Species delimitation and speciation process in the *Seriphium plumosum* L. complex (Gnaphalieae: Asteraceae) in South Africa

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Abstract

The remarkable richness of the Cape Floristic Region (CFR) and the high *in situ* diversification inferred for the region prompt interest in two key areas: first, to what extent has the true species richness of the Cape been discovered and described, and second, what are the key drivers of speciation? Steady efforts in taxonomy dating back to the early 17th century have led some to estimate that over 99% of species in the Cape flora have already been described. However, taxonomic research in the Cape has, as elsewhere, relied on morphology for delimiting species, implying that undiscovered species diversity among cryptic taxa may be substantial. Early ideas regarding the drivers of diversification in the Cape flora emphasised climatically-induced vicariant speciation. Since that time, both vicariance and ecological speciation have been invoked as drivers of diversification. However, the relative contributions of either of these modes to the richness of the flora remains unclear.

The present work focuses on *Seriphium plumosum*, a species complex in the daisy tribe Gnaphalieae with a recent evolutionary origin and a core distribution in the Cape Floristic Region. The species' problematic taxonomic history, its substantial morphological and ecological variability, as well as its large geographic distribution in southern Africa suggest that the current concept of the species houses multiple independent evolutionary species. Species limits within the complex are re-evaluated using the Bayes Factor Delimitation method of Leaché et al. (2014) within an integrative taxonomic approach, incorporating evidence from next-generation DNA sequence data, previous taxonomic treatments, morphology, ecology and geographic distribution. The drivers of lineage divergence are then investigated at the population level within selected species in the group, with a focus on neutral and adaptive processes, and the spatial contexts within which each of these processes is thought to operate.

The evidence presented here provides support for ten or eleven independent evolutionary species housed within the current concept of *S. plumosum.* The species currently considered sibling to the complex, *Seriphium cinereum,* is strongly supported as embedded within the complex, which I thus term the *S. plumosum* clade. In order to better facilitate identification of the species, several of the evolutionary species uncovered are consolidated. As such, a total of nine species are described in the taxonomic treatment of the clade, each of which is morphologically and ecologically cohesive, and five of which qualify as biological species *sensu* Mayr (1969). These include *S. plumosum*[1] and *S. cinereum* under new circumscriptions, four new species; *Seriphium alto-argillaceum*, *S. burrowsii, S. crypticum* and *S. dunensis,* and three species housed within the current concept of *S. plumosum* and formerly treated within *Stoebe; Seriphium burchellii* Levyns*, S. vulgaris* Levyns, and *S. virgatum* Thunb*.* At the population level within selected species in the *S. plumosum* clade, neutral processes and weak divergent selection within a spatially discontinuous context are supported as the key driver of differentiation. These findings suggest that phylogenetic niche conservatism and/or the limited dispersal abilities characteristic of many Cape clades have been important in diversification by neutral and adaptive processes in the region. If extrapolatable to other Cape lineages, this work implies that undiscovered species diversity in the Cape may considerably exceed recent estimates, and that the role of non-adaptive processes in generating species diversity may be considerably underestimated.

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[1] Note that the taxonomy presented in this dissertation is strictly provisional, and does not constitute a formal taxonomic revision of either *S. cinereum* or the *S. plumosum* complex.

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Chapter 1: Introduction

The Cape Floristic Region (CFR; Figure 1) is one of the world's most botanically diverse regions (Goldblatt & Manning, 2002; Manning & Goldblatt, 2012) and has been regarded as one of the world's six floral kingdoms (Takhtajan, 1969; Good, 1974). The region spans 90 000km^2, less than 4% of the total area of the southern African subcontinent, but harbours an estimated 9 383 vascular plant species (Goldblatt, 1978, 1997; Goldblatt & Manning, 2000, 2002; Manning & Goldblatt, 2012), making it substantially richer than other Mediterranean-type ecosystems (Cowling et al., 1992; Linder, 2005). The Cape flora is also highly distinct, being unusual in terms of its familial and generic composition (Goldblatt, 1997; Goldblatt & Manning, 2002). Although the first- and second- largest families in the flora, Asteraceae and Fabaceae, are species-rich in most parts of the world (Goldblatt & Manning, 2000), the next-largest families Iridaceae, Aizoaceae, Ericaceae, Scrophulariaceae, Proteaceae, and Restionaceae (Goldblatt & Manning, 2000) are poorly represented outside the CFR, the single exception being Australia, where Restionaceae and Proteaceae are also well-represented (Goldblatt & Manning, 2000, 2002). Ericaceae, Restionaceae and Proteaceae are particularly important components of the fynbos vegetation, the predominant vegetation type of the CFR, in which they are universally present (Cowling et al., 1997) and ecologically dominant (Linder, 2005). One of the defining features of the CFR is, therefore, its high level of regional endemism (Oliver et al., 1983; Goldblatt & Manning, 2000), this being reflected at the specific, generic and, to a lesser extent, family level (Oliver et al., 1983). Overall, the region is home to 957 currently-recognised genera, 20.7% of which are endemic (Goldblatt, 1978), and of the total species number, 69% are endemic (Goldblatt & Manning, 2000). The spatial distribution of this endemism, as with floral species richness (Cowling & Holmes, 1992) is concentrated in the southwestern CFR (Linder, 2003). Most endemics in the flora are recently-evolved species ("neoendemics" *sensu* Stebbins & Major, 1965) which have highly localised geographic ranges (Latimer et al., 2005) and associate with specific ecological environments (Cowling et al., 1997; Verboom et al., 2009).

The remarkable floristic diversity of the CFR, half of which can be traced to radiation events within 33 clades (Linder, 2003), and the high levels of floristic endemism, especially neoendemism at the specific and generic levels, is suggestive of considerable *in situ* speciation (Cowling et al., 1997; Warren & Hawkins, 2006; Galley et al., 2007). Naturally we next inquire: what was the dominant tempo and mode of speciation in the CFR? The most frequently invoked hypothesis regarding the tempo of speciation is that major, rapid speciation (radiation) commenced more or less simultaneously across the characteristic Cape clades in the Late Miocene (11.3 - 5.3Ma) associated with departure from the more mesic and aseasonal climate of the early- and middle-Miocene, and the onset of a strongly-seasonal, largely summer-dry, winter-rainfall climate (Adamson, 1958; Levyns, 1964; Goldblatt, 1978; Richardson et al., 2001; Linder, 2003; Verboom et al., 2009; Dupont et al., 2011; Warren et al., 2011; Verboom et al., 2015; Bouchenak-Khelladi & Linder, 2017). This trend toward an increasingly Mediterranean-type climate is thought to have resulted in the fragmentation of species ranges and subsequent rampant vicariant speciation. According to this view, speciation in the CFR has been rapid, geologically recent and largely allopatric (Cowling et al., 1992; Stock et al., 1992). This model is tied to Wiens' (2004a, 2004b) concept of ecological niche conservatism, which postulates that the tendency for lineages to retain similar ecological niches over evolutionary timescales first promotes and then maintains population isolation in the context of environmental change. Examples for this vicariance-based model of diversification include *Protea* section Exsertae (Proteaceae; Prunier & Holsinger, 2010) and *Tetraria* (Cyperaceae; Britton et al., 2014).

Ecological speciation (*sensu* Rundle & Nosil, 2005) emphasises factors driving adaptative divergence rather than those promoting vicariance (Linder, 1985, 2005). Here, new species arise in sympatry or parapatry as a result of the interplay between adaptation to their environment and reduced gene flow (Rundle & Nosil, 2005; Ellis et al., 2014). Under the "ecological speciation" model, contemporary species richness in the CFR is seen as the product of recent and ancient adaptive divergence driven, *inter alia*, by topographic, edaphic, and climatic heterogeneity, pollinator specialisation, and fire (Bolus, 1886; Goldblatt, 1978, 1997; Goldblatt & Manning, 2002; Linder, 1985; Linder & Vlok, 1991; Savolainen & Forest, 2005; Barraclough, 2006). Unlike the vicariant speciation model, the ecological model views early- and middle-Miocene climate change in the CFR as having induced widespread extinction in the existing mesic-adapted flora, which in turn precipitated the adaptive diversification of lineages pre-adapted to summer aridity into newly vacated niches (Verboom et al., 2009). Evidence from broadly divergent Cape plant lineages implies that both ecological and vicariant modes of speciation have been important in generating the diversity of the region, but a general consensus on the dominant mode appears far from being reached (Linder, 2005).

The exceptional diversity of the CFR resides not so much at the local (alpha diversity) scale, as it does at the among-site (beta diversity) level (Cowling et al., 1992; Goldblatt, 1997; Latimer et al., 2005). Beta diversity, or geographic species turnover along habitat or ecological gradients (Goldblatt & Manning, 2002), is exceptionally high in the region, and is characterised by the spatial replacement of individual species by closely-related, ecologically similar species (Linder, 1985; Richardson et al., 2001; Linder, 2005). As an example, among 188 sister-species pairs in eight Cape clades (in Orchidaceae, Brassicaceae, Polygalaceae, Geraniaceae, Poaceae, Fabaceae, and Restionaceae), van der Niet and Johnson (2009) found that 90% were either allopatric or showed some range exclusivity, while just 10% had completely overlapping distributional ranges, and similar patterns are found in the species-rich lineages of the Cape Proteaceae and Ericaceae (Linder, 2003, 2005). A natural corollary of exceptionally high species turnover in a relatively small area like the CFR is that species invariably have narrow ranges, and this is precisely what has been observed; most CFR species are highly range-restricted (Goldblatt, 1978; Latimer et al., 2005). It follows that only a small proportion of Cape species have widespread distributions that encompass a wide ecological spectrum, and among these, taxonomic revisions frequently reveal the existence of cryptic species; distinct species embedded in and classified as a single species. For example, recent studies employing morphological and traditional Sanger-sequencing of multiple DNA loci have revealed three distinct species with non-overlapping geographical ranges in the *Erica abietina* clade (Ericaceae; Pirie et al., 2017), and four or five distinct species in *Tetraria triangularis* (Cyperaceae; Britton et al., 2014). Likewise, multilocus SNP data revealed distinct genetic clusters exist in the widespread CFR species *Protea repens* (Proteaceae; Prunier et al., 2017) and microsatellite data revealed similar distinct genetic clusters in *Protea mundii* (Proteaceae; Prunier & Holsinger, 2010). In both *Protea* studies, these clusters segregate spatially and potentially represent evolutionary species. These instances of potential spatially segregated, cryptic species suggest that floristic species-level diversity in the Cape may at present be underestimated.

The daisy family Asteraceae (or Compositae) is the largest family of flowering plants and the most species-rich family in the CFR. Of the total number of vascular plant species in the region, 11.5%, or 1077 species, are daisies (Manning & Goldblatt, 2012). The Asteraceae have an estimated origin in the late Cretaceous (Barreda et al., 2015), although many authors argue for a younger age (Funk et al., 2009; Panero et al., 2014) making it an excellent system for the study of recent plant radiation (Panero & Funk, 2008). The “paper daisy” tribe Gnaphalieae is one of the largest in the Asteraceae, and has a worldwide distribution, but is particularly well-represented in southern Africa (Bremer, 1995). Biogeographic and molecular dating analyses unequivocally point to a southern African origin of the tribe in the late Eocene to Oligocene (34.5 Ma [20.6-52.3]; Bremer, 1995; Bergh & Linder, 2009; Nie et al., 2015). One of the Cape-centred clades within the Gnaphalieae is the *Stoebe-Ifloga-Metalasia* (SIM) clade first defined in Bergh et al. (2015), a clade which appears to have originated in the early Miocene (Bergh & Linder, 2009) and to have dispersed fairly recently and rapidly from out of the Cape (N.G. Bergh, unpubl.). The *Stoebe* lineage within the SIM clade houses several genera, including *Seriphium*, recently resurrected out of *Stoebe* by Koekemoer (2016). As currently circumscribed by Koekemoer (2016), *Seriphium* comprises nine species of fine-leaved shrubs, four of which are confined to the CFR, four have mostly narrow geographic distributions in tropical East

Africa, Madagascar or the Mascarene Islands, and one, *S. plumosum*, is widespread throughout southern Africa, where its habitats encompass winter- and summer-precipitation regimes; coastal, inland and montane habitats; and a range of recruitment strategies.

Seriphium plumosum L., commonly called "slangbos", was first described by Linnaeus in 1753 as one of three species in the genus *Seriphium* L. (type = *S. cinereum* L.) Subsequent taxonomists described additional species in the genus and united *Seriphium, Perotriche* Cass., and *Stoebe* L. under *Stoebe*, such that by 1937, the latter contained 34 species, including the entity formerly known as *Seriphium plumosum* L. (Levyns, 1937). In the most recent revision of this assemblage, however, Koekemoer (2016) revived *Seriphium* and placed in it *S. spirale, S. incanum, S. cinereum*, *S. cryptophyllum, S. saxatilis, S. kilimandscharica, S. pachycladum, S. passerinoides* and *S. plumosum*. Her treatment united three species; *Stoebe plumosa* L. (=*Seriphium plumosum* L.), *Stoebe vulgaris* Levyns, and *Stoebe burchellii* Levyns under the synonym *Seriphium plumosum* L. Koekemoer (2002) did, however, acknowledge that two or more good species may be housed within her concept of *S. plumosum*, although she deferred describing these as independent species pending more detailed investigation of *S. plumosum*. Phylogenetic analysis incorporating representatives of all of Levyns' (1937) species, except *S. burchellii* (N.G. Bergh, unpubl.; Figure 2) strongly support the monophyly of *Stoebe plumosa*, *Stoebe vulgaris* and *Stoebe cinereum* (as defined by Levyns, 1937), and indicates that *S. plumosa* and *S. vulgaris* are sisters and in turn form the sister group to *S. cinerea.* The node indicating the sister group to these three species is unresolved in this analysis, but could be either a small clade comprising the African members of *Seriphium* (*S. kilimandscharica, S. passerinoides* and *S. cryptophylla*), or the Cape species *S. alopecuroides.*

As currently circumscribed (i.e. *sensu* Koekemoer, 2016), *S. plumosum* has a geographic distribution that includes most of the CFR and much of eastern South Africa, the high mountains of Namibia and Angola, and parts of Zimbabwe and Mozambique (Figure 1). The distribution of *S. plumosum* both within and outside the CFR is unusual among its congeners and among Cape-centred species, which typically are locally distributed with a concentration of richness in the extreme southwestern CFR (Levyns, 1952; Linder, 2003). The species has the largest range size of all other species in *Seriphium* and the *Stoebe* clade, and one of the largest among the species that comprise the *Stoebe-Ifloga-Metalasia* (SIM) clade (Koekemoer, 2016). The ecological variation displayed by *S. plumosum* exceeds all other species in the *Stoebe* clade (*sensu* Bergh & Linder, 2009), spanning winter-, summer-, and aseasonal-rainfall regions and multiple biomes and vegetation types. The species' range also covers multiple soil types in the eastern part of South Africa, and sandstone-, limestone-, quartzite-, and shale-derived soils in the CFR (Cowling & Holmes, 1992; Cramer et al., 2014). Moreover, where *S. plumosum* is killed by fire and is perpetuated by seedlings in the western part of its range, in the east, it regenerates after fire both by resprouting from dormant buds at the base of the stems and via seedling recruitment (Cohen, 1940; Levyns, 1937). In the western part of the species' range, the seeds remain dormant for a year before germinating, while in the eastern part of the range, the seeds are capable of germinating immediately after being shed (Levyns, 1937; Cohen, 1940). *Stoebe vulgaris* Levyns, an eastern species currently included by Koekemoer (2016) in *S. plumosum,* has been proclaimed an encroacher plant in South African rangeland (Snyman, 2009) under the CARA legislation (Regulation 16 of the Conservation of Agriculture Resources Act 43; Jordaan & Jordaan, 2007). As a whole, the species complex has a ten-month-long bimodal flowering season, which exceeds that of all other species in the *Stoebe* clade (Levyns, 1937; Koekemoer, 2016).

Seriphium plumosum also displays considerable morphological variation across its geographic distribution (Figure 3), so much so that Levyns (1935) called the "slangbos" complex (then including *Stoebe cinerea*) a "heterogenous collection of plants", and contemplated the true number of good species in the complex then treated as synonyms under *S. cinerea*. Koekemoer (2016) later referred to her concept of the *Seriphium plumosum* complex as an "extremely variable taxon" without any well-defined groups. At present, *S. plumosum* follows Koekemoer's (2016) concept and is described as a perennial, much-branched woody shrub with subulate, ericoid leaves arranged in fascicles. Each plant bears several hundred capitula arranged in spicate or paniculate terminal synflorescences, and each capitulum contains a single, bisexual floret surrounded by several rows of involucral bracts (Levyns, 1937; Koekemoer, 2016). These characters apply to the assemblage of plants currently referred to as *S. plumosum*, but many other characters including leaf shape, size and orientation, branching angle, synflorescence arrangement, plant colour, and plant height are highly variable, such that the degree of morphological difference between conspecifics in the complex often parallels the degree of difference observed between other currently-recognised species in the genus.

Taken together, the broad distribution, and morphological and ecological variability of *S. plumosum* all point to the presence of multiple species in the complex. The independent species potentially housed within *S. plumosum* have, however, proven difficult to resolve using these traditional sources of evidence. Traditional taxonomic approaches could also underestimate diversity in the complex if this diversity is cryptic or semi-cryptic, and/or or produce groupings inconsistent with genealogy. An exciting solution to these problems is the application of DNA sequence data to infer the evolutionary relationships between putative species in the complex. The application of DNA data to plant systematics questions dates back to the 1980s (Mishler, 2014), with the evolutionary relationships between sets of a few closely-related species initially reconstructed using single DNA markers (Lischer et al., 2013; Rannala, 2015). A key assumption of this approach is that evolutionary relationships between copies of genes ("gene trees") approximate the evolutionary relationships between species (the "species tree", or phylogeny) (Doyle, 1992; Degnan & Rosenberg, 2009; Edwards, 2009), which, owing to factors such as introgression, incomplete lineage sorting, gene duplication and loss (Doyle, 1992; Chifman & Kubatko, 2014) and branch length heterogeneity (Edwards, 2009), is not always valid. An alternative approach is the use of multi-locus data to assess evolutionary relationships between species using information from across the genome (Lischer et al. 2013). In recent years, Single Nucleotide Polymorphisms (SNPs), single base-pair differences sampled from across the genome, are increasingly being used as a multi-locus marker for resolving phylogenetic problems and investigating population demography (Edwards, 2009; Leaché & Fujita, 2010; Bryant et al., 2012; Leaché et al., 2014; Leaché et al., 2015; Leaché & Oaks, 2017). Between several thousand and several hundred thousand SNPs are typically sequenced and identified via restriction site-associated DNA sequencing (RADseq) and genotyping-by-sequencing (GBS), substantially more than equivalent microsatellite DNA analyses (Davey et al., 2011; Gruber et al., 2017). Apart from the overall high information content, SNPs can be obtained for non-model species, are easily obtained at low-cost (per marker), and are scalable to large population sizes (Rubin et al., 2012; Leaché & Oaks, 2017).

Recent studies have used distributional, morphological, ecological and different types of multi-locus sequence data to assess species limits (Britton et al., 2014; Pirie et al., 2017) and population-level genetic differentiation (Prunier & Holsinger, 2010; Rymer et al., 2010; Lexer et al., 2014; Prunier et al., 2014) in Cape plant taxa. The present work seeks to examine the evolutionary status of the observed diversity within *Seriphium plumosum* complex, and to investigate the spatial components of the evolutionary variation in the complex. In Chapter 2, I use morphometrics, ecology, previous taxonomic treatments and genome-wide SNP data to propose different taxonomic schemes within the complex, and then use a Bayesian approach to distinguish which of these schemes is best supported under the general lineage concept of species (de Queiroz, 1998). The use of both morphology, ecology and genome data results in an integrative taxonomic approach, as advocated by Dayrat (2005) and Fujita et al. (2012). Chapter 3 presents a formal taxonomic treatment of species in the *S. plumosum* complex based on the findings presented in Chapter 2. Finally, Chapter 4 expands on the current understanding of the role of ecological versus non-ecological speciation modes in the CFR by assessing the relative contributions of topography, geography and ecology to population-level genetic differentiation in the *S. plumosum* complex.

Figures

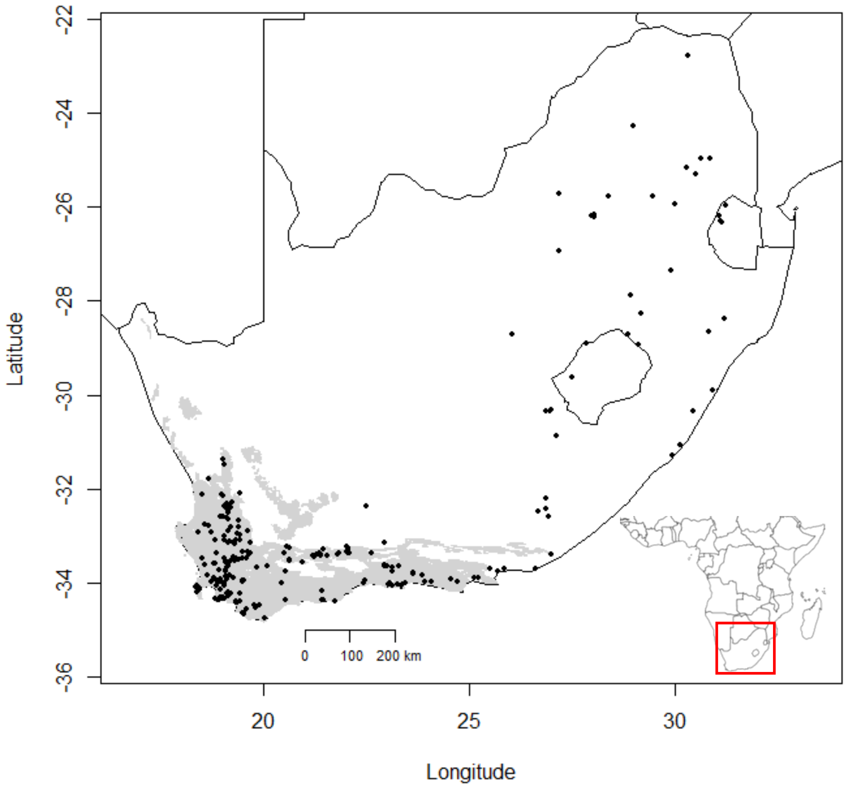

Figure 1: Map of South Africa showing the extent of the Cape Floristic Region (shaded grey) after Mucina and Rutherford (2006) and the known distribution of *Seriphium plumosum* L. in South Africa, Lesotho and Swaziland (black dots) based on herbarium specimens housed in BOL and NBG.

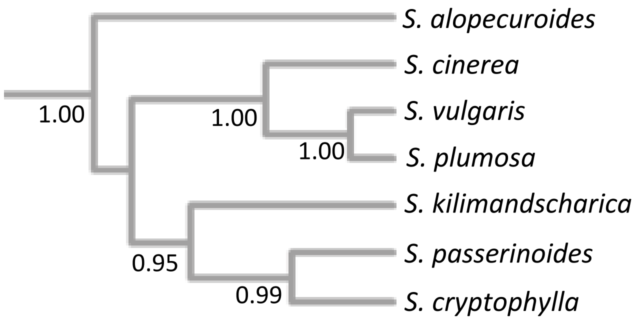

Figure 2: Part of Beast maximum clade credibility tree based on plastid spacer (psbA-trnF; ycf6-psbM), ETS and ITS sequences (N.G. Bergh, unpubl.) showing inferred relationships within the *S. plumosum* complex and closely-related species. Taxonomy according to Levyns (1937). Within the *S. plumosum* complex, *Stoebe burchellii* Levyns was not sampled. Numbers below nodes represent Bayesian posterior probabilities.

Figure 3: Morphological variation in the *Seriphium plumosum* complex. (a) *S. plumosum* on shale-derived soil on Matroosberg at 1926m elevation; (b) *S. plumosum* on quartzite-derived soil on Matroosberg at 1973m elevation; (c) *S. plumosum* (formerly *St*. *burchellii*; left) and *S. plumosum* (formerly *St. plumosa*; right) on the north side of the Swartberg Pass at 1382m elevation; (d) a colony of *S. plumosum* plants at Diaz Cross Memorial Beach, near the mouth of the Bushman's River at 12m elevation; (e) *S. plumosum* (formerly *St. vulgaris*) at the roadside between Zastron and Rouxville at 1685m elevation; (f) Invasion of *S. plumosum* (formerly *St. vulgaris*) on heavily grazed grassland (Photographs courtesy of N.G. Bergh and F. Boucher).

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Chapter 2: Integrated investigation of species limits in the *Seriphium plumosum* L. complex (Gnaphalieae: Asteraceae)

Introduction

The way species are conceptualised and discovered has dramatically changed with the recognition of species as evolutionary rather than typological entities (Darwin, 1859), the development of phylogenetic systematics, "tree thinking" (O'Hara, 1998; Mishler, 2014) and coalescent theory (Kingman, 1982; Donnelly & Tavaré, 1995), and the recent development of next-generation DNA sequencing and analytical species delimitation methods (Heled & Drummond, 2010; Yang & Rannala, 2010; Ence & Carstens, 2011; Grummer et al., 2014; Leaché et al., 2014; Jones et al., 2015; Yang, 2015). Nevertheless, the operational criteria applied by alpha taxonomists in the Cape Floristic Region (CFR) since the advent of taxonomic effort and exploration in the region in the early 17th century (Victor et al., 2016), as elsewhere (Rouhan & Gaudeul, 2014), remains largely morphology-based, sometimes with consideration of ecology, as noted by Treurnicht et al. (2017).

Operational concepts including the morphological, ecological and biological species concepts, employ specific criteria which must be met before putative species can attain species rank in the Linnaean hierarchy (Carstens et al., 2013). Each of these concepts makes the discovery of 'segments of population lineages' (Darwin, 1859) contingent on diverging lineages having acquired specific properties (de Queiroz, 2007), which may arise at different rates and in different order (de Queiroz, 1998) along the speciation continuum. This renders the decision about the species status of a particular taxon dependent on the particular operational concept employed by the researcher (de Queiroz, 2007). While operational criteria *are* essential for species delimitation, they are only useful as evidence of underlying lineage separation (de Queiroz, 2007). Over-emphasis on operational criteria, and particularly on a single criterion, can lead to taxonomies biased by easily-observed operational criteria. For example, the widespread application of the morphological species concept in the CFR flora (Smith et al., 2008; Treurnicht et al., 2017) results in the recognition only of entities with fixed, usually easily observable phenotypic characters, despite the evidence that non-adaptive and adaptive processes frequently produce cryptic or semi-cryptic species with weak phenotypic divergence but strong genetic divergence (Schönrogge et al., 2002; Linder, 2005; Bickford et al., 2007; Britton et al., 2014; Ellis et al., 2014; Verboom et al., 2015; Fišer et al., 2018). Moreover, even if species do exhibit phenotypic divergence, there is no guarantee that the particular morphological characters used in their delimitation reflect causal synapomorphic differences rather than homoplasies.

The evolutionary species concept (ESC; Simpson, 1961; Wiley, 1978) and generalised lineage concept (GLC; de Queiroz, 1992, 1998, 2005, 2007, 2011), which are essentially synonymous (Wiley & Lieberman, 2011), have directly influenced the development of analytical approaches to species delimitation based on genomic data. Analytical delimitation methods evaluate the evidence for hypothesised evolutionary species (Knowles & Carstens, 2007; Rannala, 2015) by detecting the point at which gene flow between lineages ceases at very shallow timescales (Fujita et al., 2012). These methods used multilocus DNA sequence data within a coalescent framework (Kingman, 1982; Donnelly & Tavaré, 1995) and its extension, the multispecies coalescent, which provides a framework for assessing the probabilities that gene trees associated with a set of unlinked loci have branching patterns different from the species tree topology (Degnan & Salter, 2005; Bryant et al., 2012; Degnan & Rosenberg, 2009). Existing coalescent delimitation methods include Brownie (O'Meara et al., 2006), Bayesian Phylogenetics and Phylogeography (BP&P; Yang & Rannala, 2010), SpeDeSTEM (Ence & Carstens, 2011) and DISSECT (Jones et al., 2015). One of the more recently-developed methods, the Bayes Factor Delimitation (BFD) method of Leaché et al. (2014) operates by assessing the statistical support for the observed data (*m* unlinked biallelic markers sequenced across *n* individuals) given a particular species delimitation hypothesis (Leaché et al., 2014), while simultaneously inferring the species tree using the SNAPP method of Bryant et al. (2012). This is a significant advantage over other coalescent delimitation methods, since BFD can compare non-nested models using SNP data, and integrate over independent species trees during the species delimitation procedure, removing the constraint of having to provide a (potentially incorrect) guide tree. In addition, BFD using SNAPP incorporates thousands to tens of thousands of high-throughput-sequenced, putatively unlinked single nucleotide polymorphism (SNP) or amplified fragment length polymorphism (AFLP) loci, which can be generated at significantly lower cost and effort than traditional Sanger-sequenced markers (Megens & Groenen, 2012; Rubin et al., 2012; Gautier et al., 2013; McCormack et al., 2013; Leaché et al., 2014). Despite the recent development of coalescent-based delimitation methods, their application to species discovery and validation is increasing (Leaché & Fujita, 2010; Zhang, 2011; Solis-Lemus et al., 2014) and has been hailed as the key to modernising the tree of life, and to making taxonomy a 21st century information science (Pennisi, 2003; Leaché et al., 2018a).

Using genealogical evidence from coalescent-based analytical species delimitation methods as the sole source of evidence informing species boundaries has been advocated by some researchers, since lineage independence using these methods is presented in a rigorous statistical framework (Grummer et al., 2014; Fujita et al., 2012; Solano-Zavaleta & Nieto Montes de Oca, 2018). Others have, however, argued that such approaches are likely inadequate because coalescent-based delimitation models assume that all gene tree incongruences are due only to incomplete lineage sorting, and thus that species delimitation should be conducted with the incorporation of other sources of taxonomic evidence so as to prevent “taxonomic inflation” (Knowles & Carstens, 2007; Schlick-Steiner et al., 2010). Indeed, some researchers have used evidence from morphology, ecology, biogeography, and previous phylogenies to inform species delimitation hypotheses, or else to buttress the results of genealogical species delimitations (Leaché et al., 2009; Carstens et al., 2013; Chan et al., 2017; Satler et al., 2013). This latter approach, with the explicit acceptance of the conceptual framework associated with the ESC, embodies “integrative taxonomy” (Dayrat, 2005;

Padial et al., 2009), a synthetic approach to alpha taxonomy that involves the application of multiple lines of evidence, including data from genomes, ecology, behaviour, and morphology to test species delimitation hypotheses (Sivarajan, 1991; Carstens & Knowles, 2007; Padial et al., 2010; Schlick-Steiner et al., 2010; Fujita et al., 2012; Mishler et al., 2014; Rouhan & Gaudeul, 2014; Victor et al., 2015; Victor et al., 2016; Sukumaran & Knowles, 2017; Noguerales et al., 2018; Leaché et al., 2009, 2014, 2018a). This "integrative" approach may assist in bridging the perceived divide between molecular phylogenetics and classical alpha taxonomy (O'Hara, 1998; Smith et al., 2008; Victor et al., 2015), and in so doing, reduce incongruities between genealogy and classification as advocated by Darwin (1859). Perhaps the most important benefit of the integrated approach is the possibility of diminishing the limitations associated with using any single type of taxonomic evidence. Indeed, the integrative approach is critical at the present time for generating stable taxonomies for downstream applications in conservation as biodiversity enters its sixth extinction crisis (Fujita et al., 2012; Rouhan & Gaudeul, 2014).

The *Seriphium plumosum* complex is a variable and widespread Cape species that was first described in Linnaean taxonomy by Linnaeus in his *Species Plantarum* (1753). The taxonomy of the species since that time has been problematic, different authors recognising between one and four species in the complex, prompting interest in a reassessment of species limits in the complex. In her recent revision of the genus *Seriphium*, Koekemoer (2016) used morphology in a key-character approach in the absence of any statistical analyses, and merged three previously-independent species (*Stoebe plumosa* (L.) Thunb., *Stoebe burchellii* Levyns and *Stoebe vulgaris* Levyns) under the name *Seriphium plumosum*. The result is an expanded *S. plumosum*, which includes a set of morphologically diverse plants with the largest geographic distribution of all the species included in either *Seriphium* and its putative sister genus, *Stoebe*, as these genera are currently defined. That widespread geographic distributions are uncommon among Cape floral species (Latimer et al., 2005) and that the spatial scale of gene flow in plants via pollen and seeds is often highly range-limited (Grant, 1981) questions the genetic integrity of *S. plumosum* as defined by Koekemoer (2016). The ten-month-long, bimodal flowering season of the complex (Koekemoer, 2016) further challenges its genetic cohesiveness, given that the temporal shifts in flowering time, even subtle shifts, have the potential to substantially reduce gene flow between populations (Devaux and Lande, 2009).

How many evolutionarily independent lineages are present in the *S. plumosum* complex? I reassess species boundaries within *S. plumosum* using an integrated taxonomic approach. Multivariate analysis of a number of taxonomically useful morphological characters, ecology, the work of previous taxonomists, and genetic population assignment analysis are used to formulate a series of species delimitation hypotheses for the complex. I then test these hypotheses using the Bayes Factor Delimitation method of Leaché et al. (2014). Finally, I re-examine the most favoured model in the light of the morphological and ecological data in order to devise an integrated taxonomic classification for the complex. The *S. plumosum* complex is predicted to contain at least two independent evolutionary species *sensu* Wiley (1978).

Methods

Field sampling

A total of 275 pressed specimens of *S. plumosum* and *S. cinereum* housed in BOL and NBG were examined and measurements of the leaves, flowers and involucral bracts made for each specimen under dissecting microscope using an eyepiece micrometer accurate to 0.2mm (data not presented). This data set was used as a rough guide to recognise a number of *a priori* morphological "forms" in *S. plumosum* based on the combination of characters that consistently appeared together (i.e. on visual recognition) and the geographical distribution of the specimens. These *a priori* morphological forms were in turn used as a guide to ensure that field sampling captured the full range of morphological and ecological variation within the complex. Material of *S. plumosum* was sampled from 47 localities representing the full extent of the species' distribution South Africa, while material of *S. cinereum* was obtained from a single locality near Cape Town (Figure 1; Appendix A). For each of the 48 populations, short (ca. 5cm) live leaf cuttings were collected for DNA extraction from each of six individuals spaced ≥5m apart and placed on silica-gel in an airtight container to dehydrate the material and minimise DNA degradation. Live flower and leaf material from the same individuals was also collected into FAA (60% ethanol, 25% distilled water, 10% formalin, 5% glacial acetic acid) where it was fixed for 48 hours, after which the material was transferred to a 70% ethanol solution for long-term storage. A single pressed voucher specimen for each population was deposited at the Compton Herbarium, Kirstenbosch (NBG).

Molecular data assembly

For each of the plants sampled at the 48 sampling localities (288 individuals), DNA extraction was performed within one week of collection. One hundred micrograms of silica-dried leaf material from the growing tips was ground to a fine powder using liquid nitrogen and a pestle and mortar. Genomic DNA was extracted from each powdered sample using the DNEasy plant mini kit (*Qiagen, Venlo, Netherlands*) according to the manufacturer's protocol (Qiagen, 2018), but eluted in 30 µl TE buffer instead of the recommended 100 µl in order to ensure adequate DNA concentration for GBS. Each extract was assayed with the NanoDrop ND-1000 spectrophotometer (1 µl per sample; *ThermoFisher Scientific, Waltham, MA USA*), and only samples with concentrations ≥50 ng/µl and 260/280 absorbance ratios between 1.8 and 2 were used for further analysis. Where sample DNA concentration was less than 50 ng/µl, extracts were concentrated by speed-vacuuming for 10 minutes at 43°C using the Savant SpeedVac concentrator SC210a (*ThermoFisher Scientific, Waltham, MA USA*). Following extraction, and between assaying and sequencing, all DNA extracts were stored at -80 °C, except during express-shipping (≤3 d transit) from Cape Town to Novogene Genome Sequencing Company Ltd. in Beijing, China. Here samples were for re-assayed using the NanoDrop

2000, before genotyping-by-sequencing (GBS) according to Novogene's protocol (https://en.novogene.com/), briefly outlined here. The GBS workflow generates a reduced representation library which is then high-throughput sequenced. Sample genomes were fragmented using the enzymes MseI, HaeIII, and MspI, and ligated with P1 and P2 barcodes complementary to fragment overhangs. Barcode-ligated fragments of size 350 base-pair (bp) were recovered using size selection, and these amplified by PCR. Paired-end sequencing was performed on PCR-enriched adaptor-ligated DNA fragments on the Illumina HiSeq platform, generating 200,000 150 bp reads at 8X average coverage per fragment. Raw sequence data were filtered to exclude paired reads with adaptor contamination, paired reads where ambiguously called nucleotides constitute more than 10% of the read, and paired reads where low quality nucleotides (quality score ≤5, or a 10% error rate) constitute more than 50% of either read. The filtered reads were then used to assemble a *de novo* reference genome using SOAPdenovo version 2.04 (Luo et al., 2012) with parameter settings [-K 41 -R -d 2 -p 10] with minimum quality thresholds as follows: contigs (consensus sequences) N50 ≥ 30 kilobase pairs and scaffolds N50 ≥ 1 mega bp. A single concatenated variant dataset across all 288 individuals was generated by quality-trimming and mapping all reads to the *de novo* reference genome, calling variants (SNPs and indels), and finally filtering the variants, all of which was implemented using the *dDocent* pipeline (Puritz et al., 2014a) and *R* version 3.5.1. (R Core Development Team, 2018). Quality trimming of cleaned reads was performed using Trimmomatic (Bolger et al., 2014), which removes any remaining adaptor contamination and low quality bases (below a quality score of Q20, or a 1% error rate) from the beginnings and ends of reads (where read quality tends to be poor). Trimmed reads were mapped to the *de novo* genome using the MEM algorithm of the Burrows-Wheeler Aligner, the preferred algorithm for low-divergent sequences of greater than 70bp long (Li & Durbin, 2010), with default conservative mapping parameter settings [-A 1 -B 4 -O 6]. Parallel variant calling was performed across 64 processing cores with 128 GB of memory (University of Cape Town ICTS High Performance Computing team) using FreeBayes version 1.2.0 (Garrison & Marth, 2012), and a complete variant call file (VCF) was created using VCFtools version 3.0 (Danecek et al., 2011). Genotyped variants were advance-filtered as part of the *dDocent* pipeline using VCFtools to produce a final SNP data set. Population-specific genotype call rate was set to 10% across five populations (Puritz et al., 2014a), and sequenced individuals with >30% variant missing data were discarded (instead of the default 50% used in *dDocent*; Chan et al., 2017). Only biallelic SNPs were retained (as implemented in the *R* package *dartR* version 1.0.5; Gruber et al., 2018) in order to comply with SNAPP's (Bryant et al., 2012) requirement of biallelic loci. As alleles were not phased, all SNPs were assumed to be homozygous, such that each individual was assumed to have either the reference or alternate allele state at each SNP locus. Finally, a filter was applied to the SNP data set to retain only one SNP per read, thereby reducing linkage disequilibrium and better complying with SNAPP's (Bryant et al., 2012) requirement of variant independence.

