

Evolution of South African Bruniaceae:
evidence from ecological
and geographical investigations

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1. General introduction

The South African fynbos biome is one of the hot-spots of plant diversity in the world (Cowling et al. 1992, 2009; Goldblatt 1997; Mittermeier et al. 1998; Myers et al. 2000; Goldblatt and Manning 2002; Linder 2003; Forest et al. 2007; Kreft and Jetz 2007). The term 'fynbos' is derived from the Dutch 'fijn-bosch' what means 'fine bush' and the vegetation in this biome is composed of evergreen shrub- or restioidland with Restionaceae (more than 5%), Ericaceae, other ericoid shrubs as well as Proteaceae (Cowling et al. 1997; Mucina and Rutherford 2006). Most fynbos shrub species are narrow endemics but as well can occur locally abundant (Richardson et al. 2001; Linder and Hardy 2004). In general the Cape Floristic Region in some lineages exhibits high speciation but very low migration rates due to low seed dispersal and short distance dispersal modes (ants and passive dispersal) (Campbell and van der Meulen 1980; Slingsby and Bond 1985; McDonald et al. 1995; Cowling et al. 1996; Richardson et al. 2001; Linder and Hardy 2004; Latimer et al. 2005).

A typical element of the mountain fynbos are the Bruniaceae, a member of the 33 'Cape clades' that are defined as clades with more than 50% of their species only occurring in the Cape region (Linder 2003). The (sub-) endemic Bruniaceae consist of about 80 species in 12 genera and occur throughout the Western Cape with one outlier species in KwaZulu-Natal (Pillans 1947). The ancient plant family exhibits recent speciation events (Quint and Claßen-Bockhoff 2008) and despite the low number of species, Bruniaceae are phenotypically diverse regarding growth form and inflorescence architecture (Pillans 1947, Claßen-Bockhoff 2000).

The present study shall discover biotic and abiotic factors that might have caused distribution and diversification of the Bruniaceae and can exhibit isolation mechanisms (geographical and/or ecological) causative for species evolution and species persistence. Until now the ecology of the Bruniaceae and its evolutionary background is unknown. Only one autecological study conducted by de Lange et al. (de Lange and Boucher 1990, 1993a, b; de Lange et al. 1993c, d; Wright et al. 1991) dealt with *Audouinia capitata* a species with big, brightly red flowers. The authors stated poor reproductive success which could account for the decreasing number of stable populations of *Audouinia capitata* in the Western Cape.

To discover ecological and geographical isolation mechanisms and adaptations to the fynbos biome, five hypotheses will be tested:

- 1) Bruniaceae can assumed to be generalists regarding their pollinator composition since most species have open accessible flowers.

A detailed pollinator analysis correlated with flower morphology will be conducted to detect the linkage between floral attraction for pollinators and reproductive success (Johnson 1996, 2004; Goulson 1999; Rodger et al. 2004).

- 2) If Bruniaceae are generalists regarding their pollinators it can be presumed that geitonogamy preventing mechanisms like herkogamy or dichogamy are realised.

The proceeding anthesis on population, plant, inflorescence and flower level will be observed to detect spatial and temporal mechanisms that can enhance outcrossing (Lloyd and Webb 1986; Barrett 2002, 2003; Routley et al. 2004).

- 3) The reproductive success is reflected in the genetic composition within and among populations and due to fragmented habitat structure similarities between populations decrease with increasing distance.

Since nothing is known about genetic constitution within and among populations of locally restricted and wide-spread species the present study will give an insight into haplotype distribution due to habitat fragmentation and/or gene-flow (Templeton et al. 1992; Thompson 1999; Mane et al. 2003).

- 4) As many species co-occur in the same area, niche occupation can be assumed for sympatric species.

The assumed niche occupation in areas where many species co-occur (Mayr 1996) will be tested regarding adaptation to soil (Silvertown 1983) and fire events linked with the resulting response to this disturbance. The two realised life-forms of Bruniaceae - reseeding species and species that have a lignotuber what enables them to resprout after fire (Carlquist 1978) - will be taken into account when analysing niches and population sustainability.

- 5) The niche inhabitation on small-scale level is also realised regarding the biogeography of the whole family and geographical isolation linked with the ecological adaptations of the species led to the recent distribution pattern and the morphological diversity present today.

The distribution pattern of all Bruniaceae species throughout the Western Cape will be mapped on the recent phylogeny after Quint and Claßen-Bockhoff (2006a) to illuminate evolutionary adaptation to fynbos ecology and their characteristics within the Bruniaceae. Together with the dating of genera divergence (Quint and Claßen-Bockhoff 2008) the found ecological and geographical isolation mechanisms will give the opportunity to detect species separating factors and the status quo of species stability, distribution and niche-inhabitation.

1.1 Characteristics of the species included in this study

Since this study comprises species of different genera, a short introduction to the habitus of the plants will be given to show the morphological diversity that was the initial point for the conducted analysis (see Tab. 1.1).

To focus on the most relevant species, only those being part of the pollinator observations and the breeding system analysis will be described in detail. In order to avoid redundancy as e.g. inflorescences of *Berzelia* species look similar, only inflorescence morphology will be stated and differences on species-level will be remarked.

In general five morphological levels were distinguished in the present study:

- a) the whole plant (growth form, size, branching density)
- b) inflorescence aggregates (cluster)
- c) single units of a cluster (composed of several inflorescences)
- d) the single inflorescence
- e) the flower

The morphologically diverse inflorescences of the Bruniaceae are described in detail by Claßen-Bockhoff (2000). For the present study a classification of the aggregation forms of the inflorescences will be stated using the example of *Berzelia lanuginosa* (Fig. 1.1)

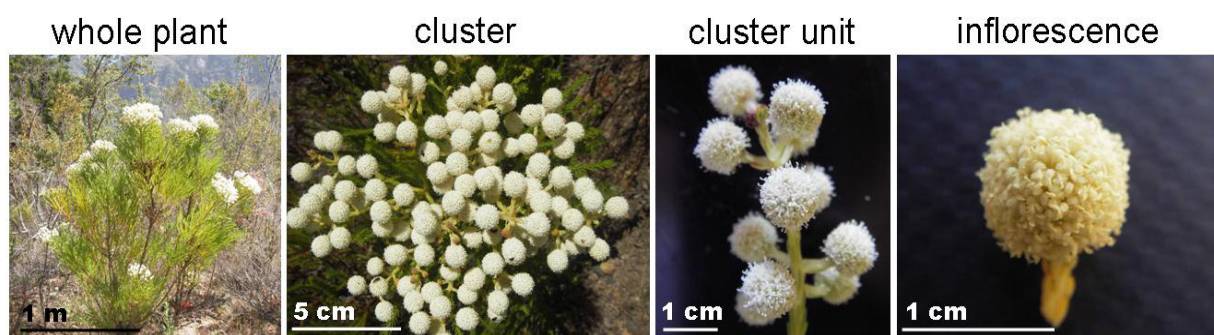


Figure 1.1: Morphological definition of inflorescence and flower aggregation expression used in the present study.

Plant morphology will not be described at this point but if necessary will be discussed in the particular analysis descriptions.

Table 1.1: Survey of the important ecological and morphological data of the species included in pollinator, anthesis and molecular analyses Life-forms refers to Carlquist (1978). Plant size after Pillans (1947).

species	life form	plant size	inflorescence	flower colour	pollinator and anthesis analysis
<i>Audouinia capitata</i> (L.) BRONGN.	resprouter	up to 0.4 m	flowers apical in dense clusters	red	
<i>Berzelia abrotanoides</i> (L.) BRONGN.	resprouter	2.0 - 3.0 m	pincushion-like	creamy white	x
<i>Berzelia lanuginosa</i> (L.) BRONGN.	reseeder	up to 5.0 m	pincushion-like	creamy white	x
<i>Berzelia squarrosa</i> (THUNB.) SOND.	reseeder	1.0 - 1.5 m	pincushion-like	creamy white	x
<i>Brunia alopecuroides</i> THUNB.	reseeder	up to 3.0 m	pincushion-like	creamy white	x
<i>Lonchostoma monogynum</i> (VAHL) PILLANS	reseeder	0.6 - 0.9 m	flowers apical in dense clusters	white	x
<i>Nebelia paleacea</i> (P.J.BERGIUS) SWEET	resprouter	0.4 - 0.6 m	pincushion-like	creamy white	x
<i>Pseudobaeckea africana</i> (BURM.F.) PILLANS	reseeder	up to 3.0 m	small flowers in an apical raceme	creamy white	x
<i>Raspalia microphylla</i> (THUNB.) BRONGN.	reseeder	0.6 - 0.9 m	small head-like	pink to light purple	x
<i>Staavia dodii</i> BOLUS	reseeder	about 0.6 m	straw-blossom like pseudanthia	pink to light purple	
<i>Staavia radiata</i> (L.) DAHL	resprouter	0.6 - 0.8 m	straw-blossom like pseudanthia	pink to light purple	x

1.2 Inflorescence types

Pincushion-like inflorescences

The pincushion-like inflorescences are composed of many single flowers, creamy white in colour (see Figure 1.2 and 1.3). The single flower is inconspicuous and without assistive optic technology hardly distinguishable. Species that were observed having pincushion-like inflorescences are: *Berzelia abrotanoides*, *Berzelia lanuginosa* and *Berzelia squarrosa*, *Nebelia paleacea* and *Brunia alopecuroides*.

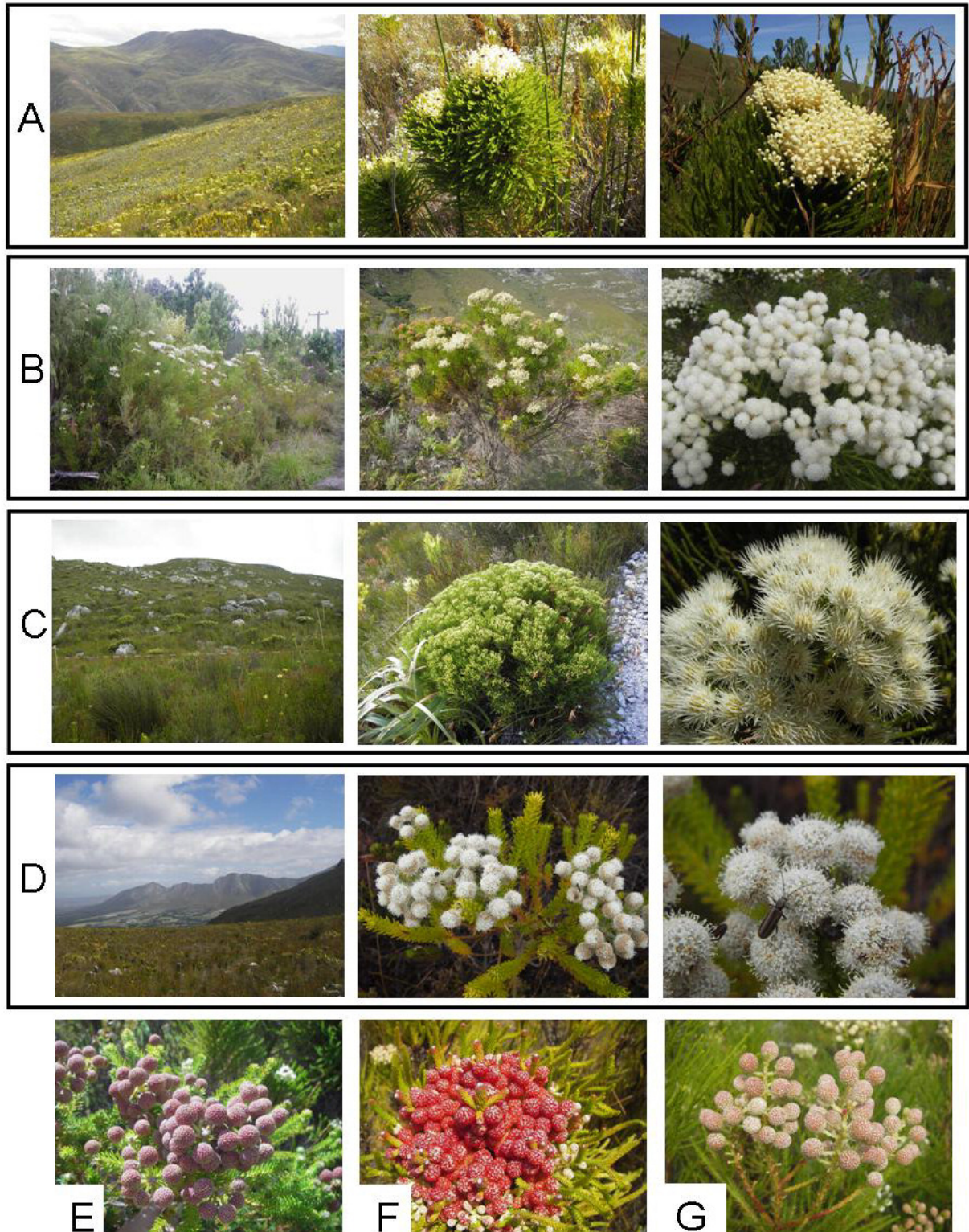


Figure 1.2: Survey of typical localities, plant morphology and inflorescence morphology of Bruniaceae included in the present study. Within boxes from the left to the right picture: habitat, plant, inflorescence.

A: *Brunia alopecuroides*; B: *Berzelia lanuginosa*; C: *Nebelia paleacea*; D: *Berzelia squarrosa*; E: fruiting inflorescence of *Berzelia squarrosa*; F: fruiting inflorescence of *Brunia alopecuroides*, G: fruiting inflorescence of *Berzelia lanuginosa*.

Straw-blossom like inflorescences

In the genus *Staavia* straw-blossom-like inflorescences are realised (Pillans 1947, Claßen-Bockhoff 2000). The bracts form an involucre that is white and surround the light purple to pink coloured flowers (see Figure 1.3K). *Staavia radiata* is the only species with a straw-blossom like inflorescence included in the pollinator and anthesis observations.

Small, head-like inflorescences

The inflorescences of *Raspalia microphylla* are composed of very little pink flowers that are separated by many hairs.

Single flowers that are composed in raceme

The single creamy white flowers of *Pseudobaeckea africana* are arranged in a raceme at the apical part of the branches.

Inflorescences composed of long-tubed flowers

Lonchostoma monogynum has apical clustered inflorescences that comprise several long-tubed densely clustered white flowers (see Figure 1.3H).



Figure 1.3: Survey of typical localities, plant morphology and inflorescence morphology of Bruniaceae included in the present study. Within boxes from the left to the right picture: habitat, plant, inflorescence.
H: *Lonchostoma monogynum*; I: *Pseudobaeckea africana*; J: *Raspalia microphylla*; K: *Staavia radiata*;
L: *Staavia dodii*; M: *Audouinia capitata*, N: *Brunia albiflora*.

1.3 The study site

Since the Bruniaceae are (sub-) endemic to South Africa, a map will help to allocate localities at the aspired observation and study sites (see Fig. 1.4).

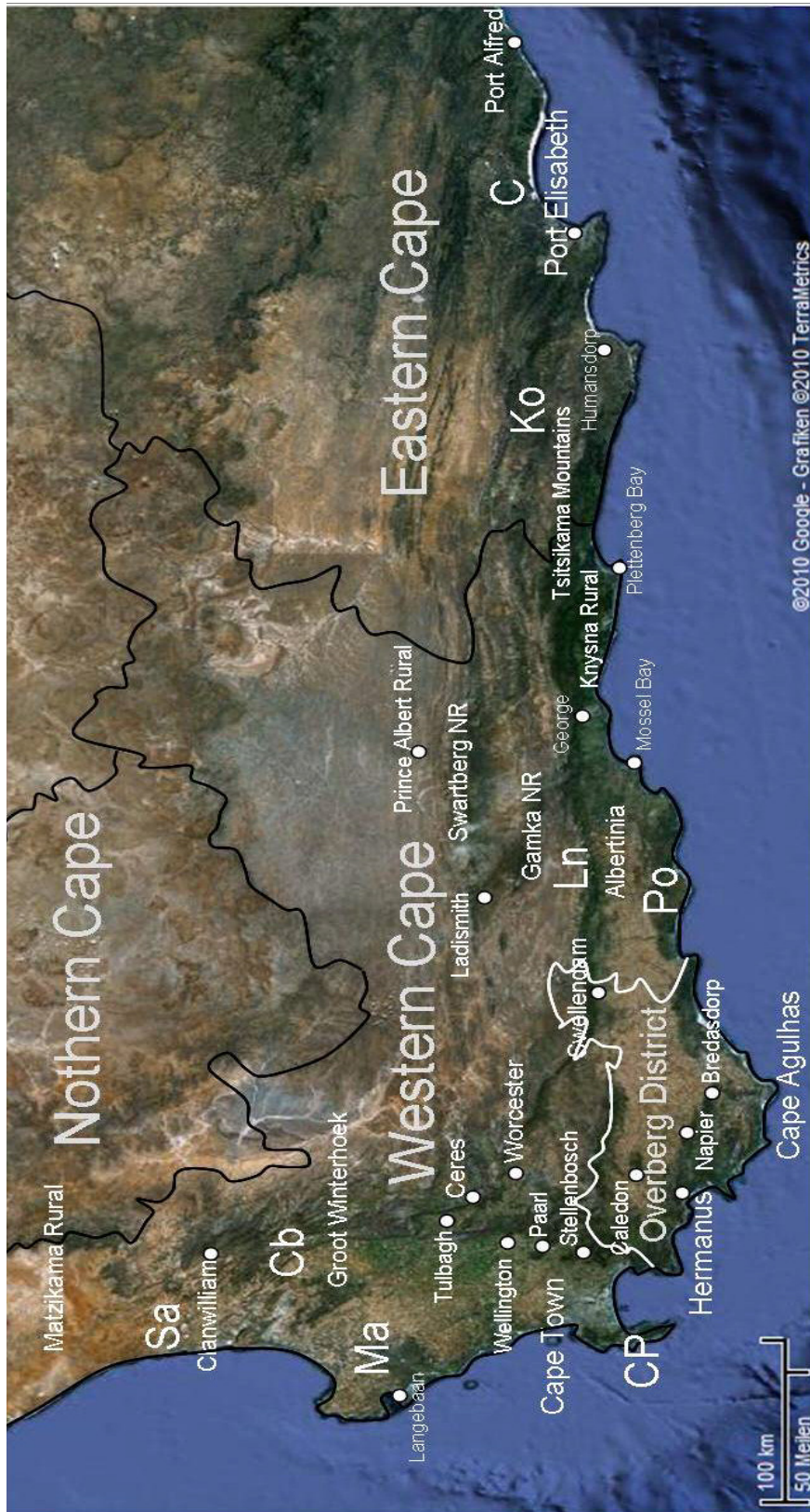


Figure 1.4: Map of South Africa. Arrow points to the main study site of the present study, Hermanus. Cb: Cederberg District, Ln: Langeberg District, Po: Potberg District, Ko: Kouga District, C: Cockscomb District, Ma: Malmesbury District, Sa: Sandverld Zone, White framed area: Overberg District.

2. Plant-pollinator interaction

Abstract

In the Bruniaceae, a great variety of inflorescence forms, flower shapes and colours is realised. To detect possible cryptic specialisation aside the assumed generalistic pollinator system of the open accessible flowers, the flower and inflorescence form, the reward types (nectar, pollen and scent) and the attracted insects were observed. The flowers and inflorescence types suggest the tendency to fly-pollination in nectar-rich species and beetle-pollination in pollen-rich species respectively. In the present study the pollinator composition and the mode and accuracy of pollen transfer with regard to the flower morphology of nine Bruniaceae species at three different localities was documented. To test the efficiency of pollen-transfer, the fruit and seed set as indicator for the reproductive success under open as well as vector-excluding conditions were tested. The results confirm the generalism of open accessible flowers and indicate that the Bruniaceae are opportunists regarding their pollinators. Only the corolla shape can exclude insects thereby leading to a specialisation on insect groups that gain access to the flower. Reproductive success is not linked with the life form (reseeder or resprouter) and contrary to the expectation species with similar morphology differ in their reproductive success.

2.1 Introduction

The Cape Province of South Africa is one of the world's hot spots of plant diversity (Cowling et al. 1992; 2009; Goldblatt and Manning 2002; Linder 2003; Kreft and Jetz 2007). Within this diverse biome the striking elements are orchids, *Gladiolus* L., *Erica* L.- and *Protea* L.-species of which many are endemic (Rebelo et al. 1985; Helme and Trinder-Smith 2006). Many studies were and are conducted illuminating their high specialisation on certain pollinator groups (Buchmann 1987; Nicolson and Fleming 2003; Ollerton et al. 2003; Collins and Rebelo 2006; Johnson et al. 2006). Referred to the number of about 80 species in twelve genera sensu Pillans (1947), the Bruniaceae are phenotypically diverse, comprising genera with big or tiny single flowers, inflorescence aggregates composed of attractive pincushion-like flowers, racemes as well as straw-blossom pseudanthia (Pillans 1947; Claßen-Bockhoff 2000).

In the present study the plant-pollinator interaction of selected Bruniaceae with very small flowers, medium-sized flowers as well as long-tubed flowers will be observed to predict a) if flower morphology attracts certain pollinator groups, b) if all Bruniaceae are generalists or if shifts to specialisation occurred that could be the reason for speciation events, c) if the effectiveness of pollinators is reflected in the reproductive success and d) if the habitat structure has an influence on the pollinator composition.

Most Bruniaceae flowers are accessible for all kind of insects and do not indicate a complex pollination mode like e.g. in *Salvia* L. (Claßen-Bockhoff et al. 2003, 2004; Claßen-Bockhoff 2007). Nevertheless it is unknown if some Bruniaceae flowers induce precise pollen deposition places on the insect body like in other hermaphroditic flower as pollen discounting prevention (Armbruster et al. 2009) what will be observed in the present study. Aside open accessible flowers Bruniaceae species with a long corolla tube like in *Lonchostoma monogynum* (VAHL) PILLANS might restrict the pollinator composition to insects that are able to enter the flower and suggest pollination by long-tongued flies, a common syndrome in South Africa (Johnson and Steiner 1997, 1998; Goldblatt et al. 1995; Manning and Goldblatt 1997; Goldblatt and Manning 2000a). Regarding the plant-pollinator interactions in Bruniaceae, the only autecological study by de Lange et al. (1990-1993) dealt with *Audouinia capitata* (L.) BRONGN., a species with brightly red and big flowers. The analysis illuminated that all kinds of insects visited the flowers with small beetles (Nitidulidae) being the most common visitor (Wright et al. 1991) wherefore no specialisation exists even though the flower morphology could indicate this. It turned out that *Audouinia* shows a low reproductive success, low pollen viability, an aberrant endosperm development, low seed set and a high mortality of the seedlings (De Lange and Boucher 1990, 1993a, b; Wright et al. 1991; De Lange et al. 1993b, c). If the reproductive mismatch of high attraction but low seed set due to inefficient pollination is a current pattern of the Bruniaceae will be clarified. Maybe other Bruniaceae species with bigger floral displays and nectar produced by nectaries at the bottom of the gynoeceum (Quint and Claßen-Bockhoff 2006b) or emanated scent can attract efficient pollinators that lead to an effective reproduction (Harder and Barrett 1995; Harder 2004).

Despite pollinator behaviour and pollinator spectrum, geomorphology can restrict genetic exchange via pollen or seed transport (Latimer et al. 2005) and can enforce the effect of possibly inefficient pollination like in *Audouinia capitata*. Metapopulations are formed that can result in either inbreeding depression or speciation events (Ellstrand and Elam 1993; Young et al. 1996). If in other Bruniaceae species like in *Audouinia capitata* outcross pollen is advantageous for fruit set (de Lange and Boucher 1993a) and since some Bruniaceae occur in small, scattered populations with only few individuals (Pillans 1947; A.V. Hall, unpublished data), the pollinator behaviour could reduce the reproductive success (Field et al. 2005).

However, aside locally restricted species there are as well wide-spread Bruniaceae species with a distribution area ranging over the whole South Western Cape (Pillans 1947; A.V. Hall, unpublished data) what raises the question if different pollinator groups vary in their efficiency as pollinators and serve as better pollen vectors (Struck 1994).

2.2 Material and methods

2.2.1 Investigation site and included plant species

Investigation area

To get a first insight into the pollinator spectrum in wide-spread *Bruniaceae* species pollinator observations were conducted at *Berzelia lanuginosa* (L.) BRONGN. in December 2006 at the Botanical Garden Kirstenbosch in Cape Town. The site in the periphery of the botanical garden consists of anthropogenic affected mountain fynbos.

Between September and December 2007 pollinator observations were conducted at seven species in six genera at the Vogelgat Nature Reserve (see Fig. 2.1). This locality was chosen since many species co-occur what enabled to observe many species that flower at the same time what avoids time-consuming changes of the locality. The Vogelgat Private Nature Reserve is located in the Overberg region, comprises a 603 ha big area and bears 14 *Bruniaceae* species sensu Pillans (1947). The Reserve is situated about 125 km east of Cape Town in the Western Cape Province and 2.4 km from the outskirts of Hermanus (see Fig. 2.1). The mountainous area is directly situated at the coast line. The altitudes range from 50 m to 820 m above sea level and the main vegetation type is mountain fynbos.

Lonchostoma monogynum was observed in June 2008 at the Fernkloof Nature Reserve that covers 1800 ha in the Kleinrivier Mountains above Hermanus and ranges in altitude from sea level to 842 m. The Reserve is situated directly west of the Vogelgat Nature Reserve. The flowering time for *Lonchostoma monogynum* cited in literature (August to January after A. V. Hall, unpublished data) deviated from the actual flowering time in the Fernkloof Nature Reserve what made this third stay necessary. All observation sites are only accessible by narrow and steep trails and species like *Pseudobaeckea africana* could only be reached after a three-hour march (see Fig. 2.1). This required a preselection of the easily reachable species and species that could only be observed sporadically (see Fig. 2.1). *Berzelia lanuginosa*, *Nebelia paleacea* and *Staavia radiata* could be found near the base camp which enabled daily observations. *Brunia alopecuroides*, *Pseudobaeckea africana* and *Raspalia microphylla* were for this reason observed as often as possible because of the far-off localities. To reach the locality of *Lonchostoma monogynum*, a more than two hours lasting march was necessary and due to daylight conditions (Southern Hemisphere winter), the observation time was restricted to 11 a.m. to 4 p.m..

In *Berzelia squarrosa* the flowering time was nearly finished at the beginning of the observations and in *Berzelia abrotanoides* anthesis has been in the very early stage for which reason the observation results will only provide a first insight in the pollinator composition.

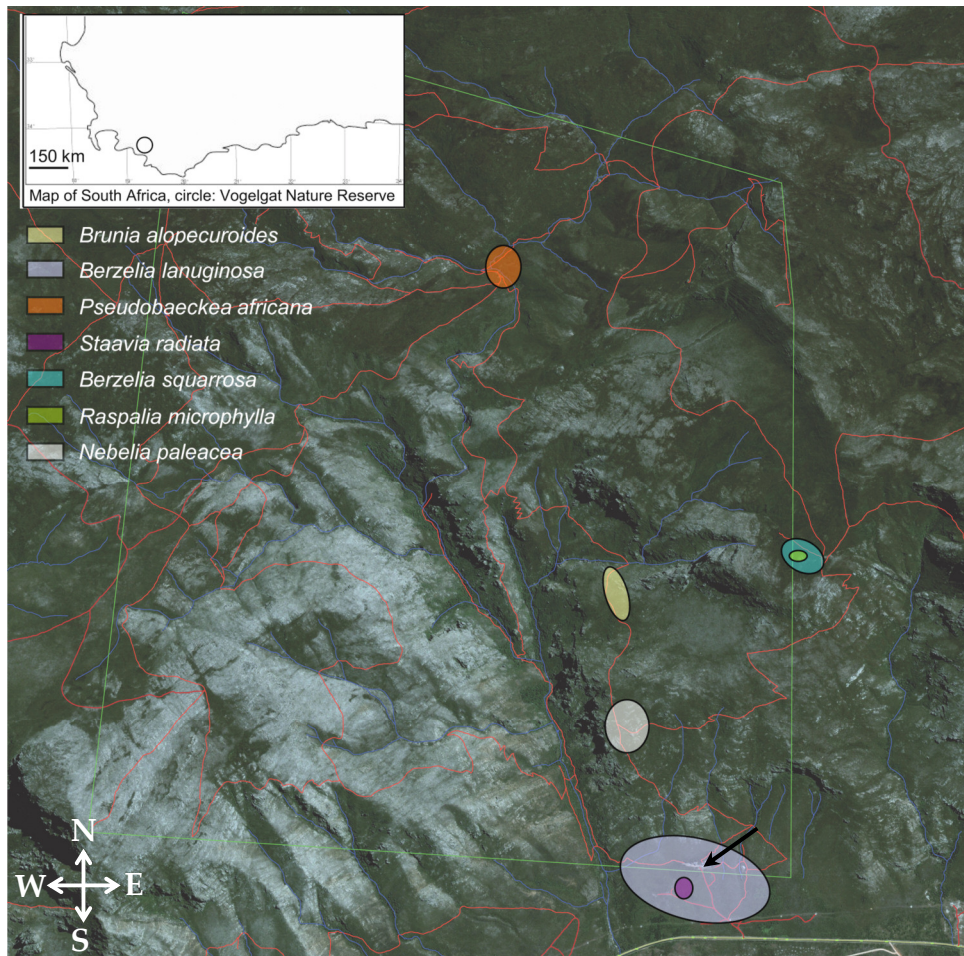


Figure 2.1: Vogelgat Nature Reserve with the localities of the observed species Green line: Reserve boundary; red lines: paths; blue lines: streams. Arrow points to the position of the base camp

2.2.2 Conducted analysis

Inflorescence and flower morphology

Insect attraction – the floral display

Ten species in seven genera could be included in the flower morphology study, the pollen/ovule calculation, and the fruit and seed set analysis (see Tab. 2.1). Due to flowering stage and locality accessibility not all species could be included in every analysis. The *Staavia dodii* site at the Cape Peninsula was burnt shortly before the analysis should have been conducted (information by the Cape Nature Conservation Board, Rondebosch, Cape Town). The beginning of the fruiting phase of *Berzelia lanuginosa* at the locality of east of George (see Fig. 1.4) was unknown and the locality was too far away to justify a trip to collect fruits of unknown maturity.

For the morphometric analysis ten randomly chosen inflorescences of each species were dissected and the containing flowers were counted to compare pollinator frequencies with the inflorescence type and to predict advantages of bigger floral displays.

To be able to predict the number of flowers per plant, all inflorescences of a medium-sized, a small and a big plant were counted, averaged, and then the number of flowers was calculated by multiplying the average number of flower per inflorescence with the found average number of inflorescences per plant.

Table 2.1: Survey of the conducted analysis. (x)*° = restricted data due to the anthesis starting* at the end of the observation period in South Africa and ending° at the beginning respectively. H: Hermanus, VNR: Vogelgat Nature Reserve, FNR: Fernkloof Nature Reserve.

species	morphometric measurements	pollinator observations	p/o ratio	fruit and seed set
<i>Berzelia abrotanoides</i> L.	x	(x)* H	x	x
<i>Berzelia lanuginosa</i> L. western morph	x	x VNR, KBG	x	x
<i>Berzelia lanuginosa</i> L. eastern morph	x	-	x	-
<i>Berzelia squarrosa</i> WENDL.	x	(x)° VNR	-	x
<i>Brunia albiflora</i> PHILL.	-	-	-	x
<i>Brunia alopecuroides</i> THUNB.	x	x VNR	x	x
<i>Nebelia paleacea</i> BERG.	x	x VNR	x	x
<i>Pseudobaeckea africana</i> BURM.F.	x	x VNR	x	x
<i>Raspalia microphylla</i> THUNB.	x	x VNR	x	x
<i>Staavia dodii</i> H.BOL.	-	-	x	-
<i>Staavia radiata</i> L.	x	x VNR	x	x
<i>Lonchostoma monogynum</i> VAHL	x	x FNR	x	x

Inflorescence position

In pincushion-like inflorescences of *Berzelia lanuginosa*, *Berzelia abrotanoides*, *Brunia albiflora* and *Nebelia paleacea* succulent structures can be found whose function is unknown. *Berzelia lanuginosa* and *Berzelia abrotanoides* exhibit these structures at the base of the inflorescence stalks. *Brunia albiflora* and *Nebelia paleacea* have smaller structures at the base of the inflorescence bracts. To test if these structures change their size, serve as food body or attraction for insects since some of them are red in colour, plants with red structures and those with green structures were compared regarding the number of pollinators. To detect possible influences of sun-exposition on the colour of the structures, three inflorescences with green structures were cut, put into water and were stored in a dark room for five days. A control experiment of three inflorescences with green structures was tagged and observed daily to check if the colour changes from green to red with ongoing sun exposition.

Since colour change might be due to the pH value of possibly containing anthocyanin the cell contents of both, red and green fleshy structures of *Berzelia lanuginosa*, *Berzelia abrotanoides* and *Brunia alopecuroides* as well as the bracteolate structures of *Nebelia paleacea* were crushed and transferred into acidic (pH=3) and alkaline (pH=9.5) media (Whiting 2001 and literature therein).

In *Berzelia lanuginosa* the inflorescences change their position during anthesis (see Fig. 2.2). To check if the inflorescence position has an influence on the pollinator attraction because of a differing inflorescence exposition, the angle between the branch axis and the inflorescence axis of 20 randomly chosen inflorescences with green as well as red succulent structures at the base of the inflorescence stalk were measured. Because the succulent structures are positioned at the base of the inflorescence stalks, a change of volume will be tested what might induce the movement. Since the structures in *Nebelia paleacea* were too small to correlate swelling and movement, the measurements were restricted to the open accessible structures of *Berzelia lanuginosa*.

The measurements were conducted for 13 days and started at the beginning of the anthesis of the single inflorescence. The measurements were completed when no angle change could be recorded any more.

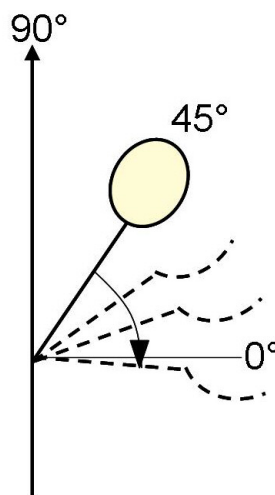


Figure 2.2: Changing inflorescence angle in *Berzelia lanuginosa*

Flower morphology

To be able to compare the flower morphological condition for the visiting insects, the corolla length, the width and the distance between anthers and stigma were measured. For the width, two different positions within the flower were chosen to define a) the possible access at the uppermost part of the flower or the corolla mouth (w1) and b) the access to the position at the basal-most position within the flower where most nectar can accumulate (w2).

Flowers of ten species including an additional *Berzelia lanuginosa* found towards the Eastern Cape in an area from George to the Tsitsikamma Mountains (see Fig. 1.4) with a variant morphology (bigger plants, long extended stamens) were analysed (see Tab. 2.1).

The measurements were conducted at ten randomly chosen mature flowers of different inflorescences and different plants per species using a calliper.

Nectar measurements

Nectar volume measurements were carried out at ten randomly taken fully flowering (corolla open, stigma visible, anthers pollen presenting) flowers of different inflorescence of different plants per species. Before the measurements the inflorescences were bagged for one hour with cotton sacs to prevent nectar loss through insect visits and to allow nectar accumulation. Because of the narrow corolla entrance and the tiny flowers, capillary tips for pipettes usually applied for enzyme analysis (Eppendorff, Hamburg, Germany) were used for the measurements. Normal glass capillary Pasteur pipettes were too big for the flowers.

Nectar sugar concentration was analysed measuring ten randomly chosen fully flowering flowers (corolla open, stigma visible, anthers pollen presenting) of each species with an Eclipse 45-81: 0-50% sucrose w/w (Bellingham and Stanley Ltd., Tunbridge Wells, Kent, UK). The measurements were carried out using bagged flowers to allow nectar accumulation for one hour.

Pollen/ovule-ratio

To analyse the number of pollen grains per ovule, ten mature but still closed randomly chosen anthers of different flowers and inflorescences per species (see Tab. 2.1) were dissected in a water drop on a slide using a dissection needle. All pollen grains were removed from the anthers and were counted using a Leica Wild M3C (Leica Microsystems, GmbH Wetzlar, Germany) stereo microscope. To ensure to count the complete amount of pollen grains, a grid was used that was made visible via a mirror normally used for stereo microscope drawings. The grid projection superposed the image of the pollen grains and allowed a precise counting of the pollen grains. The pollen grains in every square were calculated and summed up. The average value of grains was related to the number of ovules to analyse the p/o ratio per flower.

Insect morphology and pollinator observations

Pollinator observations

Pollinator observations were conducted at seven species within a general time frame of 9 a.m. to 6 p.m.. The pollinators were noted as soon as they landed on flowers. In the case of a second or third visit of the same flower, this was counted as new approach.

The insect behaviour on the flowers and inflorescences e.g. crawling, foraging for nectar, pollen or shelter, was recorded. To predict effective pollen deposition or removal the duration of the stay was noted.

Pollinator morphology

With allowance of the Vogelgat Nature Reserve single insect specimens were captured and immediately deep-frozen for several days to ensure the death of the insect specimens. The body size of the specimens was measured and the pollen deposition places on the insect body were detected. Insect species that could not be captured since they were too sensitive to approximation were recorded via photography. The size analyses of these insects were conducted using the surrounding inflorescence or flower as scale.

The study gives a qualitative survey of occurring insect groups on the plants and since nearly no determination literature allows a determination to species-level, the insects were determined as precise as possible. The insects were determined after Picker et al. (2004), Scholtz and Holm (1885), Schaefer (2000), and with help of Dr. W. Schawaller (Staatliches Naturkundemuseum Stuttgart; Germany: captured Coleoptera). The Diptera observed in *Lonchostoma monogynum* were determined by Dr. Ray M. Miller and Greg Davies (University of KwaZulu-Natal and Natal Museum, Pietermaritzburg).

Pollen-load experiments

Because of the specific morphology of *Lonchostoma monogynum* flowers having a long corolla tube, pollen load measurements were conducted to predict precision and quantity of the pollen deposition on the insects' proboscis. Therefore a dry preparation needle was inserted ten times into the same flower. A total of fifteen flowers namely three very young flowers (corolla just opened), nine flowers of medium age (corolla fully open, corolla entrance dark but with slightly pink margin, 24 to 48 hours old) and three old flowers (corolla open but corolla entrance becoming dark, older than two days) were analysed.

The insertion position of the needle has been varied between the ten measurements to imitate the natural behaviour of insects. After the needle was removed from the corolla the pollen grains were counted via a stereo microscope of unknown manufacturer immediately to prevent loss through drying.

Reproductive success

Fruit and seed set

Fruit set analyses were carried out in ten species namely *Berzelia abrotanoides*, *Berzelia lanuginosa*, *Berzelia squarrosa*, *Brunia albiflora*, *Brunia alopecuroides*, *Nebelia paleacea*, *Pseudobaeckea africana*, *Raspalia microphylla*, *Staavia radiata* and *Lonchostoma monogynum* (see Tab. 2.1). Ten randomly chosen fruiting inflorescences of different plants and of different position within the cluster per species were analysed. To check seed viability and proper endosperm formation (white, moist endosperm), fruits of altogether 14 205 flowers including those of the bagging experiments were dissected (Ooj et al. 2000). The found seed set was then related to the maximum number of ovules and thereby the maximum number of possible seeds.

Pollination and bagging experiments

To detect possible self-compatibility bagging experiments were carried out. Ten inflorescences in bud of six species each were systematically searched for insects and even smallest insects were removed to prevent pollen carryover (see Tab. 2.1). The inflorescence were bagged into a cotton-sack in order to avoid any pollination by insects. Bagged inflorescences of all experimental approaches were released after the unbagged flowers of the plant showed mature seeds. After being unbagged, fruits and seeds were tested for viability by cutting the seed to see the endosperm. The percentage of seed set was then calculated in relation to the maximum number of ovules and thereby the maximum number of possible seeds.

Pollination experiments in *Staavia radiata* were conducted to detect self-compatibility via hand-pollination. *Staavia radiata* flowers are open and pollen can be easily transferred to the stigma unlike the other flowers of the observed species that were too small to ensure proper pollen-transfer. For *Staavia radiata* two different times of hand-pollination were performed at ten buds/flowers each: buds that just had opened were pollinated with pollen of the same flower and post-male flowers were pollinated with pollen of neighbour flowers of the same plant as the pollen of the same flower is already completely dispersed in this floral stage.

After the pollination, the flowers were bagged immediately to exclude pollen transfer via insects. The following procedures were the same like in the bagging experiments mentioned above.

Fruit/seed dispersal

To test if fruits and seeds respectively are dispersed over big distances or if they just drop around the mother plant, the seed density around the plants of *Berzelia*

lanuginosa, *Brunia alopecuroides*, *Raspalia microphylla* and *Staavia radiata* were analysed since these species do not possess seritony. For comparison with species that exhibit seritony, the seed density below *Nebelia paleacea* was analysed.

Soil samples of five cm³ volume were taken in a distance of 20, 80, 150, and 200 cm around the plant (see Fig. 2.3). Three wind directions namely N, SW and SE were compared to check the influence of wind on seed dispersal, especially the occurring strong S/SE winds during the fruiting phases of the plants (Mucina and Rutherford 2006). The samples were disaggregated using a dissection pin and the seeds were counted. For an impression of the seed size see Fig. 2.3 (b).

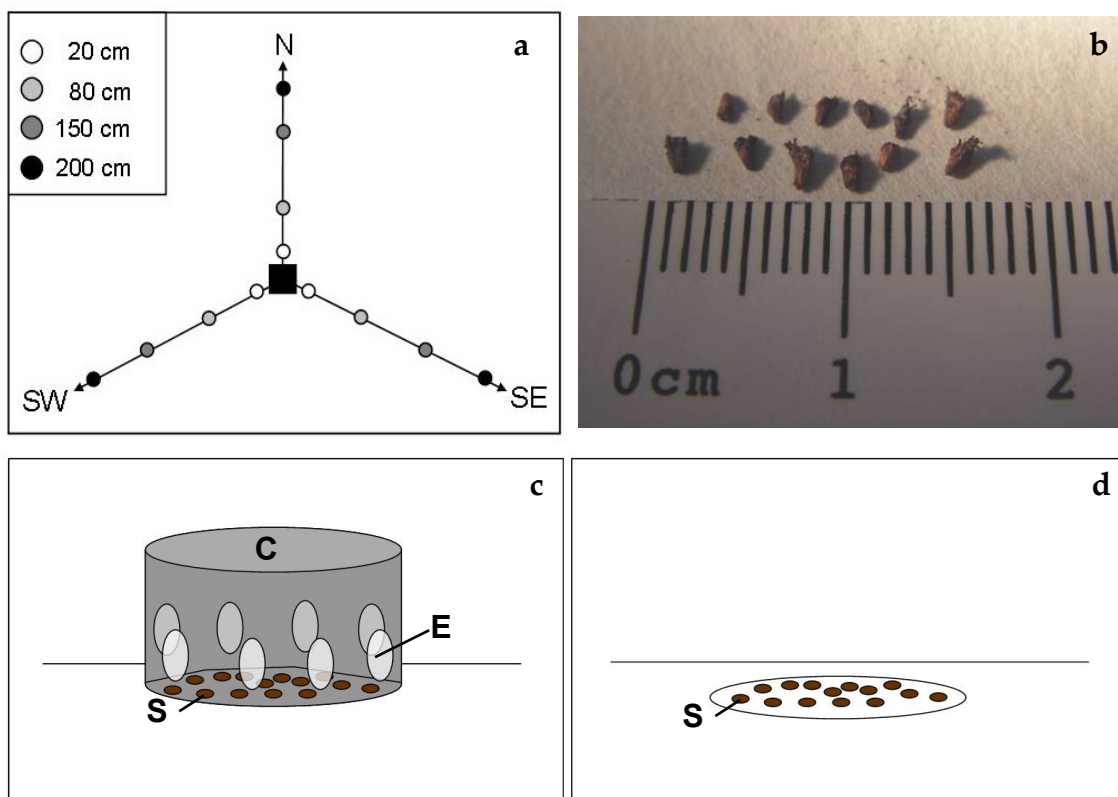


Figure 2.3: Seed analysis. a) seed density in the soil in different distances around a plant in South-Western, South-Eastern and Northern direction; b) size of *Berzelia lanuginosa* seeds c) closed trap and d) open 'trap' (C = container; E = entrance wholes for insects, S = seed).

To test seed distribution by frugivores or wind and water, two kinds of traps were built: a) traps that exclude bigger animals and only allow ants and small insects to enter and b) open traps that expose the seeds to wind, water and all kinds of animals (see Fig. 2.3). Both trap-types were loaded with one hundred viable seeds of each *Berzelia lanuginosa* and *Brunia alopecuroides* on filter paper to prevent mixture with the soil below. Three traps per trap type and species were placed in different parts of the population of each species to keep the natural conditions for seed dispersal e.g. co-

occurring insects. They were controlled every two days and removed after two weeks. The remaining seeds were counted.

Statistical analysis

The average and standard deviation was calculated for the morphometric analysis (corolla length, width 1 and 2 and stigma/anther distance), for the p/o-ratio analysis and the fruit set and seed set. Furthermore oneway ANOVA and multiple comparisons using a Post-Hoc-test with Bonferroni correction were conducted in order to compare the differences within and between the results of the species analyses.

A correlation analysis was executed to see the relation between the fruit set and seed set in open and bagged inflorescences.

The differences of the mean will be defined as significant at a level of 0.05.

All calculations were carried out using SPSS 15.0 (SPSS Incorporated, IBM Chicago, Illinois, USA) after applications described by Janssen and Laatz (2005) and Köhler et al. (2002).

Graphical material

All photographs were taken with a Pentax WP (PENTAX Europe GmbH, Hamburg, Germany). The pictures of the insects documented via stereo microscope were taken using a Leica MZ 16 A (Leica Microsystems GmbH, Wetzlar, Germany) with the camera DFC 420 C (Leica Microsystems AG Verkaufsgesellschaft, Heerbrugg, Switzerland).

2.3 Results

2.3.1 Insect attraction – the floral display

The number of inflorescences per plant range from four in *Lonchostoma monogynum* to 5796 in *Nebelia paleacea* (see Tab. 2.2).

The numbers of flowers per inflorescence range from eleven in *Lonchostoma monogynum* to 704 in *Pseudobaeckea africana* (see Tab. 2.2). The species can be subdivided into a) plants with big and dense synflorescences like *Berzelia*, *Brunia* and *Nebelia* and b) plants with sparse inflorescences like *Staavia radiata*, *Lonchostoma monogynum* and *Pseudobaeckea africana*. Pincushion-like inflorescences (*Berzelia*, *Brunia* and *Nebelia*) have more flowers than the small heads of *Raspalia* or the apical clusters of *Lonchostoma* (see Tab. 2.2). The most significant differences exhibit *Pseudobaeckea africana* owing nearly seven times more flowers per inflorescences than the pincushion-like heads of *Berzelia lanuginosa* and *B.squarrosa* (see Appendix 4). There is no significant difference among inflorescences with few flowers like those of *Lonchostoma*, *Staavia*, *Raspalia* and *Nebelia*.

Computing the number of flowers per plant a range from 44 flowers per plant in *Lonchostoma monogynum* to 272 412 flowers per plant in *Nebelia paleacea* could be observed.

The flower production of *Staavia radiata* exceeded the three months observation time. Thus it could not be clarified how many flowers are produced by one inflorescence during the anthesis of the plant. For the calculations of flowers per inflorescence the generally five present flowers per inflorescence were taken. To value the number of possibly produced flower per inflorescence the three months observation period was taken as the flowering time of a single inflorescence and multiplied with the number of flowers that were produced per day. A single flower was flowering for three days, and on one inflorescence always one bud, one to two nectar-producing flowers and one to two post-floral flowers without nectar were flowering. Ninety days for the verified flowering time of an inflorescence divided by three for the flowering time (three days per flower) and multiplied by five because there are always three to five flowering flowers add up to an assumed number of about 150 flowers per inflorescence in three months flowering time.

Table 2.2: Flowers per inflorescence (inflorescences counted: n=10), inflorescences per plant (plants counted: n=3), flowers per plant.

species	flowers/inflorescence		inflorescences/plant		flowers/plant
	m	SD	m	SD	
<i>Berzelia abrotanoides</i>	68	7	169	23	11 429
<i>Berzelia lanuginosa</i>	137	9	1593	150	218 241
<i>Berzelia squarrosa</i>	139	18	173	47	24 047
<i>Brunia albiflora</i>	77	10	123	30	9471
<i>Brunia alopecuroides</i>	58	9	130	18	7540
<i>Lonchostoma monogynum</i>	11	3	4	3	44
<i>Nebelia paleacea</i>	47	10	5796	400	272 412
<i>Pseudobaeckea africana</i>	704	95	142	18	99 968
<i>Raspalia microphylla</i>	19	2	595	65	11 305
<i>Staavia radiata</i>	5	1	3871	300	19 355

Inflorescence position in *Berzelia lanuginosa*

The inflorescence movement occurring in *Berzelia lanuginosa* shows that the angle between inflorescence branch and main axis changes during the proceeding anthesis from an average of 45° at the beginning of the anthesis to in extreme cases the point of -10°. The assumed movement inducing fleshy structures at the base of the inflorescence branch were found to be either red or green or greenish-red. With 13 red in comparison to 74 green structures on a medium-sized apical cluster, the number of green structures is higher.

The red and/or green colour changes is not due to sun exposition, age of the plant or the inflorescence position on the plant: the inflorescences that were stored under dark room conditions did not show any colour changes at the fleshy structures; the red structures appeared on young as well as on very old plants and they were not found in a certain part of the plant (e.g. only in the uppermost inflorescence branches).

The structures do not have any nectary glands or secretion aperture and therefore do not provide any liquids. No increase of insect visits at plants with red structures in comparison with those having green structures could be documented. The insects did not eat the structures or were attracted by them.

The colour is caused by anthocyanin in the vacuoles of the cell (see Fig. 2.4). A conducted pH-value measurement with pH indicator sticks (pH-Fix 0-14.0, Macherey-Nagel, Düren, Germany) and the crushed cell- content showed an acidic pH-value of 2.5 in red structures and 3.5 in green structures. In *Berzelia abrotanoides* that also bears these fleshy structures, the pH-value was 1.5, green structures had a pH-value of 3.0. A comparison with the red fruits of *Brunia alopecuroides* showed that these also have a pH-value of 2.0. The bracteolate structures of *Nebelia paleacea* that in general were green showed a pH-value of 3.0.

The volume of the fleshy structures in some cases increases with ongoing anthesis but it could not be clarified if this induces the movement as in the other species that also have these structures, no movement could be observed.

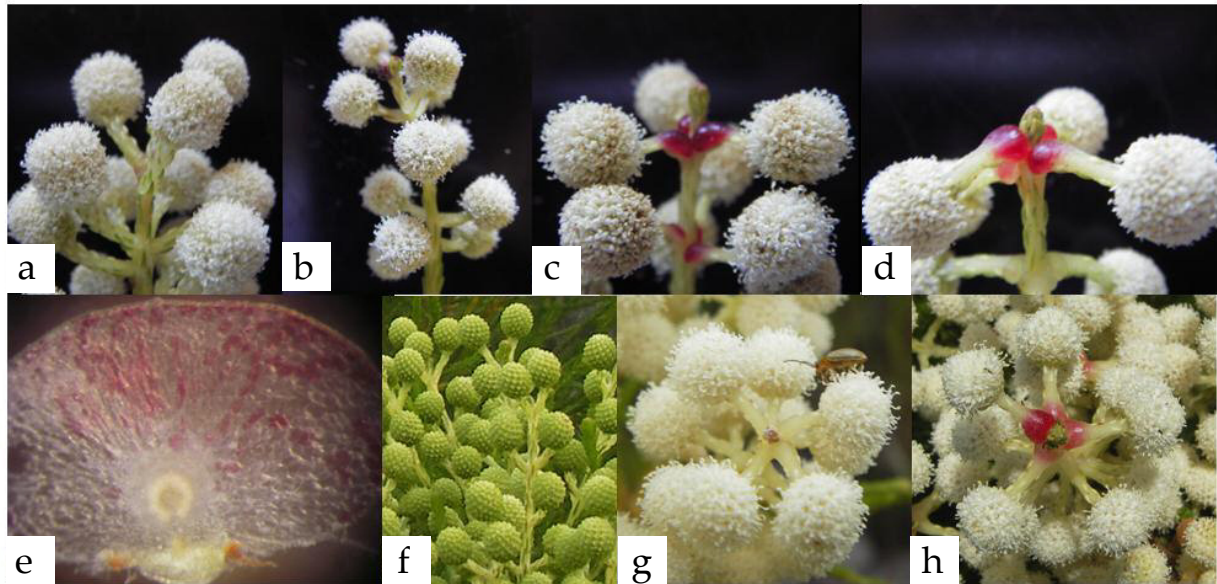


Figure 2.4: Inflorescence movement of *Berzelia lanuginosa* and red succulent structures at the base of different inflorescence stalks. a-d inflorescence movement throughout the anthesis e: section of the succulent structure with parenchymatic cells, red cell content is due to anthocyanins; f: very young inflorescences with an about 45° angle with the main axis as reference; g: view on top on an cluster unit with the inflorescences in a 0° angle position with the succulent structures being greenish white; h: view on top on an cluster unit with the inflorescences in a 0° angle position with the succulent structures being red.

Nectar measurements

Nectar measurements were conducted in *Staavia radiata*, in all other species the nectar volume was too low to be quantified. Even after extending the bagging for more than ten, hours no nectar accumulated. *Staavia radiata* showed a nectar production of $2.5 \mu\text{l} \pm 0.51$ with a sucrose content of $15\% \pm 3.10$.

For the first time scent was noticed in Bruniaceae. *Nebelia paleacea* scented of roses, *Staavia radiata* and *Pseudobaeckea africana* both emanated a honey-like scent. The intensity of the scent increased as the day progressed and reached its maximum at full sun. In *Berzelia lanuginosa* the inflorescences sometimes produced a fishy scent when many anthers exposed pollen. In *Lonchostoma monogynum* the inflorescences smelled slightly of flowery toffee when fully flowering.

pollen/ovule-ratio

The number of pollen grains per anther differed from 205 in *Pseudobaeckea africana* to 1138 in *Staavia dodii* (see Tab. 2.3). There are significant differences between the number of pollen grains per anther in the small flower inflorescences of *Pseudobaeckea*

africana, *Raspalia microphylla*, *Miniothamnea* and all other species. *Berzelia lanuginosa* and *Berzelia abrotanoides* do not show significant differences. *Berzelia lanuginosa*, *Staavia dodii* and *Berzelia abrotanoides* group together and are significantly different to all other species except the similarity between the two *Berzelia* species. The *Berzelia lanuginosa* of the Eastern Cape exhibits a significant difference to the one in the Western Cape and has only half the number of pollen grains per anther than *Berzelia lanuginosa* in the Western Cape.

The relation of pollen grains to the ovules of the flower showed that there is a correlation between anther position and P/O ratio: type 1 flowers with extended anthers had a P/O ratio of about 2125/1 to 5255/1 with the exception of *Nebelia paleacea* that has a lower P/O ratio (720/1) (see Tab. 2.3). In type 2 flowers the P/O ratio ranged from 512/1 to about 795/1. Only the *Staavia* species had a higher P/O ratio with 2107/1 to 2845/1. In the type 3 flower of *Lonchostoma monogynum* the P/O ratio of 735/1 was comparable to the P/O ratios of the type 2 flowers.

Table 2.3: P/O ratio of the observed species. Analysed anthers per species: n=10.

species	flower type	number of ovules	pollen grains per anther	P/O ratio
<i>Berzelia abrotanoides</i>	1	1	1051 ± 76	5255/1
<i>Berzelia lanuginosa</i> western morph	1	1	1025 ± 60	5125/1
<i>Berzelia lanuginosa</i> eastern morph	1	1	660 ± 35	3300/1
<i>Brunia alopecuroides</i>	1	2	810 ± 50	4250/2 ≈ 2125/1
<i>Nebelia paleacea</i>	1	2	288 ± 30	1440/2 ≈ 720/1
<i>Pseudobaeckea africana</i>	2	2	205 ± 11	1025/2 ≈ 512 /1
<i>Raspalia microphylla</i>	2	2	274 ± 16	1370/2 ≈ 685/1
<i>Staavia dodii</i>	2	2	1138 ± 74	5690/2 ≈ 2845/1
<i>Staavia radiata</i>	2	2	843 ± 49	4215/2 ≈ 2107/1
<i>Lonchostoma monogynum</i>	3	4	588 ± 37	2940/4 ≈ 735/1

2.3.2 Plant-pollinator interactions

Flower visitors

The insects observed differed due to plant species, locality and weather.

Observations took place on four to twelve different days per species. A definition of time frames within the daily observation periods did not seem reasonable as weather conditions had to be taken into account and often impeded a reasonable data acquisition.

For 265 hours daytime the insect taxa, the frequency of visitation, and insect behaviour were documented.

As very windy conditions occurred throughout the observation period, a continuous observation was only possible in 103 hours (see Tab. 2.4). During the residual time sessile insects that stayed on the inflorescences of *Berzelia lanuginosa* and *Staavia radiata* were noted every 30 to 60 minutes to check if there is any fluctuation or if the insects stay on the plants.

Table 2.4: Duration of the pollinator observations at the Vogelgat Nature Reserve in the different plant species: * = Kirstenbosch Botanical Garden; # = same number because of interval observation at both species during circuits.

species	flower type	observation time [h]	observation time [h] under windy conditions	number of observed insects
<i>Berzelia abrotanoides</i>	1	4	-	30
<i>Berzelia lanuginosa</i>	1	25 ½ hrs+ 7 hrs * in 7 days	+162 hrs # in 40 days	1378* and 711
<i>Berzelia squarrosa</i>	1	5	-	17
<i>Brunia alopecuroides</i>	1	9	-	107
<i>Nebelia paleacea</i>	1	16 ¾ hrs in 5 days	-	277
<i>Pseudobaeckea africana</i>	2	7 hrs in 5 days	-	750
<i>Raspalia microphylla</i>	2	3 ¼ hrs in 7 days	-	0
<i>Staavia radiata</i>	2	17 hrs in 7 days	+162 hrs # in 40 days	272
<i>Lonchostoma monogynum</i>	3	20 hrs in 12 days	-	57

A total of 2221 insect individuals in 103 hours of observation in all nine plant species at the Vogelgat Private Nature Reserve could be observed.

In all species a broad range of insects could be detected comprising Diptera, Coleoptera and Hymenoptera as well as some Thysanoptera, Araneae and Heteroptera (see Fig. 2.5-2.10). In contrast to this in *Pseudobaeckea africana* only a small beetle species of the Nitidulidae could be detected and the long-tubed corolla of *Lonchostoma monogynum* was only accessible for flies with a long proboscis (*Phthiria* sp., *Stomorhina* sp., *Eristalinus* sp.) Different localities possessed different pollinator composition under the same weather conditions and the same observation period of seven hours at *Berzelia lanuginosa*. At the Kirstenbosch Botanical Garden a total of 1378 insects were recorded, at the main investigation site, the Vogelgat Nature Reserve only 383 insects could be documented under the same observation conditions. While 108 butterflies were counted at Kirstenbosch, none was observed at Vogelgat.

In general few insects could be observed at the Vogelgat and Fernkloof Nature Reserve, e.g. in *Lonchostoma monogynum*, in 4.5 hours observation time, only one insect could be seen. In *Raspalia microphylla* no insects at all could be observed.



Figure 2.5: Pollinators on *Berzelia lanuginosa*: first row from the left to the right: Brachycera, *Phthiria* spec., Brachycera. Second row from the left to the right: two Formicidae, *Vanessa (Cynthia) cardui*. Third row from the left to the right: twice the same Chrysomelidae, right picture: copulating Melyridae. Fourth row from the left to the right: Galaerucinae, *Curculio* spec., Melyridae. For detailed description and body size see Appendix 1.



Figure 2.6: Pollinators on *Nebelia paleacea*: first row from the left to the right: three different Apidae. Second row from the left to the right: copulating Acanthosmatidae or Pentatomidae/Scutelleridae, two different Formicidae. Third row from the left to the right: Galaerucinae, *Anthrenus* spec. Malachiinae. Fourth row from the left to the right: Galaerucinae, *Curculio* spec., *Hoplia* spec.. For detailed description and body size see Appendix 1.



Figure 2.7: Pollinators on *Berzelia squarrosa*, *Pseudobaeckea africana* and *Brunia alopecuroides*: first row from the left to the right: on *Berzelia squarrosa*: Melyridae, *Curculio* spec., *Hoplia* spec.. Second row from the left to the right: two left pictures from *Berzelia squarrosa*: ant, Sciaridae, third picture *Pseudobaeckea africana* with copulating Scirtidae = Helodidae. Third row from the left to the right: on *Brunia alopecuroides*: *Trichostetha signata*, Sciaridae, Formicidae. Fourth row from the left to the right on *Brunia alopecuroides*: Formicidae, Apidae, Sciaridae. For detailed description and body size see Appendix 1.

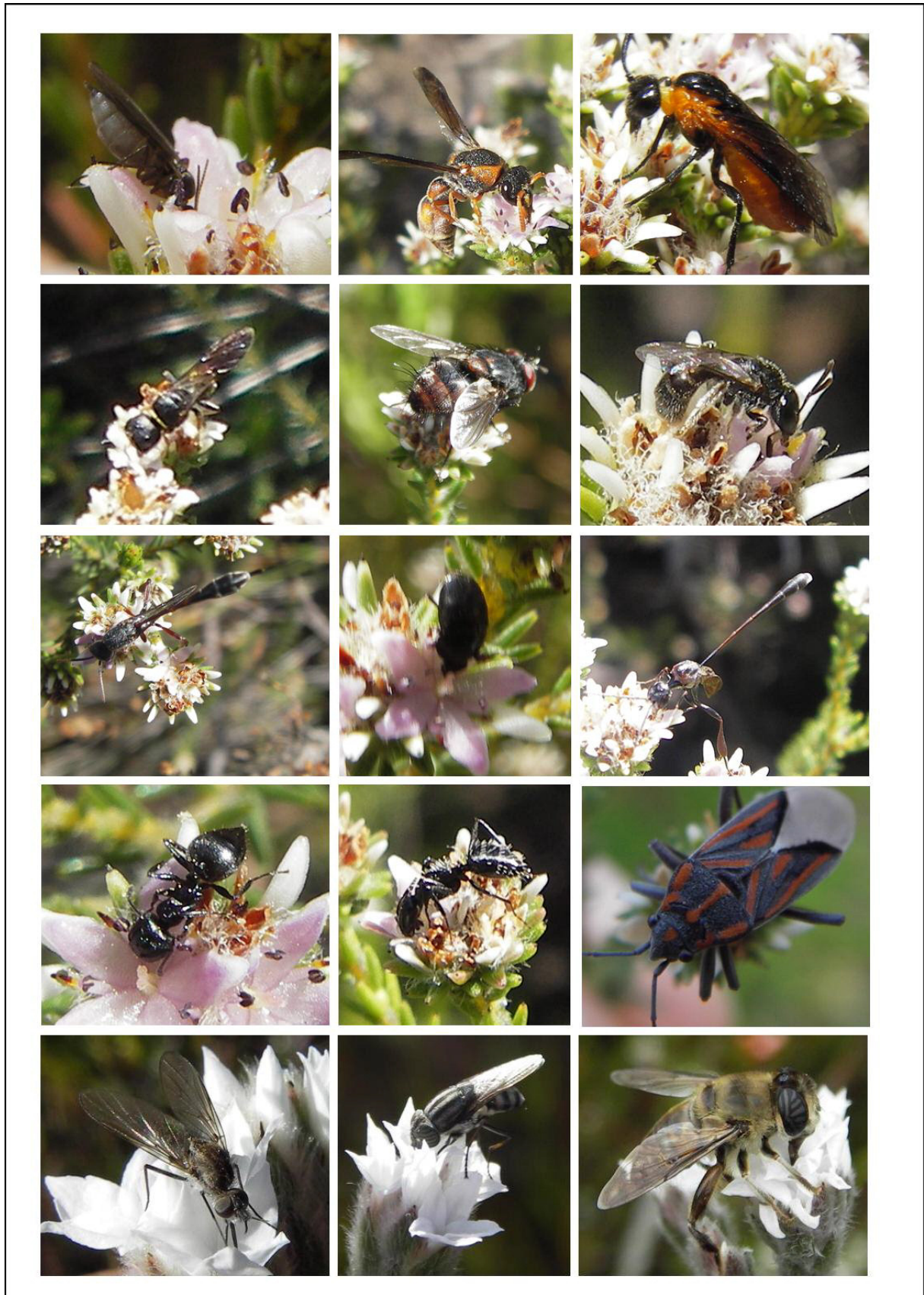


Figure 2.8: Pollinators on *Staavia radiata* and *Lonchostoma monogynum*. First three rows *St. radiata* from the left to the right: Nematocera, Vespoidea, Tenthredinoidea. Second row from the left to the right: Tenthredinoidea, Calliphoridae, Apidae. Third row from the left to the right: Spheciformes, *Meligethes* spec., Spheciformes. Fourth row from the left to the right: two Formicidae and a Lygaeidae. Fifth row: *L. monogynum*: *Phthiria* sp., *Stomorhina* sp., *Eristalinus* sp.. For detailed description and body size see Appendix 1.



Figure 2.9: Captured pollinators: first row from the left to the right: Cetoniinae, *Meligethes* spec. Formicidae. Second row from the left to the right: Brachycera, Thysanoptera, Formicidae. Third row from the left to the right: Brachycera, Diptera, Nematocera. Fourth row from the left to the right: *Meligethes* spec., Chrysomelidae, *Curculio* spec.. Fifth row from the left to the right: *Hoplia* spec., Melyridae, *Hoplia* spec. Sixth row from the left to the right: Eumolpinae, Chrysomelidae, Melyridae. Bar = 1 mm. For detailed description and body size see Appendix 1.



Figure 2.10: Captured pollinators: first row from the left to the right: Chrysomelidae from below and from above, right picture: ant. Second row from the left to the right: Formicidae, Brachycera and Apidae. Third row from the left to the right: Heteroptera, Thysanoptera, *Meligethes* spec.; Fourth row from the left to the right: Brachycera, Chrysomelidae, *Anthrenus* spec.; Fifth row from the left to the right: Malachiinae, *Curculio* spec., Auchenorrhyncha. Sixth row from the left to the right: Melyridae, Melyridae pronotum with pollen grains, Melyridae. Bar = 1 mm. For detailed description and body size see Appendix 1.

In Figure 2.11, a seven hour lasting observation period under equal weather conditions of two type 1 flower species, *Berzelia lanuginosa* and *Nebelia paleacea* and one type 2 flower, *Staavia radiata*, is illustrated (also see Table 2.6). The results show that in type 1 flowers Coleoptera represent 26 to 88% of the visitors, in the type 2 flower *Staavia radiata* only 9% of beetles could be observed. In type 2 flowers there are 27% of Diptera whilst in type 1 flowers the proportion range from 0 to 9%. Hymenoptera (excluding Formicidae) are represented in both types with 2 to 7%. Formicidae could be observed in both flower types. The data of the two chosen localities of *Berzelia lanuginosa* illustrates that Formicidae have a proportion of 24 to 56% at the Vogelgat locality, but only 1% at Kirstenbosch. In contrast in Kirstenbosch 8% of Lepidoptera occurred whilst no butterflies at all could be observed at the Vogelgat localities neither on Bruniaceae flowers nor on other plants in the area. In the Fernkloof Nature Reserve during the stay in June 2008 two butterflies could be observed that visited *Gladiolus* plants.

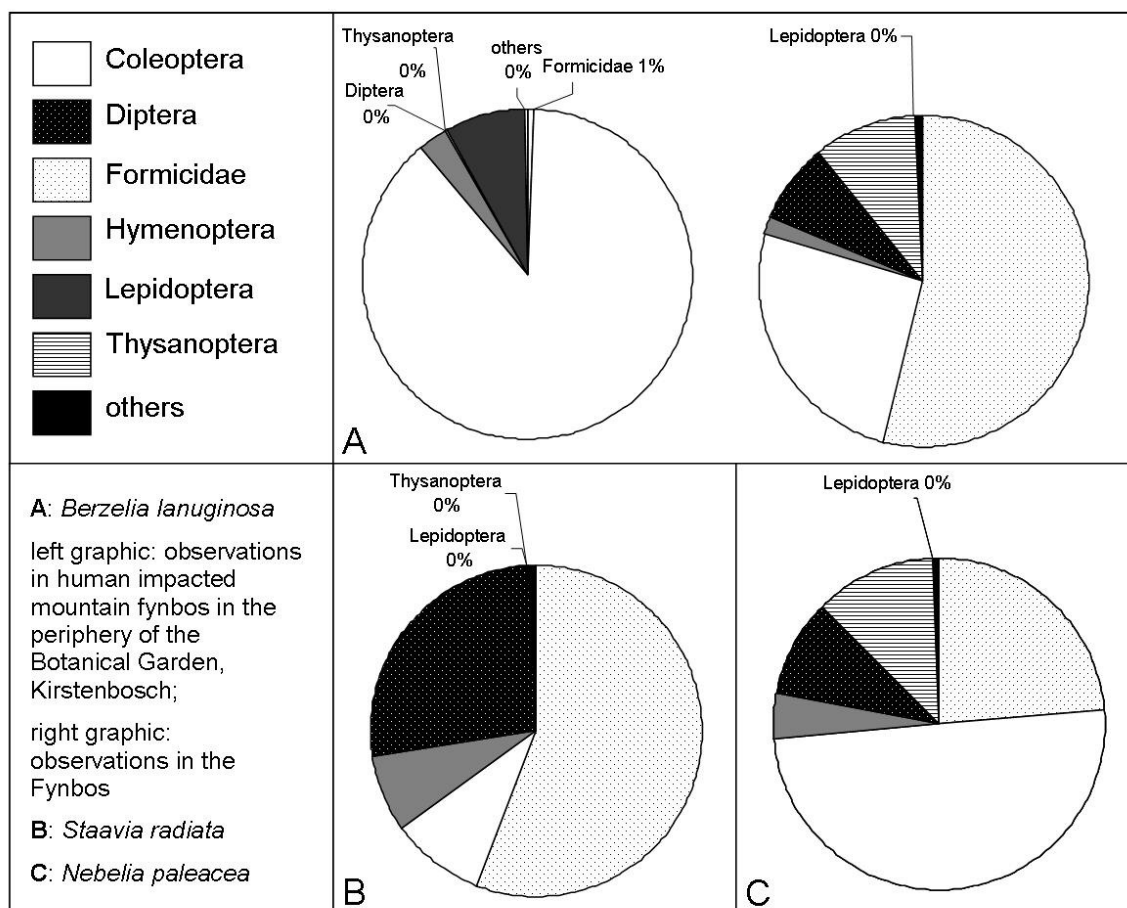


Figure 2.11: Comparison of the pollinator composition of a seven hour lasting observation at three species under equal weather circumstances (sunny, partly cloudy with windy periods). For the total numbers of observed insects see Table 2.5.

Table 2.5: Absolute numbers of observed insect individuals without Araneae and ‘others’ during a seven hour observation according to Fig 2.11 BGK= Botanical Garden Kirstenbosch. In bracts: percentage of the insects per species during the seven hours lasting observation.

	Formicidae	Coleoptera	Hymenoptera excluding Formicidae	Diptera	Thysanoptera	Lepidoptera
<i>Berzelia lanuginosa</i>	208 (54.3%)	100 (26.2%)	6 (1.6%)	30 (7.8%)	39 (10.1%)	-
<i>Berzelia lanuginosa</i> BGK	7 (0.5%)	1224 (88.7%)	39 (2.8%)	1 (0.1%)	1 (0.1%)	108 (7.8%)
<i>Nebelia paleacea</i>	73 (23.7%)	155 (50.3%)	14 (4.5%)	29 (9.4%)	37 (12%)	-
<i>Staavia radiata</i>	209 (56.2%)	35 (9.4%)	28 (7.4%)	100 (27%)	-	-

Insect morphology and behaviour

Of the observed visitors, 19 Coleoptera, four Formicidae, three Diptera, one Hymenoptera, one Heteroptera and one Thysanoptera species were captured at the Vogelgat Nature Reserve.

In Appendix 1 body size, colours and hairiness of the insects is documented. The determination was conducted as detailed as possible. In some cases no determination key allowed to specify the insects.

Coleoptera

The observed Coleoptera varied from 1.5 to 35 mm in body size. 87% beetles have a body size smaller or equal to 1 cm with some exception like big Cetoniinae and Cerambycidae. In total 43 different beetles could be observed. The biggest fraction holds the Chrysomelidae and Melyridae.

The beetles are more or less hairy with single species being covered in short, bristly hairs, others have long single hairs on the elytrons (see Fig. 2.5-2.10). The colour of the Coleoptera is mainly brownish, black, sometimes metallicly or brightly green shimmering.

The Coleoptera crawled on and between the inflorescences and in some cases changed the inflorescence cluster flying from one plant to another. The biggest distance observed was a single flight of a Cetoniinae beetle flying more than ten metres to the next *Berzelia lanuginosa* inflorescence. The beetles stayed on the inflorescences for some minutes to more than one hour, crawling around under windy conditions, too. The Chrysomelidae and Melyridae on *Berzelia lanuginosa* were also observed to copulate on the inflorescences and larvae inside of some inflorescences as well as chew marks indicate that the inflorescences are also used as a breeding ground.

Diptera and Hymenoptera (excluding Formicidae)

The 17 observed Diptera species ranged from three to 18 mm in body size. Some Diptera had single hairs on the abdomen but most of them were hairless. The dominant colour was brownish to black with the Syrphidae being yellow-brownish patterned.

A total of 14 Hymenoptera could be detected that ranged between five to 35 mm in body size. The colours of the Hymenoptera differed from black to orange or metallic shimmering. The hairiness varied from not visible to many beige bristly hairs in representatives of the Apidae.

The approach of Diptera and Hymenoptera was quite similar and took place punctiform: the insects visited one flower, flew away and settled again on another flower of the same or different inflorescence. The approach to flowers took place very directed. If there was no nectar in the flowers the Diptera and Hymenoptera quit the flower immediately.

Both insect groups also switched to other plants within a population.

Diptera and Hymenoptera stay for two seconds at one flower up to about five minutes on an inflorescence. Only single Diptera were observed to crawl between the inflorescences.

Formicidae

The body size of the five occurring Formicidae species ranged from 1.5 to 5 mm. The Formicidae are brownish to black and the biggest species with 5 mm body size has single bristly white hairs on the abdomen. The Formicidae crawled on and between the inflorescences. A change to neighbour plants could not be observed.

Formicidae sucked nectar and crawled over the inflorescences for several minutes to more than one hour under windy conditions. Between the flower visits they used to clean themselves.

Thysanoptera

The tiny, one millimetre big Thysanoptera crawled through the inflorescences and the flowers. They did not change the cluster, but moved between inflorescences. They seemed to use the inflorescences as shelter and feeding ground and therefore stayed several hours and presumably stay over night.

Heteroptera

The four Heteroptera species observed copulated and crawled around but could not be observed to search for nectar and pollen. They did not suck plant juice or damaged the plants. Heteroptera seemed to be casual visitors that could transport pollen incidentally what also holds true for the observed Arachnida.

Lepidoptera

Lepidoptera could only be observed at *Berzelia lanuginosa* at the Botanical Garden site in Kirstenbosch. The Lepidoptera searched for nectar and visited several inflorescences within a cluster. The approach is similar to the approach of Diptera and Hymenoptera and takes place punctiform. The contact places of pollen and insect are mainly the legs and the proboscis. Only accidentally the ventral body touched the anthers of *Berzelia lanuginosa*.

Flower morphology and insect behaviour

The flowers of the different species differ in size, shape and the accessibility of nectar, pollen as well as the possible contact points between insects and anthers/stigma (see Fig. 2.12 and Fig. 2.5).

Three main anther positions could be found within the observed species and will from now on be stated as flower type 1 (anthers long, far extended and with a big distance (0.2 to 3 mm) to the stigma), flower type 2 (anthers curved in, short and positioned within the flower with only 0.1 to 0.2 mm distance to the stigma) and flower type 3 (anthers hidden within the corolla, stigma with 1.62 mm in a medium distance to the anthers).

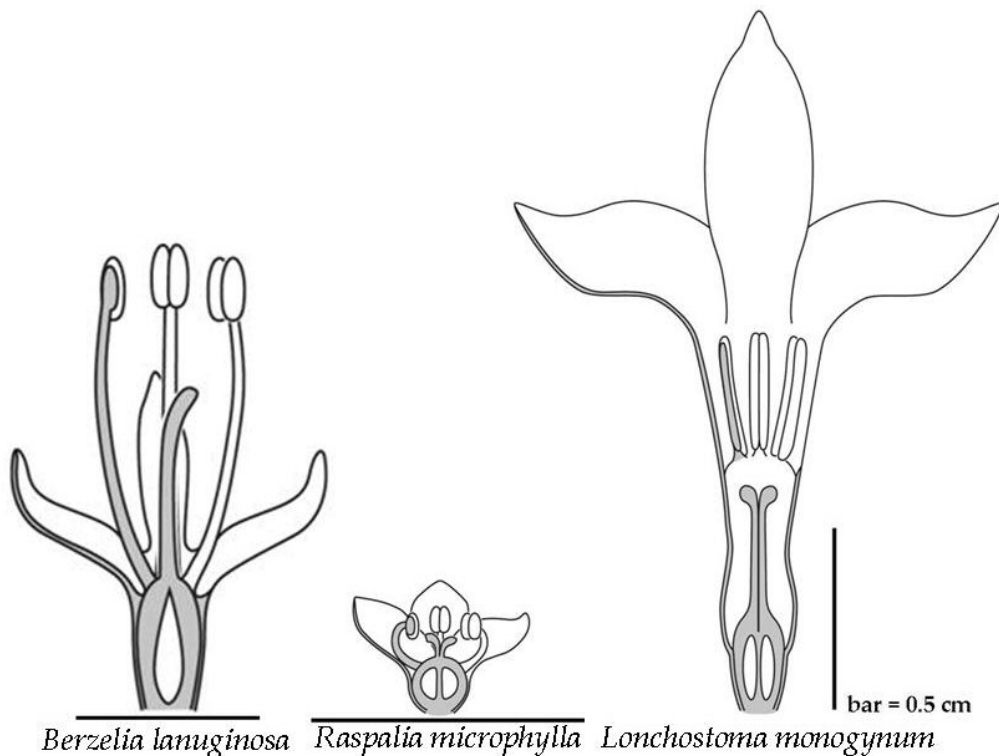


Figure 2.12: Representative longitudinal sections of the three flowers types. Flower type 1: *Berzelia lanuginosa*, flower type 2: *Raspalia microphylla* and flower type 3: *Lonchostoma monogynum*; Graphic by Linda Klöckner.

The pollen could be documented on different parts of the captured insects (see Tab. 2.7). The amount of pollen grains that got stuck differed from few grains at the joints of a Formicidae to several hundreds on very hairy beetles like *Hoplia* or *Cyrtothyrea* what was counted. The pollen deposition places on the insects were correlated with the anther position within the flower and the hairiness and behaviour of the insects. The analysis of the pollen deposition places was related to the three flower types, respectively the anther positions within the flower, and not to the observed plant species because the results of similar flower types showed more or less the same pattern.

