# Evolutionary and Biogeographic Studies in the genus Kniphofia Moench <br> (Asphodelaceae) 

A thesis submitted in the fulfilment of the requirements for the degree of<br>\title{ Doctor of Philosophy }<br>of<br>Rhodes University by<br>Syd Ramdhani<br>August 2006

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## Dedication

# To my wife, Sandhayia Kanhaye 

Durgam Kaaj Jagat Ke Jeete, Sugam Anugrah Tumhre Te Te

Goswami Tulsidas (1532-1623)


#### Abstract

Kniphofia, a genus of approximately 71 species, is almost entirely African with two species occurring in Madagascar and one in Yemen. Commonly known as 'red hot pokers' they are popular among horticulturists. The genus is also well known for its complex alpha taxonomy. To date, no studies have examined the phylogenetic relationships among species or the evolutionary history of the genus, and little work has been done on their biogeography. The main focus of this study was (i) to review the alpha taxonomy, (ii) to assess diversity and endemism in Kniphofia, (iii) to use DNA sequence data to reconstruct a specieslevel phylogeny to understand intra-generic species relationships and evolutionary processes (iv) to use phylogeographic approaches to study the biogeography and evaluate biogeographical patterns, and (v) to assess anatomical variation and determine if anatomical characters are useful for species delimitation.

It was found that the genus has six centres of diversity, five of which are centres of endemism. The South African Centre is the most speciose and is also the largest centre of endemism. Kniphofia shows a strong Afromontane grassland affinity in Tropical and East Africa. In South Africa, it is found from high altitudes to coastal habitats, with the most speciose regions being Afromontane grasslands. It is thus not considered to be an Afromontane element, but rather an Afromontane associate.


Five major evolutionary lineages were identified using cpDNA sequence data ( $\operatorname{trn} T-L$ spacer), four of which are southern African. The fifth lineage is represented by material from Madagascar, East and Tropical Africa. The nuclear ITS region failed to provide resolution, as many sequences were identical. The five lineages recovered using cpDNA showed some congruence with geographic origin rather than the taxonomic arrangement based on morphology. All of the
species with multiple samples were non-monophyletic. This could be due to hybridisation and/or incomplete lineage sorting.

The nested clade analysis, although preliminary, did not completely agree with the phylogenetic analyses. One of the three third level nested clades appears to show fragmentation between the Cape Region, KwaZulu-Natal and northern parts of southern Africa. Furthermore, another nested clades recovered suggest a range expansion and radiation from the Drakensberg into the adjacent Drakensberg-Maputoland-Pondoland transition.

Morphological species of Kniphofia exhibited substantial leaf anatomical variation and anatomical characters do not cluster samples into their morphological species. The anatomical results do not fit any geographic pattern, nor do they correspond to the lineages recovered using molecular markers or the nested clades. Leaf anatomical variation does not appear to be influenced by geographical or environmental factors. However, hybridisation may play a role but was not tested in this study.

In light of the above findings it is proposed that the evolutionary and biogeographic history of Kniphofia is strongly linked to tectonic events, and Quaternary climatic cycles and vegetation changes. Tectonic events (viz. uplifts) may have resulted in vicariance events that may account for the five cpDNA lineages recovered in phylogenetic analyses, while Quaternary climatic cycles and vegetation changes may have had a more recent impact on evolution and biogeography. It is hypothesised that the ancestral area for Kniphofia was much more widespread when Afromontane grasslands were more extensive during cooler and drier glacial episodes. Kniphofia on the high mountains of Tropical and East Africa would have tracked Afromontane grasslands as they expanded their ranges in cooler periods. While during wetter and warmer interglacial periods Kniphofia would have retreated into refugia on the mountains of Tropical and East Africa, with no gene flow possible between these refugia. In South

Africa, where latitude compensates for altitude, Kniphofia may have maintained a distribution that extended into the lowlands even during interglacials.

A cyclic climate change hypothesis implies that populations of Kniphofia (at different phases of the climatic cycle) would have experienced periods of contractions and fragmentation followed by periods of range expansion and coalescence or secondary contact. Altitudinal shifting is proposed to be the most likely mechanism for fragmentation and range expansion, and would would possibly promoted hybridisation. Within the five lineages there is evidence for recent differentiation as the branch lengths are short, there are numerous nonmonophyletic species and numerous identical haplotypes (cpDNA and ITS) which collectively indicate a recent radiation in southern Africa. A recent radiation would also account for the taxonomic confusion and difficulty in differentiating morpho-species. These climatic events may also account for the substantial anatomical variation in southern African Kniphofia species.

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## Preface

Some of the work presented in this study has been published previously:
Ramdhani, S., Barker, N.P. \& Baijnath, H. 2006. Phylogenetics of the genus Kniphofia Moench (Asphodelaceae). In: Taxonomy and ecology of African Plants: their conservation and sustainable use (Proceedings of the $17^{\text {th }}$ AETFAT Congress), eds. Ghazanfar, S.A. \& Beentje, H.J., pp. 559-573. Royal Botanic Gardens, Kew.

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## Declaration

This thesis is the result is the result of the author's original work except where acknowledged or specifically stated in the text. It has not been submitted for any other degree or examination at any other university or academic institution.

Syd Ramdhani

## Chapter 1: Introduction

### 1.1. The genus Kniphofia Moench (Asphodelaceae)

The genus Kniphofia Moench, commonly known as 'red hot pokers', comprises approximately 71 species. Kniphofia is an asphodelaceous genus characterised by a perennial, rhizomatous, usually acaulescent and herbaceous habit. Leaves are rosulate, linear and usually keeled. Inflorescences are subspicate racemes with a bract at the base of pedicels. Flowers are tubular and range from white, yellow to various shades of red which are more conspicuous at the apex of the inflorescence producing a bicolourous appearance. Flowers are three-merous, with six tepals and a hypogynous three chambered sessile ovary. Fruits are globose to ovoid capsules that house seeds that are usually flattened.

The genus is almost entirely African with two species from Madagascar and one from Yemen. Kniphofia is chiefly distributed in southern and eastern Africa, preferring temperate mountainous grassland and moist habitats (Ramdhani et al., 2006). In South Africa (SA) 48 species are currently recognised (Codd, 2005). The centre of diversity is the Drakensberg region of the KwaZulu-Natal and Eastern Cape provinces of South Africa. The genus is known for its complex alpha-taxonomy and contains several species complexes (Codd, 1968, 2005).

Kniphofia has considerable horticultural appeal because of the charismatic and conspicuous inflorescences of many members (Fig. 1.1.) and the ease with which taxa hybridise in cultivation (Taylor, 1985; Baijnath, 2004). It is a frequent subject in popular horticultural and botanical publications (McMaster, 1999; Van Jaarsveld, 2003; Baijnath, 2004; Dold and McMaster, 2005). Despite the popularity of the genus, phylogenetic relationships amongst the different species within Kniphofia have not been explored previously. The complex alphataxonomy and poorly understood phylogenetic relationships within Kniphofia


Fig. 1.1. Some representatives of Kniphofia. A. K. caulescens (N.P. Barker 1821, Sani Pass). B. K. acraea (T. Dold 4626, Mount Zebra National Park). C. K. fibrosa (P.B. Phillipson 5579, Dohne Hill). D. K. pauciflora (clone from Natal National Botanical Garden). E. K. splendida (SR 548, Haentersberg; photo by E.A. Kellogg). F. K. uvaria (SR 471, Dimbiza). G. K. schimperi (SR 391, Bale Mountains). H. K. typhoides formerly placed in Notosceptrum [portrait from www.smgrowers.com]. I. K. linearifolia (T. Dold 4638, Satans Nek).

### 1.2. Previous systematics studies in Kniphofia

The pre- and post-Linnean taxonomic history of the genus was discussed in great detail by Codd (1968), who gave an excellent overview of taxonomic work prior to his revision and it would be pointless to repeat it herein. The salient publications which have a direct bearing on the present study are dealt with below.

Bentham and Hooker (1883) described the segregate genus Notosceptrum Benth. based on floral characters. Characters used to separate Notosceptrum from Kniphofia were very long slender inflorescences bearing numerous ascending or patent flowers and a short, subcampanulate perianth, which is relatively deeply lobed in Notosceptrum (Codd, 1967).

Berger (1908) monographed Kniphofia, recognising 67 species with 13 varieties. He upheld Notosceptrum with five species. Berger divided Kniphofia into 14 formal sections. This monograph brought together the information on the genus available at the time including a comprehensive list of hybrids and garden cultivars. According to Codd (1968), Berger's work suffers two main weaknesses. Firstly, the nomenclature was not acceptable according to the International Code of Botanical Nomenclature, a criticism that is not completely justified as Berger's work was published at a time when the Code was still in early developmental stages. The second weakness was that Berger did not study the plants in the field, which made his treatment unrealistic in several respects. Codd (1968, p. 387) noted that Berger showed "no grasp of geographical distribution of species and little concept of the variation, which occurs within a taxonomic group, resulting in too many 'species' (amongst those known to him) being upheld on unreliable 'differences'".

Subsequent to Berger's (1908) monograph there was a large accumulation of collections in South Africa. However, little work was done until after the Second

World War. In 1947 Miss Eileen Bruce started a revision on the genus in South Africa. She worked actively on the genus between 1947 to 1952. She recognised three new species namely Kniphofia splendida E.A. Bruce, Kniphofia rigidifolia E.A. Bruce and Kniphofia coralligemma E.A. Bruce (Bruce, 1955). Unfortunately, her career was cut short by her death in 1955 (Codd, 1968).

During the 1950's and 1960's, Dr. L.E. Codd of the National Herbarium (PRE) contributed significantly to the taxonomy of the genus describing numerous species and resolved taxonomic problems such as typification and correct identification (Codd, 1959, 1961, 1963, 1964, 1965). Codd (1967) re-examined the status of Notosceptrum and found that the floral characters used to separate Notosceptrum and Kniphofia break down when all the known species (at that time) were considered. He thus merged Notosceptrum into Kniphofia. Codd's work on the genus culminated in a revision of the South African species in 1968. He recognised 45 species and eight subspecies. An updated revision of Codd (1968) was published posthumously in 2005 which documented 48 species and six subspecies from southern Africa. Codd passed away in 1999 but had completed the manuscript of the second revision in the late 1980's. By this time Codd was a very experienced taxonomist who had worked on a number of diverse plant groups. Codd's approach was not strictly herbarium based. He did extensive field work with numerous collections and had an excellent knowledge of Kniphofia. Despite Codd's $(1968,2005)$ revisionary efforts there still remain many taxonomic problems which require further investigation and his works are testament to the complex taxonomy and evolutionary history of this genus.

Codd's $(1968,2005)$ revisions are of vital importance, as these were used as the taxonomic framework in this study and his species delimitations were followed for southern African representatives of Kniphofia.

### 1.2.1. Problems encountered by Codd

### 1.2.1.1. The Kniphofia uvaria (L.) Oken complex

Codd (1968) did not resolve K. uvaria and its allies [Kniphofia rooperi (T. Moore) Lem., Kniphofia tysonii Baker, Kniphofia citrina Baker and Kniphofia linearifolia Baker] completely. This complex is widespread and variable. Nevertheless, he delimited five species (K. uvaria, K. rooperi, K. tysonii, K. citrina and $K$. linearifolia) on the basis of inflorescence shape and perianth length. This group varies in size and degree of variability of inflorescences and species delimitations are not clear cut, but are linked by intermediates. However, each group had a fairly well-defined geographical distribution range (Codd, 1968).

Codd (2005) retained the species in this complex but provided additional information. Overlap in distribution and intergradation of characters between $K$. uvaria and other species of the complex (except K. tysonsii) in the Eastern Cape (especially in the King Williams Town and Albany area) were noted. K. tysonii shows no intergradation with $K$. uvaria but does intergrade with $K$. linearifolia. Codd (2005) also postulated that K. linearifolia is possibly the 'parent' form of the complex. The evidence above suggests extensive hybridisation in the evolutionary history of the complex. Furthermore, a specimen from the Bouwershoekberge (Graff-Reinet, Bayliss 3917) has some unusual morphological features which requires further investigation to better understand its placement (Codd, 2005).

## K. uvaria sensu stricto

K. uvaria sensu stricto has a wide distribution from the Cape Region, extending as far north as the Kamiesberg, east towards the Albany district and inland into the foothills of the Drakensberg. Four forms were noted by Codd (2005), with slightly different morphologies and each with a fairly distinct distribution with
some overlap and intergradation. No formal rank was awarded to these forms (Codd, 2005).
K. rooperi

Poor material of this species can be confused with $K$. drepanophylla and $K$. littoralis. Several collections between Komga and Knysna, and inland (King Williams Town and Stutterheim) appear to be intermediate between K. rooperi and K. uvaria. Also specimens from Albany, Port Elizabeth and Humansdorp link K. rooperi with K. citrina (Codd, 2005).

## K. citrina

K. citrina has a distribution that overlaps with K. uvaria and intermediate collections have been reported (Codd, 2005). Despite Codd's (2005) opinion that 'separate species rank for K. citrina is scarcely justified' he retained it as a separate species.

## K. linearifolia

This is the most widespread Kniphofia species in southern Africa. Specimens that are small in stature are not easily distinguished from Kniphofia baurii Baker. K. linearifolia, K. rooperi and K. uvaria are closely related and intermediate specimens have been reported in sympatric distributions. Intermediate collections have also been reported for K. linearifolia and K. tysonii. Racemes of Kniphofia praecox Baker show close resemblance to $K$. linearifolia and it has been implicated in the hybrid ancestry of K. praecox (Codd, 2005).
K. tysonii

Codd $(1968,2005)$ recognised two subspecies of K. tysonii. Kniphofia tysonii Baker subsp. tysonii Codd is related to K. linearifolia. Intermediate specimens are also known that are difficult to place with certainty. Some specimens of Kniphofia tysonii Baker subsp. lebobomboensis Codd are difficult to separate from K. tysonii subsp. tysonii in the herbarium, although the two subspecies do
not overlap in distribution (Codd, 2005). Codd (2005) speculated that K. tysonii subsp. lebobomboensis was derived from K. linearifolia rather than K. tysonii subsp. tysonii. This implies a closer relation to a separate species rather than it sister subspecies i.e. K. tysonii is not monophyletic. Intermediates between $K$. tysonii subsp. lebobomboensis and K. linearifolia have been reported (Codd, 2005).

The above situation in the $K$. uvaria complex and $K$. uvaria sensu stricto poses complications for workers not familiar with the plants and can easily result in erroneous identifications. Thus, an intensive study on the K. uvaria complex is required before a satisfactory classification is achieved. This will entail extensive field work, identification of additional taxonomic characters and further exploration with multiple approaches.

### 1.2.1.2. Kniphofia baurii Baker

Codd $(1968,2005)$ found that the typical form of $K$. baurii may be confused in the herbarium with Kniphofia drepanophylla Baker. K. baurii occurs in two disjunct areas. One form is from Engcobo towards southern KwaZulu-Natal. The other form occurs in northern KwaZulu-Natal (Dundee inland towards the foothills of the Drakensberg and northwards towards Wakkerstroom) and is more robust.

Codd (1968) included in K. baurii a group of specimens that deviated from typical K. baurii. These specimens originate mainly from the Eastern Cape and East Griqualand. This group was characterised by a later flowering time (which is spring flowering for typical $K$. baurii) and greenish flowers with dull red buds. Apart from these differences, the plants compared well with K. baurii morphologically (Codd, 1968). Codd (2005) considered this group to belong to K. linaerifolia.

During this study difficulty was experienced at times in keying out $K$. baurii and K. uvaria as the two taxa are similar in many respects. This might indicate that $K$. baurii should be treated as part of the $K$. uvaria complex.

### 1.2.1.3. Kniphofia praecox Baker

The species concept of K. praecox is in doubt. The K. uvaria and K. praecox complexes have largely contributed to the cultivation of 'red hot pokers' in Europe. The early history of the genus is confused by the application and misinterpretation of species names within these species complexes (Codd, 1968). Until 1800 apparently only K. uvaria was known to botanists in Europe. The typification of this species is not in doubt. K. uvaria was easily available to early collectors. As botanical exploration proceeded further eastwards in South Africa, more robust Kniphofias were collected and made their way to Europe (Codd, 1968).

In the early 1800 's robust hybrids of Kniphofia started to appear in Europe (Codd, 1968, 2005). Jacquin (1809) included an illustration of a Kniphofia under the name Veltheimia uvaria Willd. The identity of the plant in this illustration was uncertain until Codd (1968) matched it with naturally occurring plants with restricted distributions in the Komga and Knysna areas [now recognised as Kniphofia bruceae (Codd) Codd, see below]. It has narrow, long-acuminate bracts that make it clearly different from members of the K. uvaria complex, which has ovate-oblong, obtuse to rounded bracts. It seems probable that these plants (i.e. K. bruceae) were widely available in Europe during the early 1800 's and superficially resembled members of the K. uvaria complex.

Baker (1870) tentatively described K. praecox. He separated K. praecox from K. uvaria mainly on the earlier flowering time of $K$. praecox. However, in subsequent revisions he included K. praecox under K. uvaria. Codd (1968) found that the type of $K$. praecox preserved in Kew Herbarium was allied to the plant
illustrated by Jacquin (1809), but was not an exact match with known wild populations associated with the plant in Jacquin's plate (i.e. K. bruceae). At the time he found it appropriate to include the wild plants (K. bruceae) under the broad concept of $K$. praecox. Codd (1968) also questioned whether the type might be of hybrid origin, as it did not match exactly with any known plants from the wild. One must take into account the history of this type, which was from garden material cultivated by W. W. Saunders. He supposedly obtained the plants from Thomas Cooper. Although Cooper made herbarium specimens of the material he collected from the wild, Codd did not trace or examine this material. Codd also suspected that the type specimen of K. praecox was possibly obtained from another source. Despite this confusion concerning the type of K. praecox, Codd (1968) maintained $K$. praecox as a separate species with two subspecies. Kniphofia praecox Baker subsp. bruceae Codd was erected based on its affinity with and possible parentage of $K$. praecox. He noted that the two subspecies could be separated on several characters, notably the narrower and more acuminate bracts, longer pedicels, the shorter perianth, which tends to be constricted above the ovary, and the well-exserted stamens in K. praecox subsp. bruceae. Codd (1987) raised $K$. praecox subsp. bruceae to species rank (= $K$. bruceae) for specimens from the Komga area. He also considered the specimens from the Knysna-Willowmore area to belong to $K$. bruceae, although they differed amongst themselves. Codd (1987) could still not locate plants in the wild that matched to the type of K. praecox. He further found that narrow bracts were recorded from three small disjunct areas (near Komga, between Plettenberg Bay and Knysna, and near Kouga).

According to Codd (2005) the species concept of $K$. praecox appears to be derived from K. bruceae crossed with another showy species (possibly K. uvaria or K. linearifolia) along the lines of his earlier work. These hybrids produced several derivatives, which became widely accepted in cultivation under epithets such as K. uvaria. The true K. uvaria was either lost or regarded as inferior to the above mentioned hybrids. Plants of this mixed nature are still grown in gardens
today but have become modified to varying degrees due to a long history of cultivation in Europe. It is evident that hybridisation with other additional species in cultivation has produced numerous horticultural and ornamental forms (C. Whitehouse, pers. comm.).

The bract shape is the only character that can separate these hybrids from $K$. linearifolia. Many illustrations published over the years cannot be confidently placed as bract characters are not depicted. The plants described as K. praecox appears to be one of these hybrids as no material collected in the wild thus far match the type of $K$. praecox. Despite the dubious and complicated history of $K$. praecox, Codd (2005) retained this species.

Furthermore, the name K. praecox still appears to be used for the KnysnaWillowmore specimens and $K$. bruceae exclusively for the Komga populations. The concept of K. praecox sensu Codd in the wild is unresolved, if such plants do exist. Although Codd (1987) regarded the Knysna-Willowmore specimens as belonging to $K$. bruceae, the name K. praecox persists and is being applied to material from the Knysna-Willowmore area (e.g. Goldblatt and Manning, 2000). It seems that this broad concept of K. praecox has been a dumping ground for material that does not key out well or fails to key out to members of the K. uvaria complex (taxa with superficially similar morphologies), but fits the geographical distribution and flowering time based on Codd's $(1968,2005)$ concept of $K$. praecox. At this point in time it is uncertain if these wild plants of so-called $K$. praecox represent a mixture of different forms. More material will have to be gathered and critically compared. A detailed study will help resolve the problems in delimiting K. praecox or validate its inclusion with the presently accepted concept of $K$. bruceae sensu stricto. I have not attempted to solve this problem in this study due to time constraints and limited field work done in the Cape Region.

### 1.2.1.4. Kniphofia brachystachya (Zahlbr.) Codd

An intermediate collection (Nicholson $s n$ ) from Karkloof between $K$. brachystachya and Kniphofia buchananii Baker was noted by Codd (2005). Codd (2005) treated it as a form of K. brachystachya until more material becomes available. This may represent a hybrid between K. brachystachya and K. buchananii.

### 1.2.1.5. Kniphofia buchananii Baker

K. buchananii is closely related to Kniphofia breviflora Harv. ex Baker. There are occasional intermediates suggesting that $K$. buchananii should be treated as a subspecies of $K$. breviflora (Codd, 2005). However, these intermediates may represent hybrids.

### 1.2.1.6. Kniphofia breviflora Harv. ex Baker

Two colour forms associated with distribution have been recorded by Codd (2005). As mentioned above K. breviflora and K. buchananii are closely related. K. breviflora also shows a close relationship with $K$. albescens Codd (see below).

### 1.2.1.7. Kniphofia albescens Codd

Codd $(1968,2005)$ noted that $K$. albescens and $K$. breviflora overlap in northern KwaZulu-Natal and it is sometimes difficult to distinguish them with certainty. $K$. albescens is a more robust plant usually growing in clusters with longer flowers and bracts.

### 1.2.1.8. Kniphofia evansii Baker

A specimen of $K$. evansii (Trauseld 741) from Giants Castle Game Reserve has a combination of characters of K. evansii and Kniphofia porphyrantha Baker. It may represent a hybrid and requires further investigation (Codd, 1968, 2005).

### 1.2.1.9. Kniphofia ichopensis Baker ex Schinz

Codd (1968) recognised this species with no infra-specific taxa. Codd (1986) described a variety of K. ichopensis viz. Kniphofia ichopensis Baker ex Schinz var. aciformis Codd. It is identical in perianth and bract characters to Kniphofia ichopensis Baker ex Schinz var. ichopensis Codd and was therefore considered to be a variety of $K$. ichopensis. However, it does have unique leaves which require further investigation and may represent a separate species (Codd, 2005). The two varieties overlap in distribution but no intermediates have been found (Codd, 2005).

### 1.2.1.10. Kniphofia fibrosa Baker

Hybrid swarms presumably derived from K. fibrosa and Kniphofia triangularis Kunth subsp. triangularis Codd have been reported from the Bushmans Nek, Upper Pholela Cave, Siponweni, Ndloveni (Hilliard and Burtt, 1987) and Mahwaqa Mountain (Anne Rennie, pers. comm.), where the putative parents are found (Codd, 1986, 2005). Both species also flower at the same time in sympatric situations on the Sunset Farm part of the Mahwaqa Mountain (personal observation).

Hilliard and Burtt (1987) also noted that Kniphofia angustifolia (Baker) Codd (= K. rufa Baker) forms hybrids with K. fibrosa and K. triangularis. K. angustifolia may have also contributed to the range of variation in K. fibrosa (Codd, 2005).

### 1.2.1.11. Kniphofia laxiflora Kunth

Codd $(1968,2005)$ found that $K$. laxiflora was a variable species with a wide distribution from Port St. Johns inland into the Drakensberg and as far north as southern Mpumalanga. He recognised three main forms (Forms A, B and C). Each form has a somewhat distinct geographical distribution but it is difficult to separate these forms in the herbarium (Codd, 2005). Thus, Codd did not award
these separate formal taxonomic rank. In southern KwaZulu-Natal K. laxiflora tends to grade with $K$. gracilis (especially in perianth length) making placement difficult (Codd, 2005). Codd $(1968,2005)$ found a few specimens with bracts of intermediate shape between $K$. laxiflora and other lax-flowering species e.g. $K$. ichopensis. These may represent hybrids.

### 1.2.1.12. Kniphofia gracilis Baker

Codd $(1968,2005)$ noted that variation within K. gracilis required further investigation. This includes examination of characters such as length and density of the inflorescences, and degree of constriction of the perianth above the ovary. In typical K. gracilis the inflorescence is short and flowers relatively dense with the perianth $14-20 \mathrm{~mm}$ long and more or less parallel-sided, expanding at the mouth. Other plants considered to belong to K. gracilis have lax, elongated inflorescences with the perianth 11-16 mm long, constricted above the ovary and expanding at about the midpoint, and varying in colour from white to yellow. Codd (1968) found several herbarium specimens that are intermediate in these inflorescence and perianth characters, making it impossible to separate the material satisfactorily into infra-specific groups in the herbarium.

In southern KwaZulu-Natal, K. laxiflora tends to intergrade with K. gracilis (especially in perianth length) (Codd, 2005). Codd (1968) found specimens of $K$. gracilis with perianths about 20 mm long, which he regarded as intermediate between K. gracilis and K. laxiflora (perianth usually $24-32 \mathrm{~mm}$ long). This renders the distinction between K. gracilis and K. laxiflora somewhat arbitrary and merging the two species would create a heterogeneous assemblage of material, which Codd considered unfavourable. Thus, Codd (1968, 2005) maintained the two species. These problematic specimens may possibly be hybrids.

The type of Kniphofia rufa Baker is a plant of uncertain origin. Furthermore, it could not be exactly matched with any material collected in the wild by Codd (1968) and may be of hybrid origin, but the evidence was inconclusive (Codd, 1968). Based on the above Codd (1968) considered discarding the name K. rufa, but found it necessary at the time to reluctantly uphold the name.

Codd (1968) found that $K$. rufa varies in colour from white to yellow or coralred. However, colours cannot be discerned in most herbarium specimens. Thus, no attempt was made to subdivide the species into varieties. Some specimens of the 'coral-red' flowering form have inflorescences that are more compact than usual and are difficult to distinguish from the Natal form of K. triangularis subsp. triangularis. Codd speculated that $K$. triangularis might have played a part in the evolution of the lax, coral-red inflorescences. However, colour is not easily observed in herbarium specimens, whereas delimitation can be made on relative inflorescence density. Thus, he included these plants as a colour form of K. rufa.

Subsequently, Codd (1986) could not find a good match of the type of K. rufa with wild material and proposed that $K$. rufa should be considered as an insufficiently known entity until matching material from the wild was found. All the other entities that did not match the type perfectly but fell under the name $K$. rufa sensu Codd (1968) were placed in K. angustifolia. Furthermore, Codd (1968) mentioned plants with longer flowers which are coral-red to orange-red may be due to hybridisation with $K$. triangularis (see above notes with regard to K. rufa). Thus, Codd (2005) considered K. rufa to be of doubtful application.

Codd (2005) noted that $K$. angustifolia exhibits some localised variation which was attributed to hybrid swarms with possibly K. triangularis subsp. triangularis. Hilliard and Burtt (1987) also noted that K. augustifolia (K. rufa) forms hyrids with $K$. fibrosa and K. triangularis. Some specimens may also be confused with K. ichopensis var. ichopensis but can be separated by leaf width.

### 1.2.1.14. Kniphofia ensifolia Baker

Codd (1968) recognised two subspecies based on flowering time (Kniphofia ensifolia Baker subsp. ensifolia Codd and Kniphofia ensifolia Baker subsp. autumnalis Codd). It appears that in the eastern Free State, K. linearifolia forms hybrids with K. ensifolia subspecies autumnalis (Codd, 2005).

### 1.2.1.15. Kniphofia coralligemma E.A. Bruce

Codd (1968) recorded three forms, which differed mainly in inflorescence colour and geographical distribution. More research is required before a decision can be reached regarding the status of these forms. Codd (2005) still recognised the three forms without formal rank being awarded.

### 1.2.1.16. Kniphofia northiae Baker

Codd (1968) noted that there were two colour forms and two leaf forms. In the typical form from the Eastern Cape, Lesotho and the Drakensberg the leaves lacked a distinct keel and were crescentiform in transverse section. In the second form (from Bergville, Estcourt and Lesotho) the leaves were broadly V-shaped with a distinct mid-rib. Codd (1968) suggested that further studies should be done in order to determine whether this species should be divided into separate taxa. Baijnath (1987) subsequently described Kniphofia albomontana Baijnath to accommodate taxa of K. northiae with V-shaped leaves using anatomical data (Baijnath, 1980) to support his decision. He also noted that K. albomontana was closely related to $K$. caulescens.

### 1.2.1.17. Kniphofia galpinii Baker

Codd (1968) noted the strong similarities between K. galpinii and K. triangularis. He considered treating K. galpinii as a subspecies of K. triangularis. However, many small differences justified retaining it as a separate species. Mention is
made of specimens that needed further investigation from Jessievale Plantation (Carolina) and the Pasture Station (Ermelo) that do not entirely match K. galpinii. Although Codd (1968) excluded these specimens from K. galpinii, he thought that they were closely related to K. galpinii and suggested that further data was needed to determine their taxonomic status. Also, some specimens cited from KwaZulu-Natal differed slightly from typical K. galpinii, but these differences were insufficient to warrant a separate status. Codd (1968) considered these to represent a form that required further investigation. Poor specimens of $K$. linearifolia may be confused with K. galpinii (Codd, 2005).

### 1.2.1.18. Kniphofia thodei Baker

Herbarium specimens of K. thodei may be confused with Kniphofia prophyrantha Baker or K. triangularis but in fresh material the floral colour of K. thodei (buds are coral red to dull red often tipped with white, while open flowers are white) helps to easily identify this species (Codd, 1968).

### 1.2.1.19 Kniphofia triangularis Kunth

Reference has already been made to $K$. triangularis subsp. triangularis under $K$. angustifolia (and K. rufa). It may have contributed to the evolution of the lax, coral-red inflorescences in the latter species. However, at times the distinction between lax and dense inflorescences are not clear cut and specimens may be difficult to place. Codd (1968) found that collections of Kniphofia triangularis Kunth subsp. obtusiloba Berger (Codd) are not clearly distinct from K. galpinii, which also occurs in Mpumalanga. Hybrid swarms presumably derived from $K$. triangularis subsp. triangularis and $K$. fibrosa have been reported above under $K$. fibrosa (Codd, 1986, 2005). In the herbarium, specimens of K. triangularis may be confused with K. porphyrantha (Codd, 2005).

### 1.2.1.20. Kniphofia porphyrantha Baker

In the herbarium it is not always easy to distinguish between specimens of $K$. porphyrantha and certain allied species with acute to acuminate bracts. However, with fresh material $K$. porphyrantha can easily be separated from $K$. triangularis subsp. obtusiloba, K. galpinii, K. thodei and Kniphofia fluviatilis Codd (Codd, 1968, 2005). Also, the closely related K. fluviatilis resembles K. porphyrantha in some respects.

### 1.2.1.21. Kniphofia littoralis Codd

This species has characteristically large fruits. When capsules are present, $K$. littoralis can be distinguished from all other species of the genus (Codd, 1968, 2005). In general appearance it resembles K. baurii, Kniphofia drepanophylla Baker and forms of $K$. rooperi. Smaller and depauperate specimens of $K$ rooperi may easily be confused with $K$. littoralis if fruits are lacking, but Codd (1968) enumerated several morphological differences. According to Codd (1968) intermediate specimens may give the impression of being hybrids, but are best considered variants of K. rooperi.

### 1.2.1.22. Kniphofia drepanophylla Baker

As noted above, the typical form of $K$. baurii may be confused in the herbarium with K. drepanophylla (Codd, 1968, 2005). Codd (2005) reported an intermediate between K. baurii and K. drepanophylla (Killick and Marais 2014) from Weza which needs further investigation.

### 1.2.2. Codd's Key

The artificial keys to species of Kniphofia that Codd $(1968,2005)$ constructed are problematic. Apart from the problems associated with the taxa discussed above, it is highly ambiguous at many dichotomies. There are at least 13 taxa that key out in more than a single couplet in the latest key (Codd, 2005). Furthermore, Codd
(2005) made extensive use of flowering times, which although useful, can be problematic as flowering periods fluctuate depending on weather patterns, especially rainfall. $K$. linearifolia is not in Codd's (2005) key which appears to be a typographical error. Kniphofia leucocephala Baijnath described by Baijnath (1992a) is wrongly placed in 'couplet 61b' which seems to best fit $K$. linearifolia.