Formulation of species delimitation hypotheses

Genomically-informed delimitation hypotheses were developed by assigning individuals to estimated ancestral gene pools using the sparse non-negative matrix factorisation (sNMF) algorithms developed by Frichot et al. (2014) applied to the SNP data set (including six individuals in each of 48 populations) as implemented in the *R* package *LEA* version 2.2.0 (Frichot & François, 2015). The sNMF approach/algorithm computes individual ancestry coefficients, which can be interpreted either as the proportion of an individual's genome originating from multiple ancestral gene pools or as the probability of an individual coming from a particular gene pool (Frichot et al., 2014), using least-squares minimisation of allele frequencies with results comparable to those generated using the likelihood-based methods STRUCTURE (Pritchard et al., 2000) and ADMIXTURE (Tang et al., 2005; Alexander et al., 2009). The sNMF method has significantly shorter runtimes than likelihood-based methods, which is an advantage where ancestry coefficients are computed for many populations and large genomic datasets. For the purposes of this analysis, each sampled locality was considered a population, and six sequenced individuals per population were included. One hundred ancestry coefficient matrices (repetitions) were computed for each value of k between k = 2 and k = 20, where k represents the number of ancestral gene pools or putative species. The optimal number of ancestral gene pools (the value of k that best explains the genotypic data) was selected using the entropy criterion computed as part of sNMF for each k value as recommended by Frichot and François (2015). The Q matrices containing ancestry coefficient estimates generated by sNMF for the best-supported value of k were summarised using Clumpak (cluster Markov packager across K; Kopelman et al., 2015) which produces major and minor solutions for each value of k to the least-squares minimisation algorithm based on the number of runs supporting the solution (i.e. the best-supported mode of clustering gene pools, followed by next-best-supported modes).

The morphology of each of the field-sampled specimens was characterised by a measuring characters of the leaves, involucral bracts and florets of each specimen under dissecting microscope. For each specimen, a single capitulum (floret and involucral bracts) and a single leaf fascicle was mounted for microscope observation in a 250:1 glycerol-fuchsin dye medium. Thirty-three quantitative and qualitative characters were measured and recorded from the fixed material using an eyepiece micrometer, accurate to 0.2 mm. Some of the characters measured were based on the characters used by Levyns (1937), the remainder being based on Anderberg's (1991) treatment of Gnaphalieae (Appendix B; detailed in Chapter 3). For the five qualitative and 12 quantitative characters showing the greatest variability (measured as standard deviation; Appendix B) and highest data completeness across the *a priori* forms in *S. plumosum,* a general pairwise Gower dissimilarity coefficient matrix was computed using the package *cluster* version 2.0.7-1 (Maechler et al., 2018) as implemented in *R*, and the scaled dissimilarity matrix subjected to principal coordinates analysis in the *R* package *ape* version 5.1 (Paradis et al., 2004). The possibility that morphological differences between low- and high-elevation populations in the Cape mountains are plastic and elevationally determined, as has been observed in *Metalasia* and *Stoebe incana* (Levyns, 1937), was explored using a linear regression of morphological difference (represented by the first PC for morphological characters above) and elevation as implemented in the built-in *R* package *stats* 3.5.1. (R core team, 2018).

Two principal aspects of abiotic environmental variation promote plant ecophysiological specialisation in the CFR: (1) a climate generally characterised by hot, dry summers contrasted with a cool, wet winter growing season and (2) the diversity of soil types, which includes highly leached, nutrient-deficient quartzite-derived soils that dominate much of the Cape mountains, and smaller areas of more nutrient rich calcrete-, granite- and shale-derived soils that dominate the valleys and flats (Goldblatt, 1978; Cowling & Holmes, 1992; Cramer et al., 2014). The spatial distribution of summer precipitation (and so of the potential degree of summertime plant water stress) across the forty-eight sampling localities was captured with three variables. Two of these were obtained from the climate layers: mean annual temperature (MAT; Hijmans et al., 2005), and precipitation of the warmest quarter (PWQ) queried using the R package *raster* version 2.6-7 (Hijmans, 2017). The third variable, elevation, was determined on-site using hand-held GPS. Soils across the forty-eight populations were characterised using simplified bedrock geology layers for the CFR and South Africa (Hoffman et al., 2015; South African Department of Mines, 1970) and field observations of geology. Ecological differences (excluding soil categories) between *a priori* forms were analysed using principal components analysis as implemented in the *R* package *ape* version 5.1 (Paradis et al., 2004).

Bayesian species delimitation

Candidate species delimitation hypotheses (Figure 2) were compared using the BFD protocol of Leaché et al (2014). Marginal likelihood and species tree estimation were implemented using SNAPP version 1.3.0 (Bryant et al., 2012) in BEAST 2 version 2.5.0 (Bouckaert et al., 2014). For all BFD model runs, sequence data for three individuals of *Seriphium cinereum* were included to represent the sibling lineage of the *S. plumosum* complex (Chapter 1, Figure 2). Single nucleotide polymorphism data in VCF format were converted to nexus format using *PGDSpider* version 2.1.1.3 (Lischer &

Excoffier, 2012). For each of the competing species delimitation hypotheses, XML files were generated in *Beauti* version 2.5.0 *Java* version 1.8.0_91 (Bouckaert et al., 2014). Marginal likelihood estimation was then performed with stepping-stone analysis using the Beast 2 application PathSampleAnalyser with 48 steps, each consisting of 100 000 generations, a pre-burnin of 10 000 generations, sampling parameters every 1000 steps, and an alpha value of 0.3 (Leaché & Ogilvie, 2016; Noguerales et al., 2018). Mutation rates *u* and *v* were both set to 1.0. I attempted to increase the number of generations to 150 000 and 200 000, respectively, in an effort to ensure that each model reached stationarity (convergence). These models, however, failed to run to completion within the 14-day timeframe imposed by the UCT High Performance Computing facility (University of Cape Town ICTS High Performance Computing team) and were aborted. The minimum 100 000 generations recommended by Leaché and Ogilvie's (2016) necessary for a thorough analysis were therefore used in this analysis. Although Leaché et al. (2014) have demonstrated that BFD is generally robust to prior misspecification, I tested two priors for expected genetic divergence (Θ): (i) the default theta prior used in SNAPP with a gamma distribution Θ ~ G(1, 250) and a mean alpha/beta = 0.004, and (ii) a theta prior estimated using pairwise sequence divergence between taxa (Leaché and Ogilvie, 2016) for the *S. plumosum* complex based on the number of confidently identified SNPs per 1000 read base pairs; Θ ~ G(1, 400) and mean 0.0025. Two gamma hyperpriors were also tested for the speciation rate parameter (λ): (i) the default prior used in SNAPP with gamma distribution (λ) ~ G(2, 200) and mean alpha x beta = 400, and (ii) a hyperprior estimated (Leaché and Ogilvie, 2016) for the *S. plumosum* complex (λ) ~ G(2, 500) and mean alpha x beta = 1000. Although models with the same species delimitation scheme but different priors appear to differ significantly (BF>10; Table 1, part ii), the BFD approach generally distinguishes between the alternative hypotheses in a consistent manner. I therefore selected the most conservative ("lumping" as opposed to "splitting") combination of priors: mean Θ = 0.04 and mean λ = 400 for the remaining model runs. In order to facilitate computation, a subset of 36 out of the total 288 sequenced individuals was used (with a minimum of two sequenced individuals per putative species;

Leaché et al., 2014), and marginal likelihood calculations were performed in parallel across two computing nodes, each with 24 processors and 1 TB memory capacity (University of Cape Town ICTS High Performance Computing team). Since the number of orthologous SNPs sampled across taxa decreases as a function of the evolutionary divergence between the taxa (Huang & Knowles, 2016; Leaché & Oaks, 2017), and since missing data (i.e. fewer orthologous SNPs sampled across taxa) have been shown to be biased toward species delimitation hypotheses with higher numbers of species (Leaché et al., 2018b), marginal likelihood estimation was performed using a random 1000-SNP subset of the SNP data set for which SNPs were captured for all 36 individuals. Although this significantly reduces the size of the SNP data set, Leaché et al (2014) have shown that BF delimitation can be confidently performed using as few as 100 SNPs, and the reduction in data set size has the advantage of both improving SNP independence as per SNAPP's requirement, and reducing the probability of inferring an incorrect species tree topology (Bryant et al., 2012; Schmidt-Lebuhn et al., 2017). Marginal likelihoods were also calculated for a different random 1000-SNP subset of the complete SNP data set with some missing data across taxa (reported below) in order to assess the effects of missing data (hierarchical and random) on model ranking and BF support (Leaché et al., 2014).

All VCF manipulation was implemented in the R package *vcfR* version 1.8.0 (Knaus & Grünwald, 2017). The level of support for competing species delimitation models was evaluated using Bayes Factors following the scale of Kass and Raftery (1995), in which 0 < BF <2 is negligible, 2 < BF < 6 is positive support, 6 < BF < 10 is strong support, and 10 < BF is decisive support. For the species delimitation model with the greatest BF support, the Beast 2 application TreeAnnotator was used to summarise the posterior distribution of species trees (Leaché & Ogilvie, 2016), identify the topology with the greatest posterior support, and estimate divergence times across all tree nodes. FigTree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) was used to generate a phylogenetic hypothesis.

Results

SNP data set

The bioinformatics workflow (read trimming, mapping, variant calling and filtering) yielded a total of 7 610 586 variants, which, after filtering for genotype depth, locus quality, minor allele frequency and genotype call depth, yielded 29 753 variants. Filtering to include only biallelic SNPs produced a 28 023-SNP data set with a mean SNP missing data level of 16.83% across 288 sequenced individuals. Filtering for 'secondary SNPs' (two or more SNPs in the same read) failed to exclude any further SNPs, since all 28 023 SNPs were sourced from unique tags. The two data sets generated from the 28 023-SNP data set for the 36 individuals used in BFD had the following number of SNPS and percentage missing data across SNPs and across sequenced individuals: 1000 SNPs with 0% missing data, and 1000 SNPs with 19.28% missing data.

Formulation of species delimitation hypotheses

Eight different species delimitation hypotheses were formulated based on genetic population assignment analysis, ecology, morphology (including *a priori* "forms" and morphometric survey data) and two taxonomic revisions of the species (Levyns, 1937; Koekemoer, 2016). Each of these sources of taxonomic evidence represents an alternative, plausible scheme for splitting the *S. plumosum* into putative species, and I consider that they cover the full range of possible delimitation schemes. Each species delimitation hypothesis is described in turn, in order from most to least complex (Figure 2).

The cross-entropy criterion reaches a minimum of 0.254 at k = 13 (Figure 3e), suggesting the presence of 13 ancestral population gene pools in *S. cinereum* and the *S. plumosum* complex (Figure 2; Figure 3a-d). Major and minor modes are largely consistent in the allocation of individuals to ancestral gene pools. All 100 sNMF runs for k = 13 support the genetic distinctiveness *Seriphium cinereum*. Six of the remaining 12 gene pools within *S. plumosum* are distributed in the Cape mountains. These correspond with: one population on the Matroosberg in the Hex River Valley (‘montane dwarf’), seven populations in the north-south trending range of the Cape mountains (‘montane N’), eight populations in the southwestern Cape (‘montane SW’), four populations in the east-west trending range of the Cape mountains (‘montane E’), two populations near the Agulhas Plain (‘montane S’) and four populations in the Little Karoo (‘Little Karoo’), the latter corresponding with *Stoebe burchellii* Levyns. With the exception of ‘Little Karoo’, the four gene pools in the Cape mountains are mixed, implying historical and/or ongoing gene flow between these populations and their genetic cohesiveness. The remaining two (Figure 3d) or three (Figure 3a-c) gene pools within the CFR are distributed along the southern coast. These gene pools correspond with: four populations on unconsolidated dunefields along the coast (‘littoral’) and six populations on coastal plains (‘coastal plain E’ and ‘coastal plain W’). Finally, there are two distinct gene pools in the summer-rainfall part of the distribution of *S. plumosum*; one with a broad distribution (‘eastern SA’) corresponding to the summer-rainfall *Stoebe vulgaris* Levyns, and one with a more limited distribution near the north-eastern part of the Great Escarpment (‘escarpment’).

The preliminary survey of the morphological diversity within *S. plumosum* based on herbarium specimens revealed six semi-distinct morphs with largely non-overlapping geographical distributions. These morphs are largely consistent with the groupings revealed by genetic population assignment analysis, except that the gene pools along the southern coast (‘littoral’ + ‘coastal plain E’ + ‘coastal plain W’) are lumped, as are the populations in the eastern part of the country (‘eastern SA’ + ‘escarpment’). The only inconsistency between the *a priori* morphs and genetic population assignment analysis is in the groupings these approaches make in the Cape mountains, where the former approach supports two morphs (a high- and low-elevation morph), and the latter supports four (‘montane N’ + ‘montane S’ + ‘montane SW’ + ‘montane E’). The high- and low-elevation *a priori* morphs distributed in the Cape mountains could be predicted from elevation ($R^2 = 0.16$, $p < 0.001$), with the two morphs overlapping in distribution between 1000 and 1400 metres elevation. Principal coordinates analysis of 17 vegetative and reproductive characters explains 59.7% of the total variance in the first two PC axes (Figure 4), and supports the gene pools along the southern coast (Figure 4, cluster a) and the gene pool ‘Little Karoo’ (Figure 4, cluster b) as somewhat morphologically distinct. There is also some support for the morphological distinctness of the forms ‘montane N’ and ‘montane SW’ (Figure 4, cluster d), and of the forms ‘eastern SA’ and ‘montane E’ (Figure 4, cluster c). The key characters causing separation along these first two principal coordinate axes are the length of the corolla lobes, the width of the corolla tube, and the shape of the leaves (Appendix B; Chapter 3). Principal components analysis based on MAT, PWQ, and elevation shows strong structuring of *S. plumosum* populations by climate, with 97.7% of the total variance captured in the first two PC axes (Figure 5). The populations in the summer-rainfall distribution of *S. plumosum* (‘escarpment’ and ‘eastern SA’) are strongly supported as ecologically distinct (Figure 6, cluster a), and there is some support for the gene pools along the southern coast as ecologically distinct and cohesive (Figure 5, cluster b). Elevation contributes the most to separation along the first principal component, while PWQ contributes the most to separation along the second principal component.

The second-least complex delimitation hypothesis is based on Levyns’ (1937) treatment of *Stoebe* and partitions *Seriphium plumosum* into three putative species (Figure 3) corresponding with *Stoebe plumosa* (L.) Thunb. (‘montane dwarf’ + ‘montane N’ + ‘montane S’ + ‘montane SW’ + ‘montane E’ + ‘littoral’ + ‘coastal plain E’ + ‘coastal plain W’), *Stoebe burchellii* Levyns (‘Little Karoo’) and *Stoebe vulgaris* Levyns (‘eastern SA’ + ‘escarpment’; Figure 2). The simplest delimitation hypothesis, based on Koekemoer’s (2016) taxonomic treatment of *Stoebe* and *Seriphium,* treats *S. plumosum* as a single species (Figure 2).

Bayesian species delimitation and species relationships

Marginal likelihood estimation computation times for each of the species delimitation models varied between 48 and 144 hours on 24 processors and used a maximum 300GB of memory. The species delimitation hypothesis representing the current taxonomy (one-species model) failed to produce a meaningful ML estimation for Bayes Factor comparison (- infinity), and thus cannot be assessed here. This is likely due to highly imbalanced sampling (A. Leaché, personal communication 2018, November 10), since in this model, *S. plumosum* is represented by 33 individuals, and *S. cinereum* by only three individuals. That species delimitation hypotheses with fewer species were associated with the lowest marginal likelihood estimates (Table 1) suggests that the one-species model would have performed even more poorly, had a meaningful marginal likelihood estimate been generated for it. It is also possible, however, that the more poorly-performing species delimitation models group populations into species in a way inconsistent with genealogy, such that the one-species model might indeed have contended with species delimitation hypotheses with the highest marginal likelihood estimates. The best supported model from the remaining set of candidate hypotheses is model 2 (Table 1), which recognises ten species within the *S. plumosum* complex, and which lumps the coastal plain gene pools. There is strong but not decisive evidence (BF < 10) against the most complex delimitation hypothesis, model 1 (Table 1), which treats 'coastal plain W' and 'coastal plain E' as distinct species. Consistent with the results obtained by Leaché et al. (2014), SNP data with missing data across individuals significantly affects model ranking (Table 1, part iii), and BFD is robust to prior misspecification, with different priors for θ and λ generally not changing species delimitation hypothesis ranking (Table 1, part ii).

The species tree associated with model 2 (Figure 6) reveals several unexpected relationships, including the nesting of *Seriphium cinereum* within the *S. plumosum* complex, which I thus term the *S. plumosum* clade. This finding casts doubt on the use of *S. cinereum* as an outgroup. The four species inferred for the Cape mountains (excluding 'Little Karoo') do not appear to be monophyletic; the gene pools 'montane N', 'montane S' and 'montane E' are supported as nested within the same clade (Figure 6, clade 2), while the gene pool 'montane SW' is supported as forming part of the clade sibling to the remainder of the complex (Figure 6, clade 1). The gene pool 'eastern SA' (formerly *St. vulgaris*) is strongly supported as embedded within the clade housing the three closely-related evolutionary species in the Cape mountains (Figure 6, clade 2), while 'Little Karoo' (formerly *St. burchellii*) is strongly supported as sibling to the coastal and near-coastal lineages (Figure 6, clade 3), which in turn are strongly supported as sister (Figure 6, clade 4). Finally, the 'montane dwarf' and 'escarpment' gene pools are recovered as embedded within the clade sibling to the remainder of the *S. plumosum* clade (Figure 6, clade 1).

Discussion

Diversity and evolution of the S. plumosum clade

Genetic data presented here reveal the potential for recognising either eleven or twelve genetically distinct lineages within the *Seriphium plumosum* clade, including *Seriphium cinereum* which is nested within it. According to the assumptions underlying the multispecies coalescent, the lineages identified are genetically cohesive and partially (Zhang et al., 2011) to completely genetically isolated (Leaché et al., 2014), and therefore qualify as distinct evolutionary species (Wiley, 1978). That several of these species, corresponding with the gene pools 'Little Karoo', 'dwarf montane', 'coastal plain', 'littoral' and ('montane N' + 'montane E' + 'montane S'), maintain their distinctness in sympatry qualifies them as biological species *sensu* Mayr (1969). Of the ten or eleven species thus identified within the current concept (Koekemoer, 2016) of *S. plumosum*, only one ('Little Karoo') is strongly discriminated using morphological data (Figure 4). The clade containing the species distributed in coastal and near-coastal regions (Figure 6, clade 4) was supported as somewhat morphologically and ecologically distinct from the remainder of the populations of *S. plumosum* (Figure 4, Figure 5), but multivariate analyses of the morphological and ecological data failed to distinguish fully the three evolutionary species ('coastal plain E', 'coastal plain W' and 'littoral') contained in this clade. Principal components analysis of the variables MAT, PWQ, and elevation (Figure 5) support the ecological distinctness populations in the summer-rainfall region of South Africa, but these data similarly failed to discriminate between the two evolutionary species ('eastern SA' and 'escarpment') that appear to be housed within this cluster. Elevation is a significant predictor of morphological difference between the low- and high-elevation a priori morphs distributed in the Cape mountains, a finding consistent with Levyns' (1937) contention that morphological differences between the low- and high-elevation populations in *Stoebe plumosa* are associated with elevation-linked moisture and/or temperature gradients. The discrepancy between these *a priori* morphs and the four species uncovered by genetic population assignment analysis (Figure 3) illustrates the difficulty in discriminating between genetic variation and phenotypic plasticity when formulating hypotheses about species boundaries.

Notwithstanding that Bayes factor support is highest for the species delimitation hypothesis which partitions *S. plumosum* into ten species, I favour a delimitation scheme that merges the three evolutionary species in the Cape Fold Belt mountains ('montane N', 'montane E' and 'montane S') based on evidence from genetic population assignment analysis of recent or ongoing gene flow between these species (Figure 3), and on the basis that the multispecies coalescent can fail to detect low levels of ongoing gene flow between species and erroneously characterise them as being genetically isolated (Zhang et al., 2011). In a similar manner, the best-supported species delimitation model (Table 1) and genetic population assignment analysis also provide support for merging the gene pools on the coastal plains (Figure 3d). Although each of the unmerged species within *S. plumosum* and *S. virgatum* (as defined here) represents an independent evolutionary species, the merged lineages are still evolutionary species in that they represent (in the former and latter case respectively) the amalgamation of paraphyletic and monophyletic lineages into species that are morphologically and ecologically similar. The final delimitation scheme presented in Chapter 3, therefore, recognises eight species in *S. plumosum*, and nine species in the *S. plumosum* clade which now includes *Seriphium cinereum*.

That most species in the *S. plumosum* clade are CFR-endemic (Chapter 3, Figure 1), and that the clade is imbedded in the more inclusive CFR-centred *Stoebe-Ifloga-Metalasia* (SIM) clade (Bergh et al., 2015) identifies the *S. plumosum* clade as most likely having a CFR, and perhaps specifically a southwestern Cape, origin. Such an interpretation with the Cape as a centre of diversification is consistent with the observation that all Cape genera exhibit concentrated species diversity in the extreme southwestern Cape (Levyns, 1952; Linder, 2003), which Levyns (1964) interpreted as evidence of this region as a “cradle of diversification” for the Cape flora, suggesting perhaps that this region is associated with an elevated diversification rate. A southwestern Cape origin for the *S. plumosum* clade implies multiple independent dispersal events into the Little Karoo and summer-rainfall region of South Africa, but only one colonisation of the coastal habitat. Patterns of repeated dispersal out of the Cape are mirrored at larger spatial scales in *Disa*, Irideae, the *Pentaschistis* clade, and Restionaceae, all of which are thought to have originated in the Cape, and to subsequently have dispersed into tropical Africa via the Drakensberg (Galley et al., 2007), and by the multiple “out of southern Africa” dispersal events inferred for in the paper daisy tribe Gnaphalieae (Bergh & Linder, 2009). A more formal analysis using ancestral area inference will, however, be required to more confidently assess the geographical origin of the *S. plumosum* clade and patterns of dispersal therefrom.

Coalescent species delimitation models and BFD

The Bayes Factor Delimitation method of Leaché et al. (2014), was applied here to assess support for the existence of multiple independent evolutionary species being nested within the *S. plumosum* complex using putatively unlinked biallelic SNP markers. The best-supported species delimitation hypothesis of those included in the candidate set was informed by genetic population assignment analysis. A similar two-step discovery-validation approach was taken by Leaché & Fujita (2010), who used genomic data for assigning individuals to putative groups using Stucturama (Huelsenbeck et al., 2011), and verified assignments using the coalescent delimitation method Bayesian Phylogenetics and Phylogeography (Yang & Rannala, 2010; Yang, 2015) for West African forest geckos. Like BP&P version 3 (Yang et al., 2015) and DISSECT (Jones et al., 2015), Bayes Factor Delimitation permits the comparison of non-nested models, which proved a necessity in the present work, where models based on previous taxonomic treatments, ecology, morphology, and genomic data differ in how they group populations into putative species (Figure 2).

The BFD method has been applied across diverse groups including flowering plants (Boucher et al., 2016; Wagner et al., 2017), reptiles (Leaché et al., 2014; Grummer et al., 2014; Mrinalini et al., 2016; Leaché et al., 2018a), insects (Noguerales et al., 2018), arachnids (Hedin et al., 2015; Bryson et al., 2018), and mammals (Hotaling et al., 2016). In general, these studies have tended to find the greatest BF support for delimitation hypotheses that reflect either one of the greatest or the greatest number of putative species. This trend toward supporting higher numbers of evolutionary species might be indicative of a systematic bias toward oversplitting (i.e. false positive detection). However, Leaché et al. (2014) have shown that BFD is able to discriminate between arbitrarily split models with, at minimum, strong support (6 < BF < 10), and Grummer et al. (2014) have shown using simulated data that, in general, rates of 'oversplitting' using BFD are negligible. The results presented in this work favour a less complex delimitation hypothesis (Table 1, model 2) over the most complex split hypothesis (Table 1, model 2) with strong support (BF = 6.04), and are thus consistent with the findings of Leaché et al. (2014) and Grummer et al. (2014). Given the apparent lack of bias toward oversplitting, the tendency for high numbers of evolutionary species supported by studies applying BFD suggests that the true diversity of evolutionary species is currently underestimated, and/or that BFD has been applied to species complexes for which there is good *a priori* evidence suggesting they house multiple independent evolutionary species.

Other potential limitations of the BFD method include that, consistent with the findings of Leaché et al. (2014), the way in which the SNP data set is filtered for missing data can substantially affect model ranking (Table 1, category iii). It is unclear, however, whether models with and without missing data can be directly compared because SNP data sets with some missing data have a lower information content (Leaché et al., 2014) and because the number of orthologous SNPs captured across taxa is positively related to the evolutionary divergence between them, resulting in patterns of hierarchical (non-random) missing data (Rubin et al., 2012; Grummer et al., 2014; Leaché et al., 2015; Leaché & Oaks, 2017). Also, highlighting the more general problem in Bayesian analysis of choosing among potentially incorrect models (Yang & Zhu, 2018), the true species delimitation hypothesis will only be selected with high probability if it is included in the set of candidate models specified by the researcher *a priori* (Grummer et al., 2014; Leaché et al., 2014). For the set of delimitation hypotheses presented here, there is no guarantee that the best-performing hypothesis is an accurate reflection of the true number of evolutionary species within *S. plumosum*. The series of species delimitation hypotheses have, however, been based on various lines of evidence, including DNA sequence data, morphology, ecology and previous taxonomic treatments.

Implications for species discovery in the CFR

The results presented here yield three important insights for alpha taxonomy in the Cape flora. First, morphology, or indeed any single type of taxonomic evidence, is likely to be inadequate for producing robust classifications that reflect the nature of species as separately evolving metapopulation lineages (Dayrat, 2005; Padial et al., 2009; Padial et al., 2010; Fujita et al., 2012; Leaché et al., 2018a; Noguerales et al., 2018). Most taxonomies produced to date for Cape lineages have relied exclusively on morphology for delimiting species (Treurnicht et al., 2017). Such an approach suggests an overemphasis on the “community service” aspect of alpha taxonomy, which prioritises naming species and providing tools for field workers to identify them, to the detriment of the core scientific element of taxonomy – delineating and classifying species (Dayrat, 2005). The argument for species that are diagnosable on the basis of morphology (Smith et al., 2008) is, however, not unfounded. Indeed, herbaria, museums and field workers require that species ideally be visually distinguishable (Smith et al., 2008). In the case of the *S. plumosum* clade, most of the evolutionary species described are morphologically distinct, and the several that are semi-cryptic or completely cryptic are lumped into morphologically cohesive and distinct species. However, in other cases where distinct evolutionary species are morphologically indistinguishable, the interests of accurately cataloguing species richness will need to be tempered by the requirement that species represent workable units. The failure to balance these apparently conflicting interests, will, in either case, compromise efforts in conservation and research that rely on robust classifications (Wheeler et al., 2004; Dayrat, 2005; Smith et al., 2008; Fujita et al., 2012).

A second insight, somewhat connected to the first, is that species diversity in the CFR, and in general, has probably been underestimated when taking into account taxonomically recalcitrant, widespread, recently-evolved and cryptic and semi-cryptic species complexes. It is probably too early to estimate the true species diversity of the CFR in a quantitative manner, but the results of the present work, and that of Britton et al. (2014) who uncovered cryptic or semi-cryptic species housed in species complex *Tetraria triangularis* (Cyperaceae), if extrapolatable to other taxa, suggests that the proportion of undescribed species in the Cape flora may greatly exceed Treurnicht et al.'s (2017) recent estimate of less than one percent.

Finally, this work highlights that the division in systematics between classical alpha taxonomy, concerned with cataloguing the diversity of life, and so-called "tree thinkers", concerned with generating phylogenies (O'Hara, 1998; Smith et al., 2008), need not exist because next-generation sequencing coupled with coalescent delimitation methods elegantly combines the delineation of evolutionary lineages (i.e. defining species) and the estimation of phylogenies. Rather than making traditional sources of taxonomic evidence obsolete, this work demonstrates that coalescent methods rely on these sources of evidence to buttress the results of genealogical species delimitations, and to inform species delimitation.

Figures

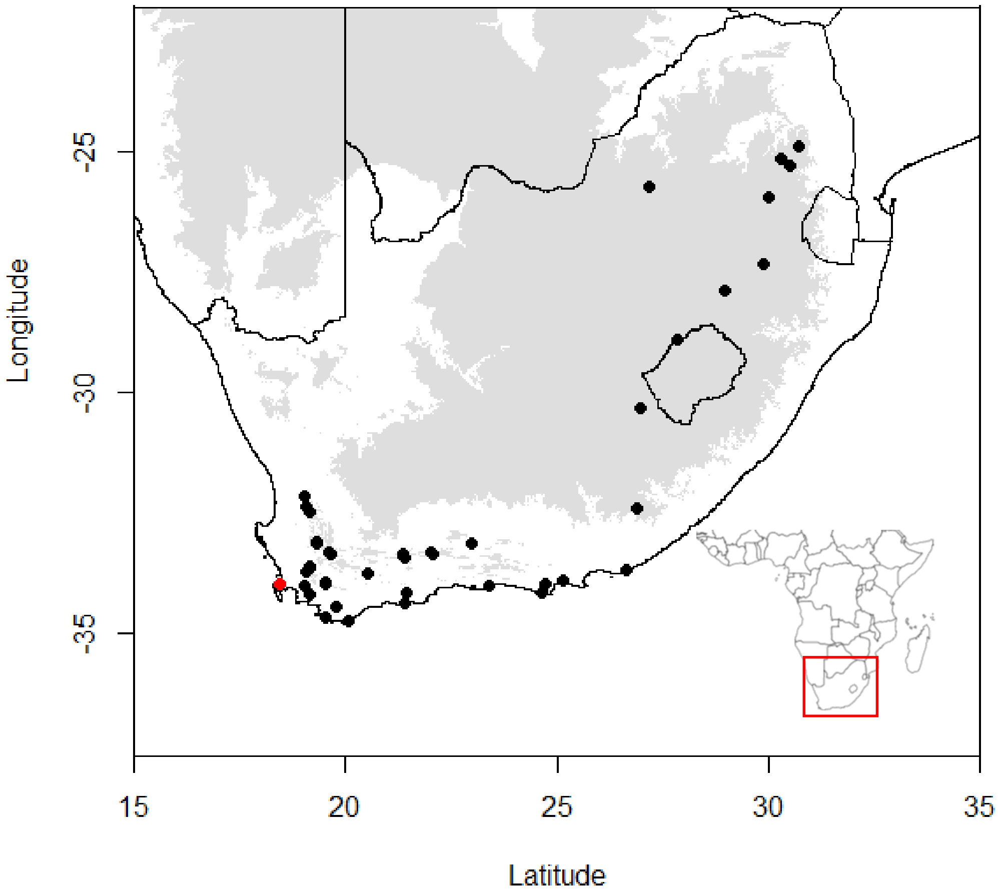


Figure 1: Map of South Africa showing the location of the forty-eight locations of *Seriphium plumosum* L. (black dots) and *Seriphium cinereum* L. (red dot) field-sampled populations superimposed on an elevation layer (shaded grey) for areas ≥ 1000 metres above sea level.

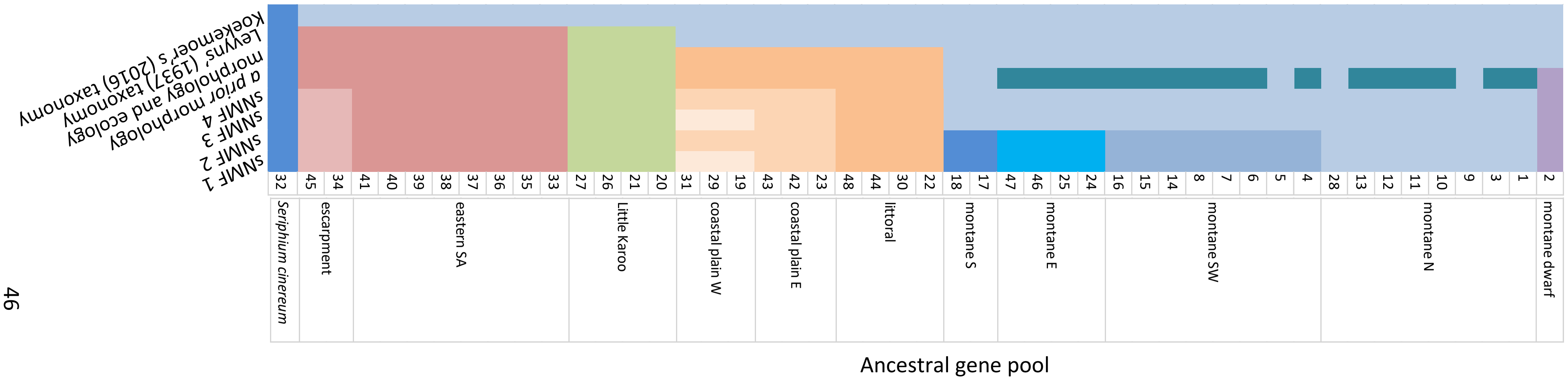
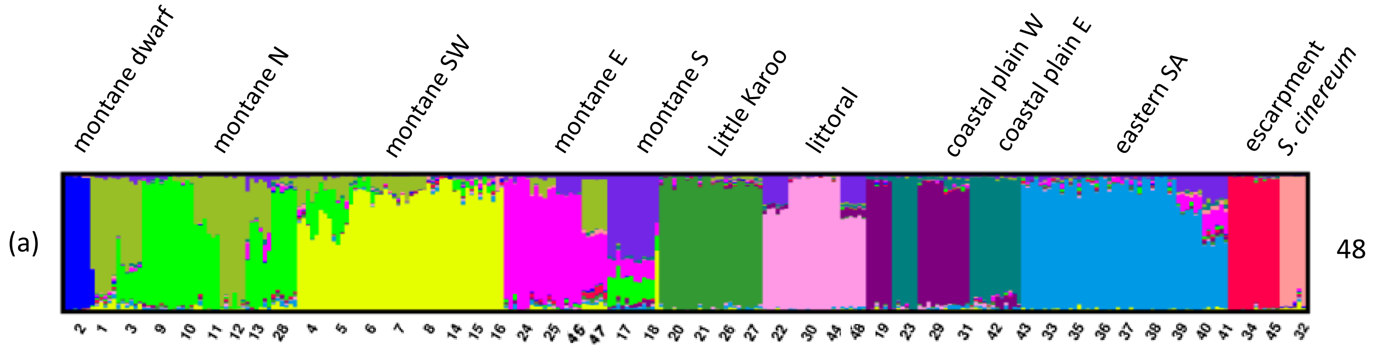
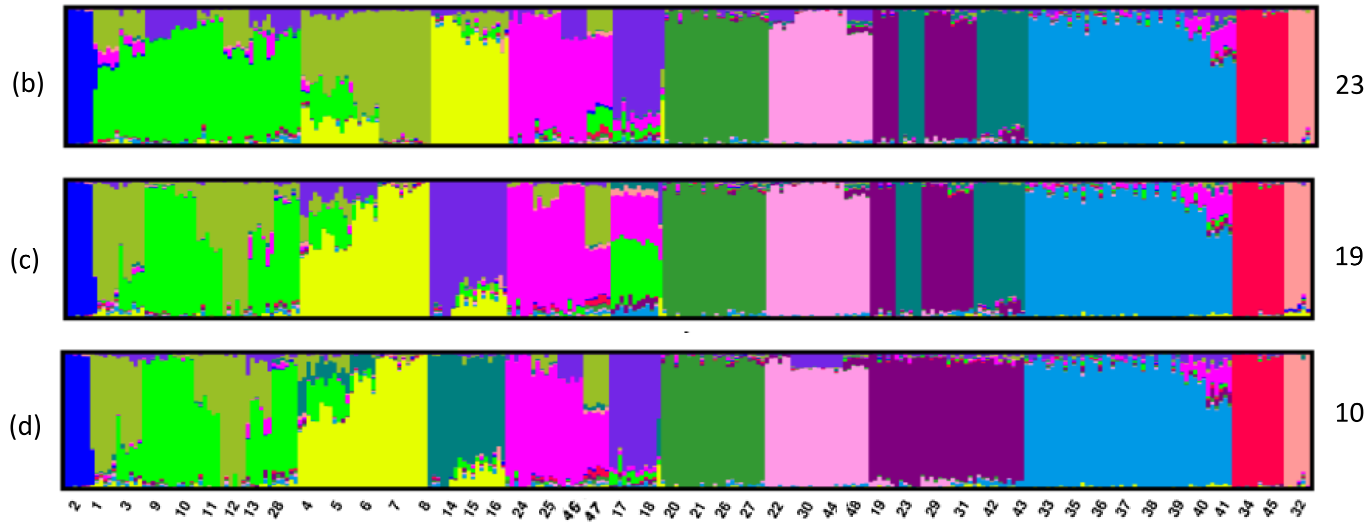


Figure 2: Summary of the eight species delimitation hypotheses for *Seriphium plumosum* informed by genetic population assignment analysis, morphology, ecology, and previous taxonomic treatments. Colour blocks indicate gene pools lumped together under each species delimitation hypothesis. Population numbers alongside colour blocks are consistent with those in Figure 4 and Appendix A.

