MORPHOLOGICAL VARIATION IN FIVE RELATED SPECIES OF *BARLERIA* (ACANTHACEAE)

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DECLARATION

I declare that this Dissertation is my own, unaided work. It is being submitted in partial fulfilment of the degree of Master of Science in the Faculty of Science, University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University. Where other workers' research findings have provided the background, or have been used for comparison, they have been duly acknowledged.

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Abstract

Variation has been known to be a result of various causes including plasticity, gene flow or the lack of it. In this work morphological variation exhibited within five species of *Barleria* (*B. bechuanensis* C.B. Clarke, *B. irritans* Nees, *B. jubata* S. Moore, *B. pungens* L.f. and *B. rigida* Nees) was studied to establish whether it was discrete or continuous. Morphological characters were examined and recorded in the form of matrices and photographs. Variation was analysed from three aspects: macro-morphology, micro-morphology and distribution of morphological characters. Cluster analysis imposed a hierarchical non-overlapping association among operational taxonomic units (OTUs) while ordination established whether the variation was discrete or continuous. Cluster analysis and ordination demonstrated that some of the species complexes exhibited discrete variation while others exhibited both continuous and discrete variation. Distribution maps which illustrated the distribution of clusters and some of the morphological characters in geographic space suggested that allopatric, parapatric and / or sympatric speciation could have occurred bringing about the differentiation. Pubescence revealed that trichome morphology and distribution can to some extent be used to define intra-specific variation. On the basis of phenetics analysis, *B. jubata* is maintained as a single species while the *B. bechuanensis* and *B. irritans* complexes are separated into two species each (differentiated by leaf size, shape and margins; and by bracteole size and number of veins in the bracteole respectively). Within the *B. rigida* complex, two species are recognised where one has lobed bracteoles and calyx lobes and the other has non-lobed bracteoles and calyx lobes. Included within the latter are plants formerly known as *B. ilicina* (E. Mey. ex T. Anders.). *Barleria pungens* is separated into two species, one with flat green leaf margins and the other with wavy green or white leaf margins.

Key words: *Barleria*, cluster analysis, distribution, microscopy, morphology, ordination, species, variation

Dedication

To my family, especially my beloved husband Edwin and my precious children Tionge, Muzang'alu, Lemekani and Zikonde, to my late mother, Mandalena and my brothers and sisters.

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CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 The study group

Barleria L., a large, polymorphic and widespread pan-tropical genus, comprises approximately 300 species (Balkwill and Balkwill, 1994; 1997; 1998). The genus is recognised by a combination of three characteristics namely: the predominance of double cystoliths in the epidermis; the 4-lobed calyx with two larger outer lobes (anticous and posticous lobe) and two smaller lateral inner lobes; and the globose, honeycomb pollen (Balkwill and Balkwill, 1997). The genus is currently divided into two subgenera (*Barleria* and *Prionitis* (Nees) C.B. Clarke) (Balkwill and Balkwill, 1997; Brummitt and Vollesen, 1992; Clarke, 1885). The subgenera are further subdivided into seven sections (*Barleria*, *Chrysothrix* M. Balkwill, *Prionitis* (Nees) Lindau, *Somalia* (Oliv.) Lindau, *Cavirostrata* M. Balkwill, *Fissimura* M. Balkwill and *Stellatohirta* M. Balkwill) based on morphological characters such as the presence or absence of sunken glands, pubescence, presence or absence of a beak in the capsule, number of seeds and inflorescence architecture among other characters (Balkwill and Balkwill, 1997; 1998; Lindau, 1895). Section *Barleria* is distinguished from the other sections by its "scarious calyx and axillary inflorescences based on scorpioid cymes" (Balkwill and Balkwill, 1997). The prickliness differentiates it from section *Chrysothrix* with which it shares the same fruit and septum morphology (Balkwill and Balkwill, 1997). Balkwill (1993) and Balkwill and Balkwill (1997) noted that section *Barleria* (in which the species in this study belong) comprised 78 species worldwide (out of which 32 are southern African) which were informally placed into five smaller subgroups based on variation of selected characters. Group 1, which has 37 species, accounts for almost half of the species placed within this Section. Species in Group 1 are characterised by almost always possessing a sharp apiculus or stiff mucronate tip on the leaf apex; bracteoles that are usually spinous and equipped with stiff or spinous teeth along the margins; an axillary spine system almost always present in the lower leaf axils; calyx margins that have stiff spines or small teeth; and a leaf surface that has dense cystoliths that are easily visible in dried specimens (Balkwill, 1993; Balkwill and Balkwill, 1997). The five species in this study, *Barleria bechuanensis* C.B. Clarke, *B. irritans* Nees, *B. jubata* S. Moore, *B. pungens* L.f. and *B. rigida* Nees belong to section *Barleria* Group 1.

Preliminary analysis (Nyirenda, 2008) revealed that *B. irritans*, which was used as a standard taxon to calibrate the character list, split into two distinct morphological and geographical entities with one of the clusters being nested within *B. pungens* (one of the two study species). However, the variation among *B. pungens* was difficult to define. It further showed that although both *Barleria pungens* and *B. irritans* exhibit geographical disjunction, the former inhabits the coast while the latter inhabits the coast and inland. Nonetheless, *B. rigida*, an inland taxon, was observed to be widespread while exhibiting differentiation within the complex (Nyirenda, 2008). It was also noted that four (4) of Balkwill's (1993) specimens cited under the informal

Group I were distributed among three different clusters within the *B. rigida* complex. This necessitated the need to re-examine the above three species. *Barleria bechuanensis* C.B. Clarke and *B. jubata* S. Moore were incorporated in the study on the basis of their morphological similarity to *B. irritans*, *B. pungens* and *B. rigida* (Obermeijer, 1933). *Barleria elegans* S. Moore another morphologically similar species to *B. jubata,* which, also belongs to section *Barleria* Group 1 (Balkwill, 1993; Obermeijer, 1933) was used as a standard taxon. This was done because further examination of the gaps and overlaps in morphology can result in recognising and circumscribing new taxa at the level of species or any other hierarchical rank lower than species.

In Africa, section *Barleria* is the most widespread and it occurs almost throughout the geographical range of the genus (Balkwill and Balkwill, 1997) although not all combinations of characters are represented in any one geographic area (Balkwill and Balkwill, 1996). Species of *Barleria*, especially the endemic ones, tend to have populations that are clustered (where they occur) as a result of soil type or short distance dispersal (Balkwill and Balkwill, 1998). In *Barleria* the forceful explosion of capsules at maturity propels seeds away from the parent plant for a relatively short distance which results in numerous seedlings around the mature plant (Long, 1976; pers. obs.). This short distance seed dispersal in *Barleria* makes it difficult for propagules to reach new habitats. Habitat specificity too may lead to clustered populations due to lack of genetic variability to allow persistence beyond the range limit (Makholela *et al.,* 2003; Samis and Eckert, 2009). Entomophylus pollination facilitates gene flow across the populations within limited distances and this reduces genetic variability among populations (Balkwill and Balkwill, 1998; Makholela *et al.,* 2003). While species collected from limited localities are considered narrow endemics (which they might be) they may be more widely distributed, but may not have been collected from other parts of their distribution range, and these may never be known due to rapid habitat destruction (Roalson *et al.,* 2002). Rare plants tend to have small population sizes (Makholela *et al.,* 2003; 2004; Rabinowitz, 1985).

Regional endemism has also been observed among many species of *Barleria* and in southern Africa estimated 45 to 70 species are endemic to the region (Balkwill and Balkwill, 1998). Some of these species have been observed to have highly restricted distributions and it is usually attributed to edaphic factors (Balkwill and Balkwill, 1998; Makholela *et al.,* 2004). Species with restricted localities may be endemic; species with widespread distribution conversely, may be frequent or occasional. In addition widespread species may be generalists. When opposing selective pressures act on individual species occupying an environment that is diverse, this can result in disruptive selection which can break up a uniform population resulting in the formation of ecotypes (Sebola and Balkwill, 2009). Depending on the genetic variability within a population, infraspecific populations adapted to particular ecological environments can be distinguished into various forms (Cron and Balkwill, 2006; Makholela *et al.*, 2003; 2004; Sebola and Balkwill, 2009).

Barleria pungens is found in the Eastern and Western Cape in the Eastern Fynbos-Renosterveld, South Coast-Fynbos and East Coast Renosterveld, whereas *B. irritans* inhabits the Albany Thicket, Eastern Fynbos-Renosterveld and Lower Karoo (Mucina and Rutherford, 2006). *Barleria bechuanensis, B. jubata* and *B. rigida* are inland taxa (Obermeijer, 1933). Preliminary distribution maps (not included) revealed that *B. bechuanensis* and *B. jubata* exhibit clustered distribution in the Northern Cape in Eastern Kalahari Bushveld and Namibia in the Mopane savanna respectively whereas *B. rigida* exhibits widespread distribution from the Northern Cape / Free State extending north-west up to the northern part of Namibia inhabiting the Dry Highveld Grassland, Upper Karoo, Bushmanland, Gariep Desert and the Southern and Central Namib Desert (Mucina and Rutherford, 2006; Obermeijer, 1933; pers. obs.). Disjunction can represent relicts of a former widespread distribution pattern or of long-range dispersal which would in turn result in divergence (Laurance, 2008; Lomolimo, 2000; Stace, 2000; Stebbins, 1967). Disjunct distribution nonetheless can also imply stepping stone speciation where the bridges that once linked a single species do not exist any longer which in turn leads to differentiation in morphology (Briggs and Walters, 1997; Masćo *et al.*, 2004; Stebbins, 1967). Geographically isolated populations that were once a single species are likely to differ as a result of diverse interactions with dissimilar environmental conditions (Laurance, 2008; Lomolimo, 2000; Sebola and Balkwill, 2009). Species can still be geographically distant and maintain similarity as was observed in *Metarungia pubinervia* (T. Anderson) Baden, another member of the Acanthaceae (Darbyshire *et al.,* 2008). Variation therefore, can be brought about by lack of gene flow or various environment-species interactions. The morphological gaps or overlaps will be used to define the observed variation.

Within *Barleria*, growth habit includes herbs, sub-shrubs, shrubs and suffrutices. The herbs are perennials, which may be erect, semi-prostrate or procumbent. The inflorescence varies from solitary to clustered flowers in cymes that are lax or congested borne in alternate axils (sometimes in opposite axils) (Balkwill and Balkwill, 1994). The inflorescence within the study group mainly differs only in the degree of compression within and between the various species. The morphological diversity in *Barleria* has been attributed to slow evolution (McDade *et al.,* 2000). The five species in this study are multi-stemmed perennial herbs and sub-shrubs whose stems may be erect, sub-erect or decumbent and spreading. The stems arise from a developed woody rootstock and exhibit variation in spinescence. There is great variation observed in form, with the *B. rigida* complex varying from dwarf cushion plants to multi-stemmed erect or spreading plants, branching from the rootstock, just above the ground or well above the ground. Other observed morphological dissimilarities within the study group include stem pubescence, leaf texture, leaf shape and margin, indumentum type on vegetative and reproductive structures, calyx size, and corolla colour among others. The number of flowers per inflorescence is highly variable within the species in this study. Flower morphology, leaf shape and margins, however, are characters that are known to be less prone to plasticity and reliably used for circumscription (Balkwill, *et al.*, 1996; Brummitt and Seyani, 1978;

Kiew and Vollesen, 1997). In view of the observed variation in a number of characters, the species in this study (except for *B. jubata*) are currently referred to as complexes.

Although pubescence density may vary with age it can be diagnostic to some extent. When indumenta type are genetically determined, they have been regarded as being of taxonomic importance as observed in *Barleria* and other genera within Acanthaceae (Darbyshire, 2008; Munday, 1980; 1984; Poriazis and Balkwill, 2008). Obermeijer (1933) based her circumscription of the species in section *Eubarleria* on vegetative morphological features such as leaf size, shape and texture; size and texture tend to be prone to plasticity and leaf texture is difficult to quantify in herbarium specimens (Watson and Estes, 1990). Among the *B. rigida* complex, leaf size, shape and margins are variable. The margins range from deeply undulate or shallowly so with or without spines or small teeth to sinuate with short or long spines. Other intra-specific differences observed among the study group include pubescence where younger stems are observed to be densely pubescent becoming light or glabrescent to glabrous with maturity. Extensive study of gaps and overlaps in morphology will be used to clarify understanding of the implications of the observed variation in morphology within the species / species complexes.

1.2 Species concepts

Although there are many species concepts, four concepts namely: the Morphological Species Concept (MSC) (Cabido *et al.*, 1992), the Biological Species Concept (BSC) (Briggs and Walters, 1997; Ereshefsky, 2001; Stace, 2000; Stebbins, 1967), Ecological Species Concept (ESC) (Ereshefsky, 2001; Van Valen, 1976[1992]) and the Phenetics Species Concept (PhSC) (Ereshefsky, 2001) will be born in mind during the interpretation of results; as there is no single species concept that is watertight. The MSC is adequate to describe species when obvious gaps or discontinuities are available in morphological characters that can be used to define particular species. It will thus be used to recognise species and other ranks. Since species are known to exist in an environment where they interact with many factors including their habitat and pollinators among others, plant breeding systems being associated with morphology plays a major role in variation within and between populations (Balkwill *et al.*, 1994; Schmidt-Lebuhn *et al.*, 2007; Vallius *et al.*, 2004). This calls for the consideration of both the ESC and BSC as the MSC is silent on breeding and interactions with the environment. Because morphology does respond to ecological changes, when morphology is combined with ecological data it can be used to determine ecotypes within species (Maśco *et al.,* 2004; Sebola and Balkwill, 2009). However, meaningful interpretations cannot be made based on the ESC when ecological studies have not been undertaken.

Reproductive organs are considered conservative and reliable (Briggs and Walters, 1997; Davis & Heywood, 1963; Stace, 2000), therefore their morphological data have been used to make deductions about breeding systems as phenetic similarity reveals functionality (Sneath and Sokal, 1973). Floral morphology can be linked to reproductive biology and thus will be used to define species biologically, thereby employing the BSC by implication as breeding and hybridisation experiments were not conducted. Phenetics will be employed to group taxa in clusters as it has been used before to recognise and circumscribe taxa at various hierarchical ranks (Cron *et al.*, 2007; Otieno *et al.*, 2006; Peres-Neto and Jackson, 2001; Schemske *et al.*, 1994; Sebola and Balkwill, 2006; 2009; Watson and Estes, 1990). Phenetics summarizes large amounts of morphological information in a few interpretable dimensions (Sokal and Sneath, 1963), and phenetically species are known to be organisms that have phenotypic similarity while appearing different from other sets of organisms (Cron *et al.*, 2007; Ereshefsky, 2001). Phenetics removes the weighting of characters as all characters are given equal weight to examine overall variation patterns to create the clusters which can in turn be used in the classification. According to the PhSC, species can be observed to form distinct clusters based on resemblance. Phenetic similarity can reveal functionality (Sneath and Sokal, 1973). This study placed emphasis on morphology in attempting to answer the questions of circumscription of the species.

1.3 Motivation for the study

It has been noted that almost every character in *Barleria* is variable, some characters being as variable within the genus as they are across the entire Acanthaceae (Balkwill, 1993; Balkwill and Balkwill, 1996; 1997; 1998; Darbyshire, 2008; Darbyshire and Ndangalasi, 2008). However not all combinations of characters are represented in one geographic area (Balkwill, 1993; Balkwill and Balkwill, 1996; 1997; 1998). In southern Africa, the last revision of the genus *Barleria* was carried out by Obermeijer in 1933. However, the genus shows large morphological diversity (Balkwill, 1993; Balkwill and Balkwill, 1996; Balkwill, 1997; 1998), much of which needs to be clearly understood and defined. This is demonstrated by the continued recognition of new species within *Barleria* (Balkwill *et al.*, 1990; 1992; Obermeijer, 1961). However, 75 years have elapsed since the last revision was carried out on the genus in southern Africa. This brings out the necessity for a formal revision and it is in view of this that this study is being undertaken, given that unnamed variation is difficult to account for and can go extinct unnoticed (Humphries, 2006; Richard and Evans, 2006). Since further examination of the extent of similarities and differences in variation of characters can lead to insights into the implication of gaps and overlaps in morphology, this study will contribute to the knowledge and understanding of the circumscription of the species and their geographic ranges.

1.4 Aim

The aim of this study is to examine the morphological variation exhibited within the five study species and establish a classification to include the observed morphological gaps and overlaps within the five species / species complexes.

1.5 Objectives and key questions of the research

The objectives of the research are:

1. To determine whether the observed variation within the five species is continuous or discrete.

Can distinct groups be identified within the species / species complexes?

What are the taxonomic implications of the gaps and / or overlaps in morphology?

2. To examine the morphology and flower colour variation within the species / species complexes in relation to distribution.

Are there clear trends in flower colour and flowering times within the species / species complexes that can suggest gaps or overlaps in gene flow?

How does morphology vary along the distribution gradient?

3. To determine whether there is cryptic variation.

Are there cryptic characters that are homogeneous within clusters?

Does the macro- and micro-morphology provide similar associations?

4. To write descriptions for species / subspecies and / or any other hierarchical rank for the taxa in this study.

5. To prepare an identification key for the resulting species within section *Barleria* Group 1.

1.6 Approach

This study was largely based on herbarium specimens held at B, BOL, C, GRA, J, K, M, NBG, NU, PRE, STEU and WIND (abbreviations for the herbaria are according to the standard codes of Holmgren *et al*. (1990)). Characters were selected to define the putative taxa and tentative character lists were made. Here a preliminary survey was done with herbarium specimens at the C.E. Moss Herbarium (J) and the National Herbarium (PRE) in Pretoria to assess the variation and distribution of the species / species complexes. Preliminary mapping was also done using approximately 160 specimens to assess the distribution of the taxa in the study. Results from this preliminary work were similar to those of an earlier study Nyirenda (2008) which had revealed that *B. irritans* (with a sample size of seven specimens), could be composed of two separate entities. This not only necessitated the need for an increased sample size but also the inclusion of *B. irritans* as part of the study group (in addition to *B. pungens* and *B. rigida*). The two other species (as earlier alluded to) were included based on morphological similarity (Obermeijer, 1933). *Barleria elegans* S. Moore, was here selected as the standard group for calibrating the character list to be used in the analyses. In addition to the tendency to inhabit well drained areas, the taxa under study were observed to exhibit either narrow or wide geographic distribution ranges (Obermeijer, 1933; pers. obs.). Examination of morphology in relation to distribution will give insights into the observed variation.

1.7 Thesis structure

The first chapter presents background information for the study group by referring to previous taxonomic studies which have been able to split the large genus into smaller logical groups. It also outlines the motivation, aim and research questions. The subsequent chapters have each focused on a particular topic. Details of the methods used to study particular themes and to analyse data sets have been included in the relevant chapters. The second chapter considers the use of morphological characters to phenetically analyse variation and place it into consistent groups. Chapter 3 includes the discussion of the distribution of morphology and flower colours. Chapter 4 discusses cryptic characters that can distinguish the groups. Chapter 5, the concluding chapter, includes formal taxonomy where new species / varieties descriptions and updates to the existing ones are noted. It also includes a key to the African species within *Barleria* section *Barleria* Group 1.

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CHAPTER 2

2.0 DETERMINATION OF MORPHOLOGICAL GAPS AND / OR CONTINUITIES WITHIN FIVE MORPHOLOGICALLY SIMILAR SPECIES OF *BARLERIA* (SECT. *BARLERIA* GROUP 1) USING QUALITATIVE AND QUANTITATIVE CHARACTERS

2.1 Abstract

The morphological variation exhibited within five species of *Barleria* (*B. bechuanensis* C.B. Clarke, *B. irritans* Nees, *B. jubata* S. Moore, *B. pungens* L.f. and *B. rigida* Nees) was studied to establish whether it was discrete or continuous. Morphological characters were used in Cluster analysis to impose a hierarchical non-overlapping association among OTUs (operational taxonomic units) and ordination to establish whether the variation was discrete or continuous. Discrete characters were determined from quantitative morphological data using box and whisker plots. Locality information for the OTUs from herbarium sheets was used to generate maps to illustrate geographic distribution of taxa. Cluster analysis and ordination demonstrated that there was discrete variation within *Barleria bechuanensis*, *B. irritans* and *B. jubata* which each split into two distinct clusters, whereas *B. pungens* and *B. rigida* exhibited both continuous and discontinuous variation as they separated into clusters with clear gaps and clusters that were not clearly differentiated. Box and whisker plots illustrated that there were few discrete quantitative characters within and between the species. Since clear morphological gaps between clusters can be deduced to represent breaks in gene flow, the distinct clusters were recognised at species level. Where clusters were not clearly differentiated but could be distinguished by at least one discrete morphological character varieties were recognised.

Key words: *Barleria*, cluster analysis, continuities, discrete, morphological characters, ordination

2.2 Introduction

2.2.1 The study group

Barleria L. is a large and widespread pan-tropical genus which comprises approximately 300 species and is currently divided into two subgenera (*Barleria* and *Prionitis* (Nees) C.B. Clarke) (Balkwill and Balkwill, 1997; 1998). Subgenus *Barleria* is further subdivided into two sections; *Barleria* and *Chrysothrix* M. Balkwill (Balkwill and Balkwill 1997). The other five sections (*Cavirostrata* M. Balkwill, *Stellatohirta* M. Balkwill, *Fissimura* M. Balkwill, *Somalia* (Oliv.) Lindau and *Prionitis* (Nees) Lindau) belong to subgenus *Prionitis.* This subdivision was done based on phylogeny using morphological characters such as the presence or absence of sunken glands, pubescence, presence or absence of a beak in the capsule, number of seeds and inflorescence architecture among other characters (Balkwill and Balkwill 1997; 1998). Section *Barleria* is a polymorphic monophyletic group comprising 78 species which Balkwill (1993) informally placed into five smaller subgroups based on variation of selected morphological characters, and Group 1 (consisting of 37 species) accounts for almost half of the species placed within section *Barleria*. The five species in this study, *Barleria bechuanensis* C.B. Clarke, *B. irritans* Nees, *B. jubata* S. Moore, *B. pungens* L.f. and *B. rigida* Nees belong to section *Barleria* Group 1 (Balkwill and Balkwill, 1994; 1997). Even though section *Barleria* is a monophyletic group (Balkwill and Balkwill, 1997), it is not obvious that the subgroups are natural as morphological similarity does not necessarily infer phyletic relatedness (Andrus *et al.*, 2004). However, morphological gaps have been observed to represent gaps in gene flow (Ereshefsky, 2001; Briggs and Walters, 1997; Jones and Luchsinger, 1979).

Although growth habit may be influenced by habitat in that specimens collected from open places are likely to exhibit erect growth whereas those growing among other vegetation tend to display a decumbent habit, plasticity in response to identical environmental constraints is likely to be species specific (Strand and Weisner, 2004). Environmental factors tend to vary with spatial scale and natural communities respond to multiple factors rather than single environmental variables (Burke, 2002; Borcard *et al.*, 1992). Because various environments trigger divergent responses in species, it has been noted that widespread taxa tend to have ecotypes as an adaptation to the wide range of environmental conditions (Burke, 2002; Heywood, 1986; Kniskern and Rausher, 2006; Samis and Eckert, 2009; Sebola and Balkwill, 2009; Strand and Weisner, 2004). Ecotypes however, have a tendency to overlap in many characters even where there is geographic isolation (Sebola and Balkwill, 2009). It is likely that *B. rigida*, a widespread species, could consist of more than one entity.

Characters such as lobing, margin serration, pubescence and shape are known to be conservative (Brummit and Seyani, 1978; Otieno *et al.*, 2006); variation in such characters is likely to provide predictable indications of taxonomic importance. Morphological variation within the five species in this study has been observed to include leaf size and shape, leaf margin, leaf texture and leaf indumentum. Other variation includes inflorescence structure, bracteole size and shape, bracteole texture and bracteole indumentum; calyx lobe size and shape, calyx lobe texture, calyx lobe indumentum, and corolla colour among others. Flowers are observed to vary from solitary to clustered in compound cymes that further differ in the degree of congestion with flowers borne in alternate (or sometimes opposite) axils. Pubescence on the other hand is observed to vary in intensity with maturity within some members of the study group or disappearing with maturity in others. This study will, therefore, only examine similarity or dissimilarity of morphological characters and consequently the gaps and overlaps in morphology will be used to determine the specific circumscriptions within the study group.

2.2.2 Problem statement

Preliminary work (Nyirenda, 2008) in this group demonstrated that *B. pungens* could possibly be split into three distinct taxonomic groups while *B. rigida* possibly comprised five entities. *Barleria rigida*, being a widespread taxon that covers more than one latitudinal zone, could possess ecotypes. *Barleria irritans* too, which was used as the standard group to calibrate the character list for cluster analysis was observed not only to be nested within *B. pungens* but also to split into two distinct groups (Nyirenda, 2008). However, the sample size was not big enough to make meaningful conclusions. This necessitated the need to examine the morphological discreteness and overlaps within the study group to understand the implications thereof. Since various species have different genetic constitutions, environmental constraints could bring about different expressions in morphology resulting in morphological gaps (Strand and Weisner, 2004). As morphological gaps could represent gaps in gene flow, depending on the morphological gaps or dissimilarities observed; phenetic clusters can be recognised at various taxonomic levels (Ensermu, 1994). Given that unnamed variation is difficult to account for, and can go extinct unnoticed (Humphries, 2006; Richard and Evans, 2006), this study is important because it will clarify the taxonomic implication of the variation observed within the study group.

2.2.3 Knowledge gap

It has been observed that almost every character in *Barleria* is variable, some characters being as variable within the genus as they are across the entire Acanthaceae (Balkwill, 1993; Balkwill and Balkwill, 1996; Balkwill & Balkwill 1997; 1998; Darbyshire, 2008; Darbyshire and Ndangalasi, 2008). In addition, not all combinations of characters are represented in one geographic area (Balkwill, 1993; Balkwill and Balkwill, 1996; Balkwill & Balkwill 1997; 1998). Nonetheless in southern Africa, the last revision of the genus *Barleria* was carried out by Obermeijer (1933). More than 78 years have elapsed since the last revision was carried out within this genus in southern Africa. However, new species have continued to be described within *Barleria* (Balkwill *et al.,* 1990; 1992; Obermeijer, 1961). Therefore, further examination of the extent of similarities and differences in the variation of morphological characters can lead to insights into the implication of the gaps and overlaps in morphology. This study will, therefore, contribute to the knowledge and understanding of the circumscription of the species and their geographic ranges.

2.2.4 Purpose statement

Since species are known to exist in an environment where they interact with many factors including their habitat, habitat factors can bring about discontinuities in morphology (Cron *et al.*, 2007; Ereshefsky, 2001). Habitat heterogeneity can lead to morphological variation because of the interaction between the genome of a plant and the conditions under which it grows (Masćo *et al.,* 2004). Where variation is environmentally induced i.e. occurs in response to environmental constraints morphological overlaps are observed in a number of characters that are not easily prone to plasticity. However, plants are known to respond to a combination of factors rather than a single variable (Burke, 2002). Variation, however, could also be genetically determined, or developmental (Makholela, 2003; McLellan, 1990). When morphological gaps are clearly distinguishable, the distinct clusters can be recognised at various levels depending on the morphological discontinuities (Cron *et al.*, 2007;

Ereshefsky, 2001; Lewis, 1972; Masćo *et al.,* 2004; Sebola and Balkwill, 2009; Stebbins, 1967; Sneath and Sokal, 1973; Sokal and Sneath, 1963). Developmental variation is brought about by changes that occur during plant maturation (McLellan, 1990). In a study based on herbarium specimens the interference of developmental variation can be avoided by examining only mature specimens.

Morphology can also be influenced by geographic separation, in which case gene flow is restricted. Similarity therefore, is maintained with the nearest population from which they originated and continue to maintain gene flow (Masćo *et al.,* 2004). Therefore, when geographic distance creates an impermeable isolating barrier against gene flow (Aizen *et al.,* 2002; Harder and Barrett, 2006), morphological divergence can occur which would then result in speciation due to the break in gene flow. When there is geographic disjunction in the distribution of clusters coupled with some degree of discreteness in several other characters, this can therefore result in the recognition of species and / or subspecies (Balkwill, *et al.,* 1992; Darbyshire, 2009; Sebola and Balkwill, 2009). Phenetic similarity can reveal functionality or adaptation (Sneath and Sokal, 1973).

When variation is observed in three or more diagnostic characters, specific recognition is accorded whereas dissimilarity in two characters coupled with distribution is recognised at subspecific level as subspecies are also considered as regional races (Clausen *et al.,* 1941). Variation in one distinct character with or without geographical isolation can be named at varietal level (Brysting and Elven, 2000). This study will seek to utilise habitat information on specimen labels together with distribution and morphological information to define the clusters. The phenetic and morphological species concepts will be employed to identify and interpret the implication of the morphological gaps and or continuities as they are observed within the species / species complexes. Therefore morphological information will be employed in identifying and defining new species, subspecies or varieties.

2.3 Materials and Methods

This study was based on herbarium specimens held at B, BOL, C, GRA, J, K, M, MO, NBG, NU, PRE, STEU and WIND and the abbreviations for the herbaria are according to the standard codes of Holmgren *et al.* (1990). A total of 153 OTUs (specimens) were examined and each gathering (including duplicates from a number of herbaria) constituted a single OTU and comparison of multiple herbarium sheets was done to assess variation if any. However, for herbarium sheets that had specimens by more than one collector, each specimen by a different collector was considered as a separate OTU. The recording of distribution data was based on Edwards and Leistner (1971) and is given in Appendix VIII.

2.3.1 Sampling strategy

A character is any attribute of the group(s), individual species / species complex or specimen(s). This study employed characters that have not been critically examined before (such as the stem shape, prominence of lateral veins on the leaf blade, petiole length, number of parallel veins on the bracteole, bracteole base shape, length of bracteole apex, nature of bracteole margin and calyx lobe margin) which, however, exhibited variation among the species / species complexes and were thus considered to possibly be of taxonomic importance. Characters that were used in previous studies (as many as possible) were also included (Moore, 1907; Obermeijer, 1933). Characters that were variable within a specimen were not included in the analyses but only taken note of, and only those that are stable within a specimen were used. Other characters also excluded from the analyses were those that were not present on all specimens creating a lot of missing information in the data matrices. In this study the term bracteole refers to a modified leaf at the base of an inflorescence in single flowers and at the base of each flower in clustered flowers of a cyme (Figure 2.1a & 2.1b respectively) (Balkwill, 1993; Balkwill and Balkwill, 1997).

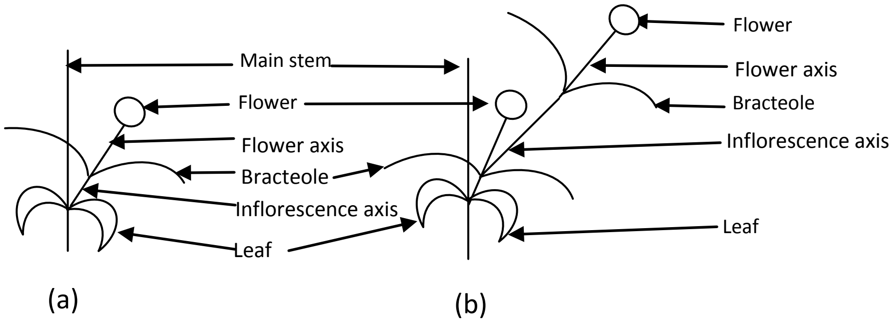

Figure 2.1. Basic inflorescence structure within the study group. *a*) solitary flower; *b*) clustered flowers. The bracteoles of a pair could be of equal or unequal lengths. Adapted from Balkwill and Balkwill (1997).

2.3.2 Selection of operational taxonomic units (OTUs)

Putative groups were established within the species / species complexes based on the observed morphological variation. OTUs were selected from the *a priori* taxa (putative groups) to reflect the distribution range for each species / species complex as far as possible. The *Barleria bechuanenesis, B. irritans* and *B. pungens* complexes were each divided into two putative taxa, while the *B. rigida* complex on the other hand was divided into three putative groups. *Barleria jubata* was not split due to limited material as there were only very few specimens at the C.E. Moss Herbarium and from among the loan specimens that were received. The study therefore comprised a total of ten putative taxa designated as: BE, BB, BI, BU, BP, PU, BR, LB, IL and JU. *Barleria elegans* which was used as a standard group was designated as EL. Type specimens (or scanned images thereof) where possible were requested from various herbaria where they are reported to be kept while others were obtained from Aluka and used to identify to which groups the names belong. The characters defining the putative taxa and preliminary character list are indicated in Appendices I and II respectively.

2.3.3 Examination of material and data recording

The number of specimens measured from putative taxa depended on the availability of specimens. Measurements were taken only from mature specimens to allow for standardised measurements and avoid confusion resulting from developmental plasticity caused by immature samples (Otieno *et al.*, 2006). For all the characters under consideration, a minimum of three replicate measurements and / or counts was taken for each OTU (Otieno *et al.*, 2006). In the case of leaves and calyx lobes the largest (and most mature) on the specimen were considered, whereas for bracteoles only the ones at the base of each inflorescence were measured and all measurements were taken in millimetres (mm) unless otherwise stated. Some of the qualitative characters were scored as binary characters while others were designated as multistate ordered characters to avoid weighting. The measurements and counts, together with observations for each qualitative character were recorded in a data matrix (Appendix 2.8.5) and where necessary photographs were also taken. Measurements and photographs were taken using a Zeiss stereo microscope (Discovery V12). Means were calculated from the replicate measurements. Although ratios were also used in the analyses, they are not given in the raw data matrix.

2.3.4 Data analysis

Matrices of qualitative, quantitative and combined qualitative and quantitative morphological characters were generated. However, not all character measurements, or counts were directly used in the analyses as some were only used to calculate ratios. Since vegetative organs are prone to plasticity, ratios were used to eliminate size differences that result from environmental modifications because ratios tend to be more stable (Watson and Estes, 1990). Where the ratio of length to width measurements was calculated and used as a character, only one, either length or width, was used with the ratio. Of the 82 characters (qualitative and quantitative) examined, only 50 (21 quantitative and 29 qualitative) characters were used in the various matrices.

Means and standard deviations of quantitative characters for all the OTUs in each putative taxon were used to make box and whisker plots to compare the degree of overlap or continuities among the quantitative characters. Characters illustrating separation or the significance of individual character differences among OTUs of the ten putative groups were identified (Figure 2.2 (i) to 2.2 (vi)). Prior to cluster analysis (CA), principal coordinate's analysis (PCO) and principal components analysis (PCA), matrices were standardised to remove the effects of characters with large variances. CA by means of UPGMA (unweighted pair group method of arithmetic averages) using correlation and average taxonomic distance coefficient as dissimilarity coefficients was performed to compute dissimilarity distances (and assess phenotypic similarity) among OTUs using NTSYS-pc version 2.2 (Rohlf, 2005). A cophenetic correlation coefficient (r) was computed from the SAHN algorithm using COPH and MXCOMP. Cophenetic correlation coefficients (r) for the resultant phenograms were used to estimate how well the phenograms fitted the data and only phenograms with an ‘r’ value greater than 0.8 were considered (Cron *et al.,* 2007; Dunn and Everitt, 1982; Gilmartin and Harvey, 1976; Lessig, 1972; Peres-Nato and Jackson, 2001). Four of the multi-state characters (5, 26, 27 & 36) involving indumentum type and intensity were coded as binary characters to represent the presence or absence of indumentum, while two quantitative characters (13 & 16) were coded as binary characters to give different size ranges, to facilitate separate analyses for the *B. pungens* and *B. rigida* complexes. Separate matrices were generated (Appendix 2.8.5b & c) and CA was separately performed on the *B. pungens* and *B. rigida* complexes (independent of the other species / species complexes and the standard taxon).

PCA was used to extract Eigen values of characters influencing the distribution of OTUs along the first three principal component axes from matrices standardised by characters. Characters with Eigen values ≥ 0.6 were further used in CA to compare the clusters (Brysting and Elven, 2000; Cron *et al.*, 2007; Işik, 1983; Lessig, 1972; Otieno *et al.,* 2006; Sebola and Balkwill, 2006). PCO was performed on standardised matrices (with combined qualitative and quantitative characters) by computing and double-centering distances across OTUs and plotting the results in two- and three-dimensional space. Eigen vectors were extracted demonstrating how variation was distributed along the coordinate axes. Matrices with only quantitative data were separately standardised and Eigen vectors were extracted which were also projected in two- and three-dimensional plots. Ordination was performed to demonstrate the tightness of and separation amongst the clusters in respect to the positions of OTUs in multivariate space.

2.4 Results

2.4.1 Variation

Although box and whisker plots (Figures 2.2 (i) – (vi)) demonstrated that a number of quantitative characters exhibit overlap within and between the species / species complexes, some pattern of character dissimilarity was also observed. Characters identified as useful were leaf length, leaf width, petiole length, length of leaf apex spine, length of leaf margin spine, ratio of leaf length to leaf width, ratio of leaf length to distance to the widest point from the base, ratio of leaf length to petiole length, length of bracteole apex spine, the total number of teeth on the upper half of the bracteole margin, ratio of bracteole length to bracteole width, ratio of bracteole length to the total number of teeth on the bracteole margin, length of apex spine on posticous calyx lobe, the total number of teeth on the upper half of the margin of the posticous calyx lobe, ratio of lobe length to lobe width of the posticous calyx lobe, ratio of length of posticous calyx lobe to total number of teeth on lobe margin, the total number of teeth on the upper half of the margin of the anticous calyx lobe, ratio of lobe length to lobe width of anticous calyx lobe and ratio of length of anticous calyx lobe to total number of teeth on calyx lobe margin.

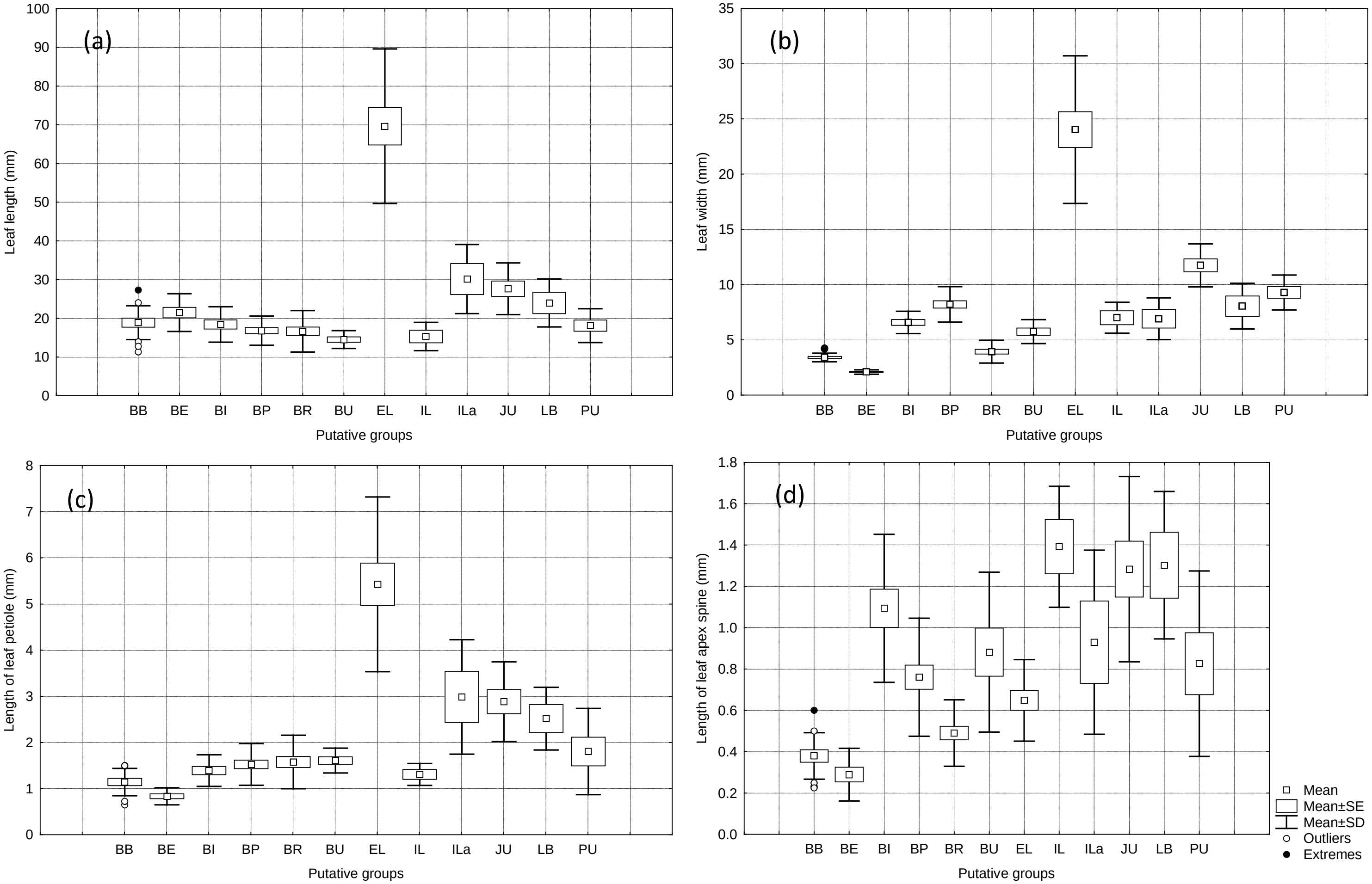

Figure 2.2 (i). Variation in four quantitative characters *a* – *d* between the putative groups. Characters: *a* = leaf length (mm), *b* = leaf width (mm), *c* = petiole length (mm), *d* = length of leaf apex spine (mm). BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*; BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida.* Abbreviations details are as outlined in Appendix I.

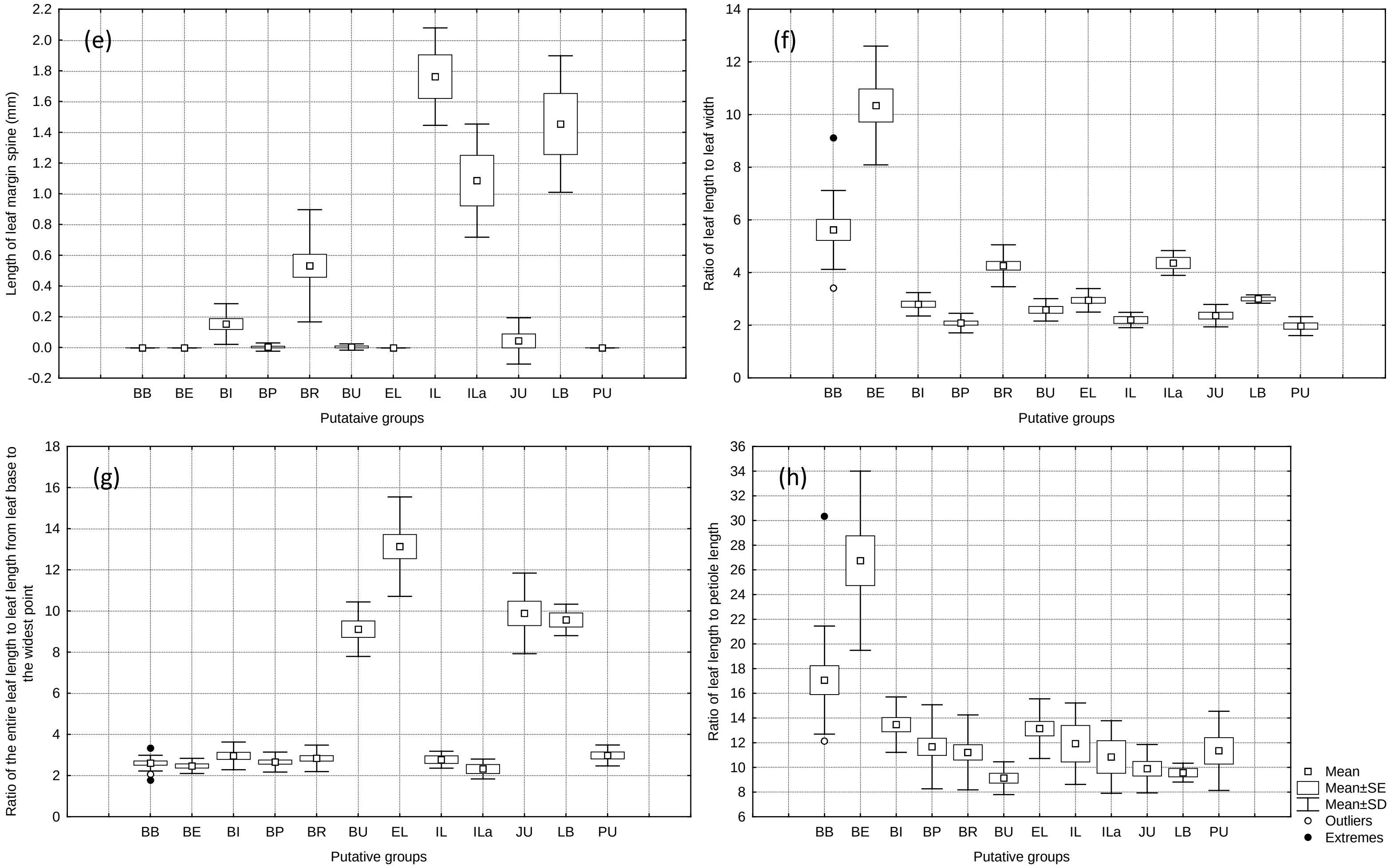

Figure 2. 2 (ii). Variation in four quantitative characters *e* – *h* between the putative groups. Characters: *e* = length of leaf marginal spine (mm), *f* = ratio of leaf length to leaf width, *g* = ratio of leaf length to length up to widest point from the base, *h* = ratio of leaf length to the petiole length. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*; BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida*. Abbreviations details are as outlined in Appendix I.

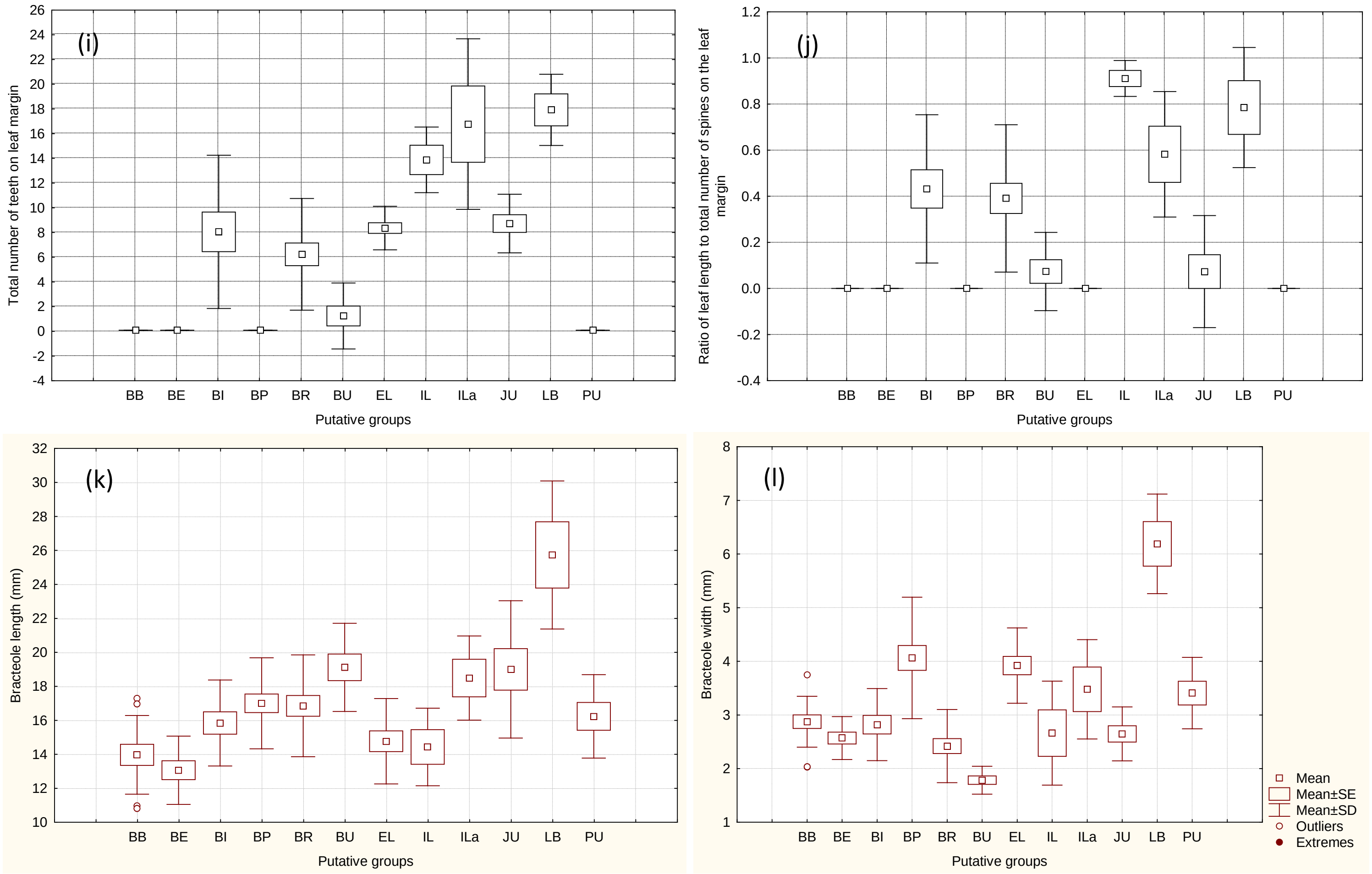

Figure 2.2 (iii). Variation in four quantitative characters *i* – *l* between the putative groups. Characters: *i* = total number of spines / teeth on leaf margin, *j* = ratio of leaf length to the total number of spines / teeth on leaf margin, *k* = bracteole length, *l* = bracteole width. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*; BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida.* Abbreviations details are as outlined in Appendix I.

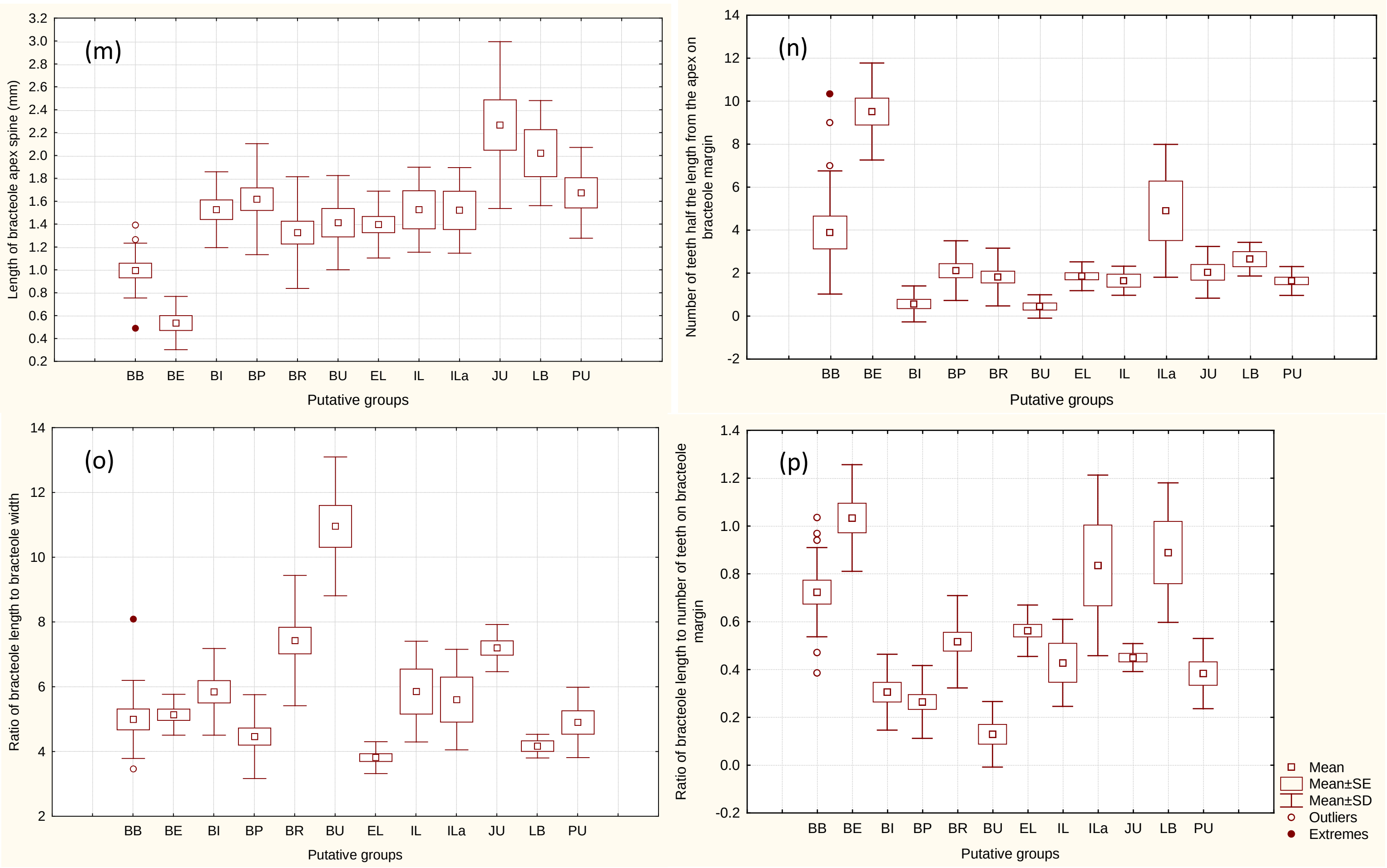

Figure 2. 2 (iv). Variation in four quantitative characters *m* – *p* between the putative groups. Characters: *m* = length of bracteole apex spine, *n* = total number of teeth on the upper half of the bracteole margin, *o* = ratio of bracteole length to bracteole width, *p* = ratio of bracteole length to the total number of teeth on bracteole margin. BB, BE = putative taxa from B. bechuanensis; BI, BU = putative taxa from B. irritans; EL = B. elegans (standard); JU = B. jubata; BP, PU = putative taxa from B. pungens; BR, IL, ILa, LB = putative taxa from B. rigida. Abbreviations details are as outlined in Appendix I.

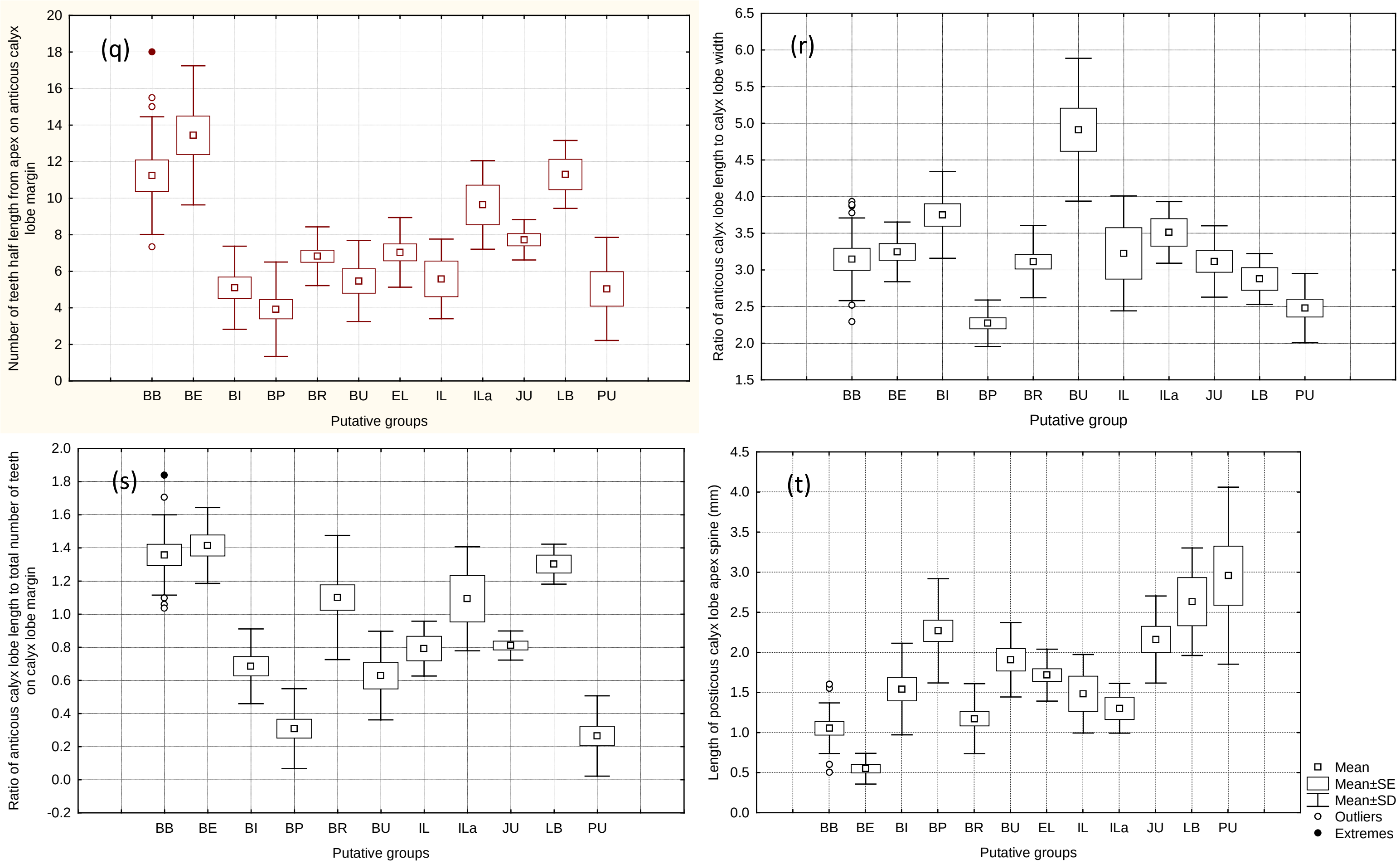

Figure 2. 2 (v). Variation in four quantitative characters *q* – *t* between the putative groups. Characters: *q* = total number of teeth on the upper half of the anticous calyx lobe margin, *r* = ratio of length of anticous calyx lobe to calyx lobe width, *s* = ratio of length of anticous calyx lobe to total number of teeth on calyx lobe margin, *t* = length of apex spine on posticous calyx lobe. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*; BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida*. Abbreviations details are as outlined in Appendix I.

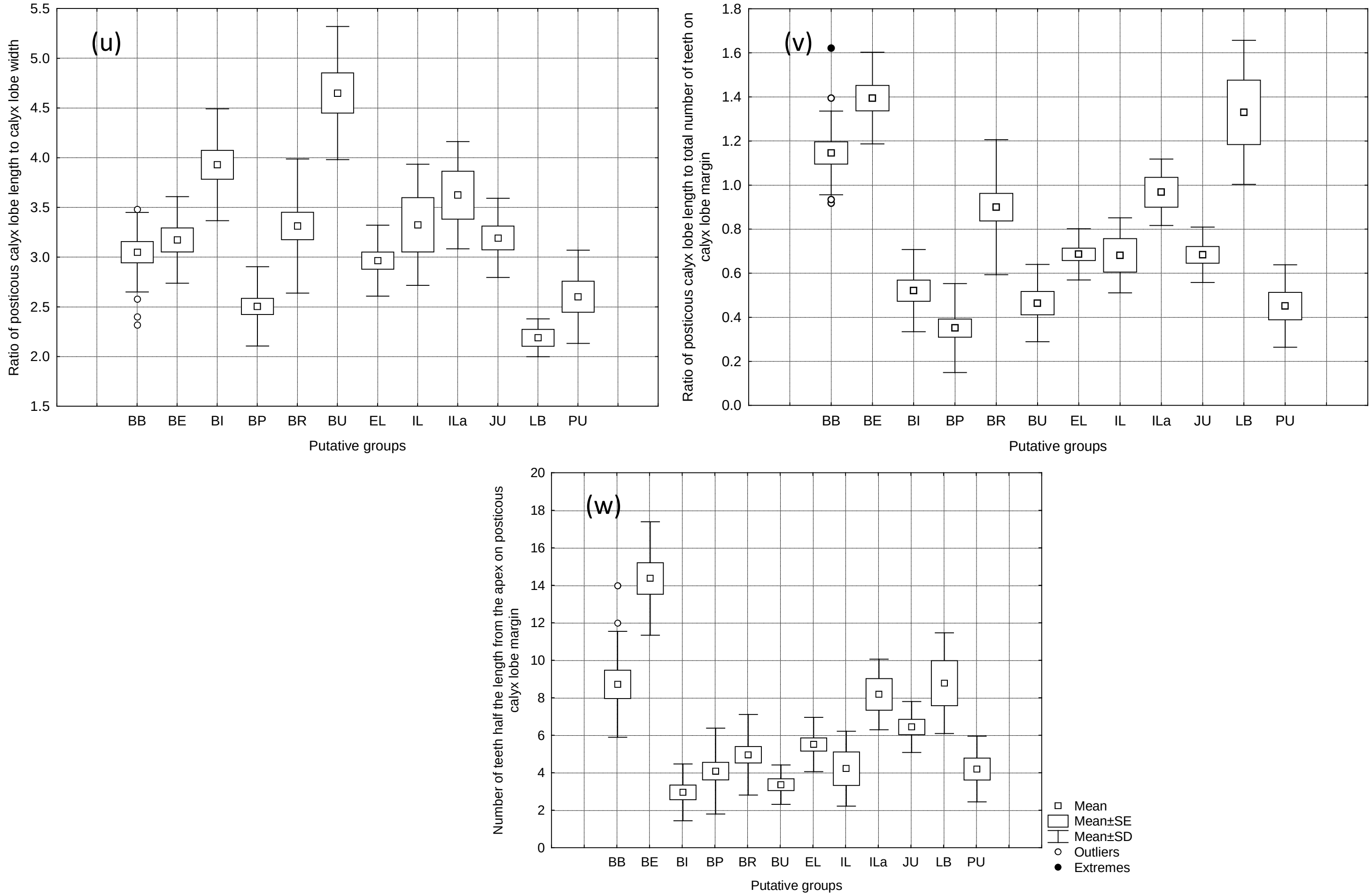

Figure 2.2 (vi). Variation in three quantitative characters *u* – *w* between the putative groups. Characters: *u* = ratio of length of posticous calyx lobe to the total number of teeth on the calyx lobe margin, *v* = the ratio of length of posticous calyx lobe to calyx lobe width, *w* = total number of teeth on posticous calyx lobe margin on the upper half from the apex. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*; BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida*. Abbreviations details are as outlined in Appendix I.

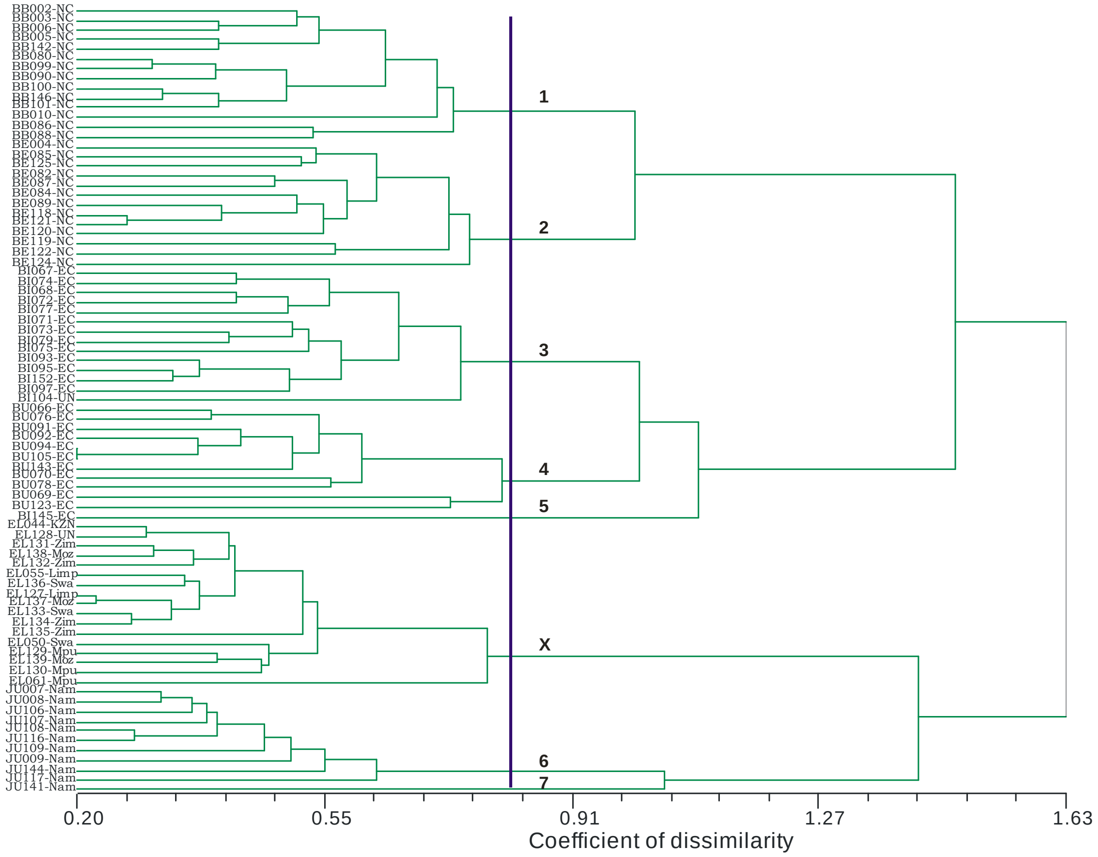

Figure 2.3a. Clustering of OTUs from a combined matrix of 35 qualitative and quantitative morphological characters with Eigen values > 0.6, r = 0.9006. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*. A phenon line is drawn at about 0.8 as the coefficient of dissimilarity just below where OTUs of the standard taxon, *B. elegans*, begin to cluster. Numbers 1 – 7 denote the distinct clusters observed and can be distinguished at species level where 5 is the standard species, 5 is an OTU distinct from the rest of BI but phenetically closer to the BU cluster and is considered as forming a distinct cluster, cluster 7 may also be referred to as JU2. 1 = *sp.1*, 2 = *sp. 2*, 3 = *sp. 3*, 4 = *sp. 4*, 5 = *sp. 5*, 6 = *sp. 6*, 7 = *sp.7*, X = *B. elegans*. Abbreviations details are as outlined in Appendix I and IV.

Leaf width and the ratio of the leaf length to the leaf width showed no overlap between the two clusters of the *B. bechuanensis* complex (Figure 2.2 (i) b and 2.2 (ii) f). However, there is overlap in the ratio of leaf length to leaf width, the ratio of leaf length to petiole length and the number of teeth on the upper half of the bracteole margin as a result of extreme values and outliers (Figures 2.2. (ii) f, 2.2 (ii) h and 2.2 (iv) n respectively). The groups within the *B. irritans* complex could be separated by the length of the teeth on the leaf margin, the ratio of the leaf length to the length from the base to the widest point, the ratio of leaf length to petiole length, bracteole width and the ratio of bracteole length to bracteole width (Figure 2.2 (ii) e, 2.2 (ii) g, 2.2 (ii) h, 2.2 (iii) l and 2.2 (iv) o). The overlap in bracteole width and ratio of leaf length to the petiole length is brought about by extreme values (Figures 2.2 (iii) l and 2.2 (ii) h).

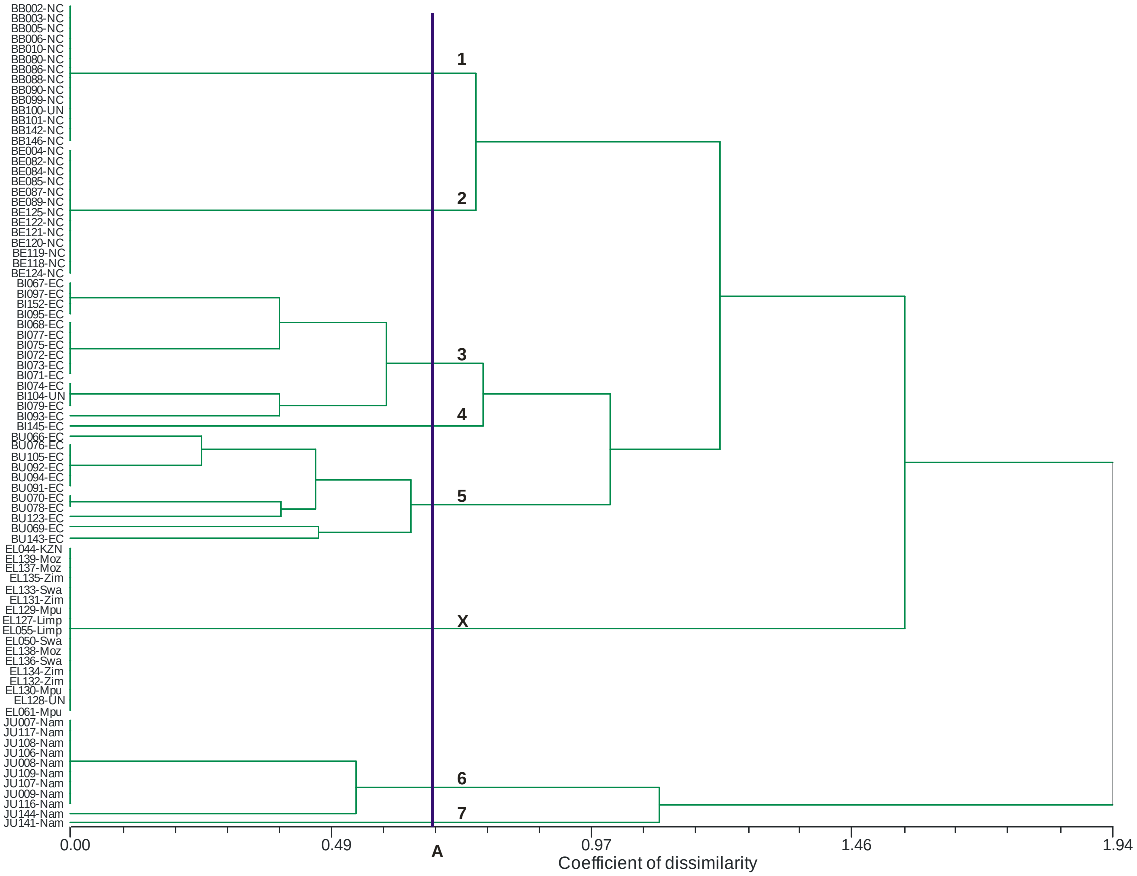

Figure 2.3b. Clustering of OTUs from a matrix of 26 qualitative morphological characters with eigen values > 0.6, r = 0.9814. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*. A phenon line is drawn at ‘A’, between 1.6 (where OTUs of the standard taxon are observed to differentiate from the rest of the study group) and 0 (where the OTUs of the standard taxon form a cluster). The qualitative characters bringing about variation among the study group do not show within group variation among the standard taxon. A = an approximate coefficient of dissimilarity estimated at 0.7 where a phenon line is drawn to denote the degree of dissimilarity (to account for the amount of variance in the sample). Numbers 1 – 7 denote the distinct clusters observed that may be distinguished at species level where X is the standard species, cluster 4 is an OTU showing clear distinction from the rest of the BI cluster, cluster 7 may also be referred to as JU2. 1 = *sp.1*, 2 = *sp.* 2, 3 = *sp. 3*, 4 = *sp.* 4, 5 = *sp. 5*, 6 = *sp. 6*, 7 = *sp. 7*, X = *B. elegans*. OTU abbreviations details are as outlined in Appendix I and IV.

Within the *B. rigida* complex, leaf width, the length of leaf apex spine, the length of the leaf margin spine, the ratio of leaf length to leaf width, the ratio of leaf length to length up to the widest point from the base, the total number of teeth on the leaf margin, bracteole length, bracteole width, ratio of bracteole length to bracteole width, the number of teeth half the length from the apex on anticous calyx lobe margin, the length of apex spine on posticous calyx lobe, and the ratio of posticous calyx lobe length to calyx lobe width can be used to clearly differentiate between BR and LB (Figures 2.2 (i) b, 2.2 (i) d, 2.2 (ii) e, 2.2 (ii) f, 2.2 (ii) g, 2.2 (iii) i, 2.2 (iii) k, 2.2 (iii) l, 2.2 (iv) o, 2.2 (v) q, 2.2 (v) t and 2.2 (vi) u). Whereas BR and IL can be separated using the leaf width, the length of the leaf apex spine, the length of leaf margin spines, the ratio of the entire leaf length to length up to widest point from the leaf base, the total number of teeth on the leaf margin and the ratio of the leaf length to the total number of spines on the leaf margin (Figures 2.2 (i) b, 2.2 (i) d, 2.2 (ii) e, 2.2 (ii) f, and 2.2 (iii) j), there is an overlap in extreme values in the number of spines on the leaf margin (Figure 2.2 (iii) i).

IL and ILa (Figure 2.4, clusters 1b and 1c) vary by the leaf length, the length of the leaf petiole, the length of the leaf margin spine, the ratio of the leaf length to leaf width, the ratio of leaf length to the total number of spines on the leaf margin and the number of teeth half the length from the apex on the posticous calyx lobe margin (Figures 2.2 (i) a, 2.12 (i) c, 2.2 (ii) e, 2.2 (ii) f, 2.2 (iii) j and 2.2 (vi) w). ILa and LB on the other hand can be separated by the ratio of leaf length to the leaf width, the ratio of the entire leaf length to the leaf length from the leaf base to the widest point, the bracteole width, the length of the posticous calyx lobe apex spine and the ratio of the posticous calyx lobe length to the calyx lobe width (Figures 2.2 (ii) f, 2.2 (ii) g, 2.2 (iii) l, 2.2 (v) t and 2.2 (vi) u). The LB and IL clusters differ by the leaf length, the length of the leaf petiole, the ratio of the leaf length to the leaf width, the ratio of the entire leaf length to the leaf length from the leaf base to the widest point, bracteole length, bracteole width, the ratio of the bracteole length to the number of teeth on the bracteole margin, the number of teeth half the length from the apex on the anticous calyx lobe margin, the ratio of the anticous calyx lobe length to the total number of teeth on the calyx lobe margin, the length of the posticous calyx lobe apex spine, the ratio of the posticous calyx lobe length to the calyx lobe width, the ratio of the posticous calyx lobe length to the total number of teeth on the calyx lobe margin and the number of teeth half the length from the base on the posticous calyx lobe margin (Figures 2.2 (i) a, 2.2 (i) c, 2.2 (ii) f, 2.2 (ii) g, 2.2 (iii) k, 2.2 (iii) l, 2.2 (iv) p, 2.2 (v) q, 2.2 (v) s, 2.2 (v) t, 2.2 (vi) u, 2.2 (vi) v and 12.2 (vi) w).

BR and ILa overlap in almost all of their quantitative characters differing only by leaf length and the total number of spines on the leaf margin (Figures 2.2 (i) a and 2.2 (iii) i) which moreover demonstrates slight overlap. BR has deeply undulate leaf margins while ILa has sinuate leaf margins. Whereas BR & LB; BR & IL; LB & IL; LB & ILa and IL & ILa differ in both quantitative and qualitative characters, BR & ILa mainly differ in qualitative characters. It was also observed that OTUs from IL, ILa and LB have leaf margins that are conspicuously spiny and sinuate while OTUs from BR have leaf margins that are shallowly undulate to deeply so with small spines or scanty teeth to almost entire. The ILa cluster appears to be an intermediate group between BR and IL. BP and PU exhibited overlaps in almost all the quantitative characters revealing very slight dissimilarity but can be differentiated by qualitative characters.

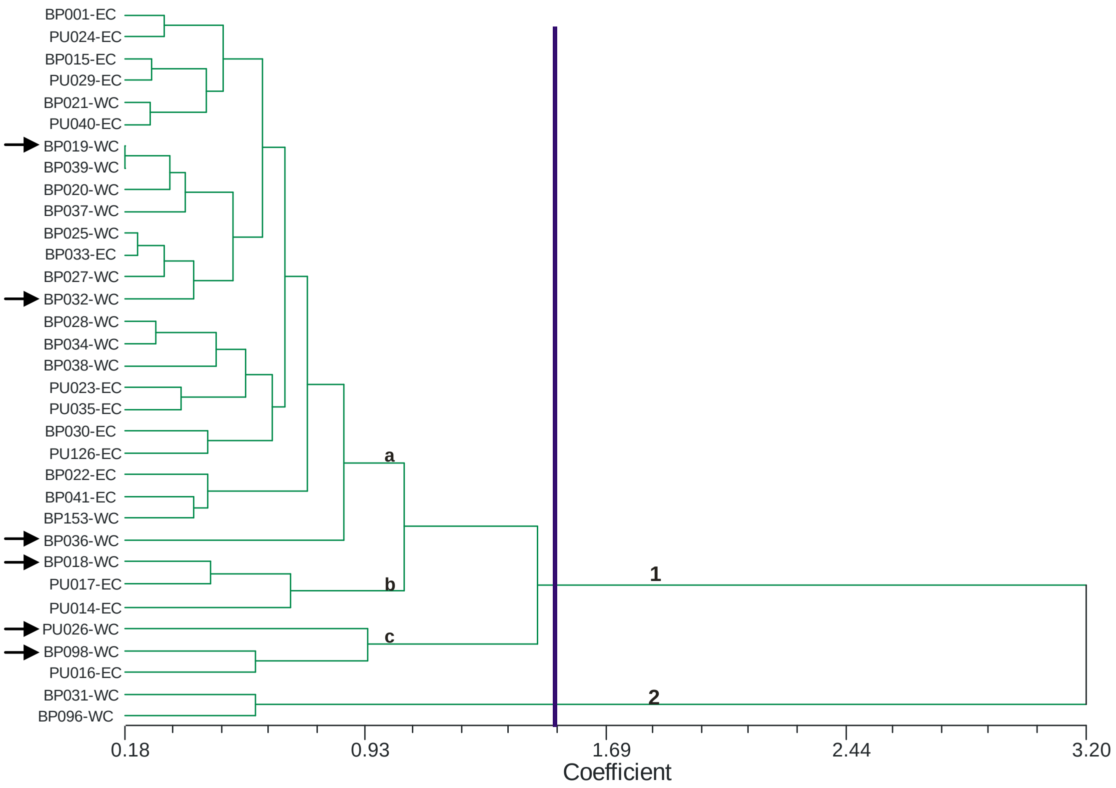

Figure 2.4a. Clustering of OTUs within the *B. pungens* complex from a combined matrix of 13 qualitative and quantitative characters, r = 0.9756. BP = *B. pungens* with green leaf margins; PU = *B. pungens* with white leaf margins. A phenon line is drawn at 1.5 just below where the first cluster is formed and showing great dissimilarity between the two clusters which form distinguishable taxa. Numbers 1 – 2 denote the major clusters that can be delimited as consistent clusters at species level. Sub-clusters ‘a’ to ‘c’ of cluster 1 have all been observed to possess OTUs with or without white leaf or bracteole margins. (Intermediate OTUs with thin white and green leaf margins are indicated by arrows). The mixing of OTUs from the two putative groups within *B. pungens* implies continuity in characters other than those used for forming the groups. 1 = *sp. 8*; 2 = *sp. 9*. OTU abbreviations details are as outlined in Appendix I and IV.

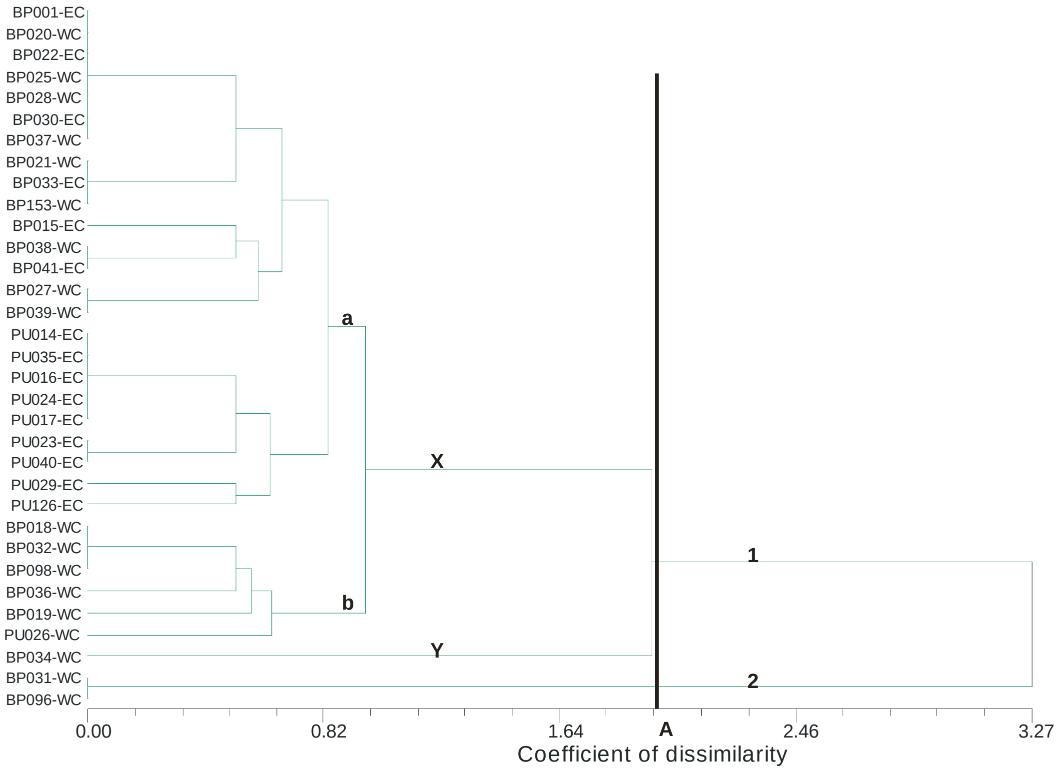

Figure 2.4b. Phenogram demonstrating the clustering of OTUs within the *B. pungens* complex from a matrix of 11 qualitative characters, r = 0.9928. BP = *B. pungens* with green leaf margins; PU = *B. pungens* with white leaf margins. A phenon line is drawn at about 2.0 based on the great dissimilarity distance from the point of differentiation and the next cluster. Numbers 1 – 2 denote the major clusters that can be delimited as consistent at species level. Cluster 'a' consists of two sub-clusters; OTUs with prominent white leaf margins in the one and OTUs with green leaf margins in the other. Cluster 'b' comprises intermediate OTUs with green and thin white leaf margins, and 'Y' has a single OTU (*Fourcade 613a* (BOL)) with green leaf margins. 1 = *sp. 8*; 2 = *sp. 9*. 'Y' cannot be recognised as a distinct cluster from X at the level of species because it only differs from the rest by the lack of an indumentum, a character that was observed to be highly variable within the *B. pungens* complex. X and Y may be recognised at the level of variety. OTU abbreviations details are as outlined in Appendix I and IV.

2.4.2 Cluster analysis

OTUs of *Barleria elegans* (the standard taxon) were observed to cluster together demonstrating that the character list was well calibrated (Figures 2.3a and 2. 3b). The point at which OTUs of the standard taxon formed a cluster was therefore used as a basis to delimit other clusters at the level of species. The five given phenograms had correlation coefficients of r = 0.9006, r = 0.9814, r = 0.9756, r = 0.9828 and 0.9872 respectively which indicate a good fit; the OTUs were separated with reliability (Figures 2.3a & b, 2.4a & b and 2.5). The analyses showed that the *B. bechuanensis* (BB and BE) and *B. irritans* (BI and BU) complexes comprised two distinct groups each. However, one OTU (BI145) was clearly distinguished from the rest of the BI cluster. *Barleria jubata* was also observed to split into two clusters, where one of the clusters only had one OTU. A phenon line drawn at approximately 0.9 as a coefficient of dissimilarity (Figure 3a), which, is just below where the OTUs of the standard taxon begin to cluster, delimited six clusters at species level from the matrix of combined qualitative and quantitative characters.

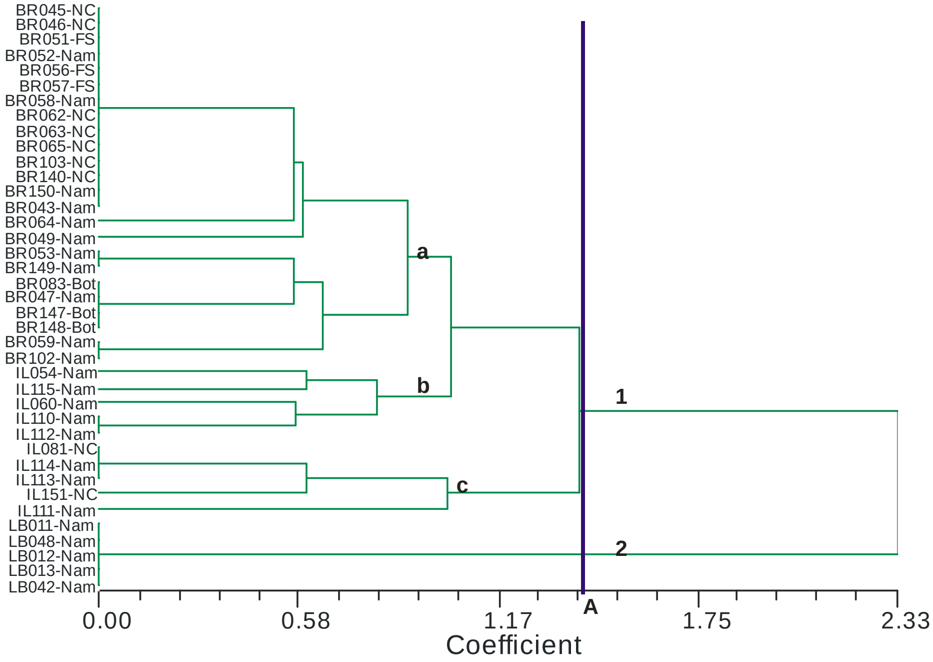

Figure 2.5. Phenogram demonstrating the clustering of OTUs within the B. rigida complex from a matrix of 12 qualitative and quantitative morphological characters, r = 0.9872. A = coefficient of dissimilarity estimated at 1.4 by drawing a phenon line just below where cluster 1 begins to form. The dissimilarity distance between where cluster 1 differentiates from cluster 2 and the point where cluster 1 begins to group can qualify them to be recognised at species level. Numbers 1 – 2 denote the major clusters delimited as consistent and identifiable and can be recognised at the level of species. Sub-clusters *a, b* and *c* of cluster 1 can be delimited at the level of variety. The IL OTUs of cluster *b* will now be referred to as ILa, while those from cluster *c* will maintain the IL. 1 = *sp. 10*, 2 = *sp. 11*, a = *sp. 10* var. *a*, *b* = *sp. 10* var. *b*, *c* = *sp. 10* var. *c*. The rest of the Abbreviations details are as outlined in Appendix I and IV.

