

## SYSTEMATIC AND ECOLOGICAL SIGNIFICANCE OF SEED COAT MORPHOLOGY IN SOUTH AFRICAN CAMPANULACEAE *SENSU STRICTO*

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The seed coat morphology of 50 species of Campanulaceae *sensu stricto*, representing all 10 South African genera, was studied by scanning electron microscopy to gauge its usefulness in the diagnosis of genera or to support clades. Possible correlations with life form (annual, herbaceous and woody perennial) and ecological requirements such as fire response, rainfall requirements, bedrock and soil preferences, as well as habitat (e.g. fynbos, strandveld, renosterveld, grassland and karoo), were also investigated. Patterns of variation are described and interpreted as conforming to two seed coat types: reticulate (Type 1) and striate/wavy (Type 2). Type 1 seeds are further divided into eight subtypes. Some general trends emerged; for example, Type 1 seeds occur in all major clades of wahlenbergioids, Type 1A with a smooth coarsely reticulate surface being predominant in fynbos taxa, all of which are woody perennials. Several of the Type 1 seeds, together with Type 2 seeds, also occur in species with wider ecological amplitude, for example in karoo, strandveld or montane grasslands. In *Siphocodon* there is remarkable disparity in seed type between species. These variations in seed type generally appear to accord with current knowledge of climatic changes and soil evolution during the Tertiary of South Africa, and may be useful indicators of generic emergence and mosaic speciation in the major lineages of wahlenbergioids. However, it was concluded that seed coat types do not correlate closely enough with specific ecological requirements or life forms to be of unequivocal predictive value. Also, apart from *Merciera* and *Treichelia*, they are of limited use as a diagnostic character for genera, but are useful for distinguishing species.

*Keywords.* Phylogeny, scanning electron microscopy, taxonomy, vegetation types, *Wahlenbergia*, wahlenbergioid.

### INTRODUCTION

In South Africa the Campanulaceae *sensu stricto* are represented by about 250 species assigned to 10 genera, of which eight are endemic to this region (Welman & Cupido, 2003). Recent molecular phylogenetic studies (Cupido, 2009) have confirmed that the

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mode of capsule dehiscence is unsatisfactory as the primary character to define broadly circumscribed genera. All the well-supported major clades revealed in these studies comprise taxa with diverse modes of capsule dehiscence or taxa assigned to different genera. Furthermore, the molecular phylogenetic trees are incongruent with trees for morphological characters, and therefore the molecular results also cannot provide a complete basis for taxonomic distinction. Above the generic level, pollen morphology and biogeography have proved useful in dividing the Campanulaceae into three lineages: the campanuloids, platycodonoids and wahlenbergioids (Eddie *et al.*, 2003; Haberle *et al.*, 2009). These criteria, plus the possession of unique reproductive morphological characters, have allowed us to recognise the distinctiveness of *Siphocodon* Turcz. and *Rhigiophyllum* Hochst. and to place them in a separate tribe, the Rhigiophylleae Eddie & Cupido (Eddie *et al.*, 2010).

Netolitzky (1926) summarised the literature on seed anatomy of angiosperms available at that time and was the first to notice that the structure of the seed coat is characteristic of a family in general and is of taxonomic value. Takhtajan (1991) also pointed out that, even for phylogenetic correlations between families and genera, the structure of the seed coat might be important. Scanning electron microscopy data may be useful to delimit taxa at the species level, or sometimes as indicators of suprageneric groupings (Barthlott, 1981, 1984; Behnke & Barthlott, 1983), while variation in seed coat surface sculpturing among taxa could be correlated to ecological aspects (Barthlott, 1981). However, according to Barthlott (1984), seed surface features are little affected by environmental conditions and are merely the expression of genetic–phylogenetic differences in the plants concerned (Haridasan & Mukherjee, 1988).

In his brief summary of seed characteristics in the family Campanulaceae, Corner (1976) listed previous literature on seed studies for this family. However, there has been no systematic study of seed surface morphology in the family as a whole, apart from the surveys of genera from the three main lineages by Eddie (1984, 1997). The results of these surveys were hard to interpret, especially for phylogenetic studies, and the conclusion was that testa pattern seems to be closely correlated with ecology and may be useful for recognition at the species level, but of little value at the generic level. Eddie found that, as a general feature of the family, seeds that are regular in symmetry, lack wings and have pronounced reticulations with high relief are more primitive than those more irregular seeds which are rather smooth or striated and possess wings.

The usefulness of seed morphology as a character in the systematics of the Campanulaceae in North America was comprehensively studied by Shetler & Morin (1986). They included representatives of all the genera of campanuloids found on the continent, reported recognisable generic patterns and speculated on the adaptive significance of seed coat sculpturing. In another broad-scale study Haridasan & Mukherjee (1988) assessed the seed coat surface features in 27 species belonging to nine genera of Indian Campanulaceae, including *Sphenoclea zeylanica* Gaertn. (now classified in the Sphenocleaceae), and found them to be useful

characters to distinguish species but not diagnostic for genera. Turkish *Campanula* L. species have been the focus of recent studies by Akcin (2009) and Alçitepe (2010), who also found seed coat useful in the identification of some species. Prior to these studies, 17 European species of *Campanula* were surveyed by Geslot (1980), who came to the same conclusions. Hong & Pan (1998) found useful characters in pollen and seed morphology to reinstate the genus *Cyclocodon* Griff. ex Hook.f. However, for African Campanulaceae, only Thulin (1974, 1975) has provided data on seed coat morphology in his studies of *Namacodon* Thulin, *Gunillaea* Thulin and *Wahlenbergia* Schrad. ex Roth. He found great variation in the pattern of the seed coat sculpturing in species of these genera, and many gaps remain in our knowledge of the utility of seed coat surface features for the systematics of the South African Campanulaceae.

The high species and ecological diversity in South African wahlenbergioids makes them uniquely suitable for the assessment of seed coat patterns. This study arose from the need to find additional morphological characters that would provide a practical basis for the delimitation of the South African wahlenbergioids, and to evaluate these characters for their usefulness in taxonomic and phylogenetic studies (i.e. whether they are useful in the circumscription of genera and whether they support clades). We also investigated whether these characters were associated with particular ecological conditions.

#### MATERIALS AND METHODS

Seed samples of 50 species from 10 genera of South African Campanulaceae were examined. The selected species are representative of the major clades revealed by molecular studies (Cupido, 2009). Seeds were taken from mature capsules collected in the field or from herbarium specimens. Species examined, and their voucher specimens, are summarised in Table 1. The seeds were studied with an FEI Quanta 200 ESEM operating under high vacuum at an acceleration voltage of 10 kV using secondary-electron detection. Life history (annual, herbaceous and woody perennial) and ecological data (habitat, bedrock, soil type, rainfall season and fire response) for each species were obtained from herbarium specimens, personal observation and the literature (Welman & Cupido, 2003; Mucina & Rutherford, 2006) (Table 2). Vegetation types (fynbos, strandveld, renosterveld, grassland and karoo) follow the definitions of Mucina & Rutherford (2006).