### 1.2.3. Other systematic studies in Kniphofia

Cufodontis (1971) described K. hildebrandtii Cufod. He also changed the invalid name Kniphofia elegans Codd to Kniphofia coddiana Cufod.

Marais (1973) revised the remaining tropical African species recognising 22 species which include:

1. Kniphofia thomsonii Baker
2. Kniphofia schimperi Baker
3. Kniphofia hildebrandtii Cufod.
4. Kniphofia princeae (Berger) Marais
5. Kniphofia pumila (Ait.) Kunth
6. Kniphofia foliosa Hochst.
7. Kniphofia splendida E.A. Bruce
8. Kniphofia dubia De Wild.
9. Kniphofia bequaertii De Wild.
10. Kniphofia linearifolia Baker
11. Kniphofia kirkii Baker
12. Kniphofia grantii Baker
13. Kniphofia nana Marais
14. Kniphofia isoetifolia Hochst.
15. Kniphofia insignis Rendle
16. Kniphofia reynoldsii Codd
17. Kniphofia benguellensis Baker
18. Kniphofia reflexa Codd
19. Kniphofia pallidiflora Baker
20. Kniphofia ankaratrensis Baker
21. Kniphofia sumarae Deflers
22. Kniphofia nubigena Mildbr.

Marais (1973) did not group taxa into infrageneric sections. Marais (1973), like Codd (1968, 2005), found difficulty with some taxa. He recognised two varieties of K. thomsonii. Marais considered K. ankaratrensis (Madagascar) to be related to K. splendida (southern Africa and Malawi) and may represent the same species. He also noted that K. sumarae from Yemen was an excellent link between taxa placed in Notosceptrum and African species such as K. pumila and K. foliosa.

Blackmore (1981a, 1981b) described Kniphofia mulanjeana Blackmore and Kniphofia monticola Blackmore from Malawi. Lavranos (1983) noted that $K$. sumarae from Yemen was closely affiliated to Kniphofia acraea Codd, K. brachystachya, Kniphofia typhoides Codd and K. umbrina from southern Africa. He also supported Marais' view that K. sumarae is a good link between taxa placed in Notosceptrum and African species such as K. pumila and K. foliosa. Codd (1985) lectotypified the type species of Notosceptrum. This was done to ensure that if at a later date the genus Notosceptrum was restored, a generic name and type species would be available. This exercise raises doubt over his earlier decision to lump Notosceptrum into Kniphofia.

Kativu (1996) treated eight species for the Flora Zambesiaca area. These included K. benguellensis, K. reynoldsii, K. dubia, K. grantii, K. mulanjeana, K. princeae, K. linearifolia and K. splendida. However, he failed to include K. monticola in this treatment. Demissew and Nordal (1997) treated seven species for the Flora of Ethiopia and Eritrea viz. K. pumila, K. foliosa, K. hildebrandtii, K. isoetifolia, K. insignis, K. schimperi and K. thomsonii. Whitehouse (2002a) recorded eight species of Kniphofia from the Flora of Tropical East Africa region.

His treatment included K. thomsonii, Kniphofia goetzei Engl., K. princeae, K. pumila, K. grantii, K. bequaertii, Kniphofia paludosa Engl. and K. reynoldsii. He maintained K. goetzei, which Marais (1973) sunk under K. thomsonii, and noted that there were taxonomic problems between these two species. Furthermore, Whitehouse (2002a) maintained K. paludosa, which Marais (1973) considered a synonym of K. kirkii. Whitehouse (2002a) relegated the name K. kirkii to nomen dubium.

Thus the genus Kniphofia, as conceived at present, contains approximately 71 species (Table 1.1.), of which 48 are southern African

Table 1.1. Currently recognised Kniphofia species with distributions. Southern African taxa are assigned to their provisional species groups proposed by Codd (1968). Author citations follow Codd (2005) for southern African representatives, and Whitehouse (2002a) and Marais (1973) for representatives from Tropical Africa, Yemen and Madagascar. Abbreviations: DRC= Democratic Republic of Congo.

| Taxon | Distribution | Informal species group recognised by Codd (1968) |
| :---: | :---: | :---: |
| 1. Kniphofia acraea Codd | South Africa | 2 |
| 2. Kniphofia albescens Codd | South Africa | 3 |
| 3. Kniphofia albomontana Baijnath | South Africa | 5 |
| 4. Kniphofia angustifolia (Baker) Codd | South Africa | 4 |
| 5. Kniphofia ankaratrensis Baker | Madagascar |  |
| 6. Kniphofia baurii Baker | South Africa | 9 |
| 7. Kniphofia benguellensis Baker | Angola, Zambia |  |
| 8. Kniphofia bequaertii De Wild. | DRC, Tanzania, Burundi, Rwanda, Uganda |  |
| 9. Kniphofia brachystachya (Zahlbr.) Codd | South Africa, Lesotho | 2 |
| 10. Kniphofia breviflora Baker | South Africa, Swaziland | 3 |
| 11. Kniphofia bruceae (Codd) Codd | South Africa | 5 |
| 12. Kniphofia buchananii Baker | South Africa | 3 |
| 13. Kniphofia caulescens Baker ex Hook. $f$. | South Africa, Lesotho | 5 |

Table 1.1. continued

| Taxon | Distribution | Informal species group <br> recognised by Codd (1968) |
| :--- | :--- | :--- |
| 14. Kniphofia citrina Baker | South Africa | 10 |
| 15. Kniphofia coddiana Cufod. | South Africa | 9 |
| 16. Kniphofia coralligemma E.A. Bruce | South Africa | 5 |
| 17. Kniphofia crassifolia Baker | South Africa | 3 |
| 18. Kniphofia drepanophylla Baker | South Africa | 5 |
| 19. Kniphofia dubia De Wild. | DRC, Tanzania, Zambia, Angola |  |
| 20. Kniphofia ensifolia Baker | South Africa | 5 |
| 21. Kniphofia evansii Baker | South Africa | 7 |
| 22. Kniphofia fibrosa Baker | South Africa | 3 |
| 23. Kniphofia flammula Codd | South Africa | 8 |
| 24. Kniphofia fluviatilis Codd | South Africa | 8 |
| 25. Kniphofia foliosa Hochst. | Ethiopia | 4 |
| 26. Kniphofia galpinii Baker | South Africa, Swaziland |  |
| 27. Kniphofia goetzei Engl. | Tanzania |  |
| 28. Kniphofia gracilis Harv. ex Baker | South Africa |  |

Table 1.1. continued

| Taxon | Distribution | Informal species group recognised by Codd (1968) |
| :---: | :---: | :---: |
| 29. Kniphofia grantii Baker | DRC, Rwanda, Burundi, Tanzania, Malawi, Zambia, Uganda |  |
| 30. Kniphofia hildebrandtii Cufod. | Ethiopia |  |
| 31. Kniphofia hirsuta Codd | South Africa, Lesotho | 5 |
| 32. Kniphofia ichopensis Baker ex Schinz | South Africa | 4 |
| 33. Kniphofia isoetifolia Hochst. | Ethiopia |  |
| 34. Kniphofia insignis Rendle | Ethiopia |  |
| 35. Kniphofia latifolia Codd | South Africa | 10 |
| 36. Kniphofia laxiflora Kunth | South Africa | 4 |
| 37. Kniphofia leucocephala Baijnath | South Africa | 3 |
| 38. Kniphofia linearifolia Baker | South Africa, Malawi, Swaziland, Zimbabwe, <br> Mozambique | 10 |
| 39. Kniphofia littoralis Codd | South Africa | 9 |
| 40. Kniphofia monticola Blackmore | Malawi |  |
| 41. Kniphofia mulanjeana Blackmore | Malawi |  |

Table 1.1. continued

| Taxon | Distribution | Informal species group recognised by Codd (1968) |
| :---: | :---: | :---: |
| 42. Kniphofia multiflora J.M. Wood \& M.S. Evans | South Africa, Swaziland | 1 |
| 43. Kniphofia nana Marais | DRC |  |
| 44. Kniphofia northiae Baker | South Africa, Lesotho | 6 |
| 45. Kniphofia nubigena Mildbr. | Sudan |  |
| 46. Kniphofia pallidiflora Baker | Madagascar |  |
| 47. Kniphofia paludosa Engl. (= K. kirkii Baker) | Tanzania |  |
| 48. Kniphofia parviflora Kunth | South Africa | 2 |
| 49. Kniphofia pauciflora Baker | South Africa | 4 |
| 50. Kniphofia porphyrantha Baker | South Africa, Swaziland, Lesotho | 8 |
| 51. Kniphofia praecox Baker | South Africa | 5 |
| 52. Kniphofia princeae (Berger) Marais | DRC, Tanzania, Malawi, Rwanda |  |
| 53. Kniphofia pumila (Ait.) Kunth | DRC, Ethiopia, Sudan, Uganda, Kenya, Sudan, Eritrea |  |
| 54. Kniphofia reflexa Codd | Cameroon |  |
| 55. Kniphofia reynoldsii Codd | Tanzania, Malawi, Zambia |  |
| 56. Kniphofia rigidifolia E.A. Bruce | South Africa | 10 |

Table 1.1. continued

| Taxon | Distribution | Informal species group <br> recognised by Codd (1968) |
| :--- | :--- | :--- |
| 57. Kniphofia ritualis Codd | South Africa, Lesotho | 5 |
| 58. Kniphofia rooperi (T. Moore) Lem. | South Africa | 10 |
| 59. Kniphofia sarmentosa (Andrews) Kunth | South Africa | 5 |
| 60. Kniphofia schimperi Baker | Ethiopia, Eritrea |  |
| 61. Kniphofia splendida E.A. Bruce | South Africa, Swaziland, Zimbabwe, Malawi | 5 |
| 62. Kniphofia stricta Codd | South Africa, Lesotho | 6 |
| 63. Kniphofia sumarae Deflers | Yemen | 4 |
| 64. Kniphofia tabularis Marloth | South Africa | 8 |
| 65. Kniphofia thodei Baker | South Africa, Lesotho | 7 |
| 66. Kniphofia thomsonii Baker | DRC, Kenya, Tanzania, Uganda, Ethiopia | 2 |
| 67. Kniphofia triangularis Kunth | South Africa, Lesotho | 10 |
| 68. Kniphofia typhoides Codd | South Africa | 2 |
| 69. Kniphofia tysonii Baker | South Africa, Swaziland | 10 |
| 70. Kniphofia umbrina Codd | Swaziland |  |
| 71. Kniphofia uvaria (L.) Oken | South Africa |  |

### 1.2.4. Attempts at Infra-generic Classification

Berger (1908) divided Kniphofia into 14 sections and maintained the genus Notosceptrum. These sections were based on floral, leaf, inflorescence and bract morphology as well as caulescence and geography (viz. Section Arabicae for $K$. sumarae). Codd (1968) grouped southern African taxa into ten informal infrageneric groups of no formal rank based on probable affinity. These sections were based on leaf, floral, inflorescence and bract morphology, stature and flowering times. He saw no advantage in Berger's (1908) sections, although he viewed them as sound, and did not support Notosceptrum as a separate genus. Codd (2005) made no mention of infra-generic groupings.

### 1.2.5. Anatomy

Baijnath (1980) investigated the leaf anatomy of 18 Kniphofia species to assess the taxonomic value of leaf anatomical characters. He found that leaf surface and internal anatomy (vascular bundles and crystals) proved to be useful characters. Anatomical data did not support Notosceptrum as a separate genus. However, in most cases multiple samples of a single species were not examined to assess intra-specific anatomical variation.

### 1.2.6. Ethnobotany

The Basutos use $K$. ritualis to prepare a decoction to cure shoulder pains. It is also used by women when girls undergo sacred initiation rites, hence the epithet 'ritualis'. K. caulescens is frequently planted around Basuto huts as a charm against lightning (Codd, 1968). Several taxa are used by the Zulus but have limited medicinal value. Infusions made from rhizomes of $K$. laxiflora and $K$. rooperi are used to treat chest aliments, while crushed roots and rhizomes of $K$. uvaria are included in enemas administered for painful menstruation. Also,
infusions of $K$. buchananii and K. parviflora are used as snake deterrents (Hutchings et al., 1996). The tough fibrous leaves of some species (e.g. K. albescens) are used as twine (Baijnath, 2004). Infusions of K. uvaria are taken orally by Xhosa women to treat infertility (Matsiliza and Barker, 2001). Xhosa mothers use pieces of dried rhizome of $K$. rooperi in necklaces to bring good fortune to their children (Baijnath, 2004).

### 1.2.7. Conservation

Many Kniphofia species are in need of conservation. A high number of South African species (25) are included in the Red Data List of Hilton-Taylor (1996). Scott-Shaw (1999) documented 17 Kniphofia taxa considered to be under threat in KwaZulu-Natal and neighbouring regions. Taxa under threat are listed in Table 1.2. Witkowski et al. (2001) examined the conservation biology of K. umbrina and found it to be critically endangered.

### 1.3. Systematic position of Kniphofia within Asphodelaceae

Asphodelaceae was placed in the order Asparagales (APG, 1998) and considered a family of the lower asparagoids, which are characterised by simultaneous microsporogenesis (Chase et al., 1995; Rudall et al., 1997, Fay et al., 2000). The Angiosperm Phylogeny Group (APG, 2003) in an apparent effort to simplify Asparagales classification, have proposed that Asphodelaceae and Hemerocallidaceae be included in Xanthorrhoeaceae sensu lato. This proposal was partly put forward to facilitate and simplify teaching asparagoid families as there are difficulties experienced by non-specialists in the group (APG, 2003; Peter Stevens, pers. comm.). In this scenario Asphodelaceae would fall into Xanthorrhoeaceae and would have to be awarded a subordinate rank. The APG classification is in a state of continual refinement and future changes are anticipated judging from the uncertainties and complexity in the classification of Asparagales.

Table 1.2. List of Kniphofia species under threat in southern African.

| Taxon | Conservation Status | Source |
| :--- | :--- | :--- |
| 1. K. acraea | Rare | Hilton-Taylor (1996) |
| 2. K. angustifolia | Not threatened; Lower risk (Least concern) | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 3. K. brachystachya | Lower risk (Least concern) | Scott-Shaw (1999) |
| 4. K. breviflora | Lower risk (Least concern) | Scott-Shaw (1999) |
| 5. K. bruceae | Rare | Hilton-Taylor (1996) |
| 6. K. buchananii | Lower risk (Least concern) | Scott-Shaw (1999) |
| 7. K. citrina | Indeterminate | Hilton-Taylor (1996) |
| 8. K. coddiana | Rare; Lower risk (Near threatened) | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 9. K. coralligemma | Rare | Hilton-Taylor (1996) |
| 10. K. crassifolia | Indeterminate | Hilton-Taylor (1996) |
| 11. K. drepanophylla | Insufficiently known; Vulnerable | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 12. K. ensifolia subsp. autumnalis | Rare | Hilton-Taylor (1996) |
| 13. K. evansii | Rare; Lower risk (Near threatened) | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 14. K. fibrosa | Not threatened | Hilton-Taylor (1996) |
| 15. K. flammula | Hilton-Taylor (1996); Scott-Shaw (1999) |  |
| 16. K. hirsuta | Vulnerable | Hilton-Taylor (1996) |

Table 1.2. continued

| Taxon | Conservation Status | Source |
| :--- | :--- | :--- |
| 17. K. ichopensis | Lower risk (Least concern) | Scott-Shaw (1999) |
| 18. K. latifolia | Endangered | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 19. K. leucocephala | Endangered; Critically endangered | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 20. K. littoralis | Not threatened; Lower risk (Near <br> threatened) | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 21. K. northiae | Lower risk (Least concern) | Scott-Shaw (1999) |
| 22. K. pauciflora | Extinct; Extinct in wild | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 23. K. rigidifolia | Rare | Hilton-Taylor (1996) |
| 24. K. rooperi | Not threatened; Lower risk (Least concern) | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 25. K. sarmentosa | Rare | Hilton-Taylor (1996) |
| 26. K. thodei | Not threatened | Hilton-Taylor (1996) |
| 27. K. triangularis subsp. obtusiloba | Rare | Hilton-Taylor (1996) |
| 28. K. typhoides | Insufficiently known | Hilton-Taylor (1996) |
| 29. K. tysonii subsp. lebomboensis | Not threatened; Data deficient | Hilton-Taylor (1996); Scott-Shaw (1999) |
| 30. K. umbrina | Endangered; Critically endangered | Hilton-Taylor (1996); Witkowski et al. (1999) |

The above proposal to recognise Xanthorrhoeaceae s.l. (including Asphodelaceae and Hemerocallidaceae) has not gained wide acceptance yet. Thus, I have taken a conservative approach and retain Asphodelaceae.

Chase et al. (2000) using chloroplast DNA sequence data found that Asphodelaceae sensu Dahlgren et al. (1985) is monophyletic, and more closely related to Hemerocallidaceae and Xanthorrhoeaceae than the morphologically similar Anthericaceae. McPherson et al. (2004) found that an intron from the 3' rps12 locus is absent in all Asphodelaceae examined and some representatives of the closely related Hemerocallidaceae. This loss can be treated as a strong molecular marker for the monophyly of Asphodelaceae. Asphodelaceae is separated from other lilioid monocot groups by the combination of the following characters: general presence of anthraquinones, lack of saponins, simultaneous microsporogenesis, atypical ovular morphology and the presences of an aril. Each of these occurs in other asparagoid groups but the above combinational features distinctively define Asphodelaceae (Chase et al., 2000). Additionally several combinational seed characters that might be useful in distinguishing Asphodelaceae include presence of an aril, an endosperm with lipids and aleurone instead of starch, and an embryo that is three-quarter of the length of the endosperm. However, all the above characters except the presence of an aril are not useful for field identifications.

Morphologically, Asphodelaceae is divided into two more or less clearly delimited subfamilies, Alooideae and Asphodeloideae. Alooideae has a distinct southern African centre of radiation with outliers in Saudi Arabia, Madagascar and the Mascarene Islands. Asphodeloideae has a predominantly Eurasian distribution with significant outliers in Africa, Australia and New Zealand (Treutlein et al., 2003). Currently, the most accepted morphological treatment of Asphodelaceae is the framework of Dahlgren et al. (1985). Although this treatment is widely accepted, there have been varying and different views on the relationships of the genera in Asphodelaceae. Despite various attempts to
stabilise classification of Asphodelaceae, generic relationships remain unresolved (Treutlein et al., 2003).

Kniphofia represents a monophyletic lineage within the Asphodelaceae. However, its subfamilial affinity has been disputed. Some workers have placed Kniphofia within Alooideae based on floral morphology and inflorescence structure (Cronquist, 1981, 1988). However, other studies using different approaches have shown that Kniphofia is best placed in Asphodeloideae, supporting the Dahlgren et al. (1985) classification. Van Staden and Drewes (1994) and Van-Wyk et al. (1995) found phytochemical evidence for a close relationship between Kniphofia and other Asphodeloideae genera (viz. Bulbine Wolf and Bulbinella Kunth). Chase et al. (2000) investigated generic relationships in Asphodelaceae using chloroplast DNA (cpDNA) sequence data and found that Kniphofia is better placed in the subfamily Asphodeloideae (with Bulbinella Kunth, Bulbine Wolf, Jodrellia Baijnath, Trachyandra Kunth, Eremurus M. Beib., Asphodeline Reichenb. and Asphodelus L.). These workers support the basic framework of Dahlgren et al. (1985). However, support for two separate subfamilies is lacking. Alooideae appears to be monophyletic, while Asphodeloideae is paraphyletic (Fig. 1.2.).

Molecular studies using cpDNA sequences by Treultein et al. (2003) concentrated on subfamily Alooideae. Increased sampling of Alooideae revealed that Aloe and Haworthia are non-monophyletic (Treultein et al., 2003). Like Chase et al. (2000), Treultein et al. (2003), found Alooideae to monophyletic while Asphodeloideae was paraphyletic. Both studies (Chase et al., 2000; Treultein et al., 2003) revealed that Bulbine was sister to Jodrellia supporting the separation of the latter from Bulbine. The segregation was originally proposed based on floral morphology by Baijnath (1976). The Bulbine-Jodrellia clade is sister to the Alooideae clade (Fig. 1.2.). Immediately sister to the above clades is a lineage composed of Kniphofia and Bulbinella i.e. Bulbinella is sister to Kniphofia (Chase et al., 2000; Treultein et al., 2003) (Fig. 1.2.).


Fig. 1.2. Phylogenetic tree showing relationships between Asphodelaceae and closely related asparagoid families based on chloroplast DNA (rbcL and trnL-F) (from Chase et al., 2000).

This is a surprising result as one would expect Kniphofia to be more closely placed to the morphologically similar Alooideae and Bulbinella to be more closely placed to other morphologically similar Asphodeloideae genera e.g. Bulbine. Further research may explain the close relationship between Kniphofia and Bulbinella.

### 1.4. The present study

Even with the vast taxonomic background that currently exists for the genus, a contemporary and cohesive revision for the entire genus does not exist. The monographic work of Berger (1908) is outdated.

Most past workers examined the genus in parts based on regional limits e.g. Codd (2005). The number of specimens collected has increased considerably, and some previously poorly collected areas have received more attention over the past few decades (e.g. Tanzania). This has not only meant that knowledge of species limits has improved, but also a number of new species have been discovered and additional distributional data has accumulated (Kativu, 1996; Demissew and Nordal, 1997; Whitehouse, 2002a).

A revision of the entire genus is thus a very difficult undertaking. It will require extensive field work (on a continental scale) to understand the biology of these plants in their natural habitats. Due to limits imposed by time and resources a revision of the entire genus was not attempted in this study. Kniphofia has a complicated taxonomic history, with numerous taxonomic problems that remain unresolved. The complex taxonomic history and problematic morphological delimitation of many taxa makes additional research necessary.

In past studies of the genus limited attempts were made to examine evolutionary relationships or produce a phylogenetic classification. Thus, phylogenetic relationships within Kniphofia (and many other genera of Asphodelaceae) are
still unsettled and continue to be disputed. Furthermore, no work has been done on the biogeography or phylogeography of the genus.

### 1.5. Aims of the present study

There are four main aims of the present study:

1. To undertake a genus level, Africa-wide, assessment of diversity and endemism in the genus (Chapter 2).
2. To use DNA sequence data from chloroplast and nuclear markers to reconstruct a species-level phylogeny for the genus Kniphofia in order to better understand intra-generic species relationships and evolutionary processes (Chapter 3).
3. To use the phylogenies and nested clades (a phylogeographic approach) generated from DNA sequence data to study the biogeography and evaluate the biogeographical patterns in the genus (Chapter 4).
4. To assess anatomical variation and determine if anatomical characters are useful for species delimitation (Chapter 5).

A synthesis of this study and conclusions are presented in Chapter 6.

# Chapter 2: Chorology and Centres of Diversity 

### 2.1. Introduction

Despite its horticultural popularity of Kniphofia, no general account of its biogeography is available. Geographic patterns combined with molecular data can provide important insights into evolutionary processes (e.g. McKinnon et al., 1999; Schaal and Olsen, 2000). In Kniphofia knowledge of geographic patterns is essential in understanding its evolution and to interpret the DNA sequence data results. Thus, it is necessary to review the biogeographical patterns in Kniphofia.

### 2.1.1. Kniphofia in African plant biogeography

According to White (1978) Kniphofia is one of several genera that are centred in southern Africa and is essentially Afromontane. Others include Alepidea F.Delaroche (Apiaceae), Bowkeria Harvey (Scrophulariaceae), Lotononis (DC) Eckl. \& Zeyh. (= Buchenroedera Eckl. \& Zeyh., Fabaceae), Styppeiochloa De Winter (= Crinipes Hochst., Poaceae), Macowania Oliv. (Asteraceae) and Rendlia Chiov. (Poaceae). Of these, Styppeiochloa, Kniphofia, Macowania and Rendlia are more or less exclusively Afromontane north of the Limpopo River (White, 1978). Furthermore, Kniphofia is centered in the eastern part of southern Africa and decreases northwards on the African mountains into south-western Arabia (Yemen) with two species from Madagascar. Other genera that have a similar distribution pattern are Alepidia, Freesia Klatt (= Anomatheca Ker Gawl., Iridaceae), Dierama K.Koch (Iridaceae), Dietes Salisb. ex Klatt (Iridaceae), Knowltonia Salisb. (Ranunculaceae) and Merxmuellera Conert (Poaceae) (Goldblatt, 1978). Beyond these general trends, no research has focused on biogeographic aspects of Kniphofia.

The findings above indicate that Kniphofia is not an Afromontane element sensu stricto. It does show a strong association with the Afromontane Region but
extends beyond the boundaries of this vegetation, especially in southern Africa. Consequently it is worth examining the Afromontane Region briefly.

### 2.1.2. The Afromontane Region: a brief overview

The Afromontane Region is an archipelago-like regional centre of endemism, consisting of c. 4000 species with $75 \%$ endemism (White, 1978, 1981, 1983) and is a hot spot of diversity (Burgoyne et al., 2005). White's figures are probably an under estimate since Hilliard and Burtt (1987) found c. 1261 species from the southern KwaZulu-Natal Drakensberg alone. Although many species are local endemics, the majority, especially the dominants, are widely distributed within the Afromontane Region (White, 1981).

Relationships to other phytochoria are considered to be complex and White (1981) considered the flora to be more complex in origin and evolutionary history than any other in the world. The 'islands' that make up the Afromontane archipelago are widely distributed over Africa. These islands extend from the Lome Mountains and Tingi Hills (Sierra Leone) in the west to the Alh Mescat Mountains (Somalia) to the east, the Red Sea Hills to the north and the Cape Region (South Africa) in the south (White, 1978, 1983). The region is very diverse in lithology and physiography. Some islands of volcanic origin are of different laval ages (White, 1983). Seven regional mountain systems are recognised (White, 1978, 1983):

1. West African
2. Ethiopian
3. Kivu-Ruwenzori
4. Imatongs-Usambara
5. Uluguru-Mulanje
6. Chimanimani
7. Drakensberg

The West African system is the most isolated and some of the mountains within it are more isolated from each other than are most mountains in other systems (White, 1978, 1983). Despite the complexity and wide distances separating the islands of the archipelago, the collective flora of the region exhibits remarkable continuity, uniformity and internal cohesiveness, making it distinct enough to separate from other adjacent phytochoria (White,1978, 1981, 1983). This high degree of homogeneity across the distributional range of the Afromontane Region is reflected ecologically and phytogeographically (Linder, 1990). The Afromontane Region was not recovered by Linder (1998) as a homogeneous unit in his study testing Frank White's phytochoria, but the importance of the Afromontane Region in contributing to species richness and endemism was demonstrated. Linder (2001) examined plant diversity and endemism in subSaharan Tropical Africa. He did not recover an Afromontane centre or centres per se, because of the coarse sampling approach but reference to the Afromontane Region is made in several instances.

On most African mountains the vegetation diminishes in stature from the lower slopes to the summit but is often modified by local features of aspect, exposure, frost, soil depth and local climatic patterns which are determined by the size and configuration of the mountain in relation to the distance from the sea or other sources of moisture (White, 1978, 1983). Climate for the region is variable. The mean rainfall in the Forest Belt is usually more than 1000 mm per year. Rainfall is lower in the drier types transitional to the lowlands. In the Afroalpine Belt above the forests, some mountains have much less than 1000 mm per year. Cloud is a feature of most mountains and frost varies considerably (White, 1983).

Each massif has a unique ecology (White, 1981). On any given mountain there is a wide range of vegetation types with a corresponding change in floristic composition (White, 1983). The vegetation may have few species in common but all the types are connected by complex series of intermediates (White, 1981). The floristic differences between the extreme vegetation types on a single mountain
are usually greater than the differences between the Afromontane assemblages as a whole on the mountain and the assemblage found on nearby or distant mountains (White, 1981, 1983). On most of the islands the vegetation consists almost predominantly of Afromontane endemic or near endemic species, but in some areas the Afromontane Region is diluted by lowland species (White, 1981).

In general the African mountains show ecological and altitudinal zonation. Three broad belts are widely recognised: the Alpine Belt, the Subalpine (Ericaceous) Belts and the Forest Belt (e.g. Hedberg, 1970; White, 1983; Linder, 1990). The mountains of Africa have large spatial separation with latitudinal influence on zonation (Linder, 1990) and each massif with its own peculiarities (White, 1978). The recognition of distinct zones is often arbitrary (White, 1978). In the tropics these belts are more or less well demarcated, but further south altitudinal belts become confusing and difficult to interpret (Linder, 1990). Zonation is more defined in the mountains of East Africa where the Forest and Ericaceous belts are reasonably distinct (White, 1978). Beneath the Forest Belt there is usually a transition zone connecting the Afromontane and lower phytochoria. In Malawi, Zimbabwe and Mozambique most of the mountains lie within the Forest Belt and in these countries the mountains are surrounded by a sea of Miombo woodland, generally with no intermingling of vegetation (White, 1978).

Extensive transitional conditions occur only in South Africa, where latitude compensates for altitude. The Afromontane vegetation transcends to almost sea level and relationships with surrounding phytochoria are complex (White, 1978). This is also because of the rich surrounding flora, the great diversity of biomes and climatic provinces (summer and winter rainfall) that the Afromontane Region transects. In the equatorial regions the Alpine Belt starts between $3300-4000 \mathrm{~m}$. In the Drakensberg it is between 2800 m to the summit ( 3484 m ). In the Cape Region the Alpine Belt is reported to be above 1700 m , but is 'scarcely known' (Linder, 1990). The Subalpine (Ericaceous) Belt lies between the upper reaches of the forest margin and the transitional to the Alpine Belt. In the tropics the

Ericaceous Belt penetrates into the forest with no clear altitudinal zonation (Linder, 1990). The Forests Belt begin at c. 2000 m in Tropical Africa, c. 800 m in Zimbabwe and reaches sea level in South Africa (Linder, 1990). There is a significant amount of mixing with the surrounding lower vegetation (Linder, 1990) and the Forest Belt is a dynamic mosaic of forests and grassland (Meadows and Linder, 1993).

Data on the distribution of Kniphofia obtained from previous revisions (Codd, 1968, 2005; Marais, 1973; Kativu, 1996; Whitehouse, 2002a; Demissew and Nordal, 1997), the Pretoria Computerised Information System (PRECIS) database and collections made during this study revealed the following patterns: in East and Tropical Africa, Kniphofia is predominantly found in the Afromontane Region supporting White's (1978) observation. Furthermore, Kniphofia exhibits a strong Afromontane Grassland affinity being found predominantly but not exclusively in Afromontane grasslands. It also occasionally occurs in lower surrounding vegetation that has a grassland element (e.g. Miombo woodland) and in higher Subalpine vegetation e.g. Bale Mountains, Ethiopia (personal observation).

Kniphofia is confined mostly to the Afromontane Region in Tropical and East Africa especially the Afromontane grasslands but occasionally occurs at lower altitudes. White (1978) noted that Afromontane species which descend into lower phytochoria are marginal intruders or do so as distant satellite populations. The distribution of Kniphofia beyond the Forest Belt in Tropical and East Africa is considered to be a marginal intrusion. This is expected when the complexity of zonation on these mountains are taken into account.

The Afromontane Region in southern Africa is centered in Lesotho and the Drakensberg Region (Cowling and Hilton-Taylor, 1997). The Drakensberg forms part of the great escarpment at the eastern periphery of the southern Africa plateau, extending from the central Eastern Cape (Barkly East) in the south to the

Wolkberg (south-east Northern Province) in the north. This spans a distance of c . 1050 km with an estimated c. 2200 angiosperms in the core of the region (sensu van Wyk and Smith, 2001). The Afromontane Region in southern Africa is unique as latitude compensates for altitude and the Afromontane vegetation descends to almost sea level with intermingling and transitional conditions between Afromontane and surrounding vegetation (White, 1978). In the Cape Region enclaves of forests are found close to sea level (White, 1978, 1983). The compensation effects of latitude results in many Afromontane species descend to sea level and the lower level of the Afromontane Region becomes blurred (Moll and White, 1978).

A few Kniphofia species are found in the Subalpine Belt e.g. K. porphyrantha and three species ( $K$. caulescens, $K$. northiae and $K$. ritualis) were noted from the Alpine Belt in the Drakensberg (Killick, 1978). In South Africa, Kniphofia occurs from the Alpine Belt in the Drakensberg and descends to the coastal regions. It appears that the compensational effects of latitude for altitude has resulted in Kniphofia spreading to lower altitudes in southern Africa.