Pollen carriers of type 1 flowers – *Berzelia lanuginosa*, *Berzelia abrotanoides*, *Berzelia squarrosa*, *Brunia alopecuroides*, *Nebelia paleacea*

Type 1 flowers are medium-sized with a corolla length of 2.00-3.58 mm, the width ranges from 1 mm to 1.95 mm (see Tab. 2.6). In type 1 flowers, pollen is easily reachable for insects, but the nectar can be inaccessible for insects with big mouthparts as the flowers are closely aggregated within the inflorescence. In *Nebelia paleacea*, a type 1 representative, the access to the flowers is additionally impeded by the spiky bracts (see Fig. 2.6). Best contact probability with anthers and stigma can be assumed for insects that are small to medium sized (see Tab. 2.6 and Appendix 1). In general in type 1 flowers pollen is deposited at random on the insect body. Regarding flower size and corolla port diameter, pollen is transported with all body parts of the insects. Especially hairy beetles can be covered with pollen grains (see Fig. 2.5-2.10). Coleoptera and Formicidae of 2 to 4 mm body size dominate the pollinator composition. The main pollen deposition places are the ventral body parts, the caput, the legs and the joints.

Diptera and Hymenoptera with only a few hairs on their body transport smaller pollen packages than the hairy beetles. Only when Diptera crawl between the inflorescences e.g. of *Brunia alopecuroides*, they can get covered in pollen. Thysanoptera that crawled between the flowers of the inflorescences could be small enough to transport pollen with the whole body.

Pollen carriers of type 2 flowers – *Pseudobaeckea africana*, *Staavia radiata*

Type 2 flowers exhibit a small to medium-long corolla (1.45-2.95 mm) (see Tab. 2.6). The widths of the uppermost part of the corolla differ within type 2 flowers: *Staavia radiata* shows an open, cup-shaped flower with a width of 2.93 mm, the tiny flowers of *Raspalia microphylla* are comparable with type 1 flowers, exhibiting a width of 1.03 mm (see Fig. 2.12 and Tab. 2.6). Insects can easily reach nectar and pollen in type 2 flowers. Pollen is released immediately after the opening of the flower. The pollen is presented punctually at the intrors opening anthers. As stigmas and anthers are adjoined, pollen can be transported to the stigma of the same flower.

In type two flowers the pollen is placed on the forefront of the insects, mainly the caput. Most insects have contact with anthers and stigma with their head (see Fig. 2.5-2.10). Bigger insects do not have contact with the pollen as they do not fit into the gap between stigma and anthers. The main visitors of the type 2 flowers of *Staavia radiata* are Diptera and Formicidae. The Diptera, Hymenoptera and Formicidae had only scattered single hairs and small pollen packages got stuck mainly at the joints of the legs or behind the caput. Exceptions are representatives of the Apidae and single hairy Diptera that have short bristly hairs and thereby can transport pollen with the abdomen.

In terms of size the caput of the Formicidae fits perfectly between the anthers and the stigma. In the type 2 flowers of *Pseudobaeckea africana* only one species of beetles could be documented. With their size of 2 to 3.5 mm they perfectly fit to the flower size and transport pollen predominantly with their caput and the front part of the abdomen.

Pollen carriers of type 3 flowers – *Lonchostoma monogynum*

The type 3 flower of *Lonchostoma monogynum* is tubular and about 9.7 mm long (see Tab. 2.6). The 0.97 mm wide flower entrance is the narrowest of all species observed but does not differ much from species like *Pseudobaeckea africana* (1.1 mm). The distance between anthers and stigma shows a significant difference to all other species (see Appendix 4). The long corolla tube combined with this narrow entrance results in a dark centre within the white corolla (see Fig. 1.3). The nectar, as well as the pollen, is not reachable for Formicidae, Coleoptera and insects with a proboscis with a diameter of more than 1 mm. In type 3 flowers, the pollen is placed precisely on the proboscis of an insect. The visiting Diptera of *Lonchostoma monogynum* do enter the flower only with their proboscis as the corolla is too narrow for all other body parts of the insects. As the anthers are fused with the corolla the insect has to visit two flowers or has to enter the same corolla twice to become a pollinator: with the first insertion of the proboscis, the insect receives pollen grains on one side of the proboscis, the stigma is on the other side. In the second insertion and in the best case a change of the insect position and the flower, the pollen can be transported to the stigma.

Table 2.6: Morphological analysis of the flowers and the resulting definition of the flower type; E = *Berzelia lanuginosa* form found in the Eastern Cape with bigger habitus of the whole plant and longer extended anthers. Sample size of all measurements per species : n=10.

species	flower type	width1 [mm]		width2 [mm]		length [mm]		stigma/anther distance [mm]	
		m	SD	m	SD	m	SD	m	SD
<i>Berzelia abrotanoides</i>	1	1.00	0.08	1.00	0.04	2.43	0.23	2.98	0.11
<i>Berzelia lanuginosa</i>	1	1.43	0.13	1.01	0.03	2.56	0.30	0.72	0.09
<i>Berzelia lanuginosa</i> E	1	1.16	0.17	1.01	0.03	2.04	0.19	1.34	0.11
<i>Berzelia squarrosa</i>	1	1.17	0.13	1.08	0.11	2.02	0.16	0.79	0.11
<i>Brunia alopecuroides</i>	1	1.46	0.08	0.98	0.06	2.00	0.06	0.24	0.05
<i>Nebelia paleacea</i>	1	1.95	0.09	0.99	0.03	3.58	0.07	2.08	0.09
<i>Pseudobaeckea africana</i>	2	1.10	0.10	1.00	0.00	2.01	0.08	0.21	0.04
<i>Raspalia microphylla</i>	2	1.03	0.06	0.48	0.07	1.45	0.09	0.13	0.03
<i>Staavia radiata</i>	2	2.93	0.12	1.05	0.08	2.95	0.20	0.21	0.03
<i>Lonchostoma monogynum</i>	3	0.97	0.09	1.00	0.04	9.70	0.67	1.62	0.13

Table 2.7: Insect groups and their behaviour as potential pollinators and pollen deposition places

insect group	pollen deposition place	behaviour	duration of stay on the inflorescences
Diptera	legs, wings, caput, abdominally, ventrally	nectar/(pollen) feeding, ± punctiform approach	seconds to minutes
Coleoptera	legs, caput, abdominally, ventrally	(nectar)/pollen feeding, crawling, copulating	minutes to hours
Hymenoptera	legs, wings, caput, abdominal, ventrally	nectar feeding, ± punctiform approach	seconds to minutes
Formicidae	legs (joints), caput, ventrally, abdominally	nectar/pollen feeding, crawling	minutes to hours
Thysanoptera	whole body	nectar/pollen feeding, crawling	minutes to hours
Lepidoptera	legs, proboscis, ventrally	nectar feeding, punctiform approach	seconds to minutes

Pollen transport distances

The distances between the plants visited by the observed insects are very different and a single shift can range from 0 (insect remains on the same cluster) to more than ten metres. The more sedentary visitors like Formicidae and Coleoptera do not cover a distance higher than within the population observed. As a population can cover whole mountain slopes, pollen transport flying Coleoptera to transport pollen can be within the whole population. Formicidae are more sedentary than beetles, they serve as close-up range pollen carriers.

Flying insects like Diptera, Hymenoptera and Lepidoptera can shift between nearby populations and can transport pollen over greater distances than Formicidae.

Strong wind had a bad influence on flying visitors which caused mainly sessile insects like Formicidae and beetles to crawl on the inflorescences during windy conditions.

Pollen-load experiments

The pollen load in *Lonchostoma monogynum* differed between the young, the old and the medium aged flowers (see Tab. 2.8). Very young flowers only had an average pollen load of 6 ± 7 pollen grains per measurement. Medium aged flowers had an average pollen load of 51 ± 50 pollen grains and old flowers had a pollen load of 6 ± 7 pollen grains per needle insertion. The maximum amount of pollen grains counted was a measurement of 202 pollen grains of a medium aged flower.

The number of pollen grains also varied between insertions of one flower. Depending on the insertion position of the needle in a medium aged flower the pollen load could range from two to 130 pollen grains in this single medium flower.

Table 2.8: Pollen load measurements of *Lonchostoma monogynum* flowers. Flower 1 to 3: very young flower, corolla just opened; flower 4 to 12: medium aged flowers, corolla fully open, center dark but with slightly pink margin; flower 13 to 15 old flower, corolla open but centre becoming dark.

measurement	1	2	3	4	5	6	7	8	9	10
flower 1-3	10	5	0	0	1	0	0	9	0	0
	15	17	11	9	27	2	3	15	0	3
	15	20	7	0	15	3	2	1	0	0
flower 4-12	78	70	51	24	39	51	70	18	0	63
	63	32	11	10	1	73	42	41	67	51
	130	141	47	37	11	21	28	13	2	3
	202	170	190	102	73	110	117	93	81	70
	107	52	47	31	7	15	0	39	71	49
	195	190	185	79	101	60	87	99	51	49
	49	70	51	62	37	10	27	14	12	4
	31	26	36	6	1	8	7	0	1	0
71	50	30	7	15	10	0	11	10	7	
flower 13-15	10	0	8	17	12	10	0	17	0	13
	2	0	3	0	1	0	4	3	0	0
	0	0	18	0	27	4	0	1	0	16

2.3.3 Reproductive success

Fruit set and seed set

The fruit set ranges from 61.2% in *Staavia radiata* to 100% in *Nebelia paleacea* and *Lonchostoma monogynum* (see Fig. 2.13 and Tab. 2.9) and there is no correlation between flower type and fruit set (see Appendix 4).

Seed set under open, unbagged conditions ranges from 0.3% in *Nebelia paleacea* to 80.8% in *Lonchostoma monogynum* (see Fig. 2.13 and Tab. 2.9). The lack of any seeds in *Raspalia microphylla* was not only found in the fruit set analysis but could be confirmed regarding the seed density below a very old under which two capsules in

935 dry flowers could be detected (see below) what in general indicates a very low seed set.

There is a significant difference of the seed set between all species (see Appendix 4).

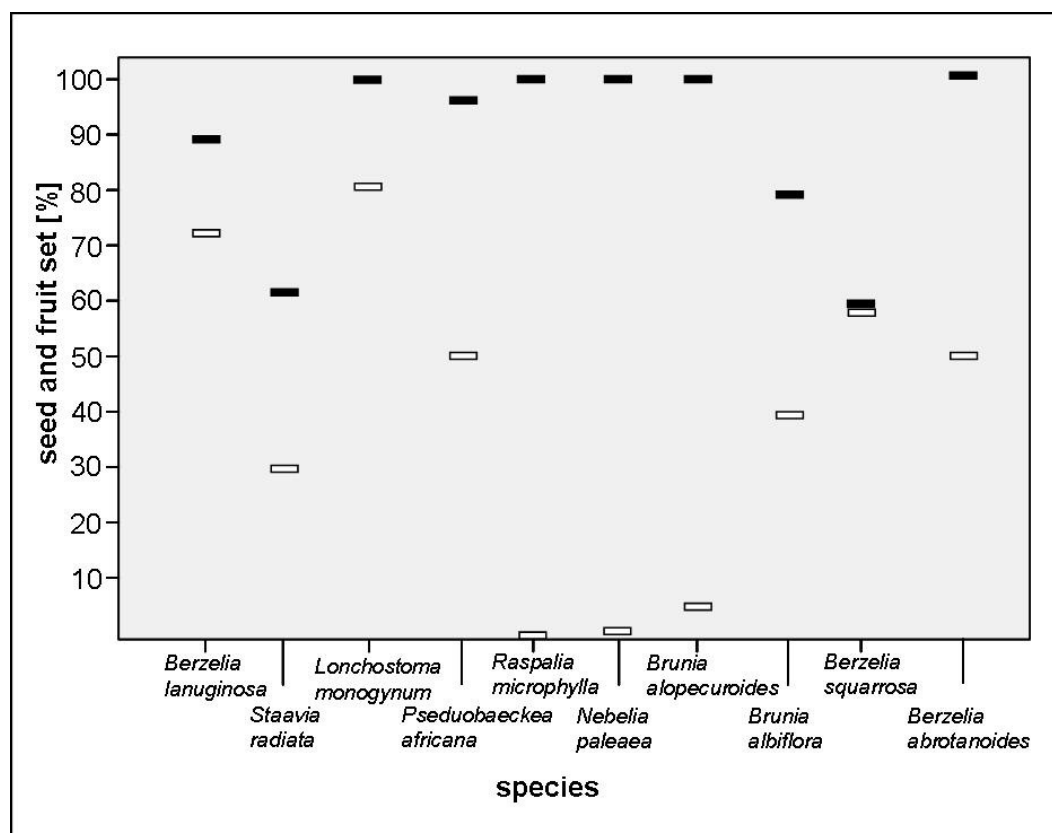


Figure 2.13: Fruit and seed set under unbagged conditions given in percentage. black bars: fruit set, white bars: seed set.

Table 2.9: Fruit and seed set of the analysed species. Seed set is calculated for the maximum of possible seeds/the number of ovules what leads to a different percentage compared to the real observed seeds per flower. Values within the brackets: percentages of fruit and seed set. All analyses were carried out with an n=10.

species	number of ovules per flower	fruit set fruits/flower	seed set seeds/flower	fruit set bagged fruits/flower	seed set bagged seeds/flower
<i>Berzelia abrotanoides</i>	1	665/676 (98%)	345/676 (51%)	-	-
<i>Berzelia lanuginosa</i>	1	1094/1236 (88.5%)	919/1236 (74.3%)	1271/1277 (98%)	23/1277 (1.8%)
<i>Berzelia squarrosa</i>	1	89/1387 (64%)	89/1387 (64%)	-	-
<i>Brunia albiflora</i>	2	608/770 (78.9%)	608/770 (39.5%)	-	-
<i>Brunia alopecuroides</i>	2	404/433 (93.2%)	53/433 (5.8%)	534/574 (93%)	10/574 (0.8%)
<i>Nebelia paleacea</i>	2	654/654 (100%)	5/654 (0.3%)	1006/1006 (100%)	17/1006 (0.8%)
<i>Pseudobaeckea africana</i>	2	554/573 (96.7%)	554/573 (48.3%)	377/498 (75.7%)	377/498 (37.8%)
<i>Raspalia microphylla</i>	2	172/172 (100%)	0/172 (0%)	158/158 (100%)	0/158 (0%)
<i>Staavia radiata</i>	2	153/250 (61.2%)	153/250 (30.06%)	0/136 (0%)	0/136 (0%)
<i>Lonchostoma monogynum</i>	4	112/448 (100%)	362/112 (80.8%)	0/133 (0%)	0/133 (0%)

Pollination and bagging experiments

Bagged and unbagged inflorescences of all species did not show any morphological difference or any treatment-caused damages or degeneration in comparison to unbagged inflorescences.

The observed Bruniaceae showed different fruit set under bagging treatment: *Berzelia lanuginosa*, *Pseudobaeckea africana*, *Raspalia microphylla*, *Nebelia paleacea* and *Brunia alopecuroides* have a fruit set that is comparable with open inflorescences (see Tab. 2.9). In *Staavia radiata* and *Lonchostoma monogynum* no fruits were set.

Regarding the seed set under bagged conditions, *Pseudobaeckea africana* showed a seed set that is only 12% lower than the open unbagged seed set. In all other species the seed set was around zero. In *Nebelia paleacea* the seed set was with 0.8% higher in the bagged than in the unbagged inflorescences that showed a seed set of only 0.3% (see Tab. 2.9).

Correlation of the seed set between bagged and unbagged plants

The correlation of seed set between open and bagged conditions differs between the species. Whilst in *Staavia radiata* and *Berzelia lanuginosa* no correlation could be stated, for *Nebelia* a positive correlation could be detected. In contrast to this, for *Brunia alopecuroides* and *Pseudobaeckea africana* a negative correlation and for *Lonchostoma monogynum* a highly negative correlation could be detected (see Appendix 4).

In *Raspalia microphylla* the correlation and significance could not be calculated because the standard error and the difference are equal to zero.

No correlation could be found between fruit set under open and bagged conditions in *Pseudobaeckea africana*, *Raspalia microphylla*, *Nebelia paleacea*, *Lonchostoma monogynum* and *Brunia alopecuroides* whilst for *Staavia radiata* a significant correlation can be stated. In *Berzelia lanuginosa* a low negative correlation between the bagging conditions and the fruit set of *Berzelia lanuginosa*.

Seed dispersal

The found propagation forms do range from dropping seeds in *Berzelia* species, *Brunia alopecuroides* and *Lonchostoma monogynum* to post-fire release of the seed in *Brunia albiflora* and *Nebelia paleacea* and an exploding capsule in *Staavia radiata*.

Below the chosen old plant individual of *Raspalia microphylla* a very dense layer of old plant material could be found. Within this material, no seeds could be found but within a total of 935 dried flowers, 2 capsules could be identified that resembled the capsules of *Staavia radiata* (see Fig. 2.14).



Figure 2.14: Capsule of *R. microphylla*

The seed density in the soil samples is highest at about 0.7 m around the mother plant.

The results showed a very low distribution radius around the plant (see Fig. 2.15), i.e. in a distance of 2 m nearly no seeds were found any more.

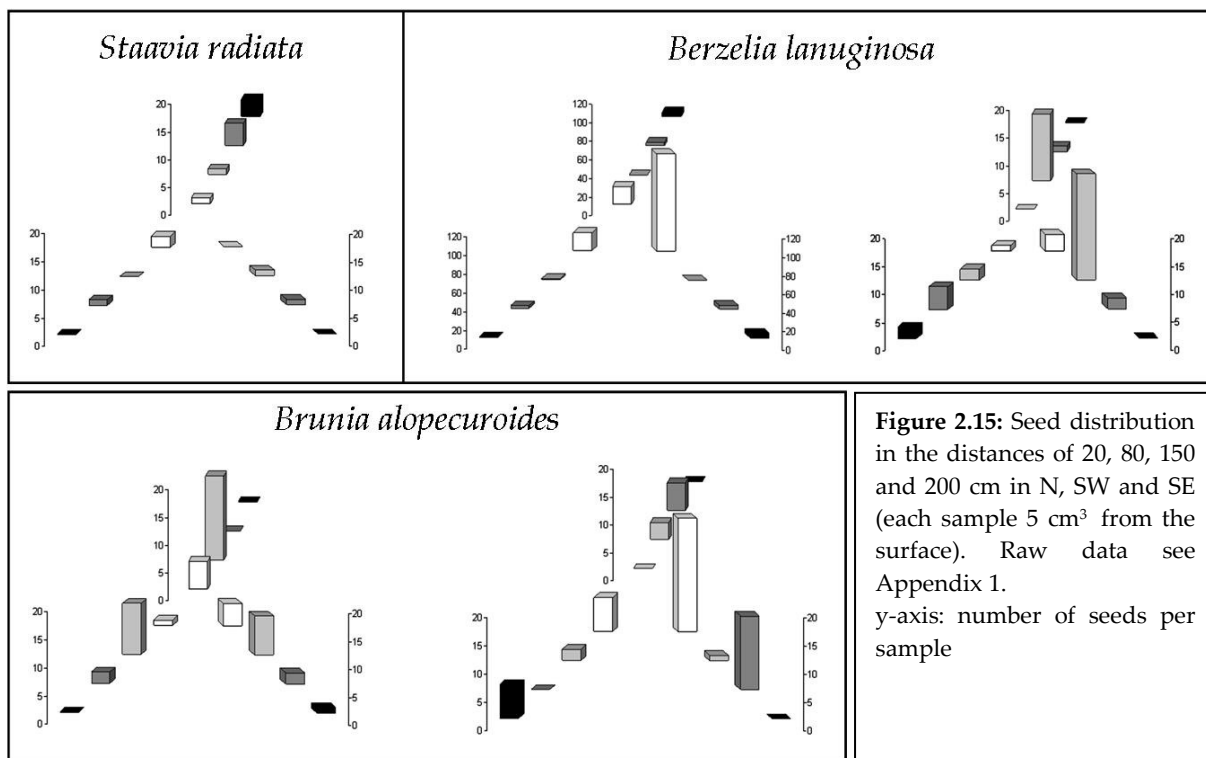


Figure 2.15: Seed distribution in the distances of 20, 80, 150 and 200 cm in N, SW and SE (each sample 5 cm³ from the surface). Raw data see Appendix 1. y-axis: number of seeds per sample

The one to three missing seeds in the traps are due to a mixture with the surrounding earth and within the traps when the traps were relieved (see Tab. 2.10). No propagation by animals or far distance dispersal by wind or through the explosive mechanism could be documented. In *Pseudobaeckea africana* the dropping seeds are transported via the streaming water they grow at/in.

Table 2.10: Seed dispersal experiment via small insects (closed) or wind/water/bigger animals (open)

species	open traps			closed traps		
	trap 1	trap 2	trap 3	trap 1	trap 2	trap 3
<i>Berzelia lanuginosa</i>	97	99	100	100	100	100
<i>Brunia alopecuroides</i>	100	100	99	100	100	97

2.4 Discussion

2.4.1 Plant-pollinator interactions

The interaction between plants and pollinators are most important for the successful pollination of flowers by insects (Mustajärvi et al. 2001; Bränn and Lehtilä 2007; Hegland and Totland 2008; Hargreaves et al. 2009; Jacobs et al. 2009). Plants that rely on insects as pollen carriers have to ensure that this transfer is conducted dependably to prevent pollen loss. To attract possible pollinators, plants apply visual, olfactory and reward based strategies (Simpson and Neff 1981; Kevan and Baker 1983; Klinkhamer and de Jong 1990). For the species observed in the present study, two types of attraction strategies could be observed: a) visually far distance attracting, pollen providing and b) olfactory attracting, nectar providing, with visual close-range effects. Some advantages and disadvantages become evident linking the far distance attraction via scent or visual effects and the morphology of the single flower. In order to interpret these advantages and disadvantages the perception of insects has to be taken into account since the analysed plant species address to the visual and olfactory system of the insects.

Visual attraction

Compared with the surrounding vegetation species like *Staavia radiata* as well as *Lonchostoma monogynum* are - at least for the human eye - more or less inconspicuous and do not outshine the occurring Restionaceae, Proteaceae and other plants of the fynbos (see Fig. 1.2 and 1.3). Distance effects like in *Berzelia* species and *Nebelia paleacea* are caused by the aggregated creamy white inflorescences that impress with their brilliancy within the greyish green fynbos vegetation and can be seen from afar (see Fig. 1.2). The compound eyes of insects allow visual images of their environment that are not as sharp as the images of vertebrate eyes (Eckert et al. 2002). The insect visual system is of a fixed-focus type, so maximum acuity occurs at a very close range and perception of details decreases with increasing distance (Prokopy and Owens 1983). As in any visual system the detectability of an object is the function of the objects dimensions, contrast against background, optical properties of the medium (in the present case air) and intensity of illumination (Hailman 1977; Lythgoe 1979). This means that the impression that the human eye generates does not automatically correspond with the insects' image of the plants. The colour receptors across insects seem to be highly diverse and the spectral range covered by these receptors differs widely between species (Briscoe and Chittka 2001). The Devonian ancestor of all pterygote insects likely had UV, blue and green receptors; red receptors evolved several times independently in the Odonata, the Hymenoptera, the Lepidoptera and the Coleoptera, Formicidae do not have UV, blue

and green receptors (Briscoe and Chittka 2001). UV-blue-green trichomats include several generalist flower visitors such as honey bees, stingless bees and bumble bees (Briscoe and Chittka 2001). Since the observed Bruniaceae represent the creamy whitish to pale pink colour range of the family, no colour specific attraction seems to be relevant for pollinator attraction. The more important factor seems to be the floral display, especially in the pollen presenting species like *Nebelia paleacea*, *Berzelia* species and *Brunia alopecuroides*.

Whether plants like *Brunia abrotanoides* show UV-pattern or if beetles are attracted via possible infra-red receptors still has to be clarified in further analysis. A first test with an improvisational infrared camera with a digital camera, an old picture slide and an enlarged exposure time did not give a hint for infrared markings. Since UV-markings like in *Gazania* GAERTN. can be found in the vegetation nearby to Bruniaceae, a detailed analysis of possible UV-patterns e.g. on the flowers of *Lonchostoma* or *Staavia* or the inflorescences of *Berzelia* might be an object of future studies.

Olfactory attraction

The olfactory system serves as a distant orientation system, olfactory receptors respond to air-transported molecules (Eckert et al. 2002). Species like *Staavia radiata*, *Pseudobaeckea africana* and *Lonchostoma monogynum* emanate a scent that is noticeable over a distance of several meters for the human olfactory receptors. As many chemosensory systems of insects are known to be very sensitive (Schneider 1969; Eckert et al. 2002) it can be assumed that the intensive honey-like scent of *Staavia radiata* also attracts the insects over several meters. As the scent is most prominent during warm periods and in full sun, bad weather conditions and cloudy sky reduce the scent emission. The attraction of pollinators is narrowed. That may be the reason why the inconspicuous but very strong scented *Staavia radiata* is visited by insect in full sun but nearly no insects could be observed under cold and cloudy conditions. Only close-by living ants and some beetle species seem to have knowledge of the nectar source and visit the flowers.

Attraction on flower-level: visual effects

On flower level visual close-range effects in *Staavia radiata* and *Lonchostoma monogynum* are caused by window effects through the tube-like corolla morphology in *Lonchostoma* and colour effects of the young opening flower with dark purple anthers in *Staavia* (see Fig.3.9). Hopliinae (monkey beetles) that could be documented on many species observed (see Fig.2.5-2.10) tend not to be attracted by the flower shape, dark centers or conspecifics but by colour (Johnson and Midgley 2001) and are pollen feeding (Johnson and Nicholson 2001). As pollinators of orchids, petaloid geophytes and other plants of the fynbos (Goldblatt et al. 1998; Steiner 1998) they

might not be specialized on a certain group of plants thereby also responding to the attraction of the Bruniaceae.

Pollen and nectar as reward

In the Bruniaceae two different reward strategies could be found: in the more inconspicuous genera like *Pseudobaeckea*, *Lonchostoma* and *Staavia*, nectar is the main reward that is linked to scent which is documented for the first time whereas in far distance attracting genera like *Berzelia*, *Nebelia* and *Brunia* pollen is the major reward. Inflorescence and flower morphology is linked with this reward systems since the pollen presenting flowers can be found in pincushion-like inflorescences (*Berzelia*) whilst nectar offering flowers are open and cup-shaped (*Staavia*). The third floral type can be found in *Lonchostoma monogynum* that offers nectar but excludes all insects but Diptera due to its long-tubed corolla. Lepidoptera could not be observed in the present study, but cannot be excluded from being potential pollinators of *Lonchostoma monogynum*.

The main reward indicates which pollinator is favourable to be attracted and nectar is said to attract a more diverse spectrum of insects than pollen (Simpson and Neff 1981). The present study could illuminate that both reward types attract the same diversity of insects and the suggestion that pollen attracts beetles and nectar attracts flies can only be stated as tendencies for the Bruniaceae, because various kinds of insects visited the flowers. These findings correspond with the results of the pollinator analysis of *Audouinia capitata* of Wright et al. (1991): all kind of insects visited the brightly red coloured flowers. The most effective pollen carriers detected in *Audouinia capitata* were small beetles of the Nitidulidae (Wright et al. 1991) that in the present study as well could be found on *Berzelia lanuginosa*, *Staavia radiata* and *Nebelia paleacea*.

Regarding the efficiency of pollinators, hairy beetles do by nature have a better adhesion precondition than flies with only several hairs on their legs since the more hairs the more pollen grains can get stuck. For the transport capacities of pollen, not only the hairiness but also the size of the insects is an important factor. Whilst very small insects do 'underpass' pollen and stigmas in *Berzelia* species, these very small individuals serve as reliable pollinators in *Staavia*, where pollen, nectar and stigmas are closely adjoined. In contrast very big insects can not reach pollen, stigmas and nectar in *Staavia* but strike off pollen from the extended anthers in *Berzelia* and touch the likewise extended anthers. Most observed insects are between 2-4 mm in body size. This seems to be the optimal size for the flower and nectar/pollen/stigma accessibility and ensures effective pollen carryover.

The definition how effective pollinators are has to be related with the pollination circumstances. Wright et al. (1991) reported the Nitidulidae to be the most effective

pollinators but in fact the exclusion of other insects in term of efficiency only because they are not frequent does not seem reasonable. The pollination conditions (weather - strong wind and rain, surrounding locality e.g. a Botanical Garden in contrast to a town or natural fynbos) as well as plant population size differ tremendously as reported in the present study. Both sessile and flying insect groups were found to be good and reliable pollinators. Beetles do ensure a big pollen portion being transported over short distances within and between inflorescences of a plant and in some cases like representatives of the Cetoniinae between plants, whereas flies bypass greater distances and can spread pollen grains within or nearby populations. Together the sessile lots of pollen transporting beetles and the lesser pollen transporting bur far transporting flies do ensure a good pollen export situation in the South African fynbos.

Morphology and reward

The morphological analysis gave rise to the definition of three flower types according and within these flower types pollen was deposited in different accuracy on the insects bodies. In species with extended anthers pollen is deposited by random on the insect body, leading to a certain degree of pollen loss but being a highly attractive feeding ground for beetles. The pollen is released during several days thereby providing a long period of pollen export what enhances good pollen-flow probabilities to other plants. Flowers whose anthers are positioned in opposite to the stigma cause pollen deposition in a more precise position on the insects which can prevent pollen loss but on the other hand requires an equally precise pollen transfer to the stigma of a neighbour flower. The pollen in these flowers is released immediately after the bud opens what reduces the potential pollen export time and nectar is the main reward for insects. Flowers of *Lonchostoma monogynum* exhibit the highest accuracy of pollen import and export. Pollen and nectar is not accessible for any insects but those with a long proboscis. Nectar robbery is impossible. The pollen deposition is restricted to the proboscis and the pollen transport to the next stigma is linked to a double visit of the insect: if the insect visits a flower once, it receives pollen on the proboscis. But the next visit does not automatically result in a pollen transfer to the stigma. Only when the insect enters the flower twice or changes the position, pollen transfer can succeed. Another species that prevents pollen-robbery is *Nebelia paleacea*. The long bracts inhibit flower access of very big insects as well as medium sized but broad beetles that have difficulties to reach nectar and pollen between the spinose bracts. The open flowers would provide access for all kind of insects without the bracts. So the restriction indicates the need of a reduction of pollen-loss through pollen-robbery. Compared with *Berzelia lanuginosa* it becomes obvious that the P/O ratio of *Nebelia* is significant lower with 720/1 in contrast to

5125/1 in *Berzelia*. This could be an indication for the bracts serving as morphological pollen discount prevention.

Since in most cases the flowers of the Bruniaceae are closely aggregated, not only the frequency but also the behaviour of the visitors determines if they act as pollen vector or not. Insects like beetles and ants crawl around and can be covered with pollen. Flies or butterflies in contrast have less contact points and thereby transport less pollen grains. This indicates that species mainly visited by beetles might to a higher proportion be exposed to possible geitonogamy whilst species that show a bigger proportion of flies as possibly outcrossing enhancing pollen vectors do have bigger chances of pollen-transfer between and not only within the cluster and the plant. Since species like *Berzelia* and *Staavia* both show a good seed set, the advantage or disadvantage of both strategies cannot be defined.

An example for an extreme mismatch between reward system and pollinator because even accidental pollen carryover is prevented are the Lepidoptera on *Berzelia lanuginosa*. The butterflies are too big in body size to ensure pollen transfer but suck nectar. Since this is an example of a 'disturbed' area in the periphery of a botanical garden which attracts insects that could not be confirmed to be natural pollinators, it cannot be stated that butterflies are disadvantageous for the reproductive success of *Berzelia lanuginosa*.

Ants are a controversially discussed to act as pollinators like in lots of plant species (Gómez and Zamora 1992, Gómez 2000; Schürch and Pfunder 2003) or rather reduce the reproductive fitness of the plants by the secretion of pollen-destructing fungicide liquids ('antibiotics hypothesis' Beattie et al. 1984, 1985; Hull and Beattie 1988; Peakall 1989; Peakall et al. 1990, 1991; Peakall and Beattie 1991). Ants can be potent pollinators which could be shown in the montane woody plant *Hormathophylla spinosa* L. (Brassicaceae) where only ants do pollinate the flowers (Gómez and Zamora 1992). Formicidae could be detected on most of the observed Bruniaceae species and can provide more than 50% of the insects visitors e.g. on *Berzelia lanuginosa* and *Staavia radiata*. The ants in the observed Bruniaceae do transport pollen on the forehead and at joints. Also frequent cleaning cannot remove all of the pollen grains which could be documented through the pollen placement analysis. As ants visit the flowers also during bad weather conditions they function as reliable pollen carriers.

Being a generalist flower – a refinement of the term generalist

With the great variety of insects attracted the hypotheses that Bruniaceae might be unspecialised regarding their pollination system could be emphasised. But this generalism bears an interesting variability.

According to the specification of the term generalization by Ollerton et al. (2007) the Bruniaceae can be subdivided into several groups of generalist flower systems. Most Bruniaceae tend to be of the ecological and functional generalist type with 'open access flowers in which all visitors provide more or less good pollination services'. But a species like *Pseudobaeckea africana* with only one visiting beetle species co-occurring in its wet, swampy habitat seems to be a functional or in this case more ecological specialist with 'open access, apparently classical generalist flowers that attract only a narrow spectrum of pollinators'. On the other hand it has to be discussed whether *Pseudobaeckea* could be comparable with the case of *Berzelia lanuginosa* where different habitats accounted for a totally different pollinator emphasis and composition that seems to be reasonable.

Berzelia lanuginosa and tend to attract mainly pollen feeding beetles *Staavia radiata* tend to only attract flies via the observed window effect (dark purple anthers in contrast to the light purple corolla) and nectar offering respectively, thereby possibly being functional and possibly ecological specialists. In fact they are opportunists, or using the definition of Ollerton et al. (2007), 'functional and ecological generalists' with 'flowers that appear to be phenotypically specialised, (...) which in fact are pollinated by whatever flower visitors are of suitable size and shape, and have appropriate behaviour'.

2.4.2 The reproductive success

The effectiveness of plant-pollinator interactions manifests itself in the reproductive success – in fruit and seed set (Waser et al. 1996). When valuating the reproductive success by the seed set, it has to be taken into account that an apparently low seed set in *Nebelia paleacea* implies 831.8 seeds in only one reproductive year of a medium sized plant with 271 282 flowers. As in *Nebelia* the fruits/seeds remain on the plant and it can be assumed that at least the last three to four years old seeds are viable, the sum of seedlings is sufficient for the maintenance of a stable population.

However, a very low seed set with self-pollen could be observed by de Lange et al. (1993c) and de Lange and Boucher (1993a), but this still indicates that the species is self-fertile. After the reproductive assurance hypothesis self-fertility may insure reproductive success, compensating failed pollination through autogamy (Schoen et al. 1997). The bagging experiments showed that only some of the observed species show autogamy or pollination through a neighbour flower. The distance between anthers and stigmas leads to a high seed set under vector-exclusion in species with low distances and a low seed set in species with big distances between stigma and anthers. In the case of a total absence of seed set like in *Staavia radiata* self-incompatibility or a very restricted phase of stigma receptivity can be assumed.

The overall results of the fruit and seed set analysis show a varying seed set in quite similar appearing plants e.g. in two species with big floral displays, creamy whitish flowers and extended anthers, namely *Berzelia lanuginosa* and *Nebelia paleacea*. The same attraction strategy does not result in a comparable reproductive success: in *Berzelia lanuginosa* seed set ranges at 74.3%, in contrast to only 0.3% in *Nebelia paleacea*. The reason for this discrepancy could be the already mentioned pollen loss prevention and thereby pollinator exclusion via the long bracts in *Nebelia*. The more probable reason is the differing life-form of the two species since *Nebelia paleacea* is a resprouter and might not be dependant on seed set (Carlquist 1978). The question about the aspired quality of seeds being either outcrossed in reseeding or selfed in resprouting species can be tried to be answered via the pollinator spectrum. Regarding flies as predominantly outcross-enhancing and beetles being more geitonogamy charging, reseeders seem to accept geitonogamy whilst the resprouter tends to outcrossing. This pattern contradicts the assumption that reseeders should enhance outcrossing to ensure a great diversity of seeds for seed bank production for the next fire event. On the other hand it becomes clear that a resprouter ensures the survival of its own genes (James 1984) and a reseeding plant has to ensure as much seeds as possible, also accepting geitonogamous offspring.

Offspring quality is not only dependant on its genetic constitution but as well on effective seed/offspring dispersal (Willson and Traveset 2000). The seed dispersal analysis indicates that the seeds are not dispersed by insects, or birds what might at first appear disadvantageous because the seeds are not dispersed over great distances. But in a fire-prone region the highest nutrition material in form of ash is available near the mother plant which enables good growing conditions for the seedlings (Keeley and Fotheringham 2000). So the observed seeds are spread of a maximum of two metres and the main seed density at about 1.8 metres seems to be reasonable and adapted to the fire-prone fynbos.

Opportunism as optimal pollination strategy

An abiotic component that influences pollination is the weather (McCall and Primack 1992). In the Western Cape the climate with winter rainfall and strong wind is an important factor and also influences the plants in the region, not only at sea level but also in the mountain area (Johnson 1992). The climate also influences the pollinator rate and composition (McCall and Primack 1992). During the study wind and rain excluded flying insects for long periods. This reduces outcrossing and forces pollen transfer within inflorescences. As only sessile insects like beetles and ants can visit the flowers meanwhile this can lead to a lack of pollen transfer between different

plants of a population. The wind that anticipates the pollination by insects can lead to nearby pollination.

Wind and insect-opportunism is the optimal adaption to the habitat fynbos, to the weather situation in the Western Cape and ensures pollination and a certain proportion of outcrossed pollen as well as an ensured fruit set within inflorescences. The question whether the resprouting and reseeding species have different pollination systems can not be answered completely.

2.4.3 Conclusion

Summing up, the Bruniaceae are opportunists regarding their pollinators. The restriction via morphology and the different emphasis of visual or olfactory attraction lead to tendencies to certain pollinator groups but the superior instance that determines the visiting insect groups is the surrounding habitat. The observations show that the plant-pollinator interaction can neither be the reason for the high variability of the flowers and inflorescences nor a mechanism for speciation in the family. It can not be dismissed that an adaptation towards a certain pollinator group might have occurred throughout the past, but the picture shown today represents the family as adaptable generalists. However the generalism seems to be a specialisation, namely the strategy to be as flexible as possible, and an adaptation that improves the chances to survive in the fynbos.

3. Architecture and breeding system

Abstract

The aim of the present study is to illuminate the breeding system and possible regulation mechanisms regarding pollen-import and pollen-export (dichogamy, herkogamy) in a generalist flower system. To test outcrossing enhancing temporal and spatial anthesis patterns that regulate pollen export and pollen import, the breeding system of eight Bruniaceae species on plant, inflorescence and flower level were observed. Receptivity tests at a total of 49 species were conducted of which 21 were documented using a scanning electron microscope to detect spatial and temporal changes of the sexual functions within the flower. The results show that the anthesis proceeds in a multicyclic or unicyclic pattern linked with dichogamy. This pattern is not uniform regarding the life-form. Beside protandry for the first time protogyny could be documented which is realised in pincushion-like inflorescences. The mapping of the stigma types on the recent phylogeny showed that the stigma types are consistent within clades except an interspersed pattern caused by *Raspalia* that again is consistent on genus level. The results lead to the conclusion that diverse morphological patterns are realised altogether enhancing outcrossing.

3.1 Introduction

Most Bruniaceae are generalists regarding their pollination syndrome (see Chapter 2). In other generalist systems like the European Apiaceae or Euphorbiaceae and a diverse breeding system is realised that enhances outcrossing and prevents geitonogamy (Lindsey and Bell 1980; Wyatt 1982; Bertin 1993; Bertin and Newman 1993; Bell 2001; Barrett 2003; Müller et al. 2006; Schardt 2006; Reuther and Claßen-Bockhoff 2010). The found P/O-ratio of the observed Bruniaceae indicates outcrossing to be the main pollination strategy (Chapter 2). Therefore it can be assumed that the pollen-export has to be optimized. As within the flowers the anther and stigma position differs and leads to different pollen deposition places on insects it could be assumed that during the anthesis a herkogamous movement separates the pollen-import and pollen-export.

Aside this spatial separation the Bruniaceae are known to flower with a spatio-temporal pattern (Claßen-Bockhoff 2000). This can be an indicator not only for occurring herkogamy but also for dichogamy. The evolutionary development of dichogamy was extensively discussed throughout the past and can be explained as a function that separates pollen-import and the pollen-export to avoid interferences and inbreeding in hermaphroditic flowers (Stout 1928; Wyatt 1983; Lloyd and Webb 1986; Bertin and Newman 1993). In the present study the focus is held on possible periodic patterns during the anthesis and the flowering sequences within and between inflorescences of eight Bruniaceae species. The flowering pattern on plant-, cluster-, inflorescence- and flower-level will be linked to the observed pollinator

behaviour (Chapter 2) what can illuminate whether the breeding system regulates pollen transfer and reduces possible pollen loss due to inefficient pollinators (Johnson and Bond 1997). Since an aspired morphological analysis of the stigma comprise species of all Bruniaceae genera, the results will be mapped on the recent phylogeny (Quint and Claßen-Bockhoff 2006a) to detect possible tendencies of possible dichogamy within and between genera.

3.2 Material and Methods

3.2.1 Plant species and observation sites:

The two observation sites were the Vogelgat Private Nature Reserve and the Fernkloof Nature Reserve, Hermanus (see Fig. 3.1). Between September and December 2007 eight species in seven genera were observed regarding the daily flowering sequence, rhythmic flowering patterns and the position of the organs (stigma and anthers) that could indicate dichogamy and herkogamy respectively (see Tab. 3.1). *Lonchostoma monogynum* was observed in June 2008.

The flowering patterns were recorded detecting possible synchrony or asynchrony on population level, on plant level, cluster level, inflorescence and flower level.

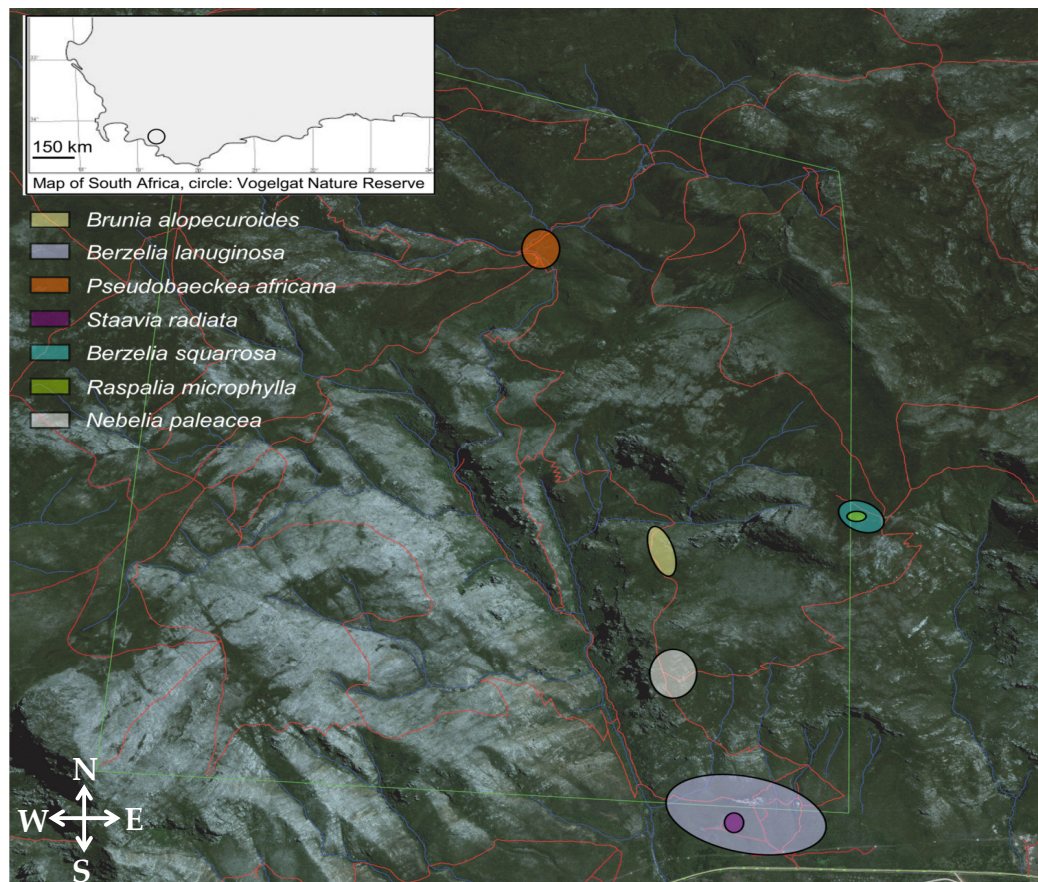


Figure 3.1: Vogelgat Nature Reserve with the particular population localities of the species observed. Green line= Reserve boundary, red line=paths, blue line=streams

Ten randomly chosen inflorescences with flowers being still in bud of different plants per species were tagged and observed daily. Since the accessibility and the flowering circumstances differed, in *Berzelia lanuginosa*, *Berzelia abrotanoides* and *Nebelia paleacea* the anthesis was recorded every day on flower and inflorescence level.

The flowering period of *Staavia radiata* exceeded the whole observation period wherefore the observations only were conducted on flower level.

Pseudobaeckea africana grows in a distant part of the Reserve and *Raspalia microphylla* as well was not reachable daily therefore both species were only observed on a weekly base. To detect the flowering pattern on flower level, inflorescences of both species were taken to the base camp and the anthesis was recorded daily. The inflorescences could not be kept alive long enough to observe the whole inflorescence anthesis. Proceeding anthesis was compared with the plants in the field to exclude possible observation errors due to changes in climate conditions or reactions to being cut.

In *Brunia alopecuroides* the inflorescences were studied at least every fifth day to get an insight into the flowering behaviour of the inflorescence.

The study site of *Lonchostoma monogynum* in the Fernkloof Nature Reserve has not been reachable every day due to bad weather circumstances therefore inflorescences were observed at least every third day.

The sexual function of the flowers had to be predefined for documentary purposes. The sexual phases in all species observed were defined as a) functional female, stigma visible, corolla open but no anthers visible/no pollen present, b) functional male, anthers visible with exposed pollen and c) post-sexual/fruitletting phase no visible pollen, dry corolla.

3.2.2 Stigma morphology analysis:

To test the stigma receptivity, plant material of 21 species which was fixed in 70% ethanol was dissected with a Leica MS5 stereo microscope (Leica, Heerbrugg, Switzerland) and dehydrated in an ascending ethanol-acetone series. The samples were incubated in every ethanol and acetone step respectively for 1

2 h in ethanol and twice for 24 h in acetone. Then the material was critical point dried with a BAL-TEC CPD030 Critical Point Dryer (, coated with gold using a BAL-TEC SCD 005 Sputter Coater (Bal-Tec AG, Liechtenstein, now Leica Microsystems AG Verkaufsgesellschaft, Heerbrugg) and observed with a Philips XL 30 ESEM (Philips Deutschland GmbH, Hamburg, Germany) scanning electron microscope.

Alcohol material of 28 other species that originated from preliminary samplings that was stored at the Johannes Gutenberg-Universität, Mainz (Marcus Quint and Claßen-Bockhoff) were observed under a stereo microscope MZ 16 A (Leica Microsystems AG Verkaufsgesellschaft, Heerbrugg Switzerland).

After analysing the receptivity and the stigmatic surface the result was mapped on the phylogenetic tree of the Bruniaceae (Quint and Claßen-Bockhoff 2006).

Table 3.1: Conducted analysis. bagging exp = bagging experiments, anthesis obs = anthesis observation.

species	SEM	stereo microscope	anthesis observation	inflorescence observation duration	flower observation duration
<i>Audouinia capitata</i> L.	X				
<i>Berzelia abrotanoides</i> L.	X		X	daily	daily
<i>Berzelia arachnoidea</i> WENDL.		X			
<i>Berzelia cordifolia</i> SCHLTDL.		X			
<i>Berzelia galpinii</i> PILLANS		X			
<i>Berzelia intermedia</i> DIETR.	X				
<i>Berzelia rubra</i> SCHLECHTD.		X			
<i>Berzelia lanuginosa</i> L.	X		X	daily	daily
<i>Brunia albiflora</i> PHILL.	X				
<i>Brunia alopecuroides</i> THUNB.	X		X	every 5 days	
<i>Brunia laevis</i> THUNB.		X			
<i>Brunia nodiflora</i> L.	X				
<i>Linconia alopecuroides</i> L.	X				
<i>Linconia cuspidata</i> L.		X			
<i>Lonchostoma esterhuyseniae</i> STRID.		X			
<i>Lonchostoma monogynum</i> VAHL	X		X		
<i>Lonchostoma myrtoides</i> VAHL		X			
<i>Lonchostoma pentandrum</i> THUNB.		X			
<i>Lonchostoma purpureum</i> PILLANS	X				
<i>Mniothamnea callunoides</i> OLIV.		X			
<i>Mniothamnea bullata</i> SCHLTR.	X				
<i>Nebelia fragarioides</i> WILLD.		X			
<i>Nebelia laevis</i> E.MEY.		X			
<i>Nebelia paleacea</i> BERG.	X		X	daily	daily
<i>Nebelia sphaerocephala</i> SOND.		X			
<i>Pseudobaeckea africana</i> BURM. F.	X		X	weekly	daily
<i>Pseudobaeckea cordata</i> BURM. F.		X			
<i>Pseudobaeckea teres</i> (OLIV.) DUMMER	X				
<i>Raspalia angulata</i> SOND.		X			
<i>Raspalia dregeana</i> (SOND.) NIED.		X			
<i>Raspalia microphylla</i> THUNB.	X		X	every 5 days	daily
<i>Raspalia oblongata</i> PILLANS	X				
<i>Raspalia phyllicoides</i> THUNB.		X			
<i>Raspalia sacculata</i> H.BOL. EX KIRCHNER	X				
<i>Raspalia staavioides</i> SOND.		X			
<i>Raspalia trigyna</i> SCHLTR.		X			
<i>Raspalia variabilis</i> PILLANS		X			
<i>Raspalia villosa</i> PRESL		X			
<i>Staavia brownii</i> DUEMMER		X			
<i>Staavia comosa</i> COLOZZA		X			
<i>Staavia dodii</i> H.BOL.	X				
<i>Staavia dregeana</i> PRESL.		X			
<i>Staavia glutinosa</i> BERG.		X			
<i>Staavia radiata</i> L.	X		X	daily	daily
<i>Staavia verticillata</i> L.F.		X			
<i>Thamnea thesioides</i> DUEMMER	X				
<i>Tittmannia esterhuyseniae</i> POWRIE	X				
<i>Tittmannia laevis</i> PILLANS		X			
<i>Tittmannia laxa</i> THUNB.		X			

3.3 Results

3.3.1 Anthesis progression

Anthesis progression on population level

In all species the plants of a population flower synchronously. Only some plants might flower several days earlier or later depending on the weather circumstances. Under warmer conditions, the plants flower 'faster', under wet and cold conditions the anthesis proceeds retarded.

The anthesis duration of the single populations differed from about four weeks in species like *Berzelia lanuginosa* to several months in *Staavia radiata*.

During the observations it became clear that the flowering times cited in literature (Goldblatt and Manning 2000b; A.V. Hall, unpublished data) have to be seen critically. The flowering time of *Berzelia lanuginosa* and *Berzelia abrotanoides* that co-occur in the observation area is quoted as September until December in *B. lanuginosa* and August until October in *B. abrotanoides* (A.V. Hall, unpublished data). In the observation area, the two species were clearly separated regarding the flowering time: *Berzelia abrotanoides* started to flower in the middle of November when the anthesis of *Berzelia lanuginosa* was already completed.

Anthesis progression on plant level

Regarding the whole plant, all species show a synchronous flowering pattern as all cluster of a plant flower at the same time (see Fig. 3.6, 3.7.).

Anthesis progression on cluster level

The single cluster of a plant flowers synchronously. Only in *Brunia alopecuroides* the whole cluster flowers centrifugally (see Fig. 3.4, 3.6). At the same time the single units of the cluster flower acropetally (see Fig. 3.4, 3.6). Moreover in the periphery of the cluster of *Brunia alopecuroides* a corona of smaller cluster units that start to flower when all other inflorescences of the plant are fruiting can be found. The flowers of these delayed inflorescences are female sterile due to degenerated gynoecium what results in a total lack of fruit set (no development of red succulent fruits) but contain fertile anthers. This leads to a male phase after all other inflorescences have already set fruit (see Fig. 3.3).

Anthesis progression on inflorescence level

The anthesis on inflorescence level proceeds in different patterns: single inflorescences within one cluster flower acropetally in all species except *Staavia radiata* which flowers basipetally.

The flowering sequence is mutlicyclic as the ring-wise and acropetal flowering patterns cause repeatedly opening flowers within one inflorescence. Because this, an overlap of female, male and post-floral phases occurs on inflorescence level cyclic in flowering species (see Fig. 3.2 and Tab. 3.2 and 3.3).

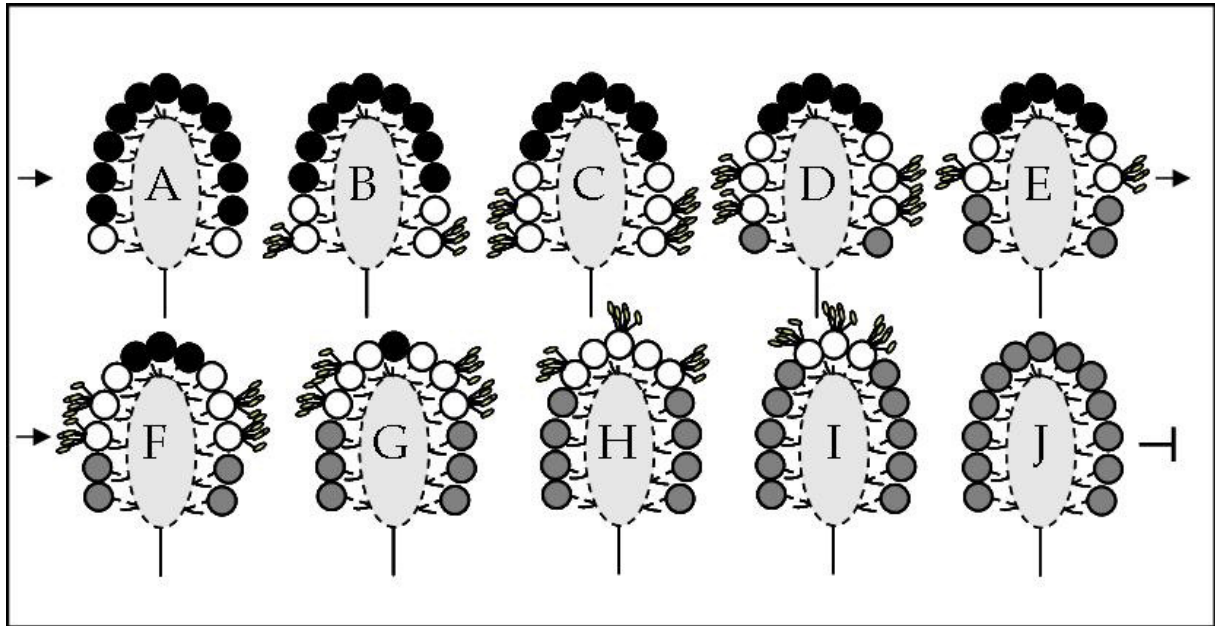
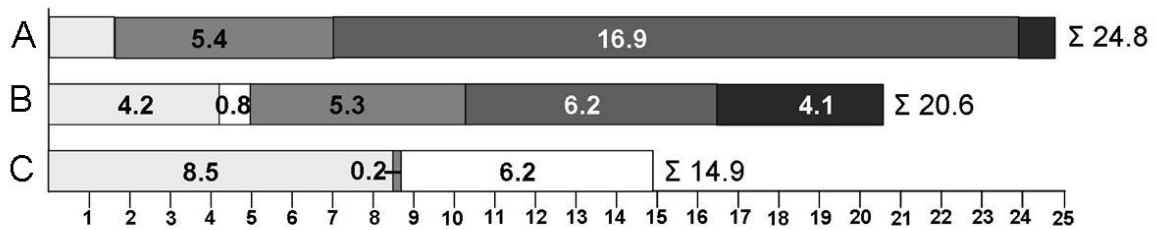


Figure 3.2: Example for the ring-wise flowering pattern on inflorescence level. Proceeding anthesis from A to J. Apical-most flower can be precedent.
 Black circle: buds; white circle: female phase; white circles with anthers: male phase; dark grey circles: post-floral/fruitletting.



<ul style="list-style-type: none"> female male female, male and postfloral postfloral 	<p>Figure 3.3: Duration of the sexual functions on inflorescence level. A: <i>Berzelia lanuginosa</i>, B: <i>Nebelia paleacea</i>, C: <i>Berzelia abrotanoides</i></p>
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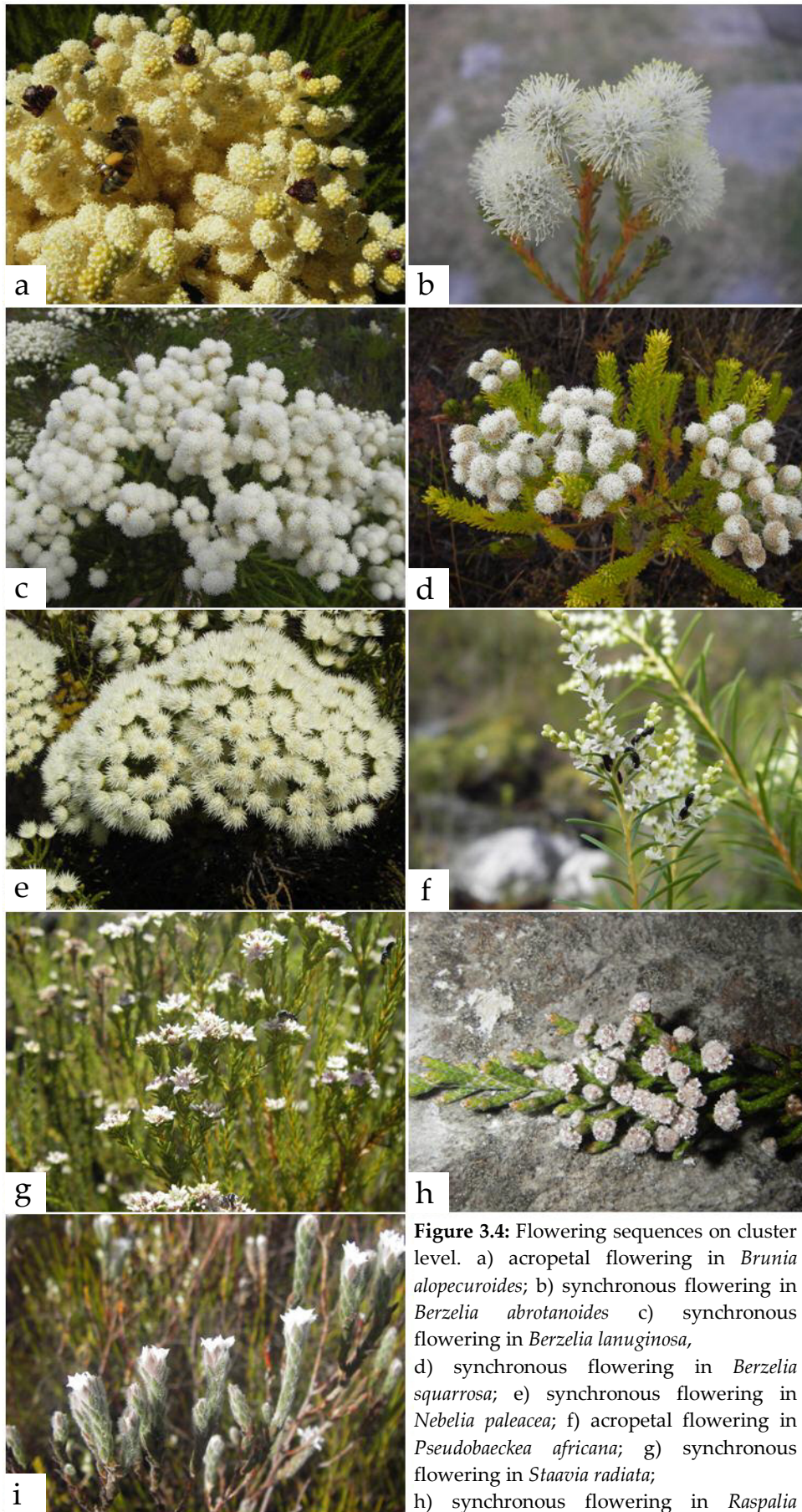


Figure 3.4: Flowering sequences on cluster level. a) acropetal flowering in *Brunia alopecuroides*; b) synchronous flowering in *Berzelia abrotanoides* c) synchronous flowering in *Berzelia lanuginosa*, d) synchronous flowering in *Berzelia squarrosa*; e) synchronous flowering in *Nebelia paleacea*; f) acropetal flowering in *Pseudobaeckea africana*; g) synchronous flowering in *Staavia radiata*; h) synchronous flowering in *Raspalia microphylla*; i) synchronous flowering in *Lonchostoma monogynum*.

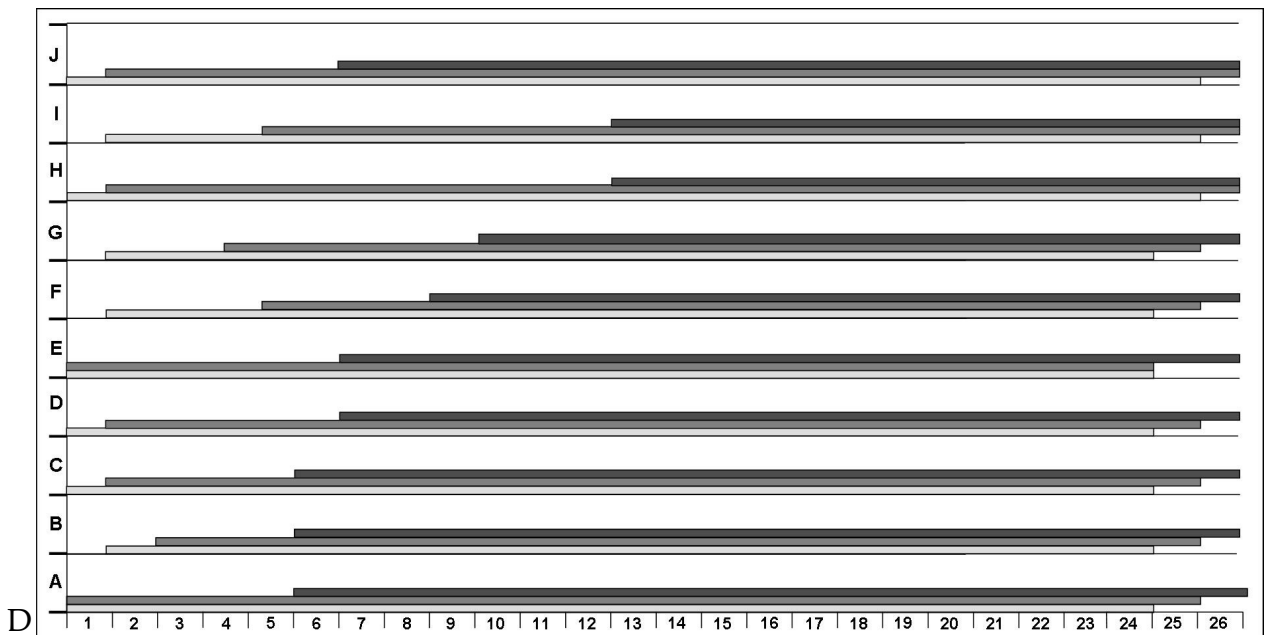
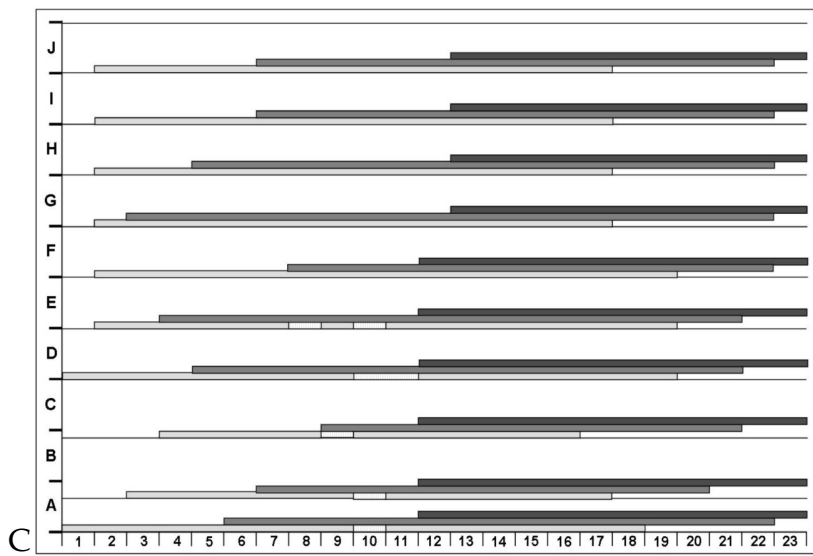
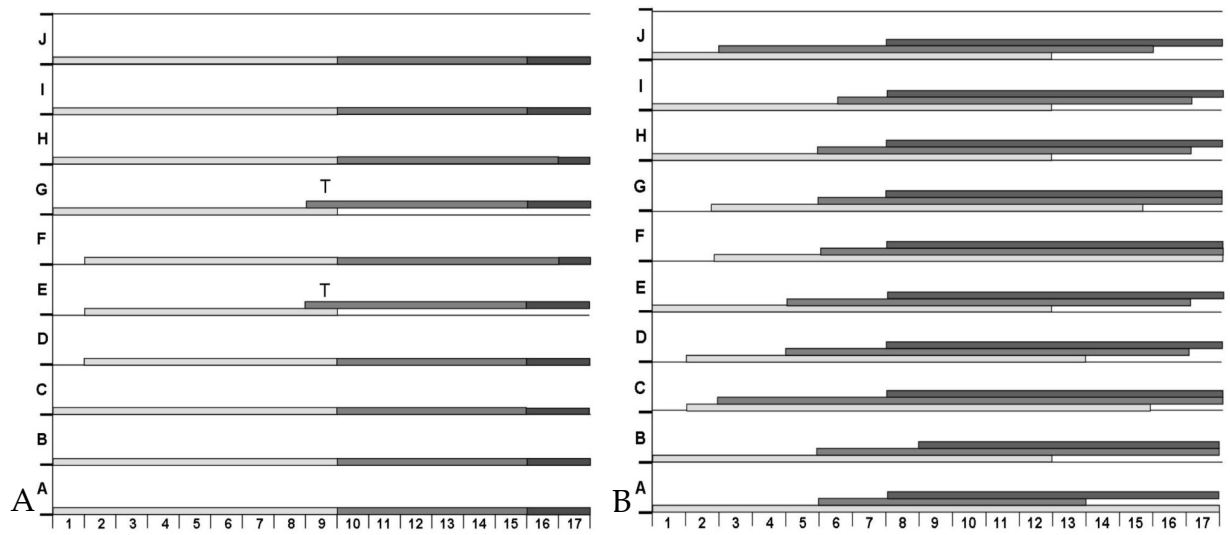


Figure 3.5: Anthesis proceeding on inflorescence level. x-axis: days; y-axis (A-J): observed inflorescences. Light grey: stigma visible, no pollen present. Grey: pollen present; dark grey: fruiting phase. A *Berzelia abrotanoides*; B *Lonchostoma monogynum*; C *Berzelia lanuginosa*; D *Nebelia paleacea*.

Berzelia abrotanoides is the only species in which no cyclic pattern could be observed. The whole inflorescence at first is functional female for about eight days before becoming functional male. In some inflorescences the terminal flower is precedent (see Tab. 3.2 and 3.3). The occurrence of assumed earlier flowering lateral patches of flowers is caused by sun-exposition: under warm conditions, the flowers start to bloom 'faster'. In fact it takes between one and two hours until all anthers of the inflorescences are extended. As all inflorescences do flower synchronously there is a period of time when the whole plant is functionally female and functionally male (see Fig. 3.5, 3.6, 3.8). The whole anthesis of a *Berzelia abrotanoides* plant lasted for 14.9 days.

In *Berzelia lanuginosa* all inflorescences synchronously flower acropetally. In some cases the terminal flower is precedent (see Fig. 3.3, 3.5, 3.8). The inflorescences do flower in a ring-wise pattern. The first basal-most rings of an inflorescence exhibit open flowers for 4.2 days before the anthers get extended (see Tab. 3.2 and 3.3). Then the anthers open in the flowers of this ring. This pattern is realised repeatedly acropetally within the inflorescences (see Fig. 3.3, 3.5, 3.8). The anthesis of the single inflorescence lasts for 20.6 days.

With 24.8 days in *Nebelia paleacea* the longest lasting anthesis of all species could be detected. The flowering pattern is similar to *Berzelia lanuginosa* but in all cases the uppermost flower is precedent (see Fig. 3.3, 3.5, 3.8). The pure sexual phases are shorter than in *Berzelia lanuginosa* and there are no pure male phases. With 16.9 days the phase in which all functional states are manifest is the longest observed for all species (see Tab. 3.2 and 3.3). The apical-most rings do flower synchronously (see Fig. 3.4).

The survey of the inflorescence flowering pattern of *Staavia radiata* shows that on one inflorescence always one bud, one early flower with nectar and one elder flower without nectar is present. The remaining flowers, respectively seeds are already dispersed. As the plants flower throughout the year, the duration of the anthesis of a single inflorescence could not be determined.

The inflorescences of *Pseudobaeckea africana* flower acropetally (see Fig. 3.7, 3.9).

In *Raspalia microphylla* the flowers open in a scattered pattern all over the head-like inflorescences. No directed or ring-wise flowering pattern could be observed (see Fig. 3.7, 3.9).

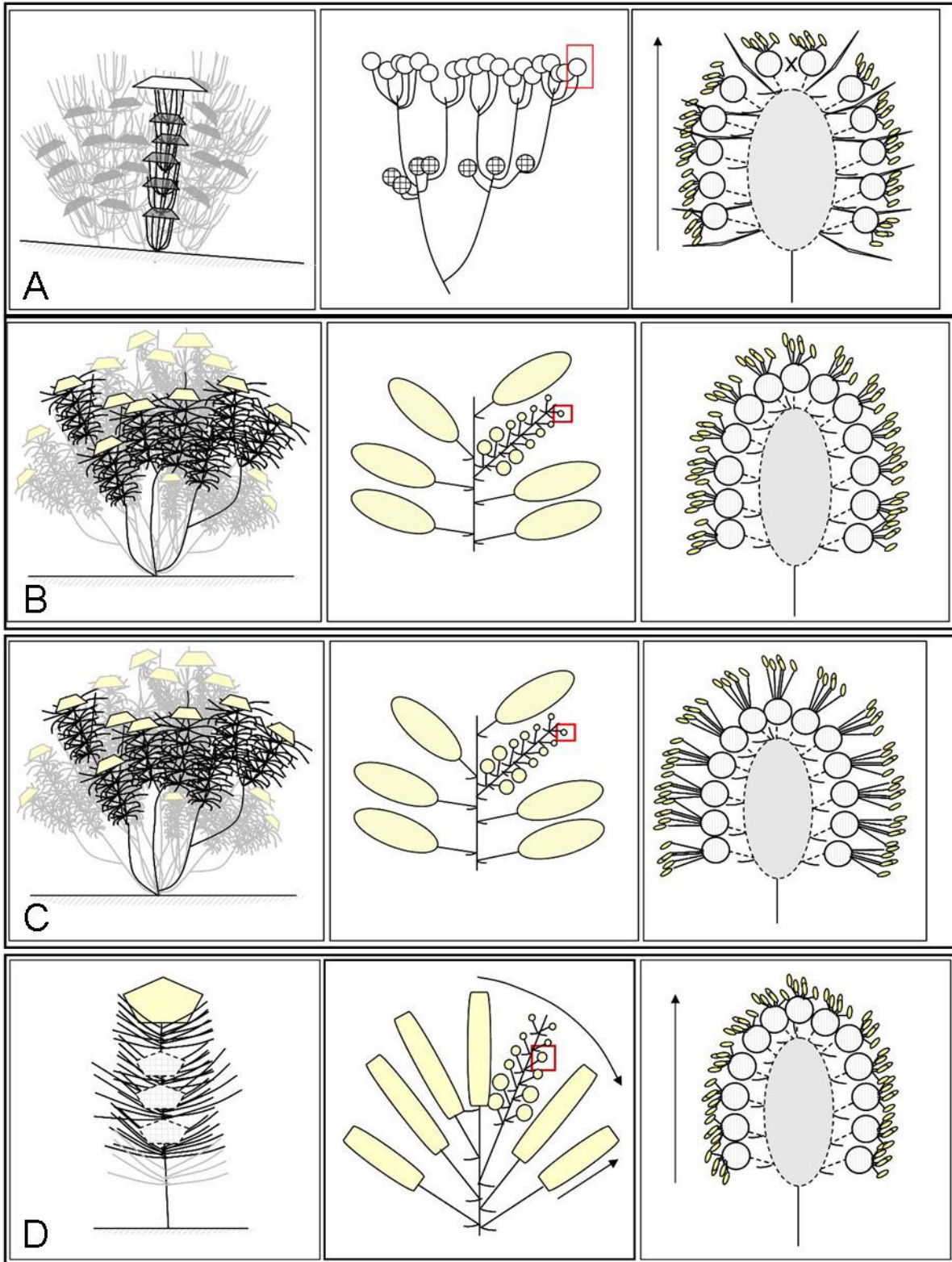


Figure 3.6: Flowering sequence on plant, cluster and inflorescence level. A: *Nebelia paleacea*, B: *Berzelia lanuginosa*, C: *Berzelia abrotanoides*, D: *Brunia alopecuroides*, Arrows indicate the spatial and temporal flowering sequence. A lack of an arrow indicates synchrony of the flowering on plant, cluster or inflorescence level.

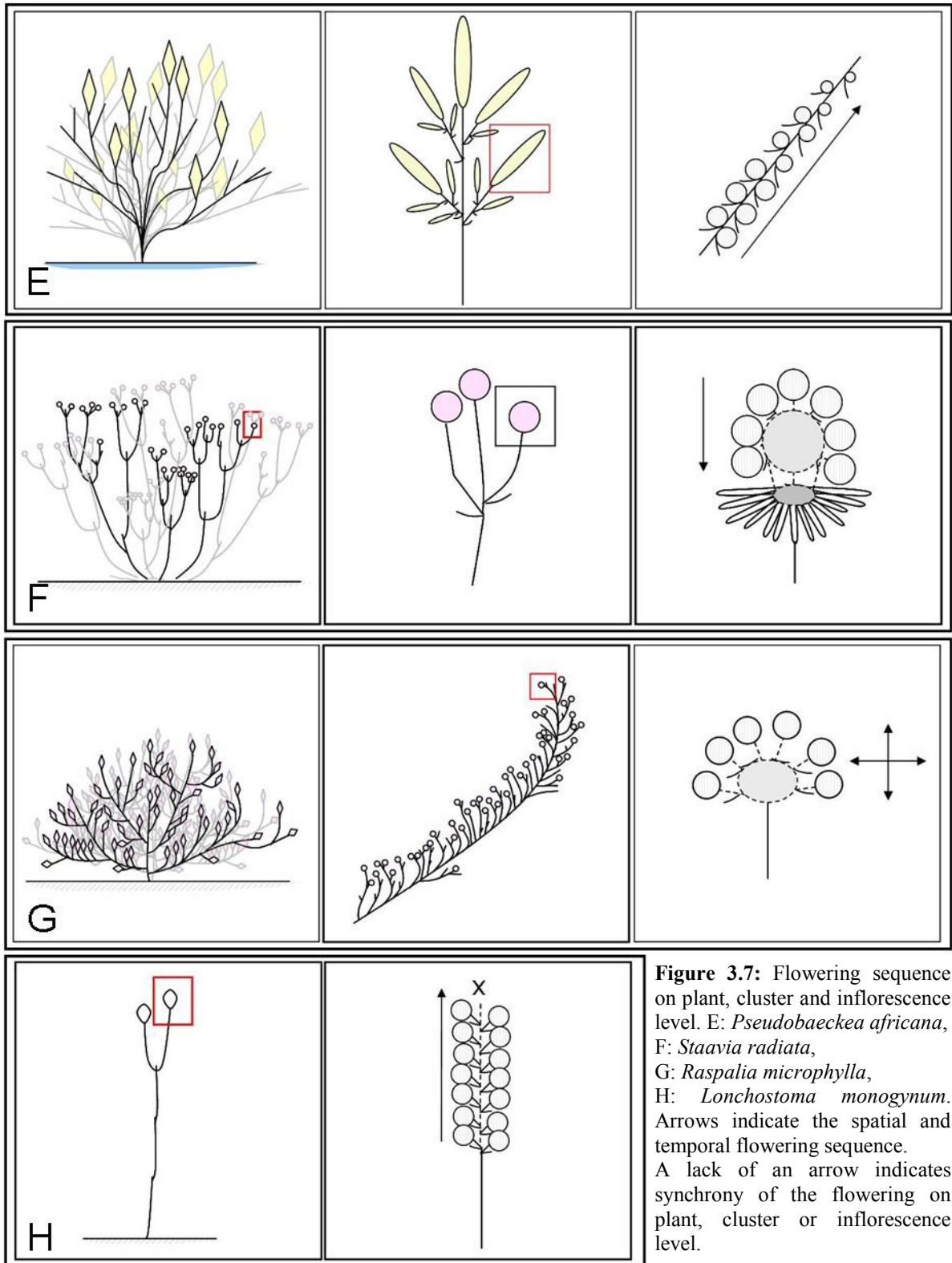


Figure 3.7: Flowering sequence on plant, cluster and inflorescence level. E: *Pseudobaeckea africana*, F: *Staavia radiata*, G: *Raspalia microphylla*, H: *Lonchostoma monogynum*. Arrows indicate the spatial and temporal flowering sequence. A lack of an arrow indicates synchrony of the flowering on plant, cluster or inflorescence level.