#### RESULTS

Scanning electron microscope studies of the secondary sculpturing (fine relief of the cell wall; Barthlott, 1981) of the seed coat patterns of 50 South African Campanulaceae species revealed differences in sculpturing patterns. The pattern of variation suggests a basic dichotomy of seed coat types. In accordance with the terminology of Barthlott (1981), these are identified as reticulate (Type 1) and striate/wavy (Type 2),

TABLE 1. List of species examined, collection data and seed characteristics. Specimens are deposited at NBG and PRE

Species	Voucher	Seed coat pattern type	Seed length (mm)
<i>Craterocapsa montana</i> (A.DC.) Hilliard & B.L.Burtt	Jacot-Guillarmod 394, Lesotho, Mamathes	1A	1.1
<i>Craterocapsa tarsodes</i> Hilliard & B.L.Burtt	Jacobsz 355, Free State, Harrismith	1A	0.5
<i>Merciera brevifolia</i> A.DC.	Cupido 235, Western Cape, Caledon	1C	1.1
<i>Merciera eckloniana</i> H.Buek	Cupido 67, Western Cape, Villiersdorp	1C	0.9
<i>Merciera leptoloba</i> A.DC.	Cupido 66, Western Cape, Hangklip	1C	1.0
<i>Microcodon glomeratus</i> A.DC.	Cupido 105, Western Cape, Kraaifontein	1D	0.6
<i>Microcodon hispidulus</i> (L.f.) Sond.	Cupido 82, Western Cape, Malmesbury	1A	0.6
<i>Prismatocarpus campanuloides</i> (L.f.) Sond.	Cupido 219, Western Cape, Genadendal	1A	1.0
<i>Prismatocarpus crispus</i> L'Hér.	Manning 2651E, Western Cape, Clanwilliam	1E	0.8
<i>Prismatocarpus diffusus</i> (L.f.) A.DC.	Cupido 220, Western Cape, Genadendal	1A	1.1
<i>Prismatocarpus fruticosus</i> L'Hér.	Cupido 118, Western Cape, Somerset West	1A	1.2
<i>Prismatocarpus nitidus</i> L'Hér.	Cupido 228, Western Cape, Cape Town	1A	1.3
<i>Prismatocarpus pedunculatus</i> (P.J.Bergius) A.DC.	Cupido 273, Western Cape, Citrusdal	1A	1.3
<i>Prismatocarpus sessilis</i> Eckl. ex A.DC.	Cupido 112, Western Cape, Bredasdorp	1A	1.1
<i>Rhigiophyllum squarrosom</i> Hochst.	Cupido 106, Western Cape, Napier	1D	1.4
<i>Roella amplexicaulis</i> Wolley-Dod	Cupido 122, Western Cape, Cape Town	1A	1.1
<i>Roella arenaria</i> Schltr.	Cupido s.n., Western Cape, Napier	1A	1.0
<i>Roella ciliata</i> L.	Cupido 213, Western Cape, Cape Town	1A	0.9
<i>Roella cuspidata</i> Adamson	Cupido 234, Western Cape, Caledon	1A	1.3
<i>Roella incurva</i> A.DC.	Cupido 200, Western Cape, Hermanus	1A	0.9
<i>Roella muscosa</i> L.f.	Cupido 232, Western Cape, Cape Town	1A	1.4
<i>Roella secunda</i> H.Buek	Cupido 285, Eastern Cape, Joubertina	1A	1.2
<i>Roella squarrosa</i> P.J.Bergius	Cupido 229, Western Cape, Cape Town	1A	1.3
<i>Siphocodon debilis</i> Schltr.	Cupido 139, Western Cape, Napier	1G	2.1
<i>Siphocodon spartioides</i> Turcz.	Cupido 133, Western Cape, Villiersdorp	2	1.2
<i>Theilera guthriei</i> (L.Bolus) Phillips	Rourke 2128, Western Cape, Calitzdorp	1A	0.9
<i>Theilera robusta</i> (A.DC.) Cupido	Cupido 317, Eastern Cape, Willowmore	1A	1.0
<i>Treichelia dodii</i> Cupido	Cupido 83, Western Cape, Malmesbury	1F	0.6

TABLE 1. (Cont'd)

<i>Treichelia longibracteata</i> (H.Buek) Vatke	Cupido 199, Western Cape, Hermanus	1F	0.8
<i>Wahlenbergia acaulis</i> E.Mey.	Cupido 267, Northern Cape, Kamiesberg	1H	0.6
<i>Wahlenbergia adpressa</i> (Thunb.) DC.	Cupido 210, Western Cape, Hopefield	1D	0.5
<i>Wahlenbergia annularis</i> A.DC.	Cupido 251, Western Cape, Elandsbaai	1D	0.7
<i>Wahlenbergia capensis</i> (L.) A.DC.	Cupido 184, Western Cape, Malmesbury	2	0.6
<i>Wahlenbergia cernua</i> (Thunb.) A.DC.	Cupido 201, Western Cape, Hermanus	1D	0.6
<i>Wahlenbergia ecklonii</i> H.Buek	Cupido 206, Western Cape, Paarl	2	0.6
<i>Wahlenbergia exilis</i> A.DC.	Cupido 81, Western Cape, Malmesbury	2	0.4
<i>Wahlenbergia fruticosa</i> Brehmer	Cupido 311, Western Cape, Riversdale	1A	1.2
<i>Wahlenbergia huttonii</i> (Sond.) Thulin	Cupido 304, KwaZulu-Natal, Himeville	1D	0.8
<i>Wahlenbergia juncea</i> (H.Buek) Lammers	Cupido 296, Eastern Cape, Sterkstroom	2	0.7
<i>Wahlenbergia krebsii</i> Cham.	Cupido 294, Eastern Cape, Hogsback	1B	0.7
<i>Wahlenbergia longifolia</i> A.DC.	Cupido 212, Western Cape, Darling	2	0.5
<i>Wahlenbergia</i> <i>neorigida</i> Lammers	Stobie 68, Western Cape, Groenfontein	1A	0.8
<i>Wahlenbergia paniculata</i> (Thunb.) A.DC.	Cupido 181, Western Cape, Yzerfontein	1D	0.4
<i>Wahlenbergia parvifolia</i> (P.J.Bergius) Adamson	Cupido 119, Western Cape, Cape Town	1D	0.8
<i>Wahlenbergia polyantha</i> Lammers	Cupido 287, Western Cape, Albertinia	1D	0.5
<i>Wahlenbergia procumbens</i> (Thunb.) A.DC.	Cupido 244, Western Cape, Napier	1D	0.6
<i>Wahlenbergia psammophila</i> Schltr.	Cupido 260, Western Cape, Vanrhynsdorp	2	0.5
<i>Wahlenbergia subulata</i> (L'Hér.) Lammers	Cupido 207, Western Cape, Somerset West	1D	0.8
<i>Wahlenbergia suffruticosa</i> Cupido	Cupido 209, Western Cape, Malmesbury	2	1.0
<i>Wahlenbergia virgata</i> Engl.	Cupido 299, KwaZulu-Natal, Himeville	1D	0.8

with further subdivision into subtypes where significant variability within types is found. Types 1 and 2 conform to those described by Haridasan & Mukherjee (1988) for the Indian Campanulaceae, and for Turkish *Campanula* species by Akcin (2009). The four seed coat types described by Murata (1992) for the Lobelioideae also fit into these types (Tables 1 and 2).