Major mode



Minor modes



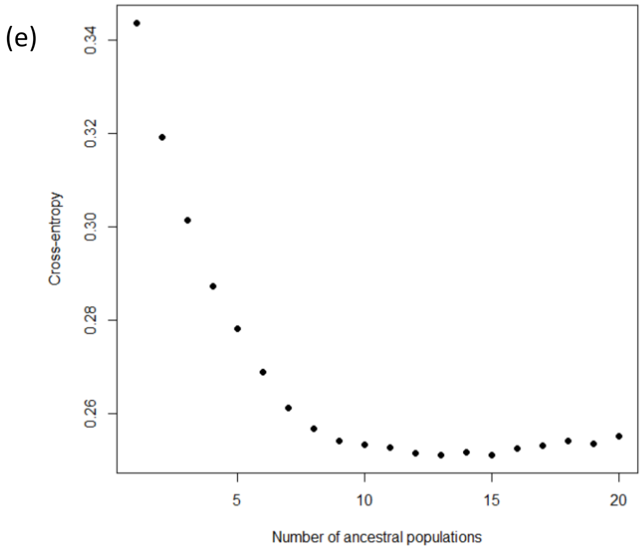


Figure 3: Plots of sNMF genomic assignment for the *Seriphium plumosum* complex for k = 13. Each individual is represented by a single vertical bar and the different ancestral gene pools are indicated by different colours. Major (a) and minor (b-d) ancestry coefficient mode summaries are shown for all sequenced individuals (n = 288) from 48 localities (locality number indicated below plot; Appendix A). Numbers to the right of boxes indicate the percentage runs out of a total of 100 runs supporting major and minor modes. Colours and gene pool names are consistent across boxes. (e) The cross-entropy criterion plotted for each value of k between k = 2 and k = 20.

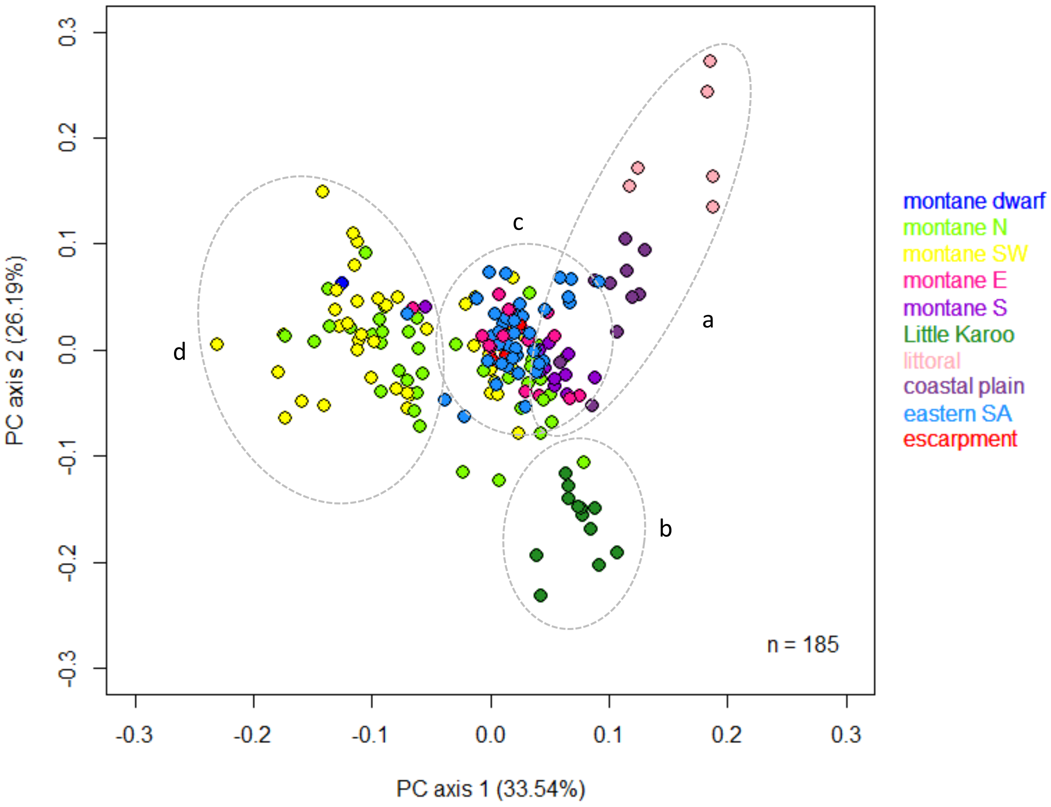


Figure 4: First two principal component axes for PCoA ordination of seventeen morphological characters recorded for the *S. plumosum* complex. The sample includes 185 of the 282 individuals sampled in 2017-18. Colours and labels are consistent with those in Figure 3.

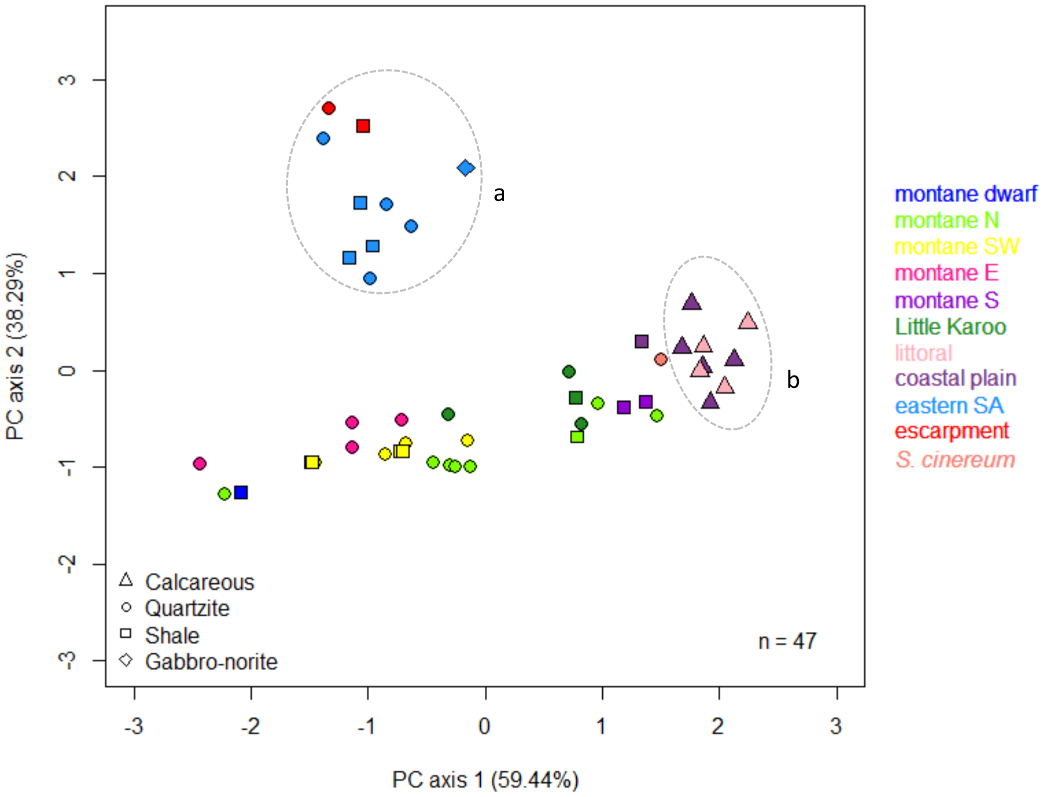


Figure 5: First two PC axes for PCA ordination of the three environmental variables (MAT, PWQ and elevation) queried for each sampled population of *S. plumosum* sampled in 2017-18 with symbols indicating soil types. Colours and labels are consistent with those in Figure 3.

Table 1: Summary of BFD results for the *Seriphium plumosum* complex. Category (i) models all implement the most conservative prior settings for a 1000-SNP data set with no missing data across taxa (see Figure 3). Category (ii) models use two arbitrarily chosen species delimitation hypotheses to test the effects of prior settings on BF support, and models in category (iii) use the most complex species delimitation model (model 1) to assess the effect of using a SNP data set with missing data on BF support.

	Species delimitation hypothesis	**Description**	**Mean Θ**	**Mean λ**	**Number of species (excl. *S. cinereum*)**	**SNP data set**	**Marginal Likelihood (ML) estimate (log$_e$)**	**Bayes Factor (BF) (2 X log$_e$)**	**Rank**
(i)	1	sNMF 1	0.0025	400	11	Random subset of 1000 SNPs, 0% missing data across taxa	-20350.01	6.04	2
	2	sNMF 2	0.0025	400	10		-20346.99	-	1
	3	sNMF 3	0.0025	400	8		-20572.85	451.72	6
	4	sNMF 4	0.0025	400	7		-20604.18	514.38	7
	5	*A priori* morphology	0.0025	400	6		-21634.06	2574.14	8
	6	Morphology and ecology	0.0025	400	4		-22521.96	4349.94	9
	7	Levyns' (1937) taxonomy	0.0025	400	3		-24633.03	8572.08	11
(ii)	8	sNMF 3	0.004	400	8		-20567.52	441.06	5
	9		0.0025	1000			-20565.25	436.52	3
	10		0.004	1000			-20566.28	438.58	4
	11	Levyns' (1937) taxonomy	0.004	400	3		-24631.87	8569.76	10
	12		0.0025	1000			-21634.06	2574.14	8
	13		0.004	1000			-22521.96	4349.94	9
(iii)	14	sNMF 1	0.0025	400	11	Alternative random subset of 1000 SNPs, 19.28% missing data across taxa	-8537.81	-	-

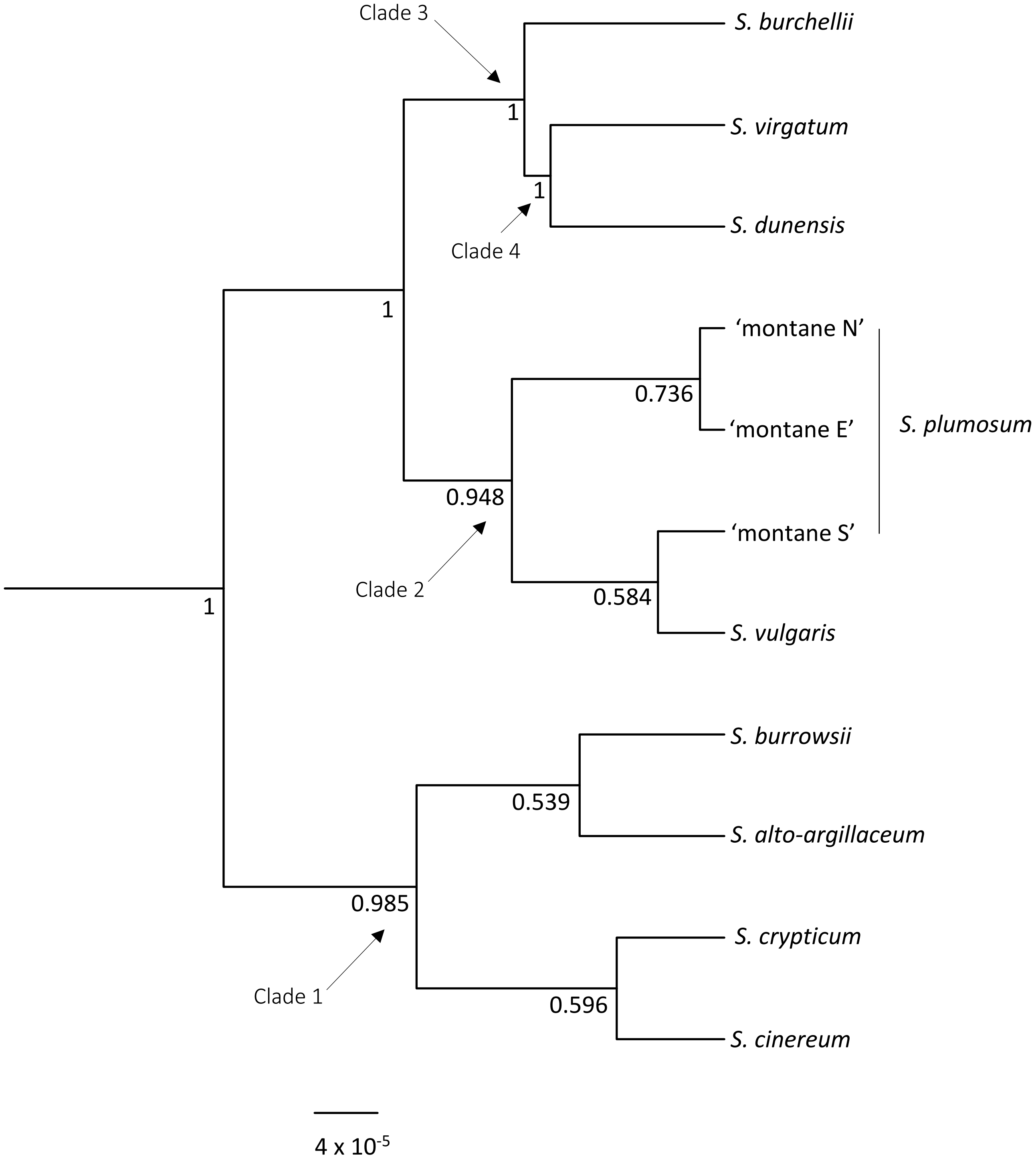


Figure 6: Beast 2 species tree for the *Seriphium plumosum* complex and *Seriphium cinereum* estimated with 1000 biallelic SNPs under the priors Θ ~ G(1, 400) and (λ) ~ G(2, 200) for the best supported species delimitation hypothesis (model 2) which partitions *S. plumosum* into ten evolutionary species. Posterior probabilities are shown on internal nodes. Shown as tip labels are the new species names presented in Chapter 3 corresponding (top-down) with the gene pools 'Little Karoo', 'coastal plain', 'littoral', ('montane N' + 'montane E' + 'montane S'), 'eastern SA', 'escarpment', 'dwarf montane', 'montane SW' and '*S. cinereum*'. Note that the gene pools 'montane N', 'montane E' and 'montane S' are lumped into *S. plumosum* (as defined in Chapter 3).

Appendix A

Coordinates and descriptions of the 48 field-sampled localities across the range of the *S. plumosum* complex in South Africa. Six individuals were sampled at each locality.

Population	Taxon	Locality	Latitude (S)			Longitude (E)		
			deg	min	sec	deg	min	sec
1	*Seriphium plumosum* L.	Matroosberg; Conical Peak	33	22	25.9	19	40	5.6
2	*S. plumosum*	Matroosberg; Conical Peak	33	22	31.6	19	39	47.7
3	*S. plumosum*	Matroosberg base	33	20	41.6	19	37	18.1
4	*S. plumosum*	Jonaskop summit	33	58	19.4	19	30	24.0
5	*S. plumosum*	Jonaskop slope	33	57	50.0	19	30	27.5
6	*S. plumosum*	Wellington Sneeukop slope	33	37	43.9	19	8	28.3
7	*S. plumosum*	Wellington Sneeukop, near summit	33	38	29.6	19	9	0.3
8	*S. plumosum*	Wellington Sneeukop, near summit	33	38	19.2	19	9	0.5
9	*S. plumosum*	Pakhuis Pass	32	8	23.7	19	1	4.0
10	*S. plumosum*	Algeria, Cedarberg	32	22	24.9	19	3	32.9
11	*S. plumosum*	Cedarberg Sneeuberg slope	32	29	28.5	19	9	12.7
12	*S. plumosum*	Mollers Berg, Skurweberge	33	7	23.2	19	18	49.3
13	*S. plumosum*	Molenrivier Farm, Skurweberge	33	8	20.9	19	18	59.2
14	*S. plumosum*	Victoria Peak summit	34	0	44.2	19	2	7.5
15	*S. plumosum*	Victoria Peak summit	34	0	42.8	19	2	8.0
16	*S. plumosum*	Victoria Peak slope	34	0	48.9	19	1	30.0
17	*S. plumosum*	Houw Hoek	34	12	33.9	19	9	24.6
18	*S. plumosum*	Napier-Elim roadside	34	28	21.4	19	46	42.8
19	*S. plumosum*	Riversdale-Albertinia roadside	34	10	57.4	21	24	56.5
20	*S. plumosum*	Mannetjiesberg	33	9	18.3	22	57	20.6
21	*S. plumosum*	Joubertina-Haarlem roadside	33	45	51.0	20	32	5.5
22	*S. plumosum*	Diaz Cross Memorial beach	33	42	37.3	26	37	12.6
23	*S. plumosum*	Humansdorp-Kruisfontein roadside	33	59	42.7	24	43	12.2
24	*S. plumosum*	Swartberg Pass, north side	33	20	25.2	22	2	6.3
25	*S. plumosum*	Swartberg Pass, top	33	21	9.0	22	2	47.2
26	*S. plumosum*	Swartberg Pass, south side	33	21	46.8	22	4	9.8
27	*S. plumosum*	Seweweekspoort roadside	33	26	2.9	21	24	24.8
28	*S. plumosum*	Du Toitskloof Pass	33	44	15.7	19	4	42.7
29	*S. plumosum*	Still Bay	34	22	54.1	21	24	54.1
30	*S. plumosum*	Struisbaai	34	46	7.8	20	2	46.9
31	*S. plumosum*	Pearly beach-Buffeljags roadside	34	39	51.7	19	32	5.3
32	*Seriphium cinereum* L.	Table Mountain slope	33	59	31.4	18	25	18.8
33	*S. plumosum*	Kgaswane N.R.	25	42	52.9	27	11	34.7
34	*S. plumosum*	Buffelskloof P.N.R.	25	17	42.4	30	31	11.9
35	*S. plumosum*	Lydenburg-Roossenekal roadside	25	8	44.2	30	18	16.1
36	*S. plumosum*	Belfast-Carolina roadside	25	55	58.0	30	1	0.2
37	*S. plumosum*	Volksrust	27	20	11.0	29	53	42.7
38	*S. plumosum*	Warden	27	52	59.5	28	56	38.3
39	*S. plumosum*	Ficksburg	28	53	36.8	27	50	46.6
40	*S. plumosum*	Zastron-Rouxville roadside	30	18	53.9	26	59	11.5
41	*S. plumosum*	Seymour-Rouxville roadside	32	25	51.2	26	52	18.6
42	*S. plumosum*	Van Stadens Wildflower Reserve	33	53	51.6	25	8	15.1
43	*S. plumosum*	Plettenberg Bay	34	1	50.7	23	22	33.5
44	*S. plumosum*	Hout Bay	34	2	42.9	18	21	2.5
45	*S. plumosum*	Pilgrim's Rest	24	52	3.8	30	41	48.4
46	*S. plumosum*	Seweweekspoortberg slope	33	22	54.6	21	21	11.4
47	*S. plumosum*	Seweweekspoortberg summit	33	23	37.3	21	21	23.6
48	*S. plumosum*	Oyster Bay	34	10	14.7	24	38	55.0

Appendix B

Morphological characters examined in the *S. plumosum* complex. Only characters 1-17 were used for in PCoA analysis. The remaining characters exhibited little variation across the complex. All measurements were made on material preserved in ethanol under a dissecting microscope using an eyepiece micrometer accurate to 0.2mm. For each character, a single measurement from each specimen was made.

	Character	Variable type	Notes
1	Presence/absence of green photosynthetic tissue on the stereome of the outer bracts	Binary	0 = absent; 1 = present
2	Presence/absence of wool on the outermost bracts	Binary	0 = glabrous; 1 = woolly
3	Presence/absence of sweeping hairs on stigmatic lobes	Binary	0 = absent; 1 = present
4	Presence/absence of apical sweeping hairs on stigma	Binary	0 = absent; 1 = present
5	Leaf succulence	Binary	0 = leaves schlerophyllous; 1 = leaves succulent
6	Number of outer bracts	Discrete	
7	Number of pappus bristles	Discrete	Refers to number of setae not number of bristles on setae
8	Length of innermost bracts	Continuous	
9	Length of outermost bracts	Continuous	
10	Length of pappus bristles	Continuous	Refers to pappus bristles, not setae on bristles
11	Length of corolla tube	Continuous	Measurement at mature female stage
12	Corolla tube diameter a base	Continuous	Measurement at mature female stage
13	Corolla lobe length	Continuous	Measurement as mature female stage
14	Length of leaf subtending fascicles	Continuous	Only mature leaves measured
15	Shape of leaves subtending fascicles (length / width)	Continuous	
16	Fascicle leaf length	Continuous	
17	Shape of leaves in fascicles (length / width)	Continuous	
18	Thickness of leaf adaxial surface tomentum	Ordinal	0-glabrous; 1-light; 2-moderate; 3-heavy
19	Thickness of leaf abaxial surface tomentum	Ordinal	0-glabrous; 1-light; 2-moderate; 3-heavy
20	Number of innermost bracts	Discrete	
21	Ovary shape	Nominal	0 = rectangular; 1 = oval; 2 = circular
22	Percentage setae cover on ovary	Ordinal	0, 25%, 50%, 75% or 100%
23	Ovary length	Continuous	Measurement at mature female stage before evidence of seed set appeared
24	Width of corolla near apex	Continuous	Measurement made immediately below corolla lobes
25	Ovary width	Continuous	
26	Style length	Continuous	
27	Length of stigma	Continuous	Length of the bifurcated stigmatic surface
28	Width of style halfway along length	Continuous	
29	Width of style at base	Continuous	
30	Length of trichomes on ovary	Continuous	
31	Length of sweeping hairs at stigma apex	Continuous	
32	Pollen grain diameter across exine	Continuous	
33	Pollen grain dimeter across intine	Continuous	

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Chapter 3: A revision of the *Seriphium plumosum* L. complex (Gnaphalieae: Asteraceae): segregation of the complex into eight species, with description of four novel species[2]

Introduction

Building on the works of Levyns (1937) and Koekemoer (2016), the current work (Chapter 2) uses a combination of genetic, morphological, ecological and geographical evidence to demonstrate that the evolutionary diversity within the *S. plumosum* clade is best described through the recognition of nine species. Although none of the taxonomic revisions of *S. plumosum* up to and including Koekemoer (2016) explicitly defined the species concepts followed, that of Levyns used consistent morphological differences coupled with ecology, while that of Koekemoer used a 'key character' approach, selecting a single character that she considered to be most discriminatory, and using geographic distribution in the absence of such a character. The species concept adopted in this work is the general lineage concept of species as defined by de Queiroz (1998). While this theoretical concept defines species as independent evolutionary entities (Fujita et al., 2012) and provides no operational criteria for developing hypotheses about species boundaries, it does not deny the existence of morphological and/or ecological differences between evolutionary species. The operational criteria used to infer species boundaries in *Seriphium plumosum* in the present work are, therefore, evidence of genetic isolation between taxa, inferred using the Bayes Factor Delimitation method developed by Leaché et al. (2014) and genetic population assignment analysis (sNMF), together with evidence of ecological and/or morphological differences (particularly synapomorphies) between taxa, as well as differences in the geographic distributions of the species. Operationally, I consider this to be an integrative approach, since a range of data types are used to inform species boundaries.

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[2] Note that the taxonomy presented in this Chapter is strictly provisional, and does not constitute a formal taxonomic revision of either *S. cinereum* or the *S. plumosum* complex. As such, type specimens are not allocated.

Species description

A conservative approach was taken in the formulation of the taxonomy of the *S. plumosum* clade so as to avoid taxonomic inflation. Cryptic evolutionary lineages were consolidated into morphologically cohesive evolutionary species. On average, 84% of specimens housed in BOL and NBG could be confidently assigned to one of the nine species in the *S. plumosum* clade on the basis of the features detailed in the taxonomic key below. The remaining specimens, resembling 2-3 species, mostly in *S. crypticum* and the coastal-Little Karoo clade (Chapter 2, Figure 6, clade 3), could not be assigned to any single species with confidence. Type specimens were consulted at BOL, NBG and PRE, or digital images were examined from B, P, SBT and UPS. In the sections that follow, I briefly outline the principal arguments justifying the recognition of nine species within the *S. plumosum* clade.

Seriphium cinereum L., the type species of *Seriphium*, was considered by Harvey (1865) in his treatment of the species in *Stoebe*, to encompass the *S. plumosum* clade as defined here. Although Harvey noted marked variation in the leaves under his concept of the species, he followed Lessing's (1832) convention in uniting the three varieties within *Stoebe cinerea* under one specific name. Levyns (1937) considered *Stoebe cinerea* to be a species distinct from the remainder of the *S. plumosum* clade, with a fairly limited distribution in the mountains of southwestern Cape. Her concept of the species included branched shrubs with spicate synflorescences and linear, twisted leaves, also characterised by transverse lamellae on the cypselae. Although I have not been able to examine the cypselae in all specimens (mature cypselae were not present), the rest of her description closely matches the lectotype for *Seriphium cinereum* (LINN 1049.3; designated in Jarvis & Turland, 1998). Koekemoer (2016) had a very different concept of the species. Her concept of *Seriphium cinereum* L. embraced a broader assemblage of plants in terms of distribution and morphology, including plants from the Little Karoo and summer-rainfall region of South Africa which were formerly included in *Stoebe burchellii* Levyns and *Stoebe vulgaris* Levyns. All of the characters used above for defining *S. cinereum* are problematic. In the case of Levyns' treatment, the lamellae near the apex of the cypselas used to define *Stoebe cinerea* are not visible in scanning electron microscope images of the cypselas (Koekemoer, 2016) and in the case of Koekemoer's treatment, the prominence of the leaf resin cavities used to define the species is problematic because size of the resin cavity can vary considerably within species in *Seriphium* as circumscribed here (personal observation), and because any assessment of what constitutes a prominent resin cavity is invariably subjective. The taxon circumscribed by Koekemoer's (2016) resin character is ecologically implausible. Almost no Cape species occur both in the winter-precipitation region of the southwestern Cape in isolated populations from the Eastern Cape escarpment, Mpumalanga and Limpopo provinces. As such, I propose that the specimens cited by Koekemoer (2016) under this taxon in the summer-rainfall region represent the larger end of the range of leaf cavity and leaf length size variation in species of *Seriphium* distributed in these areas. The concept of *Seriphium cinereum* L. presented here coincides exactly with Levyns' (1937) concept of the species, including green sub-shrubs confined to moist mountain slopes in the Western Cape (Figure 1; Figure 8) and distinguished by spicate synflorescences and linear leaves that are always reflexed (straight in the other members of the *S. plumosum* clade). This circumscription includes a suite of plants which are morphologically cohesive and which closely resemble the lectotype of the species, and is also supported by genetic population assignment analysis (Chapter 2, Figure 3) and coalescent-based delimitation (Chapter 2, Table 1) as genetically distinct.

Seriphium alto-argillaceum ('montane dwarf'), newly described in the present study, is known from a single population on the slopes of Conical Peak in the Hex River valley of the Western Cape (Figure 1, Figure 2). This species would formerly have been included in *S. plumosum*, both in Koekemoer's (2016) broad and in Levyns' (1937) narrower concepts. It resembles *Seriphium crypticum* and *Seriphium plumosum* (as defined here) in their high-elevation forms, but is distinguished by its leaves, which are imbricate, non-fascicled, and closely arranged around the stem, largely hiding the latter (Figure 3). Although imbricate leaves have been observed in *Seriphium crypticum*, *S. alto-argillaceum* is the only species in the *S. plumosum* clade which possesses all three leaf characters described above. As with *Seriphium cinereum*, this species is supported by genetic population assignment analysis (Chapter 2, Figure 3) and coalescent-based delimitation (Chapter 2, Table 1) as genetically highly distinct. Interestingly, *S. alto-argillaceum* and *Seriphium plumosum* (as defined here) are mostly separated by elevation and soil differences, co-occurring only at around 1900 metres elevation; where *Seriphium plumosum* occurs on quartzite below 1900 m elevation, *S. alto-argillaceum* occurs on shale above 1900 m elevation. The distinctness of these species in the zone of contact qualifies them as biological species *sensu* Mayr (1969), ipso facto supporting their distinctness and status as two independent evolutionary species.

Stoebe burchellii Levyns ('Little Karoo') is revived in this treatment as an independent species in the genus *Seriphium*. Plants formerly included in Levyns' (1937) species *Stoebe burchellii* are currently included, under the concepts of Koekemoer (2016), in both *Seriphium cinereum* and *Seriphium plumosum*. However, both the genetic population assignment analysis (Figure 3) and phylogenetic relationships (Figure 6) presented in Chapter 2 are inconsistent with such a grouping, instead supporting Levyns' (1937) original concept of the species. In the species tree (Chapter 2, Figure 6), this species is recovered in a clade with the species distributed along the southern coast. The concept presented here coincides exactly with Levyns' (1937) concept of the species, including green shrubs with an erect growth form (i.e. with an acute orientation of branches) and minute, ovate, obtuse leaves arranged in fascicles. *Seriphium burchellii* is supported as morphologically distinct, primarily of the basis of leaf and corolla shape (Chapter 2, Figure 4, cluster b). The species has a core distribution in the Little Karoo above 600 m elevation on shale and sandstone soils (Figure 1, Figure 4). *Seriphium burchellii* co-occurs with *Seriphium plumosum* (as defined here) on the Swartberg Pass at 1500 m elevation (Chapter 1, Figure 3c). Both species maintain their distinctness in sympatry, which identifies them as good biological species *sensu* Mayr (1969).

Seriphium burrowsii ('escarpment') is also presented here for the first time as a distinct species. It is known from Pilgrim's Rest and Buffelskloof Private Nature Reserve, both above 1600 m elevation in the far northern part of the summer-rainfall region of South Africa (Figure 1, Figure 6). This species is included in *Seriphium plumosum sensu* Koekemoer (2016), but its status as an independent species is motivated by the genetic distinctness and cohesiveness of the two populations, both of which were represented in the genetic population assignment (Figure 3) and coalescent-based delimitation analyses (Table 1) presented in Chapter 2. Many morphological similarities exist between *S. burrowsii* and *Seriphium vulgaris* (as defined here), which suggested initially that the two were likely sibling species. The tree presented in Chapter 2 (Figure 6), however, strongly supports *S. burrowsii* as being part of the clade that includes *Seriphium cinereum, S. alto-argillaceum*, and *S. crypticum*, and as more distantly related to *Seriphium vulgaris* (as defined here). Morphologically, this species is defined by its height (up 1.2 metres tall) and circumference greater than 50 centimetres due to its very ramified growth form, which makes it the largest-bodied species in the *S. plumosum* clade. The species is also defined by the right-angled orientation of its branches.

Seriphium crypticum ('montane SW'), presented in this work as a new species, is named for its strong resemblance to *Seriphium plumosum* (as defined here). The two species can only be discriminated using a combination of features of the leaves, and even these differences are subtle. Unlike *S. plumosum* (as defined here) with its distribution in much of the Cape Fold Belt mountains and over a large elevational range, *S. crypticum* is known from only a few populations distributed in the southwestern Cape mountains, from Wellington Sneeukop south to Jonaskop and Victoria Peak above 1000 m elevation (Figure 1; Figure 10). The chief motivation for the independent species status of *S. crypticum* is the genetic evidence presented in Chapter 2, where coalescent-based delimitation (Table 1) gives the strongest support for the species as being distinct from *S. plumosum* (as defined here), and very poor support for a scheme that lumps it with *S. plumosum* (as defined here). Genetic population assignment analysis (Chapter 2, Figure 3) also supports one or two distinct ancestral gene pools within the species (within the eight populations of the species included). One of the strongest arguments for the recognition of this species as separate is the topology of the species tree presented in Chapter 2 (Figure 6), where the species is placed, with high support, as only distantly related to *S. plumosum* (as defined here). Moreover, the two species appear to remain distinct in spite of their geographical proximity, although it is possible that they have elevationally non-overlapping ranges.

Seriphium dunensis (‘littoral’), is presented as a new species in this work on the basis of its ecological, morphological, and genetic distinctness. This species corresponds with the ‘possibly new coastal form’ mentioned by Koekemoer (2016), which she describes as occurring on littoral dunes and having ‘somewhat fleshy leaves of which the abaxial surface is covered with wart-like wax globules’. She, however, deferred describing it as a new species pending more detailed investigation of the *S. plumosum* complex. Based on the list of specimens cited by Levyns (1937) under *Stoebe plumosa*, she appears to have included *S. dunensis* in her concept of *Stoebe plumosa* (L.) Thunb., a grouping which is incompatible with the Beast 2 tree for the clade (Chapter 2, Figure 6), which resolves these species in two separate clades. There is a close resemblance between *S. dunensis* and its sibling species, *Seriphium virgatum* (Chapter 2, Figure 4, Figure 6), and an argument could be made for consolidating the populations distributed near the coast in the *S. plumosum* clade into a single species. Genetic population assignment (Chapter 2, Figure 3a-c) and coalescent-based delimitation analyses (Chapter 2, Table 1), however, reveal a single distinct ancestral gene pool within *S. dunensis*, and its genetic distinctness and isolation from *S. virgatum* (as defined here). *Seriphium dunensis* and *S. virgatum* have been observed in close proximity (1-2 km) at both Oyster Bay and Pearly Beach, which identifies them as biological species. *Seriphium dunensis* is also supported as distinct on the basis of its morphology (Chapter 2, Figure 4, cluster a) and ecology (Chapter 2, Figure 5, cluster b); the species is distinguished by its branches, which are supple, flexible, and whip-like; its leaves, which are always completely white, obtuse, and succulent; and by its occurrence only on unconsolidated coastal dune fields at elevations less than 10 m between Hout Bay near Cape Town in the west, to the Fish River valley in the east (Figure 1, Figure 12).

Seriphium plumosum as circumscribed by Koekemoer (2016) encompasses all of the species in the *S. plumosum* clade, excepting *Seriphium cinereum* (as defined here). Koekemoer (2016) described *Seriphium plumosum* (*sensu* Koekemoer, 2016) as “almost impossible to separate into any well-defined forms”, a view shared by Levyns (1937), even with her more limited concept of the species excluding *Stoebe vulgaris* Levyns and *Stoebe burchellii* Levyns. The genetic population assignment (Figure 3) and coalescent-based delimitation (Table 1) analyses presented in Chapter 2 identified three genetically distinct groups of populations with non-overlapping distributions in the Cape Fold Belt mountains and valleys. The first of these three groups is distributed as far north as Moltke Blick in Namibia, south to the mountains of the Cedarberg area in South Africa from Vanrhynsdorp to Dutoitskloof, the second further south from Houw Hoek to Elim, and the third in the east-west-trending range of the Cape Fold Belt mountains from the Seweweekspoortberg to the Swartberg Pass. The genetic distinctness of the three groups of populations qualifies each as evolutionary species *sensu* de Queiroz (1998), but given the limited sampling of populations in this work and the apparent existence of ongoing or ancestral gene flow between them (Chapter 2, Figure 3), the concept of *S. plumosum* embraced here consolidates the three groupings of populations (‘montane N’ + ‘montane S’ + ‘montane E’). Such a scheme reflects a conservative approach to the taxonomy of the clade containing *S. plumosum* and *S. vulgaris* (Chapter 2, Figure 6, clade 2) that is not inconsistent with the evolutionary species concept, and which also better facilitates the identification of *Seriphium plumosum* given that the three genetic groupings in my concept of the species are cryptic. The phylogenetic tree presented in Chapter 2 (Figure 6) supports *Seriphium vulgaris* (as defined here) as embedded within the clade that contains the three genetic groupings in *Seriphium plumosum*. My concept of *S. plumosum* as distinct from *Seriphium vulgaris* (as defined here), therefore, renders it, albeit with weak support, paraphyletic (Chapter 2, Figure 6).