However, the standard taxon did not exhibit within group variation from the matrix of qualitative characters, a phenon line estimated at 0.7 as a coefficient of dissimilarity (giving an allowance for within group variation among the OTUs of the standard taxon) delimited 6 distinctive and clear-cut clusters at species level (Figure

2.3b). Clusters numbered 1 – 3, 6, 7 in Figures 2.3a & b represent similar groups though the OTU arrangement varies and cluster X is the standard taxon. Clusters 4 and 5 were observed to switch positions. The *B. pungens* complex split into clusters with one group consistently possessing only two OTUs labelled BP031 and BP096 from a combined matrix of qualitative and qualitative characters and a matrix with only qualitative characters (Figures 2.4a and 2. 4b). Phenon lines drawn at approximately 1.5 and 2.0 as coefficients of dissimilarity separated the OTUs into two distinct clusters (Figures 2.4a and 2. 4b respectively). The *B. rigida* complex split into two major and distinct clusters; one with LB OTUs only and the other with IL and BR OTUs (Figure 2.5). Cluster 1 further split into three sub-clusters, two with IL OTUs and one with BR OTUs (Figure 2. 5).

2.4.3 Ordination

There were 24 characters observed to be most strongly correlated with the first principal component with values higher than 0.6 for the combined matrix in the analysis for three species / species complexes, 12 characters most strongly correlated with the first principal component for the *B. rigida* combined matrix and only six characters strongly correlated with the first axis for the combined matrix used to analyse the *B. pungens* complex (Tables 2.1, 2.2 and 2.3). Principal coordinates 1, 2 and 3 accounted for 50.4%, 18.7% and 7.1% of the variation from combined qualitative and quantitative data matrices while principal coordinates 1, 2 and 3 accounted for 33.9%, 26.6% and 14.1% of the variation from qualitative data matrices in the analyses with three species / species complexes (Tables 2.4b and 2.4a respectively). Principal coordinates 1, 2 and 3 accounted for 61.5%, 20.1% and 7.4% of the variation among the *B. pungens* complex, while principal coordinates 1, 2 and 3 accounted for 61.2%, 19.2% and 11.1% of the variation among the *B. rigida* complex (Tables 2.6 and 2.5 respectively). Characters CYS-P, Stemsurf and Babglos influenced variation along the first and second axes (Table 2.1).

The two- and three-dimensional plots for the *B. bechuanensis*, *B. jubata* and *B. irritans* species / species complexes from combined data matrices did show separation of the clusters, and the clusters formed by CA where OTUs of the same putative taxa grouped together were recovered (Figures 2.6 and 2.8 respectively). BB and BE were observed to form separate clusters within the *B. bechuanensis* complex except for one BB which was observed among the BEs and two other OTUs from BB and BE that occupied intermediate positions. There was clear separation of clusters between the BI and BU clusters of the *B. irritans* complex. The two- and three-dimensional plots from quantitative data matrices exhibited mixing of OTUs from within species complexes (BB among BE; BI at the boundary of BU cluster and vice versa) and between species / species complexes (JU amongst BI; EL amongst JU; JU at the fringe of EL) (Figures 2.7 and 2.9 respectively).

Table 2.1. Thirty six Eigen values extracted by PCA from a combined matrix of qualitative and quantitative characters in the analyses for *B. bechuanensis*, *B. irritans* and *B. jubata*. *Barleria elegans* is the standard taxon and the character loadings influencing differentiation in the first three axes are those with values greater than 0.6 (in bold). Character codes and abbreviations for all characters used in the analyses are as outlined in Appendix III.

Characters	**Dim-1**	**Dim-2**	**Dim-3**
W	**0.6409**	0.2860	0.5049
SL	0.4841	**-0.6239**	-0.0560
M	**0.6749**	0.3460	0.0611
BS	**0.6019**	-0.4106	-0.0961
B #teeth2	**-0.7705**	0.2222	-0.0200
A #teeth2	**-0.7437**	0.2234	-0.0381
CYS-P	**0.6144**	**-0.6147**	0.2200
P #teeth2	**-0.7525**	0.2837	-0.0958
L:W	**-0.8846**	0.0956	0.0829
L:PL	**-0.7880**	0.1560	0.1730
BL:BW	-0.0003	**-0.9764**	0.0041
BL:teeth	**-0.6694**	0.4538	0.0230
CYL-A:W	-0.1039	**-0.9320**	0.4115
CYL-A:teeth	**-0.7988**	0.2976	0.0422
CYL-P:W	-0.0584	**-1.0136**	0.3711
CYL-P:teeth	**-0.8457**	0.3112	-0.0368
Stemsurf	**-0.6240**	**-0.8102**	-0.3712
Stempub	**0.6651**	0.1872	0.4150
leafsurf	**0.6854**	0.3490	0.0578
Margcolr	**0.6670**	0.3173	-0.0340
Margpub	**0.6440**	0.1807	-0.2867
Hairbase	**-0.8866**	-0.7442	0.3181
Margthick	**0.6961**	0.3465	0.0064
Margent	**0.7522**	0.1945	0.1453
Margstrt	-0.3825	**-0.9076**	0.4143
Midrib	**0.7737**	-0.4100	-0.0945
Latvein	**-0.9210**	-0.2292	0.1149
Bsurfab	0.3728	-0.1697	**-0.6873**
Bsurfad	0.3728	-0.1697	**-0.6873**
Babglos	**-0.6240**	**-0.8102**	0.3712
Badglos	**-0.6166**	0.1124	0.5746
Bmargent	-0.1784	**0.6659**	-0.1654
Bmargpub	0.3728	-0.1697	**-0.6873**
Bmargspn	0.3728	-0.1697	**-0.6873**
Calpub	0.3728	-0.1697	**-0.6873**
Calmgspn	0.3778	-0.2277	**-0.6520**

Table 2.2. Thirteen Eigen values from PCA analysis extracted from a combined matrix of qualitative and quantitative characters of the *B. pungens* complex. The 13 character loadings influencing differentiation along the first three axes are those with values greater than 0.6 (in bold). Character codes and abbreviations for all characters used in the analyses are as outlined in Appendix III.

Character	**Dim-1**	**Dim-2**	**Dim-3**
CYW-A	0.4375	0.0171	**0.6287**
CYS-A	0.2419	0.1193	**-0.6119**
P #teeth4	**-0.6413**	-0.1709	-0.0269
BL:BW	-0.3642	0.4532	**-0.6723**
CYL-A:W	-0.3127	0.4794	**-0.8170**
CYL-P:W	-0.2996	0.5593	**-0.7283**
Margpub	**0.6234**	-0.5492	-0.2593
Hairbase	-0.5046	**0.9006**	0.1157
Margent	-0.5046	**0.9006**	0.1157
Margstrt	**0.6234**	-0.5492	-0.2593
Babglos	**0.6726**	-0.2329	-0.2817
Calmarg	**0.6489**	0.1264	-0.4686
Calmgspn	**0.6234**	-0.5492	-0.2593

Table 2.3. Fourteen Eigen values from PCA analysis extracted from a combined matrix of qualitative and quantitative characters of the *B. rigida* complex. The 16 characters influencing differentiation along the first three axes are those with values greater than 0.6 (in bold). Character codes and abbreviations for all characters used in the analyses are as outlined in Appendix III.

Character	**Dim-1**	**Dim-2**	**Dim-3**
M	0.2591	**0.6775**	0.1430
BW	**0.6433**	-0.0114	-0.0799
CYW-P	**0.6237**	0.2101	-0.4440
L:W	**-0.8676**	0.2369	-0.4858
BL:BW	**-0.8676**	0.2369	-0.4858
CYL-P:W	**-0.7608**	-0.1552	0.3805
Stemsurf	**0.6691**	0.0485	-0.3564
Margstrt	**-0.8487**	-0.4638	-0.5099
Bralobe	**-1.1678**	0.0920	0.1100
Bravein	**-0.7299**	-0.0147	-0.0586
Bvconsp	**-1.1678**	0.0920	0.1100
Brapex	**-1.1678**	0.0920	0.1100
Calhard	-0.0516	**0.7474**	-0.2891
Calovlap	**-0.7608**	-0.1552	0.3805

Table 2.4a. Eigen vectors obtained by Principal coordinates analysis of 17 quantitative character matrix for the three species / species complexes (*B. bechuanensis*, *B. jubata* and *B. irritans*). The table reveals that variation is concentrated in the first 9 axes; with the first three axes accounting for 76.1% of the variation.

Axis	Eigen vector	Percent total variation	Cumulative percentage
1	8.56980883	50.4106	50.4106
2	3.17483834	18.6755	69.0862
3	1.19868880	7.0511	76.1373
4	0.96720906	5.6895	81.8267
5	0.68785259	4.0462	85.8729
6	0.55446815	3.2616	89.1345
7	0.36163966	2.1273	91.2618
8	0.33980773	1.9989	93.2607
9	0.30057036	1.7681	95.0287
10	0.21258768	1.2505	96.2792
11	0.17628447	1.0370	97.3162
12	0.12799031	0.7529	98.0691
13	0.11087516	0.6522	98.7213
14	0.08183684	0.4814	99.2027
15	0.06884017	0.4049	99.6076
16	0.03892924	0.2290	99.8366
17	0.02777261	0.1634	> 100%

The JU OTUs did form a distinct but not tight cluster from both the two- or three-dimensional plots (Figures 2.7 and 2.9). EL and JU cannot be differentiated along the first and second axes from the two-dimensional plot of quantitative characters, they exhibit a narrow overlap. However, the three-dimensional plot did show separate clusters except for a few OTUs (Figure 2.9). Apart from two OTUs, the 2- and 3-dimensional plots showed no clear segregation of OTUs between BP and PU putative taxa of the *B. pungens* complex (Figures 2.10a and 2. 10b respectively). The *B. rigida* complex did not demonstrate separation of clusters in multivariate character space between OTUs from BR and some from IL which were observed to overlap but the LB taxon formed a very distinct cluster which appears as one OTU due to lack of within group variation based on the data set used (Figure 2.11).

Table 2.4b. Eigen vectors obtained from Principal coordinates analysis of combined quantitative and quantitative character matrix for the three species / species complexes (*B. bechuanensis*, *B. jubata* and *B. irritans*). Thirty three (33) Eigen values indicating that variation is concentrated in the first 6 axes; with the first three axes accounting for 74.7% of the variation.

Axis	Eigen vector	Percent total variation	Cumulative percentage
1	27.15941738	33.9493	33.9493
2	21.31458843	26.6432	60.5925
3	11.30318114	14.1290	74.7215
4	5.27872418	6.5984	81.3199
5	3.19358641	3.9920	85.3119
6	2.19900516	2.7488	88.0606
7	1.54493385	1.9312	89.9918
8	1.02448635	1.2806	91.2724
9	0.89064087	1.1133	92.3857
10	0.84869780	1.0609	93.4466
11	0.68143019	0.8518	94.2984
12	0.62401880	0.7800	95.0784
13	0.57966000	0.7246	95.8030
14	0.52976717	0.6622	96.4652
15	0.43167569	0.5396	97.0048
16	0.41513289	0.5189	97.5237
17	0.35132950	0.4392	97.9628
18	0.25727305	0.3216	98.2844
19	0.24191218	0.3024	98.5868
20	0.22273552	0.2784	98.8652
21	0.21468768	0.2684	99.1336
22	0.15394848	0.1924	99.3260
23	0.13268077	0.1659	99.4919
24	0.07694304	0.0962	99.5881
25	0.07326058	0.0916	99.6796
26	0.07036581	0.0880	99.7676
27	0.04430401	0.0554	99.8230
28	0.03380237	0.0423	99.8652
29	0.02997183	0.0375	99.9027
30	0.02728691	0.0341	99.9368
31	0.02487016	0.0311	99.9679
32	0.01922115	0.0240	99.9919
33	0.00646065	0.0081	> 100%
34	0.00000000	0.0000	> 100%
35	0.00000000	0.0000	> 100%
36	0.00000000	0.0000	> 100%

Table 2.5. Eigen vectors obtained from Principal coordinates analysis of 12 combined quantitative and qualitative character matrix for the *B. rigida* complex. Eight Eigen values indicate that variation is concentrated in the first 5 axes and the first three axes account for 91.5% of the variation.

Axis	Eigen vector	Percent total variation	Cumulative percentage
1	6.59030429	61.1624	61.1624
2	2.07437453	19.2516	80.4139
3	1.19997256	11.1365	91.5505
4	0.32178562	2.9864	94.5369
5	0.25044635	2.3243	96.8612
6	0.15080791	1.3996	98.2608
7	0.12532137	1.1631	99.4238
8	0.06208329	0.5762	> 100%

Table 2.6. Eigen vectors obtained from Principal coordinates analysis of 13 combined quantitative and qualitative character matrix for the *B. pungens* complex. Seven Eigen values indicate that variation is concentrated in the first 5 axes; with the first three axes accounting for 89.0% of the variation.

Axis	Eigen vector	Percent total variation	Cumulative percentage
1	7.03754029	61.5371	61.5371
2	2.29378296	20.0571	81.5943
3	0.85083981	7.4399	89.0341
4	0.71337843	6.2379	95.2720
5	0.36012712	3.1490	98.4210
6	0.13144330	1.1494	99.5704
7	0.04913503	0.4296	100.0000

2.4.4 Distribution

BB and BE of the *B. bechuanensis* complex overlap in geographic space, inhabiting the north-eastern part of the Northern Cape whereas BI and BU of the *B. irritans* complex exhibit differentiation in geographic space with a narrow geographic distribution overlap where BI occupies the southern coast of the Eastern Cape with one OTU at the northern border of the Eastern Cape and Northern Cape while BU occupies the inland, inhabiting the western part of the Eastern Cape (Figure 2.12a). JU is observed to be concentrated in the north central part of Namibia except for one OTU that was collected from the extreme north west of Namibia hence exhibiting disjunction in the distribution (Figure 2.12a). The BR group, though widespread from eastern Northern Cape to central Namibia, was split into two sub-clusters which overlapped in southern Namibia (Figure 2.12b). IL inhabits the southern part of Namibia at the border with the north-western tip of Northern Cape, South Africa with one OTU on the south–west coast of Namibia. ILa was observed to extend from the southern border of Namibia with South Africa up to central Namibia separating BR and LB. LB inhabits the south west part of Namibia along the coast (Figure 2.12b). Within the *B. pungens* complex BP extends from the south-central coast of Western Cape to the southern / south-east coast of the Eastern Cape, while PU exhibits disjunction with some OTUs occupying the southern coast of the Eastern Cape, and other OTUs inhabiting the central coast of the Western Cape (Figure 2.12b).

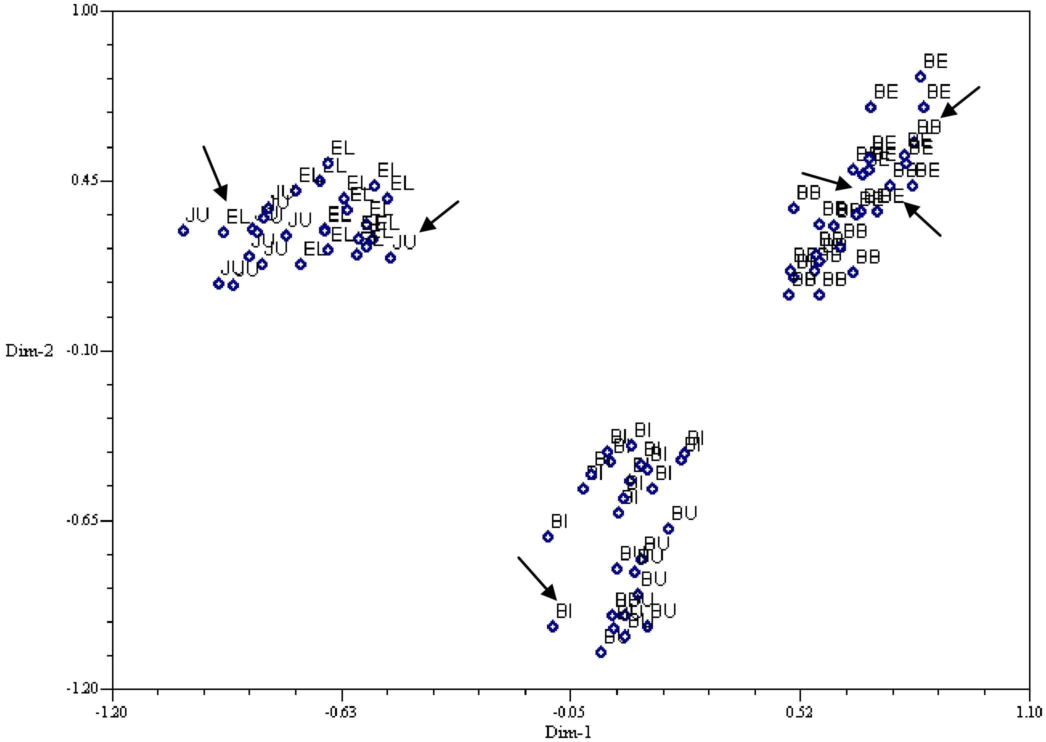

Figure 2.6. Two-dimensional scatter plot from PCO showing the distribution of the OTUs in multivariate space. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*. One OTU from the BI taxon is closer to the BU cluster a trend also observed in Figure 2.3a. Although BB and BE form distinct clusters, there is no clear gap separating the clusters along the first and second axes, and one BB OTU is observed among the BE cluster. There is also a BE and a BB in the middle of the two clusters. One JU is at the edge of the EL while one EL is among the cluster of the JUs (OTUs that are not clustering with members of the same group are indicated by arrows). OTU abbreviations details are as outlined in Appendix I.

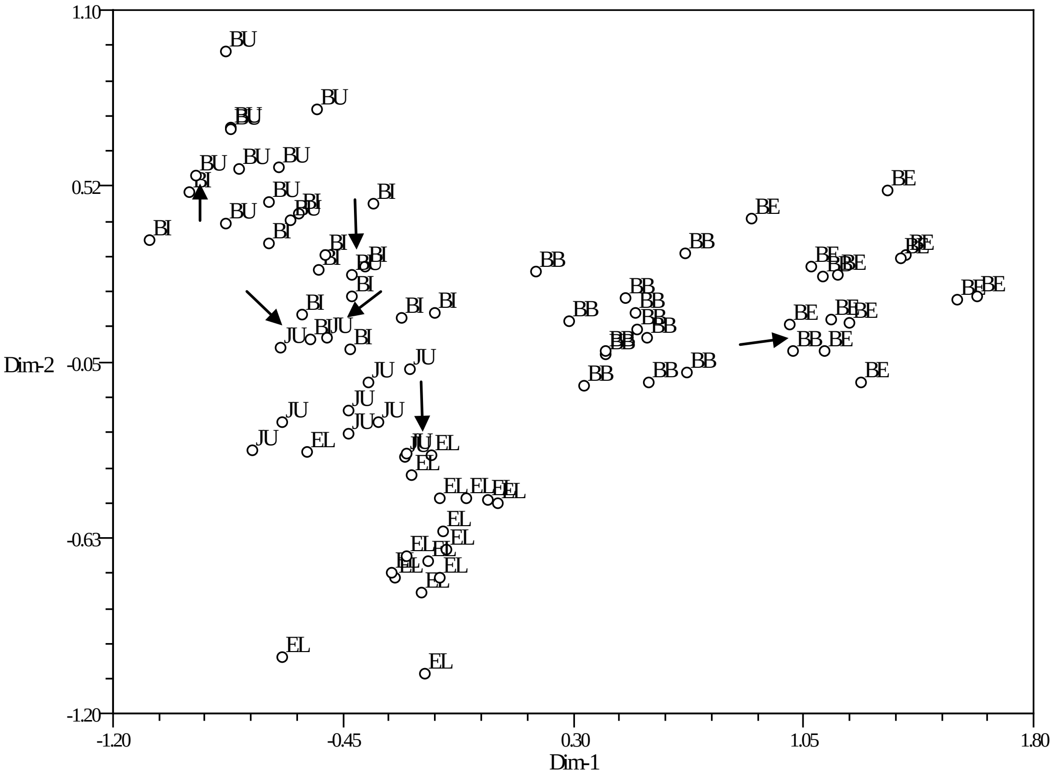

Figure 2.7. Two-dimensional scatter plot from PCA showing the distribution of the OTUs in multivariate space. There is no clear-cut distinction of clusters between some of the taxa as there is mixing of OTUs between BI and BU, between JU and EL and two JUs are observed among the BI cluster. Although one BB (indicated by an arrow) is observed among BE most of the OTUs form clear clusters. The mixing of OTUs among clearly differentiated species demonstrates that the study group cannot be adequately separated on the basis of quantitative characters alone and in only two dimensions. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata.* The OTU abbreviations details are as outlined in Appendix I.

2.5 Discussion

2.5.1 Distinct morphological characters

i) Stem and habit

Growth habit was observed to be difficult to ascertain because not all the OTUs were complete specimens. However, specimens collected from open places tend to be erect whereas those growing among other vegetation exhibit decumbent growth. Growth habit however, can be a useful taxonomic character (Balkwill *et al.*, 1996; Napper, 1970). The BB and BE taxa comprised multi-stemmed dwarf shrublets branching from the rootstock or just above the ground with bifariously hairy upright or decumbent stems, endowed with short white recurved hairs. Although BR, IL, ILa and LB are multi-stemmed dwarf shrublets branching from the rootstock or just above the ground, young stems are glabrescent with very short white recurved hairs becoming glabrous with maturity, and the hairs do not occur in furrows as observed among BB and BE. While BI are upright branching shrublets with young stems that are bifariously hairy with short white recurved hairs becoming glabrescent with maturity, BU on the other hand are multi-stemmed dwarf shrublets branching from the rootstock with scanty hairs all around young stems becoming glabrescent or glabrous with maturity.

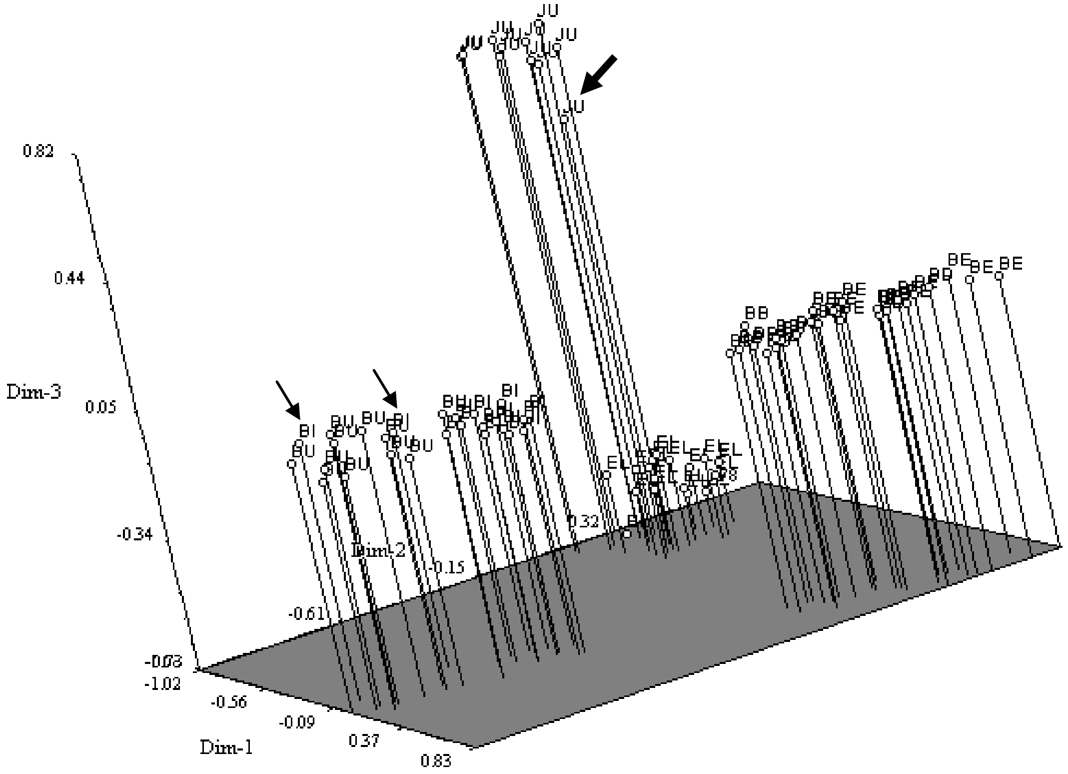

Figure 2.8. Three-dimensional scatter plot from PCO showing the distribution of the OTUs in multivariate space. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*. Two BI OTUs (with thin arrows) are among the BU cluster. Although BB and BE show a clear-cut gap with OTUs from the same putative taxon clustering together there are a few BB and BE occupying intermediate positions. JU is here clearly distinguished from EL with one OTU being distant from the major cluster (thick arrow). OTUs that are not clustering with members of the same group are indicated by arrows. OTU abbreviations details are as outlined in Appendix I.

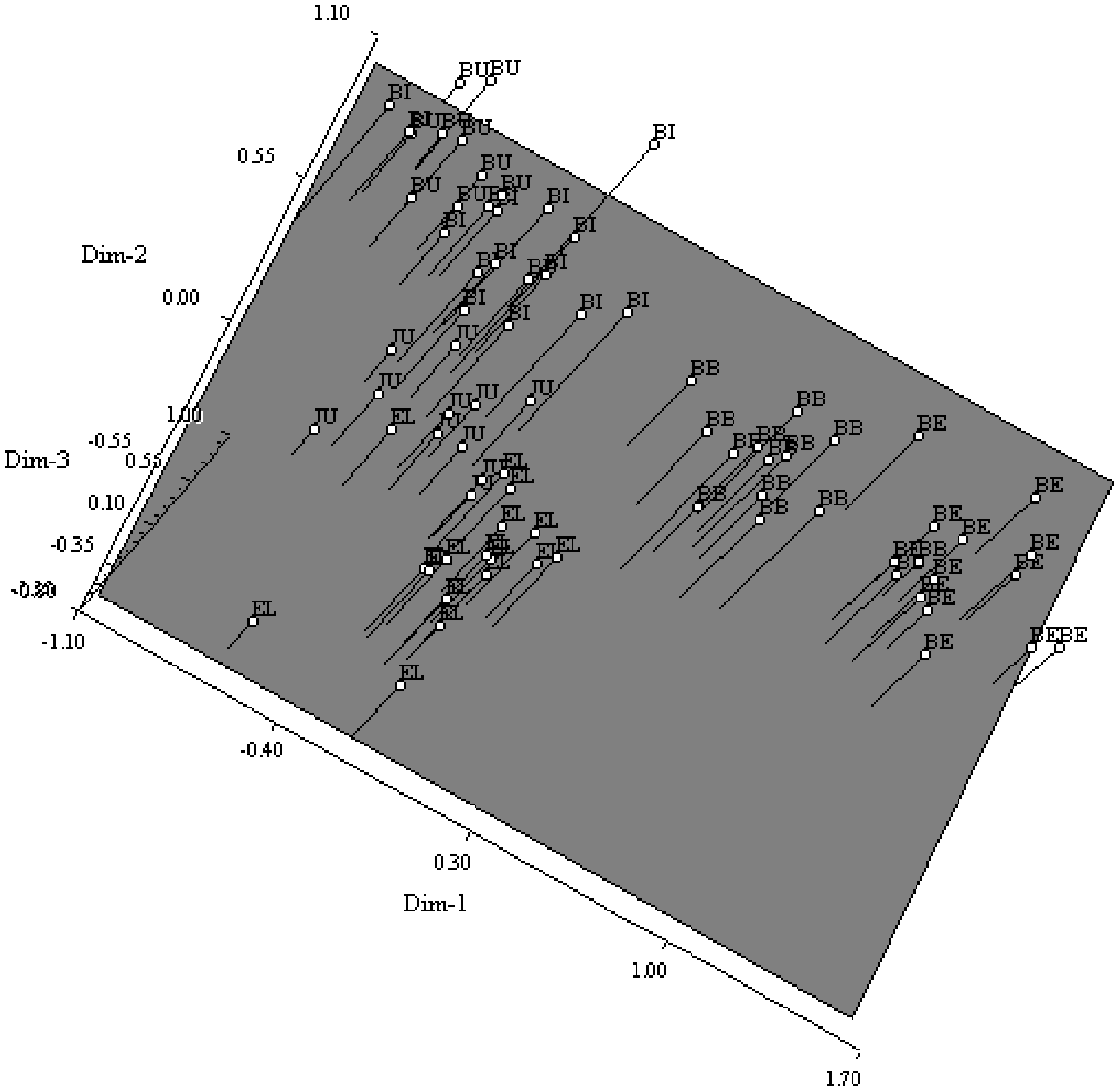

Figure 2.9. Three-dimensional scatter plot from PCA showing the distribution of the OTUs in multivariate space. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; EL = *B. elegans* (standard); JU = *B. jubata*. Although there is clear formation of clusters, one BE is observed close to the BB cluster while one BB is observed among the BE cluster indicating that there could be some overlapping quantitative characters causing this. BI and BU are clearly differentiated except for two BI OTUs observed among the BU cluster and another one at the fringe of the BU cluster. Two JUs are observed at the fringe of the EL cluster while one EL is observed among the JU demonstrating that the study group can possibly be separated on the basis of quantitative characters in three dimensions. The OTU abbreviations details are as outlined in Appendix I.

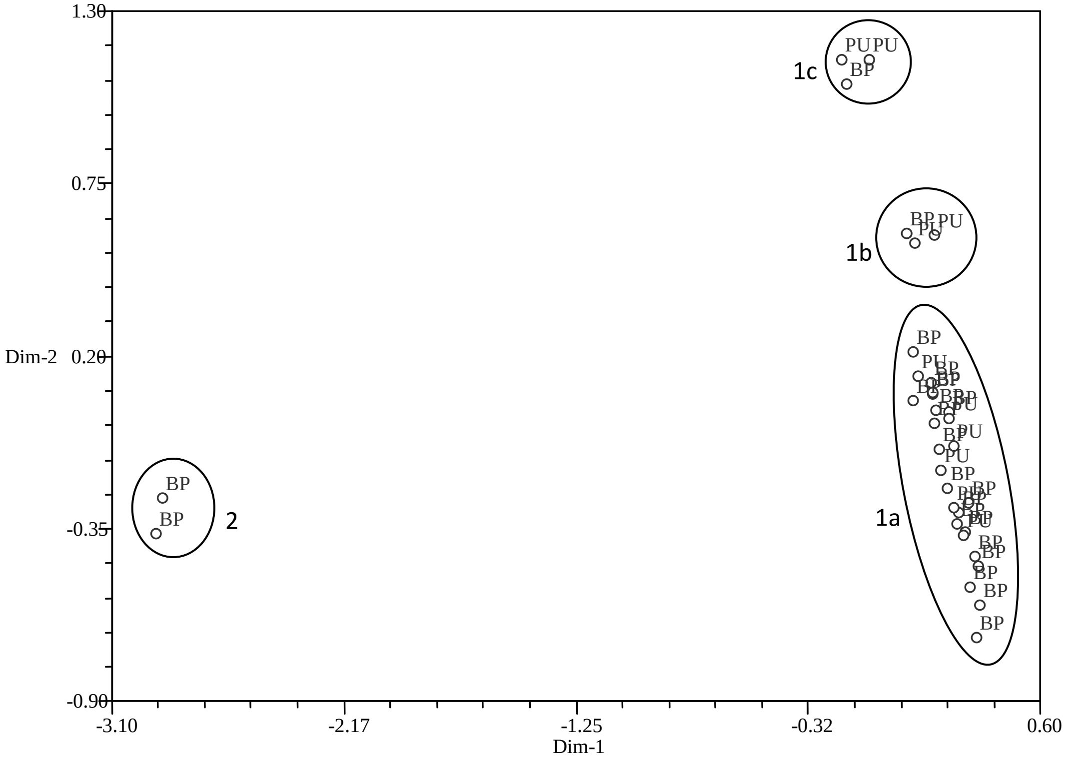

Figure 2.10a. Two-dimensional scatter plot from PCO showing the distribution of OTUs in multivariate space between BP and PU. BP = *B. pungens* with green leaf margins; PU = *B. pungens* with white leaf margins. There is no clear distinction of clusters between BP and PU except for two OTUs (cluster 2) with flat leaf margins and glabrous leaves, differentiated along the first axis. Although clusters 1a – 1c have clear gaps (along the second axis) the differentiation between the two putative groups is blurred along both the first and second axis as the clusters are formed by OTUs from the two putative groups demonstrating a considerable degree of overlap in character space. Cluster 2 will also be referred to as BP2. The OTU abbreviations details are as outlined in Appendix I.

The BP and PU are upright or decumbent shrublets with stems that are hairy all round with cream to yellow-brown straight hairs except for the two OTUs, BP031 and BP096 (designated as BP2) which have very short white hairs (Figure 2.13 e and f). Whereas the JU were observed to be upright branching shrublets with stems that are covered in erect ashy-white stiff and long yellowish eglandular hairs, one OTU JU141 has stems covered in erect cream-white stiff eglandular hairs and hairs with brown glandular heads (as opposed to the rest of the OTUs from *B. jubata*) (Figure 2.14 a and b). The variation in pubescence intensity may be considered as developmental where it varied with age (Friis and Gilbert, 1985; Strand and Weisner, 2004). The presence or absence of pubescence and the dissimilarity in hair type, hair pattern and colour, however, may be taxonomically useful and can be used to differentiate the putative taxa as evidenced in *Monechma* Hochst. (Munday, 1984).

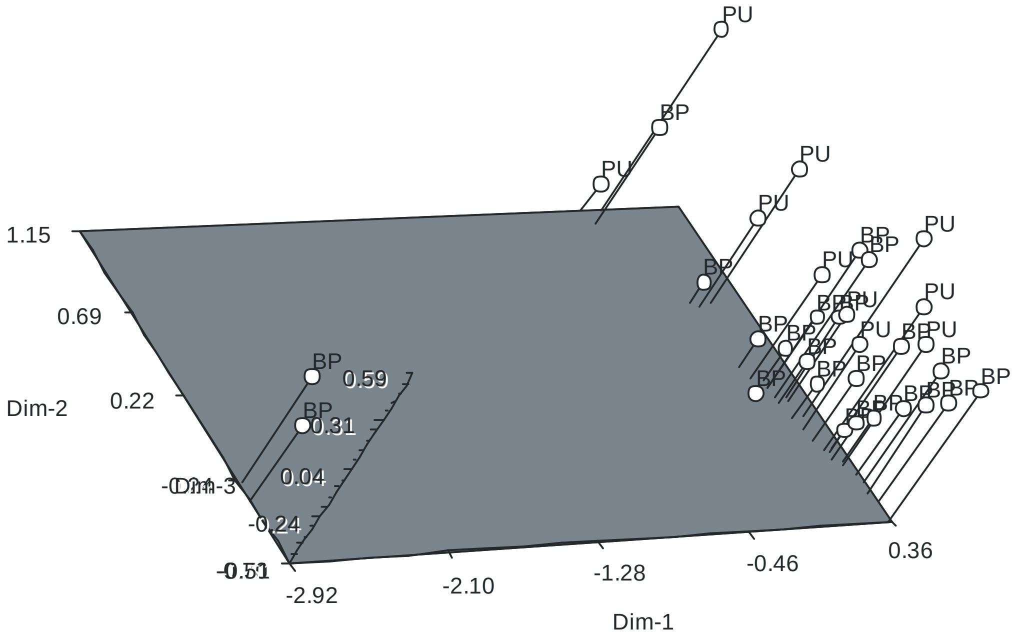

Figure 2.10b. Three-dimensional scatter plot from PCO showing the distribution of the OTUs of *B. pungens* in multivariate space. BP = *B. pungens* with green leaf margins; PU = *B. pungens* with white leaf margins. There is no clear distinction between the putative groups BP and PU except for two OTUs of BP, demonstrating that there is some overlap in characters between the two putative groups and the formation of the three clusters with mixed OTUs implies that characters other than the white colour on the margins of the leaf and bracteole could be determining the variation. The OTU abbreviations details are as outlined in Appendix I.

ii) Leaf

The leaves of the taxa in this study are herbaceous or coriaceous, variously shaped and diversely pubescent with a midrib that is prominent and terminates in a sharp apex (though texture was difficult to examine in herbarium specimens) (Figure 2.13). The leaf surface ranges from glabrous and glabrescent to pubescent while the leaf margin is glabrous or variously hairy with or without teeth / spines and with or without a prominent white colour. The leaf surface in BB, BE, BI, BR, BU, IL, ILa and LB is glabrous with no prominent lateral veins on both surfaces (or rarely with faint lateral veins on the abaxial surface as observed in BI075 and LB042 and becoming inconspicuous with maturity). However, the leaf in BB is linear-lanceolate with a shallowly wavy leaf margin while the leaf in BE is linear to linear-oblanceolate with a flat leaf margin (Figure 2.13h). The leaf is much narrower in BE than in BB and in addition, the two taxa can be differentiated by the leaf width and ratio of leaf length to leaf width (Figures 2.2 (i) b, 2. 2 (ii) f and 2.13). Therefore, leaf width, leaf margin and leaf shape are taxonomically valuable within the *B. bechuanensis* complex.

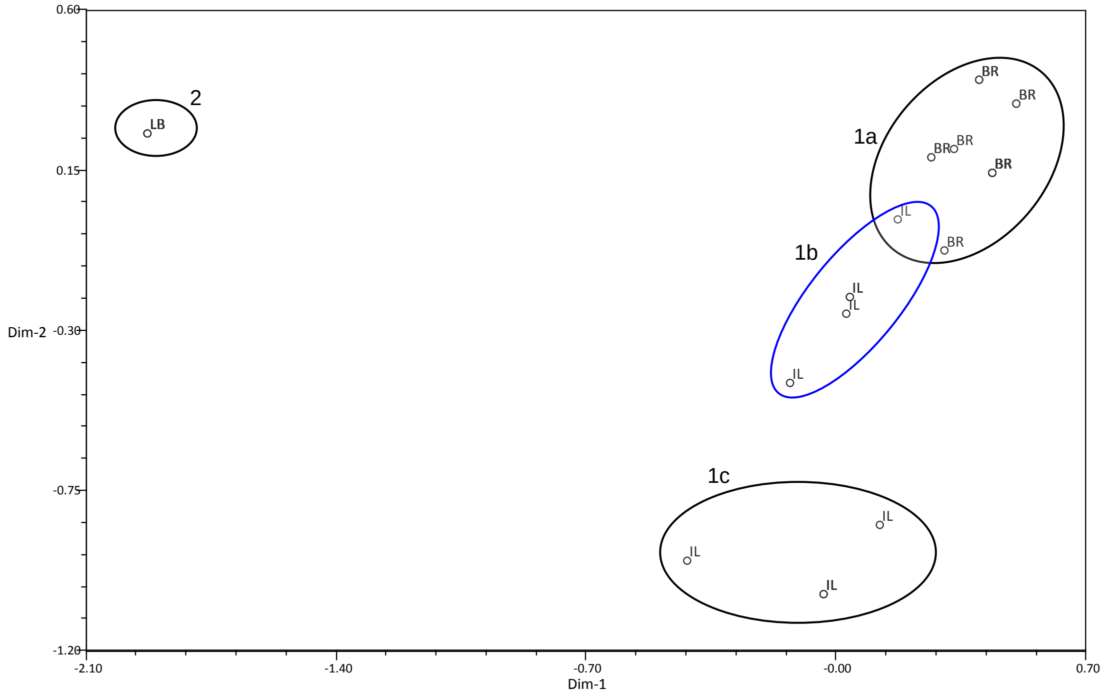

Figure 2.11. Two-dimensional scatter plot from PCO showing the distribution of the OTUs in multivariate space within the *B. rigida* complex. The LB cluster comprises five specimens that have a within cluster dissimilarity of zero based on the characters under consideration, hence the OTU positions coincide and appear as one. Although there is a clear distinction of clusters formed between BR and IL the differentiation between cluster 1a and 1b is blurred along the second axis as two OTUs from the two putative groups are observed to occupy an intermediate position demonstrating some degree of overlap. The two are however, narrowly differentiated along the first axis whereas cluster 1a and 1c are narrowly differentiated along the first axis and clear-cut along the second axis. LB and IL can be differentiated along the first and second axes while LB and BR can only be separated along the first axis. The IL OTUs of cluster 1b will now be referred to as ILa. The OTU abbreviations details are as outlined in Appendix I.

Although the leaf in BI and BU is ovate with a white very shallowly wavy leaf margin that is sparsely toothed, the two can be differentiated by the ratio of the leaf length to leaf length from base to the widest point (Figure 2.2 (ii) g). Only the leaf shape is of significance within the *B. irritans* complex. There is considerable overlap in leaf shape and leaf margin among BR, IL, ILa and LB ranging from linear, linear-lanceolate, elliptic-ovate and narrowly ovate to broadly so and leaf margins from spiny sinuate, spiny shallowly wavy / undulate and toothed undulate to entire undulate. Continuous variation is also observed in spinescence, varying from conspicuous long or short spines almost evenly distributed along the leaf margin to scanty small teeth. Entire leaf margins are common especially among the leaves with deeply undulate leaf margins.

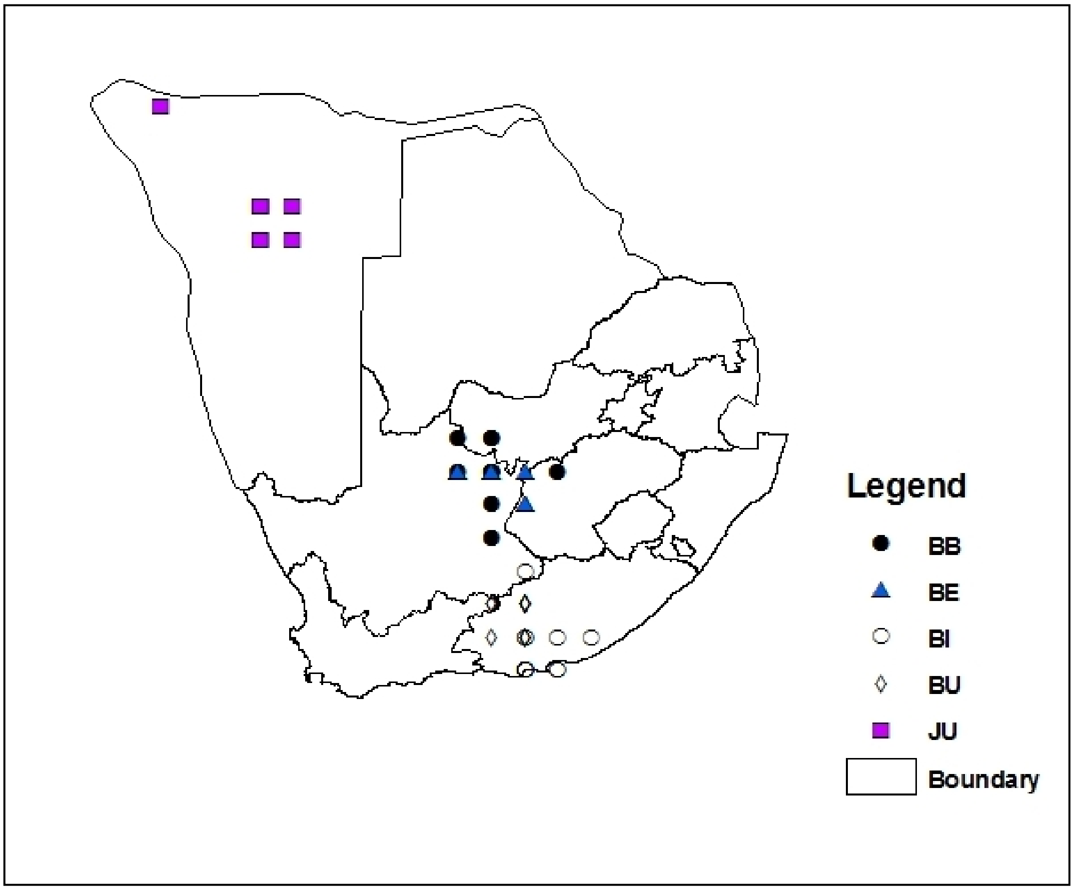

Figure 2.12a. Distribution of taxa from cluster analysis of the *B. bechuanensis*, *B. irritans* and *B. jubata* species / species complexes. BB, BE = putative taxa from *B. bechuanensis*; BI, BU = putative taxa from *B. irritans*; JU = *B. jubata*. Where BB = *sp. 1*; BE = *sp. 2*; BI = *sp. 3*; BU = *sp. 4*; JU, *sp. 6*. The one disjunct OTU of *sp. 6* may imply that *sp. 6* is a species that is differentiated into two subspecies. Details of the abbreviations are as outlined in Appendix I.

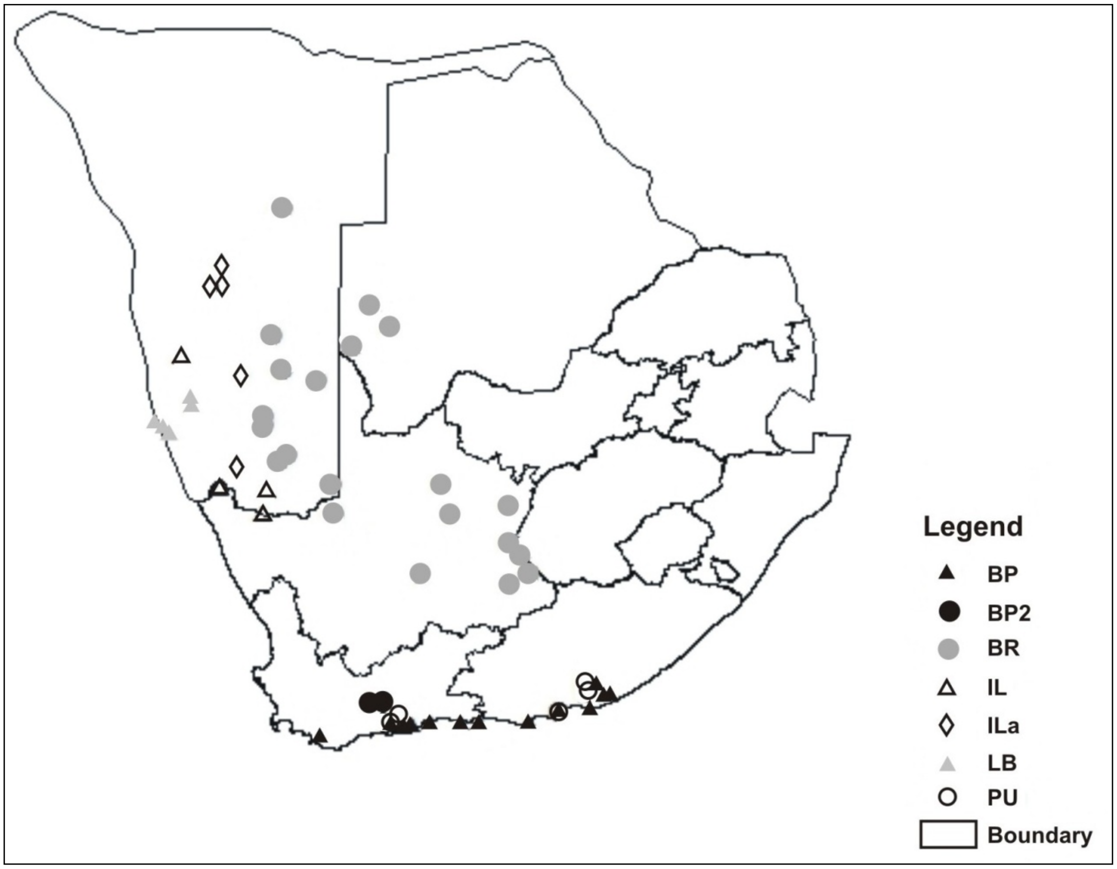

Figure 2.12b. Distribution of the taxa based on the clusters formed from cluster analysis of *B. pungens* and *B. rigida* species complexes. BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida*. BP2 denotes the two distinct OTUs (from cluster 2) with green flat leaf margins and serrate bracteole margins from the BP putative taxon (Figure 2.10a). Details of the abbreviations are as outlined in Appendix I.

Although the leaf in BP, PU and JU is pubescent (with pubescence concentrated especially on the veins), BP and PU have narrowly to broadly ovate (sometimes elliptic-ovate) leaves with lateral veins that are not easily seen in most of the OTUs and when conspicuous they exhibit brochidodromous venation as seen in BP034. BP and PU exhibited an overlap in all the quantitative leaf characters examined (Figure 2.2 (i) to 2.2 (iii) j). Overlapping characters are not taxonomically useful (Wilkin, 1999). However, two distinct BP OTUs (BP031 and BP096) have flat green leaf margins with horizontal hairs unlike the rest of the OTUs within the *B. pungens* complex which have wavy green or white leaf margins with erect hairs. In contrast, leaves in JU are larger and always with prominent lateral veins with a conspicuous reticulation of tertiary veins on the abaxial surface and leaf margins that are flat, entire and with erect hairs. Nonetheless leaves in JU144 were observed to have some of the erect hairs on the leaf margin thickening and when tips break off appearing like blunt tooth-like structures on the leaf margin, a feature that was also observed in

JU009, JU107, JU109 and JU117 (especially in OTUs collected from the Waterberg), a variation which may be in response to environmental conditions (Wilkin, 1999). Although the leaf in JU141 appeared glabrous on the surface and margins, it possessed microscopic hairs with glandular heads as opposed to the rest of the JU OTUs which possessed eglandular hairs. While characters such as leaf margin and leaf shape being less prone to plasticity (than leaf size) can reliably be used to differentiate taxa (Obermeijer, 1933), they are not taxonomically valuable among JU, BP and JU. Dissimilarity in pubescence type is considered taxonomically importance (Balkwill *et al.*, 1996; Brysting and Elven, 2000; Darbyshire, 2008; Darbyshire and Ndagalasi, 2008; Munday, 1984) and consequently can be used to differentiate taxa within *B. pungens* and *B. jubata*.

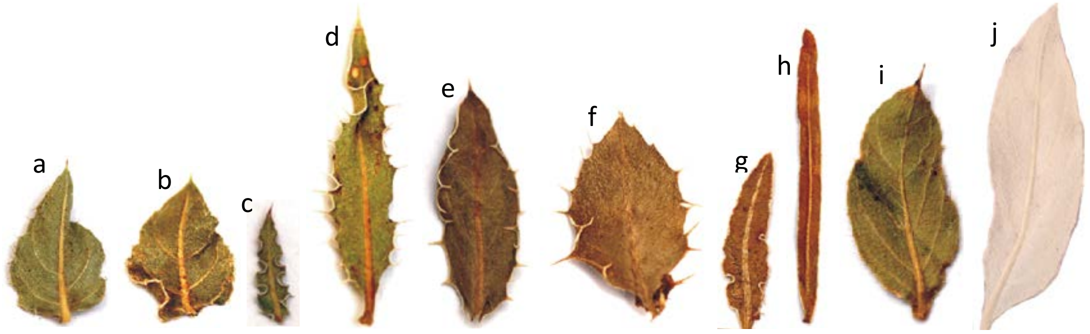

Figure 2.13. Variation in leaf shape and leaf margin between some OTUs from the putative groups. *a*) *Nyirenda 716* (J), BP with broadly ovate leaf and green leaf margins; *b*) *Dahlstrand 2715* (GRA), PU with broadly ovate leaf and white leaf margins; *c*) *Nyirenda 700* (J), BR with lanceolate-ovate leaf and undulate almost entire leaf margins; *d*) *Helary and Batault 196* (WIND), ILa with lanceolate leaf and spiny sinuate leaf margins; *e*) *Giess & van Vuuren 848* (PRE), LB with elliptic leaf and spiny sinuate leaf margins; *f*) *Schlieben 9092* (KEW), IL with elliptic-ovate leaf and spiny sinuate leaf margins; *g*) *Esterhuuysen 1008* (BOL), BB with linear-lanceolate leaf and shallowly undulate entire leaf margins; *h*) *Esterhuuysen 1007* (PRE), BE with linear (sometimes oblanceolate) leaf and flat entire leaf margins; *i*) *Wanntorp & Wanntorp 641* (J), JU with elliptic-ovate (or elliptic) leaf and flat hairy leaf margins; *j*) *Giess & Leippert 7588* (NBG), JU with elliptic leaf and flat glabrous leaf margins. Though *a*, *b* and *i* have hairy leaf margins and conspicuous lateral veins, the hairs in *a* and *b* have raised hair bases sometimes. The leaves in *c* and *g* can be further differentiated by their midribs, smooth in the one and rough in the other and the leaves in *i* and *j* vary by presence and absence of glandular hairs. Leaf shape and leaf margin is not sufficient to differentiate the groups. BB, BE = putative taxa from *B. bechuanensis*; JU = *B. jubata*; BP, PU = putative taxa from *B. pungens*; BR, IL, ILa, LB = putative taxa from *B. rigida.* Details of the abbreviations are as outlined in Appendix I.

Table 2.7. Qualitative and quantitative morphological characters that can be used to distinguish the putative taxa

Character	**BB**	**BE**	**BI**	**BP (flat leaf margin)**	**BR**	**BU**	**IL**	**ILa**	**JU**	**LB**	**BP-PU (wavy leaf margin)**
Growth form	Multi-stemmed dwarf herb	Multi-stemmed dwarf herb	Multi-stemmed, branching shrublet	Multi-stemmed shrublet	Multi-stemmed dwarf shrublet	Multi-stemmed dwarf shrublet	Multi-stemmed dwarf shrublet	Multi-stemmed dwarf shrublet	Multi-stemmed branching shrublet	Multi-stemmed dwarf shrublet	Multi-stemmed shrublet
Habit	Decumbent sometimes upright	Upright, sometimes decumbent	Upright	Upright	Upright	Upright	Upright	Upright	Upright	Upright	Upright
Stem pubescence	Bifariously hairy; short recurved coarse white	Bifariously hairy; short recurved coarse white	Bifariously hairy to glabrous; short recurved cream	Shortly pubescent; straight white	Glabrescent to glabrous; short recurved white	Bifariously hairy to glabrescent; short cream recurved	Glabrescent to glabrous; short white straight & recurved	Bifariously hairy to glabrescent; short white recurved	Pubescent; straight ashy white or cream-brown glandular	Bifariously hairy to glabrescent; short white recurved	Pubescent; straight cream to brown
Leaf shape	linear	Linear-oblanceolate	Narrowly ovate	Ovate	linear	Narrowly ovate	Ovate	Linear to elliptic-ovate	Elliptic to elliptic-ovate	Elliptic-ovate	Ovate
Leaf length	10 – 28 mm	13 – 34 mm	11 – 28.9 mm	12 – 17 mm	8.1 – 30.8 mm	9.2 – 22.7 mm	9.6 –21.7 mm	24.3 – 46.5 mm	16.6 – 42.5 mm	16.4 – 36 mm	10.5 –30 mm
Leaf width	3 – 4.7 mm	1.4 – 2.7 mm	4 – 9 mm	6 – 8 mm	1.9 – 6.7 mm	3 – 9 mm	5.6 –9.6 mm	3 –11 mm	7.48 – 16 mm	5.5 –12 mm	5.5 – 14 mm
Length to width ratio	4.1 – 9.1:1	7.2 –14:1	2.2 –3.9:1	2:1	3.1 – 6.2:1	1.8 –3.4:1	1.8 – 2.5:1	3.9 – 5.1:1	1.6 –3.4:1	2.8 –3.2:1	1 – 3:1
Leaf surface	Glabrous	Glabrous	Glabrous	Canescent	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent (glabrous in one)	Glabrous	Pubescent
Leaf margin	Shallowly wavy, white	Flat, white	Shallowly wavy, white	Flat, green	Undulate, white	Shallowly undulate, white	Undulate or sinuate, white	Sinuate, white	Flat, green	Sinuate, white	Very shallowly wavy, green

											or white
Leaf margin innovations	Entire	Entire	Sparsely toothed	Entire	Sparsely toothed to entire	Sparsely toothed	Spiny, spines 1 – 2.3 mm	Spiny, spines 0.6 – 2 mm	Entire (or occasionally toothed)	Spiny, spines 1 – 2.2 mm	Minutely toothed
Leaf margin pubescence	Glabrous	Glabrous	Glabrous	Appressed	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous	Pubescent, with raised hair bases
Inflorescence	Clustered, 1 – 4 flowers	Solitary, (rarely 2) flowers	Clustered, 1 – 6 flowers	Clustered, 1 – 4 flowers	Clustered, 1 – 8 flowers (or more)	Solitary (occasionally 2 flowers)	Clustered, 1 – 8 flowers	Clustered, 1 – 8 flowers	Clustered, 2 – 7 flowers	Clustered, 3 – 8 flowers	Clustered, 1 – 6 flowers
Bracteole shape	Lanceolate	Lanceolate to oblanceolate	Narrowly ovate to lanceolate	Narrowly ovate	Narrowly ovate to lanceolate	Narrowly ovate	Narrowly ovate	Lanceolate to ovate	Ovate or recurved	Lobed	Ovate to elliptic-ovate
Bracteole length	11 – 19mm	9.8 – 18.8mm	9 – 23 mm	16 – 23 mm	7 – 26 mm	15.5 – 24.5 mm	9.8 – 20 mm	11.5 – 22.4 mm	11– 29 mm	16.4 – 36 mm	11.9 – 22.5 mm
Bracteole width	2 – 4 mm	1.5 – 3.6 mm	1.8 – 4.5 mm	2.2 – 4 mm	1 – 4.5 mm	1 – 3 mm	1 – 4.5 mm	1.7 – 6 mm	1 – 4.5 mm	5.5 – 12 mm	2 – 8 mm
Length to width ratio	3 – 8:1	4 – 7:1	3.5 – 8:1	6 – 7:1	4.5 – 11:1	7 – 14:1	4 – 8:1	4 – 8:1	5 – 8:1	3.5 – 5:1	2 – 8:1
Bracteole veins	3	3	3	3	1 – 2	1	1	3	3	3 – 5	3 – 5
Bracteole surface	Glabrous	Glabrous	Glabrous	Canescent	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous	Pubescent to glabrescent
Bracteole margin innovations	Glabrous, spiny	Glabrous, spiny	Glabrous, spiny	Canescent, serrate	Glabrous, sparsely toothed to spiny	Glabrous, sparsely toothed	Glabrous, spiny	Glabrous, spiny	Pubescent, spiny	Glabrous, spiny	glabrescent, sparsely toothed
Bracteole margin spine / teeth pubescence	Glabrous	Glabrous	Glabrous	Canescent	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous	Pubescent to glabrescent
Calyx lobe	Ovate	Elliptic-	Ovate to	Ovate	Ovate	Narrowly	Ovate	Ovate	Ovate	Broadly	Ovate to

shape		ovate to oblanceolate	narrowly ovate			ovate to lanceolate				ovate to lobed	broadly so
Posticous calyx lobe length	14.7 – 22.8 mm	11 – 17.8 mm	18.7 – 28.6 mm	20.2 – 25 mm	13.6 – 22.5 mm	13.5 – 24.1 mm	15.6 – 20.6 mm	17.3 –22.6 mm	18 – 29.8 mm	18.7 – 26.1 mm	15.9 – 29.6 mm
Posticous calyx lobe width	4.5 – 7.6 mm	3.2 – 6.9 mm	4.2 – 8.7 mm	7 – 8 mm	3.8 – 7.5 mm	2.5 –5.3 mm	3.6 – 7.6 mm	3.9 – 7 mm	4.8 – 10.1 mm	8.7 – 13 mm	5 – 13 mm
Posticous calyx lobe length to width ratio	2.2 – 3.9:1	2.4 – 4:1	3 – 5:1	2 – 3: 1	2.4 – 6:1	3.6 – 7:1	2.6 – 4.5:1	3 – 4:1	2 – 4:1	2 – 3:1	1.8 – 4:1
Anticous calyx lobe length	13 – 19.3mm	10.4 – 17.4 mm	11 – 26.7 mm	18 – 20.5 mm	10.7 – 17.5 mm	12 – 20.7 mm	12.3 – 17 mm	15 – 18.7 mm	16 – 25 mm	16.6 – 22 mm	14 – 26 mm
Anticous calyx lobe width	4.1 – 5.7 mm	3 – 6.9 mm	3.5 – 8.1 mm	6 – 8.5 mm	3.2 – 7.0 mm	2.1 –4.7 mm	3.2 – 6.6 mm	3.4 – 6.3 mm	4 – 8.7 mm	4.7 – 8 mm	4.3 – 13 mm
Calyx lobe surface	Glabrous	Glabrous	Glabrous	Canescent	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous	Pubescent
Calyx lobe veins	Rough	Rough	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Calyx lobe margin	Glabrous, spiny from base	Glabrous, spiny bottom half almost entire	Glabrous, spiny	Canescent, serrate	Glabrous, serrate	Glabrous, spiny	Glabrous, spiny	Glabrous, spiny	Pubescent, spiny	Glabrous, spiny	Pubescent, sparsely toothed
Calyx lobe margin spine	Glabrous	Glabrous	Glabrous	Canescent to glabrescent	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous	Pubescent to glabrescent

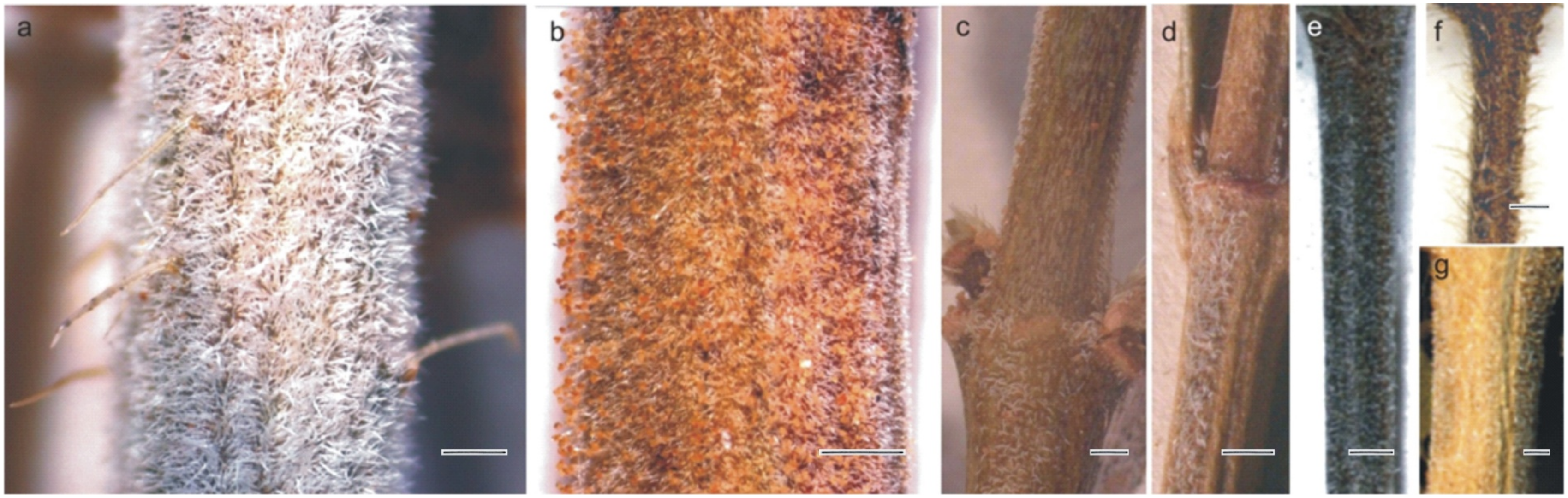

Figure 2.14. Variation in stem pubescence between the putative groups. *a*) JU: *de Winter 2739* (K) with ashy white erect eglandular hairs and scattered erect long tawny hairs; *b*) JU: *Giess and Leippert 7588* (NBG) with cream-white erect hairs with and without reddish-brown glandular heads; *c*) BI: *Hoffman 692* (GRA) with short white recurved hairs; *d*) BE: *Acocks 2121* (PRE) with short white recurved hairs; *e*) BP2: *Laidler 204* (PRE) with short white erect hairs; *f*) PU: *Chan 6* (GRA) with erect long brown hairs; *g*) IL: *Jürgens 22682* (PRE) with very short white recurved hairs. Though the stems in *a*, *b*, *e* and *f* are pubescent all round they exhibit variation in pubescence type. While the stems in *c*, *d* and *g* are bifariously hairy, with the hairs forming decussate stripes at each internode, hairs occur in longitudinal furrows in *d* as the stems in *c* and *g* become terete with age. BE = putative taxon from *B. bechuanensis*; BI = putative taxon from *B. irritans*; JU = *B. jubata*; BP2 = OTUs distinct from the rest of BP from *B. pungens*; IL = putative taxon from *B. rigida;* PU = putative taxa from *B. pungens*. Details of the abbreviations are as outlined in Appendix I. Scale bar: 2 mm.

iii) Inflorescence

The inflorescence varies from solitary flowers to clustered flowers that are lax or congested, born in alternate axils (sometimes in opposite axils). When clustered they are observed to form scorpioid cymes (Balkwill and Balkwill, 1997). The degree of compression differs more between species than within the various species / species complexes studied. While BB, BI, BP and PU have 1 – 6 flowers clustered in congested cymes in alternate axils, BE and BU have solitary, rarely 2 flowers per axil or in alternate axils, though the solitary flowers could also be found in opposite axils. 1 – 8 clustered flowers in alternate axils are observed in BR, IL, ILa and LB. Among OTUs from JU and LB no solitary flowers were observed, but only 3 – 8 congested flowers in alternate axils. Within *B. rigida* and *B. irritans* some flowers are born singly in leaf axils while in other plants within the same species, the lateral buds of the cymose cluster continue to develop forming scorpioid cymes (Balkwill and Balkwill 1994). Although the number of flowers is highly variable within most of the taxa in this study, it was observed to be stable within the BE and BU taxa and can imply that the lateral buds of the cymose cluster either have limited development or are aborted thus preventing the formation of scorpioid cymes (Balkwill and Balkwill, 1997).

iv) Bracteole

Bracteoles range from narrowly ovate to ovate, with glabrous, glabrescent or pubescent surfaces and margins that are spinous, with scanty teeth to almost entire or pubescent. The bracteole surface in BB, BE, BI, BR, BU, IL, ILa and LB is glabrous, with the bracteole margin equipped with firm glabrous spines or teeth; in BP and PU the bracteole surface is puberulus to glabrescent with the bracteole margin having coarse hairs and scanty teeth that are in turn glabrescent or pubescent with cream to yellow-brownish hairs except in BP2 (BP031 and BP096) which have short white hairs. The bracteole surface and long bracteole margin spine in JU is covered in white hairs. Variation in bracteoles include the number of veins; single-veined among BU and consistently with one or two faint or conspicuous veins parallel to the mid-vein among BB, BE, BI, BP, JU, LB and PU. Bracteole margins in BU have scanty teeth or almost entire, while bracteole margins in BI have teeth almost evenly distributed up to about half the length from the base along the margin of the 3-veined bracteoles (Figure 2.15d and e). Bracteoles of ILa can be differentiated from the rest of the *B. rigida* complex by the lanceolate leafy 3-veined lamina (Figure 2.15b). The ratio of bracteole length to the number of teeth on bracteole margin varies widely within ILa, revealing great within group variation which may be due to environmental factors (Figures 2.2 (iv) p) (Sebola and Balkwill, 2009). The bracteole in IL and BR is narrowly lanceolate-ovate and 1- or 2-veined with a hardened lamina (Figure 2.15c). Bracteole length also varies greatly. LB bracteoles have lobed or truncate bases and tapering tips and the outer veins parallel to the midrib form reticulations near the bracteole margins; and the bracteole lamina is usually broader than that of BR and IL (Figure 2.15a and c). Pubescence on bracteole surfaces and margin spines of PU and BP exhibit variation that is difficult to define, except for BP2 which are covered in very short white hairs. The number of veins appears taxonomically useful in distinguishing taxa within the *B. irritans* complex, while bracteole size (bracteole width) and shape is taxonomically valuable between taxa of the *B. rigida* complex. While the observed morphological gaps from quantitative continuous data (such as size) may suggest plasticity or the presence of ecotypes, those from quantitative discontinuous data (such as number of veins) could suggest breaks in gene flow (Darbyshire, 2008; Sebola and Balkwill, 2009).

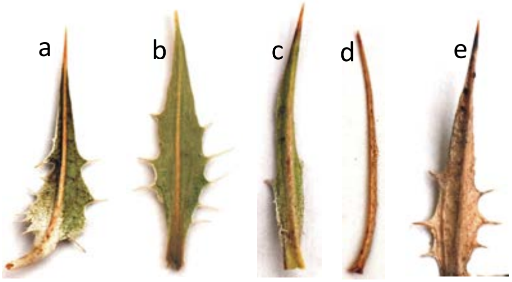

Figure 2.15. Variation in bracteole size and shape and margins between a few OTUs from the putative groups. *a*) LB: *Giess & van Vuuren 848* (PRE) with lobed base, developed lamina, spiny margin; *b*) ILa: *Giess 1787* (WIND) lanceolate, with developed lamina, spiny margin; *c*) BR: *Nyirenda 700* (J) narrowly ovate, with lamina reduced, almost entire margin; *d*) BU: *Oliver 2806* (GRA) with lamina reduced almost entirely, entire margin; *e*) BI: *Mauve 5238* (PRE) lanceolate-ovate, with developed lamina, spiny margin. BI, BU = putative taxa from *B. irritans*; BR, ILa, LB = putative taxa from *B. rigida.*

v) Calyx

The calyx in the study group is 4-lobed and the two broader outer (anticous and posticous) calyx lobes range from large broadly ovate and narrowly ovate to small lanceolate, while the two lateral lobes are small and narrowly ovate in all the taxa. However, where there is an overlap in calyx lobe size and shape within and between the species / species complexes, a distinction may be observed in calyx lobe surface and calyx lobe margin (Table 2.7). In BB the anticous and posticous lobe margins are toothed from the base while in BE they are almost entire on the bottom half and the teeth tend to be smaller. The calyx lobe margins in BR are usually serrate while in IL and ILa they are conspicuously spiny but have small delicate teeth in LB. The posticous calyx lobe is broadly ovate and lobed or truncate at the base in LB as opposed to the rest of the *B. rigida* complex where it is narrowly ovate, ovate-lanceolate or elliptic-ovate and somewhat attenuate at the base. The ratio of posticous calyx lobe length to calyx lobe width distinguishes LB from the rest of the taxa within *B. rigida*. The ratio of posticous calyx lobe length to total number of teeth on calyx lobe margin shows great within group variation (within LB) and overlaps narrowly with BR and ILa (Figure 2.2 (vi) v).

Within *B. jubata* the distinct OTU had purple-tinged calyx lobes with persistent glandular hairs on the calyx lobe surface while the rest of the JU OTUs had green calyx lobes with glandular hairs only observed on young (immature) calyx lobes of a few (not all) JU OTUs. The calyx lobe margins in BP2 are serrate and covered in white erect very short hairs and scattered long adpressed hairs while they are sparsely toothed to entire or hairy with yellowish-cream erect long hairs in the rest of BP and PU. While the dissimilarity in calyx lobe size, shape and calyx lobe margins can be used to separate putative taxa within the *B. rigida* complex, it is not taxonomically important within the *B. pungens* complex. While differentiation in pubescence type and colour the on calyx lobe surface and margins within the *B. pungens* complex is valuable, pubescence density cannot be defined.

2.5.2 Distinct clusters

Using the standard taxon as a measure for delimiting species, groups that formed clusters above or just about the point where OTUs of the standard formed a cluster were delimited at the level of species (Figure 2.3a). On the basis of X as the standard cluster, the combined data matrix of qualitative and quantitative characters revealed that clusters 1 – 7 can be delimited at the level of species. From the data matrix of qualitative characters, the standard taxon does not show within group variation among the OTUs (based on the selected characters bringing about differentiation i.e. with eigen values ≥ 0.6) which makes it difficult to decide where to draw the phenon line to delimit the species (Figure 2.3b). Clusters were, however, examined for consistency in comparison with those obtained in Figure 2.3a. The phenon line drawn at approximately 0.7 gives an approximate coefficient of dissimilarity estimate of 0.7 allowing for some amount of variance within the standard taxon because objects that share every attribute in the attribute set have a zero coefficient of dissimilarity even though they have other dissimilarities (Baulieu, 1989). The cophenetic correlation coefficients (r) above 0.8 obtained for the phenograms (Figures 2.3 to 2.5) support the association between the data and the phenograms for all the data matrices (Cron *et al.,* 2007; Dunn and Everitt, 1982; Gilmartin and Harvey, 1976; Işik, 1983; Lessig, 1972; Peres-Nato and Jackson, 2001). Therefore, clusters were delimited with confidence.

The *B. pungens* and *B. rigida* complexes when analysed with the entire study group were observed to nest among other putative taxa. In addition, OTUs from the different groups within the complex displayed mixing, presenting differentiation that could not be defined (phenogram not included). Although the number of characters reduced tremendously when the two species complexes were analysed separately (as most of the potential taxonomic characters became invariant within the two complexes), each split into two major entities. The 13 and 12 characters used were considered sufficient as few characters have been used before in analyses to distinguish taxa at the level of species and subspecies in *Merxmuellera* (Poaceae) (Barker, 1999), *Senecio* (Asteraceae) (Hodálová and Marhold, 1998), *Isoglossa* (Acanthaceae) (Darbyshire, 2009) and *Barleria* (Acanthaceae) (Darbyshire, 2009) with 8, 11, 8 and 8 characters respectively (Barker, 1999; Darbyshire, 2009; 2009; Hodálová and Marhold, 1998).

Although BI145 of the *B. irritans* complex with entire bracteole margins and entire calyx lobe margins did not cluster with the rest of the OTUs, another OTU BI079 had bracteoles with both entire and toothed margins as well as calyx lobes with toothed and almost entire margins. BI079, therefore, can be used as a bridge between BI145 and the rest of the BI cluster, even though BI145 (cluster 4) diverged from the BI / BU group at some point below where the standard taxon clusters. Therefore, only the two major clusters of BI and BU are recognised within the *B. irritans* complex. Within *B. jubata*, the dissimilarity distance between JU141 and the rest of JU cluster is long and the two groups differentiate way below the point where the standard taxon clusters, justifying the recognition of the two clusters at the level of species. Within *B. bechuanensis* two well defined clusters can be established from qualitative and combined data matrices

BP2 (represented by BP031 and BP096) formed a distinct cluster (cluster 2) while the rest of the OTUs of the *B. pungens* complex formed cluster 1 (Figures 2.4a and 2.4b. BP034 collected from Formosa, Plettenberg Bay differs from the rest of the cluster by having a glabrous bracteole surface (including on the veins), although the leaf resembles those of BP019 collected from Riversdale and BP153 collected from Bushmans River mouth, differing only in intensity of indumentum (Figure 2.4b). The morphological differentiation between BP034 and the OTUs of the rest of cluster 1 is observed in indumentum intensity, a character that is highly variable within the complex. Therefore, BP034 is still considered to be part of cluster 1 as illustrated by Figure 2.4a, resulting in the recognition of clusters 1 and 2. The *B. rigida* complex can be separated into two distinct clusters based on the long dissimilarity distance observed between clusters 1 and 2 in relation to their point of differentiation (Figure 2.5). LB formed one distinct cluster while BR and IL combined to form another major cluster. Cluster analysis therefore showed that the *B. bechuanensis*, *B. irritans*, *B. jubata*, *B. pungens* and *B. rigida* complexes could be split into two distinct groups each, which can be delimited at the level of species.

Ordination using combined data matrices however, exhibits a gap between BI145 and the rest of the major BI cluster, implying there is a morphological gap (Figure 2.6, BI with arrow). The 2- and 3-dimensional scatter plots from combined data matrices reveal two well defined clusters with clear gaps that may in turn have taxonomic implications (Figures 2.6 and 2.8). Ordination using PCO on combined matrices also demonstrated the formation of a defined JU cluster except for one distant JU as well as a BB and BE clusters (Figures 2.5 and 2.6). The gaps around the JU, BE and BB from the two- and three-dimensional scatter plots from PCO on combined matrices are likely to be of taxonomic importance as morphological gaps represent gaps in gene flow (Figures 2.6 and 2.8).

Clusters from 2- and 3-dimensional scatter plots from quantitative data matrices are not well defined. Two- and 3-dimensional plots from quantitative data matices mixing of OTUs where even OTUs of the standard taxon were observed to cluster with JU (Figure 2.7). Mixing of OTUs among clearly differentiated species suggests that there is great overlap in quantitative characters. Though EL and JU are clearly morphologically distinct, they cannot be differentiated along the first and second axes (Figure 2.7). Ordination recovered the clusters from CA and revealed an overlap between BR and IL (Figure 2.11). LB formed a distinct cluster (cluster 2) with a very clear gap surrounding the cluster (Figure 2.11). Differentiation was observed along the first axis which accounts for more than 60% of the variation. Given that the gap surrounding a cluster indicates discontinuities, the observed gap between clusters 1 and 2 can be assumed to represent morphological gaps which are taxonomically important (Dunn and Everitt, 1982; Sebola and Balkwill, 2009).

Athough BP and PU taxa were distinguished by the absence and presence of a white leaf margin respectively, intermediates with leaves with thin white leaf margins and leaves with green leaf margins were observed on the same OTU for those forming cluster ‘b’ (Figure 2.4b). Cluster ‘a’ was observed to comprise 2 sub-clusters; typical PU OTUs with a distinct white bracteole margin (in addition to the white leaf margins) or just conspicuous white teeth on bracteole margins, and typical BP OTUs with green leaf and bracteole margins and no white teeth on the bracteole margin. However, some intermediates such as PU015, BP001, BP030, PU041, BP038, BP039 and BP027 either had white bracteole margins or white teeth and green leaf margins on the same OTU while intermediates such as BP018, BP032, BP098, BP036, BP019 and BP026 had green leaf margins and very thin white leaf margins (but not bracteole margins). The integration of OTUs from the two matrices (combined qualitative and quantitative and qualitative characters) of BP and PU (except for BP031 and BP096) may imply that it may be a single species (Figures 2.4a and 2.4b).

2.5.3 Distribution

Although both BB and BE of the *B. bechuanensis* complex, have limited and clustered distributions in the Northern Cape, the two are morphologically distinct yet not geographically separated (Figure 2.12a). They however, may be spatially separated as a result of habitat differentiation, but it is not likely that the differentiation is due to phenotypic plasticity as there was no intermediate morphology observed. It can be deduced that the observed gaps in morphology are a result of breaks in gene flow (Aizen *et al.,* 2002; Maso *et al.,* 2004). Their restriction to the Thornveld may be due to habitat specificity which could in turn result in endemism (Makholela, 2003). In the absence of geographical isolation, the presence of distinct morphology could imply that other barriers exist, bringing about breaks in gene flow. It may be suggested that speciation could have occurred in sympatry (Brysting and Elven, 2000; Davis and Heywood, 1963; Stebbins, 1967; Van Valen, 1976).

BI and BU exhibit a narrow distribution range overlap though they formed morphologically discrete clusters (Figure 2.12a). The narrow geographic overlap may be due to habitat heterogeneity at point of overlap (Brysting and Elven, 2000; Richards *et al.,* 2005; Samis and Eckert, 2009; Schemske *et al.,* 1994; Strand and Weisner, 2004; Van Valen, 1976; Xu *et al.,* 2007). However, habitat varies along an environmental gradient and as species interact with their environments, the species-environment interactions in turn influence and determine their distribution, as they establish adaptive zones (Bennett, 1987; Schmidt-Lebuhn *et al.,* 2007; Van Valen, 1976). Varying selective pressures (i.e. in response to dissimilar environmental constraints) coupled with reduction in gene flow, with time can bring about diversion in morphology between populations (Laurance, 2008; Lomolimo, 2000). On the basis of distribution and distinct morphology BI and BU may be assumed to be parapatric species.

The two clusters within *B. jubata* demonstrate limited and disjunct distributions in the north-western and central Namibia (Figure 2.12a). JU141 (JU2) is not only geographically disjunct, belonging to an isolated population in Kaokoveld but is also morphologically dissimilar. JU2 could be subject to environmental conditions different from those of the Waterberg Plateau where the rest of the OTUs of the main JU cluster occur. The main JU cluster has a limited distribution of one degree north-south and two degrees east-west at altitudes ranging from 1500 to 1900m. Examination of other specimens similar to JU2 (not included in the analysis) revealed that they occurred in the Kaokoveld and surrounding areas in the Namib Desert at altitudes ranging from 300 to 1300m. The JU2 cluster has a wider distribution range spanning six degrees north-south and five degrees east-west. Although JU and JU2 appear to overlap around Otjiwarongo, the two clusters exhibit habitat differentiation as one occupies a lower altitude and the other occupies a higher altitude. The small and limited distribution range of *B. jubata* could suggest that it may be endemic to the Waterberg Plateau (Balkwill, 1993; Makholela, 2003). In view of the spatial differentiation (in addition to the morphological dissimilarity) between JU and JU2, it is likely that there is a gene flow barrier between the two populations, bringing about the observed dissimilarity (Richards *et al.*, 2005).

The limited number of OTUs within JU could imply either that this taxon is rare and has a reduced habitat in view of rapid habitat loss, or that it has simply been under-collected (Hijmans and Spooner, 2001; Makholela *et al.*, 2003; Roalson *et al.*, 2002). It can then be argued that in view of the spatial differentiation clusters 6 and 7 (Figures 2.3a and 2.3b) be recognised as two separate species (Davis and Heywood, 1963; Işik, 1983; Laurance, 2008; Lomolimo, 2000). Although ecological studies were not undertaken, it can be suggested from the differentiation in distribution ranges of JU and JU2 that, possibly parapatric speciation could have brought about the observed dissimilarities between the two taxa (Bennett, 1987; Van Valen, 1976).

A notable observation within the *B. pungens* complex was that, PU (with a distinct white leaf margin and white bracteole margins) is geographically restricted to the Eastern Cape and with a small localized distribution in the Western Cape whereas BP (with green leaf margins and bracteole margins) is widespread extending from the southern coast of the Eastern Cape to the southern coast of the Western Cape (Figure 2.12b). However, intermediate morphology (with thin white leaf or bracteole margins) also occurred throughout the range of BP; more so where the two (typical BP and PU) overlapped in their distribution ranges. The absence of PU in the intermediate zones could imply that this phenotype has a minimally adaptive zone dissimilar to that of BP with continuous distribution (Schmidt-Lebuhn *et al.*, 2005; Van Valen, 1976). The presence of BP, PU and the intermediates together could also imply that there may be habitat heterogeneity in the zone of overlap (Aizen *et al.*, 2002; Balkwill *et al.*, 1994; Brysting and Elven, 2000; Masćo *et al.*, 2004; Richards *et al.*, 2005; Samis and Eckert, 2009; Schemske *et al.*, 1994; Strand and Weisner, 2004; Van Valen, 1976; Xu *et al.*, 2007).

Although there is disjunction in the distribution of PU, the great overlap in phenotypes between BP and PU coupled with the continuous distribution of BP could imply that BP and PU may be one species with a diverse genetic variability, each of them with an adaptive zone (Schmidt-Lebuhn *et al.*, 2005; Van Valen, 1976). In addition natural communities do not respond to single environmental variables but a combination of factors resulting in intermediate morphology (Burke, 2002; Friis and Gilbert, 1985). Therefore, the lack of a clear-cut spatial and morphological separation between BP and PU argues against recognition of subspecies or varieties (Burke, 2002; Friis and Gilbert, 1985). It can be deduced then, that the observed variation between the two putative taxa may be a result of plasticity influenced by environmental conditions (Strand and Weisner, 2004). The limited number of OTUs (two) and localised distribution of BP2 at the periphery of PU could imply that this taxon is rare and has a restricted distribution or that it has been under-collected (Hijmans and Spooner, 2001; Makholela *et al.*, 2003; Roalson *et al.*, 2002).

Clusters within the *B. rigida* complex exhibited differentiated distribution with narrow overlaps. LB (cluster 2) occupies coastal sands of the South Namib Desert whereas IL (cluster 1c) extends from north of Central Namib Desert in Namibia, into South Namib, Gariep and Kalahari Deserts in South Africa at the fringe of ILa (cluster 1b) and overlapping with BR (cluster 1a) at the southern border of Namibia with South Africa (Figures 2.5, 2.11 and 2.12b) (Mucina and Rutherford, 2006). ILa (cluster 1b) inhabits the Namibian central plateau on the western side of the Kalahari Desert, an intermediate zone between BR (cluster 1a) on the eastern side and IL on the western side (Figures 2.5 and 2.11). BR (cluster 1a) is the most widespread extending from the Free State through south east Namibia into south west Botswana in the Kalahari Desert (Figure 2.12b). ILa not only exhibits intermediate distribution between BR and IL, it also displays intermediate morphology which may suggest that it could be a result of gene flow between BR and IL due to secondary contact caused by the collapse of barriers between two previously separated species (Schmidt-Lebuhn *et al.*, 2007).