A high number of Kniphofia species (18 species; $38 \%$ of Kniphofia species in SA, $25 \%$ for the entire genus) prefer high altitudes of more than 1500 m (Codd, 1968, 2005). An altitude of 1500 m in this case is used as a conservative lower altitudinal limit value for the Afromontane vegetation, considering the intermingling of vegetation in southern Africa and that the Forest Belt mostly ranges from 1 280-1 830 m in the Drakensberg (White, 1978). Thirty species (63\% of Kniphofia species in SA, 42\% for the entrie genus) occur from 0-1 500 m i.e. a substantial number of Kniphofia species occur in the Drakensberg-Maputoland-Pondoland transition. Additionally six species ( $13 \%$ of Kniphofia species in SA, $9 \%$ for the entire genus) are strictly coastal endemics (i.e. Maputoland-Pondoland coastal endemics). The six coastal endemics include $K$. littoralis, K. pauciflora, K. coddiana, K. drepanophylla, K. rooperi and K. leucocephala.

The Afromontane (Drakensberg) and Tongoland (i.e. Maputoland)-Pondoland (Drakensberg-Maputoland-Pondoland) transition is unique in that it is the only region in Africa where the Afromontane and lowland species intermingle over an extensive area (White, 1978). The Maputoland-Pondoland Region is a mosaic that displays great physiographic diversity with steep climatic gradients. It is a mosaic of forest, thicket, savanna, grassland, fynbos and swamp vegetation (Moll and White, 1978). It interfingers with elements of the Afromontane Region (Goldblatt, 1978) especially along river valleys (Cowling and Hilton-Taylor, 1997). The borders of this region are difficult to demarcate as it includes tropical, subtropical and afromontane elements. It is considered as an artifical rather than a natural floristic unit (van Wyk and Smith, 2001).

### 2.1.2.1. Afromontane Grasslands: a brief overview

Kniphofia shows a strong association with Afromontane grasslands. Thus it is worth examining these grasslands in more detail. The most extensive vegetation in the Afromontane Region is fire-maintained grassland consisting predominantly of species which are also abundant in the lowlands (White, 1978). The grasslands are perennial tussock grasslands subject to regular burning and are associated with a rich herbaceous flora. Afromontane grasslands are more extensive on the drier mountains with frequent fire (Meadows and Linder, 1993).

Evidence suggests that Afromontane grasslands are not recent (Ellery et al., 1991; Meadows and Linder, 1993), contradicting earlier views of White (1978) and Acocks (1953) who believed that these grasslands were of recent origin due to anthropogenic activity. There is a growing consensus that the current distribution of grasslands predates intensive farming by thousands of years (Ellery and Mentis, 1992; Meadows and Linder, 1993). High levels of endemism amongst herbaceous flora of grasslands (especially geophytes) also indicates a
long history for this component in the Afromontane Region (Meadows and Linder, 1993; O'Connor and Bredenkamp, 1997; Burgoyne et al., 2005).

In Tropical and East Africa most Afromontane vegetation occurs above 2000 m (White, 1983). At high altitudes Afromontane species become increasingly numerous and lowland forest gives way to transitional forest and, ultimately where massifs are high enough, to montane forests (Moll and White, 1978). The secondary grasslands of the Afroalpine Belt differ from those of the Forest Belt in both composition and chorological relationship. Grasses of the Afroalpine belts are usually confined to the high mountains (White, 1978).

The most extensive vegetation of the Forest Belt in South Africa is Themeda triandra grassland. This grassland consists predominately of species which are also abundant in the lowlands (White, 1978). In South Africa patterns are obscured because 'temperate' grasses descend much lower and the most abundant tropical grass (Tremeda triandra) ascends relatively high (White, 1983). In the Afromontane regions of southern Africa most of the endemics are associated with grasslands (Hilliard and Burtt, 1987; Meadows and Linder, 1993; Cowling and Hilton-Taylor, 1997).

Since Kniphofia has a strong Afromontane grassland affinity, factors influencing grassland distributions are presumed to influence the distribution of Kniphofia to some degree. The distribution of grasslands is the result of the subtle interplay of climate, topology, fire, and grazing. The overall extent of grasslands seems to be strongly determined by climatic variables, while fire and grazing exert considerable influence on the boundaries of the biome (O'Connor and Bredenkamp, 1997). Ellery et al. (1991) demonstrated that climate is the overriding determinant of grassland biome distribution as a whole in southern Africa. A number of grassland and savanna sites have the potential to support forest but are prevented from doing so by fire or some other disturbance. As the degree of seasonality of rainfall increases, vegetation is susceptible to burning
due to the prolonged or intense dry period. Ellery et al. (1991) have suggested that climate contributes to the maintenance of grasslands by promoting a disturbance regime that excludes woody plants. Meadows and Linder (1993) have found that for afromontane grasslands seasonality and not total rainfall is important in the shift towards grassland from forest.

Bond et al. (2003) believe that the vegetation of South Africa could be very different if fires were infrequent. The eastern half of the country could be covered with trees in the absence of fire. It appears that most of the higher rainfall southwestern and eastern parts of the country owe their current vegetation to high fire frequencies (Bond et al., 2003)

The aim of this chapter was to study the chorology of Kniphofia and to determine centres of diversity and endemism for Kniphofia. Several approaches were used to determine diversity and endemism. An Africa-wide chorological assessment was done for the entire genus. These were used to delimit centres of diversity and endemism. Subsequently, southern African was studied in greater detail. A chorological assessment was done for southern African to delimit areas of diversity and endemism. A numerical analysis was done to find areas of diversity. Additionally endemism in southern Africa was assessed using a parsimony analysis of endemicity approach and mapping of range restricted taxa.

### 2.2. Materials and Methods

Chorology in a broad sense is the study of the distribution of taxa and floristic regions and their history (see van Wyk and Smith, 2001). In a more restricted botanical sense it applies to the study of the distribution of a specific group of plants (e.g. a family or genus). A basic tenet of such a study is to determine the number of species and distribution thereof within a given area. These in turn assists in delimiting areas of diversity and endemism.

Numerical analyses can also be used to determine centres of diversity. Species richness and diversity per unit area (e.g. quarter degree grids) provides a rapid assessment of species rich areas. This data is then used to determine areas of diversity. In this approach areas with similar species composition are located by a clustering method (e.g. Linder and Mann, 1998). Clusters/groups of areas (typically grids) are then delimited and these groups are mapped to determine areas of diversity.

Areas of endemism can be located using a parsimony analysis of endemicity approach (Morrone, 1994) by subjecting distributional data to a parsimony analysis. The results are then used to determine areas of endemism. Areas of endemism can also be located by plotting the distribution of range restricted species. The distribution outlines for these taxa are mapped and areas of overlap are used to delimit areas of endemism (e.g. Linder and Mann, 1998).

### 2.2.1. Africa-wide chorological assessment for Kniphofia

Distribution data were obtained from previous revisions (Codd, 1968, 2005; Marais, 1973; Kativu, 1996; Whitehouse, 2002a; Demissew and Nordal, 1997), the PRECIS database and collections made during this study. These were plotted onto maps of Africa. Based on these distribution patterns several more or less geographically isolated distribution areas were delimited for Africa, Madagascar and Yemen. Distribution patterns were then used to produce a chorological map of Africa and Madagascar which depicted species richness by means of isochores. Centres of endemism, overlap regions and outliers were determined for these regions by comparing species lists of these areas following the method of Linder (1983). Centres of diversity were also determined from these lists. Centres of endemism are defined as regions having more than $30 \%$ endemism, outliers are areas with less than $30 \%$ endemism and where the majority of the species are held in common with a nearby centre. In overlap regions endemism is less than $30 \%$ and the non-endemic species are held in common with two other
centres (Linder, 1983). Subcentres (within centres) were also determined for diversity and endemism. Subcentres of diversity were determined from species lists and areas with more than four species were delimited as subcentres. The requirement of a minimum of four species to define a subcentre is subjective. However, it is the most convenient way of defining subcentres for Kniphofia especially in Tropical Africa were species richness is not as high as southern Africa. Subcentres of endemism were defined as areas that have $\geq 30 \%$ endemism for that centre.

### 2.2.2. Studies in Southern Africa

A more detailed phytogeographic analysis was done for southern Africa (South Africa, Swaziland and Lesotho) because of the large amount of distributional data accumulated for the region. The PRECIS data was provided in $1 / 16^{\text {th }}$ degree square (i.e. quarter degree) grids for 46 of the 48 taxa. The two exceptions were K. crassifolia and K. praecox. K. crassifolia is only known from the type collection with vague locality details. This species most likely falls within the quarter degree grid 2329DD (Houtbosch, Limpopo Province; Pieter Winter, pers. comm.). All collections of K. praecox were placed under K. bruceae in PRECIS, viz. collections from the Western Cape. The problems associated with K. praecox were outlined in Chapter 1. Codd (2005) in his latest revision upheld K. praecox, which is followed in this study. The necessary addition and modifications were done for K. crassifolia and K. praecox respectively.
2.2.2.1. Numerical analysis of distribution data to determine areas of diversity

Quarter degree grid (QDG) diversity or species richness which is the number of species per QDG was obtained from the PRECIS data and collections made during this study to determine species rich areas.

The data above was used to create a matrix for southern African Kniphofia taxa with species as characters and QDGs as terminal units (Appendix 1). Areas with similar species composition were located by using the method of Linder and Mann (1998). The data set was analysed using NT-SYS version 2.0. (Rohlf, 1998). A similarity matrix was generated using the Jaccard similarity (J) coefficient and clustering of grids was performed using UPGMA (Unweighted Pair Group Method, Arithmetic Average) clustering method. The Jaccard co-efficient disregards shared absences and is therefore suitable for biogeographical analysis where absences may either be due to 'real' absences or a result of under collection. Grids with a single species (singletons) show either $0 \%$ or $100 \%$ similarity to other grids, thus accentuating errors and distorting results (Linder and Mann, 1998). Consequently, these grids were removed prior to the analysis. Clusters/groups of grids were arbitrarily delimited by a phenon line and these groups were mapped to determine areas of diversity. This approach was also advocated at the half degree grid (HDG) scale. The data set showing Kniphofia species as characters and HDGs as terminal units (with singleton HDGs removed) is given in Appendix 2.

### 2.2.2.2. Parsimony analysis of endemicity (PAE)

Areas of endemism i.e. those grids which have at least some species resticted to them were located using a parsimony approach (Morrone, 1994). The QDG data set (Appendix 1) was subjected to a parsimony analysis conducted using PAUP* 4.0b10 (Swofford, 2002). Uninformative characters (i.e. species) were excluded and all characters were equally weighted and unordered. A randon input analysis was performed to determine if there were multiple islands of equally most parsimonious trees (Maddison, 1991). A full heuristic search was conducted on the trees found by this method with TBR branch swapping and MAXTREES set at 1000 . A strict consensus tree was constructed from all the most parsimonious trees. Groups of grids were mapped and those which have at least two species unique to them are regarded as areas of endemism. The distribution boundaries of
these endemic species are mapped to delineate the boundaries of each area (Morrone, 1994; Morrone and Crisci, 1995). This technique was also applied at the HDG scale.
2.2.2.3. Areas of endemism based on mapping of range restricted species

Areas of endemism were located by plotting the distribution of range restricted species which are defined as species with a distribution area of ten QDGs or less (Linder and Mann, 1998). The distribution outlines for these taxa are overlaid on a map and areas of overlap are used to delimit centres of endemism (Linder and Mann, 1998).

### 2.3. Results and Discussion

### 2.3.1. Africa-wide chorological assessment for Kniphofia

Six centres of diversity were delimited from the chorological analysis (Fig. 2.1.). These are Madagascar, Cameroon, Rift Valley, South-central Africa, Zimbabwe and South Africa (including Lesotho and Swaziland). These areas are based on geographic limits and species assemblages rather than political boundaries. The Rift Valley Centre includes species from Yemen, Sudan, Ethiopia, Kenya, Northeastern parts of the Democratic Republic of Congo (DRC), Uganda, Burundi, Rwanda and northern Tanzania. The South-central African Centre includes species from Angola, South-eastern parts of the DRC, Zambia, Malawi, Mozambique and south-central Tanzania. The Zimbabwe Centre includes species from Zimbabwe and a species from Mozambique. The South Africa (SA) Centre includes species from Lesotho, Swaziland and a single species from Mozambique. The number of species within these centres were represented by means of isochores (Fig. 2.1.).


Fig. 2.1. Centres of diversity recognised for Kniphofia with number of species (underlined and italised) represented by isochores. Bold numbers represent the centres of diversity: I= Madagascar, II= Cameroon, III= Rift Valley, IV= South-central Africa, V= Zimbabwe, VI= South Africa. The insert shows the vegetation map of Africa and Madagascar (White, 1978) with black areas representing the Afromontane Region. (Map source: T. Dorschied, Arizona State University ©).

The most diverse centres in descending order are: South Africa (48 species), South-central Africa (14 species), Rift Valley (11 species), Zimbabwe and Madagascar (both with two species) and Cameroon (one species). South Africa represents the centre of diversity for the genus. A summary of the results for species diversity, endemism and patterns of overlap are presented in Table 2.1.

Table 2.1. Species richness, levels of endemism and patterns of overlap among the centres of diversity for Kniphofia.

|  | Madagascar | Cameroon | Rift <br> Valley | South-central <br> Africa | Zimbabwe | South <br> Africa |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Madagascar | 2 |  |  |  |  |  |
| Cameroon | 0 | 1 |  |  |  |  |
| Rift Valley | 0 | 0 | 11 |  |  |  |
| South-central <br> Africa | 0 | 0 | 5 | 14 | 2 |  |
| Zimbabwe | 0 | 0 | 0 | 2 | 2 | 48 |
| South Africa | 0 | 0 | 0 | 2 | 38 |  |
| Percentage of <br> species in genus | 3 | 1.5 | 16 | 18 | $46(96)$ |  |
| No. of endemics <br> $(\%$ endemics) | $2(100)$ | $1(100)$ | $7(64)$ | $7(50)$ | $0(0)$ |  |

Five of the six centres are considered to represent centres of diversity and endemism for Kniphofia. The exception is the Zimbabwe Centre which has no endemics. The two species ( $K$. linearifolia and K. splendida) of this centre are found in the South Africa Centre and the South-central Africa Centre. Zimbabwe is best treated as a region of overlap. Linder (1983) also found a lack of endemism in the Chimanimani Mountains for Disinae and regarded Zimbabwe to be an overlap region.

The centres of diversity and endemism for Kniphofia have a strong Afromontane affinity. A comparison of the centres of diversity for Kniphofia with the Afromontane centres of White (1978) and the centres of diversity for Disinae recovered by Linder (1983) is given in Table 2.2.

Table 2.2. Comparisons between the centres of diversity for Kniphofia with the Afromontane centres of White (1978) and the centres of diversity for Disinae recovered by Linder (1983).

| Kniphofia: centres <br> of divesity | Afromontane centres of White <br> $(\mathbf{1 9 7 8})($ RMS regional mountain <br> system) | Disinae: centres of diversity <br> (Linder, 1983) |
| :--- | :--- | :--- |
| South Africa | Drakensberg RMS found within <br> SA Centre for Kniphofia | Cape and Natal-Transvaal <br> Centres of Linder (1983) within <br> SA Centre for Kniphofia |
| Zimbabwe | corresponds to Chimanimani RMS | corresponds to Zimbabwe <br> Centre of Linder (1983) |
| South-cental Africa | includes entire Uluguru-Mulanje <br> RMS and part of Kivu-Ruwenzori <br> RMS | mostly within South-central <br> Africa Centre of Linder (1983) |
| Rift Valley | includes part of Kivu-Ruwenzori <br> RMS, and entire Imatongs- <br> Usambara and Ethiopian RMS | corresponds mostly to Linder's <br> (1983) East Africa Centre |
| Cameroon | found within part of West African <br> RMS | found within Linder's (1983) <br> West Africa Centre |
| Madagascar | mountains of Madagascar not <br> considered as an island of the <br> Afromontane archipelago | found within Linder's (1983) <br> Madagascar Centre |

### 2.3.2. Subcentres of diversity and endemism

A summary of species richness and levels of endemism for subcentres within designated centres of diversity and endemism (using the chorological approach) are presented in Table 2.3. Eight subcentres of diversity (Fig. 2.2.) and two subcentres of endemism are proposed within the six centres of diversity. Subcentres for southern Africa are not discussed in detail in this section. It is more appropriate to discuss the subcentres for SA in section 2.3.3. (Studies in the South African Centre). Other subcentres are discussed below.

Table 2.3. Species richness and levels of endemism for subcentres of Kniphofia.

| Centre: Subcentre | Total no. of species | No. of endemics | Percentage endemism <br> (*= subcentre of <br> endemism) |
| :--- | :--- | :--- | :--- |
| Rift Valley | 11 | 7 | $64 \%$ |
| Ruwenzori | 5 | 0 | 0 |
| Ethiopia | 7 | 5 | $46^{*}$ |
| South Central Africa | 14 | 7 | $50 \%$ |
| Mulanje | 5 | 2 | 14 |
| Nyika | 4 | 0 | 0 |
| South-central Tanzania | 7 | 2 | 14 |
| South Africa | 48 | 46 | $96 \%$ |
| Cape-Karoo | 8 | 26 | $64^{*}$ |
| Extended Drakensberg | 40 | 5 | 10 |
| Northern South Africa | 15 |  |  |



Fig. 2.2. Subcentres of diversity (shaded grey) within centres of diversity recognised for Kniphofia. Bold numbers represent the centres of diversity: $\mathrm{I}=$ Madagascar, $\mathrm{II}=$ Cameroon, $\mathrm{III}=$ Rift Valley, IV= South-central Africa, V= Zimbabwe, VI= South Africa (Map source: T. Dorschied, Arizona State University ©).

### 2.3.2.1. Subcentres in the Rift Valley Centre

Two areas were delimited as subcentres of diversity: the Ruwenzori and Ethiopia subcentres. The Ruwenzori Subcentre corresponds to the Kivu-Ruwenzori regional mountain system of White (1978).

The Ruwenzori Subcentre includes the boundary regions of the following countries: DRC, Burundi, Rwanda, Uganda and Sudan. None of the species found in this subcentre are endemics as all species (K. grantii, K. pumila, K. thomsonii, K. princeae and K. bequaertii) are found in the South-central Centre and this region is considered as an area of overlap. Most of this subcentre is in the Lake Victoria Regional Mosaic. This mosaic is a junction of five distinct floras viz. Guinea-Congolian, Sudanian, Zambezian, Somali-Masai and Afromontane. The vegetation is a mosaic of improvised variants of the first four floras, in some cases with an admixture of Afromontane species (White, 1983). This may account for its overlap status.

The Ethiopia Subcentre corresponds to the Ethiopian regional mountain system of White (1978). It is also a subcentre for endemism ( $46 \%$ endemism) for Kniphofia. Only one other subcentre of endemism (the Extended Drakensberg Subcentre) was recovered for Kniphofia. The geology of Ethiopia is varied. It is a tectonically active area with the Rift Valley transecting through. About $50.4 \%$ of land above 2000 m and $79.9 \%$ landmass above 3000 m in Africa occurs in Ethiopia (Yalden, 1983). According to Brenan (1978) the size of Ethiopia (c. 1 $200000 \mathrm{~km}^{2}$ ) and the topodiversity accounts for the rich endemic element of the flora. The tectonics of the region may also play a role. These factors may also explain the regional diversity and endemism for Kniphofia in Ethiopia

### 2.3.2.2. Subcentres in South-central Africa

Three areas were delimited as subcentres of diversity: Mulanje, the Nyika Plateau and the South-central Tanzania Subcentres (Fig. 2.2). The Mulanje Subcentre represents Mount Mulanje (southern Malawi). This area is not considered a subcentre of endemism ( $14 \%$ endemism). Most of the non-endemics are found in the in the nearby Nyika Plateau Subcentre. Furthermore, K. linearifolia and K. splendida are found in the South Africa and Zimbabwe, and the region is best treated as a region of overlap. None of the species of the Nyika Plateau are endemics and most of the species are found in other subcentres of the Southcentral Africa Centre, therefore, it is considered to be a region of overlap.

The South-central Tanzania Subcentre represents south-central Tanzania, the northern tip of Malawi and part of northern Zambia. This area is not considered a subcentre of endemism ( $14 \%$ endemism). Some species (K. grantii, K. thomsonii and $K$. princeae) are found in the East Africa Centre, while other species ( $K$. renoldsii, K. grantii and K. princeae) are found in other subcentres of the Southcentral Africa Centre. Additionally K. dubia is widespread in Zambia, in areas that do not fall into a designated subcentre. It is thus best to consider this region as an area of overlap.

### 2.3.3. Studies in the South African Centre

2.3.3.1. Subcentres of diversity and endemism based on chorology

Three areas were subjectively delimited as subcentres of diversity in SA based on distribution patterns of species viz. the Cape-Karoo, the Extended Drakensberg and the Northern South Africa subcentres (Table 2.3., Fig. 2.2.). The Cape-Karoo Subcentre did not show much QDG diversity i.e. no grids had more than three species. Despite this lack of diversity, the entire area was analysed to assess
endemism of Kniphofia within this region. This was done for two reasons. Firstly the Fynbos and karooid biomes are unique within Africa, and secondly numerous workers have found links between the Cape Region and the Afromontane Region (discussed later). When the entire Cape-Karoo Subcentre is considered, eight species in total are distributed within the area of which three are endemic. It does not represent a subcentre of endemism (6\% endemism) but rather an outlier as most of the non-endemics are found in the in the nearby Extended Drakensberg Subcentre. It is found within the Cape Floristic and Succulent Karoo regions and extends eastwards into the Albany Centre (sensu van Wyk and Smith, 2001). It is also interesting to note that while Kniphofia is not widespread in the Fynbos and karooid biomes other Asphodelaceae genera (Aloe, Haworthia, Bulbine, Bulbinella, Trachyandra) are common in these regions. Also, the Disinae is most speciose in the Cape Region while Kniphofia has poor representation for this region.

Two Afromontane regions have been noted as hot spots in SA: the Drakensberg and its associated uplands falls into the Eastern Mountain Centre, and the Wolkberg Centre (Cowling and Hilton-Taylor, 1997). The two other SA subcentres for Kniphofia broadly correspond to these regions. The Extended Drakensberg Subcentre represents the Drakensberg and extended areas in Lesotho, Free State (FS), Eastern Cape (EC) and KwaZulu-Natal (KZN). This extended area represents the major diversity of the genus and is better treated as a single unit. It is also a subcentre for endemism ( $54 \%$ endemism) for Kniphofia. It mostly corresponds to the Eastern Mountain Centre (Cowling and Hilton-Taylor, 1997). It covers almost the entire Drakensberg Alpine Centre and most of the Maputoland-Pondoland Region of van Wyk and Smith (2001). It also extends into the Albany Centre (van Wyk and Smith, 2001) in the south.

The Northern South Africa Subcentre represents the area that covers the former Transvaal Province of SA, Swaziland and the southern tip of Mozambique. Additionally several endemic Kniphofia species are from this area. This area is
not considered a subcentre of endemism ( $10 \%$ endemism). Most of the nonendemics are found in the nearby Extended Drakensberg Subcentre with two species ( $K$. linearifolia and $K$. splendida) extending north to the Zimbabwe Centre and the South-central Africa Centre (Malawi). It is therefore considered to be a region of overlap. This includes the entire Wolkberg Centre (sensu Cowling and Hilton-Taylor, 1997). This region includes the entire Soutpansberg Centre, Wolkberg Centre, Sekhukhuneland Centre, Barberton Centre and northern parts of the Maputoland-Pondoland Region of van Wyk and Smith (2001).
2.3.3.2. Areas of diversity using numerical analyses

Grid diversity and species richness were based on the quarter degree scale for the SA region and is shown in Fig. 2.3.


Fig. 2.3. Quarter degree grid species richness of Kniphofia within the South Africa Centre $(\boldsymbol{\Delta}=1-3$ species, $\bullet=4-6$ species, $\llbracket=7-9$ species, $\star=10-12$ species, $\diamond=13$ species).

The 12 most species rich QDGs in descending order are:

- Champagne Castle (2929AB): $\mathrm{n}=13$
- Bushmans Nek (2929CC): $\mathrm{n}=11$
- Van Reenen (2829AD): $\mathrm{n}=10$
- Sani Pass (2929CB), Ntabamhlope (2929BA) and Witsieshoek (2828DB): $\mathrm{n}=9$
- Naudes Nek (3028CA), Estcourt (2929BB) and Cathedral Peak (2829CC): $\mathrm{n}=8$
- Weza (3029DA), Nottingham Road (2929BC) and Kamberg (2929BC): $\mathrm{n}=7$

The QDG data set (excluding singleton QDGs) used for the numerical analysis using the Jaccard similarity (J) co-efficient and UPGMA clustering contained 191 QDGs and 48 taxa. The phenogram generated is not shown as the patterns were difficult to interpret. A half degree grid approach was explored to determine if patterns were more evident at this scale. The HDG data set (excluding singleton HDGs) used for the numerical analysis using the Jaccard similarity (J) coefficient and UPGMA clustering contained 121 HDGs and 48 taxa.

The overall patterns recovered by the QDG and HDG analysis revealed similar patterns. However, distinct patterns especially within the main clusters were recovered for the HDG analysis. A possible reason for this result is that the large amount of QDG grids are too small in geographical cover and contain a fewer number of species. This results in too many QDGs with similar species composition and clustering of grids that do not necessarily reflect close proximity. At this scale there was a high degree of geographical scattering of clusters which in turn appears to reflect much geographical overlap. Consequently, this makes patterns difficult to infer. The HDG approach takes into account species in four neighbouring QDGs. Increasing the spatial area is more likely to increase the number of taxa in a HDG and also increased the likelihood
that the species composition of grids in close proximity are similar. At this scale scattering and overlap are reducded as the grids appears to reflect similar species compositions in close proximity. The HDG approach phenogram generated is shown in Fig. 2.4. Four clusters (A-D) were delimited and mapped (Fig. 2.5.).


Fig. 2.4. The phenogram of half degree grids derived from the Jaccard analysis of the data set excluding singleton HDGs. The dense black phenon line shows the main clusters while the less dense phenon line shows sub clusters. Main clusters are labeled A-D and sub clusters are numerically depicted.


Fig. 2.5. Map showing the distribution of main clusters of the phenogram derived from the Jaccard analysis of the data set excluding singleton HDGs. Main clusters are color coded: Cluster $\mathrm{A}=$ blue, Cluster $\mathrm{B}=$ red, Cluster $\mathrm{C}=$ green and Cluster $D=$ grey (see text for details).

Cluster A has a distinct Cape-Karoo distribution. It is found within the Cape Floristic and Succulent Karoo regions and extends eastwards into the Albany Centre (sensu van Wyk and Smith, 2001).

Cluster B has a distribution that covers much of northern, eastern and partly central SA, and extends into the Cape-Karoo Region in the south were it overlaps with Cluster A. An outlier of Cluster B is found in the Cape-Karoo Region. Cluster B is divided into four sub clusters (Fig. 2.6.A.).


Fig. 2.6. Map showing the distribution of main clusters with sub clusters derived from Jaccard analysis of the data set excluding singleton HDGs. A. Cluster B with sub clusters B1 (red), B2 (yellow), B3 (orange) and B4 (pink). B. Cluster C with sub clusters C1 (bright green) and C2 (dark green).

Cluster B1 is found mostly with the Maputoland-Pondoland Region of van Wyk and Smith (2001). It also extends into the Albany Centre (van Wyk and Smith, 2001) in the south. The outlier of Cluster B found in the Cape-Karoo Region is also from this sub cluster (B1). It extends from Calvinia south east to the De Doorns region. It is found within the Cape Floristic and Succulent Karoo regions of van Wyk and Smith (2001). This may represent remnants an ancestral escarpment track for Kniphofia and/or the Afromontane vegetation. Cluster B2 is found mostly with the Drakensberg Alpine Centre of van Wyk and Smith (2001). Most of western distribution of Cluster B3 is in no regions or centres of endemism as defined by van Wyk and Smith (2001). In the north and north east of its distribution, it extends partly into the Wolkberg and Sekhukhuneland centres of van Wyk and Smith (2001). In the south and south east it is found partly in the Barberton Centre and the Maputoland-Pondoland Region of van Wyk and Smith (2001). Most of the entire distribution of Cluster B4 is in no regions or centres of endemism as defined by van Wyk and Smith (2001). In the north east of the distribution of this sub cluster, it extends only partly into the Wolkberg and Sekhukhuneland centres of van Wyk and Smith (2001).

Cluster C has an eastern coast distribution with a single outlier in the Winterton region. It extends south and abuts Cluster A. The Winterton outlier has two species in the HDG ( $K$. brachystachya and $K$. gracilis) and may be an artifact of under collecting. Cluster C is divided into two sub clusters (Fig. 2.6.B.). The entire distribution of Cluster C is found in the Maputoland-Pondoland Region of van Wyk and Smith (2001). Only two HDGs (in the Mkambati region) of C1 are strictly in the Pondoland Centre of van Wyk and Smith (2001). Also only the two northern most HDGs (in the Richards Bay area) of C2 are strictly in the Maputoland Centre. All the others regions of Cluster C are in the MaputolandPondoland Region of van Wyk and Smith (2001).

Cluster D has a distinct extreme northern SA distribution. Cluster D transects through the Soutpansberg Centre of van Wyk and Smith (2001). It also marginally penetrates into the Wolkberg and Sekhukhuneland centres of van Wyk and Smith (2001) in the south.
2.3.3.3. Areas of endemism using parsimony analysis of endemicity (PAE)

A PAE approach was attempted for South African Kniphofia species. The HDG PAE analysis did not recover any areas of endemism as the consensus tree was a single large polytomy (not shown). Thus the discussion below concentrates on the QDG analysis. The data matix included 419 QDGs and 48 species. Four species were found to be parsimony uninformative viz. K. acraea, K. crassifolia, K. flammula and K. leucocephala and excluded from the analysis. A strict consensus tree based on the distribution of Kniphofia at the quarter degree scale is presented in Fig. 2.7. which is also a large polytomy. Three small clades were delimited and mapped (Fig. 2.8.). Clade A has a Cape-Karoo distribution (from Calvinia in the north extending south to Ceres, and from Citrusdal in the west towards the Sutherland region in the east). Clade B is distributed from the Underberg region northwards to Frere. Clade C is distributed from Iswepe southeast to the Wakkerstroom-Vredehof region.

Clades A and B had only one unique species and using PAE excludes these regions. Clade C had six unique species viz. K. albescens, K. baurii, K. fluvialitis, K. linearifolia, K. multiflora and K. porphyrantha. The distribution of these species were then mapped within the distribution of the clade to delimit an area of endemism ( C in Fig. 2.8.) and is termed the Iswepe-Wakkerstroom-Vredehof area of endemism. The Iswepe-Wakkerstroom-Vredehof area of endemism does not fall into any major regions or centres delimited by van Wyk and Smith (2001). It lies outside the western boundary of Maputoland-Pondoland Region. It is also in close proximity to the Barberton Centre which is to the north. This area is within the Northern SA Subcentre (of the chorological approach).


Fig. 2.7. Stict consensus tree of 1000 most parsimonious tree based on distribution of Kniphofia species at the quarter degree scale. The actual grids are not shown for each terminal as it was not possible to visually depict them in the above tree. Main clades are labeled A-C.


Fig. 2.8. Map showing the distribution of main clades of stict consensus tree based on distribution of Kniphofia species at the QDG scale. Main clades are labeled A-C (see text for details).

The PAE approach has major disadvantages as it excludes highly localised endemics confined to a single QDG that may not share this grid with other nearby endemics and/or conspecifics. These taxa are autoapomorphies which are not parsimony informative and are thus excluded from the analysis. This could potentially under estimate endemism.
K. baurii and K. linearifolia have wide distributions in SA, while K. fluvialitis and K. porphyrantha are also considered to be wides with a more restricted distribution. Thus these four species are not 'true' endemics to the Iswepe-Wakkerstroom-Vredehof area of endemism. However, K. multiflora and K. albescens are confined to the northern parts of SA. If the strict consensus tree (as above) is not well resolved then many areas rich in endemics are excluded.

Linder and Mann (1998) also found that PAE excluded regions rich in endemism. QDGs were used as infomation units and depend on presence data only. Under collection may result in grids being excluded from endemic areas (Linder and Mann, 1998). Additionally scale may be an important factor to consider. In this study the HDG PAE analysis did not recover any areas of endemism. The HDG scale may be too coarse to detect areas of endemism. The use of larger areas (e.g. whole degree grids) may reduce the noise caused by many smaller empty units and also reduces resolution (Linder and Mann, 1998).