TABLE 2. Life forms and ecological parameters scored for sampled species

Species	Life form	Vegetation type	Habitat	Bedrock	Soil type	Rainfall season	Fire response
<b>Type 1</b>							
<b>Type 1A</b>							
<i>Microcodon hispidulus</i>	A	FB	Flats, mountain slope	Sandstone, shale, granite	Sand, stony, loam, clay	W	NR
<i>Prismatocarpus campanuloides</i>	WP	FB	Flats, mountain slope	Sandstone, limestone	Sand, rocky	W	R
<i>Prismatocarpus diffusus</i>	WP	FB	Mountain slope	Sandstone, granite	Sand, stony	W	R
<i>Prismatocarpus fruticosus</i>	WP	FB, RV	Flats, mountain slope	Sandstone, shale	Sand, stony, rocky, loam, clay	W	R
<i>Prismatocarpus nitidus</i>	WP	FB	Mountain slope, crevices	Sandstone	Rocky, peat	W	R
<i>Prismatocarpus pedunculatus</i>	WP	FB, RV	Flats, mountain slope	Sandstone, shale	Loam, stony, sand	W	R
<i>Prismatocarpus sessilis</i>	HP	FB	Moist flats, mountain slope	Sandstone	Sand, rocky, gravel, loam, peat	W	R
<i>Roella amplexicaulis</i>	WP	FB	Mountain slope	Sandstone	Sand, stony	W	R
<i>Roella arenaria</i>	WP	FB	Flats, mountain slope	Sandstone	Sand, stony	W	R
<i>Roella ciliata</i>	WP	FB	Flats, mountain slope	Sandstone	Sand, stony	W	R
<i>Roella cuspidata</i>	WP	FB	Flats, mountain slope	Sandstone	Sand, stony	W	R
<i>Roella incurva</i>	WP	FB	Flats, mountain slope	Sandstone	Sand, stony	W	R
<i>Roella muscosa</i>	HP	FB	Mountain slope	Sandstone	Sand, stony	W	R
<i>Roella secunda</i>	WP	FB	Flats, mountain slope	Sandstone	Sand, stony	W, As	R
<i>Roella squarrosa</i>	WP	FB	Mountain slope	Sandstone	Sand, stony	W	R
<i>Theilera guthriei</i>	WP	FB	Mountain slope	Sandstone	Sand, stony, loam	As	R
<i>Theilera robusta</i>	WP	FB	Mountain slope, crevices	Sandstone	Rocky	As	R
<i>Wahlenbergia fruticosa</i>	WP	FB	Mountain slope	Sandstone, shale	Stony, loam	As	R
<i>Wahlenbergia neorigida</i>	WP	FB	Mountain slope	Sandstone	Sand, stony	W	?NR

TABLE 2. (Cont'd)

<i>Craterocapsa montana</i>	HP	GL	Mountain slope	Dolerite	Loam, stony	S	NR
<i>Craterocapsa tarsodes</i>	HP	GL	Mountain slope	Sandstone, shale, dolerite	Clay, loam	S	NR
Type 1B							
<i>Wahlenbergia krebsii</i>	HP	GL	Mountain slope	Mudstone, dolerite	Stony, rocky, loam, clay	S	NR
Type 1C							
<i>Merciera brevifolia</i>	WP	FB	Mountain slope	Sandstone, shale, granite	Sand, stony, loam, clay	W	R
<i>Merciera eckloniana</i>	WP	FB	Mountain slope	Sandstone	Sand, stony	W	R
<i>Merciera leptoloba</i>	WP	FB	Flats, mountain slope	Sandstone	Sand, stony	W	R
Type 1D							
<i>Microcodon glomeratus</i>	A	FB	Flats, mountain slope	Sandstone	Sand, stony	W	NR
<i>Rhigiophyllum squarrosum</i>	WP	FB	Mountain slope	Sandstone	Stony, rocky	W	R
<i>Wahlenbergia adpressa</i>	WP	FB, SV	Flats, hill slope	Sandstone	Sand	W	R
<i>Wahlenbergia annularis</i>	A	SV, K	Flats, hill slope	Sandstone, shale, granite, dolomite, quartzite	Sand	W	NR
<i>Wahlenbergia cernua</i>	A	FB, RV	Sheltered area, mountain and hill slopes	Sandstone, shale	Sand, stony, loam, clay	W	R
<i>Wahlenbergia huttonii</i>	HP	GL	Mountain slope	Sandstone, mudstone	Loam, rocky	S	NR
<i>Wahlenbergia paniculata</i>	A	FB, SV	Flats, mountain slope	Sandstone	Sand, stony	W	NR
<i>Wahlenbergia parvifolia</i>	HP	FB	Mountain slope	Sandstone	Sand, stony	W	R
<i>Wahlenbergia polyantha</i>	WP	FB, SV	Hill slope, flats	Sandstone, limestone	Sand, rocky	As	R
<i>Wahlenbergia procumbens</i>	HP	FB, RV	Moist flats, hill slope	Sandstone, shale, mudstone, dolerite	Sand, loam, clay	As, W, S	NR
<i>Wahlenbergia subulata</i>	WP	FB	Flats, mountain, hill slope	Sandstone	Sand, stony	W	R
<i>Wahlenbergia virgata</i>	HP	GL	Mountain, hill slope	Sandstone, mudstone, dolerite	Stony, rocky, loam, clay	S	NR

TABLE 2. (Cont'd)

<b>Type 1E</b>							
<i>Prismatocarpus crispus</i>	A	FB, RV	Flats, mountain slope	Sandstone, shale, granite	Sand, stony, loam, clay	W	NR
<b>Type 1F</b>							
<i>Treichelia dodii</i>	A	FB, RV	Flats, mountain slope	Sandstone, shale, granite	Loam, clay	W	NR
<i>Treichelia longibracteata</i>	A	FB	Flats, mountain slope	Sandstone	Sand, stony	W	NR
<b>Type 1G</b>							
<i>Siphocodon debilis</i>	WP	FB	Mountain slope	Sandstone	Sand, stony, peat	W	R
<b>Type 1H</b>							
<i>Wahlenbergia acaulis</i>	A	FB	Flats, mountain slope	Sandstone	Sand	W	NR
<b>Type 2</b>							
<i>Wahlenbergia capensis</i>	A	FB, SV, RV	Flats, hill slope	Sandstone, shale, granite	Sand, stony, loam, clay	W	NR
<i>Wahlenbergia ecklonii</i>	A	FB, RV, K	Mountain slope, bare rocks	Sandstone	Sand, stony, loam	W	NR
<i>Wahlenbergia exilis</i>	A	FB, RV	Flats, mountain slope	Sandstone, shale, granite	Sand, stony, loam, clay	W	NR
<i>Wahlenbergia juncea</i>	HP	GL	Mountain slope	Mudstone, dolerite	Stony, rocky, loam, clay	S	NR
<i>Wahlenbergia longifolia</i>	WP	FB, SV	Flats, hill slope	Sandstone, limestone	Sand, stony	W	R
<i>Wahlenbergia psammophila</i>	A	SV	Flats, hill slope	Sandstone	Sand	W	NR
<i>Wahlenbergia suffruticosa</i>	HP	SV	Flats	Sandstone	Sand	W	R
<i>Siphocodon spartioides</i>	WP	FB	Mountain slope	Sandstone	Sand, stony, loam	W	R

A = annual; HP = herbaceous perennial; WP = woody perennial.

GL = grassland; FB = fynbos; SV = strandveld; RV = renosterveld; K = karoo.

As = aseasonal; W = winter; S = summer.

R = resprouter; NR = non-resprouter.



### Seed coat pattern types

#### *Type 1: Reticulate*

##### *Type 1A*

Seeds are 0.6–1.4 mm long with a smooth coarsely reticulate surface. The cells are commonly laterally compressed, with the presence of protrusions from the surface at the junction of the radial and outer tangential walls. Present in all examined species of *Theilera* E.Phillips, *Roella* L., *Craterocapsa* Hilliard & B.L.Burt and *Prismatocarpus* L'Hér., except *P. crispus* L'Hér., and present in *Microcodon hispidulus* (L.f.) Sond., *Wahlenbergia fruticosa* Brehmer and *W. neorigida* Lammers (Figs 1–20).