However, intermediate morphology could also imply plasticity (in a taxon with diverse genetic variability) influenced by environmental differences (Stebbins, 1967). While BR mainly inhabits the upper karoo, IL occupies the desert and semi-desert and ILa occupies the savanna and these environmental variations could be influencing the morphology of BR, IL and ILa (Burke, 2002). The restricted occurrence of ILa in the central and southern part of Namibia at the periphery of BR may be a result of physical environmental constraints as geology can influence soil properties which in turn have an effect on the distribution of plant communities (Bennett, 1987; Boyd *et al.*, 2008; Burke, 2002; Mucina and Rutherford, 2006; Samis and Eckert, 2009; Van Valen, 1976). The geographic range of any taxon tends not to extend beyond where survival and fitness along the ecological gradient cannot sustain individuals (Bennett, 1987; Samis and Eckert, 2009). It can therefore be argued that, although there is a possibility of gene flow between BR and IL, the morphology in ILa could be influenced by edaphic factors (Borcard *et al.,* 1992; Heywood, 1986). In addition, the spatial differentiation and overlap in morphology may suggest that these three groups form a continuum that is separated by ecological conditions (Sebola and Balkwill, 2009; Stebbins, 1967).

2.5.4 Recognisable groups based on species concepts

Although BB and BE exhibit an overlap in their distribution ranges, the two clusters maintain morphological distinctness with no intermediates implying a gene flow barrier. BB and BE can be differentiated by leaf width, leaf shape, leaf margins, leaf apex, shape of the calyx lobes and the number of flowers. BB and BE can be distinguished by ordinary means on the basis of morphological dissimilarity in more than three characters. In view of the observed difference, BB and BE can be accorded the status of species based on the Morphological Species Concept (as *sp. 1* and *sp. 2* (*sp. nov.*)) (Mayr, 1970; Stebbins, 1967; Van Valen, 1976). BI and BU can be distinguished by the number of veins in the bracteole (3-veined in one and single veined in another), size and shape of the outer calyx lobes (broad and ovate in one and narrow and lanceolate in the other) and the number of flowers (1 – 6 in one and solitary or two on rare occasions in the other). BI and BU also exhibit morphological discreteness by a combination of diagnostic characters (Cabido *et al.*, 1992). The differentiation in both discrete and continuous quantitative characters between BI and BU may suggest that the dissimilarity may be due to other biological factors other than response to environmental constraints (Figure 2.12a) (Heywood, 1986; Sebola and Balkwill, 2009). Having been phenetically and morphologically categorised, BI and BU can be recognised at species level (as *sp. 3* and *sp. 4* (*sp. nov.*)) (Figure 2.3a).

Within *B. jubata,* clusters 6 and 7 with one OTU can be recognised by the presence or absence of hairs with brownish glandular heads on the stem and bracteole, glabrous leaf, recurved bracteoles, persistence of hairs with brownish glandular heads on the calyx lobe surfaces and purple tinged calyx lobes in addition to spatial disjunction. However, young calyx lobes within cluster 6 also possessed hairs with brownish glandular heads (but were absent in mature calyx lobes). When variation is observed in three or more distinct characters, specific recognition is accorded (Brysting and Elven, 2000; Cabido *et al.,* 1992; Ensermu, 1994; Stebbins, 1967). Therefore, on the basis of morphological and phenetic dissimilarity clusters 6 and 7 are recognised as separate species (*sp. 5* and *sp. 6*) (Figures 2.3a and 2.3b).

Within the *B. pungens* complex, cluster 1 (BP and PU) exhibit an overlap in almost all the characters; with no clear-cut morphological character separating them (Figure 2.2 (i) – (vi)). Cluster 2 (BP2) can be differentiated from cluster 1 by stem pubescence, leaf margin, pubescence on the bracteole and calyx lobe surface and calyx lobe margins though BP2 occurred at the western periphery of the distribution range of BP-PU creating a narrow geographic overlap (Figure 2.12b). Although cluster analysis established a hierarchical non-overlapping association among OTUs forming the BP-PU group, this cluster, however, is difficult to define (Figures 2.4a and 2.4b). Based on the morphological species concept, cluster 1 (BP-PU) is a single species exhibiting phenotypic plasticity (Balkwill *et al.*, 1994, 1996; Brysting and Elven, 2000; Mascó et al., 2004; Samis and Eckert, 2009; Schemske *et al.,* 1994; Strand and Weisner, 2004; Van Valen, 1976). Cluster 1 and 2 are morphologically distinct and can therefore be recognised at species level as *sp. 7* and *sp. 8* (Brysting and Elven, 2000).

The *B. rigida* complex, presents overlapping and non-overlapping variation (Figures 2.5 & 2.2). Discrete / discontinuous variation is observed between cluster 2 (LB) and cluster 1 (BR, IL and ILa). Even though clusters 1 and 2 have a narrow distribution range overlap, LB differs from cluster 1 by bracteole size and shape, posticous calyx lobe size and shape. In view of the morphological distinctness that LB exhibits from the rest of the *B. rigida* complex, in more than three distinct characters in the presence of geographical distribution overlap, it can be recognised as a separate species (*sp. 9*). In the absence of geographical disjunction, spatial differentiation together with discreteness in several other characters can therefore result in the recognition of species (Balkwill, *et al.,* 1992; Sebola and Balkwill, 2009; Darbyshire, 2009).

BR and IL can be differentiated by leaf shape and leaf margin; linear-lanceolate with undulate margins in the one and ovate with sinuate margins in the other, though intermediates with lanceolate-ovate leaves with shallowly undulate to sinuate leaf margins may be seen. IL and ILa on the other hand can only be distinguished one from another, by the number of veins in the bracteole as leaf shape, bracteole size and shape, and calyx lobe margins reveal some overlap. ILa and BR both of which have linear-lanceolate leaves can only be separated by the number of veins in the bracteole as the two portray a high degree of overlap in leaf margin spinescence and undulation. BR, IL and ILa may be considered as one species with diverse morphology (*sp. 10*) because overlapping characters are not taxonomically useful (Wilkin, 1999).The dissimilarity exhibited by BR, IL and ILa could be clinal, the three putative taxa can only be recognised at the level of variety within *sp. 10* (*sp. 10 var. A*, *sp. 10 var. B (var. nov. B*) and *sp. 10 var. C (var. nov. C*) repectively). In regard to the lack of sufficient clear-cut morphological distinctness to go with the spatial segregation, dissimilarity in one distinct character with or without geographical isolation can be named at the level of variety (Brysting and Elven, 2000).

2.6 Conclusion

Although taxa within complexes are difficult to distinguish due to intergradations, cluster analysis did separate the OTUs into groups that can be used in classification by imposing a hierarchical non overlapping association among OTUs (Cron *et al.,* 2007; Işik, 1983; Otieno *et al.,* 2006; Watson and Estes, 1990). In addition, ordination clearly demonstrated the gaps that represent morphological discreteness and overlaps which indicate morphological continuity (Dunn and Everitt, 1992; Sneath and Sokal, 1973; Sokal and Sneath, 1963). The persistence of isolated populations between the two taxa in the *B. bechuanensis* complex despite overlapping distribution suggests that there is no gene flow. They are therefore recognised at species level being distinguished by leaf margin, leaf shape, leaf width and the ratio of leaf length to leaf width. The taxa within the *B. irritans* complex on the other hand, can be differentiated on the basis of bracteole width, the number of parallel veins in the bracteole, calyx lobe shape, the number of flowers per axil and stem pubescence, and here too, two species are recognised.

Although the distribution of *B. jubata* and the one OTU displays disjunct distribution, suggesting that there could be allopatric speciation, however, persistence of morphological distinctness between specimens similar to JU1141 and the rest of the JU cluster suggests that the two are discernable. Therefore, two species are recognised within *B. jubata*. Apart from the two distinct OTUs *Barleria pungens* exhibits extensive variation in hair intensity on the leaf margins, midrib and leaf margin spines as well as on the bracteole and calyx lobe which is difficult to define. The presence, however, of intermediates with thinly white coloured leaf margins and green leaf margins on the same specimen could suggest that there is gene flow between specimens with green and white coloured leaf margins (Aizen, *et al.*, 2002; Brysting and Elven, 2000). Since both putative groups BP and PU exhibit variation in hair density, the variation in pubescence could be arising from plastic responses (Brysting and Elven, 2000; Samis and Eckert, 2009; Strand and Weisner, 2004; Van Valen, 1976). The variation in presence or absence of white colour on the bracteole margins may be recognised at the level of form. However, the disjunct distribution of white leaf margins could also suggest the presence of cryptic species (Boyd *et al.*, 2008; Stace, 2000). Within the *B. rigida* complex, BR / IL / ILa and LB can be recognised at species level due to their distinctness, however, it is suggested that ILa is a cline that may be associated with environmental factors and seems to be closer to BR possibly due to introgression.

2.7 References

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2.8 Appendices

2.8.1 Appendix I: Taxa abbreviations

BB: specimens from *B. bechuanensis* complex with wavy leaf margin
BE: specimens from *B. bechuanensis* complex with flat leaf margin
BI: specimens from *B. irritans* complex with broad 3-veined bracteole; bracteole margin spiny
BU: specimens from *B. irritans* complex with narrow 1-veined bracteole; bracteole margin entire or scantly toothed
BP: specimens from *B. pungens* complex with green leaf margin
PU: specimens from *B. pungens* complex with white leaf margin
BR: specimens from *B. rigida* complex with spineless leaf margin (or with minute scanty teeth); leaf narrowly ovate or linear-lanceolate; bracteole base not lobed
IL: specimens from *B. rigida* complex with spiny sinuate leaf margin; leaf ovate to elliptic; bracteole base not lobed
ILa: specimens from *B. rigida* complex with spiny sinuate leaf margin; leaf linear; bracteole base not lobed
LB: specimens from *B. rigida* complex with spiny leaf margin; leaf ovate; bracteole base lobed
JU: all specimens from *B. jubata*

2.8.2 Appendix II: Preliminary character list for the 5 Barleria species / species complexes

Stem

1. Stem surface glabrous (0), Stem surface glabrescent to pubescent (1) **Stem surf**
2. Stem surface bifariously hairy (0), Stem surface hairy all round (1), Other (2) **Stem pub**

Leaf

3. Mature leaf surface glabrous (0), Mature leaf surface sparsely pubescent, i.e. on veins only (1), Mature leaf surface markedly pubescent (2) **Leaf surf**
4. Leaf ovate (0), Leaf lanceolate (1), Leaf elliptic (2), Leaf linear (3) **Leaf shp**
5. Leaf margin white (0), Leaf margin not white (1) **Marg colr**
6. Mature leaf margin glabrous (0), Mature leaf margin with horizontal hair (1), Mature leaf margin with erect hair (2) **Marg pub**
7. Leaf margin hair on raised hair bases (0), Leaf margin hair not on raised hair bases (1), Other (2) **Hair base**

8. Mature leaf margin thick (0), Leaf margin not thick (1) **Marg thick**
9. Mature leaf margin entire (0), Mature leaf margin minutely toothed (1), Mature leaf margin conspicuously but scantly toothed to almost entire (2), Mature leaf margin conspicuously and markedly toothed (3), Other (4) **Marg ent**
10. Leaf margin flat (0), Leaf margin shallowly wavy (1), Leaf margin sinuate (2), Leaf margin undulate or deeply so (3) **Marg strt**
11. Midrib on mature leaf glabrous (0), Midrib on mature leaf glabrescent (1), Midrib on mature leaf markedly pubescent (2) **Midrib**
12. Leaf lateral veins conspicuous on lower leaf surface (0), Lateral veins inconspicuous on lower leaf surface (1) **Lat vein**
13. All leaf bases on OTU uniform (0), Leaf bases on OTU variable (1) **Leaf bas**
14. Leaf length from the base of the petiole to the leaf apex **L**
15. Leaf width measured at the widest point **W**
16. Leaf length from the base to the widest point **Leaf WP**
17. Petiole length **PL**
18. Ratio of leaf length to leaf width **L: W**
19. Ratio leaf length to length from the base to the widest point **L: Leaf WP**
20. Ratio of leaf length to petiole length **L: PL**
21. Length of leaf apex spine **SL**
22. Length of leaf margin spine/tooth **M**
23. Number of teeth at ¼ top length from apex on leaf margin **L #teeth1**
24. Number of teeth at ½ top length from apex on leaf margin **L #teeth2**
25. Number of teeth at ¼ bottom length from base on leaf margin **L #teeth3**
26. Number of teeth at ½ bottom length from base on leaf margin **L #teeth4**

Inflorescence

27. Flowers solitary (0), Flowers clustered (1) **Flrs**

Bracteole

28. Bracteole abaxial surface glabrous (0), Bracteole abaxial surface pubescent on veins only (1), Bracteole abaxial surface markedly pubescent (2) **B surfab**
29. Bracteole adaxial surfaces glabrous, (0), Bracteole adaxial surface puberulus or pubescent, (1) **B surfad**
30. Bracteole abaxial surface glossy (0), Bracteole abaxial surface not glossy (1) **B abglos**
31. Bracteole adaxial surface glossy (0), Bracteole adaxial surface not glossy (1) **B adglos**
32. Bracteole a spine or almost a spine (0), Bracteole clearly not a spine (1) **Braspn**
33. Bracteole hardened (ligneous) (0), Bracteole leafy (membranous or papery) (1) **Brahard**
34. Bracteole base lobed (0), Bracteole base not lobed (1) **Bralob**
35. Bracteole with 1 conspicuous vein (0), Bracteole with more than 1 conspicuous vein parallel to the mid-vein (1) **Bravein**
36. Bracteole parallel veins forming conspicuous reticulations near the bracteole margin (0), Bracteole parallel veins not forming conspicuous reticulations near the margin (1) **Bv consp**
37. Bracteole margin entire (0), Bracteole margin minutely toothed (1), Bracteole margin scantly toothed to becoming entire (2), Bracteole margin with conspicuous and numerous teeth/spine (3) **B margent**

38. Bracteole margin and or bracteole margin spine / tooth white (0), Bracteole margin and or bracteole margin spine / tooth not white (1) **B margcolr**
39. Bracteole margin glabrous (0), Bracteole margin puberulus (with short hair) (1), Bracteole margin pubescent (with long straight hair) (2), Bracteole margin pubescent (with short and long straight hair) (3) **B margpub**
40. Bracteole margin spine / tooth glabrous (0), Bracteole margin spine / tooth glabrescent (1), Bracteole margin spine / tooth markedly pubescent (2) **B margspn**
41. Bracteole apex with long tip (about half the bracteole length) (0), Bracteole apex with short tip (less than a third the length of the bracteole) (1) **Brapex**
42. Bracteole present (0), Bracteole absent (1) **Bractle**
43. Bracteole length from the base to the apex measured at midvein **BL**
44. Bracteole width at widest point **BW**
45. Length of bracteole from the base to the widest point **BWP**
46. Ratio of bracteole length to bracteole width **BL: BW**
47. Bracteole apex spine length **BS**
48. Ratio of bracteole apex spine length: bracteole length **BS: BL**
49. Number of teeth at ¼ top length from apex on bracteole margin **B# teeth1**
50. Number of teeth at ½ top length from apex on bracteole margin **B# teeth2**
51. Number of teeth at ¼ bottom length from base on bracteole margin **B# teeth3**
52. Number of teeth at ½ bottom length from base on bracteole margin **B# teeth4**
53. Total number of spines/ teeth on bracteole margin **B# totteeth**

Calyx

54. Abaxial surface of outer calyx lobe (anticous / posticous lobe) glabrous (0), Abaxial surface of outer calyx lobe (anticous / posticous lobe) pubescent (1) **Cal surf1**
55. Adaxial surface of outer calyx lobe (anticous / posticous lobe) glabrous (0), Adaxial surface of outer calyx lobe (anticous / posticous lobe) pubescent (1) **Cal surf2**
56. Anticous / posticous calyx lobe hardened / ligneous (0), Anticous / posticous calyx lobe membranous, papery (1) **Calhard**
57. Anticous / posticous calyx lobe margin glabrous (0), Anticous / posticous calyx lobe margin pubescent (1) **Calpub**
58. Anticous / posticous calyx lobe margin densely spiny / toothed (0), Anticous / posticous calyx lobe margin sparsely spiny / toothed (1) **Calmarg**
59. Anticous / posticous calyx lobe margin spine / tooth glabrous (0), Anticous / posticous calyx lobe margin spine / tooth glabrescent (1), Anticous/posticous calyx lobe margin spine / tooth pubescent (2) **Calmgspn**
60. Posticous calyx lobe overlapping anticous calyx lobe (0), Posticous calyx lobe overlapping anticous calyx lobe (1) **Cal ovlap**
61. Anticous calyx lobe length from base to apex **CYL-A**
62. Anticous calyx lobe width at widest point **CYW-A**
63. Anticous calyx lobe apex spine length **CYS-A**
64. Length of anticous calyx lobe from the base to widest point **CYL-AWP**
65. Ratio of anticous calyx lobe length to length of calyx lobe apex spine length **CYL-A: CYS-A**
66. Ratio of length of anticous calyx lobe to calyx lobe width **CYL-A: CYW-A**
67. Ratio of length of anticous calyx lobe to calyx lobe length from base to widest point **CYL-A: CYL-AWP**
68. Number of teeth at ¼ top length of anticous calyx lobe margin from apex **A #teeth1**
69. Number of teeth at ½ top length of anticous calyx lobe margin from apex **A #teeth2**

70. Number of teeth at ¼ bottom length of anticous calyx lobe margin from base **A #teeth3**
71. Number of teeth at ½ bottom length of anticous calyx lobe margin from base **A #teeth4**
72. Total number of teeth on anticous calyx lobe margin **A# totteeth**
73. Posticous calyx lobe length from base to apex **CYL-P**
74. Posticous calyx lobe width measured at widest point **CYW-P**
75. Posticous calyx lobe apex spine length **CYS-P**
76. Posticous calyx lobe length from base to the widest point **CYL-PWP**
77. Ratio of length of posticous calyx lobe to length of apex spine **CYL-P: CYS-P**
78. Ratio of length of posticous calyx lobe to calyx lobe width **CYL-P: CYW-P**
79. Ratio of length of posticous calyx lobe to length of calyx lobe from base to widest point **CYL-P: CYL-PWP**
80. Number of teeth at ¼ top length of posticous calyx lobe from apex P# **teeth1**
81. Number of teeth at ½ top length of posticous calyx lobe from apex P# **teeth2**
82. Number of teeth at ¼ bottom length of posticous calyx lobe from base **P# teeth3**
83. Number of teeth at ½ bottom length of posticous calyx lobe from base **P# teeth4**
84. Total number of teeth on posticous calyx lobe margin **P# totteeth**
85. Ratio of bracteole length to anticous calyx lobe length **BL: CYL-A**
86. Ratio of bracteole width to anticous calyx lobe width **BW: CYW-A**
87. Ratio of bracteole length to posticous calyx lobe length **BL: CYL-P**
88. Ratio of bracteole width to posticous calyx lobe width **BW: CYL-P**

2.8.3 Appendix III: Final list of characters used in the various analyses for the 5 Barleria species / species complexes

Stem

1. Stem surface glabrous (0), Stem surface glabrescent to pubescent (1) **Stemsurf**
2. Stem surface bifariously hairy (0), Stem surface hairy all round (1), Other (2) **Stempub**

Leaf

3. Mature leaf surface glabrous (0), Mature leaf surface sparsely pubescent, i.e. on veins only (1), Mature leaf surface markedly pubescent (2) **Leafsurf**
4. Leaf margin white (0), Leaf margin not white (1) **Margcolr**
5. Mature leaf margin glabrous (0), Mature leaf margin with horizontal hair (1), Mature leaf margin with erect hair (2) **Margpub**
6. Leaf margin hair on raised hair bases (0), Leaf margin hair not on raised hair bases (1), Other (2) **Hairbase**
7. Mature leaf margin thick (0), Leaf margin not thick (1) **Margthick**
8. Mature leaf margin entire (0), Mature leaf margin minutely toothed (1), Mature leaf margin conspicuously but scantly toothed to almost entire (2), Mature leaf margin conspicuously and markedly toothed (3), Other (4) **Margent**
9. Leaf margin flat (0), Leaf margin shallowly wavy (1), Leaf margin sinuate (2), Leaf margin undulate or deeply so (3) **Margstrt**
10. Midrib on mature leaf glabrous (0), Midrib on mature leaf glabrescent (1), Midrib on mature leaf markedly pubescent (2) **Midrib**
11. Leaf lateral veins conspicuous on lower leaf surface (0), Lateral veins inconspicuous on lower leaf surface (1) **Latvein**
12. Leaf width measured at the widest point **W**
13. Ratio of leaf length to leaf width [≤ 2.5 (0), > 2.5 (1)] **L:W**
14. Ratio of leaf length to petiole length **L:PL**

15. Leaf apex spine length **SL**
16. Length of leaf margin spine/tooth [≤ 0.9 mm (0), > 0.9 mm (1)] **M**

Bracteole

17. Bracteole abaxial surface glabrous (0), Bracteole abaxial surface pubescent on veins only (1), Bracteole abaxial surface markedly pubescent (2) **Bsurfab**
18. Bracteole adaxial surfaces glabrous, (0), Bracteole adaxial surface puberulus or pubescent, (1) **Bsurfad**
19. Bracteole abaxial surface glossy (0), Bracteole abaxial surface not glossy (1) **Babglos**
20. Bracteole adaxial surface glossy (0), Bracteole adaxial surface not glossy (1) **Badglos**
21. Bracteole margin entire (0), Bracteole margin minutely toothed (1), Bracteole margin scantly toothed to becoming entire (2), Bracteole margin with conspicuous and numerous teeth/spine (> 14) (3) **Bmargent**
22. Bracteole base lobed (0), Bracteole base not lobed (1) **Bralob**
23. Bracteole with 1 conspicuous vein (0), Bracteole with more than 1 conspicuous vein parallel to the mid-vein (1) **Bravein**
24. Bracteole parallel veins forming conspicuous reticulations near the bracteole margin (0), Bracteole parallel veins not forming conspicuous reticulations near the margin (1) **Bvconsp**
25. Bracteole apex with long tip (about half the bracteole length) (0), Bracteole apex with short tip (less than a third the length of the bracteole) (1) **Brapex**
26. Bracteole margin glabrous (0), Bracteole margin puberulus (with short hair) (1), Bracteole margin pubescent (with long straight hair) (2), Bracteole margin pubescent (with short and long straight hair) (3) [Bracteole margin glabrous (0), Bracteole margin pubescent (1)] **Bmargpub**
27. Bracteole margin spine/tooth glabrous (0), Bracteole margin spine/tooth glabrescent (1), Bracteole margin spine/tooth markedly pubescent (2) [Bracteole margin spine/tooth glabrous (0), Bracteole margin spine/tooth pubescent (1)] **Bmargspn**
28. Bracteole width at widest point **BW**
29. Ratio of bracteole length to bracteole width **BL:BW**
30. Bracteole apex spine length **BS**
31. Number of teeth at ½ top length from apex on bracteole margin **B# teeth2**
32. Ratio of bracteole length to total number of spines / teeth on bracteole margin **BL:teeth**

Calyx

33. Anticous / posticous calyx lobe hardened / ligneous (0), Anticous / posticous calyx lobe membranous, papery (1) **Calhard**
34. Anticous / posticous calyx lobe margin glabrous (0), Anticous / posticous calyx lobe margin pubescent (1) **Calpub**
35. Anticous / posticous calyx lobe margin densely spiny / toothed (0), Anticous / posticous calyx lobe margin sparsely spiny / toothed (1) **Calmarg**
36. Anticous / posticous calyx lobe margin spine/tooth glabrous (0), Anticous / posticous calyx lobe margin spine / tooth glabrescent (1), Anticous/posticous calyx lobe margin spine / tooth pubescent (2) [Anticous/posticous calyx lobe margin spine / tooth glabrous (0), Anticous / posticous calyx lobe margin spine / tooth pubescent (1)] **Calmgspn**
37. Posticous calyx lobe overlapping anticous calyx lobe (0), Posticous calyx lobe not overlapping anticous calyx lobe (1) **Calovlap**
38. Anticous calyx lobe width at widest point **CYW-A**
39. Anticous calyx lobe apex spine length **CYS-A**
40. Ratio of length of anticous calyx lobe to calyx lobe width **CYL-A:W**
41. Number of teeth at ½ top length of anticous calyx lobe margin from apex **A #teeth2**

42. Ratio of length of anticous calyx lobe to total number of teeth on anticous calyx lobe margin **CYW-A:teeth**
43. Posticous calyx lobe width measured at widest point **CYW-P**
44. Posticous calyx lobe apex spine length **CYS-P**
45. Ratio of length of posticous calyx lobe to calyx lobe width **CYL-P:W**
46. Number of teeth at ½ top length of posticous calyx lobe from apex **P #teeth2**
47. Number of teeth at ½ bottom length of posticous calyx lobe from base **P #teeth4**
48. Ratio of length of posticous calyx lobe to total number of teeth on posticous calyx lobe margin **CYL-P:teeth**

2.8.4 Appendix IV: Abbreviations for countries and South African provinces in which the study species occur

Bot – Botswana
Limp – Limpopo
Nam – Namibia
Moz – Mozambique
KZN – KwaZulu Natal
Swa – Swaziland
Mpu – Mpumalanga
Zim – Zimbabwe
Un – Unknown
EC – Eastern Cape
WC – Western Cape
NC – Northern Cape
FS – Free State

2.8.5a Appendix V: Measurements and / or counts for qualitative and quantitative characters used in the analyses

Label	L	W	PL	SL	M	leaf WP	L# teeth1	L# teeth2	L# teeth3	L# teeth4	BL	BW	BS	BWP	B# teeth1	B# teeth2	B# teeth3	B# teeth4	CYL-A	CYW-A	CYS-A	CYL-AWP	A# teeth1	A# teeth2	A# teeth3
BB002-NC	17.0	3.3	1.2	0.3	0.0	6.7	0.0	0.0	0.0	0.0	16.4	2.0	1.1	5.3	0.0	2.3	2.8	5.5	18.2	5.2	1.4	7.1	1.0	8.0	1.0
BB003-NC	20.1	3.6	1.5	0.5	0.0	8.2	0.0	0.0	0.0	0.0	11.8	2.0	1.2	4.2	0.0	1.3	2.0	7.0	15.0	5.2	0.6	5.4	3.0	15.5	1.0
BB005-NC	13.9	3.1	0.9	0.2	0.0	5.1	0.0	0.0	0.0	0.0	13.8	3.2	0.8	4.8	0.0	1.3	0.7	4.0	14.7	5.6	0.5	5.8	2.0	9.0	0.5
BB006-NC	17.0	4.2	0.9	0.4	0.0	6.3	0.0	0.0	0.0	0.0	12.5	2.9	1.4	4.4	0.0	1.7	2.7	8.7	15.0	5.3	1.1	5.3	1.5	11.5	4.0
BB010-NC	19.2	3.6	1.5	0.3	0.0	10.8	0.0	0.0	0.0	0.0	15.3	3.3	1.3	5.8	0.0	3.5	3.0	9.5	16.7	4.3	1.1	7.2	1.5	12.5	2.0
BB080-NC	21.1	3.1	1.2	0.5	0.0	6.3	0.0	0.0	0.0	0.0	12.7	3.0	1.3	5.0	1.0	4.0	1.0	4.5	13.8	4.7	1.1	6.1	3.0	10.5	1.0
BB086-NC	24.0	3.4	1.5	0.3	0.0	9.5	0.0	0.0	0.0	0.0	17.3	2.8	0.5	7.3	4.0	10.3	0.0	6.0	17.5	4.5	0.5	9.0	8.0	18.0	0.0
BB088-NC	18.0	3.2	1.0	0.4	0.0	6.5	0.0	0.0	0.0	0.0	16.0	3.3	0.8	7.5	3.5	9.0	0.0	3.0	15.8	6.3	0.5	8.5	4.5	15.0	0.0
BB090-NC	27.3	3.0	1.5	0.5	0.0	9.5	0.0	0.0	0.0	0.0	17.0	2.8	0.9	6.8	3.5	7.0	0.0	3.5	17.0	4.5	1.0	7.0	4.0	12.0	0.0
BB099-NC	20.0	3.2	1.2	0.5	0.0	7.0	0.0	0.0	0.0	0.0	13.0	3.8	1.0	3.8	1.0	3.5	0.5	5.0	15.5	5.3	0.7	6.5	4.0	10.0	1.0
BB100-UN	22.8	3.5	1.4	0.6	0.0	9.7	0.0	0.0	0.0	0.0	16.3	3.2	1.1	6.8	0.0	2.7	2.0	8.3	14.8	4.5	0.8	6.5	0.0	8.0	1.0
BB101-NC	11.3	3.3	0.9	0.3	0.0	3.8	0.0	0.0	0.0	0.0	11.0	2.8	1.1	4.2	0.0	1.7	1.3	9.0	13.5	5.3	0.9	5.5	1.5	8.0	1.0
BB142-NC	19.7	4.2	0.7	0.5	0.0	7.4	0.0	0.0	0.0	0.0	12.0	2.6	0.9	4.7	0.0	2.5	0.5	4.5	15.4	6.7	0.7	6.0	3.7	12.0	1.3
BB146-NC	12.8	3.1	0.7	0.2	0.0	6.2	0.0	0.0	0.0	0.0	10.9	2.6	1.0	4.6	0.3	3.8	1.0	7.5	13.2	4.5	0.8	5.6	1.7	7.3	0.0
BE004-NC	20.3	2.0	1.0	0.4	0.0	7.2	0.0	0.0	0.0	0.0	9.9	2.1	0.2	4.1	4.0	6.5	1.0	4.5	15.1	3.6	0.2	5.5	3.0	10.0	0.0
BE082-NC	24.9	2.3	1.0	0.3	0.0	9.6	0.0	0.0	0.0	0.0	11.5	2.3	0.3	4.8	5.0	10.5	0.0	5.0	12.7	3.6	0.5	6.7	5.0	15.0	0.0
BE084-NC	24.5	2.3	1.0	0.3	0.0	10.5	0.0	0.0	0.0	0.0	14.4	2.8	0.4	5.4	4.0	10.0	0.0	3.0	15.2	4.9	0.4	7.0	6.5	18.0	0.0
BE085-NC	16.6	1.8	0.5	0.2	0.0	8.6	0.0	0.0	0.0	0.0	13.0	2.7	0.5	5.1	2.5	7.0	0.0	7.0	13.2	4.3	0.4	6.3	4.5	12.0	0.0
BE087-NC	27.3	2.0	1.0	0.1	0.0	8.5	0.0	0.0	0.0	0.0	13.8	2.8	0.5	6.5	5.0	11.0	0.0	2.0	15.5	4.0	0.7	9.5	5.5	17.0	0.0
BE089-NC	21.2	1.8	1.0	0.4	0.0	7.8	0.0	0.0	0.0	0.0	11.5	1.8	0.6	5.8	4.5	9.0	0.0	1.0	13.5	4.5	0.4	7.3	7.0	16.0	0.0
BE118-NC	19.1	2.3	1.0	0.2	0.0	9.7	0.0	0.0	0.0	0.0	10.5	2.6	0.5	5.6	2.4	7.6	0.0	2.0	12.3	4.3	0.3	7.3	6.0	14.0	0.0
BE119-NC	25.1	2.0	0.7	0.0	0.0	11.1	0.0	0.0	0.0	0.0	16.0	2.8	0.6	6.5	5.0	10.8	0.0	9.0	15.0	5.0	0.5	7.6	4.7	15.3	0.0
BE120-NC	31.5	2.3	0.8	0.3	0.0	10.9	0.0	0.0	0.0	0.0	13.3	2.6	0.6	7.1	3.0	8.5	0.0	1.8	16.1	5.1	0.6	8.7	5.0	15.8	0.0
BE121-NC	15.4	2.1	0.9	0.3	0.0	6.7	0.0	0.0	0.0	0.0	12.0	2.1	0.4	6.7	4.0	7.5	0.0	1.0	13.2	4.4	0.5	7.4	4.0	12.7	0.0
BE122-NC	19.1	2.3	0.9	0.4	0.0	8.3	0.0	0.0	0.0	0.0	16.0	3.0	1.0	7.1	3.0	9.6	0.4	6.8	16.2	5.0	0.6	6.8	5.7	16.0	2.7
BE124-NC	15.1	2.1	0.7	0.4	0.0	6.4	0.0	0.0	0.0	0.0	12.8	3.0	0.9	6.1	3.5	15.0	0.0	4.0	9.5	3.3	0.5	4.9	1.0	5.0	5.0
BE125-NC	19.5	1.8	0.6	0.4	0.0	7.8	0.0	0.0	0.0	0.0	15.6	3.0	0.8	7.6	3.0	10.8	0.4	5.6	13.7	4.1	0.5	6.4	1.3	8.0	0.0
BI067-EC	22.6	7.4	1.6	0.7	0.2	9.1	0.0	2.7	1.7	6.0	15.1	2.5	1.5	5.1	0.0	0.0	0.5	3.5	18.0	4.3	1.8	7.0	0.0	6.7	0.7
BI068-EC	25.7	8.0	1.6	1.7	0.2	11.8	0.0	3.8	0.0	5.0	18.4	2.5	1.5	5.1	0.0	1.0	1.5	5.5	18.6	4.0	2.1	7.2	0.0	4.0	2.0
BI071-EC	17.6	6.6	1.3	1.5	0.2	5.1	0.0	3.7	1.3	6.0	17.5	3.4	1.5	3.7	0.0	1.5	3.0	7.5	17.1	4.4	1.6	6.2	0.0	2.0	2.3
BI072-EC	21.9	8.3	1.6	1.3	0.3	8.3	1.0	6.0	8.0	18.0	16.3	2.8	2.3	5.4	0.0	1.0	1.3	4.0	15.9	4.6	1.7	6.2	0.0	6.7	2.0
BI073-EC	20.1	7.0	1.7	1.2	0.3	6.6	0.0	1.5	4.0	12.0	16.3	3.9	1.1	6.0	0.0	3.0	0.0	3.5	18.4	5.5	1.6	7.0	0.0	7.0	0.0
BI074-EC	28.2	7.2	1.9	0.6	0.0	11.1	0.0	0.0	1.3	5.3	13.4	1.7	1.4	4.2	0.0	0.0	0.0	0.0	19.7	4.4	1.7	8.7	0.0	6.7	0.3
BI075-EC	16.8	7.3	1.4	1.0	0.2	6.2	2.5	4.5	2.5	7.5	15.4	1.9	1.7	4.4	0.0	1.0	2.0	4.7	17.0	4.8	1.1	6.6	0.0	5.0	2.5
BI077-EC	16.8	6.1	1.4	1.3	0.3	6.0	0.0	0.0	3.5	5.5	20.1	2.8	2.0	4.4	0.0	0.3	2.0	5.8	20.8	4.3	3.2	7.7	0.0	4.0	0.0
BI079-EC	18.6	5.6	1.9	0.8	0.0	7.3	0.0	0.0	0.0	0.0	19.6	4.2	2.0	6.7	0.0	0.0	1.5	4.0	26.0	7.9	1.4	10.6	1.0	8.0	2.0

BI093-EC	12.5	4.5	1.0	0.8	0.0	4.0	0.0	0.0	0.0	0.0	9.8	2.5	1.5	3.0	0.0	0.0	3.0	5.0	11.5	3.5	1.0	4.5	1.0	7.5	0.0
BI095-EC	12.3	5.5	0.9	0.9	0.3	3.7	0.0	0.7	4.7	9.3	15.7	2.8	1.2	5.2	0.0	0.0	2.7	5.0	17.0	5.8	1.0	7.0	0.0	3.0	3.0
BI097-EC	16.8	6.3	1.2	1.8	0.2	7.3	0.0	3.3	2.3	5.7	16.3	2.8	1.3	5.3	0.0	0.0	1.5	4.0	17.0	5.0	1.0	7.8	0.0	4.0	0.0
BI104-UN	15.8	6.3	1.2	1.0	0.0	3.3	0.0	3.5	2.3	6.0	14.7	3.4	1.3	5.1	0.0	0.3	1.0	4.5	16.9	5.7	1.7	6.6	0.5	7.0	3.0
BI145-EC	16.8	6.8	1.5	1.0	0.0	5.8	0.0	0.0	0.0	0.0	14.1	2.5	1.4	4.1	0.0	0.0	0.0	0.0	17.6	4.3	1.6	7.3	0.0	0.0	0.0
BI152-EC	13.7	5.8	0.7	0.9	0.2	3.8	0.0	1.0	0.8	2.5	15.5	2.7	1.5	3.1	0.0	0.5	4.3	7.5	15.3	4.0	0.9	5.1	0.0	5.0	1.3
BP001-EC	18.3	7.6	2.3	0.6	0.0	8.3	0.0	0.0	0.0	0.0	17.1	4.5	2.0	5.2	0.0	2.3	1.8	5.8	18.1	8.0	1.8	8.3	0.5	7.0	4.0
BP015-EC	17.9	9.0	1.3	1.1	999.0	5.2	0.0	0.0	0.0	0.0	12.2	3.6	1.6	4.1	0.0	1.3	0.3	1.8	17.1	7.7	1.3	7.7	0.0	4.0	0.7
BP018-WC	11.3	5.3	1.2	0.9	999.0	4.3	0.0	0.0	0.0	0.0	13.3	2.8	1.6	4.4	0.0	1.8	0.0	0.8	17.7	6.1	2.3	7.9	0.0	0.3	0.0
BP019-WC	17.3	8.0	0.9	0.7	999.0	7.0	0.0	0.0	0.0	0.0	15.3	3.9	0.8	5.6	0.0	0.0	0.0	0.0	21.5	10.2	1.3	9.0	0.0	0.0	0.0
BP020-WC	21.0	10.3	1.9	0.7	999.0	7.3	10.0	25.0	10.0	25.0	16.9	4.8	1.3	7.1	0.0	3.8	0.0	2.0	18.7	9.8	1.8	8.7	0.0	4.0	0.0
BP021-WC	22.2	9.9	2.0	1.0	999.0	9.0	10.0	25.0	10.0	25.0	17.8	4.1	1.5	5.0	0.3	0.3	0.0	4.7	21.2	8.3	1.8	8.2	1.3	5.0	0.0
BP022-EC	18.4	10.3	1.6	0.7	999.0	7.9	10.0	25.0	10.0	25.0	12.8	4.8	0.8	6.0	0.5	3.0	0.0	1.0	23.1	11.8	1.4	8.1	2.0	8.0	0.0
BP025-WC	19.6	9.3	1.5	1.5	999.0	6.0	0.0	0.0	0.0	0.0	18.8	4.0	1.9	6.5	0.3	2.3	0.0	2.0	19.1	7.9	1.8	8.5	0.0	1.0	0.0
BP027-WC	24.0	8.3	1.7	1.1	999.0	7.5	8.0	22.0	10.0	28.0	19.4	4.8	2.5	6.6	0.7	5.0	2.0	7.0	22.7	9.4	1.4	7.8	0.0	6.0	0.0
BP028-WC	16.5	6.3	1.2	0.4	999.0	6.2	5.0	10.7	6.7	19.7	15.8	3.4	1.7	7.1	0.0	1.0	0.0	0.5	20.5	10.5	2.2	7.6	0.3	6.7	0.0
BP030-EC	22.7	9.3	2.0	0.4	999.0	10.4	0.0	0.0	0.0	0.0	20.0	4.7	1.7	5.7	0.0	3.0	0.5	4.0	25.7	10.5	1.9	10.3	0.0	5.7	1.0
BP031-WC	13.6	6.7	1.6	0.4	999.0	7.1	0.0	0.0	0.0	0.0	18.0	2.7	1.9	4.2	0.0	3.8	1.0	6.3	19.1	6.9	0.7	7.0	0.0	2.3	1.3
BP032-WC	11.6	6.6	1.6	0.7	999.0	5.3	0.0	0.0	0.0	0.0	19.6	3.3	1.5	6.6	0.0	1.3	0.3	1.3	19.3	7.9	1.5	8.1	0.0	2.7	0.0
BP033-EC	14.1	8.2	1.1	0.9	999.0	5.1	0.0	0.0	0.0	0.0	17.9	3.8	1.8	5.6	0.0	2.7	1.7	5.7	18.0	8.0	1.5	8.0	2.0	9.0	0.0
BP034-WC	22.4	8.7	2.1	0.5	999.0	10.4	0.0	0.0	0.0	0.0	19.2	4.4	1.1	7.4	0.0	1.0	0.3	1.0	23.0	11.9	1.9	9.2	0.0	4.0	0.0
BP036-WC	12.5	7.5	1.5	0.7	999.0	4.7	0.3	3.7	1.7	7.3	13.2	3.7	1.2	5.5	0.0	1.5	0.0	2.8	15.4	5.9	0.8	6.8	0.0	1.0	0.0
BP037-WC	14.3	9.1	1.1	0.6	999.0	4.3	0.0	0.0	0.0	0.0	15.9	4.9	1.6	5.5	0.0	0.8	0.5	1.5	17.3	8.1	1.4	7.1	0.0	0.0	0.0
BP038-WC	16.8	9.8	1.2	0.5	999.0	5.2	0.0	0.0	0.0	0.0	17.8	3.3	2.1	6.2	0.0	0.8	0.3	0.5	22.5	10.2	2.3	10.6	0.0	2.3	0.0
BP039-WC	17.7	10.6	1.3	1.2	999.0	5.7	10.0	26.0	10.0	25.0	13.8	3.4	2.0	3.9	0.0	1.0	0.0	2.0	21.4	10.4	1.6	9.1	0.0	4.5	0.0
BP041-EC	15.3	10.2	1.6	0.6	999.0	4.4	10.0	25.0	10.0	10.0	16.5	6.7	2.4	5.9	0.0	1.0	0.8	3.5	18.9	9.5	1.2	7.8	0.5	6.8	1.3
BP096-WC	15.3	7.7	2.7	1.0	999.0	7.7	0.0	0.0	0.0	0.0	22.0	4.0	1.7	6.2	0.0	2.3	0.3	1.0	20.3	7.5	1.3	8.8	0.0	2.0	0.0
BP153-WC	16.5	6.3	0.9	0.5	999.0	7.2	0.0	0.0	0.0	0.0	20.0	7.1	1.2	6.7	0.5	3.5	0.0	1.0	21.5	11.6	1.0	7.7	0.0	3.0	1.0
BR043-Nam	16.1	5.1	1.7	0.5	0.8	5.4	0.7	8.0	2.3	9.7	16.3	1.7	1.2	3.6	0.0	0.0	2.0	6.0	16.1	4.5	1.8	6.4	0.0	4.0	2.0
BR045-NC	18.6	3.9	1.9	0.6	0.2	6.9	0.0	0.7	1.3	2.0	20.6	1.9	2.4	6.8	0.0	0.3	0.7	3.7	15.5	5.0	1.3	5.6	0.3	6.0	1.7
BR046-NC	17.0	4.8	1.6	0.5	0.5	5.9	0.0	0.7	2.0	4.7	17.4	3.9	1.0	7.7	0.0	1.5	2.8	8.0	15.8	6.2	0.7	7.1	2.3	6.3	1.3
BR047-Nam	10.7	3.0	0.8	0.4	0.7	4.3	0.7	6.3	3.3	9.3	17.6	3.0	0.8	6.2	0.0	2.0	3.6	8.4	15.9	5.3	1.9	6.3	2.5	8.5	3.0
BR049-Nam	31.3	6.0	2.4	0.6	1.1	12.0	0.3	4.7	2.7	7.7	16.2	1.9	1.4	4.5	0.0	0.5	2.8	6.8	16.5	4.6	1.9	6.2	1.3	6.3	0.7
BR051-FS	17.3	3.7	1.4	0.8	0.5	3.6	0.0	3.3	1.7	5.3	15.9	2.0	2.2	4.3	0.0	1.0	2.3	5.3	13.1	4.8	1.4	5.6	0.7	6.0	0.0
BR052-NC	17.2	4.8	1.9	0.6	0.5	5.9	0.0	2.0	1.3	4.7	16.9	1.7	1.6	5.1	0.0	0.7	1.0	6.0	14.9	5.5	1.8	5.4	0.0	5.7	1.3
BR053-Nam	16.1	3.3	1.9	0.4	0.3	6.2	0.0	0.0	1.3	2.0	23.1	2.9	2.5	8.4	0.0	2.0	3.0	8.0	14.8	3.4	1.4	4.9	0.0	5.3	4.0
BR056-FS	10.7	2.8	1.0	0.7	0.2	2.9	0.0	0.0	3.3	3.3	17.3	2.3	1.4	5.2	0.0	0.5	2.8	5.8	13.0	4.3	1.1	4.8	1.7	6.3	3.0
BR057-FS	8.6	2.1	0.8	0.3	0.0	3.4	0.0	0.0	1.7	2.3	12.4	1.5	1.1	5.0	0.0	2.3	1.3	4.7	14.1	4.1	1.8	5.2	0.3	5.3	0.3

BR058-Nam	11.7	3.0	1.1	0.6	0.2	4.6	0.0	0.0	0.7	4.3	17.9	2.1	1.1	6.6	0.0	2.5	0.0	4.0	16.0	6.4	1.0	6.2	1.8	6.8	0.0
BR059-Nam	15.9	3.4	2.0	0.4	1.0	5.9	0.0	1.3	3.0	6.0	16.3	1.7	1.1	6.2	0.0	4.0	3.8	9.3	15.3	4.3	1.1	6.4	4.0	10.0	4.7
BR062-NC	14.3	3.2	1.2	0.7	0.2	6.1	0.0	1.5	0.5	2.5	21.3	2.7	1.6	6.2	0.0	2.8	0.0	4.0	16.6	5.5	1.3	6.4	2.0	8.5	2.5
BR063-NC	16.6	4.8	1.7	0.4	0.6	6.0	0.0	2.0	3.0	5.5	18.8	2.4	1.5	4.9	0.0	1.0	0.5	5.0	14.4	5.5	0.9	6.6	2.0	6.0	0.0
BR064-Nam	17.9	3.2	2.8	0.3	0.4	8.8	0.0	0.0	0.0	1.0	16.7	2.3	1.2	5.8	0.7	4.3	2.0	8.0	13.1	4.0	1.3	4.8	4.0	9.7	8.0
BR065-NC	15.4	3.4	2.0	0.3	0.3	6.4	0.0	1.3	0.3	1.7	14.6	1.5	1.1	5.8	0.0	1.0	1.5	3.0	13.9	5.1	1.5	6.1	1.0	5.0	1.0
BR083-Bot	16.9	5.1	2.5	0.3	0.2	7.1	0.0	0.0	1.3	1.7	11.7	3.5	0.7	4.8	2.3	5.3	2.0	6.0	12.8	3.5	0.6	5.1	0.0	6.0	3.7
BR102-Nam	18.0	4.5	1.4	0.7	1.7	4.2	0.0	0.7	3.0	5.3	16.8	2.9	1.4	6.8	0.0	1.0	0.0	8.0	16.5	4.5	0.6	6.0	1.0	5.5	7.0
BR103-NC	18.3	4.8	1.0	0.5	0.9	5.8	0.0	0.7	1.7	5.3	20.5	3.3	1.5	7.0	0.0	2.5	4.0	11.0	12.5	3.5	1.0	6.0	2.0	8.0	8.0
BR140-NC	24.3	3.9	2.0	0.7	0.5	7.8	0.0	0.0	2.5	5.0	16.9	2.4	1.5	6.8	0.0	2.7	2.7	7.3	15.5	5.8	1.1	6.1	1.3	7.7	0.7
BR147-Bot	27.6	5.3	1.7	0.4	0.6	9.8	0.0	0.3	1.0	3.5	18.4	3.5	1.0	7.2	0.0	2.5	2.0	10.0	13.9	4.9	0.9	5.1	2.0	9.0	3.0
BR148-Bot	10.1	2.9	0.6	0.2	0.3	4.2	0.0	0.0	0.0	2.3	12.3	3.2	0.7	4.8	0.0	1.0	1.3	7.2	13.2	4.6	0.6	4.8	1.3	7.3	2.0
BR149-Nam	18.8	4.8	1.8	0.4	0.7	8.5	0.0	4.5	3.8	9.3	10.9	2.2	0.7	4.1	0.0	1.0	2.0	7.5	11.4	3.9	0.6	4.5	3.5	9.0	1.0
BR150-Nam	10.3	2.7	0.8	0.5	0.4	3.6	0.0	0.5	1.5	4.5	18.6	2.1	1.5	7.2	0.0	1.3	0.0	6.3	13.5	4.2	0.8	5.4	0.0	5.7	0.0
BU066-EC	12.9	6.8	1.8	0.7	0.0	5.5	0.0	0.0	0.0	0.0	19.6	1.5	1.5	4.3	0.0	0.0	0.8	1.0	16.2	4.0	1.5	6.5	0.3	4.7	1.3
BU069-EC	15.5	4.5	1.5	1.0	0.1	5.2	0.7	2.3	0.0	2.3	22.8	1.7	2.3	5.8	0.0	1.0	1.5	3.0	16.3	2.2	1.7	6.7	0.5	7.5	0.0
BU070-EC	13.2	4.6	1.7	1.2	0.0	5.6	0.0	0.0	0.0	0.0	16.0	1.4	1.6	3.1	0.0	0.0	0.0	0.0	17.0	3.9	2.4	8.1	0.0	3.0	0.0
BU076-EC	18.1	6.1	2.2	0.6	0.0	8.7	0.0	0.0	0.0	0.0	19.9	1.5	1.4	6.0	0.0	1.0	1.0	3.3	16.0	3.4	2.1	7.8	1.7	7.0	2.0
BU078-EC	13.3	5.0	1.7	1.8	0.0	5.5	0.0	0.0	0.0	0.0	18.3	1.6	1.4	6.4	0.3	0.8	1.0	4.0	14.1	2.8	1.2	7.2	1.5	7.5	0.5
BU091-EC	14.3	5.8	1.5	1.0	0.0	6.3	0.0	0.0	0.0	0.0	16.8	2.2	1.0	5.8	0.0	0.0	0.5	1.0	12.3	3.1	1.1	3.3	1.5	6.0	0.5
BU092-EC	13.0	5.5	1.3	1.0	0.0	4.3	0.0	0.0	0.0	0.0	15.7	2.0	1.0	4.3	0.0	0.0	0.0	0.0	17.3	3.3	1.0	7.5	0.0	3.0	0.0
BU094-EC	13.2	4.8	1.3	0.5	0.0	5.5	0.0	0.0	0.0	0.0	20.3	2.0	1.0	4.3	0.0	0.0	0.0	0.0	16.0	3.5	1.1	5.5	0.0	4.0	1.0
BU105-EC	11.5	5.1	1.4	0.5	0.0	4.7	0.0	0.0	0.0	0.0	20.0	1.7	1.2	3.9	0.0	0.0	0.0	0.0	19.2	4.4	1.6	8.3	0.0	3.8	0.0
BU123-EC	15.8	7.4	1.4	0.8	0.0	7.1	1.0	3.0	2.0	5.0	23.7	1.9	1.9	7.3	0.0	1.4	3.0	6.4	16.0	2.9	1.8	7.0	0.8	9.8	2.0
BU143-EC	18.8	7.5	1.8	0.6	0.0	7.6	0.0	0.0	0.0	0.0	17.8	2.1	1.3	4.2	0.0	0.8	1.6	5.2	18.1	3.9	1.1	8.1	0.0	4.0	0.0
EL044-KZN	78.7	22.5	7.0	1.0	999.0	36.7	0.0	0.0	0.0	0.0	15.3	3.6	1.2	4.7	0.0	1.8	3.0	6.0	15.8	6.2	1.3	6.1	2.0	6.3	2.7
EL050-Swa	76.3	28.3	6.7	0.5	999.0	33.3	0.0	0.0	0.0	0.0	13.8	5.8	1.6	4.7	0.0	3.0	2.5	7.0	18.9	5.6	1.5	7.8	1.0	6.0	2.0
EL055-Limp	58.5	19.2	4.0	0.5	999.0	30.3	0.0	0.0	0.0	0.0	12.5	3.2	0.9	5.1	0.0	3.0	2.0	5.5	16.5	5.6	1.3	6.5	1.0	7.0	1.3
EL061-Mpu	121.0	33.8	11.3	0.8	999.0	68.0	0.0	0.0	0.0	0.0	17.1	4.6	1.8	5.5	0.0	2.0	2.5	7.3	19.3	5.0	2.0	7.8	0.7	4.7	2.7
EL127-Limp	52.5	14.7	4.0	0.7	999.0	27.3	0.0	0.0	0.0	0.0	12.5	3.3	1.6	4.4	0.0	1.8	1.8	5.3	18.6	5.6	1.0	8.1	2.3	7.3	1.3
EL128-UN	68.1	24.4	6.3	0.6	999.0	36.8	0.0	0.0	0.0	0.0	15.1	3.9	1.3	5.5	0.0	1.5	3.0	4.0	21.5	7.5	1.4	9.2	0.0	5.7	4.0
EL129-Mpu	81.7	29.5	5.8	1.0	999.0	45.8	0.0	0.0	0.0	0.0	18.1	4.5	1.8	6.5	0.0	2.5	1.5	7.0	20.9	7.1	1.5	7.8	3.3	9.7	4.0
EL130-Mpu	81.0	25.7	5.0	0.5	999.0	37.5	0.0	0.0	0.0	0.0	16.1	4.5	1.1	6.6	0.3	1.8	2.5	5.5	16.3	4.8	1.3	8.3	3.5	12.5	2.5
EL131-Zim	90.0	33.7	4.8	0.5	999.0	41.7	0.0	0.0	0.0	0.0	13.4	3.6	1.1	4.5	0.0	1.7	1.3	6.3	17.8	5.8	1.3	9.0	2.3	7.5	2.5
EL132-Zim	65.8	23.7	4.0	0.8	999.0	37.3	0.0	0.0	0.0	0.0	16.2	4.1	1.3	5.3	0.0	2.0	4.0	9.5	16.3	5.6	1.4	8.7	0.0	6.0	2.7
EL133-Swa	41.8	15.3	4.0	0.5	999.0	18.3	0.0	0.0	0.0	0.0	15.3	4.2	1.4	5.2	0.0	1.8	1.5	5.8	19.1	6.6	1.5	8.3	1.0	5.7	1.3
EL134-Zim	49.3	17.0	3.7	0.6	999.0	25.5	0.0	0.0	0.0	0.0	11.0	3.5	1.2	3.6	0.0	2.5	1.0	6.0	17.6	6.5	1.8	7.7	0.7	6.0	1.3
EL135-Zim	62.5	19.2	4.7	1.0	999.0	32.2	0.0	0.0	0.0	0.0	20.4	4.3	2.0	6.5	0.0	1.0	5.0	10.0	20.3	5.4	1.6	8.1	1.3	4.7	1.0

EL136-Swa	45.0	21.7	4.8	0.5	999.0	19.0	0.0	0.0	0.0	0.0	11.0	3.0	1.5	3.7	0.0	0.5	2.0	5.0	14.4	6.4	1.3	6.3	1.0	7.3	2.7
EL137-Moz	57.0	16.3	4.3	0.5	999.0	31.7	0.0	0.0	0.0	0.0	13.0	3.1	1.5	4.2	0.0	1.0	2.0	5.0	15.3	5.3	1.1	6.5	2.7	8.3	1.0
EL138-Moz	63.7	28.3	4.7	0.5	999.0	35.0	0.0	0.0	0.0	0.0	14.3	3.7	1.2	4.5	0.0	1.7	2.0	6.3	16.1	5.3	1.6	8.3	2.0	7.3	0.7
EL139-Moz	90.7	35.3	7.2	0.5	999.0	49.7	0.0	0.0	0.0	0.0	16.5	4.0	1.6	5.9	0.0	2.3	4.0	7.5	17.7	7.3	2.0	6.8	2.3	7.7	3.0
IL081-NC	14.4	6.6	1.4	1.7	1.6	6.1	0.0	6.0	2.0	6.0	15.5	3.4	2.1	3.7	0.0	1.0	0.0	5.3	15.6	3.5	2.0	6.8	1.0	8.0	0.7
IL111-Nam	20.3	8.3	1.6	1.0	1.5	8.7	2.3	8.8	3.8	8.8	16.0	3.9	1.1	3.6	0.0	2.8	3.5	8.8	16.5	6.1	1.1	7.6	0.7	7.3	1.3
IL113-Nam	10.6	5.9	1.2	1.2	2.2	3.7	1.7	4.7	3.0	6.0	12.3	2.1	1.5	5.0	0.0	1.8	0.0	3.0	15.6	5.5	2.0	6.5	0.2	5.6	2.0
IL114-Nam	13.9	5.6	1.3	1.4	1.6	4.6	1.0	5.8	3.3	8.0	11.8	1.5	1.4	3.8	0.0	1.5	0.0	1.0	14.7	4.2	1.3	5.0	1.0	4.3	2.0
IL151-NC	17.3	8.7	1.0	1.7	2.0	5.3	0.7	6.7	2.3	8.3	16.8	2.4	1.6	4.6	0.0	1.3	2.0	5.8	15.7	6.0	1.0	5.0	0.0	2.7	1.3
ILb054-Nam	32.8	7.7	3.4	1.6	1.5	16.1	2.3	10.7	3.7	12.0	20.5	4.3	2.1	7.8	0.0	8.0	7.7	16.7	16.8	5.6	1.8	7.5	3.0	8.0	1.0
ILb060-Nam	25.2	6.3	3.3	0.5	0.5	14.6	0.0	0.3	1.3	6.0	21.7	2.8	1.3	7.2	0.0	2.0	2.3	8.7	15.2	3.9	0.9	6.5	2.3	8.3	2.3
ILb110-Nam	43.1	9.8	4.6	1.0	1.3	18.0	0.0	3.5	3.0	11.0	17.9	4.6	1.2	7.5	1.8	8.5	6.5	14.5	18.1	5.7	1.4	7.3	1.3	7.3	2.0
ILb112-Nam	30.5	6.0	2.1	0.7	1.1	12.2	1.0	10.0	5.5	13.3	16.5	2.5	1.6	6.4	0.0	3.0	2.5	7.0	15.4	4.0	1.3	6.2	3.5	12.5	5.5
ILb115-Nam	19.2	4.9	1.5	0.9	1.0	6.4	0.0	6.7	5.3	10.0	16.1	3.2	1.5	6.4	0.0	3.0	0.5	6.5	17.8	4.9	1.2	8.1	2.7	12.0	0.7
JU007-Nam	30.2	14.6	2.2	1.0	999.0	14.1	0.0	0.0	0.0	0.0	23.1	3.3	2.4	4.5	0.0	2.0	3.5	7.0	20.7	6.7	1.0	9.0	3.3	8.7	3.0
JU008-Nam	20.3	9.7	1.8	1.4	999.0	8.6	0.0	0.0	0.0	0.0	17.0	2.3	2.4	4.0	0.0	1.5	3.3	6.5	19.3	5.2	1.3	8.0	2.5	8.0	1.5
JU009-Nam	29.3	12.4	3.4	2.1	999.0	15.1	0.0	0.0	0.0	0.0	17.1	2.1	1.7	3.4	0.0	1.5	3.8	6.3	18.0	4.7	1.5	8.9	2.0	7.3	2.0
JU106-Nam	17.6	10.4	1.7	0.9	999.0	9.0	0.0	0.0	0.0	0.0	22.9	3.1	3.0	4.8	0.5	3.3	4.3	7.8	17.3	6.1	1.5	7.9	2.3	7.0	2.0
JU107-Nam	25.5	12.4	3.1	1.2	999.0	12.8	0.0	0.0	0.0	0.0	18.8	2.6	1.8	5.6	0.0	2.0	2.4	7.2	18.2	7.4	1.2	8.2	1.5	7.0	2.5
JU108-Nam	20.5	8.6	2.8	1.3	999.0	11.2	0.0	0.0	0.0	0.0	16.2	2.3	2.3	2.9	0.0	0.5	1.0	5.0	19.9	7.3	1.8	7.7	2.7	6.3	3.3
JU109-Nam	24.0	10.1	2.4	2.0	999.0	9.5	0.0	0.0	0.0	0.0	18.9	2.8	2.6	4.5	0.3	5.0	1.3	5.3	20.4	7.0	1.6	8.2	3.7	8.7	1.3
JU116-Nam	29.7	12.1	2.6	1.2	999.0	15.7	0.0	0.0	0.0	0.0	13.5	1.9	2.1	4.2	0.0	1.8	1.0	4.5	24.3	8.5	1.6	10.7	2.7	7.7	4.0
JU117-Nam	35.9	14.4	4.2	1.5	999.0	18.3	0.0	0.0	0.0	0.0	26.6	3.5	3.9	6.6	0.0	2.2	5.0	11.0	20.6	6.3	1.8	8.4	2.3	6.3	4.0
JU141-Nam	38.0	11.2	3.3	0.6	999.0	18.1	0.0	0.0	0.0	0.0	14.2	2.6	1.4	5.0	0.0	1.0	1.3	4.5	17.9	4.7	0.7	7.7	1.3	8.0	2.0
JU144-Nam	33.1	13.4	4.3	1.0	0.5	17.5	7.3	17.3	0.0	9.3	21.1	2.7	1.4	5.1	0.0	1.8	2.8	7.5	17.3	6.4	1.5	8.5	5.0	10.0	0.5
LB011-Nam	17.6	5.7	1.9	0.7	1.1	8.5	3.0	9.7	5.0	11.3	28.1	6.1	2.1	7.5	0.0	2.5	9.0	15.3	17.4	7.0	1.7	7.6	3.5	13.0	7.0
LB012-Nam	22.8	8.1	2.5	1.3	1.1	11.7	1.3	6.0	4.0	8.7	19.8	4.7	2.7	5.2	0.0	2.0	13.0	25.0	17.4	6.2	1.3	8.2	3.0	13.0	3.0
LB013-Nam	22.4	7.9	2.1	1.7	1.4	8.4	1.0	7.5	3.0	7.5	25.1	6.5	1.6	6.0	0.0	2.3	9.0	14.8	18.6	5.4	1.7	6.0	3.0	11.0	7.0
LB042-Nam	34.4	11.4	3.7	1.3	2.1	14.6	2.0	7.5	2.0	11.0	31.5	7.2	2.2	6.6	0.0	2.5	14.5	24.0	20.1	7.1	1.5	9.1	2.0	11.0	5.0
LB048-Nam	22.8	7.2	2.5	1.5	1.7	8.3	1.5	8.0	4.0	12.0	24.4	6.5	1.6	6.6	0.0	4.0	9.8	18.8	16.2	5.9	1.7	6.0	1.5	8.5	5.0
PU014-EC	18.3	7.4	2.1	1.6	999.0	5.7	0.0	0.0	0.0	0.0	19.2	3.7	2.1	5.6	0.0	1.3	0.0	4.5	24.3	9.3	3.7	7.0	0.0	3.5	1.5
PU016-EC	17.6	9.2	1.6	0.6	999.0	4.8	0.0	0.0	0.0	0.0	17.8	2.4	2.3	6.1	0.0	0.5	0.5	0.5	17.1	5.1	1.7	7.1	0.7	4.7	0.0
PU017-EC	20.8	10.4	2.3	1.1	999.0	8.0	0.0	0.0	0.0	0.0	12.5	2.7	1.7	3.6	0.0	1.3	1.0	3.8	20.8	7.7	2.7	8.7	0.0	4.0	0.0
PU023-EC	17.9	9.6	1.3	0.2	999.0	6.0	6.0	20.0	7.3	19.3	13.6	2.7	1.0	3.5	0.0	2.0	0.8	4.8	19.7	10.7	1.7	6.9	0.0	3.7	0.0
PU024-EC	19.1	9.4	1.4	0.4	999.0	8.0	10.0	25.0	10.0	25.0	18.7	4.1	1.8	5.6	0.0	2.5	2.0	7.5	16.5	7.2	1.5	6.9	1.0	5.5	1.0
BP026-WC	12.2	6.3	1.3	1.0	999.0	4.2	0.3	3.7	6.3	13.0	20.3	3.3	2.5	6.4	0.0	1.5	1.0	3.8	20.1	6.5	3.0	7.9	0.5	3.0	2.5
PU029-EC	10.8	7.5	0.8	0.7	999.0	3.3	10.0	25.0	10.0	25.0	15.9	3.8	1.3	6.8	0.3	2.5	0.5	4.0	18.2	8.5	1.3	6.8	2.3	7.3	1.3
PU035-EC	13.9	10.0	0.9	0.7	999.0	3.8	10.0	25.0	10.0	25.3	16.7	3.9	1.7	4.2	0.0	2.3	1.0	4.8	19.9	10.0	1.6	7.7	2.3	10.0	4.0

PU040-EC	26.7	12.3	3.8	0.8	999.0	11.5	0.0	0.0	0.0	0.0	13.8	3.5	1.5	5.1	0.0	2.0	0.3	2.5	20.6	8.7	2.2	8.8	0.0	0.0	0.0
BP098-WC	12.0	5.8	1.2	0.5	0.1	5.3	0.7	6.0	1.3	6.7	15.5	2.0	1.0	3.5	0.0	1.0	2.0	4.0	16.5	5.0	1.8	5.8	0.0	6.0	2.0
PU126-EC	18.0	7.9	2.2	1.4	999.0	6.4	0.0	0.0	0.0	0.0	18.2	4.1	1.8	5.8	0.2	2.8	2.3	6.5	23.8	10.9	2.3	9.4	1.7	6.7	4.3

2.8.5b Appendix V continued: Measurements and / or counts for qualitative and quantitative characters used in the analyses

Label	A# teeth4	CYL-P	CYW-P	CYS-P	CYL-PWP	P# teeth1	P# teeth2	P# teeth3	P# teeth4	FRT L	FRT W	FRT L-WP	Stemsurf	Stempub	leafsurf	L glos	Leafshp	Margcolr	Margpub	Hairbase	Margthick	Margent
BB002-NC	15.0	21.4	6.2	1.6	7.4	0.0	7.5	5.5	16.5	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BB003-NC	12.0	18.1	5.4	1.1	5.3	0.0	7.5	3.5	13.0	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BB005-NC	12.5	16.9	6.2	0.9	6.1	0.0	6.0	1.5	12.0	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BB006-NC	14.0	17.4	5.4	1.2	6.0	0.5	8.5	3.5	13.0	10.0	3.5	3.5	1	0	0	1	3	0	0	2	0	0
BB010-NC	10.0	19.6	5.8	1.6	6.9	0.0	6.0	6.0	19.5	13.0	4.7	6.8	1	0	0	1	3	0	0	2	0	0
BB080-NC	6.0	14.8	4.7	1.3	6.1	2.0	8.0	1.0	7.0	10.1	3.7	4.9	1	0	0	1	3	0	0	2	0	0
BB086-NC	8.0	18.5	5.5	0.6	9.0	2.0	14.0	0.0	16.0	8.5	4.0	3.2	1	0	0	1	3	0	0	2	0	0
BB088-NC	10.0	16.5	6.4	0.9	8.5	2.0	14.0	0.0	9.0	11.0	5.0	5.5	1	0	0	1	3	0	0	2	0	0
BB090-NC	6.0	18.0	5.5	0.5	7.0	2.0	12.0	0.0	6.0	10.0	4.0	5.5	1	0	0	1	3	0	0	2	0	0
BB099-NC	7.0	18.0	5.8	1.1	6.5	3.0	11.0	3.0	9.0	10.0	4.4	4.0	1	0	0	1	3	0	0	2	0	0
BB100-UN	10.0	18.0	5.3	1.3	7.0	0.0	7.5	0.0	9.0	10.8	4.5	4.7	1	0	0	1	3	0	0	2	0	0
BB101-NC	6.0	15.0	6.3	1.0	5.8	1.0	6.0	1.0	8.0	9.8	4.5	4.0	1	0	0	1	3	0	0	2	0	0
BB142-NC	10.0	17.0	7.3	1.0	5.6	0.7	7.0	2.7	11.3	12.1	4.5	6.3	1	0	0	1	3	0	0	2	0	0
BB146-NC	9.3	14.4	5.1	0.8	5.3	0.7	7.3	0.7	8.7	9.3	3.5	4.4	1	0	0	1	3	0	0	2	0	0
BE004-NC	7.0	15.3	3.9	0.2	6.4	3.0	10.0	0.0	9.0	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BE082-NC	0.0	14.2	3.8	0.4	6.7	7.0	17.0	0.0	6.0	9.3	2.7	4.7	1	0	0	1	3	0	0	2	0	0
BE084-NC	7.0	16.0	5.2	0.4	7.5	8.0	21.0	0.0	6.0	10.7	4.6	5.1	1	0	0	1	3	0	0	2	0	0
BE085-NC	5.5	14.6	4.6	0.4	6.8	4.0	12.0	0.0	4.0	7.6	3.3	3.8	1	0	0	1	3	0	0	2	0	0
BE087-NC	2.0	16.5	4.3	0.8	8.0	5.0	17.0	0.0	2.0	9.0	3.5	5.5	1	0	0	1	3	0	0	2	0	0
BE089-NC	4.0	14.5	4.5	0.9	7.5	4.5	12.0	0.0	5.0	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BE118-NC	6.0	12.6	5.2	0.5	7.4	6.7	15.3	0.0	4.7	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BE119-NC	8.7	15.4	5.2	0.6	8.0	4.0	14.0	0.0	9.3	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BE120-NC	8.5	16.7	5.7	0.6	9.0	6.0	15.0	0.0	4.5	10.5	3.7	5.6	1	0	0	1	3	0	0	2	0	0
BE121-NC	4.7	13.8	5.0	0.5	7.6	4.7	15.3	0.0	5.3	9.7	3.8	5.1	1	0	0	1	3	0	0	2	0	0
BE122-NC	13.3	16.8	5.6	0.8	7.1	5.0	15.3	1.3	8.7	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BE124-NC	9.0	10.7	3.6	0.6	4.6	2.5	10.5	0.0	4.0	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BE125-NC	6.5	14.3	4.6	0.6	6.2	3.5	12.5	0.5	10.5	999.0	999.0	999.0	1	0	0	1	3	0	0	2	0	0
BI067-EC	7.3	22.7	5.3	2.9	6.5	0.0	4.0	1.3	9.3	14.8	4.1	7.5	1	0	0	1	0	0	0	2	0	2
BI068-EC	10.0	23.4	5.3	2.7	8.1	0.0	3.5	3.5	8.5	14.1	4.6	6.6	1	0	0	1	0	0	0	2	0	2
BI071-EC	8.7	21.6	5.6	1.8	7.0	0.0	2.7	2.7	11.0	12.7	4.1	6.2	1	0	0	1	0	0	0	2	0	2
BI072-EC	8.0	18.7	5.5	2.8	6.4	0.0	2.0	2.7	9.7	999.0	999.0	999.0	1	0	0	1	0	0	0	2	0	2

BI073-EC	6.0	19.7	5.8	2.1	7.0	0.0	4.5	1.0	9.5	999.0	999.0	999.0	1	0	0	1	0	0	0	2	0	2
BI074-EC	7.3	24.4	5.2	2.5	7.8	0.0	3.0	2.0	10.3	999.0	999.0	999.0	1	0	0	1	0	0	0	2	0	2
BI075-EC	7.0	19.3	4.9	1.4	6.6	0.0	5.0	3.0	10.0	13.5	3.9	6.2	1	0	0	1	0	0	0	2	0	2
BI077-EC	9.7	23.9	4.8	3.4	7.9	0.0	0.0	1.3	8.7	11.7	3.6	6.0	1	0	0	1	0	0	0	2	0	2
BI079-EC	9.0	27.6	8.4	1.8	10.5	1.0	4.0	0.0	8.0	12.8	4.4	5.7	1	0	0	1	0	0	0	2	0	2
BI093-EC	3.0	13.5	4.0	1.1	4.8	0.0	2.5	1.0	7.0	8.8	3.0	4.8	1	0	0	1	0	0	0	2	0	2
BI095-EC	7.0	21.8	6.8	1.4	8.8	0.0	2.5	2.0	6.0	11.8	4.5	5.8	1	0	0	1	0	0	0	2	0	2
BI097-EC	5.0	21.0	6.0	1.8	8.0	0.0	3.0	0.0	7.0	14.5	5.0	5.5	1	0	0	1	0	0	0	2	0	2
BI104-UN	9.0	23.2	5.3	2.5	6.9	0.0	5.0	1.0	7.0	999.0	999.0	999.0	1	0	0	1	0	0	0	2	0	2
BI145-EC	0.0	21.7	4.9	2.8	8.6	0.0	0.0	0.0	0.0	13.5	3.5	7.7	1	0	0	1	0	0	0	2	0	2
BI152-EC	7.0	19.3	5.2	1.1	6.1	0.0	3.0	0.7	7.3	11.6	3.7	5.1	1	0	0	1	0	0	0	2	0	2
BP001-EC	8.0	22.9	9.2	3.1	8.3	0.0	6.5	3.0	10.0	15.0	4.6	8.7	1	1	2	1	0	1	2	0	0	1
BP015-EC	5.0	19.0	8.7	1.8	7.4	0.0	5.3	0.7	5.0	999.0	999.0	999.0	1	1	2	1	0	0	2	0	0	1
BP018-WC	0.0	20.5	6.9	3.1	7.1	0.0	0.7	0.0	0.0	999.0	999.0	999.0	1	1	1	1	0	0	2	0	0	1
BP019-WC	0.0	27.5	11.6	2.3	9.3	0.0	2.5	0.0	0.0	999.0	999.0	999.0	1	1	1	1	0	1	2	0	0	1
BP020-WC	1.0	21.8	10.6	2.3	8.9	0.5	4.5	0.0	2.0	14.4	5.9	6.8	1	1	2	1	0	1	2	0	1	1
BP021-WC	4.0	23.4	9.8	3.0	7.6	0.0	4.3	0.0	4.3	10.6	4.7	999.0	1	1	2	1	0	1	2	0	1	1
BP022-EC	4.0	27.9	12.3	2.3	8.4	2.0	7.0	0.7	4.0	999.0	999.0	999.0	1	1	2	1	0	1	2	0	0	1
BP025-WC	0.3	21.8	8.4	2.0	9.0	0.0	2.0	0.0	0.7	999.0	999.0	999.0	1	1	2	1	0	1	2	0	1	1
BP027-WC	0.0	26.0	10.2	2.4	10.2	1.3	8.7	0.0	0.7	15.2	4.3	999.0	1	1	2	1	0	0	2	0	0	1
BP028-WC	3.0	24.1	11.2	3.4	7.9	0.0	5.0	0.0	3.0	999.0	999.0	999.0	1	1	2	1	0	1	2	0	1	1
BP030-EC	6.7	29.8	10.4	2.8	9.0	0.0	6.3	1.3	7.7	999.0	999.0	999.0	1	1	2	1	0	1	2	0	0	1
BP031-WC	5.7	20.2	7.5	1.1	7.3	0.0	2.3	1.7	6.0	13.1	4.2	6.4	1	1	1	1	0	1	1	1	1	4
BP032-WC	0.3	22.9	8.8	1.7	8.7	0.0	2.7	0.0	1.0	999.0	999.0	999.0	1	1	1	1	0	0	2	0	0	1
BP033-EC	4.0	21.0	8.5	2.0	8.6	1.0	5.0	0.0	2.0	999.0	999.0	999.0	1	1	2	1	0	1	2	0	0	1
BP034-WC	2.0	24.7	12.2	2.3	9.6	0.0	2.7	0.7	3.7	999.0	999.0	999.0	1	1	1	1	0	1	2	0	1	1
BP036-WC	2.0	16.4	6.5	1.3	7.0	0.0	1.5	0.5	2.5	999.0	999.0	999.0	1	1	1	1	0	0	2	0	0	1
BP037-WC	0.0	19.4	8.7	1.6	7.8	0.0	1.7	0.0	0.3	999.0	999.0	999.0	1	1	2	1	0	1	2	0	1	1
BP038-WC	0.0	27.2	11.0	3.1	9.7	0.0	2.3	0.0	1.3	999.0	999.0	999.0	1	1	2	1	0	0	2	0	0	1
BP039-WC	2.0	24.5	11.1	2.6	8.8	0.0	4.0	0.0	0.0	14.1	5.2	6.6	1	1	2	1	0	0	2	0	0	1
BP041-EC	5.8	22.5	10.8	2.7	8.9	0.0	6.0	1.3	6.0	11.0	4.7	4.7	1	1	2	1	0	0	2	0	0	1
BP096-WC	0.0	24.3	7.3	2.0	10.5	0.0	2.0	0.0	6.0	12.3	4.5	6.5	1	1	1	1	0	1	1	1	0	4
BP153-WC	3.5	24.9	12.1	1.0	8.2	0.5	6.0	2.5	8.5	999.0	999.0	999.0	1	1	2	1	0	1	2	0	1	1
BR043-Nam	8.0	18.4	5.3	1.9	5.8	0.0	3.0	2.0	8.0	9.0	2.4	4.9	0	2	0	1	3	0	0	2	0	3
BR045-NC	6.3	16.7	5.3	1.3	6.1	0.0	3.3	0.7	5.3	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	1
BR046-NC	5.7	18.1	6.9	1.9	6.5	1.7	7.7	0.0	5.0	12.7	5.0	6.0	0	2	0	1	0	0	0	2	0	2
BR047-Nam	10.0	20.5	5.4	2.2	6.1	0.0	4.0	7.0	17.0	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	2
BR049-Nam	7.3	18.3	4.6	2.4	6.4	0.0	4.0	2.0	8.0	12.0	3.3	6.2	0	2	0	1	3	0	0	2	0	3

BR051-FS	8.0	14.2	5.5	1.8	5.3	0.0	3.3	1.3	8.0	10.0	3.5	4.8	0	2	0	1	0	0	0	2	0	2
BR052-NC	4.7	16.5	6.3	2.4	6.0	0.0	3.3	2.0	7.3	12.6	3.9	5.9	0	2	0	1	0	0	0	2	0	2
BR053-Nam	10.0	21.8	3.9	2.3	5.0	0.0	1.7	7.3	15.0	9.9	3.5	5.3	0	2	0	1	0	0	0	2	0	2
BR056-FS	9.3	14.7	5.3	1.6	5.1	0.0	4.0	2.0	8.0	8.8	2.6	4.2	0	2	0	1	0	0	0	2	0	2
BR057-FS	6.3	15.0	4.4	1.5	5.9	0.3	5.0	0.0	7.0	10.1	3.8	4.7	0	2	0	1	0	0	0	2	0	2
BR058-Nam	6.0	17.2	7.1	1.5	7.1	2.0	7.5	0.0	4.3	13.1	3.9	6.5	0	2	0	1	0	0	0	2	0	2
BR059-Nam	13.3	19.7	5.6	1.8	7.3	0.0	7.7	7.3	20.7	9.3	3.2	4.9	0	2	0	1	0	0	0	2	0	2
BR062-NC	7.5	18.9	6.5	1.7	6.4	1.0	7.0	2.0	6.5	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	2
BR063-NC	4.0	15.8	6.1	0.8	6.8	2.0	5.0	1.0	5.0	12.0	4.4	5.4	0	2	0	1	0	0	0	2	0	2
BR064-Nam	19.3	16.0	4.2	1.7	5.6	2.7	8.7	6.7	16.0	999.0	999.0	999.0	0	2	0	1	3	0	0	2	0	2
BR065-NC	7.0	15.2	5.5	1.9	6.0	1.0	5.5	1.0	9.0	10.0	4.2	4.7	0	2	0	1	0	0	0	2	0	2
BR083-Bot	11.0	14.4	4.4	0.9	5.3	0.0	5.7	4.3	13.0	10.2	4.1	5.9	0	2	0	1	0	0	0	2	0	2
BR102-Nam	13.0	21.3	5.0	1.1	7.3	0.0	10.0	9.0	21.0	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	3
BR103-NC	12.0	22.5	6.0	2.0	5.5	0.0	2.0	8.0	18.0	9.0	4.0	3.0	0	2	0	1	0	0	0	2	0	2
BR140-NC	4.7	17.0	5.4	1.1	5.8	0.0	3.3	2.0	7.3	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	2
BR147-Bot	11.0	18.4	6.2	1.3	5.3	0.0	4.0	5.0	9.0	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	2
BR148-Bot	10.5	15.9	5.1	1.0	5.4	0.0	4.8	6.5	16.0	10.1	3.6	4.5	0	2	0	1	0	0	0	2	0	2
BR149-Nam	9.0	14.3	4.5	0.8	4.7	0.5	5.8	5.5	11.5	11.2	3.7	5.0	0	2	0	1	0	0	0	2	0	2
BR150-Nam	8.0	14.7	4.7	1.1	5.6	0.0	3.3	0.0	8.7	10.9	17.0	5.1	0	2	0	1	0	0	0	2	0	2
BU066-EC	5.3	17.4	4.4	2.7	8.2	0.0	3.0	1.3	5.7	999.0	999.0	999.0	1	0	0	1	0	0	0	2	0	0
BU069-EC	4.0	19.3	4.7	2.2	7.5	0.0	4.0	0.0	4.0	999.0	999.0	999.0	1	1	0	1	0	0	0	2	0	0
BU070-EC	3.3	18.3	3.8	1.8	8.4	0.0	3.0	0.0	2.7	10.7	4.0	4.9	1	1	0	1	0	0	0	2	0	0
BU076-EC	6.7	18.0	3.5	2.5	7.7	0.0	4.7	2.0	6.0	999.0	999.0	999.0	1	1	0	1	0	0	0	2	0	0
BU078-EC	3.5	17.1	3.2	2.0	7.5	0.0	3.5	0.0	3.0	10.6	3.3	4.9	1	1	0	1	0	0	0	2	0	0
BU091-EC	5.0	13.5	3.7	1.1	5.5	0.0	3.0	1.0	5.5	999.0	999.0	999.0	1	1	0	1	0	0	0	2	0	0
BU092-EC	2.5	19.0	3.8	1.5	8.3	0.0	3.0	1.0	3.0	11.8	4.0	5.0	1	1	0	1	0	0	0	2	0	0
BU094-EC	4.5	18.0	4.5	1.8	5.5	0.0	1.5	0.0	4.0	11.3	3.3	6.3	1	1	0	1	0	0	0	2	0	0
BU105-EC	3.8	22.1	4.8	1.7	8.7	0.0	2.8	0.0	4.5	10.4	3.7	5.0	1	1	0	1	0	0	0	2	0	0
BU123-EC	8.3	18.4	3.2	2.1	7.3	1.0	5.5	2.0	10.5	7.9	2.5	3.7	1	1	0	1	0	0	0	2	0	0
BU143-EC	2.0	20.2	4.4	1.6	8.3	0.0	3.3	0.7	6.0	12.5	4.1	5.9	1	0	0	1	0	0	0	2	0	0
EL044-KZN	10.0	21.8	8.5	2.0	7.6	0.0	4.3	3.3	8.7	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL050-Swa	11.3	24.0	9.1	2.0	8.7	0.3	5.7	4.0	12.0	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL055-Limp	9.3	20.4	7.1	1.5	7.0	0.0	5.3	3.3	12.3	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL061-Mpu	9.3	25.2	7.8	2.7	8.5	0.0	4.7	1.3	8.0	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL127-Limp	6.7	22.3	6.6	1.3	8.8	1.3	6.3	2.0	9.3	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL128-UN	10.7	23.9	8.7	1.5	9.5	1.0	5.7	3.3	10.0	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL129-Mpu	11.3	23.9	7.8	1.8	9.0	2.7	9.7	4.0	11.3	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL130-Mpu	8.0	21.0	6.3	1.8	9.3	2.0	6.5	3.5	10.0	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4