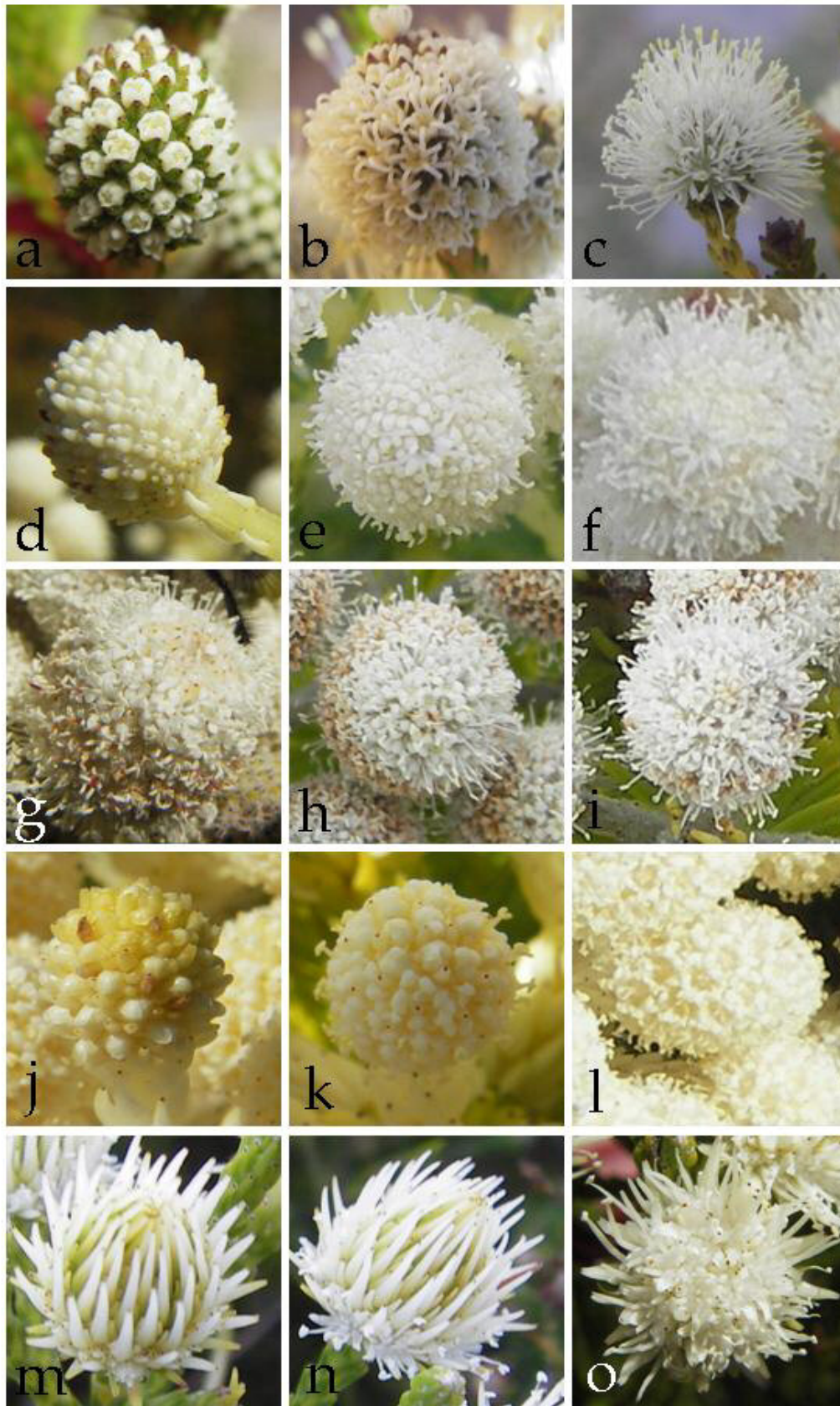


Figure 3.8: Anthesis progression on inflorescence level. Always from the left to the right: young stage, medium aged stage, old stage. a)-c) *Berzelia abrotanoides*; d)-f) *Berzelia lanuginosa*; g)-i) *Berzelia squarrosa*; j)-l) *Brunia alopecuroides*; m)-o) *Nebelia paleacea*.

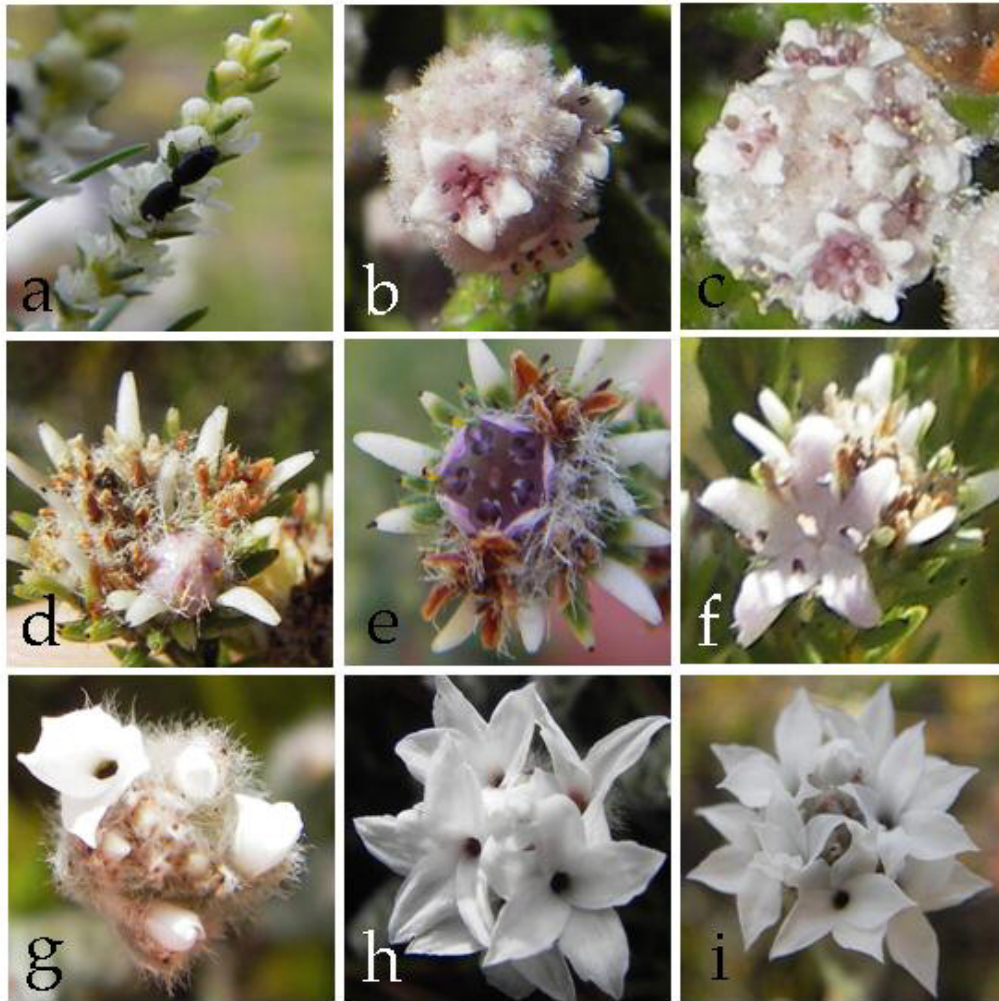


Figure 3.9 : Anthesis progression on inflorescence level. a) *Pseudobaeckea africana*; b)-c) *Raspalia microphylla*; From the left to the right: young stage, medium aged stage, old stage. d)-f) *Staavia radiata*; g)-i) *Lonchostoma monogynum*.

Table 3.2: Survey of the Anthesis duration of population, plant, cluster, inflorescence and regarding the different sexual phases.

species	population	plant	cluster	inflorescence	flower
<i>Berzelia lanuginosa</i>	~ 26	=inflorescence	=inflorescence	20.6±1.42	5.6
<i>Berzelia abrotanoides</i>	~ 15	=inflorescence	=inflorescence	14.9±0.57	8.5
<i>Nebelia paleacea</i>	~ 34	=inflorescence	=inflorescence	24.8±0.79	5
<i>Lonchostoma monogynum</i>	> 14	=inflorescence	=inflorescence	34.7±0.83	5.1
<i>Pseudobaeckea africana</i>	~ 70	-	-	-	3.6
<i>Staavia radiata</i>	> 90	-	-	-	3
<i>Raspalia microphylla</i>	~ 65	-	-	-	2.5

Table 3.3: Anthesis data of ten inflorescences observed in the field.

species	corolla open, stigma visible	pollen present and stigma visible	pollen present and stigma visible and postfloral stages	pollen present and postfloral	only pollen present	Σ
<i>Berzelia lanuginosa</i>	4.2±1.48	5.3±2.16	6.2±1.4	4.1±0.99	0.8±0.78	20.6±1.42
<i>Berzelia abrotanoides</i>	8.5±0.7	(0.2)±0.42	0±0	0±0	6.2±0.42	14.9±0.57
<i>Nebelia paleacea</i>	1.6±1.43	5.4±2.32	16.9±2.47	0.9±0.32	0±0	24.8±0.79

Table 3.4: Anthesis data number of days of floral functions within ten inflorescences

species	corolla open, but no pollen	pollen present	corolla still intact, but no pollen
<i>Lonchostoma monogynum</i>	13±1.76	11.8±1.75	9.9±0.31

Table 3.5: Average number of days of floral functions within ten flowers

species	pollen present	nectar or stigma visible, but no pollen present	postfloral
<i>Berzelia abrotanoides</i>	1.6±0.84	8.5±1.90	4.8±1.31
<i>Berzelia lanuginosa</i>	2.7±0.48	2.9±0.73	0.4±0.69
<i>Nebelia paleacea</i>	2.3±0.82	2.7±0.82	-
<i>Pseudobaeckea africana</i>	2.6±0.84	1±0	1.2±0.42
<i>Raspalia microphylla</i>	2.5±0.52	-	2.3±0.82
<i>Staavia radiata</i>	1.5±0.52	1.5±0.52	1±0
species	corolla open, but no pollen	pollen present	corolla still intact, but no pollen
<i>Lonchostoma monogynum</i>	2.7±0.82	2.4±0.96	10.1±0.74

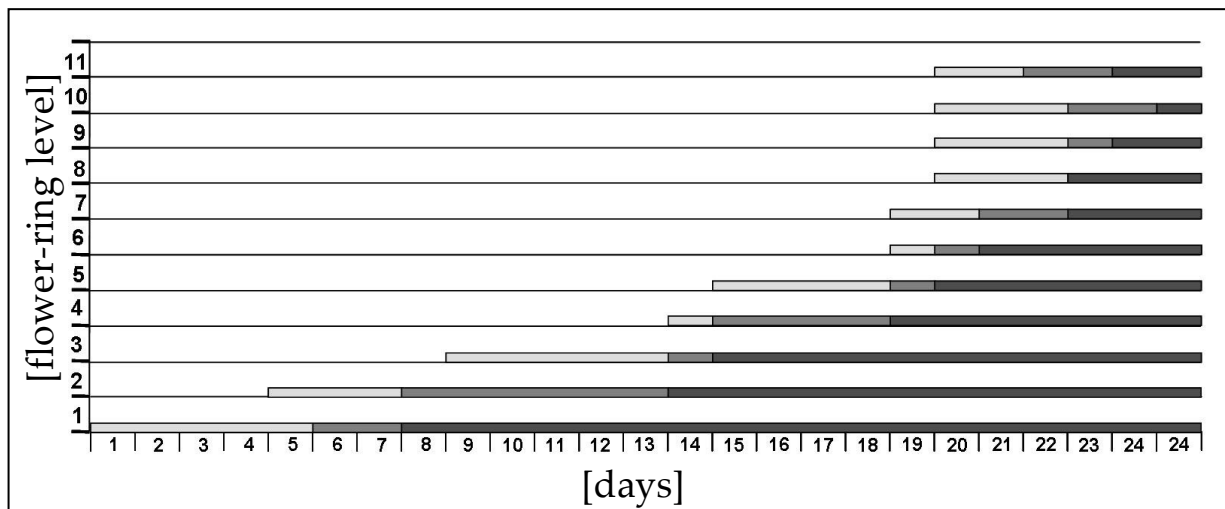


Figure 3.10: Anthesis progression within a single inflorescence of *Nebelia paleacea* on ring level. X-axis: days of the preceding anthesis; y-axis: ring of flowers (ring 1-11), that flower acropetally. Light grey: female phase; medium grey: male phase; dark grey: fruiting phase.

Anthesis progression on flower level

In flowers of the flower type 1, anthers unfold after the stigma is already present for several days. In type three flowers this growth process can be observed for the style that grows during the anthesis whilst pollen is already present.

Regarding the anthesis on flower level the flowers of *Staavia radiata*, *Pseudobaeckea africana* and *Raspalia microphylla* exhibit the same pattern: as soon as the flower opens, pollen is presented. In all other species the flowers are open for several days before the anthers are extended and present pollen.

The single flower of *Staavia radiata* blooms for four days (see Fig. 3.11 and Tab. 3.5). During 36 hours pollen is present, in another 36 hours there is no pollen, but nectar is still produced.

The flower anthesis of *Raspalia microphylla* lasts five days with a pollen presentation of two and a half days (see Fig. 3.11 and Tab. 3.5). As nectar was undetectable, it isn't clear if the nectar production is kept on until the flower is dehiscent.

In *Pseudobaeckea africana* the anthesis of a flower lasts six days with 2.6 days presenting pollen and one day where only nectar shine could be observed but no more pollen was available (see Fig. 3.11 and Tab. 3.5).

Within the pincushion-like inflorescences of *Nebelia paleacea* and *Berzelia lanuginosa*, the flowers are open for three days before another three days pollen is present.

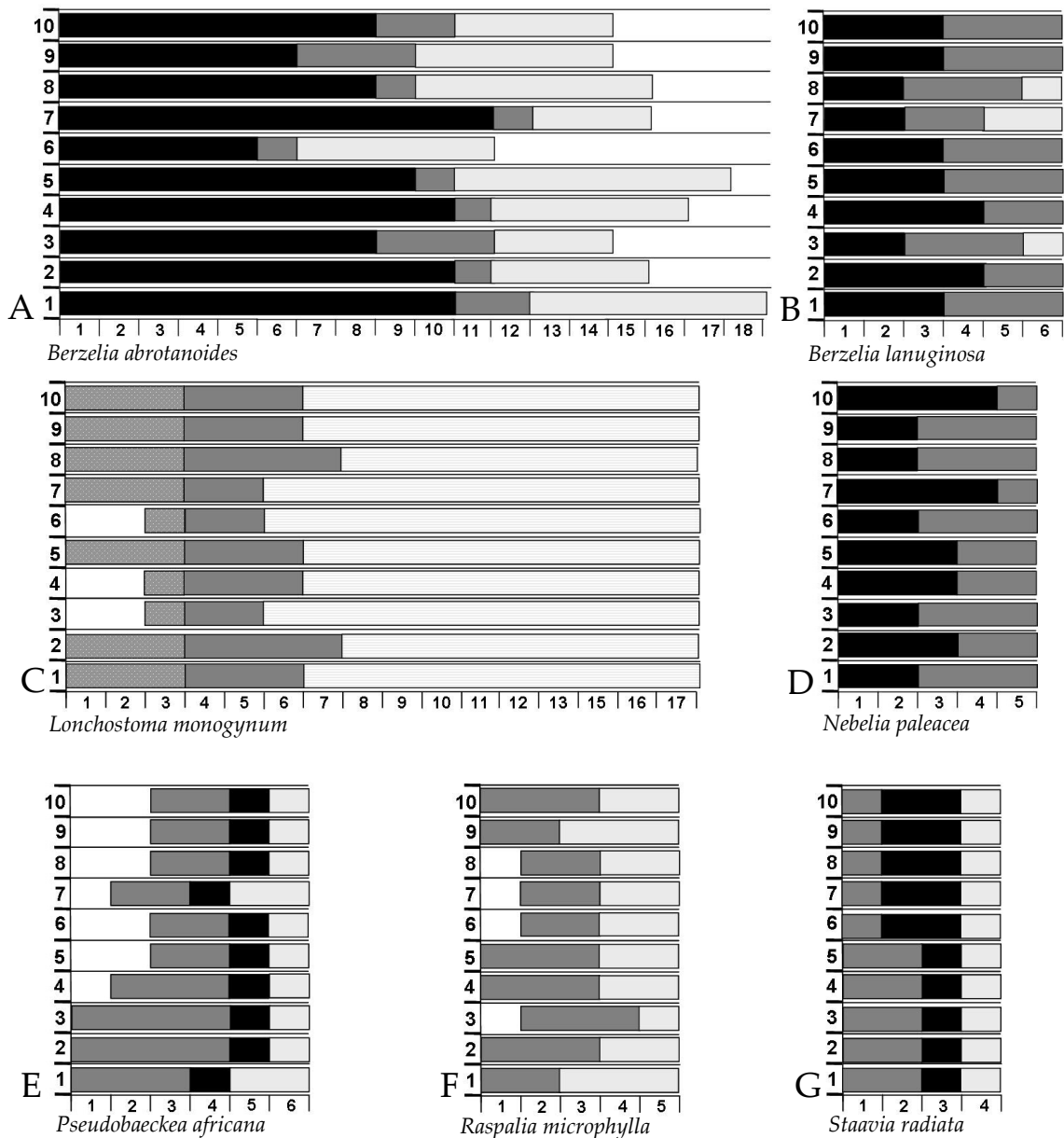


Figure 3.11: Anthesis progression on flower level. x-axis: number of days; y-axis: number of flowers observed. A *Berzelia abrotanoides*; B *Berzelia lanuginosa*; C *Lonchostoma monogynum*; D *Nebelia paleacea*; E *Pseudobaeckea africana*; F *Raspalia microphylla*; G *Staavia radiata*. black: corolla open, stigma visible/nectar present but no pollen visible; dark grey: pollen present; light grey: no pollen visible but corolla still intact/post-floral. In *Lonchostoma monogynum* the first anthesis phase had to be classified as corolla open, since the stigma lies hidden within the corolla and cannot be detected without destroying the flower, the phase after the phase with pollen presented had to be classified as corolla still intact, insects visit the flower, but no pollen is present.

3.3.2 Stigma morphology

In the observed species two different stigma types could be documented. Both types have a very small locally restricted receptive area that is located apical-lateral (see Fig. 3.12).

The difference between the two stigma types is the cellular morphology of the receptive area: the first stigma type exhibits a patch of flat slightly convex cells that burst when getting receptive and fold in when pollen was transferred to the receptive area. The second stigma type shows outgrowing papillae that can also burst and fold in. The time of secretion of the two stigma types differs since in the non-papillate type the stigma surface cells burst before anthers are exposed. In contrast in the papillate stigma type, anthers have exposed all pollen and not till then the papillae are fully grown and secretion can be detected.

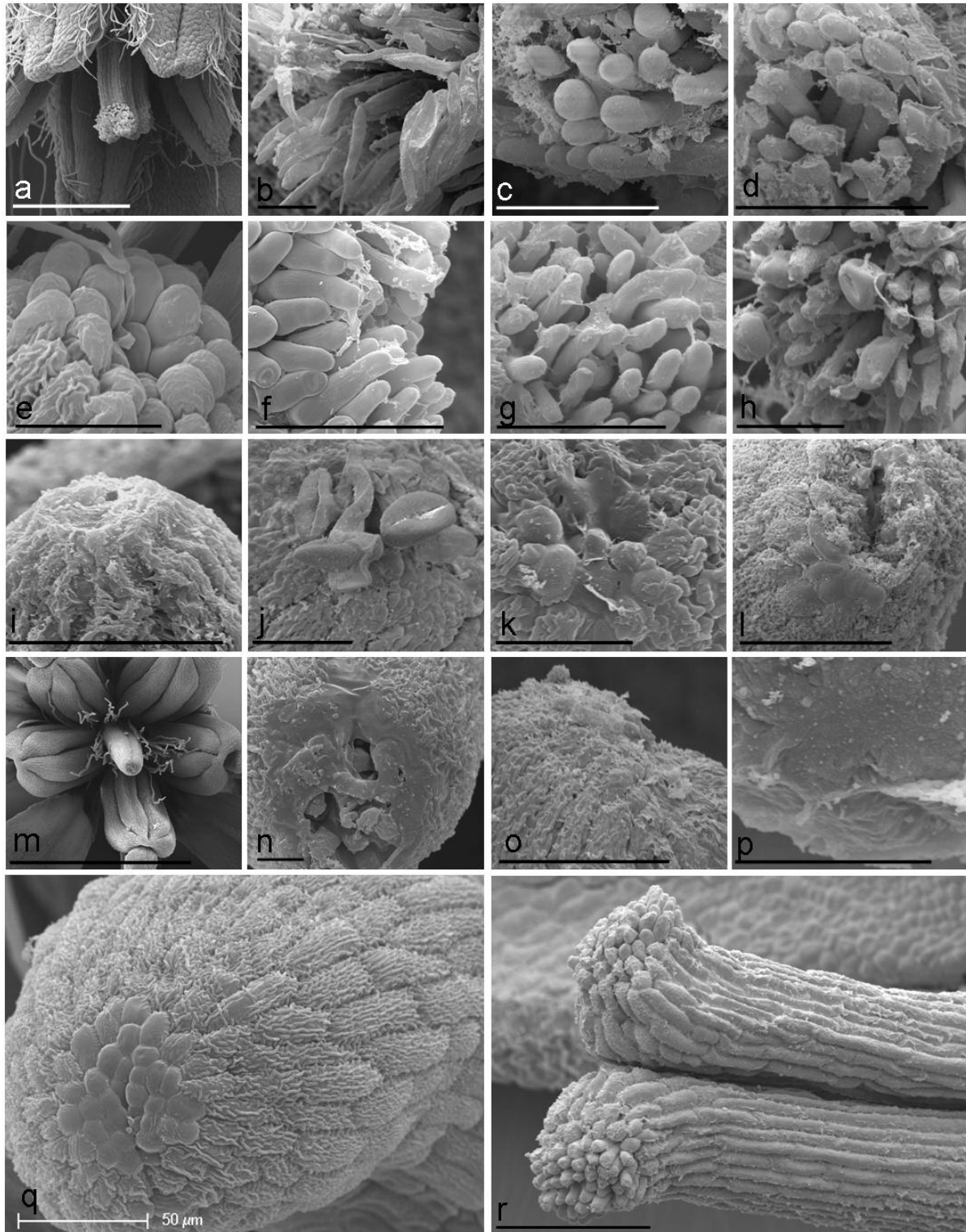


Figure 3.12: The development of the stigma surface a) bud of *Lonchostoma monogynum*, anthers closed b) stigma of an old flower of *Audouinia capitata*, papillae fully grown c) stigma of a medium aged flower stigma *Raspalia sacculata*, d) stigma of a medium aged flower of *Pseudobaeckea teres*, e) stigma of a young aged flower of *Mniothamnea bullata*, f) stigma of a medium aged flower of *Thamnea uniflora*, g) stigma of a medium aged flower of *Lonchostoma monogynum* close-up, h) stigma of an old flower of *Staavia radiata*, i) stigma of a young aged flower of *Berzelia intermedia*, j) stigma of a young aged flower of *Berzelia lanuginosa*, k) stigma of a medium aged flower of *Brunia alopecuroides*, l) stigma of an old flower of *Nebelia paleacea*, m) bud of *Berzelia abrotanoides*, anthers closed n) *Berzelia abrotanoides* close-up of the former picture, o) *Berzelia abrotanoides*, p) stigma of a young aged flower of *Brunia nodiflora*, q) *Berzelia abrotanoides*, non-sculptured cells are the receptive area r) *Staavia radiata* lateral outgrowing papillae as receptive area. Bar = 100 μm in b), h) and r); bar = 20 μm in e), j) and k); bar of all other figures = 50 μm . Second and third row from the left to the right: buds to old flowers and the development of the stigma surface.

Regarding the recent phylogeny and the morphological data of the stigma surface types, stigma morphology in general is consistent within genera. *Lonchostoma*, *Pseudobaeckea*, *Raspalia*, *Staavia*, *Thamnea* and *Tittmannia* exhibit the papillate, *Nebelia*, *Berzelia* and *Brunia* the non-papillate stigma type. The papillate type is represented in the basal clades, the Linconieae and the Audouinieae as well as in more derived *Brunia*/*Pseudobaeckea* and *Mniothamnea* clade (see Fig. 3.6).

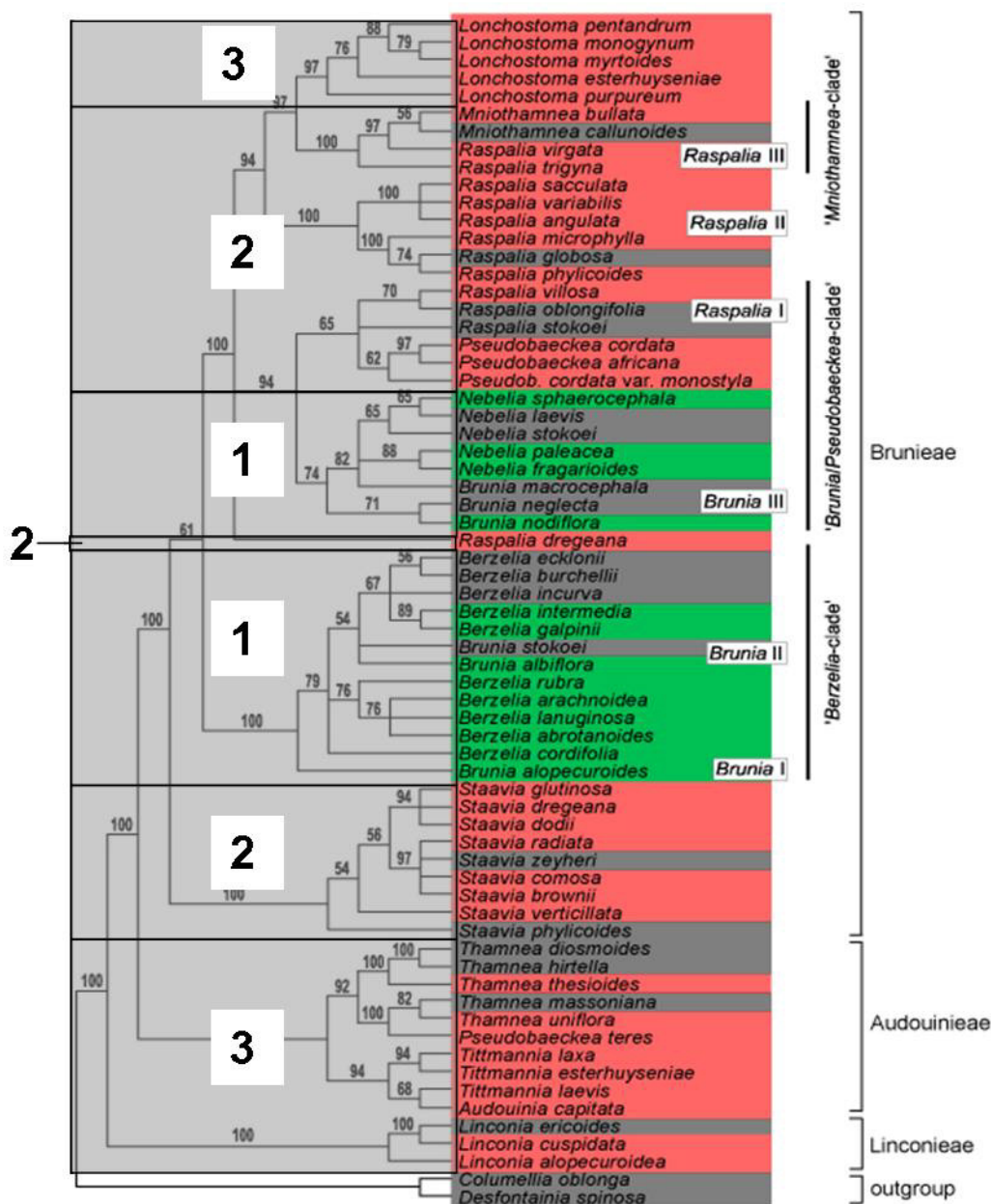


Figure 3.13: Modified phylogenetic tree after Quint et al. (2006) with the stigma types found in the present study; red fields: papiallate stigma, green field: non-papillate stigma, grey: species that were not included in the stigma analysis. Number indicate the flower types (see Chapter 2)

3.4 Discussion

3.4.1 Diverse patterns within the breeding system

Flowering pattern on population and plant level

The observed Bruniaceae supported the finding that plants pollinated by various kind of insects exhibit diverse pattern on anthesis level to attract and guide pollinators through the inflorescences (Reuther and Claßen-Bockhoff 2010, Schardt 2006). Pollinator attraction has to be optimized in plants that rely on insects as pollen vector, but at the same time this transfer has to be regulated to prevent pollen discounting in hermaphroditic flowers (Lloyd and Webb 1986, Charlesworth and Charlesworth 1987, Klinkhamer and de Jong 1990, 1993; Lloyd 1992). Since the single flower cannot be stated as functional unit of a mating system (Harder and Barrett 1996) the flowering behaviour on population and plant level has to be focused. Interestingly populations and plants of the observed Bruniaceae flower synchronously whereas variation occurs on cluster, inflorescence and flower level.

Only *Berzelia abrotanoides* exhibits a separated synchronous female and male flowering phase on inflorescence level thereby indicating temporal dioecy on population level.

The synchrony on population and plant level ensures adequate pollen flow throughout the anthesis which would be reduced in a population that flowers asynchronously thereby being reduced in size (Wright 1938; Fritz and Nilsson 1994).

Multicyclic flowering pattern on inflorescence and flower level respectively

In systems that cannot ensure restricted pollen removal or on the other hand an extensive carryover, fertility is maximised when few flowers are exposed over a longer period of time (de Jong et al. 1992, Robertson 1992). This correlates with the findings of the realised ring-wise flowering pattern in the Bruniaceae. Via the multi-cyclic pattern the sexual function is prolonged tremendously because of the periodic occurrence of female and male functions.

Because the anthesis duration is extended, bad pollinator movement due to unfavourable weather circumstances like strong wind and rain that occur frequently in the Western Cape can be balanced.

On the other hand the ring-wise flowering pattern reduces interferences between the sexual function of the actually blooming flowers. Thereby pollen discounting by insects that crawl over a fully flowering inflorescence gets avoided since only one or two rings present pollen, whilst the more apical situated flowers are still in bud. Through the ongoing cycles of opening flowers, the chance of a flower being pollinated with outcross-pollen is increased.

The flowering pattern on inflorescence and cluster level not only serves as attraction but also as guidance for the insects. Two different insect guidance forms linked to morphology could be detected: in *Berzelia lanuginosa* the acropetal guidance occurs on inflorescence-level. In *Brunia alopecuroides* the insects when following the current flowering flower the guidance takes place on cluster unit level as well as on inflorescence-level. Moreover the whole cluster of *Brunia alopecuroides* flowers centrifugally, which enforces the guidance effect because the insects are led into an additional direction.

Comparing the two cluster morphologies of the species, it becomes obvious that the cluster aggregation as well as the flowering pattern between inflorescences controls the pollen-flow. *Brunia alopecuroides* has very dense, spadix-like cluster units, in *Berzelia lanuginosa* the inflorescences are rather loosely arranged and umbel-like.

A hypothetical exchange of the two flowering patterns but still the original morphology of the species would lead to a disadvantageous pollen-transfer. If *Berzelia lanuginosa* flowered from the lowest-most inflorescences towards the cluster centre or apex (like realised in *Brunia alopecuroides*), the insects would have to fly and crawl through a labyrinth of stalks and inflorescences for a long time to reach the lowest-most flowering inflorescences. In *Brunia alopecuroides*, the spadix-like cluster enables the insects to reach fast and easy the flowering inflorescences in the lower part of the cluster.

In contrast if *Brunia alopecuroides* flowered acropetally on inflorescence level (like in *Berzelia lanuginosa*) the pollen would be exposed very punctually in the dense cluster and the insects would only be attracted by few flowers. A visit of every single level of the spadix-like units would be required to find the few open flowers per single inflorescence. In *Berzelia lanuginosa* where this pattern is realised the acropetal ring-wise flowering pattern does not cause these problems as the single inflorescences are arranged umbel-like and crawling insects do not have problems to find nectar and pollen.

The hypothetical exchange of flowering patterns demonstrated that within the apparently similar acropetal flowering a precise linkage between cluster morphology, insect behaviour and optimal pollen-transfer is realised.

3.4.2 Dichogamy and morphology

In hermaphroditic flowers without sex-separating mechanisms, the stigma can thus be pollinated by self-pollen via insects (facilitated selfing) (Lloyd and Webb 1986; Lloyd 1992) or by pollen of flowers of the same plant (geitonogamy) (Lloyd 1992; de Jong et al. 1993). Since some Bruniaceae tend to be autogamous as they produce seeds without a vector, it appears that the flowering pattern on cluster and inflorescence level is not sufficient to clearly separate the sexual phases. The ring-

wise flowering pattern seems only to reduce the interference between male and female function and incompletely separates receptive and non-receptive flowers. Maybe this is a given possibility for delayed selfing under bad weather circumstance or a lack of pollinators (Tsitrone et al. 2003)

However, the ring-wise flowering pattern of *Berzelia lanuginosa* or the spatial separation of flowers in other species like *Staavia radiata* is a rough separating factor that gets refined via the for the first detected dichogamy in Bruniaceae. Dichogamy as possible temporal separation of male and female function within a hermaphroditic flower in self-compatible systems can be discussed as inbreeding avoidance (Darwin 1876, Husband and Schemske 1996). The two realised dichogamy forms protandry (first being functionally male) and protogyny (first being functionally female) of the flowers are linked with morphology.

Protandrous flowers have anthers that are positioned within the flower (small single flowers, flower type 2, see Chapter 2). Pollen is deposited punctual and more or less precise on the insect body. In protogynous flowers the anthers are exposed and longer than the corolla the pollen gets deposited diffusely on the insects (pincushion-like inflorescences, flower type 1, see Chapter 2). This characteristic is linked with the inflorescence forms and occurred first, when on family level a shift to real inflorescences proceeded (split between the *Staavia*-branch and the *Berzelia*-clade). The anther outsourcing is a result of the narrowing spaces within the inflorescences. Only with the extended anthers a pollen transfer to the own stigma within the small flowers can be prevented and pollen-export can be ensured. Thereby protogyny guarantees a short period of possible outcrossing. Protandry in the pincushion-like species would cause a stigma covered in self-pollen and thereby pollen and seed discounting (Harder and Wilson 1998, Routley et al. 2004). In contrast protogyny in the species with non-extended anthers would lead to a high proportion of selfed seeds, because the receptive stigma would be situated directly in opposite to the released pollen. In this case only protandry leads to possible outcrossed seeds because the pollen of the flower is already released before the own stigma becomes receptive. So the inflorescence morphology and the specific anther position require protandry and protogyny respectively.

The assumption that beside the temporal separation of male and female function within a flower an additional spatial separation, namely herkogamy occurs can only be rejected for the protandrous type 2 flowers, since the anthers are opposite to the stigma. But in the protogynous type1 (pincushion-like inflorescences) as well as in the protandrous type 3 flowers (long-tubed corolla of *Lonchostoma monogynum*) a spatial separation of male and female function enforces the found dichogamy forms. In flowers of the flower type 1, anthers unfold after the stigma is already present for several days. In type three flowers this growth process can be observed for the style

that grows during the anthesis whilst pollen is already present. This indicates that growing processes take place after the female phase (stigma receptive, no pollen present) in protogynous species and the male phase (pollen present, stigma not yet receptive) in protandrous species respectively already proceed.

Interestingly the two dichogamy forms are not only linked with flower morphology but as well with stigma morphology: in protandrous Bruniaceae, papillae grow out of the receptive area, in the protogynous species the stigma surface remains smooth. Different stigma types on species level like in *Linum* L., *Villarsia* VENT. or in some Plumbaginaceae indicate self-incompatibility (Dulberger 1975, Murray 1986, Dulberger and Orndruff 2000). Since the stigma morph is consistent within the observed species, self-incompatibility can not assumed to be linked with morphology. Furthermore the present study illuminated that the Bruniaceae are self-compatible and autogamous. The found low seed set in *Audouinia capitata* was related with the need of a vector to produce seeds and due to a high percentage of pollen degeneration outcrossed pollen seems to be advantageous (Hall 1988; de Lange et al. 1993c; de Lange and Boucher 1993a). The present study supports the stated need of an insect vector since in protandrous species like *Audouinia capitata* and *Staavia radiata* the pollen of a flower is released completely before the stigma papillae are full-grown and the own stigma becomes receptive. This prevents pollen-transfer to the own stigma. Maybe the strict dichogamy includes a lesser acceptance or less pollen-germination ability of flower-own pollen compared to foreign pollen.

3.4.3 Dichogamy and life-form

The finding of both dichogamy forms in the Bruniaceae supports the observed occurrence of a dichogamy variation below family level evolving fast as response to changing ecological conditions by Bertin and Newman (1993). To analyse these ecological need, two sister protogynous species - *Berzelia lanuginosa* and *Berzelia abrotanoides* that exhibit different life forms shall be exemplarily discussed. *Berzelia lanuginosa* is a reseeder whilst *Berzelia abrotanoides* is able to resprout after fire. These life-forms suggest a certain need of reproduction effectivity as resprouters are said to be less independent on seed set as they will survive fire anyway (Carlquist 1978). However the present study showed differences in the breeding system between these sister species that in some points enforce this theory. *Berzelia abrotanoides* as resprouter exhibits a short anthesis duration (two weeks) and flowers synchronously in all parts of the plant. This leads to temporal dioecy on population level. Since pollinators movement is not guaranteed this strategy is highly uncertain in terms of ensured seed set. On the other hand a high percentage of the produced seeds will be

outcrossed because a whole plant serves as pollen donor whilst another is a pollen receptor. *Berzelia abrotanoides* can focus on outcrossing because the genes of the mother plant will most likely survive the next fire.

In contrast to this breeding system, *Berzelia lanuginosa* as a reseeder focuses on seed high set. The anthesis proceeds multicyclic on inflorescence level and lasts for about three weeks. This ensures pollination even under bad weather condition and lacking pollinators. The fact that *Berzelia lanuginosa* is autogamous under vector exclusion prefigures that even selfing is acceptable as long as many seeds ensure an adequate seed bank for the next fire event. This especially holds true as seeds on and in soil seem not to be durable for a long time due to decomposition.

However the reproductive success as well as the different anthesis durations between reseeding and resprouting species included in the present study does not reveal a clear pattern for an assumed linkage between life-form and breeding system. Since also resprouter can have cyclic flowering patterns and reseeder showed low seed set the example of *Berzelia lanuginosa* and *Berzelia abrotanoides* has to be seen an example for separating factors in the breeding system of sister species

3.4.4 Dichogamy and its distribution within the recent phylogeny

The found dichogamy forms below family level linked with inflorescence morphology suggested that protandry and protogyny occurs in different parts of the recent phylogeny by Quint and Claßen-Bockhoff (2006a). Since only members of the Bruniaceae were observed regarding the anthesis, the stigma morphology as consistent trait within genera was mapped on the phylogeny to observe the dichogamy allocation. In the Audouinieae and the Linconieae only the papillae-type is realized, which is linked to protandry whilst in the Brunieae a shift to protogyny occurred. However protandry can be assumed to be the ancestral dichogamy state although it occurs in basal as well as in more derived clades. In fact the dense aggregation of flowers in the pincushion-like species rather than the phylogenetic position seems to be the force that enhanced the development of protogyny. The pressure for anther outsourcing linked with protogyny might also be a hint of a changing pollinator composition in the past. Maybe the extended anthers in the pincushion-like inflorescences gave rise to the tendency to beetle pollination still being detectable today. Since the earliest beetle fossil is dated to 318 to 299 Mya (Béthoux 2009) and in South Africa e.g. the Hopliinae (monkey beetles) are highly radiated (Péringuey 1902, Steiner 1998) a co-evolution on the time scale of the divergence of *Berzelia* 31.7-19 Mya and *Nebelia* and *Brunia* 10.6-6.4 Mya respectively (Quint and Claßen-Bockhoff 2008) is highly speculative but may be possible.

It has still to be clarified in further investigations whether in all Bruniaceae the found papillate and non-papillate stigma morphology is linked to an outscoring enhancing

breeding system with the found dichogamy forms like in the observed species. However the present study revealed evidence for the fact that morphology and breeding system of all observed species contribute to geitonogamy prevention and enhance outcrossing. Beside the diversity in the anthesis and dichogamy forms, these differences that all contribute to the same breeding system strategy don't seem to be of sufficient importance to give reason for the great variety of inflorescences.

4. Phylogeography and genetic diversity within and among populations

Abstract

So far nothing is known about the population structure and possible gene flow between populations of Bruniaceae species along the Western Cape. The aim of the study is to test whether increasing distance implies increasing genetic difference and if intraspecific haplotype distribution differs between and within populations. Gene flow within and among populations of widespread and locally restricted species was observed using chloroplast markers. The study reveals molecular differences among the populations studied and related distances between them. The results showed that there is also little variation within populations. Most species showed a clear pattern that separated geographical isolated populations. The different haplotype proportions within and between populations suggest a genetic stability and homogeneity in the case of similar haplotypes and an assumed ongoing stabilisation or differentiation in heterogeneous populations. The question if isolation by distance or habitat fragmentation is the reason for the genetic constitution of the observed population cannot be clarified in detail.

4.1 Introduction

Population genetic and phylogeography analyses in Africa and particularly in South Africa are focussed on invasive plant species in order to predict possible endangerment for the native flora or on animals (O'Brien et al. 1985; Grant and Bowen 1998; O'Ryan et al. 1998; Christian 2001; Sakai et al. 2001; Whitehouse and Harley 2001; Richardson and van Wilgen 2004; Rambuda and Johnson 2004). But so far little is known about population genetics of endemic plant species. The phylogeography of the Cape clade families was analysed in many studies (Galley and Linder 2005; Linder 2003; Linder and Hardy 2004) but the Bruniaceae as a member of these 'Cape clades' in general are left out of detailed studies. The family has been studied phylogenetically (Quint and Claßen-Bockhoff 2006a), but nothing is known about genetic variation on the population level.

Since the Cape Floristic Region consists of mountain and hill ranges, local communities can be identified as subregions and mountain ranges seem to be a natural barrier for migration events (Latimer et al. 2005). Since the genetic consequences of small populations can be inbreeding depression and genetic bottlenecks (Sobel et al. 2009), habitat fragmentation can have an influence on plant evolution via the forming of metapopulations (Charlesworth and Charlesworth 1987; Ellstrand and Elam 1993; Young et al. 1996; Armbruster and Reed 2005; Lienert 2005; Boakes et al. 2007; Bouzat 2010). Habitat fragmentation disturbs biological and physical ecosystem processes (Saunders et al. 1991; Burgess 1988; Noss and Csuti

1994), influences the morphology of species and is also reflected on gene level (Templeton et al. 1990; Bierregaard et al. 1992; Ledig 1992; Didham et al. 1996). Therefore the distribution pattern of many Bruniaceae species might as well be reflected in the haplotype composition of the observed populations.

To test the impact of habitat structure and pollinator composition on the genetic constitution of the populations, five Bruniaceae species were chosen to be investigated regarding their genetic composition within and among population with special focus on geographic distances. Plastid DNA sequences of rare and widespread species along the Western Cape were analysed. In general chloroplast markers like the *trnT-F*-region or *rbcL* are used to clarify phylogenetic questions and relations between taxa (Müller et al. 2006; Worberg et al. 2007) but variable regions commonly show enough variation to detect intraspecific variation (Soltis et al. 1992; Soltis et al. 1997).

Chloroplast markers were the only applicable markers since the application of the AFLP method failed due to a very high content of un-removable tannins. The high tannin content as well caused difficulties in the phylogenetic ITS-analysis and seems to be a general nuclear-genome relevant problem in the Bruniaceae (Quint pers. comm.). The two markers are frequently used, generate sequences of different length (Sang et al. 1997; Tate 2002; Shaw et al. 2007) and were applied on Asteraceae, a member of the Euasterids II in which the Bruniaceae are discussed to be incorporated as sister to Asterales (Watson et al. 2000; Winkworth et al. 2008). Preliminary tests with ten different chloroplast markers indicated *psbA-trnH* and *ndhF-rpl32* to be adequate to solve our questions.

However, it cannot be precluded that close-by populations are genetically alike since habitat fragmentation and low migration rates typically for the Cape Floristic Region (Latimer et al. 2005) could have separated a former bigger population. Since no far-distance seed dispersal via insects, mammals or birds could be documented (see Chapter 2), the Bruniaceae can be assumed to disperse at the population margins. Calculating the distance of found seeds in the soil around a mother plant the maximum dispersion distance and therefore the radius of population expansion after germination is about two metres (Chapter 2). Thus it could be expected that furthestmost individuals of a population and furthestmost populations respectively show the highest genetic variability because they were and are separated for a long time (isolation by distance, Wright 1943). This would be assumable because the Bruniaceae fit to found in other species of South Africa that take place gradually along corridors and on a long-time scale since the Cape Floristic Region is an extremely migration-limited system pattern (Eeley et al. 1999, Latimer et al. 2005).

Patterns of genetic variation do not only reflect the genetic status quo but can illuminate whether Bruniaceae are still migrating or their distribution area is rather

decreasing and on which time-scale (ecological due to colonization and local extinction or macro-evolutionary) this distribution was and is shaped. (Schurr et al. 2007). Historic and recent events could have been the reasons for the genetic pattern and the distribution today, e.g. habitat fragmentation, isolation by distance or genetic bottlenecks due to extinction (Sobel et al. 2009).

The haplotype diversity between and within populations can give a hint on these influences on the genetic population composition (Templeton et al. 1995; Templeton 1998, 2008). For the calculated haplotype network, a nested clade analysis will be conducted. This method is used in studies that provide geographical data that normally are not included in plain haplotype networks and the statistical parsimony procedure is specifically designed for within-species analysis (Templeton et al. 1992, 1995). Since in South Africa the topography plays an important role that forms corridors along mountain ranges and in valleys or as well separates habitats, the usage of nested clade analysis can give spatial and temporal information about genetically changes between populations.

The data of this study will not only provide an insight in the population genetic constitution of the present populations but can also help to predict highly competitive species and if the endemic species are better adapted to the fynbos biome than invasive species with fast migration rates like *Pinus* L. (Richardson and van Wilgen 2004; Rouget et al. 2004a, 2004b).

4.2 Material and Methods

4.2.1 Plant material

We chose five species of Bruniaceae with different distribution pattern for our study, *Berzelia lanuginosa* and *Staavia radiata* as species with a large distribution area, *Lonchostoma monogynum* as a species with a disjunct area, and *Staavia dodii* and *Audouinia capitata* as restricted species only known from the Cape Peninsula (see Fig. 4.2). The populations are situated along the South Western Cape of South Africa in distances between six and 580 kilometres and were chosen due to accessibility and distribution density along the southern coast (Fig. 4.1 and Tab. 4.1).

All samples were collected in 2006 (collection permit No.17 Western Cape Nature Conservation Board for the NBI herbarium). Twelve populations were chosen: Cape Point (*Staavia dodii*), Karbonkelberg above Hout Bay harbour (*Staavia radiata*, *Audouinia capitata*), Silvermine Nature Reserve (*Berzelia lanuginosa*), Jonkershoek Nature Reserve (*Berzelia lanuginosa*), Kogelberg Nature Reserve (*Lonchostoma monogynum*), Fernkloof Nature Reserve (*Staavia radiata*, *Lonchostoma monogynum*), Vogelgat Private Nature Reserve (*Berzelia lanuginosa*, *Staavia radiata*), and three

localities of *Berzelia lanuginosa* towards the Eastern Cape (Middelarf Farm, Knysna, Storms River Village) (see Fig. 4.1).

To be able to compare sequences of the centre and the margins of a population fifteen samples were taken per population. The samples within a population were taken as far apart as possible and the plants always were separated by rocks, valleys etc. to avoid as much as possible to collect material from the same clone.

Distances between the chosen samples for the genetic analysis ranged from 0.1 m to 400 m (for details see Appendix 2). In *Staavia dodii* and *Audouinia capitata* it was only possible to collect plant material of one population since both species only occur at the Cape Peninsula (see Plate 1.I). The populations of *Audouinia capitata* shown in Fig 4.2 that are situated above Hermanus could not be reconfirmed what was approved by local botanists. Fresh leaves of all collected species were dried in silica gel.

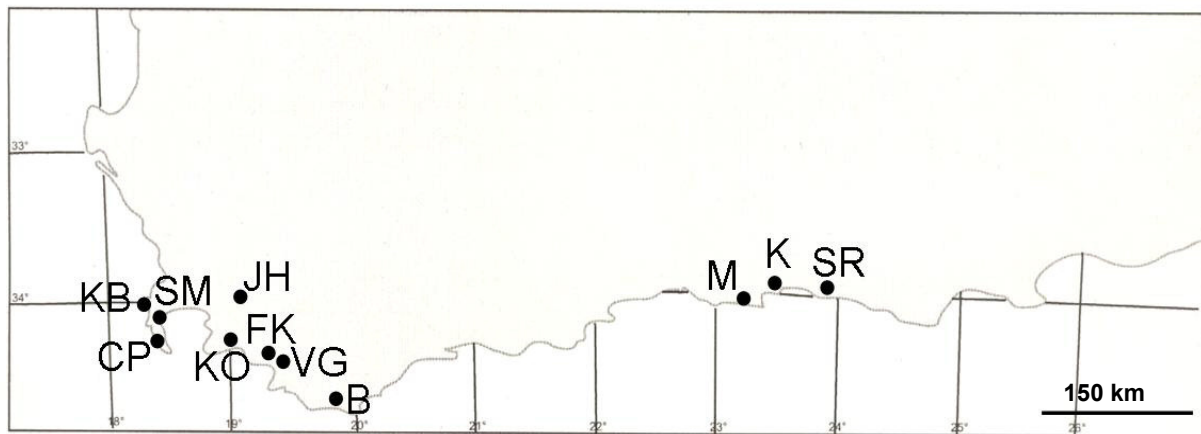


Figure 4.1: Populations included in the analysis: CP = Cape Point/Olifantsbos, KB = Hout Bay/Karbonkelberg, SM = Silvermine NR, JH= Jonkershoek NR, KO = Kogelberg NR, FK = Fernkloof NR, VG = Vogelgat Private NR, B = Bergplaas, M = Middelarf, K = Knysna, SR = Storms River Village

Between the sampled populations, unsampled populations of *Berzelia lanuginosa* and unsampled populations of *Staavia radiata* are situated (see Fig. 4.2 and Tab. 4.1). Since habitat conditions and accessibility of the populations differed, a closer sampling in many cases was impossible. The assumed number of unsampled populations referred to a distribution analysis that was part of the present dissertation and ranged from zero to 15 unsampled populations.

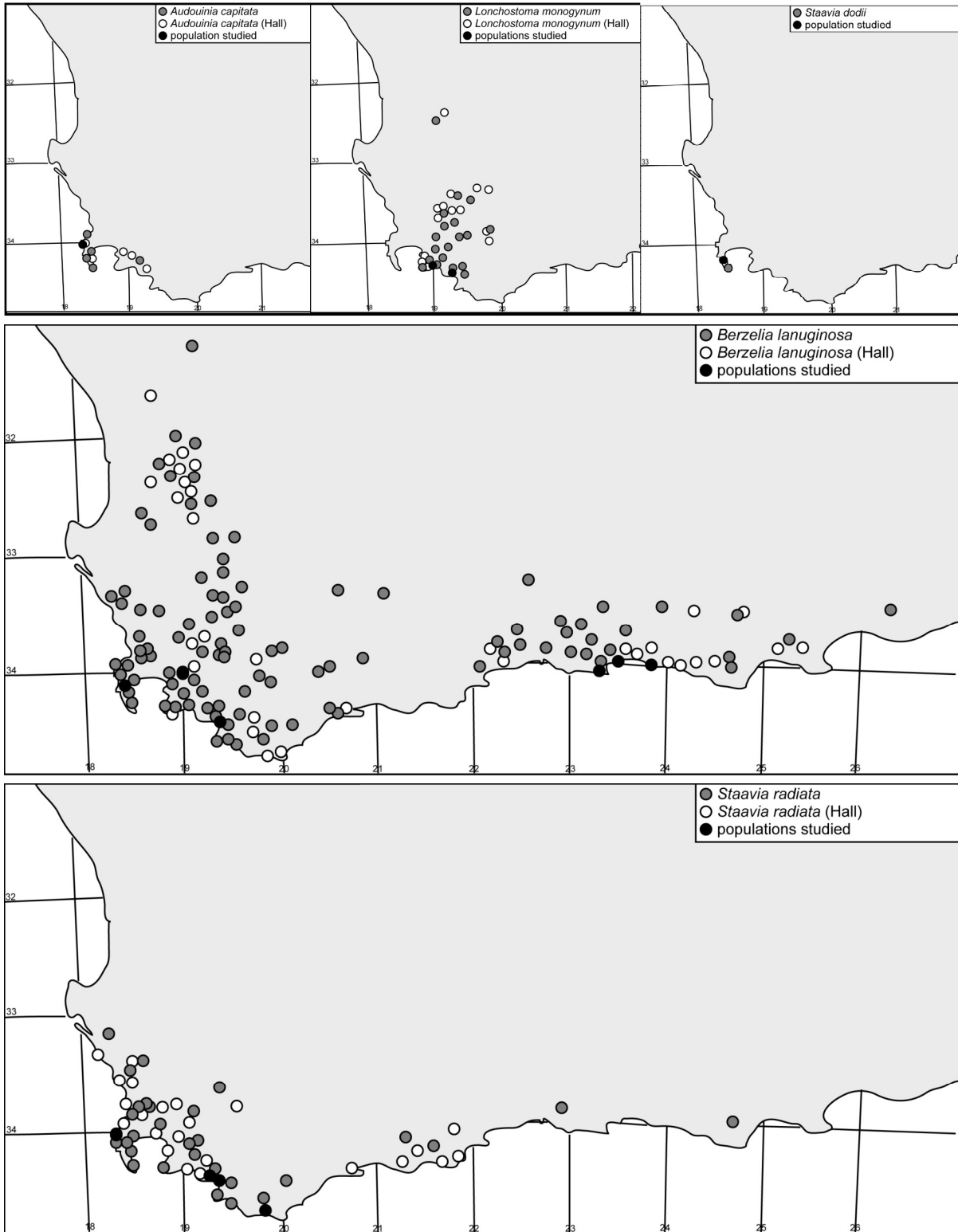


Figure 4.2: Localities of the samples study sites with unsampled populations between them (maps are created using herbarium sheet data label (grey dots) and unpublished localities after A.V. Hall (white dots). Black dots: sampled populations)

Table 4.1: Number of localities and analysed samples per marker and their coordinates. The table contains fourteen numbered localities since the Vogelgat and Karbonkelberg localities were sampled twice for two different species.

species	locality	coordinates	distances between localities	number of unsampled populations between the localities	number of analysed samples	
					<i>psbA-trnH</i>	<i>ndhF-rpl32</i>
<i>Audouinia capitata</i>	Karbonkelberg (13)	N -34.053877° E 18.330588°	-	-	6	-
<i>Berzelia lanuginosa</i>	Silvermine (1)	N -34.093702° E 18.414864°	1-2: 50 1-3: 130 1-4: 1-5: 1-6: 505	1-2: 1	3	2
	Jonkershoek (2)	N -33.992387° E 18.973577°	2-3: 110 2-4: 500 2-5: 530 2-6: 580	2-3: 4	3	2
	Vogelgat (3)	N -34.402192° E 19.318505°	3-4: 460 3-5: 490 3-6: 540	3-4: 15	3	2
	Middelarf (4)	N -34.036645° E 23.153440°	4-5: 35 4-6: 50	4-5: 1	8	2
	Knysna (5)	N -33.936008° E 23.545120°	5-6: 85	5-6: 1	2	2
	Storms River (6)	N -33.969901° E 23.885812			5	2
<i>Lonchostoma monogynum</i>	Kogelberg (7)	N -34.320670° E 18.961959°	7-8: 95	7-8: 5	4	2
	Fernkloof (8)	N -34.378551° E 19.273430°			2	2
<i>Staavia radiata</i>	Karbonkelberg (9)	N -34.051769° E 18.330331°	9-10: 160 9-11: 135 9-12: 140	9-10: 13	3	2
	Bergplaas (10)	N -34.730855° E 19.869506°	10-11: 70 10-12: 60	10-11: 4	4	3
	Hermanus (11)	N -34.400396° E 19.255463°	11-12: 6	11-12: 0	3	2
	Vogelgat (12)	N -34.403363° E 19.318782°			3	2
<i>Staavia dodii</i>	Cape Point/ Olifantsbos (14)	N -34.259865° E 18.387625°	-	-	7	4

4.2.2 Molecular methods

After preliminary tests that showed no high genetic differences within populations, we decided to reduce the number of samples per populations to a minimum of three for *psbA-trnH* and two samples per populations for *ndhF-rpl32*. In the case of lower sequences numbers, the PCR failed. If more than the three or two samples were analysed, first analysis of the sequences indicated a comparably higher variability between sequences of the same population.

Using about 10 of the silica gel dried acicular leaves, total DNA was isolated and cleaned with the DNeasy extraction kit (Qiagen, Hilden, Germany) after grinding the silica dried plant material in 2 µl tubes with 2 steel balls in a grinding mill (Retsch, Haan, Germany) for 90 seconds with three times *g*.

Two region of cpDNA were sequenced, namely the intergenic spacers *psbA-trnH* and *ndhF-rpl32* (see below). A total of 56 sequences of *psbA-trnH* and 31 sequences of *ndhF-rpl32* were generated (see Tab. 4.1)

Primers used for PCR amplification and sequencing:

<i>ndhF</i> 5'-GAA AGG TAT KAT CCA YGM ATA TT-3'	(Shaw et al. 2007)
<i>rpl32</i> -R 5'-CCA ATA TCC CTT YYT TTT CCA A-3'	(Shaw et al. 2007)
<i>psbA</i> 5'- GTT ATG CAT GAA CGT AAT GCT C-3'	(Sang et al. 1997)
<i>trnH</i> 5-R'-CGC GCA TGG TGG ATT CAC AAT CC-3'	(Tate 2002)

All amplifications were performed in 25-µL volumes containing 16.45 µL dionized sterile water, 0.6 µL of 50 mmol/L MgCl₂ solution, 2.5 µL 10x NEB buffer, 0.25 µL of a 20 mmol/L dNTP solution in equimolar ratio, 1.0 µL of each primer at 100 pmol/µL, 1 unit (0.2 µL) Taq DNA polymerase (New England Biolabs GmbH., Frankfurt, Germany), 2.0µL BSA and 1 µL genomic DNA (5-100 ng). Double-stranded DNA templates were produced in an TProfessional thermal cycler (Biometra GmbH, Göttingen, Germany) set for an initial 1 min at 94°C, followed by 35 cycles of 94°C for 0.3 min, 52°C for 0.5 min, and 72°C for 1 min, with three final incubations of 0.3 min at 94°C, 1.3 min at 52°C and a final step of 8 min at 72°C. The PCR products were cleaned with QIAquick (QIAGEN GmbH, Hilden, Germany) purification kits.

Sequencing reactions were performed with BigDye™ terminator cycle sequencing kit (Applied Biosystems, Norwalk, Connecticut, USA). Primers were the same as those of the initial PCR and used separately in forward and reverse reactions. The thermocycling profile consisted of 30 cycles of 10 s at 96°C and 4 min at 55°C.

The products were cleaned using Sephadex and analysed on a 3130 xl Genetic Analyzer (Applied Biosystems, (Hitachi), Carlsbad, California, USA).

4.2.3 Data analysis

Sequences of forward and reverse strands were automatically aligned using SEQUENCHER (version 3.0, Gene Codes Corporation, Ann Arbor, Michigan, USA) and MacCLADE (Maddison and Maddison 1992) with manual correction.

No variation was detected in the single populations of *Staavia dodii* and *Audouinia capitata*. This leads to a remaining data set of three species in *psbA-trnH* (*Berzelia lanuginosa*, *Lonchostoma monogynum*, *Staavia radiata*) and two data sets in *ndhF-rpl32* (*Berzelia lanuginosa*, *Staavia radiata*) that was used for the haplotype network analysis. Indels were coded by hand in PAUP4.0b10 (Swofford 2002) using lower cases as definition for the indel position. An indel coding method after Simmonds and Ochoterna (2000) was not useful for the later haplotype network calculation.

To test the association between genealogy and geography a nested clade analysis was conducted.

Haplotype networks, including the coordinates of the localities and permutation tests at the different nesting levels was calculated with the software GeoDis using the programme ANeCA (Panchal et al. 2007, Posada et al. 2006, Clement et al. 2000

(TCS), Posada et al. 2000 (GeoDis)) freely available at:

<http://www.rubic.rdg.ac.uk/~mahesh/software.html>.

To run the program, text-files containing the sequences and the geographic information had to be created. For the haplotype network calculation poly-nucleotide sites were reduced to one gap or in the second calculation completely eliminated. In the case of the 5th base calculation insertions or other indels also were treated as one gap.

For the calculated nested ANOVA the null hypothesis assumes that two populations have the same variance. If the null hypothesis holds true, there is no genetic difference between locally distinct populations.

A permutation test is a non-parametric test that verifies random sampling by exchanging the elements of a re-sampled group. The permutational contingency test was applied to verify randomly defined nesting levels and was based on 10000 re-samples.

Nesting levels define hierarchical relations between sequences at different scales. If a low-level nesting and a high-level nesting are outweighed by a superior level, all three levels are combined to one segment of genetic differences and thus define the hierarchy of genetic differences between populations.

The inference key determines the validity of a given process explaining the congruence between geographic distance and genetic relatedness (Templeton et al. 1995) and was run to detect differences with increasing distance. The program then computed if the genetic relatedness between populations is due to historic or present events.

4.3 Results

4.3.1 Sequence analysis

Aligned sequences of the *psbA-trnH* intergenic spacer varied from 428 bp in *Staavia radiata*, 440 bp in *Lonchostoma monogynum* to 464 bp in *Berzelia lanuginosa* (see Tab. 4.2). The *ndhF-rpl32* intergenic spacer varied from 1062 bp in *Staavia radiata* to 1113 bp in *Berzelia lanuginosa*. For detailed sequence data see Appendix 2.

Parsimony informative as well as autapomorphic sites will also be stated since both were included in the haplotype network analysis.

For both markers in *Berzelia lanuginosa* a total of 35 parsimony-informative characters including informative poly-base stretches and eleven uninformative characters/autapomorphic sites were detected. In *Staavia radiata* 27 parsimony informative and three parsimony uninformative sites were found. For *Lonchostoma monogynum* 15 variable sites and seven autapomorphic characters were detected.

Both markers showed an equal number of parsimony-informative characters in *Berzelia lanuginosa* but in *Staavia radiata* only a third of non-indel informative characters could be found. This is balanced by the high number of informative indels. Because of the lack of an assured outgroup, the informative character of indels cannot be distinguished as insertions or deletions. The autapomorphic characters are base-exchanges e.g. A→T or G→T.

Table 4.2: Results of the sequence analysis with number of informative and uninformative characters

species	marker	total number of sequences included in the analysis	length (base pairs)	number of parsimony informative characters excluding indels	number of informative indels	number of parsimony uninformative characters excluding indels	number of un-informative indels
<i>Berzelia lanuginosa</i>	<i>psbA-trnH</i>	24	464	15	1	2	3
<i>Berzelia lanuginosa</i>	<i>ndhF-rpl32</i>	12	1113	18	1	4	2
<i>Lonchostoma monogynum</i>	<i>psbA-trnH</i>	6	440	14	1	3	4
<i>Staavia radiata</i>	<i>psbA-trnH</i>	13	428	15	3	1	1
<i>Staavia radiata</i>	<i>ndhF-rpl32</i>	9	1062	5	4	0	1

4.3.2 Haplotype network analysis

Nested ANOVA

The maximum nesting levels of the haplotype networks varied between level three and level five. The observed X^2 was bigger than the random X^2 in all permutation analysis of the whole cladograms which verified that the samples were randomly taken (see Tab. 4.3). For the detailed nesting levels, see Appendix 2.

Table 4.3: Permutation analysis of the total cladograms for both calculations

species	marker	observed X^2	probability of a random X^2
<i>Berzelia lanuginosa</i>	<i>psbA-trnH</i>	3.2727	0.8981
<i>Berzelia lanuginosa</i>	<i>ndhF-rpl32</i>	12.0	0.0214
<i>Lonchostoma monogynum</i>	<i>psbA-trnH</i>	6.0	0.0662
<i>Staavia radiata</i>	<i>psbA-trnH</i>	7.6349	0.0617
<i>Staavia radiata</i>	<i>ndhF-rpl32</i>	11.7	0.0626

Berzelia lanuginosa psbA-trnH

The sequences built a total of four nesting levels. Nesting level two formed one distinct group of Knysna and Middelerf in opposite to a group of the residual Knysna and Middelerf sequences including the Storms River and Vogelgat and Silvermine sequences. A third group consists of Jonkershoek and Silvermine. Within the second group, Jonkershoek and Silvermine cluster together in a level two nesting. From nesting level four onward, a separation of Knysna and Middelerf and all other populations takes place. The remaining Knysna, Middelerf, Storms River and Vogelgat sequences form an opposing group.

Lonchostoma monogynum psbA-trnH

Within a total of four nesting clade levels, the two *Lonchostoma monogynum* populations Fernkloof and Kogelberg populations are separated on nesting level three. This holds true for both calculation types.

In lower hierarchical levels, the sequences of the populations occur separately.

Staavia radiata psbA-trnH

In the four nesting levels of *Staavia radiata* the separation of the Karbonkelberg and the Hermanus/Vogelgat sequences from the Bergplaas and the residual Hermanus/Vogelgat sequences occurs on nesting level three. The similarity of the Vogelgat and the Hermanus sequences in one and two sequences respectively that split the populations was confirmed by a second amplification and sequencing and resulted in the same split.

Berzelia lanuginosa ndhF-rpl32

Five nesting levels could be found that separate the Western Cape populations from the Eastern Cape populations. Level five consists of three relevant level four nestings. One level four cluster contains Jonkershoek and Silvermine, the second one comprises the Vogelgat sequences and the third one contains Middelerf, Storms River and Knysna.

Within the Knysna/StormsRiver/Middelerf nesting, a separation between Knysna and Storms River and Knysna and Middelerf on nesting level two can be stated.

Staavia radiata ndhF-rpl32

For the *ndhF-rpl32* sequences of *Staavia radiata* a total of three nesting levels could be found.

On level two there is a split in the Karbonkelberg group, a second Hermanus/Vogelgat group and a third Bergplaas/Hermanus/Vogelgat group. Within the Bergplaas/Hermanus/Vogelgat group, the Bergplaas sequences are separated from the Vogelgat/Hermanus sequences on lower hierarchical level.

Inference key results

The results from the inference key for the total cladogram indicate differences between the species as well as within species regarding for both applied markers (see Tab. 4.4).

For *Lonchostoma monogynum* in both markers no significant variances of the populations could be found in either marker.

In *Staavia radiata* the *psbA-trnH* analysis indicated no variance between populations whereas for *ndhF-rpl32* the outcome suggested restricted gene flow with isolation by distance.

In the case of *Berzelia lanuginosa* the results of the markers state no variance between populations in the *psbA-trnH* analysis and allopatric fragmentation for *ndhF-rpl32*.

Table 4.4: Inference key results of the total cladogram of all species and both markers

species	total cladogram	inference key conclusion
<i>Berzelia lanuginosa</i>	<i>psbA-trnH</i>	null hypothesis cannot be rejected/ the populations have the same variance
<i>Berzelia lanuginosa</i>	<i>ndhF-rpl32</i>	allopatric fragmentation
<i>Lonchostoma monogynum</i>	<i>psbA-trnH</i> <i>ndhF-rpl32</i>	null hypothesis cannot be rejected/ the populations have the same variance
<i>Staavia radiata</i>	<i>psbA-trnH</i>	null hypothesis cannot be rejected/ the populations have the same variance
<i>Staavia radiata</i>	<i>ndhF-rpl32</i>	restricted gene flow with isolation by distance

Calculated haplotype networks

Berzelia lanuginosa

In *Berzelia lanuginosa psbA-trnH* six and seven haplotypes respectively were found the latter one with poly base stretches being weighed. For the *ndhF-rpl32* sequences, nine haplotypes could be detected. The proportions of the different haplotypes within the populations differ from 16% to 100% (see Tab. 4.5 and Fig.4.3). The *psbA-trnH* sequences show the westernmost populations Silvermine, Jonkershoek and Vogelgat to have one haplotype per population. The haplotype of the Vogelgat populations is similar to the easternmost haplotype of the Storms River population. The populations Middelerf and Knysna consist of three and two haplotypes in the calculations without the poly base stretches and four and three haplotypes with the poly base stretches being weighed.

The *ndhF-rpl32* sequences show that the Silvermine and Vogelgat and the Middelerf and Knysna populations consist of two haplotypes each. Jonkershoek and Storms River consist of one haplotype each (see Tab. 4.5 and Fig. 4.3).

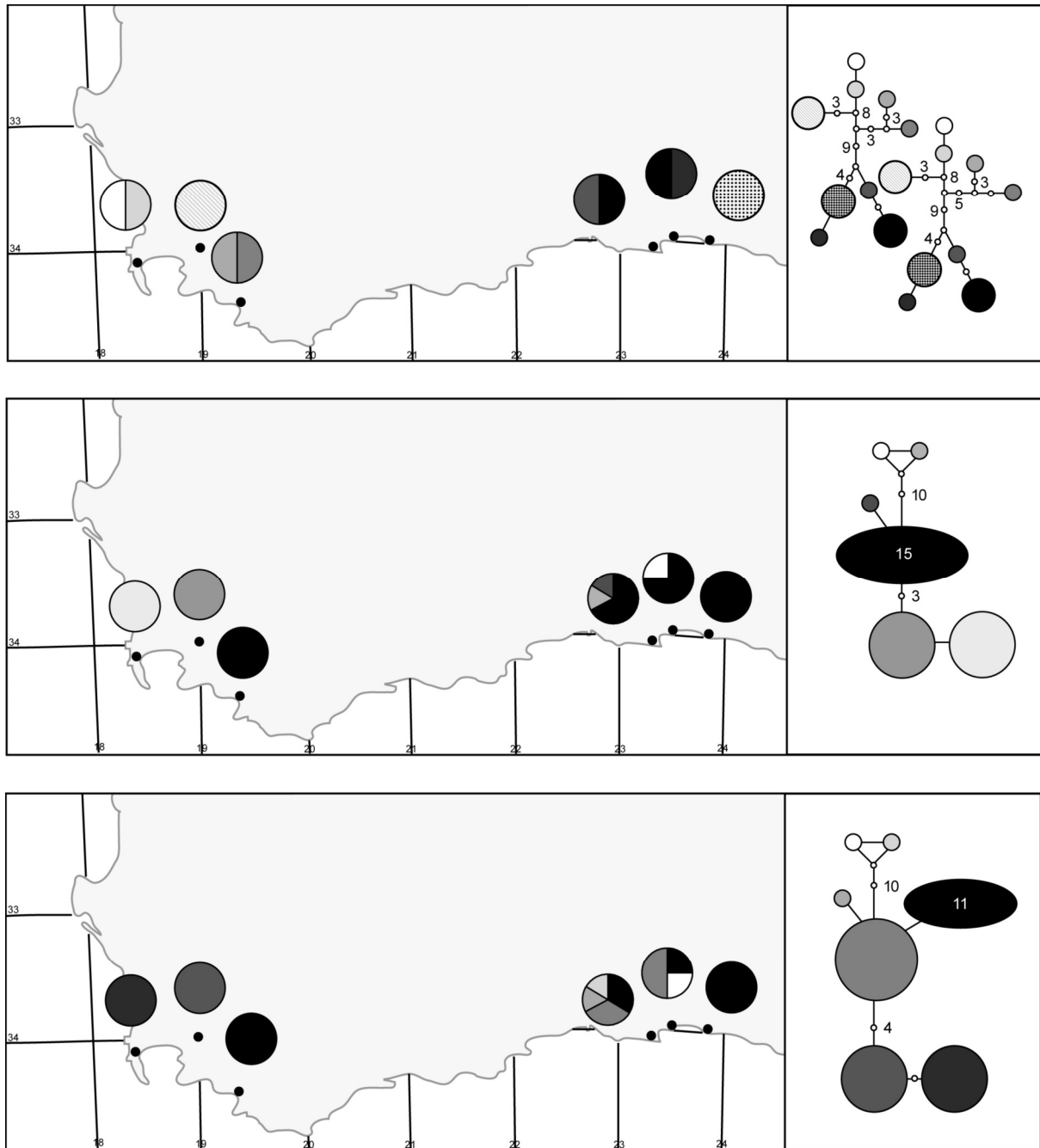


Figure 4.3: Haplotype distribution within and among populations of *Berzelia lanuginosa*; marker: first map: *ndhF-rpl32*, second map: *psbA-trnH* without poly base stretches included in the calculation and third map *psbA-trnH* with poly base stretches included in the calculation and third map

Staavia radiata

In *Staavia radiata* *psbA-trnH* seven and eight (with polybase stretches weighed) haplotypes respectively were found. For the *ndhF-rpl32* sequences, six haplotypes could be detected. The proportions of the different haplotypes within the populations differ from 25% to 100% (see Tab. 4.5 and Fig. 4.4). In both *psbA-trnH* calculations the Karbonkelberg populations consist of one haplotype, Hermanus has three and Vogelgat has two haplotypes within the population. The Bergplaas population have three, in the case of the polybase stretches included in the calculations four haplotypes.

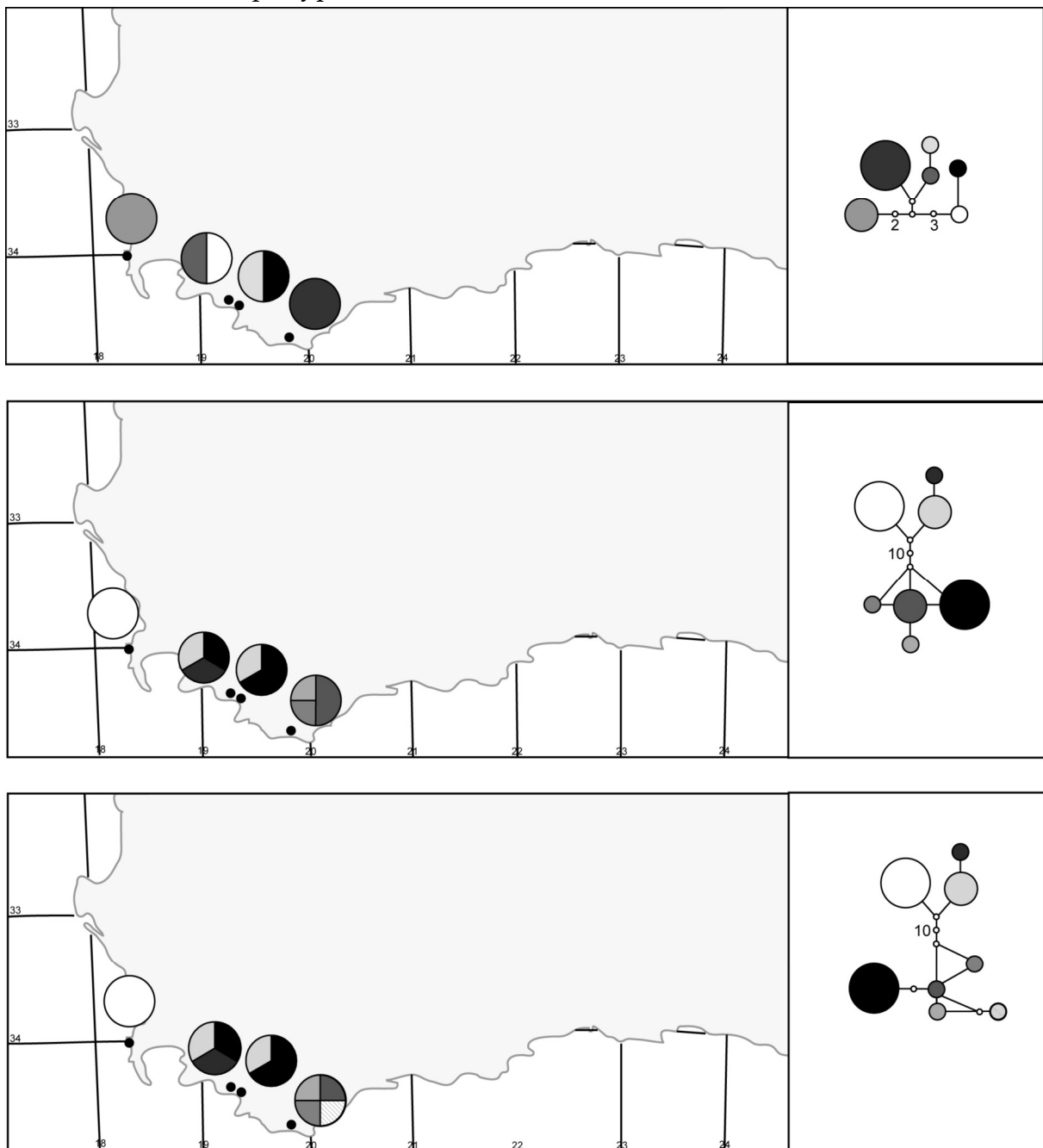


Figure 4.4: Haplotype distribution within and among populations of *Staavia radiata*; marker: first map: *ndhF-rpl32*, second map: *psbA-trnH* without poly base stretches included in the calculation and third map *psbA-trnH* with poly base stretches included in the calculation.

Lonchostoma monogynum

In *Lonchostoma monogynum* *psbA-trnH* in both calculations four haplotypes were found. Both populations consist of two haplotypes each (see Tab.4.5 and Fig.4.5).

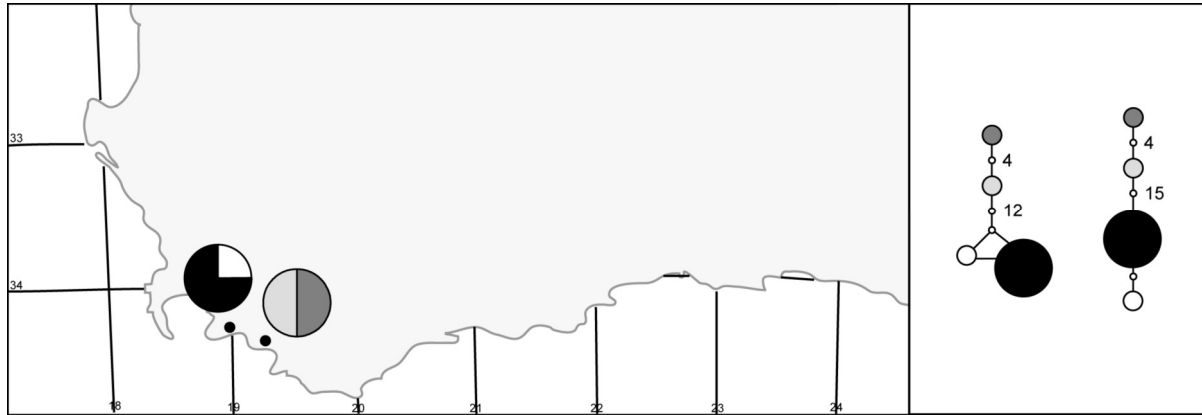


Figure 4.5: Haplotype distribution within and among populations of *Lonchostoma monogynum*; marker: *psbA-trnH*

Table 4.5: Haplotype proportions within populations.*=calculation with polybase stretches weighed as differences.

species	<i>psbA- trnH</i>	<i>ndhF-rpl32</i>	locality	<i>psbA- trnH</i>	<i>ndhF-rpl32</i>
	number of haplotypes			number and haplotype proportion [%]	
<i>Berzelia lanuginosa</i>	6	9	Silvermine	1 (100)	2 (50/50)
	7 *			1 (100)*	2 (50/50)*
<i>Lonchostoma monogynum</i>			Jonkershoek	1 (100)	1 (100)
			Vogelgat	1 (100)	2 (50/50)
			Middelarf	3 (33/33/33)	2 (50/50)
			Knysna	2 (75/25)	2 (50/50)
			Storms River	3 (50/25/25)*	2 (50/50)*
<i>Lonchostoma monogynum</i>	4	--	Kogelberg	2 (75/25)	--
	4*		2 (75/25)*	--	
<i>Staavia radiata</i>	7	6	Fernkloof	2 (50/50)	--
	8*		Karbonkelberg	1 (100)	1 (100)
<i>Lonchostoma monogynum</i>			Bergplaas	1 (100)*	1 (100)*
			Hermanus	3 (50/25/25)	2 (50/50)
			Vogelgat	3 (25/25/25/25)*	2 (50/50)*
				3 (33/33/33)	2 (50/50)
<i>Lonchostoma monogynum</i>				3 (33/33/33)*	2 (50/50)*
				2 (66/33)	1 (100)
				2 (66/33)*	1 (100)*

4.4 Discussion:

4.4.1 Genetic differences within and between populations

The results of the chloroplast sequence analysis of both markers demonstrated that generally genetic differences between populations increase with increasing distance. The choice of two plastid markers with different length that exhibit faster evolutionary development than *matK* (Shaw et al. 2007) used by Quint and Claßen-Bockhoff (2006a) for the phylogenetic study detected different haplotypes between and within populations. Comparing the resolution on population level, *psbA-trnH* seems to be more sensitive as similar data resulted in less haplotypes found using *ndhF-rpl32*.

