous deletions were aligned together not affecting the phylogenetic analyses, but coded separately. A separate matrix of coded indels was thus created and analyses were performed including and excluding this matrix.

The aligned data matrices were analysed with and without coded indels applying the Fitch parsimony criterion in PAUP* version 4.0b10 (Swofford, 2001), using rigorous heuristic searches with 100 random addition sequences and tree-bisection-reconnection (TBR) swapping. A strict consensus tree was constructed for the multiple equally most parsimonious (EMP) trees obtained for the molecular data. Branch support was estimated using Bootstrap analysis (Felsenstein, 1985) applied to the matrix excluding the coded indels. Consistency index (CI) excluding uninformative characters, retention index (RI) and tree lengths of the EMP trees are reported.

A partition homogeneity test as implemented using PAUP* (1000 replications; Farris et al., 1995) was run to assess the compatibility of the morphological and the plastid and nuclear molecular data sets (P = 0.059). The molecular data sets were subsequently combined and parsimony and bootstrap analyses were performed as described above. The morphological data set was not compatible with the molecular data sets (P = 0.01) and therefore they were not combined. Diagnostic morphological characters were mapped onto the cladogram resulting from the combined molecular analysis.

4. Results

4.1. Morphological phylogenetic analysis

Eight of the twenty-seven characters used in the morphological cladistic analysis (Table 1) were parsimony informative within Craterocapsa (Fig. 3). Fourteen of the remaining characters were useful at the generic level, and four were autapomorphic, distinguishing species (Fig. 3B).

In the single most parsimonious tree (40 steps, CI = 0.85, RI = 0.71) resulting from the analysis based on the morphological data set, Craterocapsa has ten synapomorphies supporting it (Fig. 3A). It is distinguished by its cup-shaped capsule, as opposed to Roella which has a barrel-shaped capsule (Fig. 1A, B). Other diagnostic synapomorphies of Craterocapsa are its fleshy rootstock, herbaceous stems, and the formation of persistent leaf rosettes in mature plants (Fig. 3B).

Within Craterocapsa there are two main clades (Fig. 3A). The first clade, weakly supported with bootstrap (BS) of 61%, comprises C. montana, C. tarsodes and C. congesta united by the strictly trifid stigma and trilocular ovary (Fig. 3B). C. tarsodes and C. congesta are sister species sharing a tufted growth form with rosette leaf-formation, glabrous branches, narrowly elliptic leaves and sessile flowers. Sister to this C. tarsodes–C. congesta clade is C. montana, which starts developing with the tufted growth form, but later adopts a prostrate growth form retaining the rosette leaf formation only at branch tips. In addition, C. montana has hairy branches with broadly ovate ovate-elliptical leaves which are hairy dorsally, and has flowers on pedicels (Fig. 3B).

The second clade is strongly supported (BS 98%) and comprises C. insizwae and C. alfredica, which both have either a trifid or bifid style and a trilocular or bilocular ovary. The single autapomorphy distinguishing C. insizwae from C. alfredica is the thinly hairy adaxial leaf surface of C. insizwae (Fig. 3B). Similar to C. montana, both C. insizwae and C. alfredica have hairy branches, ovate to broadly ovate leaves, and their flowers are on pedicels. The growth form of this clade is also distinctive: a low-growing spreading plant without the typical tufted habit of the other species, and a much reduced leaf rosette present only on branch tips (Fig. 3B).

Within Craterocapsa there is some variability in fruit structure and mode of dehiscence (characters 19 and 23, Fig. 3B). In C. tarsodes, C. montana, C. insizwae and C. alfredica the style widens at the base, and with the top of the ovary, forms the capsule lid or plug. In C. tarsodes a narrow, hardened ring of tissue surrounding the plug separates from the style base and calyx wall, lifts out and releases the seeds, whilst in C. montana, C. insizwae and C. alfredica this ring is absent and the plug may fall out or remain intact with the calyx rotting. In C. congesta the style base is further derived; it does not widen but is surrounded by a fleshy disc which, with the top of the ovary, forms the lid of the capsule.