EL131-Zim	8.0	22.6	7.2	1.7	10.0	1.0	6.5	2.0	8.5	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL132-Zim	8.7	19.9	6.8	1.6	8.7	0.0	4.7	4.0	10.0	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL133-Swa	6.0	22.6	7.5	1.6	9.2	0.0	6.3	1.3	8.7	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL134-Zim	7.3	22.1	7.0	1.5	8.5	0.7	6.7	3.3	9.3	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL135-Zim	6.7	24.9	6.8	2.0	9.1	0.0	3.7	2.3	8.3	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL136-Swa	8.0	20.0	8.2	1.7	7.6	0.0	4.0	5.3	11.7	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL137-Moz	6.3	20.3	6.8	1.3	7.7	0.0	4.7	1.7	7.3	14.1	4.9	6.4	0	2	1	1	2	1	1	1	1	4
EL138-Moz	5.3	19.8	6.8	1.6	7.6	0.0	4.0	1.3	6.7	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
EL139-Moz	8.3	22.2	9.7	1.6	8.8	0.0	5.3	5.3	11.3	999.0	999.0	999.0	0	2	1	1	2	1	1	1	1	4
IL081-NC	5.3	16.3	3.8	2.5	7.7	0.0	4.0	1.0	7.0	12.6	4.4	5.5	0	2	0	1	0	0	0	2	0	3
IL111-Nam	9.3	20.3	7.0	1.4	7.1	0.7	7.3	6.0	12.0	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	3
IL113-Nam	6.8	18.5	6.2	2.9	6.5	0.0	3.2	2.0	7.6	11.6	4.2	5.3	0	2	0	1	0	0	0	2	0	3
IL114-Nam	6.7	17.7	4.9	1.9	5.3	0.0	4.7	2.0	7.7	10.0	3.2	4.9	0	2	0	1	0	0	0	2	0	3
IL151-NC	6.0	20.0	7.0	1.8	6.0	0.0	2.0	2.3	8.0	999.0	999.0	999.0	0	2	0	1	0	0	0	2	0	3
ILb054-Nam	4.5	20.3	6.5	2.0	7.8	0.5	7.5	2.0	7.0	999.0	999.0	999.0	0	2	0	1	3	0	0	2	0	3
ILb060-Nam	10.3	17.6	4.4	1.0	6.7	0.0	7.0	2.7	10.0	11.2	3.8	5.4	0	2	0	1	3	0	0	2	0	1
ILb110-Nam	9.3	19.6	6.3	1.6	7.3	1.3	6.7	2.7	12.7	999.0	999.0	999.0	0	2	0	1	3	0	0	2	0	3
ILb112-Nam	11.5	19.0	4.4	1.7	7.0	0.0	8.5	6.0	12.0	999.0	999.0	999.0	0	2	0	1	3	0	0	2	0	3
ILb115-Nam	6.0	20.2	5.7	1.9	8.1	0.7	11.3	2.0	10.7	999.0	999.0	999.0	0	2	0	1	3	0	0	2	0	1
JU007-Nam	8.3	26.9	8.6	2.4	9.1	0.0	5.0	5.3	10.7	15.1	4.5	7.2	1	1	1	1	2	1	2	1	1	4
JU008-Nam	7.5	22.0	6.6	2.5	7.5	1.0	6.0	3.0	10.0	14.8	4.5	7.6	1	1	1	1	2	1	2	1	1	4
JU009-Nam	6.5	23.4	5.9	2.3	9.7	1.0	7.0	2.0	6.7	15.7	4.7	8.4	1	1	1	1	2	1	2	1	1	4
JU106-Nam	7.5	21.1	7.5	1.8	8.4	1.3	7.5	2.5	8.0	999.0	999.0	999.0	1	1	1	1	2	1	2	1	1	4
JU107-Nam	9.5	21.0	7.8	1.7	8.5	2.0	7.3	3.5	11.5	999.0	999.0	999.0	1	1	1	1	2	1	2	1	1	4
JU108-Nam	6.7	24.7	7.8	3.0	7.5	0.0	5.3	4.7	11.3	13.9	5.2	6.8	1	1	1	1	2	1	2	1	1	4
JU109-Nam	9.3	24.5	7.6	2.4	8.7	2.7	8.0	4.7	12.7	999.0	999.0	999.0	1	1	1	1	2	1	2	1	1	4
JU116-Nam	8.7	27.7	9.7	2.2	11.2	1.7	5.7	2.7	10.3	999.0	999.0	999.0	1	1	1	1	2	1	2	1	1	4
JU117-Nam	9.7	24.2	7.3	2.5	9.0	0.0	5.0	4.7	11.3	999.0	999.0	999.0	1	1	1	1	2	1	2	1	1	4
JU141-Nam	8.0	20.0	5.3	0.9	8.0	0.3	5.3	1.0	4.0	999.0	999.0	999.0	1	1	0	1	2	2	0	1	1	0
JU144-Nam	5.5	21.3	7.4	2.1	9.0	2.0	9.0	2.0	7.0	999.0	999.0	999.0	1	1	1	1	2	1	2	1	1	4
LB011-Nam	13.0	23.0	11.7	3.0	8.5	0.0	13.0	11.0	30.0	999.0	999.0	999.0	1	2	0	1	0	0	0	2	0	3
LB012-Nam	10.0	24.5	11.2	2.9	9.2	0.0	10.0	4.0	21.0	999.0	999.0	999.0	1	2	0	1	0	0	0	2	0	3
LB013-Nam	13.0	19.9	9.4	1.6	7.8	1.0	7.0	8.0	19.0	999.0	999.0	999.0	1	2	0	1	0	0	0	2	0	3
LB042-Nam	13.0	28.1	11.3	3.3	11.2	0.0	7.0	11.0	21.0	999.0	999.0	999.0	1	2	0	1	0	0	0	2	0	3
LB048-Nam	11.0	20.8	9.5	2.4	8.1	0.0	7.0	7.0	18.0	999.0	999.0	999.0	1	2	0	1	0	0	0	2	0	3
PU014-EC	5.5	28.7	9.6	4.2	8.9	0.0	2.0	0.0	3.5	15.1	5.9	6.6	1	1	2	1	0	0	2	0	0	1
PU016-EC	3.0	20.2	6.0	3.2	7.7	0.0	2.3	0.7	3.7	14.2	4.9	6.2	1	1	2	1	0	0	2	0	0	1
PU017-EC	3.0	25.3	8.1	5.1	8.7	0.5	2.5	1.0	3.5	999.0	999.0	999.0	1	1	2	1	0	0	2	0	0	1

PU023-EC	6.0	22.9	11.4	3.2	7.0	0.0	4.0	0.0	6.3	14.1	4.9	6.1	1	1	2	1	0	0	2	0	0	1
PU024-EC	7.0	20.2	7.7	1.5	6.1	1.5	4.5	2.0	8.5	13.4	4.7	7.4	1	1	2	1	0	0	2	0	0	1
BP026-WC	6.5	26.3	7.4	2.7	8.2	0.0	3.0	3.0	12.5	999.0	999.0	999.0	1	1	1	1	0	0	2	0	0	1
PU029-EC	6.7	20.1	8.7	2.2	6.8	1.3	7.0	0.7	6.0	14.7	4.5	7.0	1	1	2	1	0	0	2	0	0	1
PU035-EC	11.3	22.6	10.3	2.0	8.4	0.0	5.7	2.7	9.3	999.0	999.0	999.0	1	1	2	1	0	0	2	0	0	1
PU040-EC	5.0	22.3	9.5	2.5	7.2	0.0	4.0	0.0	4.0	999.0	999.0	999.0	1	1	2	1	0	0	2	0	0	1
BP098-WC	10.0	18.8	6.4	2.0	6.3	0.5	4.0	3.0	10.0	13.5	5.0	5.5	1	1	1	1	0	0	2	0	0	1
PU126-EC	10.0	28.1	11.7	2.7	9.3	0.7	6.0	4.3	10.0	15.9	4.9	7.8	1	1	2	1	0	0	2	0	0	1

2.8.5c Appendix V continued: Scores for binary or multistate ordered qualitative and quantitative characters used in the analyses

Label	Margstrt	Midrib	Latvein	Leafbase	Flrs	Bsurfab	Bsurfad	Babglos	Badglos	Braspn	Bmargcolr	Bralobe	Bravein	Bvconsp	Bmargent	Bmargpub	Bmargspn	Brapex	Calhard	Calpub	Calmarg	Calmgspn
BB002-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB003-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB005-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB006-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB010-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB080-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB086-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB088-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB090-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB099-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB100-UN	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB101-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB142-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BB146-NC	1	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BE004-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE082-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE084-NC	0	0	1	0	0	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE085-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE087-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE089-NC	0	0	1	0	0	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE118-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE119-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE120-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE121-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE122-NC	0	0	1	0	0	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BE124-NC	0	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0

BE125-NC	0	0	1	0	0	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BI067-EC	1	1	1	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI068-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI071-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI072-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI073-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI074-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI075-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI077-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI079-EC	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI093-EC	1	1	1	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI095-EC	1	1	1	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI097-EC	1	1	1	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI104-UN	1	1	0	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BI145-EC	1	1	1	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	2
BI152-EC	1	1	1	0	1	0	0	1	0	1	0	1	1	1	2	0	0	1	1	0	0	0
BP001-EC	1	2	0	1	1	1	0	1	0	1	0	1	1	1	2	1	1	1	1	1	1	1
BP015-EC	1	2	0	0	1	1	0	1	0	1	1	1	1	1	2	1	2	1	1	1	1	2
BP018-WC	1	1	0	0	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP019-WC	1	1	0	0	1	1	0	1	0	1	1	1	1	1	2	2	2	1	1	1	1	1
BP020-WC	1	2	0	1	1	1	0	1	0	1	1	1	1	1	2	3	2	1	1	1	1	2
BP021-WC	1	2	0	0	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP022-EC	1	2	0	1	1	1	0	1	0	1	0	1	1	1	2	1	2	1	1	1	1	2
BP025-WC	1	2	0	1	1	1	0	1	0	1	1	1	1	1	2	3	2	1	1	1	1	2
BP027-WC	1	1	0	1	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP028-WC	1	2	0	1	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP030-EC	1	2	0	1	1	1	1	1	0	1	0	1	1	1	2	1	1	1	1	1	1	2
BP031-WC	0	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	1	0	0
BP032-WC	1	1	0	0	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP033-EC	1	2	0	0	1	1	0	1	0	1	0	1	1	1	2	3	2	1	1	1	1	2
BP034-WC	1	1	0	0	1	0	0	1	0	1	1	1	1	1	2	2	0	1	1	1	1	1
BP036-WC	1	1	0	1	1	1	0	1	0	1	1	1	1	1	2	3	1	1	1	1	1	1
BP037-WC	1	2	0	1	1	1	0	1	0	1	1	1	1	1	2	3	2	1	1	1	1	2
BP038-WC	1	2	0	1	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP039-WC	1	1	0	1	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP041-EC	1	2	0	1	1	1	0	1	0	1	1	1	1	1	2	2	1	1	1	1	1	1
BP096-WC	0	1	0	0	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	1	0	0
BP153-WC	1	2	0	0	1	1	1	1	0	1	1	1	1	1	2	3	2	1	1	1	1	2

BR043-Nam	2	0	1	0	1	0	0	1	1	1	0	1	0	1	3	0	0	1	1	0	0	0
BR045-NC	3	0	1	0	1	0	0	1	1	1	0	1	0	1	2	0	0	1	1	0	0	0
BR046-NC	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BR047-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR049-Nam	2	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
BR051-FS	3	0	1	0	0	0	0	1	1	1	0	1	1	1	1	0	0	1	1	0	0	0
BR052-NC	3	0	1	0	1	0	0	1	1	1	0	1	0	1	2	0	0	1	1	0	0	0
BR053-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR056-FS	3	0	1	0	0	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	0	0
BR057-FS	3	0	1	0	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	0	0
BR058-Nam	3	0	1	0	1	0	0	1	1	1	0	1	0	1	2	0	0	1	1	0	0	0
BR059-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR062-NC	3	0	1	0	1	0	0	1	1	1	0	1	0	1	2	0	0	1	1	0	0	0
BR063-NC	3	0	1	0	0	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	0	0
BR064-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	2	0	0	1	1	0	0	0
BR065-NC	3	0	1	0	0	0	0	1	1	1	0	1	0	1	2	0	0	1	1	0	0	0
BR083-Bot	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR102-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR103-NC	3	0	1	0	1	0	0	1	1	1	0	1	0	1	3	0	0	1	1	0	0	0
BR140-NC	3	0	1	0	1	0	0	1	1	1	0	1	0	1	2	0	0	1	1	0	0	0
BR147-Bot	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR148-Bot	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR149-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	0	0	0	0
BR150-Nam	3	0	1	0	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	0	0
BU066-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU069-EC	1	1	1	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU070-EC	1	1	1	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU076-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU078-EC	1	1	1	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU091-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU092-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU094-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU105-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
BU123-EC	1	1	1	0	1	0	0	1	0	0	0	1	0	1	1	0	0	1	1	0	0	0
BU143-EC	1	1	0	0	0	0	0	1	0	0	0	1	0	1	2	0	0	1	1	0	0	0
EL044-KZN	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL050-Swa	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL055-Limp	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0

EL061-Mpu	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL127-Limp	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL128-UN	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL129-Mpu	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL130-Mpu	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL131-Zim	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL132-Zim	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL133-Swa	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL134-Zim	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL135-Zim	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL136-Swa	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL137-Moz	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL138-Moz	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
EL139-Moz	0	1	0	0	1	0	0	0	0	1	1	1	1	1	3	0	0	1	1	0	0	0
IL081-NC	2	0	1	0	1	0	0	1	1	1	0	1	0	1	3	0	0	1	1	0	0	0
IL111-Nam	2	0	1	0	1	0	0	1	1	1	0	0	1	1	3	0	0	1	1	0	0	0
IL113-Nam	2	0	1	0	1	0	0	1	1	1	0	1	0	1	3	0	0	1	1	0	0	0
IL114-Nam	2	0	1	0	1	0	0	1	1	1	0	1	0	1	3	0	0	1	1	0	0	0
IL151-NC	2	0	1	0	1	0	0	1	1	1	0	1	0	1	3	0	0	1	1	0	0	0
ILb054-Nam	2	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
ILb060-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
ILb110-Nam	2	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
ILb112-Nam	3	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
ILb115-Nam	2	0	1	0	1	0	0	1	1	1	0	1	1	1	3	0	0	1	1	0	0	0
JU007-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU008-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU009-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU106-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU107-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU108-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU109-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU116-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU117-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU141-Nam	0	0	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
JU144-Nam	0	2	0	0	1	2	2	1	1	1	1	1	1	1	3	1	2	1	1	1	0	2
LB011-Nam	2	0	1	0	1	0	0	1	0	1	0	0	1	0	3	0	0	0	1	0	0	0
LB012-Nam	2	0	1	0	1	0	0	1	0	1	0	0	1	0	3	0	0	0	1	0	0	0
LB013-Nam	2	0	1	0	1	0	0	1	0	1	0	0	1	0	3	0	0	0	1	0	0	0

LB042-Nam	2	0	0	0	1	0	0	1	0	1	0	0	1	0	3	0	0	0	1	0	0	0
LB048-Nam	2	0	1	0	1	0	0	1	0	1	0	0	1	0	3	0	0	0	1	0	0	0
PU014-EC	1	2	0	0	1	1	0	1	0	1	0	1	1	1	2	1	2	1	1	1	1	2
PU016-EC	1	2	0	0	1	1	0	1	0	1	0	1	1	1	2	2	1	1	1	1	1	1
PU017-EC	1	2	0	0	1	1	0	1	0	1	0	1	1	1	2	1	2	1	1	1	1	2
PU023-EC	1	2	0	1	1	1	0	1	0	1	0	1	1	1	2	3	2	1	1	1	1	2
PU024-EC	1	2	0	0	1	1	0	1	0	1	0	1	1	1	2	2	1	1	1	1	1	1
BP026-WC	1	1	0	0	1	1	0	1	0	1	0	1	1	1	2	2	0	1	1	1	1	1
PU029-EC	1	1	0	0	1	1	0	1	0	1	0	1	1	1	2	2	2	1	1	1	1	1
PU035-EC	1	2	0	0	1	1	0	1	0	1	0	1	1	1	2	2	1	1	1	1	1	1
PU040-EC	1	2	0	1	1	1	0	1	0	1	0	1	1	1	2	1	1	1	1	1	1	2
BP098-WC	1	1	0	0	1	1	0	1	0	1	1	1	1	1	2	1	1	1	1	1	1	1
PU126-EC	1	1	0	1	1	1	0	1	0	1	0	1	1	1	2	3	2	1	1	1	1	2

2.8.6 Appendix VI: Fifty eight qualitative and quantitative characters used in the PCO analysis for B. bechuanensis, B. irritans and B. jubata

Barleria elegans was the standard taxon, and the characters influencing differentiation in the first three axes are in bold.

Characters	**Dim-1**	**Dim-2**	**Dim-3**
W	**0.6409**	0.2860	0.5049
PL	0.5973	0.2727	0.4802
SL	0.4841	**-0.6239**	-0.0560
M	**0.6749**	0.3460	0.0611
leafWP	0.4520	0.4167	0.5535
BW	0.1834	0.4554	0.5101
BS	**0.6019**	-0.4106	-0.0961
BWP	-0.4690	-0.0210	0.3275
B #teeth2	**-0.7705**	0.2222	-0.0200
B #teeth4	0.0768	0.2961	0.0657
CYW-A	0.3649	0.3421	-0.0758
CYS-A	0.5901	-0.5538	0.3970
CYL-AWP	0.3011	-0.0856	0.0229
A #teeth2	**-0.7437**	0.2234	-0.0381
A #teeth4	-0.1028	0.2133	0.1777
CYW-P	0.4921	0.3915	0.0626
CYS-P	**0.6144**	**-0.6147**	0.2200
CYL-PWP	0.4645	-0.0544	0.1370
P #teeth2	**-0.7525**	0.2837	-0.0958
P #teeth4	-0.0225	0.1953	0.1329
L:W	**-0.8846**	0.0956	0.0829
L:PL	**-0.7880**	0.1560	0.1730
L:LWP	-0.5072	-0.5986	0.2627
BL:BW	-0.0003	**-0.9764**	0.0041
TotteethB	-0.5382	0.4335	-0.0418
BL:teeth	**-0.6694**	0.4538	0.0230
CYL-A:W	-0.1039	**-0.9320**	0.4115
TotteethA	-0.5880	0.3471	0.0199
CYL-A:teeth	**-0.7988**	0.2976	0.0422
CYL-P:W	-0.0584	**-1.0136**	0.3711
TotteethP	-0.5736	0.4304	-0.0616
CYL-P:teeth	**-0.8457**	0.3112	-0.0368
Stemsurf	**-0.6240**	**-0.8102**	-0.3712
Stempub	**0.6651**	0.1872	0.4150
leafsurf	**0.6854**	0.3490	0.0578
Leafshp	-0.5107	0.5808	-0.1434
Margcolr	**0.6670**	0.3173	-0.0340
Margpub	**0.6440**	0.1807	-0.2867
Hairbase	**-0.8866**	-0.7442	0.3181
Margthick	**0.6961**	0.3465	0.0064
Margent	**0.7522**	0.1945	0.1453

Margstrt	-0.3825	**-0.9076**	0.4143
Midrib	**0.7737**	-0.4100	-0.0945
Latvein	**-0.9210**	-0.2292	0.1149
Flrs	0.0319	0.2972	0.0609
Bsurfab	0.3728	-0.1697	**-0.6873**
Bsurfad	0.3728	-0.1697	**-0.6873**
Babglos	**-0.6240**	**-0.8102**	0.3712
Badglos	**-0.6166**	0.1124	0.5746
Braspn	-0.1626	0.4975	-0.0299
Brahard	0.1920	0.5099	-0.0899
Bravein	-0.1626	0.4975	-0.0299
Bmargent	-0.1784	**0.6659**	-0.1654
Bmargpub	0.3728	-0.1697	**-0.6873**
Bmargspn	0.3728	-0.1697	**-0.6873**
Calhard	0.3986	-0.3337	0.2247
Calpub	0.3728	-0.1697	**-0.6873**
Calmgspn	0.3778	-0.2277	**-0.6520**

2.8.7 Appendix VII: Fifty two qualitative and quantitative characters used in the PCO analysis for the B. pungens complex

Characters influencing differentiation in the first three axes are in bold.

Character	**Dim-1**	**Dim-2**	**Dim-3**
W	0.3730	0.0067	0.1194
PL	-0.0110	0.5418	-0.0891
SL	0.1997	0.3052	-0.3650
leafWP	0.1097	0.4710	0.3191
BW	0.2821	0.0332	0.5966
BS	-0.0786	0.2152	-0.4024
BWP	0.3640	0.3308	0.2503
B #teeth1	0.1584	0.0430	0.3737
B #teeth2	-0.3078	0.1742	0.4489
B #teeth3	-0.5623	-0.1515	-0.1013
B #teeth4	-0.5639	-0.1638	-0.0180
CYW-A	0.4375	0.0171	**0.6287**
CYS-A	0.2419	0.1193	**-0.6119**
CYL-AWP	0.4369	0.4611	0.1818
A #teeth1	-0.2601	-0.3711	0.1433
A #teeth2	-0.2970	-0.4285	0.1994
A #teeth3	-0.5304	-0.2367	-0.0758
A #teeth4	-0.5920	-0.4223	-0.0823
CYW-P	0.4788	-0.0364	0.5921
CYS-P	0.2973	0.1084	-0.5237
CYL-PWP	0.3393	0.5946	0.2213
P #teeth1	-0.1161	-0.1932	0.2798

P #teeth2	-0.1086	-0.3833	0.4830
P #teeth3	-0.5619	-0.2009	-0.0434
P #teeth4	**-0.6413**	-0.1709	-0.0269
L:W	0.1494	0.4104	0.0977
L:PL	0.4030	-0.1801	0.2570
L:LWP	0.3434	-0.2646	-0.3508
BL:BW	-0.3642	0.4532	**-0.6723**
TotteethB	-0.5535	-0.0797	0.1743
BL:teeth	-0.5272	-0.1694	0.1367
CYL-A:W	-0.3127	0.4794	**-0.8170**
TotteethA	-0.5171	-0.4926	0.0579
CYL-A:teeth	-0.5528	-0.5043	-0.0131
CYL-P:W	-0.2996	0.5593	**-0.7283**
TotteethP	-0.5301	-0.3312	0.2300
CYL-P:teeth	-0.5949	-0.3715	0.1558
leafsurf	0.3085	-0.3521	0.1319
Margcolr	0.1609	0.5599	0.5018
Margpub	**0.6234**	-0.5492	-0.2593
Hairbase	-0.5046	**0.9006**	0.1157
Margent	-0.5046	**0.9006**	0.1157
Margstrt	**0.6234**	-0.5492	-0.2593
Midrib	0.3233	-0.1653	0.0262
Leafbase	0.4196	0.0540	0.1984
Bsurfab	-0.0927	0.0197	-0.2843
Babglos	**0.6726**	-0.2329	-0.2817
Bmargcolr	0.3380	0.5557	0.3076
Bmargent	0.1922	-0.3719	-0.2560
Bmargspn	0.4368	-0.5190	-0.3541
Calmarg	**0.6489**	0.1264	-0.4686
Calmgspn	**0.6234**	-0.5492	-0.2593

2.8.8 Appendix VIII: Thirty four qualitative and quantitative characters used in the PCO analysis for the B. rigida complex

Characters influencing differentiation in the first three axes are in bold.

Character	Dim-1	Dim-2	Dim-3
W	0.4446	0.4266	0.3051
PL	0.2271	0.0386	0.2988
M	0.2591	**0.6775**	0.1430
SL	0.3157	0.3120	0.3041
leafWP	0.2456	0.0524	0.3844
BW	**0.6433**	-0.0114	-0.0799
BS	0.1221	0.4468	-0.2772
BWP	-0.0692	-0.2211	-0.2203
CYW-A	0.2759	0.4639	-0.5370
CYS-A	-0.0239	0.5728	-0.0425

CYL-AWP	0.3758	0.3417	-0.0437
CYW-P	**0.6237**	0.2101	-0.4440
CYS-P	0.3065	0.4261	-0.1481
CYL-PWP	0.5713	0.2577	-0.0960
L:W	**-0.8676**	0.2369	-0.4858
L:PL	-0.4465	0.0563	-0.1429
L:LWP	-0.5793	0.2124	-0.4859
BL:BW	**-0.8676**	0.2369	-0.4858
CYL-A:W	-0.4402	-0.1429	0.4637
CYL-P:W	**-0.7608**	-0.1552	0.3805
TotteethP	0.4626	-0.4636	-0.1210
Stemsurf	**0.6691**	0.0485	-0.3564
Leafshp	-0.2933	0.0510	0.4149
Margent	0.2532	0.4310	0.1838
Margstrt	**-0.8487**	-0.4638	-0.5099
Flrs	-0.0204	-0.0428	0.3589
Brahard	0.4207	0.3540	0.2883
Bralobe	**-1.1678**	0.0920	0.1100
Bravein	**-0.7299**	-0.0147	-0.0586
Bvconsp	**-1.1678**	0.0920	0.1100
Bmargent	0.1600	-0.0434	0.5120
Brapex	**-1.1678**	0.0920	0.1100
Calhard	-0.0516	**0.7474**	-0.2891
Calovlap	**-0.7608**	-0.1552	0.3805

2.8.9 Appendix IX: List of specimens examined with numbers in square brackets denoting specimen codes allocated during analysis

Namibia.

–1714 (RuacanaFalls): 20 miles southern Ruacana, Bergveld (–AD), *Giess & Leippert 7588* (NBG) [JU141]. –2016 (Otjiwarongo): Okosongomingo (–BC), 11 June1939, *Volk 2249* (M) [JU108]; 13.9 miles S. of Otjiwarongo on road to Okahanja, Farm Tweekoppies (–DA) *de Winter 2739* (K) [JU117]; Okanjanda, Erongo Mts (–DA), June – July 1916, *Pearson 9895* (BOL, NBG) [JU007]; Farm Graslaagte, on Grey Mountain (also found on the Ozondjaheberg) (–DB), 28 November 1952, *Walter 283* (B, M) [JU106]. –2017 (Waterberg): 65 km E of Otjiwarongo, Waterberg plantation, on the slope down from the plantation to the plain (–AC), 3 March 1968, *Wanntorp & Wanntorp 641* (K) [JU116]; Upper slopes at S.W. point of the Omuverume Plateau (–AC) *Rutherford 352* (WIND) [JU107]; Okarakowisa, north of Farm Bergtuine OTJ 455, on Waterberg Plateau on red sandstone cliffs (–AD) *Geiss 12375* (MO; PRE) [JU009]; Waterberg – Abhang (–AD) *Volk 2472* (M) [JU109]; Waterberg Plateau (–CA), *Boss 34948* (PRE) [JU008]; Klein Waterberg above Okosongomingo (–CA), 15 July 1954, *Schelpe 201* (J) [JU144]. –2118 (Steinhausen): Gobabis, ca 40 km NW of Gobabis, Road Steinhausen – Gobabis, Farm Hondeblaf 179 (–BA), 7 January 1968, *Wanntorp* & *Wanntorp 743* (K, PRE) [BR149]. –2217 (Windhoek): Avis, western mountain land (–CA), 7 April 1964, *Seydel 3944* (M) [ILa112]. –2316 (Nauchs): Rehoboth, Farm Gurumanas REH 306 (–BB), 10 March 1953, *Walter & Walter 1650* (WIND) [ILa115]. –

2317 (Rehoboth): Farm Bergland-Arovley, on surface lime in limestone meadow community (–AA), March 1953, *Giess 1787* (WIND, B) [ILa060].

–2418 (Stampriet): Gellapost Expt. Station (–AC), May 1949, *Liebenberg 5177* (WIND) [BR043]; 16 km from Mariental turn off to Stampriet, Opstal farm (–AD), 11 March 1983, *Germishuizen 2746* (PRE, WIND) [BR059]. –2516 (Helmering-hausen): Farm Naus 27 (–CD), 21 April 1980, *Owen-Smith 1317* (WIND) [LB013]. –2517 (Gibeon): Farm Haribes (–BC), 13 April 1956, *Volk 12391* (M) [ILa110]. –2519 (Koes): Farm Grensplaas 409, 2 kilometres south of homestead in river, 25º 29' 8" S 19º 29' 49" E (–AD), 19 April 1997, *Calitz* & *Sheuyange 104* (WIND) [BR064]. –2615 (Luderitz): 15 miles west of Aus, Dry sandy riverbed, east facing slopes of Sesselberge, (–CB), 13 August 1959, *Giess & Vuuren 848* (PRE, WIND, SRGH) [LB012]; Farm Weissenborn, in rocks behind farmhouse (–DA), 5 July 1949, *Kinges 2338 (2388)* (M, PRE) [LB042]; 48.3 m. S of Aus on road to Witputs, Coarse gravelly sand-flats and granite hills (–DD), 9 March 1963, *de Winter & Hardy 7944* (PRE) [LB048]. –2616 (Aus): Namtib, 26º 7’ 30”S 16º 22’ 30”E, cliff face (–AB), 27 May 2001, *Theile THE89* (WIND) [LB011]. –2618 (Keetmanshoop): 20 miles S.E. of Keetmanshoop on rd to Narubis (–CA), 28 April 1955, *de Winter 3267* (K, PRE, WIND) [BR049].

–2718 (Grünau): Hardap, Farm Galloway 230, about 1.2 km south of house, 25º 13' 33"S 18º 37' 51"E (–BA), 23 March 1998, *Strohbach* & *Dauth 3695* (WIND) [BR053]; Tallus, on route to Telecom Tower on Farm Rishon 365, 27º 20’ 17” S 18º 43’ 47” E, slope of Karas Mountain (–BC), 14 February 1997, *Strohbach 2856* (WIND) [BR058]; Farm Genadendal 264, 27º 30' 24" S 18º 32' 33" E, western slope of Karasberg (–DA), 16 March 1997, *Strohbach* & *Sheuyange 3308* (a) (K, WIND) [BR150]; *ibidem* (–DA), 16 March 1997, *Strohbach* & *Sheuyange 3308* (b) (WIND) [BR047]. –2816 (Oranjemund): Slate Mountains at Loreley (–BB), 21 February 1963, *Leppert 4207* (M) [IL113]; 20 miles S.E. of Keetmanshoop on rd to Narubis (–CA), 28 April 1955, *de Winter 3267* (K, PRE, WIND) [BR049]. –2818 (Warmbad): Farm Haakiesdoorn, 28º 17’ 0”S 18º 15’ 35”E (–AD), 22 April 1997, *Strohbach & Chivell 3485* (WIND, PRE) [IL114]; Farm Duvisib (–CC), 17 May 1956, *Volk 12777* (M) [IL111]; 10 miles South of Goodhouse, bushy about 30 cm flower light pink (–CC), 30 May 1961, *Schlieben 9092* (BOL, K, PRE, SRGH) [IL151].

Botswana. –2320 (Ukwi): Ghanzi & Kgalagadi, Masetleng Pan, in dwarf shrub zone, 23º 41’S 20º 51’E (–DB), 21 March 1978, *Skarpe S-289* (PRE, SRGH) [BR083]; Kalahari Sandveld, Masetleng, ± 130 km WNW of Hukuntsi (–DB), 10 March 1997, *Cole 1271* (PRE) [BR147]. –2420 (Union’s End): Kalahari Gemsbok National Park, Kudus Pump, Thornveld, clay (–CD), *van Royen & Bredencamp 372* (PRE) [BR102]. –2421 (Hukuntsi): 100 km W of Hukuntsi at Masetlheng Pan, pan fringe (–AB), 16 May 1985, *Parry 8535* (J) [BR148].

South Africa. –2723 (Kuruman): Bophuthatswana, Nyara Farm, NW-facing on slight slope of plain (–AD), 27 February 1982, *Gubb 258/39* (PRE) [BB086]; 5.3 km south south east of Kuruman (–AD), 8 April 2000, *Balkwill, McDade* and *Lundburg 11815* (J) [BE120]; 11 m N W of Kuruman, 1280 m (–AD), 19 February 1956, *Leistner 593* (PRE) [BE119]; Newstead Farm, on level plain at top of hill (–CB), 4 March 1981, *Gubb 184-41* (PRE) [BB088]; Dunmore Farm, S - facing on level talus slope (–DD), 7 March 1981, *Gubb 171-82* (PRE) [BE087]; Killarney Farm, in a pan (depression) (–DD), 19 February 1981, *Gubb 166-65* (PRE) [BE089]. –2724 (Taung): Vryburg, Kormutsetla Farm, on gently sloping plain to the W (–AA), 12 May 1982, *Gubb 323-95* (PRE) [BB099]; Dwaalvlakte (–AB), 1912, *Sharpe 8148* (PRE) [BB010]; Barkly West, Boetsap, rocky slope (–CD), 17 February 1945, *Brueckner 136* (PRE) [BB080]. –2725 (Bloemhof): Cawoods’ Hope (–DA), March 1912, *Burt-Davy 12930* (BOL) [BB002]. –2817 (Vioolsdrift): Wespental, 28.210º S 17.070º E, Richtersveld (–AC), 9 September 1987, *Jurgens 22682* (PRE) [IL081].

–2819 (Ariamsvlei): on the road to Nous, 2 – 3 km north of N14, 28º 52.05’S 19º 54.91’E (–DD), 7 April 2000, *Balkwill, McDade* and *Lundberg 11799* (J) [BR065]. –2820 (Kakamas): c 80 km from Ariamsvlei towards Upington, Karoo (–AD), 26 June 1981, *Van Wyk 4475* (PRE) [BR046]. –2822 (Glen Lyon): 6 miles N of Gemotep Pan, W of Langeberg Mountains (–BA), 21 March 1960, *Leistner 1708* (K, SRGH) [BR063]; Beeshoek Farm, steep slope (–BD), 30 April 1981, *Gubb 219/78* (PRE) [BB090]; Hay, Griquatown (–DD), Date unknown, *Wilman s.n.* (GRA) [BR140]. –2823 (Griekwastad): east of Postmasburg, west of Owendale, facing railway to Blinkklipkop Station, on the loop, 28º 14.87’S 23º 17.34’E (–AB), 8 April 2000, *Balkwill*, *McDade* and *Lundburg 11818* (J) [BE121]; about 7 m NE of Postmasburg, Blinkklipkop (–AC), 26 March 1959, *Leistner 1396* (K, SRGH, NBG, PRE, M) [BE085]; Asbestos Hills, Daniel’s Kuil (–BA), March 1939 *Esterhuysen s.n.* (BOL) [BE122]; near Daniel’s Kuil (–BA), *Rogers 10413* (BOL) [BE124]; Daniel’s Kuil on hills near Ouplaas, (–BA), April 1940, *Esterhuysen 2046* (BOL) [BE082]; Warrenton (–BB), 11 March 1902, *Adams 17* (BOL) [BE125]; Daniel’s Kuil (–DA), March 1939, *Esterhuysen 1007* (BOL) [BE084].

–2824 (Kimberly): Barkly West, Koopmansfontein, Agric. Res Station (–AA), 14 April 1964, *Nursey 133* (PRE) [BB005]; at A53 (–AA), 26 March 1937 *Acocks 2121* (PRE) [BE118]; Newlands (–AD), 17 March 1939, *Lewis s.n.* (NBG) [BB142]; Newlands (–AD), March 1939, *Esterhuysen 1008* (BOL) [BB006]; 3 mls east of Kimberly (–DA), 11 May 1961, *Schlieben 8715* (PRE) [BB101]; Riet Pan (–DB), April 1919, *Moran* 15919 (BOL) [BE004]. –2924 (Hopetown): Riet Pan, March 1920 (–CA), *Wilman 16418* (BOL) [BB003]; East of Wanda Police station, 29º 35.63’S 24º 26.29’E (–CB), 20 June 2008, *Nyirenda 706, 703, 701, 700, 705* (J); 6 Km from Wanda on road to Roodepan (–CB), 26 February 1981, *Herman 294* (PRE) [BR051]; Van der Kloofdam (–DC), 26 March 1969, *Werger 266* (PRE, SRGH) [BR045]; Precise locality unknown: date and collector unknown (curator *Pretoria 198*), (PRE) [BB100]. –3022 (Carnarvon): “Boomsynputs” north of Carnarvon (–AC), 17 May 1975, *Thompson* 3115 (NBG, PRE) [BR062]. –3024 (De Aar): Doornkloof Nature Reservet, above spruit below boundary fence 2B41 (–BD), 28 December 1982, *Handiek 129* (GRA) [BR056]; 33 Km east of De Aar on Sterkfontein road, foot of mountain, Southern slope (–CB), 4 April 1981, *Herman 561*(PRE) [BR052]. –3124 (Hanover): Noupoort, Karoo veld (–BB), December 1961, *Repton 5725* (PRE) [BI095].

–3224 (Graaff Reinet): Hillside on Farm Reitvlei (–AB), 3 March 1930, *Galpin 9972* (K, PRE) [BU070]; Spitskop (–AD), 01 April 1912, *Burtt-Davy 13503* (PRE) [BU091]; Swart River near Graaff Reinet (–BC), 03 December 1950, *Willems 703* (NBG) [BU143]; 10 miles south of Graaff Reinet (–BC), 03 January1956, *Theron 1853* (GRA, K, PRE) [BU105, BU092]; near Graaff Reinet (–BC), March 1867, *Bolus 563 &10441* (BOL) [BU066]; veld flats (–BC), 9 May 1954, *Henrici 4958* (PRE) [BU094]; Jansenville, Jockomst, in open veld (–DC), 18 April 1932, *Long 561* (PRE) [BU078]; Jansenville (–DC), 3 April 1981, *Olivier 2806* (PRE) [BU069]. –3320 (Montagu): Ladismith, Farm Comae near Plathuis station in Karroid broken veld (–DB), 09 September 1975, *Van Breda 4380* (PRE) [BP096]. –3321 (Ladismith): Farm Mannshoop, 0.8 km from first gate after homestead on link road to Barrydale, yellow-brown well drained stony loam, full sun, gentle northwest slope, Succulent Mountain scrub, alt.: 425 m (Acocks Veld Type 25), locally rare (–CA), 16 July 1982, *Laidler 204* (PRE) [BP031]. –3324 (Steytlerville): Klipplaat, central lower Karoo, Teasdale, 33º 07’S 24º 16’E (–AB), 23 April 1985, *Hoffman 692* (GRA) [BU123]; c. 5 m. S of Baroe [Farm], karoo, 2000 ft. (–BA), 27 April 1947, *Story 2312* (PRE) [BU076].

–3325 (Port Elizabeth): Kirkwood, Zuurberg National Park, 33° 18' 26" S 25° 23' 11" E, Fynbos (–AD), 17 April 1999, *Brand 133* (PRE) [BI093]; Kirkwood, Foot of Great Swartberg, above farm Groenfontain, in Renosterbosveld (–AD), December 1980, *Vlok 47*

(PRE); Cradock, near Port Elizabeth (–BA), 12 May 1902, *Galpin 6360* (PRE) [PU029]; Uitenhage, 2.5 miles from Barkley Bridge along Alexandria railway line (–CA), 10 February 1953, *Marais 162* (*i & ii*) (K, PRE) [BI145 & BI067]; Uitenhage (–CA), 13 December 1847, *Prior s.n.* (K, PRE) [BI068]; Uitenhage Cap. b. sp. (–CA), *Ecklon 384 (79.2)* (M, PRE) [BI104]; 11 km N of Uitenhage, Uitspan experimental plot of Department of Plant Protection Unit (–CD), 10 March 1979, *Mauve 5238* (PRE) [BI075]; Kommands Kraal (–CD), *Zeyher 1421* (NBG) [BI073]; Uitenhage (–CD), *Harvey s.n.* (BOL) [BI077]; Addo Elephants National Reserve, open veld (–DC), 12 February 1954, *Brynard 330* (PRE) [BI072]; (–DC), 1 February 1927, *Moss 15394* (J) [BP041]; (–DC), 31 May 1919, *Mogg 4761* (J) [PU024]; Markman Industrial area (–DC), 23 February 1971, *Dahlstrand 2715* (GRA, J) [PU016]; New Brighton (–DC), January 1901, *Galpin 5879* (PRE) [PU035].

–3326 (Grahamstwn): Albany, 5 miles NW of Grahamstown, False Renosterveld (–AB), 24 March 1950, *Accocks* 15732 (PRE) [BP017]; Hell Poort entrance slightly less than 16 m from Grahamstwn on Cradock road (–AB), 21 July 1975, *Brink 369* (PRE) [BI097]; North-eastern boundary of the farm "Mountain Top", Carlisle Bridge area, on the Fish River ridge, 33º 00'S 26º 20'E (–AB), 19 April 1995, *Bowker s.n.* (GRA) [PU126]; Double Drift, Fish River Valley, 33º 05' 20"S 26º 47' 05"E, hillside above river (–AD), 8 March 1981, *Phillipson 216* (GRA, PRE) [BI074] ; Sugar Loaf Hill (–AD), April 1950, *McGillivary 2951* (J) [PU014]; Ecca Reserve, 33º 13' 40"S 26º 38' 5"E (–BA), 6 March 1992, *Chan 6* (GRA) [PU015]; Botha's ridge (–BC), 24 March 1962, *Wells 2631* (GRA) [BP040]; Bothas Hill 11 miles from Grahamstown junction, between Karoo and Scrub veld (–BC), May 1928, *Dyer 1414a* (J) [BP030]; Peddie road 15 – 16 miles from Grahamstown junction, between Karoo and scrub veld (–BD), April 1928, *Dyer 1358* (GRA) [BP001]; Alexandria, limestone ridge S.E. of Sandflats (–CA), 2 April 1952, *Archibald 4234* (GRA, K, PRE) [BI071]; Alexandria (–CB), 24 March 1952, *Archibald 4154* (J) [BP022]. –3327 (Peddie): 2000 ft. (–AA), 1897, *Sim 20021* (NU, PRE) [BI079].

–3419 (Caledon): Hermanus, Bushman's River mouth, vacant plots at the extension (–CB), 10 January 1989, *Green 591* (J) [BP153]. –3421 (Riversdale): Soetmelksrivier, 2 km NW of Bridge (–AB), 20 February 1980, *Bohnen 7371* (PRE) [BP032 and BP037]; (–AB), 19 November 1912, *Schlechter 1985* (*1* & *2*) (BOL) [BP019, PU026]; Plateau N of Soetmelksrivier bridge on main road, (–AB), 25 February 1981, *Bohnen 7829* (PRE) [BP018]; (–AB), September 1960, *Horn 2286* (PRE) [BP036]; near settlement, 34º 5.77'S, 21º 15.24'E (–AB), 9 July 2008, *Nyirenda 716* (J) [BP025]; Albertina, about 27 km from Mossel Bay to Albertina, on N2 road, Van Rensburg Farm, 34º 10.78'S 21º 53.08'E (–AB), 8 July 2008, *Nyirenda 707, 708, 710* (J) [BP038, BP039, BP020]; about 7 km from Albertina, along N2 road, Farm Wolwekraal, 34º 12.46'S 21º 38.74'E (–CB), 8 July 2008, *Nyirenda 712* (J) [BP021]. –3422 (Mossel Bay): between George and Great Brakriver, Next to national road, near the Maalgaten river bridge (–AB), 1 January 1987, *Vlok 1844* (PRE) [PU027]. –3423 (Knysna): hills above Keurboom Strand (–AB), 30 January 1949, *Walgate s.n.* (BOL) [BP028]; Plettenberg Bay (–AB), March 1910, *Fourcade 613a* (BOL) [BP034]. –3424 (Humansdorp): between Kabeljau and Mondplaas along National Road (–BB), 11 January 1993, *Fourcade 5931* (BOL) [PU023]; (–BB), January 1912, *Burtt-Davy 12000* (BOL) [BP033].

CHAPTER 3

3.0 VARIATION AND SPATIAL DISTRIBUTION IN TWO POLYMORPHIC SPECIES, *BARLERIA PUNGENS* L.F. AND *B. RIGIDA* NEES (ACANTHACEAE)

3.1 Abstract

Earlier assessment using phenetics revealed that the two polymorphic species, *B. pungens sensu* L.f. and *B. rigida sensu* Nees exhibited both continuous and discrete variation. It was suspected that clinal variation contributed to part of the observed polymorphism among specimens of the two species complexes. The distribution of the morphological and floral variation was examined. Groups derived from phenetic analysis were maintained and used to sample leaves, bracteoles and posterior calyx lobes for pictorial maps. Flower colours were plotted on maps and corolla tube lengths and widths were plotted using Box and Whisker diagrams to illustrate floral variation. There was an association between morphology and spatial distribution within the two species complexes. The species-environmental interactions influenced most of the variation; the distinct variability was inferred to imply breaks in gene flow while the continuous variability was inferred to imply clinal variability following a distribution gradient.

Key words: Continuous, discrete, discontinuities, distribution, morphology, variation

3.2 Introduction

Species of *Barleria* L, especially the endemic ones, have localized distributions and tend to have populations that are clustered as a result of soil type or short distance seed dispersal. The short distance seed dispersal in *Barleria* limits the distance over which, seeds can be carried (Balkwill and Balkwill, 1998). Although entomophylus pollination can facilitate gene flow within populations, pollinators tend to visit plants that are near to each other hence restricting gene flow (Makholela, 2003). Endemism has been noted within the genus with different sections being concentrated in certain geographic regions (Balkwill, 1993). In Africa, *Barleria* Section I is the most widespread and it occurs almost throughout the range of the genus (Balkwill and Balkwill, 1997) and not all combinations of characters are represented in any one geographic area (Balkwill and Balkwill, 1996). It has been reported that *Barleria* floras of different regions are highly distinctive even on a considerably smaller scale and they tend to be represented by a few (or one) of the seven recognized sections (Balkwill and Balkwill, 1998; Darbyshire *et al.*, 2008). Since “characteristics which enable a plant to grow in a particular region or habitat” are difficult to quantify, one does not necessarily require rigid adherence to diagnostic characters to define variation between geographically separate species (Cabido *et al.,* 1992; Richards *et al.,* 2005). Morphological features with differences or similarities that are correlated with distribution ranges are the basis for determining relationships of taxa at various levels (Radford *et al.,* 1974). It has been observed that gaps in distribution and distinctness in any one independent character can result in the recognition of subspecies (Ensermu, 1994). Distribution information coupled with morphology therefore, can be used to determine the species geographical range and species diversity among other things, which, in turn is informative in defining species.

Ecological variations, such as moisture, wind, exposure, salinity and geology have effects on morphology (Ahmad *et al.*, 2005; Balkwill *et al.*, 1994; Vallius *et al.*, 2004). It was observed in *Senecio integrifolius* (L.) Clairv. that resource limitation influenced the propensity for individuals to flower and the number of flowering individuals per population fluctuated each year according to climatic conditions (Widén, 1991). Variation in pH may produce different flower colours: blue flowers when acidic and red flowers in basic conditions. Habitat variation could bring about phenotypic plasticity in a number of taxa (Ahmed *et al.,* 2005; Balkwill *et al.,* 1994; Snaydon, 1973; Vallius *et al.,* 2004). Graded variation can result from continuous variation in habitat factors whereas non-overlapping or discrete variation can be brought about by genetic variability or even phenotypic plasticity through adaptation where the favoured form persists (Bult and Kiang, 1992; Miner *et al., 2005*; Richards *et al.,* 2005; Widén, 1991; Xu *et al.,* 2007). It has been noted that when morphologically related taxa are found in mixed populations then it is less likely that phenotypic plasticity is an important factor in explaining the difference among the taxa (Miner *et al.*, 2005). The persistence of locally rare and geographically restricted species can depend on demographic traits and population dynamics (Schemske *et al.*, 1994; Van Tienderen, 1997) as each taxon tends to have a distinctive retention of habitat-correlated characteristics (Ellstrand, 1992). Numerically abundant species tend to be more variable than their rare relatives (Van Valen, 1965). Variation therefore, whether due to ecological, genetic, or geographical factors, can result in differentiation and this separation needs to be recorded by recognising species and or subspecies (Furnier and Adams, 1986; Makholela *et al.*, 2003; Schmidt-Lebuhn *et al.*, 2007).

Insect visitors are often determined not only by floral features, but also by a variety of local conditions such as microclimate. Insect pollinators have been noted to discriminate between conspecific plants on the basis of metric floral characters (e.g. corolla size, inflorescence height) leading to individual variation in flower visitation rates arising from differential attractiveness to pollinators (Herrera, 1993). If a certain floral trait increases the precision of pollination, then that trait may influence phenotypic selection. If that trait is genetically controlled, this may result in evolutionary response through selection for particular floral types. Such influences may reflect differences in pollinator attraction (larger flowers are more attractive). Environmental conditions influencing pollinator abundance include weather, habitat and resource availability, and plants flowering during unfavourable conditions are likely to encounter pollinator limitations due to scarcity of insect visitors (Brouat *et al.*, 2004; Guitian *et al.*, 1999). Optimally foraging pollinators will spend more time within large populations than they will in small scanty flowering populations (Ellstrand, 1992). There is more gene flow by pollen transfer from large to small populations than there is between small populations (Ellstrand, 1992). This could affect gene flow via pollen transfer among small populations of rare taxa which usually are more vulnerable in terms of genetic variability than numerically abundant taxa (Ellstrand, 1992). And because there is little or no gene flow between small populations of the same species, small populations of rare taxa tend to exhibit inter-population genetic variation which might in turn manifest in morphological variations (Ellstrand, 1992; Ellstrand *et al.*, 1989; Raybould and Gray, 1993).

Other factors that influence gene flow include differences in flowering period and pollen vectors where simultaneously flowering individuals share pollinators (Rozzi *et al.*, 1997). Differences in flight behaviour among pollinators together with pollen loads could determine variation in the quantity and effectiveness of pollination especially in areas of environmental stress and also determine how low gene flow levels must be to permit genetic differentiation. Speciation depends on the intensity of selective pressures leading to divergence (Rozzi *et al.*, 1997). If the speciation mechanisms are operating and flowering and pollinator differences increase the probability of genetic differentiation, this could contribute to high levels of plant diversity and endemism (Rozzi *et al.*, 1997). Examination of flower colours and how they are distributed along the range of taxa may clarify the taxonomic implication of the diversity in floral morphology and colour which in turn will contribute to the understanding of the circumscription of species and their geographic ranges.

Flower colours are known to influence plant-pollinator interactions which could in turn impact on the gene flow (Balkwill and Balkwill, 1994; Guitian *et al.*, 1999; Rozzi *et al.*, 1997). Overlap in flowering periods and pollen vectors are both necessary conditions for pollen exchange between individuals. It has been observed that genetic variation can shape flowering phenology in a population, influencing the timing or duration of flowering (Bennett, 1987; Widén, 1991). Since inter-specific genetic variation has an effect on phenotypic and phenological characters that determine growth, it influences when, where and how plants grow with respect to their distribution within a local plant community and at global level. This enables different species to partition the available environment sequentially and coexist within a plant community (Bennett, 1987). Although differences in flowering periods and pollinator species may be maintained from year to year, and habitat differences may be expressed by variability in the flowers and other characters which could in turn imply genetic variability, climatic patterns too could affect phenological characters (Bennett, 1987; Mayr, 1947; Rozzi *et al.*, 1997).

An array of flower colours is observed among the study group which ranged from rose, pink, mauve, lilac, violet, purple and blue to white. It is not clear whether the flower colour diversity in this group is triggered by environmental diversity, season (i.e. time of the year), soil pH or physiological changes such as beginning or end of flowering season. It is also observed that the two species complexes (*B. rigida* and *B. pungens*) exhibit leaf, bracteole and calyx lobe dissimilarity along their distribution ranges. The purpose of this study is to examine the difference in morphology and flower colour in relation to distribution and to determine how the clusters that were obtained from cluster analysis and ordination relate to distribution. Floral morphology and colour variation along with distribution can facilitate the defining of taxa. The definition of the resultant taxa at the level of species and / or subspecies, however, depends on the gaps and continuities.

3.3 Materials and Methods

3.3.1 Selection of putative taxa

Specimens were grouped based on the clusters obtained from cluster analysis and ordination during the phenetics analysis (Chapter 2) which placed the specimens in various recognisable clusters. Depending on the availability of specimens, more specimens were added to the clusters with few OTUs and 104 specimens were used in total. Specimens belonging to clusters formed within *Barleria bechuanensis, B. irritans* and *B. jubata* which only showed discrete variation from cluster analysis and ordination were, however, not included in the pictorial representation of the distribution of morphological variation, analysis and mapping floral variation. Only specimens belonging to clusters from *B. pungens* and *B. rigida* were considered and these groups were assigned temporary names (BP, PUNG, PUNW, UNBR, OVTL, SPIL, SPBL and LOBR). The distinct cluster within *B. pungens* was maintained as BP while the major cluster was split into PUNG and PUNW. Although the *B. rigida* complex had separated into four clusters from cluster analysis (chapter 2), intermediate morphology between IL and ILa was later observed which made the assigning of specimens to either IL or ILa difficult necessitating the formation of another putative group. Abbreviations and brief descriptions are given in Appendix I. Specimen distribution data was recorded according to Edwards and Leistner (1971) and is given in Appendices II – IV. Corolla tube measurements obtained in Chapter 2 were used to make box and whisker plots to illustrate variation in floral morphology between the putative taxa of the two species complexes. However, not many specimens had flowers resulting in a small sample size.

3.3.2 Morphology and distribution

Since *Barleria pungens* and *B. rigida* showed both continuous and discrete variation, drawings of leaves, bracteoles and outer calyx lobes were made to illustrate the variation exhibited within the two species complexes. The drawings were scanned for use in pictorial representations. A distribution map was made using distribution information from herbarium sheets to map the morphological variation using the drawn leaves, bracteoles and posterior calyx lobes. This illustrated how the various forms were distributed in geographic space.

3.3.3 Flower colour distribution and phenology

The dates of collection were obtained from specimens with flowers and those without, but where the collector indicated that they were flowering at the time of collection. These were used to estimate the ranges in flowering time among the groups. This information was tabulated and used to plot histograms to determine flowering time ranges. The flowering times for various forms were compared to deduce whether there was temporal isolation; and whether there was spatial isolation or overlap in distribution that could facilitate gene flow. However, most specimens lacked flowers and could not be used in the analysis due to missing data.

Flower colour information was also obtained from herbarium sheets as indicated by collectors. Since flowers are known to sometimes change colour upon drying, specimens with no colour information (indicated by the collector) were not used in this analysis. However, various collectors might have different colour perceptions resulting in flower colour diversity which in reality may not exist. The flower colour information was plotted on maps for each group to illustrate the diversity of colours and distribution within the groups. The number of specimens with flower colour information, however, varied among the putative groups.

3.4 Results

3.4.1 Floral variation

Box and whisker plots demonstrated that there was an overlap in corolla tube length between PUNG, PUNW and LOBR (Figure 3.1). Although there is also an overlap in the width of corolla tube mouth between LOBR, PUNG and PUNW, LOBR only narrowly overlaps with the two; LOBR has a wider corolla tube mouth than PUNG and PUNW. Apart from LOBR the rest of the putative taxa within the *B. rigida* complex exhibit an overlap in corolla tube dimensions (length and width). PUNG and PUNW also display overlap in both corolla tube length and width. Table 3.1 presents flower colour and ranges of corolla tube lengths and corolla tube mouth width (with the means in brackets and number of specimens from which measurements were obtained (n) in squared brackets).

3.4.2 Distribution and morphology

Pictorial plots reveal that the *B. pungens* complex exhibits intraspecific variation (Figures 3.2 to 3.4). PUNG occurred throughout the distribution range, from Grahamstown and extending westward to Swellendam and Hermanus while PUNW exhibited disjunction (Figure 3.2). Leaf size varied from small to large among PUNG and PUNW and the small and large leaves were observed to overlap in distribution (the largest were m (ii) and w, while the smallest were a, b, v and B) (Figure 3.2). Although small leaves were common among the *B. pungens* complex, they are elliptic-ovate in BP and mainly ovate in PUNG and PUNW. Ovate leaves occur among both PUNW and PUNG. Elliptic-ovate leaves only occur among PUNG and a few OTUs of PUNW with thin white leaf margins (and not among PUNW with prominent white leaf margins. Leaf ‘m’ is a mixed gathering with three stems on one herbarium sheet; one with small leaves with white margins and another with larger leaves with green and thinly white leaf margins (Fig 3.2 m (i) and m (ii)). Lateral veins were not visible on all leaves but were observed among leaves with and without white leaf margins (Figure 3.2).

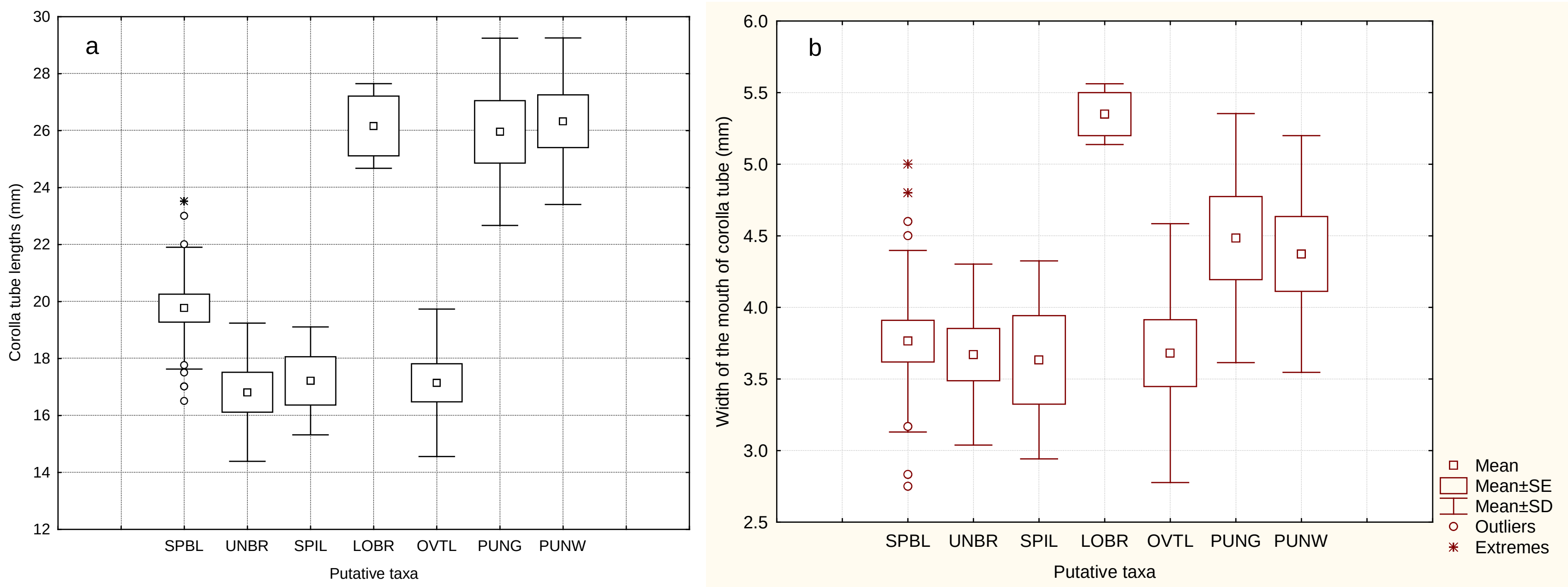

Figure 3.1. Variation in corolla tube dimensions between and among *B. pungens* L. and *B. rigda* Nees. There is no distinction in corolla tube length and corolla tube mouth width between PUNG and PUNW. LOBR is distinct in corolla tube length and corolla tube mouth width from the rest of the *B. rigda* complex. The *B. pungens* and *B. rigda* complexes (except for LOBR) exhibit an overlap in corolla tube width. The overlap in corolla tube lengths between LOBR, PUNG and PUNW can facilitate gene flow. UNBR: *B. rigida* with linear leaf, bracteole lamina reduced almost completely; SPIL: *B. rigida* with elliptic-ovate leaf, bracteole lamina reduced almost completely; OVTL: *B. rigida* with ovate leaf, bracteole lamina reduced almost completely; SPBL: *B. rigida* with linear or elliptic-ovate leaf, bracteole lamina well developed, base attenuate; LOBR: *B. rigida* with ovate or elliptic-ovate leaf, bracteole lamina well developed, base lobed or truncate; PUNG: *B. pungens* with flat and wavy green leaf margins; PUNW: *B. pungens* with white or thin white wavy leaf margins. Detailed descriptions of abbreviations are given in Appendix I.

Table 3.1. Flower colour and range in corolla tube length and width

Putative taxa	**Flower colour**	**Range of corolla tube length (mm)**	**Range of corolla tube width of the wider part (mm)**
BP	Mauve	-	-
PUNG	Blue, mauve, purple	20 – 35 (27) [9]	3 – 6 (4.4)
PUNW	Blue, mauve, purple	20 – 31 (25.5) [10]	3 – 6 (4.4)
UNBR	Pink, white, lilac, purple blue, cream-yellow	13 – 20 (16.8) [12]	2.5 – 5 (3.7)
OVTL	Pink, blue, rose, lilac, mauve, purple, pink-purple, purple-blue	12 – 23 (17) [12]	2.5 – 6 (3.7)
SPIL	Pink, blue, purple, lilac, white	14 – 20 (17) [9]	3 – 5 (3.6)
SPBL	Pink, blue, pink-lilac, lilac, purple	16 – 27 (19.8) [12]	2 – 6 (3.8)
LOBR	Blue, rose, mauve	25 – 28 (26) [5]	5 – 6 (5.0)

Although leaf pubescence is not clearly depicted in the plots it was observed to vary along the distribution gradient and was denser in specimens that occurred where PUNG and PUNW overlapped in distribution and being denser on specimens with green and / or thinly white leaf margins (Figure 3.2d, i, l, m (ii), p, y, z and A). Specimens with white leaf margins generally possess narrower bracteoles as opposed to those with green leaf margins (Figure 3.3). Although there is no clear dissimilarity in outer calyx lobe size, between PUNG and PUNW, specimens with white leaf margins tended to have longer tips as compared to specimens with green leaf margins (Figure 3.4). Leaf ‘n’ with thin white leaf margins, obtained from a specimen from New Brighton has some of its bracteoles with prominent white margins while others on the same plant are green (Figure 3.3). BP with flat green leaf margins can be distinguished from PUNG by the narrow bracteoles with serrate margins and small outer calyx lobes with short apices and serrate calyx lobe margins. BP occurred at the edge of the distribution range of PUNW and PUNG.

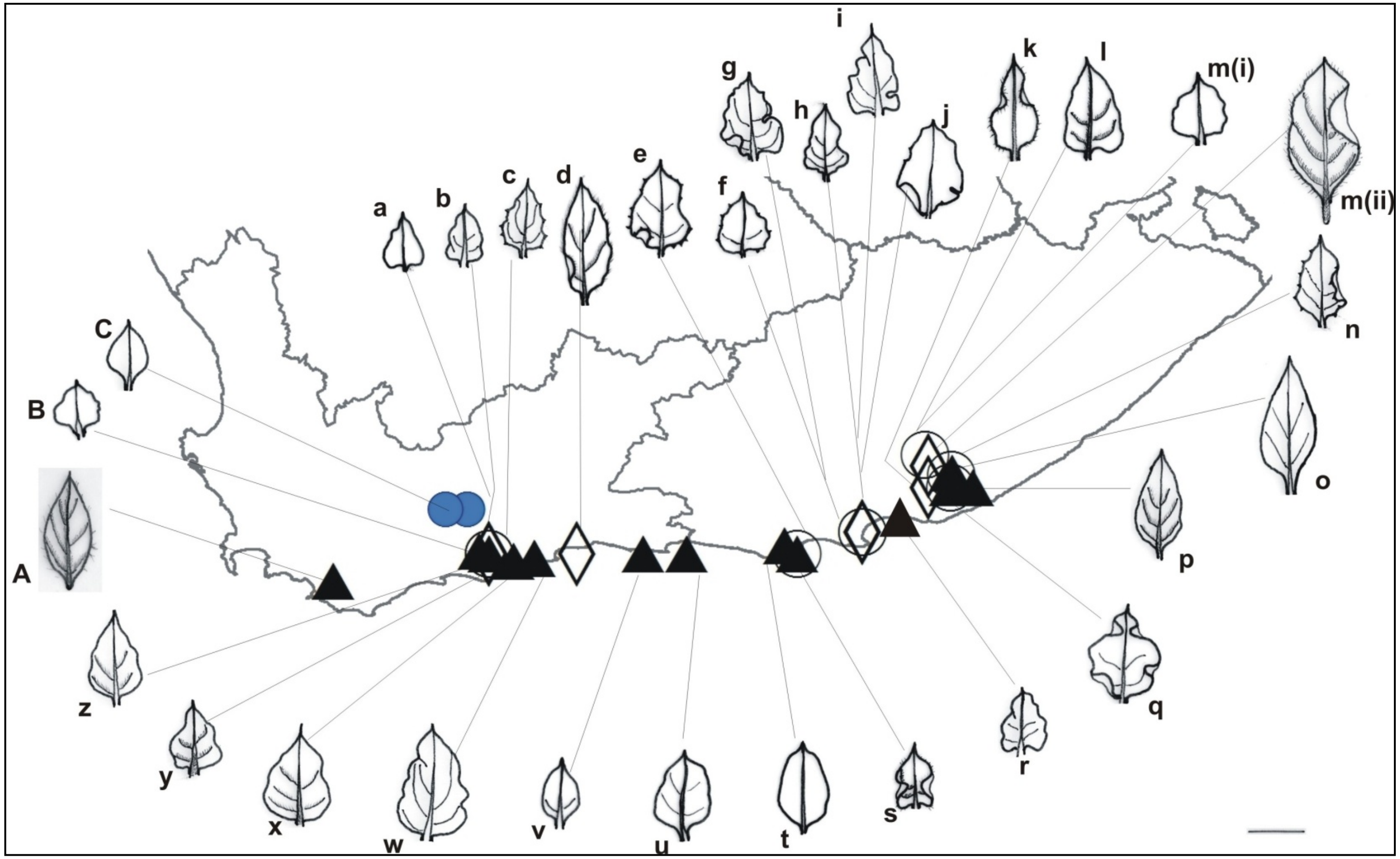

Figure 3.2. Distribution of leaf variation within *B. pungens* complex. ● and drawing C on the left of map, BP with flat green leaf margins; ▲ and drawings o – z & A below map, PUNG with green wavy leaf margins; ◊ and drawings a, b, d, h – j, k & m (ii) above map, PUNW with thin white wavy leaf margins; ○ and drawings c, e – g, l, m (i) & n above map and B on left of map, PUNW with prominent white wavy leaf margins. Both PUNG and PUNW have small and large leaves and m (i) and m (ii) are a mixed gathering on one herbarium sheet, one with white leaf margins and the other with thin white leaf margins respectively. Elliptic-ovate leaves were common among specimens with green and thin white leaf margins; leaves with prominent white margins always ovate. Scale bar, 10 mm. Specimens information is given in Appendix II with corresponding letters in square brackets.

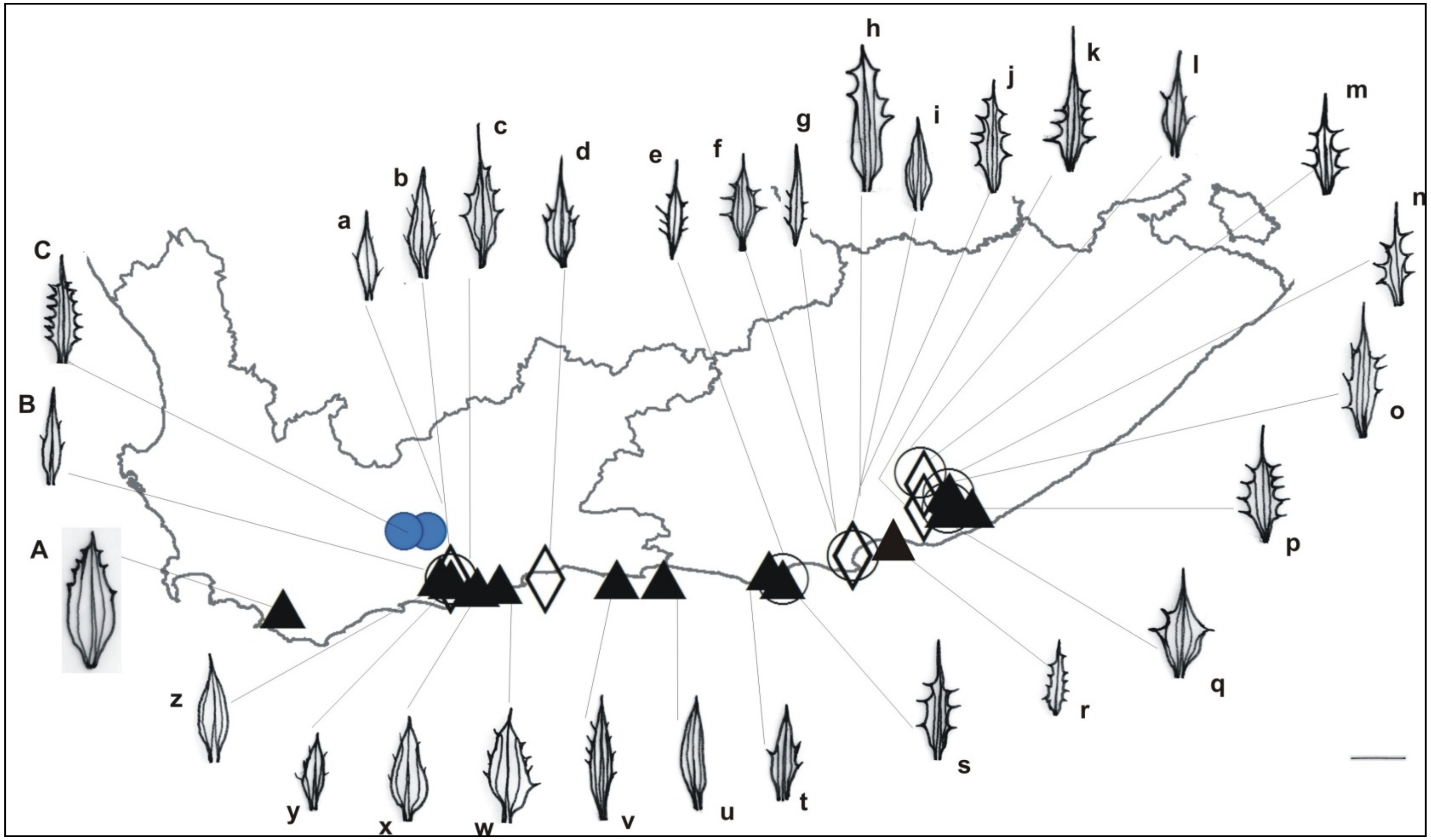

Figure 3.3. Distribution of bracteole variation within the *B. pungens* complex. ● and drawing C on the left of map is a bracteole of BP with serrate margins; ▲ and drawings o – z and A – B below map are bracteoles of PUNG with green leaf margins; ◊ and drawings a, b, d, h – k and m above map are bracteoles of PUNW with thin white leaf margins; ○ and drawings above map c, e – g, l, n & B on left of map are bracteoles of PUNW with white leaf margins. PUNG tends to have broader bracteoles than PUNW. There was no variation in bracteole size in specimen 'm' as observed in the leaf. Scale bar, 10 mm. Specimens information is given in Appendix II with corresponding letters in square brackets.

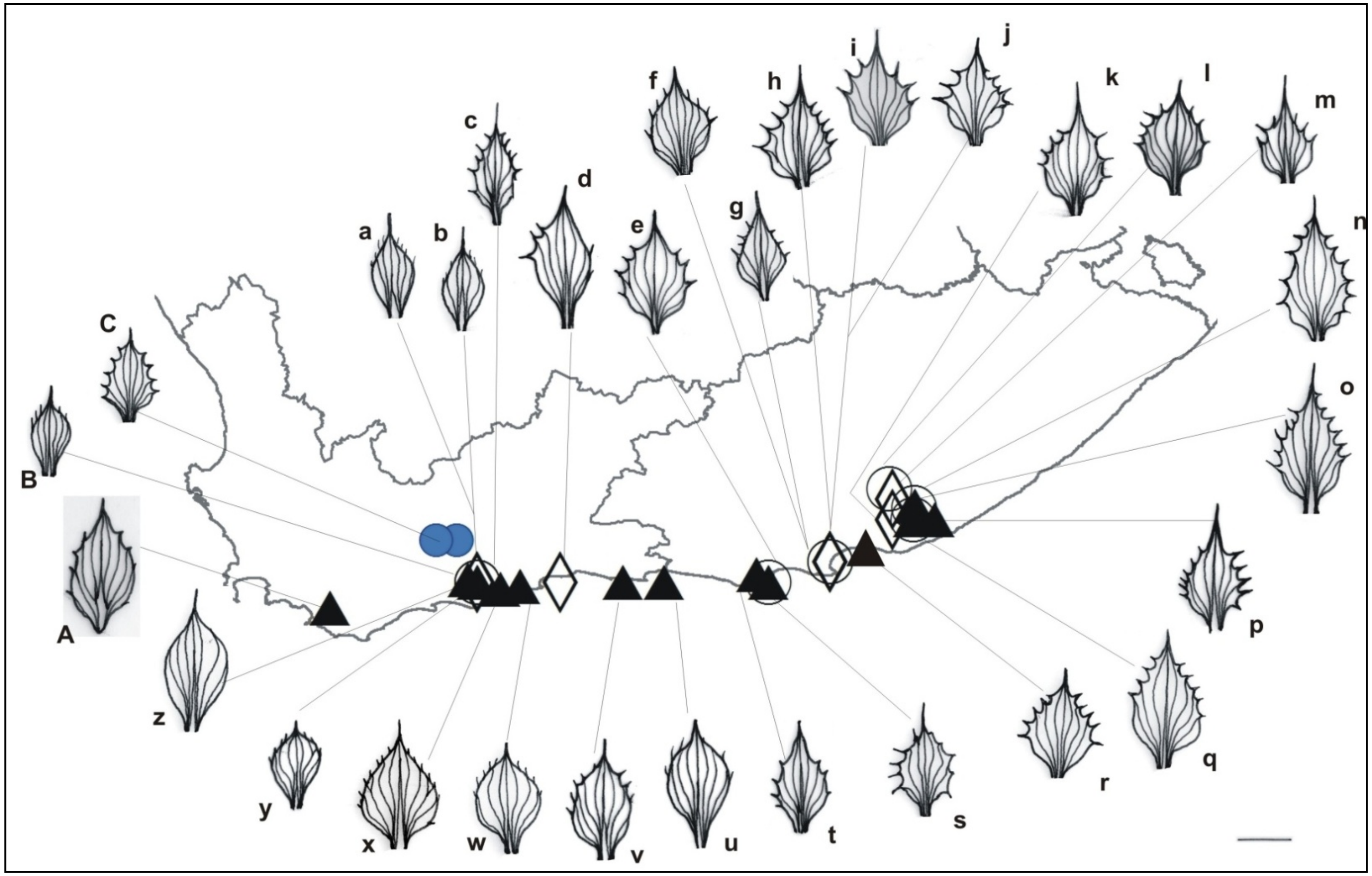

Figure 3.4. Distribution of posterior calyx lobe variation within the *B. pungens* complex. ● and drawing C on the left of map is an outer calyx lobe of BP with serrate margins; ▲ and drawings o – z and A below map, PUNG with green wavy leaf margins; ◊ and drawings a, b, d, h – k and m above map are outer calyx lobe of PUNW; ○ and drawings above map c, e – g, l, n & B are outer calyx lobes of PUNW with white leaf margins. Though calyx lobes from specimens of PUNW with prominent white margins tend to have longer tips, both PUNG and PUNW from Eastern Cape have spinier calyx lobe margins than those from Western Cape which are more hairy. Scale bar, 10 mm. Specimens information is given in Appendix II with corresponding letters in square brackets.

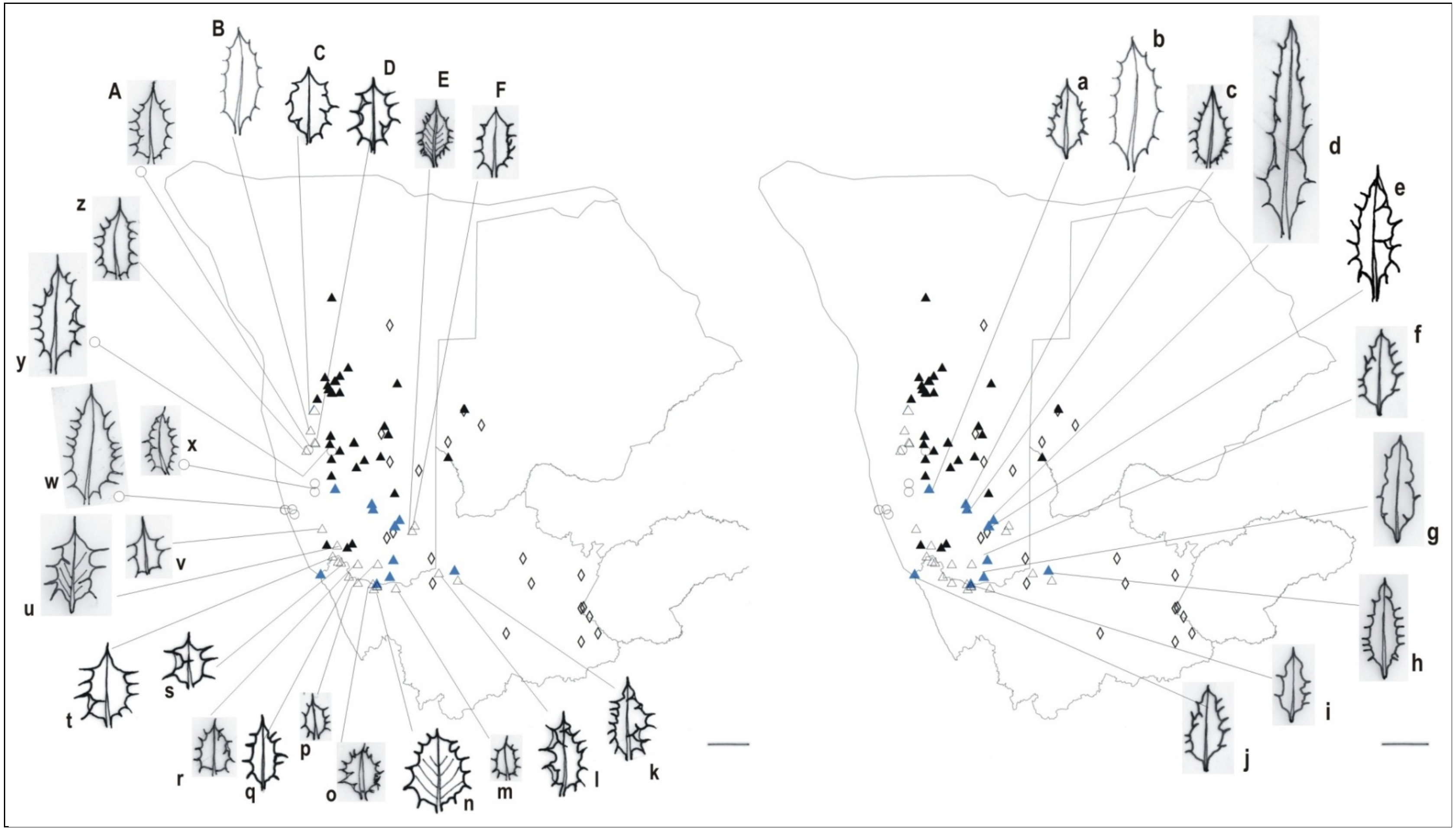

Figure 3.5. Distribution of leaf variation within the *B. rigida* complex. (i) ▲ and a – j: leaves of specimens from SPIL with narrow ovate leaves with sinuate spiny leaf margins; (ii) △ and k – v, z, B – F: leaves of specimens from OVTL with broadly ovate to elliptic-ovate leaves with sinuate spiny leaf margins; (iii) ○ and w – y & A: leaves of specimens from LOBR with elliptic-ovate leaves with sinuate spiny leaf margins (with the symbol ○ next to the leaves to distinguish them from OVTL). There is an overlap in shape where OVTL and SPIL overlap in distribution. Scale bar, 10 mm. Specimens information is given in Appendix IV with corresponding letters in square brackets.

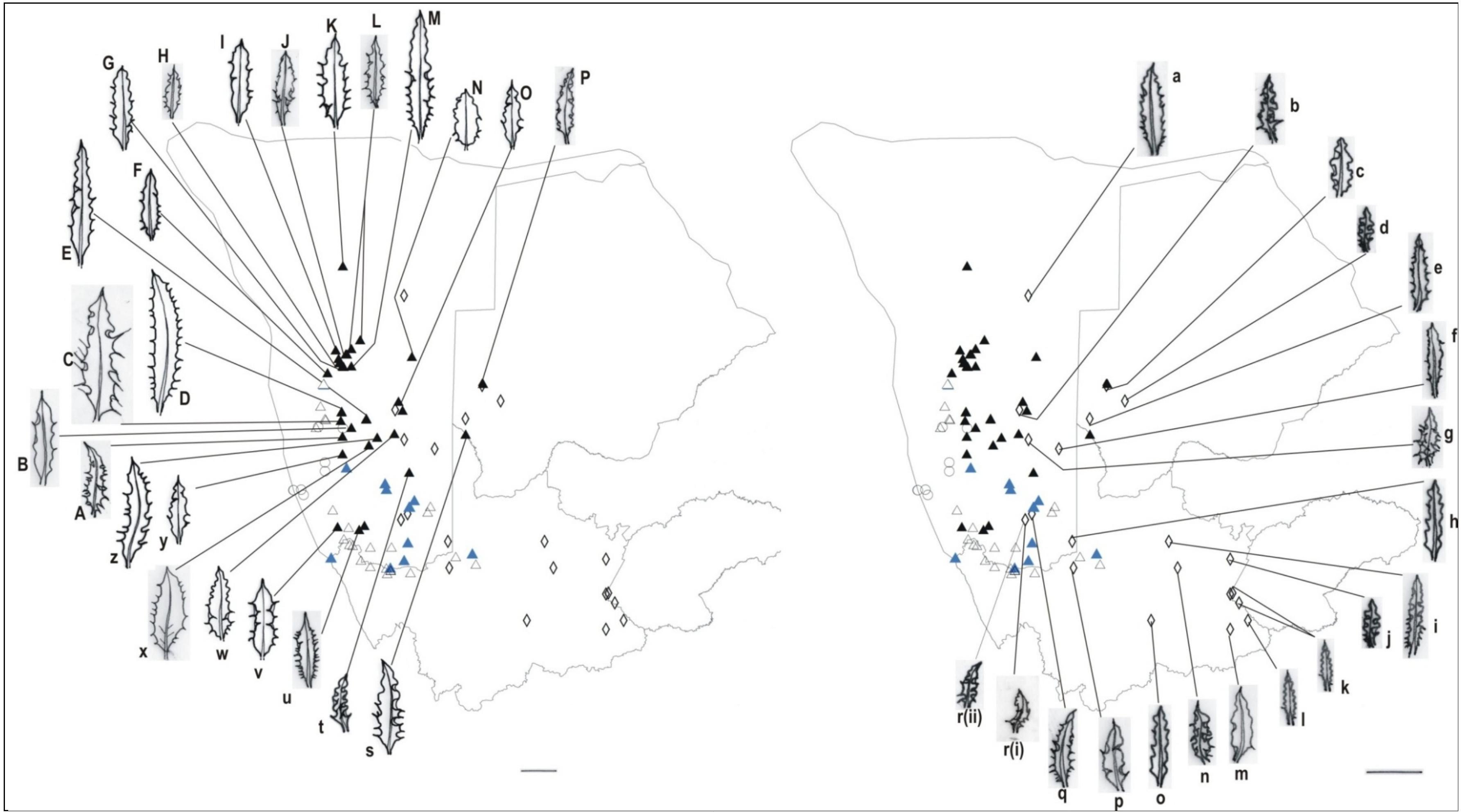

Figure 3.6. Distribution of leaf variation within the *B. rigida* complex. (i) ◊ and a – r: leaves of specimens from UNBR, with undulate leaf margins with scanty teeth to entire; (ii) ▲ and s – z and A – P: leaves of specimens from SPBL, with sinuate or undulate leaf margins with small or prominent teeth. Generally leaves of SPBL are larger and tend to be broader than those of UNBR. Scale bar, 10 mm. Specimens information is given in Appendix III with corresponding letters in square brackets.

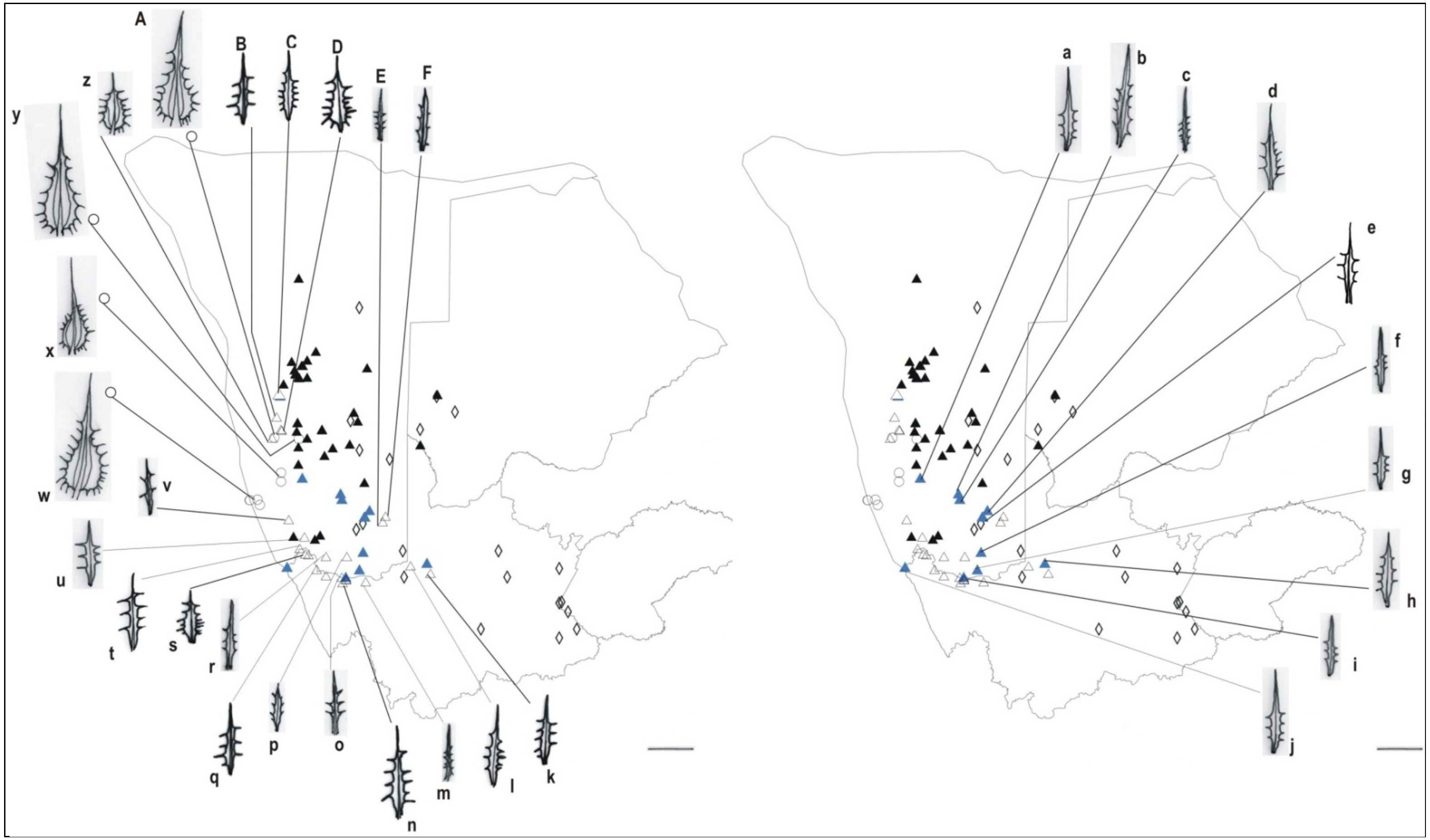

Figure 3.7. Distribution of bracteole variation within the *B. rigida* complex. ▲ and a – j: bracteoles of specimens from SPIL with narrow hardened bracteoles with one or two prominent veins; (ii) △ and k – v, z, B – F: bracteoles of specimens from OVTL with narrow hardened bracteoles usually with one or two prominent veins; (iii) ○ and w – y & A: bracteoles of specimens from LOBR with 3 (or more) parallel veins (with the symbol ○ next to the bracteoles to distinguish them from OVTL). There is an overlap in bracteole shape between LOBR and SPIL where the two closely occur together. Although both SPBL and LOBR have broad bracteoles those in LOBR have a tapering tip and a lobed or truncate base while those in SPBL have an acuminate tip and an attenuate base (see also Figure 3.8). Scale bar, 10 mm. Specimens information is given in Appendix IV with corresponding letters in square brackets.