The question arises whether these differences are the result of a historical event like habitat fragmentation or recent gene-flow between populations.

Since the sampling took place at populations of distribution hot spots of the species and the samples were only taken from localities along an east-west gradient, the present data have to be interpreted incorporating the non-sampled populations. However, the results showed that the number of haplotypes of a population changed referring to its geographic position.

Homogeneity within populations

In both markers the westernmost and easternmost populations consist of one haplotype whilst the populations in between contain several haplotypes. This pattern could be due to a long-term isolation of the western populations since the city of Cape Town restricts gene flow towards the East. Gradual isolation of populations in the western part of the Cape was also found for *Virgilia* L'HER. (Fabaceae, Podalyrieae), small trees endemic to the western and southern coastal regions of the Cape (van der Bank et al. 1996). For *Virgilia* climatic changes and niche-inhabitation led to this decrease of population connection but since the isolation is considered to have proceeded recently the populations still exhibit a low degree of genetic differentiation (van der Bank et al. 1996).

Linking this information with the found pollinators of the species, that do not exhibit far-distance pollen transfer capacities, the question if isolation or stability is the reason for the found genetic pattern remains unanswered. It cannot clearly be distinguished if a uniform haplotype pattern within populations is the result of a genetic constancy due to good genetic exchange between its plants or a high conservative gene pool that did not change for a long time. A population with a constant gene pool would consist of clones and a total lack of mutation or genetic exchange had to be stated what does not seem reasonable.

At this central point, the vegetation form of the observed species (reseeder vs. resprouter) plays an important role. Since resprouting plants regrow from the old mother root stock without changing the genetic constitution they could be very stable in their genetic constitution and non-dependant on sexual reproduction (Carlquist 1978). In contrast reseeding plants possess higher genetic variability since seeds compete via germination rate and the seedling establishment and diverse haplotypes have the ability to establish for which reason reseeder are said to speciate more rapidly than resprouter (Wells 1969).

The present study does not reveal any support for these hypotheses. *Staavia dodii* that exclusively grows in the Cape Point Nature Reserve does not exhibit any genetic differences in both observed markers and therefore is genetic uniform. Since *Staavia dodii* is the sister species to *Staavia radiata* the result cannot be due to an inappropriate chosen marker. Rather in this reseeding species the genetic constitution indicate a population of clones. Furthermore the resprouting *Staavia radiata* related to the number of populations observed exhibits high haplotype variation. The findings are supported by the fact that in South African as well as in Australian woody plants and Proteaceae no strong differences between speciation rates of reseeder and resprouter could be found since both life forms show more or less equal high diversification rates (Bell 2001; Bond and Midgley 2003; Chew and Bonser 2009).

This indicates that the life-form does not indicate haplotype diversity.

Haplotype heterogeneity

Beside homogeneous populations the heterogeneous populations are of special interest. Heterogeneous populations could be found between genetic uniform populations. This indicates that the more central localities underwent genetic changes. The low migration rates found in the Cape Floristic Region were interpreted as sufficient isolation to allow ecological drift and to cause divergence among communities since only few individuals per generation will be exchanged (Boucher 1978). But the question arises why only the sampled central populations exhibit genetic diversity. According to the present study three scenarios can be assumed for genetic exchange between populations: a) genetic exchange occurs between closeby populations but no genetic exchange is possible at a distance of more than ten kilometres or separating topology, b) the composed populations could be a result of migration events from the west to the east regarding the westernmost populations as ancestral populations and c) the composed populations are the result of two migrations: one from the west to the east and another from the east to the west.

Genetic exchange in the Western Cape of South Africa is extremely hindered via topology and the lowest migration rates found for Proteaceae illuminated that isolation over short distances (1-100 km) played a role in generating as well as

structuring the species diversity of the Cape Floristic Region (Latimer et al. 2005). In the Bruniaceae all distinct populations are separated by mountain ranges, deep valleys or in the case of the Cape Peninsula localities by cities – in this case Cape Town. Under close conditions like Vogelgat and Hermanus with a distance of only eight kilometres, recent genetic exchange between closeby populations can be the assumed reason for the sharing of haplotypes. For this locality habitat fragmentation via human impact as a historic reason for the population split can be assumed as the town Hermanus is built between both populations. This finding is supported by the fact that sequences are shared leading to a split that build two heterogeneous groups including both Vogelgat and Hermanus sequences.

In *Lonchostoma monogynum* (*psbA-trnH*) the same pattern of different haplotypes between comparably close populations could be found. As no haplotypes are shared between the Kogelberg and Fernkloof populations, the two populations seem to be separated for a long time. The population itself are heterogeneous what could be due to the habitat structure of *Lonchostoma monogynum* that inhabits insular like patches within the fynbos slopes what can lead to isolation by other vegetation and thereby to genetic isolation even of close plants.

4.4.2 Genetic constitution and possible migration events

The question of migration events – recent or in the past – can only be discussed for *Berzelia lanuginosa* since the two observed populations in *Lonchostoma* do not enable to predict any changes in distribution and in *Staavia radiata* the haplotype pattern within and between populations does not enable to predict any migration directions. In contrast in *Berzelia lanuginosa* the haplotype pattern in the *psbA-trnH* sequences requires a more detailed discussion. The Vogelgat population shares its haplotype with the haplotype of Storms River that is situated five hundred kilometres towards the east and the haplotype is represented with a 100% in both populations. The two populations in between also own this haplotype but also contain one to three other haplotypes. As well 17 unsampled populations are situated in between the Vogelgat and the Storms River population. The haplotype pattern could be due to a migration from the west to the east with the Vogelgat haplotype being the only one that has reached the eastern part of the Cape. An eastward migration can be assumed as the distribution hot spot of the Bruniaceae is situated in the south western region of the Cape (see Chapter 6). Towards the east, less competition with Bruniaceae species as well as with species of other plants families due to less species diversity (Linder 2003) can be assumed. This probably leads to a higher number of available niches than in the Western Cape for which reason an eastward migration seems reasonable. Due to the fact that many populations between the Vogelgat and the Storms River population have not been sampled, no safe assumption can be made about the

haplotype origin or migration direction. If intermediate population were similar to the Vogelgat/Storms River haplotype, the question would arise why populations between a distance of several hundred kilometres are genetic consistent whilst the surrounding populations differ. That would imply that the area between Vogelgat and Storms River is the ancestral distribution and the marginal populations underwent or still undergo speciation events.

However if the intermediate populations were genetic heterogeneous, migration from the north towards the coast has to be assumed which could have changed the genetic composition of the coastal populations.

The scenario of an assumed bi-directional migration from the west to the east and another from the east to the west is possible when a former bigger distribution area was split by e.g. habitat fragmentation or genetic bottleneck via extinction events. If so, the Vogelgat and Storms River populations contained the remaining ancient haplotype and the intermediate unsampled populations could be the result of bi-directional or even tri-directional (with migrations from the north) migration events comprising new haplotypes.

Migration events coming from the north are supported by Knysna and Middelerf that share 50% of their haplotypes in contrast to the surrounding populations. There might be an ongoing genetic exchange between Knysna and Middelerf what is very interesting since the Middelerf population comprise polyploid plants (Greilhuber pers. comm.) that still seem to be able to mate with the Knysna population that does not exhibit polyploidy.

The question whether the distribution pattern present today is the result of an expansion or retraction of the species cannot be answered completely as no long time observation analysed possible extinction events due to habitat structures and no area-wide genetic analysis was conducted.

But the habitat fragmentation of the Bruniaceae – natural and due to human impact – is obvious (see Chapter 6). Fragmented populations, badly connected through dispersal can have higher rates of local population extinction than colonisation - a non-equilibrium thereby forming metapopulations (Harrison 1991; Lienert 2005). It is still unclear how fast the Bruniaceae do colonize, if they colonize at all or if extinction and colonisation are in equilibrium. But supported by the results of the present study it can be assumed that species like *Berzelia lanuginosa* do colonize or at least keep their distribution area whilst species like *Lonchostoma monogynum* can have a higher extinction than expansion rate even though the observed *Diptera* could indicate a further genetic dispersal than e.g. the more sessile pollinators of *Berzelia lanuginosa*.

The differences between *Staavia radiata* and *Berzelia lanuginosa* as two wide-spread species indicate that the habitat fragmentation in both species could have taken place

on a different time scale: it can be assumed that the *Staavia radiata* habitat fragmentation took place earlier and the isolated populations evolved over a longer period of time, building distinct haplotypes. According to this *Berzelia lanuginosa* would have fragmented later, showing a higher similarity due to lesser time to generate different haplotypes.

Since the time-scale for haplotype evolution is unknown, it seems reasonable that other population genetic components rather than time are the reason for the haplotype constitution of both species.

However, the question if habitat fragmentation or restricted gene flow/isolation by distance led to the genetic constitution of the populations observed cannot be answered completely.

Comprising the haplotype network analysis with the pollinator data and the ecology and topology of the South African fynbos, it can be assumed that a combination of historical and recent events, namely habitat fragmentation, restricted gene flow, genetic bottleneck and migration events together led to the present haplotype pattern.

5. Ecological investigations on a small-scale level - the Bruniaceae of the Vogelgat Nature Reserve

Abstract

The present study will redefine the overall finding that Bruniaceae mainly occur on soils consisting of Table Mountain Sandstone (TMS). It shall be illuminated that aside the general tendency of the species to occur on sandstone, micro-habitats are inhabited that explain the pattern, composition and density of the plant distribution within a narrow area. Despite this refinement of soil preferences of the species, an overall vegetation zone analysis conducted at the Vogelgat Nature Reserve will allow to reconfirm population composition and vegetation zone establishment, 15 years after the first data acquisition by de Lange (1992). Eleven Bruniaceae species will be included in the analysis and disturbance factors like fire and the response of the populations will be analysed to get in insight if the plants are sensitive against these disturbances or not. The analysis illuminated that species dominance in vegetation zones changes due to soil conditions, altitude range and water supply. Fire was found to be needed for the existence of some populations and does not change the population or species composition within the Reserve. No significant shifts, diminishments or extensions of the population could be stated after 15 years.

5.1 Introduction

The Bruniaceae as a (sub-) endemic element of the fynbos biome occur throughout the Western Cape (Pillans 1947, A.V. Hall unpublished data) and show a very high species density in the Overberg region (see Chapter 6). The Bruniaceae mainly seem to be adapted to Table Mountain Sandstone (TMS) and a high annual precipitation (Mucina and Rutherford 2006). The present study shall illuminate if these overall vegetation zones have significant micro-habitat structures e.g. very stony or in contrast humous soil conditions that could allow to subdivide the vegetation zone type 'Sandstone Fynbos' in a more complex habitat. This could explain why very different Bruniaceae genera and species occur in the same area. It can be assumed that they inhabit distinct ecological niches that cannot be explained by the large-scale definition of vegetation zones. Therefore at the Vogelgat Nature Reserve that is located in the Overberg region a detailed habitat analysis will be conducted (pH-value of the soil, its carbon and nitrogen, its texture and density will be conducted) to predict low-range adaptations. At the Reserve the main soil type is derived from quartzitic sandstone but deep black coloured organic horizons develop, shale bands of finer textured and yellow and reddish coloured soils with high clay content can be found (Whittle-Herbert 1990). These soil differences within the Reserve will be analysed regarding the occurrence of Bruniaceae species.

The Vogelgat Private Nature Reserve was founded in 1971 and since then was constantly of interest for local botanists and zoologists that analysed the flora and fauna of the Reserve (Abraham 1988; De Lange 1992; Louw 1996; Anderson et al. 2003; Schönerberger and Conti 2003; Houniet et al. 2009; Measey et al. 2009).

The vegetation survey by de Lange (1992) defined nine Mesic Mountain Fynbos zones. Within five of these zones, *Bruniaceae* dominate the vegetation or at least occur as type species. All five vegetation zones are situated in the eastern part of the Reserve that was the observation site of the present study. A sixth vegetation zone with *Berzelia incurva* as type species was not included in the analysis as the zone can only be found in the western part of the Reserve.

The vegetation zones defined by de Lange (1992) will now be shortly introduced to get an overview of the topological conditions and the vegetative composition. Vegetation zone names refer to the character species that hold the biggest proportion of the plant strata. The definition of the plant stratum height (tall: > 2m; mid-high 1 m - 2 m; low 0.25 m - 1 m) and the projective canopy cover of dominant stratum (closed 74% - 100%; mid-dense 50% -75%; open 25% - 50%; sparse 5% -2 5%) by de Lange (1992) were adopted for description purposes.

1) In the proposed *Phaenocoma prolifera-Chondropetalum hookerianum* zone, an open low restioid veld, the plant community is said to be small-localised and not to have suitable conditions for more habitat sensitive plants. It is reckoned to occur on any aspect, within the mid-altitudinal range of between 300 m and 500 m and slopes are classified as steep and well drained. *Nebelia paleacea* is said to be among the type species.

2) In the mid-dense and mid-high shrubland defined as *Brunia alopecuroides–Chondropetalum deustum* vegetation type, the area is open and dominated by *Brunia alopecuroides* and *Leucodendron xanthoconus* (KUNTZE) K.SCHUM. (Proteaceae) with *Berzelia squarrosa* as a type species. After de Lange 1992 this community occurs on the limited east-north-east aspects of the reserve at altitudes of between 500 m and 600 m above sea level, with the slope varying from gentle to moderately steep with well drained soils consisting of coarse sand with numerous stones and pebbles.

3) The *Osmitopsis asteriscoides-Erica perspicua* zone after de Lange occurs in the northern part of the Reserve and is a sparse to mid-dense, mid-high to tall shrubland. Higher areas are said to subject to mist rain, soils are defined as deep, permanently wet but without stagnant water, dark brown to black, and humus rich. *Brunia alopecuroides* and *Brunia albiflora* are reckoned as dominant species.

4) In the *Erica onosmiflora-Brunia alopecuroides* zone, a restioid veld, the vegetation is said to be a mid-dense, low to mid-high ericoid and is defined to occur in the north-eastern part of the Reserve in upper altitudes (520 m to 700 m above sea level) on

moderate to steep slopes. The aspect is reckoned predominantly south-west, but varies from south-west to north on stony soils.

5) The fifth vegetation zone comprising Bruniaceae is the *Erica coccinea var coccinea-Widdringtonia cupressiodes zone*, a sparse to mid-dense, mid-high proteoid veld with *Berzelia rubra* as type species. This sub-community is defined to occur east of the main kloof, with a wide altitudinal range of 150 m to 600 m. The main aspect of the community varies between south-east and west-south-west. Slopes are moderately steep, well drained and dry. The soils here have a higher clay content and better soil moisture retention than those derive from sandstone.

The present study will try to reconfirm and if necessary to redefine the vegetation zones after de Lange (1992) to estimate how stable populations are after 15 years time. This might illuminate shifts in plant community composition and the stability after fire events since the Reserve had several fires after the vegetation zone analysis of de Lange in 1992. The vegetation zones will be specified regarding the soil components directly below the plants and the density of the occurring Bruniaceae populations.

The Reserve-own Herbarium and the recorded fire data allow a detailed analysis of the processes that influences the species composition and population stability and e.g. how fast the plants are able to become reproductive after fire. This will allow to predict the response of the plants on disturbances within the Reserve.

5.2 Material and Methods

5.2.1 The locality

The chosen investigation area, the Vogelgat Private Nature Reserve comprises different height and habitat zones and 14 Bruniaceae species in six genera of which ten were included in the analysis (see Fig. 5.1 and Tab. 5.1). *Lonchostoma monogynum* that could not be reconfirmed for the Vogelgat Nature Reserve was observed in the Fernkloof Nature Reserve that is directly situated west of the Vogelgat Nature Reserve and is the second observation site.

The Reserve is located in the Overberg region, about 125 km east of Cape Town in the Western Cape Province and 2.4 km from the outskirts of Hermanus. It covers an area of about 603 ha. The mountainous area is directly situated at the coast line with an altitudes range from 50 m to 820 m above sea level. The main vegetation type is mountain fynbos.

The Reserve is situated in the western part of the temperate winter rainfall region which has Mediterranean climate. The average annual rainfall at the lowest point of the Reserve is approximately 923 mm over a 15 year period (1988-2003) and 602.9 mm over a 51 year period (1947-98), measured at the Magnetic Observatory in

Hermanus (shortly above sea-level). The wind cycle falls into the bimodal wind regime. Predominant winds in summer are from the ESE to SE sector with an average velocity of 35 km/h, due to the South Atlantic high pressure system (management Plan of the Vogelgat Private Nature Reserve 2004, unpublished data).

In winter predominant winds are from NW and WNW sectors. Wind speeds may reach 60 km/h or more at times (Management Plan of the Vogelgat Private Nature Reserve 2004, unpublished data).

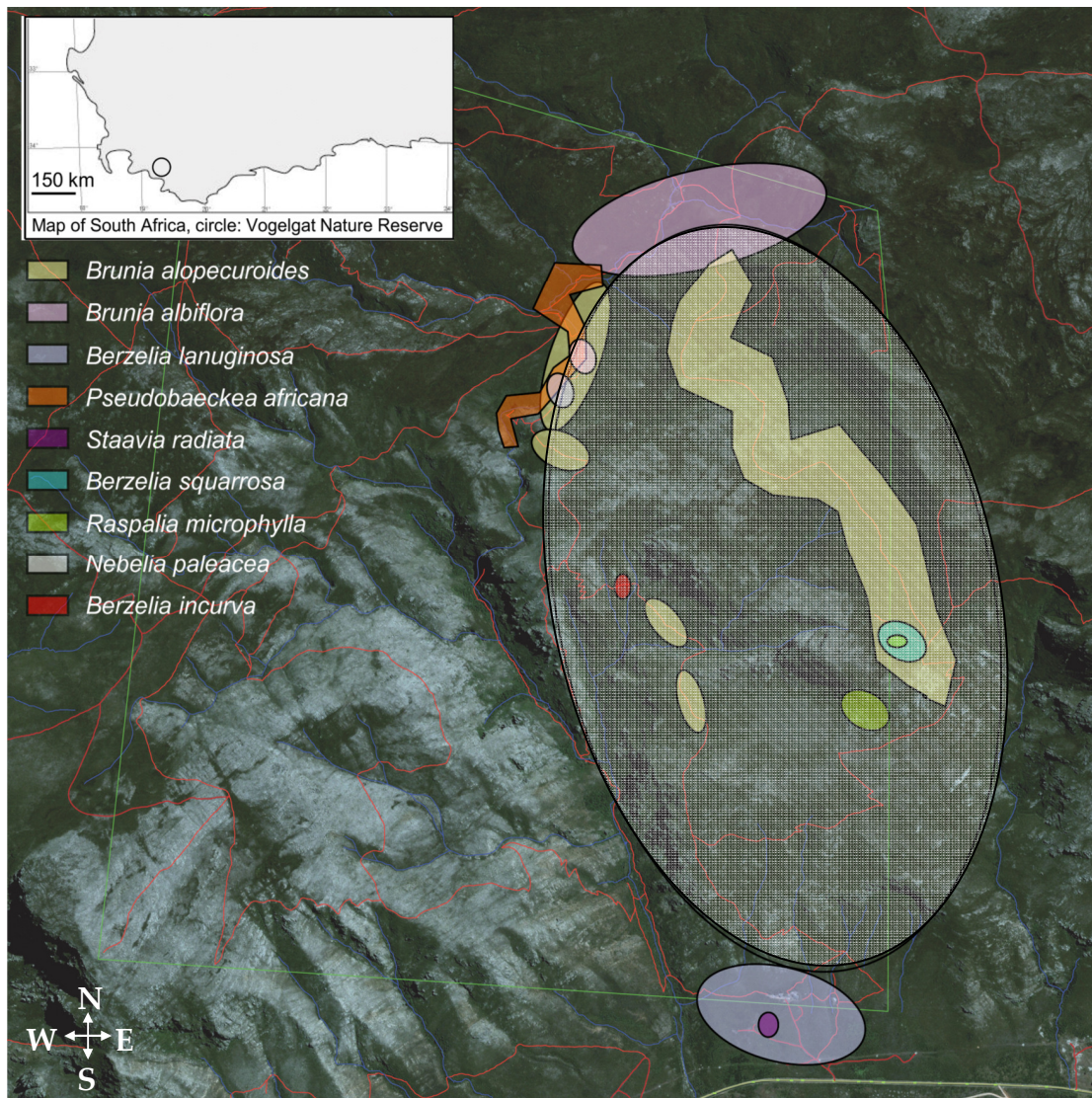


Figure 5.1: The Vogelgat Private Nature Reserve. Coloured areas: observation sites of the particular species. The size of the area does not consider single spread plant individuals which were not included in the present study.

5.2.2 The conducted analyses

All analyses were conducted between September and December 2007 in the eastern part of the Reserve since most Bruniaceae species occur there. A total of eleven species were analysed regarding a) distribution, growth form, b) age, stability, extent and density of the populations, c) soil composition, soil density, humidity, d) soil pH, C and N values, e) fire events and their influence on the populations (see Tab. 5.1). Soil samples were directly taken below the plants. In the case of *Berzelia lanuginosa* due to obvious differences in soil colour and grittiness, three soil samples were taken (see Fig. 5.3). In the other six species, only one sample was taken because no macro-morphological differences between soils that would have reasoned a bigger sampling could be observed.

Since not all species observed occur within the Reserve the macro-morphological soil analysis of *Lonchostoma monogynum* and *Berzelia abrotanoides* were conducted at the Fernkloof Nature Reserve and within a residential area in Hermanus on an open field respectively. For fire event and age analysis the data of the assumed extinct Vogelgat *Lonchostoma monogynum* population was used.

The species included in the distribution analysis within the eastern part of the Reserve were recorded as far as possible. In some cases, single plants might be missed. The distribution will be stated as an area or population when there are more than twenty plants in a spot of less than twenty metres in diameter. If single plants of another species occur within a big population, they will not be stated separately as far as they are not dominating in any parts e.g. the margin of this population.

Berzelia abrotanoides as well as *Lonchostoma monogynum* will not be included in this analysis since in both cases only a single population occurs and no possibility to compare populations under similar habitat conditions would be given.

Table 5.1: Experimental approaches and conducted analysis of the chosen species.

species	distribution	population density	soil analysis	chemical soil analysis	fire data	stability
<i>Berzelia abrotanoides</i>			X	X		
<i>Berzelia incurva</i>	X					X
<i>Berzelia lanuginosa</i> (locality 1-3)	X	X	X	X	X	X
<i>Berzelia squarrosa</i>	X		X		X	X
<i>Brunia albiflora</i>	X		X		X	X
<i>Brunia alopecuroides</i>	X	X	X	X	X	X
<i>Lonchostoma monogynum</i>			X			X
<i>Nebelia paleacea</i>	X	X	X	X	X	X
<i>Pseudobaeckea africana</i>	X		X	X		X
<i>Raspalia microphylla</i>	X		X	X		X
<i>Staavia radiata</i>	X		X	X	X	X

Population density

To detect interferences or competition of close-by growing plants the population density of different species were analysed. *Berzelia lanuginosa* and *Brunia alopecuroides* are two species with a sustained or comprehensive distribution pattern within the slopes and *Nebelia paleacea* occurs very scattered. The overall parameter is the age that had to be combined with some other factors that will be taken into account:

In *Berzelia lanuginosa* the age of the populations and the soil was the parameter that led to three chosen localities namely a) a young population in which the plants are less than 50 cm in height and are in the first or second reproductive year, b) a medium aged population with plants being up to 1 m in height, and c) an old population with the plants reaching a height of 1.60 m and more. In the young population the soil was very sandy, in the medium aged population the soil was sandy but with many rocks and pebbles. The oldest population had a very rocky soil, little sand proportion and red-brownish lime-stone elements (proven by an analysis using highly concentrated acid that resulted in gas formation in the soil probe).

For *Brunia alopecuroides* the determinant factor for the choice of the population was the soil humidity below the plants. Two populations were chosen, one with very wet soil and in some parts running water parts and of medium age (about seven reproductive years). The second locality was also very humid but no running water could be found and the population was more exposed to wind and the population had an age of about two to four reproductive years. In both cases the absence and presence of *Drosera spec.*, Restionaceae and *Osmitopsis asteriscoides* (BERG.) LESS. (Asteraceae) was documented to detect correlation between the population density of *Brunia alopecuroides* and the absence/presence of the other plants or water.

In the case of *Nebelia paleacea* three populations were chosen that exhibit different slope steepness: one slope had an angle of elevation of 7° and the population was in about the sixth reproductive year and was slightly concave, the second slope had an angle of elevation of 10° and was of the same age like the latter one. The third slope showed an angle elevation of 20° being slightly convex and the plants were in about the fourth reproductive year. The inclination was estimated using the gradient level after Finnern et al. (1994).

Since *Lonchostoma monogynum* grows in very dense populations the population density was measured taking a total of ten areas of one square meter. The plants within this square were counted.

Despite the parameters, the measurement methods differed between species due their population structure. For *Berzelia lanuginosa* and *Brunia alopecuroides* the observation was conducted using a rope that was spanned through a population forming a 1 m broad rectangle. This counting method was necessary as the populations of these two species occur in sustained populations. The rectangle was divided into square meters and the individuals within these square meters were counted. In the case of *Berzelia lanuginosa* two measurements with a length of 50 m and a third measurement with a length of 90 m was conducted. For *Brunia alopecuroides* the length was 50 m and 65 m. The distance was representative for the length of the population and therefore is variable.

In contrast to the sustained population of *Berzelia lanuginosa* and *Brunia alopecuroides*, *Nebelia paleacea* occurs throughout the slopes. Therefore three units of ten square meters were chosen and the plants within these squares were counted.

Age and stability

Age, stability and extent of the plants were documented using the more than thirty years old herbarium sheets of the Vogelgat Nature Reserve as basic data of the distribution and population size within the Reserve. The information was gained from the herbarium sheet labels on which the year of collection and a description of the status quo of the population is given.

To detect stability and enlargement or diminishment of the populations this data was compared with the present situation of the populations. The age of a plant in the field can be determined via counting the old infructescence stalks and in seritinous species the remaining fruits. These structures are clearly visible even after more than five years of ongoing reproduction.

Used herbarium sheets of the Hermanus Herbarium (HER) deposited at the Vogelgat Nature Reserve. Most herbarium sheets were collected by Dr. Ion Williams, in some cases the collector is unknown. Numbers stand for the herbarium internal numbers of the Herbarium sheets. *Berzelia* (3294): *Berzelia*

incurva (3002), *Berzelia lanuginosa* (2074), *Berzelia rubra* (2174), *Berzelia squarrosa* (3294). *Brunia* (3292): *Brunia alopecuroides* (3507, 2073) *Lonchostoma* (3286): *Lonchostoma monogynum* (3831, 3880) *Nebelia* (3289): *Nebelia paleacea* (3505, 2281), *Nebelia Pseudobaeckea* (3291), *Raspalia microphylla* (3288): *Raspalia microphylla* (2559, 2511), *Staavia* (3290): *Staavia radiata* (3858, 3039).

The data were compared with the vegetation zone analysis after de Lange (1992) to illuminate changes in population composition or migration events within the Reserve.

Soil composition

The macro-morphological soil analyses were conducted using methods described in the pedological guidance by Finnern et al. (1994) and the cited literature therein. Soil samples of about 10 cm³ were taken directly from below the plants. The parameters were categorized in three steps: very low, medium and very high according to the definition for the particular parameter after Finnern et al. (1994) (see Tab. 5.2).

The soil composition was analysed using macro-morphological parameters like flintiness, coherence, soil density, field moisture/field capacity, humidity and content of root and organic material. The soil samples were sieved using a sieve with 5 × 5 mm mesh to remove big pebbles. The amount of pebbles was counted and related to the whole volume of the sample.

The coherence and grittiness was tested by rolling and flattening a fresh soil sample in the hand. The ability of forming a cylindrical compact form gives information about the sand and clay content or humous particles that aggregate the soil. If the soil is very sandy, no such form can be built, if the soil contains much clay or viscous soil components, the built cylinder is very stable.

The density of the soil was checked by vertically cutting-off a part of the soil using a spatula. If the cutting was difficult and pressure was necessary and the cut soil border was stable, the density was high. When no pressure was used to cut the soil and no border could be formed, the density was low.

The field moisture/field capacity of the effective root zone described the amount of water that can be hold back against gravitation and is plant available.

The amount of undecomposed plant material in the soil was observed to predict the assumed nutrient poverty or richness below the plants. The root penetration intensity of fine roots (roots up to a diameter of 2 mm) was observed.

Table 5.2: Parameters of the macro-morphological soil analysis and the definition of very low, medium and very high conditions of the soil. The numbers 1), 3) and 5) will refer to the ranking in the result table.

parameter	definition of very low (1)	definition of medium (3)	definition of very high (5)
flintiness	<2% of the total volume; very lightly rocky, gritty	10-25% of the total volume; medium rocky, gritty	50-75% of the total volume; very rocky, gritty
coherence/soil density (correlates with grittiness)	no coherence of the sample/ sample not rollable	rollable, can faintly break	very coherent / sample can be rolled having a thickness of less than half a pencil without breaking
field moisture/field capacity	very low/<60 mm e.g. coarse sand and gravel	medium/140-220 mm e.g. brown earth consisting of clayey sand	very high/>300 mm e.g. black earth and luvisol
estimated humidity	dry: solid, not shapeable	moist: stiff, rollable hardly to press	wet: paste-like, being pressed it passes the space between the fingers
root penetration intensity	no roots/0 fine roots per dm ²	medium: 6-10 fine roots per dm ²	extreme amount of roots up to a root felt/>50 fine roots per dm ²
organic material	0% organic material humus free	2-4% medium humous	15-30% organic material extremely humous, peaty
humus quality (C organic /N organic)	very low C/N > 25 CEC [cmol _c /kg] <150	medium C/N 20-15 CEC [cmol _c /kg] 180-210	very high C/N <10 CEC [cmol _c /kg] >250

pH-measurements

pH value measurements, the humus content analysis and the C/N- analysis were conducted at the Johannes Gutenberg-Universität Mainz, Germany.

The pH value was measured with aqua dest. to simulate a wet soil, with CaCl₂ (0.01M) to simulate a dry soil (in Finnern et al. 1994 (DIN 19684, part1); VDLUFA method book, 1991) and with KCL (1 M) to simulate a rain-soaked soil (Meiwes et al., 1984). As *Pseudobaeckea africana* occurs in and along streams, only the measurement with aqua dest. was conducted.

The soil samples were dissolved in the different agents, stored overnight under room conditions and filtered with filter paper. The pH value of the flow-through was analysed using a DIGITAL-pH-METER 646 (Knick Elektronische Meßgeräte Berlin, Germany).

Humus-content analysis and organic/anorganic C and N values

The humus content of the samples was measured using the ignition-loss method proposed by Dean (1974). All weight measurements were conducted using an analytical balance. The empty ceramic crucibles were weighed (mass = n_1). Then the samples were placed in the crucible and both were weighed again (mass = n_2). The soil samples were burnt at 600°C for 24 hrs in a muffle furnace (M 110, Heraeus instruments, Hanau). Probes were cooled in an exsiccator containing silica gel to prevent air humidity from moistening the samples. Then the crucible with the soil was weighed (mass = n_3). The loss of ignition was calculated using the formula

$$\text{loss of ignition (weight in \%)} = 100 \times ((n_2 - n_3) / (n_2 - n_1)) \quad (\text{Dean 1974})$$

Organic and anorganic C and N value measurements were conducted at the geological laboratory of the Geographical Institute of the Johannes Gutenberg-University, Mainz in an EA/IRMS (Elementar Analysensysteme GmbH, Hanau). Half the samples were burnt and ground, the other half was ground without being burnt.

The quality of the humus will be calculated using the relation between C and N that is an indicator for the CEC (cation exchange capacity) potential (Finnern et al. 1994). For the parameter of low or high humus quality see Table 5.2.

Fire events

The fire events are recorded by the Cape Nature Conservation Board (data available at the Vogelgat Nature Reserve). The fire events included in this analysis occurred in 1982, 1986, 1990, 1992, 1996 and 2001 (see Fig. 5.2). *Berzelia incurva*, *Berzelia lanuginosa*, *Berzelia squarrosa*, *Brunia albiflora*, *Brunia alopecuroides* and *Nebelia paleacea* were analysed to show how fast the plants are able to re-establish and, if possible, how fast they start to become reproductive again after a fire event. Some plants survived fire events, which were excluded from the analysis. The status 'reproductive after fire' is reached when the first fruits or at least old inflorescence stalks are visible on the plant. In the Bruniaceae this status can easily be determined as the infructescences stay on the plants. In some cases the dried fruits drop down but the former inflorescence axis still can be detected.

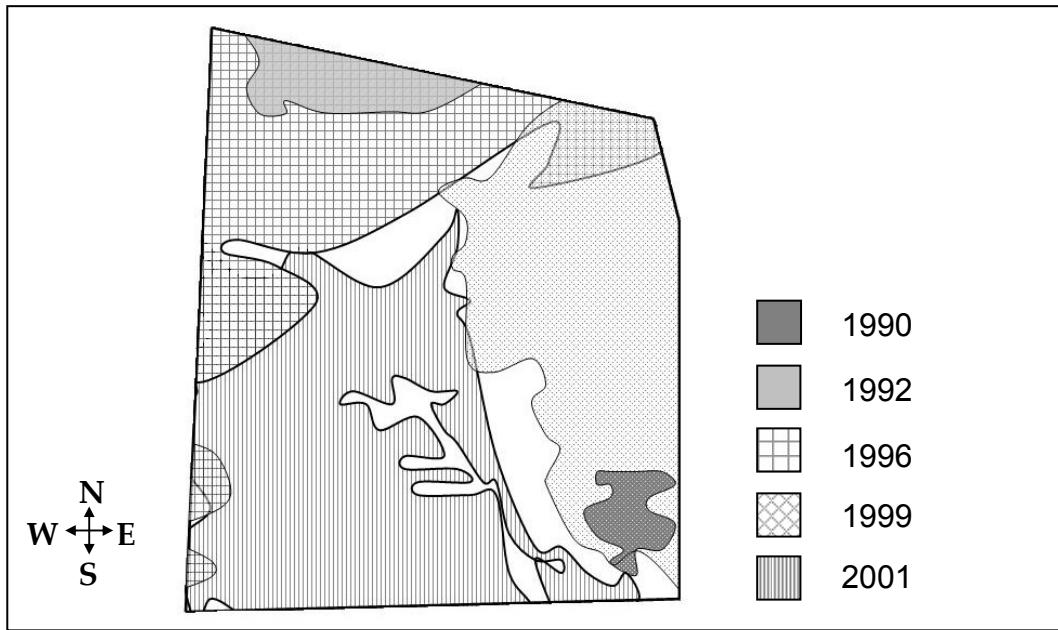


Figure 5.2: Fire events in the Vogelgat Private Nature Reserve from 1990 until 2001. Different patterns indicate the particular fire events. Since in the years 1980 and 1986 the whole Reserve burnt, these two events were not included in the graphic.

Statistical analysis

A correlation analysis was executed to define the relation between organic and anorganic C, N and the Humus content of the soils of the Vogelgat Nature Reserve. Since *Brunia alopecuroides* was observed to co-occur a) with Restionaceae under more dry soil condition but b) in another population the species co-occurs with *Drosera* and *Osmitopsis asteriscoides* under very wet conditions, the question arose if a correlation between population density and surrounding vegetation/water supply is given. Mann-Whitney U-tests were conducted to detect the possible correlation between the population density of *Brunia alopecuroides* and factors like water and the co-occurrence or absence of *Drosera*, *Osmitopsis asteriscoides* and Restionaceae. All calculations were carried out using SPSS 15.0 (SPSS Inc., IBM Chicago, Illinois, USA).

5.3 Results

5.3.1 Distribution pattern at the Vogelgat Nature Reserve

The Bruniaceae of the Vogelgat Nature Reserve show a specific pattern of distribution areas due to slope orientation, steepness and altitude (see Fig 1.2, 1.3. and Fig. 5.3).

Nebelia paleacea can be found scattered all over the slopes in all altitude ranges of the Reserve. The species does not occur in direct neighbourhood to streams. The slopes the species occurs on are of the open type with *Nebelia paleacea* with about 0.6 m in height being more or less the tallest element.

Brunia alopecuroides and *Berzelia lanuginosa* grow in big, dense populations mainly on slender slopes. *Berzelia lanuginosa* is wide-spread in the lower parts of the Reserve around the base camp, the street and the street towards Hermanus and Stanford. In higher altitudes the species only can be found scattered and mainly together with *Brunia alopecuroides* but not area-wide. *Brunia alopecuroides* occurs from an altitude of 250 m above sea-level upwards and appears area-wide in very big and dense populations of generally more than 100 plants. Most populations of *Brunia alopecuroides* appear on slender slopes. If there are no slender slopes, *Berzelia lanuginosa* and *Brunia alopecuroides* can be found stripe-like along paths or area-wide.

Brunia albiflora has its distribution area in an altitude of about 400 m above sea level. The species occurs in very dense populations and the tall plants that can be up to three metres in height do hinder the sun to reach the soil below.

Of *Staavia radiata* only two isolated plants below the base camp exist, but the species could also be found in the area above Hout Bay harbour (north-western coast of the Cape Peninsula) that showed that the population pattern is quiet similar to *Nebelia paleacea*. Above Hout Bay harbour the species occurs scattered all over the slope and can also occur in higher altitudes of more than 200 m above sea level.

Raspalia microphylla can be found spread over the slopes but in altitudes of about 400m above sea level, sometimes on plateaus, mostly in steep area.

The *Lonchostoma monogynum* population in the Fernkloof Nature Reserve shows a swampy insular-like habitat with densely surrounding Proteaceae and Restionaceae in a big patch of more than 200 plants. The locality conditions are similar to those of the assumed extinct or fire-needing site in the Vogelgat Nature Reserve that was situated in the marginal north-eastern part of the Reserve.

Berzelia abrotanoides was documented about 20 m above sea level in a residential area in Hermanus. The species occurs at the foot of a slender slope and occurs scattered.

Some of the analysed species co-occur in the same areas. In general never completely mixed populations could be observed but aside a dominant species single other species can co-occur.

Berzelia lanuginosa, *Nebelia paleacea* and *Brunia alopecuroides* inhabit the eastern marginal area of the Vogelgat Nature Reserve while in the northern border area *Brunia alopecuroides*, *Berzelia lanuginosa* and *Brunia albiflora* appear.

For all co-occurrences the dominance of the single species changes depending on the density of the other species. Under the condition of an open area, *Nebelia paleacea* and *Brunia alopecuroides* dominate whilst only single *Berzelia lanuginosa* plants can be found at the margin of the population.

Under very dense population situations of *Brunia albiflora*, *Brunia alopecuroides* and *Berzelia lanuginosa* occur with single plants at the margins of the *Brunia albiflora* population. The same holds true for *Brunia alopecuroides* populations where single *Berzelia lanuginosa* plants can occur in the outer parts of the population.

Soil composition

Soil morphology

In Table 5.3 the grade of different soil characters of the observed species were compared.

The soil below the species differed from a light grey to darkish grey-brown colour with the exception of a reddish-brown yellowish soil found at one *Berzelia lanuginosa* population (see Tab. 5.3 and Fig. 5.3).

Table 5.3: Soil characters of the observed soil types.




















species	soil colour	flintiness	coherence (low coh. = high grittiness)	field moisture/ field capacity	estimated humidity	root penetration intensity	organic material
<i>Berzelia abrotanoides</i>	 	2	1-2	1-2	1	2	1-2
<i>Berzelia lanuginosa</i>	 	2-3	1-2	1-2	1	2	1-2
<i>Berzelia squarrosa</i>	 	2	1-2	1-2	1	2	1-2
<i>Brunia albiflora</i>	 	1	3-4	3-4	3	4-5	4-5
<i>Brunia alopecuroides</i>	 	1	3-4	3-4	3-4	4-5	4-5
<i>Lonchostoma monogynum</i>	 	1	4	3	4-5	3	4
<i>Nebelia paleacea</i>	 	2-3	3	2-3	2-3	4	4
<i>Pseudobaeckea africana</i>		-	-	-	-	-	1-2
<i>Raspalia microphylla</i>	 	2	2	2	2-3	3-4	2
<i>Staavia radiata</i>	 	1	1-2	1-2	1-2	2	1-2



Figure 5.3: Soils and a survey of the Vogelgat Nature Reserve. First row: three soil types from left to the right: very sandy soil, limestone soil with many pebbles, dark peaty soil. Lower row: two impressions of the Vogelgat Nature Reserve: left picture: mist clouds at the top of Beacon Head; right picture: impression from the top of the Reserve down towards the sea.

In general three soil types in the Reserve could be defined: a) sandy soil with low undecomposed material content, b) mixture of sand and a higher amount of undecomposed to humous soil and c) highly humous to peaty soil with low content of sand. The limestone containing soil as well derived from sandstone below *Berzelia lanuginosa* (see Fig. 5.3) will not be stated as separate soil type since it only occurs in a narrow area around the base camp.

For the three found soil types different characteristic species could be found: the *Berzelia* species and *Staavia radiata* grow on sandy soils whilst *Brunia alopecuroides* and *Brunia albiflora* grow on peaty soils. *Nebelia paleacea* and *Raspalia mircophylla* can be found on intermediate soil as well at very rocky sites without much ground layer.

Pebbles could be found in all soils of the observed species. They varied from less than 1 cm in diameter up to more than 10 cm in diameter and also could be found in 10 to 20 cm soil-depth. The amount of stones within the soil samples ranged from less than 2% to about 25% of the total volume of the sample.

Coherence of the soil, field moisture/field capacity and root penetration intensity ranged from very low in sandy soils to high in the peaty soils.

The estimated humidity ranged from *Berzelia abrotanoides* with a dry soil to *Lonchostoma monogynum* with a very wet soil.

The organic material in the soil ranged from very low in sandy soils to a content of about 30% of organic material in peaty soils. In the case of *Pseudobaeckea africana* as

the plant grows in and along streams the soil is reduced to completely lacking and the roots of the plants cling to the smallest crevices.

Different localities, different soils

The composition of the soils can vary slightly due to the locality: the lower slope parts as well as stream sides were sandier than slopes of higher altitudes (400 m above sea-level and more) that exhibit more peaty components. The portion of sand or humus also varies regarding the slope inclination what leads to an accumulation of peaty soil in less steep slopes and protected areas. On paths and uncovered surfaces, sand accumulates via wind and can lead to very sandy zones on high altitude slopes within peaty soil type zones.

The humus layer differs because of the close distance to the bed rock and at times can reach some 40 cm in thickness.

pH value

The soil is slightly acidic with a pH of 5.5 to 6.5 under simulated wet conditions (aqua dest.). The simulated rain-soaked (KCL) and dry soil data (CaCl₂) confirms this finding (see Tab.5.4).

Table 5.4: pH measurements of soils on which species of the same distribution area grow. The measurements simulate wet (aqua dest.), rain-soaked (KCL) and dry soil (CaCl₂). * = species grows in the water, simulation of dry or only rain-soaked soil did not seem reasonable.

species	pH		
	aqua dest.	KCl (1M)	CaCl ₂ (0.01M)
<i>Berzelia abrotanoides</i>	5.90	4.91	5.37
<i>Berzelia lanuginosa</i>	5.90 to 6.61	4.30 to 5.55	5.09 to 5.65
<i>Brunia alopecuroides</i>	6.20	--	4.59
<i>Nebelia paleacea</i>	5.51	4.20	5.12
<i>Pseudobaeckea africana</i>	5.90	--*	--*
<i>Raspalia microphylla</i>	5.88	4.51	5.16
<i>Staavia radiata</i>	5.92	4.50	4.85

Carbon, nitrogen and humus content

The N and C analysis of the soil is linked to the humus content. *Berzelia abrotanoides* has the lowest, *Brunia alopecuroides* the highest amount of N and organic C. The high organic carbon and nitrogen content in *Brunia alopecuroides* and *Raspalia microphylla* is not due to a sampling error but to the high amount of humous soil. The quality of the humus of all species observed is low to very low. The anorganic C content in all samples is nearly zero.

Table 5.5: C and N values and humus content

sample	N _{total} (%)	C _{organic} (%)	C _{anorganic} (%)	humus content (%)	humus quality	C/N	CEC [cmol/kg]
<i>Berzelia abrotanoides</i>	0.03	0.60	0.01	1.88	low	20.00	150-180
<i>Berzelia lanuginosa</i>	0.02 to 0.13	0.68 to 2.42	0.02 to 0.03	3.66 to 10.85	low to very low	34.00 to 18.60	150-180 to <150
<i>Brunia alopecuroides</i>	0.37	13.18	0.04	24.04	very low	35.62	<150
<i>Nebelia paleacea</i>	0.14	5.12	0.02	9.97	very low	36.57	<150
<i>Pseudobaeckea africana</i>	0.08	2.57	0.02	6.37	very low	32.12	<150
<i>Raspalia microphylla</i>	0.26	7.51	0.01	17.09	very low	28.88	<150
<i>Staavia radiata</i>	0.06	1.42	0.02	4.37	very low	23.66	<150

5.3.2 Population density, age and stability

Comparing the current population sizes and distribution areas with the data from the herbarium sheets in the Vogelgat Nature Reserve, nearly no changes can be found during the last 30 years. The main occurrence areas of the species remained the same. Regarding the population density from the 1970's no accurate statement can be made, because on the herbarium sheets only approximate information is given.

The age as population density determining factor plays a role in all species but especially in the species with area-wide distribution patterns it becomes obvious. In populations of *Brunia alopecuroides*, *Brunia albiflora* or *Berzelia* species the distance between single plants averages at about 0.7 m. In younger populations e.g. with spaces of 5 cm between *Berzelia lanuginosa* plants, the density can be much higher. In general the population density of *Berzelia lanuginosa* was lower in older than in younger populations (see Fig. 5.4).

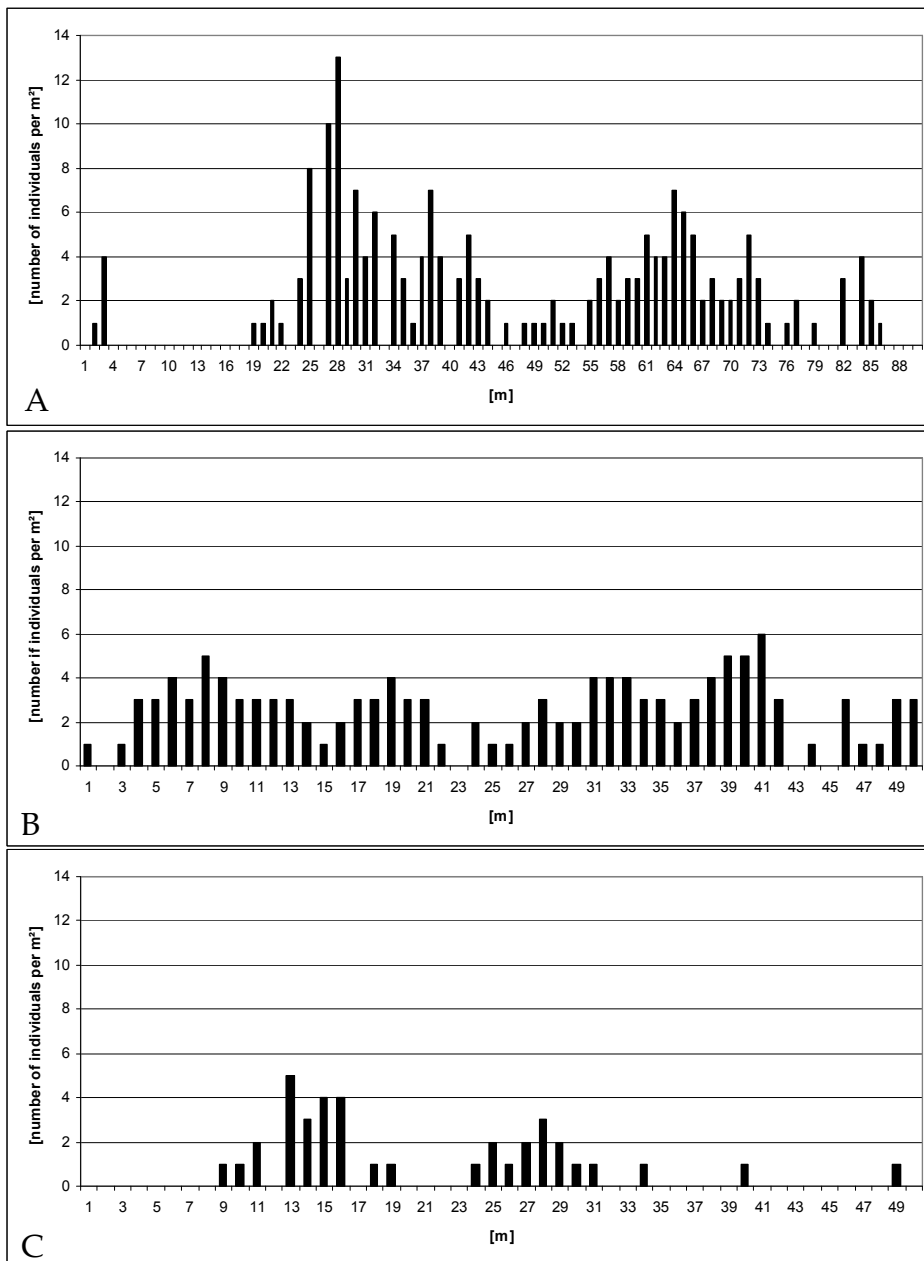


Figure 5.4: Different population densities of *Berzelia lanuginosa* due to age. A: youngest populations, about in the third reproductive year; B: older population, about in the fifth reproductive year, C: oldest population, about in the seventh reproductive year.

Although a co-occurrence between Restionaceae, *Drosera* and *Osmitopsis asteriscoides* with *Brunia alopecuroides* could be assumed for one population observed only the occurrence of *Osmitopsis asteriscoides* is positive correlated with the *Brunia* plants. Despite this, the results show that water has a high positive influence of the presence of many *Brunia alopecuroides* plants. In the second population observed, the presence of Restionaceae is related to the presence of many *Brunia alopecuroides* plants (for all statistic results see Appendix 4).

The steepness of the slope had no influence on the density of *Nebelia paleacea* populations as in the slope with low steepness 30 plants could be counted, in the middle steep slope 14 plants could be found and in the steepest slope 38 plants grew.

In *Lonchostoma monogynum* the number of plants within the square metre ranged from two to 35.

Fire events

The fire events in the Nature Reserve occurred in different intensities: in 1982 and 1986 the whole Reserve burnt with exceptions of very small patches along streams and pools. All following fire events until now only affected parts of the Reserve (see Fig. 5.2). Summing up all fire events the slopes were burnt in irregular periods. Some patches were burnt more frequently than others (see Fig.5.2). The fire events that affected the plants in the eastern part of the Reserve took place in 1986, 1996, 1999 and 2001. The reproductive ages of the observed species differed from one to six years (see Tab. 5.6). According to the fire events the estimated time needed to become reproductive again ranges from four to eight years (see Tab. 5.6).

Table 5.6: Veld age and the correlating reproductive age of some Bruniaceae. * single plants within the population that were not affected by the last fire events and therefore were not included in the time estimation.

species	years after the last fire event (date of the last fire)	reproductive age in the year 2007	estimated time to become reproductive after fire
<i>Berzelia incurva</i>	8(1999)	0	> 8 years
<i>Berzelia lanuginosa</i>	7 (2001) 11 (1986) 8 (1999)	1 4-5 3-4	4 to 6 years
<i>Berzelia squarrosa</i>	8 (1999)	1	7 years
<i>Brunia albiflora</i>	6 (2001) 8 (1999) 11 (1996) 11 (1996)	0* 3-4 4-5 5-6	4 to 6 years
<i>Brunia alopecuroides</i>	8 (1999) 8 (1999) 8 (1999) 11(1996) 11(1996)	1 1-2 3* 5 3-4	5 to 7 years
<i>Nebelia paleacea</i>	8 (1999) 8 (1999) 8 (1999) [8 (1999)	1* 4* 2-3 7*]	6 to 7 years

5.4 Discussion

5.4.1 Distribution within the Nature Reserve

After Mucina and Rutherford 2006, the Vogelgat Nature Reserve can be found in the Overberg Sandstone Fynbos zone (FFs12) which is the vegetation zone with the second-most number of Bruniaceae species (24 species). The present study illuminated that this overall vegetation zone grouping has to be refined for a precise analysis of soil preferences and micro-habitat/niche inhabitation of the Bruniaceae. At the Vogelgat Nature Reserve the high number of Bruniaceae species occurring within a small region allows not only to specify the soil adaptation of single species. As well changes in species composition due to changing soil conditions could be illuminated.

Geology, altitude and soil

The geological analysis of Whittle-Herbert (1990) in the Vogelgat Nature Reserve declared the main soil type to be derived from quartzitic sandstone and its weathering results in very poor soil development.

The residue is reckoned to be almost clay-free, shallow, coarse and sandy with a high proportion of gravel and small boulders. Soil depth is said to vary over short distances as bedrock is never far from the surface (Whittle-Herbert 1990).

Because of the acidic conditions, organic residues is said to decompose slowly what results in a very low fertility status but could contribute to water holding capacity. Whittle-Herbert interpreted this effect to be reinforced by the coarse sand blanket and to minimise moisture loss by drainage and evaporation. But in general the sandy soils of the Reserve are defined to have a low water holding capacity and low cat ion exchange capacity.

Within the Reserve, Whittle-Herbert found different aspects of soil types: a) next to valleys, very sandy alluvium is present, b) shale bands of finer texture which have a high clay content and are yellow brownish in colour can be found in between the main soil types, c) on see-facing slopes due to an accumulation of organic material deep black coloured organic horizons develop.

Whittle-Herbert distinguished between south facing and north facing slopes and found that south facing slopes the soils are deeper due to rapid chemical weathering whilst the north facing slopes have slow soil development and low water holding capacity due to the drier conditions. The present study contradicts to this finding as areas in the hinterland were observed and in these regions in more than 1 km linear distance and being not directly exposed to the coast, the soil has been peaty and black. The finding of Whittle-Herbert, that only see-facing slopes had a high decomposition rate and thereby exhibit a big layer of peat-like soil has to be

dismissed. It seems as if the slope steepness rather than the slope-orientation had an influence on the accumulation of this soil type as the slopes with the biggest peaty layers are mid-steep or the area is protected (e.g. flat valleys).

In contrast to the overall definition of north and south facing slopes and slopes orientated towards the sea, in the present study a refinement of the mountain ranges and slopes seems required. The mountain ranges could be classified in a) lower slopes and stream sides mainly with sandy soil and interspersed rocks, b) open areas and paths with a high exposure to wind being very sandy, c) gentle slopes with accumulated humous soil layers, d) valleys and protected areas in which a dense layer of humous soil could accumulate, e) very steep slopes with more rocky elements and f) the rocky or slightly sandy tops and ridges without a thick humous soil layer. The Bruniaceae occur in all these ranges in more or less dense populations. The population composition and density changes with altitude, slope steepness and orientation and soil composition.

Vegetation zones

The Bruniaceae are a characteristic element of the ericaceous mesic Mountain Fynbos (Campbell 1986). Within the Vogelgat Nature Reserve they dominate the upper and middle layer of the vegetation (de Lange 1992). With the present study vegetation zone characteristics defined by de Lange 15 years ago could be confirmed. However, in some cases a more detailed definition for soil compositions or type species definitions became necessary regarding the recent Bruniaceae populations.

Nebelia, Raspalia and Staavia

The *Phaenocoma prolifera-Chondropetalum hookerianum* vegetation zone composition that stated *Nebelia paleacea* to be a type species in general can be confirmed. But *Nebelia paleacea* also occurs between bare rocks in less drained areas and in higher and lower altitudes than the 300 m to 500 m range proposed by de Lange. As the assumed age of the plants in the lower as well as the higher parts of the Reserve do not differ from the age of those plants in the *Phaenocoma prolifera-Chondropetalum hookerianum* zone, it remains unclear if *Nebelia paleacea* enlarged its distribution within the Reserve or if the zone definition by de Lange (1992) was based on a certain population density what cannot be reconstructed by now.

Nebelia paleacea is distributed all over the slopes and occurs in differently dense populations in all altitudes and slope-inclinations so no clear adaptation could be defined. Only in stream-near areas the species could not be found what might be due to the high plant accumulation in these water rich areas or the fact that the soil is too wet for *Nebelia*.

Regarding slope steepness as a niche or micro-habitat, *Raspalia microphylla* and *Nebelia paleacea* share the same habitat. A separating factor is the very stony soil being preferred by *Raspalia*, whilst *Nebelia* grows on more peaty soil in between the rocks. Since *Nebelia* does not only grow in high altitudes on stony ground but also in about 50 m above sea level, it also shares its habitat with species that does not inhabit mountain tops. In contrast to the wide-spread *Nebelia paleacea*, *Staavia radiata* only occurs with two single plants below the base camp. These two isolated plants seem to be a relict of a former bigger distribution area as above Hermanus and eastwards in Stanford bigger populations can be found. The adaptation to sandy soils cannot be dismissed and reflects the general distribution pattern of the species.

Brunia* and *Berzelia

Brunia alopecuroides after de Lange (1992) occurs in three vegetation zones.

As the name indicates *Brunia alopecuroides* inhabits the *Brunia alopecuroides*-*Chondropetalum deustum* zone and is said to co-occur with *Berzelia squarrosa*.

The findings of the present study confirm the basic principles of the definition but *Berzelia squarrosa* only occurs in some parts of these area-wide *Brunia alopecuroides* populations and mostly the soil of *Brunia alopecuroides* also contains peaty aspects and does not only consist of pure sand. *Berzelia squarrosa* occurs on more open plateaus or slender slopes and could never be recorded to occur in a dense *Brunia alopecuroides* population. The two species rather seem to parapatrically overlap in their population margins than to co-occur sympatrically.

The *Osmitopsis asteriscoides*-*Erica perspicua* zone after de Lange includes two Bruniaceae species namely *Brunia albiflora* and *Brunia alopecuroides*.

The described area was found not to be dominated by both species but by *Brunia albiflora* whilst *Brunia alopecuroides* only occurs interspersed. Since the *Brunia albiflora* plants are very high and grow very densely, it could be assumed that the detected co-occurrence of both species with ongoing age shifts to a dominance of *Brunia albiflora*. Growth conditions and sun-exposition might be unfavourable for *Brunia alopecuroides* under competition with *Brunia albiflora*. This seems reasonable because as soon as the area becomes more open, the occurrence of *Brunia alopecuroides* strengthens again.

In the *Erica onosmiflora*-*Brunia alopecuroides* zone

As the name indicates, *Brunia alopecuroides* can be found in this aspect what could be assured in the present study. It should be mentioned that in regions higher than 500 m above sea level, the *Brunia alopecuroides* populations are less dense and the plants occur more scattered than in regions at around 200 - 300 m above sea level. This suggests the latter altitude to be the most favourable altitude range for the species

since no significant differences in slope orientation or other abiotic factors could be detected.

Pseudobaeckea

All Bruniaceae species inhabit certain areas of the Reserve but some like *Pseudobaeckea africana* occur in areas that can be called niche. Although de Lange did not define a vegetation zone with *Pseudobaeckea africana* as type species its dominance in this difficult area is obvious. The adaptation of *Pseudobaeckea africana* is the ability to grow in streams and its tolerance of regular flooding events in the rainy periods of the year. The roots of the plants build a solid anchoring in the smallest crevices. This isolates this species from all other Bruniaceae in the Reserve that need solid ground to grow. Additionally the species only occurs in the upper part at about 300 m above sea level in a calmer water section. The fact that *Pseudobaeckea africana* does not occur in lower parts along the streams could be due to the incline of the area and the disability to grow there without durable danger of being drifted by the faster flowing water. Another reason could be the existence of water falls that could also prevent a settlement in the lower stream parts. Due to the steep rock faces along the kloof in the lower parts of the stream the daylight conditions are disadvantageous compared with the lighter upper stream parts. This as well as the possibly occurring competition with the numerous ferns in the kloof could be another reason for the restricted occurrence in the upper stream parts.

Changing species dominance

The pattern of species that inhabit the same area but do not completely overlap in their distribution area can give rise to the question what conditions are favourable for each species. On the other hand if the conditions in the area are similar the ability to compete could lead to a dominance of only one species. Two wide-spread species, *Brunia alopecuroides* and *Berzelia lanuginosa* seem to be adapted to slender slopes and if these are not available, the species occur sustained along paths. *Brunia alopecuroides* occurs in very big populations in general with more than 100 plants.

The role of *Brunia alopecuroides* in the upper parts of the mountains is taken over by *Berzelia lanuginosa* in the lower parts of the Reserve, especially around the base camp and the southern border of the Reserve and the road to Hermanus. Although *Berzelia lanuginosa* in general occurs in all altitudes, within the Vogelgat Nature Reserve restricting factors seem to center its main occurrence in the lower parts. Regarding the preferred soil *Berzelia lanuginosa* is the more flexible species as *Brunia alopecuroides* only occurs on very peaty soil that even during hot periods is damp.

The question arises if these sea-facing slopes have a better humidity supply by clouds coming from the sea. Bruniaceae in general are said to occur in the mist-zone

of the mountains (Pillans 1947). Mist clouds are a common phenomenon in the Cape and at Table Mountain, the tablecloth occurs around 600 m and up to the summit at about 1000 m above sea level (Marloth 1904). If *Brunia alopecuroides* was adapted to damp soils and had an establishment advance thereby displacing *Berzelia lanuginosa* this would explain its dominance. A real mist-zone could not be detected in the Vogelgat Reserve but during the study it could be observed that fog is a very common phenomenon in the coastal area. No clear altitude pattern could be observed as the fog wafted up the slopes. But like in the case of the tablecloth of Table Mountain the fog cumulates at the top of the mountain ranges and builds mist clouds (see Fig. 5.3). Due to the wafting mist clouds, more or less the whole slopes can benefit from the humidity. For that the water holding capacity of the ground rather than the mist clouds seem to be of importance for the areas inhabited by *Brunia alopecuroides*. Nevertheless the soil leads to a competition advantage between *Brunia alopecuroides* and *Berzelia lanuginosa* leading to zones of dominance for these two species. Peaty soils are also inhabited by *Brunia albiflora*. This species occurs in an altitude of around 400 m above sea level and its very dense populations cumulate around streams. The species replaces the occurrence of *Brunia alopecuroides* and *Berzelia lanuginosa*. The plant height of in some cases more than three metres of *Brunia albiflora* leads to a lack of sun and the very dense populations enforce this effect so that smaller plants cannot compete. The separating habitat factor soil gets enforced by the morphology of *Brunia albiflora*.

Aside species with big populations and a big distribution area like *Berzelia lanuginosa*, two other *Berzelia* species, *Berzelia squarrosa* and *Berzelia incurva* occur in the Reserve. These two species occur scattered and neither build big populations nor a clear adaptation to slope orientation or soil conditions could be detected but they inhabit slopes in high altitudes. It could be assumed that their occurrence in higher altitudes is caused by an adaptation to wind exposure and thereby a reduction in plant size. This would explain why *Berzelia lanuginosa* with a much bigger plant altitude does not occur in this area. Another reason for the distribution regarding the altitude could be the influence of damaging UV-rays in higher altitudes, since *Berzelia squarrosa* and *Berzelia incurva* might benefit from their compared to *Berzelia lanuginosa* more hairy habitus in all parts of the plant since hairs can reduce the influence of UV-rays (Karabourniotis et al. 1993; Holmes and Keiller 2002). On the other hand *Berzelia lanuginosa* is known to occur in altitudes of up to 2000 m above sea-level (Pillans 1947, A.V. Hall, unpublished data) what can be definitely assumed to be more UV-ray influenced than the observation site. Therefore it should be tested in further analysis if the habitat conditions at other localities of *Berzelia lanuginosa* in high altitudes might exhibit e.g. wind-protected sites.

5.4.2 Population density and disturbance factors

The population density measurements in the three selected species and the emphasis of the chosen parameters showed that population density is influenced in many different ways. The low seed density around *Berzelia lanuginosa*, a reseeding species confirms the finding that obligate seeders have very limited primary seed dispersal (Fenner 1985; Keeley 1991; Carrington 1999; Escudero et al. 1999) and most seeds can be found directly around the adult plant (Zammit and Zedler 1988; Parker and Kelly 1989; De Luis et al. 2005).

In *Berzelia lanuginosa* and *Nebelia paleacea*, the main reason for differences in population density seems to be the age. The pattern of the population density obviously reflects the seed density pattern in showing the distribution and establishment of the individuals only in low distances of 30 to 80 cm. Neither the soil conditions in *Berzelia* nor the slope steepness in *Nebelia* seem to have an influence. In *Brunia alopecuroides* the co-occurrence of other plants and the water supply seem to have a greater influence on population density than age. Nevertheless, the observations in the field also showed that in very old *Brunia alopecuroides* populations less plants than in younger populations could be found.

An influence on population density can also be stated for the occurring fire events since after fire obligate seeder species establish very fast and increase their density immediately after fire (Lloret 1998; Ladd et al. 2005; Auld et al. 2007). After fire events the high density will decrease due to the recoverage of the resprouting plants (Mucina and Rutherford 2006) due to the ongoing increase of shadow of the vegetation cover (Keeley et al 2006) since obligate reseeder are usually shade-intolerant (Thomas and Davis 1989; Keeley et al. 2006). In the Bruniaceae both, reseeder and resprouter occur (Pillans 1947). The evolution of sprouting is correlated with frequency and intensity of disturbances: non-sprouters can only survive when the disturbances occur mild and rare enough to allow them to become mature before the next fire (Keeley and Keeley 1977; Bellingham and Sparrow 2000; Bond and Midgley 2001). Therefore resprouters occur in areas with less predictable, more frequent and more intensive disturbances and the severity of the disturbance not only its frequency influences the trait distribution of resprouting (Bellingham and Sparrow 2000, Keeley and Zedler 1998). The fire data of the Reserve and comparison with the Herbarium sheets showed that all species kept their distribution area. Only for *Lonchostoma monogynum* a lack of fire events could be assumed as in this unburnt region of the Reserve, the population could not be reconfirmed. The conducted regeneration ability analysis discovered the estimated time to become reproductive again being four to eight years depending on the species. This indicates the well-

known fact that regular fire events are necessary to keep the species diversity (Naveh 1975; Cowling 1987, Cowling et al. 1992; Uys et al. 2004; Bond and Keeley 2005).

The overall result of the comparison of the detailed population analysis of the Bruniaceae and the vegetation zone analysis of de Lange 1992 is the finding that the Overberg region is not only the species hot-spot of the Bruniaceae but the family also dominates the composition of the fynbos in the area. The populations are stable and seem not to shift their extension or dominance even after fire events.

6. Biogeography and recent distribution

Abstract

The found niche-inhabitation on small-scale level as well as the population genetic analysis including geographical data implied that the geomorphology of South Africa had a great influence on species formation and the distribution of the Bruniaceae. The assumable hypothesis for the biogeography of the Bruniaceae demands a close habitat adaptation and a distinct distribution (lateral or horizontal) or ecological adaptation of similar co-occurring species. The so far collected data will therefore be combined with the distribution and altitude ranges based on herbarium sheet data, unpublished distribution maps by A.V. Hall and flowering data to predict geographical and ecological isolation mechanism between species.

The results showed that within clades of the recent phylogeny species are separable by geographical and/or ecological factors. Only species of some clades like the *Berzelia*-clade seem not to be separable regarding both ecological and geographical parameters what leads to the conclusion that within the Bruniaceae some clades are well-established whilst others exhibit ongoing speciation processes. Geographical isolation as well as niche inhabitation is the force that led to speciation and species persistence in the Bruniaceae and determined the present distribution pattern.

6.1 Introduction

The Bruniaceae, as a relict of the late Cretaceous flora, are one of the oldest angiosperm groups found in South Africa and speciation of the early Bruniaceae took place in the Pleistocene (de Villiers 1999; Quint and Claßen-Bockhoff 2008). The (sub-) endemic family is one of the 33 'Cape floral clades' and comprises about 80 species in twelve genera sensu Pillans (Pillans 1947; Linder 2003). The characteristic element of the fynbos biome is more or less confined to Table Mountain Sandstone (TMS) with few species having a large distribution area but many local endemics (Pillans 1947; Hilton-Taylor 1996; SANBI red data list 2007). Neither the pollination syndrome nor the breeding system of the Bruniaceae exhibit a strong specialization but niche-adaptation could be shown (see Chapter 2, 3 and 5). An analysis of the biogeography of the Bruniaceae can help to illuminate the overall adaptation of species to certain geographical and ecological factors respectively. Due to the geomorphology of South Africa it seems reasonable to hypothesize that geographical isolation through valleys and mountain ranges (Latimer et al. 2005) - forming metapopulations within the fynbos slopes - led to reproductive isolation when pollinators do not ensure genetic exchange leading to speciation. The horizontal and altitudinal distribution pattern of closely related species should therefore be distinguishable. In the case of an overlapping distribution pattern ecological factors like the flowering time should be species separating factors. For species formation

two scenarios can be suggested: a) an assumed ancestral species and diverged species occur parapatrically or b) both species occur sympatrically but separating factors like flowering time, altitude preference or morphology exist.

The considered pattern of the occurrences for sympatric species would be a) a total overlap in horizontal and vertical distribution within an area, b) an overlap in marginal areas and c) a pseudo-overlap in the case of a shared distribution area but a strict vertical separation.

An interesting factor for the assumed distribution pattern is the life-form of the Bruniaceae since about 50% of the Bruniaceae have subterrestrial lignotubers which enables them to resprout after fire (Carlquist 1978; de Lange and Boucher 1993b; Pausas et al. 2006). Nothing is known about population sizes and distribution ranges in reseeded and resprouting Bruniaceae. Since resprouting and reseeded sister species exist (Quint and Claßen-Bockhoff 2006a) resprouter could be better adapted to fire resulting in a bigger distribution area what would indicate that sprouting serves as both, re-generation niche (Grubb 1977) and persistence niche (Bond and Midgley 2001, 2003). On the other hand resprouter have a mortality of less than 5% suggesting that they may be hundreds of years old (Le Maitre and Midgely 1992). In this case, big populations would be a waste of energy as stability is also secured in small populations.

The present study combines information about the distribution range, the altitude preferences and flowering times of all Bruniaceae species as well as all collected data of this dissertation. This comprehensive analysis will allow to predict geographical and ecological reasons for the recent distribution patterns and the formation of species. Habitat preferences of assumed diverged species compared to those of the ancestral species can be illuminated. Since on small-scale level adaptation to certain soils could be detected (see Chapter 5), the distribution information will be mapped on the vegetation zone analysis by Mucina and Rutherford (2006) that might show large-scale adaptations that until now could not be detected due to a lack of the overall distribution information. A mapping onto the recent phylogeny (Quint and Claßen-Bockhoff 2006a) will suggest reasons for species divergence within genera and clades.

6.2 Material and methods

6.2.1 Distribution along the Western Cape

In order to find out whether the Bruniaceae show a distribution pattern that is related to habitat conditions the localities stated on the labels of 2957 herbarium sheets of 81 Bruniaceae species were documented by drawing distribution maps. The

herbarium sheets originated from the Compton Herbarium Kirstenbosch (NBG), the South African Museum (SAM) and the Bolus Herbarium Cape Town (BOL) as well as the Herbarium at the Johannes Gutenberg University of Mainz (MJG).

Some species were listed in the Herbarium sheet information with their old unrevised name as separate species, but within this analysis they will be retracted into the recent species names. Therefore the total number of species included in the maps and the altitude range analysis is reduced to 75.

The drawn maps were compared and completed with the unpublished distribution maps of A.V. Hall (deposited at the Bolus Herbarium, Cape Town). For these data, no herbarium material was available and it is unclear how many localities found by Hall are documented by a herbarium voucher and if vouchers of these localities exist at all. All species names will correspond to the revision after Pillans (1947) and will not refer to the aspired revision of Hall after which his distribution maps are created.

Localities only documented by Hall that could not be verified via the herbarium sheets of the BOL, NBG and SAM are included in the created maps but will be marked and discussed later on.

Often the same locality was collected more than once what leads to several actually analysed herbarium sheets but only one spot in the distribution map.

The herbarium sheets of the Herbarium at Mainz will be used as latest confirmation material for the localities since it is one of the latest collected data sets. The localities will not be stated separately as no additional localities could be detected.

Clear altitude ranges were given on 1550 of the 2988 herbarium sheet labels. They will be analysed as the altitude range might be an indicator for habitat preferences. In cases of doubtful or unclear altitude information, the herbarium sheet was not included in the analysis. In the case of altitudes quoted in feet, a conversion in metres was conducted (feet divided by 3.2808).

All data included in the distribution analysis refer to the information on the herbarium sheet labels and could not be verified in detail. The number and extent of the herbarium sheet information is dependant on the collectors that knew or found localities and described them for which reason the data had to be limited to assured information (localities sampled by different collectors, plausible altitude information). If the information does not correspond with the altitude reality in this area, the information was rejected. The extent of distribution ranges will as well reflect the areas where collector have sampled, so that gaps in distribution patterns can be due to a lack of collection rather than a lack of the species in this area.

In a second step all species were mapped on the vegetation zone map by Mucina and Rutherford (2006) to detect correlation between distribution and habitat and e.g. soil preferences on vegetation zone level.

Finally all data are mapped onto the recent phylogeny of the Bruniaceae (Quint and Claßen-Bockhoff 2006a) to predict geographic patterns within clades. Species that are not included in the phylogram but were analysed in the present analysis will be stated separately.

Flowering times of the species (A.V. Hall , unpublished data) contribute to analyse ecological factors that separate species. This allows to distinguish geographical factors (distribution, altitude range) from ecological factors (flowering time) or to detect a combination of both.

6.3 Results

6.3.1 Distribution along the Western Cape

The Bruniaceae occur from the Matzikama Mountains down to the Cape Peninsula and eastwards to Port Elisabeth with *Raspalia trigyna* as known outlier in the Natal Province (Eastern coast of South Africa). Natural boundaries are the mountain ranges of the Cape (see Fig. 1.2). The easternmost locality in North-South direction are the Cold Bokkeveld Mountains. The northernmost point in East-West direction is the Swartberg Nature Reserve near to Prince Albert. The highest density of Bruniaceae species can be found in the Kogelberg/Overberg area.

On genus and clade level the distribution analysis showed that within all genera the distribution pattern can vary from wide-spread to locally restricted (see Appendix 3). Wide-spread species are distributed in a tubular N-S or E-W direction along mountain ranges, species with smaller distribution areas exhibit a more circular pattern. Nine of 75 species occur in North-South and East-West direction, 66 species occur locally restricted. For detailed information about precise distribution areas, vegetation types and altitude ranges see Appendix 3.

The altitude range analysis showed that in all clades and genera differences in the inhabited altitude amplitude occur (see Fig. 6.1). Some species of the same genus occur from 0 to 2000 m above sea level e.g. *Berzelia lanuginosa*, others show an amplitude of about 300 m *Berzelia abrotanoides*.

Thamnea is the genus with the most restricted amplitudes of all genera as all its species inhabit a very narrow altitude range (Appendix 3 and Figure 6.1)

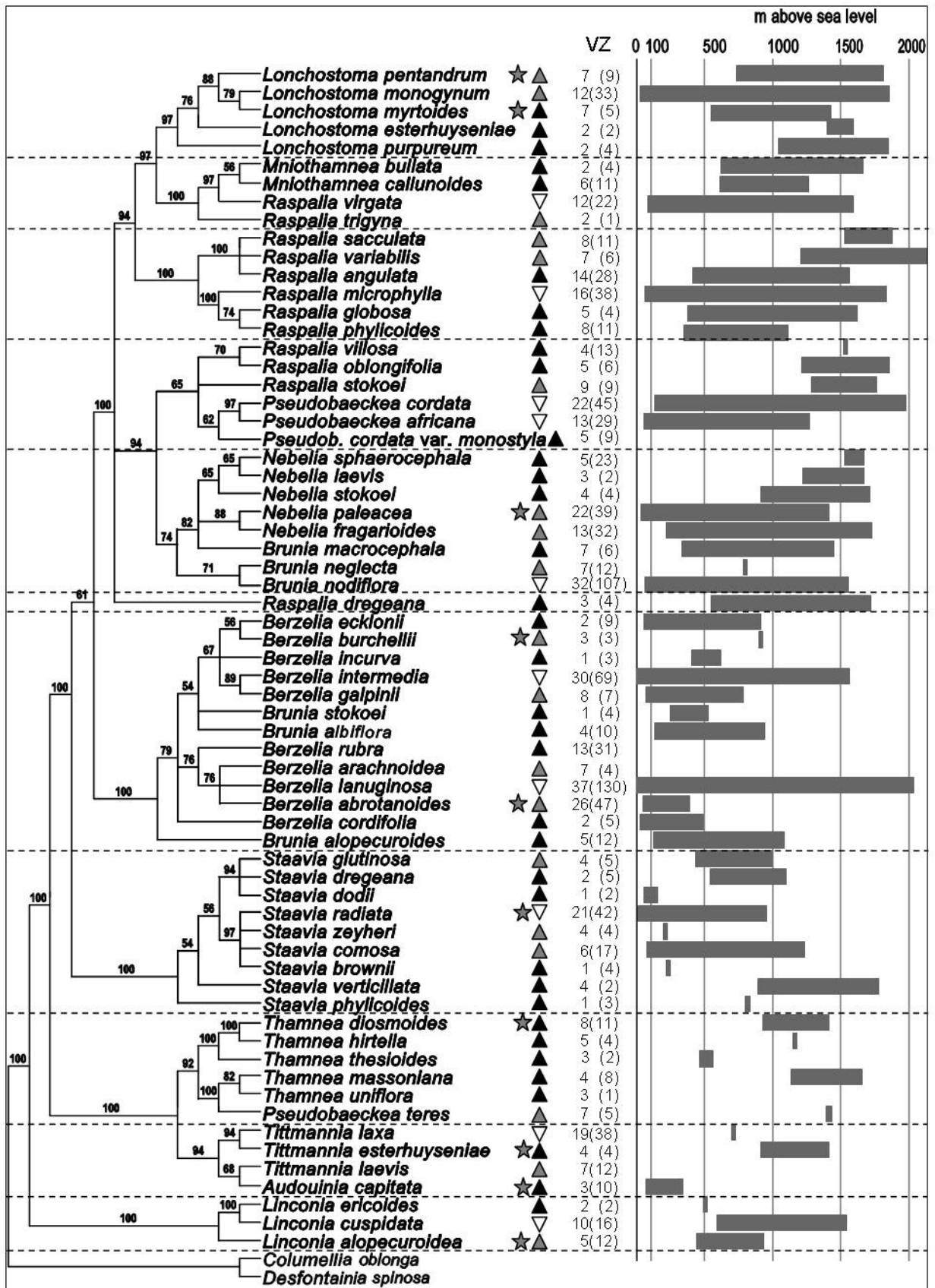


Figure 6.1: Altitudinal range and distribution information of the Bruniaceae mapped on the recent phylogeny (Quint and Claßen-Bockhoff 2006a). ★ species has a lignotuber/is a resprouter (date after Carlquist 1978); ▲ species is locally restricted (inhabits an area of less or about 10 000 km²; ▲ species distribution is extended in one direction (e.g. narrow stripe along mountain ranges, but no broad distribution) or species occurs very scattered covering a big area; ▽ wide-spread species with a distribution area bigger than 10 000 km². VZ: number of vegetation zones inhabited by the species, in bracts number of analysed localities.