##### *Type 1B*

The seed coat surface is distinctly reticulate with keeled and verrucate radial walls. Cells are distinctly or slightly elongated and have a distinct areole. The seeds are 0.7 mm long. Present in *Wahlenbergia krebsii* Cham. (Figs 21, 22).

##### *Type 1C*

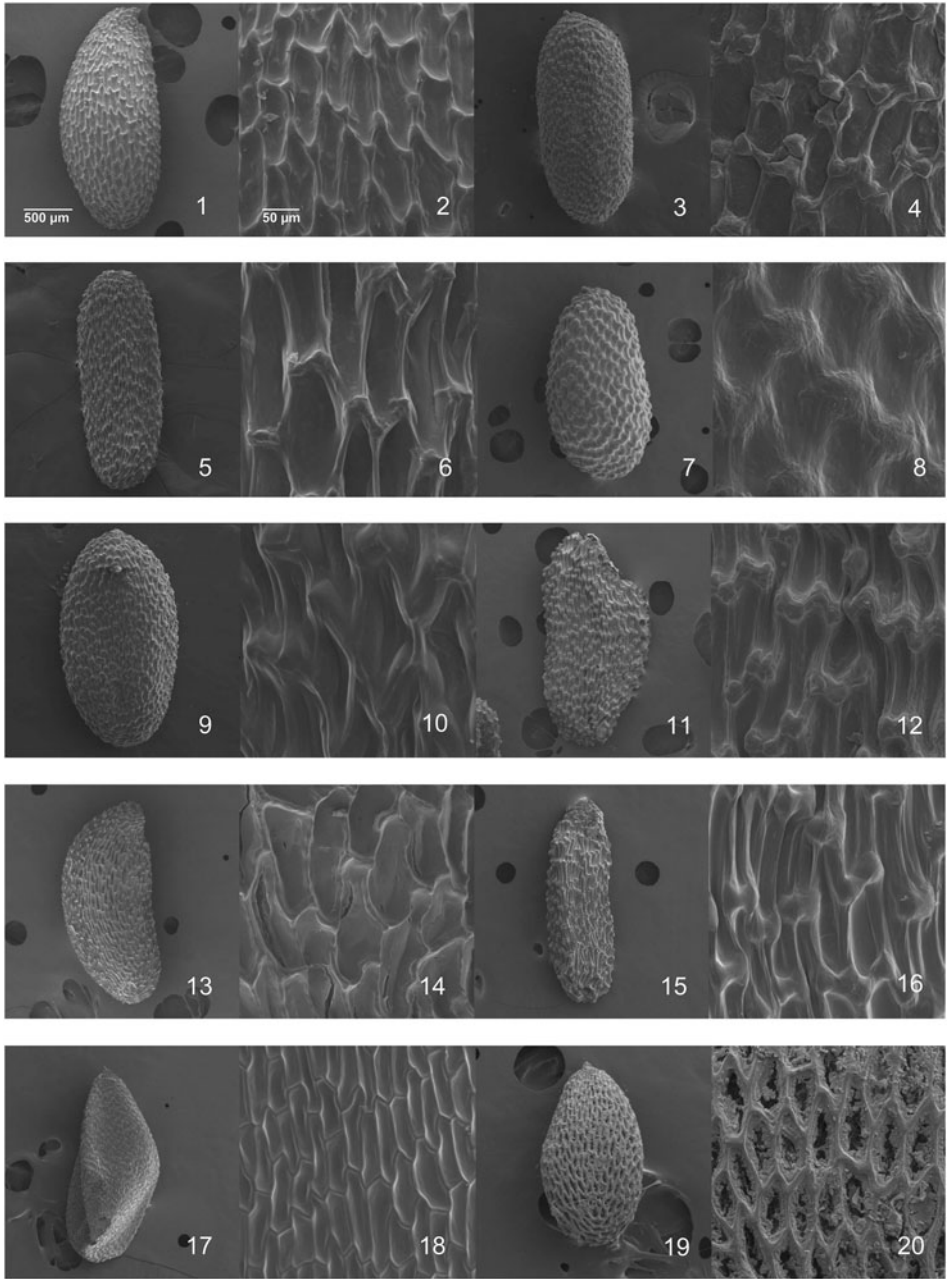
Present in all examined species of *Merciera* A.DC. and is similar to Type 1B, but with a weak reticulation formed by depressed radial walls and indistinct areole, giving the seed coat a warty appearance. Seeds are 0.9–1.1 mm long (Figs 23–26).

##### *Type 1D*

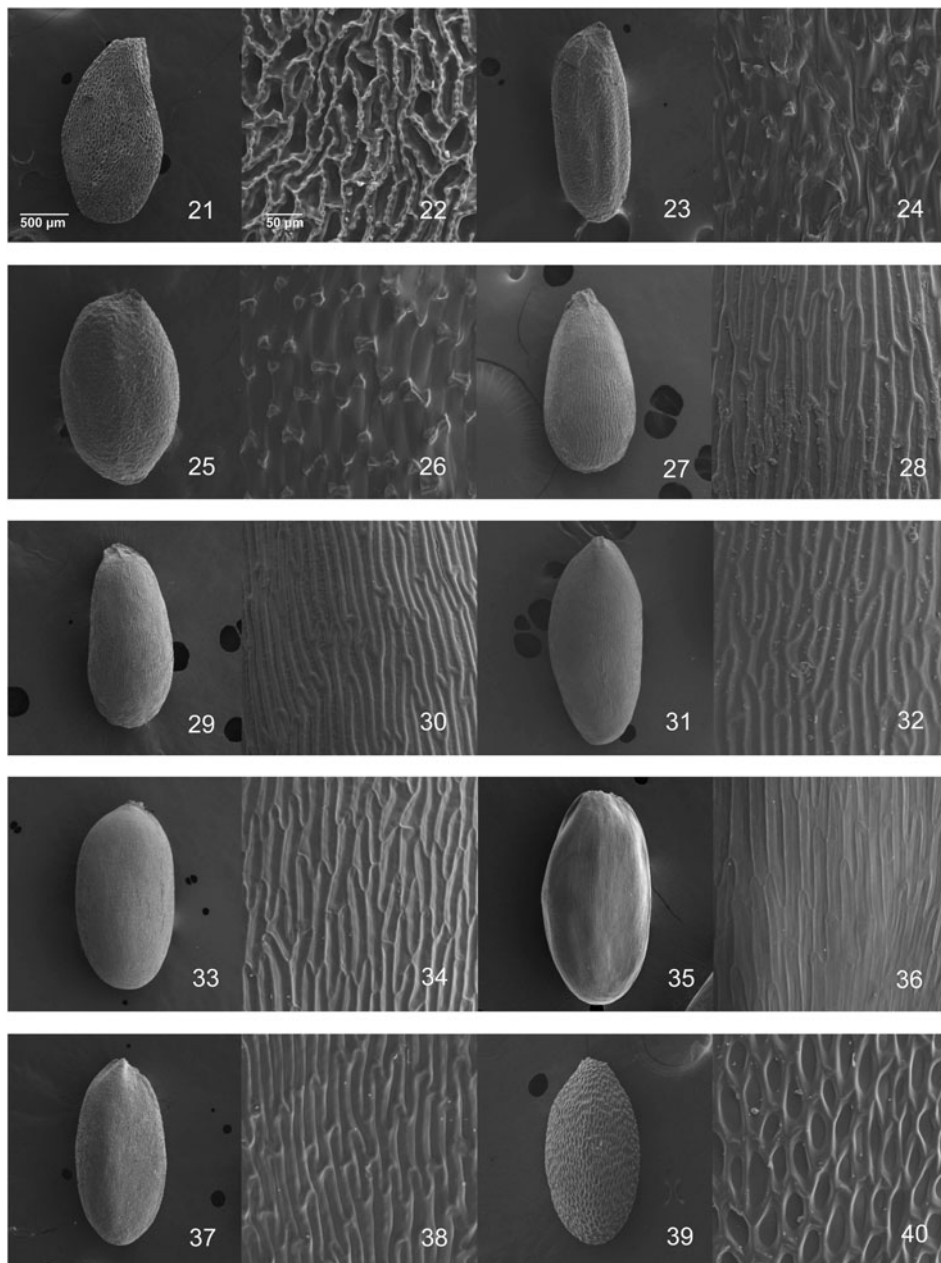
The seed coat surface is a distinctly or more or less smooth reticulum, sometimes keeled or verrucate. The cells are elongated with a distinct or indistinct areole. In species with weak reticulation the seed surface may appear striate or almost smooth. Type 1D seeds are 0.4–1.4 mm long and are present in *Wahlenbergia procumbens* (Thunb.) A.DC., *W. virgata* Engl., *W. subulata* (L'Hér.) Lammers, *W. parvifolia* (P.J.Bergius) Adamson, *W. huttonii* (Sond.) Thulin, *W. adpressa* (Thunb.) DC., *W. cernua* (Thunb.) A.DC., *W. annularis* A.DC., *W. paniculata* (Thunb.) A.DC., *Microcodon glomeratus* A.DC. and *Rhigiophyllum squarrosum* Hochst. (Figs 27–44).