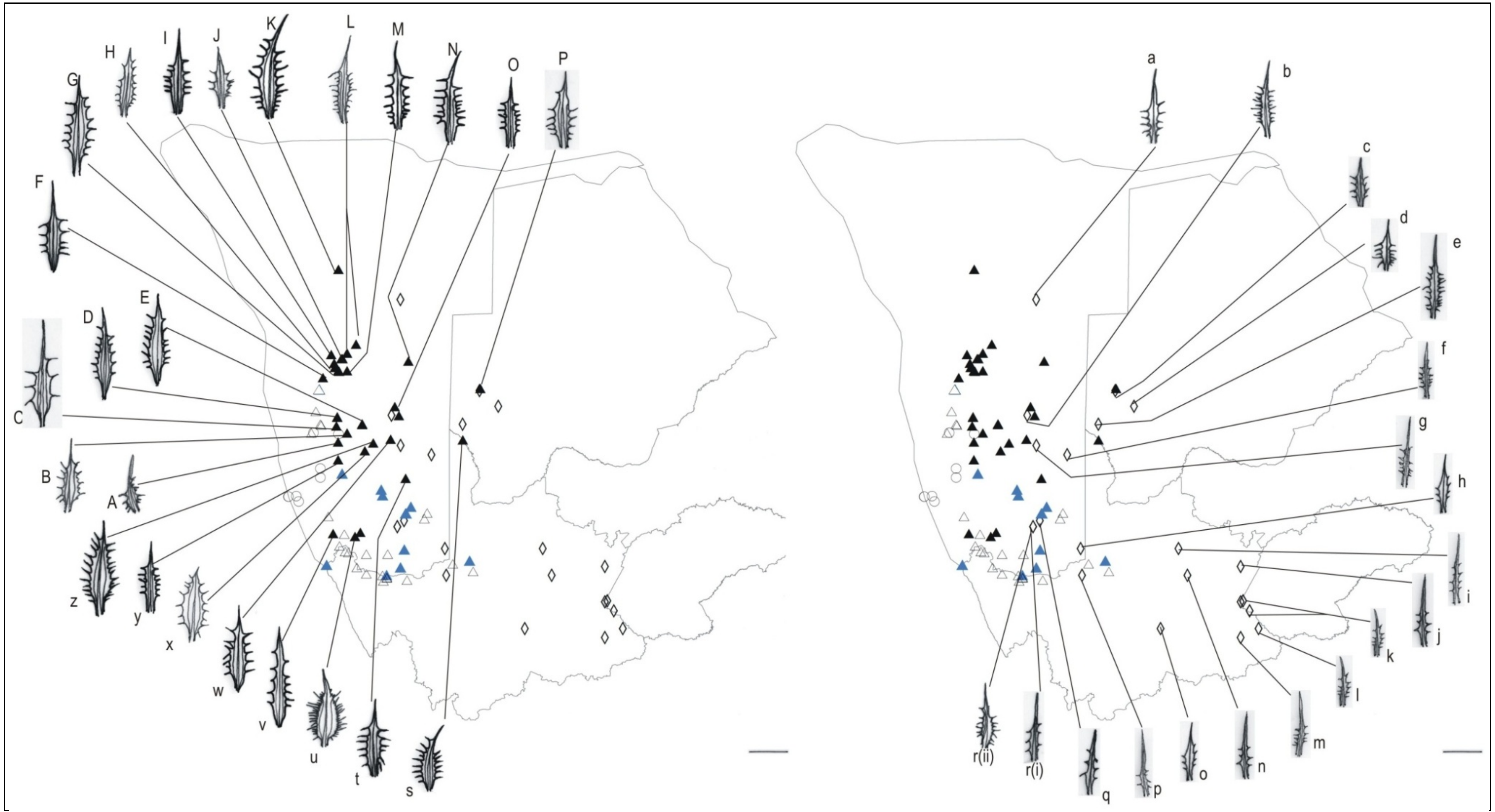

Figure 3.8. Distribution of bracteole variation within the *B. rigida* complex. (i) ◊ and a – r: bracteoles of specimens from UNBR, bracteoles usually hardened and with a reduced lamina and one or two prominent veins; (ii) ▲ and s – z and A – P: bracteoles of specimens from SPBL, bracteoles leafy and with a developed lamina and three prominent parallel veins. UNBR tends to have spinier leaf margins where they overlap with SPBL. Scale bar, 10 mm. Specimens information is given in Appendix III with corresponding letters in square brackets.

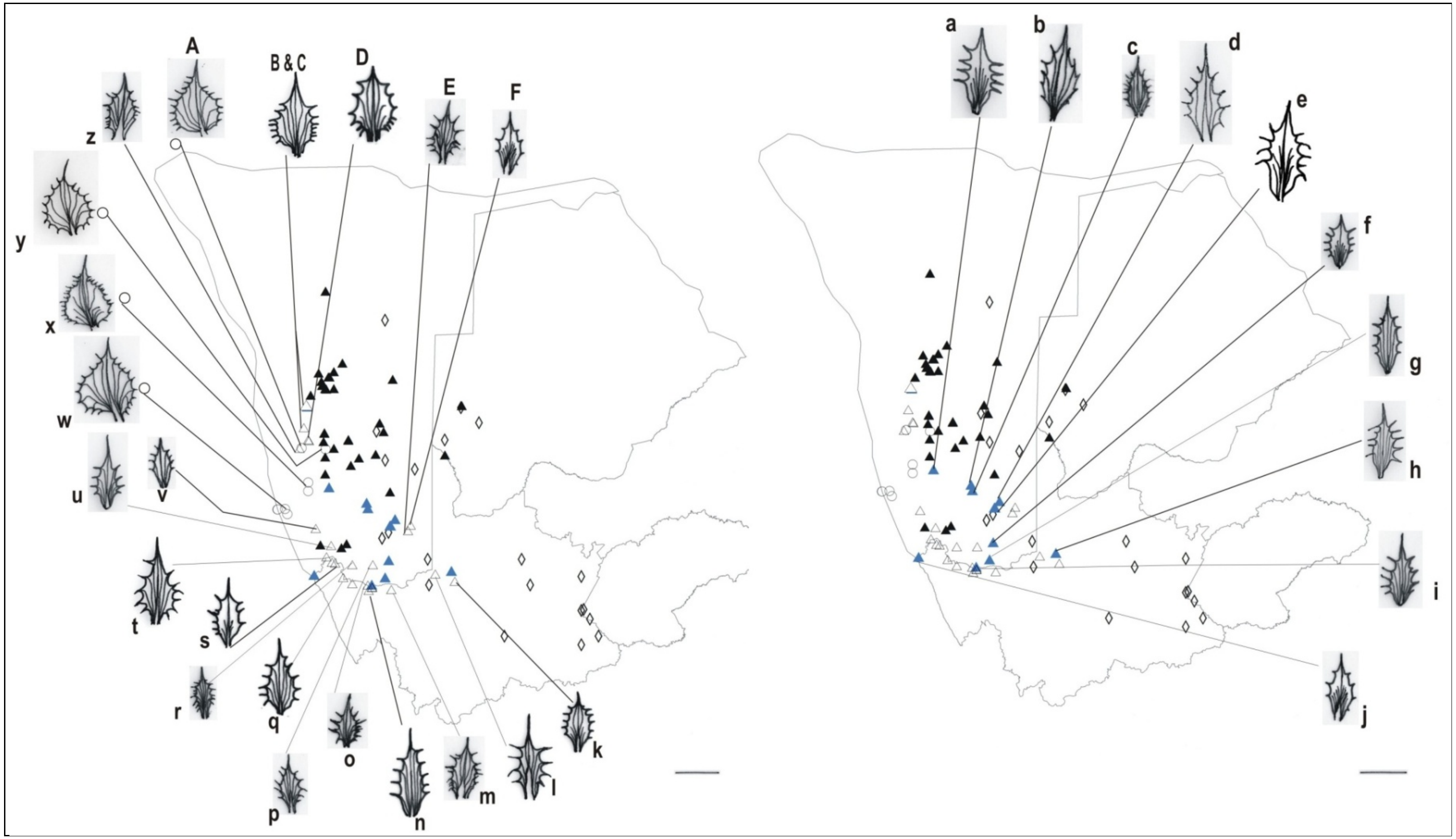

Figure 3.9. Distribution of posterior calyx lobe variation within the *B. rigida* complex. ▲ and a – j: posterior calyx lobes of specimens from SPIL with narrow ovate membranous or papery posterior calyx lobes; (ii) △ and k – v, z, B – F: posterior calyx lobes of specimens from OVTL with narrowly ovate papery posterior calyx lobes; (iii) ○ and w – y & A: posterior calyx lobes of specimens from LOBR with broadly ovate papery posterior calyx lobes with short delicate margin spines (with the symbol ○ next to the calyx lobes to distinguish them from OVTL). There is minimal variation in lobe size and shape between OVTL and SPIL (except for B – C which narrowly overlap with LOBR) while LOBR is distinct in both calyx lobe size and shape. Scale bar, 10 mm. Specimens information is given in Appendix IV with corresponding letters in square brackets.

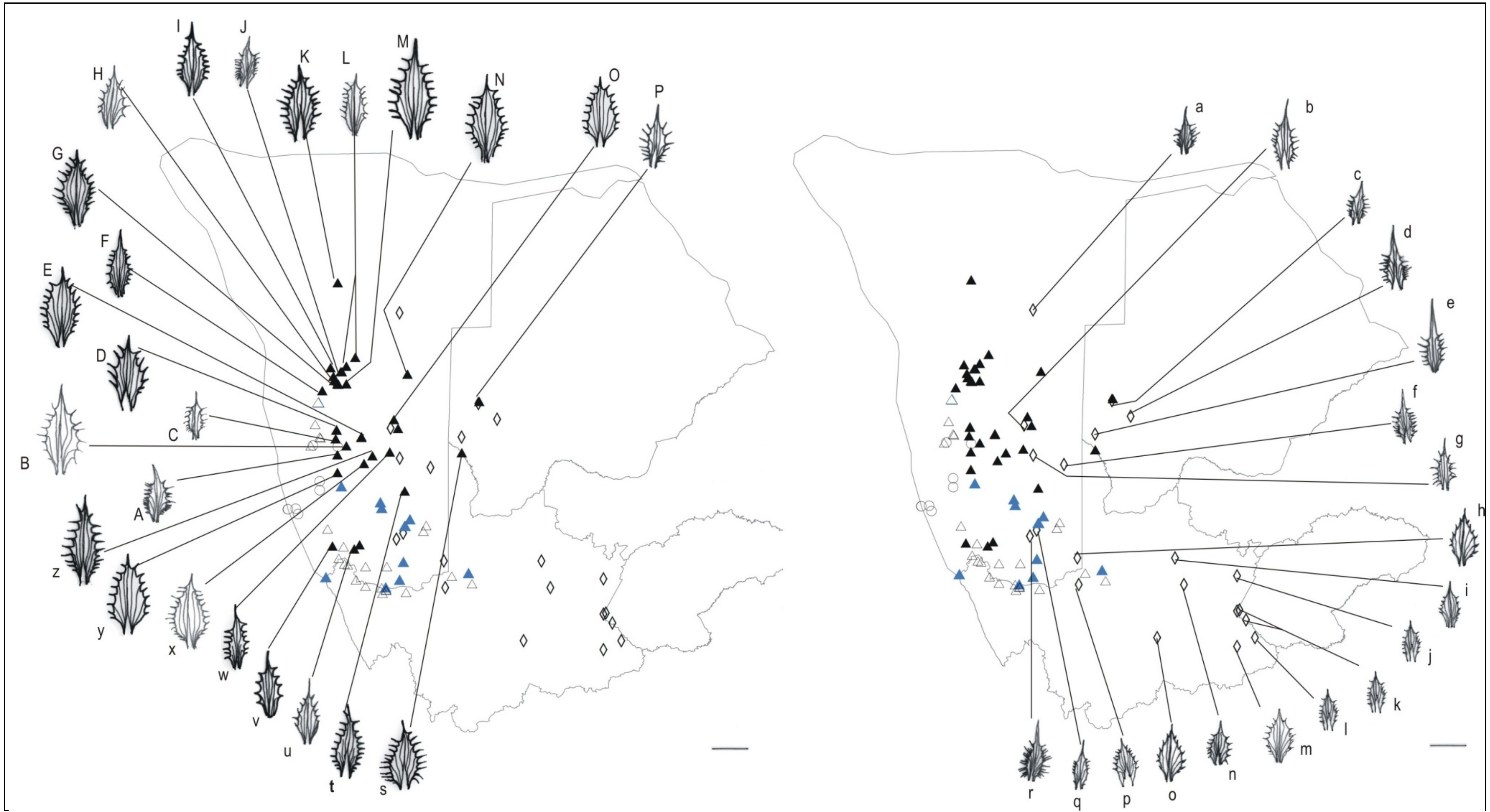

Figure 3.10. Distribution of posterior calyx lobe variation within the *B. rigida* complex. (i) ◊ and a – r: bracteoles of specimens from UNBR; (ii) ▲ and s – z and A – P: posterior calyx lobes of specimens from SPBL. SPBL generally have lager posticous calyx lobes than UNBR, though specimens occurring on the desert fringe have narrower calyx lobes than those on the plateau. Scale bar, 10 mm. Specimens information is given in Appendix III with corresponding letters in square brackets.

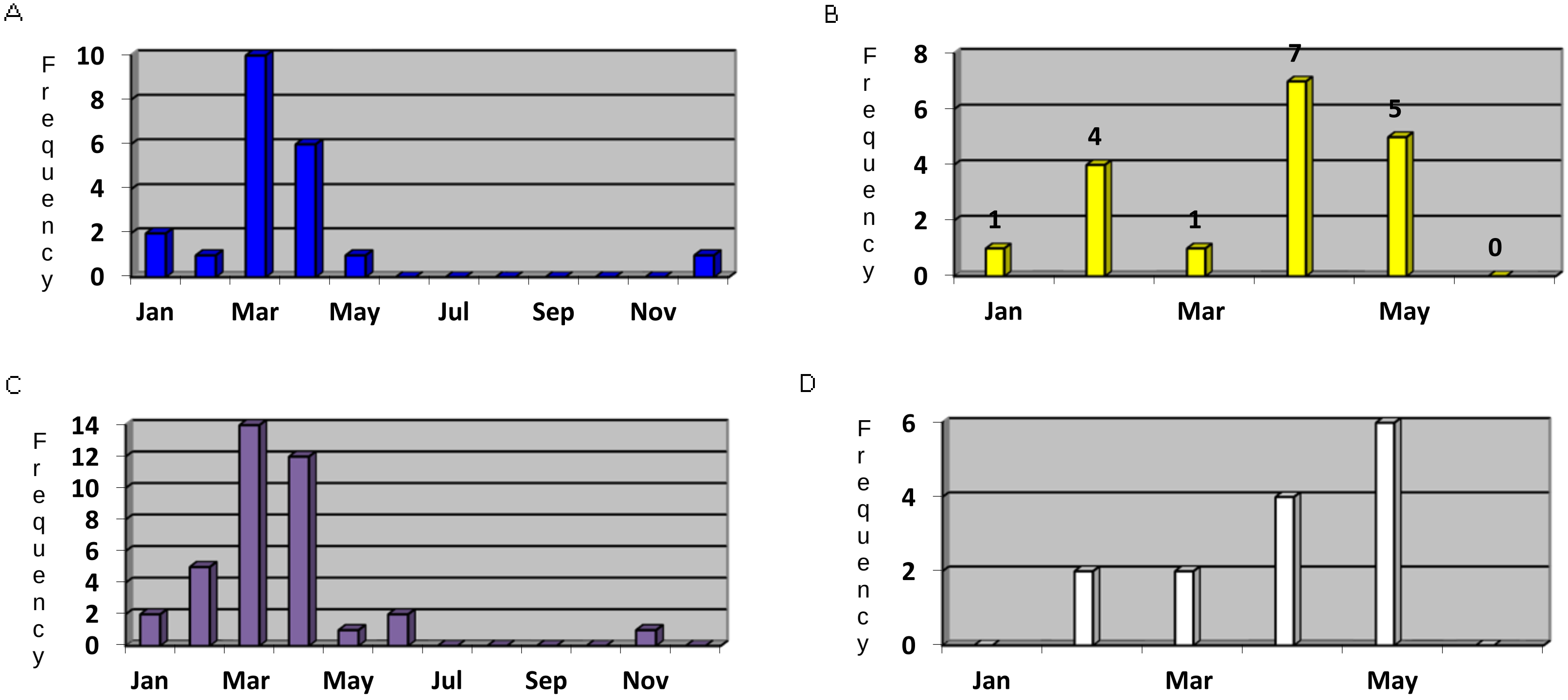

Figure 3.11. Histograms showing flowering time ranges within the *B. rigda* complex. A: UNBR, *B. rigida* with linear leaf, bracteole lamina reduced almost completely; B: OVTL: *B. rigida* with ovate leaf, bracteole lamina reduced almost completely; C: SPBL: *B. rigida* with linear or elliptic-ovate leaf, bracteole lamina well developed, base attenuate; D: SPIL: *B. rigida* with elliptic-ovate leaf, bracteole lamina reduced almost completely. UNBR flowers from December to May reaching the peak in March. OVTL flowers from January to May with a peak in April and SPIL flowers from February to May when it reaches its peak. SPBL begins to flower from November through to June with a pick in March-April. There is an overlap in flowering time among the taxa within the *B. rigida* complex with the peak from March to May. Detailed descriptions of abbreviations are given in Appendix I.

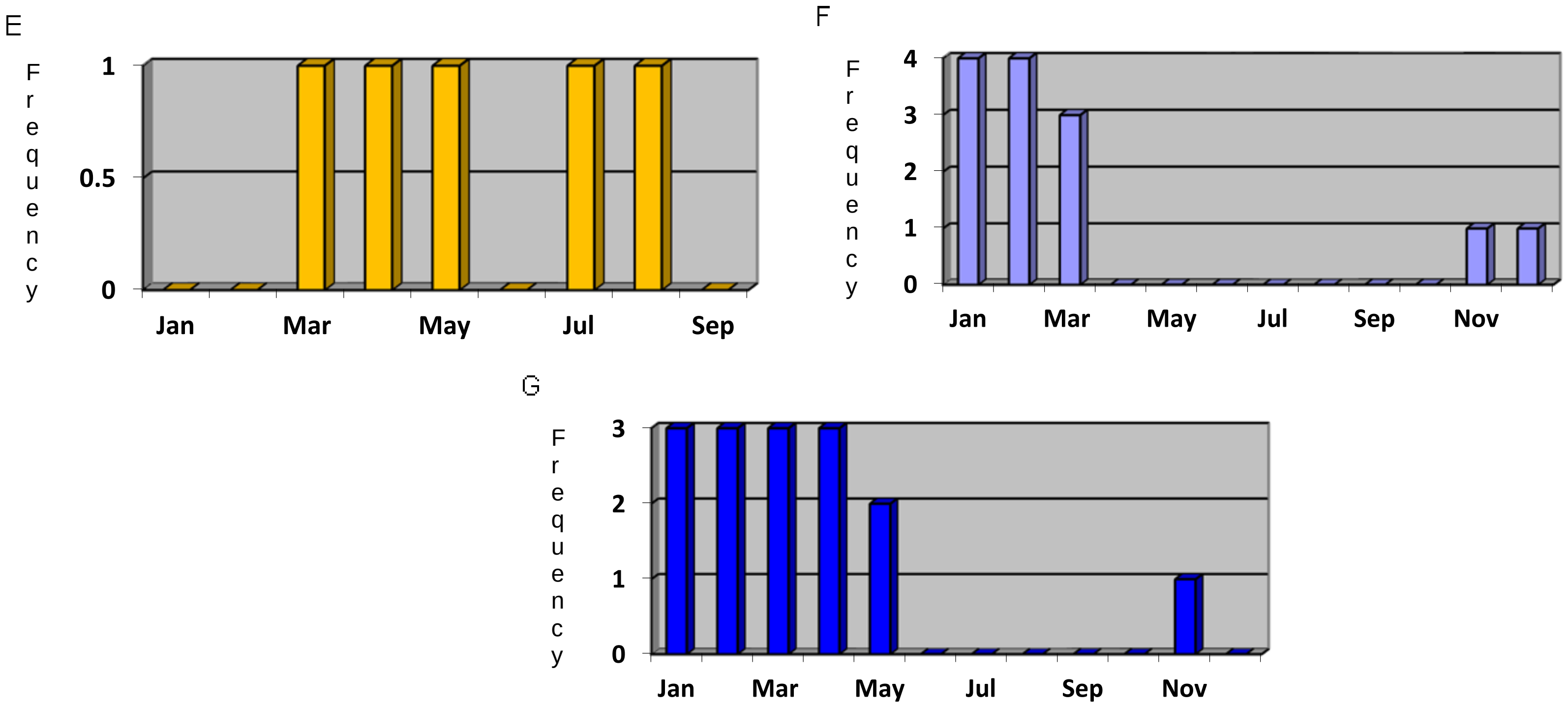

Figure 3.12. Histograms showing flowering time ranges within the *B. pungens* and *B. rigida* complexes. E: LOBR, *B. rigida* with ovate or elliptic-ovate leaf, bracteole lamina developed, base lobed or truncate; F: PUNG, *B. pungens* with green wavy leaf margins; G: PUNW, *B. pungens* with white or thin white wavy leaf margins. No peak was observed in the flowering time range for the LOBR. PUNG flowers from November to March reaching peak in January-February and PUNW flowers from November to May with a prolonged peak from January to April; exhibiting an overlap between the three groups. Detailed descriptions of abbreviations are given in Appendix I.

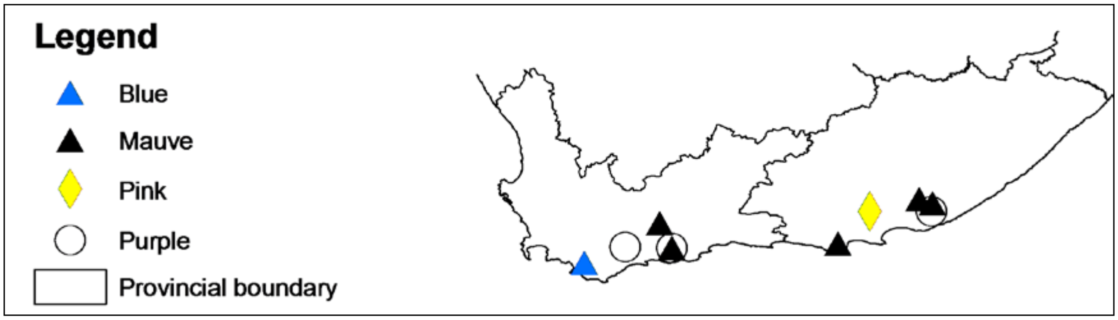

Figure 3.13. Flower colour distribution within PUNG of the *B. pungens* complex.

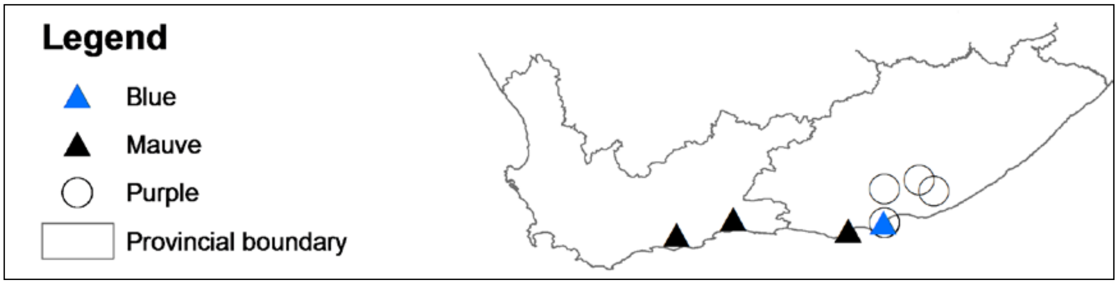

Figure 3.14. Flower colour distribution within PUNW of the *B. pungens* complex.

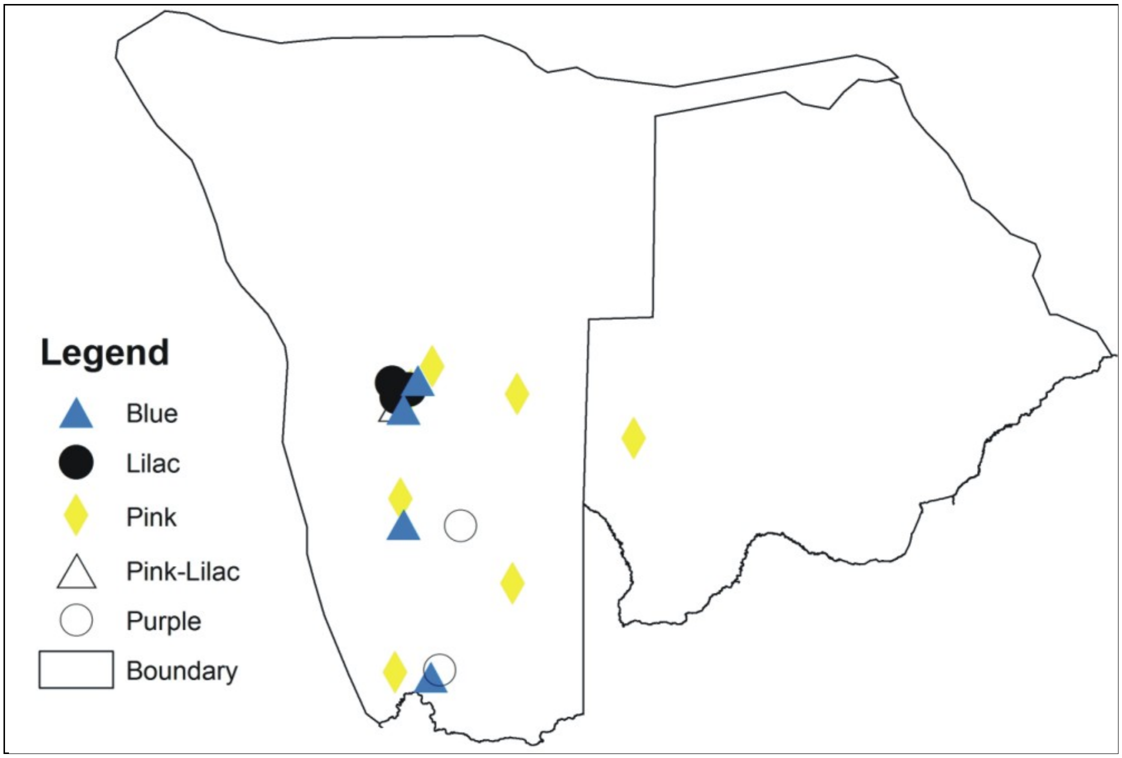

Figure 3.15. Flower colour distribution within SPBL of the *B. rigida* complex. There is an overlap in blue, purple and pink flowers with OVTL (see Figure 3.18).

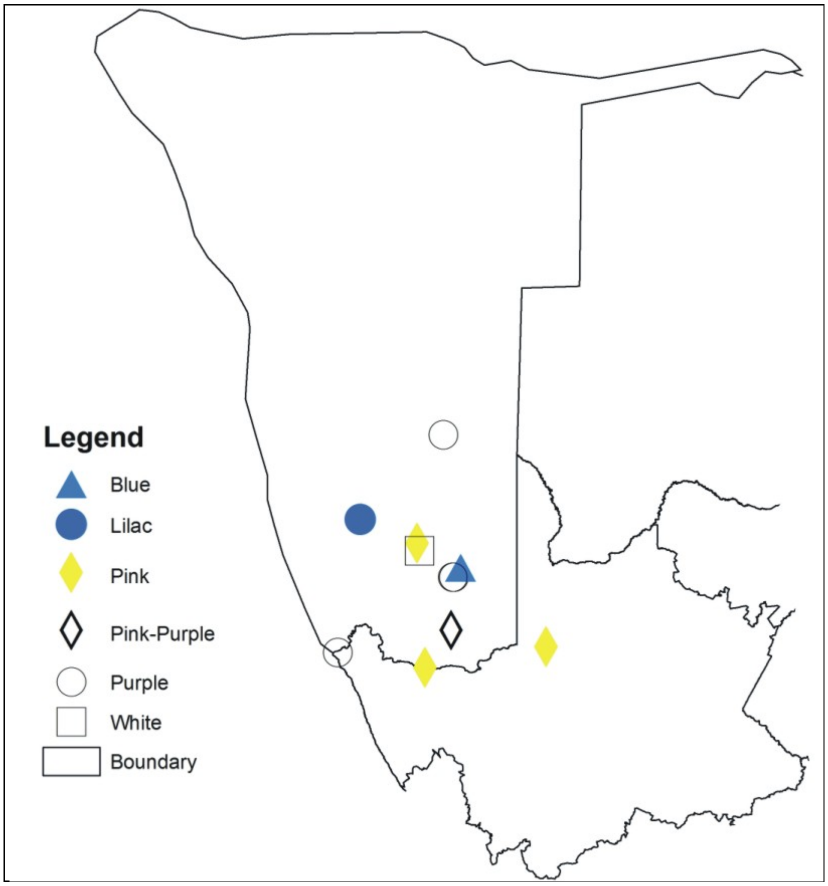

Figure 3.16. Flower colour distribution within SPIL of the *B. rigida* complex. There is an overlap in pink (with lilac) and blue flowers with that of OVTL; pink and pink-purple with that of UNBR (see Figures 3.17 and 3.18).

Specimens with intermediate leaf morphology (between OVTL and SPIL) were observed to occur in the distribution overlap zone (Figure 3.5e, f, k, l and q). Specimens of UNBR that overlap with OVTL, SPBL and SPIL have leaves that are more spine-toothed on the leaf margins than those that do not, while maintaining the narrow linear leaf shape (Figure 3.6g, p, q and r). Intermediate morphology between SPBL and SPIL has also been observed in leaf and bracteole (Figures 3.14 d & 3.16 b). SPBL bracteoles possess two conspicuous veins parallel to the mid-vein, UNBR, SPIL and OVTL usually have one conspicuous vein parallel to the mid-vein while LOBR has one (or sometimes two) shorter veins on either sides of the midvein in addition to the two longer ones. Although SPIL has bracteoles that are hardened, '*z*' and '*D*' have broad leafy bracteoles (Figure 3.7 z and D). Specimens of UNBR, SPIL and OVTL have narrow, ovate hardened bracteoles with reduced laminae while those of LOBR and SPBL have broad, ovate and lanceolate leafy or membranous bracteoles with a well developed lamina (Figures 3.7 & 3.8).

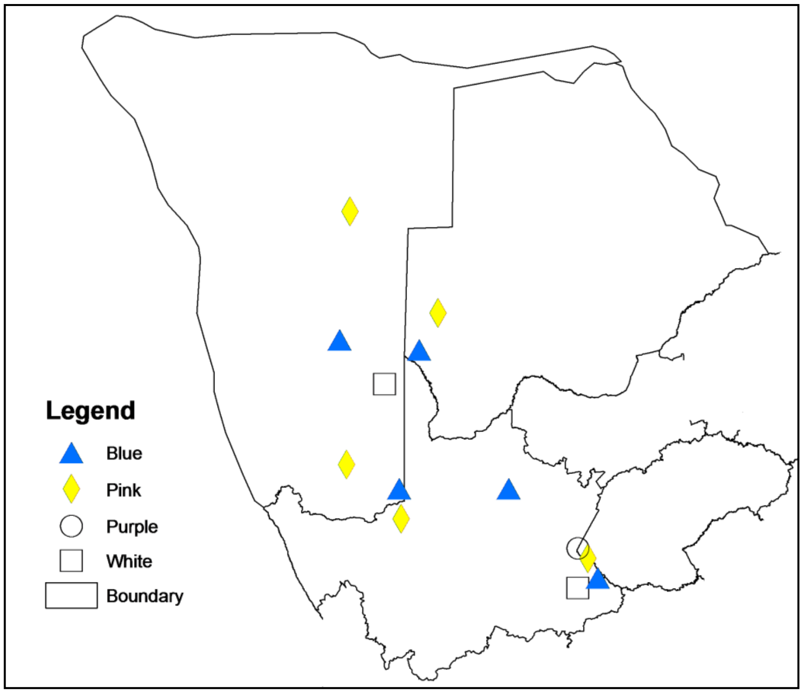

Figure 3.17. Flower colour distribution within UNBR of the *B. rigida* complex. There is an overlap in pink and blue flowers with pink and lilac of OVTL (see Figure 3.18).

Even if the bracteole labelled ‘z’ is broadly ovate with three conspicuous veins, the calyx lobe shape on the same specimen has been maintained, typical of those among OVTL (Figures 3.7 z and 3.9z). Variation is revealed in outer calyx lobe texture (sometimes difficult to ascertain), where LOBR, SPIL and OVTL have papery calyx lobes while UNBR have membranous or papery calyx lobes and SPBL have cartilaginous to indurate (or sometimes papery) calyx lobes. LOBR displays dissimilarity in bracteole and outer calyx lobe size and shape, (Figures 3.7 & 3.9). LOBR bracteoles have a long (sometimes tapering) tip and the posticous calyx lobe is almost twice the width of the anticous lobe (anticous lobe not in the map), and usually about twice the width of the posticous calyx lobes of UNBR, SPIL, SPBL and OVTL (Figures 3.9 & 3.10).

3.4.3 Flowering and flower colours

The histograms demonstrated that generally flowering occurred from November through to August, with very few flowering from June to August (Figures 3.11 and 3.12). Although the Western and Eastern Cape do receive winter rain along a narrow strip between George and Port Elizabeth (Mucina and Rutherford, 2006), all the specimens from these regions collected during winter only had mature fruit. The *B. pungens* complex was observed to flower during a period of eight months from September to May with the majority specimens with flowers collected in February and March, and exhibiting an overlap in flowering time ranges between PUNG and PUNW (Figure 3.12). The *B. rigida* complex also exhibited an overlap in flowering time ranges between all the putative taxa from March to April. Although there were no flowers observed on specimens collected in December from SPBL, it can be assumed that when flowering begins in November it continues through to June (Figure 3.11). Peak flowering time for the LOBR was difficult to predict since the only five specimens with flowers were collected in five different months (Figure 3.11). However, it can be assumed that flowering probably ranges from March to August.

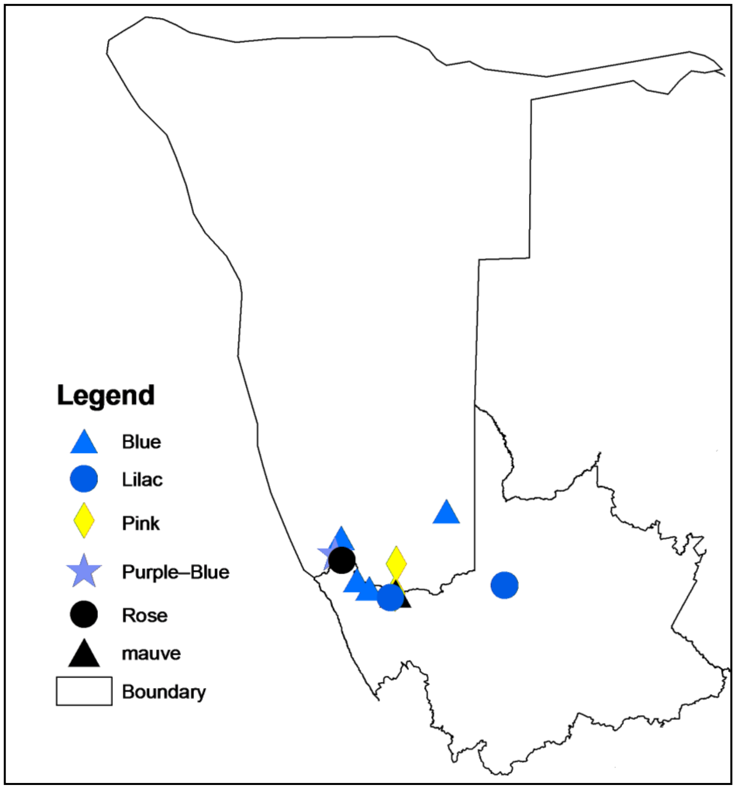

Figure 3.18. Flower colour distribution within OVTL of the *B. rigida* complex. An overlap is observed in pink and blue flowers with SPIL and blue and pink with rose with SPBL (see Figures 3.15 & 3.16).

PUNG and PUNW both have blue, mauve and purple flowers but there were no flowers on the two specimens of BP with flat leaf margins (Table 3.1 and Figures 3.13 and 3.14). Flower colour distribution revealed that mauve and purple flower colours occur at localities where the two taxa overlap in their distribution, blue flowers were only observed on two specimens (one of PUNG and PUNW). Pink flowers were only observed among PUNG. Within the *B. rigida* complex, flower colour ranges from white, pink, rose, mauve, lilac and purple to blue (Figures 3.15 to 3.19). Pink, lilac, purple and blue, are found in all the taxa within *B. rigida* (except LOBR). White flowers were only observed among UNBR and SPIL (Table 3.1 and Figures 3.16 and 3.17). Although there is a narrow distribution range overlap, there is substantial flower colour (purple, pink, blue and lilac) overlap between SPIL and SPBL. Although some flower colour variation (rose and mauve) is observed among OVTL in addition, rose colour could be classified with pink while mauve could be classified with purple.

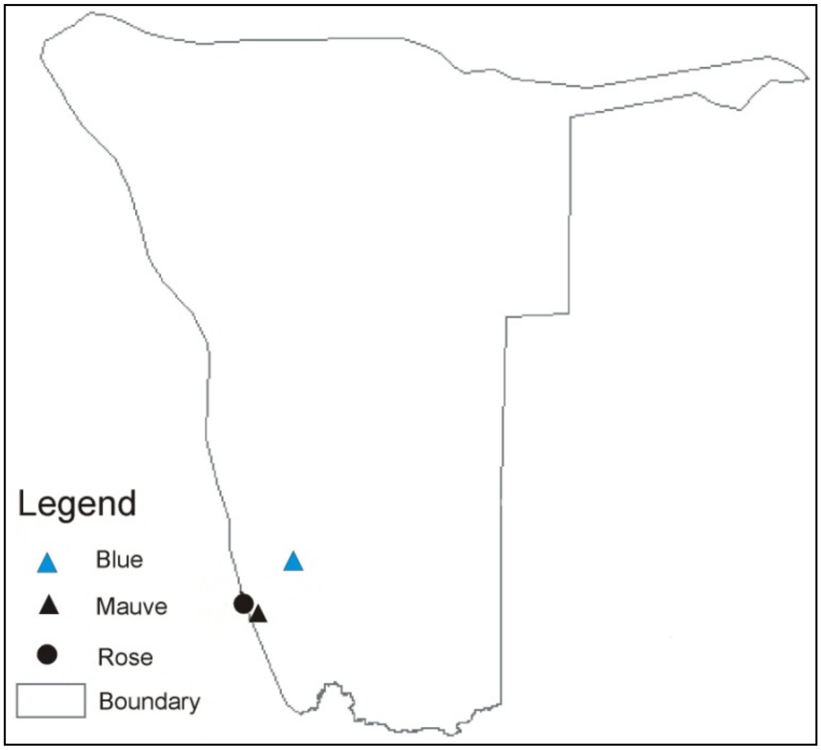

Figure 3.19. Flower colour distribution within LOBR of the *B. rigida* complex. Flower colour overlaps are observed in blue, mauve and rose with OVTL, and only blue with UNBR (see Figures 3.7 & 3.8).

3.5 Discussion

3.5.1 Floral variation

Comparison of the corolla tube length and corolla tube mouth demonstrates that these characters can be used to separate LOBR from the rest of the *B. rigida* complex. The widest part of the corolla tube (corolla tube mouth width) can also be used to differentiate LOBR from PUNG and PUNW. Although LOBR has a distribution overlap with OVTL and SPBL the discontinuity in floral characters may not facilitate gene flow, which can be deduced from the observed morphological gaps. However, corolla tube length and width was overlapping among UNBR, SPIL, OVTL and SPBL, suggesting that the two characters have some taxonomic significance (Wilkin, 1999). The dissimilarity in corolla tube dimensions can bring about breaks in gene flow whereas the overlap may facilitate gene flow among taxa in the absence of other barriers. Even if LOBR, PUNG and PUNW exhibit an overlap in corolla tube length, the geographic disjunction would prevent gene flow.

3.5.2 Distribution of the variation in morphology

Field observations revealed that specimens collected from deep soil had larger leaves as opposed to those collected from shallow rocky soil (pers. obs.). The presence of big and small leaves among BP, PUNG and PUNW therefore, could suggest plasticity. In addition, bracteole and calyx lobe morphology did not portray dissimilarity. Intra-specific variation in calyx lobe size has been observed within *Dicliptera* as a result of clinal variation (Balkwill *et al.,* 1996). PUNW, with prominent white wavy leaf margins, was restricted to Albany Thicket whereas specimens with thin white leaf margins (intermediate leaf morphology) occurred in the East Coast Renosterved and Albany Thicket, and did not occur in the intermediate Eastern Fynbos-Renosterveld. PUNG exhibited continuous and widespread distribution throughout the distribution range of the *B. pungens* complex. BP with flat green leaf margins inhabits the Rainshadow Valley Karoo at the edge of the distribution range of PUNG and PUNW in Ladismith. The presence of intermediate morphology and the absence of PUNW in the connecting area could also be attributed to plasticity influenced by genome-environment interactions. PUNG may be a generalist that is capable of inhabiting various environments while PUNW may be a specialist. In addition BP may be rare and endemic to the area around Ladismith (Makholela *et al.*, 2003). The disjunct distribution and restriction of prominent white and thin white wavy leaf margins to the Albany Thicket could be attributed to niche preference which may also be influenced by habitat fragmentation (Aizen *et al.*, 2002; Van Valen, 1965). Because ecological differences are rarely abrupt in zones of inter-gradation, there is likely to be an intermediate habitat with an intermediate population to serve as a channel for the gene flow (Delph *et al.*, 1997; Mayr, 1947). The observed variation therefore, may be attributed to spatial niche partitioning as individuals cannot thrive beyond where their survival and fitness cannot be sustained, whether due to ecological or biological factors (Bennett, 1987; Samis and Eckert, 2009; Van Valen, 1965).

Within the *B. rigida* complex, leaf size and shape does not exhibit a clear-cut differentiation. Although the leaves of OVTL are broadly ovate with sinuate spine-toothed leaf margins, at the southern border of Namibia with the Northern Cape (South Africa) and Gariep Desert, they become elliptic-ovate to narrowly ovate and become hard to distinguish from SPIL northward at the fringe of Central Plateau in Namibia, especially as both have narrow single-veined bracteoles (Figures 3.5 and 3.7). In addition the calyx lobes do not exhibit apparent or clear-cut dissimilarity between the two putative groups (Figure 3.9). LOBR presents bracteole and calyx lobe morphology that is markedly dissimilar from SPIL and OVTL (Figure 3.7 and 3.9). SPIL (with elliptic-ovate or narrowly ovate leaves) and OVTL (with broadly ovate leaves) are concentrated in regions between 400 and 1000 m (Mucina and Rutherford, 2006). OVTL occurs on the desert sands of the Namib and Kalahari Desert on the western side and southern end of the Central Plateau in Namibia and also in the Gariep Desert while SPIL occurs on the desert sands of the Southern Namib Desert and its fringes in southern Namibia toward the border of Namibia with the Northern Cape and in the Gariep Desert. At the southern fringe of the Southern

Namib Desert and in the Gariep Desert, SPIL and OVTL narrowly overlap with SPBL and UNBR (Figures 3.5, 3.7 and 3.9). LOBR occurs on the coastal desert sands of the Namib Desert overlapping narrowly with OVTL. Since the distribution range and persistence of species does depend on physiographical and climatic conditions, the phenotypic variability exhibited here may suggest there is genetic diversity and differentiation, and morphological variation may serve as an adaptation to ecological heterogeneity (Bult and Kiang, 1992; de Kroon *et al.*, 2005; Delph *et al.*, 1997; Obermeijer, 1933; Richards *et al.*, 2005; Snaydon, 1973; Strand and Weisner, 2004; van Kleunen and Fischer, 2005; Xu *et al.*, 2007). In addition, the distribution overlap might suggest the presence of intermediate habitat (Delph *et al.*, 1997), intermediate morphology between LOBR and OVTL is only observed in the bracteole of 'z' (Figure 3.7). It can then be deduced that there might not be an absolute break in gene flow between the two taxa. The intermediate leaf morphology (and overlap in bracteole and calyx lobe morphology) between OVTL and SPIL may be attributed to plasticity due to genome-environment interactions rather than genetic variation.

Even if the leaf size is relatively bigger in SPBL than in UNBR, and the margin conspicuously spinier in the one than in the other (almost entire), intermediates occur around distribution overlap zones, which may imply that the dissimilarity is due to environmental variation (Figure 3.6 b, g, and q). Bracteole lamina size, venation and texture can be used to distinguish SPBL from UNBR (Fig 3.8). UNBR generally occurs in the Upper Karoo in the Northern Cape between 400 and 1000 m while SPBL mainly occurs in the savannas on the Central Plateau and its fringes in Namibia, at altitudes between 1200 and 1800 m, but occasionally occurs between 900 and 1200 m especially where they overlap with UNBR. However, SPBL does not end abruptly but exhibits fragmented distribution into Namibia and Botswana on the Kalahari sand between 300 and 1100 m. Isolated islands of altitude of 1200 – 1500 m (surrounded by lowland between 900 and 1200 m) were observed between Haribes and Mariental from where *Volk 12171* (PRE) and *Dinter 2954a* (SAM) were collected (not in the map but with morphology similar to 'B' in Figure 3.5).

Specimens 'O' (SPBL) and 'b' (UNBR) which exhibit morphological and spatial overlap were observed at the edge of the highland 1200 – 1500 m (Figure 3.6). While 'e' (UNBR) occurs where fragments of 1100 and 1200 m land appear, and 'w' (SPBL) and 'g' (UNBR) both occur at 1100 m, 's' (SPBL) occurs on sand dunes between 1000 and 1100 m. SPBL is observed to be absent at altitudes above 2000 m resulting in fragmented and clustered distribution which may be associated with the fragmentation of suitable habitat (Aizen *et al.*, 2002; Miner *et al.*, 2005). This trend has also been noted among SPIL and OVTL which occur at 900 – 1200 m and 600 – 900 m respectively, denoting niche partitioning and preference (Aizen *et al.*, 2002). This may suggest the presence of fragments of suitable habitat. It can then be deduced that in low lying areas overlap in distribution between SPBL and UNBR only occurs in localities with fragments of high and low land implying there is selectiveness between the two taxa in response to environmental heterogeneity (Fuller and Prommer, 2000).

Two specimens ‘r (i)’ and ‘r (ii)’ on one herbarium sheet and recorded as one gathering (and representing one species), has two forms that can be differentiated by the presence of spine-toothed undulate leaf margins in one and entire undulate leaf margins in the other. Although both forms possess hardened bracteoles, one is spinier and broader than the other and occurred in an area where there is distribution overlap between OVTL, SPIL and UNBR, suggesting that they occur in mixed populations. Specimen ‘d’ was not part of a mixed gathering, but it occurs (between 800 and 900 m) where OVTL, SPIL and UNBR exhibit geographic overlap; it displays intermediate morphology by possessing a large linear-lanceolate leaf with spiny margins (as in SPBL) and a somewhat leafy but narrow bracteole with only one prominent vein (as in OVTL, SPIL and UNBR) (Figures 3.5d and 3.6d). When closely related taxa are observed growing in mixed populations then phenotypic plasticity is not likely to account for the variation (Radford, 1986). The intermediate morphology in leaf margin spinescence and bracteole lamina, therefore, could indicate the presence of gene flow among the three putative taxa (other than environmental interactions).

Bracteoles in SPBL tend to be narrower in specimens collected at altitudes between 1500 and 2000 m as observed in ‘M’ and ‘D’ and much broader in specimens collected between 1000 and 1500 m at the fringe of the plateau and the Kalahari Desert as evidenced in ‘u’, ‘x’ and z (Figure 3.8). In addition, ‘M’, ‘D’ and ‘b’ have a more dwarf habit with compacted stems than the rest, and form a shrubby cluster. The narrow bracteoles of SPBL can be recognised by their leafy nature. The presence of intermediate leaf morphology between UNBR and SPBL may suggest that the variation may be caused by environmental changes.

3.5.3 Flower colour distribution and phenology

The observed overlap in floral dimensions and flowering time between PUNG and PUNW with geographic overlap can facilitate gene flow (Figures 3.1 and 3.12). OVTL, SPBL, SPIL and UNBR exhibit an overlap in both floral dimensions and flowering time range. However, LOBR exhibits dissimilarity in floral dimensions from the rest of the *B. rigida* complex though there is an overlap in flowering time range. This discontinuity may represent differentiation and may to a great extent hinder gene flow via pollen transfer hence creating a break in gene flow. While overlapping morphology does not have taxonomic implications, discontinuous morphology may. Therefore, in the absence of other isolating mechanisms such as time of the day, gene flow via pollen transfer will occur as pollen deposition on the pollinator and effective transfer may be influenced by metric differences (Guitian *et al.,* 1999; Herrera, 1993; Kendall, 1973).

Flowering among the two putative taxa, PUNG and PUNW mainly occurs in January (with a few observed in November and December) and ends in May when temperatures begin to fall. The lack of flowers on all the specimens collected during winter from the Western and Eastern Cape which receive winter rain may imply that flowering is necessarily triggered by wetness. Of the 28 collections examined, 15 belonged to the PUNW while 13 belonged to PUNG (Figure 3.12). However, it is difficult to make meaningful conclusions on the extended peak in flowering in the absence of altitude information which was missing on most specimens. Although no flowers were present on BP, it can be deduced from the date of collection on one of the OTUs with flowering information that it flowers in July. Late flowering extending up to May in both UNBR and SPBL is observed in specimens at the edge of the Thornveld in Namibia and the Upper Karoo south-east of the Northern Cape in South Africa. Although it is possible that flowering may occur much earlier within LOBR (but collections have not been made), flowering through the cool temperatures from March to July may be influenced by the environment since it inhabits the Namib Desert which experiences relatively high temperatures in winter (Armstrong, 1990). Winter flowering is usually observed in environments where conditions are likely to be favourable in terms of pollinator availability as plants that flower during winter tend to encounter many pollination limitations due to scarcity of insect visitors (Brouat *et al.*, 2004; Guitian *et al.*, 1999).

Colour information not being present on all specimens examined had an effect on deductions on infra-specific colour variations. BP which occurs at the periphery of PUNG and PUNW is reported to have mauve flowers which are also present in PUNG and PUNW (Figures 3.2, 3.13 and 3.14). The restriction of purple flowers (in PUNG and PUNW) to localities where prominent white leaf margins occur could possibly suggest that flower colour may be influenced by spatial distribution. Apart from UNBR which did not have colour information where it overlaps with SPBL, there is overlap in flower colours among the rest of the putative taxa wherever there is range distribution overlap (Figures 3.15 to 3.19). Since colour variation is observed some localities within a putative taxon, dissimilarity in flower colours between putative taxa at localities where their distribution overlap may suggest dissimilarities between the taxa or simply colour diversity. Colour variations such as purple, lilac, mauve, pink and rose within a taxon could be attributed to differences in colour perceptions among collectors or variability in the phenotype or indeed as a result of plasticity brought about by environmental dissimilarity. Due to overlap in flower colours, LOBR, OVTL and SPIL may share the same pollinators which may be evidenced by the presence of intermediate bracteole morphology between LOBR and OVTL (Figure 3.7 z and D). However, the presence of gene flow may not necessarily suggest conspecificity, as interspecific gene flow may possibly take place (Heywood, 1986).

White flowers were only observed in SPIL and UNBR at altitudes between 1000 and 1500 m but not higher. In SPIL white flowers occurred around Karasberge – Keetmanshoop at the fringes of the Namib Desert and the Central Plateau in Namibia, and in UNBR, they occurred at the edge of the Thornveld and the Upper Karoo in De Aar in the eastern Northern Cape and at the Kalahari fringes in Mariental in Namibia (Figures 3.16 and 3.17 respectively). The absence of white flowers from the dry Namib Desert and the high altitudes (above 1500 m) of the Central Plateau could imply that they may not tolerate extreme environmental conditions. The Namib Desert is known for harsh dry environmental conditions (Armstrong, 1990). Cream-yellow flowers also occur in the Upper Karoo around Wanda. However, purple, blue, pink and lilac occur between 600 m and 1200 m whereas mauve was restricted to lower altitudes especially along the coast (except within OVTL where it occurs along the Orange River. This could suggest that the flower colour diversity may be a plastic response to environmental factors. Ecological variations in exposure, moisture, salinity and geology can have an effect on floral morphology (Ahmad *et al.*, 2005; Balkwill *et al.*, 1994; Herrera, 1995; Miner *et al.*, 2005; Vallius *et al.*, 2004). White flowers have been known to be more susceptible to environmental stress than pigmented colours (Galen, 2006; Patiño and Grace, 2002; Sapir *et al.*, 2006; Vallius *et al.*, 2004; Zhang *et al.*, 2010). In addition the persistence of white flowers, consequently, could result in a break in gene flow.

3.5.4 Recognisable groups based on species concepts

The two distinct specimens (BP) apart from being at the edge of the species distribution range have small leaves with flat leaf margins with appressed hairs, narrow bracteoles with serrate margins, glabrate to glabrous calyx lobe surface and serrate outer calyx lobe margins (Figures 3.3C & 3.4C). Material of this taxon may have a distribution overlap with the rest (PUNG and PUNW) but might just not have been collected. Although there is insufficient floral information, BP is distinct from PUNG and can be differentiated in more than three morphological characters. The difference in characters such as leaf margin, bracteole and calyx lobe margin and pubescence type can be used as taxonomic basis upon which to recognise the two specimens as a separate species morphologically (Balkwill and Balkwill, 1997; Champluvier, 2011; Darbyshire, 2008; 2009; Darbyshire and Ndagalasi, 2008; Obermeijer, 1933).

However, the two putative taxa (PUNG and PUNW) do not exhibit any clear-cut morphological differentiation, but in addition, exhibit an overlap in distribution. The dissimilarity in pubescence density where leaves with prominent white leaf margins are less pubescent than those with green or thin white leaf margins may be influenced by environmental conditions. The overlap in floral dimensions, flower colours and flowering time range, can be used to deduce that it is likely that there is gene flow between the two putative taxa based on the Biological Species Concept. In view of the Morphological Species Concept PUNW and PUNG may be a single species as continuous morphology is not taxonomically useful as the two taxa do not show clear morphological distinctness (Brouat *et al.*, 2004; Wilkin, 1999).

Within the *B. rigida* complex, specimens inhabiting the desert sand have papery calyx lobes, as opposed to those of the Karoo and Namibia’s Central Plateau which have membranous and cartilaginous lobes respectively. Generally the outer calyx lobe margins of specimens occurring on the desert sand and the Central Plateau have longer spines as opposed to those in the Upper Karoo. The variation in calyx lobe texture and calyx lobe margins may be attributed to environmental factors. Apart from LOBR and SPBL, there was very little dissimilarity in bracteole size and shape among SPIL, UNBR and OVTL. In addition LOBR differed from the rest of the taxa within the *B. rigda* complex in outer calyx lobe size and shape as well as corolla tube length and width of the wider tube. Although LOBR exhibits a narrow distribution overlap, flower colour and flowering time range overlap with SPBL, the variation in corolla tube length would inhibit gene flow to a large extent. LOBR is discernible by a combination of more than three diagnostic characters which qualify it to be recognised as a separate and distinct species morphologically (Balkwill *et al.,* 1996; Ensermu, 1994). Dissimilarity in floral characters can imply dissimilarity in functionality which in turn may hinder gene flow (Levin, 1973).

SPIL and OVTL not only overlap in distribution but also flowering time, flower colours and floral dimensions. The indistinct morphology between SPIL and OVTL in bracteole and calyx lobes and occurrence on the desert sands suggest that the two putative taxa may be one and the same such that the observed continuous leaf variation in shape may be influenced by ecological factors. Although UNBR and SPBL can be differentiated by the hardened usually 1- veined bracteole in UNBR and leafy 3-veined bracteoles in SPBL, leaf margins exhibit overlap in leaf margin spinescence and undulation. The coincidence in flower colours, flowering time in addition to overlap in floral dimensions between UNBR and SPBL may indicate that they belong to the same species. While SPIL and OVTL can be differentiated from UNBR by the leaf shape and leaf margin (ovate spiny in one and linear-lanceolate almost entire in the other), both SPIL-OVTL and UNBR have hardened narrow 1-veined bracteoles (sometimes with a faint vein parallel to the mid vein) and papery calyx lobes with overlapping size and shape. In view of lack of sufficient morphological gaps including floral dimensions, flowering time and colour SPIL-OVTL, UNBR and SPBL may be recognised as varieties of the same species since they exhibit spatial differentiation in distribution and fragmentation may alter species interaction, resulting in dissimilarity (Aizen *et al.,* 2002; Brouat, *et al.,* 2004; Sebola and Balkwill, 2009).

Although SPBL does not exhibit clear-cut spatial disjunction from UNBR and SPIL-OVTL and may not be said to have a minimally different adaptive zone, the concentration to Namibia's Central Plateau suggests it may also be an ecotype within the *B. rigida* complex (Brouat, *et al.,* 2004; Sebola and Balkwill, 2009). The presence of only one distinct morphological character in bracteole between UNBR and SPBL or SPIL-OVTL and SPBL suggests that the three are varieties that may be ecologically separated. In the absence of disjunction and at least two independent characters, SPBL cannot be recognised at the level of subspecies. SPBL is, therefore, morphologically recognised as a variety within *B. rigida* Nees.

3.6 Conclusion

It is suggested here that the two species complexes, *B. pungens* and *B. rigida* consist of distinct taxonomic entities. Apart from the two distinct specimens with green flat leaf margins, the rest of the specimens examined from the *B. pungens* complex make up one species that may consist of varieties. However, PUNG and PUNW make up a single species being influenced by ecological factors and if there be any variation, it is cryptic. The *B. pungens* with flat green leaf margins can be recognised by a shortly glabrescent leaf surface, serrate bracteole margins and glabrate to glabrous calyx lobe surface with serrate margins. Within the *B. rigida* complex LOBR can be recognised as a species based on its morphological distinctness from the rest of the *B. rigida* complex. LOBR is recognised by the lobed or truncate bracteole base, tapering bracteole apex, and broadly ovate posticous calyx lobe with lobed base. SPIL and OVTL may be recognised as one variety based on the lack of morphological distinctness as well as geographic isolation. Although there is distinct variation in bracteole morphology between UNBR and SPBL, the two have been recognised as varieties within *B. rigida* and not subspecies due to the lack of geographic isolation. Spatial niche partitioning can be said to be influencing the restriction of *B. pungens* with white margins to the Albany Thicket, SPIL-OVTL to the desert sands, UNBR to the Upper Karoo and SPBL to the Central Plateau. Apart from PUNG and LOBR the differentiation within the *B. pungens* and *B. rigida* complexes could therefore be assumed to follow ecological clines as evidenced by their distributions (although in the case of this study edaphic factors were not studied). The assumption is here based on the perception that widespread species tend to exhibit diverse morphology as an adaptation to environmental variation (Balkwill *et al.*, 1996; Furnier and Adams, 1986; Laurance, 2008; Manning and Cron, 2011). However, part of the variation could also be influenced by some other biological factors such as phenological changes which cannot be established with the available data.

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3.8 Appendices

3.8.1 Appendix I: Putative taxa

BP: specimens from the *B. pungens* complex with green flat leaf margins
PUNG: specimens from the *B. pungens* complex with green wavy leaf margins
PUNW: specimens from the *B. pungens* complex with a white wavy leaf margins
UNBR: specimens from the *B. rigida* complex with linear leaf, entire or scantly toothed undulate leaf margins; bracteole hardened
OVTL: specimens from the *B. rigida* complex with ovate leaf, spiny sinuate leaf margins; bracteole hardened
SPIL: specimens from the *B. rigida* complex with linear, elliptic-ovate leaf, spiny sinuate or undulate leaf margins; bracteole hardened
SPBL: specimens from the *B. rigida* complex with linear or elliptic-ovate leaf, spiny sinuate or undulate leaf margins; bracteole leafy
LOBR: specimens from *B. rigida* complex with ovate or elliptic-ovate leaf, spiny sinuate leaf margins; bracteole membranous, base lobed

3.8.2 Appendix II: Specimens examined from the B. pungens complex used in Figures 3.11 – 3.13

Eastern Cape. –3225 (Somerset East): Cradock, near Port Elizabeth (–BA), 12 May 1902, *Galpin 6360* (PRE) [f]. –3325(Port Elizabeth): (–DC), 1 February 1927, *Moss 15394* (J) [i]; (–DC), 31 May 1919, *Mogg 4761* (J) [j]; (–DC), 30 August 1030, *Fries, Nordlindh & Weimarck 463* (PRE) [r]; Markman Industrial area (–DC), 23 February 1971, *Dahlstrand 2715* (GRA, J) [g]; New Brighton (–DC), January 1901, *Galpin 5879* (PRE) [h]. –3326 (Grahamstown): North-eastern boundary of the farm “Mountain Top”, Carlisle Bridge area, on the Fish River ridge, 33º 00’S 26º 20’E (–AB), 19 April 1995, *Bowker s.n.* (GRA) [n]; Sugar Loaf Hill (–AD), April 1950, *McGillivary 2951* (J) [k]; Ecca Reserve, 33º 13’ 40”S 26º 38’ 5”E (–BA), 6 March 1992, *Chan 6* (GRA) [l]; Botha’s ridge (–BC), 24 March 1962, *Wells 2631* (GRA) [m]; Bothas Hill 11 miles from Grahamstown junction, between Karoo and Scrub veld (–BC), May 1928, *Dyer 1414a* (J) [o]; Peddie road 15 – 16 miles from Grahamstown junction, between Karoo and scrub veld (–BD), April 1928, *Dyer 1358* (GRA) [p]; Alexandria (–CB), 24 March 1952, *Archibald 4154* (J) [q]. –3424 (Humansdorp): Kaus and Kay, 1700 ft. (–BA), March 1026, *Fourcade 3239* (BOL) [t]; (–BB), January 1912, *Burtt-Davy 12000* (BOL) [s]; between Kabeljau and Mondplaas along Nat. Road (–BB), 11 January 1993, *Fourcade 5931* (BOL) [e].

Western Cape. –3419 (Caledon): Hermanus, Bushman’s River mouth, vacant plots at the extension (–CB), 10 January 1989, *Green 591* (J) [A]. –3421 (Riversdale): Soetmelksrivier, plateau N of bridge on main road (–AB), 25 February 1981, *Bohnen 7829* (PRE) [a]; 2 km NW of Soetmelksrivier bridge (–AB), 20 February 1980, *Bohnen 7371* (PRE) [b]; *ibidem*, 20 February 1980, *Bohnen 7371* (PRE) [y]; Riversdale (–AB), 19 November 1912, *Schlechter 1985* (BOL) [c]; *ibidem*, 19 November 1912, *Schlechter 1985* (BOL) [z]; Riversdale (–AB), September 1960, *Horn 2286* (PRE) [B]; Albertina, about 27 km from Mossel Bay to Albertina, on N2 road, Farm Van Rensburg, 34º 10.78’S 21º 53.08’E (–AB), 8 July 2008, *Nyirenda 708* (J) [x]; *ibidem*, 8 July 2008, *Nyirenda 710* (J) [w]. –3422 (Mossel Bay): between George and Great Brakriver, next to national road, near the Maalgaten river bridge (–AB), 1 January 1987, *Vlok 1844* (PRE) [d]. –3423 (Knysna): hills above Keurboom Strand (–AB), 30 January 1949, *Walgate s.n.* (BOL) [v]; Plettenberg Bay (–AB), March 1910, *Fourcade 613a* (BOL) [u]. –3424 (Humansdorp): (–BB), January 1912, *Burtt-Davy 12000* (BOL).

3.8.3 Appendix III: Specimens examined from the B. rigida complex used in the distribution of morphology among SPBL and UNBR (Figures 15, 17 & 19)

UNBR

Botswana. –2320 (Ukwi): Ghanzi & Kgalagadi, Masetleng Pan, in dwarf shrub zone, 23º 41’S 20º 51’E, (–DB), 21 March 1978, *Skarpe S-289* (PRE, SRGH) [c]; Kalahari Sandveld, Masetlheng, ± 130 km WNW of Hukuntsi (–DB), 10 March 1997, *Cole 1271* (PRE) [P].–2421 (Tshane): 100 km W of Hukuntsi at Masetlheng Pan, pan fringe (–BA), 16 May 1985, *Parry 8535* (J) [d].

Namibia. –2118 (Steinhause): ca 40 km NW of Gobabis, Farm Hondeblaf 179, Road Steinhausen – Gobabis (–BA), 7 January 1968, *Wanntorp* & *Wanntorp 743* (K, PRE) [a]. –2216 (Otjimbingwe): 20 miles from the Rehoboth border, Farm Friedental 44, Road Windhoek – Walvis Bay (–DB), 10 March 1968, *Wanntorp & Wanntorp 168* (K) [I]. –2316 (Nauchas): at edge of Farm Weissenfels REH 22 (–AD), 11 March 1953, *Walter & Walter 1690* (WIND) [F]. –2418 (Stampriet): Gellapost Expt. Station (–AC), May 1949, *Liebenberg 5177* (WIND) [e]; 16 km from Mariental turn off to Stampriet, Opstal farm (–AD), 11 March

1983, *Germishuizen 2746* (PRE, WIND) [b]. –2518 (Tses): Hardap, Farm Galloway 230, about 1.2 km south of house, 25º 13' 33"S 18º 37' 51"E (–BA), 23 March 1998, *Strohbach* & *Dauth 3695* (WIND) [g]. –2519 (Koes): Farm Grensplaas 409, 2 kilometres south of homestead in river, 25º 29' 8"S 19º 29' 49"E (–AD), 19 April 1997, *Calitz* & *Sheuyange 104* (WIND) [f]. –2716 (Witpütz): rocky hillslope facing SE, between Aus and Rosh Pinah (–DA), 8 May 1976, *Oliver & Müller 6411* (WIND) [v]. –2718 (Grünau): Tallus slope of Karas Mountain on route to Telecom Tower on Farm Rishon 365, 27º 20’ 17”S 18º 43’ 47”E (–BC), 14 February 1997, *Strohbach 2856* (WIND) [q]; Farm Genadendal 264, 27º 30' 24"S 18º 32' 33"E, western slope of Karasberg (–DA), 16 March 1997, *Strohbach* & *Sheuyange 3308* (a & b) (K, WIND) [r (i) & r (ii)].

South Africa. –2819 (Kenhardt): on the road to Nous, 2 – 3 km north of N14, 28º 52.05’S 19º 54.91’E (–DD), 7 April 2000, *Balkwill*, *McDade* and *Lundberg 11799* (J) [h]. –2820 (Upington): c 80 km from Ariamsvlei towards Upington, Karoo (–AD), 26 June 1981, *Van Wyk 4475* (PRE) [p]. –2822 (Hay): Griquatown, Hay (–DD), Date unknown, *Wilman s.n.* (GRA) [i]. –2824 (Gordonia): Kalahari Gemsbok National Park, about 120 m up Nossob from entrance gate (–BA), 18 May 1956, *Story 5529* (PRE) [j]. –2924 (Wanda): East of Wanda Police station, 29º 35.63’S 24º 26.29’E (–CB), 20 June 2008, *Nyirenda 700* (J) [k]; (Wanda): 6 Km from Wanda on road to Roodepan (–CB), 26 February 1981, *Herman 294* (PRE) [n]. –3022 (Carnarvon): “Boomsynputs” north of Carnarvon (–AC), 17 May 1975, *Thompson* 3115 (NBG, PRE) [o]. –3024 (Colesberg): Doornkloof Nature Reservet, above spruit below boundary fence 2B41 (–BD), 28 December 1982, *Handiek 129* (GRA) [l]; (De Aar): 33 Km east of De Aar on Sterkfontein road, foot of mountain, Southern slope (–CB), 4 April 1981, *Herman 561*(PRE) [m].

SPBL

Namibia. –2016 (Otjozondjupa): southern eastern corner Otjiku Plain (–BD), 16 May 2001, *Strohbach 1060* (WIND) [K]. –2217 (Windhoek): Avis, western mountain land (–CA), 7 April 1964, *Seydel 3944* (M) [L]. –2316 (Rehoboth): at edge of Farm Weissenfels REH 22 (–AD), 11 March 1953, *Walter & Walter 1690* (WIND) [F]; Farm Gurumanas REH 306 (–BB), 10 March 1953, *Walter & Walter 1650* (WIND) [H]; Khomas, Friedrichsruh, mountain slope, on koppies south of big river (–BB), 17 April 2002, *Strohbach BS5514* (WIND) [G]; F. Nautzerus, Bastardland (–CD), 15 March 1953, *Walter & Walter 1840* (B) [M]. –2317 (Mariental): Farm Bergland-Arovley, on surface lime in limestone meadow community (–AA), March 1953, *Giess 1787* (WIND, B) [J]. –2416 (Maltahöhe): Farm Nomsas MAL 26, Sign point (–BD), 16 March 1953, *Walter & Walter 1877* (WIND) [D]; N of Maltahohe about 45 km on road to Walvis Bay, 24° 40’ 495”S 16° 49’ 513”E (–DB), 11 march 1995, *Burgoyne 3465* (PRE) [C]; Grootfontein area, Farm Namseb, MAL 24 (–DD), 18 February 1962, *Giess*, *Volk and Bleissner 5189* (WIND, PRE) [B]. –2417 (Mariental): Farm Karichab, (–CC), *Koenen 65* (WIND) [E]. –2418 (Stampriet): Rohrbeck, GIB 128 (–AB), 17 April 1960, *Freyer 109* (WIND) [O]. –2516 (Helmeringhausen): Farm Lisbon, part of Farm Grootfontein MAL (–BB), 8 April 1980, *Muller 1282* (WIND) [A]; Maguams – Vrähwinkel (–DB), 23 March 1953, *Walter & Walter 2149* (B) [y]. –2517 (Gibeon): Hardap, ± 6 km west of WeiBrand along C18, 25º 9’ 4”S 17º 51’ 41”E (–BB) 24 March 1998, *Strohbach & Dauth 3738* (WIND)[z]; Farm Haribes (–BC), 13 April 1956, *Volk 12391* (M) [x]. –2518 (Tses): Farm Elandslaagte 79, 25º 2’ 50”S 18º 21’ 14”E, 15 km north of farmhouse (–AB), 24 March 1998, *Strohbach & Dauth 3719* (WIND) [w]; Omaheke, Farm Keitsaub, 25° 51’ 11”S 18° 51’ 6”E, pan area (–DD), 6 March 2002, *Uiras MU474* (WIND) [N]. –2520 (Mata-mata): Kalahari Gemsbok National Park, Koedoe bush, around abandoned mill, 25° 3’ 53”S 20° 22’ 53”E (–AB), 8 April 1997, *Mucina LM6327/8* (PRE) [s]. –2618 (Keetmanshoop): Farm

Middelplaas, east of homestead (–BB), 17 Febraury 1998, *Strohbach 3615* (WIND) [t]; Karas, Wegdraai, 27° 39’ 52”S 17° 29’ 56” E, Canyon Plateau (–CB), 4 May 2002, *Helary & Batault 196* (WIND) [u].

3.8.4 Appendix IV: Specimens examined from the B. rigida complex used in the distribution of morphology among SPIL, OVTL and LOBR (Figures 14, 16 & 18)

LOBR

Namibia. –2516 (Helmeringhausen): Farm Naus 27 (–CD), 21 April 1980, *Owen-Smith 1317* (WIND) [A]. –2615 (Luderitz): Farm Weissenborn, in rocks behind farmhouse (–DA), 5 July 1949, *Kinges 2338 (2388)* (M, PRE) [w]; 48.3 m. S of Aus on road to Witputs, Coarse gravelly sand-flats and granite hills (–DD), 9 March 1963, *de Winter & Hardy 7944* (PRE) [y]. –2616 (Aus): Namtib, 26º 7’ 30”S 16º 22’ 30”E, cliff face (–AB), 27 May 2001, *Theile THE89* (WIND) [x].

OVTL-SPIL

Namibia. –2316 (Nauchas): Farm Friedland MAL 19, high mountain (–CB), 18 March 1953, *Walter & Walter 1914* (B, WIND) [C]. –2416 (Maltahohe): Naukluft (–AC), 5 May 1974, *Meyer 503* (WIND**)** **[**B**]**; Hardap, Zebra River 122 (–CB), 13 May 1998, *Buhrmann & Buhrmann BUH1 - 42* (WIND) [D]; Farm Duvisib (–CC), 17 May 1956, *Volk 12777* (M) [z]. –2616 (Aus): Farm Moolfontein, BETH 50, edge with black lime (–DD), 26 February 1963, *Giess, Volk & Bleissner 5516* (WIND) [a]. –2618 (Keetmanshoop): West of Gellap Ost, ±15 km NW of Keetmanshoop (–CA), 8 April 1987, *Maggs108* (WIND) [d]; 20 miles S.E. of Keetmanshoop on rd to Narubis (–CA), 28 April 1955, *de Winter 3267* (K, PRE, WIND) [e]; Sandmodder, KEE 73 (–DD), 5 April 1975, *Müller 96* (WIND) [c]. –2716 (Witzpüz): Narudous Poort, between Aus and Rosh Pinah, Anusi 73, mountainside (–CC), 30 August 1989, *van Wyk 8773* (WIND) [v]. –2717 (Chamaites): Boomriver (–CC), 26 February 1992, *Strohbach 117* (WIND) [u]. –2718 (Grünau): Farm Pieterkloof (Kraaikluft), KEE370, above the water fall on a slope (–BB), 15 May 1972, *Giess* & *Müller 11994* (WIND) [b]; Farm Pieterkloof (Kraaikluft), KEE370, north of Schroffenstein Mountain (–BB), 14 May 1972, *Giess* & *Müller 11935* (WIND) [g]. –2719 (Tränental): Farm Warmfontein KEE 280, on the koppies (–AB), 5 April 1975, *Muller 81* (WIND) [F]; Karasburg, “Numdis” (–AD), January 1974, *Auret 5631* (WIND) [E]. –2816 (Orajemund): Canyon entrance, Loreley Copper Mine, on hanging granite rock (–BB), 22 February 1963, *Geiss, Volk & Bleissner 5419* (PRE, WIND) [t]; Slate Mountains at Loreley (–BB), 21 February 1963, *Leppert 4207* (M) [j]. –2817 (Vioolsdrift): Wespental, 28.210ºS 17.070ºE, Richtersveld (–AC), 9 September 1987, *Jurgens 22682* (PRE) [r]; E end of Rosyntjiesberg, Namibian side, upper south slope, Quartzitic conglomerate (–AD), 15 April 1986, *Jaarsveld, Forrester & Jacobs 8452* (NBG) [s]. –2818 (Warmbad): Farm Norachas WAR 14, top superimposed with granite blocks (–AA), 14 May 1963, *Giess*, *Volk* and *Bleissner 6909* (WIND) [f]; Farm Haakiesdoorn, 28º 17’ 0”S 18º 15’ 35”E (–AD), 22 April 1997, *Strohbach & Chivell 3485* (WIND, PRE) [p].

South Africa. –2817 (Vioolsdrift): Namaqualand (–DC), May 1984, *Preston-Mafham 15* (PRE) [q]. –2818 (Warmbad): 10 miles South of Goodhouse (–CC), 30 May 1961, *Schlieben 9092* (BOL, K, PRE, SRGH) [o]; Goodhouse Poort, rocky mountain slope (–CD), 22 June 1989, *Van Wyk 8587a* (J, PRE, WIND) [i]. –2820 (Kakamas): just to the west of the church Riemvasmaak, Deksel, on rocky hillside, (–AC), 2 October 1988, *Balkwill & Balkwill 4172* (J) [l]; 70 km NW of Upington, on road to Karasburg, rocky grassy hilltop (–BC), 4 May 1976, *Oliver* and *Steenkamp 6234* (PRE) [h]; 3 – 4 km south east of Kakamas, above Water Tunnels, 28º 45.47’S 20º 40.02’E (–DA), 4 April 2000, *Balkwill, McDade & Lundberg 11770* (J) [k]. –2918 (Gamoep): 60 km SE of intersection of Concordia – Goodhouse road and road to Pofadder, on the road to Pofadder, 29º 0.27’S 18º 48.38’E (–BB), 4 April 2000, *Balkwill, McDade & Lundberg 11792* (J) [n]. –2919 (Pofadder): Pella, on road to Orange River (–AA), 10 April 1969, *Kruger M215* (NBG) [m].

CHAPTER 4

4.0 PHENETIC ANALYSIS OF PUBESCENCE MORPHOLOGY

4.1 Abstract

Earlier assessment revealed that *B. pungens* L.f., *B. rigida* Nees and *B. jubata* S. Moore, present variation in pubescence. The three species belong to section *Barleria*, some members which are known to possess unicellular and multicellular hairs. Although trichome density and size may vary within species, variation in morphology and composition, and location can indicate differentiation. Samples from vegetative and reproductive structures obtained from herbarium specimens were examined using Scanning Electron Microscopy (SEM). Although there seems to be an overlap in ornamentation in some of the trichomes among the three species complexes, detailed morphology such as trichome size, shape, orientation and composition exhibited considerable dissimilarity between and within the species complexes. Trichome size, shape and orientation were observed to be of significant importance, resulting in the splitting of the three species complexes. Six species were identified and an artificial key was made based on trichome morphology.

Key words: *Barleria*, distinct, diversity, micro-morphology, trichome

4.2 Introduction

The genus *Barleria* L., is an assemblage of variable species (Balkwill and Balkwill, 1996) and almost every character in *Barleria* is variable, some characters being as variable within the genus as they are across the entire Acanthaceae (Balkwill, 1993; Balkwill and Balkwill, 1996; Balkwill and Balkwill 1997; 1998; Darbyshire, 2008; Darbyshire and Ndangalasi, 2008). Trichome variation, among other characters, was used to separate *Barleria* into seven sections (Balkwill and Balkwill, 1997). Trichomes generally occur in various forms, being unicellular or multicellular, glandular or eglandular, straight, spiral, tortuous or hooked, simple, stellate, peltate or capitate (Levin, 1973). Distinct trichome diversity observed in *Barleria* includes the presence of sunken glands on the abaxial surface of the leaves, dendroid and stellate hairs, biramous hairs and anvil-shaped hairs, long armed stellate hairs, and adpressed eglandular hairs with multiseriate bases (Balkwill and Balkwill 1997). *Barleria* section *Barleria*, to which the study group belongs, is known to have simple and stellate hairs (Balkwill and Balkwill, 1997).

Variation in the degree of pubescence may be developmental brought about by changes that occur during plant maturation, or may be influenced by environmental changes from moist to dry. Trichomes, however, may vary in density or form from one organ to another (Levin, 1973). Seeds of *Barleria* are usually covered in closely packed hygroscopic hairs whose ornamentation may vary (Balkwill and Balkwill, 1997). It was observed among *Stachys* (Lamiaceae) that species that are highly polymorphic morphologically tend to have morphological variations that are difficult to define (Salmaki *et al.*, 2009). Variation in trichome types may represent genetic variability (Balkwill and Balkwill, 1997; Darbyshire, 2008; Poriazis and Balkwill, 2008). When clear-cut and distinguishable morphological gaps occur they can be recognised at various levels depending on the extent of the morphological discontinuities (Cron *et al.*, 2007; Ereshefsky, 2001; Lewis, 1972; Masćo *et al.,* 2004; Sebola and Balkwill, 2009). Definite variation in trichome morphology and composition will clarify whether there is some association between trichome morphology and the clusters obtained in earlier chapters at the level of species and below.

Indumenta in Acanthaceae have been examined by a number of authors and have provided useful taxonomic information within Acanthaceae (Dipa and Daniel, 2011). Trichomes have been used to delimit infra-generic groups in *Barleria* and where they exhibit intra-species variation, it is usually in degree of pubescence rather than the presence of particular types (Balkwill *et al.*, 1996; Balkwill and Balkwill, 1997; Darbyshire, 2008; 2009; Munday, 1980; 1984; 1987; Poriazis and Balkwill, 2008). Though pubescence type (including shape, nature of trichome base and whether uni- or multi-cellular) may be genetically influenced, size and frequency variation may be environmentally influenced (Balkwill and Balkwill, 1997; Balkwill *et al.*, 1996; Barthlott, 1981). The presence or absence of particular pubescence types on certain organs and not others may also be environmentally influenced (Hossain and Emumwen, 1981). However, consistent gaps in trichome morphology of comparable (similar) organs under similar environmental conditions may also imply dissimilarity which in turn reflects genetic differences rather than environmental conditions in which a plant grows (Barthlott, 1981; Bhatt *et al.*, 2010). Within Lamiaceae, the presence or absence of glandular or eglandular trichomes, thickness of the cell walls, the number of cells (unicellular or multicellular), presence of branched (dendroid) trichomes, presence of vermiform trichomes and orientation of trichomes in relation to the epidermal surface among other trichome characters were observed to be of taxonomic interest (Salmaki *et al.,* 2009). The taxonomic importance of trichome morphology has been noted in a number of genera within Acanthaceae (Bhatt, 2010; Dipa and Daniel, 2011). Trichome morphology and composition though obscure can be used to recognize semi-cryptic species, which may be reproductively isolated but may be unrecognisable by macro-morphology alone. Examination of trichome morphology therefore, is likely to be of taxonomic importance as it may be used to delimit species.

Though trichomes have in other families also been known to be associated with water availability, geographical, ecological or altitudinal influence, insect herbivores and grazing rates (Cano-Santana and Oyama, 1992; Levin, 1973), trichome micro-morphological characters have within the Acanthaceae and other families also been associated with specific variation leading to the recognising of species (Dipa and Daniel, 2011, Hayat *et al.,* 2009). Although members of section *Barleria* have been known to generally possess simple and stellate hairs, trichomes have also been known to vary in their ornamentation which may be of taxonomic importance (Balkwill and Balkwill, 1997; Munday, 1980; 1984). Among species of *Monechma* Hochst.

(Acantahceae), trichome variation in shape, number of cells and ornamentation was used to differentiate species (Munday, 1980; 1984). Although trichome morphology has been observed to be of taxonomic importance within *Barleria* (Balkwill and Balkwill, 1997; Darbyshire, 2008; 2008; 2009; Obermeijer, 1933), detailed analysis dealing with detailed trichome morphology within the formal sub-groups of *Barleria* has not yet been accomplished at species level. The knowledge concerning detailed morphology of the trichomes in association with other morphological characters could shed light on the importance of micro-morphology in recognising species or any other taxonomic level below that of species.

During phenetic analysis, *Barleria pungens* L.f., which split into two distinct clusters (Chapter 2), had one cluster with only two specimens while the other cluster comprised the rest of the specimens in the analysis. However, the bigger cluster (of the two clusters formed) was observed to exhibit a wide range of intraspecific variation in the degree of pubescence on the leaf, bracteole and calyx lobe surface and margin, variation which was difficult to precisely define. The specimens in the smaller cluster had glabrous leaves with green flat leaf margins and glabrescent bracteoles and calyx lobes while the pubescent specimens in the larger cluster had wavy green or white leaf margins. Specimens with white leaf margins were less pubescent than those with green leaf margins, and those with white or thin white leaf margins had restricted geographic distribution (Chapter 3). *Barleria rigida* Nees, which portrayed morphological variation that suggested the presence of spatial clines, only exhibited pronounced pubescence in immature stems, leaves, bracteoles and calyx lobes when present than in mature ones (which were usually glabrous). Although this type of variation can be attributed to plant development, consistency in trichome type in regard to the clusters could be of taxonomic importance. The two clusters within *Barleria jubata* S. Moore, which showed disjunct distribution, also exhibited clear-cut difference in pubescence which included absence or presence of pubescence and a distinction in pubescence type on structures that were pubescent in both clusters by light microscopy. It has been established before within the Acanthaceae that variation in pubescence morphology can be of diagnostic importance at various levels (Balkwill and Balkwill, 1997; Darbyshire, 2008; 2009; Dipa and Daniel, 2011; Poriazis and Balkwill, 2008; Munday, 1980; 1984; 1987). It is for this reason that the three species complexes were further examined to establish the taxonomic importance of pubescence morphology among the study group.