4.2. Molecular phylogenetic analysis

4.2.1. The trnL–trnF region

The aligned trnL–trnF data matrix for Craterocapsa, Roella glomerata and Wahlenbergia krebii was 951 bases long including 14 coded indels. Within Craterocapsa and Roella, the majority of indels are below 10 bp in length, with only three indels greater than 20 bp long and the longest (in C. tarsodes from Dullstroom) is 45 bp. Analysis of the trnL–trnF region resulted in 621 equally most parsimonious trees of 115 steps (CI = 0.97 and RI = 0.96 excluding uninformative characters). The consensus tree (Fig. 4) obtained from the trnL–trnF analysis is not fully resolved, and its topology is identical to the consensus produced when R. glomerata is used to root the trees. Species clades are formed for populations of C. insizwae, C. congesta and C. montana, but C. tarsodes appears as non-monophyletic. C. alfredica groups with populations of C. tarsodes from the Central KZN Drakensberg (Cathkin) and the eastern Free State (Kerkenberg), and not with C. insizwae, which forms part of the basal polytomy. The sister relationship between C. congesta and C. montana is evident only when coded indels are included in the analysis. Similarly, the sister relationship of the two more northerly Free State populations (Clarens and Fouriesberg) of C. montana is supported by a single coded indel (Fig. 4). Relationships among the populations of C. congesta also reflect their geography with the two more northerly Lesotho populations forming a sister pair, and the more southerly Sani Top and Rama’s Gate populations sister to each other (Fig. 4).

It should be noted that one population of C. tarsodes from the Cathkin Peak region of the Central KZN Drakensberg (Cron 793) exhibited a very divergent sequence, with many more gaps (insertion/deletion events) than the other C. tarsodes populations. It was therefore very difficult to align with confidence and was not included in the analysis. A second collection (Cron 808) from the same locality was therefore used as the representative for this population.
Fig. 3. Single most parsimonious tree resulting from Fitch parsimony analysis of Craterocapsa (40 steps, CI = 0.85, RI = 0.71 excluding uninformative characters) with Roella as outgroup, based on morphological features (Table 1). A: Numbers above lines indicate bootstrap support values; numbers below lines report the minimum branch length, B: tree with non-homoplasious apomorphies plotted at nodes.
4.2. The ITS region
The total aligned length of the ITS matrix was 867 bases for Craterocapsa, R. glomerata and the outgroup W. krebsii. Fifty-six characters were variable and 33 characters were parsimony informative. Thirteen gaps ranging in size from one to 32 bases were required for alignment within Craterocapsa and with Roella. Phylogenetic analysis of the ITS region resulted in a single most parsimonious tree of 97 steps, CI of 0.96 and RI of 0.95 (excluding uninformative characters). The topology of the tree is identical to that of the combined molecular analysis (Fig. 5, described below), and identical to that produced when R. glomerata was used to root the tree. Although the trnL–trnF phylogeny is less resolved, it does not conflict with the ITS phylogeny, as confirmed by the partition homogeneity test for the two molecular regions (P = 0.06).

4.2.3. Phylogenetic analysis of the combined data
Combined analysis of the ITS and trnL–trnF regions resulted in three EMP trees of 214 steps (CI = 0.92 excluding uninformative characters and indels, RI = 0.94). The total length of the combined data set was 1818 bases and 17 coded indels. In the strict consensus tree (Fig. 5), the Craterocapsa clade is well supported with 34 synapomorphies and has the same topology as the single tree produced by the ITS analysis (Fig. 5; branch support indicated for both combined and ITS analyses).

Craterocapsa is monophyletic with two weakly supported clades (Fig. 5). The first sub-clade comprises the four populations of C. tarsodes with the populations from Cathkin Peak and Kerkenberg in Kwazulu-Natal and the eastern Free State respectively forming a well-supported clade (BS 88%). The second sub-clade includes the remaining four species of Craterocapsa, but is fairly weakly supported (BS 62%). C. alfredica diverges at the base of this clade, similar to the ITS consensus tree but not the consensus for the trnL–trnF region, where its position is unresolved. The clade comprising C. insizwae, C. montana and C. congesta is also weakly supported (BS 57%).

5. Discussion

5.1. Relationships in Craterocapsa

Within the limited sampling of this study, Craterocapsa appears to be a monophyletic group by all three molecular phylogenetic analyses, chloroplast, nuclear and combined (Figs. 4 and 5). Monophyly of all species is confirmed by the analyses, except for C. tarsodes in the phylogeny based on the plastid data set. There is insufficient variation in the plastid trnL–trnF region to resolve species relationships in Craterocapsa, however the nuclear and combined analyses corroborate the finding that C. congesta and C. montana are sister species. This is in contrast to earlier hypotheses of Hilliard and Burtt (1973) as well as the morphological parsimony analysis obtained here (Fig. 3), where C. montana and C. insizwae, and C. congesta and C. tarsodes are sister species pairs based on growth form, leaf shape, and glabrous leaves and branches. The lack of congruence between the molecular and morphological phylogenies is due to the convergence portrayed by these morphological traits. C. montana and C. insizwae occur in similar habitat conditions and a softly herbaceous,
more prostrate spreading growth form is present in both species. C. tarsodes also has prostrate growth in specimens that co-occur with C. insizwae on the adjacent outlying mountains mainly at altitudes of 1200–1350 m. However, C. tarsodes is distinctly tufted and cushion-like for the remainder of its distribution at higher altitudes and/or lower latitudes and similar morphologically to C. congesta. This illustrates the phenotypic plasticity and potential inherent in C. tarsodes. In contrast, C. congesta has little variation across its range and it occurs in very different habitats alpine vs. montane, where there is little overlap in distribution with the other species. C. congesta is exposed to more stressful environmental conditions and is adapted to these alpine conditions with a dense cushion-forming growth form and narrow leaves. Both C. tarsodes and C. congesta occur in more stressful environments that may be resource-limited at times, e.g. water and nutrients. A thickened tap root, narrow, thicker leaves and persistent rosette are adaptations for the stressful alpine environment and reflect convergence due to environmental conditions (Arroyo et al., 1982, 1985; Knox and Palmer, 1995).