6.3.2 Vegetation zone analysis

The Bruniaceae inhabit twelve vegetation zone types sensu Mucina and Rutherford (2006). Regarding the number of subtypes within the zones, the Bruniaceae grow on 20% to 93% of the vegetation subtypes (see Fig. 6.1, 6.2, 6.3 and Appendix 3). Sixty-six % of all localities analysed can be found in the Sandstone Fynbos vegetation zone. Some species like *Berzelia abrotanoides* and *Staavia radiata* show a preference to sand but in general no specific soil adaptation could be detected.

Berzelia cordifolia is said to only occur on limestone (Quint and Claßen-Bockhoff 2008) what could not be verified since referring to the herbarium sheet data the species also grows on sandstone.

Forty species occur in the vegetation zone FFs10 defined by Mucina and Rutherford (2006) that is situated in the Kogelberg region. The vegetation zones Bruniaceae grow within are: Sandstone Fynbos, Quartzite Fynbos, Sand Fynbos, Shale Fynbos, Alluvium Fynbos, Granite Fynbos, Ferricrete Fynbos, Limestone Fynbos, Shale Renosterveld, Granite Renosterveld and Western Strandveld. For detailed information about the species diversity within vegetation zones and vegetation zones inhabited by species see Appendix 3.

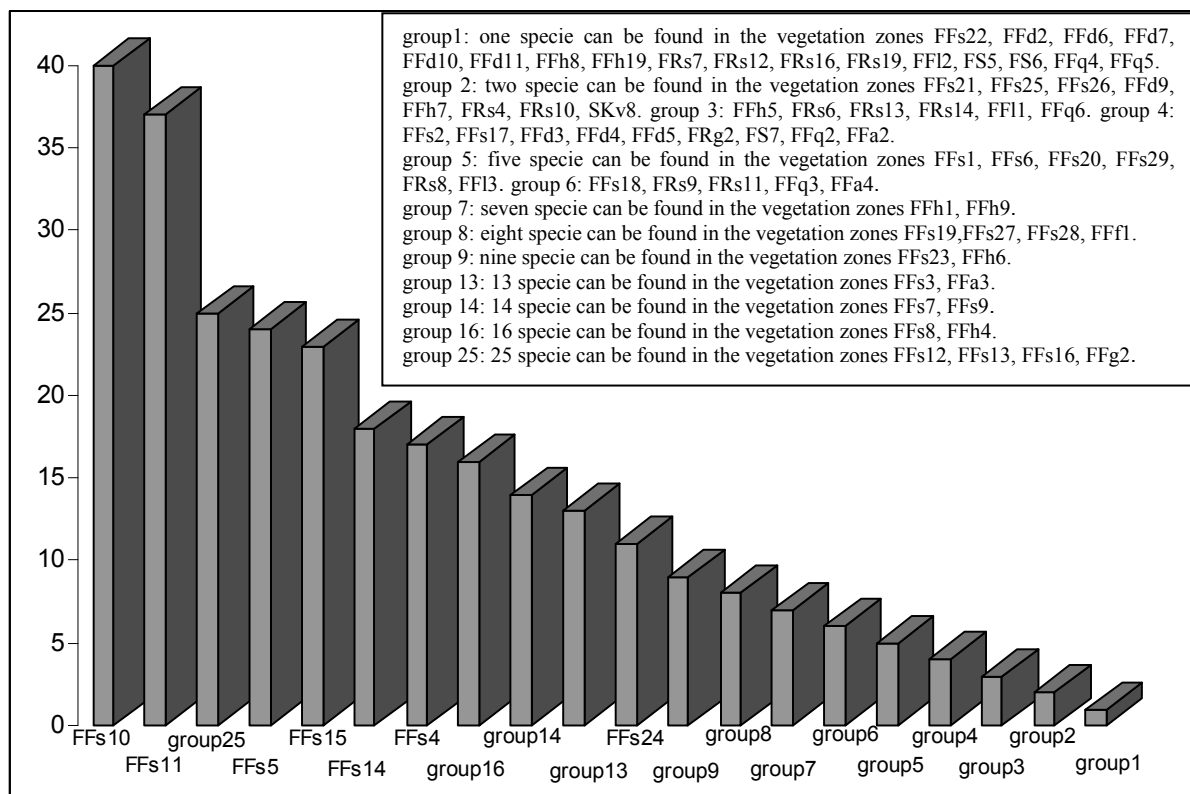


Figure 6.2: Bruniaceae species found in different vegetation zones (after Mucina and Rutherford 2006); x-axis: number of species occurring in the vegetation zones. y-axis: vegetation zones If many vegetation zones are inhabited frequently by the same number of species, they will be grouped. The group names typify the number of species that grow in this vegetation zone (e.g. group 1: one species grows in the particular vegetation zone given after the group name).

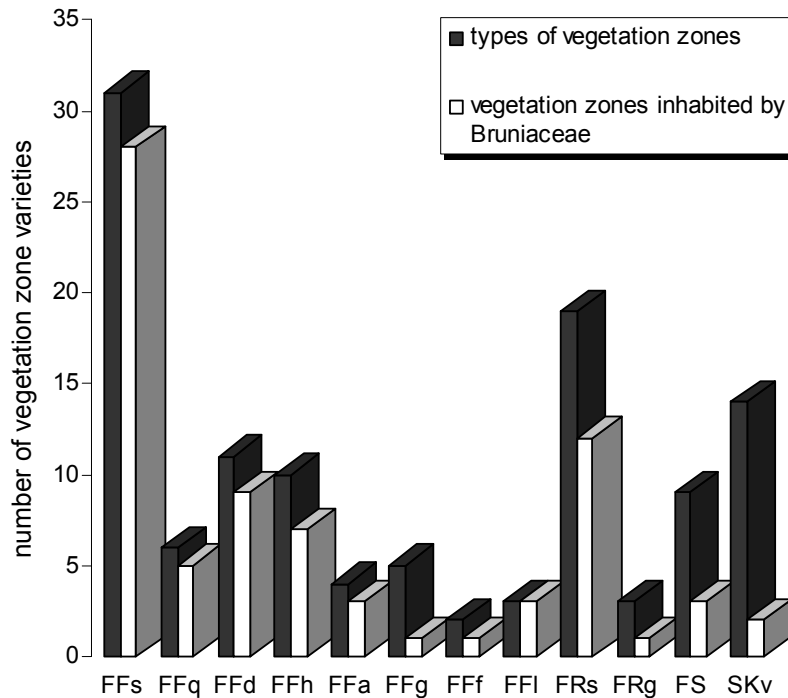


Figure 6.3: Vegetation zones after Mucina and Rutherford 2006 and their number of different varieties and number of these zones inhabited by Bruniaceae.

6.3.3 Biogeography, phylogeny and species characteristic

The data of the biogeographic information in the tables will be given referring to the species position within the clades of the phylogeny after Quint and Claßen-Bockhoff 2006a (also see Fig. 6.2).

The Linconieae

The species of the *Linconieae* are separated via their distribution area and their altitudinal range (see Tab. 6.1 and Fig. 6.1, 6.4). *Linconia cuspidata* and *Linconia alopecuroidea* are wide-spread whilst *Linconia ericoides* is only known from two localities (see Appendix 3). The found overlap of *Linconia alopecuroidea* and *Linconia ericoides* is due to a locality mentioned by Hall. *Linconia ericoides* has a narrow altitude spectrum and therefore is separated from *Linconia alopecuroidea* with a broad altitude acceptance. The flowering time of the *Linconieae* overlap but *Linconia ericoides* only flowers in October and November whilst the two other species flower from October to January and November to February.

Table 6.1: Geographical and ecological isolation between members of the Linconieae. black: strict separation on clade-level. dark grey black: weak separation on clade-level. grey: strict separation on genus-level.

species	distribution	altitude range	flowering time
<i>Linconia cuspidata</i>	Cederberg to Kogelberg area	666 m-1500 m	November-February
<i>Linconia ericoides</i>	Stormsvlei, Bonnievale	550 m	October-November
<i>Linconia alopecuroidea</i>	Villiersdorp to Riversdale	300 m-1000 m	October-January

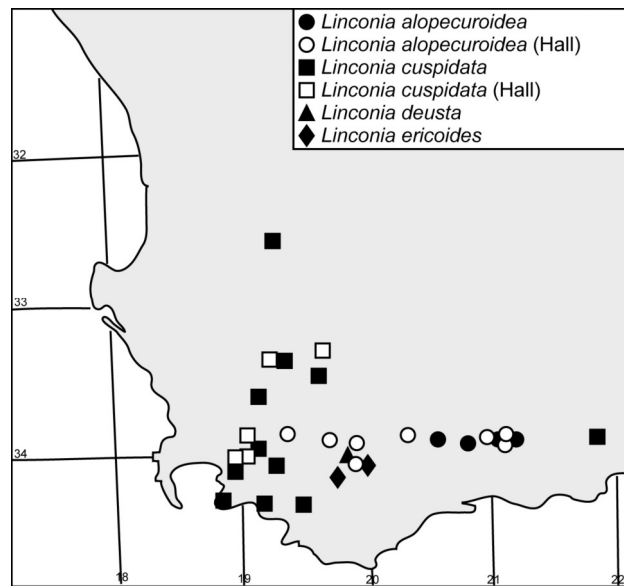


Figure 6.4: Distribution map of the Linconieae. Black signs: herbarium sheet label information, white signs: localities documented by Hall that could not be verified via herbarium data sheets

The Audouinieae

Within the Audouinieae, *Audouinia capitata* is clearly separated from the other species by its distribution area that is restricted to the whole Cape Peninsula and its altitudinal range below 100 m above sea level (see Tab. 6.2, Fig. 6.5). The three localities of which two are situated in the Kogelberg area and one above Hermanus mentioned by Hall and one locality at the Palmiet River mouth are doubtful to still exist today (information by local botanists and own field trips above Hermanus).

The *Thamnea* species exhibit an overlapping distribution but different flowering times (*Thamnea uniflora*: December-March, *Thamnea ustulata* August-November and *Thamnea hirtella* February-April). *Thamnea thesiodes* and *Thamnea diosmoides* as well are separated via altitude range: *Thamnea thesiodes* occurs between 490m -600m above sea level whilst *Thamnea diosmoides* occurs between about 800m and 1400m.

Tittmannia esterhuyseniae only flowers in March in contrast to all other species of the clade.

Pseudobaeckea teres is slightly separated via distribution and altitude range as the species occurs only above 1600m up to 2300m which is the highest populated altitude of the clade.

Table 6.2: Geographical and ecological isolation between members of the Audouinieae. black: strict separation on clade-level. dark grey black: weak separation on clade-level. grey: strict separation on genus-level. light grey weak separation on genus level. Data given in brackets refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Audouinia capitata</i>	Cape Peninsula	[5] 80 m-348 m*	May-October
<i>Tittmannia laevis</i>	Tulbagh, Ceres, Stellenbosch, Paarl	[1400] 1500 m-1933 m	October-January (March)
<i>Tittmannia esterhuyseniae</i>	Caledon, Worcester, Riviersonderend Mountains	[200]- 1000 m-1500 m	March
<i>Tittmannia laxa</i>	Cederberg Wilderness area, Paarl, Breede River	500 m-2000 m	September-January (March)
<i>Pseudobaeckea teres</i>	Grabouw, Villiersdorp, Genadendal and Ladismith	[1600] 2266 m-2300 m	February-March
<i>Thamnea uniflora</i>	Villiersdorp	[1580] 1733 m-1766 m	December-March
<i>Thamnea massoniana</i>	Kogelberg, Paarl, Stellenbosch	1333 m-1833 m	February-March
<i>Thamnea thesioides</i>	Ceres	[490] 533 m-600 m	January-March
<i>Thamnea hirtella</i>	Groot Winterhoek area, Ceres, Tulbagh	[1200] 1333 m	February-April
<i>Thamnea diosmoides</i> incl. <i>Thamnea ustulata</i>	Groot Winterhoek area, Ceres, Tulbagh	800-1400 m	September-November

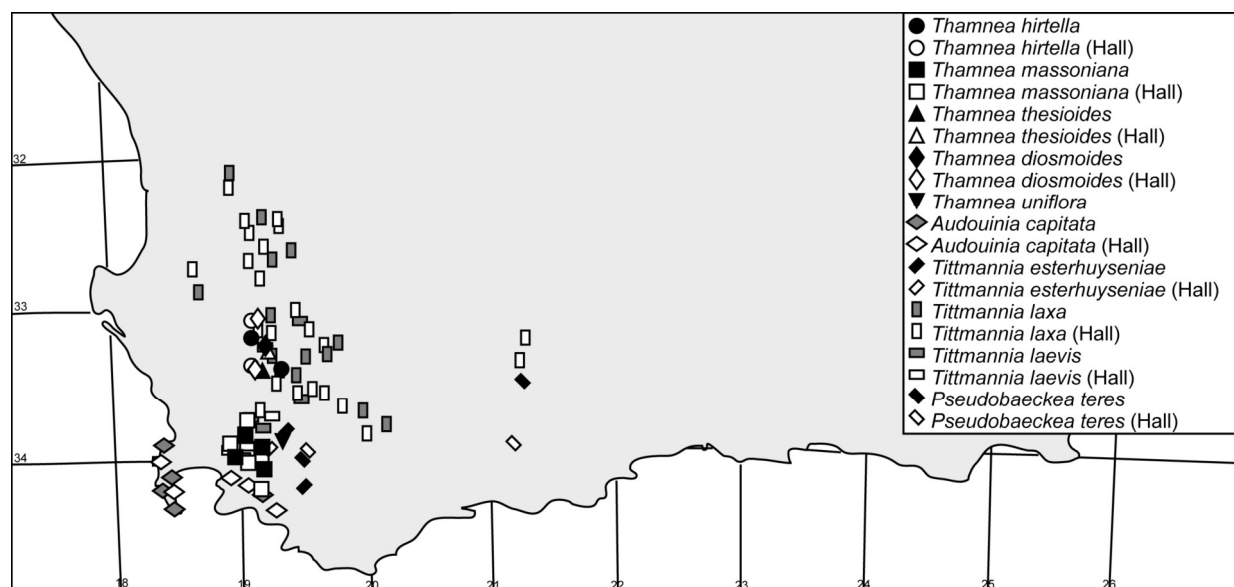


Figure 6.5: Distribution map of the Audouinieae. Black and grey signs: herbarium sheet label information, white signs: localities documented by Hall that could not be verified via herbarium data sheets

The Brunieae

The *Staavia*-branch

Staavia phyllicoides, *Staavia zeyheri* and *Staavia dodii* are separated from all other species as they only occur in the Matzikama Mountains, the Riviersonderend Mountains and

in the Cape Point Nature Reserve respectively (see Tab. 6.3 and Fig. 6.6). All other *Staavia* species do overlap in their distribution areas. *Staavia radiata* and *Staavia comosa* are the wide-spread species of the clade and both are very flexible in their altitude preference since they have an amplitude of about 1000 m, all other species are restricted to single mountain ranges.

Strictly separating altitude preferences could not be detected for any species but *Staavia verticillata* is the species that occurs with up to over 1900 m in the highest altitudes of the genus.

In general the flowering time of all species do overlap. Most species have a very long-lasting flowering time like *Staavia comosa* (throughout the year) only some species like *Staavia verticillata* flower for one to two months.

Table 6.3: Geographical and ecological isolation between members of the *Staavia*-branch. black: strict separation on clade-level. dark grey black: weak separation on clade-level. Data given in brackets refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Staavia phyllicoides</i>	Matzikama Mountains	796 m [800]	September
<i>Staavia verticillata</i>	Paarl, Worcester	900 m-1933 m	September-November
<i>Staavia brownii</i>	Kogelberg	200 m	October-February
<i>Staavia comosa</i>	Paarl, Genadendal, Elim, Bredasdorp	83 m-1333 m [1400]	throughout the year
<i>Staavia zeyheri</i>	Riviersonderend	216 m [300]	-
<i>Staavia radiata</i>	Langebaan, Bredasdorp, Humansdorp	10 m-1000 m	May-October
<i>Staavia dodii</i>	Southern Cape Peninsula	[0] 76m [200]**	April-September
<i>Staavia dregeana</i>	Cape Peninsula, one locality east of Paarl	600 m-1166 m	-
<i>Staavia glutinosa</i>	Northern Cape Peninsula	[450] 500 m-1066 m	March-October

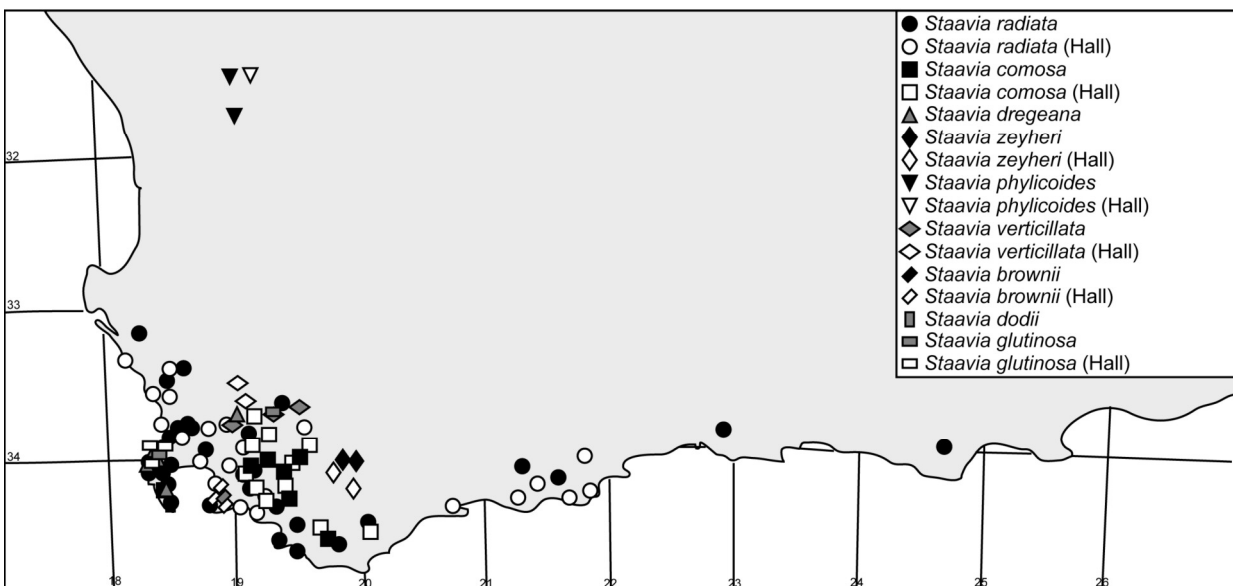


Figure 6.6: Distribution map of the *Staavia* branch. Black and grey signs: herbarium sheet label information, white signs: localities documented by Hall that could not be verified via herbarium data sheets

The *Berzelia*-clade

All *Berzelia* species overlap in their distribution area and no clear altitudinal range separation could be found (Tab. 6.4, Fig. 6.7). *Berzelia incurva* and *Berzelia burchellii* in contrast to all other species occur in a narrow altitude range. *Berzelia squarrosa*, *B. ecklonii*, *B. galpinii*, *B. incurva*, *B. burchellii* and *B. cordifolia* are locally distributed whilst the remaining four species *Berzelia commutata*, *B. intermedia*, *B. lanuginosa* and *B. abrotanoides* exhibit a rectangular distribution pattern with a northwards extension. *Berzelia incurva*, *Berzelia rubra*, *Brunia albiflora* and *Brunia stokoei* flower in summer to late summer whilst all other species of the clade mainly flower in spring.

Table 6.4: Geographical and ecological isolation between members of the *Berzelia*-clade. dark grey black: weak separation on clade-level. Data given in brackets and marked with * refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Brunia alopecuroides</i>	Stellenbosch, Caledon, Kogelberg, Stanford	[50] 140 m-1166 m	September-February
<i>Berzelia cordifolia</i>	Bree river, Potberg Nature Reserve	20 m-500 m	October-November
<i>Berzelia abrotanoides</i>	Western Cape, George	[0] 10 m-333 m [750]	August-October
<i>Berzelia lanuginosa</i>	Matzikama Mountains, Stellenbosch to Elim, Swellendam to Oudtshoorn, Port Elisabeth	3 m-2816 m	September-December
<i>Berzelia squarrosa</i>	Kogelberg ,Hermanus, Swartberg	500 m-1866 m	August-October
<i>Berzelia rubra</i>	Hermanus, Riviersonderend*	[380-969] m	February-May
<i>Brunia albiflora</i>	Kogelberg, Hermanus	[0] 15 m-1000 m	March-May
<i>Brunia stokoei</i>	Kogelberg	[50] 300 m-600 m	January-April
<i>Berzelia galpinii</i>	Langeberg Rural, Goukou River	[150] 200 m- 1166 m	August-October
<i>Berzelia intermedia</i>	Matzikama Mountains, Cape Peninsula, Stellenbosch Kogelberg, Salmondam Nature Reserve, Elim, Port Elisabeth, Grahamstown	1 m- 1666 m	November-February
<i>Berzelia incurva</i>	Hermanus	600 m- 1000 m	January-March
<i>Berzelia burchellii</i>	Riversdale, Swellendam, George	1000 m	October – November
<i>Berzelia ecklonii</i>	Kogelberg	70 m- 900 m	August-September

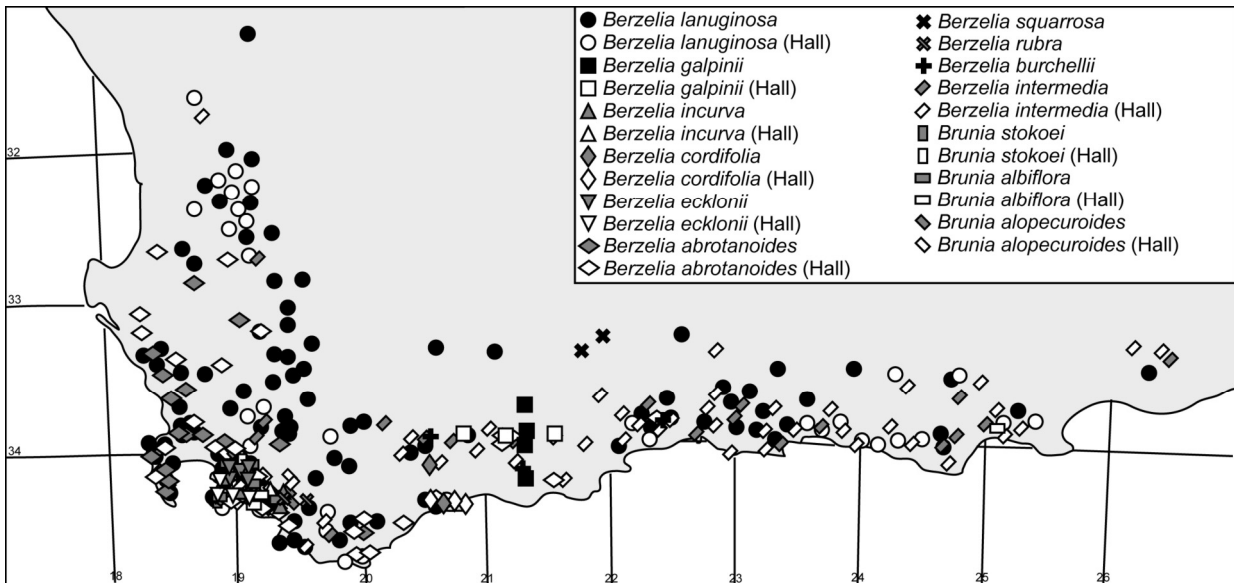


Figure 6.7: Distribution map of the *Berzelia* clade. Black and grey signs: herbarium sheet label information, white signs: localities documented by Hall that could not be verified via herbarium data sheets

Raspalia dregeana that is separately placed in the phylogeny cannot be grouped into any categories as its altitude range is very flexible and does not occur in a distinct area compared to the other *Raspalia* species. The species flowers from December until February.

The *Brunia/Pseudobaeckea*-clade

In the *Brunia/Pseudobaeckea*-clade all *Brunia* species except *Brunia macrocephala* overlap in their distribution area. *Brunia macrocephala* is the northernmost species and occurs between Ceres and the Breede River rural (see Tab. 6.5 and Fig. 6.8).

For the *Nebelia* species the same pattern holds true as all species overlap with the exception of *Nebelia stokoei* that is restricted to the Ceres Mountains.

The *Pseudobaeckea* species all overlap regarding their distribution area and altitude preference. *Pseudobaeckea africana* flowers from August to October, *P.cordata* and *P.cordata* var. *monostyla* flower from September to January and August to January respectively what distinguishes these species.

Of the three *Raspalia* species included in the clade *Raspalia villosa* differs from *Raspalia oblongifolia* and *Raspalia stokoei* by its narrow altitude range. The distribution areas of the *Raspalia* species overlap but in contrast to the other species *Raspalia oblongifolia* has a small distribution area and only occurs in the Worcester division. *Raspalia staavioides* that is not included in the phylogeny is the only species with a distinct distribution area.

Brunia nodiflora, *Brunia macrocephala* and *Nebelia sphaerocephala* start to flower from December onwards whilst all other species begin their flowering in spring.

Raspalia oblongifolia and *Raspalia stokoei* occur from an altitude of 1300 m and 1500 m respectively upwards what separates them from all other species of the clade.

Table 6.5: Geographical and ecological isolation between members of the *Brunia/Pseudobaeckea*-clade. dark grey black: weak separation on clade-level. grey: strict separation on genus-level. light grey weak separation on genus level. Data given in brackets and marked with * refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Brunia nodiflora</i>	Cederberg Wilderness, Cape Peninsula, Port Elisabeth, Bredasdorp	50 m- 1666 m	March-June
<i>Brunia neglecta</i>	Breede river, Swellendam	[100] 700 m [2400]	-
<i>Brunia macrocephala</i>	between Ceres and Breede River	400 m- 1600 m	January-June
<i>Nebelia fragarioides</i>	Salmonsdam Nature Reserve Ceres	70 m- 3450 m	August-February
<i>Nebelia paleacea</i>	Groot Winterhoek, Bredasdorp, Langebaan	50 m-1900m	September-February
<i>Nebelia stokoei</i>	Ceres mountains	[600] 1000- 1833	October-December
<i>Nebelia laevis</i>	Caledon, Worcester, Genadenal, Robertson	1500-1666	January-February
<i>Nebelia sphaerocephala</i>	Bain's Kloof, Stellenbosch	[900] 1416-2000 m	December-March
<i>Pseudobaeckea cordata</i> ssp. <i>monostyla</i>	Citrusdal, Ceres, Bain's Kloof, Du Toit's Kloof *	[900-1800]m	August-January
<i>Pseudobaeckea africana</i>	Matzikama Mountains, Cape Peninsula, Hermanus	[0] 66 m- 2000 m	August-October
<i>Pseudobaeckea cordata</i>	Cederberg, Kogelberg, Port Elisabeth	[120] 133 m- 1666 m	September-January
<i>Raspalia stokoei</i>	Cold Bokkeveld, Tulbagh, Ceres	1500 m- 2000 m	-
<i>Raspalia oblongifolia</i>	Worcester	1300 m- 2000 m	-
<i>Raspalia villosa</i>	Paarl, Cederberg	960 m-2200 m	-

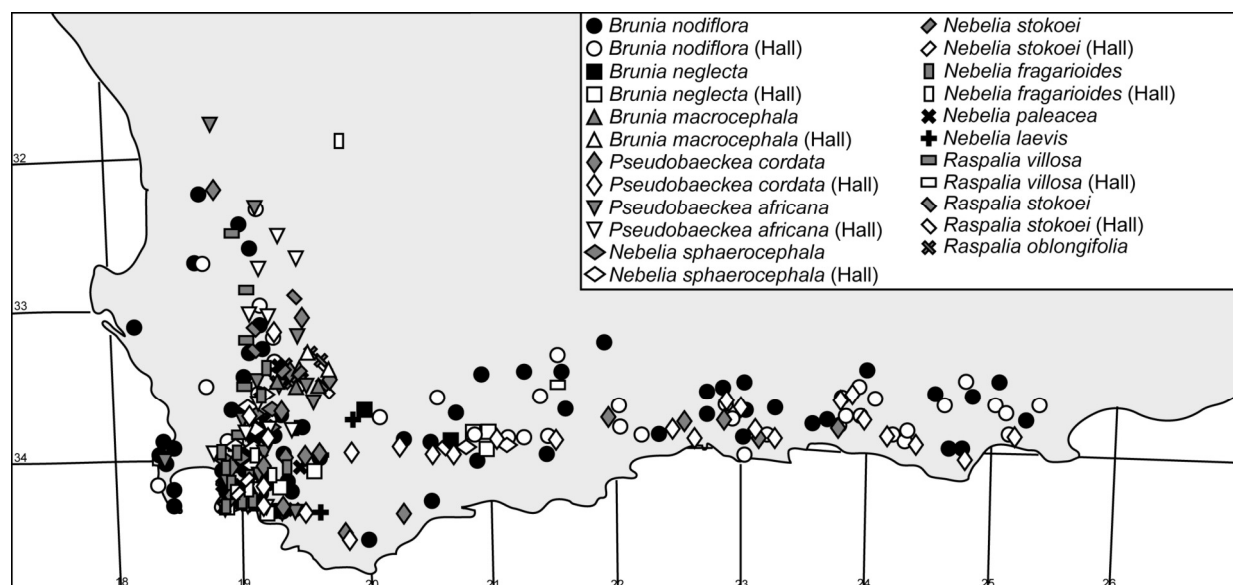


Figure 6.8: Distribution map of the *Brunia/Pseudobaeckea*-clade. Black and grey signs: herbarium sheet label information, white signs: localities documented by Hall that could not be verified via herbarium data sheets.

The *Raspalia* II-branch

All species of the *Raspalia* II branch overlap in their distribution area and altitude range (see Tab. 6.6 and Fig. 6.9). *Raspalia sacculata* only occurs above about 1666 m and *Raspalia phyllicoides* only inhabits altitudes up to 1100 m. In general the flowering seasons overlap but in the case of local co-occurrence e.g. between *Raspalia microphylla* and *Raspalia globosa* that flower from October to January and March to June respectively.

Table 6.6: Geographical and ecological isolation between members of the *Raspalia* II-branch. black: strict separation on clade-level. dark grey black: weak separation on clade-level. Data given in brackets refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Raspalia phyllicoides</i>	Kogelberg, Riviersonderend	400 m-1666 m	February-April
<i>Raspalia globosa</i>	Hottentot Holland Mountains, Ceres, Stormsvlei	400 m- 1666 m	throughout the year except the winter months
<i>Raspalia microphylla</i>	Kogelberg Stellenbosch Riviersonderend, Hottentot Holland Mountains, Montagu, Prince Albert	[0] 80 m-2000 m	September-January
<i>Raspalia angulata</i>	Cape Peninsula, Kogelberg, Stellenbosch, Ceres, Barrydale, Swartberg	400 m- 2333 m	September-April
<i>Raspalia variabilis</i>	Stellenbosch, Kogelberg, Riviersonderend, Ladismith	1333 m- 2300 m	-
<i>Raspalia sacculata</i>	Tulbagh, Paarl district, Wellington, Worcester	1666 m- 2000 m	-

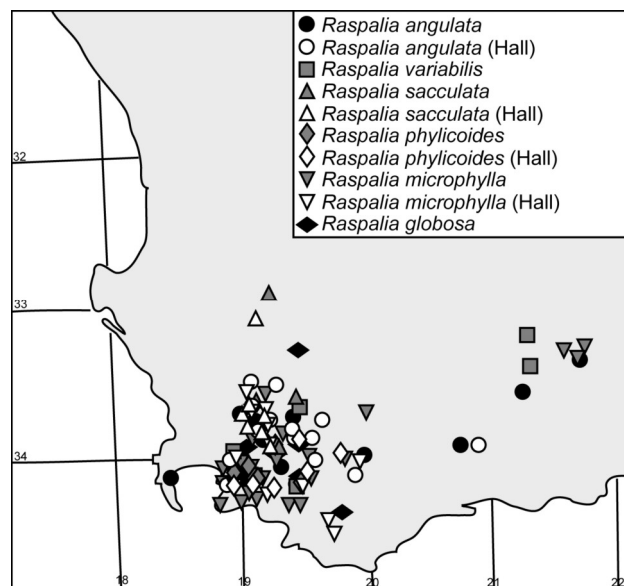


Figure 6.9: Distribution map of the *Raspalia* II-branch. Black and grey signs: herbarium sheet label information, white signs: localities documented by A.V. Hall that could not be verified via herbarium data sheets.

The *Mniothamnea*-clade

In the *Mniothamnra* clade, the *Raspalia* outlier *Raspalia trigyna* can be found than aside the Langeberg Mountains, the Eastern and Western Cape (A.V. Hall , unpublished data but without detailed localities; no herbarium sheets in the used Herbariums) also occurs in Kwazulu Natal (see Tab. 6.7 and Fig. 6.10). An additional separating factor of *Raspalia trigyna* is its flowering time from May until October whilst all other species flower in time frame from September to February. *Raspalia virgata* as well as the *Mniothamnea* species have a broad altitude range and do overlap in the altitude preference and their very scattered distribution.

Table 6.7: Geographical and ecological isolation between members of the *Mniothamnea*-clade. black: strict separation on clade-level. dark grey black: weak separation on clade-level. Data given in brackets refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Raspalia trigyna</i>	Cape, Kwazulu Natal	?	May-October
<i>Raspalia virgata</i>	Stellenbosch, Langeberg, Paarl, Robertson, Ladismith	100 m- 1733 m	September-October
<i>Mniothamnea callunoides</i>	Hottentot Holland Mountains	[600] 666 m- 1333 m	September-February
<i>Mniothamnea bullata</i>	Swellendam	[450] 666 m- 1766 m	(September) December-January

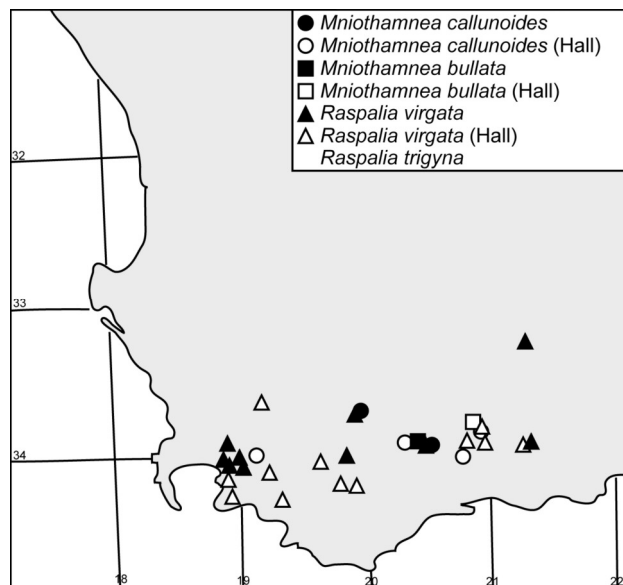


Figure 6.10: Distribution map of the *Raspalia* II-branch. Black signs: herbarium sheet label information, white signs: localities documented by A.V. Hall that could not be verified via herbarium data sheets

The *Lonchostoma*-branch

In the *Lonchostoma*-branch all species overlap in their distribution area (see Tab. 6.8 and Fig. 6.11). *Lonchostoma monogynum* inhabits the highest altitude amplitude and with six months exhibits the longest flowering time Only *Lonchostoma esterhuyseniae* and *Lonchostoma purpureum* are restricted to an altitude above 1200 m and 1100 m respectively.

Table 6.8: Geographical and ecological isolation between members of the *Lonchostoma*-branch. black: strict separation on clade-level. dark grey black: weak separation on clade-level. Data given in brackets refer to information given by A.V. Hall .

species	distribution	altitude range	flowering time
<i>Lonchostoma purpureum</i>	Stellenbosch, Kogelberg	[1100] 1166 m- 2000 m	October-December
<i>Lonchostoma esterhuyseniae</i>	Riviersonderend Mountains	[1200] 1500 m- 1733 m	November
<i>Lonchostoma myrtoides</i>	Ceres, Wellington, Clanwilliam	[550] 600 m-1500 m	October-December
<i>Lonchostoma monogynum</i>	Citrusdal, Kogelberg, Worcester, Hangklip, McGregor, Robertson	30 m-2000 m	August-January
<i>Lonchostoma pentandrum</i>	Cederberg, Stellenbosch	800 m-1750 m	September-November

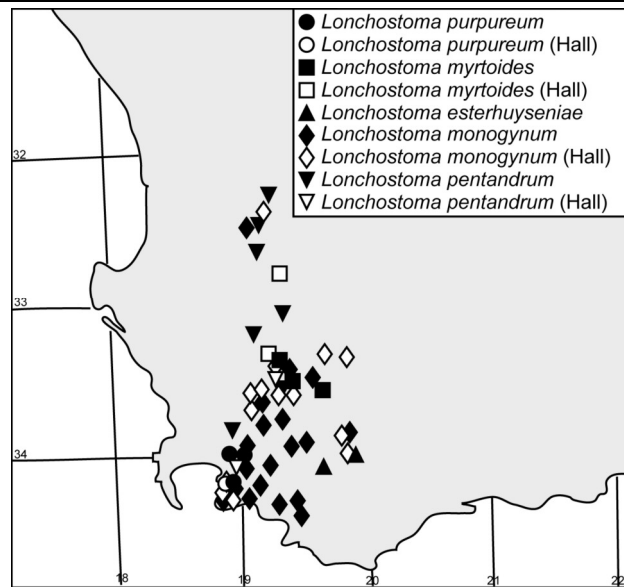


Figure 6.11: Distribution map of the *Lonchostoma*-branch. Black signs: herbarium sheet label information, white signs: localities documented by A.V. Hall that could not be verified via herbarium data sheets

6.4 Discussion

6.4.1 Adaptation to certain soils or annual precipitation

Regarding the habitat structure of the fynbos, the striking diversity is realised in terrain and thicket height, population density, exposition to sun and wind, soil composition and humidity (Stock and Lewis 1986; Campbell 1986; Cowling 1992; Cowling et al. 1997; Cowling and Heijnis 2001; Mucina and Rutherford 2006). Within this biome, the Bruniaceae are an endemic element until now has not been not analysed regarding habitat adaptation and its influence on recent distribution patterns and speciation events throughout the past.

The Kogelberg area situated in the South Western part of the Cape that was already found to be a local hotspot (Boucher 1978) within the Cape Floristic Region hotspot (Myers et al. 2000) was found to be the 'Bruniaceae hot spot' what might indicate this to be the origin of this plant family or at least the area with the best living conditions. Two main distribution patterns of the Bruniaceae species could be detected: wide-spread species are distributed in a tubular N-S or E-W direction. Species with smaller distribution areas exhibit a more circular pattern. The reason for this distribution patterns can be a) the adaption to certain soil conditions, b) the dependence on a certain precipitation, c) the occurrence in ecological niches.

Adaption to certain soil conditions

Since 66% of all localities analysed occur on Sandstone Fynbos, the establishment on other soil types can be an assumed flexibility regarding the soil conditions. No species could be found that exclusively grows on other soils. *Berzelia cordifolia* was said to be a limestone-endemic (Quint and Claßen-Bockhoff 2008) what could not be verified since referring to the herbarium sheet analysis and the mapping on the vegetation zone analysis of Mucina and Rutherford (2006) this species as well occurs on sandstone soil. Further analyses have to show if there are low-range patches of differing soil in the overall sandstone layer that would verify the assumed adaptation to limestone. The local analysis at the Vogelgat Nature Reserve would stand as an example for soil adaptation on low-range level that cannot be resolved by the vegetation zone analysis of Mucina and Rutherford (2006)

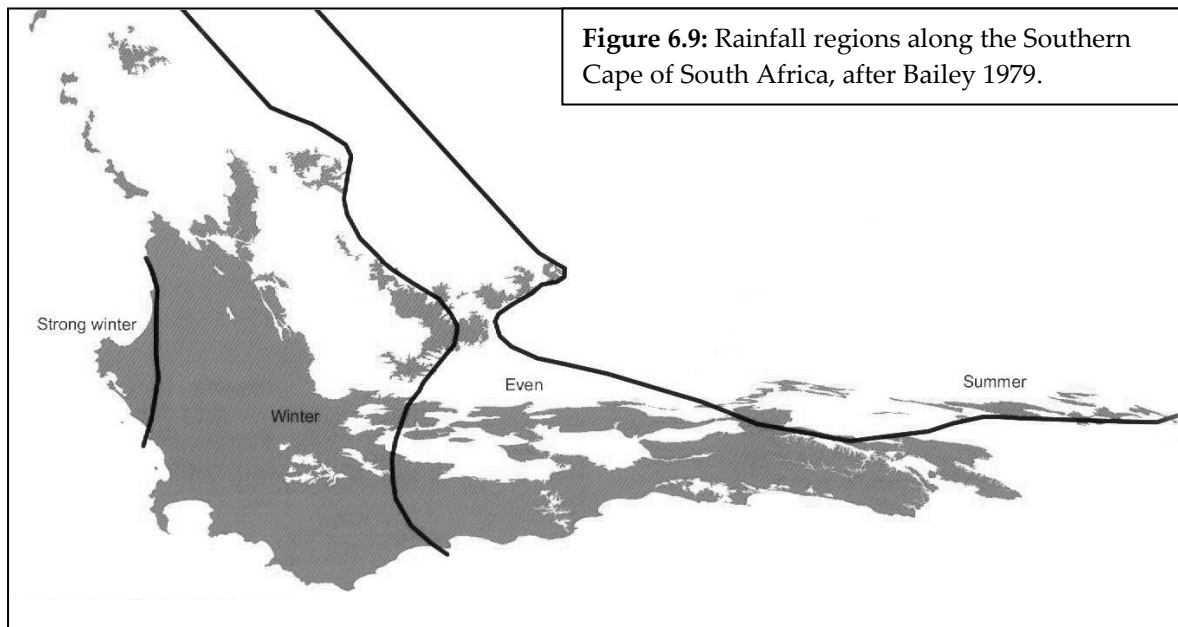
Dependence on a certain precipitation

Regarding the rainfall regions, most species mainly occur in the winter-rainfall regions (Mucina and Rutherford 2006). The Bruniaceae 'hot spot', the Kogelberg area shows the highest mean annual rainfall (> 1300 mm) in the Western Cape (Mucina and Rutherford 2006) and rainfall is highly dependant on slope orientation and altitude (Boucher 1982). Wide-spread species of the genus *Berzelia* also occur

frequently along the even rainfall zones and few *Berzelia*-species occur in the summer rainfall areas (see Fig. 6.9, after Bailey 1979).

Water availability seems to be a key element in determining the occurrence and distribution of species and aside rainfall, mist is an important factor in the mountains of South Africa since it occurs in summer especially under southeasterly conditions (Schulze 1997; Mucina and Rutherford 2006).

Bruniaceae are an element of the ericaceous fynbos (Campbell 1986). This fynbos type is an extremely mesic community mainly occurring on coastal south-facing slopes with much summer precipitation in form of mist lacking the summer-drought of many regions of the Cape (Campbell and Werger 1988). A study about the fog-water availability along the West Coast of South Africa showed that the main fog zone can be found below the 200 m contour line with fog frequency decreasing with latitude within this zone (Olivier 2002). In contrast to this, the fog zone of the Table Mountain, the tablecloth occurs around 600 m and up to the summit at about 1000 m above sea level (Marloth 1904). Regarding the analysis of altitude ranges of the Bruniaceae there is no correlation between the 600 m mist zone observed at Table Mountain and the occurrence of Bruniaceae species. The species show a very flexible altitude range and do not cumulate around the 600 m contour line. But it cannot be dismissed that mist appearing dependently on the local mountain range altitudes and therefore occurring in different altitudes accounts for better moisture supply for the species in this area.



6.4.2 Occupation of niches and the influence of habitat structures on speciation and migration

Detected species isolating factors in Bruniaceae can be subdivided in geographical (distribution, altitude) and ecological (pollinators, morphology, flowering times). However, since many species of the same genus co-occur, the question arises if niche-formation occurs.

A niche is defined as the sum of biotic and abiotic conditions that allows a species to persist and maintain a stable population (Hutchinson 1957).

Niches can be conservative (Peterson et al. 1999; Prinzig et al. 2001) or evolutionary labile (Böhning-Gaese et al. 2003; Losos et al. 2003). The question if a niche in a fire-prone region can be stable is answered when fire is a factor for niche-formation (Cowling 1987; Johnson 1996). The fact that two species occur in the same habitat can be an indicator that the split of both species a) took place in the nearer past and is not performed completely, b) no split was necessary as adaptations of both species allow the co-existence without any disadvantages for their reproductive success. In the case of *Berzelia lanuginosa* and *Berzelia abrotanoides* it can be presumed that no split was necessary as the species are reseeder and resprouter and more important the altitude amplitude where they occur differs significantly. While *Berzelia lanuginosa* occurs from 0 to 2000 m above sea level, *Berzelia abrotanoides* occurs between 10 and 300 m above sea level. This pattern of altitude range differences between species of the same genus can be found in nearly all clades of the Bruniaceae. This could be an indicator that periodic fire events and the ongoing colonisation and retraction of distribution areas especially took place along an altitude gradient. The resulting altitude ranges of species of one clade could be the consequence of speciation events of altitudinal isolated populations. This indicated that the proceeded altitudinal zonation is the main cause for possible speciation events and soil preferences of species are of lower importance.

A scenario for the forming and colonisation of niches in Bruniaceae could be the existence of wide-spread ancestral prototype species that were sub-divided into metapopulations due to recurrent fire, human impact or both. This could have lead to a permanent isolation of populations from the ancestral prototype species and in these isolated populations to an adaptation to niches, resulting in speciation events. Isolation effects can get enforced by the absence of far flying insects.

Analyses that correlate species diversity and distribution can manifest speciation events and the effects of geography on evolutionary processes (Nelson and Platnik 1981; Barraclough and Nee 2001). Habitat isolation via topology enforced by events has already been discussed as presumed force that drove speciation in the

Bruniaceae (Chapter 4 and 5). Now the present data enables to discuss speciation events as a cause of isolation mechanisms and niche inhabitation.

Regarding the distribution pattern present today, different possibilities of speciation and migration processes could be supposable:

1) species with big distribution areas are the ancestral prototype that has been stable enough to keep its distribution area until today; species with a very scattered distribution area are the result of migration and retraction of the ancestral species thereby forming metapopulations that diversified. The species can occur either allopatrically or parapatrically due to once again habitat fragmentation or retraction and sympatrically.

2) the locally restricted species are the prototype species and genetic events like polyploidisation or mutations enabled the new species to enlarge its distribution area; recent species with a wide-spread area would then be the migrating progeny of the locally still restricted ancestral species. Both can occur allopatrically, parapatrically as well as sympatrically.

3) the third possibility is a secondary conjunction of species that were formerly separated and now occur sympatrically. The sympatry can comprise scattered and wide-spread species, different scattered species or different wide-spread species.

The found biogeography of the Bruniaceae implies that all three patterns are imaginable and the recent distribution does not entirely exclude any of these scenarios. Assuming allopatric speciation, the underlying geographic separation would be associated with niche conservatism (Wiens 2004). In the case of sympatric speciation conditions an adaptation to micro-habitats within the distribution area could be the reason for diversification and sympatrically occurring species can become parapatric if the adaptation to a certain area excludes the ancestral species and forms separate populations (Wiens and Graham 2005). Since the distribution pattern shows distinct as well as sympatrically occurring species e.g. in the genus *Berzelia*, for Bruniaceae both scenarios are assumable. For the genus *Berzelia* this would indicate that after the first hypothesis e.g. *Berzelia lanuginosa* as a wide-spread species could be the ancestral state, after the second hypothesis the ancestral species situation is not clear. Regarding the phylogeny after Quint and Claßen-Bockhoff 2006a no locally restricted species has a position that would clearly indicate an ancestral species possibility. *Berzelia cordifolia* that could be discussed as basal within the *Berzelia*-clade is locally restricted but its localities can be found more than 100 km eastwards from the Bruniaceae hot spot, the Kogelberg area. In the Kogelberg area *Berzelia ecklonii* and *Berzelia incurva* occur but the *matK*/ITS data of Quint and Claßen-Bockhoff 2006a do not provide evidence these two species to be basal. The question if any of these species is the ancestral one and if wide-spread or locally restricted is the

ancestral state cannot be answered since no fossil record could account for the absence of extinct species that as well could be an ancestral stage. The example showed that in general it is highly speculative to predict an ancestral species and isolating mechanisms that led to speciation without focussing on ecological mechanisms that separates species.

6.4.3 Ecological and geographical separation within clades

Geographical and ecological differences between species of clades of the recent phylogeny could be detected. Distribution pattern, distinct locality inhabitation and altitude preferences as well as the flowering time are separating factors were detected with different specificity. In clades like the Linconieae all species are perfectly distinguishable referring to their ecology or distribution. Otherwise the *Berzelia*-clade can not be separated via distribution and there are only some weak differences in altitude range and flowering time.

These differences between the clades could be due to their age since after the 'age and area hypothesis' the older a species is, the more time did it have to cover an area and the age of a species is of great importance for its geographical distribution (Willis 1922). In contrast for Proteaceae ecological rather than macro-evolutionary time-scale seems to be the determining factor of range dynamics (Schurr et al. 2007). The divergence time of the clades indicates that some genera are very young (Quint and Claßen-Bockhoff 2008). The found results allow the assumption that *Berzelia* with a divergence time of only 5-3 Mya (Quint and Claßen-Bockhoff 2008) is not completely speciated. *Berzelia* species are neither geographically, nor ecologically, nor in some cases morphologically distinguishable what supports the theory that the species are not yet segregated. The genus seems to be stable, but described hybrids (A.V. Hall, unpublished data) indicate that on species level reproductive isolation is not yet realised. In contrast to *Berzelia*, earlier diverged genera like *Linconia* (99.5-59.7 Mya, Quint and Claßen-Bockhoff 2008) have well established species that are geographically and ecologically distinct. Thereby the ecological and geographical data of the present study support the assumption that the Bruniaceae are an ancient family, but exhibits recent speciation events (Quint and Claßen-Bockhoff 2008).

The present chapter illuminated that species persistence of the Bruniaceae is due to niche-inhabitation and geographical isolation (horizontal and altitudinal). Ecological isolation mechanisms like pollinator composition and variations regarding the breeding system, seed propagation and life-form are adaptations to the ecosystem Fynbos that enhance species persistence but are not the causal reason for species sustainability.

7. General discussion

The present study illuminated that the species diversity of the observed Bruniaceae is driven by geographical preconditions and niche-inhabitation. Plant-pollinator interactions and breeding system diversity have to be seen as adaptation to the Fynbos biome and enhance survival in this fire-prone habitat but cannot be determined as speciation inducing factors. An important factor influenced by the Fynbos ecology is the occurrence of the two life-form reseeding and resprouting.

7.1 Resprouter vs. reseeder: the breeding system

Being a resprouter a plant is able to survive fire without being exclusively dependant on high seed set (Carlquist 1978). The own genes will survive anyway and the breeding system can be directed to outcrossing. In the case of the resprouting species observed in this study, two different patterns of breeding systems could be found. In *Berzelia abrotanoides*, the temporal dioecy on population-level combined with a short anthesis approves the theory that the reproduction can focus on fast but maybe inefficient anthesis in resprouting plants. However two other resprouting species, *Nebelia paleacea* and *Staavia radiata* have a cyclic thereby elongated anthesis and a very long flowering time respectively. This would contradict to the 'reproduction-independence' theory that holds true for *Berzelia abrotanoides*.

In contrast the reseeding species should show an anthesis that focuses on an assured reproduction. All reseeding species observed except *Brunia alopecuroides* showed a high percentage of seed set and all exhibited an elongated anthesis via cyclic flowering pattern on inflorescence level. Thus they are enabled to generate a big seed stock below the mother plant until the next fire. For species like *Berzelia lanuginosa* it can be assumed that since they are self-compatible and the cyclic flowering pattern proceeds within narrow spaces, geitonogamy has not entirely to be precluded. Under unfavourable weather circumstances or insufficient pollination, geitonogamy ensures at least a certain quantity of seeds (Finer and Morgan 2003). The same holds true for *Pseudobaeckea africana* since the species grows in water and many seeds will be drifted without establishing in the flooding water. Under pollinator-exclusion 50% of the regularly seed set could be documented in *Pseudobaeckea africana*.

7.2 Fynbos ecology and distribution

The very diverse habitat structures ranging from seaside habitats, mountain fynbos and steep, rocky mountainous areas in closest proximity facilitate niche inhabitation. The found characteristics of these habitats and the species composition therein illuminated the establishment of niches or at least dominance of species under certain conditions. The Overberg region is the "hot spot" and therefore an assumed optimal

habitat for Bruniaceae. However wide-range and long-term analyses have to show if the found habitat flexibility of some species in contrast to the strict niche inhabitation of others is representative for all members of the family. This can suggest if niche-inhabitation or flexibility is the more successful or advantageous state within the Bruniaceae.

Some species are said to be highly endangered what has to be put into perspective and surely cannot be generalised. *Audouinia capitata*, a species that is listed on the Red Data list (SANBI 2007) is extinct in some parts of the Cape (e.g. above Hermanus) but can be found with hundreds of individuals in the Hout Bay area. This illuminates that detailed studies of shifting habitat conditions or the lack of fire as revitalising force have to be conducted. A lack of fire can also be stated for *Lonchostoma monogynum* in the Vogelgat and Maanskynekop Nature Reserve as the populations decreased in size throughout the last years and in the Vogelgat Reserve could not be reconfirmed at all. The ability of fynbos species to recover after fire and to form stable populations again has to be taken into account when declaring species as endangered.

7.3 Speciation and species persistence

The phylogeographic analysis showed that reseeders and resprouters cannot be distinguished via diverse or uniform haplotypes within populations respectively. However increasing distance between populations results in increasing genetic differences.

Especially fire events and thereby the forming of metapopulations seems to be a key factor for speciation via isolation resulting in allopatric speciation in the Bruniaceae. This is reflected in the distribution pattern that shows species of one genus to occur wide-spread as well as locally restricted and distinct.

As many species of the same genus inhabit the same area, it has to be taken into account that speciation in the Bruniaceae could as well have proceeded sympatrically or at least could have started sympatrically (Kondrashov and Mina 1986 and literature therein). The fact that many locally restricted species co-occur with wide-spread species might speak for sympatric speciation (Tauber and Tauber 1977 a, 1977b).

After all the so far discovered facts about Bruniaceae do not allow to commit to either sympatrically or allopatrically speciation. It can be assumed that a mixture or alternation of both speciation forms seem reasonable for the Bruniaceae.

This supports the finding that speciation events in the fynbos are said to be a gradual in situ process rather than a migration event what means that speciation in the fynbos has proceeded after the primary dispersal of the relevant ancestral taxa (Deacon 1983). The more recent species evolved from an assumed ancestral species

via habitat isolation, changing flowering times, changing morphology as adaptation to an altitude shift and also as a possible adaptation to a at this time dominant pollinator. An ancestral species being a member of the recent a) wide-spread type or b) locally restricted type cannot be defined as both scenarios seem plausible.

The result of clearly species-separating geographical and ecological factors in only some clades of the recent phylogeny (Quint and Claßen-Bockhoff 2006a) leads to the conclusion that the forming of stable and distinct species has not been completed for all clades.

An example for a clade that can not be distinguished via strict ecological or geographical facts and as well in some cases is hardly to determine morphologically is the *Berzelia* clade.

Berzelia after Quint and Claßen-Bockhoff 2008 assumingly diverged 5-3 million years ago and thereby is a very recent evolved genus. In contrast to the *Berzelia* clade, the Linconieae as the basal most clade diverged 99.5-59.7 million years ago (Quint and Claßen-Bockhoff 2008). The fact that all Linconieae are geographically and/or ecologically separable argues for the longer period of time of species stabilisation. However, the Linconieae possibly due to extinction events have much less species than the *Berzelia* clade, what is an irresolvable question because of lacking fossils.

The results of the phylogeographic analysis indicate that the young genus *Berzelia* undergoes niche-shift what means the genetic refinement that integrates this population in its new habitat is still proceeding (Levin 2003). A *Berzelia lanuginosa* populations of the eastern part of the Cape, is genetically still connected with the Western Cape type but already shows morphological differences due to polyploidisation (Greilhuber pers.comm.).

Further analyses have to show how stable the species of the *Berzelia* clade are and which factors can lead to a steady formation of distinguishable species.

In conclusion the Bruniaceae has to be stated as a well-adapted plant family of the fynbos, a biome whose ecology predefined the life-form of its members. The breeding system, the mainly generalistic pollination and the seed dispersal are affected by this life-form that as well is reflected in distribution and phylogeography. As ecological isolation mechanisms they enhance the establishment of species but the causal reason for species diversity are niche-occupation and geographical isolation (horizontal and altitudinal). Bruniaceae are a valuable member of the fynbos biome and a fascinating example of the evolution of the Cape flora. Further investigations surely will discover even more details of other genera of the Bruniaceae and can help to support the conservation of this (sub-) endemic family of the Cape flora.

7.4 Summary

The central aim of the present study was to analyse ecological and geographical mechanisms that led to the species diversity and distribution pattern of the (sub-) endemic Bruniaceae shown today. To answer the question if the endangerment of some species and the sometimes restricted distribution area is due to an incongruence of pollination and breeding system, pollinator observations and the underlying breeding system were analysed.

The effectiveness of the plant-pollinator interactions should be reflected in the reproductive success wherefore fruit set analyses were carried out. The genetic constitution of distant and close-by populations along a spatial gradient should illuminate gene-flow or habitat isolation that could have led to the species diversity. Since niche-inhabitation could be shown in the present study, an overall biogeographical analysis illuminated the distribution pattern on family level and the geographical as well as ecological factors that led to species persistence.

The study illuminated that the plant-pollinator interactions and the breeding system are adaptations to the fynbos biome but can not be defined as factors that drove speciation or have tremendous influence on distribution of Bruniaceae. In fact the geography of South Africa with its fragmented landscape as well as close niche-inhabitation of co-occurring species is the reason for species diversity. The projection of the present data on the phylogeny illuminated that Bruniaceae comprise well-established clades as well as clades that right now undergo detectable speciation processes.

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Annotation: Shortly after this study had been submitted to the deanery of the department of biology (Johannes-Gutenberg Universität Mainz, Germany), a new nomenclature of the Bruniaceae has been accepted:

Claßen-Bockhoff, R., Oliver, E.G.H., Hall, A.V., Quint, M. 2010 (accepted). New classification of the Bruniaceae based on molecular and morphological data. *Taxon*.

Since the new nomenclature had not been accepted until the submission date of this study, the genus and species names according to Pillans (1947) will be retained.

Appendix 1 Plant -pollinator interactions

Table 1: Descriptions of the observed insects at all localities. K= locality at the Kirstenbosch Botanical Garden; E = locality Eastern Cape, Storms River Village. C = captured.

Coleoptera	size (length)	special trait, colour, hairs, body shape	plant species the insects were captured on
Curculionidae, Curculioninae, <i>Curculio</i> spec. C	1.8	greyish/whitish brown patterned elytrons	<i>Berzelia lanuginosa</i>
Rutelidae, Hopliinae, <i>Hoplia</i> spec. C	8 mm	many black hairs, brownish	<i>Berzelia lanuginosa</i>
Chrysomelidae, Eumolpinae C	2mm	metallic shining, crooked	<i>Berzelia lanuginosa</i>
Rutelidae, Hopliinae <i>Hoplia</i> spec. C	8 mm	stripped elytrons	<i>Berzelia lanuginosa</i>
Chrysomelidae, C Eumolpinae	3 mm	green metallic shining, long antennae, brown legs	<i>Berzelia lanuginosa</i>
Chrysomelidae, C Eumolpinae	2-3 mm	green metallic shining, spheroidal	<i>Berzelia lanuginosa</i>
Chrysomelidae, C Eumolpinae	2 mm	black metallic shining elytrons	<i>Berzelia lanuginosa</i>
Melyridae C	4 mm	spheroidal, shining greenish/reddish metallic	<i>Berzelia lanuginosa</i>
Curculionidae, Curculioninae, <i>Curculio</i> spec.	~ 3-5 mm	reddish brown	<i>Berzelia lanuginosa</i>
Scarabaeidae, Cetoniinae, <i>Pachnoda sinuata</i>	25 mm	black-yellow patternend	<i>Berzelia lanuginosa</i>
Meligethinae, Melyridae	8 mm	beige/brown-black striped elytrons	<i>Berzelia squarrosa</i>
Nitidulidae, Meligethinae <i>Meligethes</i> spec.	2.5 to 3.5 mm	dark brown to black, spheroidal	<i>Berzelia squarrosa</i>
Curculionidae, Curculioninae, <i>Curculio</i> spec.	3 mm	dark brown	<i>Berzelia squarrosa</i>
Rutelidae, Hopliinae, <i>Hoplia</i> spec.	8 mm	covered in black hairs, goldish brown elytrons	<i>Berzelia squarrosa</i>
K Scarabaeidae Cetoniinae, <i>Cyrtothyrea marginalis</i>	5 mm	black elytrons, white dots	<i>Berzelia squarrosa</i>
K Mordellidae	3 mm	black	<i>Berzelia lanuginosa</i>
K Cerambycidae, <i>Promeces longipes</i> cf.	35 mm	bright green metallic	<i>Berzelia lanuginosa</i>
K Melyridae, Malachiinae	4 mm	green metallic shining, red pronotum, single hairs	<i>Berzelia lanuginosa</i>
K Scarabaeidae, Cetoniinae <i>Cyrtothyrea capensis</i> cf.	13 mm	red elytrons with whitish dots; hairy	<i>Berzelia lanuginosa</i>
K Nitidulidae, Meligethinae <i>Meligethes</i> spec.	2-3 mm	dark brown to black	<i>Berzelia lanuginosa</i>
K Dermestidae <i>Anthrenus</i> spec.	2 mm	black/white patterned, short hairs all over the body	<i>Berzelia lanuginosa</i>
K Chrysomelidae, Galaerucinae	4-5 mm	gold olive, apical antennae and tarsae black, all other extremities ochery	<i>Berzelia lanuginosa</i>
K Rutelidae, Hopliinae <i>Hoplia</i> spec.	6 mm	very hairy	<i>Berzelia lanuginosa</i>
K Curculionidae, Curculioninae, <i>Curculio</i> spec.	4 mm	mint green	<i>Berzelia lanuginosa</i>
E Mordellidae	3 mm	black with a spine at the tibia	<i>Berzelia lanuginosa</i>
E Chrysomelidae, Galaerucinae	4-5 mm	gold olive, apical antennae and tarsae black, all other extremities ochery	<i>Berzelia lanuginosa</i>
Coleoptera	size (length)	special trait, colour, hairs, body shape	plant species the insects were captured on

E Melyridae	8 mm	beige/brown-black elytrons with single hairs, punctured	<i>Berzelia lanuginosa</i>
E Scarabaeidae, Melolonthinae	8 mm	dark to light brown, fan-shaped antennae	<i>Berzelia lanuginosa</i>
1 Nitidulidae, Meligethinae <i>Meligethes</i> C spec.	2-3 mm	dark brown to black	<i>Brunia alopecuroides</i>
Scarabaeidae, Cetoniinae, <i>Trichostetha signata</i> FABRICIUS	25-30 mm	brown elytrons with single white dots and a black stripe in between, hairy in all body parts, hairs white, black scutellum	<i>Brunia alopecuroides</i>
Oedemeridae cf.	22-25 mm	green blue-ish elytrons, expanded tibiae, legs and head orange brown	<i>Brunia alopecuroides</i>
Chrysomelidae, C Galaerucinae	4-5 mm	gold olive, apical antennae and tarsae black, all other extremities ochery	<i>Nebelia paleacea</i>
Dermestidae <i>Anthrenus</i> spec. C	2 mm	black/white patterned, short hairs all over the body	<i>Nebelia paleacea</i>
Nitidulidae, Meligethinae <i>Meligethes</i> C spec.	1.5 mm	dark brown	<i>Nebelia paleacea</i>
Chrysomlidae C	8 mm	black with golden beige stripes	<i>Nebelia paleacea</i>
Melyridae, Malachiinae C	4 mm	green metallic shining, red pronotum, single hairs	<i>Nebelia paleacea</i>
Curculionidae, Curculioninae, <i>Curculio</i> spec. C	2 mm	anthracite, short silvery hairs	<i>Nebelia paleacea</i>
Melyridae C	8 mm	beige/brown-black striped elytrons, red pronotum	<i>Nebelia paleacea</i>
Melyridae C	8 mm	reddish brown-black striped elytrons, black pronotum	<i>Nebelia paleacea</i>
Rutelidae, Hopliinae, <i>Hoplia</i> spec.	7 mm	black with few hairs, red elytrons	<i>Nebelia paleacea</i>
Scirtidae = Helodidae C	2-3.5 mm	black, metallic shining elytrons	<i>Pseudobaeckea africana</i>
Nitidulidae, <i>Meligethes</i> C spec.	2.5 to 3.5 mm	dark brown to black, more spheroidal than 1	<i>Staavia radiata</i>
Scarabaeidae, Cetoniinae <i>Cyrtothyrea marginalis</i> SWARTZ cf.	6 mm	black elytrons with white dots	<i>Berzelia abrotanoides</i>
Thysanoptera			
C	1 mm	black	<i>Nebelia paleacea</i> , <i>Berzelia lanuginosa</i> , <i>Brunia alopecuroides</i>
Brachycera Bombyliidae, Phthiriinae C <i>Phthiria</i> sp. EVENHUIS	12 mm	black wings, acute abdomen	<i>Staavia radiata</i>
Brachycera, Tephritidae	10 mm	black wings with white dots, metallic shining eyes	<i>Staavia radiata</i>
Brachycera Calliphoridae	18 mm	reddish brown, rotund fly, single long black hairs	<i>Staavia radiata</i>
Nematocera Sciaridae C	3-8mm	black greyish with acuminate abdomen	<i>Nebelia paleacea</i> , <i>Brunia alopecuroides</i> , <i>Staavia radiata</i>
Brachycera	~ 13 mm	conical, hairy abdomen, dark brownish black	<i>Berzelia lanuginosa</i>

Diptera	size (length)	special trait, colour, hairs, body shape	plant species the insects were captured on
Brachycera	~ 18 mm	abdomen black with lateral brown markings	<i>Berzelia lanuginosa</i>
K Brachycera, Tephritidae	10 mm	black wings with white dots, metallic shining eyes	<i>Berzelia lanuginosa</i>
K Brachycera Syrphidae	8 mm	black-yellow striped abdomen	<i>Berzelia lanuginosa</i>
K Nematocera Sciaridae C	7 mm	black greyish with acuminate abdomen	<i>Berzelia lanuginosa</i>
K Nematocera Bibionidae	8 mm	whole body black, hairy in parts e.g. pronotum	<i>Berzelia lanuginosa</i>
Nematocera Sciaridae cf.	~ 3 mm	black wings, red abdomen	<i>Berzelia squarrosa</i>
Brachycera Bombyliidae	3-8mm	black greyish with acuminate abdomen	<i>Brunia alopecuroides</i>
Nematocera Sciaridae	3 mm	greyish black	<i>Brunia alopecuroides</i>
Brachycera Bombyliidae, <i>Phthiria</i> sp. EVENHUIS	5 mm	dark grey, mosquito-like body	<i>Lonchostoma monogynum</i>
Calliphoridae: <i>Stomorhina</i> sp. RÓNDANI	6 mm	dark and light grey striped pronotum, short grey bristly hairs, wings folded back	<i>Lonchostoma monogynum</i>
Brachycera Syrphidae, <i>Eristalinus</i> sp.	12 mm	big, brownish beige, hairy	<i>Lonchostoma monogynum</i>
Hymenoptera	size (length)	special trait, colour, hairs, body shape	plant species the insects were captured on
Apocrita, Apoidea, Apidae C	5 mm	black shining, without body hairs	<i>Brunia alopecuroides</i>
Apocrita, Apoidea, Apidae	5 mm	black, blue metallic shining head, abdomen with short beige hairs	<i>Nebelia paleacea</i>
Apocrita, Apoidea, Apidae	5 mm	black shining, without body hairs	<i>Nebelia paleacea</i>
Apocrita, Apoidea, Apidae	15 mm	brown with beige hairs	<i>Nebelia paleacea</i>
Apocrita, Apoidea Spheciformes	25 mm	orange red wings, black body	<i>Nebelia paleacea</i>
Symphyta, Tenthredinoidea	10 mm	orange body, black wings, black legs, black pronotum, no hairs	<i>Staavia radiata</i>
Apocrita, Apoidea, Apidae	5 mm	black, blue metallic shining head, abdomen with short beige hairs	<i>Staavia radiata</i>
Symphyta, Tenthredinoidea	8 mm	black yellow head, head broader than the rest of the body, beige stripes in the abdomen	<i>Staavia radiata</i>
Apocrita, Apoidea Spheciformes Gasteruptiidae cf.	30 mm	black to dark brown, dark reddish-brown legs, long thin abdomen with a long spine	<i>Staavia radiata</i>
Apocrita, Apoidea Spheciformes	30 mm	black to dark brown, dark reddish-brown legs, long thin abdomen	<i>Staavia radiata</i>
Apocrita Vespoidea	27 mm	orange, black patterned body, short bristly hairs	<i>Staavia radiata</i>
Apocrita, Apoidea Spheciformes	35 mm	dark brown, black stripe in the abdomen	<i>Staavia radiata</i>

Hymenoptera	size (length)	special trait, colour, hairs, body shape	plant species the insects were captured on
K Symphyta, Tenthredinoidea	10 mm	orange body, black wings, black legs, black pronotum, no hairs	<i>Berzelia lanuginosa</i>
K Apocrita, Apoidea, Apidae	5 mm	black-brown abdomen with short beige hairs	<i>Berzelia lanuginosa</i>
Apocrita, Vespoidea Formicidae Myrmicinae C	3.5 mm	brownish black	<i>Berzelia lanuginosa</i>
Apocrita, Vespoidea Formicidae Ponerinae C	5 mm	black with single white hairs on the abdomen	<i>Berzelia lanuginosa</i> , <i>Brunia alopecuroides</i> , <i>Staavia radiata</i> <i>Berzelia squarrosa</i>
Apocrita, Vespoidea Formicidae Formicinae C	1.5 mm	black	<i>Brunia alopecuroides</i>
Apocrita, Vespoidea Formicidae Formicinae	3 mm	black	<i>Brunia alopecuroides</i>
Apocrita, Vespoidea Formicidae Myrmicinae C	3 mm	black, heart-shaped abdomen	<i>Staavia radiata</i>
K	4 mm	black	<i>Berzelia lanuginosa</i>
Heteroptera			
Pentatomidae or Scutelleridae C		red-brown with black-framed scutellum with two white lateral markings	<i>Brunia alopecuroides</i>
Cixiidae, Auchenorrhyncha	3.5 mm	grey whitish marbled	<i>Berzelia lanuginosa</i>
Acanthosomatidae or Pentatomidae/Scutelleridae	12 mm	beige-brown grey striped	<i>Nebelia paleacea</i>
Lygaeidae cf.	18 mm	black red striped,	<i>Staavia radiata</i>
Lepidoptera			
K Papilionoidea Nymphalidae Heliconiinae, Acraeini <i>Acraea horta</i> L.	60 mm wing-span	orange, wings with transparent parts	<i>Berzelia lanuginosa</i>
K Papilionoidea Nymphalidae Nymphalinae, Nymphalini, <i>Vanessa (Cynthia) cardui</i> L.	55 mm wing-span	orange, white, black, grey patterned hairy body	<i>Berzelia lanuginosa</i>
Araneida			
Thomisidae	10 mm	three types: white, green, brownish	<i>Berzelia lanuginosa</i>
K Thomisidae	10 mm	green brownish	<i>Berzelia lanuginosa</i>

Table 2: Seed density in the soil samples in different radii below the plant species according to the directions North, South-East and South-West.

	radius [cm]	<i>Berzelia lanuginosa</i> 1	<i>Berzelia lanuginosa</i> 2	<i>Brunia alopecuroides</i> 1	<i>Brunia alopecuroides</i> 2	<i>Nebelia plaeacea</i>	<i>Raspalia microphylla</i>	<i>Staavia radiata</i>
N	20	0	19	5	0	0	0	1
	80	12	0	15	3	0	0	1
	150	1	3	0	5	0	0	4
	200	0	5	0	0	0	0	3
SO	20	3	105	4	22	0	0	0
	80	19	0	7	1	0	0	1
	150	2	4	2	13	0	0	1
	200	0	5	1	0	0	0	0
SW	20	1	19	1	6	0	0	2
	80	2	1	9	2	0	0	0
	150	4	3	2	0	0	0	1
	200	2	0	0	6	0	0	0

Appendix 2 Phylogeography

Distances between samples/plants used for the phylogeographic analysis

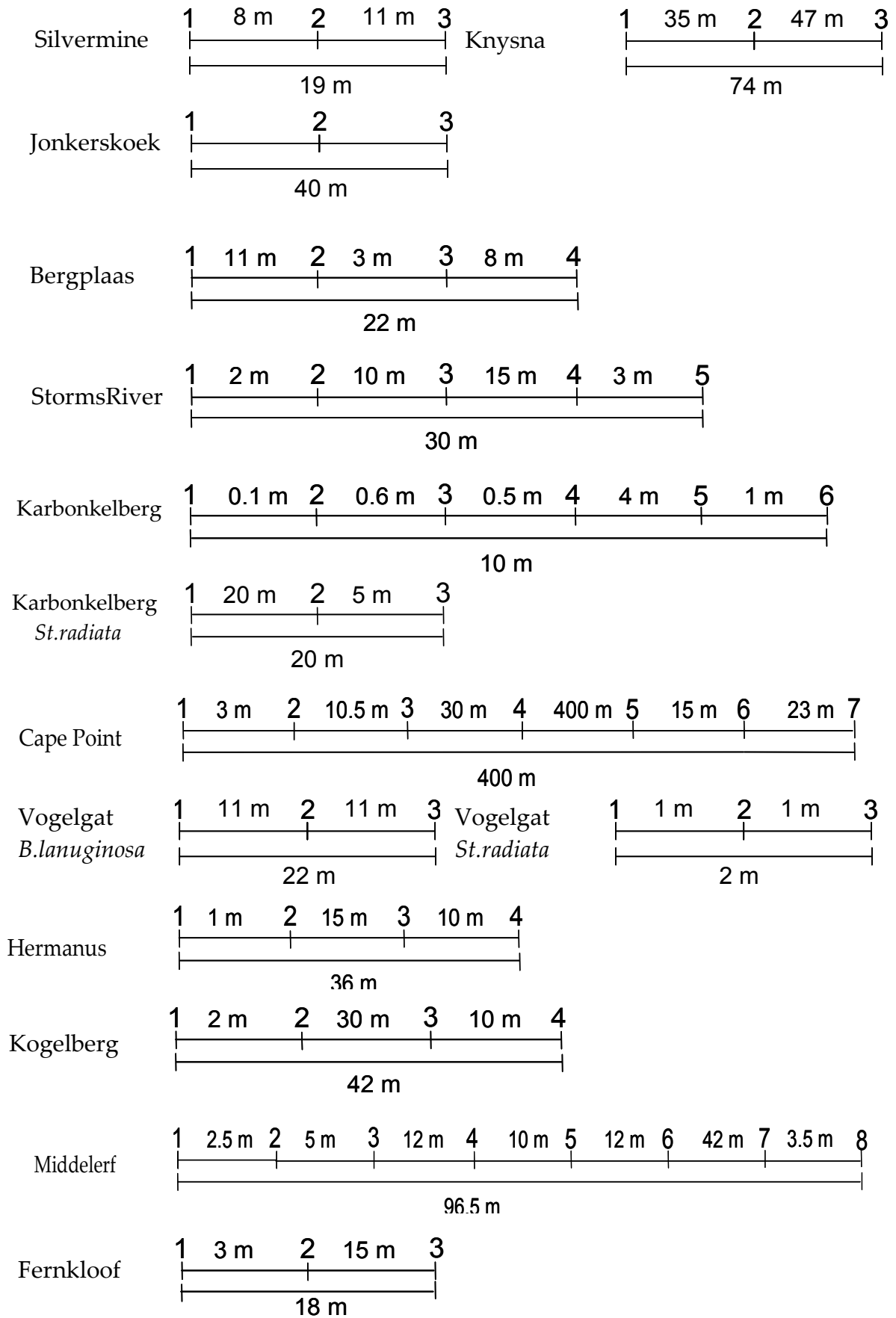


Table1: Distance matrices of the haplotype networks declaring differences between sequences of the localities. ‘With poly-base stretches’: the haplotype network was calculated with the poly-base stretches being weighed as indel.

real distance matrix <i>psbA-trnH Lonchostoma monogynum</i> without poly-base stretches				
locality	1	2	3	4
[Fernkloof 1.1] 1	--	5	19	19
[Fernkloof 2.1] 2	5	--	14	14
[Kogelberg 1.2] 3	19	14	--	1
[Kogelberg 2.2] 4	19	14	1	--
computed from the network distance matrix				
locality	1	2	3	4
[Fernkloof 1.1] 1	--	5	19	19
[Fernkloof 2.1] 2	5	--	14	14
[Kogelberg 1.2] 3	19	14	--	1
[Kogelberg 2.2] 4	19	14	1	--
difference matrix				
locality	1	2	3	4
[Fernkloof 1.1] 1	--	0	0	0
[Fernkloof 2.1] 2	0	--	0	0
[Kogelberg 1.2] 3	0	0	--	0
[Kogelberg 2.2] 4	0	0	0	--
real distance matrix <i>psbA-trnH Lonchostoma monogynum</i> with poly-base stretches				
locality	1	2	3	4
[Fernkloof 1.1] 1	--	5	22	21
[Fernkloof 2.1] 2	5	--	17	16
[Kogelberg 1.2] 3	22	17	--	2
[Kogelberg 2.2] 4	21	16	2	--
computed from the network distance matrix				
locality	1	2	3	4
[Fernkloof 1.1] 1	--	5	23	21
[Fernkloof 2.1] 2	5	--	18	16
[Kogelberg 1.2] 3	23	18	--	2
[Kogelberg 2.2] 4	21	16	2	--
difference matrix				
locality	1	2	3	4
[Fernkloof 1.1] 1	--	0	1	0
[Fernkloof 2.1] 2	0	--	1	0
[Kogelberg 1.2] 3	1	1	--	0
[Kogelberg 2.2] 4	0	0	0	--

real distance matrix <i>psbA-trnH Staavia radiata</i> without poly-base stretches							
locality	1	2	3	4	5	6	7
[Bergplaas 1.1] 1	--	1	1	1	14	13	13
[Bergplaas 2.1] 2	1	--	2	2	14	13	13
[Bergplaas 4.1] 3	1	2	--	2	15	14	14
[Hermanus 1.2] 4	1	2	2	--	14	13	13
[Hermanus 2.2] 5	14	14	15	14	--	1	3
[Hermanus 3.2] 6	13	13	14	13	1	--	2
[Karbonkelberg 1.3] 6	13	13	14	13	3	2	--
computed from the network distance matrix							
locality	1	2	3	4	5	6	7
[Bergplaas 1.1] 1	--	1	1	1	14	13	13
[Bergplaas 2.1] 2	1	--	2	2	14	13	13
[Bergplaas 4.1] 3	1	2	--	2	15	14	14
[Hermanus 1.2] 4	1	2	2	--	14	13	13