4.3 Materials and Methods

4.3.1 Sampling strategy

A phenetic analysis (Chapter 2) demonstrated that *B. jubata*, *B. pungens* and *B. rigida* are complexes which can be split into separate entities at various levels. Chapter 3 revealed that the continuous variation within the *B. pungens* and *B. rigida* complexes was generally influenced by genotype-environmental interactions triggered by environmental variation. In this chapter, specimens were grouped based on the clusters obtained in Chapter 2. *Barleria pungens* was divided into two, namely: *B. pungens1,* with wavy leaf margins and *B. pungens2,* with flat leaf margins

(Chapter2, Figure 2.4b, clusters 1 and 2 respectively). *Barleria rigida* was also subdivided into *B. rigida1,* with non-lobed bracteoles and *B. rigida2,* with lobed bracteoles (Figure 2.5, clusters 1 and 2 respectively). *Barleria pungens1* and *B. rigida1* were further subdivided based on the observed variation depicted by the sub-clusters and were assigned temporary names (BP, PG, PT, PW, BR, IL, ILa and LB) as illustrated in Figures 4.1 and 4.2. Characteristics defining the groups and abbreviations are given (Appendix I). Since *Barleria jubata* had split into two groups, eglandular (*B. jubata1*) and glandular (*B. jubata2*) form at least one specimen was selected from each sub-cluster of the clusters to give a representation of pubescence variation between species. Though *B. rigida* specimens appear glabrous they have inconspicuous, minute and scanty microscopic hairs. Representative specimens from the *B. rigida* complex were included in order to ascertain whether micro-variation in pubescence type could be of any taxonomic significance.

Samples were obtained from dried herbarium material and were mounted on aluminium stubs using graphite, and then coated with gold-palladium and examined using a FEI QUANTA 400 ESEM. Only up to three samples per putative group representative specimens were examined as prior permission to remove parts had not been sought from the herbaria from which most specimens were borrowed. A total of 53 samples (including stem bark, seeds, leaves, bracteoles and posterior calyx lobes) were scanned, photographed, saved, and were later compared. This was to establish whether trichome variation (trichome micro-morphology) on the leaf, bracteoles, outer calyx lobes (and seed where possible), within and between clusters could be taxonomically useful at species level (i.e. conform to the phenetics groups in Chapter 2). Herbarium abbreviations are according to the standard codes of Holmgren *et al.* (1990) and the specimens examined are in Appendix II, recorded according to Edwards and Leistner (1971).

4.3.2 Data recording and analysis

Trichome types were identified and trichome information was obtained, coded and recorded in data matrices. Character codes and specimen abbreviations for the OTUs (operational taxonomic units) are given in Appendices I and III. Cluster analysis was performed on the trichome characters using UPGMA (unweighted pair group method of arithmetic averages) using correlation and average taxonomic distance coefficient as coefficients of dissimilarity to compute dissimilarity distances and assess trichome phenotypic similarity among OTUs using NTSYS-pc version 2.2 (Rohlf, 2005). Although 48 characters were assessed, only 25 characters with Eigen values ≥ 0.6 were considered as the ones influencing variation along the first three axes and used in the analysis with combined trichome characters from leaf, bracteole and calyx lobe surfaces. These were used to plot the final phenogram of combined leaf, bracteole and calyx lobe characters. However, phenograms with leaf characters and bracteole and calyx lobe characters were plotted separately to test whether trichome characters from vegetative and reproductive organs can yield similar clusters as those from a combined matrix. For the separate analyses (which had fewer characters), characters with an Eigen value ≥ 0.6 were considered to bring about the variation. Ordination was not performed since the data sets only constituted qualitative characters. It was also difficult to accurately obtain frequencies and trichome sizes as it was not clear at what developmental stages the trichomes were, from the various structures. Plates were made to illustrate the trichome variation between and within the putative taxa.

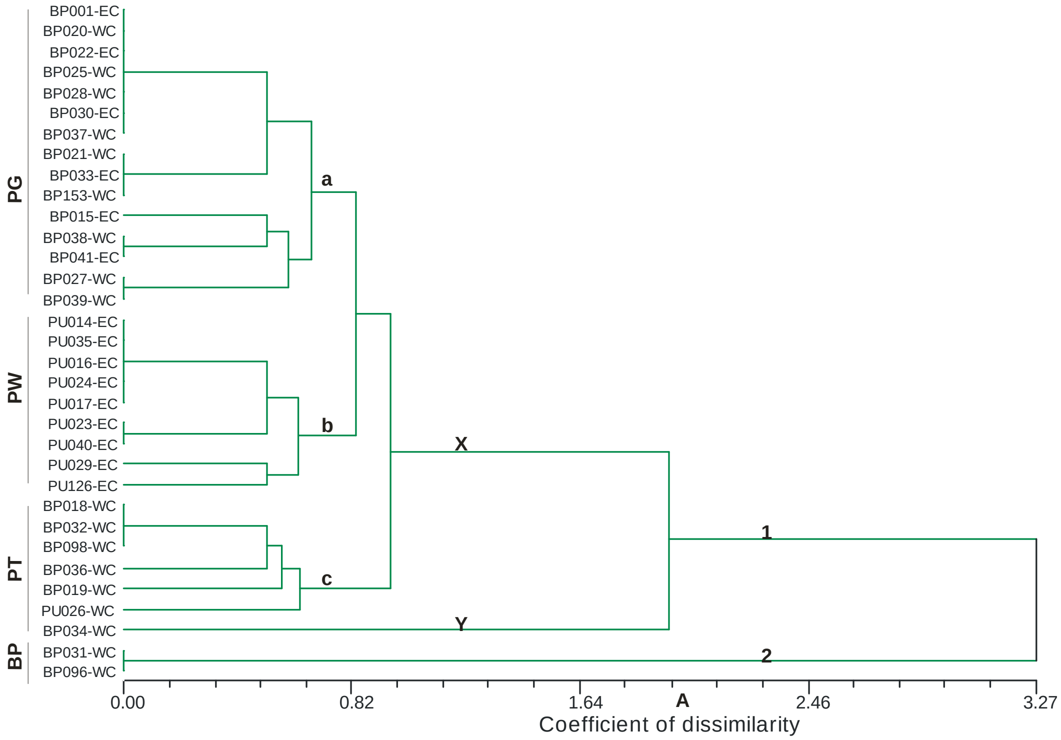

Figure 4.1. Phenogram demonstrating the allocation of OTUs of the *B. pungens* to respective putative taxa for use in pubescence analyses. Abbreviations details are given in appendix I.

4.4 Results

Trichome morphology in the study group may be described as single, multicellular (multicellular may be jointed with swollen junctions), glandular, eglandular, ornamented or non-ornamented. The trichome base may have a raised disc, flat disc or no disc at all. Trichomes may be sessile, stalked, simple or capitate and the capitate head may be single-celled or multicellular. Additional variation observed between the trichomes includes size, shape, orientation and ornamentation. Though conical trichomes were observed among all the putative taxa, it is not clear whether the short ones were immature forms of the long conical trichomes or immature forms of the multi-cellular trichomes. The conical trichomes were with or without a disc at the base. It was also not established whether all the collapsed trichomes (or collapsed cells of multicellular trichomes) do occur as such in nature or collapsed during the preparation (except for the long trichome type 2a which was seen as long thin flat twisting or slightly coiled hairs under the compound microscope) or as a result of being dried for herbarium purposes. In addition, it is likely that the flat twisting trichomes (trichome type 2a) could have died and collapsed before preparation. The short collapsed trichomes were either ornamented or non-ornamented, and with or without a disc at the trichome base. Table 4.1 gives a description of the trichome types observed while Table 4.2 gives the description and codes used in the analyses. Tables 4.4 and 4.5 summarise the distribution of the observed diversity in trichome morphology among the putative taxa, indicating some of the trichome characters which can be used to differentiate them into species. Plates 4.1 – 4.9 illustrate the distribution and variation in morphology (orientation, size and shape) of representative trichome types and detailed ornamentation of the long conical unicellular trichomes on the leaf, bracteole and calyx lobe surfaces. Where the bracteole and calyx lobe surfaces had similar trichome composition or where a trichome type was common in more than one sample, only one of the two (bracteole or calyx lobe) was presented for a representative trichome type.

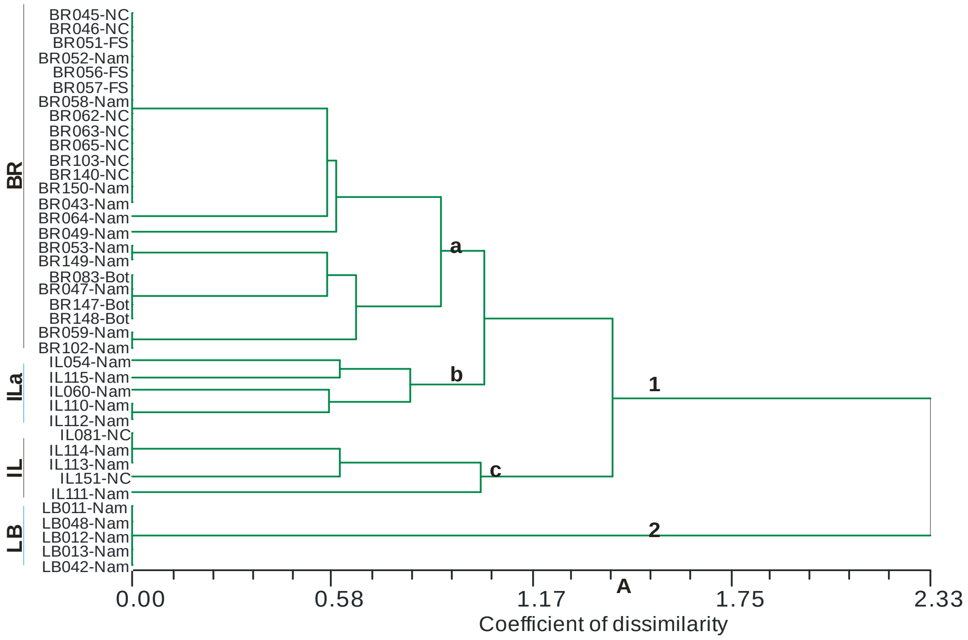

Figure 4.2 Phenogram demonstrating the allocation of OTUs of the *B. rigida* complex to respective putative taxa for use in pubescence analyses. Abbreviations details are given in appendix I.

Table 4.1. Types of trichomes recognised in the study group.

Trichome type	**Trichome description**
1	*Unicellular non-collapsed ornamented trichomes*
1a	Trichome conical, base supported by 1-celled disc
1b	Trichome conical, base supported by multi-celled disc
1c	Trichome conical, base not supported by a disc
1d	Trichome conical, curved
1e	Trichome appressed, ornamented
1f	Trichome horizontal
2	*Unicellular collapsed trichomes*
2a	Trichome ornamented
2b	Trichome non-ornamented
2c	Trichome curved
2d	Trichome non-ornamented, base supported by a disc
2e	Trichome ornamented, base not supported by a disc
3	*Multi-cellular trichomes*
3a	Trichome 2-celled, cells differentially ornamented, both cells not collapsed
3b	Trichome 2-celled, cells differentially ornamented, both cells collapsed
3c	Trichome 2-celled, cells differentially ornamented, basal cells collapsed
3d	Trichome 2-celled, cells differentially ornamented, apical cell collapsed
3e	Trichome 2-celled, cell ornamentation alike (or both cells not ornamented), both cells collapsed
3f	Trichome 2-celled, cell ornamentation alike (or both cells not ornamented), both cells not collapsed
3g	Trichome 3-celled, cells differentially ornamented, two lower cells collapsed
3h	Trichome 3-celled, cells differentially ornamented, all cells collapsed
3i	Trichome 3-celled, cells differentially ornamented, cells not collapsed
3j	Trichome multi-cellular, cells not ornamented
4	*Glandular trichomes*
4a	Trichome sessile, gland-like
4b	Trichome multi-cellular with globose or capitate head, non-ornamented

Table 4.2. Trichome descriptions and abbreviations used in the analyses. Coding of character states is given in appendix III.

No	Character abbreviation	Trichome description
1.	Sht	Trichome short
2.	Con	Trichome conical
3.	Curv	Trichome curved
4.	Non-orn	Trichome non-ornamented
5.	Disc	Trichome base supported by single-celled disc
6.	Disc2	Trichome base supported by multi-celled disc
7.	LW1	Trichome long (L/W ratio 1:3 – 6)
8.	LW2	Trichome long (L/W ratio > 1: 6)
9.	c-cel1	Trichome collapsed
10.	c-cel2	Trichome not collapsed
11.	Appr	Long trichome erect or sub-erect / horizontal / appressed / absent
12.	2-cel	Trichome 2-celled differentially ornamented
13.	3-cel	Trichome 3-celled differentially ornamented
14.	2-cel1	Trichome cell ornamentation alike (or both cells not ornamented) (2-)
15.	2-cel2	Trichome cells differentially ornamented (3-)
16.	c-cel3	Basal cell of trichome collapsed
17.	c-cel4	Apical cell of trichome collapsed
18.	c-cel5	Trichome multi-cellular, cells not collapsed / all cells collapsed / cells collapsed and not collapsed together
19.	Sess	Trichome sessile (saucer-like gland)
20.	Stal	Trichome stalked (with saucer-like or globose head)

On the BR leaf surface, the 2-celled trichomes have both cells collapsed; only the basal cell collapsed or both cells not collapsed whereas the 3-celled trichomes have all the cells collapsed (Plate 4.3a & b, 4.4b). In addition, the 2-celled trichomes were non-ornamented (or very lightly so) or differentially ornamented while the 3-celled trichomes were similarly ornamented or only the basal cell was lightly ornamented (or non-ornamented) (Plate 4.3d, 3-celled similarly ornamented trichome not given). The bracteole and calyx lobe surfaces of BR lacked the non-ornamented trichomes. While IL only had 2-celled differentially ornamented trichomes with collapsed basal cells on the leaf surface (and not on the bracteole or calyx lobe surface), ILa had 3-celled differentially ornamented trichomes with two collapsed cells, on the bracteole and calyx lobe (and not on leaf) surfaces (Plate 4.4c). Two-celled trichomes with non-collapsed cells were observed on the leaf, bracteole and calyx lobe surfaces of PG, while trichomes with collapsed apical cells were observed on the bracteole and calyx lobe surfaces (Plate 4.5a and 4.6e). However, on PW the 2-celled differentially ornamented trichomes with non-collapsed cells were only observed on the leaf surface (and not on the bracteole and calyx lobe surfaces). On the other hand, PT only had 2-celled collapsed differentially ornamented trichomes on the bracteole and calyx lobe (but not on the leaf) surfaces.

Table 4.3. Twenty five trichome characters influencing variation in pubescence morphology obtained from a combined matrix of leaf, bracteole and calyx lobe surface characters. The highlighted (in bold) Eigen values ≥ 0.6 along the first three axes are influencing the variation, mainly along the first axis. Character codes preceeded by 'L' were scored from the bracteole and calyx lobe surfaces.

Characters	**Dim-1**	**Dim-2**	**Dim-3**
Con	0.2832	0.3936	0.1193
Sht	**-0.6549**	-0.5570	0.1049
Curv	0.4019	0.0036	0.4381
Non-orn	**-0.7737**	-0.3933	0.0264
c-cel1	**-0.7737**	-0.3933	0.0264
c-cel2	-0.3579	**0.7434**	0.5347
Disc	**-0.9555**	0.0094	0.1024
Disc2	**0.9674**	0.0402	-0.0918
LW1	**0.6128**	-0.3284	0.2215
LW2	**0.8180**	0.5224	-0.0954
2-cel	**-0.7661**	0.3412	0.0950
2-cel1	**-0.7189**	0.3562	-0.2891
2-cel2	-0.4490	0.1584	-0.4396
3-cel	**-0.7189**	0.2867	-0.3836
c-cel3	**-0.6187**	0.3644	-0.3199
c-cel4	-0.3523	0.3437	-0.3376
c-cel5	-0.2987	**0.8932**	-0.0048
Stal	-0.1806	-0.0027	0.8949
Appr	**0.9060**	0.2737	-0.2603
L-sht	**-0.6054**	0.0034	0.0600
L-curv	0.3741	-0.1902	-0.1018
L-non-orn	-0.3579	**0.7434**	0.5347
L-c-cel1	0.1806	0.0027	**-0.8949**
L-c-cel2	**0.9006**	0.0392	0.2532
L-disc	**-0.9555**	0.0094	0.1024
L-disc2	0.5202	-0.0376	0.0329
L-LW1	**0.7521**	-0.1040	0.2779
L-LW2	**0.8180**	0.5224	-0.0954
L-2-cel	0.0249	0.4233	0.0343
L-2-cel1	-0.2987	**0.8932**	-0.0048
L-2-cel2	0.4605	0.4743	0.0665
L3-cel	**-0.6398**	0.3169	-0.3063
L-c-cel3	0.0058	0.3085	-0.4821
L-c-cel4	0.5422	0.5038	-0.4244
L-c-cel5	-0.5701	-0.1349	-0.4555
L-Sess	-0.1104	0.0872	0.0417
L-Stal	-0.3579	**0.7434**	0.5347
L-Appr	**0.8447**	0.2731	-0.2600

The leaf surface of *B. jubata1* had 2- and 3-celled trichomes with non-collapsed cells, and 2-celled trichomes with short non-collapsed basal cells and collapsed curved apical cells (Plates 4.1a). The bracteole and calyx lobe surfaces had 2-celled trichomes with collapsed cells (Plates 4.2e). Trichomes with short non-collapsed basal cells and collapsed curved apical cells were absent on the bracteole and calyx lobe surface of *B. jubata1* which predominantly had collapsed cells (Plate 4.2e). The leaf surface of *B. jubata2* lacked the 2-celled differentially ornamented trichomes (with non-collapsed cells) only present on the bracteole and calyx lobe surfaces (Plates 4.1c & 4.2a). The multicellular differentially ornamented trichomes with non-collapsed cells were observed on the leaf surfaces of PG, BR, PW and *B. jubata1* as well as on the calyx lobe surfaces of *B. jubata2* and BR, though their detailed morphology such as size and number of cells varied (Plates 4.1a, 4.2a & b, and 4.5a). Multicellular trichomes with non-collapsed cells that are similarly ornamented were observed on the leaf surface of PW and on the bracteole and calyx lobe surfaces of BP while those with differentially ornamented cells occurred on the leaf surfaces of *B. jubata1* and on the bracteole and calyx lobe surfaces of *B. jubata2*.

Although the collapsed trichomes were observed on almost all the OTUs they differed in size and shape, ornamentation and distribution (whether on the leaf, bracteole or calyx lobe surface) (Plates 4.3e & f, 4.4d & e, 4.5b & c, 4.6a, c & d and 4.8b – e). The short conical or collapsed ornamented trichomes on the leaf, bracteole or calyx lobe surfaces of BR, IL and ILa possessed or lacked a disc at the base whereas those on LB almost always possessed a disc and those of BP always lacked a disc at the base (Plates 4.4d & e, 4.7b – f). Although short collapsed non-ornamented trichomes with or without a disc at the base, were also present on the bracteole and calyx lobe surfaces of IL, they were absent on the bracteole and calyx lobe surfaces of BR, ILa and LB. The short conical trichomes were notably absent on the bracteole and calyx lobe surfaces of *B. jubata1*. The long twisting collapsed trichomes were only observed on some of the leaf and calyx lobe surfaces of PG and PW and they were seen only on some calyx lobe surfaces of PT and occurred on the bracteole surface (in addition to leaf and calyx lobe) of one PW (Plate 4.5 b & c). The long erect conical and collapsed (non-twisting) ornamented trichomes were on all OTUs of PG, PT and PW but not on BP (Plates 4.6c & d, 4.8a – c). Trichomes were also observed to break off from the bracteole and calyx lobe surfaces of PT, LB and BP. The short conical and collapsed trichomes were common on the glabrous to glabrescent OTUs (BP, BR, IL, ILa & LB), though they occasionally occurred on the bracteole and calyx lobe surfaces of BP while the longer ones were observed among the pubescent OTUs (PG, PT, PW, *B. jubata1* & *B. jubata2*). Generally the short erect conical and collapsed trichomes differed only in the ornamentation (light or heavy) and whether they possessed a disc at the trichome base.

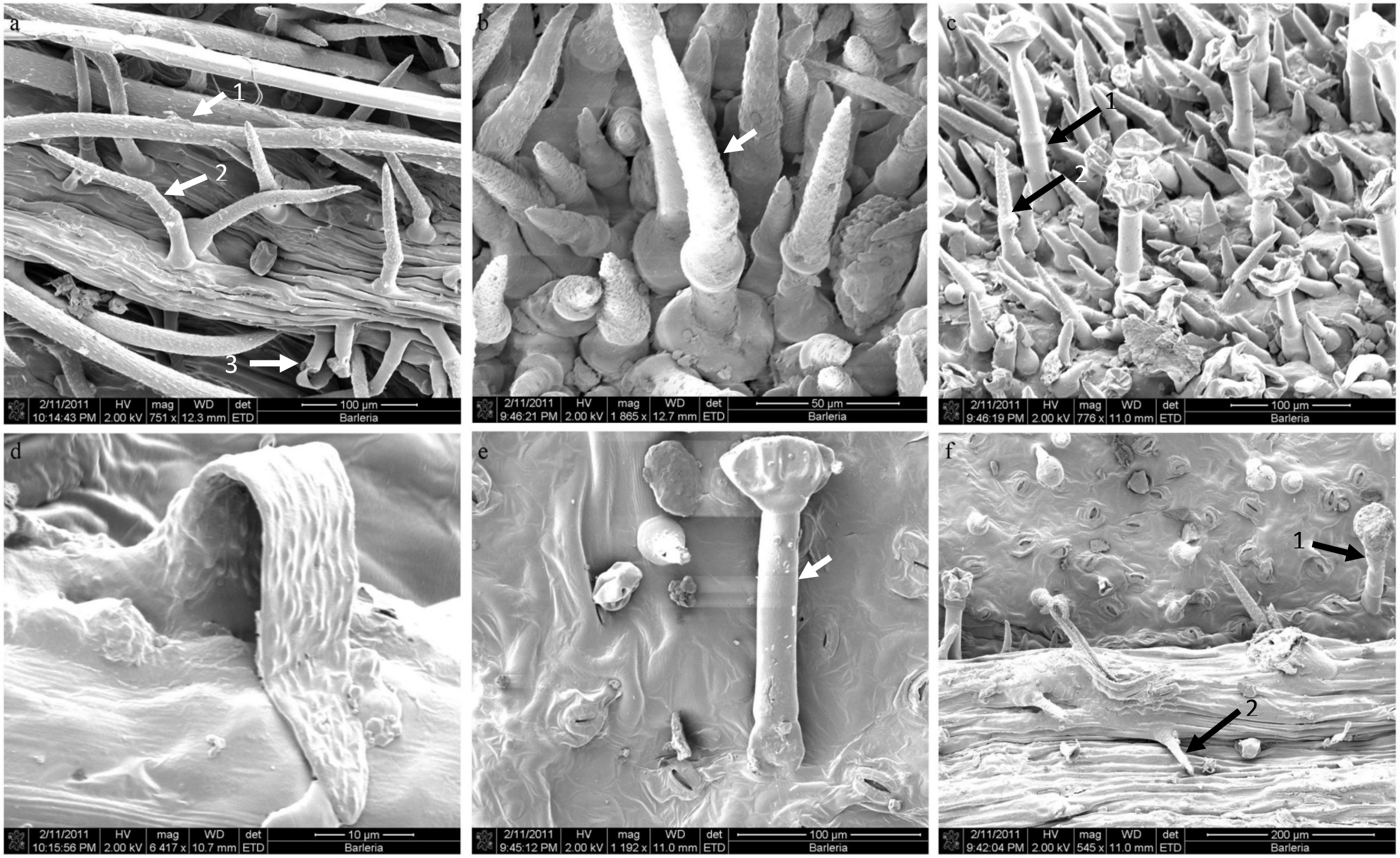

Plate 4.1. Distribution and diversity of trichome morphology between *B. jubata1* and *B. jubata2*. *a*) three trichome types shown by arrow, 1f (1), 3i (2) and 3d (3) on leaf midrib of JUE, *Boss 34948* (PRE); *b*) trichome type 3i on the stem surface of JUE, *Boss 34948* (PRE); *c*) trichome types 4b (1) and 3a (2) on stem surface of JUG, *Giess & Leippert 7588* (NBG), *d*) trichome type 2c on the calyx lobe surface of JUG, *Giess* & *Leippert 7588* (NBG); *e*) enlarged trichome type 4b on leaf lamina of JUG, *Giess & Leippert 7588* (NBG); *f*) trichome type 4b (1) and 1d (2) on leaf midrib and lamina of JUG, *Giess & Leippert 7588* (NBG). 3d, 3i: multi-celled differentially ornamented trichomes; 4b: non-ornamented trichome with glandular head; 1d, 1.f, 2c, 11: single-celled ornamented trichomes. There is variation in trichome composition between stem and leaf surfaces of JUE and JUG. JUE: *B. jubata1*; JUG: *B. jubata2*. See Table 4.2 and Appendix I for detailed trichome descriptions and specimen codes respectively.

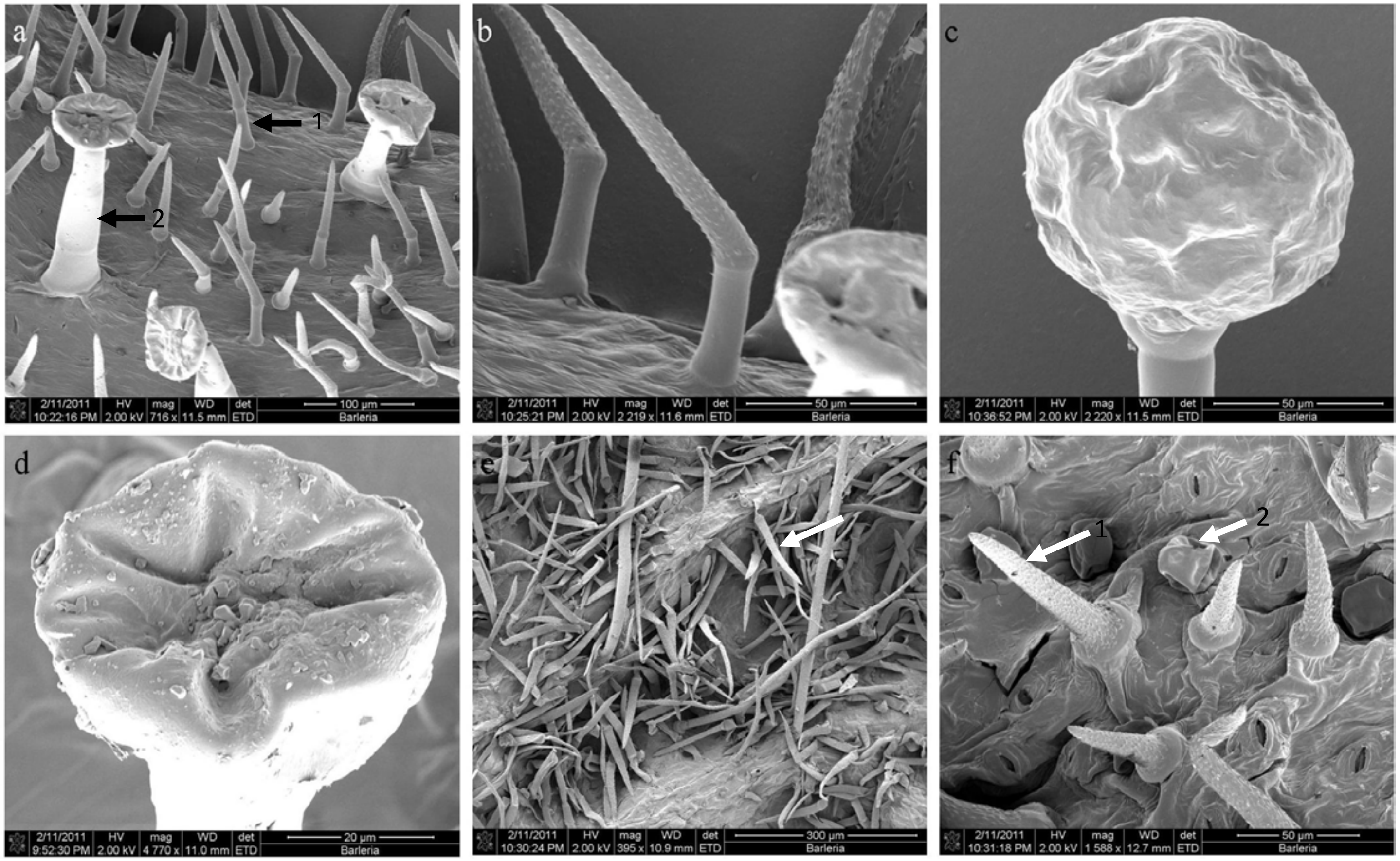

Plate 4.2. Distribution and diversity of trichome morphology in *B. jubata1* and *B. jubata2*. *a*) trichome type 3a (1) and 4b (2) on calyx lobe surface of JUG, *Giess & Leippert 7588* (NBG); *b*) differential ornamentation in trichome type 3a on the calyx lobe surface of JUG, *Giess & Leippert 7588* (NBG); *c* & *d*) secreting and collapsed gland of trichome type 4b on the calyx lobe surface of JUG, *Giess & Leippert 7588* (NBG); *e*) distribution of trichome type 3e on calyx lobe surface of JUE, *Boss 34948* (PRE); *f*) trichome type 1a (1) and 4a (2) on the leaf surface of JUE, *Boss 34948* (PRE). 1a: 1-celled ornamented trichome; 3e: 2-celled with like ornamented collapsed trichomes; 3a: 2-celled differentially ornamented trichomes; 4b: non-ornamented trichome with glandular head. Although multicellular trichomes are present on the bracteole and calyx lobe surfaces of JUE and JUG, their sizes and shapes vary. JUE: *B. jubata1*; JUG: *B. jubata2*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

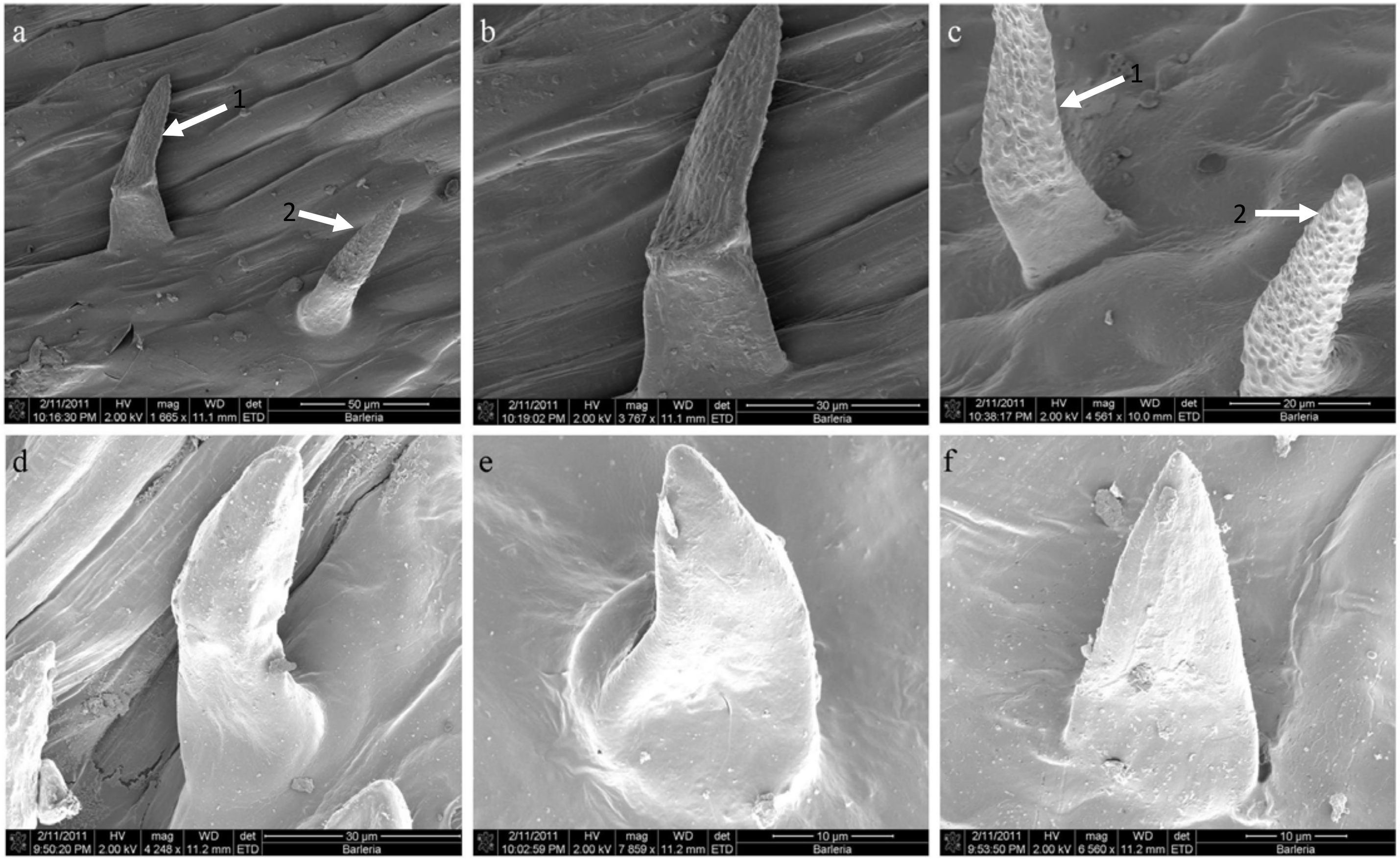

Plate 4.3. Variation in trichome morphology on leaf, bracteole and calyx lobe surfaces of *B. pungens1* and *B. rigida1*. *a*) trichome type 3b (1) and 3a (2) on bracteole surface of BR, *Germishuizen 2746* (PRE); *b*) differential ornamentation in trichome type 3b on the bracteole surface of BR, *Germishuizen 2746* (PRE); *c*) trichome type 3b (1) and 2a (2) on calyx surface of PT, *Moss 15394* (J); *d*) type 3e on the leaf surface of BR, *de Winter 3267* (PRE); *e*) type 2d on the leaf surface of IL, *Jurgens 22682* (PRE); *f*) type 2b on the leaf surface of BR, *de Winter 3267* (PRE). 2b, 2d: collapsed non-ornamented trichomes; 2c: collapsed ornamented trichome; 3e: 2-celled non-ornamented trichome: 3b: 2-celled differentially ornamented trichomes. Although collapsed non-ornamented trichomes are present on the leaf surfaces of BR and IL, one has a disc at the base and the other has not. BR, IL: *B. rigida1*; PT: *B. pungens1*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

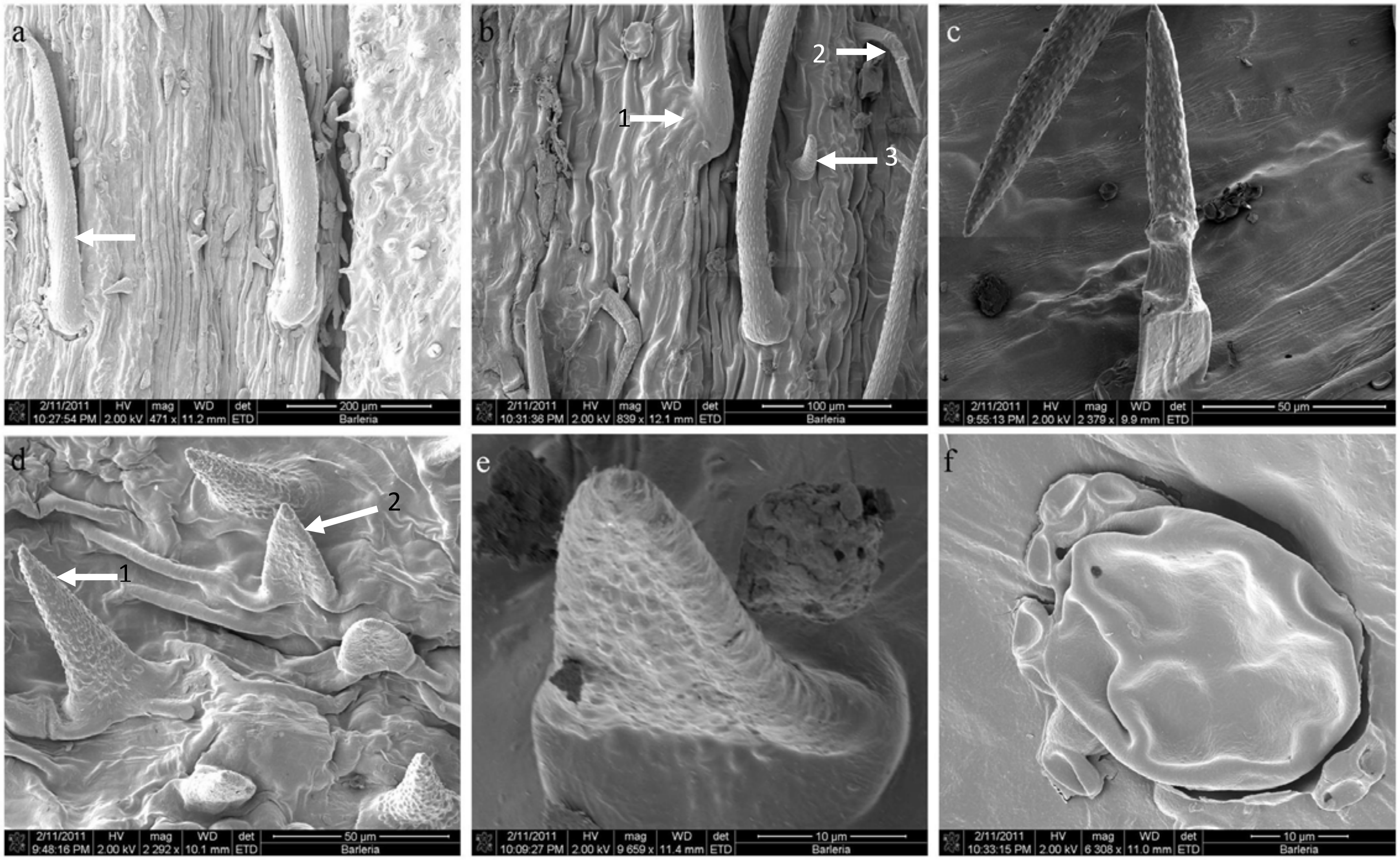

Plate 4.4. Variation and distribution of trichome morphology on leaf, bracteole and calyx lobe surfaces of *B. rigida1* and *B. pungens2*. *a*) trichome type 1e on leaf surface of BR, *de Winter 3267* (PRE); *b*) base of trichome type 1e (1), type 3b (2), 1a (3), on the calyx lobe surface of BR, *Germishuizen 2746* (PRE); *c*) trichome type 3g on calyx lobe surface of ILa, *Giess 1787* (WIND); *d*) trichome type 1c (1) and 2a (2) on the leaf surface of BP, *Laidler 204* (PRE); *e*) trichome type 2d on the leaf surface of BR, *Germishuizen 2746* (PRE); *f*) collapsed trichome type 4a on the bracteole surface of ILa, *Giess 1787* (WIND). 1c: ornamented conical trichomes; 2a, 2d: collapsed ornamented trichomes; 3g: 3-celled differentially ornamented trichome; 4a: sessile gland; 1e: appressed ornamented trichome. Trichome types 1e and 4a are present on leaf and calyx lobe surfaces of *B. rigida* whereas trichome types 2a & 1c are only common on leaf surfaces of both *B. pungens2* and *B. rigida1*. BR, ILa: *B. rigida1*; PG: *B. pungens2*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

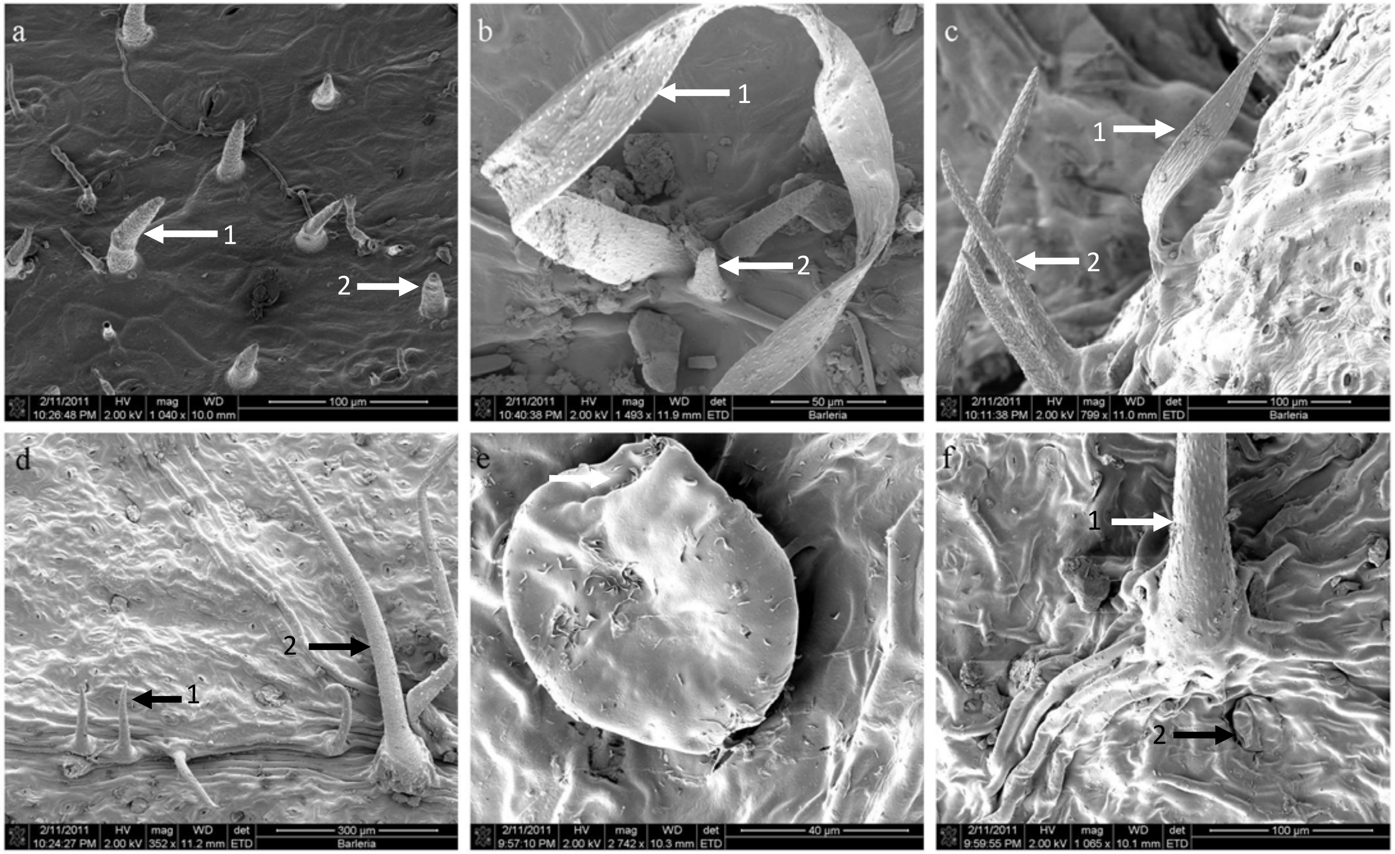

Plate 4.5. Trichome diversity and distribution within *B. pungens1*. *a*) trichome type 2a (1) and 3e (2) on bracteole surface of BP, *Bohnen 7371* (PRE); *b*) long trichome type 2a (1) and trichome type 1c (2) on the calyx lobe surface of PW, *Acocks 15732* (PRE); *c*) long trichome type 2a (1) and trichome type 1c (2) on leaf surface of PT, *Moss 15394* (J); *d*) long and short trichome type 1b on leaf midrib of PW, *Dahlstrand 2715* (J); *e*) secreting gland of trichome type 4a on the leaf surface of PW, *Horn 2286* (PRE); *f*) base of trichome type 1b (1) and type 4a (2) on the leaf surface of PW, the base raised by a group of specialised supporting cells, and a collapsed gland, trichome type 4a, *Horn 2286* (PRE). 1b, 1c: ornamented conical trichome; 2a: ornamented collapsed trichomes; trichome; 4a: sessile gland. While trichome type 2a is present on both leaf and calyx lobe surfaces of *B. pungens1*, there is variation in size. PG, PT, PW: *B. pungens1*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

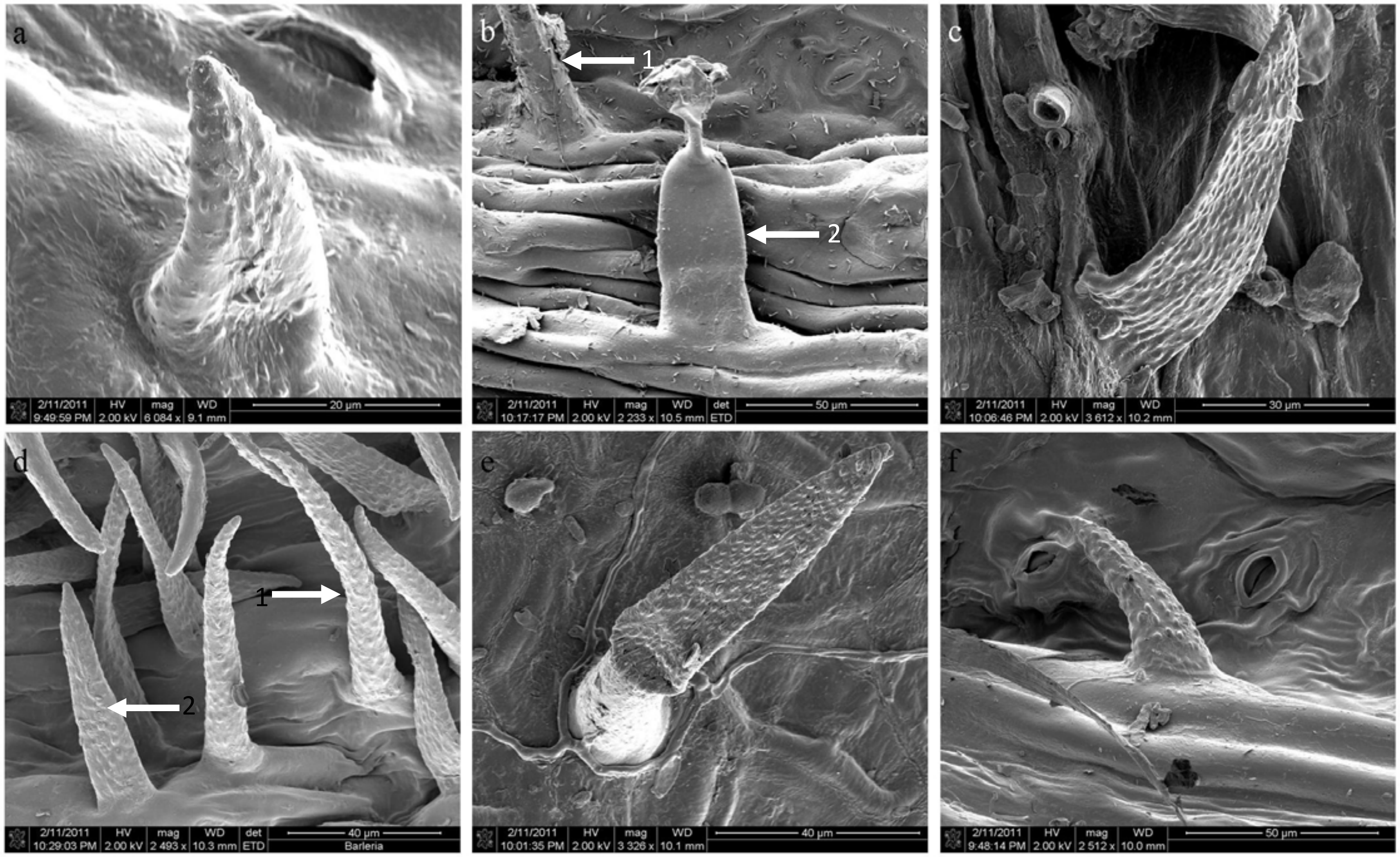

Plate 4.6. Diversity in trichome morphology and distribution on leaf and calyx lobe surfaces of *B. pungens1*. *a*) trichome type 2c on leaf surface of PG, *Bohnen 7371* (PRE); *b*) base of trichome type 1c (1) and trichome type 3j (2) on the leaf surface of PW, *Horn 2286* (PRE); *c*) trichome type 2c on calyx lobe surface of PG, *Bohnen 7371* (PRE); *d*) trichome types 1c (1) and 2a (2) on calyx lobe surface of PT, *Moss 15394* (J); *e*) trichome type 3d on the calyx lobe surface of PG, *Bohnen 7371* (PRE); *f*) trichome type 1d on the calyx lobe surface of PG, *Nyirenda 712* (J). 2a: collapsed ornamented trichomes; 1b: conical ornamented trichomes; 3j: multi-cellular non-ornamented trichome; 2c: collapsed curved ornamented trichome. There is variation in size and shape of collapsed trichomes on calyx lobe surfaces of PG, and PT and the leaf surface of PG. PG, PT, PW: *B. pungens1*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

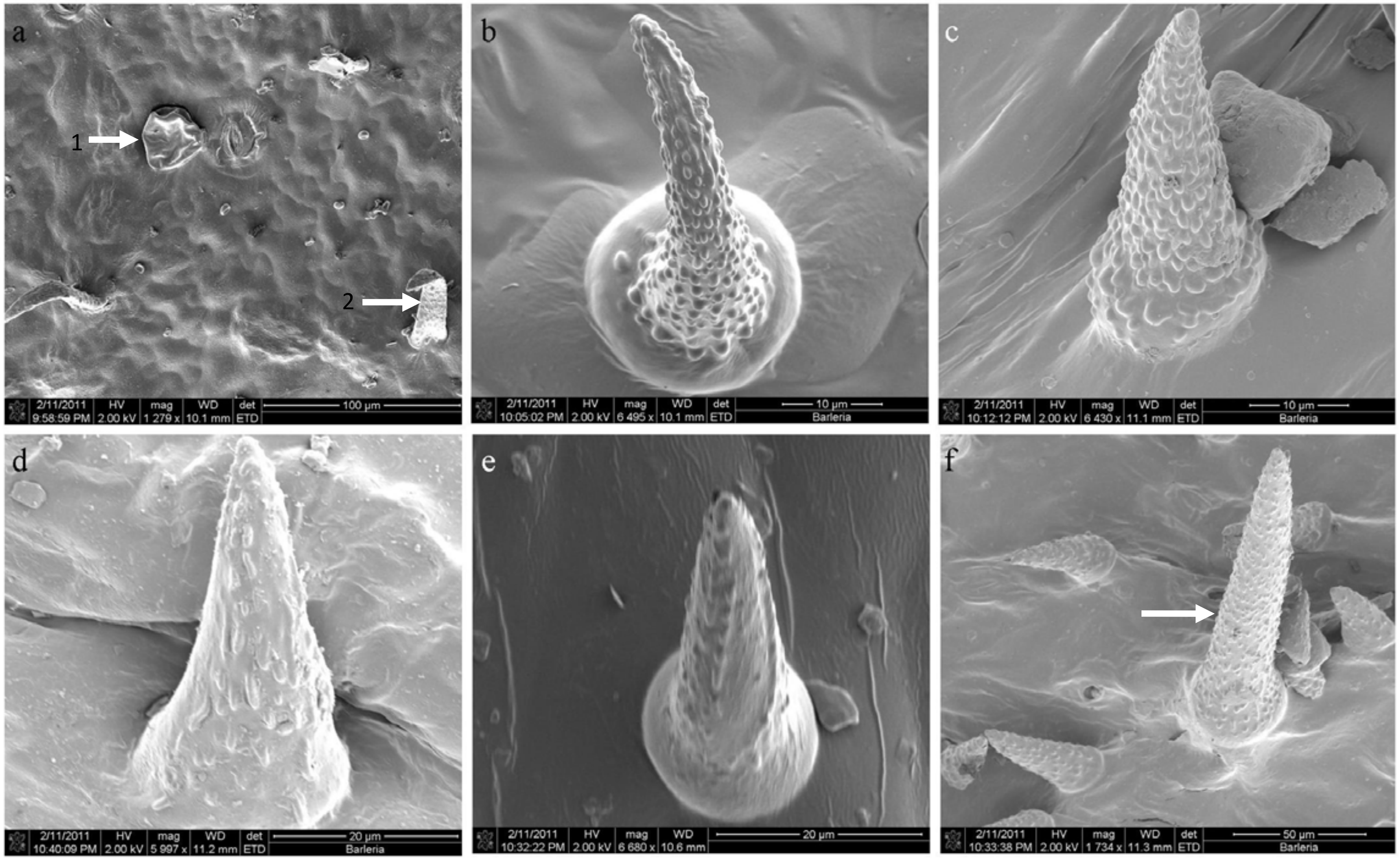

Plate 4.7. Diversity in trichome morphology and distribution on leaf, bracteole and calyx lobe surfaces of *B. rigida1* and *B. rigida2*. *a*) trichome type 4a (1) and 2c (2) on the calyx lobe surface of ILa, *Giess 1787* (WIND); *b*) trichome type 1a with a somewhat swollen base and non-ornamented disc on the calyx lobe surface of LB, *Theile THE89* (WIND); *c*) trichome type 1c with an ornamented raised base on the bracteole surface of BR, *de Winter 3267* (PRE); *d*) trichome type 1c on the leaf surface of BR, *de Winter 3267* (PRE); *e*) trichome type 1a on the bracteole surface of IL, *Volk 12777* (M); *f*) trichome type 1a on the leaf surface of IL, *Jürgens 22682* (WIND). 1a, 1c, conical ornamented trichomes; 4a: sessile gland. Though conical trichomes occur in *B. rigida1* and *B. rigida2* they exhibit variation in their bases and ornamentation pattern. BR, IL: *B. rigida1*; LB: *B. rigida2*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

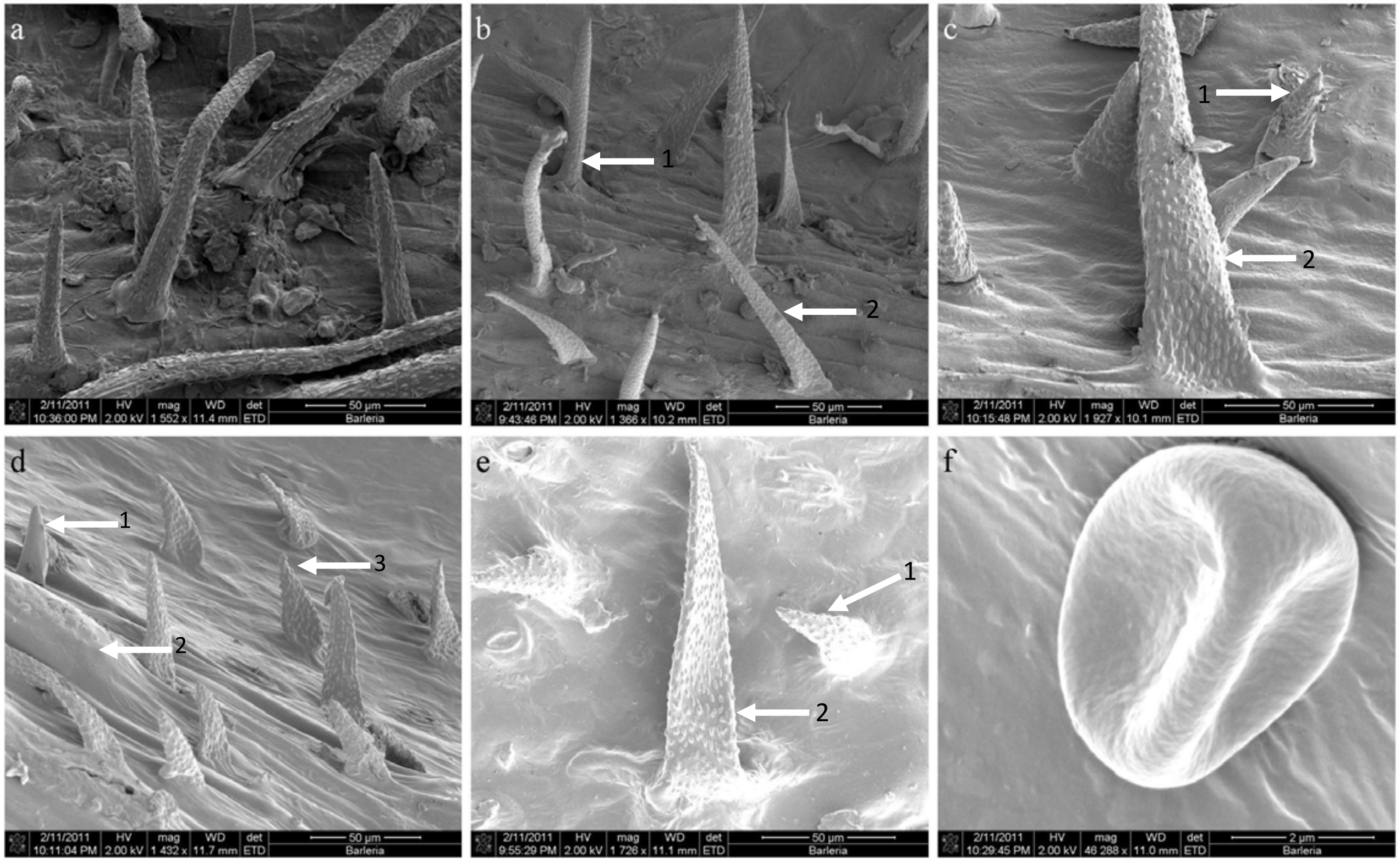

Plate 4.8. Trichome morphology and distribution on leaf, bracteole and calyx lobe surfaces of *B. rigida1*, *B. pungens1* and *B. pungens2*. *a*) trichome type 1c on the calyx lobe surface of BP, *Nyirenda 710* (J); *b*) trichome types 1c (1) and 2a (2) on the calyx lobe surface of BP, *Nyirenda 712* (J); *c*) trichome type 1c (trichome breaking (1) and base partially collapsed (2)), on the bracteole surface of BP, *Laidler 204* (PRE); *d*) trichome type 2b (1), base of type 1e (2) and trichome type 2a (3), on the bracteole surface of IL, *Jürgens 22682* (PRE); *e*) trichome types 1c (1) and 2a (2) on the leaf surface of IL, *Jürgens 22682* (PRE); *f*) young collapsed trichome on the bracteole surface of ILa, *Giess 1787* (WIND). 1c: conical ornamented trichomes; 2a: collapsed non-ornamented trichome; 2a: collapsed ornamented trichomes; 1e: appressed ornamented trichome. Though collapsed trichomes occur on BP, PG and IL they exhibit variation in their sizes. PG: *B. pungens1*; IL, ILa: *B. rigida1*; BP: *B. pungens2*. Detailed trichome descriptions and specimen codes are as outlined in Table 4.2 and Appendix I.

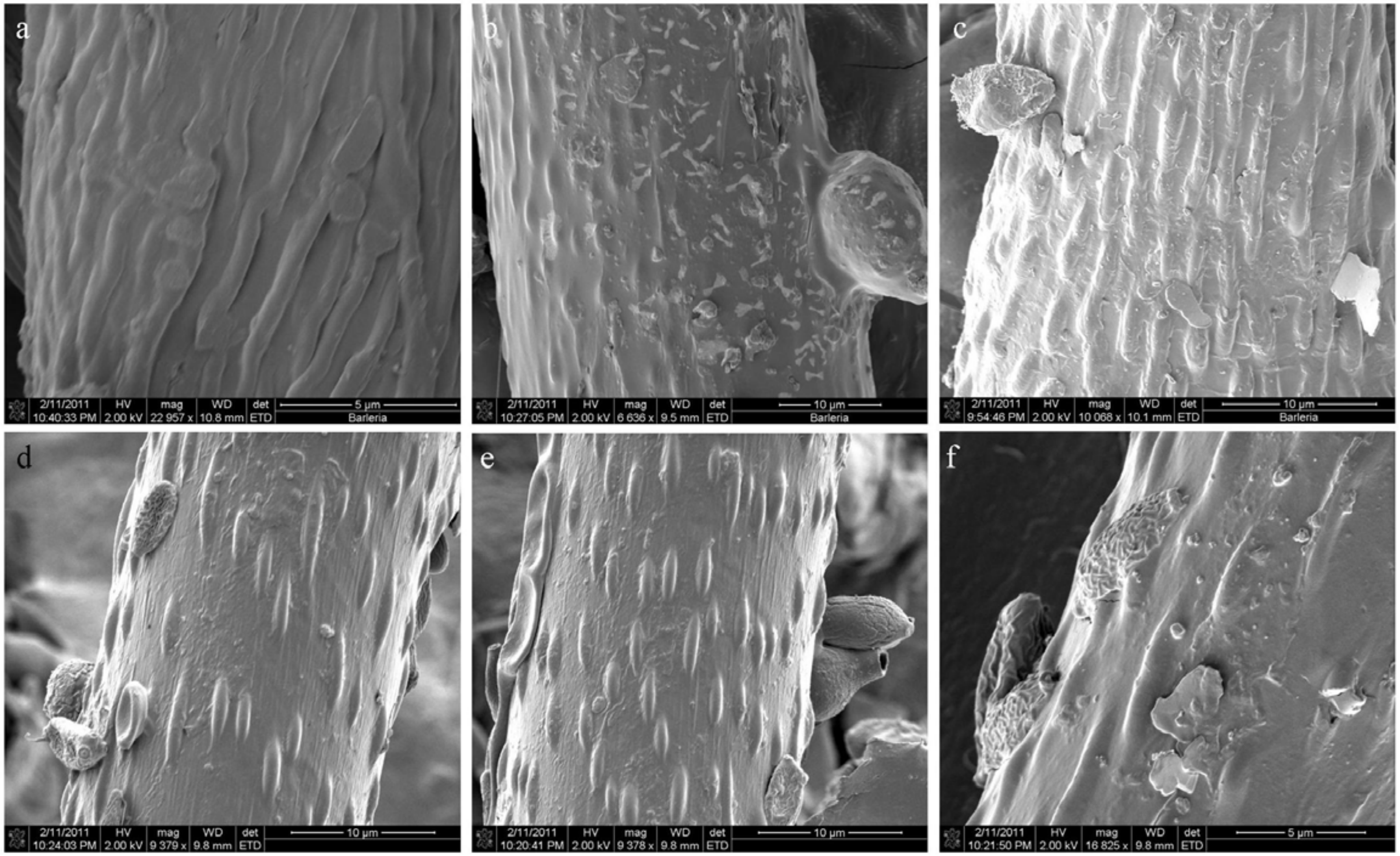

Plate 4.9. Variation in trichome ornamentation of trichome type 1b, 1e and 1f on the calyx lobe surfaces of *B. pungens* (*1* & *2*), *B. jubata1* and *B. rigida* (*1* & *2*). *a*) ornamentation on trichome type 1c on calyx lobe surface of JUE, *Boss 34948* (PRE); *b*) ornamentation on trichome type 1e on calyx lobe surface of LB, *Theile THE89* (WIND); *c*) ornamentation on trichome type 1e on calyx lobe surface of BP, *Laidler 204* (PRE); *d* & *e*) ornamentation on trichome type 1b on calyx lobe surface of PG, *Nyirenda 717* (J); *f*) ornamentation on trichome type 1e on calyx lobe surface of ILa, *Giess 1787* (WIND). 1c: long erect conical ornamented trichome; 1e: appressed ornamented trichome; 1c: long horizontal non-collapsed ornamented trichome. Apart from variation in the orientation, there is variation in the detail of the ornamentation. PG: *B. pungens1*; IL, ILa: *B. rigida1*; JUE: *B. jubata1*; BP: *B. pungens2*. Specimen abbreviations are as outlined in Appendix I.

Table 4.4. Trichome distribution on leaf surfaces of the putative groups. (Where - denotes absence; + denotes presence; * denotes scanty)

Taxa	**Unicellular appressed**	**Short conical**	**Short conical - disc**	**Short collapsed ornamented**	**Short collapsed non-ornamented**	**Long collapsed ornamented**	**Long erect conical ornamented**	**Long erect disc-base**	**Multicellular non-ornamented**	**Long horizontal ornamented**	**Stalked gland**	**Differently ornamented multi-cellular**
PG	+	+	-	+	-	-	-	-	-	-	-	-
BP	-	+	-	+	-	+	+	+	-	-	-	*+
PT	-	+	-	-	-	+	+	+	-	-	-	+
PW	-	+	-	-	-	+	+	+	+	-	*+	+
BR	+	+	+	+	+	-	-	-	+	-	-	+
IL	+	+	+	+	+	+	-	-	-	-	-	+
ILa	+	+	+	+	-	-	-	-	-	-	-	+
LB	+	+	+	*+	-	-	-	-	-	-	-	+
JUE	-	-	+	-	-	+	-	-	-	+	-	+
JUG	-	+	+	-	-	-	-	-	-	-	+	-

Table 4.5. Trichome distribution on the bracteole and calyx lobe surfaces of the putative groups. (Where - denotes absence; + denotes presence)

Taxa	**Unicellular appressed**	**Short conical**	**Short conical - disc**	**Short collapsed ornamented**	**Short collapsed non-ornamented**	**Long collapsed ornamented**	**Long erect conical ornamented**	**Curved collapsed ornamented**	**Long horizontal ornamented**	**Sessile gland**	**Stalked gland**	**Differently ornamented multi-cellular**
PG	+	+	-	+	-	-	-	-	-	-	-	-
BP	-	+	+	+	-	+	+	+	+	+	-	+
PT	-	+	+	-	-	+	+	+	+	+	-	+
PW	-	+	+	-	-	+	+	+	-	+	-	-
BR	+	+	+	+	+	-	-	-	-	+	-	+
IL	+	+	+	+	+	+	-	-	-	+	-	+
ILa	+	+	+	+	-	-	-	+	-	+	-	+
LB	+	-	+	+	-	-	-	-	-	-	-	+
JUE	-	-	+	-	-	+	-	-	+	+	+	+
JUG	-	-	+	-	-	-	-	-	-	+	+	+

The long erect or sub-erect conical trichomes with a base raised by supportive cells were observed among almost all the specimens from the *B. pungens* complex (except BP with long trichomes adpressed to the surface) (Plates 4.5d & f). The long erect or sub-erect conical trichomes were also absent among the members of the *B. rigida* complex which instead possessed long trichomes adpressed to the surface and *B. jubata1* which possessed long trichomes that were horizontal to the surface (Plates 4.1a, 4.4a & b). The sessile saucer-like trichomes were present in all the leaf samples differing only in size and shape (Plates 4.2f, 4.4f, 4.5e & f, and 4.7a). While *B. jubata2* possessed multicellular non-ornamented trichomes with glandular heads on the stem, leaf, bracteole and calyx lobe surfaces, they were only observed on the young calyx lobe surfaces of *B. jubata1* and very sparsely distributed or absent in old calyx lobes (Plates 4.1c, e & f, 4.2a – d). It was generally observed that the trichome composition on the leaf surfaces of specimens from the same putative taxa was different from that on the bracteole and calyx lobe surfaces, with overlap in one or two trichomes while trichome composition between bracteole and calyx lobe remained consistent, differing only in the frequency rather than type (except for some OTUs between BP and PT). Within *B. jubata* where stem surface was also examined, the leaf and stem had similar trichome composition in *B. jubata1* but not in *B. jubata2* (Plate 4.1a – f).

Two phenograms obtained from cluster analysis using pubescence characters from leaf surface and a combination of pubescence characters from bracteole and calyx lobe surfaces, had correlation coefficients of r = 0.97059 and 0.96508 respectively. A phenogram obtained by using pubescence characters from a combined matrix of leaf, bracteole and calyx lobe surface trichome characters had a correlation coefficient of r = 0.97163. Though cluster analysis may sometimes impose a hierarchical segregation which is not practical in nature, values above 0.8 indicate a good fit; implying that the OTUs were reliably separated (Cron *et al.*, 2007; Dunn and Everitt, 1982; Gilmartin and Harvey, 1976; Lessig, 1972; Peres-Nato and Jackson, 2001). The two phenograms with separate trichome characters from vegetative and reproductive organs demonstrate that the three species complexes can be separated into five and four distinct clusters respectively, based on the qualitative trichome characters on the leaf surfaces and clusters based on trichome morphology on the bracteole-calyx lobe surfaces. In addition a combined matrix of leaf, bracteole and calyx lobe surface characters also separates the three species complexes into four clusters. It can also be noted that based on the bracteole and calyx lobe trichome characters and the combined matrix, cluster 2 consists of OTUs from three well separated taxa, varying only in OTU arrangement (Figures 4.4 & 4.5). Leaf pubescence separates clusters 1, 3, 4 and 5 into clearly recognisable groups though cluster 2 comprises OTUs from three separate entities that have already been convincingly differentiated based on other morphological characters (Chapter 2) (Figure 4.3). Nevertheless cluster 2 in all three phenograms consists of OTUs that have glabrous to glabrescent leaves, bracteoles and / or calyx lobe surfaces.

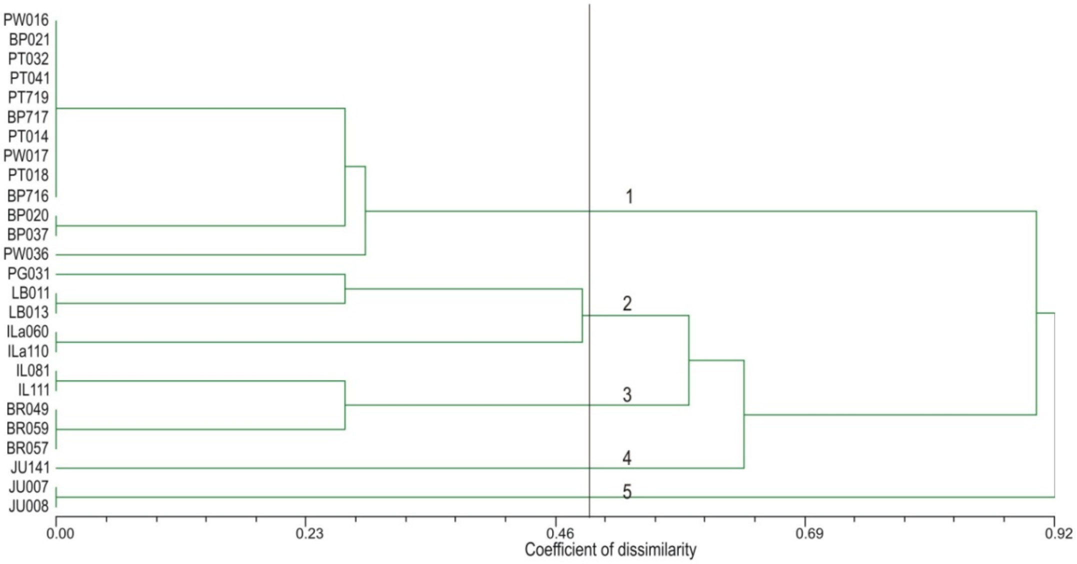

Figure 4.3. Clustering of putative taxa based on 15 qualitative characters of leaf surface pubescence. Cophenetic correlation coefficient, r = 0.97059. A phenon line is drawn at about 0.9 just below where the first cluster with short dissimilarity distance between OTUs begins to form. Numbers 1 – 5 denote clusters that can be recognised as species based on the dissimilarity distance. The phenogram reveals that there is a lot of mixing of OTUs from morphologically distinct taxa using qualitative leaf trichome characters and therefore cannot be independently used to define and demarcate groupings at species level. BR: *B. rigida* with linear leaves, narrow 1- or 2-veined non-lobed bracteoles; ILa: *B. rigida1* with linear leaves, broad 3-veined non-lobed bracteoles; IL: *B. rigida1* with ovate leaves, narrow 1- or 2-veined non-lobed bracteoles; LB: *B. rigida2* with elliptic-ovate leaves, broad lobed bracteoles: PG: *B. pungens2* with flat green leaf margins; BP: *B. pungens1* with wavy green leaf margins; PT: *B. pungens1* with thin wavy white leaf margins; PW: *B. pungens1* with prominent wavy white leaf margins; JUE: *B. jubata1*; JUG: *B. jubata2*. Detailed OTU descriptions and abbreviations are as outlined in Appendix I.

4.5 Discussion

4.5.1 Distinct trichome morphology

Variation in hair density on the laminae, margins, mid-veins and margin spines of the leaf, bracteole and posticous calyx lobe was difficult to define from light microscopy due to limited depth of field. Although hair density may depend on environmental conditions, this is not easy to establish here as it was challenging to accurately examine the frequency of trichomes on the structures. Although the depth was improved with Scanning Electron Microscopy (SEM), it was not clear whether the collapsed trichomes were originally cylindrical but only collapsed during preparation. It was also not clear whether what appeared to be different types of trichomes (e.g. the short conical trichomes) were simply different developmental stages of the complex trichomes (such as the multicellular ones). In addition dissimilarity in trichome size was not definite which posed a source of inaccuracies as trichomes were only recorded as present or absent because it was not clear whether they were mature or immature.

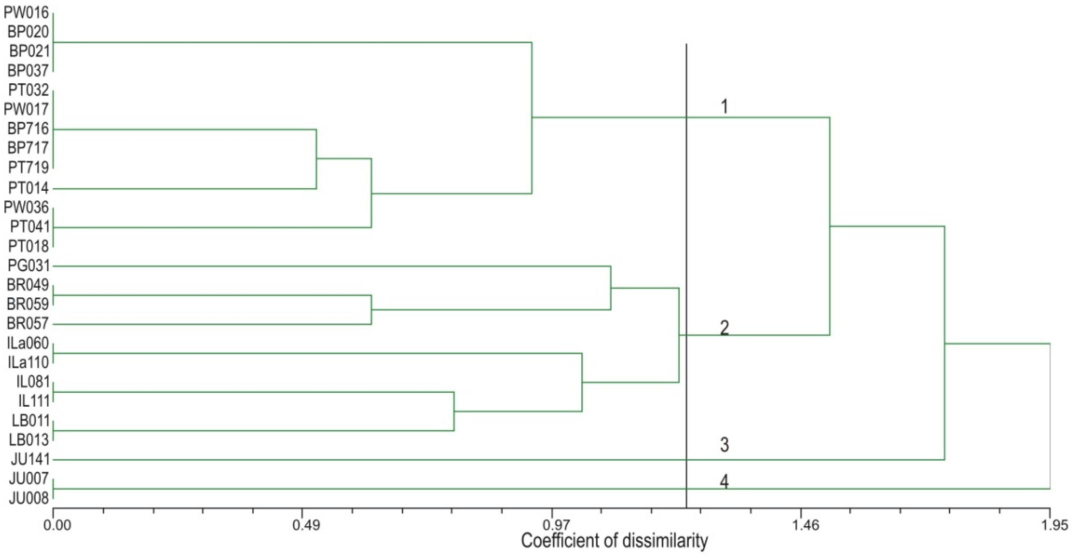

Figure 4.4. Clustering of putative taxa based on 10 combined characters of bracteole and calyx lobe surface pubescence. Cophenetic correlation coefficient, r = 0.96508. A phenon line is drawn just about 1.2 (i.e. just below where the first cluster with short dissimilarity distance between OTUs of a cluster, begins to form). Numbers 1 – 4 denote clusters recognised as species based on the dissimilarity distance. Bracteole and calyx lobe trichome morphology appears less reliable in separating the species than leaf trichome morphology. BR: *B. rigida* with linear leaves, narrow 1- or 2-veined non-lobed bracteoles; ILa: *B. rigida1* with linear leaves, broad 3-veined non-lobed bracteoles; IL: *B. rigida1* with ovate leaves, narrow 1- or 2-veined non-lobed bracteoles; LB: *B. rigida2*: PG: *B. pungens2* with flat green leaf margins; BP: *B. pungens1* with wavy green leaf margins; PT: *B. pungens1* with thin wavy white leaf margins; PW: *B. pungens1* with prominent wavy white leaf margins; JUE: *B. jubata1*; JUG: *B. jubata2*. Detailed OTU descriptions and abbreviations are as outlined in Appendix I.

The long unicellular trichomes, type 1b and 1c (erect to sub-erect) present among *B. pungens1* (PG, PT and PW) can be differentiated from type 1e (adpressed to the surface) present among the *B. rigida* complex and BP by their orientation and base (Plates 4.4a & b and 4.5d). Type 1e trichomes tend to have bent tips in BP (*B. pungens2*) and remain straight among the *B. rigida* complex. In addition to variation in orientation, trichome type 1f exhibited dissimilarity in the ornamentation detail from trichome types 1b, 1c (of *B. pungens1*) and type 1e (of *B. rigida1* and *2*) which have similar ornamentation (Plate 4.9a – f). Trichome type 1a on the leaf lamina of *B. jubata1* has the base raised by supportive cells which is absent in the rest (Plate 4.7a – f). The short conical trichomes of LB possess a swollen base and non-ornamented disc while those of BR, *B. jubata1* and IL do not have a swollen base but they may have a non-ornamented disc (Plate 4.7). Although the short collapsed trichomes occur among *B. rigida1*, *B. rigida2* and *B. pungens2*, those of *B. pungens2* (and occasionally *B. pungens1* when present) lack a disc at the base whereas those of *B. rigida1* (BR, IL, ILa) may or may not possess a disc at the base (Plate 4.3e & f and Plate 4.4d & e). Although widely distributed among the *B. pungens* complex, the conical trichomes (type 1c), have somewhat collapsed bases on BP (Plate 4.8c).

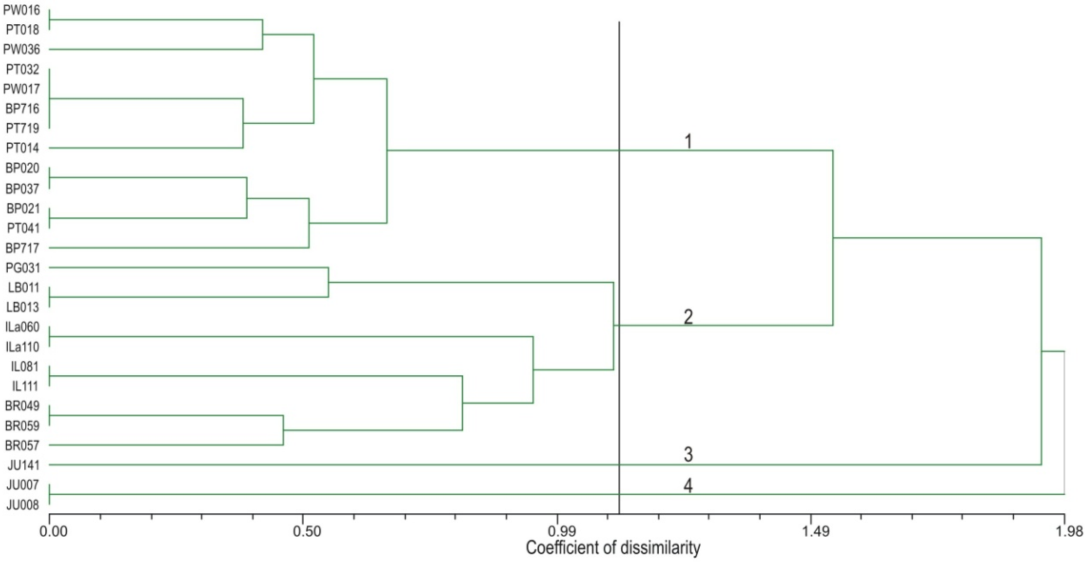

Figure 4.5. Clustering of putative taxa based on 25 combined trichome characters from leaf, bracteole and calyx lobe surfaces. Cophenetic correlation coefficient, r = 0.97163. A phenon line is drawn at about 1.0 just below where the first cluster begins to form. Numbers 1 – 4 denote clusters that can be recognised as species based on the dissimilarity distance. Combined trichome morphology alone cannot reliably separate taxa at species level. OTU abbreviations are the same as in Figure 4.2

4.5.2 Distribution of trichome morphology

There was variation in trichome morphology and composition between vegetative and reproductive structures within the *B. rigida, B. pungens* and *B. jubata* complexes. The IL putative taxon does not show marked variation in trichome morphology between leaf and bracteole (or calyx lobe) surfaces, except for variation in the ornamentation pattern of the conical trichomes and non-ornamented conical trichomes with a somewhat collapsed base. In addition, the collapsed trichomes are shorter on the bracteole and calyx lobe surfaces and longer on the leaf surface (Plates 4.7e & f and 4.8d & e). Ornamented collapsed trichomes were not observed on the bracteole and calyx lobe surfaces of *B. rigida2* (LB) though oval holes representing where trichomes could have fallen off occurred. However, it cannot be ascertained whether these trichomes were ornamented or non-ornamented. The breaking off of trichomes from the bracteole and calyx lobe surfaces of LB, PT and PG may also account for the lack of certain trichome types and ultimately the glabrescent and / or glabrous nature of *B. pungens2* and *B. rigida2*. Long collapsed trichomes of type 2a were common on the calyx lobe surface towards the calyx lobe margin on specimens of PG, PT and PW from Riversdale and Port Elizabeth and sparsely on leaf surface of PT from Port Elizabeth but were not observed on the PG occurring where PT and PW does not occur. While the possession of particular features or trichomes and their caducous nature may be genetically influenced, their density and size could be environmentally triggered, which could be the reason why within *B. pungens1*, the PG cluster has a higher trichome density than the PT and PW (which sometimes had very long collapsed trichomes).

Though conical and collapsed trichomes were observed among the *B. pungens* and *B. rigida* complexes, the short conical ornamented trichomes with and without a disc at the base (type 1a and 1c) and collapsed ornamented and non- ornamented trichomes (type 2a, 2b, 2d and 2e) occur among the *B. rigida* complex while type 1d, 2a, 2b, 2e, 3a and 3b occur among the *B. pungens* complex with an overlap in type 2b and 3a. Type 1a, 2b and 2d were notably absent on *B. pungens1* and *B. pungens2* while 1b and long 2a occur exclusively among *B. pungens1*. The long erect collapsed trichomes (type 2a) were denser on the bracteole and calyx lobe veins than on the lamina of *B. pungens1* but were absent from *B. pungens2*. Trichome type 1d only occurred on *B. pungens1* and *B. jubata2* (Plate 4.6f). The short collapsed ornamented trichomes without a disc at the base were prominent among specimens of *B. rigida1* and *B. pungens2* while the much longer collapsed ornamented trichomes without a disc at the base were prominent among *B. pungens1*. Trichome composition between *B. pungens1* and *B. pungens2* exhibited variation in size of the collapsed trichomes and orientation of the long conical (unicellular) trichomes.

The multicellular trichomes also have a wide distribution though variation was observed in the ornamentation, trichome base, number of cells, shape and size of the cells (Plates 4.1a – 1c, 4.2a & b, 4.3a – d, 4.4c, 4.5a and 4.6e,). Though multicellular trichomes occur on leaf, bracteole and calyx lobe surfaces among the *B. jubata, B. pungens* and *B. rigida* complexes, type 3a, 3b and 3e is seen on BR, type 3b and 3g is found on ILa, type 3e on *B. jubata2*, and type 3a and 3d on PG while type 3a, 3f and 3i occur on *B. jubata1*. Even though BR and *B. jubata1* possess 2-celled trichomes with like cell ornamentation with both cells collapsed (3a) they are lightly or non-ornamented and shorter within BR and ornamented and much longer within *B. jubata1*. The multicellular trichomes with short non-collapsed basal cells and collapsed apical cells had the collapsed cells form an angle of more than 90º in respect to the erect basal cell in *B. pungens1* while the collapsed cells curved downward in *B. jubata1*. Trichome type 3e varied between BR and *B. jubata2* based on the size (Plates 4.2b and 4.3a). BR and ILa possessed 2- to 3-celled differentially ornamented trichomes (whose basal cells were non-ornamented or very lightly so) on the leaf, bracteole and calyx lobe surface. While 3-celled trichomes were present on the leaf surface of *B. jubata1* they were absent on the bracteole and calyx lobe surface of *B. jubata1*. The 2-celled differentially ornamented trichomes on the bracteole surfaces of BR have both cells collapsed or only the basal cell collapsed whereas *B. pungens1* have only the apical cell collapsed (or showing no clear cell demarcation when both cells are collapsed) and *B. jubata2* have both cells non-collapsed. Although *B. pungens1* like *B. jubata2* also possesses 2-celled trichomes with non-collapsed cells, they are similarly ornamented (Plates 4.2a & b, 4.3a – c, 4.5a and 4.6e). The 2-celled differentially ornamented trichomes on the bracteole surfaces of BR, *B. pungens1* and *B. jubata2* therefore, differ in cell size and detailed cell morphology. However, multicellular trichomes are altogether absent on IL and LB leaf and bracteole (may occasionally occur on LB calyx lobe).