Despite the fact that the two populations of C. insizwae (from Hebron in the Mt. Currie district, and Zuurberg in the Mt Ngeli district) exhibit the bifid and trifid style states and the bilocular and trilocular ovary respectively, they form a well supported monophyletic clade (Figs. 4 and 5). This confirms Hilliard and Burtt’s (1973) interpretation of these features as variable within C. insizwae. It therefore negates the recognition of C. alfredica on the basis of the tri-lobed style and bilocular ovary as compared to C. insizwae which is bi-lobed and trilocular according to Hong (2002). Clearly these characters are variable within C. insizwae, and the odd combination used to distinguish C. alfredica is dubious (see below). As noted previously, variability in stigma lobe numbers is not unusual for the Campanulaceae, for example Eddie and Ingrouille (1999) note a range between 3 and 5 stigmatic lobes in a single population of Campanula scopoletia.

The placement of C. alfredica is equivocal in the phylogenies resulting from the various analyses performed here. It is sister to C. insizwae in the phylogeny based on morphological data (Fig. 3), but is sister to the clade comprising C. montana–C. congesta–C. insizwae in the phylogeny resulting from the nuclear and combined nuclear and plastid parsimony analyses (Figs. 5 and 4, respectively). However, this relationship is only weakly supported (BS < 50%) by a few characters unlike the more substantial support for the other relationships hypothesised here. In the phylogeny based on plastid data (Fig. 4), C. alfredica is grouped in a weakly supported clade (~50% BS) with populations of C. tarsodes from Cathkin and Kerkenberg, the most southerly collections used in the molecular analysis. The remaining populations of C. tarsodes are part of a polytomy at the base of the Craterocapsa clade (Fig. 4). However, C. tarsodes is morphologically quite distinct from C. alfredica as they differ in growth form, leaf shape, and indumenta of leaves, branches and calyx.

5.2. Recognition of C. alfredica

The continued recognition of C. alfredica is thus contentious. Morphologically it does not appear to be distinct from the range of variation expressed in C. insizwae (pers. obs., and Fig. 3). The main morphological characters distinguishing C. alfredica from C. insizwae are tenuose: C. alfredica was diagnosed as having a trifid style and bilocular ovary (Hong, 2002), however inspection of the isotype (NU) specimen revealed it to be trilocular and bifid. In their description of Craterocapsa, Hilliard and Burtt (1973) noted that the tissues separating the locules are delicate and often degenerate. As C. alfredica is constituted by only the type specimens collected 40 years ago, it is probable that the tissues have disintegrated in the interim and this is not a reliable diagnostic character. The distinction in leaf shape between C. alfredica and C. insizwae is also highly dubious as that described for C. alfredica falls within the range observed for C. insizwae. Thus, the morphological features of C. alfredica do not separate it from C. insizwae.

The lack of congruence between plastid and nuclear topologies in regard to the placement of C. alfredica suggests that a hybridization event may have occurred, resulting in the specimens on which the name C. alfredica is based. C. alfredica diverges between C. tarsodes and the clade with C. insizwae in the combined and nuclear analyses (Fig. 5), i.e. sister to the clade comprising C. insizwae, C. montana and C. congesta. In contrast, the maternal lineage indicates a relationship between C. alfredica and two populations of C. tarsodes (Fig. 4) and the morphology confirms a close relationship with C. insizwae. It is possible that C. insizwae and C. tarsodes (which co-occur in the Weza region) hybridised to produce a highly localised population of plants, with C. tarsodes providing the maternal component in the hybrid population.

Introgression is often given as a reason to account for discord in nuclear and chloroplast data (Okuyama et al., 2005). Whilst the ITS region is thought to represent the biparental history, concerted evolution can obscure the evidence. The discord between chloroplast and nuclear data or simply the chloroplast data may remain as the only evidence of an introgression/hybridisation event (Okuyama et al., 2005). In the case of C. alfredica it is possible that introgression occurred with C. insizwae as the morphology of both taxa is indistinguishable, however the molecular evidence indicates a mixed parental history.