##### *Type 1E*

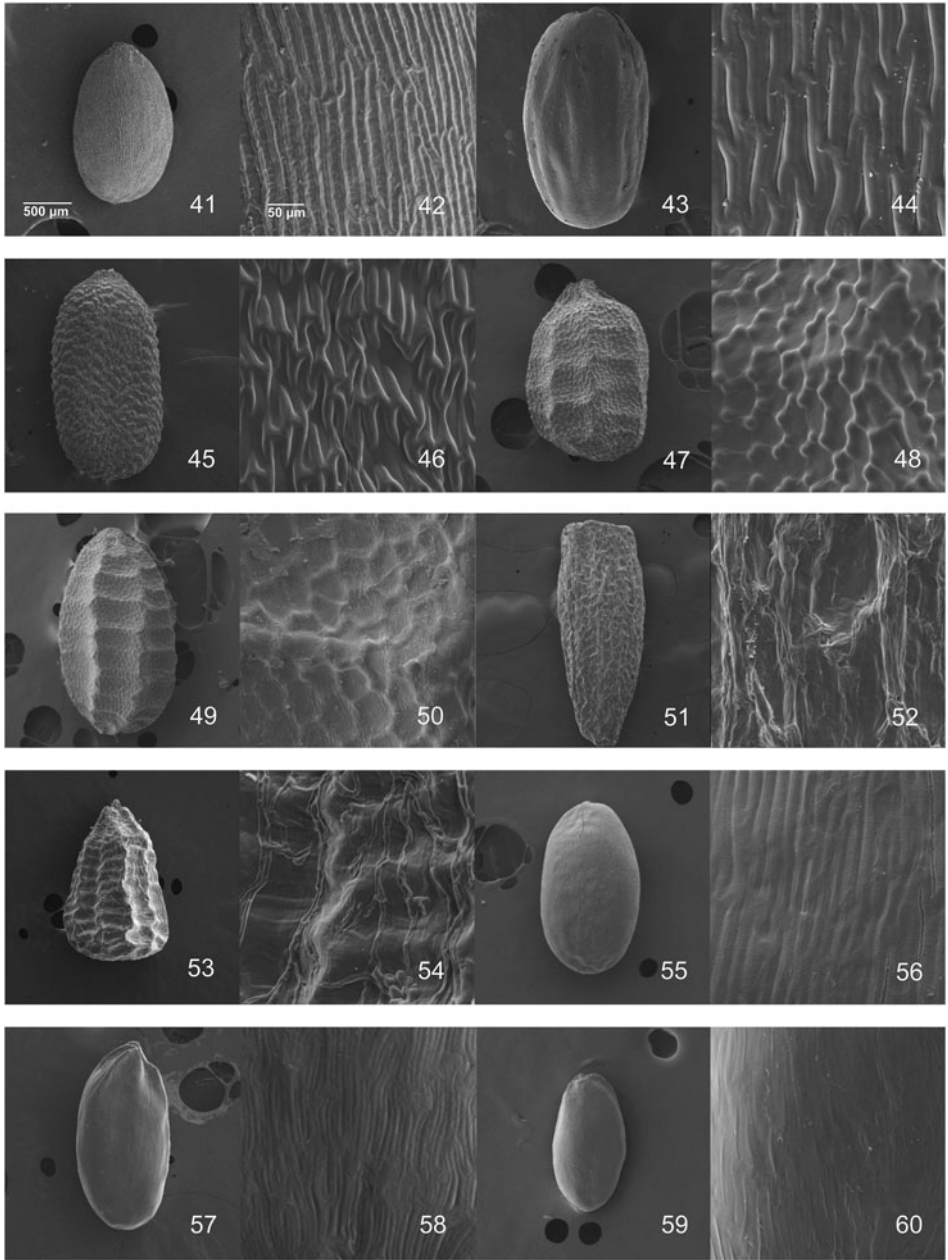
Type 1E seeds are present in *Prismatocarpus crispus* (Figs 45, 46) and resemble the seed coat pattern in *Wahlenbergia huttonii* (Type 1D; Figs 37, 38) in having a smooth reticulum with a linear areole. However, irregularly depressed radial walls create ridges that run more or less perpendicular to the long axis of the seed, giving the seed coat a rugose appearance. Seeds are 0.8 mm long.



FIGS 1–20. Scanning electron micrographs of selected seeds of Campanulaceae. 1 & 2: *Microcodon hispidulus*. 3 & 4: *Prismatocarpus diffusus*. 5 & 6: *P. pedunculatus*. 7 & 8: *Roella ciliata*. 9 & 10: *R. secunda*. 11 & 12: *Theilera guthriei*. 13 & 14: *T. robusta*. 15 & 16: *Wahlenbergia fruticosa*. 17 & 18: *W. neorigida*. 19 & 20: *Craterocapsa montana*. Whole seed: Figs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 (scale bar = 500 µm). Detail of seed coat: Figs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 (scale bar = 50 µm).



FIGS 21–40. Scanning electron micrographs of selected seeds of Campanulaceae. 21 & 22: *Wahlenbergia krebsii*. 23 & 24: *Merciera brevifolia*. 25 & 26: *M. leptoloba*. 27 & 28: *Microcodon glomeratus*. 29 & 30: *W. adpressa*. 31 & 32: *W. subulata*. 33 & 34: *W. parvifolia*. 35 & 36: *W. annularis*. 37 & 38: *W. huttonii*. 39 & 40: *W. procumbens*. Whole seed: Figs 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 (scale bar = 500 µm). Detail of seed coat: Figs 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 (scale bar = 50 µm).