Notwithstanding the more limited assemblage of plants included in this concept of *Seriphium plumosum* relative to the treatments of Levyns (1937) and Koekemoer (2016), the species is still the most morphologically variable in the *S. plumosum* clade, and is only distinguishable on the basis of multiple, often subtle, characters of the leaves (detailed below). The species' extensive geographical distribution in much of the Cape Fold Belt mountains at between 100 and 2000 m elevation (Figure 1, Figure 14), relative to the more limited known distributions of *S. crypticum* and *S. alto-argillaceum* in the southwestern Cape above 1000 m elevation, also facilitates discrimination between the species.

Stoebe virgata Thunb. ('coastal plain E' + 'coastal plain W') is revived in this treatment as an independent species in the genus *Seriphium*. The species was first described by Thunberg (1800) as a shrub with linear, obtuse, tomentose leaves, and terminal, spicate synflorescences, a description consistent with the lectotype of the species designated by Koekemoer (2016). Ninety percent of the genetic population assignment solutions (Figure 3a-c) and the coalescent-based delimitation analysis (Table 1) presented in Chapter 2 support two distinct lineages that qualify as evolutionary species *sensu* de Queiroz (1998) distributed on the coastal plains within the *S. plumosum* clade. These two lineages have non-overlapping distributions on the southern coast of South Africa; one being distributed between Pearly Beach and Still Bay, and the other being distributed between Plettenberg Bay and Van Stadens River near Humansdorp. Because the two lineages are completely cryptic, the concept of *Seriphium virgatum* embraced in this work includes both the east- and west-distributed lineages. The phylogenetic tree associated with the most complex species delimitation hypothesis (Chapter 2, Table 1; phylogeny not presented) supports the two lineages as sibling, making *S. virgatum* (as defined here) a monophyletic evolutionary species. As for most of the other species in the *S. plumosum* clade*, S. virgatum* is defined by its leaves, which are non-succulent, acuminate or obtuse, and grey to white. These features, in addition to the species' occurrence only on consolidated calcareous substrates on coastal plains between 10 m and 550 m elevation (Figure 1; Figure 17), facilitates discrimination between *S. virgatum* and its closest relative, *S. dunensis.*

Finally, *Stoebe vulgaris* Levyns (‘eastern SA’) is revived in this work as an independent species in the genus *Seriphium*. Levyns (1937) first described *Stoebe vulgaris* as a new species on the basis of its acuminate bracts, its pappus with approximately 12 bristles, and its leaves, which are usually less than three millimetres long. This description is problematic because all of these features are observed in other species in *Stoebe* as defined by Levyns (1937). Levyns’ (1937) confidence in the independence of *Stoebe vulgaris* appears to have been based instead on her assessment of the phenological and ecological differences between *Stoebe plumosa* (*sensu* Levyns) and *Stoebe vulgaris*. She noted that while *Stoebe vulgaris* grows in regions with summer rainfall, regenerates by resprouting after fire, and has cypselas that germinate immediately after being shed, *Stoebe plumosa* (*sensu* Levyns) grows in mainly in regions with winter rainfall, regenerates after fire by reseeding, and has cypselas that remain dormant for a year before they germinate. Although Levyns (1937) noted that *Stoebe vulgaris* has belowground root storage structures which are absent in *Stoebe plumosa*, she failed to cite the storage structures as a defining feature of *Stoebe vulgaris* in the key she developed for the genus *Stoebe*. The concept of *Seriphium vulgaris* adopted here coincides with Levyns’ (1937) original concept of the species. *Seriphium vulgaris* has a distribution extending from near Umtali in Zimbabwe, south through the summer-rainfall region of South Africa to near Queenstown in the eastern Cape (Figure 1, Figure 19), making it the most widespread species in the *S. plumosum* clade. Although there is some support for ancestral or ongoing gene flow between *Seriphium vulgaris* and *Seriphium plumosum* (as defined here) in the southern part of the distribution of *Seriphium vulgaris* (Chapter 2, Figure 3), overall, the genetic population assignment and the coalescent-based delimitation analyses presented in Chapter 2 support *Seriphium vulgaris* as a distinct and genetically cohesive species. The independence of *S. vulgaris* is further supported by its morphological distinctness (Chapter 2, Figure 4, cluster c), the defining feature of the species under this circumscription being the presence of belowground root storage structures.

Morphological features of the *S. plumosum* clade

Habit: The plants are all perennial much-branched shrubs or sub-shrubs between 15 cm and 2 m tall. The branches are oriented at right angles (divaricately) or at angles less than 90 degree (erect), and are either rigid, or supple, flexible and whip-like.

Leaf arrangement, shape and size: The leaves are highly variable in their shape, size and arrangement, and often form the basis for distinguishing between species in the *S. plumosum* clade. The leaves are either borne singly or arranged in brachyblasts/fascicles. The leaves subtending the fascicles are up to 7 mm long, while the leaves within fascicles (if present) are up to 3 mm long. The leaf blades are either involute or straight, and succulent in *S. dunensis,* and schlerophyllous in other species within the *S. plumosum* clade. In S. cinereum, the leaf blade reflexed, while in the other species in the clade, the leaves are non-reflexed. In *S. alto-argillaceum* and certain individuals in *S. crypticum*, the leaves are imbricate, while in all other species in the *S. plumosum* clade, the leaves are non-imbricate. The leaf apex is either mucronate or obtuse. Leaf shape is ovate, triangular (awl-shaped) or linear.

Resin cavities: Two resin cavities are embedded at the base of the leaves, one on either side of the leaf midrib. The presence of resin cavities was used by Koekemoer (2016) to define *S. cinereum,* but this feature is universally present among all species within the *S. plumosum* clade. When well developed, the cavities are conspicuous on the abaxial surface of mature leaves.

Indumentum: The stems and abaxial and adaxial surfaces of the leaves are covered by an indumentum than ranges from light to dense. The density of the indumentum gives the entire plant an overall green or grey/white appearance.

Capitulum sex: Each capitulum contains a single bisexual floret.

Arrangement of synflorescence: The arrangement of the synflorescence has been used as a taxonomic character by Linnaeus (1753), Gaertner (1791), Thunberg (1800), Lessing (1832) for defining *S. cinereum* and *S. plumosum*. The capitula are numerous, being arranged into loose clusters which in turn are arranged into a loose paniculate. Alternatively, the capitula are compacted and form glomerules, which are arranged along the stem to form a continuous or interrupted spike.

Involucral bracts: The involucral bracts are arranged in a few rows. The bracts are free and acuminate, with an opaque, orange-brown lamina and an undivided stereome. The innermost bracts are papery and membranous and up to 5.1 mm long. The outermost bracts are up to 4.2 mm long and have a papery and membranous lamina, and a cartilaginous or leaf-like stereome with or without a light tomentum.

Corolla: The corolla is 5-lobed, tubular and up to 2.3 mm long. The corolla is white-yellow all along its length and pink-plum coloured at the apex at anthesis. The corolla lobes are small, triangular, and erect. In rare cases, there are trichomes on the external surface of the corolla lobes.

Style: The style is bifid, and exerted and reflexed at maturity. The stylar apex (the apex of the two stigmatic arms) is either smooth or furnished with fine sweeping hairs. The style base often has a conspicuous swelling, but the swelling is generally absent. The stigmatic lobes also often have sparse sweeping hairs, but generally these are absent.

Stamens: There are five stamens. The anthers have lanceolate basal appendages.

Pollen: Pollen is abundant. The surface of the pollen grains is spinulose.

Cypselae: The cypselae are minute, 5-ribbed, elliptical and scabrid. Levyns (1937) used the presence of transverse lamellae on the cypselae to define *S. cinereum*. I consider that the lamellae referred to by Levyns are in fact trichomes, which are either present near the cypsela apex, or distributed over the entire surface of the cypsela. In rare cases, the trichomes are absent.

Pappus: The pappus is plumose and has up to 16 bristles, each of which is up to 3.4 mm long. The bristles are free or basally connate. The pappus remains loosely attached to the seeds when the seeds are shed. The pappus bristles may be basally fused to form a small rim termed an *annulus.* Lessing (1832) and Candolle (1838) used the presence of the annulus in *Stoebe*, and its absence in *Seriphium*, for separating the two genera. The annulus is highly variable, being present and absent in the same population (personal observation). As such, I agree with Harvey (1865) in considering it to be an uninformative taxonomic character.

Roots: A rootstock is present in post-fire resprouters in species distributed in the summer-rainfall part of South Africa, and absent in reseeders.

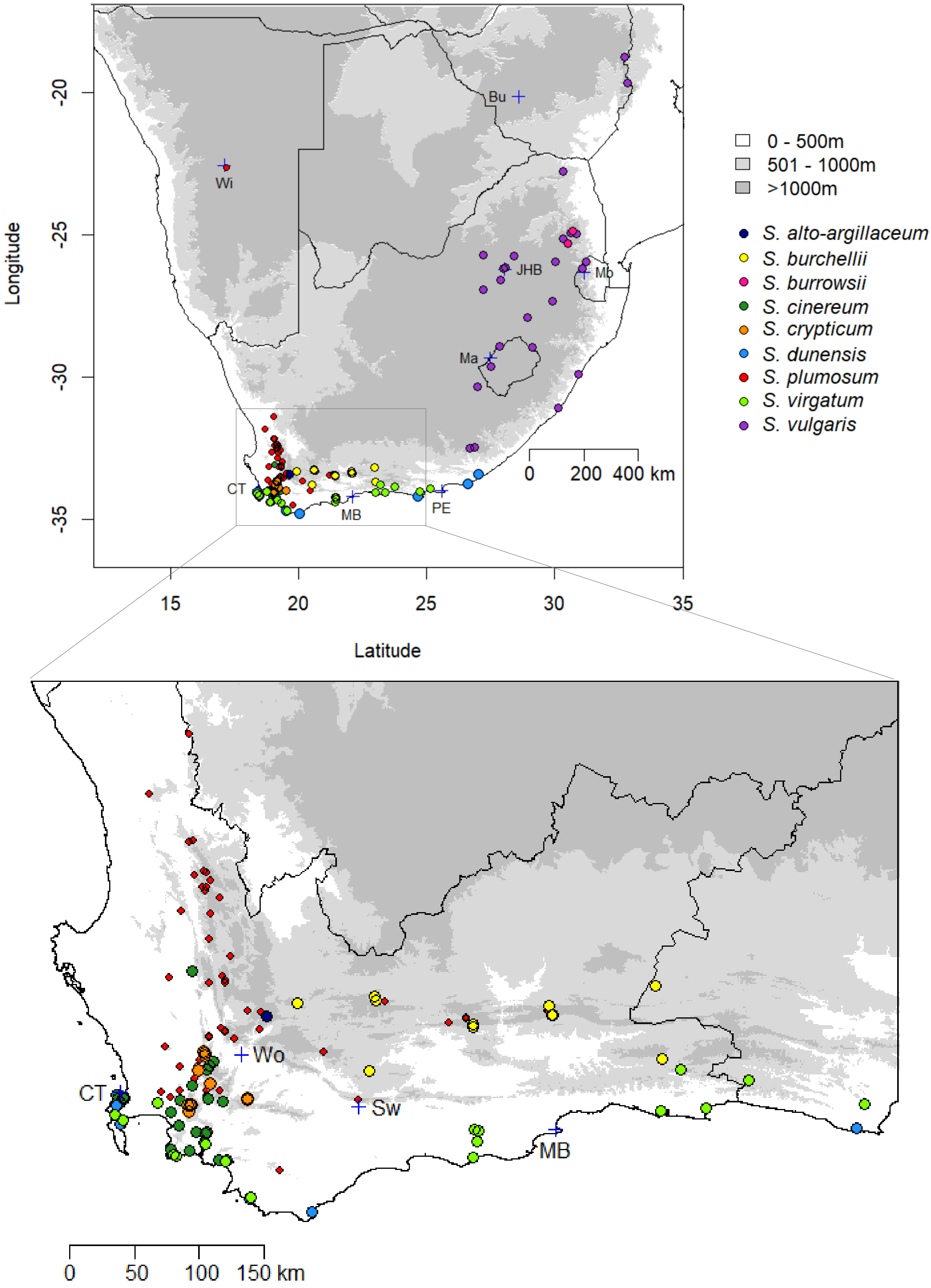

Figure 1: Known distribution of the nine proposed species in the *S. plumosum* clade in South Africa, Lesotho, Namibia, Swaziland and Zimbabwe. Shadings of the elevation layer represent 500 m intervals. Major cities and towns are also shown (CT – Cape Town, Wo – Worcester, Sw – Swellendam, MB – Mossel Bay, PE – Port Elizabeth, Ma – Maseru, JHB – Johannesburg, Mb – Mbabane, Bu – Bulawayo, Wi - Windhoek).

Key to the species

1.) Branches supple, flexible, and whip-like, leaves succulent, confined to littoral dune habitats.......... **6.** *Seriphium dunensis*

Branches rigid, leaves not succulent, not occurring in littoral dune habitats.......... 2

2.) Leaves reflexed.......... **4.** *S. cinereum*

Leaves not reflexed 3

3.) Leaves not in fascicles, adpressed, densely imbricate and obscuring the stem .... **1.** *S. alto-argillaceum*

Leaves in fascicles, erect to spreading, loosely imbricate or non-imbricate, stem visible between leaves 4

4.) Belowground rootstock present, plant usually with multiple slender resprouting stems.......... **9.** *S. vulgaris*

Belowground rootstock absent, plant usually branching from near the base.......... 5

5.) Dense, tangled dark green shrub, adult plants ≥1 m tall and ≥50 cm in circumference, branching at 90° .... **3.** *S. burrowsii*

Loosely or densely branched shrub or sub-shrub, light green or grey-green, <1 m tall and <50 cm in circumference, branching angle acute, ≤90°.......... 6

6.) Leaves lightly tomentose, never densely tomentose, green, minute, ovate, up to 1.3mm long .... **2.** *S. burchellii*

Leaves lightly to densely tomentose, green, grey or white, linear or triangular, longer than 1.3mm 7

7.) Plant height up to 1.5 m, leaves lightly tomentose, green or grey, synflorescence paniculate **8.** *S. virgatum*

Plant height less than 1 m, leaves lightly to densely tomentose, synflorescences spicate or paniculate.......... 8

8.) Leaves and stem with dense tomentum, leaves in poorly-defined fascicles, imbricate or non-imbricate, orientation of branches at less than right angles, synflorescences spicate.......... **5.** *S. crypticum*

Leaves and stem with light to dense tomentum, leaves in well-defined fascicles, non-imbricate, orientation at right angles or lower than right angles, synflorescence spicate or paniculate.......... **7.** *S. plumosum*

Species treatment[3]

1. *Seriphium alto-argillaceum* Z. Shaik, sp. nov. ms.

Compact, single-stemmed grey-green sub-shrub, up to 15 cm tall. *Branching angle* acute. *Branches* rigid, densely tomentose; terminal and secondary branches terminating in synflorescences. *Leaves* fascicled or non-fascicled, densely imbricate and adpressed to stem, up to 7 mm long, linear, straight; apex sharply mucronate or obtuse; both leaf surfaces densely tomentose; non-succulent. Leaves within fascicles (if present) ovate and up to 1.8 mm long; basal resin cavities not visible on outside of leaf, dead leaves near base of plant retained, leaves above photosynthetic. *Synflorescence* a continuous, erect spike, terminal on primary and secondary branches. *Outer involucral bracts* up to 5, up to 4.1 mm long with cartilaginous stereome bearing a light tomentum. *Inner involucral bracts* up to 5, up to 5.1 mm long. *Corolla* tubular, up to 2.7 mm long. Style up to 3.1 mm long, apex with sweeping penicillate hairs, stigmatic lobes without hairs. *Pappus* comprising up to 14 bristles, each up to 2.5 mm long. *Cypselae* ovate with a dense covering of penicillate trichomes. *Flowering* observed in April.

Diagnostic characters: Closely resembles the high elevation forms of *Seriphium plumosum* and *Seriphium crypticum,* but is distinguished from both by its leaves, which are always densely tomentose, linear, generally not fascicled, and closely adpressed to the stem, which is completely obscured by the leaves. *Seriphium alto-argillaceum* is also distinguished from these two species by its smaller stature and more compact form.

Distribution and ecology: Known from a single locality in the Hex River mountains of the Western Cape, on a north- and northeast-facing slope of Conical Peak near to Matroosberg above 1900 m elevation. This population comprises approximately 100 plants within an area of about 500m^2 confined to dark grey, thinly laminated to massive shale soils.

Notes: Upon discovery of this unique population, similar high-elevation (1900 m – 2000 m) shale habitats in the Hex River Mountains were sought that might harbour other populations. The only other peak with a high-elevation shale band in the Hex River Mountains accessible by hiking is Groothoek Peak, west of the Matroosberg. An expedition to Groothoek Peak in April 2018 revealed no other populations.

Specimens examined: South Africa. Western Cape: 3319 (Ceres): Conical Peak, Hex River valley (-BC), 29 April 2017, *Shaik 2* (NBG).

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[3] Note that the taxonomy presented in this Chapter is strictly provisional, and does not constitute a formal taxonomic revision of either *S. cinereum* or the *S. plumosum* complex. As such, type specimens are not allocated. The abbreviation “ms” indicates manuscript name.

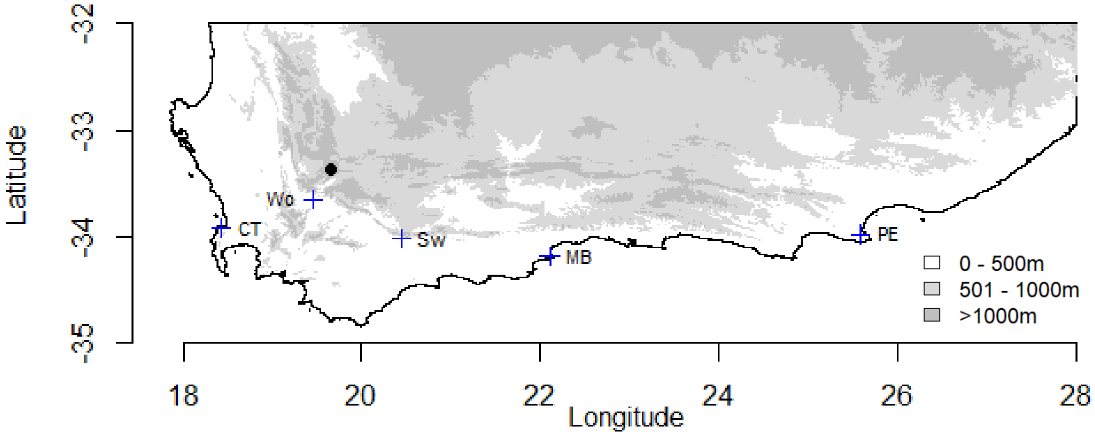


Figure 2: Distribution of Seriphium *alto-argillaceum* (circle). Major towns are indicated with crosses (CT - Cape Town, Wo – Worcester, Sw – Swellendam, MB – Mossel Bay, PE – Port Elizabeth).

Figure 3: Scanned image *Seriphium alto-argillaceum* (*Shaik 2*, NBG). Note the grey-green foliage, very small branching angle, lack of brachyblasts, twigs that appear thick due to the adpressed, imbricate leaves, and uninterrupted spicate synflorescences.

2. *Seriphium burchellii* (Levyns) Z. Shaik, comb. nov. ms. *Stoebe burchellii* Levyns. J. S. Afr. bot. p. 18 (1937).

Single-stemmed, much-branched, woody, green shrub, up to 2 m tall. *Branching angle* acute. *Branches* rigid; lightly tomentose. *Leaves* minute, ovate, straight; apex obtuse, lightly tomentose adaxial surface, glabrous abaxial surface; non-succulent; in alternately-arranged fascicles; leaves subtending fascicles up to 1.3 mm long, leaves within fascicles up to 1.1 mm long; basal resin cavities prominent. *Synflorescence* a panicle. *Outer involucral bracts* up to 21, up to 1.3 mm long with leaf-like stereome, glabrous or hairy. Inner involucral bracts up to 7, up to 3 mm long. *Corolla* up to 2.1 mm long. *Style* up to 2.5 mm long; apex with or without sweeping penicillate hairs; stigmatic lobes without hairs. *Pappus* with up to 15 bristles, each up to 1.9 mm long. *Cypselas* with penicillate trichomes near apex and glabrous below. *Flowering* March-June, but also recorded in November and January.

Diagnostic features: This species is defined by minute, lightly tomentose, green, ovate leaves generally less than 1mm long, and synflorescences that are exclusively paniculate. *Seriphium burchellii* closely resembles *Seriphium virgatum*, but is distinguished by its ovate leaves, which are less than 1.3 mm long, the leaves in *Seriphium virgatum* being ovate or linear and generally longer than 2.0 mm.

Distribution and ecology: On mountain slopes in and around the Little Karoo from near the Touws River, south and east to the Langkloof, above 500 m elevation on shale and sandstone soils. Although the distribution is large, it is highly fragmented as the species does not extend to the valley bottoms or the mountain tops.

Notes: A written correspondence between Margaret Levyns and Mrs Milne-Redhead at Kew Gardens, housed in the *S. plumosum* collection at the Bolus Herbarium, details the lack of agreement (in morphology) between the type for *Stoebe virgata* Thunb. and a fragment of *Stoebe burchellii* Levyns sent to Kew. Levyns (1937) appears to have relied on this account in describing *S. burchellii* as a new species.

Specimens examined: South Africa. Eastern Cape: 3324 (Hankey): Baviaanskloof, Eland vlakte (-CB), 17 Jan 1978, *Bond 1311* (NBG). **Western Cape: 3319 (Ceres)**: Gouronna, kloof in Bonteberg (-BD), 3 Nov 1940, Esterhuysen 3717 (BOL); 50km out of Ceres along R46/R355 Kareekloof Private Nature Reserve (-BA), 16 July 2003, *Murray HMM 119* (NBG). **3319 (Montagu)**: Koo mountains (-DB), 28 Dec 1940, *Bond 801* (NBG); Robertson, Montagu (-CC), Moss 5382 (J). **3320 (Ladismith)**: Touwsberg, S slopes (-DB), 1 June 1956, *Esterhuysen 25909* (BOL); Kloof on S slopes of the Anysberg (-DA), 22 May 1950, *Esterhuysen 17076* (BOL). **3320 (Laingsburg)**: Witteberg, Maggisfontein (-BA), *Rehmann 2931* (BOL); Witteberg kloof, along stream and alluvium (-BC), 16 July 1923, *Compton 2504* (BOL). **3321 (Calitzdorp/Oudtshoorn)**: Calitzdorp district, Gamka Reserve (-DB), 25 May 1975, *Esterhuysen 33864* (BOL). **3321 (Ladismith)**: Seven Weeks Poort, roadside (-AD), July 1936, *Levyns 5525* (BOL); Swartbergen [Swartberg] lower S slopes below Towerkop (-AC), 23 Apr 1951, *Esterhuysen 18555* (BOL); Seven Weeks Poort (-AD), 23 March 1940, *Compton 8607* (NBG). **3321 (Laingsburg)**: Seven Weeks Poort (-AD), Dec 1946, *Stokoe 9412* (BOL); Seven Weeks Poort (-AD), Dec 1946*, Stokoe 9412* (BOL). **3322 (Prince Albert)**: Zwartberg Pass, N slopes (-AC), July 1935, *Levyns 5036* (BOL); on N side of Swartberg Pass, base of road few kilometres above Malvadraai (-AC), 27 June 2005, *Bergh 1465b* (NBG). **3322 (Oudtshoorn)**: Klein Swartberg, Swartberg Pass, southern slopes of mountain on pass road (-AC), 10 Apr 2004, *Bergh 1289* (NBG); Swartberg Pass, southern slopes of mountain on southern slopes (-AC), 10 Apr 2004, *Bergh 1292* (NBG). **3323 (Joubertina)**: Louterwater, Joubertina (-DC), 2 Jan 1953, *Compton 23895* (NBG); Die Hoek, N foot of Tsitsikamma mountains near Joubertina (-DC), March 1954, *Esterhuysen 22819* (BOL); Louterwater (-DC), 27 Jan 1941, *Esterhuysen 4620* (BOL). **3323 (Uniondale)**: top of pan between Avontuur and Uniondale (-CA), March 1922, *Fourcade 2097* (BOL); Kammanassie valley, 8 miles from Uniondale (-CA), Jan 1928, *Fourcade 3602* (BOL).

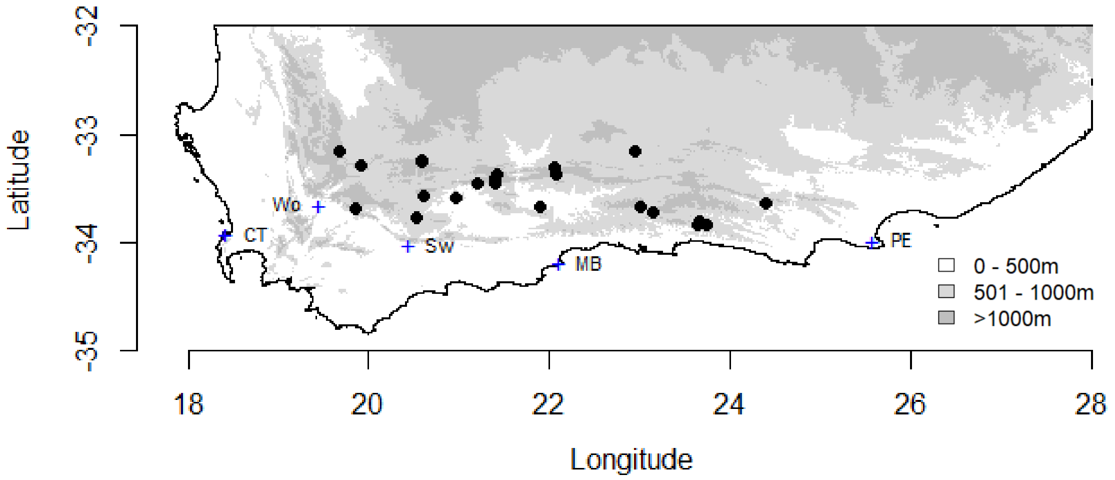
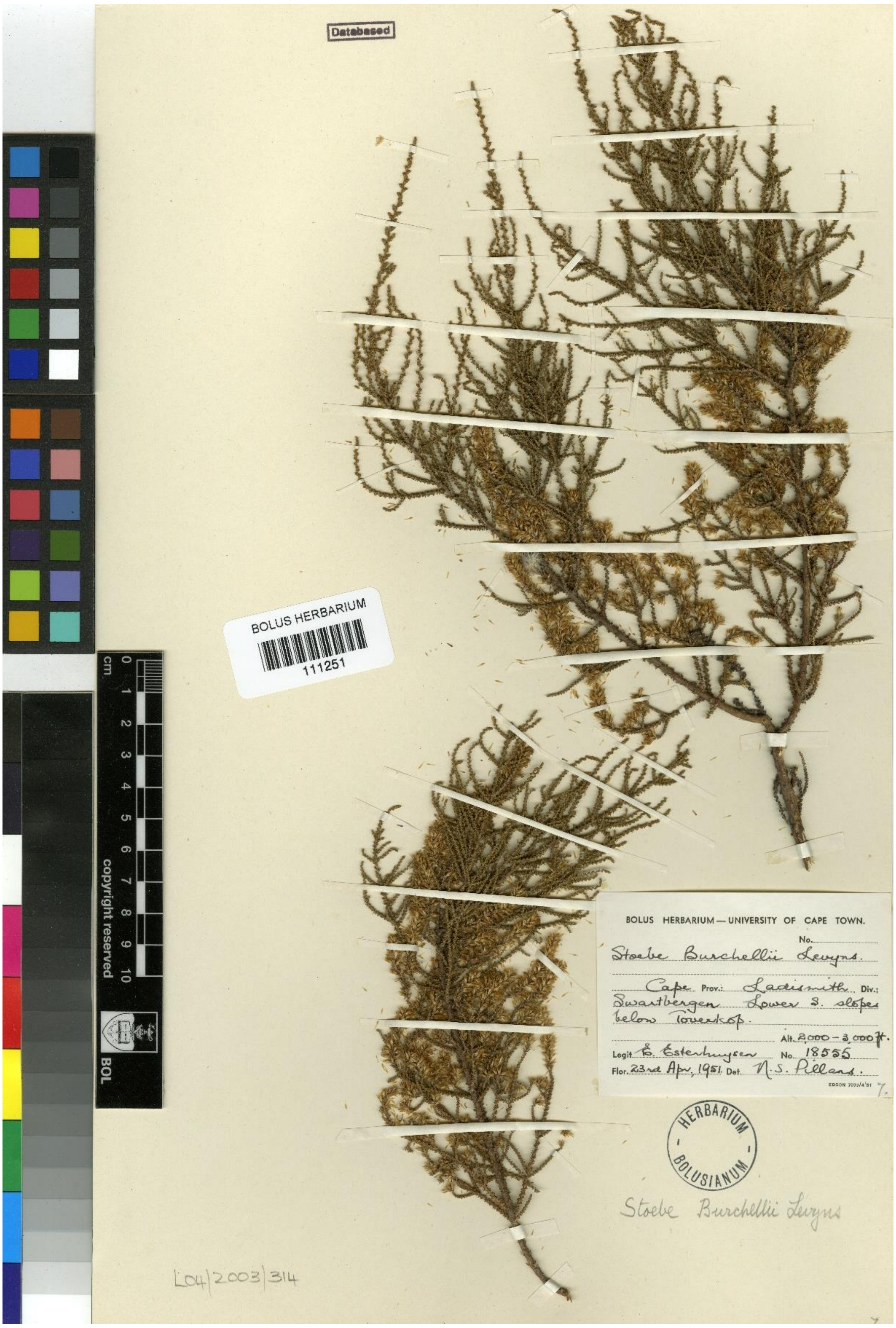


Figure 4: Distribution of *Seriphium burchellii* (circles) from verified herbarium specimens and collections. Major towns are indicated with crosses.



BOLUS HERBARIUM — UNIVERSITY OF CAPE TOWN.
No.
Stoebe Burchellii Levyns.
Cape Prov.: Ladismith Div.:
Swartbergen Lower S. slopes
below Toverkop.
Alt. 2,000 - 3,000 ft.
Legit E. Esterhuysen No. 18555
Flor. 23rd Apr, 1951. Det. N.S. Pillans.
EDSON 7000/4'51 7.

Figure 5: Scanned image of *Seriphium burchellii* (*Esterhuysen 18555*, BOL). Note the acute branching angle, dark-green colour, very tiny leaves and diffuse and paniculate synflorescence arrangement.

3. *Seriphium burrowsii* Z. Shaik, sp. nov. ms.

Much-branched woody green shrub up to 1.5 m tall. *Branching angle* 90°. *Branches* rigid, glabrous. *Leaves* in alternately-arranged fascicles, linear, involute; adaxial surface densely tomentose, abaxial surface glabrous; non-succulent; leaves subtending fascicles up to 4 mm long, leaves within fascicles up to 3 mm long; basal resin cavities small. *Synflorescence* a continuous or interrupted spike, terminal on primary and secondary branches forming continuous or interrupted spikes. *Outer involucral bracts* up to ten, up to 1.6 mm long with leaf-like stereome bearing a tomentum. *Inner involucral bracts* up to 8, up to 4 mm long. *Corolla* up to 2.7 mm long. *Style* up to 2.6 mm long; apex with sweeping penicillate hairs; stigmatic lobes without hairs. *Pappus* with up to 16 bristles, each up to 2.3 mm long. *Cypselas* with dense covering of penicillate trichomes. *Flowering* August to September.

Diagnostic features: Closely resembles *Seriphium cinereum* and *Seriphium vulgaris*, but is distinguishable from the former having non-reflexed leaves, and from the latter by the 90° branching angle.

Distribution and ecology: Known so far from two localities in Mpumalanga, Buffelskloof Private Nature Reserve and Pilgrim's Rest, on shale and quartz soils respectively, near the escarpment edge of the north-eastern portion of the Great Escarpment above 1600 m elevation. The escarpment-edge habitat may offer a peculiar climatic and/or edaphic niche.

Notes: This species is named after John and Sandie Burrows of Buffelskloof Private Nature Reserve who, with Ms Barbara Turpin, first proposed that these specimens represent a distinct species.

Specimens examined: South Africa. Mpumalanga: 2430 (Lydenburg): Pilgrim's Rest, Robber's Pass, opposite the entrance to Crystal Springs Resort (-DC), 5 Apr 2018, *Burrows 15445* (BNRH, NBG). **2530 (Lydenburg)**: Buffelskloof Nature Reserve (-BC), 9 May 2013, *Burrows 4701* (BNRH); High forest stream, Buffelskloof Nature Reserve (-BC), 27 Aug 2006, *Turpin 2* (BNRH); Zig-zag road, Buffelskloof Nature Reserve (-BC), 12 June 2000, *Burrows 6962* (BNRH, J, PRE); Buffelskloof Nature Reserve, Lydenburg (-BC), 13 Sept 2017, *Turpin & Burrows 1569* (BNRH, WAG).

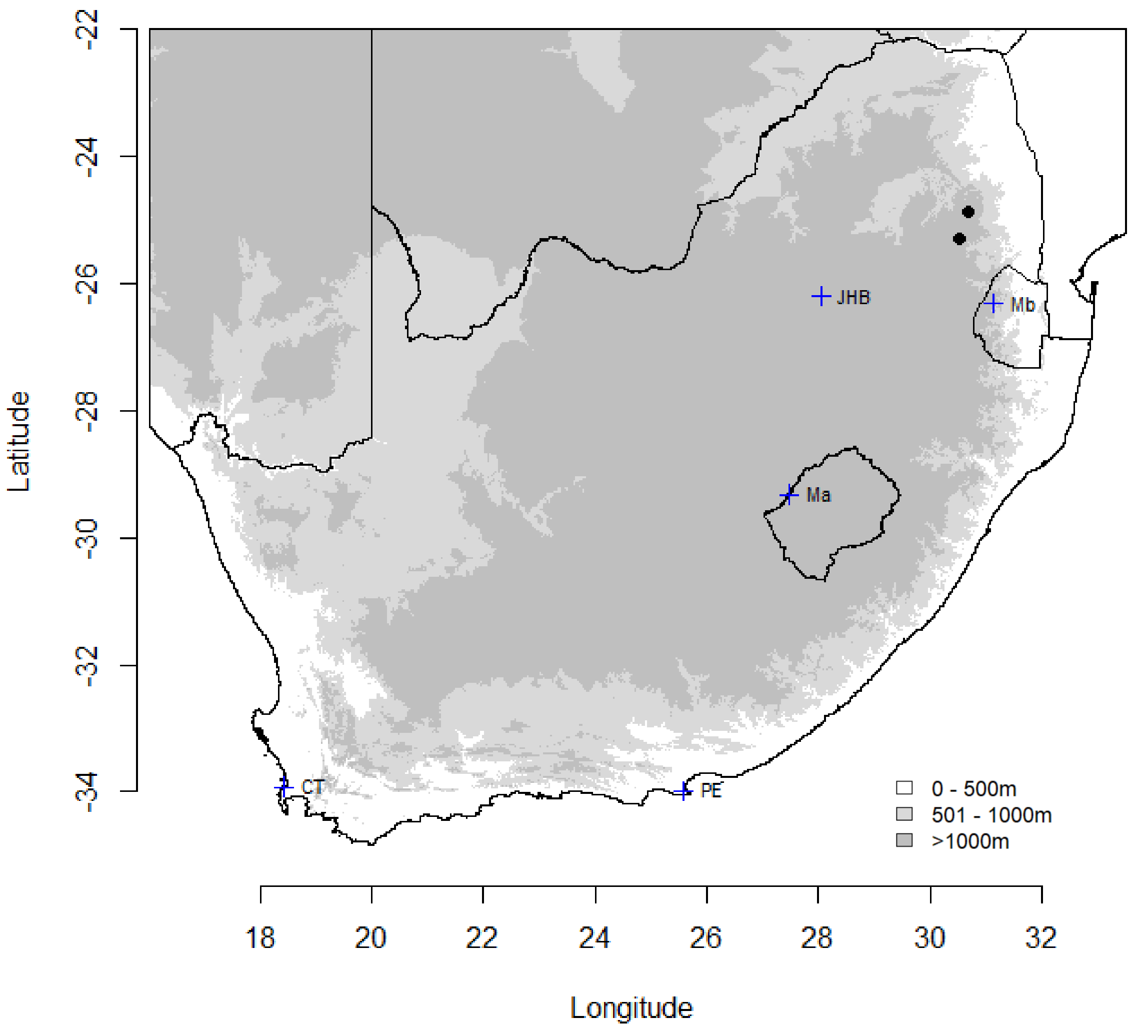
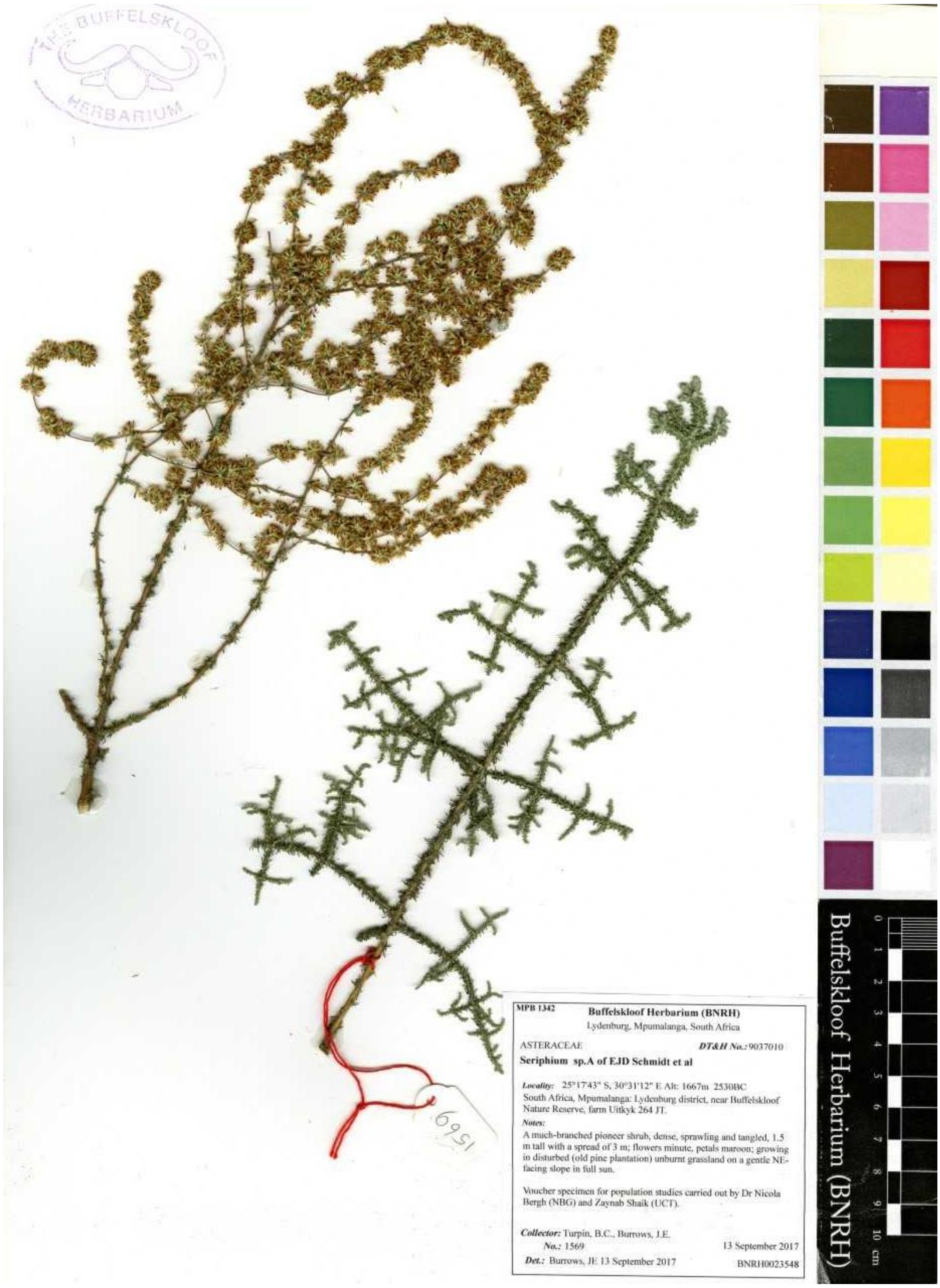


Figure 6: Distribution of *Seriphium burrowsii* (circles) based on collections. Major towns and cities are indicated with crosses (CT – Cape Town, PE – Port Elizabeth, JHB – Johannesburg, Mb – Mbabane, Ma - Maseru).