### 2.3.3.4. Areas of endemism found by mapping of range restricted species

Twenty-one Kniphofia species are found in ten or less QDGs and were considered to be range restricted (Table 2.4).

Table 2.4. Kniphofia species occurring in ten or less QDGs.

| Number $\quad$ of | Kniphofia species |
| :--- | :--- |
| 1 | K. acreae, K. crassifolia, K. leucocephala, K. flammula |
| 2 | K. bruceae, K. drepanophylla, K. evansii, K. <br> pauciflora, K. umbrina |
| 4 | K. hirsuta, K. latifolia |
| 5 | K. coddiana |
| 7 | K. praecox, K. tabularis |
| 8 | K. brachystachya, K. breviflora, K. rigidifolia |
| 9 | K. albomontana, K. angustifolia, K. sarmentosa |
| 10 | K. fibrosa |

The mapped distribution of these range restricted species are shown in Fig. 2.9.A. Areas of endemism were determined from this map and are defined by contours of overlapping distributions (Fig. 2.9.B.).


Fig. 2.9. A. Map showing the outlines of distribution of range restricted Kniphofia species. B. The dense black out lines show areas of endemism (A-E) which are defined by the overlapping distributions of range restricted Kniphofia species (see text for details).

Five areas of endemism (Fig. 2.9.B.) were determined using this method viz.:
A. Cape area of endemism (defined by K. sarmentosa and K. tabularis): extends from Calvinia to Groenfontein in the south.
B. Albany area of endemism (defined by K. fibrosa and K. bruceae): centred in the Kei Road-Komga region.
C. Pondoland Coast area of endemism (defined by $K$. coddiana and $K$. drepanophylla): centred in the Mkambati-Kanyayo region.
D. Northern KZN area of endemism (defined by K. fibrosa and K. latifolia): distributed in the Nkandla-Bobanango region.
E. Drakensberg-Maputoland-Pondoland area of endemism (defined by $K$. drepanophylla, K. hirsuta, K. fibrosa, K. albomontana, K. brachystachya, K. angustifolia, K. breviflora and K. evansii) is the largest area. It extends from the southern Free Sate into the Eastern Cape (including parts of Lesotho) in a southern direction, and eastwards into KZN and the Eastern Cape.

The Cape area of endemism is found within the Cape Floristic and Succulent Karoo regions of van Wyk and Smith (2001) and does not fall into any areas of diversity and endemism found by Linder and Mann (1998) for Thamnochortus. The Albany area of endemism falls into a very small part of the Albany Centre of van Wyk and Smith (2001). The Pondoland Coast area of endemism represents a small portion of the Pondoland Centre of van Wyk and Smith (2001). The Northern KZN area of endemism represents a very small area of the MaputolandPondoland Region of van Wyk and Smith (2001). The Drakensberg-MaputolandPondoland area of endemism covers a large part of the Drakensberg Alpine Centre and the Maputoland-Pondoland Region of van Wyk and Smith (2001).

This approach also has disadvantages. Range restricted species are considered to be taxa within ten or less QDGs. Under collecting may result in a taxon to be considered range restricted. Range restricted status may not be due to 'real' absences but a result of under collection. Highly localised range restricted taxa
that are not sympatric in QDGs with other range restricted species are also not taken into account as overlapping of range restricted species defines this method. Eight species ( K. acreae, K. crassifolia, K. flammula, K. latifolia, K. leucocephala, K. pauciflora, K. praecox and K. rigidifolia) with restricted ranges do not have distributions that overlap with other range restricted taxa and were not used to define areas of endemism. Furthermore taxa within ten or less QDGs may have a wide distribution and are not a 'true' range restricted species. In this study, K. fibrosa which is present in ten QDGs has a range from northern KZN to the Albany region. Excluding this species results in only three areas of endemism (Cape, Pondoland Coast and Drakensberg-Maputoland-Pondoland).
2.3.3.5. Comparison of areas of diversity and endemism in the South African Centre

Several approaches were explored to determine areas of diversity and endemism in SA. Areas of diversity were found by plotting distributions of species and then subjectively delimiting subcentres of diversity (chorological approach). A numerical analysis was also done to find areas of diversity. Delimiting areas of diversity in the SA region presented a formidable challenge.

The Cape-Karoo Region was recovered as an area of diversity in both approaches viz. Cluster A of the numerical analysis and the Cape-Karoo of the chorological approach. Furthemore an outlier of Cluster B (B1) is found in the Cape-Karoo Region.

A Northern SA area of diversity was also recovered by both approaches. The area delimited by the numerical approach (Cluster D) was far smaller in geographical cover and at the extreme north when compared with the chorological approach (Northern SA Centre). The rest of the Northern SA Centre is represented by two sub clusters from Cluster B (B3 and B4) which are north of the $28^{\circ}$ S latitude.

This is very interesting as the $28^{\circ} \mathrm{S}$ latitude is also the approximate southern boundary of the Northern SA Centre (chorological approach).

The Extended Drakensberg Subcentre (chorological approach) is represented by Cluster C and two sub clusters of Cluster B (viz. B1 and B2) of the numerical approach. Cluster B1 (excluding the Cape-Karoo outlier) fits a MaputolandPondoland Region distribution but extends into the Albany Centre (van Wyk and Smith, 2001) in the south. Cluster B2 fits a Drakensberg Alpine Centre distribution of van Wyk and Smith (2001). While Cluster C (excluding the Winterton outlier) is found in the coastal regions of the Maputoland-Pondoland Region of van Wyk and Smith (2001).

It is difficult to delimit clear cut and distinct areas of diversity as there is a high degree of overlap using the two approaches (above). The transitional nature of southern African grasslands, the many ecotones and altitudinal transgression of Afromontane Grassland may be responsible for large number of overlapping Kniphofia distributions. These factors (especially in the greater Afromontane and Maputoland-Pondoland regions) may account for the above results.

Endemism was determined by three methods. In the first approach the subcentres of diversity from the chorological approach were analysed for levels of endemism. Using this approach only the Extended Drakensberg Subcentre (with $54 \%$ endemism within SA) was recognised as a subcentre of endemism. The second approach using PAE recovered only one area of endemism. The Iswepe-Wakkerstroom-Vredehof area of endemism is defined by six species i.e. $13 \%$ endemism within SA. The third approach entailed mapping range restricted species and using overlapping distributions to determine areas of endemism. This approach recovered five areas of endemism viz. a Cape, Albany, Pondoland Coast, Northern KZN and a Drakensberg-Maputoland-Pondoland area. The Cape, Albany, Pondoland Coast and Northern KZN areas of endemism were each defined by two endemics i.e. each has $4 \%$ endemism within SA. The

Drakensberg-Maputoland-Pondoland area of endemism was defined by eight endemics i.e. $17 \%$ endemism within SA.

The PAE approach recovered the Iswepe-Wakkerstroom-Vredehof area of endemism which is within the Northern SA Subcentre (of the chorlogical approach). The mapping of range restricted approach recovered a Cape area of endemism falls in a small part of the Cape-Karoo Subcentre found in the chorological approach. Additionally the Albany, Pondoland Coast, Northern KZN and Drakensberg-Maputoland-Pondoland areas of endemism are found within the boundaries of the Extended Drakensberg Subcentre recovered in the chorological approach.

The PAE and mapping of range restricted species approaches did not recover exactly the same centres but some interesting patterns are worth noting. The Cape, Albany and Pondoland Coast areas of endemism are too far south and with a different suite of species for a detailed comparison with the Iswepe-Wakkerstroom-Vredehof area of endemism i.e. only the Drakensberg-Maputoland-Pondoland and Northern KZN areas of endemism are worth considering with the Iswepe-Wakkerstroom-Vredehof area of endemism.

The Drakensberg-Maputoland-Pondoland area of endemism (obtained using the mapping of range restricted species method) is the largest of the three areas and covers a large part of the Drakensberg Alpine Centre and the MaputolandPondoland Region of van Wyk and Smith (2001). The Northern KZN area of endemism (mapping of range restricted species method) represents a very small area of the Maputoland-Pondoland Region of van Wyk and Smith (2001) and is the smallest of the three areas of endemism under consideration. The Iswepe-Wakkerstroom-Vredehof area of endemism (PAE approach) lies outside the extreme inland (western) boundary of the Maputoland-Pondoland Region and the Barberton Centre is in close proximity to the north. The boundaries of the Barberton Centre have not been mapped in detail (van Wyk and Smith, 2001) and
the possibility that the Iswepe-Wakkerstroom-Vredehof area of endemism is within this centre cannot be ruled out. The Barberton Centre has a strong afromontane affinity in terms of the vegetation and flora (van Wyk and Smith, 2001) and has been treated as part of the Drakenberg Regional Mountain System by White (1978). Floristically the Barberton Centre is part of the Afromontane Region and mountainous areas in the Barberton Centre may have served as afromontane refugia (van Wyk and Smith, 2001). The Iswepe-WakkerstroomVredehof area of endemism appears to reflect this afromontane affinity.

The Drakensberg-Maputoland-Pondoland area of endemism is defined by $K$. drepanophylla, K. hirsuta, K. fibrosa, K. albomontana, K. brachystachya, K. angustifolia, K. breviflora and K. evansii. The Northern KZN area of endemism is defined by K. fibrosa and K. latifolia. The Iswepe-Wakkerstroom-Vredehof area is defined by K. albescens, K. baurii, K. fluvialitis, K. linearifolia, K. multiflora and $K$. porphyrantha. Suprisingly not a single species is shared by all three regions of endemism. Only K. fibrosa is shared by the Drakensberg-Maputoland-Pondoland and the Northern KZN areas of endemism. Also none of the areas of endemism recovered by PAE and mapping of range-restricted taxa show any degree of overlap. These patterns are indeed very hard to comprehend.

It is difficult to assess the above findings in a broader context as no studies exist that have defined areas of diversity endemism in the Drakensberg, the Maputoland-Pondoland Region, the Drakensberg-Maputoland-Pondoland transition and northern parts of SA. Also no studies could be found on a particular plant group that has examined areas of diversity and endemism on a fine scale (as above) for these particular regions. Delimiting areas of diversity and endemism for Kniphofia in SA presents an arduous task as shown above. Perhaps it may be more convenient to express areas of diversity and endemism in terms of a core area, regions of overlap and outlier regions. In this scenario the Drakensberg-Maputoland-Pondoland region will be designated as the core area of
diversity and endemism, with the Northern SA and Cape-Karoo regions as outliers and the regions in between treated as regions of overlap.

### 2.4. Conclusions

Kniphofia has a strong Afromontane Grassland affinity in Tropical and East Africa but occasionally extends beyond the boundary of the Afromontane vegetation. In South Africa it is found from high altitudes to coastal habitats but the most speciose regions for Kniphofia are Afromontane grasslands. The compensation of latitude for altitude may largely explain this pattern in southern Africa. It is thus not considered to be an Afromontane element, but rather an Afromontane associate. Other factors such as fire and climate which influence grassland distribution in southern Africa may also influence the distribution of Kniphofia. In the Drakensberg-Maputoland-Pondoland transition soil and geology may be more important factors to understand distribution patterns in Kniphofia. However, this will entail a detailed study of soil and lithology that are required for the different species of Kniphofia.

Kniphofia has six centres of diversity, five of these are centres of endemism. The South African Centre is the most diverse, species rich and the largest centre of endemism for Kniphofia. Delimitation of areas of diversity and endemism within these centres is more challenging as demonstrated by a more detailed study of the SA region. However, eight subcentres of diversity are proposed of which only two are considered subcentres of endemism (the Extended Drakensberg and Ethiopia subcentres) at this stage. A more comprehensive study for the entire genus will require the loan of material from southern Africa, African and international herbaria to verify identifications and locality data. This could not be done in this study due to time limitations. Thus, the data presented here should be regarded as preliminary attempt to better understand the biogeographical patterns for the entire genus.

The different approaches used to study diversity and endemism of Kniphofia in SA produced varying results. PRECIS data is known to have a $10 \%$ error factor. Specimen identifications and locality data were not checked and verified for the PRECIS data. Hence the data and results presented here may have some degree of error and may partly account for the results obtained. Another major issue in these studies, especially endemism, is collecting intensity. The PRECIS data may not include collections from smaller regional herbaria that may have a higher localised collecting intensity and more local Kniphofia collections. Also there may be over collecting in accessible areas and more importantly under collecting in areas that are inaccessible for which data is critically needed. This is of particular importance in Kniphofia. Although the genus has a wide distribution in SA, endemic species frequently occurs in mountainous terrain in areas that are not well collected. The lack of appropropraite data may partly account for the differing results.

## Chapter 3: Phylogenetic Reconstruction

### 3.1. Introduction

Phylogeny reconstruction using DNA sequence data provides plant systematists with unrivaled scope for investigating relationships and evolutionary processes. Since the 1980's the molecular phylogenetic approach has re-shaped and revolutionised our understanding of relationships and evolution at all taxonomic levels in plants (Crawford, 2000; Soltis and Soltis, 2000a; Borsch et al., 2005). Initial molecular systematic studies focussed on resolving higher level (suprageneric) relationships (e.g. Chase et al., 1993). However, more recently specieslevel molecular phylogenies of plants have become more common in the literature as DNA regions suitably variable at the species level have been found for various groups (e.g. van der Niet et al., 2005; Archibald et. al., 2005).

DNA sequence data can be obtained from three sources in angiosperms: chloroplast DNA (cpDNA), nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). These sources each have advantages and disadvantages for the taxonomic level of study and the nature of the evolutionary problem (Soltis and Soltis, 1998). Numerous regions of the chloroplast and nuclear genomes are routinely used for phylogenetic inference in plants. Mitochondrial DNA sequences are also now being used more frequently (Chat et al., 2004; Bakker et al., 2005; Guo and Ge, 2005). Plant molecular systematists have relied heavily on the chloroplast genome. Chloroplast markers are still the primary source of data for inferring phylogenies followed by the nuclear ribosomal internal transcribed spacer (ITS) region (Shaw et al., 2005).

Genomes are composed of different segments of DNA (genes, introns, spacers). These regions mutate at different rates due to functional constraints and each region generally has a typical taxonomic range of application. However, rates of evolution for a specific marker may vary between groups (Doebley et al., 1990;

Bousquet et al., 1992) and the range of application may vary vastly from group to group (Soltis and Soltis, 1998). Thus, the mode and tempo of DNA sequence evolution varies for different markers.

### 3.1.1. The Chloroplast Genome

The chloroplast genome is circular, ranging in size from 120-217 kilobase pairs (Kbp) (Palmer, 1987; Dowling et al., 1990). The chloroplast genome contains (with few exceptions) two duplicate regions in reverse orientation, called the inverted repeats (IR). The inverted repeats range from $12-25 \mathrm{Kbp}$ in length, is structurally conserved and accounts for length variation between different groups (Kim and Lee, 2005). The occurence of insertions or deletions (indels) of nucleotides which results in length mutations are common in cpDNA. The majority of these length mutations are extremely short ( $1-10 \mathrm{bp}$ ) and occur mostly in non-coding regions (Palmer, 1987). The two IR regions separate the remainder of the molecule into the large single-copy (LSC) and the small single copy (SSC) regions (Kim and Lee, 2005).

The chloroplast genome shows a high degree of conservation in size, structure, gene content and linear order of genes (Palmer, 1987; Downie and Palmer, 1992; Kim and Lee, 2005). Despite the general assumption that the chloroplast genome is conservative, some studies have shown that its composition is not static. It can lose segments over time by gene deletion and intron loss. For example McPherson et al. (2004) have found that an intron from the 3 ' rps12 locus is absent in all Asphodelaceae examined (including Kniphofia) and some representatives of the closely related Hemerocallidaceae. This loss can be treated as a marker supporting the monophyly for Asphodelaceae and these Hemerocallidaceae representatives.

The abundance of cpDNA in leaf cells, its ability to be isolated easily, its small size and the highly conserved mode of cpDNA evolution have made cpDNA
useful for systematic comparisons (Palmer, 1987). The technical ease of working with cpDNA is another advantage and many molecular phylogenies for plants are still based solely on chloroplast markers (e.g. Scheen et al., 2004). Consequently, this has created a bias towards the use of cpDNA in molecular systematic studies (Harrison, 1991; Morton, 2000). The extensive use of the chloroplast genome has led to the construction of many primers for both coding and non-coding regions of this genome (e.g. Taberlet et al., 1991; Sang et al., 1997; Shaw et al., 2005), which has greatly facilitated phylogenetic studies.

The chloroplast genome evolves slowly at the nucleotide sequence level and generally evolves more slowly than nuclear DNA. In some cases the rates of substitution are so slow that data from multiple regions have to be combined to gain phylogenetic insights at lower levels (Goldblatt et al., 2002; Butterworth and Wallace, 2004; Barfuss et al., 2005; Shaw et al., 2005).

Different parts of the chloroplast genome evolve at different rates (Palmer, 1987). As a result, a wide range of possibilities exist for resolving relationships using data from the chloroplast genome, from the level of species to family, and even higher levels (Soltis and Soltis, 1998). The conservative nature of the chloroplast genome limits applicability, potentially excluding studies of closely related species and intra-specific populations. However, several studies have successfully used cpDNA variation to examine population-level relationships and evolutionary processes within species (e.g. Saltonstall, 2002; Huang et al., 2002; Honjo et al., 2004; Zhang et al., 2005).

The genetic data present in cpDNA is of great interest in phylogeny and population genetics mainly because of the non-mendelian mode of inheritance of the genome. Chloroplasts are usually maternally inherited in angiosperms (Palmer, 1987; Harris and Ingram, 1991; Korpelainen, 2004). Uniparental transmission of cpDNA provides information unavailable from the nuclear genome, which is bi-parentally inherited.

Hybridisation is an important phenomenon in angiosperm evolution and speciation (Vriesendorp and Bakker, 2005). Hybridisation has played a vital role in the evolution of many plant lineages throughout their histories (Church and Taylor, 2005; Okuyama et al., 2005) and it is becoming more apparent that reticulate evolution is more frequent than previously thought (Ainouche et al., 2003). As the chloroplast genome is uniparentally inherited and haploid, it reveals only half the parentage in angiosperms. If hybridisation is followed by introgression and subsequent fixation of alien cpDNA, then the phylogeny will resolve the maternal lineage (Small et al., 2004). A central assumption in phylogenetics is that terminal taxa are the product of bifurcating lineage splitting events, rather than the products of reticulation. The uniparental, usually maternal inheritance of the chloroplast genome is bifurcating rather than reticulate. The chloroplast genome is also haploid with no intra-individual allelic variation (Small et al., 2004).

If chloroplast capture (the movement of a chloroplast genome from one species to another by introgression) is undetected it will bias phylogenetic estimates. It can lead to erroneous phylogenetic conclusions, as only the maternal lineage is reconstructed (Rieseberg and Soltis, 1991; Cronn et al., 2002), but when recognised it can be very informative about evolutionary processes (Soltis and Soltis, 1998). At higher taxonomic levels, where hybridisation is unlikely, organellar and nuclear phylogenies should be concordant (Palmer, 1987). Thus, any conclusions drawn from cpDNA phylogenies have to be preliminary and tentative until they are compared with nuclear data (Cronn et al., 2002).

### 3.1.1.1. Non-coding regions in the chloroplast genome

In general non-coding DNA regions [introns and intergenic spacers (IGS)] have been viewed as more variable than coding regions due to fewer functional constraints (Taberlet et al., 1991; Gielly and Taberlet, 1994). Non-coding regions
tend to evolve more rapidly than coding regions, by the accumulation of indels at a rate equal to that for nucleotide substitution and have become very useful below the family level (Gielly and Taberlet, 1994). Gielly and Taberlet (1994) found that non-coding cpDNA (trnL-F region) evolved faster than coding cpDNA ( $r b c L$ ). Studies of coding and non-coding regions show that the variable sites in $r b c L$ change faster than those of the non-coding $\operatorname{trnL}-\operatorname{trn} F$ region, however, the trnL-trnF region has more variable sites than rbcL (Chase et al., 2000; Richardson et al., 2000). Reeves et al. (2001) found that protein coding regions (rbcL and rps4) had fewer variable sites overall but those present changed more frequently than the more numerous variable sites in the $\operatorname{trnL}-F$ region. These results may be explained by non-coding regions being constrained by their own secondary structures (Asmussen and Chase, 2001).

However, in some cases non-coding regions do not have enough variation and have to be combined with coding regions to provide desired resolution (Goldblatt et al., 2002; Butterworth and Wallace, 2004; Barfuss et al., 2005). Also Sauquet et al. (2003) reported that coding $n d h F$ and matK regions were as or more variable than non-coding regions in Magnoliales and Myristicaceae. Third codon positions in coding regions like $r b c L$ have been shown to be under less functional constraint as they change more often than first and second codon positions (Kellog and Juliano, 1998).

Despite all the research on non-coding cpDNA evolution, we still know very little (Bremer et al., 2002). Non-coding cpDNA regions have been thought to be unsuitable for resolving deep level phylogenies because of high mutational and evolutionary rates (Nickrent et al., 2004). The fast rate of evolution has lead to the notion that most of the sites in non-coding DNA will be saturated when used at higher levels in phylogenetic reconstruction (Borsch et al., 2003). However, Bremer et al. (2002) have shown that non-coding regions are almost as good as coding regions in higher ranks of the asterids. Borsch et al. (2003) demonstrated
that a large number of variable sites change only once in non-coding regions and these regions could be used at higher levels to infer basal angiosperm relationships. Non-coding DNA can have secondary structures, regulating regions and different functions that constrain DNA. It consists of independently and randomly evolving parts as well as constrained parts (Bremer et al., 2002). Knowledge of these features could optimise phylogenetic reconstruction.

Choosing an appropriate non-coding region for a particular taxonomic level is essential for maximising its utility as a phylogenetic tool (Kelchner, 2000). Shaw et al. (2005) compared relative rates of evolution among non-coding regions between broad taxonomic groups. The phylogenetic utility of different noncoding cpDNA regions within a group can vary tremendously (Shaw et al., 2005). In some plant groups that have undergone recent radiation it may be difficult to generate sufficient phylogenetic signal due to the relatively slow rate at which mutations accumulate, even for rapidly evolving non-coding regions (Small et al., 1998).

In non-coding cpDNA microstructural changes of four to six nucleotides are frequent (Kelchner, 2000; Borsch et al., 2003). Many of these are simple sequence repeats (SSR). In some positions these changes can occur repeatedly, completely or partly. These require attention in homology assessment (Borsch et al., 2005). Slipped-stranded mispairing, stem-loop secondary structures and mutational triggers should be recognised. Kim and Lee (2005) have reported the widespread presence of many small inversions in non-coding regions of cpDNA of many land plants. These should also be recognised in phylogenetic reconstruction (Kim and Lee, 2005). Presently, phylogenetic approaches do not routinely take these factors into account even though these phenomena could enhance the phylogenetic utility and accuracy of non-coding cpDNA data (Kelchner, 2000).

Despite these drawbacks, Shaw et al. (2005) found that since 1995 studies using non-coding cpDNA are increasing every year, with the continued reliance on a few selective regions. Most of these studies have employed some portion of the $\operatorname{trn} L-F$ region or the trnK-matK region despite these markers having fewer potentially informative characters than other options. Very few investigators are using other non-coding cpDNA regions from the numerous possibilities that exist. Shaw et al. (2005) attributes this trend to the lack of knowledge of the relative evolutionary rates of different non-coding cpDNA regions.

### 3.1.2. The Nuclear Genome

Plants have nuclear genomes that are complex. This genome has extensive structural variation in size, chromosome number, number and arrangement of genes and number of genome copies per nucleus (Kellogg and Bennetzen, 2004). Nuclear DNA other than ribosomal DNA (rDNA) exists either as high copy repetitive DNA or low to moderate copy DNA elements. Distinguishing sequences related by descent (orthologues) from a massive pool of related but non-orthologous sequences is challenging. Low copy nDNA typically evolves independently of paralogous sequences and tend to be stable in position and copy number facilitating identification and isolation of orthologous sequences (Small et al., 2004).

In land plants rRNA genes are organised into two distinct tandem arrays. One is composed of 5S rRNA genes and intergenic spacers in tandem arrays at one or more chromosomal loci. The second is the 18S-5.8S-26S rDNA cistron (Small et al., 2004). This marker is by far the most frequently used nuclear marker (Álvarez and Wendel, 2003; Small et al. 2004). Recently more studies are beginning to use low copy nuclear genes because of the problems and limitations imposed by cpDNA and nDNA (Mort and Crawford, 2004; Small et al., 2004). Low copy nuclear genes are limitless and generally evolve faster than cpDNA and ITS sequences (Small et al., 1998; Cronn et al., 2002). Faster evolutionary
rates result in greater reward for sequencing effort as more variation is found per unit sequence than in organellar genes. In numerous studies low copy nuclear genes have proven to be more useful than chloroplast and/or ITS sequence data (Baumel, et al. 2002; Ingram and Doyle, 2003; Levin et al., 2005).

Nuclear genes also provide numerous independent unlinked loci for comparative phylogenetic inference with cpDNA markers. The combination of maternally inherited cytoplasmic markers with biparentally inherited nuclear markers provides a more precise and accurate determination of parentage (Ainouche et al., 2003). This can assist with inference of hybridisation, introgression and lineage sorting (Doyle et al., 2003; Small et al., 2004).

However, even at this early stage of usage, several limitations of nDNA have been noted (see Small et al., 2004 for review). These included complex architecture and dynamics. Genes have a tendency to exist as a family, which leads to difficulty in identifying and isolating orthologous genes.

Plants hybridise and undergo polyploidy processes that are frequent modes of evolution in plants (Baumel, et al. 2002, Ainouche et al., 2003, Soltis et al. 2003; Doyle et al. 2003). It is well known that hybridisation can result in polyploidy. Polyploidy is common in plants with estimates ranging from $30-80 \%$ of angiosperms being polyploid (Hegarty and Hiscock, 2005). Polyploidisation can have major effects on genome structure and function (Soltis et al., 2003; Adams and Wendel, 2005). It can also have major effects on the evolution and ecology of taxa involved (Soltis et al., 2003). The results from nuclear markers may be more complex than those of chloroplast markers in cases of polyploidy because nuclear markers can reflect multiple donors to a polyploid taxon (Mason-Gramer, 2004). The complex architecture and dynamics of the nuclear genome coupled with factors such as hybridisation and polyploidy could make identification and isolation of orthologous genes difficult.

Other issues (that to varying degrees may be compounded by hybridisation and/or polyploidy) include concerted evolution, paralogous sequences and the presence of intra-specific, intra-populational and intra-individual variation (heterozygosity). Moverover, the target nDNA occurs in relatively low quantities, consequently the amplification of single-copy genes can be problematic.

### 3.1.3. Regions of DNA employed in this study

It is often necessary to use multiple independent data sets to gain insights on phylogenetic relationships (Cronn et al., 2002). Independent sources of data improve the tracking of species rather than gene phylogenies. This approach allows for phylogenies generated from independent data sources to be compared and to test for congruency. Comparing phylogenies at lower taxonomic levels can be invaluable to explain evolutionary processes that cannot be achieved by either genome alone (Baumel et al., 2002; Cronn et al., 2002; Doyle et al. 2003; Ingram and Doyle, 2003). DNA sequence data from both the chloroplast and nuclear genome were utilised for this study. It was also necessary to ensure that gene regions sampled had adequate variation, to produce informative phylogenies.

Choosing an appropriate marker for molecular phylogenetics is of fundamental importance (Knoop, 2005). A priori selection of appropriate markers for phylogenetic studies is often difficult (Gielly and Taberlet, 1994; Pelser et al., 2003, Knoop, 2005). There is a paucity of information on the relative tempo of evolution among different regions (Shaw et al., 2005) and the phylogenetic resolution of different markers is highly dependent on the taxonomic group (Nickrent et al., 2004). Furthermore, it is difficult to predict from character variability or sequence divergence what or how much sequence data will be adequate for any given phylogenetic problem (Bohs, 2004) as variability alone does not always constitute phylogenetic usefulness. For the purposes of this study, four non-coding chloroplast regions (trnT-L spacer, trnL intron, rps16
intron and $p s b A-t r n H$ spacer) and one nuclear region (ITS) were screened for phylogenetic usefulness.

### 3.1.3.1. The $\operatorname{trn} T-\operatorname{trn} F(\operatorname{trn} T-F)$ region

Among the first non-coding regions to be exploited for lower level systematic studies was the trnT-F region. The trnT-F region is located in the large single copy region of the cp genome, about eight Kbp downstream from rbcL. Three highly conserved transfer RNA (tRNA) genes are found in this region: threonine (UGU), leucine (UAA) and phenylalanine (GAA). These genes are separated by intergenic spacers of several hundred base pairs (Taberlet et al., 1991). The entire trnT-F region is composed of seven units (Taberlet et al., 1991):
(i) a small portion of the 3 ' end of the threonine (UGU) gene
(ii) the trnT-L intergenic spacer (IGS)
(iii) the 5' exon of $\operatorname{trnL}$
(iv) the trnL intron
(v) the 3' exon of $\operatorname{trn} L$
(vi) the trnL-F IGS
(vii) a small portion of the phenylalanine (GAA) gene

The primers designed by Taberlet et al. (1991) were situated in conserved regions and demonstrated amplification in diverse land plants ranging from mosses (e.g. Stech, 2004) to angiosperms. The non-coding portions are the $\operatorname{trn} L$ intron, as well as two intergenic spacers between $t r n T-L$ and $\operatorname{trnL} L-F$.

The $\operatorname{trnL}$ intron and trnL-F spacer have become the most popular and widely used non-coding cpDNA markers in plant systematics (Shaw et al., 2005; Kim and Lee, 2005). Initial comparisons suggest that these regions evolve at rates similar to $r b c L$, but can evolve at rates up to three times faster than $r b c L$, depending on the study group (Soltis and Soltis, 1998).

Bakker et al. (2000) found that nucleotide substitutions in the $\operatorname{trnL}$ intron accumulated in a more uniform pattern than the trnL-F spacer in angiosperms, suggesting differing functional constraints between the two regions. The trnL intron showed sequence conservation in the regions flanking the trnL exons while the central part was variable. Bakker et al. (2000) assumed that since the trnL-F spacer had no secondary structural elements, the $\operatorname{trn} L$ intron and $t r n L-F$ spacer where probably co-transcribed. Bakker et al. (2000) concluded that in the trnL-F region there was neutrality in substitutions i.e. the $\operatorname{trn} L$ intron and $t r n L-F$ spacer evolve neutrally. The spacers of the trnT-F region are not required for processing of tRNA and the trnL gene maintains secondary structure and sequence elements critical for self-splicing from precursor RNAs. No promoter elements were found in the trnT-L and trnL-F spacer regions in Gnetales (Won and Renner, 2005). The absence of conserved promoter elements for tRNA genes from these spacer regions suggests that these spacers are not under functional constraint. This contradicts Bakker et al. (2000) suggestions that the trnL-F spacer (but not the $\operatorname{trn} T-L$ spacer) is under differing functional constraint. Won and Renner (2005) suggested that the absence of promoter elements upstream of the genes relieves the spacers from functional constraint that would otherwise be necessary if promoters were present. Release from the tRNA promoting/processing mechanism may explain why the spacers are less conserved with many indels and point mutations (Won and Renner, 2005). No studies of this nature were found for angiosperms where a similar scenario may apply.

Usually these regions are used in studies of closely related species or genera. However, Bremer et al. (2002) and Borsch et al. (2003) demonstrated that the trn $T$ - $F$ region could infer relationships at higher levels. Fay et al. (2000) used a combined analysis of four plastid regions $(r b c L, a t p B, \operatorname{trnL}$ intron and $\operatorname{trn} L-t r n F$ spacer) to resolve relationships in the Order Asparagales. Renner and Chanderbali (2000) used the trnT-L region in a combined approach to resolve relationships in Hernandiaceae, Lauraceae and Monimiaceae (Order Laurales).

Sauquet et al. (2003) examined relationships in Magnoliales using multiple cpDNA regions including the $t r n T-F$ region.

This region has also been found to be informative at the family level. Bayer and Starr (1998) used the $t r n L$ intron and the $\operatorname{trnL}-F$ intergenic spacer to resolve relationships among tribes of Asteraceae. Bayer et al. (2000) used trnL-F sequences to reconstruct the phylogenetic relationships in Gnaphalieae (Asteraceae). Chase et al. (2000) investigated generic relationships in Asphodelaceae using chloroplast DNA ( $r b c L$ and $t r n L$-trnF) sequence data. These authors found that $t r n L-F$ region only marginally out-performed $r b c L$. In a comparative study of coding and non-coding regions in palms, Asmussen and Chase (2001) found that trnL-F sequences were almost as useful as the rps16 intron sequences in phylogenetic inferences. This study focussed on subfamilial and tribal delimitations in Arecaceae.