A closer look at the plastid sequence data reveals that the Cathkin and Kerkenberg populations of C. tarsodes and C. alfredica share two apomorphies, whereas C. insizwae has 10 autapomorphies. No apomorphies are shared between C. alfredica, C. insizwae and C. tarsodes, nor exclusively by C. alfredica and C. insizwae, i.e. there are no characters grouping these species together. However in the nuclear data, a single point mutation is shared by C. alfredica, C. tarsodes and C. insizwae. C. alfredica has four polymorphisms in the ITS nuclear regions, however none are shared by either C. tarsodes or C. insizwae. These polymorphisms do not reflect different bases in the putative parents as C. tarsodes and C. insizwae do not differ at these positions. Nonetheless, polymorphisms are not common in the genus and those that do occur tend to be limited to a single population within a species. Their existence in C. alfredica may support the theory of a hybrid origin. We therefore here exclude C. alfredica, concluding that it should no longer be recognised as a distinct species in Craterocapsa.

5.3. Evolutionary trends

Craterocapsa appears to have evolved in response to mainly abiotic factors which are reflected in its growth form, leaf shape and other vegetative features, rather than reproductive ones. This suggests that it has adapted to the physical environment rather than to biotic factors (e.g. pollinators), which is in contrast to most genera of the Campanulaceae where pollinators have been heavily implicated in their evolution (Eddie et al., 2003; Cupido, 2009). As noted previously, leaf rosette formation appears to be characteristic of Craterocapsa and is most notable in the tufted growth form of C. congesta, the only species found within the environmentally stressful alpine zone of the DAC. The DAC has extreme temperature fluctuations (Grab, 1998) and during winter water can be scarce (Fleming et al., 1999; Mucina and Rutherford, 2006). The tufted growth is compact, saving on metabolically expensive, exposed branches. Cushion-forming vegetation is a common feature of alpine vegetation, often forming a distinct band (Johnson and Billings, 1962). The rosette leaf formation complements the tufted growth compacting leaves and protecting branches with the remains of the overlapping peduncle. These adaptations protect individual plants from the environment. For example, Helichrysum species occurring in the DAC are often small and tufted (Pooley, 2003), and rosette-forming herbs, e.g. Viola species, are also common in the high Andes (Ferreira et al., 2006).

The prostrate (as opposed to tufted) growth form appears to have arisen independently or the underlying potential was brought to expression at least twice in the genus: C. montana and C. insizwae
(and C. alfredica) share this prostrate growth form (Hilliard and Burtt, 1973; Hong, 2002). They occur within the montane belts of the Eastern Cape and Free State, respectively. Within this altitudinal range a predominantly sandstone substrate is found (Hilliard and Burtt, 1973; Clark et al., 2009). The temperatures are not as extreme as in the DAC and snow is an uncommon occurrence (Clark et al., 2009). Furthermore, C. insiszwae only has the typical rosette formation on the tips of branches unlike C. montana which has a distinct rosette-tufted growth form similar to C. tarsodes and C. congesta in the early stages of development. However at maturity C. montana mirrors C. insiszwa and has rosettes limited to the tips of branches. C. montana has a much larger distribution than C. insiszwa, similar to C. tarsodes, and the initial tufted-rosette growth form may be an advantage in surviving a dispersal event. The initial tufted-rosette growth is probably more resilient to adverse environmental conditions allowing the plant to establish itself.

C. tarsodes has the greatest morphological variability among the five species (Hilliard and Burtt, 1973). It has hairy lateral branches in populations in the Drakensberg and outlying mountains at the boundary of the Eastern Cape and KwaZulu-Natal, where the climate is less stressful, being both wetter and having milder temperatures in the montane zone where it occurs. However, in the Mpumalanga populations, the lateral branches are absent (Fig. 1G).

Leaf shape and width correlate well with the growth form: a much broader leaf occurs in the prostrate C. montana, C. insiszwa (and C. alfredica), whereas C. tarsodes and C. congesta have elongated linear leaves. The leaves of C. montana, C. insiszwa and C. alfredica are also more delicate and hairy occurring alternately and more spread out on the prostrate branches, whereas the leaves of C. congesta and C. tarsodes are more rigid and glabrous and the margins are toothed. However, C. congesta has a dense, small leaf rosette at branch tips while C. tarsodes has leaves which vary in size (length and breadth) and do not form a dense rosette.

5.4. Speciation

The southern African genera of the Campanulaceae are centred in the Cape Floristic Region (CFR), and molecular dating suggests that the southern African Campanulaceae arose in the early to mid Oligocene (33–28 mya) (Cupido, 2009, Fig. 3.11: Eddie pers. comm.). Furthermore, the Craterocapsa clade is hypothesised to have diverged as recently as 1.8 mya (Cupido, 2009), concurrent with the changing summer-rainfall to winter-rainfall regime that resulted in the Mediterranean climate of the CFR (Coetzee, 1983; Cupido, 2009).