MPB 1342

Buffelskloof Herbarium (BNRH)
Lydenburg, Mpumalanga, South Africa

ASTERACEAE **DT&H No.:** 9037010

Seriphium sp.A of EJD Schmidt et al

Locality: 25°17'43" S, 30°31'12" E Alt: 1667m 2530BC
South Africa, Mpumalanga: Lydenburg district, near Buffelskloof Nature Reserve, farm Uitkyk 264 JT.

Notes:
A much-branched pioneer shrub, dense, sprawling and tangled, 1.5 m tall with a spread of 3 m; flowers minute, petals maroon; growing in disturbed (old pine plantation) unburnt grassland on a gentle NE-facing slope in full sun.

Voucher specimen for population studies carried out by Dr Nicola Bergh (NBG) and Zaynab Shaik (UCT).

Collector: Turpin, B.C., Burrows, J.E.
No.: 1569 13 September 2017
Det.: Burrows, JE 13 September 2017 BNRH0023548

Figure 7: Scanned image of *Seriphium burrowsii* (*Turpin & Burrows 1569*, BNRH, WAG). Note the 90° branching angle, small linear dark-green leaves strongly arranged into fascicles, and the diffuse synflorescence comprising loose aggregations of spicately-arranged glomerules

4*. Seriphium cinereum* L. Sp. Pl. p. 928 (1753). *Stoebe cinerea* (Linn.) Thunb. Prod. p. 169 (1800). *Seriphium cinerum* (L.) var. *cinereum* Less. Syn. Comp. p. 350 (1832). *Stoebe cinerea* Thunb. var. *cinerea* Harv. Fl. Cap. III. P. 284 (1865).

Much-branched green sub-shrub, up to 40 cm tall. Branching angle 90° or acute. *Branches* rigid, glabrous. *Leaves* in alternately-arranged fascicles, linear, involute, reflexed and twisted; apex mucronate; adaxial surface densely tomentose, abaxial surface glabrous; non-succulent; up to 5 mm long, basal resin cavities prominent. *Synflorescence* an interrupted of continuous spike, terminal on primary and secondary branches forming continuous or interrupted spikes. *Outer involucral bracts* up to 9, each up to 4.1 mm long with cartilaginous or leaf-like stereome, with or without tomentum. Inner involucral bracts up to 5, up to 4.2 mm long. *Corolla* up to 2.2 mm long. *Style* up to 2.5 mm long; apex with sweeping penicillate hairs; stigmatic lobes with or without hairs. *Pappus* with up to 13 bristles, each up to 2 mm long. *Cypselas* with dense covering of penicillate trichomes. *Flowering* March to May, sporadic flowering observed throughout the year.

Diagnostic features: *Seriphium cinereum* is the only species in the *S. plumosum* clade with reflexed leaves, and the leaves are longer than most other species in the complex. It most closely resembles *S. burrowsii* and *S. vulgaris* (as defined here), but neither of these species have reflexed leaves.

Distribution and ecology: *Seriphium cinereum* has a fairly broad distribution within the southwestern Cape mountains, from the Franschhoek Pass south to Hermanus, and west to Cape Town on moist mountains slopes on sandstone and shale soils above 100 m elevation. A single isolated population is known from the Grootwinterhoek Mountains.

Specimens examined: South Africa. Western Cape: 3318 (Cape): on Table Mountain (-CD), Nov 1885, nn (BOL); in heath near the foot of Devil’s peak (-CD), 1898, *Bolus 3962* (BOL); slopes of Lion’s head (-CD), 19 Sept 1933, *Levyns 4710* (BOL); Cape Peninsula, UCT grounds (-CD), 7 May 1957, nn *27238* (BOL); top of woody ravine, Table mountain (-CD), 1 May 1933, *Levyns 4241* (BOL); Kirstenbosch, lower slopes north of Window stream (-CD), Apr 1950, *Esterhuysen 16935* (BOL); University grounds, Rondebosch (-CD), 29 Apr 1931, *Levyns 3128* (BOL); slope of Table mountain above Platteklip gorge (-CD), Sept 1837, *Pappe 17426* (NBG); Cape, mountain near Cape Town (-CD), *Ecklon nn* (NBG); Devil’s peak, first waterfall ravine (-CD), 9 May 1980, *le Maitre 100* (NBG). **3318 (Stellenbosch)**: Jonkershoek, west of “Hangrivier” source (-DD), 26 March 1931, *Jordaan 33256* (NBG); Jonkershoek (-DD), 16 Mar 1946, *Jordaan nn* (NBG). **3319 (Paarl)**: Franschhoek pass, Villiersdorp side (-CA), 3 May 1936*, Levyns 5638* (BOL); Franschhoek, Houmoed, Wemmershoek (-CA), 4 March 1947, *Heyns 26778* (BOL); Summit of DuToiskloof pass (-CC), Feb 1962, Walters 186 (NBG); Du Toits berg. Fisantkraal, west-facing slopes (-CC), 21 Feb 2004, *Bergh 1185* (NBG); Paarl, Frnaschhoek, Houwhoek, Wemmershoek (-CA), 4 Mar 1947, *Heyns 26778* (NBG). **3319 (Piketberg)**: Cockscomb, Grootwinterhoek mountains (-AA), 30 Nov 1978, *nn 28085* (BOL). **3319 (Villiersdorp)**: Silverstream near Villiersdorp, mountain slopes (-CD), *Goldblatt 1819.* **3319 (Wellington)**: Summit of DuToitskloof pass (-CA), Feb 1962, *Walters 186* (NBG). **3418 (Caledon)**: top of Sir Lowry’s pass (-BB), 24 Apr 1974, *Goldblatt 1677* (BOL); Kogelberg State Forest (-BD), 16 Mar 1992, *Kruger 441* (NBG). **3418 (Simon’s town)**: Kalk Bay, mountain ridge (-AB), 22 May 1933, *Levyns 4240* (BOL); Kalk Bay mountain, Cape Peninsula (-AB), 6 Apr 1974, *Goldblatt 1363* (BOL). **3418 (Somerset west)**: Somerset west (-BB), 20 March 1940, *Parker 3471* (BOL). **3419 (Caledon)**: Houwhoek mountain, on northern side of valley above Houwhoek Inn (-AA), 18 Mar 2004, *Bergh 1210* (NBG); Kogelberg State Forest (-AC), 9 Apr 1975, *van Wilgen 29* (NBG); Elgin Basin, Arieskraal (-AA), 16 June 1995, nn *312* (NBG).

3419 (Hermanus): Fernkloof Nature Reserve (-AD), 2 Apr 1980, *Orchard 548* (NBG); Vogelgat, James' kloof (-AD), 20 Mar 1980, *Williams 2996* (NBG). **3419 (Paarl)**: Houhoek, Houtech terrain (-AA), 29 Mar 1988, *Boucher & Stindt 5412* (NBG); Lebanon State Forest, Jakkalsrivier catchment (-AA), 29 Mar 1973, *Haynes 782* (NBG).

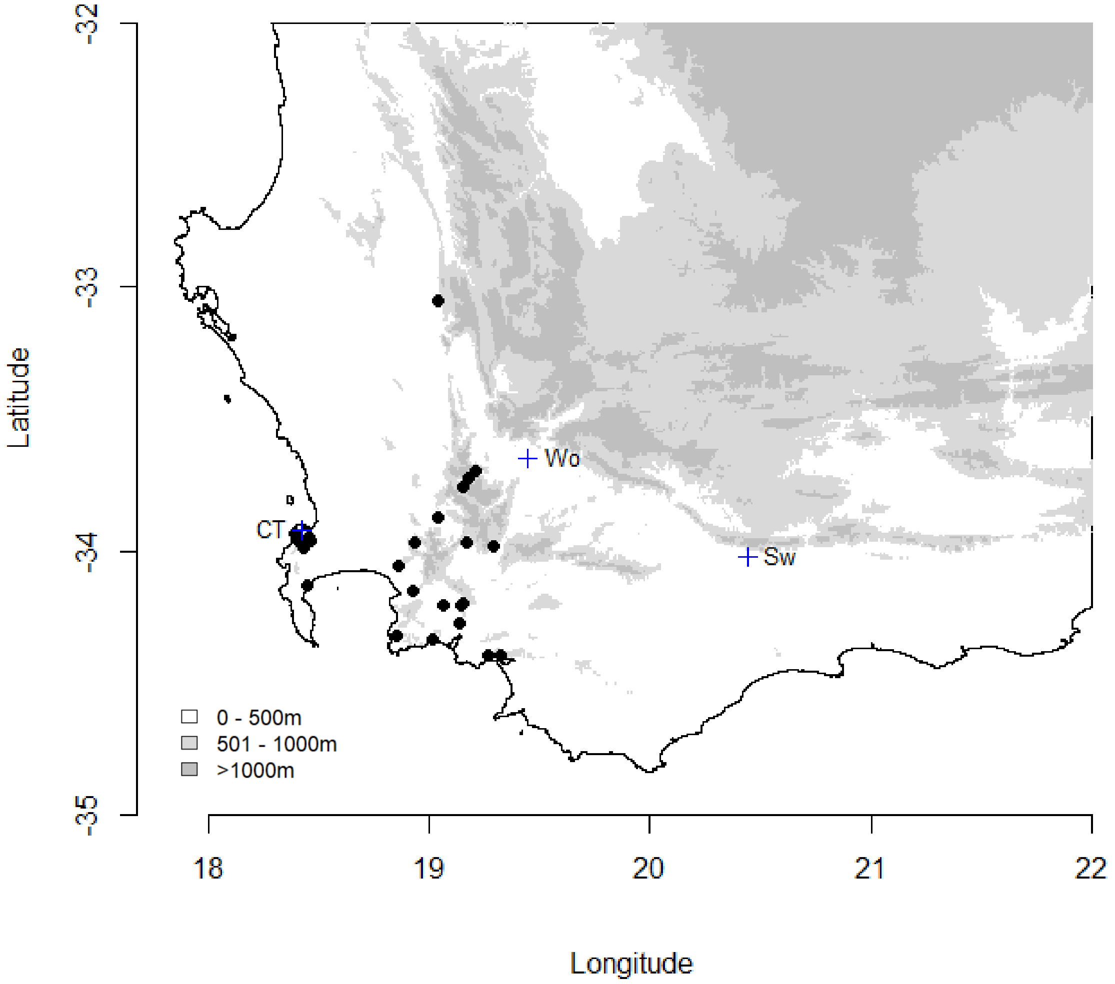


Figure 8: Distribution of *Seriphium cinereum* (circles) from verified herbarium specimens and collections. Major towns are indicated with crosses.

Figure 9: Scanned image of *Seriphium cinereum* (*Esterhuysen 27238*, BOL). Note the acute branching angle, very long reflexed dark green leaves, and densely congested spicate synflorescences.

5. *Seriphium crypticum* Z. Shaik, sp. nov. ms.

Single-stemmed, much-branched, woody grey shrub up to 30 cm tall. *Branching angle* acute. *Branches* rigid, densely tomentose. *Leaves* in alternately-arranged poorly-defined fascicles, linear, straight; both surfaces densely tomentose; non-succulent; apex obtuse; leaves subtending fascicles up to 4.6 mm long, leaves within fascicles up to 2.7 mm long; basal resin cavities small. *Synflorescences* an interrupted spike, terminal on primary and secondary branches. *Outer involucral bracts* up to 13, each up to 3.7 mm long with cartilaginous or leaf like stereome, glabrous or hairs. Inner involucral bracts up to 7, each up to 5 mm long. *Corolla* up to 2.8 mm long. *Style* up to 3.5 mm long; apex with sweeping penicillate hairs; stigmatic lobes glabrous or hairy. *Pappus* plumose with up to 19 bristles, each up to 2.4 mm long. *Cypselas* with dense covering of penicillate trichomes. *Flowering* April to June.

Diagnostic features: This species is very difficult to distinguish from *Seriphium plumosum* (as defined here). A combination of features is required to discriminate them. A proportion of specimens will not be distinguishable by my key, and it is possible that only genetic markers can distinguish these. In *S. crypticum*, the leaves are grouped into poorly-defined fascicles and may be imbricate or widely-spaced, are always densely tomentose, and the branching angle is always acute. In *S. plumosum* (as defined here), on the other hand, the leaves are always arranged in well-defined fascicles, are never imbricate, vary between being glabrous to densely tomentose, and the branching angle ranges from acute to right-angled.

Distribution and ecology: *Seriphium crypticum* occurs on the slopes and summits of the mountains in the extreme southwestern Cape, from Wellington Sneeukop to the Stellenbosch mountains and south to Wemmershoek Peak and Jonaskop at elevations between 1000m and 1700 m. It is found on both shale and quartz soils.

Notes: This species is named for its close resemblance to *Seriphium plumosum* (as defined here).

Specimens examined: South Africa. Western Cape: 3318 (Malmesbury): Upper Wellington, Sneeuwkop (-AD), 31 May 1946, *Esterhuysen 12810* (BOL). **3318 (Stellenbosch)**: Dwarsberg, Jonkershoek (-DD), 17 Feb 1952, *Esterhuysen 1257* (BOL); Wemmershoek Peak (-DD), 2 May 1945, Esterhuysen 11570 (BOL); valley between Stellenbosch range and Jonkersberg range (-DD), 3 Sept 1996, *Puttock SAF-96081* (NBG). **3319 (Worcester)**: Jonaskop summit (-DC), 28 Apr 2004, *Bergh 1613* (NBG). **3320 (Ladismith)**: Haelhoeksneeukop, upper slopes (-DA), 4 Apr 1942, *Esterhuysen 7718* (BOL). **3419 (Caledon)**: source of Palmiet River slopes, Nuweberg State Forest (-AA), 21 May 1960, *Stehle 115* (NBG); Jonkershoek State Forest, Victoria Peak (-AA), 12 April 1983, Forsyth 245 (NBG); Victoria Peak (-AA), 1 June 2017, *Shaik 14* (NBG, PRE, BOL).

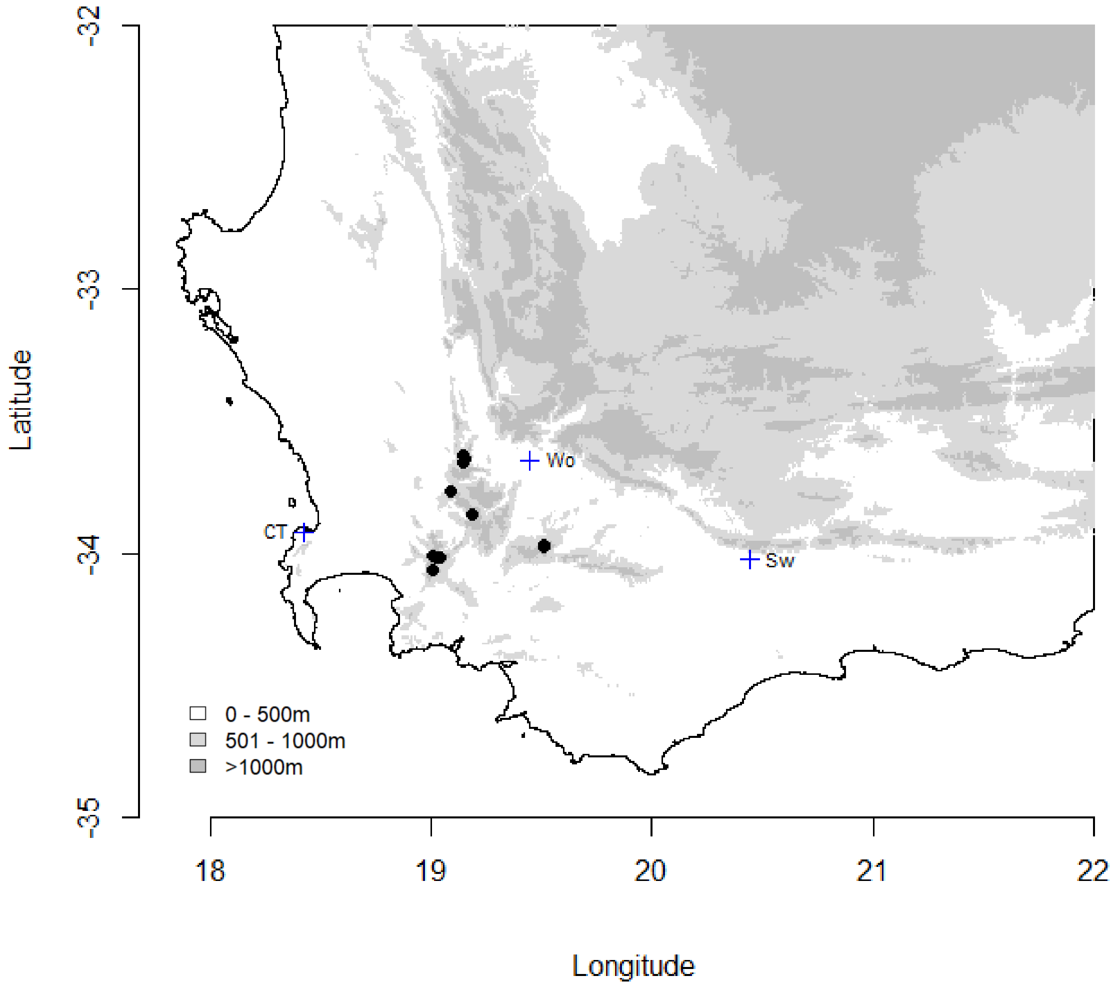


Figure 10: Distribution of *Seriphium crypticum* (circles) from verified herbarium specimens and collections. Major towns are indicated with crosses.

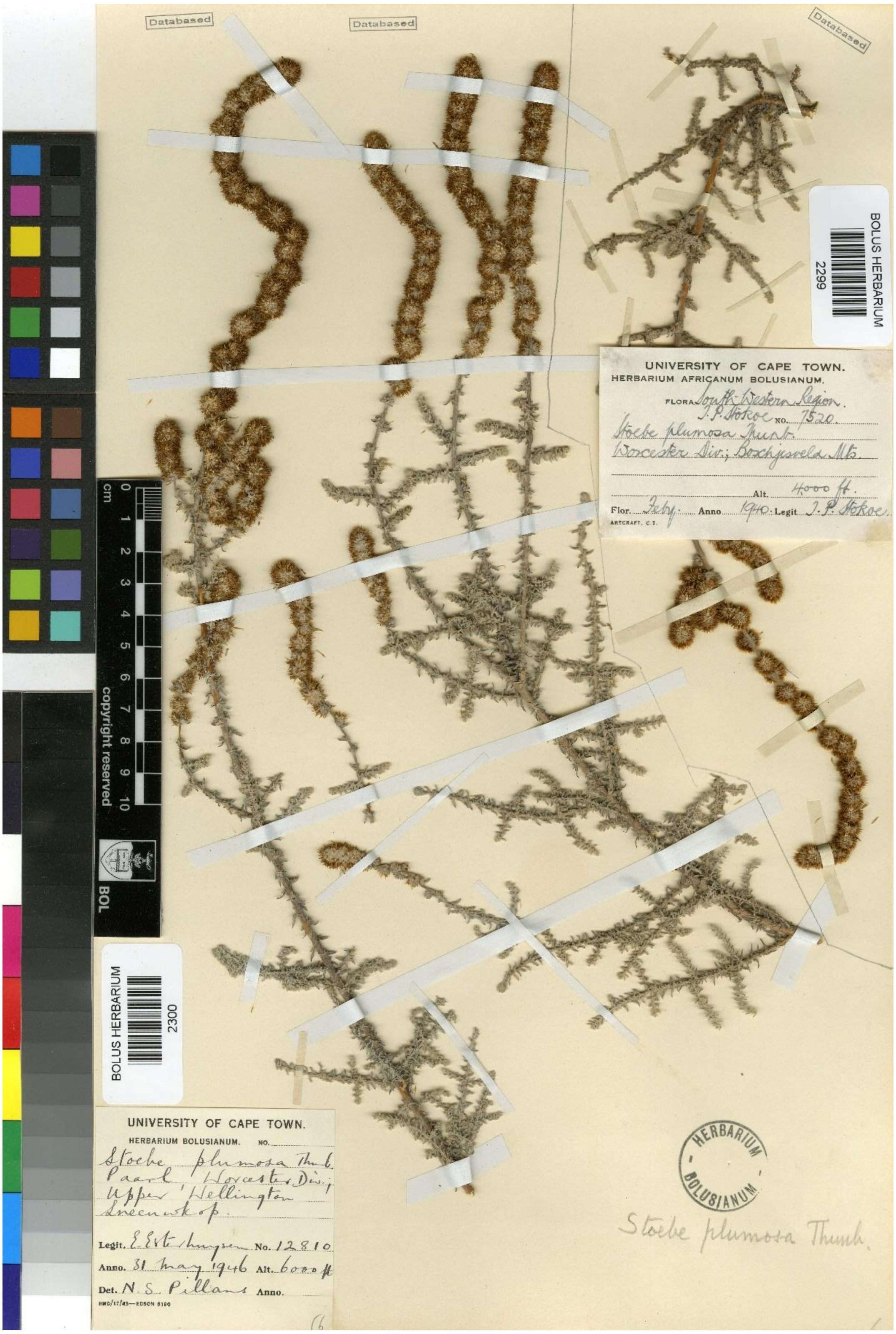


Figure 11: Scanned image of *Seriphium crypticum* (left and centre specimens; *Esterhuysen 12810*, BOL). Note the densely tomentose leaves and uninterrupted spicate synflorescences.

6. *Seriphium dunensis* Z. Shaik, sp. nov. ms.

Multi-stemmed white-grey shrub, up to 1.3 m tall. *Branching angle* acute. *Branches* supple, flexible, and whip-like; densely tomentose. *Leaves* in alternately-arranged fascicles, linear, straight, apex obtuse, adaxial and abaxial surfaces densely tomentose; succulent; leaves subtending fascicles up to 2.9 mm long, leaves within fascicles up to 2.1 mm long; basal resin cavities small. *Synflorescences* a panicle, terminal on primary and secondary branches but borne below the level of the uppermost leaf shoots. *Outer involucral bracts* up to seven, up to 2.7 mm long, with leaf-like stereome bearing a tomentum. Inner involucral bracts up to 11, up to 5 mm long. *Corolla* up to 3.7 mm long. *Style* up to 3.6 mm long; apex with sweeping penicillate hairs; stigmatic lobes glabrous or hairy. *Pappus* with up to 15 bristles, up to 3.4 mm long. *Cypselas* with or without covering of penicillate trichomes. *Flowering* February to May, also observed in September.

Diagnostic features: *Seriphium dunensis* closely resembles *S. virgatum*, but is distinguished by its supple, flexible and whip-like branches, and its leaves which are always grey-white and succulent. In *S. virgatum*, the branches are rigid, and the leaves are grey-white or green and non-succulent.

Distribution and ecology: This species forms stands on unconsolidated coastal dunefields and in dune slacks and is distributed from Hout Bay in the Western Cape, eastwards along the southern coast to the Fish River valley in the Eastern Cape below 10 m elevation.

Notes: This species corresponds to the coastal form mentioned by Koekemoer (2016).

Specimens examined: South Africa. Eastern Cape: 3327 (Bathurst): Peddie district, Fish River valley (-AC), Aug 1943, *nn 2039/34181* (NBG). **Western Cape: 3418 (Caledon)**: Pringle Bay (-BD), 27 Jan 1936, *Compton 6080* (NBG). **3418 (Simon's town)**: Cape Peninsula, Fish Hoek dunes (-AB), March 1903, *Marloth 2845* (NBG); Fish Hoek dunes (-AB), 28 March 1947, nn *22384* (NBG). **3419 (Bredasdorp)**: Pearly Beach, littoral dunes near mouth of stream (-DA), 4 Sept 1971, *Boucher 1604* (NBG); Pearly Beach, on sand dunes close to coast (-CB), 19 Jan 1998, Koekemoer 1361 (PRE). **3421 (Riversdale)**: coast at Still Bay (-AD), May 1943, nn (BOL). **3423 (Knysna)**: Plettenberg Bay, sand flats along coast (-AB), 25 Feb 1955, *Esterhuysen 24205* (BOL).

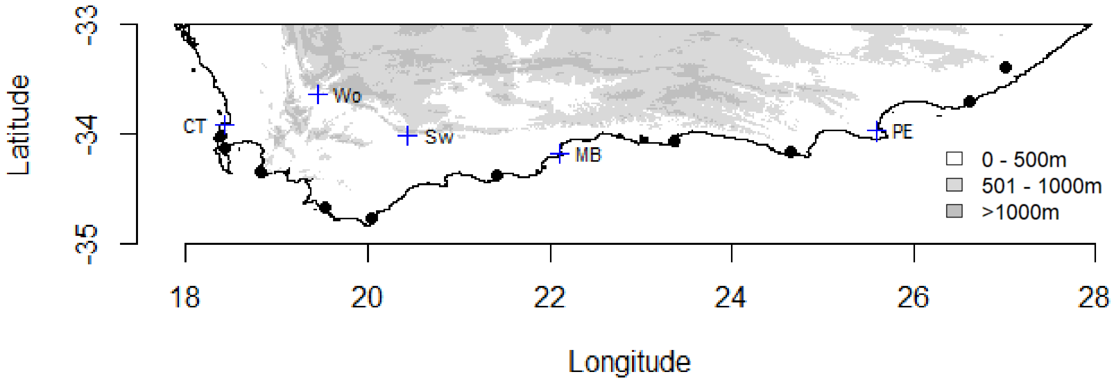


Figure 12: Distribution of *Seriphium dunensis* (circles) from verified herbarium specimens and collections. Major towns are indicated by crosses.

Figure 13: Scanned image of *Seriphium dunensis* (*Koekemoer 1361*, PRE). Note the slender, flexible branches, the fine, grey-green leaves, and the paniculate synflorescences borne below the level of the uppermost leaf shoots.

7. *Seriphium plumosum* L. Sp. Pl. p. 928 (1753). *Stoebe plumosa* (Linn.) Thunb. Prod. p. 169 (1800). *Seriphium cinerum* (L.) var. *plumosum* Less. Syn. Comp. p. 350 (1832). *Stoebe cinerea* L. var. *plumosa* Harv. Fl. Cap. III. P. 284 (1865).

= *Seriphium plumosum* L. var. *glabriusculum* DC. Prod. VI. p. 262 (1837). Type: South Africa. Hottentots Holland mountains, Drège 860 (G-DC; G00451587, lectotype; designated by Koekemoer, 2016)

= *Stoebe fasciculata* Cassini (1827: 62). Type not known.

= *Elytropappus ruschianus* Dinter Repert. Spec. Nov. Regni Veg. p. 88 (1932). Type: Namibia. Lichtenstein, Dinter 3513 (M, holotype; PRE, SAM, BOL, isotypes)

Single-stemmed, much-branched woody grey-green shrub, up to 1.3 m tall. *Branching angle* 90° or acute. *Branches* rigid; densely tomentose. *Leaves* in alternately-arranged fascicles, linear or ovate, straight; apex obtuse or mucronate; densely tomentose adaxial surface, abaxial surface tomentose or glabrous; non-succulent; leaves subtending fascicles up to 4.2 mm long, leaves within fascicles up to 1.8 mm long; basal resin cavities prominent. *Synflorescences* terminal on primary and secondary branches forming a continuous or interrupted spike, or panicle. *Outer involucral bracts* up to 14, up to 2.7 mm long, with cartilaginous or leaf-like stereome, with or without tomentum. *Inner involucral bracts* up to 8, up to 4.8 mm long. *Corolla* up to 2.9 mm long. *Style* up to 3.1 mm long; apex with sweeping penicillate hairs; stigmatic lobes without hairs. *Pappus* with up to 17 bristles, each up to 2.8 mm long. *Cypselas* with dense covering of penicillate trichomes. *Flowering* February to July.

Diagnostic features: This species is morphologically highly variable and is associated with two fairly distinct elevational morphs. The low-elevation morph (Figure 15) occurs between 100 m and 1400 m elevation, and is characterised by branches oriented at right angles, short (~1 mm long) leaves subtending the fascicles, and synflorescences arranged into loose panicles or interrupted spikes. The high-elevation morph (Figure 16) occurs above 1000 m elevation, and is characterised by branches oriented at an angle less than 90°, long (up to 4.2 mm) leaves subtending the fascicles, and synflorescences arranged into interrupted or continuous spikes. The species closely resembles *S. alto-argillaceum* and *S. crypticum*, but can be distinguished from these species using several features of the leaves (see “diagnostic features” under those species treatments).

Distribution and ecology: Core distribution in the mountains and valleys of the Cape Fold Belt, from Vanrhynsdorp south through the Cedarberg to Cape Town and eastwards along the Cape Fold Belt mountains to the Swartberg Pass on shale, sandstone and quartz soils at elevations between 100 m and 2000 m elevation. A single population is known from Moltke Blick in Namibia, but the species is likely under-collected between Namibia and Vanrhynsdorp. Distribution large but populations often highly localised and possibly confined to moist habitats.

Notes: The concept of *S. plumosum* presented here is far narrower than that of any previous author, including Levyns’ (1937), whose concept was the narrowest to date. Linnaeus (1753) does not mention the number of florets in the capitula in his original description of *Seriphium plumosum* L. In *Mantissa Plantarum* (1771) and *Systema Naturae* (1796), *S. plumosum* is described as having five- or six-flowered capitula. These descriptions are inconsistent with Linnaeus’ herbarium specimens of *S. plumosum*, all of which represent plants with single-flowered capitula.

Specimens examined: Namibia. 2217 (Khomas): Moltke Blick, Aus mountains (-CA), 19 July 1925, *Rennie 1982* (BOL).