FIGS 41–60. Scanning electron micrographs of selected seeds of Campanulaceae. 41 & 42: *Wahlenbergia paniculata*. 43 & 44: *Rhigiophyllum squarrosum*. 45 & 46: *Prismatocarpus crispus*. 47 & 48: *Treichelia dodii*. 49 & 50: *T. longibracteata*. 51 & 52: *Siphocodon debilis*. 53 & 54: *W. acaulis*. 55 & 56: *W. longifolia*. 57 & 58: *W. ecklonii*. 59 & 60: *W. exilis*. Whole seed: Figs 41, 43, 45, 47, 49, 51, 53, 55, 57, 59 (scale bar = 500 µm). Detail of seed coat: Figs 42, 44, 46, 48, 50, 52, 54, 56, 58, 60 (scale bar = 50 µm).

*Type 1F*

The seeds are 0.6–0.8 mm long with the seed coat surface distinctly reticulate which follows the contour of pronounced longitudinal and transverse ridges. The cells are more or less isodiametrical with a distinct areole and the presence of protrusions from the surface at the junction of the radial and outer tangential walls. This type is present in the species of *Treichelia* Vatke (Figs 47–50).

*Type 1G*

Seeds are 2.1 mm long, characterised by a weakly reticulate surface with the radial walls irregularly elevated, giving the seed a wrinkled appearance. This type is present in *Siphocodon debilis* Schltr. (Figs 51, 52).

*Type 1H*

This type, present in *Wahlenbergia acaulis* E.Mey. (Figs 53, 54), resembles Type 1E in its pronounced longitudinal and transverse ridges. However, the seed coat surface has a very fine reticulate pattern formed by irregularly shaped radial walls superimposed on the ridges. Seeds are generally 0.6 mm long.

***Type 2: Striate or wavy***

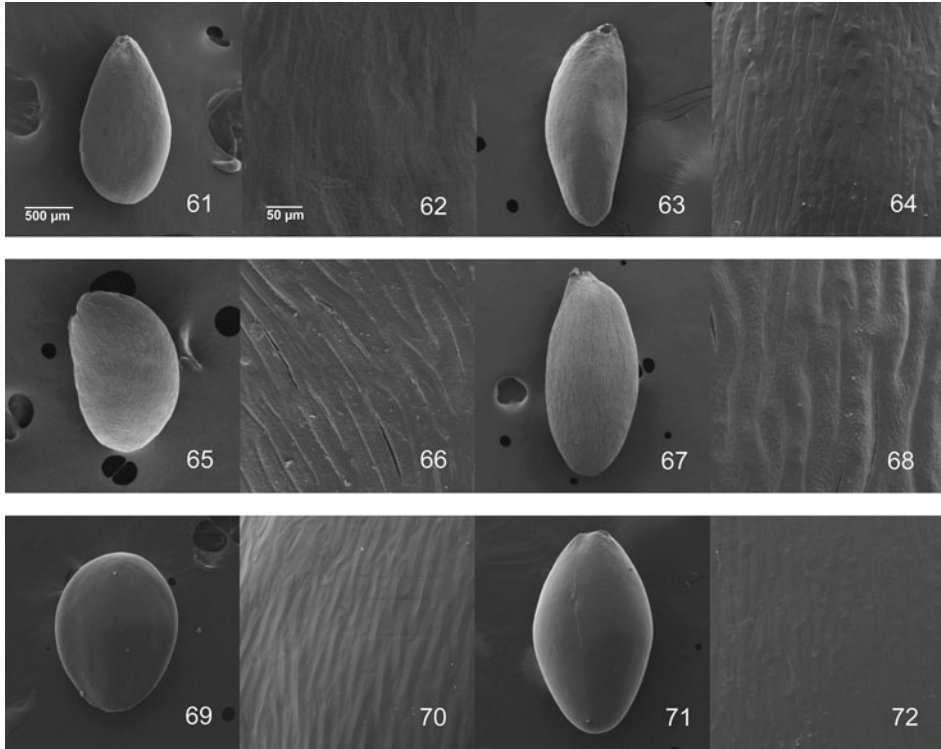
The seeds are 0.4–1.2 mm long and the cell outlines are indiscernible. The seed surface is characterised by longitudinal interrupted grooves or a wavy-striped appearance. Present in *Wahlenbergia longifolia* A.DC., *W. ecklonii* H.Buek, *W. exilis* A.DC., *W. psammophila* Schltr., *W. juncea* (H.Buek) Lammers, *W. polyantha* Lammers, *W. suffruticosa* Cupido, *W. capensis* (L.) A.DC. and *Siphocodon spartioides* Turcz. (Figs 55–72).

***Seed coat sculpturing and ecological and life history patterns***

Among the species sampled, none of the seed coat types or subtypes here described is uniquely associated with any specific ecological factor or life form, although four types are found only in single species (Table 2). However, some general trends can be seen, ranging from uniform to highly diverse patterns.

*Restricted to fynbos*

The majority of fynbos species had seed Type 1A (reticulate). All are woody perennials except *Microcodon hispidulus* (annual), *Prismatocarpus sessilis* Eckl. ex A.DC. (herbaceous perennial) and *Roella muscosa* L.f. (herbaceous perennial). *Microcodon hispidulus* is found on shale, sandstone and granite derived soils (sand,



FIGS 61–72. Scanning electron micrographs of selected seeds of Campanulaceae. 61 & 62: *Wahlenbergia psammophila*. 63 & 64: *W. juncea*. 65 & 66: *W. polyantha*. 67 & 68: *W. suffruticosa*. 69 & 70: *W. capensis*. 71 & 72: *Siphocodon spartioides*. Whole seed: Figs 61, 63, 65, 67, 69, 71 (scale bar = 500 µm). Detail of seed coat: Figs 62, 64, 66, 68, 70, 72 (scale bar = 50 µm).

stony, clay and loam) of mountains and flats, whereas its congener, *M. glomeratus*, has seed Type 1D and prefers sandstone derived soils (sand and stony) of mountains and flats. Among the *Prismatocarpus* species, *P. sessilis* seems to have a wide soil-type tolerance, including peat and loam. *Roella secunda* H.Buek has similar soil requirements to other fynbos *Roella* species, but its distribution extends to aseasonal rainfall areas. The two species of *Theilera* are restricted to aseasonal rainfall areas, but *T. guthriei* (L.Bolus) Phillips has a wider soil-type tolerance than *T. robusta* (A.DC.) Cupido. *Wahlenbergia fruticosa* and *W. neorigida* are among the *Wahlenbergia* species with reticulate seed Type 1A, with the former species occurring in areas with aseasonal rainfall. Two species of *Wahlenbergia* with reticulate Type 1D seeds seem to be confined to purely fynbos vegetation: *Wahlenbergia parvifolia* (herbaceous perennial) and *W. subulata* (woody perennial). The three species of *Merciera* are resprouting, woody perennials and all have reticulate Type 1C seeds. All of them occur on mountain slopes and require sandy, stony soils. *Merciera*

*brevifolia* A.DC. may have a requirement for more moisture since it occurs on clay soils also. *Rhigiophyllum squarrosum* has reticulate Type 1D seeds. It is a fynbos species of stony or rocky hill and mountain slopes with sandy soils. *Siphocodon debilis* (woody perennial) has seeds of reticulate Type 1G whereas its congener, *S. spartioides* (woody perennial), has seeds of Type 2. Both are fynbos species of sandy mountain slopes on loam or peat. *Wahlenbergia acaulis* has reticulate seeds of Type 1H. It too is a fynbos species of mountain slopes and flats, on sand.