In the phylogeny of Eddie et al. (2010, Fig. 7) the clade B2a comprising Craterocapsa, Theliera and some Wahlenbergia species are all Western Cape and Western and Eastern Cape endemics, except for Craterocapsa. The clade B2b (Eddie et al., 2010, Fig. 7), sister to that containing Craterocapsa, comprises species of Wahlenbergia with distributions in the Western Cape, Northern Cape and Namibia. In addition, the Cape near-endemic Roella and endemic genera Prismatesiopsis and Merciera, form part of the clade (B1, Eddie et al., 2010, Fig. 7) sister to the above-mentioned clades.

As Craterocapsa is nested in a predominantly CFR clade (Eddie et al., 2010, Fig. 7), a Cape origin seems highly probable with subsequent dispersal northwards. During the late Miocene, major uplift events were thought to give rise to gullies and promote gorge incision in the Drakensberg/EMR as part of the processes within the Quaternary (King and King, 1959; McCarthy and Rubidge, 2005; Grab, 2010), thereby creating a diversity of habitats along the escarpment. However, recent studies based on in situ-produced cosmogenic isotopes (Fleming et al., 1999) suggest a long-term escarpment retreat rate and low summit denudation rates, which suggests that these habitats have been available with very little change for the last few million years.

Stepping stones for dispersal from the Cape into the Drakensberg have been proposed in the form of the Sneueberg–Nuwevedelberg–Hantam–Roggeveld (SNRH) mountain ranges which act as high altitude islands (Clark et al., 2009, 2011). Genera which have migrated from the CFR to the EMR include Disa, Moreae, Pentaschistis, and at least 18 migration events from the CFR to the EMR appear to have occurred (Linder and Kurzweil, 1994; Carbutt and Edwards, 2001; Galley and Linder, 2006; Galley et al., 2007). Wahlenbergioid/campanulid genera which occur in the CFR, EMR and the SNRH include Roella and Wahlenbergia (Carbutt and Edwards, 2001; Pooley, 2003; Clark et al., 2011).

Craterocapsa is a grassland genus and knowledge of the origin of grasslands in the EMR and along the SNRH would assist in informing the speciation ‘story’. Pollen analysis of late Tertiary deposits from the Namib coast suggests that southern African grasslands were well established by the Miocene. The Quaternary is noted for its glacial and interglacial periods and a general cooling, with grassland species adapting to these conditions (Scott, 2002). C3 grasses are found predominantly in the winter-rainfall regions of the Cape and along a north-eastward extension on the high-lying escarpment into Lesotho, across regions that include a variety of substrates. It can therefore be inferred that the distribution of these grasses is determined by a cool growing season: winter-rainfall or temperate conditions (Scott, 2002).

Grasslands appear to have consistently been present in the interior of South Africa for the last 300,000 years (Scott, 2002), however, there is a lack of fossil evidence and dating has been limited to noting that these grasslands predate anthropological influence i.e. before 12,000 BP (Meadows and Linder, 1993). Isotope studies on bone from the Melikane Cave, Lesotho suggest that C3 plants became more prominent in the diet of animals during the late Pleistocene due to the lowering of the upland grassland. In addition, evidence of pollen grains from grassland species vegetation during the Holocene was also found in the contemporary grassland biome at Inyanga mountains, Zimbabwe, the KwaZulu-Natal and Lesotho Drakensberg range, the Free State and the Eastern Cape (Meadows and Linder, 1993; Scott et al., 1997).

Craterocapsa has speciated across the Eastern Mountain Region. According to topologies of the combined and nuclear phylogenetic analyses presented here, the most widespread species C. tarsodes diverged first, followed by C. insiszwa which has the most localised distribution. C. tarsodes and C. insiszwa are altitudinally sympatric, however they have not been found to co-occur, inhabiting different microhabitats: C. insiszwa occurring on moister, darker, richer soil and C. tarsodes on drier, sandier soil (pers. obs.). Ecological speciation in sympatry or parapatry may have resulted in the morphologically distinct C. insiszwa and C. tarsodes. It would appear that more than one dispersal event has resulted in C. tarsodes’ distribution range extending northwards to the eastern mountains of Mpumalanga as the Dullstroom and Lydenberg populations do not form a monophyletic group (Fig. 5), but further sampling is necessary to confirm this hypothesis. Alternatively, vicariance may have occurred as the grasslands were more widespread during the Late Pleistocene (Scott et al., 1997) and ancestral populations may have been contiguous. However, it seems unlikely that contiguous montane grasslands with ancestral populations of C. tarsodes extended as far north as Mt. Inyangani in Zimbabwe, and its occurrence here is more likely to be due to a past dispersal event. (No populations have to date been found at intermediate localities such as the Chimanimani mountains.)