[Hermanus 2.2] 5	14	14	15	14	--	1	3
[Hermanus 3.2] 6	13	13	14	13	1	--	2
[Karbonkelberg 1.3] 6	13	13	14	13	3	2	--
difference matrix							
locality	1	2	3	4	5	6	7
[Bergplaas 1.1] 1	--	0	0	0	0	0	0
[Bergplaas 2.1] 2	0	--	0	0	0	0	0
[Bergplaas 4.1] 3	0	0	--	0	0	0	0
[Hermanus 1.2] 4	0	0	0	--	0	0	0
[Hermanus 2.2] 5	0	0	0	0	--	0	0
[Hermanus 3.2] 6	0	0	0	0	0	--	0
[Karbonkelberg 1.3] 6	0	0	0	0	0	0	--

real distance matrix <i>psbA-trnH Staavia radiata</i> with poly-base stretches								
locality	1	2	3	4	5	6	7	8
[Bergplaas 1.1] 1	--	1	2	1	2	14	13	13
[Bergplaas 2.1] 2	1	--	3	2	3	14	13	13
[Bergplaas 3.1] 3	2	3	--	2	5	17	16	16
[Bergplaas 4.1] 4	1	2	2	--	3	15	14	14
[Hermanus 1.2] 5	2	3	5	3	--	15	14	14
[Hermanus 2.2] 6	14	14	17	15	15	--	1	3
[Hermanus 3.2] 7	13	13	16	14	14	1	--	2
[Karbonkelberg 1.3] 8	13	13	16	14	14	3	2	--
computed from the network distance matrix								
locality	1	2	3	4	5	6	7	8
[Bergplaas 1.1] 1	--	1	2	1	2	14	13	13
[Bergplaas 2.1] 2	1	--	3	2	3	14	13	13
[Bergplaas 3.1] 3	2	3	--	2	4	16	15	15
[Bergplaas 4.1] 4	1	2	2	--	3	15	14	14
[Hermanus 1.2] 5	2	3	4	3	--	16	15	15
[Hermanus 2.2] 6	14	14	16	15	16	--	1	3
[Hermanus 3.2] 7	13	13	15	14	15	1	--	2
[Karbonkelberg 1.3] 8	13	13	15	14	15	3	2	--
difference matrix								
locality	1	2	3	4	5	6	7	8
[Bergplaas 1.1] 1	--	0	0	0	0	0	0	0
[Bergplaas 2.1] 2	0	--	0	0	0	0	0	0
[Bergplaas 3.1] 3	0	0	--	0	-1	-1	-1	-1
[Bergplaas 4.1] 4	0	0	0	--	0	0	0	0
[Hermanus 1.2] 5	0	0	-1	0	--	1	1	1
[Hermanus 2.2] 6	0	0	-1	0	1	--	0	0
[Hermanus 3.2] 7	0	0	-1	0	1	0	--	0
[Karbonkelberg 1.3] 8	0	0	-1	0	1	0	0	--

real distance matrix <i>psbA-trnH Berzelia lanuginosa</i> without poly-base stretches						
locality	1	2	3	4	5	6
[Jonkershoek 1.1] 1	--	4	16	16	5	1
[Knysna 1.2] 2	4	--	12	12	1	5
[Knysna 2.2] 3	16	12	--	1	13	17
[Middelarf 2.3] 4	16	12	1	--	13	17
[Middelarf 4.3] 5	5	1	13	13	--	6
[Silvermine 1.4] 6	1	5	17	17	6	--
computed from the network distance matrix						
locality	1	2	3	4	5	6
[Jonkershoek 1.1] 1	--	4	16	16	5	1
[Knysna 1.2] 2	4	--	12	12	1	5

[Knysna 2.2] 3	16	12	--	1	13	17
[Middelerf 2.3] 4	16	12	1	--	13	17
[Middelerf 4.3] 5	5	1	13	13	--	6
[Silvermine 1.4] 6	1	5	17	17	6	--
difference matrix						
locality	1	2	3	4	5	6
[Jonkershoek 1.1] 1	--	0	0	0	0	0
[Knysna 1.2] 2	0	--	0	0	0	0
[Knysna 2.2] 3	0	0	--	0	0	0
[Middelerf 2.3] 4	0	0	0	--	0	0
[Middelerf 4.3] 5	0	0	0	0	--	0
[Silvermine 1.4] 6	0	0	0	0	0	--

real distance matrix <i>psbA-trnH Berzelia lanuginosa</i> with poly-base stretches							
locality	1	2	3	4	5	6	7
[Jonkershoek 1.1] 1	--	6	17	5	17	6	2
[Knysna 1.2] 2	6	--	13	1	13	2	8
[Knysna 2.2] 3	17	13	--	12	1	13	19
[Knysna 3.2]4	5	1	12	--	12	1	7
[Middelerf 2.3] 5	17	13	1	12	--	13	19
[Middelerf 4.3] 6	6	2	13	1	13	--	8
[Silvermine 1.4] 7	2	8	19	7	19	8	--
computed from the network distance matrix							
locality	1	2	3	4	5	6	7
[Jonkershoek 1.1] 1	--	6	17	5	17	6	2
[Knysna 1.2] 2	6	--	13	1	13	2	8
[Knysna 2.2] 3	17	13	--	12	1	13	19
[Knysna 3.2]4	5	1	12	--	12	1	7
[Middelerf 2.3] 5	17	13	1	12	--	13	19
[Middelerf 4.3] 6	6	2	13	1	13	--	8
[Silvermine 1.4] 7	2	8	19	7	19	8	--
difference matrix							
locality	1	2	3	4	5	6	7
[Jonkershoek 1.1] 1	--	0	0	0	0	0	0
[Knysna 1.2] 2	0	--	0	0	0	0	0
[Knysna 2.2] 3	0	0	--	0	0	0	0
[Knysna 3.2]4	0	0	0	--	0	0	0
[Middelerf 2.3] 5	0	0	0	0	--	0	0
[Middelerf 4.3] 6	0	0	0	0	0	--	0
[Silvermine 1.4] 7	0	0	0	0	0	0	--

real distance matrix <i>ndhF-rpl32 Berzelia lanuginosa</i> without poly-base stretches									
locality	1	2	3	4	5	6	7	8	9
[Jonkershoek 1.1] 1	--	21	18	16	5	6	20	13	10
[Knysna 1.2] 2	21	--	9	7	18	19	1	18	15
[Knysna 2.2] 3	18	9	--	2	15	16	8	21	18
[Middelerf 2.3]4	16	7	2	--	13	14	6	19	16
[Silvermine 1.4] 5	5	18	15	13	--	1	17	10	7
[Silvermine 2.4] 6	6	19	16	14	1	--	18	10	8
[StormsRiver1.5]7	20	1	8	6	17	18	--	17	14
[Vogelgat1.6] 8	13	18	21	19	10	10	17	--	5
[Vogelgat2.6] 9	10	15	18	16	7	8	14	5	--
computed from the network distance matrix									
locality	1	2	3	4	5	6	7	8	9
[Jonkershoek 1.1] 1	--	21	18	16	5	6	20	13	10
[Knysna 1.2] 2	21	--	9	7	18	19	1	24	15

[Knysna 2.2] 3	18	9	--	2	15	16	8	21	18
[Middelarf 2.3]4	16	7	2	--	13	14	6	19	16
[Silvermine 1.4] 5	5	18	15	13	--	1	17	10	7
[Silvermine 2.4] 6	6	19	16	14	1	--	18	11	8
[StormsRiver1.5]7	20	1	8	6	17	18	--	23	20
[Vogelgat1.6] 8	13	24	21	19	10	11	23	--	5
[Vogelgat2.6] 9	10	21	18	16	7	8	20	5	--
difference matrix									
locality	1	2	3	4	5	6	7	8	9
[Jonkershoek 1.1] 1	--	0	0	0	0	0	0	0	0
[Knysna 1.2] 2	0	--	0	0	0	0	0	6	6
[Knysna 2.2] 3	0	0	--	0	0	0	0	0	0
[Middelarf 2.3]4	0	0	0	--	0	0	0	0	0
[Silvermine 1.4] 5	0	0	0	0	--	0	0	0	0
[Silvermine 2.4] 6	0	0	0	0	0	--	0	1	0
[StormsRiver1.5]7	0	0	0	0	0	0	--	6	6
[Vogelgat1.6] 8	0	6	0	0	0	1	6	--	0
[Vogelgat2.6] 9	0	6	0	0	0	0	6	0	--

real distance matrix <i>ndhF-rpl32 Berzelia lanuginosa</i> with poly-base stretches									
locality	1	2	3	4	5	6	7	8	9
[Jonkershoek 1.1] 1	--	21	18	16	5	6	20	15	13
[Knysna 1.2] 2	21	--	9	7	18	19	1	20	18
[Knysna 2.2] 3	18	9	--	2	15	16	8	23	21
[Middelarf 2.3]4	16	7	2	--	13	14	6	21	19
[Silvermine 1.4] 5	5	18	15	13	--	1	17	12	10
[Silvermine 2.4] 6	6	19	16	14	1	--	18	12	11
[StormsRiver1.5]7	20	1	8	6	17	18	--	19	17
[Vogelgat1.6] 8	15	20	23	21	12	12	19	--	6
[Vogelgat2.6] 9	13	18	21	19	10	11	17	6	--
computed from the network distance matrix									
locality	1	2	3	4	5	6	7	8	9
[Jonkershoek 1.1] 1	--	21	18	16	5	6	20	15	13
[Knysna 1.2] 2	21	--	9	7	18	19	1	26	24
[Knysna 2.2] 3	18	9	--	2	15	16	8	23	21
[Middelarf 2.3]4	16	7	2	--	13	14	6	21	19
[Silvermine 1.4] 5	5	18	15	13	--	1	17	12	10
[Silvermine 2.4] 6	6	19	16	14	1	--	18	13	11
[StormsRiver1.5]7	20	1	8	6	17	18	--	25	23
[Vogelgat1.6] 8	15	26	23	21	12	13	25	--	6
[Vogelgat2.6] 9	13	24	21	19	10	11	23	6	--
difference matrix									
locality	1	2	3	4	5	6	7	8	9
[Jonkershoek 1.1] 1	--	0	0	0	0	0	0	0	0
[Knysna 1.2] 2	0	--	0	0	0	0	0	6	6
[Knysna 2.2] 3	0	0	--	0	0	0	0	0	0
[Middelarf 2.3]4	0	0	0	--	0	0	0	0	0
[Silvermine 1.4] 5	0	0	0	0	--	0	0	0	0
[Silvermine 2.4] 6	0	0	0	0	0	--	0	1	0
[StormsRiver1.5]7	0	0	0	0	0	0	--	6	6
[Vogelgat1.6] 8	0	6	0	0	0	1	6	--	0
[Vogelgat2.6] 9	0	6	0	0	0	0	6	0	--

real distance matrix <i>ndhF-rpl32 Staavia radiata</i> with and without poly-base stretches						
locality	1	2	3	4	5	6

[Bergplaas 1.1] 1	--	6	2	5	7	3
[Hermanus 1.2] 2	6	--	6	7	1	7
[Hermanus 2.2] 3	2	6	--	5	7	1
[Karbonkelberg 1.3] 4	5	7	5	--	8	6
[Vogelgat 4.4] 5	7	1	7	8	--	8
[Vogelgat 5.4] 6	3	7	1	6	8	--
computed from the network distance matrix						
locality	1	2	3	4	5	6
[Bergplaas 1.1] 1	--	6	2	5	7	3
[Hermanus 1.2] 2	6	--	6	7	1	7
[Hermanus 2.2] 3	2	6	--	5	7	1
[Karbonkelberg 1.3] 4	5	7	5	--	8	6
[Vogelgat 4.4] 5	7	1	7	8	--	8
[Vogelgat 5.4] 6	3	7	1	6	8	--
difference matrix						
locality	1	2	3	4	5	6
[Bergplaas 1.1] 1	--	0	0	0	0	0
[Hermanus 1.2] 2	0	--	0	0	0	0
[Hermanus 2.2] 3	0	0	--	0	0	0
[Karbonkelberg 1.3] 4	0	0	0	--	0	0
[Vogelgat 4.4] 5	0	0	0	0	--	0
[Vogelgat 5.4] 6	0	0	0	0	0	--

Table 2: Sequences included in haplotypes of the populations. Numbers indicate the haplotypes. The first sequence name is the one which the programme chose as haplotype name. *=calculation with polybase stretches weighed as differences

species	marker	sequences within haplotypes of the populations
<i>Lonchostoma monogynum</i>	<i>psbA-trnH</i> and <i>psbA-trnH*</i>	1) Fernkloof 1.1 2) Fernkloof 2.1 3) Kogelberg 1.2 4) Kogelberg 2.2, Kogelberg 3.2, Kogelberg 4.2
<i>Staavia radiata</i>	<i>ndhF-rpl32</i> and <i>ndhF-rpl32*</i>	1) Bergplaas 1.1, Bergplaas 2.1, Bergplaas 3.1 2) Hermanus 1.2 3) Hermanus 2.2 4) Karbonkelberg 1.3, Karbonkelberg 2.3 5) Vogelgat 4.4 6) Vogelgat 5.4
<i>Staavia radiata</i>	<i>psbA-trnH</i>	1) Bergplaas 1.1, Bergplaas 3.1 2) Bergplaas 2.1 3) Bergplaas 4.1 4) Hermanus 1.2, Vogelgat 4.4, Vogelgat 6.4 5) Hermanus 2.2 6) Hermanus 3.2, Vogelgat 5.4 7) Karbonkelberg 1.3, Karbonkelberg 2.3, Karbonkelberg 3.3
	<i>psbA-trnH*</i>	1) Bergplaas 1.1 2) Bergplaas 2.1 3) Bergplaas 3.1 4) Bergplaas 4.1 5) Hermanus 1.2, Vogelgat 4.4, Vogelgat 6.4 6) Hermanus 2.2 7) Hermanus 3.2, Vogelgat 5.4 8) Karbonkelberg 1.3, Karbonkelberg 2.3, Karbonkelberg 3.3
<i>Berzelia lanuginosa</i>	<i>psbA-trnH</i>	1) Jonkershoek 1.1, Jonkershoek 2.1, Jonkershoek 3.1 2) Knysna 1.2, Knysna 3.2, Knysna 4.2, Middelerf 1.3, Middelerf 3.3, Middelerf 5.3, Middelerf 6.3, Stormsriver 1.5, Stormsriver 2.5 Stormsriver 3.5 Stormsriver 4.5 Stormsriver 5.5, Vogelgat 1.6, Vogelgat 2.6, Vogelgat 3.6 3) Knysna 2.2 4) Middelerf 2.3 5) Middelerf 4.3 6) Silvermine 1.4, Silvermine 2.4, Silvermine 3.4
	<i>psbA-trnH *</i>	1) Jonkershoek 1.1, Jonkershoek 2.1, Jonkershoek 3.1 2) Knysna 1.2 Middelerf 3.3, Middelerf 5.3, Stormsriver 1.5, Stormsriver 2.5 Stormsriver 3.5 Stormsriver 4.5 Stormsriver 5.5, Vogelgat 1.6, Vogelgat 2.6, Vogelgat 3.6 3) Knysna 2.2 4) Knysna 3.2, Knysna 4.2, , Middelerf 1.3, Middelerf 6.3 5) Middelerf 2.3 6) Middelerf 4.3 7) Silvermine 1.4, Silvermine 2.4, Silvermine 3.4
<i>Berzelia lanuginosa</i>	<i>ndhF-rpl32</i> and <i>ndhF-rpl32*</i>	1) Jonkershoek 1.1, Jonkershoek 2.1 2) Knysna 1.2 3) Knysna 2.2, Middelerf 2.3 4) Middelerf 1.3 5) Silvermine 1.4 6) Silvermine 2.4 7) Stormsriver 1.5, Stormsriver 2.5 8) Vogelgat 1.6 9) Vogelgat 2.6

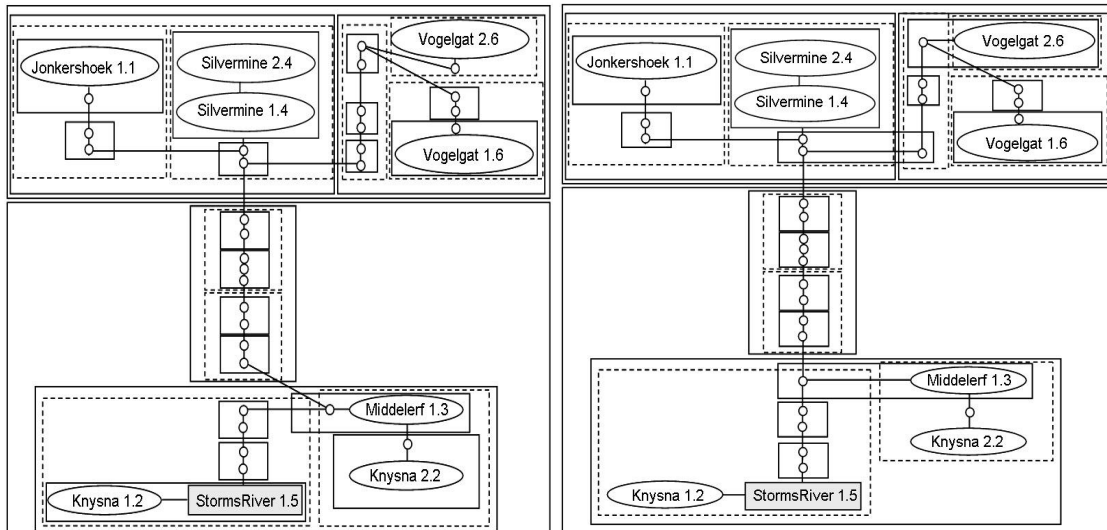


Figure 1: Nested design of the haplotype network of *Berzelia lanuginosa* ndhF-rpl32. left graphic: without polybase stretches, right graphic: with polybase stretches included in the analysis. Oval boxes: sequences; white circles: hypothetical intermediate haplotypes; grey box: biggest possible outgroup. Innermost lines: one-step clades, dashed lines: two-step clades, outer lines: three-step clades, outermost lines: four-step clades.

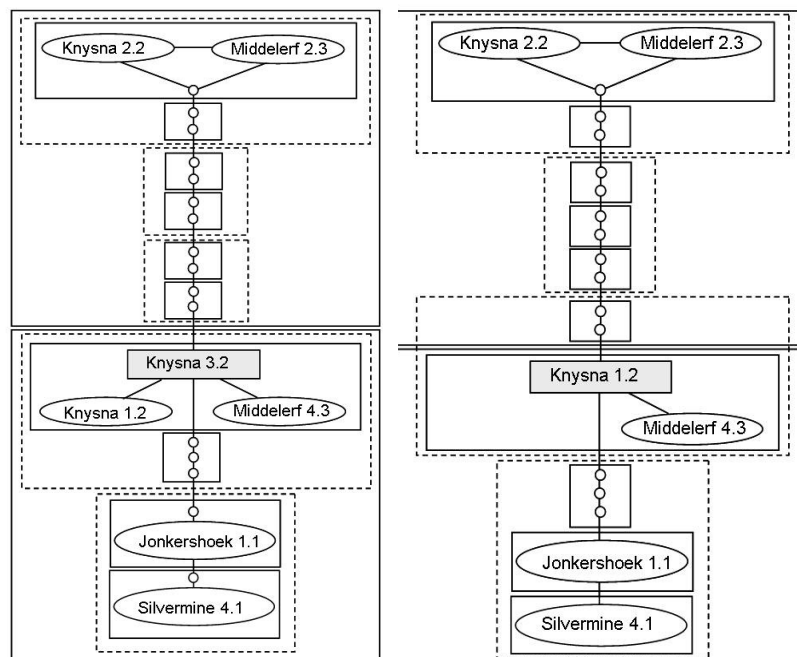


Figure 2: Nested design of the haplotype network of *Berzelia lanuginosa* psbA-trnH. left graphic: without polybase stretches, right graphic: with polybase stretches included in the analysis. Oval boxes: sequences; white circles: hypothetical intermediate haplotypes; grey box: biggest possible outgroup. Inner lines: one-step clades, dashed lines: two-step clades, outer lines: three-step clades.

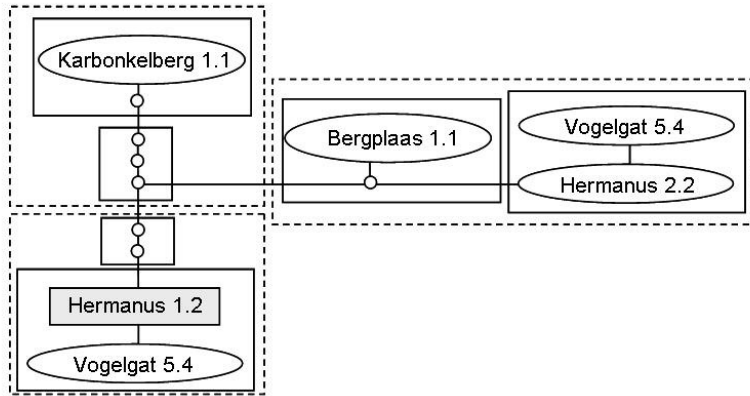


Figure 3: Nested design of the haplotype network of *Staavia radiata* ndhF-rpl32. left graphic without polybase stretches, right graphic: with polybase stretches included in the analysis. Oval boxes: sequences; white circles: hypothetical intermediate haplotypes; grey box: biggest possible outgroup. Inner lines: one-step clades, dashed lines: two-step clades.

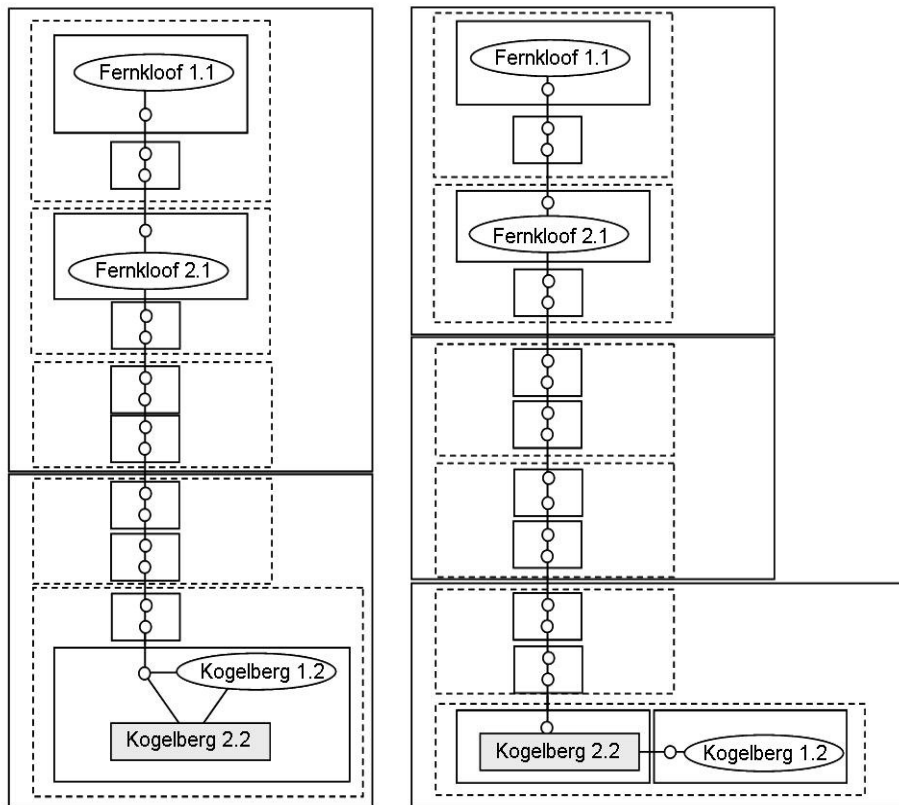


Figure 4: Nested design of the haplotype network of *Lonchostoma monogynum* psbA-trnH. left graphic without polybase stretches, right graphic: with polybase stretches included in the analysis. Oval boxes: sequences; white circles: hypothetical intermediate haplotypes; grey box: biggest possible outgroup. Inner lines: one-step

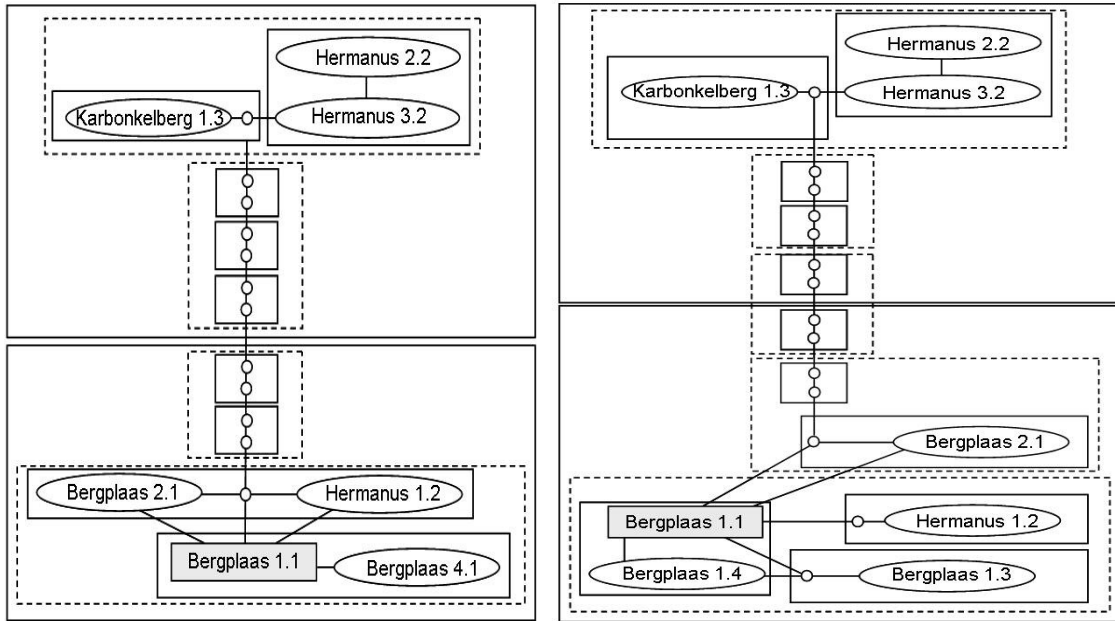


Figure 5: Nested design of the haplotype network of *Staavia radiata* psbA-trnH. left graphic without polybase stretches, right graphic: with polybase stretches included in the analysis. Oval boxes: sequences; white circles: hypothetical intermediate haplotypes; grey box: biggest possible outgroup. Inner lines: one-step clades, dashed lines: two-step clades, outer lines: three-step clades.

Jonkershoek1.1 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Jonkershoek2.1 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Knyrna1.2 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Knyrna2.2 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Middel erf2.3 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Middel erf1.3 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Middel erf1.4 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Middel erf2.4 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Middel erf1.4 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Stormsriver1.5 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Stormsriver2.5 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Vogelgat1.6 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag
Vogelgat2.6 AAACAGATTAATTAACHTAGTCTCAITTTGAAATTTTGAA... ttaettatag

Jonkershoek1.1 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Jonkershoek2.1 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Knyrna1.2 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Knyrna2.2 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Middel erf1.3 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Middel erf1.4 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Middel erf1.5 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag
Middel erf1.6 ATGTAAATTAATA - TGTPAGAA CGACGAAACTAGACAGAG... ttaettatag

Jonkershoek1.1 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Jonkershoek2.1 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Knyrna1.2 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Knyrna2.2 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Middel erf2.3 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Middel erf1.3 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Middel erf1.4 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Middel erf1.5 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag
Middel erf1.6 CCACGTTTTAAITCAAATAC - AAATTTTTTCTTCTTACA... ttaettatag

Jonkershoek1.1 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Jonkershoek2.1 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Knyrna1.2 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Knyrna2.2 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Middel erf2.3 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Middel erf1.3 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Middel erf1.4 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Middel erf1.5 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag
Middel erf1.6 GAAATAAATAGAGAAATCAAATAGTAAAGGAGATTCGTTT... ttaettatag

Bergplaas1.1 TTTTAGGAAAAAGTTGGGTTTTTCGATGTTTTTTTATTAGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Hermanus1.2 TT- - - - AAAGTTTCGGTTTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Hermanus2.2 TTTTAGGAAAAAGTTTCGATGTTTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Karbonkelberg1.3 TTTTAGGAAAAAGTTTCGATGTTTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 VogeIgat4.4 TT- - - - AAAGTTTCGGTTTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 VogeIgat5.4 TTTTAGGAAAAAGTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Bergplaas2.1 TTTTAGGAAAAAGTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Bergplaas3.1 TTTTAGGAAAAAGTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Karbonkelberg2.3 TTTTAGGAAAAAGTTTCGATGTTTTTTTATTAGATGATGTAATAATGTAGAAACGACCGAAAAATAGACACAGAGATAGAAAAGGAGGGGTATAGATATTTTTGAAATGATAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Bergplaas1.1 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - TCAA
 Hermanus1.2 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATC- - - - -
 Hermanus2.2 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - - TCAA
 Karbonkelberg1.3 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - - TCAA
 VogeIgat4.4 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATAGTCAATAGTCAATAG
 VogeIgat5.4 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - - TCAA
 Bergplaas2.1 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - - TCAA
 Bergplaas3.1 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - - TCAA
 Karbonkelberg2.3 CTTAGAAAATATTGTTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC- AAAAAAAAAATCA CATATCTTTTCAATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAG- - - - - TCAA
 Bergplaas1.1 GGACGATTCGTT
 Hermanus1.2 - - - - -
 Hermanus2.2 GGACGATTCGTT
 Karbonkelberg1.3 GGACGATTCGTT
 VogeIgat4.4 GGACGATTCGTT
 VogeIgat5.4 GGACGATTCGTT
 Bergplaas2.1 GGACGATTCGTT
 Bergplaas3.1 GGACGATTCGTT
 Karbonkelberg2.3 GGACGATTCGTT

Bergplaas1.1 TTTTAGGAAAAAGTTGGGTTTTTCGATGTTTTTTTATTTAGATGTAATAATGTAGAAAGGAGGTAGACAGAGATAGAAAAGGAGGCGTATAGATATTTTTGAATGATAAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
 Hermanus1.2 TTTTAGGAAAAAGTTGGGTTTTTCGATGTTTTTTTATTTAGATGTAATAATGTAGAAAGGAGGTATAGACAGAGATAGAAAAGGAGGCGTATAGATATTTTTGAATGATAAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
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 Vogei1gat4.4 TTTTAGGAAAAAGTTGGGTTTTTCGATGTTTTTTTATTTAGATGTAATAATGTAGAAAGGAGGTATAGACAGAGATAGAAAAGGAGGCGTATAGATATTTTTGAATGATAAAAGAAATGAGAAAATACAAAGTATTAATCAATACAAAATTCCTTCCTTT
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 Bergplaas1.1 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC - AAAAAAAAAATCA CATATCTTTTTCACATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Hermanus1.2 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAACAAAATAAATAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - tcaa
 Hermanus2.2 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC - AAAAAAAAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Karbonkelberg1.3 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAACAAAATAAATAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Vogei1gat4.4 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAACAAAATAAATAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Vogei1gat5.4 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC - AAAAAAAAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Bergplaas2.1 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC - AAAAAAAAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Bergplaas3.1 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAAC - AAAAAAAAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATAGAGAAATCAATA - TCAA
 Karbonkelberg2.3 CTTAGAAAATATTTGTATGGAAATAACCCGCCCTTTTTTGGATTTTTGAAAAACAAAATAAATAAATCA CATATCTTTTCA CATATCCCATTTTTTATGAGAAAAAGAAATCTCTATTTTTCTTAGAGAAATAAATAGAGAAATCAATA - TCAA
 Bergplaas1.1 GGACGATTCGTT
 Hermanus1.2 **ggacgat**tcggt
 Hermanus2.2 GGACGATTCGTT
 Karbonkelberg1.3 GGACGATTCGTT
 Vogei1gat4.4 GGACGATTCGTT
 Vogei1gat5.4 GGACGATTCGTT
 Bergplaas2.1 GGACGATTCGTT
 Bergplaas3.1 GGACGATTCGTT
 Karbonkelberg2.3 GGACGATTCGTT

Alignment *psbA-trnH Lonchostoma monogynum*

Fernk1.oof1.1 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTGTGAAAT - AAAAAAGGAGCAAATACCAATTTTCTTCTATCAAGA CCGCGGTATTGCTCCTTTTTTATTTAGTATGTAATTTGACTTTACAC
Fernk1.oof2.1 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTGTGAAAT - AAAAAAGGAGCAAATACCAATTTTCTTCTATCAAGA CCGCGGTATTGCTCCTTTTTTATTTAGTATGTAATTTGACTTTACAC
Kogel.berg1.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTGTGAAAT - AAAAAAGGAGCAAATACCGCCGTCTTGA TAGAAGAAATGGTATTCCTCCTTTTTTATTTAGTATGTAATTTGACTTTACAC
Kogel.berg2.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTGTGAAAT - AAAAAAGGAGCAAATACCGCCGTCTTGA TAGAAGAAATGGTATTCCTCCTTTTTTATTTAGTATGTAATTTGACTTTACAC
Kogel.berg3.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTGTGAAAT - AAAAAAGGAGCAAATACCGCCGTCTTGA TAGAAGAAATGGTATTCCTCCTTTTTTATTTAGTATGTAATTTGACTTTACAC
Kogel.berg4.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTGTGAAAT - AAAAAAGGAGCAAATACCGCCGTCTTGA TAGAAGAAATGGTATTCCTCCTTTTTTATTTAGTATGTAATTTGACTTTACAC
Fernk1.oof1.1 AGTTTCTTTAAA GAAAAAAA - - GTGGATTTTCTGATTTGCTCATGATTCAGTGTCAATGTTGATATAGAAATACCAATATATATACTTTCTTTACCAAATTTTGTGTAAGTTCGTTA TTTGCCCATTTA AAAAAAGAAAAG
Fernk1.oof2.1 AGTTTCTTTAAA GAAAAAAA - - GTGGATTTTCTGATTTGCTCATGATTCAGTGTAGAAATACCAATATATATACTTTCTTTACCAAATTTTGTGTAAGTTCGTTA TTTGCCCATTTA AAAAAAGAAAAG
Kogel.berg1.2 AGTTTCTTTAAA GAAAAAAAAGTGGATTTTCTGATTTGCTCATGATTCAGTGTAGAAATACCAATATATATACTTTCTTTACCAAATTTTGTGTAAGTTCGTTA TTTGCCCATTTA AAAAAAGAAAAG
Kogel.berg2.2 AGTTTCTTTAAA GAAAAAAAAGTGGATTTTCTGATTTGCTCATGATTCAGTGTAGAAATACCAATATATATACTTTCTTTACCAAATTTTGTGTAAGTTCGTTA TTTGCCCATTTA AAAAAAGAAAAG
Kogel.berg3.2 AGTTTCTTTAAA GAAAAAAAAGTGGATTTTCTGATTTGCTCATGATTCAGTGTAGAAATACCAATATATATACTTTCTTTACCAAATTTTGTGTAAGTTCGTTA TTTGCCCATTTA AAAAAAGAAAAG
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Fernk1.oof1.1 CAAAAATTTGGAAATTTTGTGCTTACTTTTCTAATTTGTATGTCCAGAAAATAAGAAAAGTGAATAGTCCGAAAATTTGAAACCTTTTTTT - ATTTGATGAAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGTGGATG
Fernk1.oof2.1 CAAAAATTTGGAAATTTTGTGCTTACTTTTCTAATTTGTATGTCCAGAAAATAAGAAAAGTGAATAGTCCGAAAATTTGAAACCTTTTTTT - ATTTT - TAAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC - - - - -
Kogel.berg1.2 CAAAAATTTGGAAATTTTGTGCTTACTTTTCTAATTTGTATGTCCAGAAAATAAGAAAAGTGAATAGTCCGAAAATTTGAAACCTTTTTTTATTTT - TAAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC - - GGATG
Kogel.berg2.2 CAAAAATTTGGAAATTTTGTGCTTACTTTTCTAATTTGTATGTCCAGAAAATAAGAAAAGTGAATAGTCCGAAAATTTGAAACCTTTTTTTATTTT - TAAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC - - GGATG
Kogel.berg3.2 CAAAAATTTGGAAATTTTGTGCTTACTTTTCTAATTTGTATGTCCAGAAAATAAGAAAAGTGAATAGTCCGAAAATTTGAAACCTTTTTTTATTTT - TAAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC - - GGATG
Kogel.berg4.2 CAAAAATTTGGAAATTTTGTGCTTACTTTTCTAATTTGTATGTCCAGAAAATAAGAAAAGTGAATAGTCCGAAAATTTGAAACCTTTTTTTATTTT - TAAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC - - GGATG

Alignment *psbA-trnH Lonchostoma monogynum* without plybase stretches being weighed

Fernk1.oof1.1 ATCTACAAATGGGATAAAGATCCCTTTCTTAAGTGTATATGTAATGTATATGGGATA CGGGTTTTTTGAAATAAAAAAGAGACAAATACCAATTTTCTTCTATCAAGA CGGCGGTATTTGCTCCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Fernk1.oof2.1 ATCTACAAATGGGATAAAGATCCCTTTCTTAAGTGTATATGTAATGTATATGGGATA CGGGTTTTTTGAAATAAAAAAGAGACAAATACCAATTTTCTTCTATCAAGA CGGCGGTATTTGCTCCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg1.2 ATCTACAAATGGGATAAAGATCCCTTTCTTAAGTGTATATGTAATGTATATGGGATA CGGGTTTTTTGAAATAAAAAAGAGACAAATACCGCGGTATTTGCTCCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg2.2 ATCTACAAATGGGATAAAGATCCCTTTCTTAAGTGTATATGTAATGTATATGGGATA CGGGTTTTTTGAAATAAAAAAGAGACAAATACCGCGGTATTTGCTCCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg3.2 ATCTACAAATGGGATAAAGATCCCTTTCTTAAGTGTATATGTAATGTATATGGGATA CGGGTTTTTTGAAATAAAAAAGAGACAAATACCGCGGTATTTGCTCCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg4.2 ATCTACAAATGGGATAAAGATCCCTTTCTTAAGTGTATATGTAATGTATATGGGATA CGGGTTTTTTGAAATAAAAAAGAGACAAATACCGCGGTATTTGCTCCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Fernk1.oof1.1 AGTTTTCTTTAAAAGAAAAAaaAGTTTTCTGATTTTTCATGATTTGAGTGTCAATGCTTTTTCTGATTTGGATATAGAAATATCCAATATATATACTTTCTTTA CCAAATTTTGTGAAAGTTCGTTAATTTGCCCATTTAAAAAGAAAAAG
Fernk1.oof2.1 AGTTTTCTTTAAAAGAAAAAaaAGTTTTCTGATTTTTCATGATTTGAGTGTCAATGCTTTTTCTGATTTGGATATAGAAATATCCAATATATATACTTTCTTTA CCAAATTTTGTGAAAGTTCGTTAATTTGCCCATTTAAAAAGAAAAAG
Kogel.berg1.2 AGTTTTCTTTAAAAGAAAAAaaAGTTTTCTGATTTTTCATGATTTGAGTGTCAATGCTTTTTCTGATTTGGATATAGAAATATCCAATATATATACTTTCTTTA CCAAATTTTGTGAAAGTTCGTTAATTTGCCCATTTAAAAAGAAAAAG
Kogel.berg2.2 AGTTTTCTTTAAAAGAAAAAaaAGTTTTCTGATTTTTCATGATTTGAGTGTCAATGCTTTTTCTGATTTGGATATAGAAATATCCAATATATATACTTTCTTTA CCAAATTTTGTGAAAGTTCGTTAATTTGCCCATTTAAAAAGAAAAAG
Kogel.berg3.2 AGTTTTCTTTAAAAGAAAAAaaAGTTTTCTGATTTTTCATGATTTGAGTGTCAATGCTTTTTCTGATTTGGATATAGAAATATCCAATATATATACTTTCTTTA CCAAATTTTGTGAAAGTTCGTTAATTTGCCCATTTAAAAAGAAAAAG
Kogel.berg4.2 AGTTTTCTTTAAAAGAAAAAaaAGTTTTCTGATTTTTCATGATTTGAGTGTCAATGCTTTTTCTGATTTGGATATAGAAATATCCAATATATATACTTTCTTTA CCAAATTTTGTGAAAGTTCGTTAATTTGCCCATTTAAAAAGAAAAAG
Fernk1.oof1.1 CAAAAATTTGGAAATTTTTTTCGTTA CTTTTCTAATTTTGTATGTCAGAAAAAAGAAAAACTGATGAAATAGTCGAAATTTGAAACCTTTTTTTTtATTTTGAATAAATGTACCTAATTTTTTAAATGTACCTAAGAGTAGGGGGC-**gggagt**
Fernk1.oof2.1 CAAAAATTTGGAAATTTTTTTCGTTA CTTTTCTAATTTTGTATGTCAGAAAAAAGAAAAACTGATGAAATAGTCGAAATTTGAAACCTTTTTTTTtATTTTt-**TAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC-**gggagt****
Kogel.berg1.2 CAAAAATTTGGAAATTTTTTTCGTTA CTTTTCTAATTTTGTATGTCAGAAAAAAGAAAAACTGATGAAATAGTCGAAATTTGAAACCTTTTTTTTtATTTTT-**TAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC-**gggagt****
Kogel.berg2.2 CAAAAATTTGGAAATTTTTTTCGTTA CTTTTCTAATTTTGTATGTCAGAAAAAAGAAAAACTGATGAAATAGTCGAAATTTGAAACCTTTTTTTTtATTTTT-**TAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC-**gggagt****
Kogel.berg3.2 CAAAAATTTGGAAATTTTTTTCGTTA CTTTTCTAATTTTGTATGTCAGAAAAAAGAAAAACTGATGAAATAGTCGAAATTTGAAACCTTTTTTTTtATTTTT-**TAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC-**gggagt****
Kogel.berg4.2 CAAAAATTTGGAAATTTTTTTCGTTA CTTTTCTAATTTTGTATGTCAGAAAAAAGAAAAACTGATGAAATAGTCGAAATTTGAAACCTTTTTTTTtATTTTT-**TAATGTACCTAATTTTAAATGTACCTAAGAGTAGGGGGC-**gggagt****

Alignment *psbA-trnH Lonchostoma monogynum* with plybase stretches being weighed

Fernk1.oof1.1 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTTTTGAAAT - AAAAAAAGGAGCAAATACCAATTTTCTTCTCTATCAAGA CGGCGGTATTGCTCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Fernk1.oof2.1 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTTTTGAAAT - AAAAAAAGGAGCAAATACCAATTTTCTTCTCTATCAAGA CGGCGGTATTGCTCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg1.1.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTTTTGAAAT - AAAAAAAGGAGCAAATACCGGCCGTCTTGA TAGAAGAAGAAATGGTATTGCTCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg2.1.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTTTTGAAAT - AAAAAAAGGAGCAAATACCGGCCGTCTTGA TAGAAGAAGAAATGGTATTGCTCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
Kogel.berg3.1.2 ATCTACAAAATGGATAAGATCCCTTCCTTAGTGTATATGTAATGTATATGGATACGGGTTTTTTGAAAT - AAAAAAAGGAGCAAATACCGGCCGTCTTGA TAGAAGAAGAAATGGTATTGCTCCTTTTTTTATTTAGTATGTAATTTGACTTACAC
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Fernk1.oof1.1 AGTTTTCTTTAAAGAAAAAa - GTGGATTTTTCTGA TTTTTCATGATTCAGTGTCAATGTTGCTCTATTTGGATATAGAAATACCAATATATATACTTTCTTTACCAATTTTGTGAGTTCGTTA TTTGCCCATTTAAAAAAGAAAAAG
Fernk1.oof2.1 AGTTTTCTTTAAAGAAAAAa - GTGGATTTTTCTGA TTTTTCATGATTCAGTGTCAATGTTGCTCTATTTGGATATAGAAATACCAATATATATACTTTCTTTACCAATTTTGTGAGTTCGTTA TTTGCCCATTTAAAAAAGAAAAAG
Kogel.berg1.1.2 AGTTTTCTTTAAAGAAAAAAGTGGATTTTTCTTA TTTTGTTCATGATTCAGTGTCAATGTTGCTCTATTTGGATATAGAAATACCAATATATATACTTTCTTTACCAATTTTGTGAGTTCGTTA TTTGCCCATTTAAAAAAGAAAAAG
Kogel.berg2.1.2 AGTTTTCTTTAAAGAAAAAAGTGGATTTTTCTTA TTTTGTTCATGATTCAGTGTCAATGTTGCTCTATTTGGATATAGAAATACCAATATATATACTTTCTTTACCAATTTTGTGAGTTCGTTA TTTGCCCATTTAAAAAAGAAAAAG
Kogel.berg3.1.2 AGTTTTCTTTAAAGAAAAAAGTGGATTTTTCTTA TTTTGTTCATGATTCAGTGTCAATGTTGCTCTATTTGGATATAGAAATACCAATATATATACTTTCTTTACCAATTTTGTGAGTTCGTTA TTTGCCCATTTAAAAAAGAAAAAG
Kogel.berg4.1.2 AGTTTTCTTTAAAGAAAAAAGTGGATTTTTCTTA TTTTGTTCATGATTCAGTGTCAATGTTGCTCTATTTGGATATAGAAATACCAATATATATACTTTCTTTACCAATTTTGTGAGTTCGTTA TTTGCCCATTTAAAAAAGAAAAAG
Fernk1.oof1.1 CAAAAATTTGGAAATTTTTTGCTTACTTTTCTATTTGTATGTCAGAAATAAGAAAAGTGAATAGTTCGAAATTTGAACTTTTTT - ATTTTGAATAATGTACCTA TTTTFTTAAATGTACCTAAGAGTAGGGGGCTGGATG
Fernk1.oof2.1 CAAAAATTTGGAAATTTTTTGCTTACTTTTCTATTTGTATGTCAGAAATAAGAAAAGTGAATAGTTCGAAATTTGAACTTTTTT - ATTTT - TAAATGTACCTA TTTTFTTAAATGTACCTAAGAGTAGGGGGC - gggatg
Kogel.berg1.1.2 CAAAAATTTGGAAATTTTTTGCTTACTTTTCTATTTGTATGTCAGAAATAAGAAAAGTGAATAGTTCGAAATTTGAACTTTTTT - TAAATGTACCTA TTTTFTTAAATGTACCTAAGAGTAGGGGGC - gggatg
Kogel.berg2.1.2 CAAAAATTTGGAAATTTTTTGCTTACTTTTCTATTTGTATGTCAGAAATAAGAAAAGTGAATAGTTCGAAATTTGAACTTTTTT - TAAATGTACCTA TTTTFTTAAATGTACCTAAGAGTAGGGGGC - gggatg
Kogel.berg3.1.2 CAAAAATTTGGAAATTTTTTGCTTACTTTTCTATTTGTATGTCAGAAATAAGAAAAGTGAATAGTTCGAAATTTGAACTTTTTT - TAAATGTACCTA TTTTFTTAAATGTACCTAAGAGTAGGGGGC - gggatg
Kogel.berg4.1.2 CAAAAATTTGGAAATTTTTTGCTTACTTTTCTATTTGTATGTCAGAAATAAGAAAAGTGAATAGTTCGAAATTTGAACTTTTTT - TAAATGTACCTA TTTTFTTAAATGTACCTAAGAGTAGGGGGC - gggatg

Appendix 3 Habitat and Distribution

Detailed information about species distribution

The data will not be arranged regarding the clades of the phylogeny after Quint and Claßen-Bockhoff (2006) since species that are not included in this phylogeny would have to be stated separately. Therefore data order is based on the genus classification after Pillans (1947).

Audouinia

Audouinia capitata occurs on the whole Cape peninsula. Three localities of which two are situated in the Kogelberg area and one above Hermanus mentioned by A.V. Hall and one locality at the Palmiet River mouth are doubttable to still exist today (information by local botanists and own field trips above Hermanus).

Berzelia

The genus is subdivided into two distribution patterns: the species *Berzelia squarrosa*, *B. ecklonii*, *B. galpinii*, *B. incurva*, *B. burchellii* and *B. cordifolia* are punctually distributed. The remaining four species *Berzelia commutata*, *B. intermedia*, *B. lanuginosa* and *Berzelia abrotanoides* exhibit a more or less tubular pattern.

Berzelia ecklonii is restricted to the Kogelberg area. *Berzelia incurva* occurs in the Overberg Mountains above Hermanus. *Berzelia cordifolia* occurs along the Bree River and in the Potberg Nature Reserve. *Berzelia galpinii* shows a cross-like distribution pattern along the Langeberg Rural and punctually at the Goukou River. *Berzelia burchellii* occurs at three localities at south-facing mountain ranges along the Western Cape. The distribution of *Berzelia squarrosa* is split in two areas that are about 200 km apart from each other and are situated in the Kogelberg and Hermanus area and in the Swartberg Mountains.

Berzelia commutata is located along and at the mountain ranges from the Matzikama Mountains, the Cederberg Mountains, the Kogelberg area and then eastwards in the Langeberg rural, the Jonkersberg State Forest, the Baavianskloof Wilderness area to Port Elisabeth and Grahamstown.

Berzelia intermedia is distributed from the western Matzikama Mountains, the Cape Peninsula, the Stellenbosch mountains and the Kogelberg area. Eastwards the species occurs in the Salmondam Nature Reserve and around Elim and then without gaps to Port Elisabeth. The eastern-most locality is the area around Grahamstown.

Nearly the same distribution pattern like *Berzelia intermedia* is shown in *Berzelia lanuginosa*. A distribution center of *Berzelia lanuginosa* seems to be the western part of the Cape. *Berzelia lanuginosa* also inhabits the higher mountain ranges from Swellendam to Oudtshoorn. There is a distribution gap in a broad stripe from Albertinia to Mossel Bay; no localities are documented in the herbaria.

Berzelia abrotanoides occurs on lower altitudes compared to *Berzelia lanuginosa* but also can occur in mountainous area. In contrast to the other far distributed *Berzelia* species, *Berzelia abrotanoides* is restricted to the western part of the Cape and the most eastwards occurring locality got documented in the area of George.

Berzelia rubra occurs above Hermanus and the Riviersonderend Mountains (data by A.V. Hall).

Berzelia burchellii occurs in Riversdale, Swellendam and George.

Brunia

Like in the genus *Berzelia*, in *Brunia* there are species that are distributed punctually, others are far distributed. The area with the highest species density is the Kogelberg area.

The four species with a restricted occurrence area are *Brunia albiflora*, *Brunia alopecuroides*, *Brunia macrocephala* and *Brunia stokoei*.

Brunia albiflora only occurs in the Kogelberg area and eastwards to Hermanus. There is another locality described by A.V. Hall between Jeffreys Bay and Port Elisabeth, but as no locality got documented in between it might be a human induced occurrence.

Brunia alopecuroides is restricted to an area ranging from Stellenbosch to Caledon and from the Kogelberg area to Stanford.

Brunia stokoei only occurs in the Kogelberg area.

The species with the northernmost punctual distribution area is *Brunia macrocephala* which occurs between Ceres and the Breede River rural.

The three *Brunia* species, *Brunia nodiflora*, *Brunia neglecta* and *Brunia laevis* have a wider distribution range.

The species with the biggest occurrence area is *Brunia noduliflora* which ranges from the Cederberg Wilderness area down to the Cape Peninsula and eastwards to Port Elisabeth. The southernmost locality is Bredasdorp.

Brunia neglecta occurs in the Breede river region and in the Swellendam Mountains. Hall also documented localities in Stellenbosch, the Kogelberg area, Sand Bay and Greyton.

Brunia laevis is documented in a broad stripe from Stellenbosch to the Kogelberg area and downwards to the Cape Agulhas rural. Single localities also got documented north of Heidelberg and the Kannaland rural. One locality got recorded in the Groendal Wilderness area near to Port Elisabeth by Hall.

Linconia

The genus *Linconia* exhibits different distribution patterns: *Linconia cuspidata* and *Linconia alopercurioides* are further distributed. *Linconia ericoides* is only known from two localities at Stormsvlei and the area of Bonnievale. *Linconia deusta* is only known from one locality at Riviersonderend.

The two further distributed species *Linconia cuspidata* and *Linconia alopecuroidea* show two different patterns: *Linconia cuspidata* occurs from the Cederberg wilderness area down to the Kogelberg area. The eastern-most locality got documented in the Mossel Bay rural. In contrast *Linconia alopecuroidea* is distributed like pearls on a string along the mountains from Villiersdorp to Riversdale.

Lonchostoma

In *Lonchostoma*, the same pattern like in *Linconia* is realised: there are two further distributed and three locally restricted species.

Lonchostoma monogynum, one of the further distributed species can be found from the Citrusdal area down to the Kogelberg area. The distribution center is between Worcester and Hangklip. The eastern-most locality lies between McGregor and Robertson.

Lonchostoma pentandrum is the second species that can be called further distributed compared with the other species of the genus. There is a clear N/S pattern ranging from the Cederberg Wilderness area to the Stellenbosch rural.

The three restricted species are *Lonchostoma esterhuyseniae* that only occurs in the Riviersonderend Mountains, *Lonchostoma myrtooides* that occurs in the Clanwilliam, Ceres and Wellington division and *Lonchostoma purpureum* which occurs in the Stellenbosch Mountains and the Kogelberg area.

Mniothamnea

Mniothamnea only occurs locally restricted: *Mniothamnea bullata* and *Mniothamnea* can be found at the mountains of Swellendam. For *Mniothamnea callunoides*, Hall documents another locality in the Hottentot Holland Mountains.

Nebelia

All four *Nebelia* species are restricted to an area ranging from the Groot Winterhoek wilderness area down to the Kogelberg area and eastwards to Bredasdorp.

The exception is *Nebelia sphaerocephala* that occurs from The Bain's Kloof pass down to Stellenbosch but after Hall has another two localities in the Langeberg rural which are the easternmost localities of the whole genus.

Nebelia stokoei is restricted to the Ceres mountains but Hall found two localities in the Kogelberg area.

Nebelia paleacea can be found in a broad stripe from the Groot Winterhoek wilderness area to Bredasdorp. The westernmost locality can be found at the Langebaan lagoon.

Nebelia fragarioides shows a distribution ranging from the Kogelberg area to the Salmonsdam Nature Reserve. The northernmost point is the Ceres district.

Nebelia laevis can be found in Caledon, Worcester, Genadendal and Robertson.

Pseudobaeckea

The four *Pseudobaeckea* species are distributed punctually and wide-spread. *Pseudobaeckea teres* occurs scattered at Grabouw and Villiersdorp and at Genadendal and Ladismith.

Pseudobaeckea cordata occurs throughout the Western Cape with the Cederberg rural locality as northernmost point. The distribution ranges from the Kogelberg area eastwards to Port Elisabeth.

Pseudobaeckea africana occurs in a broad stripe from the Matzikama mountains down to the Cape peninsula and Hermanus.

Pseudobaeckea cordata var. *monostyla* can be found in Citrusdal, Ceres, Bains Kloof and Du Toit's Kloof (data by A.V. Hall).

Raspalia

In *Raspalia*, the species are distributed scattered, wide-spread or locally restricted.

Raspalia virgata is distributed from Stellenbosch to the Langeberg rural with single localities in the Paarl district, Robertson and north of Ladismith.

Raspalia stokoei occurs in the Cold Bokkeveld, the Tulbagh and Ceres area and after Hall above Swellendam.

Raspalia variabilis occurs scattered with localities in the Stellenbosch and Kogelberg area, the Riviersonderend mountains and the Ladismith area.

Raspalia sacculata is distributed north of Tulbagh and in the Paarl district, upper Wellington and the Worcester district.

Raspalia staavioides occurs in the Clanwilliam and Wuppertal district.

Raspalia phyllicoides can be found in the Kogelberg area, the Riviersonderend Mountains and Hall documented one locality in the Cold Bokkeveld.

The species *Raspalia oblongifolia* is restricted to the Worcester division.

Raspalia microphylla inhabits the Kogelberg and Stellenbosch area, the Riviersonderend Mountains, the Hottentot Holland Mountains, one locality at Montagu and another spot in the Prince Albert district.

Raspalia palustris can be found in the Tulbagh area and Prince Alfred.

Raspalia angulata occurs at the Cape Peninsula, The Kogelberg and Stellenbosch area up to Ceres and eastwards to Barrydale and the Swartberg mountains.

Raspalia barnardii occurs in the Swellendam district.

Raspalia villosa occurs in a narrow stripe from Paarl up to north of the Cederberg wilderness area and Hall documented one eastern locality north of Calitzdorp.

Raspalia globosa occurs in the Hottentot Holland mountains in the Ceres area and at Stormsvlei.

Raspalia dregeana occurs scattered from the Ceres district, the Clanwilliam division and Wellington.

Staavia

The distribution area of the species of the genus *Staavia* differs from locally restricted to wide spread.

Staavia radiata is the far-most distributed species and occurs from the Langebaan area over Bredasdorp to Humansdorp.

Staavia dregeana is restricted to the Cape peninsula and one other locality east of Paarl.

Staavia glutinosa is restricted to the northern, *Staavia dodii* to the southernmost part of the Cape peninsula.

Staavia comosa occurs from Paarl to Genadendal, to Elim and Bredasdorp.

Staavia brownii is restricted to the coastal Kogelberg area.

Staavia zeyheri occurs in the mountains near Riviersonderend and after Hall in the flats below.

Staavia verticillata shows a distribution from the Paarl District and Worcester.

Staavia phyllicoides is the northernmost *Staavia* species and occurs in the Matzikama mountains.

Thamnea

All six *Thamnea* species show a very local distribution. *Thamnea hirtella* and *Thamnea diosmoides* occur in the Groot Winterhoek wilderness area, the Ceres Mountains and the Tulbagh area.

Thamnea uniflora occurs above Villiersdorp.

Thamnea massoniana shows a distribution in the Kogelberg and Stellenbosch mountains and the Paarl district.

Thamnea gracilis exhibits a split pattern with one locality above Swellendam and another in the Ceres area.

Thamnea thesioides occurs in Ceres.

Tittmannia

The genus *Tittmannia* exhibits both distribution patterns, locally restricted and wide-spread.

Tittmannia laxa is distributed from the Cederberg Wilderness area down to the Paarl district and eastwards to the Breede River. Hall documented another two localities in the area around Ladismith.

Tittmannia esterhuyseniae occurs in the Caledon district, the Riviersonderend Mountains and the Worcester district.

Tittmannia laevis can be found in the Tulbagh and Ceres area, in Stellenbosch and the Paarl district.

Tittmannia hispida is represented with two localities, one in the Cold Bokkeveld and one in the Worcester division.

Vegetation zone analysis

The genera show different preferences to certain vegetation zone types. The vegetation zones will be stated by decreasing frequency.

Berzelia

Berzelia is represented with 140 localities and grows in ten different vegetation zones comprising Sandstone Fynbos (FFs), Shale Fynbos (FFh), Sand Fynbos (FFd), Limestone Fynbos (FFl), Quartzite Fynbos (FFq), Shale Renosterveld (FRs), Western Strandveld (FS), Granite Fynbos (FFg), Ferricrete Fynbos (FFf) and the Rainshadow Valley Karoo Bioregion (SKv) (see Appendix and Fig.). 65% of the localities occur on Sandstone Fynbos.

Staavia

Staavia is represented with 47 localities and inhabits eleven vegetation zones: Sandstone Fynbos (FFs), Sand Fynbos (FFd), Shale Renosterveld (FRs), Granite Fynbos (FFg), Alluvium Fynbos (FFa), Limestone Fynbos (FFl), Western Strandveld (FS), Granite Renosterveld (FRg), Shale Fynbos (FFh) and Quartzite Fynbos (FFq). 44% of the localities occur on Sandstone Fynbos.

Thamnea

Thamnea is represented with 29 localities and occurs in five different vegetation zones: Sandstone Fynbos (FFs), Alluvium Fynbos (FFa), Shale Fynbos (FFh), Shale Renosterveld (FRs) and Granite Fynbos (FFg). 68% of the localities occur on Sandstone Fynbos.

Tittmannia

Tittmannia is represented with 35 localities and grows in seven vegetation zones: Sandstone Fynbos (FFs), Shale Fynbos (FFh), Shale Renosterveld (FRs), Quartzite Fynbos (FFq), Granite Fynbos (FFg), Alluvium Fynbos (FFa) and Sand Fynbos (FFd). 65% of the localities occur on Sandstone Fynbos.

Brunia

Brunia is represented with 76 localities and inhabits ten vegetation zones: Sandstone Fynbos (FFs), Shale Renosterveld (FRs), Granite Fynbos (FFg), Ferricrete Fynbos (FFf), Shale Fynbos (FFh), Alluvium Fynbos (FFa), Sand Fynbos (FFd), Quartzite Fynbos (FFq), Western Strandveld (FS) and the Rainshadow Valley Karoo Bioregion (SKv). 76% of the localities occur on Sandstone Fynbos.

Linconia

Linconia is represented with 18 localities and occurs in four vegetation zones: Sandstone Fynbos (FFs), Ferricrete Fynbos (FFf), Granite Fynbos (FFg) and Shale Fynbos (FFh). 83% of the localities occur on Sandstone Fynbos.

Lonchostoma

Lonchostoma is represented with 30 localities and grows in five vegetation zones: Sandstone Fynbos (FFs), Granite Fynbos (FFg), Alluvium Fynbos (FFa), Ferricrete Fynbos (FFf) and Shale Fynbos (FFh). 80% of the localities occur on Sandstone Fynbos.

Mniothamnea

Mniothamnea is represented with 8 localities and inhabits three vegetation zones: Sandstone Fynbos (FFs), Shale Fynbos (FFh) and Shale Renosterveld (FRs). 75% of the localities occur on Sandstone Fynbos.

Nebelia

Nebelia is represented with 41 localities and grows on eight vegetation zones: Sandstone Fynbos (FFs), Shale Fynbos (FFh), Alluvium Fynbos (FFa), Granite Fynbos (FFg), Ferricrete Fynbos (FFf), Sand Fynbos (FFd), Shale Renosterveld (FRs) and Western Strandveld (FS). 53% of the localities occur on Sandstone Fynbos.

Pseudobaeckea

Pseudobaeckea is represented with 48 localities and inhabits eight vegetation zones: Sandstone Fynbos (FFs), Shale Fynbos (FFh), Shale Renosterveld (FRs), Alluvium Fynbos (FFa), Ferricrete Fynbos (FFf), Granite Fynbos (FFg), Quartzite Fynbos (FFq) and Granite Renosterveld (FRg). 77% of the localities occur on Sandstone Fynbos.

Raspalia

Raspalia is represented with 102 localities and grows on seven vegetation zones: Sandstone Fynbos (FFs), Shale Fynbos (FFh), Alluvium Fynbos (FFa), Shale Renosterveld (FRs), Granite Fynbos (FFg), Quartzite Fynbos (FFq) and the Rainshadow Valley Karoo Bioregion (SKv). 65% of the localities occur on Sandstone Fynbos.

Table 1: Vegetation zones after Mucina et al. (2006) combined with the distribution maps of A.V. Hall (unpublished data), completed with the herbarium sheets of the NBG and BOL and SAM.

species	vegetation/habitat zone	habitat zones per species	localities analysed
<i>Audouinia capitata</i> (L.) BRONGN.	FFs9,11,12	3	10
<i>Berzelia abrotanoides</i> (L.) BRONGN.	FFs5,6,9,10,11,12,13,15,16,17,18,19, FFd2,3,4,5,9, FFg2, FFh5,8,9, FF11,2,3, FRg2,FS7	26	47
<i>Berzelia burchellii</i> DUMMER	FFs15,18,FF13	3	3
<i>Berzelia commutata</i> SOND.	FFs1,3,4,11,19,20,23,24,27,28,29,FFh9,FRs16,FFq6	14	22
<i>Berzelia cordifolia</i> SCHLTDL.	FFs16,17	2	5
<i>Berzelia ecklonii</i> PILLANS	FFs11, FFh6	2	9
<i>Berzelia galpinii</i> PILLANS	FFs15,16,21,22,23,24, FF13, Skv8	8	7
<i>Berzelia incurva</i> PILLANS	FFs12	1	3
<i>Berzelia intermedia</i> (D.DIETR.) SCHLTDL.	FFs1,3,4,9,10,11,12,15,16,17,18,19,20,23,24,25,27,28,29, FF11,3, FRs8,9,13,14, FFh9,10, FFd10,11, FFq6	30	69
<i>Berzelia lanuginosa</i> (L.) BRONGN.	FFs1,3,4,5,6,8,9,10,11,12,13,15,16,17,18,19,20,21,23,24,26,27,28,29, FRg2, FFd3,4,5, FFg2, FFh6, FF11, FRs9,FFq2,3,5,6,FS7,	37	130
<i>Berzelia squarrosa</i> (THUNB.) SOND. (incl. <i>Berzelia rubra</i> SCHLTDL.)	FFs11,12,23,24,27, FFh6, FFf1 (FFs3,4,5,9,10,11,12,13,14,16, FFh1,4, FFq3)	7 (13)	4 (31)
<i>Brunia albiflora</i> E.PHILLIPS	FFs11,12,27,28	4	10
<i>Brunia alopecuroides</i> THUNB.	FFs11,12, FFg2, FFf1, FRs11	5	12
<i>Brunia laevis</i> (THUNB.)	FFs11,12,14,15,16,23,24,27,28,FFg2,FS7,FFf1	12	29
<i>Brunia macrocephala</i> WILLD.	FFs6,7,10,11, FRs4, FFs7,8	7	6
<i>Brunia neglecta</i> SCHLTR.	FFs11,12,13,15,16, FFg2, Skv8	7	12
<i>Brunia noduliflora</i> GOLDBLATT & J.C.MANNING	FFs2,3,4,5,6,9,10,11,12,13,14,15,16,18,19,20,23,24,25,26,27,28,29 FFg2, FFh4,9, FFd5,FRs10,FFa4,FFq3,FRs13,14	32	107
<i>Brunia stokoei</i> E.PHILLIPS	FFs11	1	4
<i>Linconia alopecuroidea</i> L.	FFs10,13,14,15,16	5	12
<i>Linconia cuspidata</i> (THUNB.) SW.	FFs4,5,7,10,11,12,19, FFg2,FFf1,FFh1	10	16
<i>Linconia deusta</i> (THUNB.) PILLANS	FFs13	1	1
<i>Linconia ericoides</i> E.G.H.OLIV.	FFs13,14	2	2
<i>Lonchostoma esterhuyseniae</i> STRID	FFs13,14	2	2
<i>Lonchostoma</i>	FFs3,4,7,8,10,11,12,13,16,FFh6,FFf1,FFg2	12	33

<i>monogynum</i> (VAHL) PILLANS			
<i>Lonchostoma myrtoides</i> (VAHL) PILLANS	FFs4,5,7,8,10,15,16	7	5
<i>Lonchostoma pentandrum</i> (THUNB.) DRUCE	FFs3,4,5,10,11,FFa3,FFg2	7	9
<i>Lonchostoma purpureum</i> PILLANS	FFs11, FFg2	2	4
<i>Mniothamnea bullata</i> SCHLTR. (incl. <i>Raspalia bullata</i>)	FFs15,16 (FFs15,16)	2 (2)	2 (2)
<i>Mniothamnea callunoides</i> (OLIV.) NIED. (incl. <i>Raspalia callunoides</i>)	FFs10,15,16,FRs13 (FFs11,15,16, FFh9)	4 (4)	6 (5)
<i>Nebelia fragarioides</i> (WILLD.) KUNTZE (incl. <i>Brunia globosa</i> (THUNB.)) [incl. <i>Brunia fragarioides</i>]	FFs10,11,14,FFd6,FFa4,FFg2 (FFs5,7,10,11, FFh5) [FFs10,11,12,14, FFh4,6, FFf1]	6 (5) [3]	16 (7) [9]
<i>Nebelia laevis</i> (E.MEY.) KUNTZE (incl. <i>Brunia latebracteata</i>)	(FFs13,14, FFh7)	(3)	(2)
<i>Nebelia paleacea</i> (P.J.BERGIUS) SWEET (incl. <i>Brunia paleacea</i>)	FFs5,10,11,12, FFh1,6, FFa4, FFg2 (FFs5,9,10,11,12,13,14, FRs9,FFa3,4, FFg2, FFh4, FFf1,FS5)	8 (14)	14 (25)
<i>Nebelia sphaercephala</i> (SOND.) KUNTZE (incl. <i>Brunia sphaerocephala</i>)	FFs10,11,15,16,FFg2 (FFs10,11,15,16 FFg2)	5 (5)	13 (10)
<i>Nebelia stokoei</i> PILLANS	FFs10,11,FRs11,FFh6	4	4
<i>Pseudobaekkea africana</i> (BURM.F.) PILLANS	FFs1,3,4,5,7,8,9,10,11,12,FFa4,FRg2,FFq2	13	29
<i>Pseudobaekkea cordata</i> (BURM.F.) NIED.	FFs2,4,5,10,11,12,13,14,15,16,18,19,20,27,28,29, FFg2,FFf1,FRs8,19,FFh4,9	22	45
<i>Pseudobaekkea cordata subsp. monostyla</i> PILLANS	FFs5,10,13,15, FFh4	5	9
<i>Pseudobaekkea stokoei</i> PILLANS	FFs12	1	2
<i>Pseudobaekkea teres</i> (OLIV.) DUMMER	FFs10,13,14,15,16,24,FFh9	7	5
<i>Raspalia angulata</i> (SOND.) NIED.	FFs8,9,10,11,13,14,15,16,23,24, FFg2,FRs11,FFa2,3	14	28
<i>Raspalia barnardii</i> PILLANS	FFs15,16	2	3
<i>Raspalia compacta</i> HALL	FFs3,4,5,13,14, FFh1	6	6
<i>Raspalia dregeana</i> (SOND.) NIED.	FFs7,8, FFq2	3	4
<i>Raspalia globosa</i> (LAM.) PILLANS incl. <i>squalida</i>	FFs10,11,12, FRs12, FFg2	5	4
<i>Raspalia microphylla</i> (THUNB.) BRONGN. (incl. <i>Staavia pinifolia</i> (WILD.))	FFs10,11,12,13,14,15,16,23,24,FFa2,3,4,FFh4,6,FFg2 (FFs9)	15 (1)	35 (3)

<i>Raspalia oblongifolia</i> PILLANS	FFs5,7,FFh1,4,FRs4	5	6
<i>Raspalia palustris</i> (SCHLTR. EX KIRCHN.) Pillans	FFs5,FFh4	2	3
<i>Raspalia phyllicoides</i> (THUNB.) ARN. incl. <i>R. passerinoides</i> C.PRESL	FFs5,10,11,12,13,14,FFg2,FRs11	8	11
<i>Raspalia sacculata</i> (BOLUS EX KIRCHN.) PILLANS	FFs4,5,7,8,10, FFa2,3,FFg2	8	11
<i>Raspalia staavioides</i> (SOND.) PILLANS (incl. <i>Staavia</i> <i>staavioides</i>)	FFs2,3,4 (FFs4)	3 (1)	3 (4)
<i>Raspalia stokoei</i> PILLANS	FFs5,7,8,15,16,FFa3,FRs7,FFh1,4	9	9
<i>Raspalia trigyna</i> DUMMER	FFs16, FFh9	2	1
<i>Raspalia variabilis</i> PILLANS	FFs11,14,FRs6,11,FFa3,FFq3,Skv6	7	6
<i>Raspalia villosa</i> C.PRESL	FFs3,4,5, FFq3	4	13
<i>Raspalia virgata</i> (BRONGN.) PILLANS	FFs10,11,12,13,14,15,16,FFh6,7,FRs6,11,FFa3	12	22
<i>Staavia brownii</i> DUMMER	FFs11	1	4
<i>Staavia comosa</i> COLOZZA	FFs10,11,12,FFg2,FFa2,3	6	17
<i>Staavia dodii</i> BOLUS	FFs9	1	2
<i>Staavia dregeana</i> C.PRESL	FFs9,10	2	5
<i>Staavia glutinosa</i> (L.) DAHL	FFs9,10, FFh5,FRs10	4	5
<i>Staavia phyllicoides</i> PILLANS	FFs1	1	3
<i>Staavia radiata</i> (L.) DAHL (incl. <i>Staavia</i> <i>pinifolia</i> WILLD.)	FFs8,9,10,11,12,15,19,28, FFd3,4,5,7,9, FFg2, FF11,3, FS6,7,FRg2,FRs9,14 (FFs9)	21 (1)	39 (3)
<i>Staavia verticillata</i> (L.F.) PILLANS	FFs10,13, FFg2,FFq4	4	2
<i>Staavia zeyheri</i> SOND.	FFs11,13,14,FRs8	4	4
<i>Thamnea diosmoides</i> OLIV. (<i>Thamnea ustulata</i> HOFFM.)	FFs5,10,FFa3,FFd4 (FFs3,4,5,7,8)	4 (5)	3 (8)
<i>Thamnea gracilis</i> (KUNTZE) OLIV.	FFs5,10,11,13,16,FFh4	6	3
<i>Thamnea hirtella</i> OLIV.	FFs5,10,FFh4,FFa3,FRs9	5	4
<i>Thamnea massoniana</i> DUMMER	FFs10,11,FFg2,FFa3	4	8
<i>Thamnea matroosbergensis</i> HALL	FFs8	1	1
<i>Thamnea thesioides</i> DUMMER	FFs5,FRs8,FFh4	3	2
<i>Thamnea uniflora</i> SOL. EX BRONGN.	FFs10,13,14	3	1
<i>Tittmannia</i>	FFs7,8,10,12	4	4

<i>esterhuyseniae</i> POWRIE			
<i>Tittmannia hispida</i> PILLANS	FFh4,FFs7,8	3	2
<i>Tittmannia laevis</i> PILLANS	FFs5,8,10,11, FFg2,FFa3,FFh4	7	12
<i>Tittmannia laxa</i> (THUNB.) C.PRESL	FFs2,3,4,5,6,7,8,10,15,16,24, FFq2,3,FFh1,4,FRs6,8,9,FFd3	19	38
<i>Staavia capitella</i>	FFs10,13	2	2

Bruniaceae of the Vogelgat Nature Reserve

Table 2: Common names and origin of genus names of the Bruniaceae observed in the Vogelgat Nature Reserve. In: Jackson, W.P.U 1990. Origin of South African plant genera, UCT Ecolab Rondebosch)

species	common name	origin of genus names
<i>Berzelia incurva</i> PILLANS	Knipknopbossie	honouring Count Jacob J. Berzelius, 1779-1845 renowned swedish chemist (also professor of medicine), founder of chemical symbols
<i>Berzelia lanuginosa</i> L.	Kolkol	
<i>Berzelia rubra</i> SCHLECHTD.	splender buttons	
<i>Berzelia squarrosa</i> WENDL.	spider bush	
<i>Brunia albiflora</i> PHILL.	coffee bush	often said to commemorate Dr.Alexander Brown, a ship's surgeon and plant collector who worked in the East Indies around 1690, but more likely after Linnaeus' contemporary the apothecary, Dr.Cornelius Brun who travelled in Russia and the Levant
<i>Brunia alopecuroides</i> THUNB.	red berries	
<i>Brunia laevis</i> THUNB.	Stompie	
<i>Brunia nodiflora</i> L.	Volstruisies	
<i>Lonchostoma monogynum</i> VAHL	?	from the Greek <i>lonche</i> , <i>lance</i> (stoma, mouth), alluding to the lance-shaped sepals and tepals of some species
<i>Nebelia paleacea</i> BERG.	Bergstompie	From the Greek <i>nebel</i> , a harp-like instrument, from Hebrew <i>nebbel</i>
<i>Pseudobaeckea africana</i> BURM.F.	stream bush	"false" Baeckea (or Baekea) a genus in Myrtaceae, after a Swedish physician, Baeck
<i>Pseudobaeckea cordata</i> BURM.F.	heart-leaf Brunia	
<i>Raspalia microphylla</i> THUNB.	false cedar	after one "M.Raspail", a french botanist
<i>Staavia radiata</i> L.	Altydbossie	after one Martin Staaf, a correspondent of Linnaeus