The presence of many small inversions appears to be a common feature of the trnT-F region, which may account for a large number of sequence differences. A single inversion (i.e. a single evolutionary event) may be interpreted as many point mutations. This could lead to erroneous phylogenetic reconstruction (Kim and Lee, 2005). Indels, which are characteristic of non-coding regions, are also common in this region (Bakker et al., 2000; Borsch et al., 2003). These are derived from either deletion or duplication of adjacent sequences or occur in nonrepetitive regions of the spacer (Goldenberg et al., 1993). Borsch et al. (2003) examined numerous basal angiosperm families and had to first assess the primary homology of indels. Thereafter substitutions in indels and overlapping indels were treated.

### 3.1.3.1.1. The $\operatorname{trn} T-L$ spacer

The $\operatorname{trn} T-L$ spacer is not as frequently used as the $\operatorname{trnL}-F$ regions (Perret et al., 2003; Shaw et al., 2005), although it is the most variable of the three markers
(Small et al., 1998, Neves et al., 2005), with the trnL-F spacer generally more variable than the $\operatorname{trnL}$ intron (e.g. Bayer and Starr, 1998; Bayer et al., 2000). The $\operatorname{trn} T-L$ spacer varies in plants from c. 400 to 1500 bp . The unpopularity of the $\operatorname{trn} T-L$ spacer is due to difficulties associated with amplification. It also frequently has large AT rich regions that may be difficult to align (Shaw et al., 2005).

Despite the limited application of the $\operatorname{trn} T-L$ spacer, it was found to be variable in Kniphofia and is thus reviewed in detail here. In many studies this spacer has been used in combination with sequences from other cpDNA regions (particularly adjacent trnL-F sequences) to infer phylogenetic relationships at various levels. The high variability of the spacer has resulted in the application of this region to inferring relationships at the species and genus level. However, it has been used at higher levels in several combinational studies (Bremer et al., 2002; Borsch et al.; 2003; Fay et al., 2000; Renner and Chanderbali, 2000; Sauquet et al., 2003).

The $\operatorname{trn} T-L$ spacer has also proven useful at various infra-familial levels (generic, tribal and subtribal limits) in Apocynaceae (Liede, 2001; Liede and Täuber, 2002; Liede et al., 2002; Liede and Kunze, 2002; Meve and Liede, 2004a; Meve and Liede, 2004b; Liede-Schumann et al., 2005), Cactaceae (Applequist and Wallace, 2002), Cyperaceae (Roalson et al., 2001), Atherospermataceae (Renner et al., 2000), Gesneriaceae (Perret et al., 2003); Rubiaceae (Razafimandimbison and Bremer, 2002; Lantz and Bremer, 2004; Alejandro et al., 2005; Lantz and Bremer, 2005) and Scrophulariaceae (Kornall and Bremer, 2004).

It has been used in many species level studies to gain insights into phylogenetic relationships at the species level. Some of these are summarised in Table 3.1.

It has also proven in some instances to be variable at intra-specific level e.g. Cyclobalanopsis glauca [Fagaceae, Huang et al. (2002)], Phragmites australis
[Poaceae, Saltonstall (2002)] and Primula sieboldii [Primulaceae, Honjo et al. (2004)]. Mason-Gamer (2004) used the trnT-F region (along with other markers) to examine the evolutionary history of Elymus repens (Poaceae). Zhang et al. (2005) found the trnT-F region useful in inferring the phylogeography of Juniperus przewalskii (Cupressaceae).

Nickrent et al. (2004) could not obtain sequences in trnT-F regions (regions not specified) for many taxa in Arceuthobium. This problem was attributed to sequence divergence at the primer binding sites caused by either substitutional mutations or deletions. Thus, these workers have questioned the universal applicability of the primers of Taberlet et al. (1991).

Table 3.1. Examples of phylogenetic studies that have utilised the $\operatorname{trn} T-L$ spacer at the species level.

| Study | Genus studied | Makers used | Comments |
| :---: | :---: | :---: | :---: |
| Böhle et al. (1996) | Echium | ITS, trnT-L, trnL, trnLF | ITS most divergent; trnT-F treated as a single unit, combined analysis used to infer relationships |
| Small et al. (1998) | Gossypium | Seven cpDNA markers $\& A d h$ | $A d h$ most informative, only two cpDNA markers (trnT-L \& rpl16) with informative characters |
| Fukuda et al. (2001) | Lycium | matK, trnT-L, trnL, $\operatorname{trnL}-F$ | trnL-F most informative, but trnT-L evolving more rapidly, combined analysis used to infer relationships |
| Mummenhoff et al. (2001) | Lepidium | trnT-L, $\operatorname{trn} L, \operatorname{trn} L-F$ | trnT-L most informative |
| Baumel et al. (2002) | Spartina | ITS, trnT-L, waxy | waxy most informative, trnT-L least informative |
| Mast and Givnish (2002) | $\begin{array}{lr} \hline \text { Banksia } & \& \\ \text { Dryandra } & \end{array}$ | $\operatorname{trn} T-L, \quad \operatorname{trn} L, \quad \operatorname{trn} L-F$, rps16, psbA-trnH | Not all data for markers given but trnT-L appears to be one of the more informative markers |
| $\begin{array}{lll} \hline \text { Yang } & e t & a l . \\ (2002) & & \end{array}$ | $\begin{array}{ll} \hline \text { Brassica } \& \\ \text { Raphanus } \end{array}$ | $\begin{array}{ll} \operatorname{trn} T-L, & \operatorname{trn} L, \quad \operatorname{trn} L-F, \\ \operatorname{trn} D-T & \end{array}$ | trnD-T most informative |
| Patterson and <br> Givnish (2003) | Calochortus | trnT-L, trnL, trnL-F, rpl16, psbA-trnH | Data for markers not given, combined analysis used to infer relationships |

Table 3.1. continued

| Study | Genus studied | Makers used | Comments |
| :---: | :---: | :---: | :---: |
| Valcárcel et al. (2003) | Hedera | ITS, trnT-L | ITS more informative, but trnT-L with enough resolution to serve as an independent source to infer ancestry |
| Bohs (2004) | Solanum | ITS, trnT-L, trnL, trnLF | trnT-L with greatest resolving power |
| Gravendeel et al. (2004) | Pleione | ITS, trnT-L, trnL, $\operatorname{trnL}$ F, matK | ITS most informative, trnT-F more variable than matK, trnT-F treated as a single unit |
| Nickrent et al. (2004) | Arceuthobium | ITS, trnT-L, trnL, trnLF | ITS most informative, trnT-F treated as a single unit |
| $\begin{array}{lll} \hline \text { Mast } & \text { et } & \text { al. } \\ (2004) & & \\ \hline \end{array}$ | Dodecatheon <br> \& Primula | matK, rpl16, rps16, $\operatorname{trn} T-L, \operatorname{trn} L, \operatorname{trn} L-F$ | matK most informative, combined analysis used to infer relationships |
| Levin et al. (2005) | Solanum | ITS, $\operatorname{trn} T-L, \operatorname{trnL}, \operatorname{trn} L-$ F, waxy | waxy most informative, trnT-F treated as a single unit |

The trnT-L spacer can be problematic in terms of amplification especially the 5 , end (Lantz and Bremer, 2004). Renner and Chanderbali (2000) found that the 5, end is highly variable and had to be excluded because of ambiguity and alignment problems. It appears that this region (especially the 5 ' end of $\operatorname{trn} T-L$ spacer, viz. the priming site for Taberlet's primer ' $a$ ') is problematic in many different plant groups (Shaw et al., 2005). Cronn et al. (2002) found it necessary to design a new primer. In several studies internal primers had to be designed to amplify and sequence this region (Razafimandimbison and Bremer, 2002; Fukuda et al., 2001; Valcárcel et al., 2003).

The region is prone to large AT rich hotspots (Sauquet et al. 2003; Perret et al., 2003; Mast and Givnish, 2002; Mummenhoff et al., 2001), poly-T regions (Perret et al., 2003) and poly-A chains (Liede et al., 2002; Liede and Täuber, 2002). Stech (2004) found small indels of $1-4 \mathrm{bp}$ within the $\operatorname{trnT-L}$ spacer. These short indels were mostly simple sequence repeats (A, T, AA, TT, AT, ATTT) situated in poly A/poly T stretches. Borsch et al. (2003) showed that high length sequence variability is confined to mutational hotspots in the trnT-F region, which were common. Most other microstructural changes present in the intergenic spacers were simple repeat motifs ( $4-6 \mathrm{bp}$ ).

These nucleotide repeats result in indels of dubious homology, length variation and ambiguous alignments. These segments have to be frequently excluded from analyses (Sauquet et al., 2003; Perret et al., 2003; Liede et al., 2002; Liede and Täuber, 2002; Liede and Kunze, 2002; Mummenhoff et al., 2001; Mast and Givnish, 2002). Frequent large indel events can result in loss of data and possibly increase noise (Neves et al., 2005). Mast and Givnish (2002) and Mummenhoff et al. (2001) have also reported that poly-AT tracks cause sequencing problems. These factors impact negatively on the trnT-L spacer's potential in phylogenetic reconstruction.

These regions of simple repetitive DNA (A, T, AT) are most likely the result of slipped-strand mispairing (Levinson and Gutman, 1987). Valcárcel et al. (2003) examined the sequence substitution and the secondary structure of the $\operatorname{trn} T-L$ foldings in Hedera (Araliaceae). Loops, stem-loops and nucleotide domains failed to reveal any hot spots for mutation or mutational triggers. These workers dismissed slipped-strand mispairing or intra-molecular recombination as factors accounting for these sequence repeats and concluded that secondary structure formation does not appear to significantly affect the evolution of this non-coding chloroplast spacer and that the indels were not homoplasious in nature.

Few studies have shown the limited use of the trnT-L spacer. Neves et al. (2005) found that although the $\operatorname{trn} T-L$ spacer was the most variable of the non-coding regions in their study ( $\operatorname{trn} T-F$ ), but most of the variation was autoapomorphic.

Nickrent et al. (2004) have reported that a single species (Arceuthobium douglasii) has a large deletion in the $\operatorname{trn} T-L$ spacer, which spanned into the $\operatorname{trn} L$ intron. This species lacked the $\operatorname{trnL} 5^{\prime}$ exon but retained the 3 ' exon, which was considered to be a 'pseudogene'.

### 3.1.3.1.2. The trnL Intron

This is a Group I intron and ranges from c. 250 to 1400 bp in plants (Shaw et al., 2005). The trnL intron shows sequence conservation in the regions flanking the $\operatorname{trn} L$ exons while the central part is variable (Bakker et al., 2000). Bakker et al. (2000) found that nucleotide substitutions in the $\operatorname{trn} L$ intron accumulated in a more uniform pattern than the $t r n L-F$ spacer in angiosperms.

The trnL intron contains several highly conserved motifs in Gnetales (Won and Renner, 2005). The conservative nature of this intron is attritubuted to the maintenance of secondary structure and sequence elements of the trnL gene which are critical for self-splicing from precursor RNAs (Simon et al., 2003).

The trnL intron is the oldest intron transmitted from cyanobacteria to the chloroplast. Its conserved nature and high AT content may explain why it evolves more slowly than other non-coding chloroplast sequences (Yang et al., 2002). Yang et al. (2002) found the rate of nucleotide substitution in the $\operatorname{trn} L$ intron was less than the surrounding spacers. This was presumably due to the greater functional constraints imposed on the intron. Borsch et al. (2003) found that the $\operatorname{trnL}$ intron was less variable than the surrounding spacers due to the fewer length mutational changes in the intron. Length conservation of the intron may also relate to the role of the intron in splicing during mRNA processing. It also appears that the $\operatorname{trn} L$ intron and the $\operatorname{trnL}-F$ spacer evolve in concert. This may be because the tRNA genes of the intron and spacer are transcribed in the same direction (Borsch et al., 2003 and references therein).

Sequences of this trnL intron are usually co-amplified with the trnL-F spacer (Shaw et al., 2005) and together these two regions have become the most popular and widely used non-coding cpDNA markers (Shaw et al., 2005; Kim and Lee, 2005). The $\operatorname{trnL}-F$ spacer is usually shorter than the intron ranging from less than 100 to 500 bp (Shaw et al., 2005). The number of parsimony informative characters in the $\operatorname{trn} L-F$ spacer is usually greater than the $\operatorname{trn} L$ intron, despite the intron being usually larger (Shaw et al., 2005).

In numerous studies the $t r n L$ intron has been used to complement, support and/or enhance the phylogenetic signal and potential of the $\operatorname{trnL} L-F$ spacer (e.g. Udovicic and Ladiges, 2000; Reeves et al., 2001; Morton et al., 2003; Scheen et al., 2004; Mols et al., 2004; van der Niet et al., 2005). In some studies the trnL intron has closely equalled or marginally out-performed the trnL-F spacer (e.g. Mummenhoff et al., 2001; Bellstedt et al., 2001; Razafimandimbson and Bremer, 2002; Oberlander et al., 2004; van den Berg et al, 2005). This is due partly to greater length of the intron in these studies.

Based on these findings, the trnL intron was used in this study to determine if it would support and/or enhance the phylogenetic signal of the trnT-L spacer. In addition the amplification approach (see Materials and Methods) resulted in coamplified PCR products for both the $\operatorname{trn} T-L$ spacer and $\operatorname{trn} L$ intron, thus facilitating sequencing.

### 3.1.3.2. The $p s b A$-trnH intergenic spacer

Sang et al. (1997) were the first to use this region in phylogenetic studies in Paeonia (Paeoniaceae). It is a short region found between two highly conserved genes and is therefore, easily amplified. This spacer region lies in the inverted repeat region of the cp genome adjacent to the $\operatorname{trn} K$ gene. The two flanking genes are the psbA and trnH gene (Chandler et al., 2001). Sang et al. (1997) found that the $p s b A-\operatorname{trnH}$ sequences evolved slightly less rapidly than ITS sequences, but over three times faster than the matK coding region. However, matK sequences served as a better phylogenetic marker from the cp genome.

Although this region has a very high percentage of variable characters (Hamilton et al., 2003), it is usually coupled with other markers as it is too short and may not provide enough characters to build a well-resolved phylogeny (Shaw et al., 2005). Shaw et al. (2005) found the average length to be 465 bp and ranges from 198 to 1077 bp . Although this spacer was the second most variable, it is relatively short and provides few overall characters (Shaw et al., 2005).

The region has proven to be useful in combined approaches at the species level (Udovicic and Ladiges, 2000; Chandler et al., 2001; Mast and Givnish, 2002; Patterson and Givnish, 2003; Scheen et al., 2004; Butterworth and Wallace, 2004; Yamashiro et al., 2004). It has also been used with other markers at the infra-specific level (Holderegger and Abbott, 2003; Honjo et al. 2004; McKinnon et al., 2004; Howis et al., in prep.).

In some studies at higher taxonomic levels larger parts of the spacer were difficult to align and were excluded from analyses (Renner, 1999; Renner and Chanderbali, 2000; Soltis et al., 2001). Schönenberger and Conti (2003) and Klak et al. (2003a) found this region to be of limited use. Initial screening of this region in Kniphofia found little sequence divergence and the option of exploring this marker was not pursued further.

### 3.1.3.3. The rps 16 intron

The plastid ribosomal protein 16 small subunit (rpsl6) gene has a group II intron that was first used by Oxelman et al. (1997) to construct relationships in the tribe Sileneae (Caryophyllaceae). The intron averages 846 bp in length and ranges from 784 to 946 bp (Shaw et al., 2005). Schönenberger and Conti (2003) found that rps16 was one of the more informative markers for Penaeaceae, Oliniaceae, Rhynchocalyaceae and Alzateaceae. It has proven to be useful at the generic level (Asmussen et al., 2000; Asmussen and Chase, 2001; Morton et al., 2003).

This region cannot be used in certain groups as all or a portion of the rps16 gene is absent in some angiosperm families (Doyle et al., 1995). The rpsl6 intron often does not provide enough characters to resolve relationships below generic levels (e.g. Ingram and Doyle, 2003; Wanntorp et al., 2001; Goldblatt et al., 2002; Muellner et al., 2005). Initial screening of this region in Kniphofia found little sequence divergence and the option of exploring this marker was not pursued further.

### 3.1.3.4. Nuclear DNA: the $18 \mathrm{~S}-5.8 \mathrm{~S}-26 \mathrm{~S}$ rDNA cistron

This region is part of a transcription unit of nDNA. The spacer sectors are not incorporated into mature ribosomes. ITS 1 and ITS 2 of the nDNA transcript appear to function at least in part of the maturation of nRNAs (Baldwin et al., 1995). This region was used to obtain a data set independent from the cpDNA
markers in Kniphofia, therefore, the use of this spacer in phylogenetic studies is reviewed.

The wide phylogenetic use of the 18S-5.8S-26S rDNA cistron is due to its structure and molecular evolution (Baldwin et al., 1995; Álvarez and Wendel, 2003). It is structured in tandem arrays at one or more chromosomal loci. The basic structure of a transcribed single repeat unit of rDNA is as follows: an external transcribed spacer (ETS); the $18 S$ gene; an internal transcribed spacer (ITS 1); the 5.8 S gene; a second internal transcribed spacer (ITS 2); the $26 S$ gene and an intergenic spacer ( $I G S$ ). Each unit is repeated thousands of times in most plant genomes and separated from the next by the IGS. The tandem repeat structure and high copy number faciliates amplification and sequencing.

Other factors that make this region popular and advantageous according to Álvarez and Wendel (2003) are biparental inheritance, universality of primers for amplification, intragenomic uniformity, intergenomic variability and low functional constraints.

### 3.1.3.4.1. Use of ITS sequence data in lower level plant systematics studies

Preliminary studies indicated that ITS was conservative in length with high sequence variability, suggesting that the spacers would be easily alignable and variable enough to address lower level (various intra-familial levels) phylogenetic issues (Balwin et al., 1995). The $18 S$ and 26S genes are highly conserved and useful for higher taxonomic levels (family and above) of phylogeny reconstruction. The more rapidly evolving segments, ITS1 and ITS2 (commonly called the ITS region along with the $5.8 S$ gene) is the most utilised nuclear region for phylogenetic inference at lower levels (genus and below) (Álvarez and Wendel, 2003; Small et al., 2004). In many studies ITS has out-performed cpDNA markers (e.g. Razafimandimbison and Bremer, 2002; Verboom et al.,

2003; Nickrent et al., 2004; van der Niet et al., 2005; Muellner et al., 2005; van den Berg et al., 2005; Chen et al., 2005).

The ITS region has proven useful at various infra-familial levels (generic, tribal and subtribal limits): Agavaceae (Bogler and Simpson, 1996), Rubiaceae (Razafimandimbison and Bremer, 2002; Lantz and Bremer, 2004) and Saxifragaceae (Soltis et al., 2001).

The ITS region has also proven useful in many species level studies, a few of which are summarised in Table 3.2. It has also proven in some instances to be variable at infra-specific level (e.g. McKinnon et al., 2004; Barker et al., 2005).

### 3.1.3.4.2. Problems with the ITS region

While the ITS region has contributed much to the field of plant phylogenetics, there are some major disadvantages (Álvarez and Wendel, 2003; Small et al. 2004; Mort and Crawford, 2004) which will be briefly reviewed below.

A fundamental pre-requisite and frequent assumption for historical inference is that the genes compared are orthologous as opposed to paralogous. Genes are considered orthologous if their relationships originated from organismal cladogenesis. This a misleading view as diploid individuals can contain two different orthologues. Their history may reflect divergence events among species. However, if there has been a history of gene/sequence duplication then the duplicated sequences are considered paralogous. When paralogous sequences are included in phylogenetic reconstruction the resulting phylogeny will confound divergence events by tracking the history of duplication. Incorrect assessment of orthology and paralogy leads to phylogenetic incongruence. This is also the consequence if sampling includes a mixture of orthologous and paralogous sequences (Álvarez and Wendel, 2003).

Table 3.2. Examples of phylogenetic studies that have utilised the ITS region at the species level.

| Study | Genus studied | Makers used | Comments |
| :--- | :--- | :--- | :--- |
| Adams et al. (2000) | Aloe | ITS only | Limited sampling, no other study for comparison |
| Baumel et al. (2002) | Spartina | ITS, trnT-L, waxy | waxy most informative, followed by ITS, trnT-L least <br> informative |
| Samuel et al. (2003) | Hypochaeris | ITS, trnL, trnL-F, <br> matK | ITS most informative |
| Verboom et al. (2003) | Ehrharta | ITS, trnL-F | ITS more informative than trnL-F |
| Barker et al. (2004) | Leucadendron | ITS only | No other marker for comparison |
| Gravendeel et al. (2004) | Pleione | ITS, trnT-L, trnL, <br> trnL-F, matK | ITS most informative |
| Nickrent et al. (2004) | Arceuthobium | ITS, trnT-L, trnL, <br> trnL-F | ITS most informative |
| Muellner et al. (2005) | Aglaia | ITS, rpll6 | ITS more informative |
| van der Niet et al. (2005) | Satyrium | ITS, matK, trnL, <br> trnL-F | ITS most informative |
| Archibald et al. (2005) | Zaluzianskya | ITS, rpll6, trnL, <br> trnL-F | ITS most informative |

Nuclear genes have the ability to evolve in unison. Instead of each gene copy accumulating unique sequence variation via accumulation of mutations all repeat copies in the array may jointly share the same set of mutations resulting in intergenic sequence homogenisation i.e. concerted evolution (Álvarez and Wendel, 2003; Small et al., 2004). Mechanisms such as high frequency crossing over or gene conversion are implicated in this process (Baldwin et al., 1995). Concerted evolution was initially considered to be advantageous, as it would eliminate paralogous sequences and facilitate the inference of true homology in phylogenetic reconstruction (Álvarez and Wendel, 2003). However, the presence or absence of both or multiple copies per array and multiple arrays per genome and the presence, absence or variable extent of concerted evolution may homogenise sequences within and sometimes between arrays. Sequence variants can arise and be maintained within and between arrays resulting in distantly related nDNA types within individuals. Such erratic variation may be the norm rather than the exception but is often ignored, underreported or undetected. Thus, when concerted evolution is incomplete it results in a mixture of multiple divergent copies, which constitute paralogues and orthologues.

This complicates phylogenetic reconstruction. Even when sampling measures are taken, one or more repeat types may be lost in one or more descendant taxa thus resulting in a loss of possible historical evidence (see Álvarez and Wendel, 2003 for review). Evidence of ITS paralogues has been reported in several studies (Mayol and Rosselló, 2001; van den Berg et al., 2005; Neves et al., 2005). Multiple copies of ITS have been reported by some workers (Goldblatt et al., 2002; Liede and Täuber, 2002; Okuyama et al., 2005). Paralogues could lead to spurious phylogenetic estimates, thus ITS data need to be treated with caution and compared with other sources of data (Barker et al., 2004; Neves et al., 2005).

Multiple nDNA arrays may arise from hybridisation, polyploidisation, gene/chromosome duplication and various forms of recombination. It cannot be
assumed that strict orthology has been maintained for sequences amplified among a set of taxa. Direct sequencing may not detect paralogues and may require an extensive cloning approach (Rauscher et al., 2002). Reticulation, introgression or polyploidisation may give rise to the co-existence of divergent ITS repeat types and the direction of sequence homogenisation may be different for the different lineages. This becomes an important factor and consideration in groups with hybridisation and polyploidisation (Álvarez and Wendel, 2003). This could lead to incorrect estimates of relationships in studies using ITS sequences at lower taxonomic levels (Soltis and Soltis, 1998).

In the evolution of polyploids concerted evolution plays an important role in the maintenance of sequence homogeneity of multigene families (Zhang and Sang, 1999). The mode and rate of concerted evolution in rDNA varies in different groups (Li and Zhang, 2002). The degree to which concerted evolution may cause homogenisation depends on the extent to which the initial parents have diverged and the time of polyploidisation. Polyploids may exhibit additivity of parental rDNA sequences e.g. Paeonia (Sang et al., 1995). Concerted evolution may homogenise rDNA towards one parent in a hybrid genome while the other parental rDNA type is eliminated. In Aegilops concerted evolution of rDNA in allopolyploid species is uni-directional (Wang et al., 2000). Li and Zhang (2002) found that in Thinopyrum ponticum (a decaploid) rDNA has experienced unidirectional concerted evolution towards diploid relatives. In some cases concerted evolution of rDNA may be bi-directional e.g. Gossypium (Wendel et al., 1995).

An alternate explanation for sequence homogenisation is the loss of rDNA loci of one or more genomes following the polyploidisation (Li and Zhang, 2002). Concerted evolution may fail to homogenise sequences in certain case e.g. recent hybridisation, development of pseudogenes, large number of rDNA repeats and asexual reproduction (Li and Zhang, 2002).

Several studies have used ITS sequences with other markers and/or morphological evidence to gain insights into evolutionary processes e.g. hybridisation and lineage sorting (e.g. Sang et al., 1995; Sang et al., 1997; Aguilar et al., 1999; Comes and Abbott, 2001; Goldman et al., 2004; Howarth and Baum, 2005).

Not all 18S-5.8S-26S repeats remain functional and some may degenerate into pseudogenes (Álvarez and Wendel, 2003; Small et al., 2004). Non-functional variants of ITS (pseudogenes) have been reported (Neves et al., 2005). Putative ITS pseudogenes have been found to be useful for phylogenetic inference in three species of Rubiaceae (Razafimandimbison et al., 2004).

Bell (2004) reported that ITS sequence data does not provide support for relationships within Valerianaceae. This was because the region appeared to be evolving too fast and suffers from alignment problems. Plana et al. (2004) found that large segments of ITS had high sequence divergences and were difficult to align in Begonia. In many studies ITS sequence data showed low divergence and has been implicated in recent rapid radiation (Baldwin and Sanderson, 1998; Harris et al., 2000; Malcomber, 2002; Klak et al.; 2003b; Richardson et al., 2001; Warwick et al., 2004; Howarth and Baum, 2005).

Based of the usefulness of the markers screened the trnT-L spacer, trnL intron and ITS region were used to examine phylogenetic relationships in Kniphofia. Initial ITS results showed little sequence divergence (below). Despite this, further investigations were done as this marker as it was the only independent nuclear marker that could be easily amplified and sequenced routinely.

### 3.2. Materials and Methods

### 3.2.1. Sampling strategy, Collection and Preservation

Whitehouse (2002b) has suggested that at the species level, an understanding of the taxonomy of the genus and variation within species needs to be developed prior to selection of exemplar collections to reliably represent a species. He also recommended that more than one collection should be chosen to cover the range of morphological and geographic diversity found within the species. If knowledge of the genus is superficial, an arbitrary choice of a specimen could result in one that lies at the boundary between two species or represents a case where introgression has occurred with a sympatric species (Whitehouse, 2002b). This is of particular importance, as species of Kniphofia can hybridise and are sometimes sympatric. However, Kniphofia has c. 71 species, a complex taxonomy and a wide distribution extending from southern to eastern Africa, Yemen and Madagascar. Understanding of species concepts and collecting material required a substantial amount of field work and time. Due to the limitations imposed by the number of taxa in Kniphofia, flowering times and resources, samples were sequenced as and when material became available. As many southern African species were sampled as possible to obtain species coverage and geographical coverage (at the quarter degree scale).

Moreover, based on a pilot study (Ramdhani et al., 2006) it became apparent that multiple samples per species had to be sequenced because many taxa appeared to be non-monophyletic. Thus a multiple exemplar approach was advocated. Every attempt has been made to include as many samples representing as many morphological species sensu Codd $(1968,2005)$ as possible. Four southern African species were not included in this study. K. flammula is a highly restricted species only known from the Glencoe region of KwaZulu-Natal. It was last collected in 1968. Attempts were made over two flowering seasons to locate living material but to no avail. K. evansii is also a restricted species found only in
the Bergville District in high montane grasslands. I have tried to locate this species three times in the Catheral Peak area but have failed. K. tabularis is restricted to the Cape fold mountains. This species was not encountered in the limited time spent sampling in the Western Cape. K. crassifolia is only known form the type collection made by the Austrian botanist A. Rehmann. It was collected in 1880 from the Houtbosch region in Limpopo Province. No attempt was made to find this species as the original locality probably has been transformed by timber plantations (Pieter Winter, pers. comm.).

Material from other parts of Africa proved more difficult to obtain. Field work was done in the Bale Mountains in Ethiopia and the area around Addis Ababa (Tatek) in September 2003. Limited material was obtained from Kenya, Tanzania, Malawi and Madagascar from collecting done by colleagues. DNA was obtained from a single sample of herbarium origin (K. splendida, Chapman \& Chapman 9061) from Mt Mulanje (Malawi). Table 3.3. lists the details of the samples used in this study.

Most leaf samples were dried with silica gel (Chase and Hills, 1991). In some cases plants collected in the field were cultivated and fresh material was used to extract DNA. Most samples collected have an accompanying herbarium voucher, which is housed at the Selmar Schonland Herbarium (GRA). Specimens collected in Ethiopia are housed at the National Herbarium of Ethiopia (ETH, Addis Ababa). In some instances DNA material was collected with no accompanying herbarium voucher, as the sites were visited when plants were not flowering. In these instances specific DNA collections were only included if the species in question were known to occur at the site from previous collections and/or field observations by other botanists. These were not assigned collection numbers and tagged as sine numero (sn). Likewise material collected by other collectors with no herbarium voucher were tagged sine numero. Subspecies, variant and forms were specified only when confidently known by the author.

Specimens obtained from botanical gardens were tagged with the reference number of the particular garden. In the case of K. leucocephala the only material available was from the Natal National Botanical Garden's living collection in Pietermaritzberg. These plants were clones (via tissue culture) of specimens collected at the type locality due to its restricted distribution and critically endangered conservation status (Scott-Shaw, 1999).

Table 3.3. List of specimens used in phylogenetic reconstruction with locality details. Notes to abbreviations: Collectors initials, $\mathrm{HB}=\mathrm{H}$. Baijnath, $\mathrm{NPB}=\mathrm{N} . \mathrm{P}$. Barker, $\mathrm{JB}=\mathrm{J}$. Burrows, $\mathrm{TD}=\mathrm{T}$. Dold, JMG= J.M. Grimshaw, CK= C. Kayombo, RAL= R.A. Lubke, RJM= R.J. McKenzie, $\mathrm{AMM}=\mathrm{A} . \mathrm{M}$. Muasya, $\mathrm{AN}=\mathrm{A}$. Nicholas, $\mathrm{CP}=\mathrm{C}$. Peter, $\mathrm{PBP}=\mathrm{P} . \mathrm{B}$. Phillipson, $\mathrm{JP}=\mathrm{J}$. Pote; $\mathrm{SR}=\mathrm{S} . \operatorname{Ramdhani}, \mathrm{AR}=\mathrm{A} . \mathrm{Rennie}, \mathrm{BT}=$ B. Tarr; Botanical garden material: NNBG= Natal National Botanical Garden (Pietermaritzburg); sn=unnumbered collections with no herbarium voucher. Herbarium abbreviations follow Holmgren et al. (1994).