As noted above, C. alfredica co-occurs with its putative parent species, C. tarsodes and C. insiszwa, and is known only from a single locality in the Weza region. Morphologically similar to C. insiszwa, it grouped with C. tarsodes in the plastid phylogeny but is positioned between C. tarsodes and C. insiszwa in the nuclear and combined phylogenies (Fig. 5). The putative parent species are sympatric and a hybridisation event is quite feasible. The high density of species and the probability of a hybridisation event found in this region suggest that it is a centre of speciation for the genus (Rieseberg et al., 1998).

The pollinators visiting the flowers in this genus are likely to be bees, a pollinator also noted for Wahlenbergia (Welford and Johnson, 2011).
and as the flowers of the species of Craterocapsa are nearly identical (Fig. 1), reproductive isolation via pollinator specificity seems unlikely. Nonetheless, in Mediterranean species of Campanula there is a great variation in the shade of blue between species, and altitudinal separation of bee species has apparently promoted population differentiation within single species of Campanula (Blonis and Vokou, 2002). The same could be true for altitudinally separated sister species of Craterocapsa, such as C. montana and C. congesta, the clade that diverges next. C. congesta is mainly alpine in its altitudinal distribution and occurs on basalt as it is centred in the high KwaZulu-Natal Drakensberg and Maloti Mountains of Lesotho, only found below 1500 m on the outlier mountains near Weza–Kockstad (e.g. on Mount Ngeli’s summit) (Hilliard and Burtt, 1973; Fleming et al., 1999). C. montana occurs on the sandstone ridges, often associated with seepage zones as water is forced out due to the exposed rock (Hilliard and Burtt, 1973; pers. obs.). Prior to 16,000 BP a drier cooler climate predominated in the Drakensberg (Partridge, 1993). During drier periods, plants seek refuge in the moister climates found higher up the mountains (Knox and Palmer, 1995). A possible scenario is that during the cooler, drier climate some ancestral populations moved up into the basalt of the alpine region, and some populations remained in the lower sandstone regions in the montane belt, thereby resulting in both allopatric and ecological speciation.

The origin of the disjunct distribution of C. montana however is debatable. In our opinion [and supported by the divergence of the more northerly populations first in this limited sample (Fig. 5)], the populations from the Free State are more likely to represent the centre of origin of the species, as they are closer to the Drakensberg and Maloti massif, with subsequent dispersal southwards to the Eastern Cape mountains such as the Bosshoek and Winterberg. Intermediate populations of C. montana are known from Roma (near Blue Mountain Pass) and Mamathe (towards the south-western border) in Lesotho, and may represent a dispersal route towards the Eastern Cape. Alternatively, populations may have been more widespread during cooler times with less marked seasonal rainfall patterns (e.g. in the Pleistocene) when the grasslands are thought to have extended down the mountains (Scott et al., 1997; Burgoyne et al., 2005), and these populations may represent relics of this larger ancestral population. Further sampling of populations within the species is needed to resolve this issue.

5.5. Conservation

All of the species of Craterocapsa are termed of ‘least concern’ in the IUCN Red list of threatened species (Daniels, 2005; Foden and Potter, 2005). However, many plant species in South Africa have historically been assigned this status simply due to a lack of current information (Raimondo, 2011). We noted with concern that the species often were not located at previously recorded localities. Grazing seems to negatively affect their distribution: on fairly to heavily grazed sites in the Eastern Cape and Lesotho, no Craterocapsa species were found. In addition, C. insizwaec, which Hilliard and Burtt (1973) noted to be very common on the ‘Zuurberg’ (near Weza State Forest), was located there at only two places: a small population on a steep bank next to the mountain track and two single plants near the hill top, and it was noted during subsequent fieldwork (a year later) that only the small population on the steep bank remained. C. alfredica was not found at the type locality which now appears to be overgrown with grasses and creepers. It will be important to actively monitor Craterocapsa species’ populations in data collections/recorded data by groups such as Custodians of Rare and Endangered Wildflowers (CREW).

5.6. Conclusion

Craterocapsa is a monophyletic genus with four species and C. alfredica is suggested to have resulted from a hybridisation event between C. tarsodes and C. insizwaec and is here excluded from the genus. Incongruence in the phylogenies based on molecular and morphological data indicate convergent adaptation in growth form and morphology, and support the theory of possible hybridisation/introgression having occurred in its evolutionary history. The centre of diversity of Craterocapsa occurs in the mountains around Kokstad, including Mt Ngeli, Mt Insizwa, Mt Currie and Mt Ayliff, which appear to be a speciation hotspot for the genus. A Cape ancestor is hypothesised for Craterocapsa, with a sympatric ecological speciation event likely/possible for C. tarsodes and C. insizwaec, and ecological speciation in allopatry for the sister species C. congesta and C. montana.