South Africa. Northern Cape: 3119 (Calvinia): near top of Van Rhyns pass, west of Niewoudtville (-AC), 4 Apr 1974, *Goldblatt 1335* (NBG). **Western Cape: 3118 (Van Rhynsdorp)**: Gift Berg (-DC), 17 Sept 1911, *Phillips 7398* (BOL). **3218 (Piketberg)**: Kapteinskloof mountain(-DC), 23 May 1980, *Linder 2319* (BOL); Greys Pass, Piquetberg [Piketberg] (-DB), 14 May 1935, *Sallis 5294* (BOL); Grootwinterhoek, E facing slope on voorberg above Groot Kliphuis River (-AA), 21 May 1928, *Low 1312* (NBG); Porterville mountains, Kliphuisvlakter old werf, abandoned orchard, 20 Sept 1973, *Taylor 8599* (-AA); summit of Zebra Kop, Piquetberg [Piketberg] (-DB), 10 Nov 1934, nn *7299* (BOL). **3219 (Clanwilliam)**: Wuppertal, Amon jeep track, N Cedarberg, near "crossroads camp", near TRO53 (-AC), 9 May 1989, Taylor 12059 (NBG); Slangvlei, "Duiwlesgat", Cedarberg (-AC), 17 Sept 1973, *Andrag 257* (NBG); Central Cedarberg, Algeria, Sneeuwberg, on path up from Eikeboom (-AC), 7 Apr 2004, *Bergh 1001* (NBG); Elandskloof, Cold Bokkeveld (-CA), 20 Apr 1935, *Levyns 4905* (BOL); Elands kloof, Citrusdal side (-CA), Oct 1924, *Levyns 4782* (BOL); Elands kloof, Cold Bokkeveld (-CA), 20 Apr 1935, *Levyns 4882* (BOL); Cedarberg (-CB), 28 June 1972*, Andrag 95* (NBG); Gonnafontein (-CB), 30 March 2000, *Pond UP39* (NBG); Cedarberg, Crystal Pool (-AC), Sept 1930*, Levyns 2986* (BOL); Cedarberg, Middelberg mountain slopes (-AC), Oct 1923, *Pocock 131* (NBG); summit Sneeuwkop, Cedarberg (-AC), 13 Oct 1923, *Pocock 210* (NBG); summit Sneeuwkop, Cedarberg (-AC), 18 Oct 1923, *Pocock 362* (NBG); Sneeuwberg, S Cedarberg (-CA), 12 Apr 1952*, Esterhuysen 20051* (BOL); Elands Kloof, Cold Bokkeveld (-CA), 21 Apr 1935*, Levyns 4930* (BOL); Elands kloof, Cold Bokkeveld (-CA), 21 Apr 1935, *Levyns 4918* (BOL); Tafelberg, Cedarberg, rocky W slopes (-AC), 3 May 1953, *Esterhuysen 21367* (BOL). **3219 (Ceres)**: Houdenbek, koue bokkeveld (-CD), May 1928, *Levyns 2408* (BOL); Ceres div., E foot of Schurweberg, cold Bokkeveld (-CD), 19 Apr 1946*, nn 12659* (BOL); Zandfontein (-CB), 6 Apr 1947, *Compton 19444* (BOL); Tuinskloof farm, Op Die Berg (-CA), 8 Jan 2003, *Botha (3)013* (NBG); Bokkeveld Tafelberg SE slopes (-CD), 8 Dec 1940, *Esterhuysen 3891* (BOL); Schoongezight peak, Cold Bokkeveld mountains, ledges SW side below summit (-CC), 4 Apr 1953, *Esterhuysen 21284* (BOL); Schoongezicht Peak (-CC), 4 Apr 1953, *Esterhuysen 21300* (BOL). **3318 (Malmesbury)**: Riebeek's kasteel (-BD), 14 May 1934*, Levyns 3111* (BOL); south side of summit of Paardeberg (-DB), nn *Pillans* 6324 (BOL). **3318 (Moorreesburg)**: Piketberg, Bridgetown, sandy alluvial banks of Berg River (BB), 29 May 1952, *Esterhuysen 20154* (BOL). **3318 (Paarl)**: Paarlberg (-DB), 28 July 1962, *Krugen m35* (NBG); Paarlberg (-DB), 29 March 1962, *van de Merwe 1028* (NBG). **3318 (Stellenbosch)**: Stellenbosch Wolwedans, plot located on slope adjacent drainage line (-DD), 5 July 1994, *Nel and Boucher 0204* (NBG); Jonkershoek valley, lower slopes of Swartboskloof (-DD), 16 Apr 1990, *Buys 162* (NBG); Stellenbosch mountain (-DD), 1991, *Green 11* (NBG); Clanwilliam div., Pakhuis, 23 Aug 1941, *Esterhuysen 5922* (BOL); Lemoenkloof farm, NE of Stellenbosch, ridge directly W of Silverminekloof (-DD), 17 Apr 1990, *Buys 169* (NBG); Stellenbosch mountain between winelands and fynbos (-DD), 12 June 1963, *Bos 36* (NBG); Dalsig (-DD), 25 Aug 1960, *van Rensberg 346* (NBG); valley between Stellenbosch range and Jonkersberg range (-DD), 3 Sept 1996, *Puttock SAF-96081* (NBG). **3319 (Ceres)**: Worcester, Waaihoek Peak, at Hoare hut, 23 March 2004, Bergh 1243 (-AD); Warme Bokkeveld, farm Erfdeel, gorge at bottom of the Spekrivier Valley, 17 March 2006, *Mucina and Jakubowsky 170306/09* (-BC); Matroosberg, N slopes, on grassy tussock veld in seep along 4x4 track, 9 Dec 2003, *Bergh 1048* (-BC). 3319 (Caledon): Kaaimansgat. Kroonlandplek, on dirt track up to "Daniel Roux Kamplek" around 10km NW of Villiersdorp (-CD), 21 May 2005, *Bergh 1464* (NBG); Hansiesberg, Cold Bokkeveld (-AB), 18 Apr 1956, *Esterhuysen 25730* (BOL); Conical Peak (-BC), Dec

1940, *Hokoe 8150* (BOL); Matroosberg, on path to main peak after having left jeep track (-BC), 9 Dec 2003, *Bergh 1050* (NBG); Matroosberg, slopes on W side below nek between mountain and Conical Peak (-BC), 7 Apr 1956, *Esterhuysen 27701* (BOL); Hansiesberg, Cold Bokkeveld, W slopes (-AB), 18 Apr 1956, nn *25729* (BOL); Rocklands farm about 30km N of Ceres (-AB), 10 March 1981, *Hugo 2515* (NBG); Hex River mountains along road to Ceres at turnoff to Klipfontein roadside (-BC), 5 Sept 1996, *Bayer SAF-96116* (NBG). **3319 (Paarl)**: Franschhoekbosreservaat-Puratory outspan (-CC), NE aspect,22 May 1969, *Stehle TS164* (NBG); Paarl div., top of Franschhoek pass (-CC), 2 May 1945, *Esterhuysen 11595* (BOL); Du Toits kloof, New Years Peak, SANBI-UK research site (-CA), Nov 2006, *Dorrat-Haaksma EDH-O25* (NBG); La Motte Forest Station, Zachariashoek, Kasteelkloof (-CC), 5 May 1982, *Viviers 234* (NBG). **3319 (Worcester)**: Hex River mountains, on vlakte behind Prospect Peak (-BC), 30 May 1949, nn *15411* (BOL); Jonas Kop, N slopes (-DC), 25 Sept 1965, *Pycroft 2887* (NBG); Bains Kloof (-CA), 29 May 1946, *Compton 18006* (NBG); Hex River mountains, near orchard, lower slopes (-BC), July 1944, *Esterhuysen 10304* (BOL); Waaihoek Plateau (-CB), May 1942, *Hokoe 8720* (BOL); Jonaskop summit parking area at FM tower (-DG), 15 March 1975, *Boucher 2736* (NBG); Matroosberg slopes (-BC), Sept 1924, *Levyns 970* (BOL); Fonteintjiesberg (-CB), 28 March 1952, *Esterhuysen 20005* (BOL). **3319 (Tulbagh)**: Moist slope at Sneeuwgat, Thulbagh (-AA), 8 Apr 1950, nn *16912*; Winterhoek mountain, tip of rocky summit (-AA), Nov 1879, *Bolus 5078* (BOL). **3319 (Tulbagh/Worcester)**: on mountains near Breede River, Darling Bridge (-CA), Oct 1893, nn *3171* (BOL). **3319 (Tulbagh/Ceres)**: Mostert’s Hoek twins, rocky slopes (-AD), 9 Apr 1955, *Esterhuysen 24265* (BOL). **3319 (Worcester)**: Waaihoek Peak (-AD), 10 May 1955, nn *24293* (BOL); Boschjesveld mountains (-DC), Feb 1940, *Hokoe 7520* (BOL). **3319 (Wellington)**: Bain’s kloof (-CA), July 1940, *Kies 36* (NBG); Pic Blanc (-CA), March 1940, *Esterhuysen 1656* (BOL). **3320 (Ladismith)**: Swellendam div., southern slopes of the Anysberg (-DA), 2 May 1950, *Esterhuysen 17053* (NBG); Anysberg (-BC), 3 June 1956, *Esterhuysen 25984* (BOL). **3320 (Laingsberg)**: top of Witteberg (-BC), 21 May 1925, *Compton 2972* (BOL). **3320 (Montagu)**: Pypsteelfontein, Waboomsberge N of the Koo valley (-CA), 3 March 1986, *Moffet and Steensma 4080* (NBG). **3320 (Swellendam)**: along N side of Langeberg range, 10 o’ clock, 11 o’ clock and Crown Peaks (-CD), 14 June 1952, *Wurts 176* (NBG). **3321 (Ladismith)**: Towerkop, Swartbergen rocky slopes at summit (-AC), 22 Apr 1951, nn *18520* (BOL); Seven Weeks Poort Berg (-AD), 1 Apr 1975, nn *33797* (BOL). **3321 (Laingsburg)**: Rietvleikloof (-AC), 23 March 1940, *Compton 8621* (NBG); Seven Weeks Poort Berg, N slopes (-AD), 30 March 1959, *Esterhuysen 28287* (BOL). **3322 (Prince Albert)**: Zwartberg Pass [Swartberg Pass] (-AC), July 1935, *Levyns 5039* (BOL); Swartberg Pass (-AC), 10 Apr 2004, *Bergh 1291* (NBG).

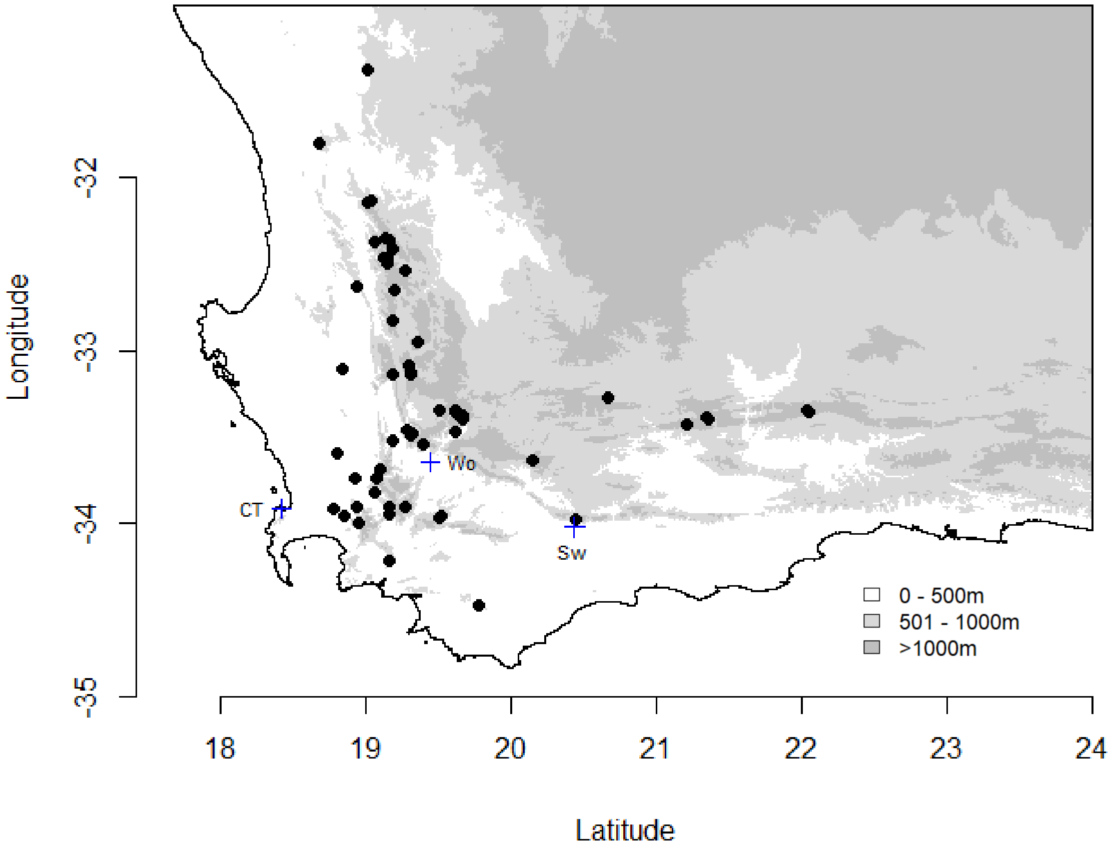


Figure 14: Distribution of *Seriphium plumosum* (circles) from verified herbarium records and collections. Major towns are indicated with crosses (CT – Cape Town, Wo – Worcester, Sw – Swellendam). A single record for the species from Namibia is not shown (see “specimens examined” under *S. plumosum*).

Figure 15: Scanned image of *Seriphium plumosum* (*Esterhuysen 12659*, BOL), lower-elevation form. Note the minute, grey-green leaves.

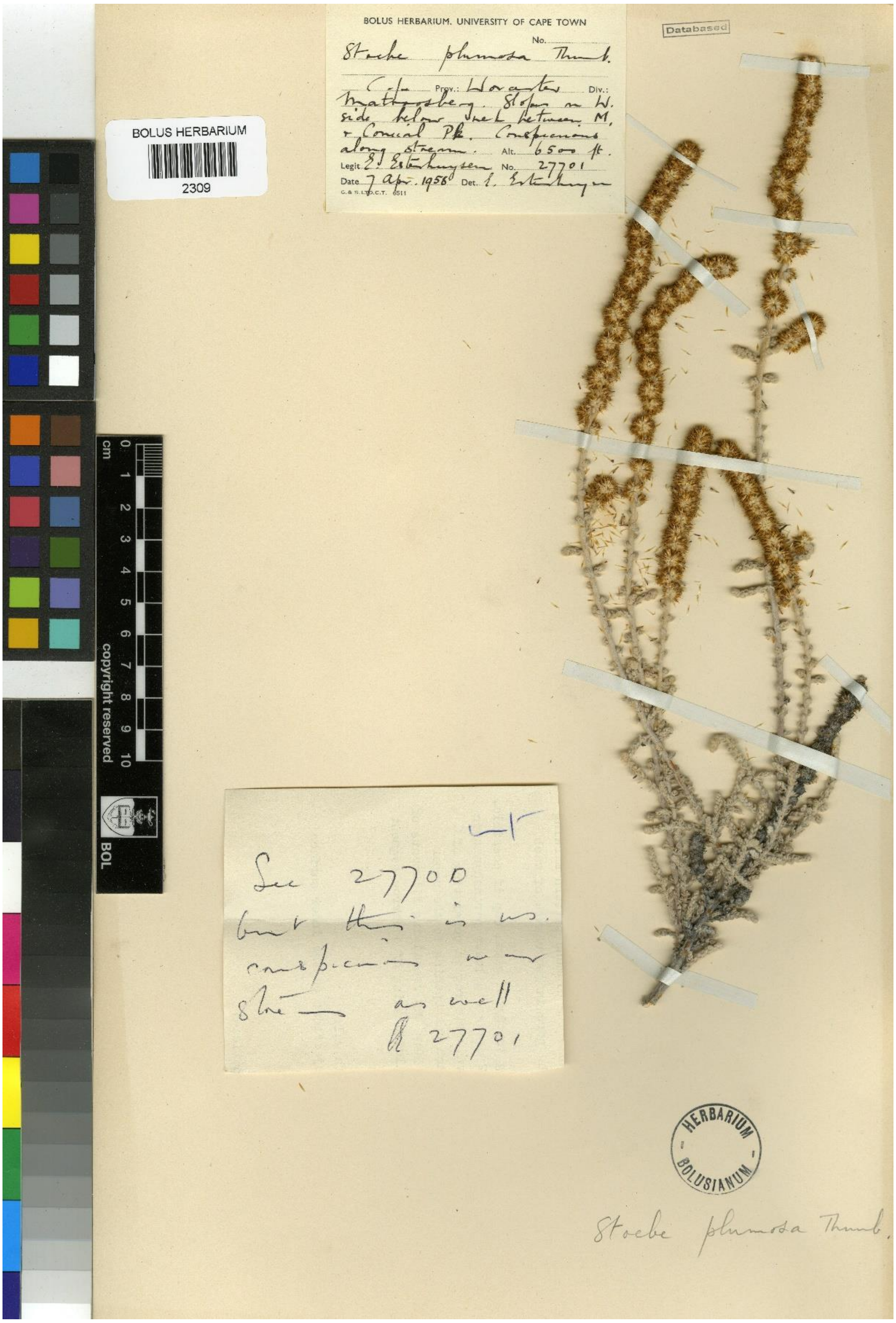


Figure 16: Scanned image of *Seriphium plumosum* at high elevation (*Esterhuysen 27701*, BOL), higher-elevation form. Note the white leaves, which are longer than in the low-elevation morph of the species.

8. *Seriphium virgatum* (Thunb.) Z. Shaik, comb. nov. ms. *Stoebe virgata* Thunb. Prod. p. 170 (1800). *Seriphium cinerum* L. var. *virgatum* Less. Syn. Comp. p. 350 (1832). *Stoebe cinerea* L. var. *virgata* Harv. Fl. Cap. III. P. 284 (1865).

= *Artemisia vermiculata* L. Mant. Pl. p. 281 (1771). *Seriphium vermiculatum* (L.) DC. Prod. p. 263 (1837). Type: LINN 988.1 (LINN)

= *Seriphium plumosum* L. var. *canescens* DC. Prod. VI. p. 262 (1837). Type: South Africa. Uitenhage, Port Elizabeth, Ecklon 1143 (G-DC; G00451545, lectotype; designated by Koekemoer, 2016)

Single-stemmed, much-branched woody grey-green shrub, up to 1.3 m tall. *Branching angle* 90°. *Branches* rigid, lightly tomentose. *Leaves* in alternately-arranged fascicles, linear or ovate, straight; apex obtuse or mucronate; densely tomentose adaxial surface, abaxial surface tomentose or glabrous; non-succulent; leaves subtending fascicles up to 3.1 mm long, leaves within fascicles up to 1.9 mm long; basal resin cavities small. *Synflorescences* on primary and secondary branches forming a continuous or interrupted spike, or panicle approaching a spike. *Outer involucral bracts* up to 14, up to 2.3 mm long, with leaf-like stereome with or without tomentum. Inner involucral bracts up to 7, up to 5 mm long. *Corolla* up to 3.1 mm long. *Style* up to 3.4 mm long; apex with or without sweeping penicillate hairs; stigmatic lobes glabrous or hairy. *Pappus* with up to 18 bristles, each up to 3 mm long. *Cypselas* with or without dense covering of penicillate trichomes. *Flowering* November to January, sporadic flowering at other times of the year.

Diagnostic features: Most closely resembles *S. dunensis* but is distinguished by features of the leaves and the branches (see "diagnostic features" under *Seriphium dunensis*). *Seriphium virgatum* is also distinguished by its habitat on consolidated calcareous soils, where *S. dunensis* occurs on unconsolidated coastal dunefields.

Distribution and ecology: From Noordhoek near Cape town east along coastal margins on consolidated calcareous substrates to Uitenhage near Port Elizabeth between elevations of 10 m and 550 m.

Notes: One of the oldest specimens of *S. virgatum* (*Thunberg 20986*) may have been collected at Noordhoek beach (south-west of Cape Town) in 1773, as Thunberg recorded seeing *Seriphium plumosum* (which he calls *slangebosch*) at this locality in his travelogue (Thunberg, 1793).

Specimens examined: South Africa. Eastern Cape: 3323 (Humansdorp): Storms River (-DD), 6 Nov 1951, *Taylor 3739* (BOL). **3323 (Joubertina)**: Die Hoek, N foot of Tsitsikamma mountains near Joubertina (-DC), 2 Nov 1949, *Esterhuysen 16344* (BOL). **3324 (Humansdorp)**: Rietvlei (-DC), 11 Nov 1941, *Esterhuysen 6626* (BOL); Witte Els Bosch flats (-CC), 18 Nov 1941, *Esterhuysen 6823* (NBG). **3325 (Port Elizabeth)**: Near Port Elizabeth, Uitenhage district, van Stadens (-BC), 14 Sept 1930, *Holland 119* (NBG). **Northern Cape: 3119 (Calvinia)**: Niewoudtville, Oorlogskloof Nature Reserve (-AC), 24 Aug 1988, *Pretorius 95* (BOL). **Western Cape: 3318 (Kuils River)**: Penhill, Eersterivier (-DC), 19 May 1979*, Raitt 373* (NBG). **3318 (Malmesbury)**: Darling Flower Reserve (-AD), 24 May 1956, *Plenderleith 3* (NBG); Paardeberg, Modderkloof (-DB), 17 May 2011, *Nicholson and Roets 55* (NBG). **3318 (Stellenbosch)**: Dwarsriviershoek, Banghoek (-DD), 9 July 1978, *Rycroft 3261* (NBG). **3322 (George)**: Kleinplaat locality (-DC), 27 Aug 1968, *Morze 2231* (NBG). **3322 (Uniondale)**: Mannetjiesberg (-DB), 5 Nov 1941, *Esterhuysen 6440* (BOL); Mannetjiesberg (-DB), 5 Nov 1941, *Esterhuysen 6441* (BOL); Cloete's Pass in Kammanassie mountains (-DB), Jan 1940, *Hokoe 7293*

(BOL). **3323 (Knysna/Uniondale)**: Knysna/Unindale division, on mountain (-CC), Jan 1940*, Hokoe 7283* (BOL). **3323 (Uniondale)**: top of old pass between Uniondale and Avontuur (-CA), Apr 1928, *Fourcade 3801* (NBG); top og Prince Albert's Pass (-CC), 28 Sept 1961, *Pycroft 2316* (NBG). **3418 (Caledon)**: Stellenbosch, Grietjiesgat, between Lowry's Pass and Palmiet River (-BB), May, *Ecklon and Zeyher 497* (NBG); roadside between Pringle Bay and Betty's Bay (-BD), 10 Dec 2005, *Bergh 1479* (NBG); Bus kop slopes, Betty's Bay (-BD), 5 Jan 1971, *Boucher 1449* (NBG). **3418 (Simon's town)**: Nordhoek [Noordhoek], high up on Nordhoek berg rise (-AB), 3 March 1929, *Gillett 3570* (NBG); Nouwhoek (Noordhoek) high up on Nouwhoek Berg (-AB), 3 March 1929, *Gillett 3573* (NBG); Kalk Bay mountain, slope above Boyes drive (-AB), 6 Apr 1974, *Goldblatt 1426* (NBG); Cap. B. Spei, *Thunberg 20986* (UPS). **3419 (Caledon)**: Houwhoek, Houtech terrain (-AA), 6 May 1988, *Boucher and Stindt 5417* (NBG); Bot Rivier Vlei (-AC), 16 Aug 1982, *O'Callaghan 239* (NBG); Kleinmond vlakte, N of vlei, S of road (-AC), 30 Apr 1949, *de Vos 863* (NBG); Klein River mountains (-AD), 2 Apr 1956, *Stokoe 66241* (NBG); Kleinmond, flats after Heuningklip (-AC), 5 Jan 1948, *de Vos 806* (NBG). **3419 (Hermanus)**: Vogelgat (-AD), 11 Jan 1980, *Williams 2970* (NBG). **3420 (Bredasdorp)**: The Poort (-CA), Sept 1933, *Levyns 4463* (BOL). **3421 (Riversdale)**: Takiesfontein farm, on flats (-AD), 18 March 1980, *Bohnen 7471* (NBG); Dekriet station in front of Soutpan shop (-AB), 10 Apr 1979, *Bohnen 5532* (NBG). **3422 (George)**: Pacaltzdorp (-AB), 5 Nov 1928, *Hutchinson 1233* (BOL). **3422 (Knysna)**: Belvedere hillsides (-BB), Jan 1922, *Duthie STE29226* (NBG). **3423 (Knysna)**: Knysna (-AA), Jan 1922, nn 728 (BOL); hill near Karwelkop, Concordia State Forest (-AA), 19 Dec 1977, *Boucher 3418* (NBG).

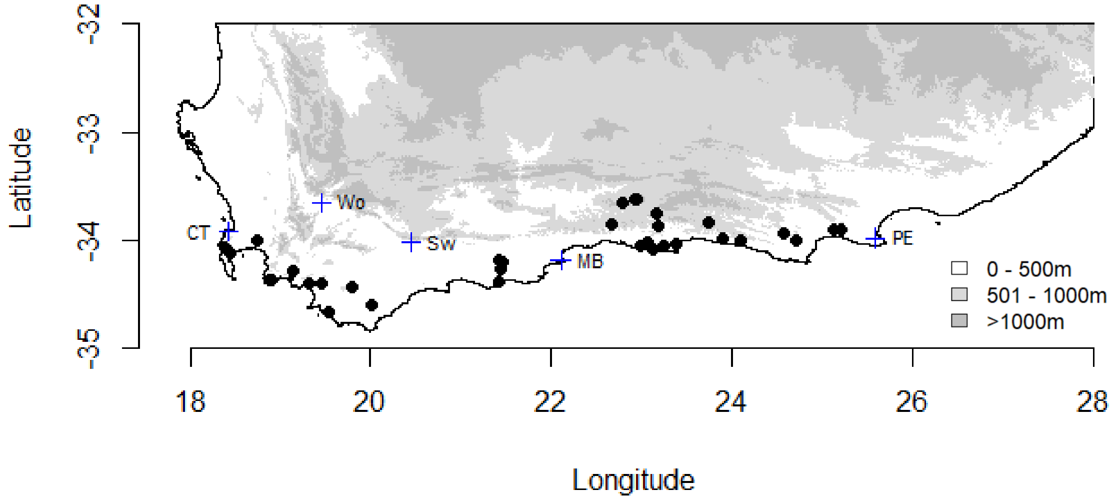


Figure 17: Distribution of *Seriphium virgatum* (circles) from verified herbarium records and collections. Major towns are indicated with crosses.

Figure 18: Scanned image of *Seriphium virgatum* (*Esterhuysen 6823*, BOL). Note the rigid branches and grey-white densely tomentose leaves.

9. *Seriphium vulgaris* (Levyns) Z. Shaik, comb. nov. ms. *Stoebe vulgaris* Levyns. J. S. Afr. bot. p. 16 (1937).

Multi-stemmed much-branched grey-green shrub, up to 70 cm high. *Branching angle* acute. *Branches* rigid, fine, lightly tomentose. *Leaves* in alternately-arranged fascicles, linear, straight; apex obtuse or mucronate; lightly tomentose adaxial surface, abaxial surface tomentose or glabrous; non-succulent; leaves subtending fascicles up to 3.9 mm long, leaves within fascicles up to 2.4 mm long; basal resin cavities small. *Roots* with storage structures just below ground level. *Synflorescences* terminal on primary and secondary branches forming a continuous or interrupted spike, or panicle approaching a spike. *Outer involucral bracts* up to 12, up to 2.5 mm long with leaf-like stereome, with or without tomentum. *Inner involucral bracts* up to 8, up to 4.7 mm long. *Corolla* up to 3.1 mm long. *Style* up to 3.9 mm long; apex with sweeping penicillate hairs; stigmatic lobes glabrous. *Pappus* with up to 19 bristles, each up to 2.7 mm long. *Cypselas* with or without dense covering of penicillate trichomes. *Flowering* March to July.

Diagnostic features: This species resembles the low-elevation morph of *Seriphium plumosum* (as defined here), but has a subterranean rootstock from which new stems resprout after fire. These are absent in *S. plumosum*, which is killed by fire and relies on seed for regeneration. Where *Seriphium vulgaris* has not yet burned and resprouted, the plants resemble *S. burrowsii* and *S. cinereum* (as defined here). In such cases, the species can be discriminated on the basis of features of the leaves and the orientation of branches (see “diagnostic features” under *Seriphium burrowsii*).

Distribution and ecology: Widespread and forming dense stands in Lesotho, Swaziland, Zimbabwe, and the eastern summer-rainfall region of South Africa, especially in grazed areas, from Umtali in Zimbabwe, south to Hogsback in South Africa. Perhaps due to its notoriety as a weed, this species is seriously under-collected within the confines of its distribution, and is likely much more densely distributed than shown in Figure 19.

Notes: *Seriphium vulgaris* is an encroacher in natural rangeland in the savannah and grassland biomes of South Africa, largely as a result of agricultural overgrazing, and is significantly reducing the grazing potential of these areas, with an estimated 11 million hectares already affected by the species (Avenant, 2015).

Specimens examined: Lesotho. 2927 (Maseru): Morija (-DA), Apr 1919, *Page 15974* (BOL).

South Africa. Eastern Cape: 3027 (Lady Grey): farm “De Kraal” (-CC), 9 Feb 1986, *Welman 745* (BOL). **3130 (Bizana)**: Pondoland, Umtamvana gorge (-AA), 7 Dec 2004, *Gwynn-Evans 2156* (NBG). **3226 (Mpofu)**: Near “Apple Tree”, Katberg (-BC), 27 November 1928, *Hutchinson 1665* (BOL); Mitchell’s Pass from Seymour (-DB), 18 July 1952, *Levyns 9850* (BOL). **3226 (Victoria East)**: Hogsback (-DB), 15 Apr 1935, Noel 1225 (BOL); Hogsback, below road east of Hobbiton (-DB), 10 Jan 2004, *Bergh 1081* (NBG). **Free State: 3026 (Zastron)**: road from Zastron to Rouxville (-BD), Jan 2004, *Bergh 1079* (NBG). GAUTENG: **2528 (Pretoria)**: Pretoria, along R104 (-CD), 22 March 2011, *Maurin OM3453* (NBG). **2627 (Vereeniging)**: Houtkop 3, Langerand hills, 5-6 miles NNW of Vereeniging (-DB), 31 March 1953, *Mogg 21031* (BOL). **2628 (Johannesburg)**: Johannesburg (-AA), July 1936*, Cohen 5830* (BOL); Johannesburg (-AA), 22 March 1935, *Cohen 4938* (BOL); Johannesburg (-AA), July 1936, *Cohen 5831* (BOL); Johannesburg (-AA), July 1936, *Cohen 5832* (BOL); Johannesburg, Melville Koppies (-AA), 26 Feb 1962, *MacNae nn* (BOL); Houghton Ridge, Johannesburg (-AA), *Moss 2850* (J). **Kwa-Zulu Natal: 2828 (Witzieshoek)**: W facing slope near the Pudding, Bethlehem (-DB), 22 Sept 1983, *Roux 1583*

(NBG). **2829 (Bergville)**: Cathedral Peak area, Drakensberg (-CC), 13 Aug 1947, *Levyns 8310* (BOL); Cathedral Peak (-CC), Aug 1947, *Levyns 8264* (BOL); Cathedral Peak area (-CC), July 1949, *Esterhuysen 15473* (BOL). **2930 (Chatsworth)**: Hill crest, Umhlatuzane valley (-DD), Sept 1917, *Thode 2915* (NBG). **2831 (Mtonjaneni)**: Melmoth, Zululand (-CB), 1 July 1945, *Smith 8803* (BOL). **Limpopo: 2230 (Mutale)**: Gogogo mountain, Gorongoza (-CD), 5 July 1955, *Schelpe 490* (BOL). **2428 (Waterberg)**: near Waterberg-Nylstrom River (-CB), Apr 1994, *Schlechte 4783* (BOL). **Mpumalanga**: **2430 (Lydenburg)**: Ohrigstad Nature Reserve (-DC), 20 Apr 1976, *Theron 3574* (NBG); Kowyn's Pass near Graskop (-DD), 19 July 1949, *Levyns 9406* (BOL). **2529 (Middelburg)**: Middelburg (-CD), 12 Feb 1922, *Rudatis 2447* (NBG). **North West Province: 2627 (Potchefstroom)**: Vredefort Dome before Leeufontein turn off (-CC), 16 Nov 2011, *Komape, Mabe and Siebert KMS183* (NBG).

Swaziland. 2531 (Hhohho): Barberton, road left Piggs Peak mine (-CC), 22 July 1953, *Levyns 9976* (BOL). **2631 (Mbabane)**: Hawane Falls (-AA), 30 May 1958, *Compton 27864* (NBG); Ukutula (-AC), 26 Oct 1954, *Compton 24580* (NBG); Racecourse (-AC), 29 Aug 1962, *Dlamini PRE44417* (NBG).

Zimbabwe. 1832 (Nyanga): Chipunsu falls, Umtali (-DC), 14 July 1955, *Schelpe 558* (BOL). **1932 (Chimanimani)**: Cashel-Melsettin road (-DC), 11 July 1953, *Levyns 9944* (BOL).

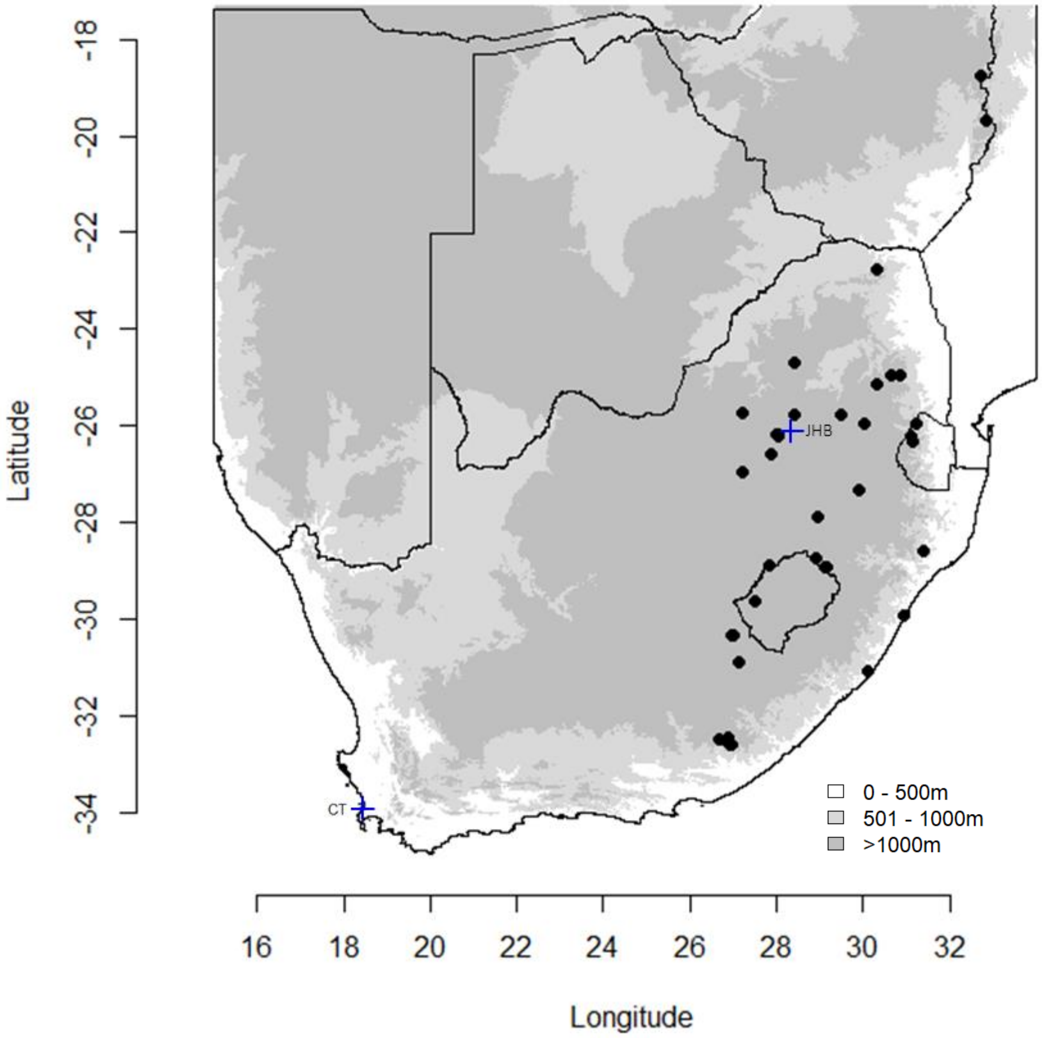


Figure 19: Distribution of *Seriphium vulgaris* (circles) from verified herbarium records and collections. Major towns are indicated with crosses.

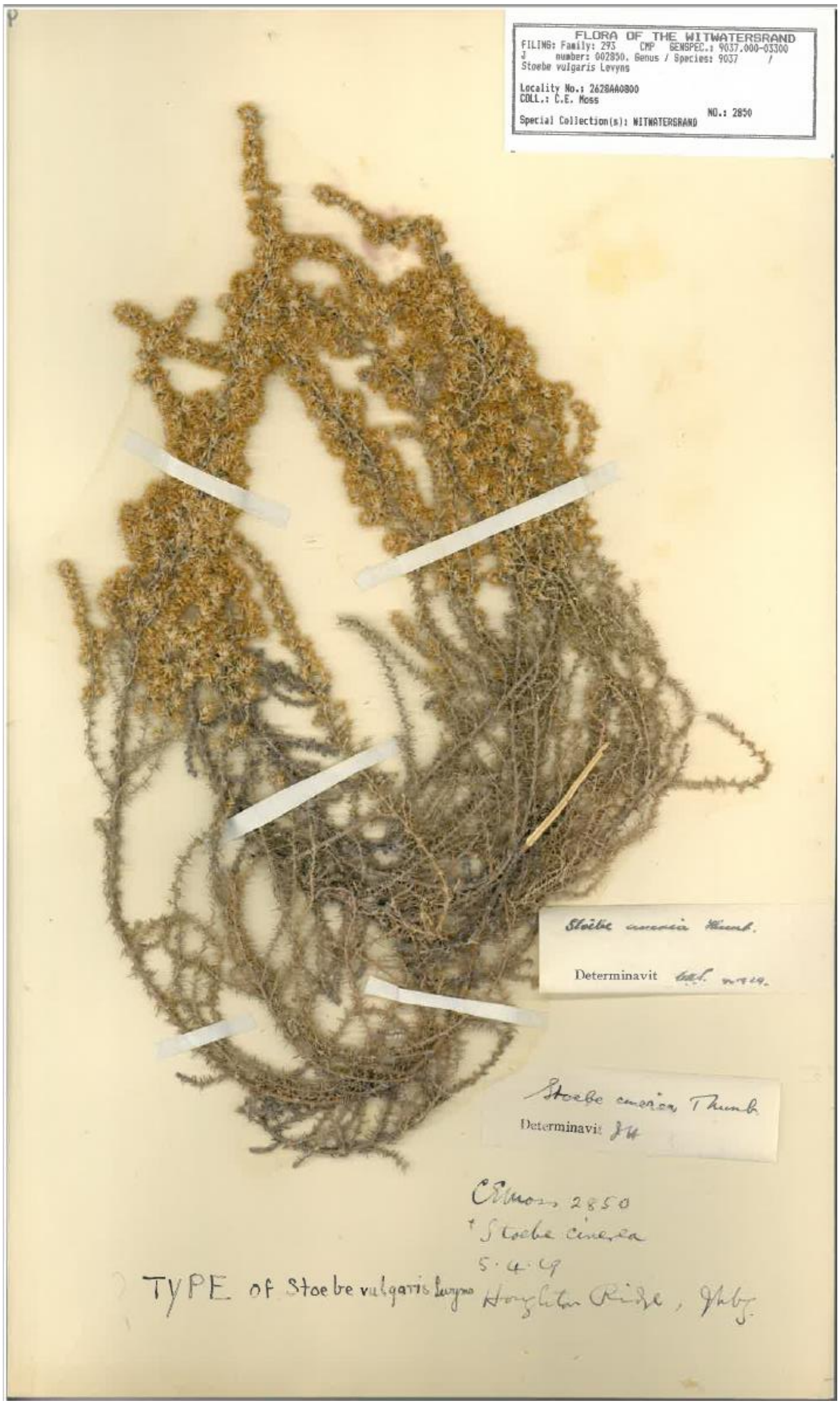


Figure 20: Scanned image of *Seriphium vulgari*s (*Moss 2850*, J). Note the slender main stem (the result of recent resprouting), linear, grey-grey leaves, and loose spicate synflorescences.

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Chapter 4: Divergent selection and drift as drivers of population-level genetic differentiation in the *Seriphium plumosum* clade (Gnaphalieae: Asteraceae)

Introduction

The Cape Floristic Region (CFR) of South Africa supports approximately 9 383 flowering plant species (Manning & Goldblatt, 2012), more than two thirds of which are endemic to the area (Cowling et al., 1992). The region's high level of endemism and the fact that over half of its floral diversity can be traced to radiation events within just 33 clades (Linder, 2003) imply that *in situ* diversification has played an important role in the evolution of its diversity (Oliver et al., 1983; Cowling et al., 1997; Onstein et al., 2014). A range of mechanisms have been proposed as possible drivers of the massive diversification of the CFR, including topographical complexity, steep local climatic gradients, edaphic heterogeneity, frequent fires, pollinator specialisation, short dispersal distances and long-term climatic stability (Cowling & Lombard, 2002; Goldblatt & Manning, 2002; Barraclough, 2006; Ellis et al., 2014). In some of the earliest works on the subject of speciation in the Cape, climate change-induced vicariance was emphasised as the dominant driver of speciation (Adamson, 1958; Levyns, 1964), a view supported by later workers on the basis of the recurring pattern of allopatry between closely-related species (Goldblatt, 1978, 1997; van der Niet & Johnson, 2009). This idea has become known as the "classical theory" for speciation at the Cape - that is - that speciation in the CFR has been largely non-adaptive (Ellis et al., 2014), with examples recently described in *Tetraria* (Britton et al., 2014; Verboom et al., 2015) and *Protea* (Prunier & Holsinger, 2010; Prunier et al., 2017). Much recent research, however, emphasises the role of divergent selection in promoting the formation of barriers to gene flow (ecological speciation; Rundle & Nosil, 2005) as the dominant driver of species diversification in the Cape flora (Linder, 1985; Linder & Vlok, 1991; Goldblatt & Manning, 2002; Savolainen & Forest, 2005; Barraclough, 2006). Overall, adaptive and non-adaptive processes, acting independently or in concert, appear to have been important in generating the massive diversity of the region (Linder, 2003), but the relative influence of either of these processes on patterns of gene flow in the CFR remains unclear (Hoffman et al., 2015; Ellis et al., 2014; van der Niet & Johnson, 2009).