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :--- | :--- | :--- | :---: | :---: | :---: |
| K. acraea | TD 4626 (GRA) | Mountain Zebra National <br> Park | X | X | X |
| K. albescens | SR \& JB 314 (GRA) | Dirkiesdorp | X | X | X |
| K. albomontana | SR \& AN 149 (GRA) | Greytown | X |  | X |
| K. angustifolia | SR 542 (GRA) | Cathedral Peak Nature <br> Reserve | X |  | X |
| K. angustifolia | SR 453 (GRA) | Cathedral Peak Nature <br> Reserve | X |  | X |
| K. ankaratrensis | PBP 5676 (P) | Madagascar | X |  | X |
| K. baurii | SR 174 (GRA) | Humansdorp | X |  |  |
| K. baurii | Kareedouw |  |  |  |  |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | $t r n L$ intron | ITS |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. baurii | SR 275 (GRA) | Elands Heights | X |  | X |
| K. baurii | SR 285 (GRA) | Naudes Nek | X | X | X |
| K. baurii | SR 360 (GRA) | Port Elizabeth | X |  | X |
| K. baurii | SR 382 (GRA) | Kenton-on-Sea | X |  | X |
| K. baurii | SR 398 (GRA) | Mooi River | X |  |  |
| K. baurii | RJM 1026 (GRA) | Natures Valley | X |  |  |
| K. baurii | NPB 1923 (GRA) | Alicedale | X |  | X |
| K. brachystachya | SR sn (GRA) | Estcourt | X |  | X |
| K. breviflora | SR 452 (GRA) | Oliviershoek Pass | X | X | X |
| K. bruceae | SR \& NPB 171 (GRA) | Komga | X | X | X |
| K. buchananii | SR \& BT 305 (GRA) | Greytown | X |  |  |
| K. buchananii | SR \& BT 307 (GRA) | Greytown | X |  | X |
| K. buchananii | SR 458 (GRA) | Howick | X |  |  |
| K. caulescens | SR 270 (GRA) | Elands Heights | X |  | X |

Table 3.3. continued

| Taxon | Voucher (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. caulescens | SR 278 (GRA) | Naudes Nek | X | X | X |
| K. caulescens | NPB 1821 (GRA) | Sani Pass | X |  | X |
| K. caulescens | RJM 974 (GRA) | Carlisles Hoek | X |  |  |
| K. citrina | SR 176 (GRA) | Humansdorp | X |  | X |
| K. coddiana | SR $s n$ | Umtamvuna Nature Reserve | X |  | X |
| K. coralligemma | SR 549 (GRA) | Iron Crown (Wolkberg) | X | X | X |
| K. drepanophylla | RAL 4816 (GRA) | Mkambati | X |  | X |
| K. drepanophylla | RJM 1100 (GRA) | Mkambati | X |  |  |
| K. ensifolia subsp. ensifolia | JB $s n$ | Witbank | X |  | X |
| K. ensifolia subsp. autumnalis | SR 448 (GRA) | Harrismith | X |  | X |
| K. fibrosa | SR \& AR 297 (GRA) | Pervensey | X |  | X |
| K. fibrosa | PBP 5579 (GRA) | Dohne Hill | X |  |  |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. fluviatilis | SR $s n$ | Verloren Vallei |  |  | X |
| K. foliosa | SR 383 (ETH) | Gafesar Dam, Ethiopia | X |  | X |
| K. foliosa | SR 387 (ETH) | Bale, Ethiopia | X |  | X |
| K. foliosa | SR 389 (ETH) | Goba, Ethiopia | X |  | X |
| K. foliosa | SR 390 (ETH) | Bale, Ethiopia | X |  | X |
| K. foliosa | JMG 034 (ETH | Sebese Washi, Ethiopia | X | X | X |
| K. foliosa | JMG 038 (ETH) | Bale, Ethiopia | X |  | X |
| K. galpinii | SR 312 (GRA) | Long Toms Pass, Lydenberg | X |  | X |
| K. gracilis | SR \& HB 321 <br> (GRA)   | Durban | X |  | X |
| K. gracilis | SR 561 (GRA) | Park Rynie | X |  |  |
| K. gracilis | NNBG 77/99 | Draycott | X |  | X |
| K. grantii | CP 4154 (GRA) | Nyika Plateau, Malawi | X |  | X |
| K. hirsuta | SR 282 (GRA) | Naudes Nek | X |  | X |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | $t r n L$ intron | ITS |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. ichopensis var. ichopensis | SR 242 (GRA) | Nottingham Road | X |  | X |
| K. ichopensis var. ichopensis | SR 286 (GRA) | Giants Castle Nature Reserve | X |  | X |
| K. ichopensis var. ichopensis | SR 289 (GRA) | Cathedral Peak Nature   <br> Reserve   | X |  | X |
| K. ichopensis var. ichopensis | SR 409 (GRA) | Balgowan | X |  |  |
| K. insignis | SR $s n$ | Tatek, Ethiopia | X | X | X |
| K. isoetifolia | SR 386 (ETH) | Bale Mountains, Ethiopia | X |  | X |
| K. isoetifolia | SR 388 (ETH) | Goba, Ethiopia | X | X | X |
| K. isoetifolia | SR 393 (ETH) | Kofele, Ethiopia | X |  | X |
| K. latifolia | RSS sn | Greytown | X |  | X |
| K. laxiflora form B | SR 295 (GRA) | Kamberg Nature Reserve | X |  | X |
| K. laxiflora form B | NPB 1810 (GRA) | Bushmans Nek | X |  | X |

Table 3.3. continued

| Taxon | Voucher (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. laxiflora form B | SR 441 (GRA) | Nottingham Road | X |  |  |
| K. laxiflora form B | SR 442 (GRA) | Michaelhouse | X |  |  |
| K. laxiflora form B | SR 467 (GRA) | Weza | X | X | X |
| K. laxiflora form B | SR 468 (GRA) | Weza | X | X | X |
| K. laxiflora form C | SR $s n$ (GRA) | Wakkerstroom | X |  | X |
| Kniphofia sp. cf. laxiflora | SR 283 (GRA) | Naudes Nek | X |  | X |
| K. leucocephala | NNBG | Richards Bay (clone of type) | X |  | X |
| K. linearifolia | SR \& NPB 170  <br> (GRA)   | Komga |  |  | X |
| K. linearifolia | SR 269 (GRA) | Hogsback (Seymour) | X |  | X |
| K. linearifolia | SR 287 (GRA) | Loskop | X |  |  |
| K. linearifolia | SR 290 (GRA) | Rosetta | X |  | X |
| K. linearifolia | SR 291 (GRA) | Kamberg Nature Reserve | X |  | X |
| K. linearifolia | SR $\quad \&$ JB 311 <br> (GRA)   | Lydenberg | X | X | X |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :--- | :--- | :--- | :---: | :---: | :---: |
| K. linearifolia | SR 313 (GRA) | Sabie |  |  | X |
| K. linearifolia | SR 328 (GRA) | Mt. Currie Nature Reserve | X |  | X |
| K. linearifolia | SR 343 (GRA) | Hogsback (Seymour) | X |  | X |
| K. linearifolia | SR 558 (GRA) | Underberg | X |  |  |
| K. linearifolia | JP sn (GRA) | Stutterheim | X |  |  |
| K. linearifolia | TD 4638 (GRA) | Satans Nek | X |  | X |
| K. linearifolia | SR 400 (GRA) | Mooi River | X |  | X |
| K. littoralis | SR \& HB 200 (GRA) | Silverglen Nature Reserve | X |  | X |
| K. multiflora | SR \& JB 310 (GRA) | Lydenberg | X | X |  |
| K. multiflora | SR \& JB 315 (GRA) | Dirkiesdorp | X |  | X |
| K. northiae | SR 263 (GRA) | Hogsback (Seymour) | X |  |  |
| K. northiae | SR 274 (GRA) | Naudes Nek | X | X |  |
| K. northiae | SR 446 (GRA) | Katberg | X | X | X |
| K. parviflora | SR 268 (GRA) | Hogsback (Seymour) | X |  |  |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. parviflora | SR 330 (GRA) | Mt. Currie Nature Reserve | X | X | X |
| K. pauciflora | HB $s n$ (GRA) | Durban | X |  | X |
| K. porphyantha | SR $s n$ | Verloren Vallei | X |  | X |
| K. praecox | SR 529 (GRA) | Jefferys Bay | X |  | X |
| K. praecox | SR 530 (GRA) | Elandsbos River | X |  |  |
| K. praecox | SR 532 (GRA) | Haroldsbaai | X |  |  |
| K. rigidifolia | SR $s n$ | Lydenberg | X |  | X |
| K. ritualis | SR 300 (GRA) | Pervensey | X | X | X |
| K. rooperi | SR 237 (GRA) | East London | X |  | X |
| K. rooperi | TD 4559 (GRA) | Mkambati | X |  |  |
| K. rooperi | SR 485 (GRA) | Riet River | X |  | X |
| K. rooperi | RAL 4229 (GRA) | Morgans Bay | X |  |  |
| K. rooperi | SR 528 (GRA) | Van Stadens River | X |  |  |
| K. sarmentosa | SR 207 (GRA) | Hex River Pass | X |  | X |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :--- | :--- | :--- | :---: | :---: | :---: |
| K. schimperi | SR 391(ETH) | Sebsebe Washe, Ethiopia | X |  | X |
| K. schimperi | JMG 036 (ETH) | Sebsebe Washe, Ethiopia | X | X | X |
| K. stricta | SR 548 (GRA) | Haenertsberg | X |  | X |
| K. splendida | EG Chapman \& JD <br> Chapman <br> (FHO) | Mt. Mulanje, Malawi |  |  |  |$\quad$|  |  |  |
| :---: | :---: | :---: |
| K. stricta | SR 279 (GRA) | Rhodes |
| K. thodei | SR 407 (GRA) | Kamberg Nature Reserve |
| K. thomsonii | JMG 031 (ETH) | Senatti Plateau, Ethiopia |
| K. thomsonii | AAM 2647 (EA) | Mt. Elgon, Kenya |
| K. thomsonii | CK 4821 (GRA) | Mt. Kilimanjaro, Tanzania |
| K. triangularis <br> triangularis | subsp. | SR 264 (GRA) |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :--- | :--- | :--- | :---: | :---: | :---: |
| K. triangularis subsp. <br> triangularis | SR 266 (GRA) | Hogsback (Seymour) | X |  | X |
| K. triangularis subsp. <br> triangularis | SR 299 (GRA) | Pervensey | X | X | X |
| K. triangularis subsp. <br> obtusiloba | SR sn | Kemps Heights | X |  | X |
| K. typhoides | NNBG 139/99 | Vryheid | X | X | X |
| K. typhoides | JB 8084 (GRA) | Witbank | X | X | X |
| K. tysonii subsp. tysonii | SR 302 (GRA) | Creighton | X | X |  |
| K. tysonii subsp. tysonii | SR 460 (GRA) | Balito | X | X |  |
| K. umbrina | R Gama sn | Forbes Reef, Swaziland |  |  |  |
| K. uvaria | SR \& NPB 166 | Port Elizabeth |  |  |  |

Table 3.3. continued

| Taxon | Voucher <br> (Herbarium) | Locality | trnT-L spacer | trnL intron | ITS |
| :--- | :--- | :--- | :---: | :---: | :---: |
| K. uvaria | SR \& NPB 172 <br> (GRA) | Post Wellington | X |  |  |
| K. uvaria | SR 186 (GRA) | Kurlandsdorp | X | X | X |
| K. uvaria | SR 201 (GRA) | Cape St. Francis | X |  | X |
| K. uvaria | SR 203 (GRA) | Elim | X |  | X |
| K. uvaria | SR 211 (GRA) | Clarkson | X | X | X |
| K. uvaria | SR 337 (GRA) | Hogsback (Seymour) | X |  |  |
| K. uvaria | SR 342 (GRA) | Hogsback (Seymour) | X |  | X |
| K. uvaria | SR 344 (GRA) | Grahamstown | X |  | X |
| K. uvaria | SR 471 (GRA) | Dimbaza | X |  |  |
| K. uvaria | SR 477 (GRA) | Grahamstown | X |  | X |
| K. uvaria | TD 4477 | Port Elizabeth | X |  |  |

### 3.2.2. DNA Extraction, Amplification and Sequencing

Total genomic DNA was extracted using a modified hot CTAB method of Doyle and Doyle (1987). Polymerase Chain Reaction (PCR) amplifications were conducted either on a ThermoHybaid PCR Sprint Temperature Cycling System or a Corbett Research PC-960G Microplate Gradient Thermal Cycler using the following conditions: $95^{\circ} \mathrm{C}$ for 45 seconds, $52-55^{\circ} \mathrm{C}$ for 45 seconds and $72^{\circ} \mathrm{C}$ for three minutes repeated between $30-35$ cycles (depending on the necessary number of cycles needed for suitable amplification). Annealing temperature was also manipulated to obtain optimal PCR product. A 10 minute $72^{\circ} \mathrm{C}$ extension was included at the end of the PCR program. The PCR reagents and volumes are shown in Appendix 3.

### 3.2.2.1. The trn $T$-trnL region

The trnT-L spacer was initially amplified by means of the PCR using primers ' $a$ ' and 'b' (Taberlet et al., 1991). The forward primer 'a' proved to be problematic in amplification. As mentioned in the introduction, this region is noted for being troublesome (e.g. Lantz and Bremer, 2004; Renner and Chanderbali, 2000). It appears that this region (especially the 5 ' end of $\operatorname{trn} T-L$ spacer, viz. the priming site for ' $a$ ') is problematic in many different plant groups (Shaw et al., 2005). To circumvent this problem, an alternative internal primer, 'Knip1' was designed (Ramdhani et al., 2006). Subsequently, the Knip1-d primer combination proved to be more efficient for amplification. Primers Knip1-b and c-d were used to sequence the spacer and inton respectively (refer to Figure 3.1. and Table 3.4. for details).


Figure 3.1. Schematic representation of the trnT-L region (spacer and intron) with primers (after Taberlet et al., 1991). Arrows indicate approximate starting points and directions of primers.

### 3.2.2.2. The ITS region

The ITS region (ITS1, 5.8S and ITS2) was amplified using the primers 'ITS 18 ' and 'ITS 26' (Käss and Wink, 1997; modified by Lavin). These were used as flanking primers for amplification. Internal primers (both forward and reverse) were used for sequencing. These were primers 'ITS 1' and 'ITS 4' of White et al. (1990), as well as primers 'Chrys 5.8 F ' (Barker et al., unpublished) and ‘Chromo5.8R’ (Barker et al., 2005). Primers ITS 1-Chromo5.8R were used to sequence ITS1 and part of the $5.8 S$ gene, while primers Chrys5.8F-ITS 4 were used to sequence the $5.8 S$ gene and ITS2 (refer to Figure 3.2. and Table 3.4. for details).


Figure 3.2. Schematic representation of the ITS region with primers used. Arrows indicate approximate starting points and directions of primer.

Table 3.4. Details of chloroplast and nuclear primers used in $P C R *$ and sequencing ${ }^{\#}$ ( $F=$ forward, $R=$ reverse ).

| Primer | Direction | Reference | Length (bp) | Sequence |
| :---: | :---: | :---: | :---: | :---: |
| a | F | Taberlet et al. (1991) | 21 | 5'CATTACAAATGCGATTGCTCT3' |
| Knip1** | F | Ramdhani et al. (2006) | 18 | 5'CTACCGGATCTTAGGTAT3' |
| $\mathrm{b}^{\text {* }{ }^{\text {¢ }}}$ | R | Taberlet et al. (1991) | 20 | 5'TCTACCGATTTCGCCATATC3' |
| $\mathrm{c}^{* \text { ®\# }}$ | F | Taberlet et al. (1991) | 20 | 5'CGAAATCGGTAGACGCTACG3' |
| d **\# | R | Taberlet et al. (1991) | 20 | 5'GGGGATAGAGGGACTTGAAC3' |
| ITS 18* | F | Käss and Wink (1997), modified by Lavin (pers. comm.) | 26 | 5'GTCCACTGAACCTTATCATTTAGAGG3' |
| ITS 26* | R | Käss and Wink (1997), modified by Lavin (pers. comm.) | 26 | 5'GCCGTTACTAAGGGAATCCTTGTTAG3' |
| $\mathrm{ITS}_{1}{ }^{\#}$ | F | White et al. (1990) | 19 | 5'TCCGTAGGTGAACCTGCGG3' |
| Chromo $5.8 \mathrm{R}^{\#}$ | R | Barker et al. (2005) | 15 | 5'GATTCTGCAATTCAC3' |
| Chrys 5.8R | F | Barker et al. (unpublished) | 20 | 5'GACTCTCGGCAACGGATATC3' |
| ITS $4{ }^{\text {\# }}$ | R | White et al. (1990) | 20 | 5'TCCTCCGCTTATTGATATGC 3' |

### 3.2.2.3. Visualisation of PCR products

The PCR products were run on $1 \%$ agarose gels, which consisted of 0.5 g agarose in 50 ml TBE buffer [10.8g Tris (hydroxymethyl) aminomethane, 5.5 g Boric Acid and 0.93 g EDTA (ethylene diamine tetra-acetic acid di-sodium) made up to one litre with distilled water]. Five to seven microlitres of ethidium bromide was added to the molten solution and then left for 15 minutes to set. Ten microlitres of PCR product were mixed with five microlitres of loading buffer (bromophenol blue and xylene cyanol in glycerol). Samples were loaded onto a gel and left to run in a gel rig to run for c . ten minutes at 150 volts. PCR products were visualised with a UV transilluminator.

A clean bright band was taken as a positive result. Smearing indicated an unsatisfactory (negative) result. In these cases PCR conditions were manipulated (changing the number of cycles, annealing temperature or amount of DNA template) to obtain satisfactory bands.

### 3.2.2.4. PCR product purification

PCR products were purified with Promega Magic PCR Preps ${ }^{\text {TM }}$, QIAGEN® QIAquick ${ }^{\text {TM }}$ or Promega Wizard ® kits following the manufacturers instructions. The purified PCR product was finally eluted with $25 \mu 1$ nuclease free water. Two microlitres of eluted purified product was then checked on a $1 \%$ agarose gel (see above).

### 3.2.2.5. DNA Sequencing

Purified PCR product was sequenced using an ABI Prism BigDye Terminator v3.0 or v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) according to manufacturers instructions with primers for the respective marker (see above). Sequencing reactions usually contained: $2 \mu 1$ sequence mix, $3 \mu 1$ sequence buffer, $0.5 \mu \mathrm{l}$ primer, $2.5 \mu \mathrm{l}$ purified DNA template and $12 \mu \mathrm{l}$ nuclease free water to make a total reaction volume of $20 \mu \mathrm{l}$. The amounts of
template and water were adjusted depending on the quality of the template with the final reaction volume remaining $20 \mu 1$.

Sequencing reactions were conducted on a ThermoHybaid PCR Sprint Temperature Cycling System using the following conditions: $95^{\circ} \mathrm{C}$ for 45 seconds, $50^{\circ} \mathrm{C}$ for 45 seconds and $60^{\circ} \mathrm{C}$ for three minutes repeated for 30-35 cycles. The annealing temperature was also manipulated $\left(50-52^{\circ} \mathrm{C}\right)$ in some instances to obtain optimal product.

Cycle sequencing products were then precipitated as follows: $20 \mu 1$ of sequence product was added to $50 \mu \mathrm{l} 100 \%$ ethanol, $2 \mu \mathrm{l} 125 \mathrm{mM}$ EDTA solution and $2 \mu \mathrm{l} 3 \mathrm{M}$ sodium acetate solution. The mixture was shaken and left to stand for at least 15 minutes before centrifuging at 10000 rpm for 15 minutes. The liquid was discarded leaving the pellet undisturbed. One hundred and fifty microlitres of $70 \%$ ethanol was added and samples were centrifuged for a further ten minutes. The supernatent was discarded leaving the pellet undisturbed and left to air-dry, and resuspended in Hi-Dye formamide. Sequencing was done on a ABI 3100 Genetic Analyzer at Rhodes University's Sequencing Unit.

### 3.2.3. Sequence Editing and Alignment

Sequences (forward and reverse) were assembled, checked and edited using Sequencher ${ }^{\mathrm{TM}}$ version 3.1.1 and version 4.2.2. (Gene Codes Corporation). Consensus sequences were exported from Sequencher into MacClade version 4.06 (Maddison and Maddison, 2000).

Phylogenetic analyses based on DNA sequences are dependent on sequence alignment, and it is therefore important that the evolutionary events, which cause length variation, are recognised and used during the alignment of length-variable sequences (Asmussen and Chase, 2001). Non-coding regions such as those used here are known to have a higher substitution rate than coding sequences and also accumulate insertions/deletions (indels) at a faster rate. Non-coding DNA experiences a high frequency of indel mutations of varying lengths making sequence alignment problematic (Small et al., 1998). Additional confounding factors are:
i. homology of short repeats or individual nucleotides runs via slipped-strand mispairing.
ii. homoplasy resulting from the reduction in character states caused by high AT content.
iii. the multiple origin of small inversions that are localised in the loop of the stem-loop secondary structures.
iv. bias nucleotide substitutions in AT rich regions.

All alignments were done manually using MacClade. Gaps corresponding to indels were positioned to minimise the number of nucleotide differences among sequences.

Sequences were not submitted to GenBank and will be submitted at a later date when data will be published in full. The full alignment of the $\operatorname{trn} T$ - $L$ spacer, $\operatorname{trn} L$ intron and ITS sequences are provided in Appendix 4, 5 and 6 respectively.

### 3.2.4. Phylogenetic Analyses

Several analytical approaches were used in phylogenetic reconstruction viz. distance, parsimony and bayesian methods. Out-groups varied slightly for each data set as outlined below.

Coding of indels should be awarded on the merits of the indel, when it has been established (with some degree of confidence) that the indel is homologous (sequences identical in length and sequence; Nickrent et al., 2004). A conservative approach was taken and indels were not coded and scored. Single base pair substitutions/mutations and indels associated with mononucleotide runs and repeat regions were re-checked against the original sequence chromatograms for verification after alignment.
3.2.4.1. Neighbor Joining (NJ)

The neighbor joining method (Saitou and Nei, 1987) was used to construct distance trees. Coding regions, exons, introns and intergenic spacers do not have the same molecular evolutionary constraints and these regions need to be portioned in model based approaches. The most appropriate model of sequence evolution for a given partition was determined using the Akaike Information Criterion (AIC) as implemented in the program MODELTEST version 3.06 (Posada and Crandall, 1998). The chloroplast genome of Zea mays L. from GenBank (accession X86563) was used as a guide to determine limits of the trnT-L spacer and $\operatorname{trnL}$ intron to partition these markers prior to NJ and Bayesian Inference analyses (see below). The model was incorporated into reconstructions performed using PAUP* version 4.0 b 10 (Swofford, 2002) for NJ analyses. Uncombined and combined partitions were treated as a single unit and a single model was implemented. The same approach was applied to all the data sets viz.: the $\operatorname{trn} T-L$ spacer, the $\operatorname{trn} L$ intron, combined $\operatorname{trn} T-L$ spacer and $\operatorname{trn} L$ intron, a subset of $\operatorname{trn} T-L$ sequences that had matching sequences to the $\operatorname{trn} L$ intron, the ITS region, and combined ITS and trnT-L spacer sequences. A subset of $\operatorname{trn} T-L$ sequences that had complementary sequences to the $\operatorname{trn} L$ intron matrix was analysed to establish if the phylogenies recovered for the $\operatorname{trn} L$ intron was a result of sample selection and if the $\operatorname{trn} T-L$ spacer subset matrix reflected the same topology as the larger (entire) $\operatorname{trn} T-L$ spacer matrix.

Nodal support was evaluated by generating 1000 neighbor joining bootstrap replicates. Bulbine latifolia (SR 61) was used as the out-group with the exception of the trnL intron analysis. Four additional trnL intron sequences of Bulbine and Bulbinella retrieved from GenBank were included [Bulbine semibarbata (AJ290259), Bulbine succulenta (AJ290260), Bulbine weisei (AJ290261) and Bulbinella cauda-felis (AJ290262)]. All Bulbine samples mentioned above and Bulbine latifolia (SR 61) were used as the out-group.

### 3.2.4.2. Maximum Parsimony (MP)

Maximum parsimony analysis was conducted using PAUP* 4.0b10 (Swofford, 2002) for all data sets. Uninformative characters were excluded and all nucleotide characters were equally weighted and unordered. Gaps were treated as missing data. A random input analysis was performed to determine if there were multiple islands of equally most parsimonious trees
(Maddison, 1991) with 1000 replicates with one tree kept at each replicate. A full heuristic search was conducted on the trees found by this method with TBR branch swapping and MAXTREES set at 10000 . A strict consensus tree was constructed from all the most parsimonious trees. Bootstraps values were obtained from 1000 heuristic bootstrap replicates with MAXTREES set at 100. Bulbine latifolia (SR 61) was used as the out-group.

The same approach was applied to all the data sets with the exception of the $\operatorname{trn} L$ intron analysis. Four additional $\operatorname{trn} L$ out-groups sequences were included and all Bulbine samples were used as the out-group.

### 3.2.4.3. Bayesian Inference (BI)

The chloroplast genome of Zea mays from GenBank (accession X86563) was used as a guide to determine limits of the $\operatorname{trn} T-L$ spacer and $\operatorname{trnL}$ intron to partition these markers. The ITS partitions were determined using the ITS sequence of Bulbine weisii (GenBank accession AF234350). The matrix was partitioned into three regions (ITS1, 5.8S and ITS2). MrModel version 2.2. (Nylander, Uppsala University, Sweden) was used to obtain the best model for the data sets and these models were incorporated into the BI analyses.

Bayesian inference was performed using MrBayes version 3.1.1 (Huelsenbeck and Ronquist, 2001). The analysis was conducted with four Monte Carlo Markov Chains (three heated and one cold). Chains were run for 3000000 generations and sampled every $100^{\text {th }}$ generation. An a proiri burn in was not specified. Upon completion of analysis, the output files were examined to determine when log likelihood values stablised and determine the burn in. These burn in generations were excluded when constructing the Bayesian Inference trees. Posterior probabilities (PP) were estimated by constructing a $50 \%$ majority rule consensus tree in PAUP* 4.0b10 (Swofford, 2002). BI trees were retrieved using TREEVIEW (Page, 1996).

Bulbine latifolia (SR 61) was used as the out-group. The same approach was applied to all the data sets with the exception of the $\operatorname{trn} L$ intron analysis. Four additional trnL out-groups sequences were included. All Bulbine samples were used as the out-groups.

### 3.2.4.5. Partition homogeneity (or ILD) test for combined data sets

In this study a subset of $\operatorname{trn} T-L$ spacer and $\operatorname{trn} L$ intron sequences were combined. Due to the differences in lineages recovered a partition homogeneity (or ILD) test (Farris et al., 1994) which tests congruence in the phylogenetic signal of the two separate partitions was implemented. The ILD test addresses whether two data sets are arbitrary subdivisions of what should be a single larger data set. Tree comparisons and the ILD tests are useful initial comparisons. The ILD test was implemented in PAUP* 4.0 b 10 (Swofford, 2002) with 1000 replicates, maximum trees set at 1000 and TBR branch swapping.

The $\operatorname{trn} T$ - $L$ spacer and ITS sequences were also combined. The ILD test was implemented as described above. This proved to be computationally prohibitive and the test was implemented with 100 replicates and maximum trees set at 100 .

In both the above cases visual examination of nodes that are not well supported (bootstrap values of $<70 \%$ ) are considered non-conflicting and warranted merger of data sets (MasonGamer and Kellogg, 1996).

### 3.2.4.5.2. Sequencing to assess intra-population cpDNA polymorphisms

The trnT-L spacer was sequenced to check for intra-population polymorphisms in two taxa representing two populations [K. northiae (SR 274) from Naudes Nek and K. rooperi (SR 237) from East London]. Multiple individuals of each assession were sampled. Five and four samples were sequenced for $K$. northiae and $K$. rooperi respectively.

### 3.3. Results

### 3.3.1. Sampling

DNA samples of 51 of the 71 Kniphofia species ( $72 \%$ species coverage) were obtained during the course of the study. A total of 50 species were sequenced for the $t r n T-L$ spacer and 51 species were sequenced for the ITS region. Four of the 48 southern Africa taxa were not included (i.e. $92 \%$ species coverage for southern Africa). Specimens of K. evansii, K. tabularis, K. crassifolia and K. flammula were not obtained.

Samples of seven of the 23 Malagasy, Tropical and East African species were obtained (30\% species coverage). Only the Bale Mountains (Ethiopia) received sufficient sampling attention. Uncollected species were difficult to obtain because of limited budgets and time constraints. Limited attempts were made to use herbarium material for DNA extractions. Herbarium material often go brown and do not appear to retain usable DNA. Herbarium material was only requested from one foreign herbarium (ETH). DNA material for most Ethiopian taxa were collected while doing field work in Ethiopia and these were used instead of herbarium material for DNA studies.

### 3.3.2. Phylogenetic analysis of $\operatorname{trn} T$ - $L$ spacer data (Fig. 3.3.-3.5.)

trnT-L sequences were obtained for 125 samples of Kniphofia representing 50 species (Table 3.3.). A single Bulbine and Bulbinella sequence were also generated for this marker.

As discussed in the materials and methods section, Taberlet's primer 'a' was problematic and an internal primer, 'Knip1' was used. The priming site for 'Knip1' is approximately 945 bp upstream from the priming site of primer ' $b$ '. Following alignment it was found that the initial part of this matrix was rich in $(\mathrm{AT})_{\mathrm{n}}$ and $(\mathrm{T})_{\mathrm{n}}$ repeats, which proved difficult to align in certain regions. In order to avoid alignment related homology assessment problems associated with these repeats the first 317 bp were excluded from analyses i.e. most of the problematic regions in terms of alignment were omitted. Effectively this meant that the trnT-
$L$ spacer matrix started 628 bp upstream from the priming site of 'b'. Thus only the 3 ' region of the $\operatorname{trn} T-L$ spacer was used (hereafter referred to the $\operatorname{trn} T-L$ spacer).

The final aligned matrix with Bulbine and Bulbinella included was 609 bp in length (Appendix 4). Eighty eight ( $14.4 \%$ ) characters were variable and 31 (5.1\%) were parsimony informative with the out-groups included. When the out-groups were excluded these values reduced to 39 ( $6.4 \%$ ) variable and 20 ( $3.3 \%$ ) parsimony informative characters (Table 3.5.). Additional data (RI, CI and tree lengths values) are given in Table 3.6.

The $\operatorname{trn} T-L$ spacer supports the recognisition of five lineages for the NJ, MP and BI analyses (Fig. 3.3-3.6). These lineages are hereafter termed Groups 1-5 and labelled as such in the figures. In a preliminary investigation using the $\operatorname{trn} T-L$ spacer (Ramdhani et al., 2006), three lineages were recovered. Two of these were characterised by insertions. With a more thorough sampling and a trimmed data set used here, one of these lineages (Group 5) is still characterised by a nine bp insertion (position 544-552). The other group (Group 4) was characterised by a six bp insertion (position 87-92) except for a single sample (SR 453, K. angustifolia) for which the insertion is not present.

Of the five clades, Group 1 is the smallest with two species ( $K$. splendida and $K$. coralligemma) and is not retrieved in the NJ and MP analyses (Fig. 3.3. \& 3.4.). However, in the BI analysis this group is poorly supported with a posterior probability ( PP ) $=0.78$ (Fig. 3.5.).

Group 2 has 17 samples representing eight species (K. typhoides, K. brachystachya, K. uvaria, K. praecox, K. rooperi, K. citrina, K. sarmentosa, K. baurii and K. grantii). This group is not well supported by the $\mathrm{NJ}[\mathrm{BS}$ (bootstrap)=54\%)] (Fig. 3.3.) and BI analyses ( $\mathrm{PP}=0.87$ ) (Fig. 3.5.). It was better supported by the MP analysis $(\mathrm{BS}=71 \%)$ (Fig. 3.4.).

Table 3.5. Nucleotide sequence characteristics of the $\operatorname{trn} T-L$ spacer and $\operatorname{trn} L$ intron.

| Region | No. of Base pairs (bp) | No. of Variable bp (\%) | Parsimony informative bp (\%) | No. of samples (no. of taxa) |
| :---: | :---: | :---: | :---: | :---: |
| trnT-L spacer including out-groups | 609 | 88 (14.4\%) | 31 (5.1\%) | 127 (Kniphofia-50, <br> Bulbinella-1, <br> Bulbine-1) |
| trnT-L spacer excluding out-groups |  | 39 (6.4\%) | 20 (3.3\%) | 125 (50) |
| trnL intron including outgroups | 571 | 72 (12.6\%) | 39 (6.8\%) | 33 (Kniphofia-27, <br> Bulbinella-2, <br> Bulbine-4) |
| trnL intron excluding outgroups |  | 36 (6.3\%) | 25 (4.4\%) | 27 (24) |
| $\operatorname{trn} T-L$ spacer subset of $\operatorname{trn} L$ intron including out-groups | 598 | 74 (12.4\%) | 27 (4.5\%) | 29 (Kniphofia-27, <br> Bulbinella-1, <br> Bulbine-1) |
| $\operatorname{trn} T$ - $L$ spacer subset of $\operatorname{trn} L$ intron excluding out-groups |  | 20 (3.3\%) | 16 (2.7\%) | 27 (24) |
| Combined trnT-L spacer and trnL intron including out-groups | $\begin{aligned} & 1159 \\ & (598+561) \end{aligned}$ | $\begin{aligned} & 113 \text { (9.8\%) } \\ & (74+59) \end{aligned}$ | $\begin{aligned} & 54(4.7 \%) \\ & (27+27) \end{aligned}$ | 29 (Kniphofia-27, <br> Bulbinella-1, <br> Bulbine-1) |
| Combined trnT-L spacer and $\operatorname{trn} L$ intron excluding out-groups |  | $\begin{aligned} & 56(4.8 \%) \\ & (20+36) \end{aligned}$ | $\begin{aligned} & 41(3.5 \%) \\ & (16+25) \end{aligned}$ | 27 (24) |

Table 3.6. Summary statistics of data sets analysed using MP and the resulting tree statistics.

| DNA marker | No. of <br> trees | CI | RI | Tree <br> length |
| :--- | :--- | :--- | :--- | :--- |
| trnT- $L$ spacer | 10000 | 0.630 | 0.949 | 54 |
| trnL intron | 10000 | 0.596 | 0.777 | 77 |
| trnT-L subset of trnL intron | 10000 | 0.829 | 0.933 | 35 |
| Combined trnT-L and trnL intron | 228 | 0.626 | 0.803 | 99 |
| ITS | 36 | 0.947 | 0.880 | 100 |
| Combined trnT-L and ITS | 10000 | 0.750 | 0.921 | 160 |

Group 3 has 17 samples representing seven species from Madagascar, Tropical and East Africa (K. foliosa, K. isoetifolia, K. schimperi, K. thomsonii, K. splendida, K. insignis and K. ankaratrenesis). The group is weakly supported by the NJ analysis ( $\mathrm{BS}=56 \%$ )(Fig. 3.3.) and MP analysis ( $B S=63 \%$ ) (Fig. 3.4.). However, for the BI analyses it is well supported with a $\mathrm{PP}=1.00$ (Fig. 3.5.).