6. Excluded species


Morphologically not distinguishable from C. insizwaec and molecular analyses suggest that it may be of hybrid origin: C. insizwaec × C. tarsodes.

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Appendix A

Specimens of Craterocapsa and Roella used for molecular and morphological studies. Specimen vouchers for the molecular work indicated with an asterisk in front of the species’ name. Genbank Accession numbers are for the ITS and trnL–trnF regions, respectively. All locations are in South Africa unless otherwise indicated.

*Craterocapsa alfredica* KWAZULU-NATAL, Alfred district, near Weza, 3029DA; O. Hilliard & B. Burtt 7227 (E, MO, NU); JX536223; KC147783

*Craterocapsa congesta* LESOTHO, Blue Mountain Pass, 2928AC; E. Uys & G. Cron 4 (J); JX536230; KC147788

*Craterocapsa congesta* LESOTHO, Oxbow, 2828DC; E. Uys & N. Ives 15 (J); JX536227; KC147787

*Craterocapsa congesta* LESOTHO, Sani Top, 2929CB; E. Uys & N. Ives 16 (J); JX536228; KC147786

*Craterocapsa congesta* LESOTHO, Rama’s Gate, E. Uys & J. Arnestad 38 (J); JX536229, KC147785

Craterocapsa congesta KWAZULU-NATAL, Plateau Summit of Giant’s Castle Pass, 2929AD; F. Wright 1060 (E); Craterocapsa congesta; LESOTHO, Hlatimba Pass, 2929AD; F. Wright 1317 (E, NU);

Craterocapsa congesta EASTERN CAPE, Naude’s Nek Pass, between Malpas and Nek, 3028CA; T. Dold & M. Cocks 2057 (GRA);

Craterocapsa congesta; EASTERN CAPE, Tiffendell Ski Resort, Southern Slopes of Ben McHdui, 3027DB; T. Dold & M. Cocks 3413 (GRA);

Craterocapsa congesta; LESOTHO, Leribe district, 2928AB; B. Phillips, C. Mokuku, R. Judd & C. Hobson 4656 (GRA);

Craterocapsa congesta; EASTERN CAPE, Mount Ayliff District, Inziswa Mountain, 3029BA; O. Hilliard & B. Burtt 6563 (NU);