*Primarily fynbos but with wider ecological amplitude*

These included species that have wider ecological amplitude and occur in two or more vegetation types including fynbos. Those with Type 1D seeds are the woody perennials *Wahlenbergia adpressa* and *W. polyantha* (both strandveld), the herbaceous perennial *W. procumbens* (renosterveld), and the annuals *W. cernua* (renosterveld) and *W. paniculata* (strandveld). *Prismatocarpus crispus* has seeds of reticulate Type 1E, whereas the two species of *Treichelia* have reticulate Type 1F seeds. All of these taxa are found on sandy slopes of mountains or flats. *Prismatocarpus crispus* and *Treichelia dodii* Cupido also occur on clay soils derived from shale. They may have greater moisture requirements, and therefore also occur in renosterveld. Species of *Wahlenbergia* with Type 2 seeds are the woody perennial *W. longifolia* on sandstone or limestone (extending also to strandveld), the annual *W. capensis* on sands, loams and clays (strandveld and renosterveld), the annual *W. ecklonii* on sandy loam among bare rocks (renosterveld and karoo), and the annual *W. exilis* on sands, loams and clays (renosterveld).

*Montane grasslands*

The two species of *Craterocapsa*, and *Wahlenbergia krebsii*, are plants of montane grasslands. *Craterocapsa* has reticulate seeds of Type 1A while *Wahlenbergia krebsii* has seeds of Type 1B. All three are herbaceous perennials and all require a loamy soil. *Craterocapsa tarsodes* Hilliard & B.L.Burt and *Wahlenbergia krebsii* appear to tolerate clay soils and may require more soil moisture. *Wahlenbergia huttonii* and *W. virgata* are herbaceous perennials with seeds of Type 1D. They prefer grassy mountain slopes in sandy, stony or loamy soils on sandstone, mudstone or dolerite. *Wahlenbergia juncea* is also a herbaceous perennial, and grows in a similar habitat, but has Type 2 seeds. All the species associated with grasslands occur in the summer rainfall areas.

*Strandveld and karoo*

Three *Wahlenbergia* species are unique to these vegetation types. The herbaceous perennial *Wahlenbergia suffruticosa* (strandveld) and the annual *W. psammophila* (strandveld) have Type 2 seeds. The annual *Wahlenbergia annularis* (strandveld and karoo) has seed Type 1D. All these species prefer sandy soil on flats.

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## DISCUSSION AND CONCLUSIONS

The seed coat surface patterns found in the South African Campanulaceae appear to be variations of a reticulate pattern. In its most basic organisation, this reticulum is formed by a raised network of cells. However, by a combination of various degrees of depression of the radial and tangential walls, lateral compression of cells, and the development of secondary wall features, a number of recognisable seed coat patterns are established. These are in essence a transformation series among homologues. The resulting seed surface characters are complex. Although many of the characters appear to be highly adapted for dormancy, dispersal, water-uptake, and so on, the adaptive significance of seed coat sculpturing and ornamentation has been little studied at the autecological level. Shetler & Morin (1986) concluded that the seeds of the Campanulaceae in North America show degrees of specialisation in the seed coat although it was not clear whether these specialisations have evolved in response to ecological or other selective factors, or show patterns found elsewhere in the family. The patterns found in the South African samples are discussed below in relation to the current genera, ecological and life history traits, and phylogenetic groups from molecular data.

### *Distinction of current genera in relation to seed types*

Among the 10 genera, species of *Merciera* and *Treichelia* are uniform in seed type – Types 1C and 1G, respectively – with subtle variation between individual species. The remaining seed types are species specific or include species from more than one genus. Type 1A seed, corresponding to Type A in Murata (1992), is reportedly only found in the Lobelioideae. However, it is also characteristic of the Campanuloideae genera *Craterocapsa*, *Theilera*, *Roella*, *Prismatocarpus* (except *P. crispus*), and one *Microcodon* and two *Wahlenbergia* species. The species of *Wahlenbergia* are most diverse in seed types, ranging from coarsely reticulate to almost smooth in surface features. The unique seed type of *Wahlenbergia acaulis* is associated with a mode of capsule dehiscence, via protruding folds, found nowhere else in the genus. A similar association between seed type and capsule dehiscence is found in *Prismatocarpus crispus*. This species differs from the usual longitudinally dehiscent capsule in *Prismatocarpus* in having what appears to be an indehiscent capsule. The two species of *Siphocodon* have distinctly different seed types to each other although, together with *Rhigiophyllum squarrosom*, they share unique pollen morphology (Eddie *et al.*, 2010). *Rhigiophyllum squarrosom* has a similar seed surface morphology to *Microcodon glomeratus* and species of *Wahlenbergia*, some of which were previously classified in *Lightfootia* L'Hér., nom. illeg.

### *Ecology and life history in relation to seed types*

It has been suggested that the remarkably diverse South African landscape, with its associated climatic conditions and soil types, has created numerous niches that have



in turn led to high species diversity. These factors partly explain the high level of endemism and species diversity of the Cape flora (Goldblatt, 1978; Linder, 2003). Elsewhere, Givnish *et al.* (1995) found a striking correlation between niche and phenotypic diversification within genera for the Hawaiian lobelioids. They found that species occupying habitats such as sea-cliffs, wet forest and mesic forest have altered growth forms.

Although most of the herbarium specimens used in our study generally lacked detailed ecological data, the broad categories used permit us to see general trends in relation to seed types. Species with Type 1A seeds have a more restricted ecological tolerance, whereas those with seed Types 1D and 2 have wider tolerance and are also likely to include diverse life forms. In the North American study, Shetler & Morin (1986) suggested that annual species have seed surface features comparable to those present in Type 1D, which perhaps have selective advantages in dry or disturbed habitats by reducing water loss. Our study offers little evidence that seed types are closely correlated to unique ecological factors present in the vegetation types, such as the regular occurrence of fire, nutrient-poor soils, winter or summer rainfall season, soil type or even life form. Typically in South Africa, annuals occur in the winter-wet south Western Cape, where the seed germinates during this wet period and survives the dry summer in the soil. In the same area, the perennial species flower during the dry summer months, with their seeds maturing on the plant through most of the hot dry conditions and then being released during the cooler autumn months. As with the ecological factors, our study does not show a correlation between life form and seed type despite these different strategies. Instead the seeds have an equal tolerance to different pH levels in soils derived from different bedrock types, or equal tolerance to the higher moisture levels in renosterveld or lower moisture levels in fynbos.

#### *Phylogenetic patterns in relation to seed types*

In recent phylogenetic studies of the South African Campanulaceae using DNA sequence data (Cupido, 2009), the 50% majority rule consensus tree inferred from the Bayesian analysis reveals that *Wahlenbergia* is paraphyletic and forms three major clades (A, B and C; see Fig. 73) with one minor outlier (*W. krebsii*). Reticulate seed (Type 1) is distributed in all three clades and occurs in all genera. Type 1A occurs predominantly in clade C comprising *Craterocapsa*, *Theilera* and *Wahlenbergia* (the majority of the species in this clade being formerly treated as *Lightfootia*, nom. illeg.), and also in clade A comprising *Roella*, *Prismatocarpus* and *Merciera*. No genus is characterised by possession of striate or wavy seed (Type 2). This seed type occurs in clade C (*Wahlenbergia ecklonii*, *W. longifolia*, *W. exilis*) and clade B (*W. capensis*), but does not occur in clade A. Eddie (1997) concluded that the distribution of character states in the seeds of the Campanulaceae is reticulate (tokogenetic) across taxa, making cladistic analysis difficult. Nevertheless, from a phylogenetic perspective it would appear that a transition series of testa surface relief can be distinguished. The reticulate pattern appears to be the ancestral