Group 4 is the largest lineage with 70 samples representing 33 species. The species included: K. albescens, K. baurii, K. drepanophylla, K. rooperi, K. linearifolia, K. coddiana, K. hirsuta, K. laxiflora, K. ichopensis, K. parviflora, K. littoralis, K. fibrosa, K. stricta, K. latifolia, K. buchananii, K. ensifolia, K. triangularis, K. leucocephala, K. bruceae, K. tysonii, K. galpinii, K. multiflora, K. porphyrantha, K. breviflora, K. thodei, K. angustifolia, K. ritualis, K. pauciflora, K. albomontana, K. gracilis, K. uvaria, K. rigidifolia and K. umbrina. Group 4 is weakly supported by the NJ analysis (BS=63\%) (Fig. 3.3.) and MP analysis (BS= $62 \%$ )(Fig. 3.4.). The BI analysis supported this group with a $\mathrm{PP}=0.95$ (Fig. 3.5.).

Group 5 has 19 samples representing nine species (K. acraea, K. northiae, K. linearifolia, K. triangularis, K. parviflora, K. fibrosa, K. uvaria, K. rooperi and K. caulescens). Group 5 was well supported for the $\mathrm{NJ}(\mathrm{BS}=74 \%)$ (Fig. 3.3.) and $\mathrm{BI}(\mathrm{PP}=0.96)$ (Fig. 3.5.) analyses, but not for the MP analysis ( $\mathrm{BS}=51 \%$ ) (Fig. 3.4.).

It is interesting to note that species with multiple samples in Group 2 (K. uvaria, K. praecox and K. baurii), Group 3 (K. foliosa, K. isoetifolia, K. schimperi, K. thomsonii), Group 4 (K. baurii, K. drepanophylla, K. rooperi, K. linearifolia, K. laxiflora, K. ichopensis, K. buchananii, K. ensifolia, K. triangularis, K. tysonii, K. angustifolia, K. gracilis and K. uvaria) and Group 5 (K. northiae, K. linearifolia, K. triangularis, K. uvaria and K. caulescens) do not cluster to form monophyletic species lineages. In addition eight species were placed in more than one group (K. baurii, K. fibrosa, K. linearifolia, K. parviflora, K. rooperi, K. splendida, K. triangularis and K. uvaria). The implications of this will be discussed in detail later.

_ 0.0005 substitutions/site
Fig. 3.3. Neighbor joining tree based on $\operatorname{trn} T-L$ spacer sequences, obtained using the $\mathrm{K} 81 \mathrm{uf}+\mathrm{G}$ model (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.4. Strict consensus tree of 10000 most parsimonious trees based on $\operatorname{trn} T-L$ spacer sequences obtained from the maximum parsimony analysis. Length $=54$; $\mathrm{CI}=0.630$; RI $=0.949$. Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.5. Bayesian tree of the $\operatorname{trnT} T-L$ spacer sequences estimated using the GTR $+G$ model (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Numbers above the branches represent posterior probability values.

### 3.3.3. Phylogenetic analysis of trnL intron data (Fig. 3.6.-3.8.)

Sequences of the $\operatorname{trnL}$ intron were obtained for 27 samples of Kniphofia representing 24 species (Table 3.3.). A single Bulbine and Bulbinella sequence was also generated for this marker. A restrictive budget and time did not allow for all the $\operatorname{trn} T$ - $L$ spacer samples to be sequenced for the $\operatorname{trn} L$ intron. Samples chosen had complementary sequences for the trnT-L spacer and were representative of the main lineages recovered by the $\operatorname{trn} T-L$ spacer. Four sequences of Bulbine and Bulbinella retrieved from GenBank provided additional out-groups.

The final aligned matrix with Bulbine and Bulbinella included was 571 bp in length (Appendix 5). Messy 3' ends were trimmed and the 5' $\operatorname{trn} L$ exon excluded. This region corresponds to positions 671 to 1231 in the full alignment. Seventy-two (12.6\%) characters were variable and 39 ( $6.8 \%$ ) were parsimony informative with out-groups included. When the out-groups were excluded these values reduced to 36 (6.3\%) variable and 25 (4.4\%) parsimony informative characters (Table 3.5.). Additional data (RI, CI and tree lengths values) are given in Table 3.6.

The $\operatorname{trnL}$ intron had no indels that characterised lineages retrieved by the $\operatorname{trn} T-L$ spacer. A single sample had a unique 82 bp deletion (K. ritualis, SR 300). Large deletions are often problematic as they remove large amount of potentially informative sites (Asmussen and Chase, 2001). This sample was placed as sister to all the other Kniphofia samples in the NJ analysis (Fig. 3.6.). The deletion may account for this placement but in the MP and BI analyses this sample was nested in Group 4 (Fig. 3.7. \& 3.8.).

The $\operatorname{trn} L$ intron data failed to recover the same groups of taxa that were found in the $\operatorname{trn} T-L$ spacer analyses. Group 1 had only a single sample (K. coralligemma) for the intron. Several attempts to sequence the intron for the other sample (K. splendida, SR 548) that was placed into Group 1 failed. Group 2 has three samples representing two species ( $K$. typhoides and $K$. uvaria). Group 2 collapsed with one sample (K. uvaria, SR 211) not clustering with the two other samples in the NJ analysis (Fig. 3.6.). Group 2 also collapsed in the MP (Fig. 3.7.) and BI analyses (Fig. 3.8.), and was resolved as a polytomy with other groups.

Group 3 has five samples representing five species (K. foliosa, K. thomsonii, K. insignis, K. schimperi and $K$. isoetifolia). The group is well supported for both the NJ (BS=74\%) (Fig. 3.6.) and $\mathrm{BI}(\mathrm{PP}=1.00)$ (Fig. 3.8.) analyses, while the support for the MP analysis was weaker ( $\mathrm{BS}=69 \%$ ) (Fig. 3.7.).

Group 4 had 13 samples representing 12 species (K. albescens, K. baurii, K. stricta, K. bruceae, K. tysonii, K. breviflora, K. laxiflora, K. triangularis, K. parviflora, K. linearifolia, K. multiflora and $K$. ritualis). This group forms a separate lineage with the exception of two samples [SR 300 (K. ritualis) and RG sn (K. umbrina)] in the NJ analysis (Fig. 3.6.), but with no support. In the MP analysis (Fig. 3.7.) Group 4 collapsed and was resolved as a polytomy with other groups. All samples that characterise this group clustered together in the BI analysis with weak support ( $\mathrm{PP}=0.53$ ) (Fig. 3.8.).

Group 5 has four samples representing four species (K. acraea, K. parviflora, K. northiae and K. caulescens). This group collapsed in all the analytical approaches with no support (Fig. 3.6.-3.8).


Fig. 3.6. Neighbor joining tree based on $\operatorname{trnL}$ intron sequences, obtained using the TVM $+\mathrm{I}+\mathrm{G}$ model (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text). Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.7. Strict consensus tree of 10000 most parsimonious trees based on $\operatorname{trnL}$ intron sequences obtained from the maximum parsimony analysis. Length $=77$; $\mathrm{CI}=0.597 ; \mathrm{RI}=0.777$. Major groups are denoted by bars to the right (discussed in text). Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.8. Bayesian tree of the $\operatorname{trn} L$ intron sequences estimated using the GTR $+\mathrm{I}+\mathrm{G}$ model (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text). Numbers above the branches represent posterior probability values.

### 3.3.4. Phylogenetic analysis of trnT-L spacer subset of $t r n L$ intron data (Fig. 3.9.-3.11.)

A subset of only $\operatorname{trn} T-L$ sequences that matched sequences to the $\operatorname{trnL}$ intron matrix were analysed separately. This was done to establish if the phylogenies recovered for the trnL intron was a result of sample selection and if the $\operatorname{trn} T-L$ spacer subset matrix reflected the same topology as the larger (entire) trnT-L spacer matrix.

A total of 27 samples representing 24 species of Kniphofia were obtained. A single Bulbine and Bulbinella sequence was also generated. The final aligned matrix with Bulbine and Bulbinella included was 598 bp in length. Seventy-four (12.4\%) characters were variable and $27(4.5 \%)$ were parsimony informative. When the out-groups were excluded these values reduced to 20 (3.3\%) variable and 16 ( $2.7 \%$ ) parsimony informative characters (Table 3.5.). Additional data (RI, CI and tree lengths values) are given in Table 3.6.

The same five lineages found by the $\operatorname{trn} T-L$ spacer analyses (entire matrix) were also found. Group 4 and 5 are characterised by the same insertions found in the spacer (discussed above).

Group 1 has only a single sample ( $K$. coralligemma) (Fig. 3.9.-3.11.). Group 2 were recovered as a single lineage, with good support in the NJ ( $\mathrm{BS}=73 \%$ ) (Fig. 3.9.) and MP ( $\mathrm{BS}=75 \%$ ) (Fig. 3.10.) analyses, while in the BI analysis this group also had fairly good support ( $\mathrm{PP}=0.94$ ) (Fig. 3.11.). Group 3 had good support in the NJ ( $\mathrm{BS}=90 \%$ ) (Fig. 3.9.), MP ( $\mathrm{BS}=89 \%$ ) (Fig. 3.10.) and $\mathrm{BI}(\mathrm{PP}=1.00)$ (Fig. 3.11.) analyses. Group 4 was recovered as a single lineage with poor support in the $\mathrm{NJ}(\mathrm{BS}=66 \%)$ (Fig. 3.9.) and MP $(\mathrm{BS}=63 \%)$ (Fig. 3.10.) analyses. However, in the BI analysis it was well supported ( $\mathrm{PP}=0.97$ ) (Fig. 3.11.). Group 5 had good support for the $\mathrm{NJ}(\mathrm{BS}=96 \%)$ (Fig. 3.9.), MP $(\mathrm{BS}=95 \%)$ (Fig. 3.10.) and $\mathrm{BI}(\mathrm{PP}=1.00)$ (Fig. 3.11.) analyses.


Bulbinella cauda-felis SR204

Fig. 3.9. Neighbor joining tree based on $\operatorname{trn} T-L$ spacer (subset of $\operatorname{trn} L$ intron) sequences, obtained using the K81uf + I model (determined by the Akaike Information Criterion) for the entire matrix. Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.10. Strict consensus tree of 10000 most parsimonious trees based on on $\operatorname{trn} T-L$ spacer (subset of $t r n L$ intron) sequences obtained from the maximum parsimony analysis. Length $=35 ; \mathrm{CI}=0.892$; $\mathrm{RI}=0.933$. Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.11. Bayesian tree of the $\operatorname{trn} T-L$ spacer (subset of $\operatorname{trn} L$ intron) sequences estimated using the GTR + I model (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Numbers above the branches represent posterior probability values.

### 3.3.5. Phylogenetic analysis of combined $\operatorname{trn} T-L$ spacer and $\operatorname{trn} L$ intron data (Fig. 3.12.3.14.)

Visual examination of the bootstrap values for the trnL intron NJ and MP phylogenies showed that none of the clades had bootstrap values $>70 \%$, with the exception of Group 3 in the NJ analysis (Fig. 3.6.). This group was not a source of conflict with the trnT-L spacer. The ILD test gave a value of 0.1650 with no significant incongruence in tree length between the data sets. Thus, the $\operatorname{trn} L$ intron and $\operatorname{trn} T-L$ spacer data sets were combined.

Combined sequences were obtained for 27 samples representing 24 species of Kniphofia (Table 3.3.). A single Bulbine and Bulbinella sequence was also generated. The final aligned matrix with Bulbine and Bulbinella included was 1159 bp in length. One hundred and thirteen $(9.8 \%)$ characters were variable and $54(4.7 \%)$ were parsimony informative. When the out-groups were excluded these values reduced to 56 ( $4.8 \%$ ) variable and 41 (3.5\%) parsimony informative characters (Table 3.5.). Additional data (RI, CI and tree lengths values) are given in Table 3.6.

Group 5 and 4 are characterised by the same insertions found in the spacer (discussed above). The sample with the unique 82 bp deletion in the intron (K. ritualis, SR 300) did not affect the outcome of the analyses and was placed in Group 4. The analyses retrieved the same five lineages as the $\operatorname{trn} T$ - $L$ data above, irrespective of the reconstruction approach.

Group 1 has only a single sample (K. coralligemma). Group 2 has no support in the NJ analysis (Fig. 3.12.) and weak support in the MP analysis ( $\mathrm{BS}=66 \%$ ) (Fig. 3.13.), while in the BI analysis this group had good support ( $\mathrm{PP}=0.99$ ) (Fig. 3.14.). Group 3 has good support in the $\mathrm{NJ}(\mathrm{BS}=97 \%)$ (Fig. 3.12.), MP $(\mathrm{BS}=97 \%)$ (Fig. 3.13.) and $\mathrm{BI}(\mathrm{PP}=0.99)$ (Fig. 3.14.) analyses. Group 4 has poor support in the NJ analysis ( $\mathrm{BS}=67 \%$ ) (Fig. 3.12.). However, in the MP analysis and BI analysis it was well supported with BS $=82 \%$ (Fig. 3.13.) and $\mathrm{PP}=1.00$ (Fig. 3.14.) respectively. Group 5 has good support for the $\mathrm{NJ}(\mathrm{BS}=$ $86 \%$ ) (Fig. 3.12.), MP (BS=89\%) (Fig. 3.13.) and $\mathrm{BI}(\mathrm{PP}=1.00)$ (Fig. 3.14.) analyses.


Fig. 3.12. Neighbor joining tree based on combined $\operatorname{trn} T-L$ spacer and $t r n L$ intron sequences, obtained using the GTR + I + G model (determined by the Akaike Information Criterion) for the entire matrix. Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.13. Strict consensus tree of 228 most parsimonious trees based on combined trnT-L spacer and trnL intron sequences obtained from the maximum parsimony analysis. Length $=99 ; \mathrm{CI}=0.626 ; \mathrm{RI}=$ 0.803 . Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.14. Bayesian tree of the combined $\operatorname{trn} T-L$ spacer and $\operatorname{trn} L$ intron sequences estimated using the following models: GTR $+\mathrm{G}(\operatorname{trn} T-L$ spacer) and GTR $+\mathrm{I}+\mathrm{G}(\operatorname{trn} L$ intron) (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Numbers above the branches represent posterior probability values.

### 3.3.6. Phylogenetic analysis of ITS data (Fig. 3.15.-3.17.)

The only nuclear marker screened for this study was the ITS region. Initial results showed little sequence divergence. Despite this, further investigations were done as this marker provided an independent and unlinked source of DNA sequence data and it was the only independent nuclear marker available that could be easily amplified and sequenced routinely.

ITS sequences were obtained for 99 samples representing 51 species of Kniphofia (Table 3.3.). A single Bulbine and Bulbinella sequence was also generated.

PCR products generated for the ITS region were resolved as single bands with no size difference. In addition no double peaks or ambiguous base calls were found in electrophenograms/trace files of ITS sequences, suggesting that there is no evidence of paralogues. No attempts at cloning were pursued as this is expensive and time consuming especially in studies with a large amount of samples or taxa.

The final aligned matrix with Bulbine and Bulbinella included was 830 bp (Appendix 6). Position 1-395 represents the ITS1 spacer, positions 396-566 the 5.8S gene and positions 567830 the ITS2 spacer. One hundred and seventy-six ( $21.1 \%$ ) characters were variable and 72 ( $8.7 \%$ ) were parsimony informative. When the out-groups were excluded these values reduced to 63 ( $7.6 \%$ ) variable and 28 ( $3.4 \%$ ) parsimony informative characters (Table 3.7.). Additional data (RI, CI and tree lengths values) are given in Table 3.6.

The ITS region had no indels that characterised lineages. The ITS data was of limited use as many sequences were identical. The analyses recovered a large polytomy of samples from Groups 1-5 with little structure and resolution (Fig. 3.15.- Fig. 3.17.). Most samples of Group 3 (representatives from Ethiopia), denoted as Clade A in the cladograms, formed a well supported lineage in the $\mathrm{NJ}(\mathrm{BS}=99 \%)$ (Fig. 3.15.), MP ( $\mathrm{BS}=100 \%$ ) (Fig. 3.16.) and BI $(\mathrm{PP}=0.99)$ (Fig. 3.17.) analyses.

Table 3.7. Nucleotide sequence characteristics of the ITS region, and the combined $\operatorname{trnT}-L$ spacer and ITS region.

| Region | No. of Base pairs (bp) | No. of Variable bp (\%) | No. of Parsimony informative bp (\%) | No. of samples (No. of taxa) |
| :---: | :---: | :---: | :---: | :---: |
| ITS1 spacer | 395 | 89 (10.7\%) | 41 (5.0\%) |  |
| 5.8S gene | 170 | 36 (4.3\%) | 14 (1.7\%) |  |
| ITS2 spacer | 264 | 51 (6.1\%) | 17 (2.0\%) |  |
| ITS: Total <br> including out- <br> groups  <br>   | 830 | 176 (21.1\%) | 72 (8.7\%) | 101 (Kniphofia- <br> 51, Bulbinella- 1, <br> Bulbine-1) |
| ITS: Total <br> excluding out- <br> groups  |  | 63 (7.6\%) | 28 (3.4\%) | 99 (51) |
| Combined trnT-L spacer and ITS including outgroups | $\begin{aligned} & 1434 \\ & (604+830) \end{aligned}$ | $\begin{aligned} & \hline 260(\mathbf{1 8 . 1 \%}) \\ & (84+176) \end{aligned}$ | $\begin{aligned} & 101(7.0 \%) \\ & (29+72) \end{aligned}$ | 96 (Kniphofia- 50, <br> Bulbinella- <br> 1, <br> Bulbine-1) |
| Combined trnT-L spacer and ITS excluding outgroups |  | $\begin{aligned} & 95(6.6 \%) \\ & (32+63) \end{aligned}$ | $\begin{aligned} & 47 \text { (3.2\%) } \\ & (19+28) \end{aligned}$ | 94 (50) |

Two K. thomsonii samples from Group 3, denoted as Clade B in cladograms, formed a well supported lineage the $\mathrm{NJ}(\mathrm{BS}=96 \%)$ (Fig. 3.15.), MP $(\mathrm{BS}=95 \%)$ (Fig. 3.16.) and $\mathrm{BI}(\mathrm{PP}=$ $0.98)$ (Fig. 3.17.) analyses. Samples of K. caulescens and K. stricta from Group 5, denoted as Clade C in cladograms, had good support in the $\mathrm{NJ}(\mathrm{BS}=88 \%)$ (Fig. 3.15.) and MP ( $\mathrm{BS}=$ $96 \%$ ) (Fig. 3.16.) analyses. In the BI analysis this lineage was recovered with some support $(\mathrm{PP}=0.94)$ (Fig. 3.17.). Some samples (K. galpinii, K. triangularis, K. fluviatalis, K. multiflora, K. umbrina) from Group 4 (notably from Mpumalanga and Swaziland), denoted as Clade D in figures, formed a weakly supported lineage in the NJ ( $\mathrm{BS}=57 \%$ ) (Fig. 3.15.), $\mathrm{MP}(\mathrm{BS}=62 \%)($ Fig. 3.16.) and $\mathrm{BI}(\mathrm{PP}=0.73)$ (Fig. 3.17.) analyses.


Fig. 3.15. Neighbor joining tree based on ITS sequences, obtained using GTR + G model for the entire matrix (determined by the Akaike Information Criterion). Groups denoted by bars to the right are discussed in the text. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.16. Strict consensus tree of 36 most parsimonious trees based on ITS sequences obtained from the maximum parsimony analysis. Length $=100 ; \mathrm{CI}=0.880 ; \mathrm{RI}=0.947$. Groups denoted by bars to the right are discussed in the text. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.17. Bayesian tree for ITS sequences estimated using the following models: GTR + G (ITS1), HKY + I ( $5.8 S$ ) and GTR (ITS2) (determined by the Akaike Information Criterion). Groups denoted by bars to the right are discussed in the text. Numbers above the branches represent posterior probability values.

### 3.3.7. Phylogenetic analysis of combined trnT-L spacer and ITS data (Fig. 3.18.-3.20.)

Visual examination of the ITS phylogeny shows a large polytomy with no bootstrap values for the nodes defining the $\operatorname{trn} T$ - $L$ groups. The ILD test for the combined $\operatorname{trn} T-L$ spacer and ITS gave a value of 0.100 indicating no significant incongruence in tree length between the data sets. Based on the above findings, the two markers were joined despite the limited potential of the ITS region to provide phylogenetic signal. Ninety-four combined sequences representing 50 species of Kniphofia were obtained (Table 3.3.). A single Bulbine and Bulbinella sequence were also included.

The final aligned matrix with Bulbine and Bulbinella included was 1434 bp in length. Two hundred and sixty ( $18.1 \%$ ) characters were variable and 101 ( $7.0 \%$ ) were parsimony informative. When the out-groups were excluded these values reduced to 95 (6.6\%) variable and 47 (3.2\%) parsimony informative characters (Table 3.7.). Additional data (RI, CI and tree lengths values) are given in Table 3.6.

The large number of identical ITS sequences effectively meant that the $\operatorname{trn} T-L$ spacer signal dominated the low signal in the ITS data in the combined analyses and the resultant topology in most respects reflects the same lineages retrived by the $\operatorname{trn} T-L$ spacer alone, with exceptions noted below.

Group 1 is weakly supported in the $\mathrm{NJ}(\mathrm{BS}=66 \%)$ (Fig. 3.18.), MP $(\mathrm{BS}=61 \%)$ (Fig. 3.19.) and BI ( $\mathrm{PP}=0.91$ ) (Fig. 3.20.) analyses. Group 2 was not supported for the NJ (Fig. 3.18.) and MP (Fig. 3.19.) analyses as it forms a grade at the base of these topologies. However, the BI analysis recovered the group as a single lineage with $\mathrm{PP}=0.62$ (Fig. 3.20.). Group 3 was poorly supported in the $\mathrm{NJ}(\mathrm{BS}=60 \%)$ (Fig. 3.18.) and MP ( $\mathrm{BS}=59 \%$ ) (Fig. 3.19.) analyses, while this group was well supported in the $\mathrm{BI}(\mathrm{PP}=0.98)$ (Fig. 3.20.) analysis. Group 4 was recovered as a single lineage but with no support in the NJ analysis (Fig. 3.18.). In the MP analysis (Fig. 3.19.), Group 4 formed a polytomy with some samples of K. caulescens from Group 5. Group 4 was recovered as a single lineage with fairly poor support in the BI analysis ( $\mathrm{PP}=0.74$ ) (Fig. 3.20.). Group 5 was recovered as a single lineage but with no
support in the NJ analysis (Fig. 3.18.), while this group was weakly supported in the BI (PP= 0.81 ) (Fig. 3.20.) analysis. As mentioned above, in the MP analysis (Fig. 3.19.) some samples of $K$. caulescens from Group 5 formed a polytomy with Group 4. The remaining samples representative of Group 5 formed a weakly supported lineage ( $B S=53 \%$ ).

All species with multiple samples in Group 2 (K. uvaria), Group 3 (K. foliosa, K. isoetifolia, K. schimperi, K. thomsonii), Group 4 (K. baurii, K. linearifolia, K. triangularis, K. uvaria, K. angustifolia, K. ensifolia, K. gracilis, K. ichopensis, K. laxiflora and K. tysonii) and Group 5 (K. linearifolia, K. triangularis, K. northiae and K. caulescens) failed to be resolved as monophyletic species lineages.

### 3.3.8. Sequencing of the trnT-L spacer to assess for intra-population cpDNA polymorphisms

Five samples from the population of K. northiae represented by SR 274 had identical sequences. This was also the case for $K$. rooperi (SR 237) with four samples sequenced. These two populations showed no evidence of intra-population cpDNA polymorphisms. The significance of these findings are dicussed later.


Fig. 3.18. Neighbor joining tree based on combined $\operatorname{trn} T-L$ spacer and $I T S$ sequences, obtained using the TIM + I + G model for the entire matrix (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.19. Strict consensus tree of 10000 most parsimonious trees based on of combined trnT-L spacer and $I T S$ sequences obtained from the maximum parsimony analysis. Length $=160 ; \mathrm{CI}=0.750$; $\mathrm{RI}=0.921$. Major groups are denoted by bars to the right (discussed in text). Bootstrap values ( 1000 replicates) of $>50 \%$ are indicated above the relevant nodes.


Fig. 3.20. Bayesian tree for the combined trnT-L spacer and ITS sequences estimated using the following models: GTR + G (trnT-L spacer), GTR + G (ITS1), HKY + I (5.8S) and GTR (ITS2) (determined by the Akaike Information Criterion). Major groups are denoted by bars to the right (discussed in text) and numbers below the group labels show position of insertions with bp size in parentheses. Numbers above and below the branches represent posterior probability values.

### 3.4. Discussion

### 3.4.1. Comparisons of chloroplast and nuclear markers

Of the regions used in this study, the $\operatorname{trn} T-L$ spacer was the most informative, providing more resolution than the other markers sequenced. As already stated, five lineages were recovered using the spacer. The $\operatorname{trn} L$ intron failed to recover the same topology as the trnT-L spacer with the exception of Group 3. A possible reason why the trnL intron did not recover a similar topology may be due to a lack of characters that define key nodes.

The ITS data were of limited use as many sequences were identical. The three analytical approaches failed to recover the same lineages as the $\operatorname{trnT-L}$ spacer and only parts of some groups were supported. Low sequence divergence of ITS has been reported in several studies especially in cases were a recent rapid radiation has been implicated (Baldwin and Sanderson, 1998; Harris et al., 2000; Richardson et al., 2001; Malcomber, 2002; Klak et al.; 2003b; Howarth and Baum, 2005). Other reasons for the lack of sequence divergence could be unidirectional homogenisation towards one type of sequence type via concerted evolution. Extensive gene flow via hybridisation (akin to a panmixis scenario) may also explain the predominance of only one sequence type. These aspects will be discussed in more detail later.

It is commonly assumed that combined analyses will reduce the differences between individual data sets thus providing a better approximation of organismal phylogeny (Borsch et al., 2005). Combining data sets is not always recommended as they may reflect different histories (Smith and Sytsma, 1990) and combining them results in the loss of this data. In some situations it is difficult to judge which data set performed better and a combined approach is favoured (Roalson et al., 2001). The combined chloroplast data sets (spacer and intron) generally recovered the same five lineages found by the $\operatorname{trn} T-L$ analyses, in all reconstruction approaches. In the combined trnT-L spacer and ITS data set Groups 1, 3, 4 and 5 were recovered with Group 2 not supported in the NJ and MP analyses. In the MP analysis, Group 4 formed a polytomy with some samples from Group 5 (viz. K. caulescens). However,
in the BI analysis all five groups were recovered. This indicates that the $\operatorname{trn} T-L$ spacer data contributes most of the phylogenetic signal.

Due to the limited applicability of the $\operatorname{trnL}$ intron and the $I T S$ region, the discussion below will be based primarily on the results of the $\operatorname{trn} T-L$ spacer (viz. the five groups recovered by this marker).

### 3.4.2. Non-monophyly of species

Despite a general lack of robust support, the most intriguing result in the phylogenetic reconstruction of Kniphofia is that all of the species with multiple samples were resolved as non-monophyletic. Some species had samples placed in more than one of the major lineages. In addition, several species with multiple samples, within a single major lineage failed to form monophyletic species clades (Table 3.8.). It should be emphasised that six species ( $K$. fibrosa, K. linearifolia, K. parviflora, K. rooperi, K. triangularis and K. uvaria; Table 3.8.) are in different lineages characterised by major deletions (Groups and 5) i.e. their sequences are very different. In order to test the extent of species non-monophyly at the local scale one population of each of two species were examined for intra-population polymorphisms. Multiple samples of populations of K. northiae (represented by SR 274) and K. rooperi (represented by SR 237) revealed no polymorphisms within each population. The samples sizes (K. northiae: $\mathrm{n}=5$ and K. rooperi: $\mathrm{n}=4$ ) for this exercise were small. The possibility of intra-population polymorphisms cannot be excluded but these results suggest that populations are monomorphic.

What explanations are there for this unusual result (i.e. non-monophyly of species)? Three (not mutually exclusive) explanations are:

1. the alpha taxonomy is not correct and species are not adequately delimited.
2. the evolutionary history of the DNA regions used here are not reflecting the history of the morphological entities.
3. sequence divergence in chloroplast and nuclear markers are to too low to detect monophyly in morphological species.

Table 3.8. Species of Kniphofia with multiple samples which were placed in more than one major group delimited using the trnT-L spacer, also included are species with multiple samples, which are placed within a major single group but not clustering together to form monophyletic species clades.

| Taxon | Group/s | Samples |
| :---: | :---: | :---: |
| 1. K. baurii | $\begin{aligned} & 2 \\ & 4 \end{aligned}$ | SR 202, RJM 1026 <br> SR 174, SR 398, SR 275, SR 285, SR 360, 382, NPB 1923 |
| 2. K. fibrosa | $\begin{aligned} & 4 \\ & 5 \end{aligned}$ | $\begin{aligned} & \hline \text { SR } 297 \\ & \text { PBP } 5579 \end{aligned}$ |
| 3. K. linearifolia |  | SR 269, SR 343 <br> SR 287, SR 290, SR 291, SR 558, JP sn, SR 400, SR328, TD 4638, SR 311 |
| 4. K. parviflora |  | $\begin{aligned} & \hline \text { SR } 268 \\ & \text { SR } 330 \end{aligned}$ |
| 5. K. rooperi | $\begin{aligned} & 5 \\ & 4 \\ & 2 \end{aligned}$ | SR 237 <br> TD 4559, RAL 4227, SR 485 <br> SR 528 |
| 6. K. splendida | $\begin{aligned} & \mathbf{1} \\ & \mathbf{3} \end{aligned}$ | SR 548 <br> Chapman 9061 |
| 7. K. triangularis | $\begin{aligned} & 5 \\ & 4 \end{aligned}$ | SR 264, SR 266 <br> SR 299, SR sn K. triangularis subsp. obtusifolia |
| 8. K. uvaria | $\begin{aligned} & 2 \\ & 5 \\ & 4 \end{aligned}$ | SR 166, SR 186, SR 201, SR 203, SR 211, TD 4477 <br> SR 337, SR 342, SR 471, SR 172 <br> SR 477, SR 344 |
| 9. K. angustifolia | 4 | SR 542, SR 453 |
| 10. K. buchananii | 4 | SR 305, SR 307, SR 458 |
| 11. K. caulescens | 5 | SR 270, SR 278, RJM 974, NPB 1821 |
| 12. K. drepanophylla | 4 | RAL 4816, RJM 1100 |
| 13. K. ensifolia | 4 | JB $s n$, SR 448 |
| 14. K. foliosa | 3 | JMG 034, SR 387, JMG 038, SR 398, SR 390, SR 383 |
| 15. K. gracilis | 4 | SR 321, SR 561, NNBG |
| 16. K. ichopensis | 4 | SR 242, SR 289, SR 286, SR 407 |
| 17. K. isoetofolia | 3 | SR 388, SR 393, SR 386 |
| 18. K. laxiflora | 4 | SR sn K. laxiflora form C, SR 295, SR 441, SR 442, SR 468, SR 467, SR 283, NPB 1820 |
| 19. K. northiae | 5 | SR 274, SR 446, SR 263 |
| 20. K. praecox | 2 | SR 529, SR 530, SR 532 |
| 21. K. schimperi | 3 | JMG 036, SR 391 |
| 22. K. thomsonii | 3 | AMM 2647, JMG 031, CK 4821 |
| 23. K. tysonii | 4 | SR 302, SR 303, SR 460 |

As noted in the introduction of this study, the alpha taxonomy and species delimitations in this genus are difficult in many instances. Hybridisation has been invoked by taxonomists that have worked on the genus (Codd, 1968, 2005; Marais, 1973). However, in at least some instances non-monophyly is also found in readily identifiable species e.g. $K$. northiae and $K$. parviflora suggesting that possible mis-identification of species alone cannot be used to explain this result. Hybridisation and lineage sorting could be playing a role in the evolution of species in Kniphofia.