Speciation occurs within a geographical context (Levin, 1993) and involves the genetic divergence of lineages. Under ecological speciation, ecologically-based divergent selection, adaptation, and ecological specialisation drive the evolution of barriers to genetic exchange between populations (Rundle & Nosil, 2005). This type of lineage divergence is typically thought to be initiated in allopatry, or at least in parapatry (Coyne & Orr, 2004; Rundell & Price, 2009), but differentiation of populations due to divergent selection is also considered possible with populations in sympatry (Rundle & Nosil, 2005; Nosil, 2012). Speciation by random genetic drift involves the fixation of alternative alleles, the neutral divergence of populations, and the subsequent formation of distinct lineages (Nosil et al., 2009; Nosil, 2012; Ellis et al., 2014). Although speciation by uniform selection (Nosil, 2012), polyploidisation, and by random genetic drift are all considered to be non-ecological modes of speciation (Sobel et al., 2010), speciation by drift is often treated as synonymous with non-ecological divergence (Nosil et al., 2009; Rundell & Price, 2009). Unlike ecological speciation, lineage divergence by genetic drift will only proceed where populations are geographically isolated (Sobel et al., 2010; Nosil, 2012; Ellis et al., 2014), and where consequently, the homogenising effect of gene flow is expected to be small or absent (Sobel et al., 2010; Ellis et al., 2014).

Sobel et al. (2010) have argued that geographic isolation is a largely neglected isolating barrier in speciation research, a view shared by recent workers who have emphasised neutral processes in the presence of weak divergent selection as the driver of lineage divergence in the CFR among taxa with phylogenetically-conserved ecological niches (*sensu* Wiens, 2004) and fragmented ranges (Britton et al., 2014; Verboom et al., 2015). Verboom et al. (2015) proposed that adaptive and non-adaptive processes play separate roles in the mountains versus lowlands in the CFR, the predominant mode of speciation being non-ecological (non-adaptive) in the archipelago-like high-elevation zone of the Cape Fold Belt mountains, but ecological (adaptive) in the more continuous, edaphically heterogeneous lowland habitats of the CFR. These authors, and Britton et al. (2014), emphasise the discontinuity of the high-elevation zone habitat as the driver of species diversification by drift, a process probably strengthened by the tendency for taxa associated exclusively with high-elevation habitats to retain their ecological niches over evolutionary timescales (Wiens, 2004). It seems reasonable, however, to infer that non-adaptive processes (i.e. drift) might be equally responsible for species diversification in other discontinuously-distributed habitats (Wiens, 2004) such as coastal, riverine or Afromontane forest habitats. Undoubtedly, the short dispersal distances characteristic of many Cape plants (Linder & Vlok, 1981; Linder, 1985; Latimer et al., 2005; Latimer et al., 2009) also plays a role in producing phylogeographic discontinuities in species' distributions in the absence of physical barriers to gene flow (Irwin, 2002), thereby promoting genetic isolation between populations.

The mechanisms generating plant diversity can be studied at multiple points along the speciation continuum; at the population genetic level prior to speciation, and retrospectively, after speciation has occurred (Goldblatt, 1978; Schaal et al., 1998; Via, 2009; Nosil, 2012; Ellis et al., 2014). Much research in the CFR investigating the drivers of lineage divergence has studied the process of speciation at the species level, revealing support for both adaptive (Linder & Vlok, 1991; van der Niet & Johnson, 2009) and non-adaptive (Prunier & Holsinger, 2010; Britton et al., 2014; Verboom et al., 2015) modes of speciation. Relatively few studies, however, have investigated the drivers of lineage diversification as an "active process" at the population level (Ellis et al., 2014; but see Rymer et al., 2010; Lexer et al., 2013; Lexer et al., 2014; Prunier et al., 2017), an approach referred to by Via (2009) as the 'magnifying glass' approach. Given that population-level differentiation within existing species is a necessary prerequisite for the formation of species in allopatry, parapatry or sympatry (Darwin, 1859; Givnish, 2010), assessing the degree and distribution of genetic differentiation between populations in relation to putative neutral and ecological drivers of the differentiation may offer valuable insights into modes of speciation in the CFR, particularly where such research investigates lineage diversification across multiple species (Orsini et al., 2013). Moreover, an evaluation of environmentally- and geographically-linked drivers of genetic divergence at the population level before speciation has occurred may clarify the causal relationship between barriers to gene flow and adaptive divergence (Linder, 1985; Coyne & Orr, 2004; Nosil et al., 2009; Nosil, 2012; Prunier et al., 2017).

Although previous studies of population-level genetic structure in the CFR have focused on animals (Lexer et al., 2013), a handful have evaluated the drivers of population-level genetic structure in flowering plants. These have used signatures of isolation-by-environment (IBE) and isolation-by-distance (IBD) as signatures of ecological and vicariant divergence, respectively. Isolation-by-environment describes patterns of gene flow where populations inhabiting different ecological environments are more genetically diverged, leading to a positive correlation between genetic and environmental distance between diverging populations, regardless of the geographical distance and/or geographical discontinuities between them (Rundle & Nosil, 2005; Wang et al., 2013). On the other hand, IBD describes patterns of gene flow where remote individuals experience reduced gene flow, regardless of the environmental distance between them (Wright, 1943, 1946; Orsini et al., 2013). Geographical distance and geographical isolation have been identified as drivers of population divergence in both *Erica coccinea* (Ericaceae; Segarra-Moragues & Ojeda, 2010) and the white *Protea* clade (Proteaceae; Prunier & Holsinger, 2010), while adaptive divergence is supported as the driver of population differentiation in the *Gladiolus carinatus* species complex (Iridaceae; Rymer et al., 2010). Only two studies to date in the CFR have investigated population-level divergence within both an ecologically *and* spatially explicit context, with contrasting results: where Lexer et al. (2014) identified isolation-by-environment, potentially due to differences in water availability and soil type, as the dominant driver of population divergence in the widespread CFR endemic *Restio capensis* (Restionaceae), Prunier et al. (2017) found greater support for isolation-by-distance, and only limited support for isolation-by-environment in the widespread *Protea repens*

(Proteaceae). Prunier et al. (2017) suggest that differences in the way the species are pollinated (anemophily in *R. capensis* and ornithophily/entomophily in *P. repens*; Lexer et al., 2014) and intrinsic differences in the species' responses to environmental gradients may explain the contrasting patterns of IBE and IBD as dominant drivers of population divergence. A comparison of these latter two studies yields two important insights; the first is that the spatial scale of gene flow, and therefore the potential strength of IBD, is highly taxon-specific, and the second is that intrinsic biological differences between taxa determine their evolutionary responses to environmental gradients (Rundell & Price, 2009). Although none of these studies investigated population genetic structure within the context of the lowland/montane predictions made by Verboom et al. (2015), it is interesting to note that in *Erica coccinea*, patterns of isolation-by distance are stronger for populations distributed in the mountains of the Cape Fold Belt than for those distributed in the Cape lowlands, a finding the authors (Segarra-Moragues & Ojeda, 2010) attribute to the shorter generation times associated with lowland (seeder) populations relative to the montane (resprouter) populations.

The nine species that comprise the *Seriphium plumosum* clade form an ideal system for exploring drivers of population differentiation and speciation in the Cape flora. Most of the species in this clade have geographic distributions that span steep climatic, topographic, and edaphic gradients over tens to hundreds of square kilometres (Figure 2). In addition, the species have geographic distributions that are fragmented and discontinuous to a lesser of greater degree, and the discontinuities may have different structures (e.g. linear discontinuity versus high-elevation archipelago). Where *Seriphium plumosum, S. burchellii* and *S. crypticum* associate predominantly with the Cape Fold Belt mountains, *S. virgatum* and *S. dunensis* inhabit the coastal plains of the southern part of the CFR, and *Seriphium vulgaris* has an extensive distribution outside the CFR in the summer-rainfall region of South Africa. The species in the *S. plumosum* clade all have very similar floral structures, a likely indication of very similar breeding system and pollen dispersal dynamics. In addition, they have similar pappus structures, seed numbers, seed sizes and weights, and similar release heights, all of which imply similar seed dispersal dynamics. Although the pappus remains loosely attached to the mature seed after shedding, it has a “parachute” rather than a long-distance dispersal function, such that seeds are passively dispersed, with dispersal distances being typically short, save in exceptional cases (Cohen, 1940). A typical leptokurtic pattern of seed dispersal suggests a possible role for the formation of phylogeographic discontinuities, with implications for non-adaptive divergence among populations not separated by physical gene flow barriers.

Within the context of the above, I propose that habitat discontinuity is an important driver of population structure in the Cape flora, and that among highly fragmented and discontinuously-distributed populations, drift, rather than divergent selection, is the strongest driver of genetic divergence. Conversely, divergence along environmental gradients may drive population structure in populations with more continuous distributions. Focusing on six of the species in the *S. plumosum* clade with broad geographic distributions for which genomic data were captured; *Seriphium burchellii*, *S. crypticum, S. dunensis, S. plumosum, S. virgatum,* and *S. vulgaris*, I test the following predictions:

- Within species having discontinuous ranges, population genetic differentiation is expected to be stronger, and driven by genetic drift, while within species with continuous ranges, population differentiation is expected to be weaker, and driven by adaptive processes.
- Within species with discontinuous distributions, geographical distance and/or habitat continuity is expected to be the strongest predictor of population structure, while within species with continuous distributions, ecoclimatic distance is expected to more strongly predict population structure.

Methods

Sampling and population genetic characterisation

Population sampling, DNA extraction, GBS, and single nucleotide polymorphism (SNP) marker calling and filtering are detailed in Chapter 2. Genetic differentiation was captured using F_{ST}, which estimates the proportion of total genetic variance attributable to between-population variance (Holsinger & Weir, 2009). Since Wright's (1943) F_{ST} has been shown to overestimate population genetic divergence (Willing et al., 2012), Weir and Cockerham's (1984) fixation index (hereafter F_{st}^{WC}) was used to generate estimates of pairwise population divergence. Although population samples were small, population genetic divergence has been shown to be accurately estimated on the basis of small samples (n = 2-6) where a large number (>500) of biallelic unlinked SNPs is used (Willing et al., 2012; Nazareno et al., 2017). Weir and Cockerham's F_{st} was estimated for six sequenced individuals from each of four populations of *Seriphium burchellii*, eight of *S. crypticum*, four of *S. dunensis*, fourteen of *S. plumosum*, six of *S. virgatum*, and eight of *S. vulgaris*, each species being characterised using between 27 672 and 28 023 confidently identified biallelic SNP markers (Figure 3). Three distinct lineages were revealed (Chapter 2, Table 1 and Figure 6) within *S. plumosum* as defined in Chapter 3 of this work, each of which could be analysed independently for patterns of spatially- or environmentally-determined population genetic structure. However, gaps in sampling may have created the appearance of genetic discontinuities where there are none, such that a single analysis for this species was preferred. The possibility that the three lineages within *S. plumosum* (as defined in Chapter 3) are truly discontinuous, and therefore deserving of independent analysis, will be further explored when this chapter is prepared for publication. For each of the six species, the complete clade-wide SNP data set was filtered to exclude non-polymorphic SNPs (i.e. to include only SNPs for which there is at least one polymorphism at each SNP among each of the six sequenced individuals) and converted to genlight format in the package *vcfR* version 1.8.0 (Knaus & Grünwald, 2017) as implemented in *R* version 1.1.423 (R Core Team, 2018). For each of the species, F_{st}^{WC} was calculated using the filtered and converted SNP data sets using the R package *hierFstat* version 0.04-22 (Goudet & Jombart, 2015). Population genetic structure within each species was summarised using principal coordinates analysis applied to the SNP data set for each species using the R package *dartR* version 1.0.5 (Gruber & Georges, 2018). Previous authors (Lexer et al., 2014; Prunier et al., 2017) have assumed loci having F_{ST} values significantly different from the mean ("outlier loci") are loci potentially under divergent selection, using them as the response variable when assessing patterns of IBD and IBE. Even when very stringent criteria are used to infer outlier loci as a precaution against including non-outlier loci ($p < 0.0001$; Prunier et al., 2017), the assumption that outlier F_{ST} loci are under selection is problematic because loci with high F_{ST} values can also arise by drift, as well as by selection on loci not relevant to speciation (Via, 2009; Cruikshank & Hahn, 2014; Wolf & Ellegren, 2017). The analyses presented here, therefore, use genetic differentiation as captured by F_{st}^{WC} across all loci as the response variable.

Adaptive and non-adaptive drivers of population genetic structure

Three potential spatial determinants of population divergence were examined: linear geographical distance between populations, ecoclimatic distance, and habitat continuity. Geographical distance was investigated as a predictor of population differentiation within the conceptual framework provided by the isolation-by-distance (IBD) model (Wright, 1943, 1946). Linear geographical distance between sampled populations was calculated using the *R* package *geosphere* version 1.5-5 (Hijmans, 2017a). The natural logarithm of geographical distance is traditionally used as the explanatory variable when assessing IBD (Orsini et al., 2013), but since this transformation and untransformed geographical distances yielded similar results, untransformed geographical distance was used here instead.

Incipient ecological speciation may be revealed by patterns of isolation-by-environment (Nosil et al., 2009). Ecoclimatic distance between each population was calculated as pairwise Euclidean distance using 17 environmental variables selected from a broader suite of nineteen temperature and moisture variable layers (Hijmans et al., 2005) and 25 soil variable layers (Hengl et al., 2015). Variables were selected by retaining a single variable for clusters of variables with multi-collinearity values (Pearson's correlation coefficient) exceeding 0.75, estimated using the *R* package *virtualspecies* version 1.4-4 (Leroy et al., 2015). The selected variables relate to temperature (annual mean temperature, maximum temperature of the warmest month, isothermality, maximum temperature of the driest quarter, maximum temperature of the wettest quarter, temperature seasonality, maximum temperature of the warmest month; Hijmans et al., 2005), precipitation (precipitation of the wettest month, precipitation of the coldest quarter, precipitation seasonality, precipitation of the driest month, Hijmans et al., 2005), soil characteristics (cation exchange capacity of soil [cmolc/kg], clay content [percentage mass fraction], coarse fragments [percentage volume], pH, silt content [percentage mass fraction]; Hengl et al., 2015), and elevation (Hijmans et al., 2005). Soil layers with 250m^2 resolution were bilinear-resampled using the *R* package *raster* version 2.6-7 (Hijmans, 2017b) to standardise all layers to 30 arc-second resolution. The value of each environmental variable for each species occurrence record (Appendix A) was queried using the *R* package *raster*. In order to ensure that environmental variables with large values do not contribute disproportionately to ecoclimatic distances, the variables were scaled by subtracting the variable mean from each value, and then dividing by the standard deviation of the variable. A matrix of pairwise environmental Euclidean distances between populations was then calculated for each species using the built-in *R* package *stats* version 3.5.1. (R Core Development Team, 2018).

Habitat continuity between localities was estimated by assessing the continuity of areas having a ≥0.5 probability of species' occurrence, with the known distribution of each species based on occurrence records used to model the potential range. Occurrence records for each species (Appendix A) were obtained from field-sampled localities supplemented with herbarium records, where the latter could confidently be assigned to species and manually georeferenced to ≤2000 m. Most species had more than ten occurrence records, although only eight were available for *S.*

dunensis and more than 60 were obtained for *S. plumosum*. The potential range of each species was modelled using MaxEnt (maximum-entropy species niche and distribution modelling; Phillips et al., 2004) implemented in the *R* package *dismo* version 1.1-4 (Hijmans et al., 2017) using the 30 arc-second resolution environmental variable layers above. A pairwise habitat continuity matrix was then developed for each species (using the *R* package *raster*) as illustrated in Figure 1. Each species' modelled range was subsampled to include only areas with a ≥ 0.5 probability of species occurrence, which were then used to generate a layer of polygons corresponding to the perimeter of each range fragment. The identity of the polygon containing each species occurrence record was then queried in order to construct a pairwise continuity matrix for each pair of locality points. The matrix was generated by allocating a value of one to pairs of occurrence records from the same polygon, and values of zero to pairs of records from different polygons.

Because habitat continuity as described above is captured only as a pairwise matrix comparison and may fail to capture more nuanced aspects of the habitat continuity of species, an additional metric, the range continuity index (RCI), was computed for each species as a measure of habitat/potential range continuity. The RCI for each species was calculated by projecting one hundred random points onto the modelled layers for ≥ 0.5 probability (i.e. only within the bounds of the modelled areas) of species occurrence generated above (Figure 1). Habitat continuity was then calculated as:

$$\text{Range continuity index (RCI)} = \frac{\text{Number of point pairs on the same polygon}}{\text{Total number of pairwise point comparisons}} \qquad \text{Eq. 1}$$

Where RCI = 1 implies that all 100 sampling points are connected by suitable habitat, and RCI = 0 implies that all sampling points are separated from all others by areas of unsuitable habitat which may function as barriers to dispersal. The same range continuity index calculations were performed using georeferenced occurrence records for each species (Figure 1; Appendix A).

Population-level analysis

For each species, the effects of linear geographical distance, habitat continuity, and ecoclimatic distance on genetic differentiation between populations were jointly evaluated using partial Mantel tests (Mantel, 1967) with the statistical significance of the coefficients assessed by randomly permuting the rows and columns of the response (genetic distance) matrix 9999 times, a procedure that has the effect of randomly assigning genetic distances to populations while preserving predictor values. Partial Mantel tests were performed using the R package *phytools* version 0.6-44 (Revell, 2012). For species whose habitat continuity matrix contained only zeros (i.e. where all populations are apparently isolated), habitat continuity was excluded from partial Mantel regression as an uninformative explanatory variable.

Species-level analysis

Across the six species, maximum range extent, ecoclimatic volume, and habitat continuity were investigated as predictors of population-level genetic differentiation using simple linear regression. The largest linear geographic distance between sampled populations (for which sequence data were obtained) and the maximum ecoclimatic distance between sequenced populations were used to estimate the maximum range extent and ecoclimatic range respectively. The range continuity indices (described above) were used to estimate the habitat continuity of each species. Following the predictions made by Verboom et al. (2015), elevation was also evaluated as a predictor of population-level differentiation across the species. Linear regression was implemented using the built-in *R* package *stats* version 3.5.1.

Results

Patterns of population-level diversity and differentiation

Estimates of population-level genetic structure (Figure 3) ranged from 0.0043 in *S. burchellii* to 0.0606 in *S. dunensis*. This indicates very weak genetic differentiation among populations in *Seriphium burchellii* and moderate differentiation among populations in *S. dunensis*, with intermediate levels of differentiation in *S. crypticum, S. plumosum, S. virgatum,* and *S. vulgaris*. Overall, principal coordinates analysis of SNP data revealed patterns of inter- and intra-population genetic divergence largely concordant with the estimates of F_{st}^{WC} for each species (Figure 3). For example, in *S. burchellii*, the first two PCos for genomic differentiation explain only 15.6% of the total variance and reveal that individuals within the same population are frequently more genetically divergent than individuals from different populations, a result consistent with the low F_{ST}^{WC} for *S. burchellii* which implies little inter-population differentiation relative to intra-population divergence (Figure 3a; Wright, 1943, 1978; Fumagalli et al., 2013). On the other hand, the first two PC axes for *S. dunensis* explain over 50% of the variance and differentiate strongly between populations, with individuals from the same population clustering close together (Figure 3f), a pattern consistent with the F_{ST}^{WC} for this species, which implies little intra- relative to inter-population differentiation.

Determinants of population genetic structure within species

Weir and Cockerham's F_{ST} was significantly related to untransformed geographical distance in *Seriphium plumosum*, *S. virgatum*, and *S. vulgaris,* but not in *S. burchellii*, *S. crypticum*, or *S. dunensis* (Table 1). A positive relationship between ecological distance and F_{ST}^{WC} is observed across all six species, although across all six species these patterns lacked statistical support (Table 1). Populations isolated on 'islands' of suitable habitat are frequently more genetically diverged that those connected by areas of suitable habitat (Figure 4, Figure 5), but these patterns also lacked statistical support across all six species (Table 1). Note that the statistical power of the partial Mantel test is somewhat limited for species with few pairwise population comparisons. Limited power may explain why spatial isolation was not revealed as a significant predictor of genetic differentiation across all species, but especially for *S. crypticum* and *S. dunensis*, which have the least continuous ranges (the lowest range continuity indices; RCI) of all six species when RCI is calculated using random points projection, and some of the least continuous ranges when RCI is calculated using the known locations of populations (Figure 4a-b). Consistent with our expectations, there appears to be a general trend toward isolated populations being more genetically differentiated than those connected by areas suitable for occupation (Figure 4, Figure 5).

Correlates of population genetic structure across species

Weak positive relationships are revealed between population differentiation and both ecoclimatic volume (Figure 6a) and maximum range extent (Figure 6b), but these patterns lack statistical support. Population genetic structure tends to decline as a function of elevation, but this relationship also lacks statistical support (Figure 6e). Genetic differentiation tends to decline as a function of habitat continuity (Figure 6c-d), a relationship which is statistically supported when habitat continuity is estimated using true species occurrence records (Figure 6d), but not when RCI is calculated using random points projection (Figure 6c). The range continuity index based on random points projection is likely more reliable given that RCI based on true species occurrence records vulnerable to the non-random sampling of points, and to biases related to the different numbers of records for each species (Appendix A).

Discussion

The present work provides evidence for range discontinuity as the strongest correlate of differentiation in the *S. plumosum* clade, with little evidence for adaptive divergence as a driver of population differentiation within the six species included in this comparison. In general, populations isolated by zones of unsuitable habitat exhibit stronger population genetic differentiation than those connected by areas of suitable habitat (Figure 5, Figure 6d). This pattern is almost certainly attributable the effect of habitat barriers on gene flow, with only the most continuously-distributed species showing clear signatures of IBD indicative of ongoing gene flow (Table 1; Figure 4; Wright, 1943, 1946). These findings suggest a greater role for neutral processes in driving divergence among discontinuously-distributed taxa where the homogenising effects of gene flow are minor or absent. Overall, the results presented are more consistent with the classical theory that speciation in the CFR as largely driven by geography and is non-adaptive and vicariant, and counter the idea that the massive floristic diversity of the region is powered by divergent adaptation along ecological gradients (Linder, 1985; Linder & Vlok, 1991; Linder, 2005; van der Niet & Johnson, 2009; Carlson & Holsinger, 2012; van der Niet et al., 2014).

The high level of population genetic differentiation (Figure 3f) and extreme habitat discontinuity in *S. dunensis* (Figure 4a), and conversely, the very low level of population genetic differentiation (Figure 3a) and high habitat continuity in *S. burchellii* (Figure 4f) support the prediction made in this work that discontinuously-distributed taxa should show the highest levels of genetic differentiation (Figure 6d). The inverse relationship between genetic differentiation and the continuity of species' habitats holds generally for *S. plumosum, S. virgatum* and *S. vulgaris* (Figure 3, Figure 4), with the single exception of *S. crypticum*, which, despite its highly fragmented distribution (Figure 4b), shows limited population genetic differentiation (Figure 3c). Note, however, that F_{ST}^{WC} for *S. crypticum* was estimated using several spatial clusters of "populations" which may in fact comprise a single population (Figure 2), and that this may have depressed the F_{ST}^{WC} estimate for the species. Although the within-species analyses of population-level structure provided no statistical support for habitat continuity as a predictor of genetic differentiation (Table 1), habitat continuity represented as a binary predictor variable probably captures habitat continuity poorly. Based on the patterns of greater genetic differentiation among isolated populations (Figure 5) and on the relationship between habitat isolation and population genetic differentiation at the population level across species (Figure 6c-d), I propose that for *S. crypticum* and *S. dunensis* (the most discontinuously-distributed taxa; Figure 4), non-adaptive (neutral) processes drive population divergence in allopatry, and that these patterns are maintained by low seed dispersal capabilities (Cohen, 1940) and/or ecological niches that are phylogenetically conserved (Wiens, 2004).

Previous studies (Lexer et al., 2014; Prunier et al., 2017) have interpreted patterns of isolation-by-distance as support for lineage divergence by drift. However, given that neutral divergence leading to speciation is only expected to proceed between allopatric populations (Sobel et al., 2010; Nosil, 2012; Ellis et al., 2014), and that patterns of IBD only exist between individuals distributed continuously in space or between populations connected by gene flow (Wright, 1943, 1946; Orsini et al., 2013), the assumption that patterns of IBD at the population level can be interpreted as evidence of lineage divergence leading to speciation is potentially misguided (Levin, 1993). The use of IBD as a proxy of differentiation by random genetic drift is further questioned by the observation that the species with the most fragmented ranges as captured by RCI within the *S. plumosum* clade do not exhibit patterns of IBD. Patterns of IBD are interpreted here instead as evidence of recent or ongoing (albeit spatially structured) gene flow that simultaneously erodes population genetic structure (or indeed precludes the formation of such structure) and maintains the genetic cohesiveness of populations. The IBD patterns reported here reveal interesting relationships between genetic differentiation, distance, and the habitat continuity of species. In *Seriphium crypticum* and *Seriphium dunensis*, linear geographical distance between populations fails to explain population-level genetic differentiation (Table 1), most likely because among genetically-isolated populations, the geographic distance between populations is expected to be of little consequence to population differentiation, and the sole determinant of genetic similarity between the populations is expected to be time to shared ancestry (Schaal et al., 1998). In *Seriphium burchellii*, on the other hand, low levels of population differentiation (Table 1) and high levels of habitat continuity (Figure 4a) suggest the absence of IBD reflects a lack of dispersal limitation and widespread gene flow among populations (i.e. panmixia). The IBD patterns uncovered in *Seriphium plumosum, S. virgatum,* and *S. vulgaris*, and the finding that ecoclimatic distance does not appear to drive genetic differentiation among populations within these species (Table 1) implies that limited dispersal (Cohen, 1940) and neutral divergence rather than adaptation along environmental gradients powers population divergence in these species.

In taxa both with continuous and discontinuous distributions, no support was found for ecoclimatic distance as a driver of population genetic differentiation (Table 1; Figure 6a). The failure to detect a significant relationship between ecoclimatic distance and genetic distance across all six species could be attributed to the possibility that the ecoclimatic distance matrix developed here fails to capture aspects of the abiotic environment that promote divergent selection. However, the ecoclimatic distance matrix used here takes into account aspects of the contemporary moisture, temperature and edaphic environment, all of which are considered to be key variables promoting the ecophysiological specialisation of plants in the CFR (Cramer et al., 2014). Given that most environmental variables change across the CFR (Cramer et al., 2014), it is likely that weak divergent selection is indeed driving population genetic divergence among continuously- and discontinuously-distributed taxa, but that the effect size is so small as to be undetectable given the small sample sizes used here.

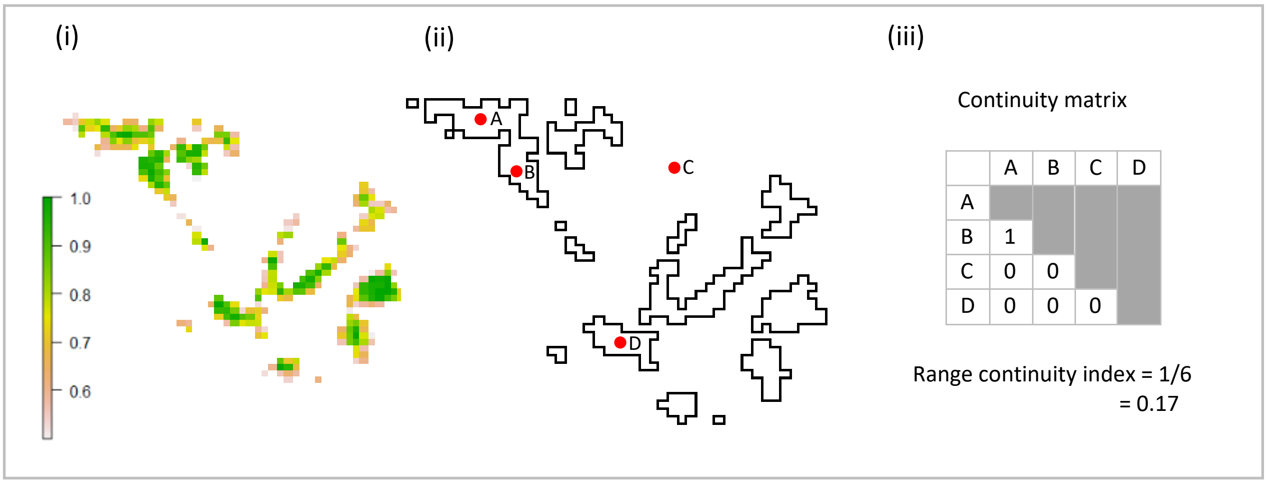
Several methodological limitations of the approach taken in this work merit recognition. First, population sampling was non-random, and different numbers of populations were sampled for each species, which likely biased the estimation of F_{st}^{WC}, the modelled potential ranges of each species, and the estimates RCI using species occurrence records for each species. A linear regression of population number against F_{st}^{WC}, however, shows that the number of populations used to estimate F_{st}^{WC} do not predict F_{st}^{WC} ($R^2 = 0.02$, $p = 0.80$). Second, the use of IBD for assessing patterns of gene flow between remote individuals, though ideal for taxa with linear distributions, is poorly suited to taxa with more complex distributions, such as those associated with the Cape Fold Belt mountains (Irwin, 2002). Third, the Mantel test, and specifically the partial Mantel test, despite its widespread application (Diniz-Filho et al., 2013) has been criticized for its high Type I (false positive) error rates (Guillot & Rousset, 2013). Evidence of recent and/or ongoing, albeit spatially-constrained, gene flow among populations within *S. plumosum, S. virgatum* and *S. vulgaris* revealed here by partial Mantel tests, however, appears to be corroborated by the tendency for neighbouring populations of these species to show greater genetic affinities when population genetic structure is summarised using principal coordinates analysis (Figure 2; Figures 3d-f).

Although not a central aim of this Chapter, the estimate of population-level differentiation in *Seriphium vulgaris* (*Stoebe vulgaris* Levyns) presented here contributes to an improved understanding of the species' history as an encroacher in South African rangelands. The encroachment of the species into agricultural land has long been recognised as a problem in the summer-rainfall region of South Africa, which early authors attributed to overgrazing (Cohen, 1935; Levyns, 1937; Cohen, 1940; Snyman, 2009). Although the limited genetic differentiation among populations within *Seriphium vulgaris* would appear to support an evolutionarily recent invasion of the summer-rainfall region, F_{ST}^{WC} in the species is not outside the range of values obtained for other species in the *S. plumosum* clade (Figure 3). Given that *S. vulgaris* is the only species in the clade known to resprout after fire (Levyns, 1937), low population differentiation in the species could be associated with the species' relatively longer generation times (cf. reseeding species in the clade; Cowling & Lombard, 2002; Barraclough, 2006). A longer evolutionary history of the species in the summer-rainfall region of South Africa cannot, I propose, be discounted for the species.

The present work highlights the value of studying differentiation at the population level as a means of gaining insight into the relative contributions of adaptive and non-adaptive processes in driving speciation. Although there is no guarantee that population divergence leads to speciation (Via, 2009), this approach avoids the difficulties encountered when studying speciation retrospectively at the species level, where changes in geographic distribution and/or the evolution of ecological differences obscure the relative influences of adaptive and non-adaptive processes to speciation

(Linder et al., 1985; Coyne & Orr, 2004; Nosil et al., 2009; Nosil, 2012; Prunier et al., 2017). Consistent with the classical theory of speciation emphasising the importance of vicariance in the CFR (Adamson, 1958; Levyns, 1964; Goldblatt, 1978, 1997, van der Niet & Johnson, 2009; Britton et al., 2014; Verboom et al., 2015), the findings presented here imply an important role for range discontinuity in restricting gene flow between populations, and little evidence for speciation along environmental gradients (at least as concerns gradients of soil, moisture and temperature). Such an interpretation, if applicable to Cape lineages generally, accords with Levin's (1993, 2000) view that plant speciation involves the isolation of populations through either the contraction of species' ranges, or through dispersal outside the existing species range. It also explains the high beta diversity of the Cape flora (Cowling et al., 1992), which Latimer et al. (2005) similarly attributed to isolation and speciation over small spatial scales (1 – 100 kilometres). This interpretation is also consistent with the species tree for the *S. plumosum* clade presented in Chapter 2 (Figure 6) and the distribution of the species (this chapter; Figure 2), showing that sister species almost always have non-overlapping distributions. Notwithstanding the evidence presented for neutral divergence as the primary driver of population differentiation, vicariant speciation cannot be considered entirely non-ecological since the conservation of ecological niches over evolutionary timescales contributes to the creation and then the maintenance of range discontinuity (Wiens, 2004), and because ecological conditions are highly unlikely to be identical between any two diverging lineages, such that weak divergent selection (which, independently, would not be adequate to drive lineage divergence in the face of gene flow) likely contributes to the genetic divergence of vicariant lineages (Sobel et al., 2010).

Figures



	A	B	C	D
A				
B	1			
C	0	0		
D	0	0	0	

Range continuity index = 1/6
= 0.17

Figure 1: Hypothetical modelled species distribution (i) showing areas with ≥ 0.5 probability of species occurrence, which can be used to construct polygons of continuous habitat (ii). A habitat continuity matrix (iii) can then be generated by querying the identity of the polygon into which points (true species occurrence records or random projected points) fall. The habitat continuity matrix can then be used to calculate the range continuity index RCI; the proportion of pairwise comparisons for which records fall into the same polygons (ones) out of the total number of pairwise non-self comparisons (Equation 1). Note that points outside the modelled species range (in this example, point C) are treated as falling within unique polygons.

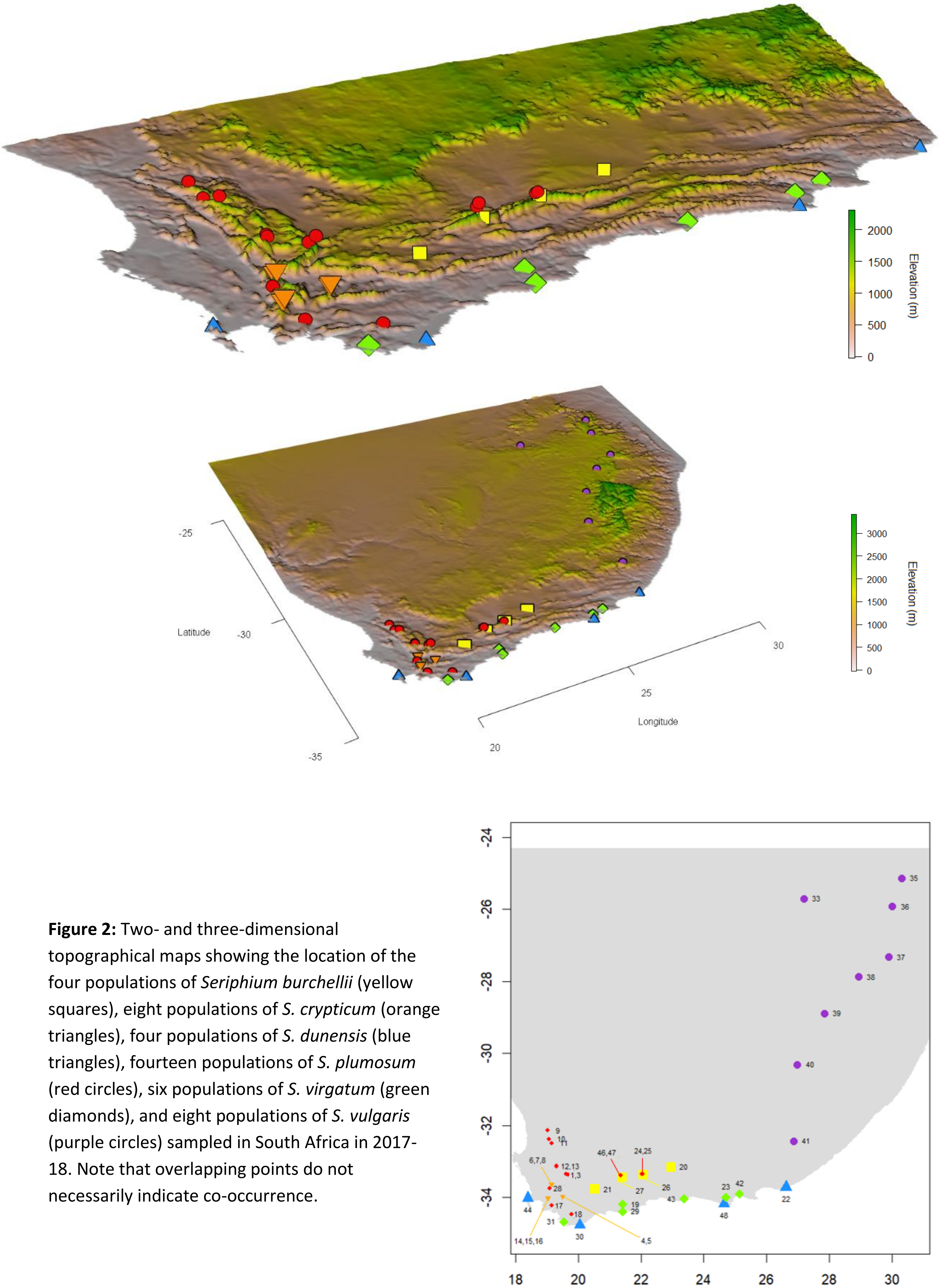


Figure 2: Two- and three-dimensional topographical maps showing the location of the four populations of *Seriphium burchellii* (yellow squares), eight populations of *S. crypticum* (orange triangles), four populations of *S. dunensis* (blue triangles), fourteen populations of *S. plumosum* (red circles), six populations of *S. virgatum* (green diamonds), and eight populations of *S. vulgaris* (purple circles) sampled in South Africa in 2017-18. Note that overlapping points do not necessarily indicate co-occurrence.