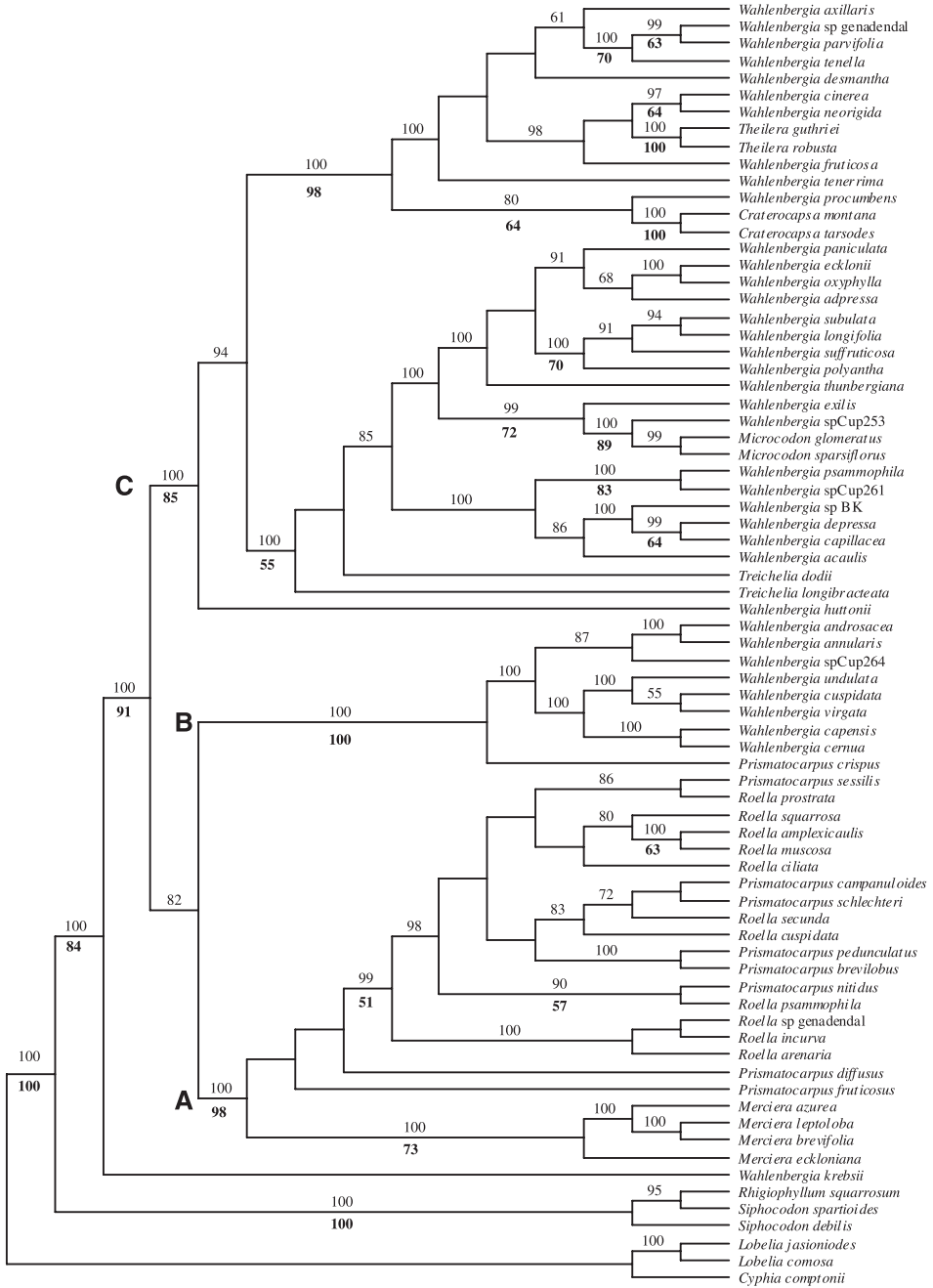


FIG. 73. Fifty per cent majority rule consensus of trees retained in the Bayesian analysis of the combined *trnL-F* and ITS data sets for 72 taxa of the Campanulaceae and three Lobeliaceae (outgroup). Numbers above branches indicate posterior probability values expressed as percentages. Bootstrap values  $\geq 50\%$  are indicated below the branches.

condition whereas striated arrangements are more derived. The general picture that is emerging is of the reticulate seed (Type 1) being found in all genera, with subtype 1A being the most ancestral and predominant in the majority of fynbos taxa. There is a departure from the ancestral reticulate type towards a number of reticulate subtypes until we reach the derived striated or wavy type (Type 2). This conclusion would accord with what is known of the gradual aridification of the South African climate since the Oligocene and the subsequent proliferation of mosaic speciation across a diversity of soil types and microclimatic conditions associated with the vicissitudes of the mid-late Tertiary. Many of the unique wahlenbergioid genera of the 'fynbos' vegetation may be relictual palaeoendemic elements of a formerly more widespread afro-montane flora. This sclerophyllous heath vegetation is not restricted to the Cape but occurs elsewhere in southern Africa, in Pondoland and the Drakensberg, and throughout sub-Saharan Africa, Madagascar and the Mascarene Islands, in cool, montane environments where rainfall is high and soils are highly leached (Linder, 1990; Carbutt & Edwards, 2001).

The molecular studies further revealed five assemblages, each comprising all, or some, of the species of each genus (Fig. 73): 1. *Wahlenbergia*–*Theilera*–*Microcodon*–*Craterocapsa*–*Treichelia* (clade A); 2. *Wahlenbergia*–*Prismatocarpus crispus* (clade B); 3. *Roella*–*Prismatocarpus*–*Merciera* (clade A); 4. *Wahlenbergia krebsii*; 5. *Rhigiophyllum*–*Siphocodon*. *Wahlenbergia* resolved as paraphyletic, and except for *W. krebsii* none of the lineages that involve *Wahlenbergia* species can be correlated with any unique seed type. The isolated position of *Wahlenbergia krebsii*, already reported by Thulin (1975), is supported by its possession of Type 1B seeds while all the other *Wahlenbergia* lineages possess diverse seed-type patterns. The inclusion of *Wahlenbergia krebsii* in *Wahlenbergia* requires further investigation. The common possession of Type 1A seeds supports the close relationship between *Theilera*, *Craterocapsa*, *Wahlenbergia fruticosa* and *W. neorigida*, but the sharing of this seed type with *Prismatocarpus* and *Roella* is surprising. The species of *Prismatocarpus* and *Roella* appear in a thoroughly mixed clade which is sister to *Merciera*, the entire clade being well supported. The monophyly of *Merciera* revealed by the molecular data is supported morphologically by Type 1C seeds.

In *Prismatocarpus*, *Siphocodon* and *Microcodon* two distinct seed types are evident. The placement of *Prismatocarpus crispus* is corroborated by increasing morphological evidence, including its unique seed type, suggesting the re-classification of this species or even the formation of a new genus. Seed-type patterns do not support the close relationship between *Siphocodon* and *Rhigiophyllum* as revealed by molecular and pollen data, but rather the uniqueness of the individual species. Similarly, the phylogenetic pattern found for the two sampled species of *Microcodon* contradicts their species-specific seed-type pattern.

Seed coat morphology in the South African wahlenbergioids is of limited use as a diagnostic character to circumscribe genera or as a synapomorphy to define clades. It does, however, appear to be useful to distinguish species, a conclusion also arrived at by Haridasan & Mukherjee (1988) for Indian Campanulaceae and by Eddie (1984,

1997) for all three main lineages of the family. Differences in seed coat sculpturing patterns are not correlated with specialised ecological requirements or life history traits, but instead indicate adaptability for survival in a set of diverse environmental conditions.

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