Craterocapsa congesta; LESOTHO, Black Mountains, 2929CA; O. Hilliard & B. Burtt 8763 (E, NU, PRE)
Craterocapsa congesta; LESOTHO, Sani Top, 2929CB; O. Hilliard & B. Burtt 8837 (E, NU);
Craterocapsa congesta; LESOTHO, Maseru District, Blue Mountain Pass, 2928AC; O. Hilliard & B. Burtt 12029 (NU);
Craterocapsa congesta; EASTERN CAPE, Barkley East District, Ben McDhui, 3027DB; O. Hilliard & B. Burtt 16446 (E, NU);
Craterocapsa congesta; EASTERN CAPE, Engleksu Nek, 3028AD; O. Hilliard & B. Burtt 18675 (E, MO, NU);
Craterocapsa congesta; EASTERN CAPE, Mount Currie District, Farm Hebron; E. Uys, G. Cron & T. Malebu 11 (J); JX536222, KC147784
Craterocapsa congesta; WAZULU-NATAL, Alfred District, Weza, Zuurberg, 3029BC; E. Uys, G. Cron & T. Malebu 12 (J);
Craterocapsa congesta; WAZULU-NATAL, Mount Currie District, Farm Hebron; E. Uys & J. Arnestad 35 (J); JX536221, KC147784
Craterocapsa congesta; WAZULU-NATAL, Hela Hela Mountains, Game Valley Estates, 2930CC; B. Schrire 953 (NU) Durban;
Craterocapsa congesta; WAZULU-NATAL, Alfred District, Weza, Zuurberg, 3029BC; O. Hilliard 5467 (E, MO);
Craterocapsa congesta; WAZULU-NATAL, Ngeli Mountain above Rinkhals bank, 3029DA; A. Abbott 5491 (NU) Durban;
Craterocapsa congesta; WAZULU-NATAL, Alfred District, Ngeli Slopes, 3029BC; R.G. Strey 6319 (NU);
Craterocapsa congesta; WAZULU-NATAL, Alfred District, Zuurberg, 3029BC; O. Hilliard & B. Burtt 7545 (NU, PRE);
Craterocapsa congesta; WAZULU-NATAL, Isopo district, Farm Lynn Avis, 3030AA; O. Hilliard & B. Burtt 11212 (NU);
Craterocapsa montana; FREE STATE, Clarens, ridge behind town, 2828CB; E. Uys & G. Cron 1 (J); JX536225, KC147790
Craterocapsa montana; FREE STATE, Clarens, ridge behind town, 2828CB; E. Uys & G. Cron 2 (J);
Craterocapsa montana; FREE STATE, Clarens, ridge between town, 2828CB; E. Uys & G. Cron 3 (J);
Craterocapsa montana; FREE STATE, Fouriesberg, Hike Kututsa, 3321DD; E. Uys & N. Ives 5 (J);
Craterocapsa montana; FREE STATE, Fouriesberg, Hike Kututsa, 3321DD; E. Uys & N. Ives 6 (J); JX536226, KC147789
Craterocapsa montana; EASTERN CAPE, Amatole Mountains, below Gaikas Kop, 3226DB; H. Furness & P. Phillipson 68 (MO);
Craterocapsa montana; LESOTHO, Mamathe, 2927BB; A. Jacot-Guillarmod 96 (GRA, PRE);
Craterocapsa montana; EASTERN CAPE, Somerset East, Boschberg NR, Boscherberg summit, 3119DD; V. Clark, R. Daniels, M. Fabricius & J. Le Roux 282 (GRA); JX536224, KC147791
Craterocapsa montana; EASTERN CAPE, Old Hogsback Road, 3227CA; O. Hilliard & B. Burtt 10905 (NU);
Craterocapsa montana; EASTERN CAPE, Stutterheim, Keiskammahoek, “Garden Castle NR, Valley of Umzimkulu, 2929CC; O. Hilliard & B. Burtt 6898 (NH) Durban;
Craterocapsa montana; FREE STATE, Harrismith, Flatberg, Gibson Dam, 2829AC; O. Hilliard 5258 (NU);
Craterocapsa montana; EASTERN CAPE, Hogsback, Little Tuinbus, 3227CA; O. Hilliard & B. Burtt 10905 (NU);
Craterocapsa montana; EASTERN CAPE, Somerset East, Boschberg, 3119DD; O. Hilliard & B. Burtt 13221 (E, NU);
Craterocapsa montana; EASTERN CAPE, Stockenstrom District, Katberg Pass, 3226BC; O. Hilliard & B. Burtt 13262 (E, NU);
Craterocapsa montana; FREE STATE, Ficksburg, Farm Strathcoma, 2827DD; E. Galpin 13847 (PRE);
Craterocapsa montana; FREE STATE, Witsieshoek, 2828DB; E. Goldblatt 6849;
Craterocapsa montana; FREE STATE, East of Karkenber, 2829AC; G. Cron & M. Goodman 809 (J); JX536233, KC147793
Craterocapsa montana; WAZULU-NATAL, Lions River District, boundary gate between Roslyn & Silverdale, 2930AC; F. Wright 1346 (NU);
Craterocapsa montana; WAZULU-NATAL, Weza State Forest, grassland next to Bangeni Forest near forestry guard hut, 2930DA; A. Nichols 2091 (NH);
Craterocapsa montana; WAZULU-NATAL, Ithala NR, Louwsberg plateau, 2731CB; K. MacDevette 2160 (NU);
Craterocapsa montana; MPUMALANGA, Carolina, Athole Experimental Farm, 2630CB; A. Balsinha 2955 (PRE);
Craterocapsa montana; WAZULU-NATAL, Ngome-Bona Esperanza, 2731CD; T. Edwards 3302 (NU);
Craterocapsa montana; WAZULU-NATAL, Harding, Above Umkilo cutout, Ngeli Forest, 3029BC; A. Abbott 6898 (NH) Durban;
Craterocapsa montana; WAZULU-NATAL, Alfred District, Weza, Zuurberg, 3029AD; O. Hilliard & B. Burtt 7708 (E, NU, PRE);
Craterocapsa montana; WAZULU-NATAL, Lions River District, Farm Roslyn, 2930AC; O. Hilliard & B. Burtt 8718 (NU, MO);
Craterocapsa montana; WAZULU-NATAL, Lions River District, Farm Allendale near Kamberg Reserve, 2930AC; O. Hilliard & B. Burtt 8745 (E, NU);
Craterocapsa montana; WAZULU-NATAL, Underberg District, Garden Castle NR, Valley of Umzimkulu, 2929CC; O. Hilliard & B. Burtt 8897 (E, NU);
Craterocapsa montana; EASTERN CAPE, Umzimkulu District, Farm Ebuta, Mount Malowe summit, 3029BD; O. Hilliard & B. Burtt 11221 (E, MO, NU);
Craterocapsa montana; EASTERN CAPE, Umzimkulu District, Farm Ebuta, Mount Malowe summit, 3029BD; O. Hilliard & B. Burtt 11221 (E, MO, NU);
Craterocapsa montana; FREE STATE, Harrismith District, Kerkenber, 2829AC; O. Hilliard & B. Burtt 11927 (E, NU);
Craterocapsa montana; LIMPOPO PROVINCE, Polokwane, Haenertsburg from Crown Mountain, 2329DD; A. Mogg 16641 (PRE);
References