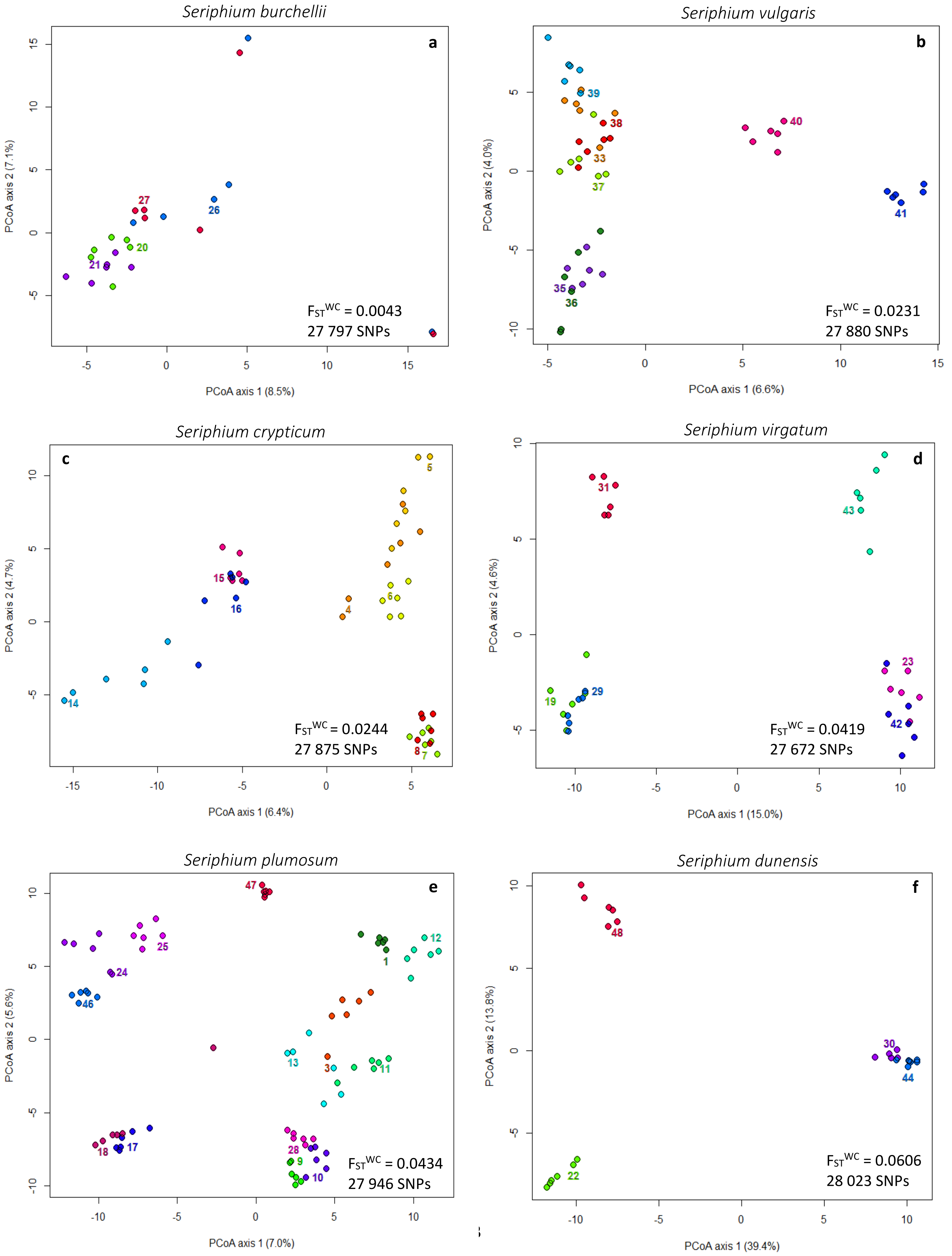

Seriphium burchellii
a
27
26
20
21
FSTWC = 0.0043
27 797 SNPs
PCoA axis 1 (8.5%)
PCoA axis 2 (7.1%)
Seriphium vulgaris
b
39
38
33
37
40
41
35
36
FSTWC = 0.0231
27 880 SNPs
PCoA axis 1 (6.6%)
PCoA axis 2 (4.0%)
Seriphium crypticum
c
5
15
16
4
6
14
8
7
FSTWC = 0.0244
27 875 SNPs
PCoA axis 1 (6.4%)
PCoA axis 2 (4.7%)
Seriphium virgatum
d
31
43
19
29
23
42
FSTWC = 0.0419
27 672 SNPs
PCoA axis 1 (15.0%)
PCoA axis 2 (4.6%)
Seriphium plumosum
e
47
25
24
46
1
12
13
3
11
18
17
28
9
10
FSTWC = 0.0434
27 946 SNPs
PCoA axis 1 (7.0%)
PCoA axis 2 (5.6%)
Seriphium dunensis
f
48
30
44
22
FSTWC = 0.0606
28 023 SNPs
PCoA axis 1 (39.4%)
PCoA axis 2 (13.8%)

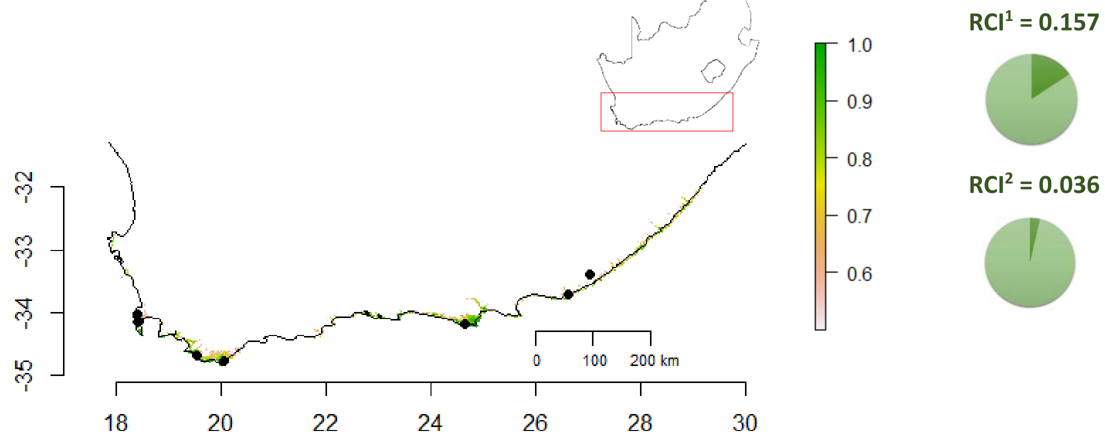
Figure 3: Population-level genetic differentiation summarised using principal coordinates analysis. The two PC axes for PCoA ordination using biallelic SNPs as attributes are shown for (a) *Seriphium burchellii*, (b) *S. vulgaris*, (c) *S. crypticum*, (d) *S. virgatum*, (e) *S. plumosum* and (f) *S. dunensis* in order of increasing F_{ST}^{WC}. Each individual is shown with a single point, and population numbers coloured by points are consistent with those in Figure 1. Weir and Cockerhams' F_{ST} and the number of SNPs polymorphic for at least one individual per species used in each ordination is shown in individual boxes. Colours are not consistent with Figure 2.

Table 1: Results of partial Mantel regression tests assessing the relationship between Weir and Cockerhams' fixation index (F_{ST}^{WC}) estimated from biallelic SNP markers and linear geographical distance, environmental distance, and effective population isolation between populations for selected species in the *S. plumosum* clade. Coefficient significance was evaluated by permuting the rows and columns of the genetic distance matrix 9999 times while controlling for the effects of second and third explanatory variable matrices.

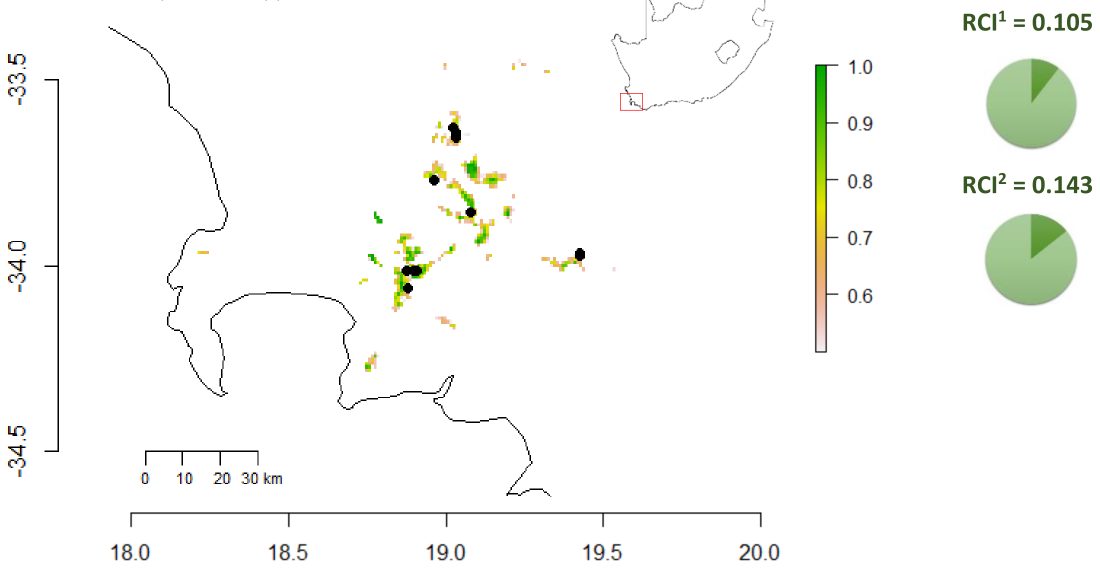
Taxon	**Number of populations**	**Geographical distance β**	**Environmental distance β**	**Habitat continuity β**	**Multiple R^2**
Seriphium burchellii	4	1.27 x 10^{-4}	8.68 x 10^{-3}	2.08 x 10^{-3}	0.67
S. crypticum	8	2.70 x 10^{-4}	-2.27 x 10^{-5}	8.59 x 10^{-4}	0.17
S. dunensis	4	1.07 x 10^{-4}	-1.17 x 10^{-2}	-	0.92
S. plumosum	14	9.30 x 10^{-5} *	2.87 x 10^{-3}	7.73 x 10^{-3}	0.39 ***
S. virgatum	6	9.69 x 10^{-5} *	3.60 x 10^{-4}	-1.51 x 10^{-2}	0.66 **
S. vulgaris	8	3.89 x 10^{-5} **	1.86 x 10^{-4}	-4.11 x 10^{-3}	0.54 **

* indicates co-efficient p-value < 0.05
** indicates co-efficient p-value < 0.01
*** indicates co-efficient p-value < 0.001

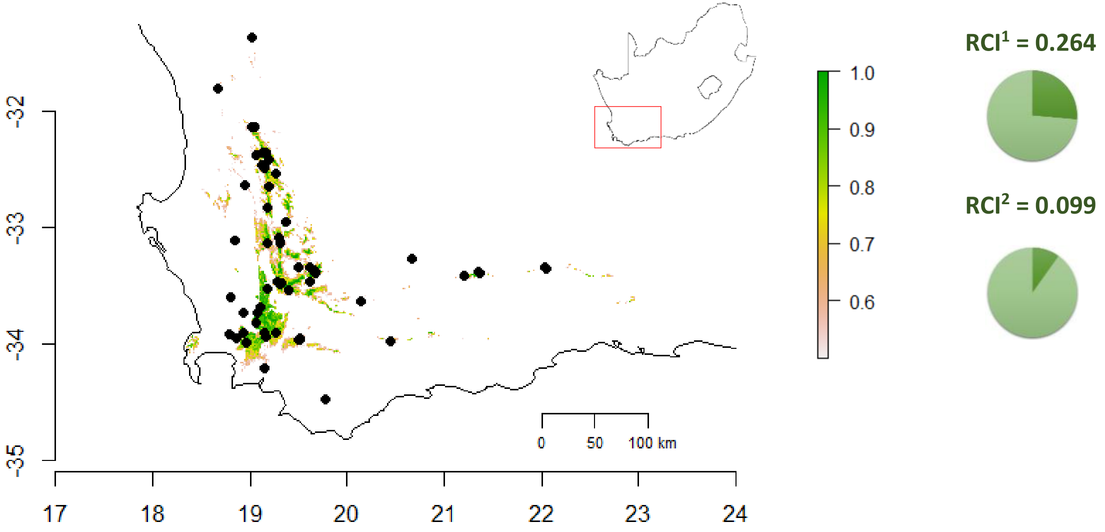
(a) *Seriphium dunensis*



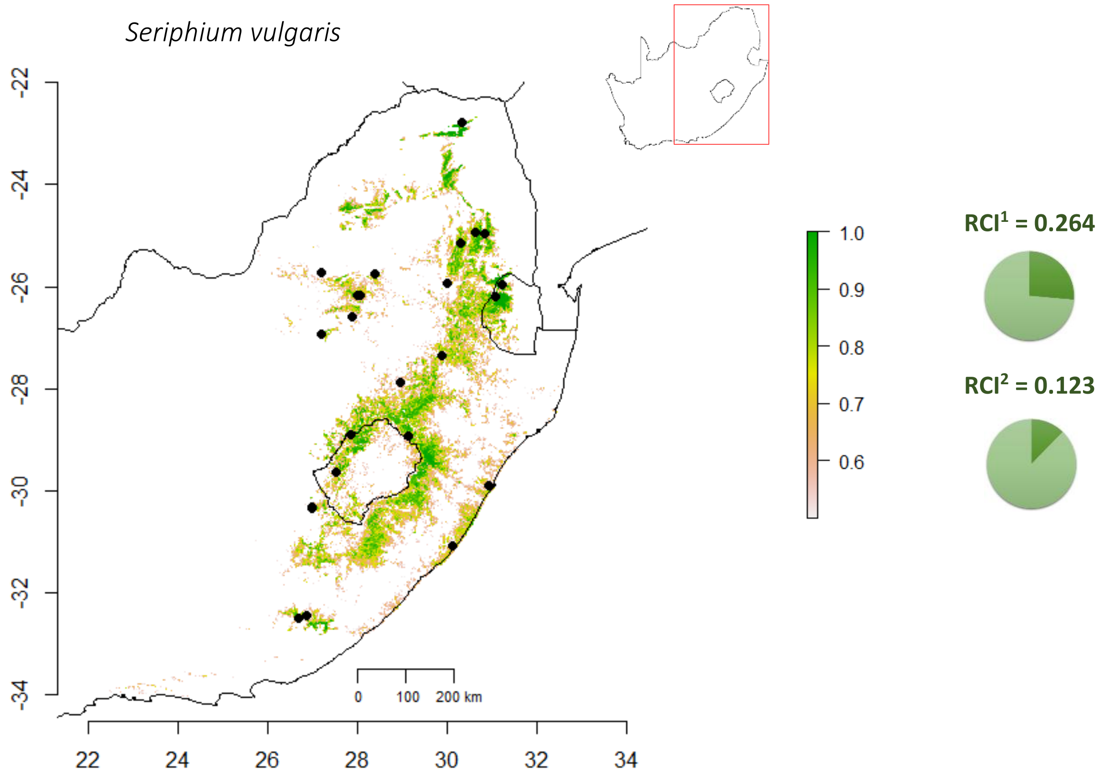
(b) *Seriphium crypticum*



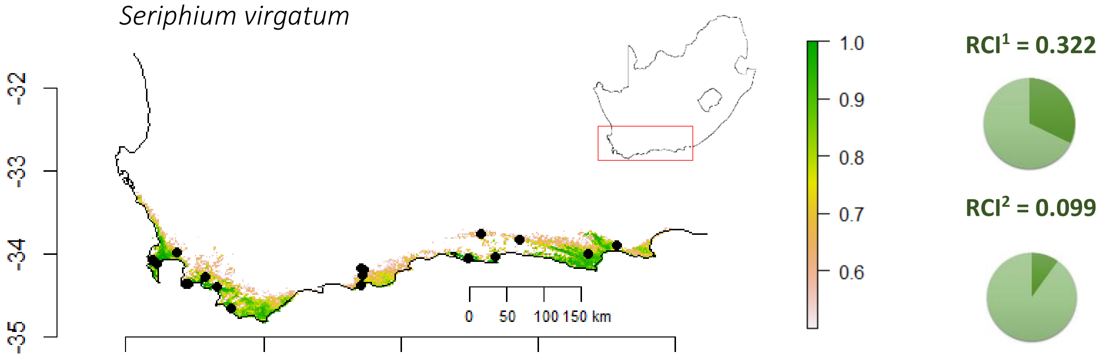
(c) *Seriphium plumosum*



(d) *Seriphium vulgaris*



(e) *Seriphium virgatum*



(f) *Seriphium burchellii*

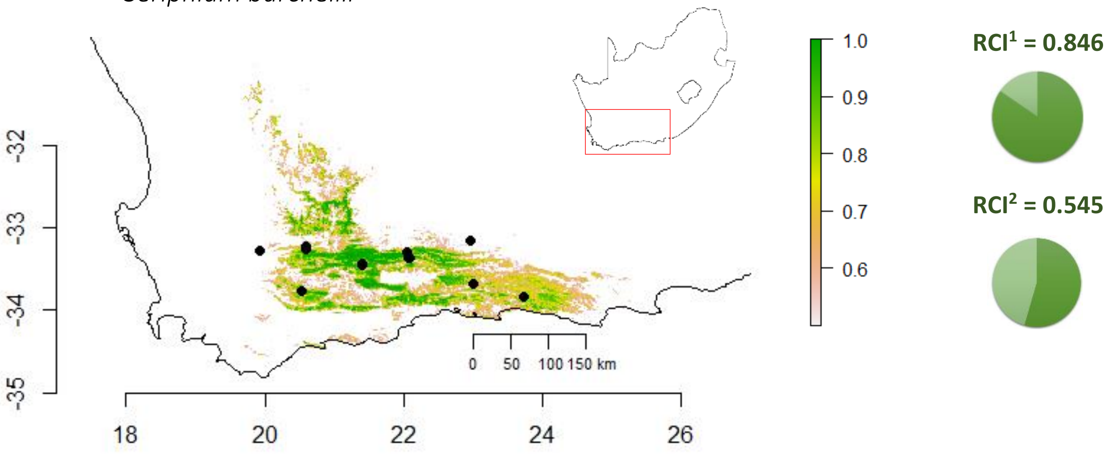


Figure 4: MaxEnt-modelled species distributions based on soil, precipitation, temperature, and elevation data shown for (a) *Seriphium dunensis*, (b) *S. crypticum*, (c) *S. plumosum*, (d) *S. vulgaris*, (e) *S. virgatum* and (f) *S. burchellii* in South Africa in order of increasing RCI. Black circles indicate the species occurrence records used for generating the modelled distributions where occurrence records could be georeferenced to ≤2000m. Legends are identical across species and indicate the modelled probability of species occurrence where there is a species occurrence probability of at least 0.5. Also shown are the range continuity indices for each species; RCI^1 indicates the range continuity index calculated by random points projection and RCI^2 indicates the range continuity index calculated using georeferenced points.

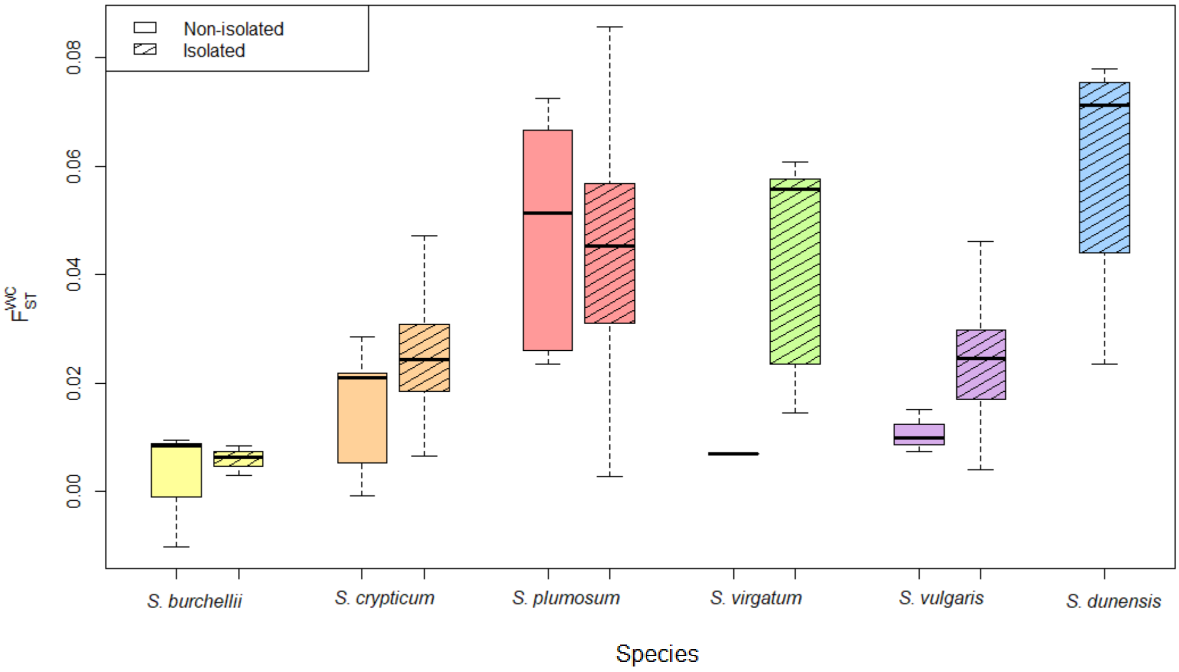


Figure 5: Boxplots of genetic distance between spatially isolated and non-isolated populations based on the continuity matrices for each species. Central bars indicated the median and ends of boxes indicate the 25th and 75th percentiles. Whiskers extend to the minimum and maximum points (excluding outliers). Colours are consistent with those in Figure 2.

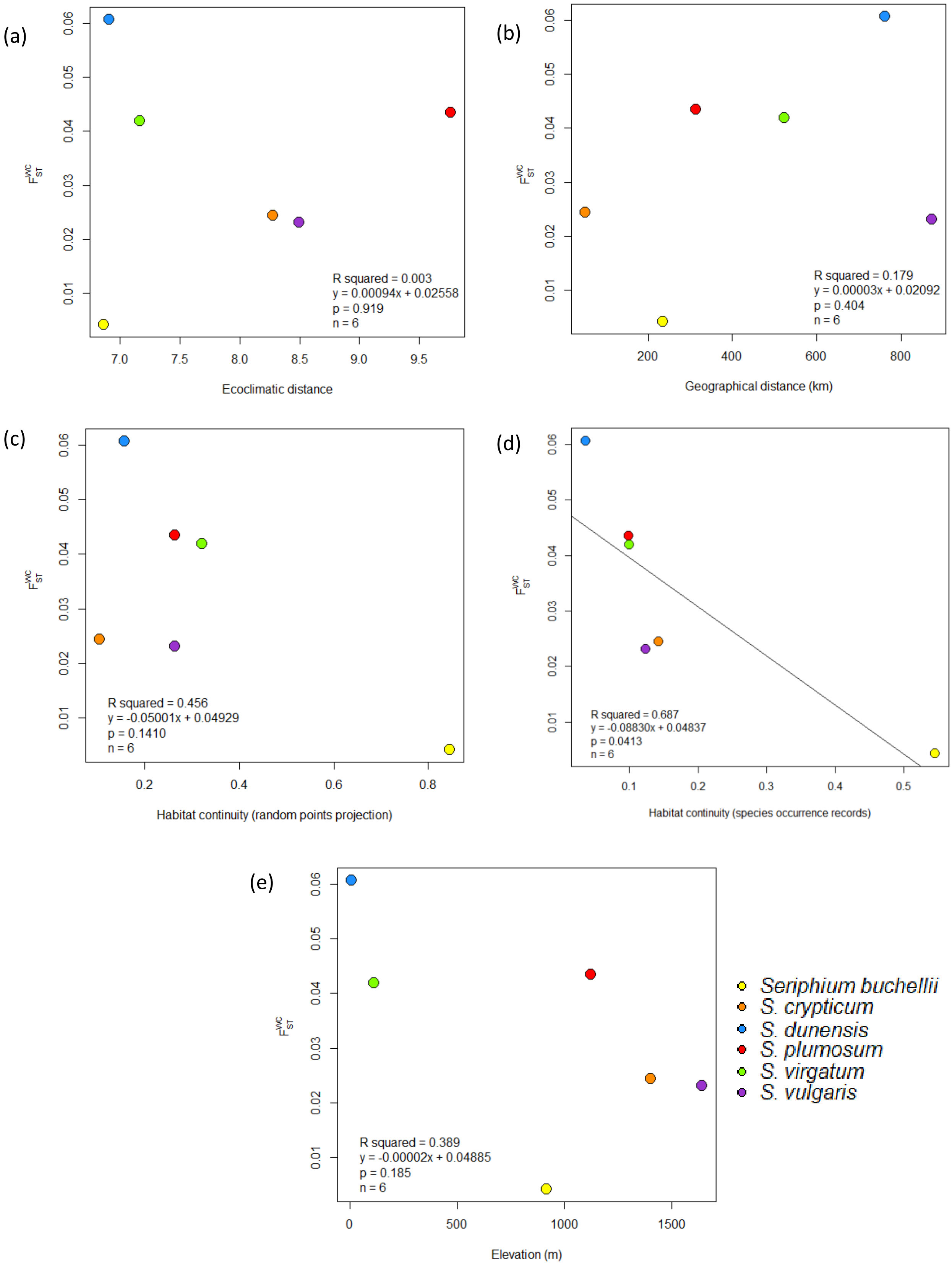


Figure 6: Drivers of population-level genetic divergence across selected species in the *S. plumosum* clade. Shown are linear regressions of the mean genetic differentiation between populations within species estimated using Weir and Cockerham's fixation index (F_{ST}^{WC}) against (a) ecoclimatic volume; (b) maximum range extent; (c) the range continuity index for each species, estimated using random points projection; (d) the range continuity index for each species, estimated using species occurrence records; and (e) elevation. Colours are consistent with those in Figure 2 and Figure 5.

Appendix A

Occurrence records used for MaxEnt species distribution modelling.

Species	**Resolution of georeferenced species records**			**Total**
	Exact (field sampling, and herbarium records with GPS)	< 500 m	500 – 2000 m	
Seriphium burchellii	4	0	8	12
S. crypticum	8	1	5	14
S. dunensis	4	1	3	8
S. plumosum	20	9	36	65
S. virgatum	6	3	10	19
S. vulgaris	12	1	12	25

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Chapter 5: Synthesis

To what extent can the species richness of the Cape Floristic Region (CFR) be considered 'adequately known' (Treurnicht et al., 2017)? Over the more than 250 years of steady taxonomic effort in the region starting with the publication of Linnaeus' *Species Plantarum* (1753), 9 383 vascular plant species have been described in the CFR (Manning & Goldblatt, 2012), making it the third richest but by far the smallest of the world's 34 biodiversity hotspots (Myers et al., 2000). Treurnicht et al. (2017) recently estimated that over 99% of species in the flora have already been discovered, under which estimate fewer than one hundred species remain undiscovered. This seems encouraging, especially since in other biodiversity hotspots, the estimated proportion of undiscovered flowering plant species is 15% (Joppa et al., 2011b). Several authors (Padial et al., 2010; Bacher, 2012; Rouhan & Gaudeul, 2014; Treurnicht et al., 2017) have advocated for the recruitment of more taxonomists and increased funding for taxonomic research as a means of accelerating the capture of the last remaining undiscovered plant species. However, increasing the rate of discovery will not address the bias toward morphologically diagnosable taxa that likely characterises existing classification in the CFR, given that the history of taxonomy in the region has essentially been one of morphology-based operationalism (Treurnicht et al., 2017). The evidence presented in this study (Chapter 2), and in Britton et al. (2014) and Pirie et al. (2017), if extrapolatable to other Cape species complexes, provides strong support for the idea that a substantial portion of the Cape flora remains undescribed. This implies that the proverbial 'goal posts' for the complete cataloguing of the species richness of the CFR have shifted, and that a greater diversity of tools may be required to discover and document the 'missing' species. This study (Chapter 2) constitutes the first application of Bayesian species delimitation to a Cape floral taxon, and demonstrates the utility of next-generation-sequenced DNA markers as a new source of information for addressing the problem of species delimitation. As the cost of Sanger and high-throughput sequencing declines (Rubin et al., 2012; Willing et al., 2012; Rouhan & Gaudeul, 2014; Leaché et al., 2017), as analytical delimitation methods improve (Yang, 2015; Leaché et al., 2018), and as the utility of these methods across diverse groups becomes apparent (Hedin et al., 2015; Hotaling et al., 2016; Boucher et al., 2016; Mrinalini et al., 2016; Wagner et al., 2017; Bryson et al., 2018), these methods should become more widely applied in South African systematics.

Accurate species inventories have important downstream applications, including in evolutionary biology (Dayrat, 2005; Fujita et al., 2012; Ely et al., 2017; Noguerales et al., 2018), where classification affects the inferences made regarding speciation process (Dayrat, 2005; Fujita et al., 2012; Rouhan & Gaudeul, 2014). Much current research in the CFR (Linder, 1985; Linder & Vlok, 1991; Goldblatt & Manning, 2002; Savolainen & Forest, 2005; Barraclough, 2006) and more broadly (Rundell & Price, 2009; Via, 2009) is dominated by the view that ecological speciation (Rundle & Nosil, 2005) is primarily responsible for the generation of species diversity. This seems hardly surprising given that much existing classification for the Cape flora (as elsewhere; Rouhan & Gaudeul, 2014) is largely morphology-based (Treurnicht et al., 2017) and that ecological speciation is expected to generate morphologically distinct species (Ellis et al., 2014) for which clear phenotype-environment correlations can be observed (Schluter, 2000; Ellis et al., 2014). With the more widespread application of synthetic taxonomy, classification less biased toward morphologically distinguishable taxa may provide further support for non-adaptive speciation, since this mode of speciation is expected to yield weakly phenotypically diverged species (Linder, 2005; Britton et al., 2014; Ellis et al., 2014; Verboom et al., 2015). The compelling evidence supporting vicariance as a key driver of genetic differentiation both in this study (Chapter 4) and others (Prunier & Holsinger, 2010; Segarra-Moragues & Ojeda, 2010; Britton et al., 2014; Prunier et al., 2017) further argues for the importance of vicariant speciation in the CFR. These sources of evidence, coupled with the mounting evidence questioning the likelihood of speciation with gene flow (Fitzpatrick et al., 2008; Rundell & Price, 2009; Via, 2009; Wolf & Ellegren, 2017), particularly among plants (Levin, 1993, 2000, 2003), suggest that a revival of the vicariant theory of speciation for the Cape may be imminent.

Our incomplete knowledge of the world's flora, the so-called 'taxonomic impediment' is seriously compromising efforts in conservation (Rouhan & Gaudeul, 2014), because conservation requires that species first be discovered and described (Ely et al., 2017). Species delimitation which relies on a single type of taxonomic evidence may lack the power to resolve species if species simply have not acquired specific properties during the course of speciation (de Queiroz, 2007). For example, species boundaries inferred using only morphology may fail to discriminate cryptic evolutionary species, either because cryptic species go undetected or because genetic variation is difficult to discriminate from phenotypic plasticity (Sivarajan, 1991; Frankham et al., 2012). Taxonomies based on a single type of evidence may also lack the power resolve evolutionary species housed within taxonomically problematic species complexes, which, in the CFR, are concentrated among widespread and variable species (Pirie et al., 2017). The greater confidence inherent in classifications resulting from consilience among multiple lines of taxonomic evidence (Dayrat, 2005; de Queiroz, 2007; Fujita et al., 2012; Solis-Lemus et al., 2014; Noguerales et al., 2018) should reduce both taxonomic instability and the frequency of species delineations that synonymise good species (Frankham et al., 2012). This in turn should lead to better-informed decisions in conservation (Frankham et al., 2012). This point is well-illustrated by the stark contrast between the key-character approach taken by Koekemoer (2016) in formulating her concept of *Seriphium plumosum*, and the integrative approach applied in this work which reveals nine species within the *S. plumosum* clade. Where *S. plumosum* as defined by Koekemoer (2016) is categorised by the Red List of South African Plants as 'least concern' (Kumandi & Victor, 2011), several of the species within the *S. plumosum* clade likely qualify as being of conservation concern. Based on their very limited geographic distributions and small population sizes, I consider that *S. alto-argillaceum* and *S. burrowsii* are of conservation concern. *Seriphium dunensis,* despite its large geographic range, may also be of conservation concern, given its highly fragmented distribution along the southern coast of South Africa, much of which is under threat by development (South African National Biodiversity Institute, 2013). On the other hand, the weedy species *S. vulgaris* as defined here likely warrants being placed in the Red List category 'least concern' given its large population sizes and extensive geographical distribution. As biodiversity enters its sixth extinction crisis (Kolbert, 2014; Rouhan & Gaudeul, 2014), and as most species still undiscovered are housed in biodiversity hotspots (Joppa et al., 2011b), conservation will undeniably benefit from a more accurate species inventory of the Cape flora.

Future research

Future work should be aimed towards developing a complete catalogue of the species richness of the CFR within the integrative taxonomic framework advocated by Dayrat (2005) and Fujita et al. (2012), ideally incorporating multilocus DNA sequence data and analytical species delimitation methods. I propose that taxonomically problematic, recently-evolved, and geographically widespread taxa, as well as taxa specifically prioritised for taxonomic research (von Staden et al., 2013) form the focal groups for taxonomic revisions applying these methods. Revisionary research incorporating analytical delimitation methods should be informed by existing taxonomies in which there is reasonable confidence, since the decision about the specific taxa to include in an analysis depends on correct information about closely related species. Moreover, the assumptions made by specific delimitation methods (e.g. SNAPP's assumption of marker independence; Bryant et al., 2012) and the specific properties of marker data sets (e.g. hierarchical missing data; Leaché et al., 2017) should be accounted for as far as possible so that the results of these analyses reflect the reality of species limits and evolutionary relationships, rather than statistical artefacts. Users of analytical delimitation methods should also note that these methods make no distinction between 'good species' and population-level lineages that qualify as evolutionary species, but then neither does the evolutionary species (Simpson, 1961; Wiley, 1978) and generalised lineage (de Queiroz, 1998) concepts, or indeed Darwin's concept of species in *The Origin* (1859). Importantly, users of analytical delimitation methods should acknowledge that the performance of these methods depends of the candidate delimitation hypotheses being well-informed. It seems tempting to simply test every possible arrangement of sequenced individuals in every possible number of species, but even with model runs accelerated by high performance computing, this is computationally intractable for any analysis with more than a few sequenced individuals. I consider the most advisable course of action to be (1) to develop a set of well-informed delimitation hypotheses based on as many types of taxonomic evidence as are available (de Queiroz, 2007); (2) to select a single species delimitation method based on its statistical properties (Rannala, 2015); and (3) to reassess the results of the best-supported hypothesis in the light of morphological differences (specifically, synapomorphies), ecology and geographic distribution *a posteriori.* This approach represents a practical means of using multiple operational criteria integratively for inferring the underlying evolutionary species, and ensures that every type of taxonomic evidence available to the researcher informs the delineation of species, as has been advocated by de Queiroz (2007).

Concerning speciation process, I propose that speciation be studied at the population level within a spatially explicit context. Ideally, such research should quantify the continuity of species distributions, and, as far as possible, clearly differentiate between populations in sympatry and those in micro-allopatry, since these two geographical settings are expected to impose contrasting limits on gene flow (Fitzpatrick et al., 2008). Such an approach should circumvent the difficulty in discriminating between pre- and post-speciational changes in species' distribution and ecology, and provide clearer insights into the relative contributions of divergent selection and drift to genetic differentiation. Models such as IBD should be replaced by models that account for complex (non-linear) species distributions, and the use of patterns of IBD as a proxy of divergence by drift should be interrogated given that IBD appears to signal ongoing, spatially-restricted gene flow rather than differentiation by drift (Chapter 4). If ecological speciation is as pervasive a mode of speciation in the CFR as is currently thought by some researchers, future research should also seek to elucidate the genetic basis of differentiation in sympatry, and specifically, the mechanisms by which so-called ‘speciation islands’ across the genome remain intact in the face of the homogenising effects of gene flow (Nosil et al., 2009; Via, 2009; Feder et al., 2012; Nosil, 2012; Arnegard et al., 2014).

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