Herbarium sheet data

Audouinia capitata (L.) BRONGN.: Morgan 4821 (BOL); Ecklon & Zeyher 1085 (BOL); Guthrie 913 (BOL); Guthrie 3283 (BOL); Powrie 255 (BOL); Powrie 65 (BOL); Esterhuysen 12933 (BOL); Compton 5312 (BOL); Bolus 23048 (BOL); Wolley-Dod 1556 (BOL); Powrie 13 (BOL); Salter 3949 (BOL); Peers 23047 (BOL); Bean 244 (BOL); Pillans s.n. (BOL); Levyns 6736 (BOL); Caporn s.n. (BOL); Page s.n. (BOL); Taylor s.n. (BOL); De Lange 8 (NBG); MacOwan 1459 (NBG); Marloth 91 (NBG); De Lange 7 (NBG); Taylor 9299 (NBG); De Lange s.n. (NBG); De Lange 22 (NBG); Morgan 4821 (NBG); Goldblatt 1790 (NBG); Esterhuysen 12933 (NBG); Compton 5312 (NBG); De Lange s.n. (NBG); De Lange s.n. (NBG); Bond 435 (NBG); Compton 8916 (NBG); De Lange 8 (NBG); Helme 3401 (NBG); MacOwan 1459 (SAM); Ecklon & Zeyher 1079 (SAM); Zeyher 1085 (SAM); Minicki s.n. (SAM); Quint 29 (MJG); *Berzelia abrotanoides* (L.) BRONGN.: Powrie 127 (BOL); Powrie s.n. (BOL); Clayton s.n. (BOL); Powrie 25 (BOL); Pillans 2774 (BOL); Esterhuysen 33331 (BOL); Schlechter 9365 (BOL); Bolus 2786 (BOL); Bolus 3266 (BOL); Powrie 37 (BOL); Esterhuysen 35089 (BOL); Levyns s.n. (BOL); Collector unknown (BOL); Esterhuysen 4346 (BOL); Esterhuysen 2946 (BOL); Kensit s.n. (BOL); Powrie 158 (BOL); Powrie 48 (BOL); Schlechter 4971 (BOL); Levyns 5691 (BOL); Pillans 1763 (BOL); Powrie 253 (BOL); Grant 2543 (BOL); Leighton 1481 (BOL); Paleker 15 (BOL); Esterhuysen s.n. (BOL); Esterhuysen 30812 (BOL); Smuts 1130 (BOL); Pillans 9965 (BOL); Celliers 4734 (BOL); Powrie 126 (BOL); MacOwan 1050 (BOL); Levyns 10845 (BOL); Burt Davy 18702 (BOL); Leighton 2313 (BOL); Claßen-Bockhoff 1545 (BOL); Froebe & Claßen-Bockhoff 1307 (BOL); Froebe & Claßen-Bockhoff 1381 (BOL); Powrie 163 (BOL); Bean 1134 (BOL); Collector unknown (BOL); Hennecart 6864 (BOL); Levyns 5323 (BOL); Pillans 9964 (BOL); Leighton 666 (BOL); Compton 18819 (BOL); Powrie 148 (BOL); Collector unknown (BOL); Pillans s.n. (BOL); Levyns 6551 (BOL); Froebe 1290 (BOL) Helme 1964 (NBG); *Berzelia intermedia* (D. DIETR.) SCHLTDL.: Powrie 1290 (BOL) Rogers 10502 (BOL); Esterhuysen 31983 (BOL); Powrie 156 (BOL); Esterhuysen 7817 (BOL); Pillans 9824 (BOL); Leighton 23027 (BOL); Powrie 10408 (BOL); Stokoe 8263 (BOL); Esterhuysen 10809 (BOL); Powrie 10720 (BOL); Esterhuysen 31708 (BOL); Esterhuysen 9967 (BOL); Levyns 9752 (BOL); Esterhuysen 4625 (BOL); Levyns 677 (BOL); Fourcade 2530 (BOL); Liebenberg 146 (BOL); Levyns 4368 (BOL); Hutchinson 1189 (BOL); Pillans 3496 (BOL); Esterhuysen 35171 (BOL); Esterhuysen 12517 (BOL); Esterhuysen 11615 (BOL); Bolus 1573 (BOL); Hops 72 (BOL); Rennie & Rennie 209 (BOL); Powrie 155 (BOL); Leighton 2562 (BOL); Rodin 1194 (BOL); Rodin 1299 (BOL); Rodin 1101 (BOL); Hall 1278 (BOL); Hutchinson 1266 (BOL); Esterhuysen 10665 (BOL); Esterhuysen 10853 (BOL); Muir s.n. (BOL); Esterhuysen 10589 (BOL); Esterhuysen 6479 (BOL); Esterhuysen 4714 (BOL); Cassidy 124 (BOL); Hall 1335 (BOL); Powrie 254 (BOL); Stokoe 8262 (BOL); Esterhuysen 13577 (BOL); Bean & Viviers 1189 (BOL); Powrie 157 (BOL); Leipoldt 17088 (BOL); Hall 1294 (BOL); Esterhuysen 10666 (BOL); Hall 1295 (BOL); Esterhuysen 33304 (BOL); Fourcade 2882 (BOL); Fourcade 6409 (BOL); Hall 1297 (BOL); Esterhuysen 12581 (BOL); Esterhuysen 10032 (BOL); Compton 17654 (BOL); Pillans 9974 (BOL); Stokoe 8821 (BOL); Stokoe 8904 (BOL); Pillans 9859 (BOL); Esterhuysen 12964 (BOL); Stokoe 7332 (BOL); Parker 4783 (BOL); Esterhuysen 10017 (BOL); Leighton 2313 (BOL); Esterhuysen 13071 (BOL); Esterhuysen 7999 (BOL); Fourcade 804 (BOL); Strid & Strid 37963 (NBG); *Berzelia burchellii* DUMMER: Powrie 151 (BOL); Powrie 154 (BOL); Hall 1286 (BOL); Muir 102 (BOL); Muir 5096 (BOL); *Berzelia commutata* SOND.: Hutchinson 1189 (BOL); Fourcade 3543 (BOL); Tolken 3014 (BOL); Vlok 1391 (BOL); Esterhuysen 27146 (BOL); Pillans 734 (BOL); Vlok 1765 (BOL); *Berzelia cordifolia* SCHLTDL.: Pillans 9330 (BOL); Esterhuysen 23278 (BOL); Pillans 9352 (BOL); Vlok 1710 (BOL); Pillans 9511 (BOL); Esterhuysen 29365 (BOL); Levyns 8379 (BOL); Boucher 3749 (NBG); Burgers 1109 (NBG); Taylor 7179 (NBG); Taylor 4005 (NBG); Univ. students 9 (NBG); Rycroft 1743 (NBG); Pillans 9352 (NBG); Morley 94 (NBG); O'Callaghan 396 (NBG); Van Wyk 1760 (NBG); Rourke 952 (NBG); Rourke s.n. (NBG); Oliver 3217 (NBG); Pillans 9352 (NBG); Helme 3556 (NBG); Ecklon & Zeyher 1060 (SAM); Ecklon & Zeyher s.n. (SAM); Lewis 3024 (SAM); Quint 48 (MJG); *Berzelia dregeana* COLOZZA: Stokoe 7333 (BOL); Stokoe 6023 (BOL); Stokoe 18360 (BOL); Stokoe 7334 (BOL); Collector unknown (BOL); Stokoe 6006 (BOL); Collector unknown (BOL); Stokoe 996 (BOL); Drège s.n. (BOL); Stokoe 17734 (BOL); Stokoe 17441 (BOL); Stokoe 17734 (BOL); Stokoe 6024 (BOL); Stokoe 6026 (BOL); *Berzelia ecklonii* PILLANS: Hall 1331 (BOL); Esterhuysen 35568 (BOL); Stokoe 17321 (BOL); Stokoe 8264 (BOL); Stokoe 7142 (BOL); Powrie 162 (BOL); Powrie 49 (BOL); Leighton 757 (BOL); Hall 1277 (BOL); Powrie 83 (BOL); Stokoe 8820 (BOL); Esterhuysen 9967 (BOL); Esterhuysen 9967 (BOL); Stokoe 17869 (BOL); Rourke 983 (NBG); Compton 16527 (NBG); Taylor 3885 (NBG); Rycroft 1362 (NBG); Compton 14120 (NBG); Compton 16452 (NBG); Compton 16455 (NBG); Powrie 52 (NBG); Kruger 1151 (NBG); Stokoe s.n. (NBG); Stirton 9977 (NBG); Grobler 30307 (NBG); Le Maitre 416 (NBG); Le Maitre 407 (NBG); Kruger 902 (NBG); Grobler 15134 (NBG); Boucher 876 (NBG); Stokoe s.n. (SAM); Stokoe 7142 (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher 1067 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); *Berzelia galpinii* PILLANS: Hall 1296 (BOL); Collector unknown (BOL); Claßen-Bockhoff 1518 (BOL); Powrie 153 (BOL); Levyns 8987 (BOL); Collector unknown (BOL); Muir 3319 (BOL); Froebe & Claßen-Bockhoff 1320 (BOL); Claßen-Bockhoff 1518 (BOL); Haynes 1394 (NBG); Hubbard 32 (NBG); Bohnen 8712 (NBG); Burger 23 (NBG); Burger 6 (NBG); Powrie 391 (NBG); Fellingham 444 (NBG); Van Wyk 695 (NBG); Marsh 1163 (NBG); Rourke 2011 (NBG); Rourke 1879 (NBG); Stokoe s.n. (SAM); Claßen-Bockhoff 4028 (MJG); *Berzelia incurva* PILLANS: Paterson 18879 (BOL); Stokoe 8801 (BOL); Powrie 171 (BOL); Esterhuysen 26970 (BOL); Burman 1274 (BOL); Esterhuysen 33577 (BOL); Esterhuysen 4984 (BOL); Stokoe 7425 (BOL); Orchard 410 (NBG); Williams 3002 (NBG); Williams 2269 (NBG); Esterhuysen 4984 (NBG); Thorne s.n. (SAM); Stokoe s.n. (SAM); Quint 45 (MJG); *Berzelia lanuginosa* (L.) BRONGN.: Paleker 16 (BOL); Burl 4 (BOL); Levyns 4801 (BOL); Pillans 7437 (BOL); Fourcade 350 (BOL); Stokoe 7625 (BOL); Compton 4469 (BOL); Levyns 2945 (BOL); Pillans 9085 (BOL); Esterhuysen 11394 (BOL); Esterhuysen 11529 (BOL); Bolus 1574 (BOL); Parker 4776 (BOL); Leighton 1574 (BOL); Hutchinson 347 (BOL); Levyns 3298 (BOL); Pillans 9865 (BOL); Vlok 811 (BOL); Vlok 917 (BOL); Esterhuysen 12229 (BOL); Levyns 3297 (BOL); Leighton 1348 (BOL); Esterhuysen 11975 (BOL); Esterhuysen 4271 (BOL); Esterhuysen 11145 (BOL); Powrie 122 (BOL); Van Niekerk 23208 (BOL); Leighton 1925 (BOL); Collector unknown (BOL); Fourcade 5303 (BOL); Fourcade 4570 (BOL); Fourcade 4113 (BOL); Muir 13673 (BOL); Hall 1309 (BOL); Hall 1311 (BOL); Pillans 9876 (BOL); Esterhuysen 31330 (BOL); Hall 1312 (BOL); Hall 1332 (BOL); Hall s.n. (BOL); Hall 1325 (BOL); Hall 1324 (BOL); Hall 1323 (BOL); Hall 1321 (BOL); Hall 1320 (BOL); Hall 1322 (BOL); Hall 1334 (BOL); Hall s.n. (BOL); Hall 1304 (BOL); Hall 1306

(BOL); Hall 1313 (BOL); Hall 1315 (BOL); Hall 1308 (BOL); Hall 1318 (BOL); Hall 1302 (BOL); Hall 1314 (BOL); Bean 691 (BOL); Michell s.n. (BOL); Cassidy 43 (BOL); Levyns 6794 (BOL); Levyns 5199 (BOL); Levyns s.n. (BOL); Pillans s.n. (BOL); Powrie 140 (BOL); Stokoe 8257 (BOL); Esterhuysen 7362 (BOL); Barnes 23028 (BOL); Drake 8606 (BOL); Lewis 22068 (BOL); Esterhuysen 122 (BOL); Gray 12 (BOL); Esterhuysen13374 (BOL); Esterhuysen 4815 (BOL); Phillips 7551 (BOL); Schlechter 8606 (BOL); Middlemost 19280 (BOL); Drege s.n. (BOL); Gillett 984 (BOL); Esterhuysen 2621 (BOL); Vlok, Van Wyk & Schutte 89 (BOL); Stokoe 9218 (BOL); Stokoe s.n. (BOL); Stokoe 6031 (BOL); Stokoe 6029 (BOL); Galpin 12862 (BOL); Stokoe 6032 (BOL); Esterhuysen 2895 (BOL); Gilmore 2504 (BOL); Schlechter 9561 (BOL); Stokoe 6030 (BOL); Levyns 3295 (BOL); Wolley-Dod 2733 (BOL); Bolus 2611 (BOL); Schlechter 1281 (BOL); Pillans 9885 (BOL); Parker 3930 (BOL); Esterhuysen 10120 (BOL); Middlemost 18447 (BOL); Esterhuysen 12098 (BOL); Esterhuysen 9971 (BOL); Collector unknown (BOL); Rodin 3182 (BOL); Esterhuysen 11513 (BOL); Hutchinson 310 (BOL); Esterhuysen 10968 (BOL); Esterhuysen 12064 (BOL); Powrie 129 (BOL); Froebe & Claßen-Bockhoff 1457 (BOL); Collector unknown (BOL); Levyns 9828 (BOL); Collector unknown (BOL); Stokoe s.n. (BOL); Powrie 35 (BOL); Stokoe 7034 (BOL); Paterson 35 (BOL); Compton 36884 (BOL); Esterhuysen 1694 (BOL); Gillett 3602 (BOL); Esterhuysen 9152 (BOL); Burman 1207 (BOL); Esterhuysen 34000 (BOL); Esterhuysen 1347 (BOL); Leighton 1347 (BOL); Esterhuysen 1640 (BOL); Esterhuysen 1664 (BOL); Claßen-Bockhoff 1410 (BOL); Stokoe 2503 (BOL); Powrie 139 (BOL); Esterhuysen 9719 (BOL); Froebe & Claßen-Bockhoff 1291 (BOL); Froebe & Claßen-Bockhoff 1371 (BOL); Froebe & Claßen-Bockhoff 1089 (BOL); Esterhuysen 8274 (BOL); Powrie 159 (BOL); Stokoe 6025 (BOL); Powrie 131 (BOL); Esterhuysen 9800 (BOL); Stokoe 8905 (BOL); Stokoe 2739 (BOL); Powrie 32 (BOL); Esterhuysen 2922 (BOL); Esterhuysen 4989 (BOL); Esterhuysen 8988 (BOL); Powrie 244 (BOL); Stokoe 508 (BOL); Stokoe 8258 (BOL); Hall 1290b (BOL); Claßen-Bockhoff 1530 (BOL); Hall 1292 (BOL); Hall 1299 (BOL); Powrie 170 (BOL); Esterhuysen 11442 (BOL); Stokoe 6007 (BOL); Levyns 3296 (BOL); Smuts s.n. (BOL); Hall 1289B (BOL); Esterhuysen 23404 (BOL); Compton 4135 (BOL); Esterhuysen 13128 (BOL); Leighton 21604 (BOL); Levyns 4661 (BOL); Esterhuysen 13416 (BOL); Schlechter 7778 (BOL); Schlechter 8926 (BOL); Hutchinson 602 (BOL); Esterhuysen 6180 (BOL); Esterhuysen 11443 (BOL); Fourcade 804 (BOL); Wurts 2144 (NBG); MacPherson 35 (NBG); Compton 9674 (NBG); Mathews 33 (NBG); Grobler 493 (NBG); Strid & Strid 37230 (NBG); Grobler 22225 (NBG); Fourcade 804 (NBG); Fourcade 4113 (NBG); Walters 1940 (NBG); Barker 3085 (NBG); Kruger 283 (NBG); Compton 15970 (NBG); Taylor 6923 (NBG); Haynes 1403 (NBG); Duthie 725 (NBG); Fourcade 2530 (NBG); Taylor 5013 (NBG); Gillett 3602 (NBG); Hugo 1305 (NBG); Forsyth 119 (NBG); Maguire 49 (NBG); Dahlstrand 1298 (NBG); Dahlstrand 630 (NBG); Hubbard 337 (NBG); Taylor 5013 (NBG); Boucher 435 (NBG); Runnalls 548 (NBG); Rode 421 (NBG); Pretorius 487 (NBG); Collector unknown (NBG); Gillett 1794 (NBG); De la Bat s.n. (NBG); De Vos 555 (NBG); O'Callaghan 1447 (NBG); De la Bat s.n. (NBG); Boucher 1434 (NBG); Chater & Hansford 137 (NBG); Boucher 3678 (NBG); Boucher 2307 (NBG); Boshoff P289 (NBG); McDonald 1344 (NBG); Compton 6519 (NBG); Taylor 5901 (NBG); Fourcade 6409 (NBG); Cassidy 43 (NBG); Barker 4155 (NBG); Maguire 2618 (NBG); Taylor 6454 (NBG); Rourke 1761 (NBG); Hugo 1354 (NBG); Andreae 1087 (NBG); Walters 296 (NBG); Leith 65 (NBG); Paterson-Jones 91 (NBG); Topper 507 (NBG); Williams 2244 (NBG); Goldblatt 1564 (NBG); Williams 819 (NBG); Powrie 25 (NBG); Parker 4783 (NBG); Rycroft 1822 (NBG); Middlemost 1679 (NBG); Compton 6108 (NBG); Compton 19002 (NBG); Barker 4048 (NBG); Rourke s.n. (NBG); Heyns s.n. (NBG); Raitt 343 (NBG); Gillett 3512 (NBG); Van Wyk 873 (NBG); Smuts s.n. (NBG); Malherbe s.n. (NBG); Orchard 337 (NBG); Boucher 3573 (NBG); Fellingham 305 (NBG); Van der Kooij 6 + 19 (NBG); Burgers 2834 (NBG); Rode & Boucher 71 (NBG); Bohnen 8838 (NBG); Andreae 218 (NBG); Marloth 3254 (NBG); Daneel 4 (NBG); Haynes 900 (NBG); Taylor 9794 (NBG); Taylor 4383 (NBG); Taylor 4894 (NBG); Smuts s.n. (NBG); Boucher 2854 (NBG); Thompson 2643 (NBG); Montgomery 267 (NBG); Esterhuysen 35245 (NBG); Middlemost 2262 (NBG); Compton 16088 (NBG); Compton 11961 (NBG); Compton 11985 (NBG); Compton 16175 (NBG); Esterhuysen 31689 (NBG); Rourke 2150 (NBG); Rourke 2105 (NBG); Williams 2037 (NBG); Compton 6424 (NBG); Compton 4135 (NBG); Rourke 1098 (NBG); Walters 566 (NBG); Taylor 5013 (NBG); Rourke 824 (NBG); De Villiers s.n. (NBG); Stokoe 6030 (NBG); Esterhuysen 4989 (NBG); Herre s.n. (NBG); Herre s.n. (NBG); Markötter s.n. (NBG); Jordaan 817 (NBG); Esterhuysen 31690 (NBG); Collector unknown (NBG); Rourke 1296 (NBG); Smuts s.n. (NBG); Williams 2558 (NBG); Williams 2174 (NBG); Walters 235 (NBG); Van der Merwe 1796 (NBG); Orchard 463 (NBG); Stokoe 63918 (NBG); Oliver 8687 (NBG); Jordaan 830 (NBG); Jacobs 8 (NBG); Pocock 83 (NBG); Andreae 157 (NBG); Walters 1194 (NBG); Walters 4 (NBG); Walters 271 (NBG); Walters 1169 (NBG); Taylor 10308 (NBG); Fellingham 499 (NBG); Boucher 2634 (NBG); Oliver 6055 (NBG); Orchard 274 (NBG); Stirton 5866 (NBG); Andrag 253 (NBG); McDonald 601 (NBG); Van der Merwe 2180 (NBG); Thode 4761 (NBG); Taylor 6591 (NBG); Thode 8151 (NBG); Haynes H.54 (NBG); Pretorius 174 (NBG); Barker 591 (NBG); Compton 21803 (NBG); Williams 2286 (NBG); Compton 7725 (NBG); Ecklon & Zeyher 715 (NBG); Schlechter 745 (NBG); Boucher 408 (NBG); Boucher 1333 (NBG); Van der Walt 385 (NBG); Barker 7689 (NBG); Bond 891 (NBG); Rycroft 2611 (NBG); Compton 4469 (NBG); Lewis 5402 (NBG); Braun 7 (NBG); Williams 2285 (NBG); Kruger 1320 (NBG); Taylor 10052 (NBG); Van der Merwe 197 (NBG); Kruger 1195 (NBG); Lamb 199 (NBG); McDonald 1138 (NBG); Wagner s.n. (NBG); Andreae 340 (NBG); Rourke 933 (NBG); Henderson 1487 (NBG); Barker 7147 (NBG); Barker 6889 (NBG); Heginbotham 333 (NBG); Wurts 319 (NBG); Esterhuysen 13309 (NBG); Esterhuysen 10408 (NBG); Stokoe s.n. (NBG); Rourke 1756 (NBG); Oliver 5667 (NBG); McDonald 1553 (NBG); Stephensen s.n. (NBG); Esterhuysen 10032 (NBG); Fellingham 1579 (NBG); Compton 13068 (NBG); Barker 10162 (NBG); Rourke s.n. (NBG); Barker 5245 (NBG); Willems 95 (NBG); Taylor 4894 (NBG); Schonland 3621 (NBG); Balkema s.n. (NBG); Campbell 13481 (NBG); Parsons 138 (NBG); Kapp 12 (NBG); Middlemost 1926 (NBG); Henderson 1412 (NBG); Taylor 5924 (NBG); Scott 397 (NBG); Van Zinderen Bakker 47 (NBG); Stokoe s.n. (NBG); Barker 611 (NBG); Van Jaarsveld 7749 (NBG); Boucher 2710 (NBG); Liebenberg 6365 (NBG); Fourcade 4570 (NBG); Fourcade 5303 (NBG); Rycroft 2611 (NBG); Andreae 1262 (NBG); Rourke 1898 (NBG); Rourke 1905 (NBG); Exhibitions 89 (NBG); McDonald 1536 (NBG); Du Plessis 20 (NBG); McDonald 1670 (NBG); Walters 2345 (NBG); Bond 1739 (NBG); Marsh 1343 (NBG); Van Wyk 1134 (NBG); Williams 2617 (NBG); Stokoe 420 (NBG); Markötter s.n. (NBG); Du Plessis 48 (NBG); Thompson 2065 (NBG); Marais s.n. (NBG); Levyns & Levyns 677 (NBG); Taylor 4249 (NBG); Bohnen 5992 (NBG); Boucher 3407 (NBG); Morley 182 (NBG); O'Callaghan 290 (NBG); Stokoe s.n. (NBG); Compton 7222 (NBG); Barker 4603 (NBG); De Vos 1131 (NBG); De Vos 410 (NBG); De Vos 332 (NBG); Van Wyk 944 (NBG); Boucher & Shepherd 4564 (NBG); Kruger 1609 (NBG); Stokoe 1465 (NBG); Compton 8726 (NBG); Compton 9020 (NBG); Compton 18819 (NBG); Bond 516 (NBG); Compton 13879 (NBG); Barker 4310 (NBG); Burgers 17262 (NBG); Boucher 3288 (NBG); Thompson 563 (NBG); Van Wyk 1643 (NBG); Montgomery 267 (NBG); Bohnen 5531 (NBG); Muir 102 (NBG); O'Callaghan, Fellingham & van

Wyk 82 (NBG); Campbell 14834 (NBG); Rourke 1904 (NBG); Parker 3930 (NBG); Guthrie 2486 (NBG); Barker 6889 (NBG); Walters 1101 (NBG); Michell s.n. (NBG); Ebersohn 7/68 (NBG); Wasserfall 549 (NBG); Compton 18614 (NBG); Walters 571 (NBG); Walters 234 (NBG); Rode & Boucher 168 (NBG); Compton 13606 (NBG); Parker 4776 (NBG); Boucher & Stindt 5318 (NBG); Compton 23681 (NBG); Cohen s.n. (NBG); Compton 22924 (NBG); Walters 268 (NBG); Walters 1418 (NBG); Tymens s.n. (NBG); Williams 850 (NBG); Williams 2067 (NBG); Williams 2074 (NBG); Goldblatt 2900 (NBG); Paterson-Jones 587 (NBG); Steiner 2600 (NBG); Walters 399 (NBG); Orchard 377 (NBG); De Vos 69 (NBG); Boucher 540 (NBG); Lamb 137 (NBG); Davies 16 (NBG); Kruger KR.927 (NBG); Pappe s.n. (NBG); Marloth 9558 (NBG); De Kock s.n. (NBG); Rode & Boucher 28 (NBG); Tromp 11 (b) (NBG); O'Callaghan 289 (NBG); Compton 10602 (NBG); Boucher 574 (NBG); Woodvine 23 (NBG); Grobler 715 (NBG); Moffett 2 (NBG); Burgers 1053 (NBG); Gillett 420 (NBG); Taylor 5063 (NBG); Rossouw s.n. (NBG); Thode 846 (NBG); Fellingham 1291 (NBG); Haynes 844 (NBG); Gillett 658 (NBG); Steyn s.n. (NBG); Van Wyk 603 (NBG); Jacobs 16 (NBG); Rogers 17515 (NBG); Fourcade 2829 (NBG); Scharf 1785 (NBG); Scharf 1744 (NBG); Muir 732 (NBG); Forsyth 119 (NBG); Kruger 1062 (NBG); Kruger KR. 1034 (NBG); Phillips 7551 (NBG); Fellingham 306 (NBG); Dahlstrand 1347 (NBG); McKinnon 189 (NBG); Phillips 7551 (NBG); Van der Merwe 24-32 (NBG); Morrison s.n. (NBG); Thode 8149 (NBG); Nel s.n. (NBG); Keet s.n. (NBG); Stauffer & Esterhuysen 5039 (NBG); Taylor 10514 (NBG); Van der Walt 416 (NBG); Van der Merwe 2046 (NBG); Kruger 328 (NBG); Bos 285 (NBG); Boucher 2627 (NBG); Van Wyk 2034 (NBG); Dahlstrand 2222 (NBG); Marloth 1087 (NBG); Stokoe s.n. (NBG); Taylor 8483 (NBG); Meyer s.n. (NBG); Collector unknown (NBG); Hubbard 229 (NBG); Gillet 12 (NBG); Durand 85 (NBG); Gillett 1511 (NBG); Taylor 8101 (NBG); Downing 377 (NBG); Andrag 148 (NBG); Grobler 365 (NBG); Van der Hoven 30 (NBG); McDonald 1507 Bos 744 (NBG); Smuts s.n. (NBG); Smuts s.n. (NBG); Taylor 5063 (NBG); Pocock 595 (NBG); Boucher 573 (NBG); Boucher 687 (NBG); Oliver 3304 (NBG); Kruger 1390 (NBG); Kruger 1508 (NBG); Boucher 17 (NBG); Keet s.n. (NBG); Thompson 1606 (NBG); Pond UP 230 (NBG); Pond UP 110 (NBG); Botha (3)008 (NBG); Botha (3)015 (NBG); Botha (3)074 (NBG); Martínez-Azorín 66 (NBG); Helme 4380 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher 1060 (SAM); Ecklon & Zeyher 1058 (SAM); MacOwan 1610 (SAM); Bolus 3266 (SAM); Lamb 1827 (SAM); Ecklon & Zeyher 1060 (SAM); Potts 1555 (SAM); Pappe s.n. (SAM); Thorne s.n. (SAM); Muir 102 (SAM); Lewis 1722 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Purcell 317 (SAM); Pappe s.n. (SAM); Ecklon & Zeyher 1610 (SAM); Phillips 1125 (SAM); Stokoe s.n. (SAM); Stokoe 17734 (SAM); Lamb 2970 (SAM); Zeyher s.n. (SAM); Zeyher s.n. (SAM); Paterson 1916 = 887 (SAM); MacOwan 1092 (SAM); Rogers 29216 (SAM); Stokoe s.n. (SAM); Phillips 1126 (SAM); Stokoe s.n. (SAM); Phillips 7551 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Pappe s.n. (SAM); Pappe s.n. (SAM); Rogers 17515 (SAM); Zeyher 2642 (SAM); Ecklon & Zeyher 1053 (SAM); Stokoe s.n. (SAM); Pappe s.n. (SAM); Zeyher 728 (SAM); Ecklon & Zeyher 1054 (SAM); Ecklon & Zeyher 1053 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Rogers 28752 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Barnard 422 (SAM); Bolus 2611 (SAM); Tyson 788 (SAM); Ecklon & Zeyher s.n. (SAM); Ecklon & Zeyher 1056 (SAM); Ecklon & Zeyher 2641 (SAM); Ecklon & Zeyher 1057 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher (SAM); 1055 Stokoe s.n. (SAM); Phillips s.n. (SAM); Salisbury s.n. (SAM); Barnard s.n. (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Lewis 3025 (SAM); Claßen-Bockhoff 4013 (MJG); Claßen-Bockhoff 4046a (MJG); Claßen-Bockhoff 4046b (MJG); Quint 59 (MJG); **Berzelia rubra** SCHLTR.: Quint 12 (MJG); Quint 21 (MJG); Quint 10 (MJG); Quint 54 (MJG); Quint 56 (MJG); Quint 57 (MJG); Quint 58 (MJG); **Berzelia squarrosa** (THUNB.) SOND.: Bolus 11493 (BOL); Esterhuysen 9964 (BOL); Boucher 7089 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); **Brunia albiflora** E.PHILLIPS: Stokoe 8259 (BOL); Pillans 8218 (BOL); Collector unknown (BOL); Guthrie 16936 (BOL); Collector unknown (BOL); Collector unknown (BOL); Middlemost s.n. (BOL); Stokoe 17429 (BOL); Stokoe 8260 (BOL); Esterhuysen 9965 (BOL); Michell 15847 (BOL); Michell 15276 (BOL); Stokoe 17217 (BOL); Claßen-Bockhoff 1520 (BOL); Esterhuysen 33576 (BOL); Burt Davy 18560 (BOL); Stokoe 17709 (BOL); Collector unknown (BOL); Levyns 2674 (BOL); Smuts 1202 (BOL); Burman 1115 (BOL); Behr 1094 (NBG); Rode629 (NBG); Botha (2)060 (NBG); **Brunia alopecuroides** THUNB.: Bean 694 (BOL); Powrie 46 (BOL); Burman 1182 (BOL); Burman 1260 (BOL); Collector unknown (BOL); Stokoe 23036 (BOL); Cuthbert 8604 (BOL); Stokoe 2581 (BOL); Esterhuysen s.n. (BOL); Esterhuysen 2894 (BOL); Froebe & Claßen-Bockhoff 1363 (BOL); Hall 1329 (BOL); Froebe & Claßen-Bockhoff 1364 (BOL); Stokoe 7035 (BOL); Esterhuysen 9720 (BOL); Hall 1307 (BOL); Powrie 95 (BOL); Leighton 872 (BOL); Pillans 8571 (BOL); Levyns 10445 (BOL); Levyns 3289 (BOL); Esterhuysen 9962 (BOL); Esterhuysen 2620 (BOL); Botha (2)062 (NBG); Quint 55 (MJG); **Brunia fragarioides** WILLD.: Esterhuysen 7998 (BOL); Esterhuysen s.n. (BOL); Haynes H. 274 (NBG); Van Wyk 974 (NBG); Viviers 667 (NBG); Haynes 698 (NBG); Van der Merwe 1233 (NBG); Stokoe s.n. (NBG); Marloth 5331 (NBG); Fellingham 498 (NBG); Kruger 1147 (NBG); Compton 7726 (NBG); Marloth 2492 (NBG); Forsyth 14 (NBG); Kruger KR. 122 (NBG); Van der Merwe 923 (NBG); Grobler 15132 (NBG); Compton 7726 (NBG); Compton 9227 (NBG); Compton 6418 (NBG); Compton 13529 (NBG); Rourke 1097 (NBG); Hansford & Hansford 24 (NBG); Scott 305 (NBG); Van Wyk 1172 (NBG); McDonald 612 (NBG); Kruge 1802 (NBG); Andreae 1058A (NBG); Andreae 1058B (NBG); NBG s.n. (NBG); Expedition Parker 4800 (NBG); Boucher 418 (NBG); Hubbard 438 (NBG); Ecklon & Zeyher 1081 (SAM); Stokoe s.n. (SAM); Zeyher 2650 (SAM); Lamb 2974 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher 1081 (SAM); Stokoe s.n. (SAM); Zeyher 2650 (SAM); Stokoe s.n. (SAM); **Brunia laevis** THUNB.: Burman 1278 (BOL); Leighton 352 (BOL); Bolus 20536 (BOL); Collector unknown (BOL); Schlechter 7331 (BOL); Collector unknown (BOL); Bolus 7387 (BOL); Bolus 5349 (BOL); Levyns 10658 (BOL); Claßen-Bockhoff 1542 (BOL); Esterhuysen 4969 (BOL); Gilmore 2501 (BOL); Froebe & Claßen-Bockhoff 1368 (BOL); Levyns 4534 (BOL); Leighton 1578 (BOL); Leighton 2581 (BOL); Stirton 11322 (BOL); Rode & Boucher 11 (NBG); Van Breda 1661 (NBG); Rycroft 1828 (NBG); Downing 344 (NBG); Jordaan 822 (NBG); Jordaan 821 (NBG); Cowling 1722 (NBG); Hugo 1547 (NBG); Beyers 9 (NBG); Nel 17259 (NBG); Lotter 17100 (NBG); Orchard 361 (NBG); Van der Walt 388 (NBG); Bolus 5349 (NBG); Van Wyk 601 (NBG); Thomas s.n. (NBG); Winkler 7 (NBG); Bond 770 (NBG); De Klerk 5 (NBG); Boucher & Stindt 5380 (NBG); Guthrie 3571 (NBG); Van Wyk 800 (NBG); Mathews 34 (NBG); Williams 2274 (NBG); Williams 2072 (NBG); Paterson-Jones 372 (NBG); Boucher & Stindt 5397 (NBG); Marsh 1199 (NBG); Boucher 1777 (NBG); Marsh 1199 (NBG); De Vos 494 (NBG); Hugo 1734 (NBG); Van der Kooij & Van der Kooij 2027 (NBG); De Kock s.n. (NBG); Rycroft 1828 (NBG); Rycroft 1017 (NBG); Compton 19011 (NBG); Rourke 1691 (NBG); Williams 2855 (NBG); Compton 21941 (NBG); Walters 355 (NBG); Guthrie s.n. (NBG); Collector unknown (NBG); Esterhuysen 4959 (NBG); Van der Merwe 239 (NBG); Kruger 901 (NBG); Grobler 240 (NBG); Bohnen 8665 (NBG); Esterhuysen 34932A (NBG); Johnson 18 (NBG); Compton 14250 (NBG); Barker 7200 (NBG); De Villiers NBG3298/32 (NBG); Thunberg 5740 (NBG); Acocks 22826 (NBG); Boucher 641 (NBG); Botha (2)093 (NBG); Lewis 3026

(SAM); Bolus 5349 (SAM); Stokoe s.n. (SAM); Zeyher 2640 (SAM); Ecklon & Zeyher 1081 (SAM); Stokoe s.n. (SAM); Zeyher 2640 (SAM); Rogers 29136 (SAM); *Brunia macrocephala* WILLD.: Marloth 2353 (BOL); Collector unknown (BOL); Marloth 2353 (NBG); Schonken 290 (NBG); Esterhuysen 10352 (NBG); Esterhuysen 30.223 (NBG); Taylor 6446 (NBG); Marloth 2355 (NBG); McDonald 1700 (NBG); Marloth 2353 (NBG); Marloth 2353 (SAM); Barnard s.n. (SAM); Stokoe s.n. (SAM); Quint 17 (MJG); *Brunia marlothii* SCHLTR.: Bolus 6364 (BOL); Hutchinson 631 (BOL); Esterhuysen 35424 (BOL); Esterhuysen 10352 (BOL); Esterhuysen 34763 (BOL); Esterhuysen 30223 (BOL); Bolus 6364 (BOL); Esterhuysen 12519a (BOL); Powrie 61 (BOL); Schlechter 7297 (BOL); Esterhuysen 34932a (BOL); Esterhuysen 35382 (BOL); Powrie 110 (BOL); Esterhuysen 34962 (BOL); Esterhuysen 4959 (BOL); Esterhuysen 11980 (BOL); Stokoe 7330 (BOL); Levyns 6334 (BOL); Hall 1333 (BOL); *Brunia neglecta* SCHLTR.: McDonald 1252 (NBG); Quint 25 (MJG); *Brunia noduliflora* GOLDBLATT & J.C.MANNING: Esterhuysen 25765a (BOL); Esterhuysen 10746 (BOL); Esterhuysen 1114 (BOL); Smuts 1078 (BOL); Fourcade 149 (BOL); Burman 768 (BOL); Rodin 3305 (BOL); Leighton 1005 (BOL); Pillans 7845 (BOL); Esterhuysen 25765a (BOL); Esterhuysen 10746 (BOL); Esterhuysen 1114 (BOL); Smuts 1078 (BOL); Fourcade 149 (BOL); Burman 768 (BOL); Rodin 3305 (BOL); Leighton 1005 (BOL); Pillans 7845 (BOL); Esterhuysen 7845 (BOL); Esterhuysen 9690 (BOL); Esterhuysen 33824 (BOL); Esterhuysen 17155 (BOL); Esterhuysen 33842 (BOL); Esterhuysen1894 (BOL); Esterhuysen 4754 (BOL); Pillans 7343 (BOL); Esterhuysen 8989 (BOL); Esterhuysen 24307 (BOL); Esterhuysen 25901 (BOL); Esterhuysen 13064 (BOL); Collector unknown (BOL); Levyns 7452 (BOL); Levyns 9080 (BOL); Hutchinson 47(BOL); Powrie 64 (BOL); Claßen-Bockhoff & Froebe 1265 (BOL); Bolus 7299 (BOL); Pillans 9590 (BOL); Stokoe 17500 (BOL); Esterhuysen 35430 (BOL); Esterhuysen 650 (BOL); Archibald 3495 (BOL); Esterhuysen 10808 (BOL); Esterhuysen 12829 (BOL); Esterhuysen 11597 (BOL); Levyns 7440 (BOL); Levyns 11535 (BOL); Pocock S15 (BOL); Rodin1121 (BOL); Fourcade 149 (BOL); Compton 4210 (BOL); Esterhuysen 4567 (BOL); West 259 (BOL); Fourcade 6530 (BOL); Compton 23577 (BOL); Pillans s.n. (BOL); Wurts 594 (BOL); Whitworth 23035 (BOL); Esterhuysen 10177 (BOL); Bean1403 (BOL); Cassidy 169 (BOL); Guthrie 480 (BOL); Kensit s.n. (BOL); Middlemost 1925 (NBG); Behr 1132 (NBG); Chater & Hansford 139 (NBG); Grobbelaar 2146 (NBG); Forrester 1101 (NBG); Van Wyk 1009 (NBG); Moffett 2671 (NBG); Van Wilgen 84 (NBG); Manson 51 (NBG); Schonken 4 (NBG); McKinnon 188 (NBG); Viviers 1328 (NBG); Du Plessis 19 (NBG); Taylor 6399 (NBG); Van Wyk 1241 (NBG); Van der Merwe 21-81 (NBG); Smuts s.n. (NBG); Smuts s.n. (NBG); Vlok 2125 (NBG); Vlok 2103 (NBG); Taylor 12118(NBG); (NBG); Kruger 1285 (NBG); Wurts 594 (NBG); Strid & Strid 37247 (NBG); Kruger 1042 (NBG); Moffett 277 (NBG); Andreae 335 (NBG); Fourcade s.n. (NBG); Van der Merwe 142 (NBG); Keet 719 (NBG); Cooper 1591 (NBG); Thode A 101 (NBG); Pillans s.n. (NBG); Smuts s.n. (NBG); D'Ewes s.n. (NBG); Roux s.n. (NBG); Forest s.n. (NBG); Van der Merwe 57 (NBG); Hugo1168 (NBG); Dahlstrand 2228 (NBG); Hanekom 1368 (NBG); Bos 240 (NBG); Keet 495 (NBG); Thode 5813 (NBG); Haynes 896 (NBG); Campbell 13961 (NBG); Kruger 509 (NBG); Stauffer & Levyns 5007 (NBG); Oliver 4873 (NBG); Haynes 613 (NBG); Van der Merwe & Van Rensburg 2010 (NBG); Hugo 2499 (NBG); De Vos 875 (NBG); Davies 55 (NBG); Hanekom 3019 (NBG); Rode & Boucher 21 (NBG); Jordaan 1167 (NBG); Kerfoot 5092 (NBG); Taylor 9661 (NBG); Thode 8148 (NBG); Gillett 104 (NBG); Dahlstrand 629 (NBG); Hubbard 340 (NBG); Middlemost 1925 (NBG); Cattell & Cattell 4 (NBG); Smuts s.n. (NBG); McDonald 652 (NBG); Schlechter 4806 (NBG); Hugo 2596 (NBG); Smuts s.n. (NBG); Braun 4 (NBG); McDonald 2376 (NBG); McDonald 2443 (NBG); Van Jaarsveld & Kotze 7695 (NBG); Boucher 3595 (NBG); Van Rensburg 295 (NBG); Gillett 13 (NBG); Hugo 3025 (NBG); Van der Merwe 1014 (NBG); Stehle TS 151 (NBG); Gillett s.n. (NBG); Haynes H.129 (NBG); Taylor 4676 (NBG); Wurts 1625 (NBG); Morris 72 (NBG); Esterhuysen 33824 (NBG); Scharf 1691 (NBG); Scott 304 (NBG); Morris 73 (NBG); Wurts 1288 (NBG); Powrie 395 (NBG); Barker 971 (NBG); Cohen s.n. (NBG); Compton 24091 (NBG); Batten 4.PL106 (NBG); Loots s.n. (NBG); Collector unknown (NBG); Rycroft 2953 (NBG); Rode & Boucher 31 (NBG); Goldblatt 1727 (NBG); Esterhuysen s.n. (NBG); Compton 4210 (NBG); Williams 1999 (NBG); Compton 8801 (NBG); Williams 3241 (NBG); Walters 844 (NBG); Beyers 49 (NBG); Rycroft 2679 (NBG); Middlemost 2199 (NBG); Leach & Bayliss 13607 (NBG); Wurts 2258 (NBG); Walters 835 (NBG); Herre s.n. (NBG); Pocock S15 / S 228 (NBG); Williams 2284 (NBG); Rycroft 2973 (NBG); Rycroft 1387 (NBG); Rycroft 2226 (NBG); Compton 23577 (NBG); Compton 19548 (NBG); Barker 7863 (NBG); Phillips s.n. (NBG); Morris 251 (NBG); Cassidy 169 (NBG); Jordaan J1262 (NBG); Botha (2)027 (NBG); Pappé s.n. (SAM); Drege s.n. (SAM); Collector unknown (SAM); Zeyher s.n. (SAM); Ecklon & Zeyher 1062 (SAM); Zeyher 497 (SAM); Zeyher 2639 (SAM); Thorne s.n. (SAM); Stokoe s.n. (SAM); Purcell s.n. (SAM); Phillips 1440 (SAM); Phillips 1739 (SAM); Lewis 3896 (SAM); Stokoe s.n. (SAM); Quint 60 (MJG); Quint 13 (MJG); Claßen-Bockhoff 4019 (MJG); *Brunia paleacea* P.J.BERGIUS: Walters 189 (NBG); Walters 1694 (NBG); Compton 12288 (NBG); Morris 17 (NBG); Rourke s.n. (NBG); MacPherson s.n. (NBG); Williams 2281 (NBG); Compton 8327 (NBG); Rycroft 3184 (NBG); Grisbrook s.n. (NBG); Esterhuysen 9796 (NBG); Martin 308 (NBG); Martin 555 (NBG); Compton 23669 (NBG); Compton 8175 (NBG); Barker 10372 (NBG); Nel 17296 (NBG); Versluis 11708 (NBG); Thode 7978 (NBG); Student 17444 (NBG); Andreae A.730 (NBG); Gillett 1066 (NBG); Van der Merwe 1814 (NBG); Van der Merwe 1207 (NBG); Andreae 874 (NBG); Andreae 730 (NBG); Barker 1839 (NBG); Esterhuysen 4074 (NBG); Bolus s.n. (NBG); Guthrie 3878 (NBG); Compton 12979 (NBG); Parker 4726 (NBG); Parker 4666 (NBG); Ebersohn 26/69 (NBG); Fellingham 1356 (NBG); Koegelenberg s.n. (NBG); Forsyth 427 (NBG); Fellingham 503 (NBG); Van der Walt 475 (NBG); Fellingham 496 (NBG); Van der Kooij & Van der Kooij 2015 (NBG); Strid & Strid 37872 (NBG); Van Rensburg 296 (NBG); Van der Merwe 2040 (NBG); Kruger KR.206 (NBG); Van der Merwe 2108 (NBG); Kruger 619 (NBG); Van der Merwe 2040 (NBG); Jordaan 826 (NBG); Grobler 359 (NBG); Kruger 843 (NBG); Buys 205 (NBG); Kerfoot 5091 (NBG); Haynes 901 (NBG); Taylor 4673 (NBG); Boucher 26 (NBG); Viviers 743 (NBG); Andreae 1067 (NBG); Hubbard 380 (NBG); Grobler 10. Jan (NBG); Le Maitre 26 (NBG); Paterson-Jones 250 (NBG); Bos 281 (NBG); Van Wyk 976 (NBG); Hugo 2646 (NBG); Stokoe s.n. (NBG); Schmidt 20 (NBG); Gillett 959 (NBG); Van Wilgen 45 (NBG); Haynes 931 (NBG); Haynes 998 (NBG); Jordaan 825 (NBG); MacOwan 1458 (NBG); De Vos 569 (NBG); Orchard 320 (NBG); Forsyth 15 (NBG); Campbell 10641 (NBG); Van der Merwe 2108 (NBG); Schlechter 5428 (NBG); Jordaan 824 (NBG); Kruger 1514 (NBG); Stokoe s.n. (SAM); Barnard 421 (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Ecklon & Zeyher 1080 (SAM); Stokoe s.n. (SAM); Muir 856 (SAM); Stokoe s.n. (SAM); Taylor s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Pappé s.n. (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher 1080 (SAM); Phillips 1123 (SAM); Potts s.n. (SAM); *Brunia stokoei* E.PHILLIPS: Levyns 5394 (BOL); Burman 1279 (BOL); Stokoe 23038 (BOL); Stokoe 23037 (BOL); Collector unknown (BOL); Pillans 8240 (BOL); *Linconia alopecuroidea* L.: Stokoe s.n. (BOL); Collector unknown (BOL); Galpin 4044 (BOL); Stokoe 7428 (BOL); Stokoe 6756 (BOL); Schlechter 2050 (BOL); McDonald & Morley 1055 (NBG); Taylor 4247 (NBG); Rourke 1895 (NBG); Galpin 4044 (NBG); Wurts 402 (NBG); Taylor 4247 (NBG); Lamb 3669 (SAM);

Pappe s.n. (SAM); Quint 15 (MJG); *Linconia cuspidata* (THUNB.) SW.: Stokoe s.n. Stokoe 3550 (NBG); Stokoe 7631 (NBG); Compton 16547 (NBG); Goldblatt 6924 (NBG); Stokoe 6016 (NBG); Viviers 829 (NBG); Stokoe s.n. (NBG); Esterhuysen s.n. (NBG); Stokoe 4006 (BOL); Stokoe 3550 (BOL); Stokoe 64090 (BOL); Esterhuysen 31670 (BOL); Esterhuysen 33149 (BOL); Esterhuysen 2619 (BOL); Esterhuysen12492 (BOL); Esterhuysen 28679a (BOL); Esterhuysen 2618 (BOL); Esterhuysen 33380 (BOL); Stokoe 17366 (BOL); Stokoe 2852 (BOL); Esterhuysen 31639 (BOL); Esterhuysen 35113a (BOL); Esterhuysen 27070 (BOL); Stokoe 17366 (BOL); Stokoe 2852 (BOL); Schlechter 9273 (BOL); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Schlechter 9273 (SAM) Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Zeyher 2651 (SAM); Stokoe s.n. (SAM); Quint 51 (MJG); *Linconia deusta* (THUNB.) PILLANS: Zeyher 2651 (BOL); *Linconia ericoides* OLIV.: Langeveld s.n. (NBG); Oliver 11200 (NBG); *Lonchostoma esterhuyseniae* STRID: Esterhuysen 31811 (BOL); Esterhuysen 31406 (BOL); Powrie 108 (BOL); Dahlgren & Strid 4233 (BOL); Turner 863 (NBG); Strid & Dahlgren 4233 (NBG); Oliver 11231 (NBG); Quint 67 (MJG); *Lonchostoma monogynum* (VAHL) PILLANS: Esterhuysen 8644 (BOL); Esterhuysen 12462 (BOL); Esterhuysen 9660 (BOL); Esterhuysen 35101 (BOL); Esterhuysen 15620 (BOL); Stokoe1256 (BOL); Linder 4431 (BOL); Stokoe 6037 (BOL); Esterhuysen 14593 (BOL); Krige 8062 (BOL); Compton 4120 (BOL); Powrie 23 (BOL); Stokoe 6034 (BOL); Stokoe 17740 (BOL); Esterhuysen 32676 (BOL); Esterhuysen 28635 (BOL); Stokoe 7624 (BOL); Levyns 3292 (BOL); Grisbrook 8062 (BOL); Schlechter 9823 (BOL); Levyns s.n. (BOL); Leighton 975 (BOL); Esterhuysen 34605 (BOL); Esterhuysen 17630 (BOL); Collector unknown (BOL); Esterhuysen 12809 (BOL); Leighton 2157 (BOL); Stokoe 6036 (BOL); Adamson 3623 (BOL); Esterhuysen 8487 (BOL); Stokoe 6035 (BOL); Stokoe 6033 (BOL); Leighton 976 (BOL); Powrie 256 (BOL); Esterhuysen 10980 (BOL); Adamson 1470 (BOL); Esterhuysen 5434 (BOL); Esterhuysen 15789 (BOL); Esterhuysen 8533 (BOL); Esterhuysen 8434 (BOL); Smuts 23050 (BOL); Marloth 658 (BOL); Esterhuysen 23049 (BOL); Powrie 59 (BOL); Esterhuysen 9493 (BOL); Esterhuysen 26529 (BOL); Le Roux 11473 (NBG); Forsyth s.n. (NBG); Hansford & Hansford 97 (NBG); Oliver & Oliver 11939 (NBG); Andreae 871 (NBG); Pocock 382 (NBG); Kruger 1052 (NBG); Van der Kooij 1301 (NBG); Stokoe 397 (NBG); Andrag 283 (NBG); Powrie s.n. (NBG); Boucher 1123 (NBG); Stokoe s.n. (NBG); Collector unknown (NBG); Esterhuysen 10980 (NBG); Collector unknown (NBG); Oliver 10841 (NBG); Van der Merwe 891 (NBG); Williams 3831 (NBG); Snijman 53 (NBG); Walters 183 (NBG); Compton 14530 (NBG); Compton 13518 (NBG); Goldblatt 1812 (NBG); Rourke 1100 (NBG); Stokoe 56805 Goldblatt 2071 (NBG); Esterhuysen 10980 (NBG); Rourke 1344 (NBG); Kies s.n. (NBG); Compton 4120 (NBG); Stokoe 6186 (NBG); Forsyth 302 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Linley s.n. (SAM); Stokoe s.n. (SAM); Stokoe 7624 (SAM); Thorne s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Zeyher s.n. (SAM); Zeyher s.n. (SAM); Phillips s.n. (SAM); Phillips 2025 (SAM); Quint 37 (MJG); Quint 64 (MJG); Quint 65 (MJG); *Lonchostoma myrtooides* (VAHL) PILLANS: Leighton 501 (BOL); Esterhuysen 33358 (BOL); Esterhuysen 32675 (BOL); Lavis & Compton 3989 (BOL); Guthrie 3233 (BOL); Esterhuysen s.n. (BOL); Esterhuysen 14914 (BOL); Eyre 4736 (BOL); Guthrie 3233 (BOL); Compton 16225 (BOL); Collector unknown (BOL); 10697 Marloth (NBG); 10697 Marloth 6154 (NBG); Compton 11988 (NBG); Compton 3989 (NBG); Grant 3471 (NBG); Compton 16225 (NBG); Lewis s.n. (SAM); Guthrie 3233 (SAM); Collector unknown (SAM); Quint 38 (MJG); *Lonchostoma pentandrum* (THUNB.) DRUCE: Esterhuysen 13084 (BOL); Leighton 2300 (BOL); Tredgold L.722 (BOL); Compton 3988 (BOL); Esterhuysen 3376 (BOL); Compton 18801 (BOL); Esterhuysen 13817 (BOL); Schlechter 10057 (BOL); Leighton 500 (BOL); Esterhuysen 13856 (BOL); Esterhuysen 29679 (BOL); Esterhuysen 33012 (BOL); Esterhuysen 12504 (BOL); Powrie 59 (NBG); Wasserfall 630 (NBG); Compton 11980 (NBG); Kotze 338 (NBG); Compton 6697 (NBG); Compton 16172 (NBG); Compton 10025 (NBG); Compton 18801 (NBG); Esterhuysen 13084 (NBG); Leighton 2300 (NBG); Marloth 658 (NBG); Taylor 6596 (NBG); Cillie 29 (NBG); Taylor 10693 (NBG); Drake 10057 (NBG); Brown 502 (NBG); Cillie s.n. (NBG); Andrag 102 (NBG); Hanekom 1 (NBG); Oakes 27 (NBG); Schlechter 10057 (SAM); Lewis s.n. (SAM); Marloth 658 (SAM); *Lonchostoma purpureum* PILLANS: Esterhuysen 12504 (BOL); Stokoe 3760 (BOL); Stokoe 4002 (BOL); Glover 10753 (BOL); Esterhuysen 8253 (BOL); Stokoe 6018 (BOL); Esterhuysen 33313 (BOL); Esterhuysen 2617 (BOL); Stokoe 970 (BOL); Stokoe 8903 (BOL); Esterhuysen 17583 (BOL); Stokoe 17279 (BOL); Esterhuysen 31379a (BOL); Esterhuysen 9163 (BOL); Stokoe 6018 (NBG); Esterhuysen 12809 (NBG); Esterhuysen 9163 (NBG); Esterhuysen 17583 (NBG); Stokoe 8903 (NBG); Boucher 1241 (NBG); Oliver 3013 (NBG); Forsyth 105 (NBG); Kruger KR.1049 (NBG); Foster s.n. (NBG); Goldblatt 1646 (NBG); Boucher 2205 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Esterhuysen 12809 (SAM); Quint 9b (MJG); *Mniothamnea bullata* SCHLTR.: Esterhuysen 10479 (BOL); Esterhuysen 30879a (BOL); Carlquist s.n. (BOL); Zeyher 1410 (BOL); Esterhuysen 10479 (BOL); Esterhuysen 30879a (BOL); *Mniothamnea callunoides* (OLIV.) NIED.: Esterhuysen 4802 (BOL); Burchell 7382 (BOL); Esterhuysen 10407 (BOL); Schlechter 2040 (BOL); Ecklon & Zeyher 312 (BOL); Quint 47 (MJG); *Nebelia fragarioides* (WILLD.) KUNTZE: Stokoe 9223 (BOL); Esterhuysen 34092 (BOL); Bodkin 7480 (BOL); Esterhuysen 9395 (BOL); Esterhuysen 2623 (BOL); Powrie 60 (BOL); Schelpe s.n. (BOL); Pillans 8270 (BOL); Collector unknown (BOL); Esterhuysen & Claßen-Bockhoff 1412 (BOL); Powrie 123 (BOL); Esterhuysen & Claßen-Bockhoff 1412 (BOL); Powrie 278 (BOL); Hall 1330 (BOL); Powrie 28 (BOL); Stokoe 6009 (BOL); Stokoe 17501 (BOL); Stokoe 9132 (BOL); Hubbard 438 (BOL); Stokoe 8907 (BOL); Schlechter 7228 (BOL); Collector unknown (BOL); Levyns 3290 (BOL); Stokoe 16952 (BOL); Stokoe s.n. (BOL); Stokoe 14198 (BOL); Esterhuysen 9970 (BOL); Leighton 1006 (BOL); Behr 1131 (NBG); Rode 469 (NBG); Quint 62 (MJG); Quint 23 (MJG); *Nebelia laevis* (E.MEY.) KUNTZE: Stokoe 7457 (BOL); Esterhuysen 35633 (BOL); Stokoe 6008 (BOL); Trinder-Smith 211 (BOL); Stokoe 7329 (BOL); Stokoe 26083 (BOL); Levyns 5467 (BOL); Bolus 7386 (BOL); Quint 49 (MJG); *Nebelia paleacea* (P.J.BERGIUS) SWEET: Rogers 17865 (BOL); Compton 5348 (BOL); Esterhuysen 1158 (BOL); Schlechter 7500 (BOL); Hall 1326 (BOL); Powrie 47 (BOL); Esterhuysen 1620 (BOL); Esterhuysen 33376 (BOL); Esterhuysen 1642 (BOL); Esterhuysen 1646 (BOL); Esterhuysen 12327 (BOL); Esterhuysen 13733 (BOL); Powrie s.n. (BOL); Esterhuysen 4988 (BOL); Esterhuysen 4256 (BOL); Compton 12979 (BOL); Bolus 7385 (BOL); MacOwan & Bolus 137 (BOL); Schlechter 9919 (BOL); Stokoe 8819 (BOL); Esterhuysen 11969 (BOL); Esterhuysen 12516 (BOL); Esterhuysen 8271 (BOL); Esterhuysen 9128 (BOL); Burman 1007 (BOL); Rodin 3260 (BOL); Esterhuysen 4074 (BOL); Pattison 14473 (BOL); Guthrie 23059 (BOL); Levyns 7776 (BOL); Levyns 3291 (BOL); Bolus 4179 (BOL); Schlechter 5428 (BOL); Esterhuysen 9796 (BOL); Esterhuysen 7825 (BOL); Powrie s.n. (BOL); Esterhuysen 11483 (BOL); Esterhuysen 23061 (BOL); Claßen-Bockhoff & Froebe 1345 (BOL); Esterhuysen 9963 (BOL); Esterhuysen 10090 (BOL); Zeyher 23060 (BOL); Pillans 8277 (BOL); Powrie 277 (BOL); Runnalls 645 (NBG); Behr 1129 (NBG); Rode 540 (NBG); Chater & Hansford 138 (NBG); Forest et al. 561 (NBG); Quint 10a (MJG); Quint 14 (MJG); *Nebelia sphaerocephala* (SOND.) KUNTZE: Esterhuysen 12419 (BOL); Esterhuysen 9661 (BOL); Esterhuysen 9147 (BOL);

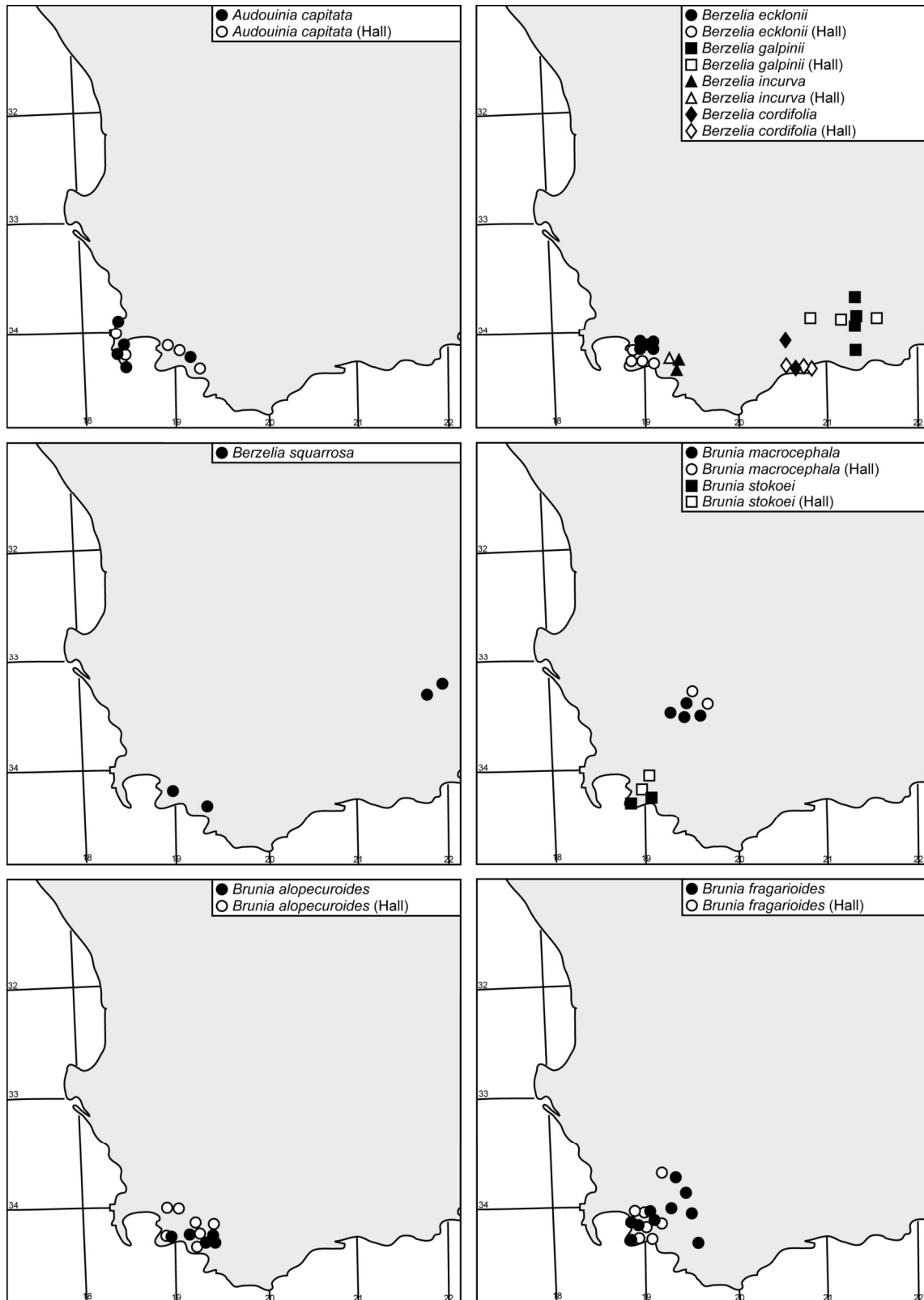
Esterhuysen 1708 (BOL); Esterhuysen 10016 (BOL); Esterhuysen 8270 (BOL); Esterhuysen 9671 (BOL); Esterhuysen 2622 (BOL); Esterhuysen 15239 (BOL); Esterhuysen 9662 (BOL); Esterhuysen 33737 (BOL); Esterhuysen 9478 (BOL); Smuts 23062 (BOL); Marloth 2492 (BOL); Stokoe 5022 (BOL); Stokoe s.n. (BOL); *Nebelia stokoei* PILLANS: Esterhuysen 33362 (BOL); Esterhuysen 30824a (BOL); Esterhuysen 7802 (BOL); Compton s.n. (BOL); *Pseudobaeckea africana* (BURM.F.) PILLANS: Edwards 16154 (BOL); Esterhuysen 11169 (BOL); Hall 1319 (BOL); Bolus 5048 (BOL); Esterhuysen 8275 (BOL); Powrie 82 (BOL); Powrie 125 (BOL); Levyns 9331 (BOL); Levyns 2673 (BOL); Hall 1316 (BOL); Hall 1301 (BOL); Burman 1234 (BOL); Stokoe 490 (BOL); Levyns 3294 (BOL); Esterhuysen 3375 (BOL); Levyns 4672 (BOL); Leighton 1346 (BOL); Esterhuysen 13552 (BOL); Guthrie 18582 (BOL); Schlechter 9346 (BOL); Gray 4 (BOL); Hall 1291b (BOL); Leighton 958 (BOL); Powrie 29 (BOL); Compton 3437 (BOL); Schelpe 4180 (BOL); Levyns 7650 (BOL); Esterhuysen 12326 (BOL); Stokoe 7036 (BOL); Compton 17336 (BOL); Marloth 2751 (BOL); Compton s.n. (BOL); Compton s.n. (BOL); Rourke 2147 (NBG); Hanekom 3063 (NBG); Kruger 757 (NBG); Kruger 578A (NBG); Oliver 10765 (NBG); Collector unknown (NBG); Taylor 4478 (NBG); Stokoe 1012 (NBG); Haynes 1551 (NBG); Boucher 144 (NBG); Tymens 12 (NBG); Collector unknown (NBG); Pocock 499 (NBG); Kruger 1422 (NBG); Van Wyk 986 (NBG); Taylor 12245 (NBG); Thompson 1456 (NBG); Van der Merwe 1205 (NBG); Stokoe s.n. (NBG); Stokoe s.n. (NBG); Geldenhuys 1344 (NBG); Gillett s.n. (NBG); Taylor 8580 (NBG); Taylor 9749 (NBG); De Vos 943 (NBG); Collector unknown (NBG); Van Wyk 982 (NBG); Low 1010 (NBG); Viviers 688 (NBG); Helme 1364 (NBG); Williams 2373 (NBG); Rourke 660 (NBG); Compton 9677 (NBG); Compton 18611 (NBG); Compton 11931 (NBG); Rycroft 1367 (NBG); Compton 5757 (NBG); Stokoe 490 (NBG); Compton 13538 (NBG); Rourke 1249 (NBG); Taylor 8580 (NBG); Nänni & Manning 335 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Ecklon & Zeyher 1068 (SAM); Drege s.n. (SAM); Marloth s.n. (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher 1069 (SAM); Stokoe s.n. (SAM); *Pseudobaeckea cordata* (BURM.F.) NIED.: Viviers & Vlok 22 (BOL); Esterhuysen 2616 (BOL); Esterhuysen 9165 (BOL); Stokoe 9131 (BOL); Esterhuysen 33438 (BOL); Claßen-Bockhoff & Esterhuysen 1411 (BOL); Stokoe 7427 (BOL); Drege 1077 (BOL); Stokoe 17120 (BOL); Hall 1300 (BOL); Holland 3662 (BOL); Esterhuysen 2615 (BOL); Vlok 1370 (BOL); Esterhuysen 11051 (BOL); Bolus 8609 (BOL); Esterhuysen 12285 (BOL); Esterhuysen 28105 (BOL); Powrie 53 (BOL); Esterhuysen 28710 (BOL); Esterhuysen 30358 (BOL); Stokoe 6005 (BOL); Stokoe 6004 (BOL); Esterhuysen 30373 (BOL); Esterhuysen 15674 (BOL); Stokoe 1302 (BOL); Esterhuysen 28228 (BOL); Powrie 53 (BOL); Powrie 54 (BOL); Esterhuysen 21785 (BOL); Edwards 16156 (BOL); Esterhuysen 10729 (BOL); Vlok 897 (BOL); Stokoe 8911 (BOL); Stokoe 8911 (BOL); Stokoe 6021 (BOL); Stokoe 7331 (BOL); Stokoe 8906 (BOL); Pillans 29224 (BOL); Esterhuysen 4603 (BOL); Fourcade 2829 (BOL); Esterhuysen 13574 (BOL); Kennedy 215 (BOL); Schlechter 9288 (BOL); Esterhuysen 13305 (BOL); Esterhuysen s.n. (BOL); Esterhuysen 33310 (BOL); Esterhuysen 2896 (BOL); Stokoe 8266 (BOL); Schlechter 2099 (BOL); Ecklon, Zeyher & Drege s.n. (BOL); Stokoe 7426 (BOL); Fourcade 3210 (BOL); Phillips 29 (BOL); Collector unknown (BOL); Esterhuysen 10730 (BOL); Fourcade 3210 (NBG); Vlok 795 Keet s.n. (NBG); Oliver 3020 (NBG); Oliver 11005 (NBG); Kruger 493 (NBG); Stokoe 10721 (NBG); Stokoe 6004 (NBG); McDonald 1693 (NBG); Powrie 54 (NBG); Kruger 129 (NBG); Powrie 55 (NBG); Williams 2556 (NBG); Esterhuysen 12091 (NBG); Stokoe 56838 (NBG); Kruger 245 (NBG); Bolus 8609 (NBG); Middlemann s.n. (NBG); Williams 3557A (NBG); Kruger 195 (NBG); Taylor 8659 (NBG); Bond 897 (NBG); Boucher 1331 (NBG); De Kock 151 (NBG); Van der Merwe 41 (NBG); Marloth 10721 (NBG); Kerfoot s.n. (NBG); Stokoe s.n. (NBG); Oliver & Oliver 11754 (NBG); Packer 673 (NBG); Kruger KR 826 (NBG); McDonald 2040 (NBG); Kerfoot K 5512 (NBG); Collector unknown (NBG); Viviers 540 (NBG); Bond 1653 (NBG); Viviers & Vlok 22 (NBG); Van der Merwe 1589 (NBG); Marloth 8805 (NBG); Helme 3287 (NBG); Stokoe 6004 (SAM); Drege 6856 (SAM); Zeyher 2226 (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Stokoe s.n. (SAM); Rogers 29224 (SAM); Stokoe s.n. (SAM); Taylor 1126 (SAM); Edwards 16156 (SAM); Thorne s.n. (SAM); Barnard 480 (SAM); Mundt s.n. (SAM); Zeyher 2225 (SAM); Ecklon & Zeyher 1071 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher 1072 (SAM); Drege 6853 (SAM); *Pseudobaeckea stokoei* PILLANS: Stokoe 16886 (BOL); Stokoe16612 (BOL); Stokoe7458 (BOL); Marloth 9553 (NBG); Stokoe s.n. (SAM); *Pseudobaeckea teres* (OLIV.) DUMMER: Esterhuysen 33463 (BOL); Esterhuysen 28252 (BOL); Burchell 7700 (BOL); *Raspalia angulata* (SOND.) NIED.: Esterhuysen 13857 (BOL); Stokoe s.n. (NBG); Boucher 594 (NBG); Rourke 711 (NBG); Rycroft 2894 (NBG); Rourke 348 (NBG); Manning 2136 (NBG); Kerfoot 6617 (NBG); Rycroft 2894 (NBG); Van der Merwe 264 (NBG); Manning s.n. (NBG); Rourke 714 (NBG); Andrae 1243 (NBG); Esterhuysen 11345 (NBG); Wasserfall 563 (NBG); Esterhuysen 11569 (NBG); Taylor 6597 (NBG); Wasserfall 636 (NBG); Esterhuysen 12446 (NBG); Kies s.n. (NBG); Forsyth 103 (NBG); Oliver 7 (NBG); Oliver 3066 (NBG); Oliver 5524 (NBG); De Lange 6 (NBG); Rourke 2146 (NBG); Taylor 2636 (NBG); Helme & Turner 3393 (NBG); Helme 3418 (NBG); Barnard 471 (SAM); Thorne s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Drege 6868 (SAM); Barnard s.n. (SAM); Esterhuysen 12446 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Tyson 2648 (SAM); *Raspalia barnardii* PILLANS: Barnard 28912 (BOL); Esterhuysen 36154 (BOL); Esterhuysen 33405 Helme & Turner 3789 (NBG); *Raspalia dregeana* (SOND.) NIED.: Esterhuysen 33019 (BOL); Esterhuysen 33179 (BOL); Esterhuysen 18063 (BOL); Stokoe 4531 (BOL); Esterhuysen 13802 (BOL); Esterhuysen 7589 (BOL); Stokoe 7627 (BOL); Bolus 6362 (BOL); Marloth 2255 (BOL); Bolus 4404 (BOL); Esterhuysen 1520 (BOL); Adamson 3077 (BOL); Oliver 10667 (NBG); Marloth 2363 (NBG); Compton 8375 (NBG); Esterhuysen 7589 (NBG); Kruger KR.978 (NBG); Stehle TS.99 (NBG); Paterson-Jones 733 (NBG); Oliver 4258 (NBG); Stokoe 6020 (NBG); Snijman 61 (NBG); Kruger 1569 (NBG); Boucher 3058 (NBG); Oliver 11075 (NBG); Rourke 1319 (NBG); Helme 1242 (NBG); Powrie 503 (NBG); Bond s.n. (NBG); Du Toit s.n. (NBG); McDonald 952 (NBG); Marloth 2255 (SAM); Drege s.n. (SAM); Esterhuysen 7589 (SAM); Stokoe s.n. (SAM); Phillips 2024 (SAM); Stokoe s.n. (SAM); *Raspalia globosa* (LAM.) PILLANS: Powrie 173 (BOL); Esterhuysen 32832 (BOL); Collector unknown (BOL); Esterhuysen & Claßen-Bockhoff 1533 (BOL); Paterson-Jones 733 (BOL); Esterhuysen 33526 (BOL); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Bolus 135 (SAM); Stokoe s.n. (SAM); Ecklon & Zeyher s.n. (SAM); *Raspalia microphylla* (THUNB.) BRONGN.: Stokoe 56829 (BOL); Esterhuysen & Claßen-Bockhoff 1532 (BOL); Esterhuysen 4985 (BOL); Pillans 8216 (BOL); Stokoe 8817 (BOL); Stokoe 9242 (BOL); Esterhuysen 9968 (BOL); Stokoe 4031 (BOL); Rycroft 1425 (BOL); Salter 6521 (BOL); Stokoe 3193 (BOL); Esterhuysen 11525 (BOL); Bolus 7388 (BOL); Cuthbert 3293 (BOL); Levyns 5395 (BOL); Bolus 5547 (BOL); Stokoe 7327 (BOL); Powrie 38 (BOL); Stokoe 8915 (BOL); Burman 1233 (BOL); Schlechter 5389 (BOL); Schlechter 9563 (BOL); Stokoe s.n. (BOL); Schlechter 9850 (BOL); Leighton 760 (BOL); Galpin 4039 (BOL); Bolus 100 (BOL); Vlok, Van Wyk & Schutte 94 (BOL); Stokoe 6012 (BOL); Esterhuysen