The long twisting collapsed trichome within *B. pungens1* occurred in specimens from Grahamstown, Port Elizabeth and Riversdale, where the three forms (PG, PT & PW) have a distribution overlap (Chapter 3), which could be influenced by environmental factors. The multicellular trichomes with non-collapsed cells only occurred in leaves of specimens of PG and PW from Riversdale. The variation of the multicellular trichomes among the calyx lobes of *B. pungens1* could be a result of the collapsing of trichomes during preparation or a variation that actually exists in nature (Plates 4.3c, 4.5a and 4.6e). While *B. jubata2* possesses multicellular non-ornamented trichomes with glandular heads on leaf, bracteole and calyx lobe surface, they were only observed on the young calyx lobe surfaces within *B. jubata1* (on a few OTUs, not all) and very sparsely distributed or absent in mature calyx lobes (Plates 4.1a & f, 4.2a – d and 4.5a,). The long unicellular tawny or cream horizontal ornamented non-collapsed trichomes (type 1f) of *B. jubata1* were absent in *B. jubata2*. The long unicellular tawny or cream ornamented non-collapsed trichomes (type 1b and 1e) are erect to sub-erect with a base raised by a group of supporting cells within *B. pungens1* while they are adpressed to the surface within *B. pungen2, B. rigida1* and *B. rigida2*. The leaf surfaces of *B. jubata1* were dominated by non-collapsed trichomes while the bracteole and calyx lobe surfaces were dominated by collapsed trichomes (Plates 4.1a and 4.2e). The variation between leaf and bracteole (or calyx lobe) in trichome composition emphasises the need for similar organs to be compared.

4.5.3 Distinct and recognisable clusters

Barleria rigida1, B. rigida2 and *B. jubata2* possess short conical trichomes with a disc at the base on their calyx lobe surfaces, and in addition *B. jubata2* also has non-ornamented trichomes with capitate heads. The base of the trichomes of *B. rigida2* is somewhat rounded just above the disc and the lack of 2-celled differentially ornamented trichomes on the calyx lobe surface differentiates it from *B. rigida1* and *B. jubata2*. Within *B. rigida1,* IL differs from BR and ILa by the lack of differentially ornamented multicellular trichomes. BR and ILa can be differentiated by the non-ornamented collapsed trichomes that are absent in ILa. Though BR and IL possess non-ornamented collapsed trichomes, IL lacks the 2-celled differentially ornamented trichomes on the leaf, bracteole and calyx lobe surfaces. LB can be differentiated from *B. rigida1* by the lack of differentially ornamented multicellular trichomes or non-ornamented multicellular trichomes, the lack of non-ornamented collapsed trichomes, the presence of caducous trichomes (which are persistent in *B. rigida1*) and the possession of conical trichomes with a swollen base. LB differs from *B. rigida1* by at least three trichome characters. The continuous variation observed between the putative taxa within *B. rigida1* could suggest that the slight differences may be environmentally influenced.

The dissimilarity in trichome morphology between *B. rigida1* and *B. rigida2* within the *B. rigida* complex could imply that there is differentiation. Though the observed trichome morphological dissimilarity may imply that there is some measure of genetic variation, the discontinuity between ILa, IL and BR within *B. rigida1* is in only one or two trichome characters which can only be recognised at the level of variety. LB can be differentiated from BR, IL and ILa by at least three characters and may be recognised as a morphologically distinct species, due to the observed discontinuity in trichome morphology which may represent gaps in gene flow. Although there is a possibility that the non-ornamented collapsed and conical trichomes and multicellular trichomes may occur on LB, but could have broken off, the caducous nature of trichomes is likely to be genetically influenced. However, these conclusions cannot be made with certainty due to the small sample size.

The observed caducous nature of trichomes of BP and PT unlike PG might contribute to the reduced trichome density among the specimens with thin white leaf margins. Although multicellular trichomes with differential ornamentation were observed throughout *B. pungens1,* trichomes with all cells collapsed on calyx lobes of PT were from Port Elizabeth while those with non-collapsed basal cells and collapsed apical cells on calyx lobes of PG and PW were from Riversdale (Plates 4.3c, 4.5a and 4.6a). The leaf surfaces of PG, PT and PW from Riversdale, Grahamstown and Port Elizabeth possessed differentially ornamented multicellular trichomes with collapsed basal cells and non-collapsed apical cells in addition to the conical and collapsed ornamented trichomes. The similarity in trichome morphology and distribution on the leaf surfaces of *B. pungens1* may suggest that the observed variation in trichome morphology and distribution on the calyx lobe surfaces could probably have been brought about by environmental factors as they do not exhibit a definite discontinuity between the putative taxa. Although this kind of variation was attributed to ecological variation within *Nelsonia* (Acanthaceae) (Hossain and Emumwen, 1981), it is difficult to make such a conclusion here due to small sample size and unknown environmental conditions under which the specimens grew.

However, *B. pungens1* and *B. pungens2* can be differentiated by the presence of long erect or sub-erect unicellular trichomes with or without a group of specialised supporting cells at the base, the presence of multicellular ornamented trichomes, and presence of long ornamented collapsed trichomes in one, as opposed to the presence of long adpressed trichomes, the lack of multicellular ornamented trichomes, the lack of long ornamented collapsed trichomes but instead the presence of short caducous collapsed and conical trichomes in the other. Therefore *B. pungens2* and *B. pungens1* can be recognised as separate species based on the observed differentiation in trichome morphology and distribution. Though *B. pungens2* was observed to cluster together with *B. rigida1* and *B. rigida2* (due to the presence of short conical and collapsed trichomes and long adpressed trichomes; the presence of short conical and collapsed trichomes and long adpressed trichomes and the lack of multicellular trichomes respectively), *B. pungens2* can be differentiated from *B. rigida1* and *B. rigida2* by the lack of a disc at the base of conical or collapsed trichomes, the lack of non-ornamented collapsed trichomes, the lack of multicellular trichomes and the presence of caducous trichomes (Figures 4.3 & 4.4). The observed discontinuities which include trichome ornamentation, trichome size and shape between the above three putative taxa are discernible and can be recognised at species level as ornamentation and trichome morphology have been used before in *Monechma, Barleria* and other species within Acanthaceae (Dipa and Daniel, 2011; Munday 1980; 1984; 1987; van der Bank *et al.,* 2009).

Even though both *B. jubata1* and *B. jubata2* possess non-ornamented trichomes with capitate heads on the calyx lobe surfaces, only *B. jubata2* has the non-ornamented trichomes with capitate heads on the leaf and bracteole surface. While the 2-celled differentially ornamented trichomes occur on the calyx lobe surface in *B. jubata2,* they in addition to 3-celled differentially ornamented trichomes occur on the leaf surface in *B. jubata1* and the calyx lobe surfaces are predominantly covered in collapsed trichomes. The leaf surface of *B. jubata2* possesses short conical trichomes without a disc at the base whereas *B. jubata1* possesses short conical trichomes with a non-ornamented disc at the base. In addition *B. jubata1* has long horizontal unicellular ornamented trichomes, which, are absent in *B. jubata2*. Although this variation may be environmentally influenced it is likely that there is also genetic differentiation represented by the clear gaps in trichome morphology and distribution. The dissimilarity, therefore, in trichome morphology in a number of trichome characters (including glandular ones) could here imply taxonomic variation and the definite distinction in at least 4 trichome characters between *B. jubata1* and *B. jubata2* can qualify them to be separated into discrete species morphologically as morphological discreteness in trichome characters is known to represent gaps in gene flow (Levin, 1973; Lewis, 1972; Masćo *et al.,* 2004; Munday, 1984).

The observed mixing of OTUs from distinct and separate species in clusters labelled 2 of the phenograms may be arising from the lumping of characters such as presence or absence of multicellular trichomes, and presence or absence of non-ornamented trichomes, with varying sizes and detailed shape (Figures 4.3, 4.4 and 4.5). Though the sessile saucer-like glandular trichomes were in abundance on the leaf surfaces of all specimens examined, their size and shape varied and their distribution on the bracteole and calyx lobe surfaces also varied from sparse to absent. However, the clusters were more discernable using trichome characters from leaf surface with only *B. pungens2* and *B. rigida1* clustering together (which are glabrescent to glabrous species) which suggests the reliability of leaf trichome characters. This also suggests that qualitative characters of leaf trichome morphology on their own may be sufficient to differentiate the above taxa meaningfully into separate species. However, a combination of qualitative and quantitative trichome morphology may be of significant value. Reproductive characters may tend to be reliable at various taxonomic levels because of their stability and usefulness and their direct association with the floral and fruit complexes, on one hand, and characteristic relationships with pollinators and seed dispersers on the other (Levin, 1973). However, a combination of leaf, bracteole and calyx lobe characters yielded a phenogram similar to that obtained from bracteole and calyx lobe surface trichome characters. This suggests that there were more bracteole and calyx lobe surface characters influencing the variation; and that bracteole and calyx lobe surface trichome morphology presents a lot of intermingling of qualitative characters among the species.

4.6 Conclusion

Although Section *Barleria* has been known to have unicellular and multicellular hairs, detailed morphology of size, shape, ornamentation and trichome distribution has in this study been observed to vary between putative taxa while maintaining some consistency within particular putative taxa. Stellate hairs were however, not observed in this study. While the density of trichomes varied within putative taxa, clear dissimilarity in trichome morphology and distribution indicated differentiation. Although there was an overlap in ornamentation and general trichome morphology among the putative taxa, particular trichome distributions were specific, which can be used as a taxonomic character. Detailed morphology and orientation of the trichomes have been observed to vary between clusters that can be recognised as species. Variation within species only exhibited small limited gaps. Trichome orientation in addition to size was of taxonomic significance between *B. pungens1* on the one hand and *B. pungens2*, *B. rigida1* and *B. rigida2* on the other. Detailed trichome morphology and distribution was also useful in differentiating between *B. jubata1* and *B. jubata2.* However, due to the lack of evidence as to whether the collapsed trichomes and collapsed trichome cells came about during the preparation of samples or whether they exist in nature; and the lack of certainty that the trichomes that remained intact are non-collapsible, the use of such characters is likely to be erroneous. Comparison of trichome morphology from vegetative and reproductive structures separately can yield clear-cut differences among species. However, in the event that there is insufficient floral or fruiting herbarium material, comparison of qualitative trichome morphology of different specimens could be misleading. Although only five clusters at the most were obtained by cluster analysis, the three species complexes may be separated into six recognisable groups at species level to conform to the clusters from Chapter 2.

4.6.1 Artificial key using trichome characters for the identification of species

1a. Leaf surface glabrous, or glabrescent, with scattered very short conical or collapsed trichomes ... 2

1b. Leaf surface pubescent, with long, erect, sub-erect or horizontal trichomes ... 5

2a. Adpressed trichomes absent on leaf, bracteole and calyx lobe surfaces, possess two- or three-celled non-ornamented trichomes with capitate or globose glandular heads on leaf, bracteole and calyx lobe surfaces ... ***B. jubata2***

2b. Adpressed trichomes present on leaf, bracteole and calyx lobe surfaces, two- or three-celled non-ornamented trichomes with capitate or globose glandular heads absent on leaf, bracteole and calyx lobe surfaces ... 3

3a. Stem covered in short straight white hairs, leaf, bracteole and calyx lobe surfaces with long adpressed trichomes usually bending at the tips, short conical and collapsed trichomes without a disc at the base, trichomes caducous ... ***B. pungens2***

3b. Stem bifariously hairy with 2- or 3-celled recurved white coarse hairs, long adpressed trichome with straight tips, short conical and collapsed trichomes usually with a disc at the base ... 4

4a. Short conical trichomes somewhat swollen at the base, caducous leaving small holes on the bracteole or calyx lobe surface ... ***B. rigida2***

4b. Short conical trichomes not swollen at the base, persistent on the bracteole or calyx lobe surface ... ***B. rigida1***

5a. Two- and three-celled differentially ornamented trichomes present on leaf surface, two-celled trichomes with similar cell ornamentation dominating on the bracteole and calyx lobe surfaces, long erect ornamented conical (unicellular) trichomes absent ... ***B. jubata1***

5b. Only two-celled differentially ornamented trichomes (or with similar cell ornamentation) may be present on the leaf surface, long erect ornamented conical or collapsed (unicellular) trichomes in addition to two-celled trichomes present on the bracteole or calyx lobe surfaces ... ***B. pungens1***

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4.8 Appendices

4.8.1 Appendix I. Specimen codes and abbreviations given to the specimens used in the analysis

BR denotes specimens belonging to *B. rigida1* of the *B. rigida* complex with linear leaves and narrow hardened non-lobed bracteoles
ILa denotes specimens belonging to *B. rigida1* of the *B. rigida* complex with linear leaves and broad leafy non-lobed bracteoles
IL denotes specimens belonging to *B. rigida1* of the *B. rigida* complex with ovate leaves and narrow hardened non-lobed bracteoles
LB denotes specimens belonging to *B. rigida2* of the *B. rigida* complex with elliptic-ovate leaves and broad leafy lobed bracteoles with tapering tips
BP denotes specimens belonging to *B. pungens2* a cluster within the *B. pungens* complex with green flat leaf margins
PG denotes specimens belonging to *B. pungens1* a cluster within the *B. pungens* complex with green wavy leaf margins
PT denotes specimens belonging to *B. pungens1* a cluster within the *B. pungens* complex with thin white wavy leaf margins
PW denotes specimens belonging to *B. pungens1* a cluster within the *B. pungens* complex with prominent white wavy leaf margins
JUE denotes specimens belonging to *B. jubata1* a cluster within *B. jubata* with eglandular hairs
JUG denotes specimens belonging to *B. jubata2* a cluster within *B. jubata* with glandular hairs

4.8.2 Appendix II. Coding of the 20 characters used in cluster analyses

1. Presence of short ornamented trichomes (1), absence of short ornamented trichomes (0) **Sht**
2. Presence of conical ornamented trichomes (1), absence of conical ornamented trichomes (0) **Con**

3. Presence of curved ornamented trichomes (1), absence of curved ornamented trichomes (0) **Curv**
4. Presence of non-ornamented trichomes (1), absence of non-ornamented trichomes (0) **Non-orn**
5. Presence of trichomes with base supported by single-celled disc (1), absence of trichomes with base supported by single-celled disc trichomes (0) **Disc**
6. Presence of trichomes with base supported by multi-celled disc (1), absence of trichomes with base supported by multi-celled disc (0) **Disc2**
7. Presence of long (L/W ratio 1:3 – 6) trichomes (1), absence of long (L/W ratio 1:3 – 6) trichomes (0) **LW1**
8. Presence of long (L/W ratio > 1: 6) trichomes (1), absence of long (L/W ratio > 1: 6) trichomes (0) **LW2**
9. Presence of collapsed trichomes (1), absence of collapsed trichomes (0) **C-cel1**
10. Presence of non collapsed trichomes (1), absence of non collapsed trichomes (0) **C-cel2**
11. Presence of long erect or sub-erect trichomes (3), presence of long horizontal trichomes (2), presence of long appressed trichomes (1), absence of long trichomes (0) **Appr**
12. Presence of 2-celled trichomes (1), absence of 2-celled trichomes (0) **2-cel**
13. Presence of 3-celled trichome (1), absence of 3-celled trichome (0) **3-cel**
14. Presence of 2-celled trichome with like cell ornamentation or both cells not ornamented (1), absence of 2-celled trichome (0) **2-cel1**
15. Presence of multi-celled differentially ornamented trichome (1), absence of multi-celled trichome (0) **2-cel2**
16. Presence of 2-celled trichome with basal cell collapsed (1), absence of 2-celled trichome (0) **C-cel3**
17. Presence of 2-celled trichome with apical cell collapsed (1), absence of 2-celled trichome (0) **C-cel4**
18. Presence of 3-celled trichome with all cells collapsed (3), presence of 3-celled trichome with cells not collapsed (2), presence of 3-celled trichome with only two cells collapsed (1), absence of 3-celled trichome (0) **C-cel5**
19. Presence of sessile saucer-shaped glands (1), absence of sessile saucer-shaped glands (0) **Sess**
20. Presence of multicellular non-ornamented trichomes with saucer-like or globose head (1), absence of multicellular non-ornamented trichomes with saucer-like or globose head (0) **Stal**

4.8.3 Appendix III. Specimens examined

Namibia. –1714 (Ruacana Falls): Kaokoveld, 20 miles southern Ruacana, Bergveld (–CD), 14 June 1963, *Giess & Leippert 7588* (NBG) [JU141]. –2016 (Otjiwarongo): Okanjande, Erongo Mts (–DA), June – July 1916, *Pearson 9895* (NBG) [JU007]. –2017 (Waterberg): Waterberg Plateau (–CA), December 1935, *Boss 34948* (PRE) [JU008]. –2416 (Maltahohe): Farm Duvisib (–CC), 17 May 1956, *Volk 12777* (M) [IL111]. –2418 (Stampriet): 16 km from Mariental turn off to Stampriet, Opstal farm (–AD), 11 March 1983, *Germishuizen 2746* (PRE) [BR059]. –2616 (Aus): Namtib, cliff face, 26º 7’ 30”S 16º 22’ 30”E (–AB), 27 May 2001, *Theile THE89* (WIND) [LB011]. –2618 (Keetmanshoop): 20 miles S.E. of Keetmanshoop on rd to Narubis (–CA), 28 April 1955, *de Winter 3267* (PRE) [BR049]. –2817 (Vioolsdrift): Wespental, 28.210º S 17.070º E, Richtersveld (–AC), 9 September 1987, *Jurgens 22682* (PRE) [IL081].

South Africa. –3321 (Ladismith): Farm Mannshoop, 0.8 km from first gate after homestead on link road to Barrydale, yellow-brown well drained stony loam, full sun, gentle northwest slope, succulent mountain scrub (Acocks Veld Type 25), locally rare, alt.: 425 m (–CA), 16 July 1982, *Laidler 204* (PRE) [BP031]. –3325 (Port Elizabeth): (–DC), 1 February 1927, *Moss 15394* (J) [PT041]; Markman Industrial area (–DC), 23 February 1971, *Dahlstrand 2715* (J) [PW016]. –3326 Albany, 5 miles NW of Grahamstown, False Renosterveld (–AB), 24 March 1950, *Accocks* 15732 (PRE) [PW017]; Sugar Loaf Hill (–AD), April 1950, *McGillivary 2951* (J) [PT014]. –3421 (Riversdale): Soetmelksrivier, Plateau N of bridge on main road (–AB), 25 February 1981, *Bohnen 7829* (PRE) [PT018]; (–AB), September 1960, *Horn 2286* (PRE) [BP036]; Soetmelksrivier, 2 km NW of Bridge (–AB), 20 February 1980, *Bohnen 7371* (PRE) [BP037]; near settlement, on brown loam, 34º 5.79’S 21º 15.30’E (–AB), 9 July 2008, *Nyirenda 717*(J); Albertina, about 27 km from Mossel Bay to Albertina, on N2 road, Van Rensburg Farm, 34º 10.78’S 21º 53.08’E (–AB), 8 July 2008, *Nyirenda 710* (J) [BP020]; about 7 km from Albertina, along N2 road, Farm Wolwekraal, 34º 12.46’S 21º 38.74’E (–CB), 8 July 2008, *Nyirenda 712* (J) [BP021].

4.8.4 Appendix IV.Ttrichomes data matrix. Trichome codes beginning with ‘L’ denote trichomes of the bracteole nd calyx lobe surfaces while the rest denote those observed on the bracteole and calyx lobe surfaces.

Combined matrix of 38 leaf, bract and calyx lobe characters

Leaf	con	sht	cur	non-orn	c-cel1	c-cel2	disc	disc2	LW1	LW2	2-cel	2-cel1	2-cel2	3-cel	c-cel3	c-cel4	c-cel5	Stal	Appr	L-sht	L-cur	L-non-orn	L-c-cel1	L-c-cel2	L-disc	L-disc2	L-LW1	L-LW2	L-2-cel	L-2-cel1	L-2-cel2	L3-cel	L-c-cel3	L-c-cel4	L-c-cel5	L-Sess	L-Stal	L-Appr
PW016	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	0	0	0	1	1	0	0	1	1	0	0	1	0	0	1	0	1	0	3
BP020	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0	1	0	3
BP021	1	0	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	3	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0	1	0	3
PG031	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	1	0	0	0	1
PT032	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	1	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	0	0	3
PW036	1	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	0	0	3	0	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	3
BP037	1	1	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	3	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0	1	0	3
PT041	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	0	1	0	1	1	0	1	1	1	1	0	1	0	1	1	0	1	0	3
PT014	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	1	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	1
PW017	1	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	3	1	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	3
PT018	1	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	3	0	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	3
ILa060	0	1	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	1
ILa110	0	1	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	1
IL081	1	1	0	1	1	0	1	0	1	0	1	1	1	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1
IL111	1	1	0	1	1	0	1	0	1	0	1	1	1	0	1	1	0	0	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1
LB011	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1
LB013	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1
BR049	1	1	0	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	0	0	0	1	0	1	1	1	1	3	1	0	1
BR057	1	1	0	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	0	0	0	1	0	1	0	1	1	3	1	0	1
BR059	1	1	0	1	1	0	1	0	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	1	0	0	0	1	0	1	1	1	1	3	1	0	1
BP716	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	1	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	3
BP717	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	1	1	0	1	1	0	1	1	1	1	0	1	0	1	1	0	1	0	3
PT719	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	3	1	1	0	1	1	0	0	1	1	1	0	1	0	1	1	0	1	0	3
JU007	1	0	0	0	0	1	1	0	0	1	1	1	1	1	1	1	2	0	2	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1	2
JU008	1	0	0	0	0	1	1	0	0	1	1	1	1	1	1	1	2	0	2	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1	2
JU141	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1	0	1	1	1	0	1	1	0	1	0	1	0	1	0	0	0	0	1	1	0

CHAPTER 5

5.0 A TAXONOMIC TREATMENT OF FIVE SOUTHERN AFRICAN SPECIES OF *BARLERIA* SECTION *BARLERIA* GROUP 1 (ACANTHACEAE)

5.1 Abstract

Five species, *Barleria bechuanensis* C.B. Clarke, *B. irritans* Nees, *B. jubata* S. Moore, *B. pungens* L.f. and *B. rigida* Nees, which belong to *Barleria* section *Barleria*, Group 1 have been investigated. Examination of the range of intraspecific variation exhibited within the five species resulted in recognising new species and other infraspecific taxa. *Barleria bechuanensis* exhibits distinct morphological variation and limited distribution and the taxon is recognised at the level of species, clearly differentiated by leaf shape and leaf margins. *Barleria jubata* exhibits distinct intraspecific variation with limited but disjunct distribution and two species are recognised, one with glabrous leaves and glandular and eglandular hairs on other parts of the plant and the other with eglandular hairs on the whole plant. *Barleria irritans*, which occurs along the coast and extending inland, exhibits well defined intraspecific variation and differentiated distribution with a narrow overlap around Steytlerville and Kirkwood, is separated into two species based on bracteole size and the number of veins in the bracteole. *Barleria pungens* is limited to the coast extending from the central south coast of the Eastern Cape to the south west coast of the Western Cape, and exhibits a range of morphological variation, and is distinguished into two species, one with green flat leaf margins and the other with green or white wavy leaf margins. *Barleria rigida* exhibits a very wide range of morphological variation and has a widespread distribution extending from Free State through Northern Cape to Namibia and the south-west border area of Botswana. Within the *B. rigida* complex, two species and three varieties are recognised. Of the two species one has lobed bracteoles. Among the three varieties within the species with the non-lobed bracteoles, are plants formerly known as *B. ilicina.*

Key words: Acanthaceae, *Barleria*, morphology, southern Africa, variation.

5.2 Introduction

Barleria L., comprising approximately 300 species, is well represented in southern Africa where there are approximately 70 species out of which 32 belong to section *Barleria* (Balkwill and Balkwill, 1997; 1998). *Barleria* is currently divided into seven sections: *Barleria, Chrysothrix* M. Balkwill, *Prionitis* (Nees) Lindau, *Somalia* (Oliv.) Lindau, *Cavirostrata* M. Balkwill, *Fissimura* M. Balkwill and *Stellatohirta* M. Balkwill and section *Barleria* is further subdivided into five informal groups (Balkwill and Balkwill, 1997). Group 1 of section *Barleria* includes 37 species which are distinguished by almost always possessing a sharp apiculus or stiff mucronate tip on the leaf apex; bracteoles that are usually spinous and equipped with stiff or spinous teeth along the margins; an axillary spine system almost always present in the lower leaf axils; calyx margin that has stiff or delicate spines or small teeth; and the leaf surface has dense cystoliths that are easily visible in dried specimens (Balkwill and Balkwill, 1998). The five species in this study, *Barleria bechuanensis* C.B. Clarke, *B. irritans* Nees, *B. jubata* S. Moore, *B. pungens* L.f. and *B. rigida* Nees are among other species that belong to Group 1 of Section *Barleria* (Balkwill and Balkwill, 1994; 1997).

In the phenetics chapter, it was established that morphology varied greatly within the already known and circumscribed species revealing some allopatric, parapatric and sympatric forms. Leaf size and shape, bracteole size and shape and calyx lobe size and shape were the most important diagnostic characters. The *Barleria pungens* complex exhibited great diversity in indumenta on the stem and leaves in addition to leaf shape and leaf margins. Bracteole margins were entire, hairy or toothed (the teeth resulting from the breaking off of the long cream-brown single-celled hairs on raised bases), with or without hairs on the spines when spine-toothed; and the calyx lobe margins had stiff hairs or scanty thick sharp teeth. Two species within the *Barleria pungens* complex were separated on the basis of leaf margin, bracteole and calyx lobe margin. The *B. rigida* complex exhibited great variation in leaf size and shape, leaf margin and bracteole and calyx lobe size and shape. Within the *B. rigida* complex, bracteole size and shape, calyx lobe size and shape were the important distinguishing characters between the two species recognised. The *B. bechuanensis* complex was split into two species differentiated by leaf size and shape, leaf margin as well as calyx lobe margin while the *B. irritans* complex was split into two species differentiated by the leaf margin, bracteole size and the number of veins parallel to the midvein in the bracteole. The two clusters within the *B. jubata* complex exhibited differentiation in pubescence, by possessing or lacking glandular hairs, presence or absence of hairs on leaf surface and bracteole shape in addition to disjunct distribution. These were recognised at the level of species. In addition, during the preparation of the key, more specimens were discovered which matched the one specimen that formed its own cluster from the rest of *B. jubata*; belonging to a recognised species *B. damarensis* T. Anders. which exhibits spatial disjunction though it appears to display a narrow distribution overlap with *B. jubata*.

In the chapter further examining the distribution of the observed variation, *B. rigida* complex is observed to be a widespread and polymorphic species, occurring inland from the Free State through the Northern Cape, extending north-west into Namibia on the western coast of the southern Namib Desert and eastward up to the south-west of Botswana bordering with Namibia. In the Northern Cape in the Upper Karoo, Eastern Kalahari Bushveld and the south-west edge of the Dry Highveld the outer calyx lobes are wider than those of specimens from the Kalahari Desert and Semi-desert but the leaves are linear and leaf margins have scanty teeth or are almost entire. At the fringe of Bushmanland and West Griqualand extending into the Gariep Desert the leaves are ovate and the leaf margins are conspicuously spine-toothed. In the Eastern Kalahari Bushveld and the South, Central and North Namib Desert and Semi-desert, the leaves are linear and the leaf margins are conspicuously spine-toothed. In Namibia, on the Central Plateau, in the Dwarf Shrub Savanna, Highland Savanna and Thornbush Savanna, leaves are linear and the leaf margins are conspicuously spine-toothed, in addition the bracteoles are broader with developed laminae. Although the distinctions between these variants are clear, in the Dwarf Shrub Savanna where the ovate and linear leaf forms with spine-toothed leaf margins overlap, the distinction becomes less clear between the two leaf forms (ovate and linear leaf forms) as both tend to be elliptic-ovate. On the basis of the observed variation in leaf shape, leaf margin, bracteole and calyx lobe size and shape and distribution the *Barleria rigida* complex is split into two species, where one comprises three ecological forms recognised as varieties (Sebola and Balkwill, 2009).

The *B. pungens* complex has a coastal distribution inhabiting the Western and Eastern Cape. *Barleria pungens* with white wavy leaf margins is restricted to the Albany Thicket, while *B. pungens* with green wavy leaf margins occurrs throughout the distribution range of the species complex. *Barleria pungens* with green flat leaf margins inhabits the Rainshadow Valley Karoo at the edge of the distribution range of *B. pungens* in Ladismith. The *B. pungens* specimens with flat green leaf margins was recognised as a separate species by a shortly glabrescent leaf surface, serrate bracteole margins and glabrate to glabrous calyx lobe surface with serrate margins. The two forms with green and white wavy leaf margins did not exhibit any clear-cut variation by which varieties could be recognised.

During the analysis of trichome variation in chapter 4, ornamentation and general trichome morphology overlapped among the putative taxa. However, detailed trichome morphology (such as size, number of cells in a trichome, whether or not cells are collapsed, presence or absence of differential ornamentation, trichome base and trichome orientation) and trichome distribution were observed to vary between clusters that can be recognised as species. There was variation in trichome orientation, trichome size and distribution between *B. pungens* with green flat leaf margins and *B. pungens* with green or white leaf margins which differentiated the two into separate species. Alhough *B. pungens* with green flat leaf margins and *B. rigida* complex have similar trichome morphology in a number of trichomes, their distribution varied. Trichome morphology and distribution was also useful in differentiating between *B. jubata* and *B. damarensis.*

5.3 Materials and methods

Analyses performed in earlier chapters (Chapters 2, 3 and 4) grouped the specimens into clusters. Scanned images of type specimens and descriptions were used to assign the names to the appropriate clusters to which they belonged (Anderson, 1863; Balkwill and Balkwill, 1997; Clarke, 1901; De Candolle, 1847; Linnaeus, 1781; Linnaea, 1841; 1850; Moore, 1907; Obermeijer, 1933; 1934; Thunberg, 1794). The remaining unnamed clusters were then recognised at various suitable ranks. The Herbarium abbreviations are according to the standard codes of Holmgren *et al.* (1990) and the recording of specimen distribution data was according to Edwards and Leistner (1971).

5.4 Taxonomy

Barleria L., consists of perennial herbs, suffrutices, shrubs and subshrubs, with aborted inflorescences in leaf axils, simple axillary inflorescence based on scorpioid cymes and stigmas with 2-subconfluent capitate lobes (Balkwill and Balkwill, 1997; Obermeijer, 1933). The habit ranges from erect to semi-prostrate or procumbent. Bracteoles are often stiff, spinous with serrate spinous margins or sometimes entire. Bracteole lamina is sometimes almost completely reduced but at other times leaf-like and unarmed. Calyx is scarious with inner and outer lobe differentiated in size and shape, outer lobes often spine tipped and margins rarely entire (Balkwill and Balkwill, 1997). Group 1 can be recognised by cystoliths that are often extremely dense and easily visible in dried specimens, a sharp apiculus or stiff mucronate tip always present in the leaf, bracteoles that are usually spinous and equipped with stiff hairs or spinous teeth along the margin and an axillary spine system that is almost always present in lower leaf axils (Balkwill and Balkwill, 1997).

The genus exhibits variation in almost all the characters, with some characters being as variable as they are across the family (Balkwill and Balkwill, 1997). Variation observed within the species in this study includes leaf size and shape, leaf margin, bracteole texture, bracteole size and shape, calyx lobe size and shape, calyx lobe texture, and flower colour. The flowers vary from solitary to clustered, in compound cymes that further differ in the degree of congestion with flowers borne in alternate (or sometimes opposite) axils. Other differences include presence or absence of glandular or eglandular hairs on parts of the plant or the whole plant; with pubescence varying in intensity with maturity within some members of the study group or disappearing with maturity in others. Table 5.1 gives some of the morphological characters used to distinguish the intraspecific variation and to recognize the taxa at the level of species in this study. Table 5.2 is a schematic summary and is given to show how groups were arrived at. A key to the African species of Group 1 is given to show which species of section *Barleria* belong to Group 1 and to illustrate how the study group (in bold) corresponds with the rest, followed by a detailed account for the study group.

5.4.1 Taxonomic gaps for future consideration

Information obtained from herbarium specimens, morphology and flower colour shows that there is morphological and flower colour diversity. However, it is difficult to deduce from available data whether the observed array of flower colours within the study group is due to differences in colour perceptions and terminology by the various collectors while describing similar colours or that variation actually exists in nature. It is also not clear whether the flower colours are influenced by environmental changes. To establish whether there is an association between flower colour diversity and environmental differences, field examination with appropriate equipment (and not just visual observation) would need to be carried out to be able to adequately determine the variation in flower colour intensity while at the same time taking note of the ecological or environmental conditions. If it is verified that the distribution of flower colour is environmentally or geographically influenced, it may be possible to circumscribe formal groups on that basis.

Trichome examination further revealed that there is dissimilarity in detailed morphology and distribution within this group, though the general hair type (simple hairs) remained consistent. However, during the preparation of a key to the African species of Group 1, specimens of *Barleria nyasensis* C.B. Clarke examined possessed stellate hairs. Although Balkwill and Balkwill (1997) noted that section *Barleria* possesses simple and stellate hairs, only one species within Group 1 had stellate hairs. Detailed examination of trichome morphology within Group 1 in Africa may therefore, clarify the taxonomic implications thereof and ultimately the circumscription of this informal group in the African context.

Table 5.1. Some morphological characters that can be used to distinguish between the examined *Barleria* species

Character	***B. bechuanensis***	***B. levitundulatus***	***B. irritans***	***B. uninervis***	***B. jubata***	***B. damarensis***	***B. pungens***	***B. foliciliosus***	***B. rigida***	***B. bracteilobis***
Leaf shape	linear-oblanceolate	linear	ovate	ovate	elliptic-obovate	Linear to linear-obovate	ovate	ovate	linear	elliptic-ovate
Leaf surface	glabrous	glabrous	glabrous	glabrous	pubescent	glabrous	pubescent	glabrous	glabrous	glabrous
Leaf margin	flat or very shallowly undulate	undulate	shallowly undulate	shallowly undulate	flat	flat	shallowly undulate	flat	undulate or sinuate	sinuate
Leaf margin indentation	entire	entire	toothed	toothed or entire	entire	entire	toothed or entire	entire	spine-toothed or entire	spine-toothed
Leaf margin pubescence	glabrous	glabrous	glabrous	glabrous	pubescent (erect)	glabrous	pubescent (erect)	glabrescent (horizontal)	glabrous	glabrous
midrib	glabrous	glabrous	glabrous	glabrous	pubescent	glabrous	pubescent	pubescent	glabrous or glabrescent	glabrous
Flowers per axil	1 (rarely 2)	1 – 4	1 – 6	1 (rarely 2)	2 – 7	2 – 6	1 – 6	1 – 4	1 – 8 (10)	3 – 8
Bract shape	lanceolate	lanceolate	ovate	narrow ovate	ovate-lanceolate	ovate & recurved	ovate or lanceolate	ovate	ovate or lanceolate	broad ovate
Bract base	not lobed	not lobed	not lobed	not lobed	not lobed	not lobed	not lobed	not lobed	not lobed	lobed
Bract apex	not tapering	not tapering	not tapering	not tapering	not tapering	tapering	not tapering	not tapering	not tapering	spine-tipped, tapering
Bract veins	3	3	3	1	3	3	3 – 5	3	1 – 3	3 – 5
Posticous calyx lobe shape	ovate	elliptic-ovate	ovate	lanceolate-ovate	ovate	ovate	ovate	ovate	ovate	broad ovate
Calyx lobe major veins	rough, glabrous	rough, glabrous	smooth, glabrous	smooth, glabrous	smooth, pubescent	smooth, pubescent	smooth, pubescent	smooth, cillate	smooth, glabrous	smooth, glabrous

Table 5.2. A schematic summary for the study group

Species	**Chapter 1 (putative taxa)**	**Chapter 2**	**Chapter 3**	**Chapter 4**	**Chapter 5**
B. bechuanensis	BB, BE	BB and BE recognised as Sp. 1 and Sp. 2	-	-	BB (*B. sp. nov. 1*) BE (*B. bechuanensis*)
B. irritans	BI, BU	BI and BU recognised as Sp. 3 and Sp. 4	-	-	BI (*B. irritans*) BU (*B. sp. nov. 2*)
B. jubata	JU	JU split into JU (Sp. 5) and JU2 (Sp. 6)	-	JU and JU2 maintained	JU (*B. jubata*) JU2 (*B. damarensis*)
B. pungens	BP, PU	BP and PU split into BP (Sp. 7) and BP-PU complex (Sp. 8)	BP maintained BP-PU = PUNG & PUNW	BP maintained BP-PU split into PG, PT & PW	BP (*B. sp. nov.3)* PG-PT-PW (*B. pungens*)
B. rigida	BR, IL, LB	BR, IL, LB separated into LB (Sp. 9) and BR-IL-ILa (Sp. 10)	BR = UNBR (Sp. 10 var. 1) IL = OVTL & SPIL (Sp. 10 var. 2) ILa = SPBL (Sp. 10 var. 3) LB = LOBR (Sp. 9)	LB maintained as Sp. 9 BR-IL-ILa form one species (Sp. 10)	LB (*B. sp. nov 4*) BR (*B. rigida var.rigida*) IL (*B. rigida var.ilicina*) ILa (*B. rigida var.nov.*)

5.4.2 Key to African species in Group 1 of Section Barleria

1a. Posticous calyx lobe margin entire ... 2
1b. Posticous calyx lobe margin spinoso-dentate or with stiff hairs ... 6

2a. Flowers always solitary ... 3
2b. Flowers usually clustered (but with solitary flowers in some axils) ... 4

3a. Stem glabrous, leaf linear to linear-oblanceolate, glabrous, leaf margin white, entire or sparsely spinous and shallowly undulate ... 1. *B. argillicola*
3b. Stem thinly bifariously hairy with very short curved white hairs or glabrate, leaf obovate to oblanceolate, pubescent on the veins with long grey-white coarse hairs, leaf margin green, entire and flat ... 2. *B. cyanea*

4a. Bracteole margin entire ... 5
4b. Bracteole margin sparsely toothed ... 6

5a. Leaf ovate or oblong-ovate with truncate base, leaf margin flat ... 3. *B. greenii*
5b. Leaf linear with attenuate base, leaf margin undulate ... 32

6a. Stem bifariously pubescent or forming stripes of long and short hairs, bracteole and outer calyx lobe with glandular hairs ... 7
6b. Stem pubescent all round, bracteole and outer calyx lobe covered in eglandular hairs ... 11

7a. Stem hairy, leaf linear or linear-oblanceolate, glabrous, corolla 50 – 70 mm long, with glandular hairs confined to the base ... 4. *B. lanceolata*
7b. Stem villous, leaf ovate or elliptic-ovate, pubescent with long erect glandular hairs on both surfaces, corolla about 100 mm long or more, covered in long glandular hairs ... 5. *B. longissima*

8a. Flowers solitary in opposite or alternate axils (rarely 2) ... 9
8b. Flowers usually clustered ... 23

9a. Stem pubescent all round ... 10
9b. Stem bifariously hairy with short or long hairs ... 19

10a. Posticous calyx lobe margin entire or sometimes sparsely toothed ... 11
10b. Posticous calyx lobe margin conspicuously spine-toothed ... 12

11a. Stem hispid, leaf pubescent with short and long hairs, posticous calyx lobe margin usually weakly dentate in the upper half to almost entire ... 6. *B. grandicalyx* var. *vix-dentata*
11b. Stem covered in short white hairs, leaf pubescent with short hairs only, posticous and anticous calyx lobe margins usually entire but sometimes with one or two small teeth in the upper half ... 7. *B. stimulans*

12a. Leaf longer than 3cm ... 13
12b. Leaf up to 2.5 cm long (usually shorter) ... 14

13a. Flowers pedicillate, solitary, in opposite axils, leaf elliptic or elliptic-ovate, distinctly petiolate, apex acute to obtuse ... 8. *B. grandicalyx* var. *grandicalyx*
13b. Flowers sessile, solitary, in alternate axils, leaf obovate, very shortly petiolate, apex truncate ... 9. *B. mucronata*

14a. Stem covered in white hairs, including glandular hairs (very thin multicellular hairs with glandular heads) ... 10. *B. eylesii*
14b. Stem pubescent or glabrescent with eglandular hairs ... 15

15a. Old stem glabrescent, young stems pubescent, leaf petiolate, leaf margin white, bracteoles straight ... **11. *B. univervis***
15b. Old and young stems pubescent, leaf sessile, leaf margin green, bracteoles may be somewhat recurved ... 16

16a. Stem with sub-erect or appressed hairs ... 17
16b. Stem with erect hairs ... 18

17a. Leaf sessile, posticous calyx lobe about 12 mm long and 8 mm wide, bracteole shorter than posticous calyx lobes (8 mm long), bracteoles somewhat recurved ... 12. *B. ramulosa*
17b. Leaf subsessile or shortly petiolate, posticous calyx lobe about 20 mm long and 12 mm wide, bracteole equal to or longer than posticous calyx lobes, bracteoles straight ... 13. *B. lanceata*

18a. Leaf linear-oblanceolate, bracteole 1 – 1.7 cm long, posticous calyx lobe 8 – 12 mm long, 4 – 6 mm wide, stellate hairs absent ... 14. *B. transvaalensis*
18b. Leaf elliptic-ovate or ovate, bracteole usually less than 1 cm long, posticous calyx lobe 18 – 25 mm long, 12 – 15 mm wide, brown stellate hairs on the leaf and abaxial surfaces of bracteole and calyx lobes ... 15. *B. nyasensis*

19a. Leaf linear or linear-oblanceolate, leaf surface thinly strigose or glabrous ... 20
19b. Leaf linear-lanceolate or obovate, leaf surface densely pubescent ... 22

20a. Stem with long coarse white erect hairs forming opposite longitudinal stripes (not in furrows), bracteole usually shorter than the posticous calyx lobe ... 16. *B. virgula*
20b. Stem with very short or recurved coarse white hairs forming longitudinal opposite stripes in furrows on the stem, bracteole usually longer than the posticous calyx lobe ... 21

21a. Leaf linear or linear-oblanceolate, 13 – 34 x 1 – 3 mm, leaf surface glabrous, leaf margin white, bracteole linear to oblanceolate, 9 – 19 x 1.5 – 4 mm, bracteole lamina developed, bracteole margin with delicate teeth ... **17. *B. bechuanensis***
21b. Leaf elliptic or obovate, 15 – 35 x 5 – 10 mm, leaf surface glabrescent, leaf margin green, bracteole very narrowly ovate, 15 – 22 x 0.7 – 1 mm, bracteole

lamina almost completely reduced, bracteole margin with stiff sharp teeth ... 18. *B. merxmuelleri*

22a. Leaf obovate, bracteole pinnatifid with 2 – 3 thick long sharp margin spines, posticous calyx lobe lanceolate, about 3 mm wide, and with 2 – 4 long thick spines on each side of the calyx lobe margin ... 19. *B. spinisepala*
22b. Leaf linear or narrowly ovate, bracteole almost a spine occasionally with 1 – 2 very short margin teeth, posticous calyx lobe ovate, about 8 mm wide, and with 6 – 10 or more long thin spines on each side of the calyx lobe margin ... 20. *B. oxyphylla*

23a. Leaf margin flat ... 24
23b. Leaf margin wavy or sinuate ... 32

24a. Stem pubescent with short hairs and with scattered long brownish hairs, leaf margin appearing spine-toothed due to long margin hairs ... 21. *B. meeuseana*
24b. Stem pubescent with either short or long white rigid hairs, leaf margin entire ... 25

25a. Stem bifariously hairy or with alternating hairy and glabrous stripes on an angular stem which becomes terete with age ... 26
25b. Stem hairy all round, stem usually cylindrical ... 27

26a. Stem covered in white straight rigid hairs, leaf oblong to lanceolate, 20 – 30 mm long, 10 – 20 mm wide, bracteole and calyx lobe dotted with erect hairs with glandular heads especially on the margin and margin spines ... 22. *B. saxatilis*
26b. Stem covered in white recurved rigid hairs, leaf elliptic, 40 – 125 mm long, 15 – 40 mm wide, bracteole and calyx lobe glabrous otherwise dotted with long 1-celled cream appressed hairs ... **23. *B. elegans***

27a. Stem with very short erect hairs ... 28
27b. Stem with long erect or recurved hairs ... 29

28a. Leaf obovate or elliptic-ovate, 10 – 16 mm long, posticous calyx lobe 21 – 25 mm long, 7 – 8 mm wide, covered in very short white hairs, calyx lobe margin serrate ... **24. *B. foliciliosus***
28b. Leaf elliptic-obovate, 25 – 50 mm, posticous calyx lobe 16 – 19 mm long, 10 – 13 mm wide, sparsely hairy, calyx lobe margin entire or ciliate, hardly toothed ... 25. *B. acanthoiodes*

29a. Bracteole recurved outward, bracteole with a short tapering tip, bracteole lamina developed, leaf appearing glabrous but with scattered microscopic glandular hairs, stem pubescence consisting of glandular and eglandular hairs ... 26. *B. damarensis*
29b. Bracteole straight or slightly recurved outward, bracteole with a short abrupt tip, bracteole lamina developed or almost completely reduced, leaf pubescent, stem pubescence consisting of eglandular hairs only ... 30

30a. Stem and inflorescence covered in erect or deflexed white ashy hairs, bracteole lamina developed, and posticous calyx lobe longer than 2 cm ... **27. *B. jubata***
30b. Stem and inflorescence covered in white or grey villous or short uncurved hairs, bracteole lamina may be almost completely reduced, posticous calyx lobe less than 2 cm ... 31

31a. Stem pubescent with short straight hairs, bracteole margin conspicuously spine-toothed, leaf elliptic-obovate, about 16 – 19 mm long, 10 – 13 mm wide, leaf apex somewhat truncate with a mucro in the centre, posticous calyx lobe 17 – 19 mm long, 6 – 8 mm wide, corolla tube about 30 mm long ... 28. *B. homoiotricha*
31b. Stem pubescent with long villous hairs, bracteole margin with one or two teeth to entire, leaf elliptic, about 8 – 15 mm long, 8 – 9 mm wide, leaf apex somewhat apiculate, posticous calyx lobe 20 – 30 mm long, 10 – 15 mm wide, corolla tube about 40 mm long ... 29. *B. mucronifolia*

32a. Stem pubescent all round with whitish or cream-brown erect long hairs, leaf margin pubescent ... **30. *B. pungens***
32b. Stem glabrous or glabrescent with scattered short white erect or recurved hairs (may be bifariously hairy in young stems), leaf margin glabrous ... 33

33a. Calyx lobe surface shortly pubescent with glandular hairs ... 31. *B. papillosa*
33b. Calyx lobe surface glabrous or with scattered eglandular hairs ... 34

34a. Bracteole base lobed or truncate, posticous calyx lobe 8 – 13 mm wide (almost twice the width of the anticous calyx lobe) ... **32. *B. bracteilobis***
34b. Bracteole base attenuate, posticous calyx lobe 3 – 9 mm wide (equal to or slightly wider than the anticous calyx lobe) ... 35

35a. Leaf linear, 10 – 28 mm long, 3 – 5 mm wide, leaf margin entire ... **33. *B. levitundulatus***
35b. Leaf linear, ovate or elliptic-ovate, 10 – 50 mm long, 3 – 11 mm wide, leaf margin minutely toothed or spine-toothed ... 36

36a. Species restricted to the Eastern Cape, leaf ovate not longer than 29 mm long, leaf margin shallowly undulate and with scattered minute teeth ... **34. *B. irritans***
36b. Species widely distributed from the Northern Cape of South Africa into Namibia and Botswana, leaf shape various and of varying lengths, 10 – 50 mm long, when linear leaf margins are shallowly undulate or deeply so and with scattered minute teeth or conspicuously spine-toothed, when ovate or elliptic-ovate, leaf margins are sinuate and conspicuously spine-toothed ... 37

37a. Bracteole ovate, narrow, with 1 prominent vein, bracteole lamina almost completely reduced, bracteole v-shaped in cross-section, flowers may be solitary or clustered … 38

37b. Bracteole lanceolate, broad, lamina well developed, usually with 3 parallel veins, bracteole somewhat flat, flowers always clustered ... **35. *B. rigida* var. *latibracteatus***

38a. Leaf margin undulate usually with very few small scanty teeth on the lower half of the leaf margin to entire (sometimes with teeth evenly distributed along the undulate leaf margin) ... **36. *B. rigida* var. *rigida***

38b. Leaf margin sinuate with small spines or well developed spines evenly distributed along the leaf margin ... **37. *B. rigida* var. *ilicina***

5.4.3 Detailed account of the study species

5.4.3.1 Barleria uninervis

B. uninervis *N*yirenda *sp. nov.* affinis *B. irritans* Nees, similis sed floribus solitarius ad alternus vel oppositus axillis. Bractea venis solitarius lamina omnino reductus. Calyce lobis exteriora lanceolata vel lanceolata-ovata differt.

Type: [South Africa, Eastern Cape], Graaff Reinet and Jansenville, by the Sundays River, 1500 – 2000 ft (–DA), *Drège* (*Drège s.n.* in h. Benth.) [non h. Hook.] (K, lectotype, here designated).

B. stimulans E. Mey. ex Nees var. *macracantha* Nees in DC. Prod. 11: 241 (1847), synon. nov.

Multi-stemmed dwarf shrublets, upright or sub erect, to about 50 cm high, arising from a woody rootstock. *Stems* bifariously hairy or stem entirely lightly pubescent in young stems (with very short white recurved coarse hairs) but glabrescent to glabrous in old stems, with a greyish-brown bark. *Leaves* 9 – 23 × 3 – 9 mm, narrowly ovate, glabrous, leaf surface rough, not glossy, apex acute, distinctly mucronate, base attenuate, petiole short 1 – 2.5 mm long, appearing rusty brown, with scattered tawny hairs; midrib glabrescent; leaf margin white, shallowly wavy, minutely toothed to almost entire; spine present in sterile leaf axils. *Flowers* solitary, in alternate (sometimes opposite) axils. *Bracteoles* paired at the base of each flower, 15 – 25 × 1 – 3 mm, single-veined, lamina very much reduced almost entirely, very narrowly ovate, margins toothed, teeth glabrous. Outer *calyx* lobes lanceolate or lanceolate-ovate, anticous calyx lobe 12 – 21 × 2 – 5 mm, posticous calyx lobe 13 – 25 × 2 – 5.5 mm, margin spine-toothed, teeth glabrous; inner calyx lobes narrowly ovate about half the length of posticous lobe. *Corolla* tube 16.5 – 19 mm long, the lower narrower tube of almost equal length to the upper wider tube, flowers white or blue. Flowering from December to April. *Fruit* elliptic, 7 – 13 × 2 – 4.5 mm.

Distribution and habitat

Barleria uninervis is known from collections that are all from the Eastern Cape mostly around Graaff Reinet in the Lower Karoo (Figure 5.1). It exhibits distribution overlap with *B. irritans* to which it is morphologically similar around Steytlerville which is in the Sundays Noorveld vegetation with underlying shale mudstone and sandstones of the Karoo (Mucina and Rutherford, 2006). The distribution indicates that this species is restricted to the Lower Karoo. It frequently grows on stony soil, at altitudes of 600 – 800 m above sea level.

Diagnostic features

Barleria uninervis resembles *B. irritans* in that both species have ovate leaves with white wavy margins endowed with minute teeth. However, *B. uninervis* can be distinguished from *B. irritans* by a narrow 1-veined bracteole (with a highly reduced lamina), narrow lanceolate external calyx lobe and solitary flowers. It can be differentiated from *B. eylesii* which also has a narrow 1-veined bracteole by its glabrous stem and leaves and narrow lanceolate external calyx lobes and solitary flowers.

Notes

Specimens of *B. uninervis* have been filed under the name *B. irritans.* Obermeijer (1933) overlooked the single vein and reduced lamina in the bracteole and the narrower lanceolate external calyx lobes of *B. uninervis* in the two specimens by *Bolus 563* (BOL) and *Galpin 9972* (K, PRE) which she annotated and identified as *B. irritans sensu* Obermeijer. Although both *B. irritans* and *B. uninervis* have tawny or cream hairs on the leaf veins of young leaves, Nees (1847) could have overlooked some of the salient features such as the shortly pubescent leaves with green leaf margins, ovate calyx lobe with entire or almost entire calyx lobe margins in *B. stimulans* and glabrous leaves (except on midrib of young leaves) with a white leaf margin, a much narrower lanceolate calyx lobe with spiny calyx lobe margins in *B. uninervis*. *Drège s.n.* (K), a specimen from Sundays River, Graaff Reinet in Bentham’s Herbarium is morphologically dissimilar to *Drège s.n.* (K) from the same locality but in Hooker’s Herbarium. Although the two specimens were considered to be of the same species, they exhibit dissimilarity in calyx lobe size and shape, calyx lobe margins and the number of flowers per axil, clustered in the one and solitary in the other. This morphological gap is distinct enough to be recognised at the level of species. The single vein and reduced lamina of the bracteole and the narrower lanceolate external calyx lobes in addition to solitary flowers are of taxonomic importance in differentiating this specimen (*Drège s.n.* from Bentham’s Herbarium) from *B. irritans.*

Barleria macracantha R. Br., (Salt, 1790) a synonym for *B. trispinosa* Vahl (Nees, 1847) is sometimes wrongly labelled as *B. macrantha* R. Br.; as observed on *Petit s.n.* (BM), which is one of the specimens cited under *B. trispinosa.* However, two names, *Barleria macracantha* R. Br. and *B. trispinosa*, are also noted on this specimen. Nonetheless, *B. macracantha* R. Br. is a different species which was published as a naked name and is now a synonym of *B. trispinosa* Vahl, a member of section *Prionitis* (Cufodontis, 1964). *Salt s.n.* (BM) also cited under *B. trispinosa* was annotated by Clarke (1897) as *B. macracantha* R. Br. Although Clarke (1900) excluded *B. stimulans* E. Mey. ex Nees var. *macracantha* Nees from *B. trispinosa* Vahl, Nees’ (1847) variety name (*macracantha*) could not be used as a species epithet for this species as this would create a later homonym. A new epithet is here suggested. The specimens, *Bolus 563* (BOL), *Drège s.n.* (K) from Bentham’s Herbarium and *Galpin 9972* (K, PRE) and others with the same characters, are here recognised as *B. uninervis.*

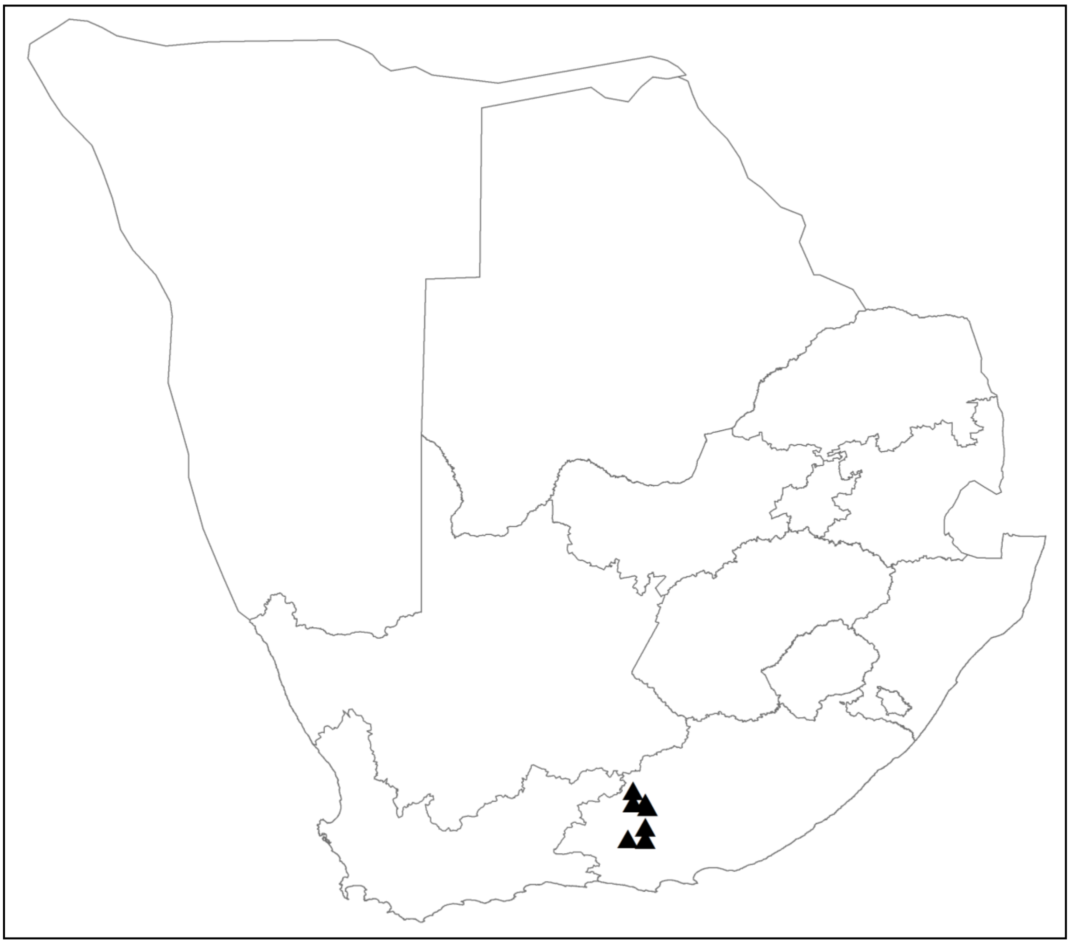

Figure 5.1. Distribution of *Barleria uninervis* in southern Africa.

Additional specimens examined

South Africa. –3224 (Graaff Reinet): Hillside on Farm Reitvlei (–AB), 3 March 1930, *Galpin 9972* (K, PRE); Spitskop (–AD), 01 April 1912, *Burtt-Davy 13503* (PRE); Swart River near Graaff Reinet (–BC), 03 December 1950, *Willems 703* (NBG); 10 miles south of Graaff Reinet (–BC), 03 January1956, *Theron 1853* (GRA, K, PRE); near Graaff Reinet (–BC), March 1867, *Bolus 563* &*10441* (BOL); Veld flats (–BC), 9 May 1954, *Henrici 4958* (PRE); Jansenville, Jockomst, in open veld (–DC), 18 April 1932, *Long 561* (PRE); Jansenville (–DC), 3 April 1981, *Oliver 2806* (PRE). –3324 (Steytlerville): Klipplaat, Teasdale, 33º 07’S 24º 16’E, central lower Karoo, (–AB), 23 April 1985, *Hoffman 692* (GRA); c. 5 m. s of Baroe [Farm], karoo, 2000 ft. (–BA), 27 April 1947, *Story 2312* (PRE).

Figure 5.2. Scanned specimen (non type) of *Barleria uninervis*, *Theron 1853* (K). Scale bar: 1cm.

5.4.3.2 Barleria bechuanensis

B. bechuanensis C.B. Clarke in Flora Capensis 5: 48 (1901); Obermeijer in Annals of Transvaal Museum 16: 134 (1933). Type: South Africa, Bechuana Country [Northern Cape], Batlapin Territory, May 1883, *Holub s.n.* (K, holo., photograph seen on ALUKA).

Barleria bechuanensis C.B. Clarke var. *espinulosa* C.B. Clarke in Flora Capensis 5: 48 (1901); Oberm. in Annals of Transvaal Museum 16: 134 (1933); Oberm. in Journal of Botany 72: 277 (1934). Type: [South Afica], Griqualand West, By the Vaal River at the Diamond, August 1880, *Nelson 151* (K, holo., photograph seen on ALUKA).

Multi-stemmed occasionally branched perennial herb, upright or decumbent, arising from a woody rootstock; stems bifariously hairy with short white recurved hairs, with the two longitudinal furrows decussate at each successive node. *Leaves* 13 – 34 × 1.4 – 3 mm, linear-oblanceolate, glabrous, apex narrow or somewhat rounded, mucronate or inconspicuously mucronulate, leaf base cuneate; petiole very short, inconspicuous, almost sessile; midrib glabrous; leaf margin white, flat or shallowly undulate; spine absent in sterile leaf axils. *Flowers* axillary, solitary, sometimes paired at opposite axils (rarely two in a single axil). *Bracteoles* 9 – 19 × 1.5 – 4 mm, linear to oblanceolate, a pair at the base of each flower, of equal or unequal lengths, lamina developed, bracteole margins with scanty teeth. External *calyx* lobes elliptic-ovate; anticous calyx lobe 10 – 17 × 3 – 7 mm, posticous calyx lobe 11 – 18 × 3 – 7 mm, calyx lobe margin spine-toothed, teeth concentrated on the upper half of the lobe margin. *Corolla* tube 14 – 21 mm long, with the lower narrower portion and upper wider portion almost of equal length; corolla lobes orbiculate, 8 – 10 × 5 – 7 mm; flowers reddish-mauve or white. Flowers from February to April. *Fruit* elliptic, 7 – 11 × 2.5 – 5 mm.

Distribution and Habitat

Barleria bechuanensis is known from more than ten collections and they are limited to the Upper Karoo and the Ghaap Plateau in the Northern Cape and border areas with the North-West Province (Figure 5.3). The species grows on shallow sandy brown soil with pebbly surface from ironstone gravel, with underlying shale and dolomite of the Schmidtsdrift Zone (Mucina and Rutherford, 2006). It also occurs on pans underlain with calcrete. Specimens have been collected from bushveld dominated by *Acacia mellifera* (Vahl) Benth. and *Tarchonanthus camphorates* L., at the foot of hill slopes and level surfaces on fine-grained sediments of the Karoo subgroup. The species occurs at altitudes ranging from 1200 to 1700 m.

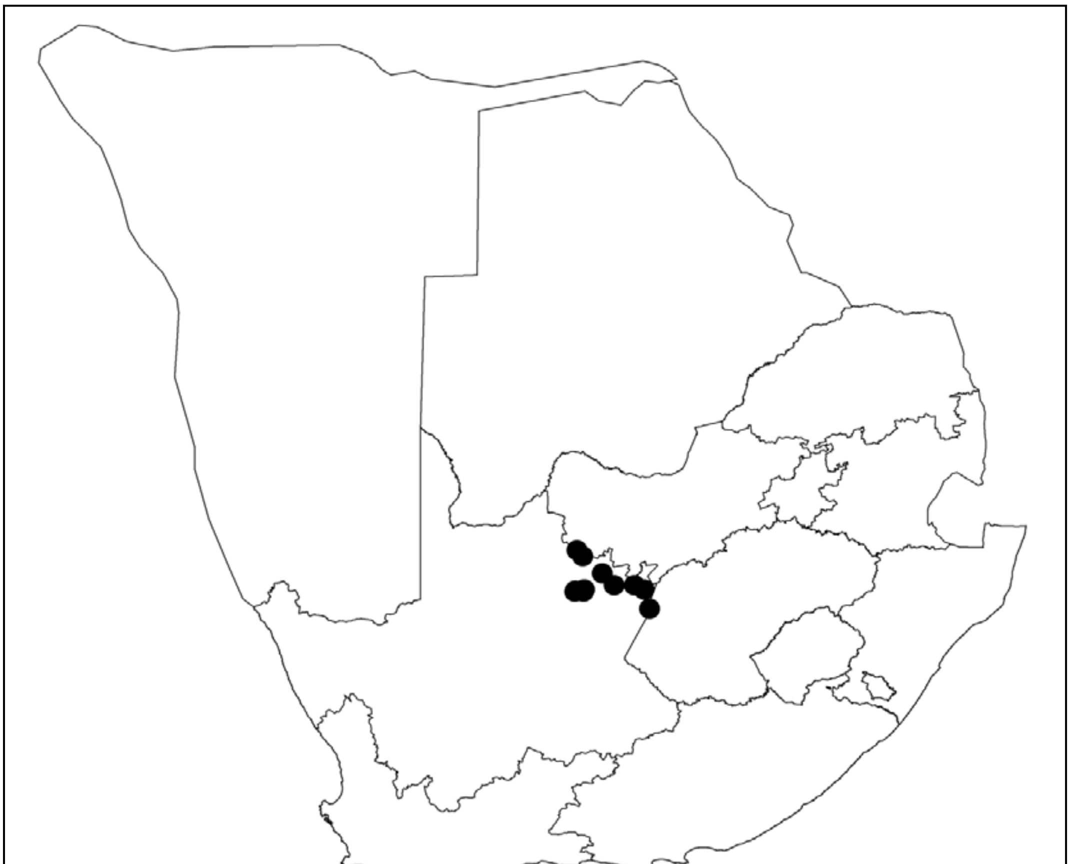

Figure 5.3. Distribution of *Barleria bechuanensis* in southern Africa.

Diagnostic features

Barleria bechuanensis has flat leaf margins or sometimes (but not always) shallowly undulate leaf margins and can be confused with *B. levitundulata.* However, *B. bechuanensis* has narrower linear-oblanceolate leaves with cuneate bases, solitary (rarely two) flowers in an axil and outer calyx lobe margins that are entire at the base (to about one third the length of the lobe). *Barleria bechuanensis* may also be mistaken for *B. rigida* which always has undulate leaf margins but *B. bechuanensis* is recognised by the lack of teeth on the leaf margins, the roughness of veins on the bracteoles and outer calyx lobes, and the lack of conspicuous reticulation on the external calyx lobes. In addition *B. bechuanensis* can be differentiated from *B. rigida* by two longitudinal furrows on the stem, decussate at each successive node and each furrow has short recurved white hairs.

Notes

Clarke (1901) in his description of *Barleria bechuanensis* and the variety *B. bechuanensis* var. *espinulosa* only cited two specimens (*Holub s.n.* and *Nelson 151* respectively) which, are morphologically similar and cannot be recognised at variety level. Clarke's (1901) type specimen *Holub s.n.*, (the only specimen cited in his description) has entire (not spine-toothed) leaf margins.

Additional specimens examined

South Africa. –2723 (Kuruman): 11 m N W of Kuruman, 1280 m (–AD), 19 February 1956, *Leistner 593* (PRE); 5.3 km south south east of Kuruman (–AD), 8 April 2000, *Balkwill, McDade* and *Lundburg 11815* (J); Dunmore Farm, S - facing on level talus slope (–DD), 7 March 1981, *Gubb 171-82* (PRE); Killarney Farm, in a pan (depression) (–DD), 19 February 1981, *Gubb 166-65* (PRE). –2823 (Griekwastad): east of Postmasburg, west of Owendale, facing railway to Blinkklipkop Station, on the loop, 28º 14.87'S 23º 17.34'E (–AB), 8 April 2000, *Balkwill, McDade* and *Lundburg 11818* (J); about 7 m NE of Postmasburg, Blinkklipkop (–AC), 26 March 1959, *Leistner 1396* (K, SRGH, NBG, PRE, M); Postmasburg, Daniel's Kuil, Asbestos Hills (–BA), March 1939, *Esterhuysen s.n.* (BOL); near Daniel's Kuil (–BA), *Rogers 10413* (BOL); Daniel's Kuil (–DA), March 1939, *Esterhuysen 1007* (BOL); Daniel's Kuil on hills near Ouplaas (–BA), April 1940, *Esterhuysen 2046* (BOL). –2824 (Kimberly): at A53 (–AA), 26 March 1937 *Acocks 2121* (PRE); Warrenton (–BB), 11 March 1902, *Adams 17* (BOL); Riet Pan (–DB), April 1919, *Moran* 15919 (BOL).

Figure 5.4. Scanned specimen (non type) of *Barleria bechuanensis*, Esterhuysen 1007 (PRE). Scale bar: 2 cm.

5.4.3.3 Barleria elegans

B. elegans S. Moore in Journal of Botany 269 (1880); C. B. Clarke in Flora of Tropical East Africa 5: 154 (1900); C.B. Clarke in Flora Capensis 5: 49 (1901). Type: Angola, Luanda: 1858, *Welwitsch 5068* (K, lectotype, here designated; BM, C, LISU, P, isolecto., photographs seen on JSTOR).

B. pungens L. var. *macrophylla* Nees in *D.C. Prod*. 11: 237 (1847) non L.

Herb about 60 cm or higher. *Stem* sparsely pubescent with simple cream-brownish hairs or glabrous with age. *Leaves* 40 – 120 × 15 – 40 mm, elliptic, margin entire, cuneate at the base; petiole 4 – 12 mm long. *Flowers* 2 – 8 in congested cymes, 20 – 30mm long. *Bracteoles* of a pair of unequal length, 10 – 22 × 3 – 6 mm wide, lanceolate-ovate, bracteole margin spine-toothed, margin spine 2 – 6 mm long. Posticous *calyx* lobe 19 – 26 × 6 – 11 mm, elliptic-ovate, margin strongly spine-toothed, margin spine glabrous about 4 – 9 mm long. *Corolla* blue, mauve or white; corolla tube 18 – 25 mm long (narrower tube almost the same length as the wider tube), corolla lobes 8 – 14 mm long. Flowers from February to May. *Fruit* about 14 mm long.

Distribution and habitat

This species is widespread occurring in and outside tropical Africa, from Mozambique in Maputo, through South Africa, Swaziland and Zimbabwe into Angola. In South Africa, it occurs from KwaZulu-Natal and extends north-west into Mpumalanga and northward into Limpopo. It generally occurs in places with a high water table, such as on river banks and in the flood plain usually among rocks. It has also been collected at the foot of mountains and occasionally near the margin of riverine forest fringes. It grows on orange sand and red-brown loamy soil with altitudes ranging from 300 to 600 m.

Diagnostic features

Barleria elegans can be differentiated from a morphologically similar species *B. saxatilis* Oberm. by its distribution along the coast and also in more humid places with a high water table. Its stems have sparse erect or sub-erect tawny hairs in alternate rows (or alternate glabrous rows) with coarse recurved hairs and lack the dense coarse erect white hairs observed in *B. saxatilis*. *Barleria elegans* can also be identified by its lack of hairs with glandular heads on the calyx lobe surface and calyx lobe margin spines. Flowers range from 2 to 8 or more in sessile or sub-sessile cymes as opposed to 1 to 4 flowers in *B. saxatilis*.

Notes

Although Nees (1847) alleged that this species is morphologically close to *B. pungens* L., which has much smaller ovate to elliptic-ovate leaves (10 – 30 mm long) with toothed (as a result of hairs breaking off) flat or wavy leaf margins, *B. elegans* has much larger elliptic leaves (40 – 120 mm long) with entire leaf margins. The stems of *B. elegans* are sparsely pubescent while those of *B. pungens* are densely pubescent. *Barleria pungens* has long erect unicellular trichomes all round the stem as opposed to *B. elegans* which has recurved multicellular hairs forming longitudinal stripes along the stem. *Barleria pungens* possesses erect or suberect brown hairs on leaf veins while *B. elegans* possesses suberect white hairs. Calyx lobe margins have long wiry spines on *B. elegans* whereas they have short teeth or stiff hairs on *B. pungens*. The two species, therefore, bear no superficial resemblance.

Additional specimens examined

Angola. Precise locality unknown: Loanda, 13 April 1874, *Soyaux 26* (K), *Welwitsch 5187* (K, LISU); Bumbo, October 1859, *Welwitsch 5034* (LISU); Cazango, June 1855, *Welwitsch 5114* (LISU); *Welwitsch 5122* (LISU); Ambriz, *Monteiro* (K).

Mozambique. –2332 (Mabalane): (–DC), 4 June 1959, *Grandvaux*, *Barbosa and de Lemos 8613* (PRE). –2532 (Lourenco Marques): (–DC), 6 June 1920, *Borle 1103* (PRE); Maputo (–DC), 2 April 1947, *Hornby 2640* (SRGH); Delagoa Bay [Maputo], *Forbes s.n.* (K).

South Africa. –2230 (Messina): (–AC), March 1918, *Rogers 20785* (PRE). –2330 (Tzaneen): Hans Merensky Nature Reserve, Gallery forest, by pumping station, sandy soil, N aspect, slight garden slope (–DA), 25 May 1983, *du Toit 1* (PRE). –2430 (Pilgrim's Rest): Harmonie, 140T, Kampvuur SE of Tzaneen (–AB), 26 May 1974, *de Sousa 473* (PRE).–2530 (Lyndenburg): Abel Erasmus Pass (–DA), 19 April 1960, *Schleiben & Strey 8397* (PRE, SRGH); Nelspruit, Kaapsehoop (–DB), *Rogers 20783* (PRE). –2531(Komatipoort): White River, Kruger National Park, Seekoeigat (–BD), 24 February 1954, *Van Der Schijf 2885* (PRE); Barberton, beside road from Barberton to Kaapmuiden, 100 m south of "Jock's Tree", 620 m (–CA), 26 April 1984, *Balkwill 1481* (NU); Hyslops Creek (–CB), 30 March 1956, *Clarke 50* (PRE). –2632 (Bela Vista): Ndumo Game Reserve, Ndumo Hill (–CD), 20 April 1969, *Pooley 470* (NU). –2731 (Louwsburg): Lebombo Mts. east foot hills, rivulet bank (–DB), 9 April 1966, *Strey 6585* (PRE).

Swaziland. –2531 (Komatipoort): Thabankulu, banks of Umbeluzi River (–BB), 19 May 1971, *Nel 125* (PRE). –2631 (Mbabane): Mlalume, between Komati and Mbuluzi Rivers, at the foot of Managa Mountain, Swaziland Irrigation Scheme (–BB), 20 March 1967, *Clarke 534* (PRE); St Phillips (–DD), 13 March 1956, *Murdoch 56* (PRE).

Zimbabwe. –2130 (Lundi): Lundi River (–BC), 23 April 1962, *Drummond 7701* (PRE). –2131 (Chiredzi): N bank of Lundi River 5 km E of Chironga Camp (–BA), 28 May 1975, *Muller s.n.* (PRE, SRGH). –2132 (Espungabora): Sabi-Lundi junction (–AD), 31 May 1971, *Mavi 1272* (PRE, SRGH). –2230 (Messina): Bubye River, near Bubye Ranch homestead (–BB), 8 May 1958, *Drummond 5688* (PRE).

5.4.3.4 Barleria foliciliosa

B. foliciliosa Nyirenda *sp. nov.* affinis *B. pungens* L., sed caulibus ad 15 cm altus, pilis in caulibus erectus brevis albis veteribus cortice cinereus. Foliis ovato-obovatus glabrescens margine planus. Bractea et calyce lobis glabrecens margine serratus (non spinoso-dentatus) differt.

Type: [South Africa, Western Cape], Ladismith District, Farm Mannshoop, 0.8 km from first gate after homestead on link road to Barrydale, Mountain, alt.: 425 m (Acocks Veld Type 25), locally rare, 16 July 1982, *Laidler 204* (PRE, holo.! NBG, iso).

Figure 5.5. Scanned type specimen of *Barleria foliciliosa*, *Laidler 204* (PRE, Holotype!). Scale bar: 1 cm.

Multi-stemmed loosely branched perennial upright woody dwarf shrublet, stems arising from a woody rootstock. *Stems* up to 15 cm high, endowed with short cream-white erect hairs, old bark greyish-brown. *Leaves* 10 – 16 × 6 – 8 mm, ovate or obovate, attenuate at the base, obtuse at the apex, sharp mucronate tip; leaf surface almost glabrous but covered in very short white microscopic hairs, lateral veins inconspicuous on abaxial and adaxial surface; midrib with appressed hairs; margin entire, flat, with hairs appressed to the margin; petiole glabrescent. *Flowers* 1 – 3 in loosely arranged cymes. *Bracteoles* 12 – 16 × 2 – 3.5 mm, narrowly ovate, glossy yet covered in short white microscopic hairs, with 2 conspicuous veins parallel to the mid-vein, margin serrate; apex spinous. Outer *calyx* lobes ovate or elliptic-ovate, shiny, also covered in short white microscopic hairs, margin serrate to spinous, posticous lobe 18 – 22 × 4 – 6 mm and anticous lobe 17 – 19 × 4 – 5 mm. Inner lobes narrow, ovate with single vein, apex sharp, and margin entire. *Corolla* mauve (though no flowers seen on herbairum sheet). Flowers in July. *Fruit* 11 – 14 × 4 – 5 mm wide.

Distribution and habitat

This species is known from two specimens which, were collected from Ladismith, in the Succulent Mountain Scrub (Figure 5.10). The species may be confined to the Rainshadow Valley Karoo of the Western Gwarriveld with underlying sandstones, siltstones and mudstones. The species occurs on stony yellow-brown loamy-clay and altitudes ranging from 120 – 800 m (Mucina and Rutherford, 2006).

Diagnostic features

Barleria foliciliosa resembles *B. pungens* in that they both have pubescent stems and ovate leaves but differs from *B. pungens* by possessing short white hairs on the stem and flat green leaf margins. Although the leaves of *B. foliciliosa* may be confused with those of *B. meeuseana, B. merxmuelleri* and *B. stimulans,* *B. foliciliosa* can be differentiated by its 3-veined bracteole with serrate margins. *Barleria foliciliosa* has outer calyx lobe margins that are serrate while *B. stimulans* has outer calyx lobe margins that are entire (or may possess 1 – 2 minute teeth on the upper half of the calyx lobe margin) and even though the bracteole margins of *B. foliciliosa* may resemble those of *B. stimulans*, they can be differentiated by the number of veins in the bracteole.

Notes

Barleria foliciliosa has been filed under the name *B. pungens* and is represented by only two collections (*Laidler 204* (PRE) and *Van Breda 4380* (PRE)) both of which were separately collected from Ladismith in the Rainshadow Valley Karoo at the edge of the distribution range of *B. pungens*.

Additional specimen examined

South Africa. –3320 (Montagu), Farm Comae near Plathuis station in Karroid broken veld, Ladismith (–DB), 09 September 1975, *Van Breda 4380* (PRE).

5.4.3. 5 Barleria damarensis

B. damarensis T. Anders. in Bot. Jahrb. 10: 262 (1888); Anderson in Journal Linn. Soc. 7: 29 (1863); Obermeijer in Journal of Botany 72: 278 (1934). Type: [Namibia], Damaraland: May 1886, *Marloth 1315* (GRA, holo., photographs seen on JSTOR; SAM, iso.).

Erect, loosely branched shrublet of about 1 m high. *Stems* pubescent, endowed with cream-brown erect hairs with glandular heads and whitish eglandular hairs, old bark light gray. *Leaves* bluish-green, appearing glabrous (but possess microscopic hairs with glandular heads), 28 – 42 × 7 – 13 mm, elliptic, leaf margin glabrous, petiole glabrous. *Flowers* 2 – 6 in congested lateral cymes. *Bracteoles* recurved (longitudinally outward), hairy, 11 – 14.5 × 2 – 3.5 mm, with a short tapering spinous apex. Outer *calyx* lobes ovate, surface and margin pubescent, with glandular and eglandular hairs; margin sparsely toothed or almost entire, posticous calyx lobe 17 – 21 × 4 – 6 mm, anticous calyx lobe 16 – 18 × 3.5 – 5 mm. *Corolla* pale mauve, light or dark purple to bright blue, with glandular hairs especially on the corolla tube. *Fruit* not seen.

Distribution and habitat

This species is known from more than ten specimens collected from the Kaokoveld and its fringes (Figure 5.7). The Kaokoveld which occupies much of the Northern Namib Desert consists of harsh arid gravel plains and shifting sand dunes, and where the dry riverbeds are a source of moisture (Armstrong, 1990). However, the dry sandy soils are calcareous or with calcareous crusts (Armstrong, 1990). Although this species mainly occupies the desert sands of the Namib Desert (where specimens sometimes occur in dense clusters), it has also been collected from surrounding areas on sandy soils with surface limestone, from limestone cliff faces as well as on the slopes of dolomite koppies. The species also grows on sand flats and between coarse rocks of river banks and spans altitudes of 300 – 1300 m.

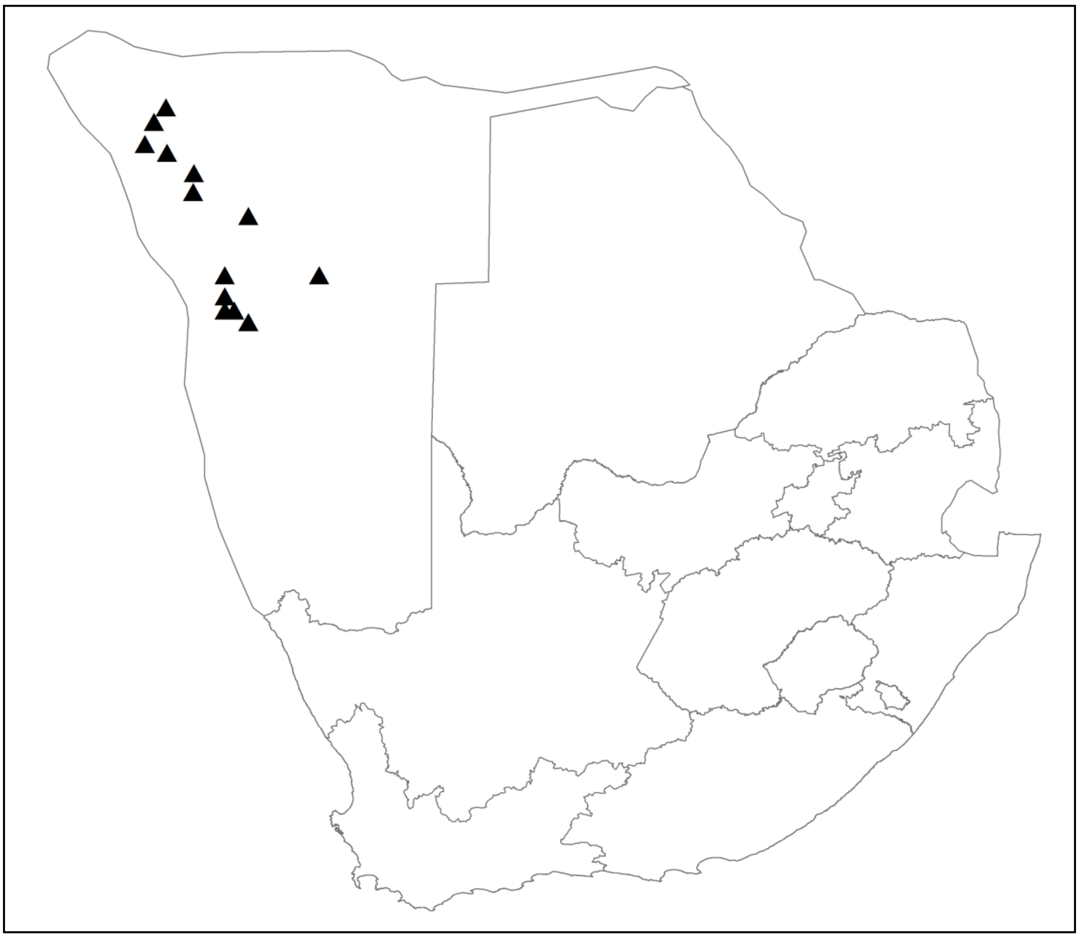

Figure 5.6. Distribution of *B. damarensis* in southern Africa

Diagnostic features

Barleria damarensis may be confused with *B. jubata* which also has pubescent stems. However, the two species can be differentiated by the presence of hairs with brownish glandular heads in addition to the white straight eglandular hairs (on the stem, leaf, bracteole and calyx), in *B. damarensis.* The leaves appear glabrous (though they possess microscopic hairs) and bracteoles are recurved in *B. damarensis* while the leaves are conspicuously pubescent and bracteoles are straight in *B. jubata*. However, *B. jubata* may sometimes possess glandular hairs on immature calyx lobes but they disappear with maturity. The recurved bracteoles also differentiate it from *B. stimulans* which it superficially resembles by possessing almost entire calyx lobe margins. This species is confined to the Kaokoveld and its fringes.

Additional specimens examined

Namibia. –1714 (Ruacana Falls): Kaokoveld, 20 miles south of Ruacana, Bergveld (–CD), 14 June 1963, *Giess & Leippert 7588* (NBG). –1813 (Ohopoho): Kaokoveld Reserve, more or less 4 m. N. of Ombombo Waterhole, dolomite koppie (–BD), 17 May 1957, *de Winter & Leistner 5818* (PRE). –1814 (Otjitundua): Otjitoko, 35 miles S. of Ohopoho (–AA), 3 June 1957, *de Winter & Leistner 5910A* (PRE, WIND); 3 miles south of Otjitjekua (–CD), 5 June 1963, *Giess & Leippert 7340* (WIND). –1914 (Kamanjab): 50 miles from Kamanjab on the road to Ohopoho (–DA), *Davies*, *Thompson & Miller 23* (WIND). –1915 (Okaukuejo): Etosha Pan, 1 km south of the pan (–DC), 18 April 1978, *Giess 15085* (PRE, WIND). –2016 (Otjiwarongo): 3 miles north of Otjovasandu (–AA), Joubert 165 (WIND). –2115 (Karibib): Ameib NC (–DC), March 1972, *Jensen s.n.* (WIND); (–DC), 22 April 1972, *Jensen 49/1434* (WIND). –2117 (Otjosondu): on the mountain caves, ca 1300 m (–BD), 16 March 1967, *Seydel 5501* (SRGH). –2215 (Trekkopje): Farm Tsabichab (Part of Navachab) (–BA), 10 May 1973, *Giess 12743* (PRE); Namibland, Okongava, at the foot of the Sargdeckel Mountain, 329 m (–BB), 11 April 1962, *Seydel 3100* (SRGH). –2216 (Otjimbingwe): Sandflats, 8.6 miles N. of Otjimbingwe, 900 – 1200 m (–AC), 5 May 1955, *de Winter 2650* (PRE, WIND).

5.4.3.6 Barleria jubata

B. jubata S. Moore in Journal of Botany 45: 229 (1907). Obermeijer in Annals of the Transvaal Museum 16: 154 (1933). Type: [Namibia], Damaraland, *Een s.n.* (BM, lecto.).