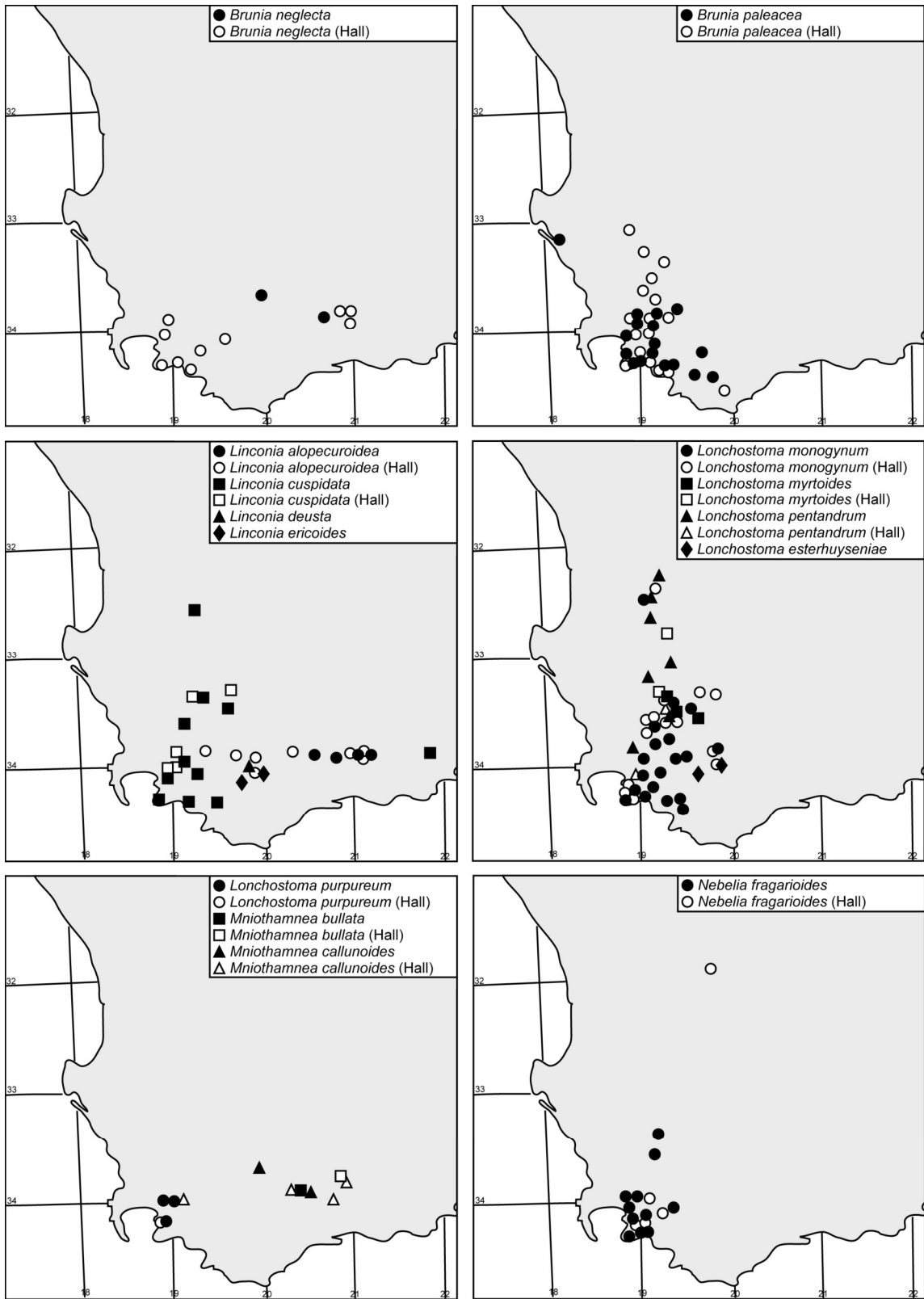
81412 (BOL); Stokoe 7430 (BOL); Hall 1279 (BOL); Cuthbert 8599 (BOL); Stokoe 8816 (BOL); Bodkin 6905 (BOL); Stokoe 8914 (BOL); Hutchinson 504 (BOL); Stokoe 7326 (BOL); Esterhuysen 1709 (BOL); Esterhuysen 8523 (BOL); Esterhuysen 1718 (BOL); Esterhuysen 7725 (BOL); Esterhuysen 13905 (BOL); Esterhuysen 12385 (BOL); Esterhuysen 12384 (BOL); Stokoe 6041 (BOL); Stokoe 6039 (BOL); Stokoe 6040 (BOL); Stokoe 9220 (BOL); Stokoe 4040 (BOL); Stokoe 6038 (BOL); Stokoe 17871 (BOL); Stokoe 2741 (BOL); Stokoe 1803 (BOL); Stokoe 8913 (BOL); Esterhuysen 12816 (BOL); Esterhuysen 11345 (BOL); Esterhuysen 9472 (BOL); Esterhuysen 9663 (BOL); Esterhuysen 35403 (BOL); Stokoe 2741 (BOL); Esterhuysen 4104 (BOL); Esterhuysen 35410 (BOL); Esterhuysen 32689 (BOL); Stokoe 8912 (BOL); Esterhuysen 9744 (BOL); Oliver s.n. (BOL); Stokoe 7632 (BOL); Powrie 109 (BOL); Stokoe 8815 (BOL); Collector unknown (BOL); Stokoe 7432 (BOL); Powrie 41 (BOL); Stokoe 8912 (BOL); Esterhuysen 11569 (BOL); Esterhuysen s.n. (BOL); Esterhuysen s.n. (BOL); Stokoe 7405 (BOL); Esterhuysen 33448 (BOL); Esterhuysen 30 357 (BOL); Esterhuysen 35404 (BOL); Esterhuysen 28230 (BOL); Esterhuysen 11569 (BOL); Esterhuysen 12446 (BOL); Esterhuysen 8679 (BOL); Smuts 23039 (BOL); Adamson 3622 (BOL); Drege 6868 (BOL); Esterhuysen 1648 (BOL); Esterhuysen 32689a (BOL); Esterhuysen 32689b (BOL); MacPherson s.n. (NBG); Helme 2325 (NBG); Lamb 130 (NBG); Taylor 11985 (NBG); Boucher 24 (NBG); Thompson 492 (NBG); Taylor 7226 (NBG); Oliver 10764 (NBG); Orchard 457 (NBG); De Vos 1553 (NBG); Haynes 1238 (NBG); Van der Merwe 922 (NBG); Andreae 1066 (NBG); Durand 387 (NBG); Esterhuysen 12446 (NBG); Viviers 766 (NBG); Helme 1244 (NBG); Boucher 2046 (NBG); Maguire 2656 (NBG); Esterhuysen 12385 (NBG); Ebersohn 214 (NBG); Compton 6741 (NBG); Bolus 6905 (NBG); Esterhuysen 4985 (NBG); Compton 6108 (NBG); Bond 1536 (NBG); Rourke 2054 (NBG); Oliver 11218 (NBG); Boucher 2238 (NBG); Bos 649 (NBG); Hall 724 (NBG); Grobler 368 (NBG); Oliver 5858 (NBG); MacPherson s.n. (NBG); Kruger 187 (NBG); Stokoe s.n. (NBG); Compton 16463 (NBG); Rycroft 1237 (NBG); Williams 2511 (NBG); Compton 16871 (NBG); Williams 3587 (NBG); MacPherson (NBG); 17 Stokoe 66466 (NBG); Gillett 862 (NBG); Lamb 130 (NBG); Schlechter 152 (NBG); Stirton 10003 (NBG); Van der Merwe 1224 (NBG); Boucher 2626 (NBG); Parker 4708 (NBG); Stokoe 56830 (NBG); Rycroft 1425 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Barnard 667 (SAM); Stokoe s.n. (SAM); Bolus 5547 (SAM); Drege s.n. (SAM); Ecklon & Zeyher 1073 (SAM); Bolus 100 (SAM); Schlechter 5339 (SAM); *Raspalia oblongifolia* **PILLANS:** Esterhuysen 28151 (BOL); Esterhuysen 27711 (BOL); Esterhuysen 28626 (BOL); Esterhuysen 29883 (BOL); Esterhuysen 8321 (BOL); Esterhuysen 122388 (BOL); Esterhuysen 29924 (BOL); Esterhuysen 32557 (BOL); Esterhuysen 31646 (BOL); Esterhuysen 14271 (BOL); Esterhuysen 30406 (BOL); Esterhuysen 30089a (BOL); Esterhuysen 14865 (BOL); *Raspalia palustris* (**SCHLTR. EX KIRCHN.**) **PILLANS:** Schlechter 10055 (BOL); Hansford 58 (NBG); *Raspalia passerinoides* **C.PRESL:** Powrie 174A (BOL); Pillans 6299 (BOL); Bolus 5488 (BOL); Ecklon & Zeyher 1064 (BOL); Schlechter 7339 (BOL); Powrie 174B (BOL); *Raspalia phyllicoides* (**THUNB.**) **ARN.:** Esterhuysen 34203 (BOL); Powrie 174B (BOL); Esterhuysen 35583 (BOL); Powrie 174A (BOL); Bolus 135 (BOL); *Raspalia sacculata* (**BOLUS EX KIRCHN.**) **PILLANS:** Esterhuysen 35409 (BOL); Esterhuysen 36263 (BOL); Esterhuysen 35100 (BOL); Esterhuysen 8643 (BOL); Bolus 1154 (BOL); Esterhuysen 28557 (BOL); Esterhuysen 12458 (BOL); Esterhuysen 32807 (BOL); Esterhuysen 32405 (BOL); Esterhuysen 31669a (BOL); Esterhuysen 17781 (BOL); Esterhuysen 35257 (BOL); *Raspalia sp.:* Esterhuysen 31628 (BOL); Esterhuysen 34250 (BOL); Esterhuysen 32619 (BOL); Esterhuysen 32160 (BOL); Esterhuysen 29432 (BOL); *Raspalia squalida* **DUMMER:** Esterhuysen 4763 (BOL); Stokoe 6017 (BOL); Stokoe 23041 (BOL); Stokoe 4033 (BOL); Bolus 5327 (BOL); Stokoe 8818 (BOL); Stokoe 9219 (BOL); Stokoe 6020 (BOL); Stokoe 4033 (BOL); Esterhuysen 2658 (BOL); *Raspalia staaivooides* (**SOND.**) **PILLANS:** Esterhuysen 23757 (BOL); Leipoldt 3331 (BOL); Esterhuysen 12138 (BOL); Leipoldt 3331 (BOL); Esterhuysen 12175 (BOL); Esterhuysen 3378 (BOL); Drege s.n. (BOL); Schlechter 10802 (BOL); Esterhuysen 7506 (BOL); Powrie 136 (BOL); Lavis 19633 (BOL); *Raspalia stokoei* **PILLANS:** Stokoe 346 (BOL); Esterhuysen 31648 (BOL); Esterhuysen 28512 (BOL); Esterhuysen 28585 (BOL); Esterhuysen 34177 (BOL); Esterhuysen 8457 (BOL); Esterhuysen 28533 (BOL); Esterhuysen 31915 (BOL); Stokoe 7626 (BOL); Esterhuysen 33018 (BOL); *Raspalia variabilis* **PILLANS:** Esterhuysen 33464 (BOL); Esterhuysen 18521 (BOL); Esterhuysen 13930 (BOL); Vlok 1406 (BOL); Esterhuysen 32734 (BOL); Esterhuysen 9969 (BOL); Esterhuysen 2614 (BOL); Esterhuysen 9162 (BOL); Esterhuysen 8584 (BOL); *Raspalia villosa* **C.PRESL:** Esterhuysen 7376 (BOL); Nieuwoudt 23046 (BOL); Esterhuysen 2513 (BOL); Esterhuysen 7561 (BOL); Esterhuysen 7257 (BOL); Stokoe 7429 (BOL); Stokoe 6042 (BOL); Esterhuysen 34679 (BOL); Powrie 137 (BOL); Stokoe 7368 (BOL); Esterhuysen 12091 (BOL); Esterhuysen 2475 (BOL); Esterhuysen 32434 (BOL); Esterhuysen 12182 (BOL); Esterhuysen 9221 (BOL); Esterhuysen 7502 (BOL); Esterhuysen 13843 (BOL); Esterhuysen 7368 (BOL); Stokoe 49628 (BOL); Stokoe 49628 (BOL); Esterhuysen 7376 (BOL); Esterhuysen 7257 (BOL); Stokoe 346 (BOL); Esterhuysen 35076 (BOL); Esterhuysen 35076 (BOL); Stokoe 6754 (BOL); Esterhuysen 7257 (BOL); Esterhuysen 22511 (BOL); Esterhuysen 32434 (BOL); Esterhuysen 12094 (BOL); Oliver 11501 (NBG); Hanekom 941 (NBG); Esterhuysen 35702 (NBG); Taylor 11642 (NBG); Esterhuysen 12458 (NBG); Taylor 11106 (NBG); Taylor 6150 (NBG); Stokoe s.n. (NBG); Powrie 137 (NBG); Esterhuysen 7376 (NBG); Stokoe 1803 (NBG); Pocock 95 (NBG); Schlechter 10153 (NBG); Stokoe s.n. (NBG); Viviers 1232 (NBG); Forsyth 520 (NBG); Esterhuysen 17781 (NBG); Schlechter 2099 (NBG); Schlechter 10055 (NBG); Helme 2990 (NBG); Barnard 2912 (SAM); Stokoe s.n. (SAM); Bolus 1154 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Adamson s.n. (SAM); Esterhuysen 12458 (NBG); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Thorne s.n. (SAM); Helme 4127 (NBG); *Raspalia virgata* (**BRONGN.**) **PILLANS:** Esterhuysen 9771 (BOL); Esterhuysen 10476 (BOL); Stokoe 8908 (BOL); Esterhuysen 9155 (BOL); Esterhuysen 9160 (BOL); Esterhuysen 8254 (BOL); Esterhuysen 33312 (BOL); Esterhuysen 2613 (BOL); Esterhuysen 2612 (BOL); Stokoe 17741 (BOL); Stokoe 7433 (BOL); Ecklon & Zeyher 1065 (BOL); Galpin 4046 (BOL); Powrie 39 (BOL); Galpin 4045 (BOL); Zeyher 2652 (BOL); Stokoe 64104 (BOL); Stokoe 2484 (BOL); Stokoe 6044 (BOL); Powrie 33 (BOL); Pillans 15698 (BOL); Stokoe 2742 (BOL); Stokoe 9364 (BOL); Oliver 3023 (NBG); Helme 2324 (NBG); Helme 1408 (NBG); Stokoe 6045 (NBG); Stokoe 6043 (NBG); Wurts 423 (NBG); Wurts 321 (NBG); Stokoe 8908 (NBG); Stokoe s.n. (NBG); Stokoe 6045 (NBG); Stokoe s.n. (NBG); Oliver & Oliver 11753 (NBG); McDonald 1156 (NBG); Haynes 1497 (NBG); McDonald 1610 (NBG); Oliver 3012 (NBG); Le Maitre 373 (NBG); Boucher 2206 (NBG); Boucher 915 (NBG); Van der Merwe 152 (NBG); Kruger KR.1046 (NBG); Lamb 195 (NBG); Ecklon & Zeyher 1065 (SAM); Ecklon & Zeyher 1066 (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Lamb 2978 (SAM); Zeyher 2652 (SAM); Stokoe s.n. (SAM); *Staavia brownii* **DUMMER:** Levyns 4954 (BOL); Stokoe 66471 (BOL); Rourke 2117 (NBG); Stokoe s.n. (SAM); Kew Herbarium s.n. (SAM); *Staavia capitella* (**THUNB.**) **SOND.:** Paterson-Jones 870 (NBG); Oakes & Oakes 15 (NBG); *Staavia comosa* **COLOZZA:** Esterhuysen 36373 (BOL); Esterhuysen 34131 (BOL); Esterhuysen 1886 (BOL); Schlechter 9842 (BOL); Esterhuysen 33264 (BOL);

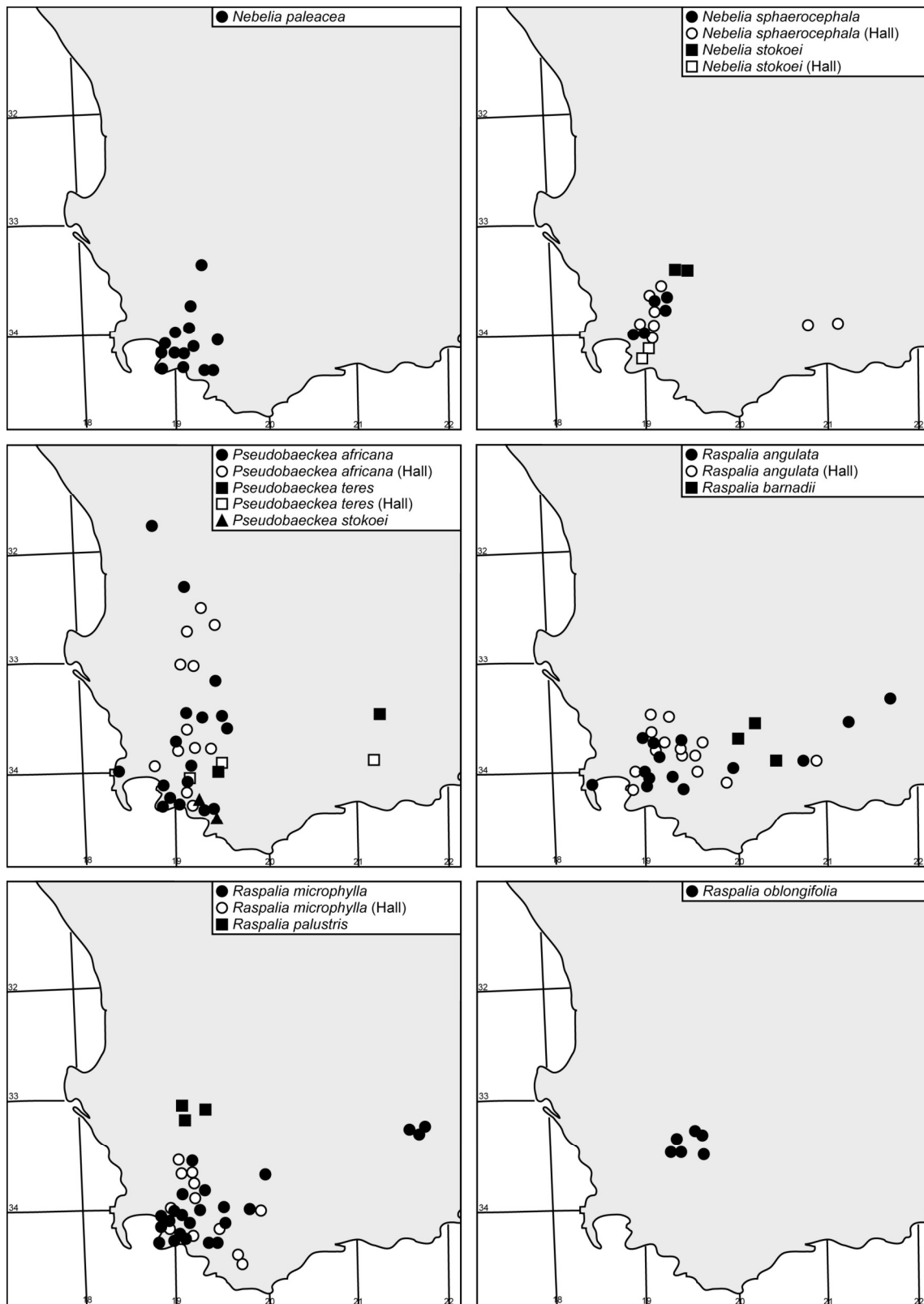
Esterhuysen 32067 (BOL); Esterhuysen 32691 (BOL); Esterhuysen 15617 (BOL); Esterhuysen 32063 (BOL); Hall 1288b (BOL); De Villiers s.n. (BOL); Stokoe 2501 (BOL); Stokoe 2740 (BOL); Stokoe 7328 (BOL); Stokoe 3183 (BOL); Schlechter 9391 (BOL); Bolus 5049 (BOL); Bodkin 6906 (BOL); Levyns 2138 (BOL); Bolus 1155 (BOL); Bodkin 9218 (BOL); Schlechter 5460 (BOL); Esterhuysen 29632 (BOL); Compton 8806 (NBG); Goldblatt 4340 (NBG); Esterhuysen 1886 (NBG); Bolus 9606 (NBG); Goldblatt 1813 (NBG); Bolus 1155 (NBG); Bolus 6907 (NBG); Esterhuysen 15617 (NBG); Beyers 51 (NBG); Schlechter 5460 (NBG); Kruger 1206 (NBG); Bolus s.n. (NBG); Boucher 2528 (NBG); Schlechter 9842 (SAM); Stokoe 7328 (SAM); Stokoe s.n. (SAM); Bolus 5049 (SAM); Ecklon & Zeyher 1079 (SAM); Ecklon & Zeyher 1077 (SAM); Zeyher 2647 (SAM); Bolus 1155 (SAM); Schlechter 9842 (SAM); *Staaivia dodii* **BOLUS**: Levyns s.n. (BOL); Esterhuysen 31684 (BOL); Powrie 69 (BOL); Taylor 6765 (BOL); Esterhuysen 32158 (BOL); Bodkin 7988 (BOL); Esterhuysen 3107220 (BOL); Gonbimis 23042 (BOL); Leighton 23043 (BOL); Wolley-Dod 2641 (BOL); Powrie 12 (BOL); Barker 706 (NBG); Goldblatt 1791 (NBG); Compton 14554 (NBG); Compton 19369 (NBG); Goulimis s.n. (NBG); Minicki s.n. (NBG); Taylor 6765 (NBG); Low 91 (NBG); Taylor 9300 (NBG); Hall s.n. (NBG); Taylor 6797 (NBG); Helme 3840 (NBG); Phillips s.n. (SAM); Bodkin 7988 (SAM); *Staaivia dregeana* **C.PRESL**: Esterhuysen 7647 (BOL); Drege 15 (BOL); Pappe 23303 (BOL); Levyns 6576 (BOL); Esterhuysen 31715a (BOL); Pillans 7122 (BOL); Esterhuysen 30055 (BOL); Bolus 4490 (BOL); Collector unknown (BOL); Dummer 641 (SAM); *Staaivia glutinosa* **(L.) DAHL**: Esterhuysen 7082 (BOL); Collector unknown (BOL); Esterhuysen 7095 (BOL); Wolley-Dod 1689 (BOL); Bolus 45799 (BOL); Dummer s.n. (BOL); Ecklon & Zeyher 1207 (BOL); Esterhuysen 11406 (BOL); Hallack 3008 (BOL); Bolus 4579 (BOL); Bodkin 2500 (BOL); Esterhuysen 29645 (BOL); Ashton 445 (BOL); Claßen-Bockhoff & Froebe 1094 (BOL); Meterlekamp 239 (BOL); Pillans 3682 (BOL); Powrie 15 (BOL); Cameron & Manchip 7 (BOL); Esterhuysen 35637 (BOL); Esterhuysen 32145 (BOL); Esterhuysen 17623 (BOL); Bayer & Puttock 96039 (NBG); Esterhuysen 35637 (NBG); McKinnon 149 (NBG); Andreae 307 (NBG); Thode 5814 (NBG); Esterhuysen 11406 (NBG); Esterhuysen 35637 (NBG); Bodenstein 164 (NBG); Barker 329 (NBG); Froembling 528 (NBG); Esterhuysen 35431 (NBG); McKinnon 28X (NBG); Phillips 261 (SAM); Stokoe s.n. (SAM); Barnard s.n. (SAM); Drege s.n. (SAM); Ecklon & Zeyher 1074 (SAM); Pappe s.n. (SAM); *Staaivia phyllicoides* **PILLANS**: Pretorius 579 (NBG); *Staaivia radiata* **(L.) DAHL**: Guthrie 397 (BOL); Brown 100 (BOL); Levyns 9160 (BOL); Guthrie 23045 (BOL); Parker 4741 (BOL); Barker 5283 (BOL); Van Niekerk 454 (BOL); Hall 1287 (BOL); Pillans 4763 (BOL); Schelpe 107 (BOL); Lewis s.n. (BOL); Ecklon 1075 (BOL); Stokoe 6014 (BOL); Leighton 3035 (BOL); Pillans 9862 (BOL); Esterhuysen 31325 (BOL); Kensi s.n. (BOL); Claßen-Bockhoff 1540 (BOL); Burman 1189 (BOL); Barker 5543 (BOL); Levyns 3177 (BOL); Kruger 714 (BOL); Leighton 1577 (BOL); Bodkin 6907 (BOL); Boisvin 695 (BOL); Esterhuysen 4053 (BOL); Bolus 7933 (BOL); Pappe 650 (BOL); Compton 18213 (BOL); Collector unknown (BOL); Froebe & Claßen-Bockhoff 1264 (BOL); Barker 5370 (BOL); Hutchinson 70 (BOL); Arbuthnot 23044 (BOL); Powrie 14 (BOL); Schlechter 212 (BOL); Wolley-Dod 259 (BOL); Bolus 7933 (BOL); Esterhuysen 12934 (BOL); Pillans 2894 (BOL); Pillans 1924 (BOL); Trelawney s.n. (BOL); Leighton 1482 (BOL); Esterhuysen 32466 (BOL); Esterhuysen 10021 (BOL); Esterhuysen 3856 (BOL); Leighton 967 (BOL); Powrie 63 (BOL); Kensit s.n. (BOL); Trelawney s.n. (BOL); Bosenberg 1759 (NBG); Boucher 5207 (NBG); Taylor 4339 (NBG); Thompson 2627 (NBG); Van Wilgen 172 (NBG); Barker 726 (NBG); Rode 437 (NBG); Palmer 67 (NBG); Bond 194 De Vos 1236 (NBG); Kruger 724 (NBG); De Vos 193 (NBG); Hugo 2347 (NBG); Bohnen 5173 (NBG); Paterson-Jones 395 (NBG); Kruger 1105 (NBG); Bohnen 6053 (NBG); Thompson 3982 (NBG); Pool 82 (NBG); Fellingham 286 (NBG); Van Wyk 839 (NBG); Andreae 246B (NBG); Parker s.n. (NBG); Kruger 652 (NBG); Fellingham 73 (NBG); Viviers 1171 (NBG); Van der Merwe 148 (NBG); Schlechter 5304 (NBG); Duthie 646A (NBG); Grobler 870 (NBG); Taylor 3479 (NBG); McKinnon 184 (NBG); Fellingham 1079 (NBG); Le Maitre 492 (NBG); Le Maitre 450 (NBG); Markötter s.n. (NBG); Andreae 246 (NBG); Taylor 4928 (NBG); Gillett 3466 (NBG); Gillett 762 (NBG); Oliver 3327 (NBG); Thompson 3735 (NBG); Taylor 9478 (NBG); Luckhoff s.n. (NBG); Collector unknown (NBG); Compton 8274 (NBG); Goldblatt 2596 (NBG); Rycroft 3070 (NBG); Rourke 351 (NBG); Compton 18213 (NBG); Barker 5283 (NBG); Barker 5543 (NBG); Cross 36 (NBG); Isaac s.n. (NBG); Cohen s.n. (NBG); Hugo 1254 (NBG); Wilson S.A. 112 (NBG); Montgomery 291 (NBG); Boucher 3312 (NBG); Axelson 22 (NBG); Boucher 523 (NBG); Thode 5815 (NBG); Markötter s.n. (NBG); Pappe s.n. (NBG); Low 503 (NBG); Heginbotham 247 (NBG); Raitt 293 (NBG); Low 505 (NBG); Low 590 (NBG); Low 525 (NBG); Van der Merwe 1617 (NBG); Compton 8488 (NBG); Rycroft 2380 (NBG); Maguire 57 (NBG); Andreae 246 (NBG); Jordaan 828 (NBG); Jordaan 533 (NBG); Jordaan 827 (NBG); Grobler 870 (NBG); Van der Merwe 1610 (NBG); Van Wilgen 172 (NBG); Van der Walt 148 (NBG); Van der Walt 287 (NBG); Willemsse 31 (NBG); Van der Merwe 6 (NBG); Pillans s.n. (NBG); Boucher 1968 (NBG); McDonald 1375 (NBG); Taylor 9478 (NBG); Cowling 1687 (NBG); Rycroft 3070 (NBG); Phillips 3070 (NBG); Parker 4741 (NBG); Bos 342 (NBG); Forsyth 270 (NBG); Thode A 103 (NBG); Bolus 7933B (NBG); Muir 4801 (NBG); Malherbe 27 (NBG); Smuts s.n. (NBG); Smuts s.n. (NBG); De Kock 136 (NBG); Smuts s.n. (NBG); Boucher & Shepherd 4300 (NBG); Viviers 1113 (NBG); Viviers 1171 (NBG); Lewis s.n. (NBG); Hugo 2685 (NBG); Oliver 5696 (NBG); Compton 8488 (NBG); Phillips 217 (NBG); Penfold 238 (NBG); Walgate 232 (NBG); Walters 3 (NBG); Pearson 52 (NBG); Bayliss 9950 (NBG); Williams 2308 (NBG); Barker 9607 (NBG); Middlemost 2089 (NBG); Taylor 3841 (NBG); Compton 9129 (NBG); Parker 4110 (NBG); Parker 3500 (NBG); Barker 1861 (NBG); Compton 14776 (NBG); Martin 554 (NBG); Barker 5893 (NBG); Rycroft 2380 (NBG); Heginbotham 247 (NBG); Walters 260 (NBG); Williams 3039 (NBG); Axelson 22 (NBG); Taylor 4339 (NBG); Taylor 4073 (NBG); Maguire 81 (NBG); Maguire 57 (NBG); Martin 3 (NBG); Barker 5370 (NBG); Barker 5360 (NBG); Barker 1756 (NBG); Compton 7399 (NBG); Steytler s.n. (NBG); Barker 372 (NBG); Werner 28 (NBG); Boucher 2756 (NBG); Smuts s.n. (NBG); De Vos 193 (NBG); Hugo 580 (NBG); Thompson 2015 (NBG); Kruger 714 (NBG); Oliver 3354 (NBG); Gillett 471 (NBG); Taylor 2545 (NBG); Stauffer & Esterhuysen 5050 (NBG); Thompson 754 (NBG); Botha (2)97 (NBG); Stokoe s.n. (SAM); Lewis 3898 (SAM); Lewis 3899 (SAM); Phillips s.n. (SAM); Purcell 316 (SAM); Phillips s.n. (SAM); Phillips 225 (SAM); Lewis 3087 (SAM); Lewis 3897 (SAM); Stokoe s.n. (SAM); Sidey 2195 (SAM); Phillips s.n. (SAM); Stokoe s.n. (SAM); Stokoe s.n. (SAM); Gill s.n. (SAM); Pappe s.n. (SAM); Zeyher 768 (SAM); Zeyher 726 (SAM); Ecklon & Zeyher 1075 (SAM); Drege s.n. (SAM); Phillips s.n. (SAM); *Staaivia verticillata* **(L.) PILLANS**: Esterhuysen 32301 (BOL); Esterhuysen 16538 (BOL); Powrie 58 (BOL); Primos 11668 (BOL); Esterhuysen 35268 (BOL); Esterhuysen 23935 (BOL); Powrie 58 (NBG); Esterhuysen 35268 (NBG); Helme 2991 (NBG); Ecklon & Zeyher 1067 (SAM); Drege s.n. (SAM); *Staaivia zeyheri* **SOND.**: Powrie 273 (BOL); Powrie 21 (BOL); Zeyher 2648 (BOL); Stokoe 9273 (BOL); Goldblatt 2061 (NBG); Williams 752 (NBG); Stokoe s.n. (NBG); Stokoe s.n. (SAM); Zeyher 2648 (SAM); *Thamnea diosmoides* **OLIV.**: Esterhuysen 23758 (BOL); Esterhuysen 35045 (BOL); Powrie 257 (BOL); Bolus 3386 (BOL); Helme 69 (BOL); Collector unknown (BOL); Marloth 2506 (BOL); Hall 1292 (BOL); Esterhuysen 8015 (BOL); Compton 3685 (BOL); Esterhuysen 8961 (BOL);

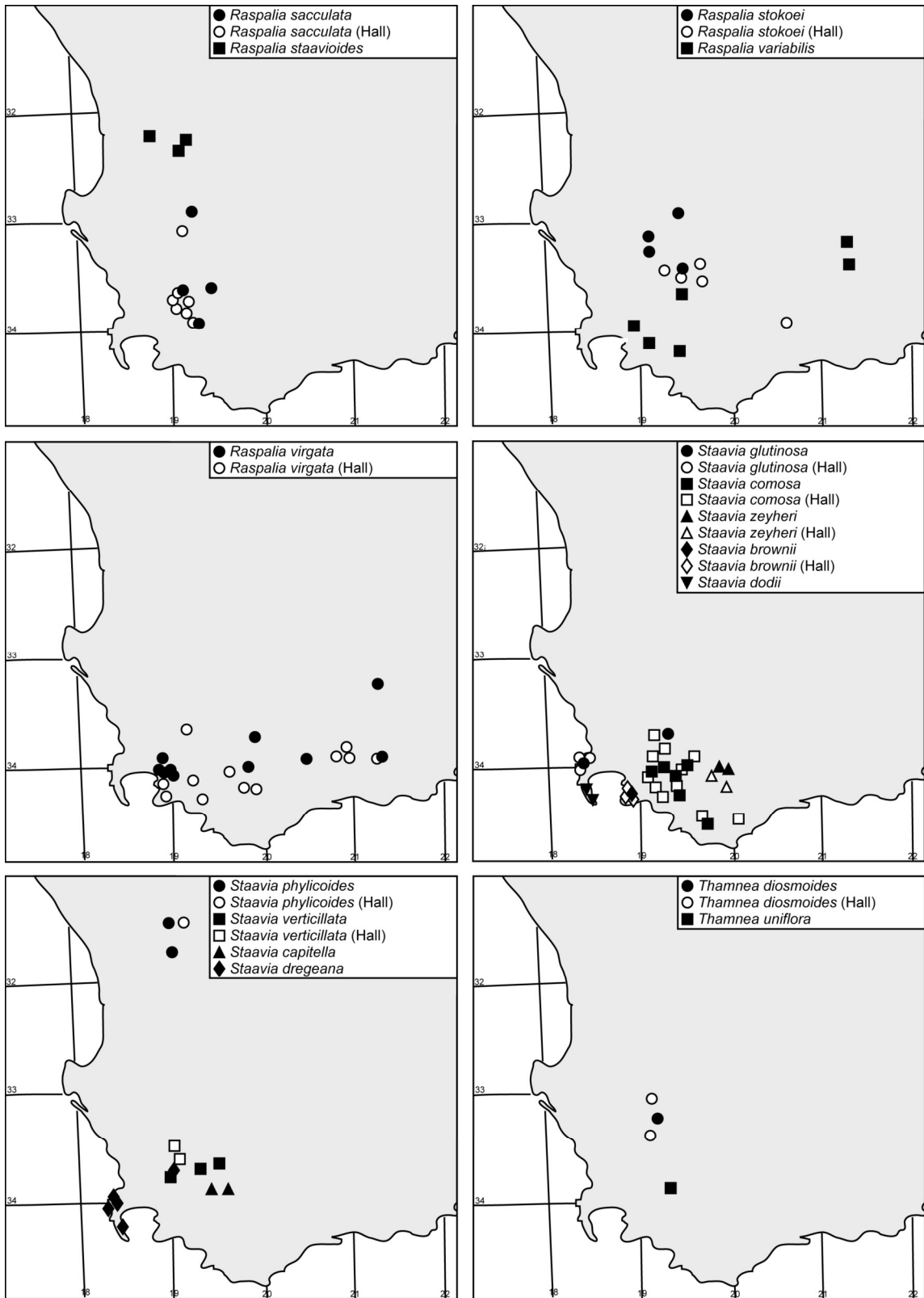
Esterhuysen 22242 (BOL); Schlechter 10700 (BOL); Schlechter 1662 (BOL); Bolus 7479 (BOL); MacOwan 3088 (SAM); Schlechter 1662 (SAM); Bolus 7479 (SAM); *Thamnea gracilis* (KUNTZE) OLIV.: Burchell 7342 (BOL); Esterhuysen 35408 (NBG); Goldblatt 1648 (NBG); Bolus 4952 (NBG); Taylor 7377 (NBG); Kerfoot 5104 (NBG); Kerfoot 5097 (NBG); *Thamnea hirtella* OLIV.: Esterhuysen 25765 (BOL); Powrie 252 (BOL); Rycroft 2711 (BOL); Burchell 8685 (BOL); *Thamnea massoniana* DUMMER: Esterhuysen 35390 (BOL); Esterhuysen 9638 (BOL); Esterhuysen & Claßen-Bockhoff 2853 (BOL); Stokoe 5003 (BOL); Esterhuysen s.n. Esterhuysen s.n. (BOL); Stokoe 8910 (BOL); Esterhuysen 31935 (BOL); Esterhuysen 9745 (BOL); Esterhuysen 33276a (BOL); Esterhuysen 34188 (BOL); Esterhuysen 34194 (BOL); Stokoe 8909 (BOL); Stokoe 8909 (SAM); Stokoe s.n. (SAM); *Thamnea* sp.: Esterhuysen 27710 (BOL); Esterhuysen 29877 (BOL); Esterhuysen 28150 (BOL); *Thamnea thesioides* DUMMER: Bolus 1152 (BOL); Bolus 5490 (BOL); Bolus 1152 (NBG); Bolus 1152 (SAM); MacOwan 2714 (SAM); *Thamnea uniflora* SOL. EX BRONGN.: Esterhuysen 35584 (BOL); Esterhuysen 35408 (BOL); Esterhuysen 35105 (BOL); *Tittmannia esterhuyseniae* POWRIE: Esterhuysen 32117 (BOL); Esterhuysen 33370a (BOL); Esterhuysen 29771 (BOL); Claßen-Bockhoff & Esterhuysen 1527 (BOL); Esterhuysen 31832 (BOL); Esterhuysen 32117 (NBG); *Tittmannia hispida* PILLANS: Esterhuysen 34564 (BOL); Bean & Viviers 1957 (BOL); Esterhuysen 18207 (BOL); Esterhuysen 24306 (BOL); Stokoe 18936 (BOL); Esterhuysen 22663 (BOL); Stokoe 8814 (BOL); *Tittmannia laevis* PILLANS: Stokoe 1300 (BOL); Wordsdell 22947 (BOL); Esterhuysen 33272 (BOL); Esterhuysen s.n. (BOL); Esterhuysen 32113 (BOL); Esterhuysen 33015 (BOL); Esterhuysen 17780 (BOL); Esterhuysen 13535 (BOL); Esterhuysen 9629 (BOL); Esterhuysen 27936 (BOL); *Tittmannia laxa* (THUNB.) C.PRESL: Leighton 2230 (BOL); Stokoe 6013 (BOL); Linder 4416 (BOL); Esterhuysen 4172 (BOL); Esterhuysen 20486 (BOL); Esterhuysen 13063 (BOL); Bolus 13549 (BOL); Esterhuysen 6156 (BOL); Esterhuysen 23034 (BOL); Esterhuysen 10154 (BOL); Esterhuysen 23426 (BOL); Hutchinson 1029 (BOL); Esterhuysen 3377 (BOL); Esterhuysen 12145 (BOL); Esterhuysen 31627 (BOL); Esterhuysen 2539 (BOL); Esterhuysen 31162 (BOL); Esterhuysen 33931 (BOL); Esterhuysen 29808 (BOL); Stokoe 2822 (BOL); Stokoe 2630 (BOL); Stokoe 2650 (BOL); Schlechter 8874 (BOL); Bodkin 1153 (BOL); Compton s.n. (BOL); Compton 3978 (BOL); Esterhuysen 7406 (BOL); Esterhuysen s.n. (BOL); Stokoe 7325 (BOL); Stokoe 2648 (BOL); Schlechter 257 (BOL); Oliver s.n. (BOL); Esterhuysen 2462 (BOL); Bolus 6363 (BOL); Bolus 23033 (BOL); Collector unknown (BOL); Compton 3686 (BOL); Drege s.n. (BOL); Esterhuysen 28625 (BOL); Esterhuysen 3657 (BOL); Bolus 6707 (NBG); *Tittmannia laxa* (THUNB.) C.PRESL var. *langebergensis* PILLANS: Esterhuysen 23823 (BOL); Esterhuysen 32042 (BOL); Bolus 6707 (BOL).

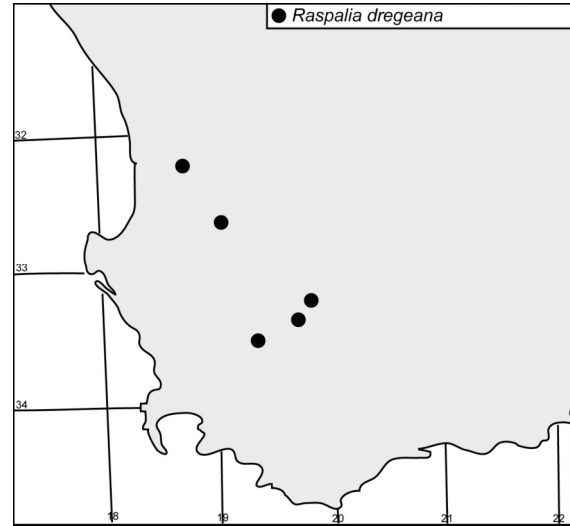
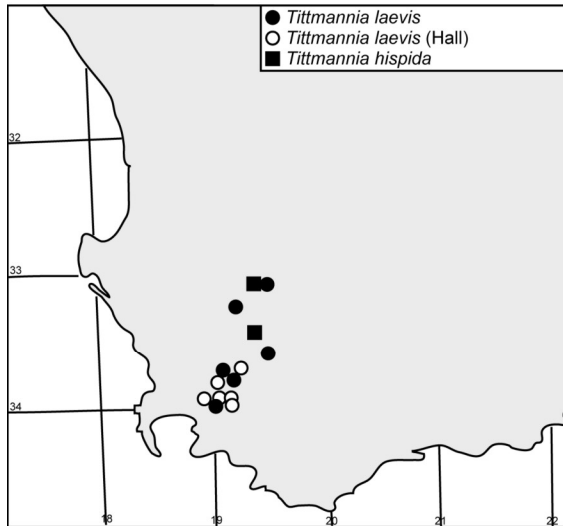
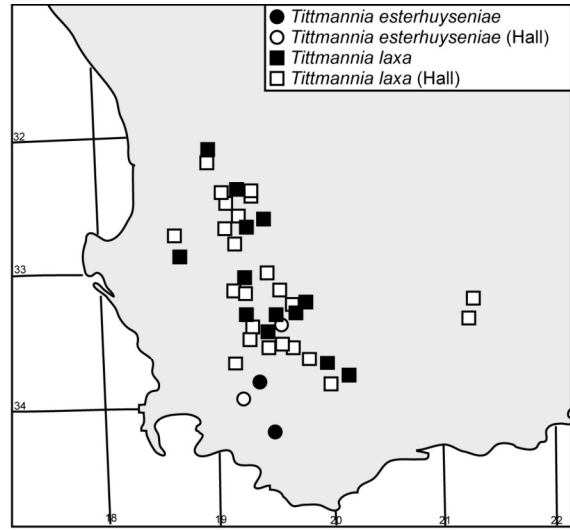
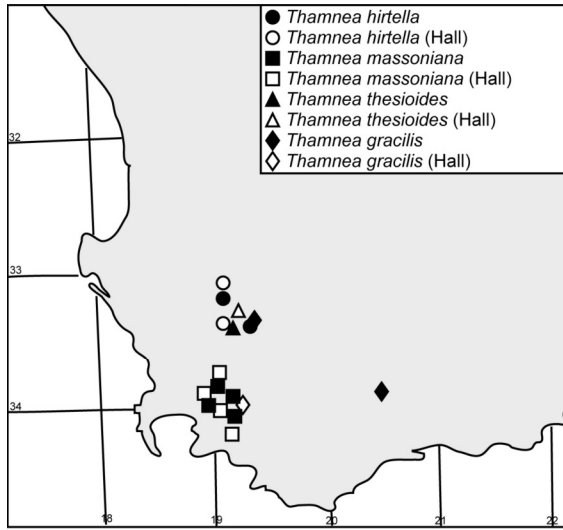
Distribution maps of all Bruniaceae species according to herbarium sheet label data (black signs) and unpublished localities after Hall (white signs).

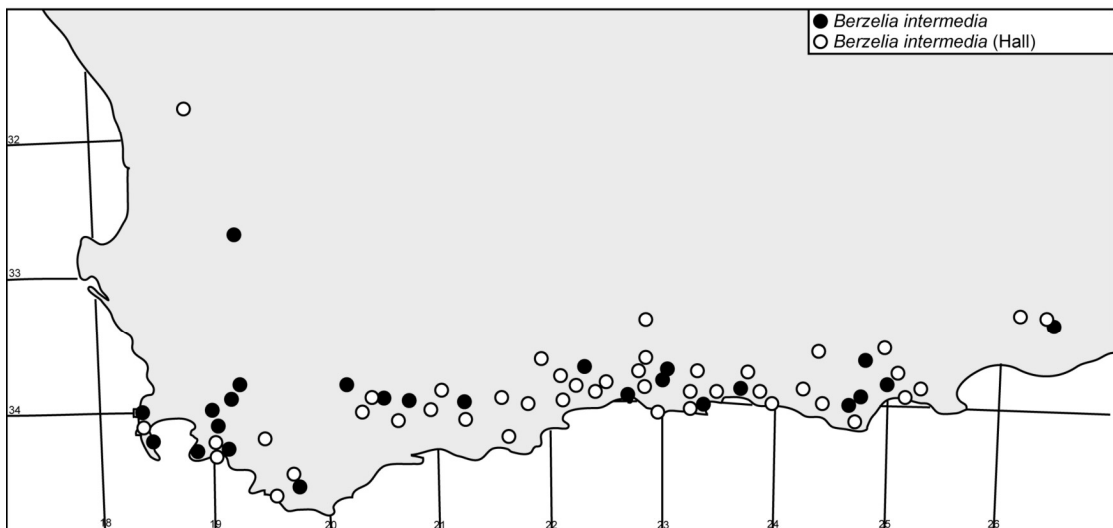
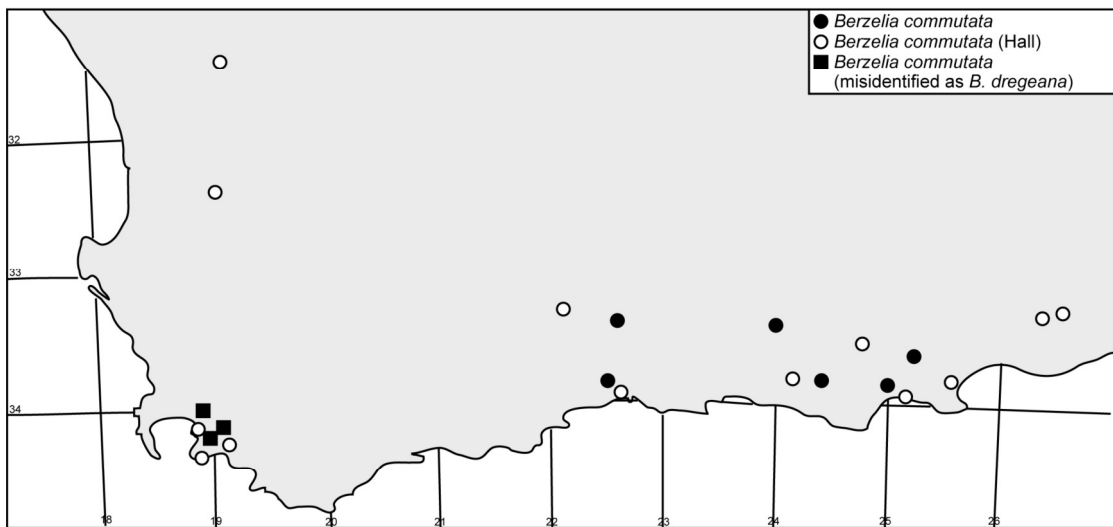
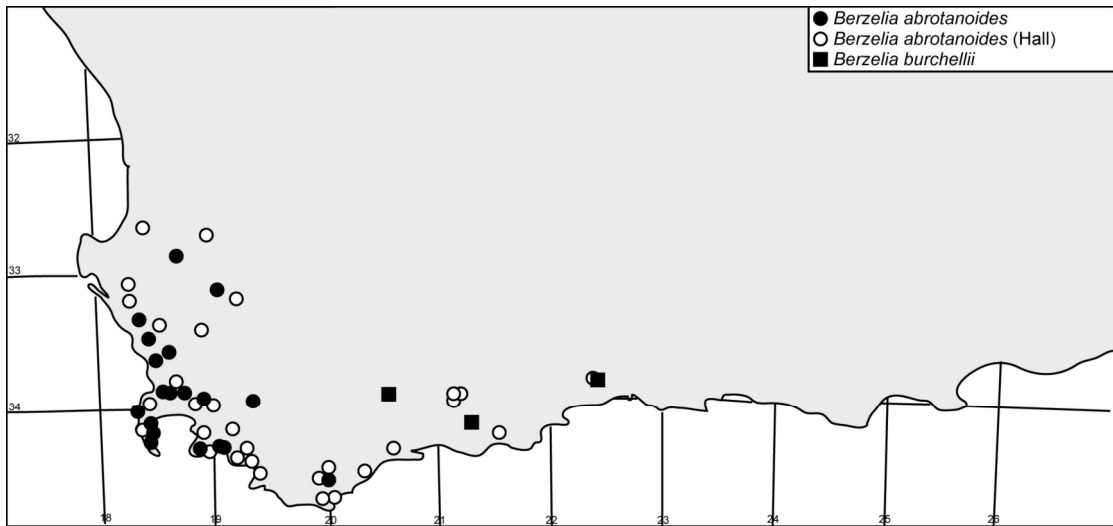


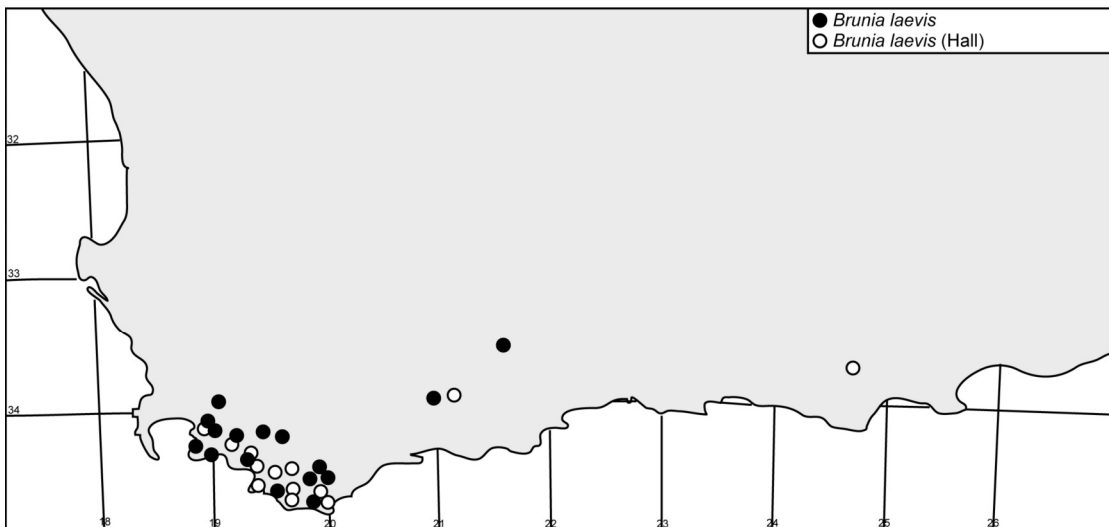
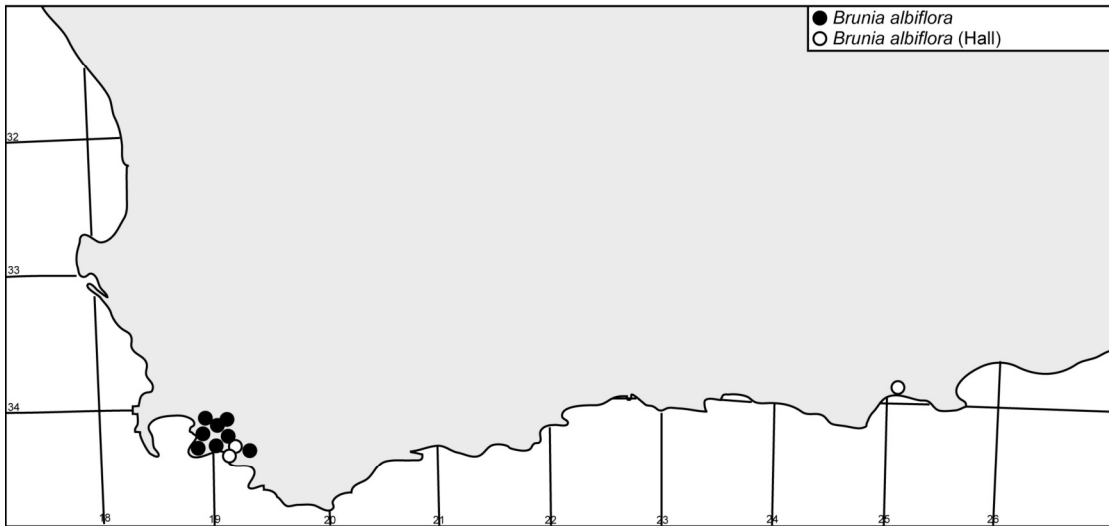
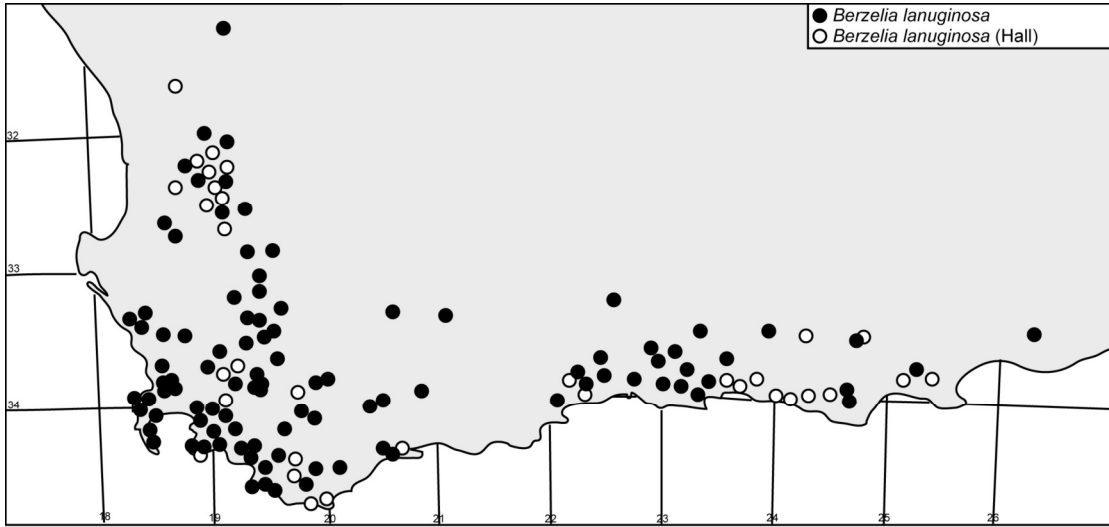


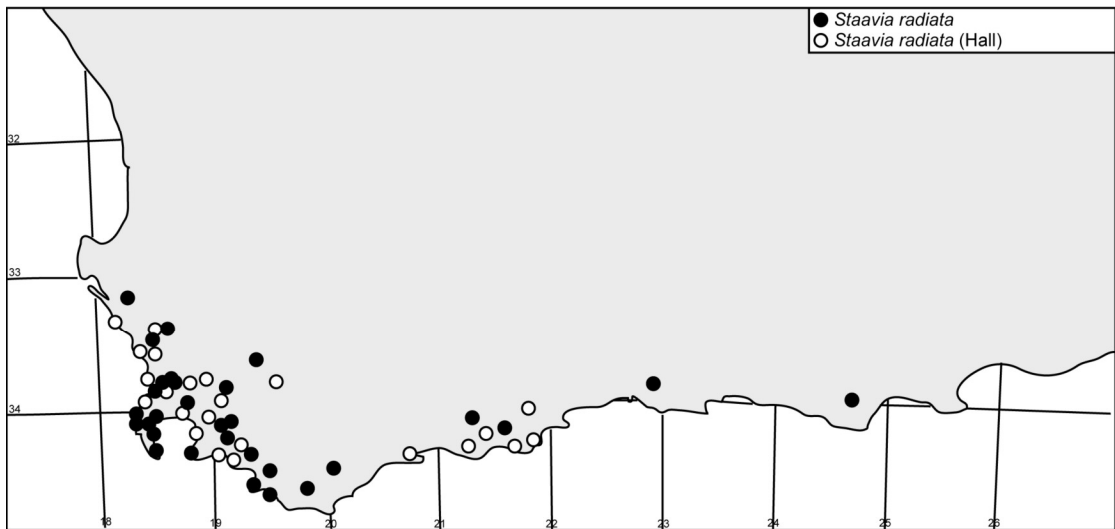
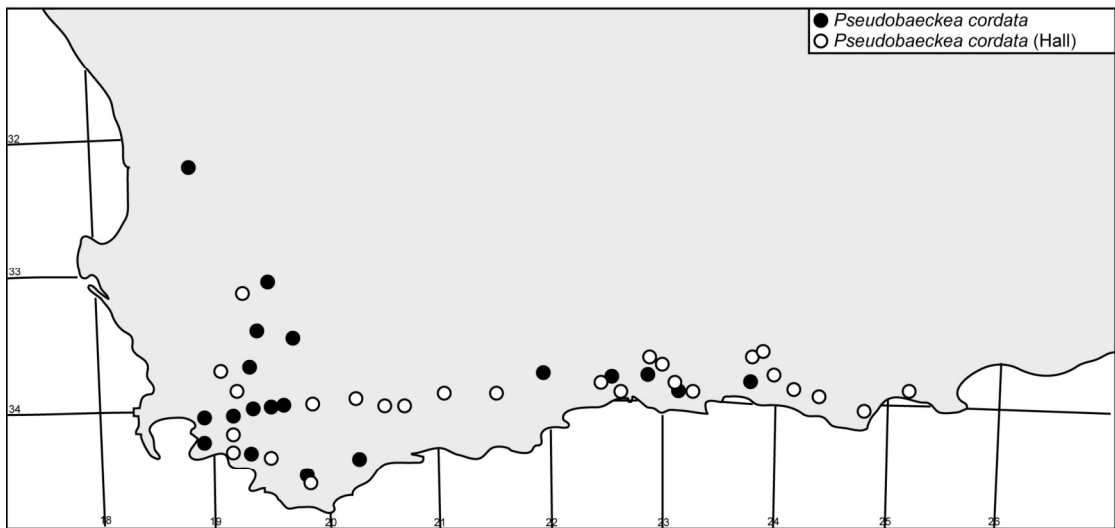
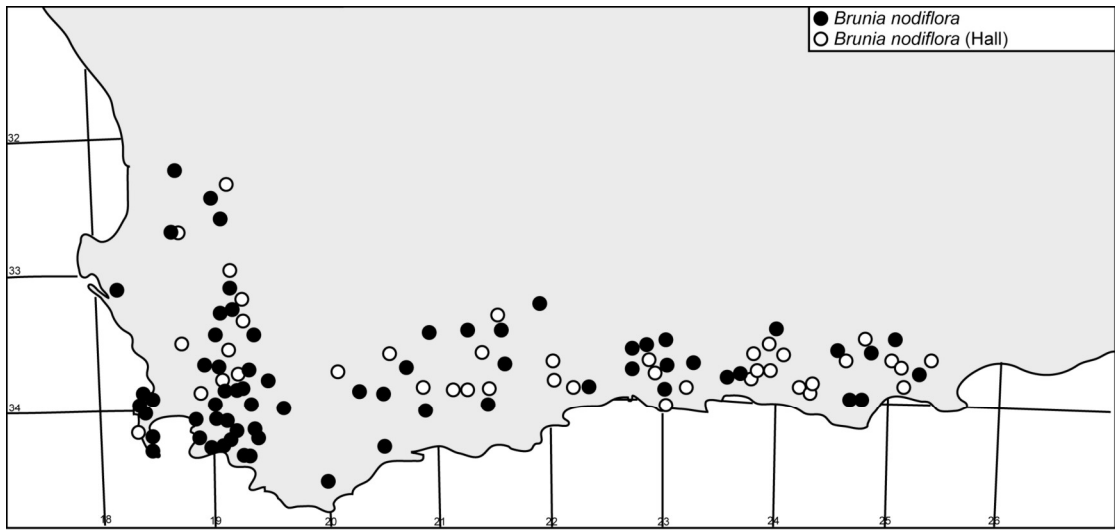












Population density at the Vogelgat Nature Reserve

Population density *Berzelia lanuginosa*

A: 0, 1, 4, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 2, 1, 0, 3, 8, 0 10, 13, 3, 7, 4, 6, 0, 5, 3, 1, 4, 7, 4, 0, 3, 5, 3, 2, 0, 1, 0, 1, 1,
 1, 2, 1, 1, 0, 2, 3, 4, 2, 3, 3, 5, 4, 4, 7, 6, 5, 2, 3, 2, 3, 5, 3, 1, 0, 1, 2, 0, 1, 0, 0, 3, 0, 4, 0, 1, 0, 0, 0, 0.
 B: 0, 0, 0, 0, 0, 0, 1, 1, 2, 0, 5, 3, 4, 0, 1, 1, 0, 0, 0, 1, 2, 1, 2, 3, 2, 1, 10, 0, 1, 0, 0, 1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0,
 0, 1, 0.

C: 1, 0, 1, 3, 3, 4, 3, 5, 4, 3, 3, 3, 3, 2, 1, 2, 3, 3, 4, 3, 3, 1, 0, 2, 1, 1, 2, 3, 2, 2, 4, 4, 3, 3, 2, 3, 4, 5, 5, 6, 3, 0, 1, 0, 3, 1, 1, 3, 3.

Population density *Brunia alopecuroides*

A: 0, 1, 5, 6, 0, 3, 0, 0, 1, 2, 1, 2, 4, 0, 1, 1, 1, 3, 6, 9, 9, 4, 2, 0, 9, 2, 12, 12, 10, 14, 22, 12, 4, 0, 0, 0, 0, 0, 7, 0, 11, 9, 0, 6, 8,
 3, 1, 0, 0, 0.
 B: 23, 14, 9, 15, 13, 8, 8, 11, 11, 10, 5, 7, 12, 7, 2, 2, 2, 3, 10, 4, 4, 5, 6, 9, 11, 5, 4, 7, 10, 16, 7, 7, 3, 11, 13, 17, 14, 24, 18, 19,
 11, 13, 16, 14, 13, 15, 11, 6, 15, 17, 13, 15, 22, 12, 9, 13, 5, 4, 4, 16, 36, 8, 4, 2.

Population density *Lonchostoma monogynum*

- A: 25
- B: 31
- C: 15
- D: 5
- E: 31
- F: 7
- G: 2
- H: 35
- I: 20
- J: 11

Population density *Nebelia paleacea*

- A: 30
- B: 14
- C: 38

Appendix 4 Statistic

Correlation analysis of seed set under bagged and unbagged conditions

T-TEST of *Staavia radiata* seed set open and seed set bagged, Criteria = CI(.95)

	mean	N	sd	standard error of the mean
seed_open	30,0000	10	,74536	,23570
seed_bagged	,0000	10	,00000	,00000

Correlation of paired random samples

	N	correlation	significance
seed_open & seed_bagged	10	.	.

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
seed_open - seed_bagged	30,00000	,74536	,23570	29,46680	30,53320	127,279	9	,000

T-TEST of *Brunia alopecuroides* seed set open and seed set bagged, Criteria = CI(.95)

	mean	N	sd	standard error of the mean
seed_open	5,8000	10	,14907	,04714
seed_bagged	,8000	10	,14907	,04714

Correlation of paired random samples

	N	correlation	significance
seed_open & seed_bagged	10	-,300	,400

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
seed_open - seed_bagged	5,00000	,24037	,07601	4,82805	5,17195	65,779	9	,000

T-TEST of *Lonchostoma monogynum* seed set open and seed set bagged CRITERIA = CI(.95)

	mean	N	sd	standard error of the mean
seed_open	80,7100	10	,75638	,23919
seed_bagged	,0110	10	,03143	,00994

Correlation of paired random samples

	N	correlation	significance
seed_open & seed_bagged	10	-,758	,011

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit				
	lower bound	upper bound	lower bound	upper bound	lower bound			
seed_open - seed_bagged	80,69900	,78046	,24680	80,14069	81,25731	326,976	9	,000

T-TEST of *Nebelia paleacea* seed set open and seed set bagged, Criteria = CI(.95)

	mean	N	sd	standard error of the mean
seed_open	,3000	10	,10541	,03333
seed_bagged	,8000	10	,06667	,02108

Correlation of paired random samples

	N	correlation	significance
seed_open & seed_bagged	10	,316	,373

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit				
	lower bound	upper bound	lower bound	upper bound	lower bound			
seed_open - seed_bagged	-,50000	,10541	,03333	-,57541	-,42459	-15,000	9	,000

T-TEST of *Pseudobaeckea africana* seed set open and seed set bagged. Criteria= CI(.95)

	mean	N	sd	standard error of the mean
seed_open	48,3000	10	,10541	,03333
seed_bagged	37,8000	10	,06667	,02108

Correlation of paired random samples

	N	correlation	significance
seed_open & seed_bagged	10	-,474	,166

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
seed_open - seed_bagged	10,50000	,14907	,04714	10,39336	10,60664	222,739	9	,000

T-TEST of *Berzelia lanuginosa* seed set open and seed set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
seed_open	74,3000	10	,66667	,21082
seed_bagged	1,8000	10	,06667	,02108

Correlation of paired random samples

	N	correlation	significance
seed_open & seed_bagged	10	1,000	,000

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
seed_open - seed_bagged	72,50000	,60000	,18974	72,07079	72,92921	382,109	9	,000

T-TEST of *Raspalia microphylla* seed set open and seed set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
seed_open	,0100(a)	10	,03162	,01000
seed_bagged	,0100(a)	10	,03162	,01000

(a) correlation and T-test cannot be calculated as the standard error and the standard difference are equal to zero.

Correlation analysis of fruit set under bagged and unbagged conditions

T-TEST of *Lonchostoma monogynum* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	99,5000	10	3,02765	,95743
fruit_bagged	,0200	10	,04216	,01333

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	,000	1,000

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
fruit_open - fruit_bagged	99,48000	3,02794	,95752	97,31394	101,64606	103,893	9	,000

T-TEST of *Nebelia paleacea* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	99,5000	10	3,02765	,95743
fruit_bagged	100,0000	10	3,59011	1,13529

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	,000	1,000

Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
fruit_open - fruit_bagged	-,50000	4,69633	1,48511	-3,85956	2,85956	-,337	9	,744

T-TEST of *Staavia radiata* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	61,2050	10	,03028	,00957
fruit_bagged	,0200	10	,04216	,01333

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	-,087	,811

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)	
	mean	sd	standard error of the mean	95% confidence limit					standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound				upper bound
fruit_open - fruit_bagged	61,18500	,05401	,01708	61,14637	61,22363	3582,627	9	,000	

T-TEST of *Raspalia microphylla* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	100,0000	10	,00000	,00000
fruit_bagged	100,0000	10	1,49071	,47140

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	.	.

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)	
	mean	sd	standard error of the mean	95% confidence limit					standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound				upper bound
fruit_open - fruit_bagged	,00000	1,49071	,47140	-1,06639	1,06639	,000	9	1,000	

T-TEST of *Pseudobaeckea africana* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	96,5950	10	,03028	,00957
fruit_bagged	75,7000	10	,14907	,04714

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	,000	1,000

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
fruit_open - fruit_bagged	20,89500	,15211	,04810	20,78618	21,00382	434,381	9	,000

T-TEST of *Berzelia lanuginosa* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	88,5080	10	,02974	,00940
fruit_bagged	98,2000	10	1,31656	,41633

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	-,244	,497

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
fruit_open - fruit_bagged	-9,69200	1,32413	,41873	-10,63923	-8,74477	-23,146	9	,000

T-TEST of *Brunia alopecuroides* fruit set open and fruit set bagged. Criteria = CI(.95)

	mean	N	sd	standard error of the mean
fruit_open	92,8000	10	1,81353	,57349
fruit_bagged	99,5000	10	3,02765	,95743

Correlation of paired random samples

	N	correlation	significance
fruit_open & fruit_bagged	10	,000	1,000

T-Test of paired random samples

	paired differences					T	df	Significance (2-sided)
	mean	sd	standard error of the mean	95% confidence limit		mean	sd	standard error of the mean
	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound	lower bound	upper bound
fruit_open - fruit_bagged	-6,70000	3,52924	1,11604	-9,22467	-4,17533	-6,003	9	,000

Flowers per inflorescence

Table 1: Univariate analysis (oneway ANOVA)

	square sum	df	median square	F	significance
between groups	3906146.360	9	434016.262	445.497	.000
within groups	87680.600	90	974.229		

Table 2: Post-Hoc-Test Flowers per inflorescence with Bonferroni correction: $\alpha = 0.05$; *the differences of the mean is significant at 0.05 standard error = 13,95872

	<i>Berzella lanuginosa</i> (1)	<i>Staavia radiata</i> (2)	<i>Lonchostoma monogynum</i> (3)	<i>Berzella abrotanoides</i> (4)	<i>Pseudobaeckea africana</i> (5)	<i>Raspalia microphylla</i> (6)	<i>Nebelia paleacea</i> (7)	<i>Brunia alopecuroides</i> (8)	<i>Brunia albiflora</i> (12)
<i>B. lanuginosa</i> (1)	-	-	-	-	-	-	-	-	-
<i>S. radiata</i> (2)	131.20 (*)	-	-	-	-	-	-	-	-
<i>L. monogynum</i> (3)	125.40 (*)	-5.80	-	-	-	-	-	-	-
<i>B. abrotanoides</i> (4)	69.00 (*)	-62.20 (*)	-56.40 (*)	-	-	-	-	-	-
<i>P. africana</i> (5)	-567.80 (*)	-699.00 (*)	-693.20 (*)	-636.80 (*)	-	-	-	-	-
<i>R. microphylla</i> (6)	117.40 (*)	-13.80	-8.00	48.40 (*)	685.20(*)	-	-	-	-
<i>N. paleacea</i> (7)	89.80 (*)	-41.40	-35.60	20.80	657.60(*)	-27.60	-	-	-
<i>Br. alopecuroides</i> (8)	78.30 (*)	-52.90 (*)	-47.10 (*)	9.30	646.10(*)	-39.10	-11.50	-	-
<i>Br. albiflora</i> (12)	59.60 (*)	-71.60 (*)	-65.80 (*)	-9.40	627.40(*)	-57.80(*)	-30.20	-18.70	-
<i>B. squarrosa</i> (13)	-2.10	-133.30 (*)	-127.50 (*)	-71.10(*)	565.70(*)	-119.50(*)	-91.90(*)	-80.40(*)	-61.70(*)

Pollen/ovule ratio

Table 3: Univariate analysis (oneway ANOVA)

	square sum	df	median square	F	significance
between groups	11860014.091	10	1186001.409	527.684	.000
within groups	222508.600	99	2247.562		

Table 4: Post-Hoc-Test Pollen/ovule ratio with Bonferroni correction: $\alpha = 0.05$; *the differences of the mean is significant at 0.05 standard error 21,20171

	<i>Berzella lanuginosa</i> (1)	<i>Staavia radiata</i> (2)	<i>Lonchostoma monogynum</i> (3)	<i>Berzella abrotanoides</i> (4)	<i>Pseudobaeckea africana</i> (5)	<i>Raspalia microphylla</i> (6)	<i>Nebelia paleacea</i> (7)	<i>Brunia alopecuroides</i> (8)	<i>Brunia albiflora</i> (12)
<i>B. lanuginosa</i> (1)	-								
<i>S. radiata</i> (2)	182,10 (*)								
<i>L. monogynum</i> (3)	436,80(*)	254,70(*)							
<i>B. abrotanoides</i> (4)	-25,90	-208,00(*)	-462,70(*)						
<i>P. africana</i> (5)	820,10(*)	638,00(*)	383,30(*)	846,00(*)					
<i>R. microphylla</i> (6)	750,40(*)	568,30(*)	313,60(*)	776,30(*)	-69,70				
<i>N. paleacea</i> (7)	736,70(*)	554,60(*)	299,90(*)	762,60(*)	-83,40(*)	-13,70			
<i>Br. alopecuroides</i> (8)	214,50(*)	32,40	-222,30(*)	240,40(*)	-605,60(*)	-535,90(*)	-522,20(*)		
<i>Br. albiflora</i> (12)	-112,90(*)	-295,00(*)	-549,70(*)	-87,00(*)	-933,00(*)	-863,30(*)	-849,60(*)	-327,40(*)	
<i>B. squarrosa</i> (13)	364,80(*)	182,70(*)	-72,00	390,70(*)	-455,30(*)	-385,60(*)	-371,90(*)	150,30(*)	-477,70(*)

Distance between stigma/anther and corolla length

Table 5: Univariate analysis (oneway ANOVA) A: stigma/anther and B: corolla length

A	square sum	df	median square	F	significance
between groups	82.818	9	9.202	1119.157	.000
within groups	.740	90	.008		

B	square sum	df	median square	F	significance
between groups	519.572	9	57.730	780.139	.000
within groups	6.660	90	.074		

Table 6: Post-Hoc-Test Lower part of the table: distance between stigma/anther and corolla; upper part of the table: length with Bonferroni correction: $\alpha = 0.05$; *the differences of the mean is significant at 0.05 standard error 21,20171

	<i>Berzelia lanuginosa</i> (1)	<i>Staavia radiata</i> (2)	<i>Lonchostoma monogynum</i> (3)	<i>Berzelia abrotanoides</i> (4)	<i>Pseudobaeckea africana</i> (5)	<i>Raspalia microphylla</i> (6)	<i>Nebelia paleacea</i> (7)	<i>Brunia alopecuroides</i> (8)	<i>Berzelia lanuginosa/leost</i> (10)	<i>Berzelia squarrosa</i> (13)
<i>Berzelia lanuginosa</i> (1)	-	-.39	-7.14(*)	.13	.55(*)	1.11(*)	-1.02 (*)	.56 (*)	.52 (*)	.54 (*)
<i>Staavia radiata</i> (2)	.51 (*)	-	-6.75(*)	.52(*)	.94(*)	1.50(*)	-.63 (*)	.95 (*)	.91 (*)	.93 (*)
<i>Lonchostoma monogynum</i> (3)	-.90(*)	-1.41(*)	-	7.27(*)	7.69(*)	8.25(*)	6.12 (*)	7.70 (*)	7.66 (*)	7.68 (*)
<i>Berzelia abrotanoides</i> (4)	-2.26(*)	-2.77(*)	-1.36(*)	-	-.42(*)	.56(*)	-1.57 (*)	.01	-.03	-.01
<i>Pseudobaeckea africana</i> (5)	.51(*)	.00	1.41(*)	2.77(*)	-	-.56(*)	-2.13 (*)	-.55 (*)	-.59 (*)	-.57 (*)
<i>Raspalia microphylla</i> (6)	.59(*)	.08	1.49(*)	2.85(*)	.08	-	-2.13 (*)	-.55 (*)	-.59 (*)	-.57 (*)
<i>Nebelia paleacea</i> (7)	-1.36(*)	-1.87(*)	-.46(*)	.90(*)	-1.87(*)	-1.95(*)	-	1.58 (*)	1.54 (*)	1.56 (*)
<i>Brunia alopecuroides</i> (8)	.48(*)	-.03	1.38(*)	2.74(*)	-.03	-.11	1.84 (*)	-	-.04	-.02
<i>Berzelia lanuginosa/Ost</i> (10)	-.62(*)	-1.13(*)	.28(*)	1.64(*)	-1.13(*)	-1.21(*)	.74 (*)	-1.10 (*)	-	.02
<i>Berzelia squarrosa</i> (13)	-.07	-.58(*)	.83(*)	2.19(*)	-.58(*)	-.66(*)	1.29 (*)	-.55 (*)	-.55 (*)	-

Width 1 and 2 of the corolla

Table 7: Univariate analysis (oneway ANOVA) A: width 1 and B: width 2

A	square sum	df	median square	F	significance
between groups	33.262	9	3.696	287.237	.000
within groups	1.158	90	.013		

B	square sum	df	median square	F	significance
between groups	2.640	9	.293	77.647	.000
within groups	.340	90	.004		

Table 8: Post-Hoc-Test Lower part of the table: width; upper part of the table: width 2 with Bonferroni correction: $\alpha = 0.05$; *the differences of the mean is significant at 0.05 standard error width 1: .05073; standard error width 2: .02749

	<i>Berzelia lanuginosa</i> (1)	<i>Staavia radiata</i> (2)	<i>Lonchostoma monogynum</i> (3)	<i>Berzelia abrotanoides</i> (4)	<i>Pseudobaeckea africana</i> (5)	<i>Raspalia microphylla</i> (6)	<i>Nebelia paleacea</i> (7)	<i>Brunia alopecuroides</i> (8)	<i>Berzelia lanuginosa/Ost</i> (10)	<i>Berzelia squarrosa</i> (13)
<i>Berzelia lanuginosa</i> (1)	-	-.04	.01	.01	.01	.53 (*)	.02	.03	.00	-.07
<i>Staavia radiata</i> (2)	-1.50 (*)	-	.05	.05	.05	.57 (*)	.06	.07	.04	-.03
<i>Lonchostoma monogynum</i> (3)	.46 (*)	1.96 (*)	-	.00	.00	.52 (*)	.01	.02	-.01	-.08
<i>Berzelia abrotanoides</i> (4)	.43 (*)	1.93 (*)	-.03	-	.00	.52 (*)	.01	.02	-.01	-.08
<i>Pseudobaeckea africana</i> (5)	.33 (*)	1.83 (*)	-.13	-.10	-	.52 (*)	.01	.02	-.01	-.08
<i>Raspalia microphylla</i> (6)	.40 (*)	1.90 (*)	-.06	-.03	.07	-	-.51 (*)	-.50 (*)	-.53 (*)	-.60 (*)
<i>Nebelia paleacea</i> (7)	-.52 (*)	.98 (*)	-.98 (*)	-.95 (*)	-.85 (*)	-.92 (*)	-	.01	-.02	-.09
<i>Brunia alopecuroides</i> (8)	-.03	1.47 (*)	-.49 (*)	-.46 (*)	-.36 (*)	-.43 (*)	.49 (*)	-	-.03	-.10 (*)
<i>Berzelia lanuginosa/Ost</i> (10)	.27 (*)	1.77 (*)	-.19 (*)	-.16	-.06	-.13	.79 (*)	.30 (*)	-	.07
<i>Berzelia squarrosa</i> (13)	.26 (*)	1.76 (*)	-.20 (*)	-.17	-.07	-.14	.78 (*)	.29 (*)	-.01	-

Fruit and seed set (unbagged)

Table 9: Univariate analysis (oneway ANOVA) A: fruit set and B: seed set

A	square sum	df	median square	F	significance
between groups	19822.048	9	2202.450	210.660	.000
within groups	940.949	90	10.455		

B	square sum	df	median square	F	significance
between groups	781632.837	9	86848.093	12188.270	.000
within groups	641.299	90	7.126		

Table 10: Post-Hoc-Test Lower part of the table: fruit set; upper part of the table: seed set with Bonferroni correction: $\alpha = 0.05$;

*the differences of the mean is significant at 0.05 fruit set standard error = 1.44603, seed set standard error = 1.19378

	<i>Berzelia lanuginosa</i> (1)	<i>Staavia radiata</i> (2)	<i>Lonchostoma monogynum</i> (3)	<i>Berzelia abrotanoides</i> (4)	<i>Pseudobaeckea africana</i> (5)	<i>Raspalia microphylla</i> (6)	<i>Nebelia paleacea</i> (7)	<i>Brunia alopecuroides</i> (8)	<i>Berzelia lanuginosa/Ost</i> (10)	<i>Berzelia squarrosa</i> (13)
<i>Berzelia lanuginosa</i> (1)	-	-.04	.01	.01	.01	.53 (*)	.02	.03	.00	-.07
<i>Staavia radiata</i> (2)	-1.50 (*)	-	.05	.05	.05	.57 (*)	.06	.07	.04	-.03
<i>Lonchostoma monogynum</i> (3)	.46 (*)	1.96 (*)	-	.00	.00	.52 (*)	.01	.02	-.01	-.08
<i>Berzelia abrotanoides</i> (4)	.43 (*)	1.93 (*)	-.03	-	.00	.52 (*)	.01	.02	-.01	-.08
<i>Pseudobaeckea africana</i> (5)	.33 (*)	1.83 (*)	-.13	-.10	-	.52 (*)	.01	.02	-.01	-.08
<i>Raspalia microphylla</i> (6)	.40 (*)	1.90 (*)	-.06	-.03	.07	-	-.51 (*)	-.50 (*)	-.53 (*)	-.60 (*)
<i>Nebelia paleacea</i> (7)	-.52 (*)	.98 (*)	-.98 (*)	-.95 (*)	-.85 (*)	-.92 (*)	-	.01	-.02	-.09
<i>Brunia alopecuroides</i> (8)	-.03	1.47 (*)	-.49 (*)	-.46 (*)	-.36 (*)	-.43 (*)	.49 (*)	-	-.03	-.10 (*)
<i>Berzelia lanuginosa/Ost</i> (10)	.27 (*)	1.77 (*)	-.19 (*)	-.16	-.06	-.13	.79 (*)	.30 (*)	-	.07
<i>Berzelia squarrosa</i> (13)	.26 (*)	1.76 (*)	-.20 (*)	-.17	-.07	-.14	.78 (*)	.29 (*)	-.01	-

Seed set bagged and unbagged

Table 11: Univariate analysis (oneway ANOVA) A: seed set bagged and B: seed set unbagged

A	square sum	df	median square	F	significance
between groups	11908.604	6	1984.767	3118990.82	.000
within groups	.040	63	.001		

B	square sum	df	median square	F	significance
between groups	40239.122	9	4471.014	22355067.66	.000
within groups	.008	40	.000		

Table 12: Post-Hoc-Test Lower part of the table: seed set unbagged; upper part of the table: seed set bagged with Bonferroni correction: $\alpha = 0.05$; *the differences of the mean is significant at 0.05 standard error seed unbagged = ,00894; standard error open seed bagged = .01128

	<i>Berzella lanuginosa</i> (1)	<i>Staavia radiata</i> (2)	<i>Lonchostoma monogynum</i> (3)	<i>Berzella abrotanoides</i> (4)	<i>Pseudobaeckea africana</i> (5)	<i>Raspalia microphylla</i> (6)	<i>Nebelia paleacea</i> (7)	<i>Brunia alopecuroides</i> (8)	<i>Brunia albiflora</i> (12)
<i>B. lanuginosa</i> (1)	-	1.80 (*)	1.80 (*)	-	-35.99 (*)	1.80 (*)	1.00 (*)	1.00 (*)	-
<i>S. radiata</i> (2)	44.30 (*)	-	.00	-	-37.80 (*)	.00	-.80 (*)	-.80 (*)	-
<i>L. monogynum</i> (3)	-6.50 (*)	-50.80 (*)	-	-	-37.80 (*)	.00	-.80 (*)	-.80 (*)	-
<i>B. abrotanoides</i> (4)	23.30 (*)	-21.00 (*)	29.80 (*)	-	-	-	-	-	-
<i>P. africana</i> (5)	26.00 (*)	-18.30 (*)	32.50 (*)	2.70 (*)	-	37.80 (*)	37.00 (*)	37.00 (*)	-
<i>R. microphylla</i> (6)	74.28 (*)	29.98 (*)	80.78 (*)	50.98 (*)	48.28 (*)	-	-.80 (*)	-.80 (*)	-
<i>N. paleacea</i> (7)	74.00 (*)	29.70 (*)	80.50 (*)	50.70 (*)	48.00 (*)	-.28 (*)	-	.00	-
<i>Br. alopecuroides</i> (8)	68.50 (*)	24.20 (*)	75.00 (*)	45.20 (*)	42.50 (*)	-5.78 (*)	-5.50 (*)	-	-
<i>Br. albiflora</i> (12)	34.80 (*)	-9.50 (*)	41.30 (*)	11.50 (*)	8.80 (*)	-39.48 (*)	-39.20 (*)	-33.70 (*)	-
<i>B. squarrosa</i> (13)	10.30 (*)	-34.00 (*)	16.80 (*)	-13.00 (*)	-15.70 (*)	-63.98 (*)	-63.70 (*)	-58.20 (*)	24.50 (*)

Non-parametric Mann-Whitney-Test for *Brunia alopecuroides*

BINOMIAL (.50) = plants *Drosera*

	<i>Drosera</i>	N	mean rank	rank sum
plants	1.00	29	30.40	881.50
	2.00	21	18.74	393.50

Statistik for Test(a)

	plants
Mann-Whitney-U	162.500
Wilcoxon-W	393.500
Z	-2.844
asymptotic significance (2-sided)	.004

a group variable: *Drosera*

BINOMIAL (.50) = plants *restios*

	<i>Restios</i>	N	mean rank	rank sum
plants	1.00	23	30.67	705.50
	2.00	27	21.09	569.50

Statistik for Test(a)

	plants
Mann-Whitney-U	191.500
Wilcoxon-W	569.500
Z	-2.361
asymptotic significance (2-sided)	.018

a group variable: *restios*

BINOMIAL (.50) = plants *water*

	<i>water</i>	N	mean rank	rank sum
plants	1.00	22	25.77	567.00
	2.00	28	25.29	708.00

Statistik for Test(a)

	plants
Mann-Whitney-U	302.000
Wilcoxon-W	708.000
Z	-.120
asymptotic significance (2-sided)	.905

a group variable: *water*

BINOMIAL (.50) = plants *Osmitopsis asteriscoides*

	<i>Osmitopsis asteriscoides</i>	N	mean rank	rank sum
plants	1.00	15	27.13	407.00
	2.00	35	24.80	868.00

Statistik for Test(a)

	plants
Mann-Whitney-U	238.000
Wilcoxon-W	868.000
Z	-.529
asymptotic significance (2-sided)	.597

a group variable: *Osmitopsis asteriscoides*

Non-parametric Mann-Whitney-Test for a second population of *Brunia alopecuroides* with dry soil

BINOMIAL (.50)= plants restios

	restios	N	mean rank	rank sum
plants	1.00	32	36.33	1162.50
	2.00	22	14.66	322.50

Statistik for Test(a)

	plants
Mann-Whitney-U	69.500
Wilcoxon-W	322.500
Z	-4.984
asymptotic significance (2-sided)	.000

a group variable: restios