Erect branching shrublet, 60 – 120 cm high, stems arising from a woody rootstock. *Stems* endowed with long whitish-silver erect hairs all round, old bark whitish-grey. *Leaves* 16 – 40 × 7 – 16 mm, elliptic or elliptic-obovate, bluish-green, attenuate at the base); petiole pubescent, hispid at the base; lateral veins conspicuous on abaxial surface, leaf surface pubescent; midrib and lateral veins with horizontal and sub-erect hairs; leaf apex obtuse, with a sharp mucronate tip; leaf margin entire, flat, with erect hairs (sometimes hairs harden and appear tooth-like). *Flowers* 2 – 6 (may be more but usually the seventh is undeveloped. *Bracteoles* pubescent, 11 – 29 × 1.5 – 4.5 mm, lanceolate to narrowly ovate, with 2 conspicuous outer veins parallel to the mid-vein; margin spiny, with a straight spinous apex. Outer *calyx* lobes ovate, pubescent (pubescence more intense at the base), posticous lobe 19 – 30 × 5 – 11 mm; anticous lobe 16 – 25 × 4 – 9 mm; outer calyx lobe margin spinous. Inner calyx lobes half the length of the posticous lobe and two thirds the length of the anticous lobe, narrowly ovate with a single vein, sharp apex and entire margin. Flowers in December, April to July. *Corolla* pubescent, blue, bright violet or purple. *Fruit* 13 – 16 × 4 – 6 mm.

Distribution and habitat

Barleria jubata is known from specimens that were all collected from and around Otjiwarongo on the Waterberg Plateau and surrounding areas (Figure 5.9). The Waterberg is situated on the western edge of the Kalahari Sandveld and consists of Karoo sandstone underlain by shales and mudstones. It is surrounded by Thornbush savanna and attains altitudes of 1500 – 1900 m (Wardell-Johnson, 2000). This species can be said to be confined to the Karoo sandstone and high altitudes.

Diagnostic features

Although *Barleria jubata* may be confused with another morphologically similar species, *B. elegans*, it can be recognised by its ashy white pubescence on the stems, bracteoles and calyx lobes. *Barleria jubata* can also be differentiated from *B. damarensis* by its pubescent leaves, the lack of glandular hairs on stems, leaves, bracteoles and mature calyx lobes in addition to the lack of recurved bracteoles. It is confined to the Waterberg in Namibia.

Figure 5.7. Scanned specimen of *Barleria damarensis*, *Giess* and *Leippert 7588* (NBG). Scale: 2 cm.

Additional specimens examined

Namibia. –2016 (Otjiwarongo): Okosongomingo (–BC), 11 June1939, *Volk 2249* (M); 13.9 miles S. of Otjiwarongo on road to Okahandja, Farm Tweekoppies (–DA), 15 March 1955, *de Winter 2739* (K); Okanjande, Erongo Mts (–DA), June – July 1916, *Pearson 9895* (BOL, NBG); Farm Graslaagte, on Grey Mountain (also found on the Ozondjaheberg) (–DB), 28 November 1952, *Walter 283* (B, M). –2017 (Waterberg): 65 km E of Otjiwarongo, Waterberg plantation, on the slope down from the plateau to the plain (–AC), 3 March 1968, *Wanntorp & Wanntorp 641* (K); Waterberg – Abhang (–AD), 25 July 1939, *Volk 2472* (M); (Okarukuvisa): Waterberg Plateau, north of Farm Bergtuine: OTJ 455, on red sandstone cliffs (–AD), 21 July 1972, *Geiss 12375* (MO, PRE); upper slopes at S.W. point of the Omuverume Plateau (–CA), 22 April 1971, *Rutherford 352* (WIND); Waterberg Plateau (–CA), December 1935, *Boss 34948* (PRE); Klein Waterberg above Okosongomingo (–CA), 15 July 1954, *Schelpe 201* (J).

5.4.3.7 Barleria pungens

B. pungens Linn. f. in Suppl., 290 (1781); Thunb. in Prod. 1: 98 (1794); Linnaea in Journal of Botany 15: 358 (1841); Nees in DC., Prod. 11: 236 (1847) excluding *B. pungens* var. *macrophylla*; Anderson in Journal Linn. Soc. 7: 28 (1863); C.B. Clarke in Flora Capensis 5 (1): 47 (1901); Oberm. in Annals of Transvaal Museum 16: 152 (1933). Type: [South Africa, Eastern Cape], area between Kabeljous and Sondags Rivers, *Thunberg s.n.* (C, holo.! photograph seen at J; BM, homo.).

Barleria irritans Nees var β, Nees in DC., Prod. 11: 236 (1847). Type as above.

Crabbea pungens Harv. Gen. S. Afr. Pl. 276 (1838).

Perennial multi-stemmed spreading shrublet. *Stems* sub-erect or decumbent, up to 50 cm high, stems arising from a woody rootstock, with brownish-cream erect hairs all round. *Leaves* 10 – 30 × 5 – 14 mm, elliptic-ovate, ovate to broadly so, midrib with erect hairs; apex sharp; petiole very short, hispid; leaf margin shallowly wavy, with or without white colour, leaf margin minutely toothed or hairy, hairs on raised bases. *Bracteoles* 11 – 22.5 × 2 – 8 mm, lanceolate to ovate, thinly hairy, with 2 conspicuous veins parallel to the mid-vein (sometimes with 4 where the two outermost are very short), tip spinous; margin with a few scanty teeth, teeth pubescent to glabrescent. Outer *calyx* lobes broadly ovate, spine tipped; anticous calyx lobe 14 – 26.5 × 4 – 13 mm, posticous calyx lobe 15 – 30 × 5 – 13.5 mm; inner calyx lobes narrowly ovate, with single vein and sharp apex. *Corolla* tube 24 – 40 mm long, with lower narrower tube almost twice the length of the upper wider tube. *Flowers* blue, pink, mauve, lavender, violet, or purple. Flowers in November, January to June. *Fruit* 10 – 15 × 4 – 5 mm.

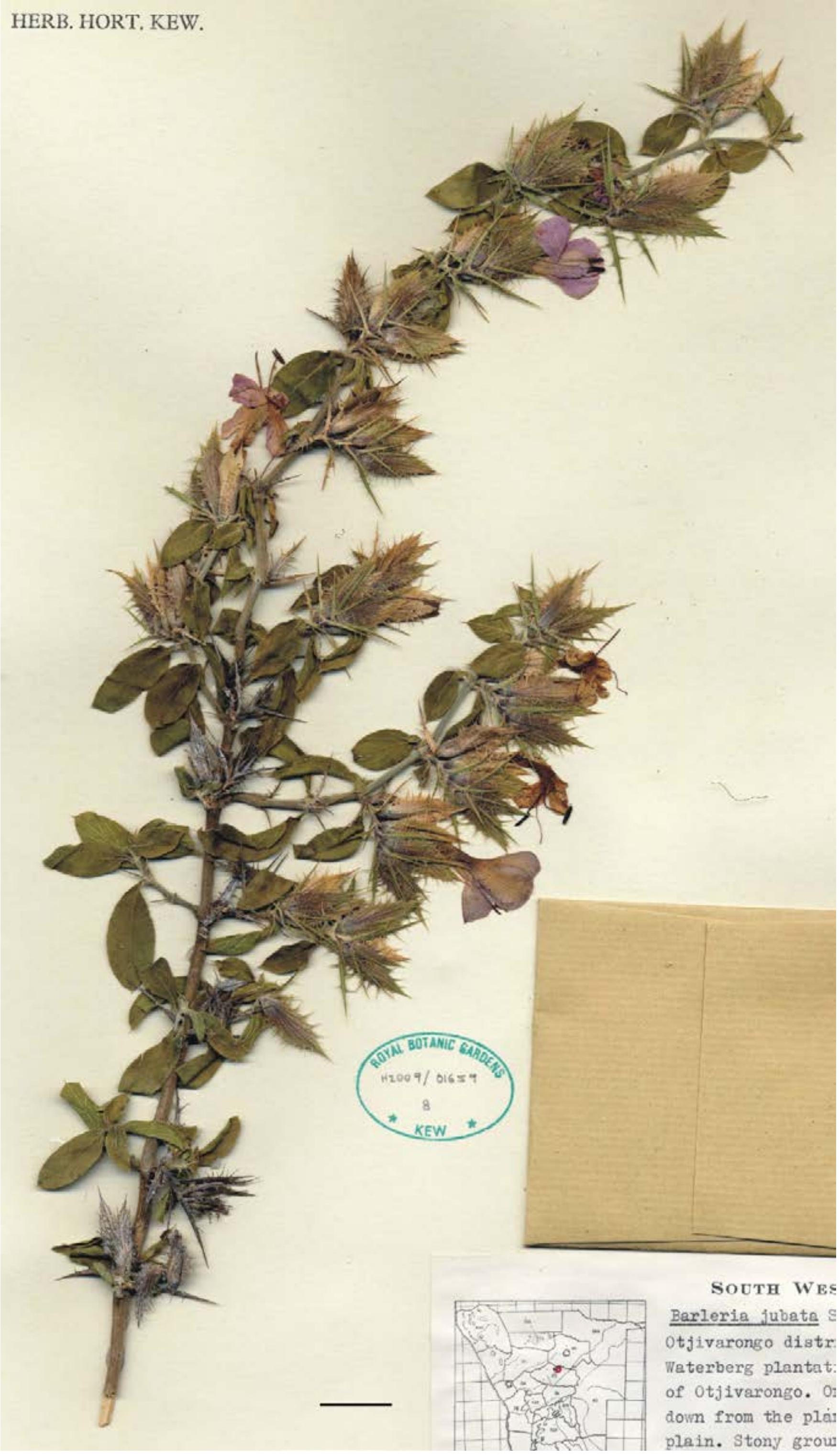

Figure 5.8. Scanned specimen of *Barleria jubata*, *Wanntorp and Wanntorp 641* (K). Scale: 2 cm.

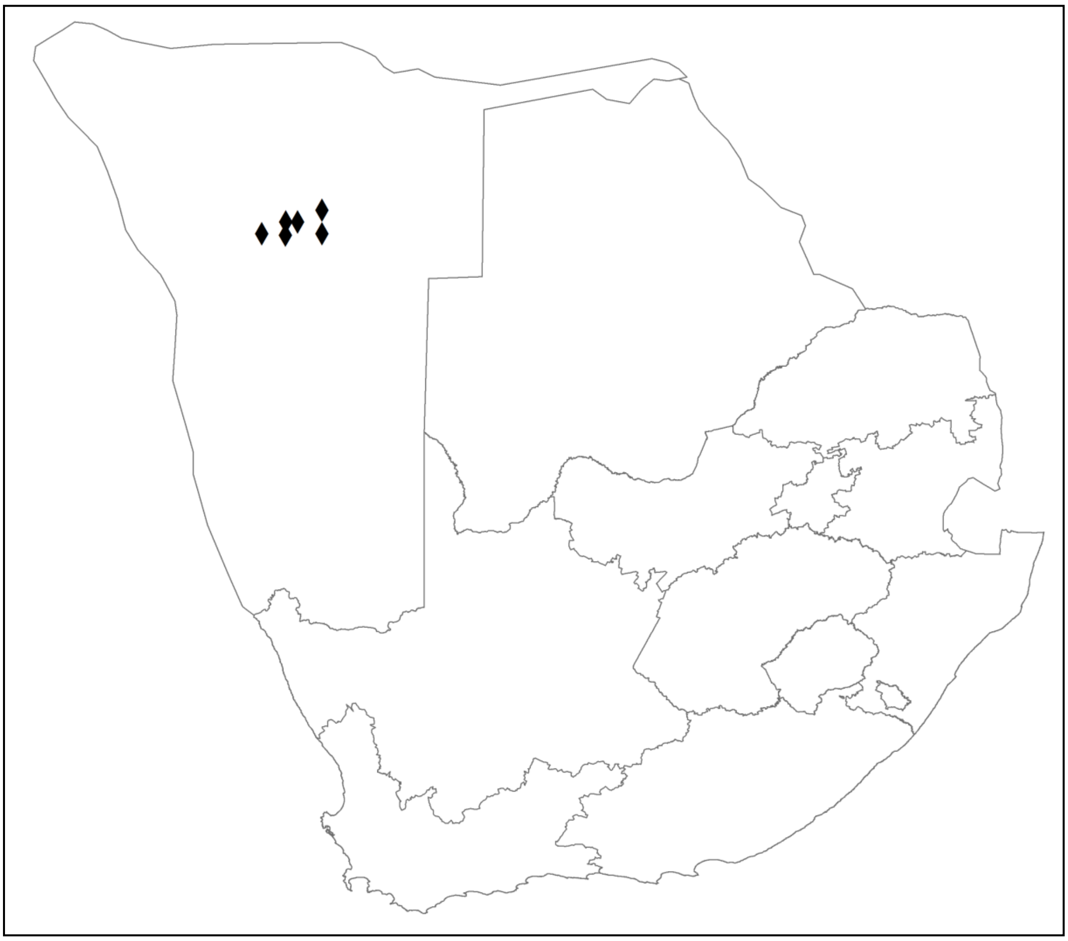

Figure 5.9. Distribution of *Barleria jubata* in southern Africa.

Distribution and habitat

Barleria pungens has been observed to occupy the southern coast of Western and Eastern Cape in the southern Fynbos, East Coast Renosterveld, South Coast Fynbos, Eastern Fynbos-Renosterveld and Albany Thicket (Figure 5.10). *Barleria pungens* is widespread and variable within its range. Specimens with prominent white leaf margins and toothed or stiff-haired bracteole margins occur in the Albany Thicket, and those with thin white leaf margins and toothed or stiff-haired bracteole margins inhabit the East Coast Renosterveld and Albany Thicket and do not occur in the intermediate Eastern Fynbos-Renosterveld. Specimens with green leaf margins and entire or stiff-haired bracteole margins occur throughout the species distribution range. This species grows on dry stony and shallow soil over weathering granites with varying amount of gravel and surface limestone, which may be on gentle or flat slopes. The species inhabits varying altitudes from 0 to 1000 m.

Diagnostic features

Barleria pungens resembles *B. irritans, B. uninervis* and *B. foliciliosa* by having ovate leaves. However, the leaves in *B. pungens* are pubescent with erect hairs on raised bases with the pubescence being more prominent on the leaf margins, midrib and lateral veins, the leaves in *B. foliciliosa* are pubescent with very short hairs in addition to the inconspicuous appressed hairs on the leaf margins, midrib and lateral veins, while the leaves in *B. irritans* and *B. uninervis* are glabrous. *Barleria pungens* can also be differentiated from *B. foliciliosa* by its wavy leaf margins.

Notes

Barleria pungens is a polymorphic species with various leaf shapes ranging from broadly ovate leaves with cordate bases to narrowly ovate or elliptic-ovate leaves with attenuate bases and white or green wavy leaf margins. Intergradations of leaf margins from green to thin white may also be found on one specimen. Other forms include inconspicuous or conspicuous lateral veins on the abaxial surface of the leaf and when lateral veins are conspicuous they exhibit brochidodromous venation as seen in *Fourcade 613a* (BOL).

Additional specimens examined

South Africa. –3225 (Somerset East): Cradock, near Port Elizabeth (–BA), 12 May 1902, *Galpin 6360* (PRE). –3325 (Port Elizabeth): (–DC), 1 February 1927, *Moss 15394* (J); (–DC), 31 May 1919, *Mogg 4761* (J); (–DC), 30 August 1030, *Fries, Nordlindh & Weimarck 463* (PRE); Markman Industrial area (–DC), 23 February 1971, *Dahlstrand 2715* (GRA, J); New Brighton (–DC), January 1901, *Galpin 5879* (PRE). –3326 (Grahamstown): Albany, 5 miles NW of Grahamstown, False Renosterveld (–AB), 24 March 1950, *Accocks 15732* (PRE); north-eastern boundary of the farm “Mountain Top”, Carlisle Bridge area, on the Fish River ridge, 33º 00’S 26º 20’E (–AB), 19 April 1995, *Bowker s.n.* (GRA); Sugar Loaf Hill (–AD), April 1950, *McGillivary 2951* (J); 20 km from Grahamstown towards Fort Beaufort, Kaap (–BA), 26 February 1976, *von Teichman 361* (PRE); Ecca Reserve, 33º 13’ 40”S 26º 38’ 5”E (–BA), 6 March 1992, *Chan 6* (GRA); Botha’s ridge (–BC), 24 March 1962, *Wells 2631* (GRA); Bothas Hill 11 miles from Grahamstown junction, between Karoo and Scrub veld (–BC), May 1928, *Dyer 1414a* (J); Peddie road 15 – 16 miles from Grahamstown junction, between Karoo and scrub veld (–BD), April 1928, *Dyer 1358* (GRA); Alexandria (–CB), 24 March 1952, *Archibald 4154* (J). –3419 (Caledon): Hermanus, Bushman’s River mouth, vacant plots at the extension (–CB), 10 January 1989, *Green 591* (J). –3420 (Bredasdorp): Swellendam, in the paddock near the gate, close to the roadside, 34º 5.9’S 20º 25.4’E, on gentle slope (–AB), 11 July 2008, *Nyirenda 719* (J). –3421 (Riversdale): Soetmelksrivier, plateau N of bridge on main road (–AB), 25 February 1981, *Bohnen 7829* (PRE); 2 km NW of Soetmelksrivier bridge (–AB), 20 February 1980, *Bohnen 7371* (PRE); Riversdale (–AB), 19 November 1912, *Schlechter 1985* (BOL); Riversdale (–AB), September 1960, *Horn 2286* (PRE); near settlement, 34º 5.77’S 21º 15.24’E (–AB), 9 July 2008, *Nyirenda 716* (J); Albertina, about 27 km from Mossel Bay to Albertina, on N2 road, Farm Van Rensburg, 34º 10.78’S 21º 53.08’E (–AB), 8 July 2008, *Nyirenda 707, 708, 710* (J); about 7 km from Albertina, along N2 road, Farm Wolwekraal, 34º 12.46’S 21º 38.74’E (–CB), 8 July 2008, *Nyirenda 712* (J). –3422 (Mossel Bay): between George and Great Brakriver, next to national road, near the Maalgaten river bridge (–AB), 1 January 1987, *Vlok 1844* (PRE). –3423 (Knysna): hills above Keurboom Strand (–AB), 30 January 1949, *Walgate s.n.* (BOL); Plettenberg Bay (–AB), March 1910, *Fourcade 613a* (BOL). –3424 (Humansdorp): Kaus and Kay, 1700 ft. (–BA), March 1026, *Fourcade 3239* (BOL); Humansdorp (–BB), January 1912, *Burtt-Davy 12000* (BOL); between

Kabeljau and Mondplaas along Nat. Road (–BB), 11 January 1993, *Fourcade 5931* (BOL).

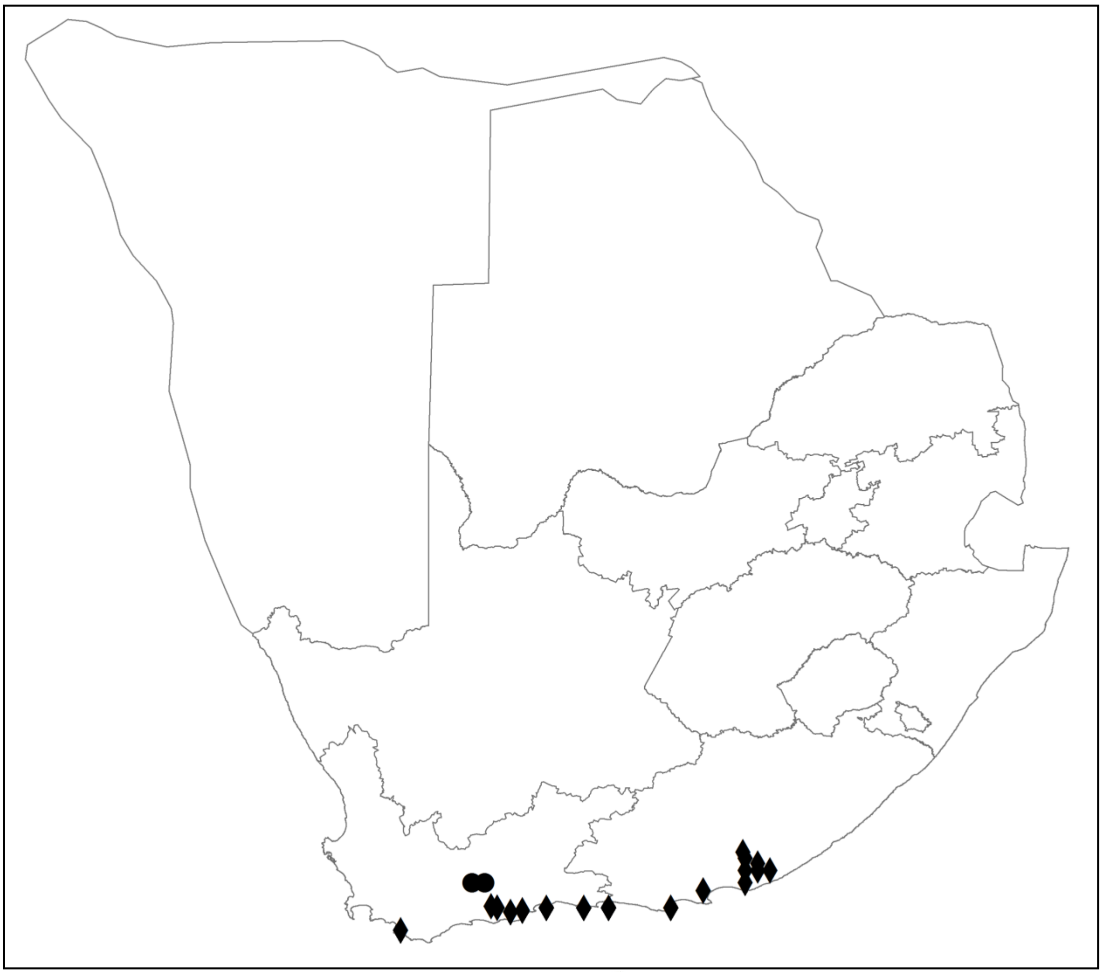

Figure 5.10. Distribution of *Barleria pungens* (♦) and *Barleria foliciliosus* (●) in southern Africa.

5.4.3.8 Barleria bracteilobis

B. bracteilobis Nyirenda *sp. nov.* affinis *B. rigida* Nees, sed foliis 16 – 36 mm longa, (non 7 – 15, 37 – 55 mm), bracteis latus 3 – 10 mm lata basibus lobatus apicibus acuminatus, calyce lobis exteriora late ovatus 8 – 13 mm lata differt. Type: Namibia, [Luderitz], desert, cliff face, sandy soil, 26º 7' 30"S 16º 22' 30"E, 27 May 2001, *Theile THE89* (WIND, holo.!).

Perennial heavily branched shrublet. *Stems* upright, up to about 60cm high, glabrous to glabrescent; young stems bifariously hairy. *Leaves* narrowly ovate to elliptic-ovate, 16 – 36 × 4 – 12 mm, glabrous; midrib with short brownish hairs. Leaf apex with a spine, leaf margin thick, white, sinuate, with well developed straight spines, 0.5 – 3 mm long. Leaf petiole, 1.5 – 4 mm long. *Flowers* 3 – 8, in compact lateral cymes. *Bracteoles* membranous, 17 – 22 × 3 – 10 mm, ovate, glabrous, lamina with 3 or more conspicuous parallel veins, forming reticulations toward the margin, margin spinous with delicate spines almost at right angles to the margin, bracteole base lobed, bracteole apex with a spineless tapering tip, half to one third the length of the bracteole. Outer *calyx* lobes thin papery, broadly ovate, glabrous, calyx lobe margins with thin delicate spines at right angles to the margin; posticous lobe broad, 18 – 27 × 8 – 13 mm, almost twice the width of the anticous lobe and partially enveloping the edges of the anticous lobe; anticous lobe 16 – 22 × 4 – 8 mm wide. *Corolla* tube about 25 – 27 mm long, glabrous, mauve, rose or bright blue. Flowers March to August. *Fruit* not seen.

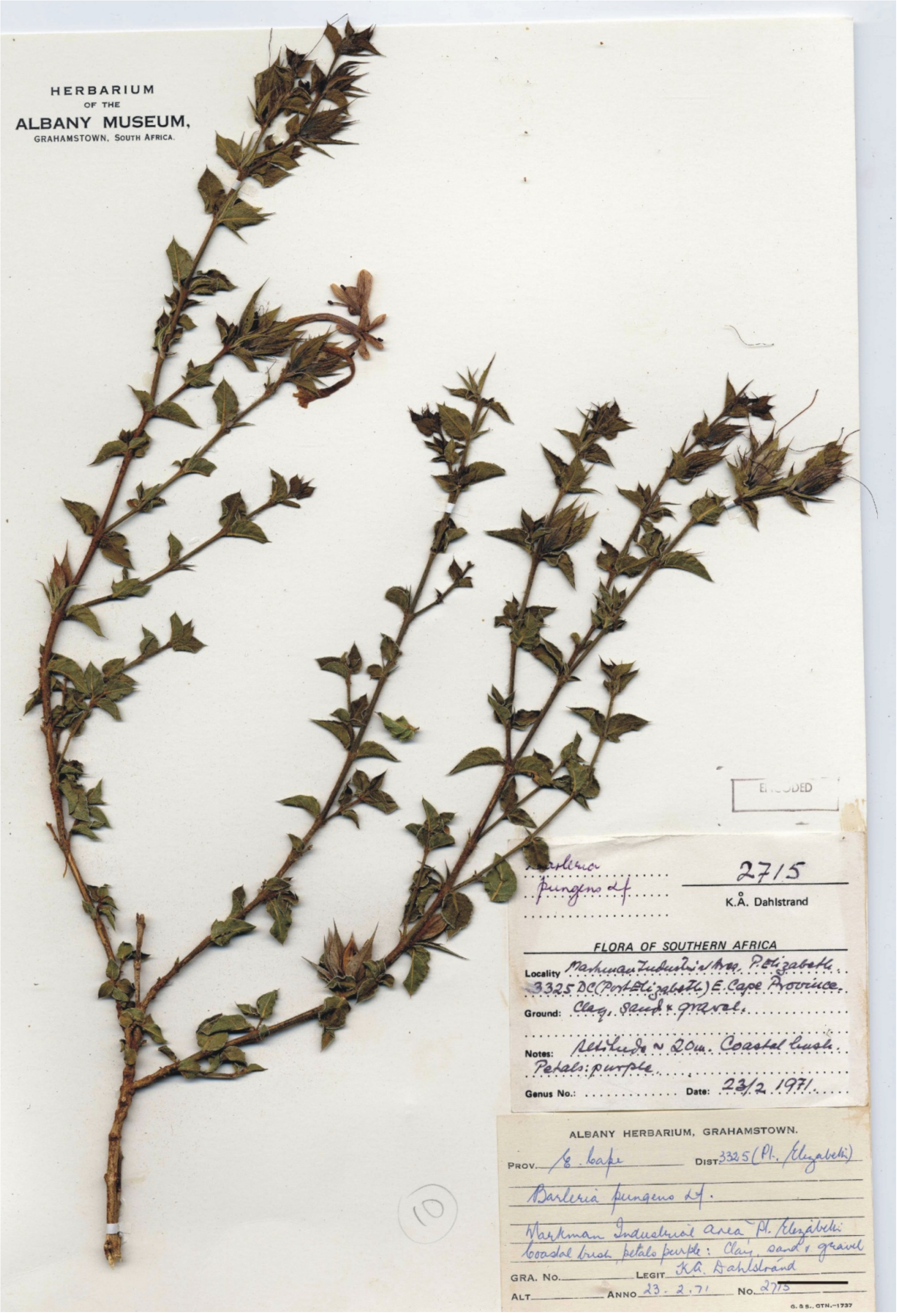

Figure 5.11. Scanned specimen of *Barleria pungens*, Dahlstrand 2715 (GRA). Scale bar: 2 cm.

Distribution and habitat

This species is known from seven specimens, occurring along coastal areas on sandy and rocky soils of the southern Namib Desert (Figure 5.13). It has been collected within three degrees north to south and two degrees east to west. It is recorded from dry pans, and on the cliff face of granite hills in Luderitz, in rocky watercourses in Bethanien and on slopes of basalt outcrops in Maltahöhe. It also grows on dry rocky gravelly sand underlain with granite, at altitudes of 400 – 1500 m. Its distribution range could therefore be assumed to be narrow and restricted until it is reported from other areas. This species can be assumed to be rare and endemic to this area of the southern Namib Desert.

Diagnostic features

Barleria bracteilobis has leaves that are narrowly elliptic or elliptic-ovate with sinuate leaf margins and can be confused with *B. rigida* var. *ilicina*. However, *B. bracteilobis* has characteristically broad ovate bracteoles with lobed bases and tapering tips and broadly ovate posticous calyx lobes almost twice the size of the anticous lobe, which, is absent in *B. rigida* var. *ilicina* and the other members of the *B. rigida* complex. In addition *B. bracteilobis* can be differentiated from *B. rigida* var. *rigida,* to which it is superficially similar in terms of habit by, the leaf margins which are undulate and entire (or with scanty teeth) in *B. rigida* var. *rigida*.

Notes

Although *B. bracteilobis* is highly distinctive and not likely to be confused with *B. rigida* with which it has a superficial resemblance, it has remained undescribed until now. Despite the earliest collections of *B. bracteilobis* having been done in July 1949 by *Kinges 2338* (typed *2388*) (M, PRE), the species has remarkably remained unknown and has been filed under the name *B. rigida* Nees.

Additional specimens examined

Namibia. –2416 (Maltahöhe): slope of Basalt outcrop (–DD), 10 April 1979, *Lind 406* (WIND); Farm Hammerstein MAL 102 (–CC), 17 February 1963, *Leippert 4895* (WIND). –2516 (Helmering-hausen): Farm Naus 27 (–CD), 21 April 1980, *Owen-Smith 1317* (WIND). –2615 (Luderitz): Farm Weissenborn, in rocks behind farmhouse (–DA), 5 July 1949, *Kinges 2338 (2388)* (M, PRE); 15 miles west of Aus, east facing slopes of Sesselberge, dry sandy riverbed (–CB), 13 August 1959, *Giess & van Vuuren 848* (PRE, WIND, SRGH); 48.3 m. S of Aus on road to Witputs, Coarse gravelly sand-flats and granite hills (–DD), 9 March 1963, *de Winter & Hardy 7944* (PRE).

Figure 5.12. Scanned type specimen of *Barleria bracteilobis*, *Theile THE89* (WIND, Holotype!). Scale bar: 1 cm.

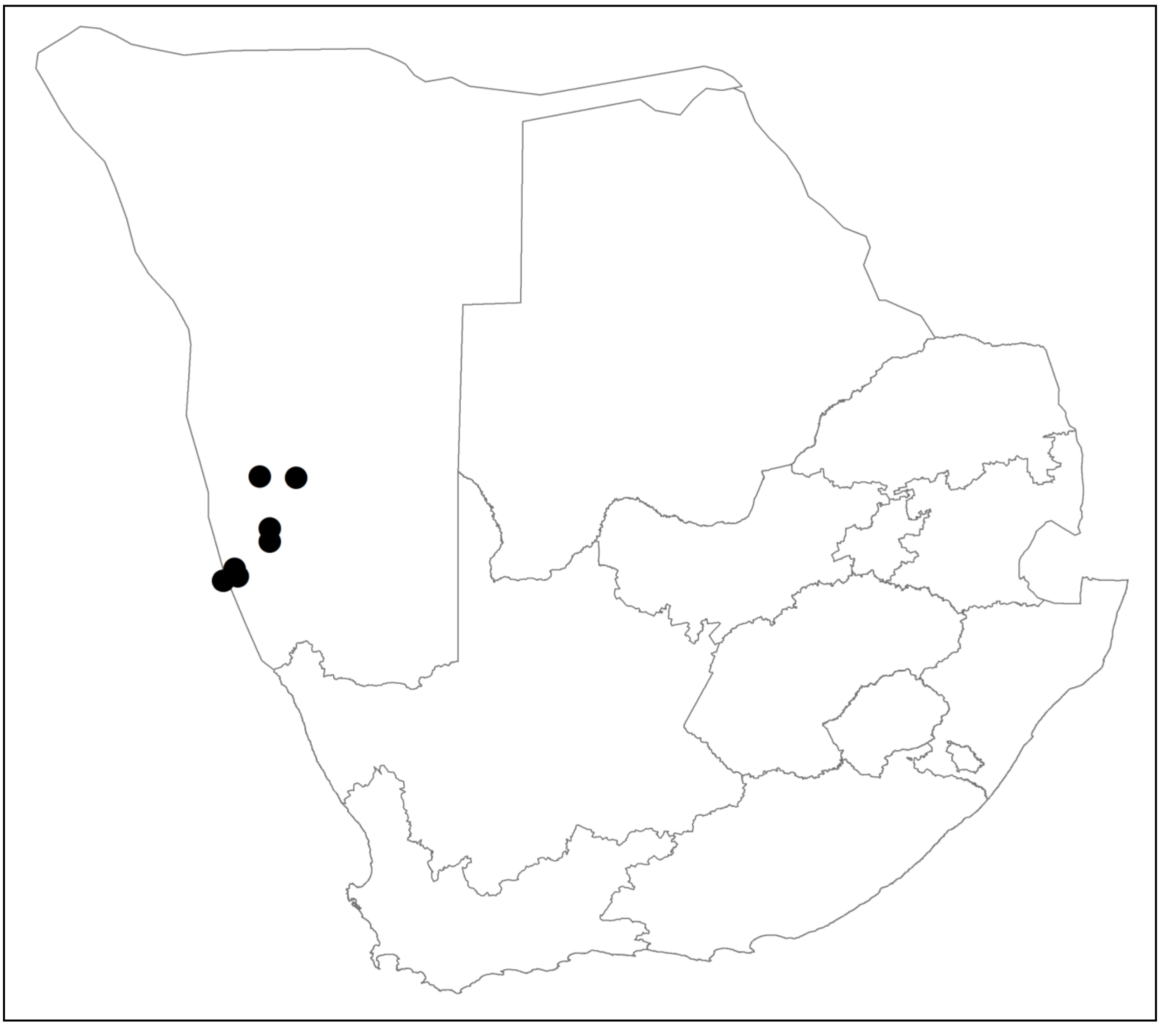

Figure 5.13. Distribution of *Barleria bracteilobis* in southern Africa.

5.4.3.9 Barleria levitundulata

B. levitundulata Nyirenda *sp. nov. affinis B. bechuanensis* C.B. Clarke sed foliis linearis (non oblanceolatus), latius [3 – 5 mm lata, non (1.4 –) 1.8 – 2.5 (3) mm], margine leviter undulata (non spinoso-dentatis), floribus aggregata 1 – 4, calycibus lobis exteriora margine dentatis spina de-basi differt. Type: [South Africa] North West, –2724 Vryburg, Dwaalvlakte (–AB), 1912, *Sharpe 8148* (PRE, holo.!).

B. bechuanensis sensu Oberm. in Annals of Transvaal Museum 16: 153 (1933), non *B.bechuanensis* C.B. Clarke Fl. Cap. (Harvey) 5(1): 48 (1901). synon. nov

Multi-stemmed perennial shrublet, prostrate, decumbent or upright to about 20 cm high. *Stem* bifariously hairy, arising from a woody rootstock. *Leaves* 10 – 28 × 3 – 5 mm, linear, glabrous, apex narrow, with mucronate tip, midrib glabrous, leaf margin cartilaginous, white, shallowly wavy, entire; spine absent in sterile leaf axils. *Flowers* clustered, 1 – 4, in opposite axils. *Bracteoles* of a pair at the base of each flower, equal or conspicuously unequal, lanceolate, 11 – 19 × 2 – 4 mm, bracteole margins sharply spine-toothed. Outer *calyx* lobes ovate or elliptic-ovate, calyx lobe margin spine-toothed from the base, teeth white, anticous calyx lobe 13 – 20 × 4 – 6 mm, posticous calyx lobe 15 – 23 × 4 – 8 mm. *Corolla* pink or white, corolla tube up to 24 mm long, lower narrow tube almost equal in length to the upper wider tube, corolla lobes orbiculate, 9 – 11 × 6 – 9 mm. Flowers February to May. *Fruit* elliptic, 8.5 – 12 × 3.5 – 5 mm.

Figure 5.14. Scanned type specimen of *Barleria levitundulata*, *Sharpe 8148* (PRE, Holotype!). Scale bar: 1 cm.

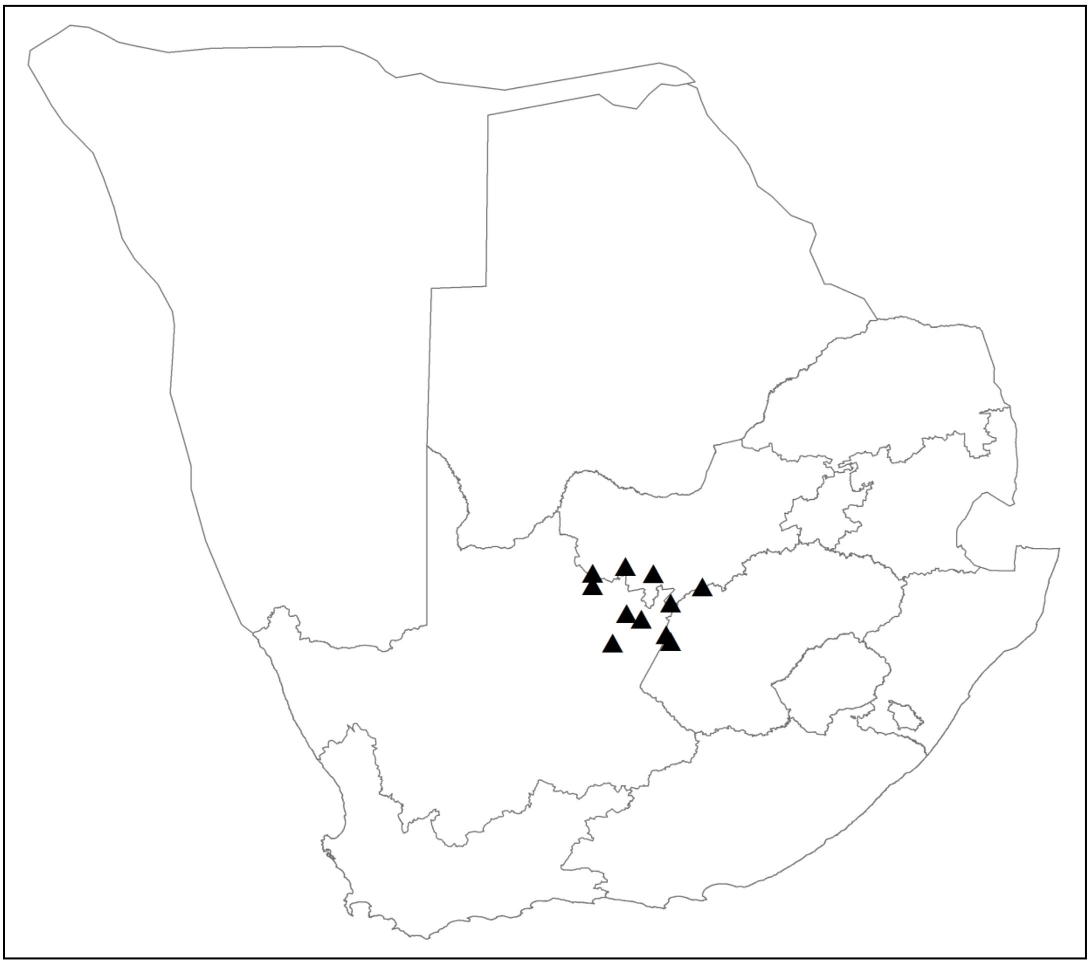

Figure 5.15. Distribution of *Barleria levitundulata* in southern Africa

Distribution and habitat

This species is known only from South Africa. It inhabits the Schmidtsdrift Thornveld vegetation on shale and dolomite of the Schmidtsdrift Subgroup (Figure 5.15). It also occurs on the Ghaap Plateau Vaalbosveld with surface limestone, and dolomite and chert of the Campbell group (Mucina and Rutherford, 2006) in addition to soils underlain by calcrete and dolerite. *Barleria levitundulata* grows on level hill tops as well as rocky slopes on shallow sand enriched with iron ore. It has been collected at altitudes between 1100 and 1500 m.

Diagnostic features

Barleria levitundulata resembles *B. rigida* in that it always has linear leaves with undulate leaf margins but can be distinguished from *B. rigida* by the lack of teeth on the leaf margin, the presence of rough veins on the bracteole and external calyx lobes, and the external calyx lobes lack a conspicuous reticulation. *Barleria levitundulata* can be differentiated from *B. bechuanensis* which sometimes (but not always) has wavy leaf margins by its wider linear leaves with attenuate bases, external calyx lobe margins that are spinous from the base and 1 – 4 flowers loosely arranged in lateral cymes.

Notes

Barleria levitundulata, though with restricted distribution, has been well collected and is here known from 14 specimens filed under the name *Barleria bechuanensis* C.B Clarke. The specimens *Wilman 16418* (BOL) and *Burtt-Davy 12930* (BOL) that Obermeijer (1933) identified as *B. bechuanensis* C.B Clarke *sensu lato,* however, are here recognized as *B. levitundulata sensu stricto.*

Additional specimens examined

South Africa. –2723 (Kuruman): Bophuthatswana, Nyara Farm, NW-facing on slight slope of plain (–AD), 27 February 1982, *Gubb 258/39* (PRE); Newstead Farm, on level plain at top of hill (–CB), 4 March 1981, *Gubb 184-41* (PRE). –2724 (Taung): Kormutsetla Farm, on gently sloping plain to the W (–AA), 12 May 1982, *Gubb 323-95* (PRE); Boetsap, rocky slope (–CD), 17 February 1945, *Brueckner 136* (PRE). –2725 (Bloemhof): Cawoods' Hope (–DA), March 1912, *Burt-Davy 12930* (BOL). –2822 **(**Glen Lyon): Beeshoek Farm, steep slope (–BD), 30 April 1981, *Gubb 219/78* (PRE). –2823 (Griekwastad): Herbert, 6 km east of Campbell, 28º 47.80'S 23º 47.28'E, Karoo type vegetation, on limestone (–DD), 3 April 2000, *Balkwill and McDade 11756* (J). –2824 (Kimberly): Koopmansfontein, Agric. Res. Station (–AA), 14 April 1964, *Nursey 133* (PRE); Barkly West, Newlands (–AD), 17 March 1939, *Lewis s.n.* (NBG); Newlands (–AD), March 1939, *Esterhuysen 1008* (BOL); Riet Pan, March 1920 (–CA), *Wilman 16418* (BOL); 3 mls east of Kimberly (–DA), 11 May 1961, *Schlieben 8715* (PRE). Precise locality unknown: date and collector unknown (curator *Pretoria 198*), (PRE).

5.4.3.10 Barleria irritans

B. irritans Nees in Linn. 15: 359 (1841); Nees in DC., Prod. 11: 236 (1847); Linnaea in Journal für die Botanik 23: 92 (1850); Anderson in Journal Linn. Soc. 7: 28 (1863); C.B. Clarke in Flora Capensis 5: 47 (1901); Obermeijer in Annals of Transvaal Museum 16: 133 (1933). Type: [South Africa], Uitenhage, Addo, Ecklon pl. cap. (S, holo., photograph seen on ALUKA; GZU, iso., photograph seen on JSTOR; SAM, iso).

Multi-stemmed dwarf shrublets, upright or sub-erect, arising from a woody rootstock. *Stems* bifariously hairy or lightly pubescent when young, glabrescent when mature. *Leaves* 9 – 29 × 3 – 9 mm, narrowly ovate, glabrous; leaf apex narrow, acute, distinctly mucronate; leaf petiole short, smooth; midrib glabrous; leaf margin white, shallowly wavy, minutely toothed; sterile bracteoles present in leaf axils. *Flowers* in clusters of 1 – 8 in alternate axils. *Bracteoles* a pair at the base of each flower, of equal or unequal length, 9 – 23 × 1.8 – 4.5 mm, lanceolate or narrowly ovate and with 3 parallel veins, glabrous, margins spine-toothed, teeth glabrous. Outer *calyx* lobes elliptic-ovate, anticous calyx lobe 11 – 27 × 3 – 8.5 mm wide, posticous calyx lobe 19 – 29 × 4 – 9 mm, calyx lobe margin spine-toothed. *Corolla* blue, mauve or purple; corolla tube 23 – 28 mm long, the upper wider tube about twice the length of the lower narrower tube. Flowers from December to April. *Fruit* elliptic, 11 – 15 × 3 – 5.5 mm.

Distribution and habitat

Barleria irritans occurs from the southern coast of Eastern Cape and extends inland north-west to Kirkwood, although one collection *Repton 5725* (PRE) is recorded as having been collected from Nauwpoort at the northern border of the Eastern Cape with the Northern and Western Cape in the Lower Karoo revealing some disjunction (Figure 5.17). This species has been recorded in the Albany Thicket from Gamka Thicket, Groot Thicket to Sundays Noorveld (Mucina and Rutherford, 2006). Although there appears to be a distribution range overlap around Steytlerville and Kirkwood with a morphologically similar species, *B. uninervis* which occurs in the Lower Karoo, fragments of the Albany Thicket, Lower Karoo, Rainshadow Karoo and the Eastern Fynbos-Renosterveld occur around this area (Mucina and Rutherford, 2006). The species is known to grow on well drained sand between rocks on river banks and hillsides, and in disturbed areas such as the roadside, lying on limestone ridges and quartzite base. *Barleria irritans* mainly occurs on steep slopes at altitudes ranging from 100 to 700 m.

Diagnostic features

Barleria irritans may be confused with *B. uninervis* and *B. pungens* in that they all have ovate leaves with wavy and minutely toothed margins. However, *B. irritans* can be differentiated from *B. pungens* by the glabrescent stem and glabrous leaf surface and leaf margins. *Barleria irritans* can be differentiated from *B. uninervis* by its 3-veined broader bracteole.

Additional specimens examined

South Africa.–3124 (Hanover): Noupoort, Karoo veld (–BB), December 1961, *Repton 5725* (PRE). –3325 (Port Elizabeth): Kirkwood, Zuurberg National Park, 33° 18' 26" S 25° 23' 11" E, Fynbos (–AD), 17 April 1999, *Brand 133* (PRE); Kirkwood, Foot of Great Swartberg, above Farm Groenfontain, in Renosterbosveld (–AD), December 1980, *Vlok 47* (PRE); Uitenhage Cap. b. sp. (–CA), *Ecklon 384 (79.2)* (M, PRE); Uitenhage (–CA), 13 December 1847, *Prior s.n.* (K, PRE); Uitenhage, 2.5 miles from Barkley Bridge along Alexandria railway line (–CA), 10 February 1953, *Marais 162* [*1 & 2*] (K, PRE); 11 km N of Uitenhage, Uitspan experimental plot of Dept. Plant Protection Unit (–CD), 10 March 1979, *Mauve 5238* (PRE); Kommands Kraal (–CD), *Zeyher 1421* (NBG); Uitenhage (–CD), *Harvey s.n.* (BOL); Addo Elephant Nattional Park, open veld (–DC), 12 February 1954, *Brynard 330* (PRE). –3326 (Grahamstown): Albany, Hell Poort entrance slightly less than 16 m from Grahamstwn on Cradock road (–AB), 21 July1975, *Brink 369* (PRE); (Double Drift): Fish River Valley, 33º 05’ 20”S 26º 47’ 05”E, hillside above river (–AD), 8 March 1981, *Phillipson 216* (GRA, PRE); Alexandria, limestone ridge S.E. of Sandflats (–CA), 2 April 1952, *Archibald 4234* (GRA, K, PRE). –3327 (Peddie): 2000 ft. (–AA), 1897, *Sim 20021* (NU, PRE).

Figure 5.16. Scanned specimen of *Barleria irritans*, *Mauve 5238* (PRE). Scale bar: 1 cm.

5.4.3.11 Barleria rigida

B. rigida Nees in DC., Prod. 11: 242 (1847); Linnaea in Journal für die Botanik 23: 92 (1850); Anderson in Journal Linn. Soc. 7: 28 (1863); C.B. Clarke in Flora Capensis 5 (1): 48 (1901); Obermeijer in Annals of the Transvaal Museum 16: 152 (1933). Type: [South Africa], Grootriviers Poort in prom. B. Sp., *Burchell 1991* (K, holo., microfiche seen at J).

B. stimulans E. Mey. ex Nees var. *macracantha* Nees in DC., Prod. 11: 241 (1847) (*Burchell 1621* in h. Hook.) *Burchell 1621* (K, photograph seen on JSTOR).

B. irritans Nees var. β *rigida* C.B. Clarke, Flora Capensis 5 (1): 48 (1901).

B. rigida Nees var. *ilicina* (E. Mey. ex T. Anders.) Oberm. in Annals of the Transvaal Museum 16: 153 (1933). Type: [South Africa], Garip in Africa australi, 19 September 1830, *Drège s.n.* (S, holo.).

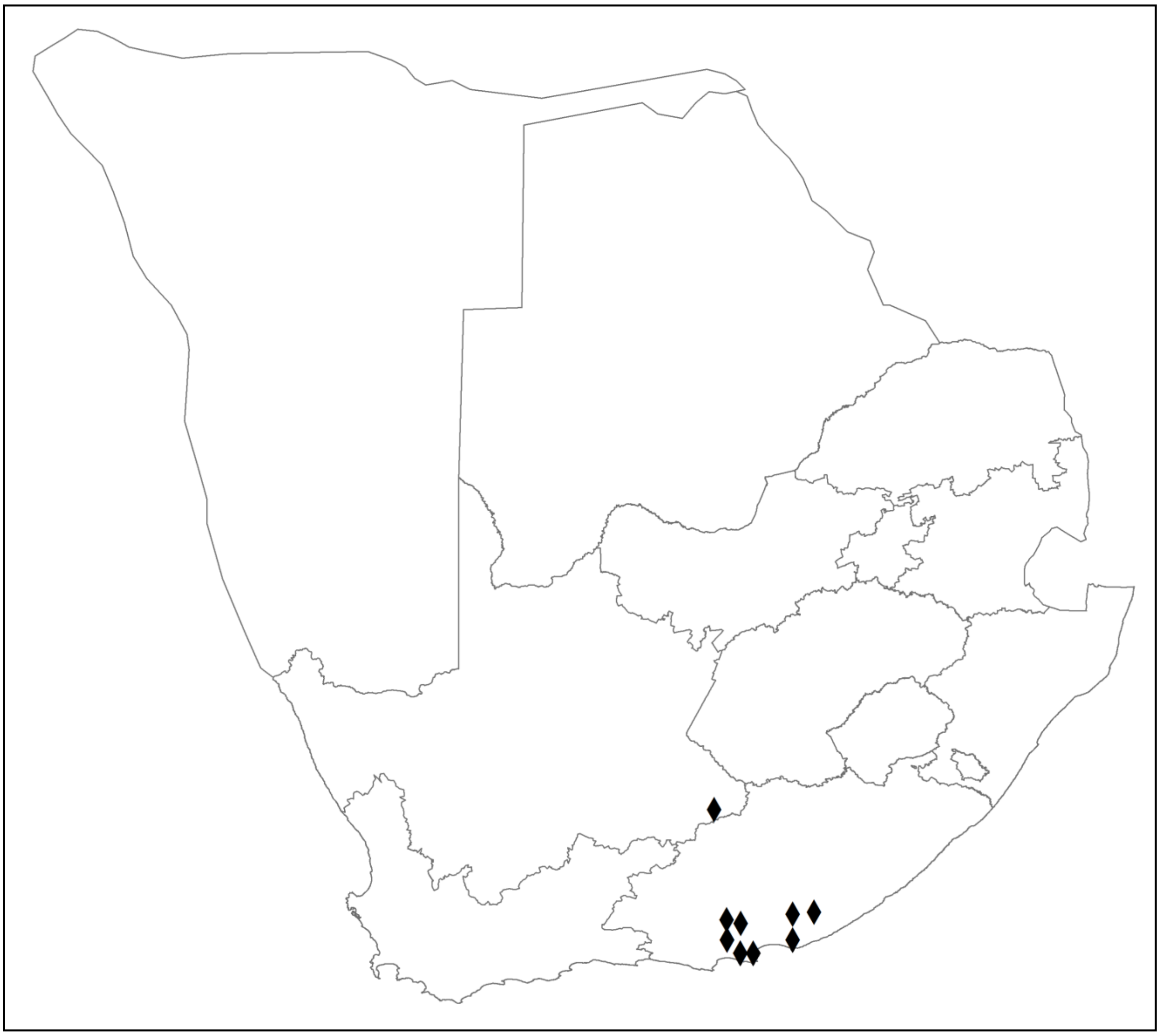

Figure 5.17. Distribution of *Barleria irritans* in southern Africa

Perennial woody shrublet. *Stems* arising from a woody rootstock and branching above the ground, up to 30 cm high or more; old stems glabrescent to glabrous, young stems may be bifariously hairy or covered in very short hairs. *Leaves* linear, elliptic-ovate or ovate, glabrous 7 – 55 × 1.5 – 11 mm; midrib glabrous; apex spinous; leaf margin white, thickened, undulate or sinuate, entire or spine-toothed, spine 0.4 – 3 mm long; petiole short 0.5 – 5 mm long. *Flowers* solitary or clustered in lateral cymes of 1 – 10 flowers, in alternate or opposite axils. *Bracteoles* 7 – 26 × 1 – 6 mm, glabrous, margin spinous or with a few scattered teeth to almost entire; bracteole lamina developed or reduced to almost a spine. Outer *calyx* lobe ovate, glabrous, with or without a purplish tinge on veins, outer calyx lobe margins serrate or spine-toothed; posticous lobe 13 – 23 × 3 – 9 mm, anticous lobe 10 – 21 × 3 – 9 mm. Corolla glabrous, flowers white, cream, pink, lilac, mauve, blue, or purple. Flowers from January to June. *Fruit* 8 – 13 × 3 – 5 mm.

Three forms exist within *B. rigida* which exhibit some morphological and distributional dissimilarity (though with narrow overlaps) and have been recognised at the level of variety. A key to the varieties is given below followed by a detailed account for each variety.

5.4.4 Key to the varieties within Barleria rigida

1a. Bracteole ovate, narrow, lamina reduced almost completely, with 1 prominent vein, v-shaped in cross-section … 2

1b. Bracteole lanceolate, somewhat flat, broad, lamina well developed (leafy), usually with 3 parallel veins ... ***B. rigida*** **var.** ***latibracteatus***

2a. Leaf linear, leaf margins undulate, with very few small scanty teeth (on the lower half of the leaf) to entire ... ***B. rigida*** **var.** ***rigida***

2b. Leaf ovate or elliptic-ovate, leaf margins usually sinuate and with small spines or well developed spines evenly distributed along the leaf margins ... ***B. rigida*** **var.** ***ilicina***

5.4.4.1 Barleria rigida var. rigida

B. rigida Nees in DC., Prod. 11: 242 (1847); Linnaea in Journal für die Botanik 23: 92 (1850); Oberm. in Annals of the Transvaal Museum 16: 152 (1933). Type: [South Africa], Grootriviers Poort in prom. B. Sp. *Burchell 1991* (K, holo.; J, microfiche!).

B. stimulans E. Mey. ex Nees var. *macracantha* Nees (*Burchell 1621* in h. Hook.) Kalahari Region [Northern Cape], Prieska Div.: Keiskamma Poort (Moddergat Poort), *Burchell*, *1621* (K, photograph seen on JSTOR).

Leaves linear, usually glabrous, 7 – 30 × 1.5 – 7 mm; midrib glabrous or with scattered short hairs or long cream appressed hairs; leaf margin undulate entire or with small scanty teeth. Petiole short, 0.5 – 1.5 mm long. *Flowers* usually solitary, sometimes clustered with 1 – 5 in alternate axils. *Bracteole* narrow, hardened, v-shaped in cross section, with a single major thick vein, accompanied by one faint vein on one side; bracteole margin with a few small scattered teeth (occasionally entire). Outer *calyx* lobe ovate, membranous, reticulate, glabrous, with or without purplish tinge on veins, outer calyx lobe margins serrate (sometimes, but rarely entire). Corolla with thin microscopic hairs on corolla tube. Flowers from February to June.

Distribution and habitat

Barleria rigida var. *rigida* is known from a large number of specimens (over 20) with widespread distribution occurring mainly in the Upper Karoo and part of the Gariep Desert and Kalahari Karroid Shrubland (Figure 5.18) (Mucina and Rutherford, 2006). The variety grows on deep or shallow red-yellow sandy soils, or gravelly stony / rocky light grey soils with underlying dolerite or calcrete and components of the shales and mudstones. This species occupies altitudes ranging from 400 to 1500 m. Plants near the type locality occur in the transitional fragmented Upper Karoo and Dry Highveld Grassland, in the Besemkaree Koppies Shrubland near where the Northern Cape and North-West Province borders with the Eastern Cape. The Besemkaree Koppies Shrubland consists of dolerite koppies and soils embedded within Karoo Supergroup sediments (Mucina and Rutherford, 2006).

Diagnostic features

The leaves of *B. rigida* var. *rigida* usually have undulate leaf margins with small scanty teeth or entire leaf margins which distinguishes it from *B. rigida* var. *ilicina* which have sinuate leaf margins with well developed spines or teeth. This variety differs from *B. rigida* var. *latibracteatus* by its hardened bracteole, while it can be recognised from *B. rigida* var. *ilicina* by its linear leaves and undulate leaf margins. *B. rigida* var. r*igida* can be differentiated from *B. levitundulatus* which also has wavy leaf margins by its conspicuous vein reticulation in the outer calyx lobes.

Notes

Specimens with indurate external calyx lobes usually possess 3 – 5 flowers and rarely have a conspicuous reticulation on the external calyx lobes while those with membranous calyx lobes usually possessed 1 – 2 flowers (very rarely 3) and have prominent vein reticulation in the outer calyx lobes. Indurate calyx lobes are common on the desert sand of the Bushmanland while membranous calyx lobes are common in the Upper Karoo.

Additional specimens examined

Botswana. –2320 (Ukwi): Ghanzi & Kgalagadi, Masetleng Pan, in dwarf shrub zone, 23º 41’S 20º 51’E, (–DB), 21 March 1978, *Skarpe S-289* (PRE, SRGH); Kalahari Sandveld, Masetlheng, ± 130 km WNW of Hukuntsi (–DB), 10 March 1997, *Cole 1271* (PRE). –2420 (Union’s End): Kalahari Gemsbok National Park, Kudus Pump, thornveld, clay (–CD), *van Royen & Bredencamp 372* (PRE). –2421 (Tshane): 100 km W of Hukuntsi at Masetlheng Pan, pan fringe (–BA), 16 May 1985, *Parry 8535* (J).

Namibia. –2118 (Steinhausen): Road Steinhausen – Gobabis, ca 40 km NW of Gobabis, Farm Hondeblaf 179 (–BA), 7 January 1968, *Wanntorp* & *Wanntorp 743* (K, PRE). –2216 (Otjimbingwe): Road Windhoek – Walvis Bay, 20 miles from the Rehoboth border, Farm Friedental 44 (–DB), 10 March 1968, *Wanntorp & Wanntorp 168* (K). –2418 (Stampriet): 16 km from Mariental turn off to Stampriet, Opstal farm (–AD), 11 March 1983, *Germishuizen 2746* (PRE, WIND). –2518 (Tses): Hardap, Farm Galloway 230, about 1.2 km south of house, 25º 13' 33"S 18º 37' 51"E (–BA), 23 March 1998, *Strohbach* & *Dauth 3695* (WIND). –2519 (Koes): Farm Grensplaas 409, 2 kilometres south of homestead in river, 25º 29' 8" S 19º 29' 49" E (–AD), 19 April 1997, *Calitz* & *Sheuyange 104* (WIND). –2718 (Grünau): Tallus slope of Karas Mountain on route to Telecom Tower on Farm Rishon 365, 27º 20’ 17” S 18º 43’ 47” E (–BC), 14 February 1997, *Strohbach 2856* (WIND); Farm Genadendal 264, western slope of Karasberg, 27º 30' 24" S 18º 32' 33" E (–DA), 16 March 1997, *Strohbach* & *Sheuyange 3308* (a) (K, WIND).

South Africa. –2819 (Ariamsvlei):Kenhardt,on the road to Nous, 2 – 3 km north of N14, 28º 52.05’S, 19º 54.91’E (–DD), 7 April 2000, *Balkwill*, *McDade* and *Lundberg 11799* (J). –2820 (Kakamas): c 80 km from Ariamsvlei towards Upington, Karoo (–AD), 26 June 1981, *Van Wyk 4475* (PRE). –2822 (Glen Lyon): Postmasburg, 6 miles N of Gemotep Pan, W of Langeberg Mountains (–BA), 21

March 1960, *Leistner 1708* (K, SRGH); Griquatown, Hay (–DD), Date unknown, *Wilman s.n.* (GRA). –2824 (Kimberly): Gordonia, Kalahari Gemsbok National Park, about 120 m up Nossob from entrance gate (–BA), 18 May 1956, *Story 5529* (PRE); on the Farm Klipfontein, at junction of Paardeberg East Road with Schmidt's Drift to Olierivier Road (–CA), *Balkwill and McDade 11753* (J); Schmidt's Drift (–CA), *MacDonald 77/68* (J). –2924 (Hopetown): Wanda, East of Wanda Police station, 29º 35.63'S 24º 26.29'E (–CB), 20 June 2008, *Nyirenda 700, 701, 703, 705, 706* (J); 6 km from Wanda on road to Roodepan (–CB), 26 February 1981, *Herman 294* (PRE); Van der Kloofdam (–DC), 26 March 1969, *Werger 266* (PRE, SRGH). –3022 (Carnarvon): "Boomsynputs" north of Carnarvon (–AC), 17 May 1975, *Thompson* 3115 (NBG, PRE). –3024 (De Aar): Colesberg, Doornkloof Nature Reserve, above spruit below boundary fence 2B41 (–BD), 28 December 1982, *Handiek 129* (GRA); 33 Km east of De Aar on Sterkfontein road, southern slope, foot of mountain (–CB), 4 April 1981, *Herman 561*(PRE).

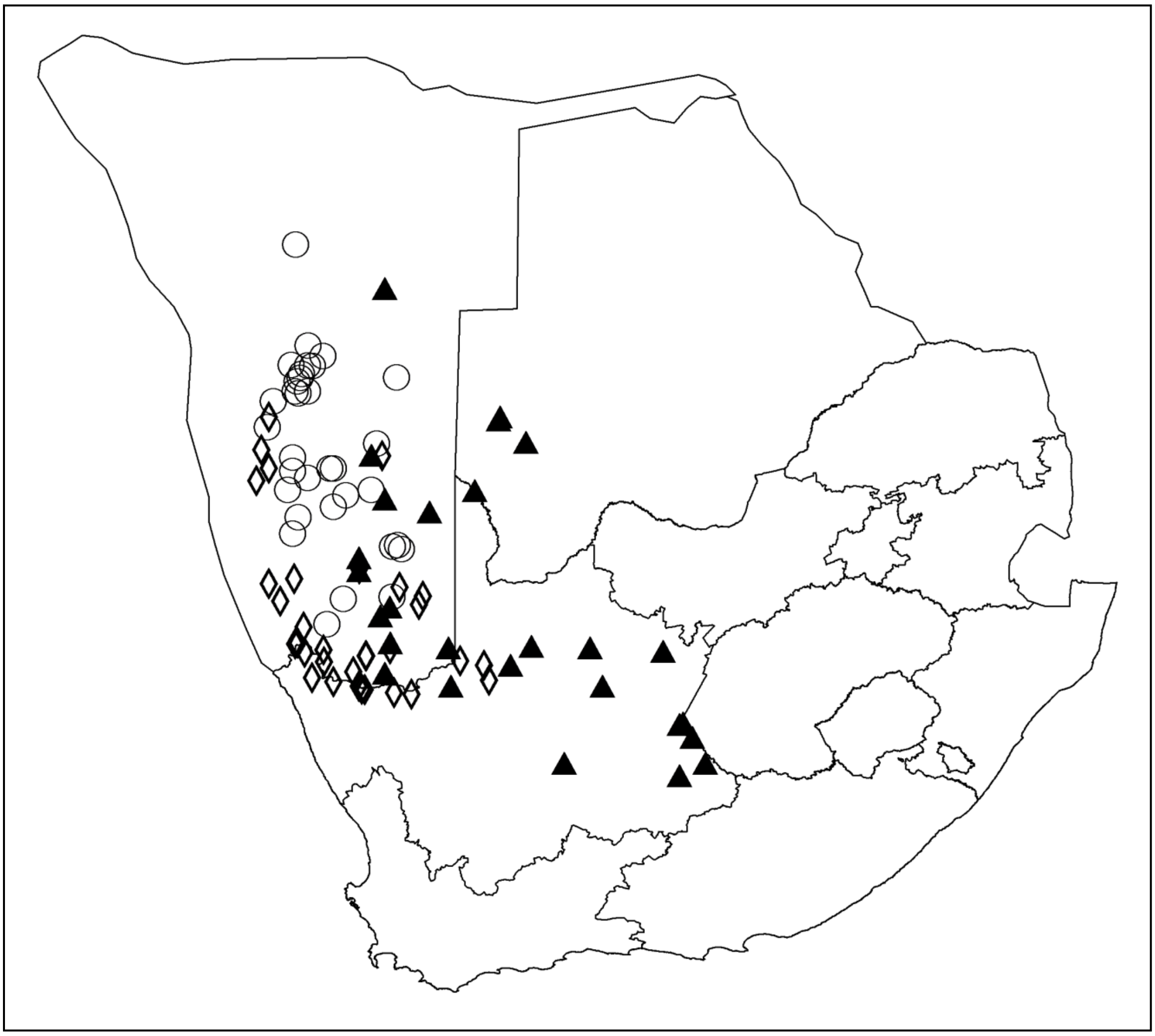

Figure 5.18. Distribution of *Barleria rigida* in southern Africa. (▲) *B. rigida* var. *rigida*, (◊) *B. rigida* var. *ilicina*, (○) *B. rigida* var. *latibracteatus*.

Figure 5.19. Scanned specimen of *Barleria rigida* var. *rigida*, *Herman 294* (PRE). Scale bar: 1cm.

5.4.4.2 Barleria rigida var. ilicina

B. rigida Nees var. ***ilicina*** (E. Mey. ex T. Anders.) Oberm. excl. var. *latibracteatus* (emend. Nyirenda). Type: [South Africa], Garip in Africa australi, 19 September 1830, *Drège s.n.* (S, holo., photograph seen on ALUKA).

Barleria ilicina E. Mey. ex T. Anders., Journal Linn. Soc. 7: 28 (1863).

B. irritans Nees var. β rigida C.B. Clarke in Flora Capensis, 5 (1): 48 (1901).

Barleria rigida Nees var. *ilicina* (E. Mey. ex T. Anders.) Oberm. in Annals of the Transvaal Museum 16: 153 (1933) excl. var. *latibracteatus* (emend. Nyirenda).

Perennial shrublet 40 cm or more high, branching above the ground. Young *stems* may be hairy. *Leaves* 9 – 45 × 4 – 10 mm, linear or narrowly elliptic-ovate to ovate; leaf margins sinuate, evenly spine-toothed, spines 0.6 – 2.5 mm long; petioles 0.4 – 5 mm long. *Flowers* solitary or clustered with 1 – 5 in alternate axils. *Bracteoles* 9 – 23 × 1 – 4 mm, glabrous, hardened, v-shaped in cross-section, with a reduced lamina and with or without one conspicuous vein parallel to the mid-vein, apex spine tipped not tapering; bracteole margin spiny, with spines almost at right angles to the margin. Outer *calyx* lobe ovate, glabrous (sometimes hairy at the base especially when young), membranous or papery, posticous lobe 15 – 23 × 3.5 – 8 mm, anticous lobe 11 – 19 × 3 – 7 mm, with or without purple tinge on veins; outer calyx lobe margins with long or short thin spines; inner lobes very narrow, single-veined. *Corolla* glabrous; flower colours ranging from white, pink, lilac and pale violet to blue. Flowers from January to May. *Fruit* 9 – 13 × 3 – 5 mm.

Distribution and habitat

Barleria rigida var. *ilicina* occurs in arid areas and is known from collections occurring in Namibia from north of the Central Namib Desert and extending south to the South Namib Desert and Gariep Desert, at the edge of Bushmanland and then eastward into the Kalahari Desert in South Africa (Figure 5.18). It inhabits the semi-desert and desert sand from Naulkluft, and Dwarf Shrub Savanna in Maltahöhe and extends southward on desert fringes and the semi-desert in Vioolsdrift as its southern limit, through Goodhouse up to around Upington as its eastern limit. It grows on gravelly / stony sandy-loam with underlying sandstone and calcrete. It has also been collected from hard clay in pans or depressions. *Barleria rigida* var. *ilicina* also inhabits shallow sand on rocky granite koppies, on sandstone found in deep rock fissures as well as river sand on Quartzite conglomerate. It occurs at altitudes between 200 and 1400 m. Although a few specimens have been reported from the fringes of the Central Plateau, *B. rigida* var. *ilicina* occurs on the arid desert sands on the eastern and western sides of the Central Plateau.

Diagnostic features

Barleria rigida var. *ilicina* with elliptic-ovate or ovate leaves appears to be an intermediate taxon between *B. rigida* var. *latibracteatus* and *B. rigida* var. *rigida.* It is differentiated from *B. rigida* var. *latibracteatus* by its hardened narrow single-veined bracteoles while it is differentiated from *B. rigida* var. *rigida* by its ovate leaves and conspicuously spiny sinuate leaf margins (with spines evenly distributed).

Notes

Barleria rigida var. *ilicina* (E. Mey. ex T. Anders.) Oberm. *sensu lato* as previously defined included specimens with narrowly elliptic, elliptic-ovate and linear leaves with spiny leaf margins (Figures 5.20 and 5.21) and was based on *Drège s.n.* (S). It also included plants with bracteoles with well developed laminae with three conspicuous parallel veins and those with reduced laminae with one or two parallel veins. However, Drège's specimen, the type for *B. ilicina* E. Mey, on which Anderson (1863) based his description of *B. ilicina,* represents plants with broad ovate leaves with long spines / teeth on the leaf margin and bracteoles with reduced laminae with one conspicuous vein. *Barleria ilicina* E. Mey. does not have sufficient distinct characters that can adequately differentiate it from *B. rigida* at the level of species, it is therefore reduced in rank to variety and the plants thereof are recognised as belonging to variety *B. rigida* var. *ilicina. Barleria rigida* var. *ilicina* (E. Mey. ex Anders.) Oberm. *pro parte* and *B. ilicina* E. Mey. ex T. Anders., are here maintained as *B. rigida* var. *ilicina* (E. Mey. ex Anders.) Oberm. excl. *Dinter 4358* (SAM), *Range 487* (SAM) (emend. Nyirenda).

Figure 5.20. Scanned specimen of *Barleria rigida* var *ilicina*, *Jurgens 22682* (PRE) with broadly ovate leaves. Scale bar: 1 cm.

Additional specimens examined

Namibia. –2316 (Nauchas): at edge of Farm Weissenfels REH 22 (–AD), 11 March 1953, *Walter & Walter 1690* (WIND); high mountain, Farm Friedland MAL 19 (–CB), 18 March 1953, *Walter & Walter 1914* (B, WIND). –2416

(Maltahöhe): Naukluft (–AC), 5 May 1974, *Meyer 503* (WIND**)**; Hardap, Zebra River 122 (–CB), 13 May 1998, *Buhrmann & Buhrmann BUH1 - 42* (WIND); Farm Duvisib (–CC), 17 May 1956, *Volk 12777* (M). –2418 (Stampriet): Gellapost Expt. Station (–AC), May 1949, *Liebenberg 5177* (WIND). –2616 (Aus): Kahanstal (–CD), 4 December 1934, *Dinter 8161* (B); Bethanien, Farm Mooifontein BETH 50, edge with black lime (–DD), 26 February 1963, *Giess, Volk & Bleissner 5516* (WIND). –2618 (Keetmanshoop): West of Gellap Ost, ±15 km NW of Keetmanshoop (–CA), 8 April 1987, *Maggs108* (WIND); 20 miles S.E. of Keetmanshoop on rd to Narubis (–CA), 28 April 1955, *de Winter 3267* (K, PRE, WIND); Sandmodder, KEE 73 (–DD), 5 April 1975, *Müller 96* (WIND). –2716 (Witpütz): Narudous Poort, between Aus and Rosh Pinah, Anusi 73, mountainside (–CC), 30 August 1989, *van Wyk 8773* (WIND); North of Witpütz, between Aus and Rosh Pinah, rocky hillslope facing SE (–DA), 8 May 1976, *Oliver & Müller 6411* (WIND). –2717 (Chamaites): Boomsriver (–CC), 26 February 1992, *Strohbach 117* (WIND). –2718 (Grünau): north of Schroffenstein Mountain, Farm Pieterkloof (Kraaikluft), KEE370 (–BB), 14 May 1972, *Giess* & *Müller 11935* (WIND); western slope of Karasberg, Farm Genadendal 264, 27º 30' 24" S 18º 32' 33" E (–DA), 16 March 1997, *Strohbach* & *Sheuyange 3308* (b) (WIND). –2719 (Tränental): Farm Warmfontein KEE 280, on the koppies (–AB), 5 April 1975, *Muller 81* (WIND); Karasburg, “Numdis” (–AD), January 1974, *Auret 5631* (WIND). –2816 (Orangemund): Loreley Copper Mine, Canyon entrance, on hanging granite rock (–BB), 22 February 1963, *Geiss, Volk & Bleissner 5419* (PRE, WIND); Slate Mountains at Loreley (–BB), 21 February 1963, *Leppert 4207* (M). –2817 (Vioolsdrift): Richtersveld, Wespental, 28.210º S 17.070º E (–AC), 9 September 1987, *Jurgens 22682* (PRE); E end of Rosyntjiesberg, Namibian side, upper south slope, Quartzitic conglomerate (–AD), 15 April 1986, *van Jaarsveld, Forrester & Jacobs 8452* (NBG). –2818 (Warmbad): Farm Norachas WAR 14, top superimposed with granite blocks (–AA), 14 May 1963, *Giess, Volk* and *Bleissner 6909* (WIND); Karasburg, Farm Haakiesdoorn, 28º 17’ 0”S 18º 15’ 35”E (–AD), 22 April 1997, *Strohbach & Chivell 3485* (WIND, PRE); Keetmans, Farm Lovedale WAR 32, on sandstone mountainside (–BC), 20 May 1963, *Giess, Volk* and *Bleissner 7134* (WIND); Farm Witpütz WAR 258, Quartzite basins with succulent vegetation (–CA), 15 May 1963, *Geiss, Volk and Bleissner 6941* (WIND, PRE, MO); Horechab (–DA), 24 March 1985, *van Ee BLFU10224* (BLFU); foot of Fish River Canyon, large space at foot of black lime-ridge (–DA), 30 March 1953, *Walter & Walter 2322* (B).

South Africa. –2817 (Vioolsdrift): Mountain W of the confluence of Stinkfontein River and Orange River, south and upper slopes, Namaqualand broken veld (–CB), 14 April 1986, *Jacobs 8407* (NBG); (–DC), May 1984, *Preston-Mafham 15* (PRE); 6 miles S of Vioolsdrift, N Namaqualand (–DD), *Thorne 51598* (NBG / SAM). –2818 (Warmbad): about 1 km South of Goodhouse, 28º 56.56’S, 18 º 14.36’E, 400 m, on koppie and near base, in cracks in rock or on scree slopes (–CC), 5 April 2000, *Balkwill, McDade & Lundberg 11789a* (J); 10 miles South of Goodhouse (–CC), 30 May 1961, *Schlieben 9092* (BOL, K, PRE, SRGH); Goodhouse Poort, rocky mountain slope (–CD), 22 June 1989, *Van Wyk 8587a* (J, PRE, WIND); 8 miles S by W of Goodhouse (–DD), 30 May 1961, *Leistner 2592* (PRE). –2820 (Kakamas): Riemvasmaak, Deksel, just to the west of the church, on rocky hillside (–AC), 2 October 1988, *Balkwill &*

Balkwill 4172 (J); 70 km NW of Upington, on road to Karasburg, rocky grassy hilltop (–BC), 4 May 1976, *Oliver* and *Steenkamp 6234* (PRE); 3 – 4 km south east of Kakamas, above Water Tunnels, 28º 45.47’S 20º 40.02’E (–DA), 4 April 2000, *Balkwill, McDade & Lundberg 11770* (J). –2918 (Gamoep): 60 km SE of intersection of Concordia – Goodhouse road and road to Pofadder, on the road to Pofadder, 29º 0.27’S 18º 48.38’E (–BB), 4 April 2000, *Balkwill, McDade & Lundberg 11792* (J). –2919 (Pofadder): Pella, on road to Orange River (–AA), 10 April 1969, *Kruger M215* (NBG).

5.4.4.3 Barleria rigida var. latibracteatus

B. rigida Nees var. ***latibracteatus*** Nyirenda *var. nov.* affinis *B. rigida* var. *ilicina* (E. Mey. ex T. Anders.) Oberm. sed bractea foliosus venis tria lamina latus differt. Type: South West Africa [Namibia], Maltahöhe, Farm Namseb MAL 24: Grootfontein area, 18 February 1962, *Giess, Volk and Bleissner 5189* (WIND, holo.! PRE, iso.!).

Barleria rigida var. *ilicina* (E. Mey. ex Anders.) Oberm. in Annals of the Transvaal Museum 16: 153 (1933). excl. var. *ilicina* (emend. Nyirenda).

Shrublet, 30 cm high or more, beginning to branch above the ground. Tufts of long single-celled cream or white hairs may be observed around the nodes and at the base of petioles. *Leaves*18 – 55 × 4 – 10 mm, linear, glabrous or dotted with cream-yellow appresed hairs on the abaxial surface; leaf margins sinuate or shallowly undulate, spine-toothed; leaf margin spines 0.6 – 3 mm long; petiole, 2 – 5 mm long, glabrous or with short white recurved hairs at the base, adaxial surface of midrib may possess white recurved hairs while the abaxial surface may possess cream-yellow appressed hairs. *Flowers* clustered with 3 – 8 (sometimes up to 10 but not solitary). *Bracteoles* 16 – 23 × 3 – 6 mm, glabrous or dotted with cream-yellow appresed hairs, leafy, with two faint or conspicuous veins parallel to the mid-vein, apex spine-tipped, short, not tapering; margin spine-toothed, with spines almost at right angles to the margin. Outer *calyx* lobes ovate, glabrous or dotted with cream-yellow appresed hairs, cartilaginous or indurate, posticous lobe 17 – 22 × 5 – 9 mm, anticous lobe 15 – 19 × 4 – 7 mm, without purple tinge on veins; outer calyx lobe margin with thin spines, inner lobes single-veined. Corolla glabrous; flower colours ranging from white, pink, lilac and purple to blue. Flowering from January to April. *Fruit* 12 × 3 – 4 mm.

Distribution and habitat

B. rigida var. *latibracteatus* occurs on an extensive central plateau on the western edge of the Kalahari Desert and eastern edge of the Namib Desert (Figure 5.18). However, it has not been recorded on the Brandberg west of the central plateau. It also occurs in Mopane Savanna, in Otjondjupa and extends through the Thornbush Savanna, Highland Savanna, Camelthorn Savanna and Dwarf Shrub Savanna vegetation in Karasberg, as well as at the edge of the Kalahari Desert in Northern Cape, South Africa. It grows on well drained rocky / stony sandy-loam, underlain by calcrete. In the Kalahari Desert, it occurs on gentle slopes of the calcareous pans on sandy soils in partial shade of other plants. It occurs at altitudes between 1000 and 2000 m.

Figure 5.21. Scanned specimen of *Barleria rigida* var. *ilicina, Maggs 108* (WIND) with linear or narrowly elliptic leaves. Scale bar: 2 cm.

Diagnostic features

Barleria rigida var. *latibracteatus* may sometimes (but not always) possess leaves that are elliptic-ovate, resembling *B. rigida* var. *ilicina* and *B. bracteilobis*, but it can be identified from *B. rigida* var. *ilicina* by lanceolate bracteoles with broad leafy laminae with three parallel veins and attenuate bracteole bases; and from *B. bracteilobis* by attenuate bracteole bases and short spinous apices (not lobed or truncate bracteole bases and tapering tips). It can be distinguished from

B. rigida var. *rigida* by its linear leaves with evenly spine-toothed leaf margins. Where an overlap in leaf margins has been observed, the two are identified by the 3-veined bracteole which also tends to have much longer margin spines up to 3 mm long (only up to 1 mm long in *B. rigida* var. *rigida*).

Notes

Although specimens of *B. rigida* var. *latibracteatus* have been filed under the name *B. rigida* var. *ilicina,* bracteole dissimilarity and distribution qualifies it to be recognised as distinct at the level of variety. While the two veins parallel to the bracteole mid-vein may sometimes not be conspicuous, this variety does not bear solitary flowers (flowers are always clustered).

Additional specimens examined

Namibia. –2016 (Otjiwarongo): Otjozondjupa, southern eastern corner Otjiku Plain (–BD), 16 May 2001, *Strohbach 1060* (WIND).–2216 (Otimbingwe): mountain peak, east of house, gentle slope (–DD), 15 April 2002, *Strohbach BS5427* (WIND); Khomas, Farm Lichtenstein 366 next to road near house (–DD), 25 February 1990, *Kolberg & Mannheimer 419* (WIND). –2217 (Windhoek): Neudam Exp. Farm (–AD), 25 February 1960, *van Vuuren 995* (WIND); Avis, western mountain land (–CA), 7 April 1964, *Seydel 3944* (M) [ILa112]; (Windhoek): Avis bei, foothills of the Auas Mountains (–CA), 12 April 1960, *Seydel 2283* (WIND, B); 12 km east of Windhoek, Auas Mountains, quartzite slopes (–CA), 15 January 1916, *Pearson 9795* (K); Farm Lichtenstein West, 22° 47.233'S 17° 0.154'E (–CC), 14 March 2001, *Uiras MU256* (WIND). – 2218 (Gobabis): Omaheke, Farm Keitsaub, 25° 51' 11"S 18° 51' 6"E, pan area (–DD), 6 March 2002, *Uiras MU474* (WIND). –2316 (Nauchas): Rehoboth, Farm Gurumanas REH. 306 (–BB), 10 March 1953, *Walter & Walter 1650* (WIND); Friedrichsruh, mountain slope, on koppies south of big river (–BB), 17 April 2002, *Strohbach BS5514* (WIND); Hardap, Farm Duruchaus, among Karoo vegetation (–BB), 3 July 2004, *Wittneben WO4-269* (WIND); F. Nautzerus, Bastardland (–CD), 15 March 1953, *Walter & Walter 1840* (B). –2317 (Rehoboth): Farm Bergland-Arovley, on surface lime in limestone meadow community (–AA), March 1953, *Giess 1787* (WIND, B). –2416 (Maltahöhe): Farm Nomsas MAL 26, Sign point (–BD), 16 March 1953, *Walter & Walter 1877* (WIND); N of Maltahohe about 45 km on road to Walvis Bay, 24° 40' 495"S 16° 49' 513"E (–DB), 11 March 1995, *Burgoyne 3465* (PRE). –2417 (Mariental): Haribes, Farm BEZ Mariental 18/19 (–DA), April 1956, *Volk 12171* (WIND). – 2417 (Mariental): Haribes - Mariental (–DA), 27 April 1913, *Dinter 2954a* (SAM); Farm Karichab, (–CC), *Koenen 65* (WIND). –2418 (Stampriet): Rohrbeck, GIB 128 (–AB), 17 April 1960, *Freyer 109* (WIND). –2516 (Helmeringhausen): Farm Lisbon, part of Farm Grootfontein MAL (–BB), 8 April 1980, *Muller 1282* (WIND); Maguams – Vrähwinkel (–DB), 23 March 1953, *Walter & Walter 2149* (B).

Figure 5.22. Scanned type specimen of *Barleria rigida* var. *latibracteatus*, *Giess, Volk and Bleissner 5189* (WIND, Holotype!). Scale bar: 2 cm.

–2517 (Gibeon): Hardap, ± 6 km west of WeiBrand along C18, 25º 9’ 4”S 17º 51’ 41”E (–BB), 24 March 1998, *Strohbach & Dauth 3738* (WIND); Farm Haribes (–BC), 13 April 1956, *Volk 12391* (M) [ILa110]. –2518 (Tses): Farm Elandslaagte 79, 25º 2’ 50”S 18º 21’ 14”E, 15 km north of farmhouse (–AB), 24 March 1998, *Strohbach & Dauth 3719* (WIND). –2520 (Mata-Mata): Kalahari

Gemsbok National Park, Koedoe Bush, around abandoned mill, 25° 3’ 53”S 20° 22’ 53”E (–AB), 8 April 1997, *Mucina LM6327/8* (PRE). –2618 (Keetmanshoop): Farm Middelplaas, east of homestead (–BB), 17 Febraury 1998, *Strohbach 3615* (WIND). –2717 (Chamaites): Mara (–BB), 30 June 1986, C*raven 2516* (WIND); Canyon Plateau, Wegdraai, 27° 39’ 52”S, 17° 29’ 56” E (–CB), 4 May 2002, *Helary & Batault 196* (WIND). –2718 (Grünau): Farm Pieterkloof (Kraaikluft) KEE370, above the water fall on a slope (–BB), 15 May 1972, *Giess* & *Müller 11994* (WIND).

South Africa. –2821 (Upington): 32 m. E. of Aranos, Farm Lekkerwater (–AB), 26 April 1960, *van Vuuren & Giess 1105* (PRE); Edge of Kalahari (–AC), 21 May 1973, *Bayliss 5790* (MO).

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