In some plant groups that have undergone recent radiation it may be difficult to generate sufficient phylogenetic signal due to low sequence divergence even for rapidly evolving regions (Small et al., 1998). In many studies low sequence divergence and has been implicated in recent rapid radiations (Baldwin and Sanderson, 1998; Harris et al., 2000; Malcomber, 2002; Klak et al.; 2003b; Richardson et al., 2001; Warwick et al., 2004; Howarth and Baum, 2005). This could result in non-monophyletic species (e.g. Harris et al., 2000; Malcomber, 2002; Warwick et al., 2004; Howarth and Baum, 2005). Additionally, fixation and rapid concerted evolution following hybridisation may account for the predominance of a single ITS type (Álverez and Wendel, 2003). However, lack of sequence divergence is unlikely to exclusively explain the extensive non-monophyly in Kniphofia especially for the $\operatorname{trn} T-L$ cpDNA marker.

### 3.4.3. Hybridisation

Hybridisation is an important phenomenon in angiosperm evolution and speciation (Vriesendorp and Bakker, 2005; Church and Taylor, 2005; Okuyama et al., 2005). Comparative molecular phylogenetics studies are showing that hybridisation is more prominent than previously thought. For example, Cronn et al. (2003) reported the role of hybridisation in the evolutionary history of Gossypium gossypioides. This study showed introgression in two stages in this species. Ainouche et al. (2003) studied hybridisation in Spartina, a genus with well documented cases of hybridisation involving small species groups.

Kniphofia is noted for hybridisation in horticulture, contributing to its horticultural appeal and numerous artificial hybrids are in cultivation (Taylor, 1985). Hybridisation (past and/or present) could be more extensive in wild populations and could account for the observed results as hybridisation can yield complex patterns of relationships and processes that are difficult to infer and explain.

The following species from southern Africa that were found to be non-monophyletic in this study have all been implicated in hybridisation events: K. uvaria, K. rooperi, K. tysonii, K. linearifolia, K. praecox, K. baurii, K. drepanophylla, K. triangularis, K. fibrosa, K. angustifolia, K. buchananii, K. ensifolia, K. laxiflora, K. gracilis and K. ichopensis (Codd, 1968, 2005). Taxonomic problems associated with these taxa were reviewed in detail earlier (Chapter 1). Two of the four Tropical African species (K. schimperi and K. thomsonii) that were found to be non-monophyletic in this study have also been implicated in hybridisation (Marais, 1973).

It is well documented that natural hybridisation leads to intermediate morphology, and this may explain the numerous cases of intermediate and integrading morphology, and species complexes with extensive variation reported by Codd (1968, 2005). A recent radiation suggests that not enough time has passed for currently delimited morphological species to differentiate fully and develop reproductive barriers, thus promoting hybridisation and mixing of haplotypes. Incomplete or weak reproductive barriers in taxa of complexes may promote hybridisation. Back-crossing could further complicate the issue. A highly reticulate evolution may explain why variation patterns in morphology become blurred (Mummenhoff et al., 2004). Many problematic Kniphofia taxa appear to be either incipient species or populations sensu Lu (2001) that are in the process of diverging to the point of speciation but still have the potential to interbreed.

Hybridising species are often sympatric, share pollinators and/or flowering phenology (Chruch and Taylor, 2005). This description applies to many problematic species of Kniphofia from southern Africa, which are often wide-ranging species, display sympatry and
flower contemporaneously. However, there is no robust support and conflict in the results to test hybridisation issues.

### 3.4.3.1. ITS and Hybridisation

Relying solely upon ITS to reveal phylogenetic patterns in complex genera is insufficient and is likely to be misleading in some respects of evolutionary history (Chase et al., 2003). Lack of phylogenetic signal has important consequences (Comes and Abbott, 2001). As found in Kniphofia, McKinnon et al. (2001) found ITS sequence data in Eucalyptus to be homogeneous, suggesting a low rate of sequence evolution, recent speciation and hybridisation between species resulting in homogenisation of sequences.

Gene flow can potentially disrupt the divergence of nuclear sequences (see Barker et al., 2005). Divergent rDNA copies may experience a variety of fates following merger in a single genome after reticulation. One possible outcome following hybridisation is that one paralogue becomes fixed within a genome as a consequence of concerted evolution (Álverez and Wendel, 2003). Rampant/extensive hybridisation (i.e. panmixis) could have promoted gene flow and rapid concerted evolution, and may account for the results i.e. the predominance of a single ITS type in Kniphofia.

Wang et al. (2000) showed that rDNA is homogenised uni-directionally after hybridisation and polyploidisation. The process of concerted evolution can be rapid and can occur in one generation after the combination of parental ITS types (Aguilar et al., 1999). Concerted evolution of ITS has also been reported to be rapid in the silversword alliance, which is of hybrid ancestry (Barrier et al., 1999).

Presently we have very little understanding on the features that may affect crossing over and gene conversion, but presumably the genomic location and number of arrays play an important role. Generation times and time since reticulation may also be important factors to consider (Álverez and Wendel, 2003).

As noted previously there was a lack of multiple peaks and ambiquous calls in the ITS electrophenograms/trace files, which would indicate the possibility of different paternal and maternal copies from hybridisation events. However, the lack of ITS variability makes this test difficult to apply in the case of Kniphofia, as hybridising individuals may contribute identical ITS sequences to the hybrid progeny.

### 3.4.4. Lineage sorting

The evolutionary history of cpDNA represents one particular 'gene genealogy' (matriarchal phylogeny) within an organismal pedigree. Stochastic matriarchal lineage sorting from a polymorphic ancestral gene pool can account for the discordance between species affiliations (conventional morphological taxon boundaries) and cpDNA genotype (Avise et al., 1990). The persistence and sorting of ancestral polymorphisms may predate the divergence of the taxonomic lineages (Olsen and Schaal, 1999). It is biologically plausible that some individuals may be more closely related to a member of another species than to their conspecifics, solely due to patterns of maternal lineage survival and extinction accompanying the speciation process (Avise et al., 1987). A consequence of lineage sorting is that species will not cluster on a phylogeny and appear non-monophyletic. Recent speciation could result in morphologically different species possessing undifferentiated cytotypes i.e. a species may have more than one cytotype (McKinnon et al., 1999).

Thus reticulation and/or incomplete lineage sorting can explain the non-monophyly of Kniphofia species. Reticulation and incomplete lineage sorting may not be mutually exclusive and difficult to disentangle (Comes and Abbott, 2001; Goldman et al., 2004; Church and Taylor, 2005).

Lineage sorting (instead of hybridisation) requires the maintenance of ancestral cpDNA polymorphisms through one or more speciation events (Comes and Abbott, 2001). Random sorting will have to occur with several losses of the polymorphic state. Determining the influence of lineage sorting requires the estimation of the age of species. This will show if haplotype divergence pre- or post dates speciation. If divergence predated speciation, then
lineage sorting could account for haplotype sharing from a polymorphic ancestor. However, if speciation pre-dates haplotype divergence, interspecific hybridisation and introgression must be considered (McKinnon et al., 2001).

An indication of lineage sorting is the presence of polymorphisms within a population. Once this is established more detailed studies at the population level are required (e.g. Chiang et al., 2004). Multiple samples of populations of K. northiae and K. rooperi revealed no intrapopulation polymorphisms. These findings do not negate the possibility of incomplete lineage sorting and the results in this study may also be attributed to incomplete lineage sorting. However, it is impossible to determine whether haplotype divergence pre- or post-dated speciation. Determining age estimates in a phylogeny on a continental scale can be difficult (Plana et al., 2004). This is challenging when there is no fossil evidence or geological events cannot confidently be used to calibrate the phylogeny. Tree caliberation can be achieved by using sequence divergence rates from other groups with similar life histories, or closely related lineages. However, such results have to be considered as tentative. Different lineages may evolve at diffent rates depending on intrinsic and extrinsic factors. Caution must also be exercised in groups where hybridisation has been implicated. The age/s of speciations anatomises in the reticulations and only heterogenous assemblages can be dated, reflecting time of hybridisation rather than speciation. Moreover, this approach is not applicable when phylogenies are not robust with poorly supported nodes.

Lineage sorting and hybridisation are not mutually exclusive. The results obtained may be due to a combination of both phenomena. The extent of lineage sorting is usually determined by genetic heterogeneity within populations and migratory modes, which is associated with gene flow and natural hybridisation as well the geological time that the species have evolved through (Chiang et al., 2004). With the existing information it is impossible to distinguish the relative impact of hybridisation versus lineage sorting in Kniphofia.

Thus, the results of this study may reflect the combinational effects of a recent radiation, hybridisation, concerted evolution and/or incomplete lineage sorting.

### 3.4.5. Molecular phylogenies versus existing classification

Berger (1908) upheld Notosceptrum and divided Kniphofia into 14 formal sections. Codd (1968) grouped taxa into ten informal sections and did not support Notosceptrum. Marais (1973) and Codd (2005) did not apply any infra-generic groupings. Species included in this study are from all of Codd's (1968) informal sections. None of the sections delimited by Codd (1968) correspond to the lineages recovered from the nuclear and chloroplast markers for southern African material.
K. typhoides, K. umbrina and K. brachystachya of the segregate genus Notosceptrum where included in this study and results obtained here do not support the monophyly of this genus. K. typhoides and K. brachystachya are nested in Group 2, while K. umbrina is nested in Group 4. These results support Codd's (1967) inclusion of Notosceptrum in Kniphofia.

### 3.4.6. Geographical interpretation of phylogeny

If there has been gene flow and/or lineage sorting then conspecific individuals may be scattered in different lineages, but geographically localised genotypes should be shared between the different species (Whittemore and Schaal, 1991). Mapping samples of the five groups (Fig. 3.21.A.) revealed several geographic patterns. Composition of the five groups showed some congruence with geographic origin rather than the systematic arrangement based on morphology. Additionally these groups show some correspondence to the centres of diversity recovered by the numerical analysis (Chapter 2) that appears to suggest a historic link between the DNA signature and distribution patterns.

Group 1 contained samples from the Northern Province. Geographically most of the samples from Group 2 are from the Cape Region with outliers in KwaZulu-Natal (K. typhoides, K. brachystachya) and Malawi (K. grantii). A northern South Africa and Cape area of diversity were also recovered in the numerical analysis (Chapter 2). Group 3 is represented by material from Tropical and East Africa [with the exception of $K$ grantii (Group 2) from Malawi], and

Madagascar. Species from Ethiopia (K. foliosa, K. isoetifolia, K. schimperi, K. thomsonii and K. insignis), Kenya (K. thomsonii), Tanzania (K. thomsonii), Malawi (K. splendida) and Madagascar (K. ankaratrenesis) are in this group. Group 4 includes samples predominantly from KwaZulu-Natal and the Eastern Cape, with some samples from Mpumalanga and Swaziland. Group 5 is represented by samples mostly from the Eastern Cape and KwaZuluNatal. Groups 4 and 5 appears to reflect the distribution of the Cluster B (numerical analysis, Chapter 2), an area of diversity that covers much of northern, eastern and partly central SA. However, the boundaries of the three areas of diversity (from South Africa) mentioned above are not exactly the same as the distribution of the cpDNA groups because the distributional data has much wider geographical coverage.

Figure 3.21.B. shows a more detailed map of samples of the different groups from southern Africa. The above results viz. non-monophyly of species, the correlation of the five clades to geographic regions and the correlation of some of the clades to areas of diversity suggest that the results and data should be analysed from a phylogeographic perpective, rather than a phylogenetic one. These aspects are explored in the next chapter.


Fig. 3.21. A. Map showing distribution of cpDNA groups in Africa overlaid on the vegetation map of Africa (White, 1983), B. detail map of cpDNA groups in southern Africa (pink= Group 1, red= Group 2, blue= Group 4 and green= Group 5).

## Chapter 4: Phylogeography

### 4.1. Introduction

Phylogeography is the study of the principles and processes governing the geographical distributions of genealogical lineages, especially at the intra-specific level (Avise et al., 1987, 1998). However, this approach has been used at the inter-specific level especially when species show close relationships (Larena et al., 2002; Dobeś et al., 2004; Hughes et al., 2005). It is an integrative discipline in evolutionary biology usually requiring input from molecular genetics, population genetics, phylogenetics, demography and historical biogeography (Avise, 1998, 2000).

In recent years there has been an increase in the number of studies using phylogeograhical approaches in plants, with investigations concentrated on the floras of the Northern Hemisphere (Hewitt, 2001). Several studies have, however, been done in the American tropics (Schaal and Olsen, 2000; Collevatti et al., 2003; Lorenz-Lemke et al., 2005) and the Southern Hemisphere (McKinnon et al., 2004; Gardner et al., 2004). A few phylogeographic studies exist for the African flora (Médail et al., 2001; Hughes et al., 2005; Barker et al., 2005; Howis et al., in prep.).

Of the African studies, Médail et al. (2001) examined the phylogeography of subspecies of Olea europaea to gain clarity on the systematics, ecology and biogeography of Olea europaea subsp. maroccana. It was found that $O$. europaea subsp. maroccana is a well differentiated relictual taxon that possibily originated from an ancestral unit within Tropical Africa. The results also indicated that $O$. europaea subsp. maroccana is not an intermediate between $O$. europaea subsp. europaea and O. europaea subsp. laperrinei as previously suggested. Barker et al. (2005) examined the phylogeography of two asteraceous species, Chromolaena odorata (a invasive species in South Africa) and Chrysanthemoides monilifera (a natural species to South Africa)
and demonstrated that ITS sequence data can be a useful marker for phylogeographic studies. Chromolaena odorata showed little geographic or morphological correlation with genetic diversity, while considerable genetic structure was correlated to morphology and geography in Chrysanthemoides monilifera. Hughes et al. (2005) examined the phylogeography of Streptocarpus primulifolius and Streptocarpus rexii in relation to forest fragmentation in the eastern parts of South Africa. S. primulifolius was found to have high nDNA and cpDNA diversity which supported the hypothesis that this species is a coastal Pleistocene relict. S. rexii, which extends from high to low altitudes, has no or little nuclear diversity and most populations of $S$. rexii share a common chloroplast haplotype. Low genetic diversity and homogeneity indicated that $S$. rexii attained its current distribution during the Holocene. Hughes et al. (2005) considered this to be a consequence of forest habitat fragmentation.

A major difficulty in the phylogeographic study of plants is finding suitable genealogical markers. Most plant studies are phylogeographic in a broad sense, detecting patterns of genetic variation and geography and do not incorporate a genealogical perspective (Schaal and Olsen, 2000). Both nuclear and chloroplast markers have been used in plant phylogeographic studies. Nuclear ITS has proven useful in several studies (Barker et al., 2005; Hughes et al., 2005; Lorenz-Lemke et al., 2005). Schaal and Olsen (2000) have used the low copy gene G3pdh to study the phylogeography of Manihot esculenta and close relatives. Chloroplast markers have also proven to be useful (e.g. Noguchi et al., 2004; Dobeś et al., 2004; McKinnon et al., 2004; Gardner et al., 2004; DeChaine and Martin, 2005).

It is important to mention some of the differences in approach between phylogenetics and phylogeography. Phylogeography is a broad field with many methodologies (Avise, 2000), and the comparisons will concentrate on network methods as this was used in the present study (below). Phylogenetic methodologies are usually designed to determine evolutionary relationships above the species level. Species in this context are regarded as products of reproductive isolation and population fission during which mutations combined with population
divergence leads to fixation of different alleles and eventually to non-overlapping gene pools. In a broad sense relationships are considered to be hierarchical (Posada and Crandall, 2001).

Phylogeographic approaches are targeted at the intra-specific level. Relationships are not considered hierarchical, as they are the result of sexual reproduction among individuals with a smaller number of recent mutations and frequent recombination. At the intra-specific level, the more traditional approaches used to infer inter-species relationship estimates (such as maximum likelihood and maximum parsimony) are not applicable as some of their underlying assumptions are violated. Evolutionary processes at the population level such as recombination and hybridisation of lineages generate reticulate relationships. Traditional phylogenetic methods are bifurcating, and make no allowance for such reticulations (Posada and Crandall, 2001). Additionally in natural populations haplotypes exist as sets of multiple identical copies. When one copy mutates it is unlikely that other copies of the ancestral haplotype mutates or that all copies of the ancestral haplotypes becomes extinct. This situation results in sampling of ancestral haplotypes persisting with descendants in a population characterised by multifurcations (Posada and Crandall, 2001).

Alternate approaches need to be followed to take into account the phenomena experienced at the population level or intra-specific level (Posada and Crandall, 2001). One solution is to apply network-based approaches. Networks can account for the processes acting at the species level and might be able to incorporate predictions from population genetics theory. Most network based approaches are distance methods with a common theme of minimising the distance (number of mutations) among haplotypes (Posada and Crandall, 2001). The network algorithm employed in this study is statistical parsimony as implemented in the program TCS (Clements et al., 2000). It estimates the maximum number of differences among the haplotypes as a result of single substitutions (i.e. those that are not multiple substitutions at a single site) with $95 \%$ statistical confidence. This number is called the parsimony limit. Haplotypes differing by one change are then connected, and then those differing by two and so on until all the haplotypes are
connected into a single network or when the parsimony limit is reached. This method emphasises what is shared among haplotypes that differ minimally rather than the differences among haplotypes and provides an empirical assessment of deviations from parsimony (Posada and Crandall, 2001).

Nested clade analyses (NCA) aim to assess the historic causes of geographic variation. In the NCA a haplotype tree is used to define a nested series of branches (clades). This allows for an evolutionary nested analysis of the spatial distribution of genetic variation. NCA can discriminate between phylogeographic associations due to recurrent but restricted gene flow versus historical events operating at the population level (Templeton, 1998). Nesting starts with the tips of the network and moves one mutational step into the interior uniting all haplotypes that are connected by this prodecure into ' 1 -step clades' or first level clades. Thereafter these ' 1 -step clades' are pruned off. The prodecure is repeated on the more interior portions of the network if needed until all haplotypes are placed in ' 1 -step clades'. The next level (second level) uses 1 -step clades as base units rather than haplotypes applying the rules above to determine 2 -step clades. This process is repeated until the original network falls into a single category (Templeton, 1998).

Several phylogeographic studies have found the trnT-L spacer (used in this study) to be useful at the intra-specific level (Huang et al., 2002; Saltonstall, 2002; Honjo et al., 2004; Zhang et al., 2005). Huang et al. (2002) examined the phylogeography of Cyclobalanopsis glauca (Fagaceae) incorporating 32 populations with 140 samples. A combined analysis was used with the trnT-L spacer being the most variable and informative. Huang et al. (2002) did not use TCS or phylogenies but concentrated on a genetic diversity approach.

Saltonstall (2002) studied Phragmites australis (Poaceae) world wide using 345 populations represented by 345 samples. A combined analysis was used with $\operatorname{trn} T-L$ spacer and rbcL-psaI sequence data. Not all the data for these markers were provided. Saltonstall (2002) used TCS to determine haplotypes but no NCA or phylogenies were presented. Twenty-seven haplotypes
were recovered with a single haplotype ( M ) currently being the most common in North America, Europe and Asia. It is closely related to haplotypes from Europe, Asia and Africa, and is also predicted to be the ancestral haplotype. Haplotpye M is the most common and widely distributed in North America but is not closely related to other North American haplotypes. Saltonstall (2002) results indicate a cryptic invasion is occurring in North American Phragmites australis by a non-native genotype (viz. haplotype M ), which is highly competitive with broad ecological tolerances. It has spread throughout North America displacing native types and has spread to regions not previously occupied by Phragmites australis.

Honjo et al. (2004) examined 66 populations ( $\mathrm{n}=275$ ) of Primula sieboldii (Primulaceae) using several markers (the $\operatorname{trn} T-L$ spacer, the $\operatorname{trn} L$ intron, the $\operatorname{trnL}-F$ spacer, the $\operatorname{trn} D-T$ spacer and the trnH-psbA spacer). A combined analysis was presented and not all the data for these markers were given. Honjo et al. (2004) used TCS to determine the cpDNA haplotypes ( $\mathrm{n}=22$ ), but a NCA was not done. In order to infer relationships among the haplotypes MP and NJ were used, with only the MP phylogeny presented. Most haplotypes were geographically confined but one was widely distributed throughout northern Japan, while several others were found in geographically distant regions. Three major phylogenetic clades were recovered and none of the haplotypes recovered were placed in more than one clade. Clade I was distributed in Kyushu and central Honshu, Clade II in western Honshu and Hokkaido and Clade III in central Honshu and Hokkaido.

Zhang et al. (2005) examined the phylogeography of Juniperus przewalskii (Cupressaceae) on the Qinghai-Tibetan Plateau. Twenty populations represented by 392 samples were studied using the $\operatorname{trn} T-L$ spacer, the $\operatorname{trn} L$ intron, the $\operatorname{trn} L-F$ spacer and the $t r n S-G$ region. A combined analysis was used with the $\operatorname{trnS}-G$ region being the most variable and informative. TCS was used to determine the haplotypes and a NCA was performed. Phylogenetic relationships among the haplotypes were determined using NJ, MP and Maximum-Likelihood, which resulted in the same topologies. Six haplotypes (A-F) were found which nested into three first level clades. Clade 1-1
contained haplotypes C, D and E, Clade 1-2 contained haplotypes E and B while Clade 1-3 contained only haplotype F . The nested clades corresponded to the main lineages recovered by the phylogenetic analyses and none of the haplotypes recovered were placed in more than one clade. The most widely distributed haplotype (A) was hypothesised not to be the ancestral haplotype. Zhang et al. (2005) findings indicate that the Qinghai-Tibetan Plateau was recolonised by J. przewalskii during the most recent post-glacial period by a post-glacial range expansion from the edge of the Qinghai-Tibetan Plateau. This was followed by recent fragmentation which was proposed to explain the current distribution of cpDNA haplotypes.

Based on the results presented in Chapter 3 it was deemed necessary to explore the trn $T-L$ sequence data from a phylogeographic perspective rather than a strict phylogenetic context in an effort to understand and explain the geographical and phylogenetic patterns recovered. The ITS data was not analyses in this manner because of the low sequence divergence and recombination.

### 4.2. Materials and Methods

### 4.2.1. Data Sets

Two analyses were conducted. The first (Analysis I) was done on the entire trnT-L matrix with the following modifications: the out-groups (Bulbine and Bulbinella) and three Kniphofia sequences (SR 342 = K. uvaria, J. Pote $s n=$ K. linearifolia, TD $4559=$ K. rooperi), which had substantial regions of missing data, were excluded from the analysis. Messy ends were trimmed at the 3 ' end of the spacer. Once the out-groups and incomplete sequences were removed, the matrix was re-checked and redundant gaps were removed to minimise internal node haplotypes. This approach was done primarily to compare results of the phylogenetic and phylogeographic approaches. In the second analysis (Analysis II) only South African (SA) samples were included. The matrix was then trimmed and edited as described above. This data set was subjected to a
nested clade analysis. It is important to note that samples in the matrices analysed are not population samples but are treated in this study as such.

### 4.2.2. Nested Clade Analysis (NCA)

Haplotype networks were constructed using TCS version 1.13 (Clements et al., 2000). Gaps were treated as missing data. Haplotypes were nested into hierarchically interlocking groups i.e. nested clades for both data sets.

A quantitative analysis of geographical data (i.e. NCA), as described by Templeton (1998) was performed using GeoDis version 2 (Posada et al., 2000). GeoDis is a program that allows to test a null hypothesis of no association between geography and the inferred gene tree (Avise, 2000). Acceptance of the null hypothesis may be due to biological factors viz. high contemporary gene flow or recent historical association. The null hypothesis may also be accepted due to insufficient power of the test because of small samples sizes or poor sampling. When the null hypothesis is rejected the program can be used to gain insights on causes of associations between the phylogeny and geography (Avise, 2000). Statistically significant large and small $D_{c}$ values (a measure of the geographical range of a particular clade), $\mathrm{D}_{\mathrm{n}}$ values (the geographical range of a particular clade relative to its closest sister clades), (I-T) $\mathrm{Dc}_{\mathrm{c}}$ (the $\mathrm{D}_{\mathrm{c}}$ of the interior minus the tip clades) or (I-T) Dn ( $D_{n \text { interior }}-D_{n \text { tip }}$ ) were interpreted using the inference key (available from http://inbio.byu.edu/Faculty/kac/crandal_lab/geodis.htm; Templeton, 1998).

This analysis was only done for SA samples, as the large spatial separation of Tropical and East African samples and the lack of sampling over the entire distributional range were deemed unsuitable for further analysis. The output files and the GeoDis inference key were used to determine possible biogeographic scenarios.

The TCS and GeoDis software is used mainly for population level studies with typically more than one sample per population. Throughout this study only one sample per population was analysed as it was initially anticipated that species would cluster along morphological lines and not geographically. Thus the original sampling approach was to collect as many morphological species as possible and to cover geographic distribution for SA and strictly speaking the sampling above is not suitable for a NCA. However, it was pursued to test if there was any association between DNA sequence data and geography, and determine the nature of the association. These aspects were done in an effort to better understand the biogeography and evolutionary history of Kniphofia.

An approach using a single sample per population recovered a haplotype network but problems were encountered using GeoDis. Initial attempts which treated each sample (and locality) as a separate population failed to recover statistical values to use the GeoDis inference key. Populations were then grouped at the quarter dergree grid (QDG) scale i.e. samples within a given QDG were treated as a single population. This also did not recover statistically usable values. Samples were then grouped at the half degree grid (HDG) scale. Single samples in QDG that were the only representative for a HDG were grouped with adjacent and immediate HDGs. This was done to minimise the number of populations and group samples that would otherwise cluster if a strict HDG clustering approach was not implemented. In several cases single samples (populations) for a QDG were not immediately adjacent to a designated HDG and prevented incorporation. These were treated as independent ('lone') HDGs. A similar approach was used for full degree grids to explore and compare the outcome at a different spatial scale.

### 4.3. Results

### 4.3.1. Analysis I: southern, East and Tropical African samples

The matrix consisted of 122 sequences representing 50 Kniphofia species and was 545 bp in length. The network recovered (Fig. 4.1.) had a total of 56 haplotypes of which 15 were internal nodes (i.e. unsampled haplotypes).

The remaining 41 haplotypes are listed in Appendix 7. Twenty-three first level clades were recovered of which four were internal node clades i.e. clades with no representative samples. Ten second level clades and three third level clades were recovered (Fig. 4.1.). Nesting at the fourth level incorporated the entire network. The loops are no described in both analyses as these contained internal node clades. Additionally loops can be broken down depending on what haplotype is basal in equally parsimonious solutions.


Fig. 4.1. Haplotype network with nested clades based on the trnT-L spacer of Kniphofia. Colors represent groups obtained from the phylogenetic analyses (Chapter 3) (Green= Group 1, Blue= Group 2, Orange= Group 3, Red= Group 4 and Pink= Group 5; refer to text for details).
4.3.1.1. Comparison of haplotype network and the lineages recovered by phylogenetic approaches

It is useful to compare nested clades with the phylogenetic groups recovered as most studies find that there is a correspondence (e.g. Zhang et al., 2005). The bayesian inference tree which generally had good support for the five major groups (Chapter 3) is used below to compare nested clades and groups recovered in this study. The first level clades are too numerous to make any detail comparisons. All the first level nested clades (excluding internal node clades) were group specific (Fig. 4.1.) i.e. all first level clades contained samples from only one group as resolved by the phylogenetic methods (Chapter 3). All the second level nested clades were also group specific except Clade 2-10 which is composed of two first level clades, Clades 1-16 and 118 (Fig. 4.1.). Clade 1-16 has two samples from Group 4 while Clade 1-18 has one sample from Group 3 (SR 383= K. foliosa from Ethiopia).

The third level nested clades do not strictly reflect the same lineages recovered by the phylogenetic analyses (Fig. 4.2.). Clade 3-1 ( $\mathrm{n}=62$ ) is composed of five second level clades and has samples from all five lineages recovered in the phylogenetic analyses (Fig. 4.1., 4.2. and 4.3.). Clade 2-1 is composed of all samples from Group $1(\mathrm{n}=2)$. Clade 2-4 is composed of all samples from Group $2(\mathrm{n}=17)$. Clade $2-5$ is composed of all samples from Group $3(\mathrm{n}=16)$, except SR 383 ( $K$. foliosa) which is placed in Clade 3-2. Clade 2-8 is composed of all samples from Group $5(\mathrm{n}=18)$. Clade 2-7 is composed of some samples from Group $4(\mathrm{n}=9)$.

Clade 3-2 $(\mathrm{n}=48)$ is composed of three second level clades and has samples from two lineages recovered in the phylogenetic analyses (Fig. 4.1., 4.2. and 4.4.). Clade 2-2 ( $\mathrm{n}=35$ ) and Clade 2-6 $(\mathrm{n}=10)$ are composed of samples from Group 4. However, Clade 2-10 is composed of samples from two phylogenetically delimited groups, Group $4(\mathrm{n}=2)$ and Group $3(\mathrm{n}=1)$. Clade 3-3 ( $\mathrm{n}=$ 12) has two second level clades (Fig. 4.1., 4.2. and 4.5.) and contained only representatives from Group 4: Clade 2-3 $(\mathrm{n}=4)$ and Clade 2-9 $(\mathrm{n}=8)$.


Fig. 4.2. Third level clades plotted on bayesian tree of the $\operatorname{trn} T-L$ spacer. Major cpDNA groups are denoted by bars to the right and numbers below the group labels show indel positions and size (outgroups not shown). Clade $3-1=$ red, Clade $3-2=$ blue and Clade $3-3=$ green.


Fig. 4.3. Second level clades of Clade 3-1 plotted on bayesian tree of the trnT-L spacer. Major cpDNA groups are denoted by bars to the right and numbers below the group labels show indel positions and size (out-groups not shown). Clade 2-1= pink, Clade 2-4= red, Clade 2-5= brown, Clade 2-7= green and Clade $2-8=$ blue.
$\qquad$

Fig. 4.4. Second level clades of Clade 3-2 plotted on bayesian tree of the trnT-L spacer. Major cpDNA groups are denoted by bars to the right and numbers below the group labels show indel positions and size (out-groups not shown). Clade 2-2= blue, Clade 2-6= red and Clade 2-10= green.


Fig. 4.5. Second level clades of Clade 3-3 plotted on bayesian tree of the trnT-L spacer. Major cpDNA groups are denoted by bars to the right and number below the group labels show indel positions and size (out-groups not shown). Clade 2-3= blue and Clade 2-9= red.

### 4.3.2. Analysis II: NCA of South African samples

The second matrix (only SA samples) consisted of 104 sequences representing 44 Kniphofia species and was 539 bp in length. The network recovered (Fig. 4.6.) had 42 haplotypes were recovered of which nine were internal node haplotypes. The remaining 33 haplotypes are characterised in Appendix 8. Eighteen first level clades recovered of which three were internal node clades i.e. clades with no representative samples. Seven second level clades and three third level clades were recovered. Nesting at the forth level incorporated the entire network.

It is not possible to directly compare the nested clades with the lineages recovered using phylogenetic approaches as no phylogenetic reconstructions were done exclusively on SA samples.

For the GeoDis analysis a total of 39 HDGs were delimited. Details of samples grouped into HDGs are given in Appendix 9. A total of 25 full degree grids were delimited (Appendix 10). The GoeDis output files are given in Appendix 11 and 12 for the half and full degree grid analyses respectively. The results discussed here focused only on the third level nested clades as these allowed for meaningful comparisons (see above). The inference key results are presented below for the half and full degree grid analyses. Statistically significant large and small $\mathrm{D}_{\mathrm{c}}, \mathrm{D}_{\mathrm{n}}$, $(\mathrm{I}-\mathrm{T})_{\mathrm{Dc}}$ and (I-T) $\mathrm{Dn}_{\mathrm{Dn}}$ values from the GeoDis output file were used with the inference key in a stepwise manner to determine possible biogeographic scenarios. The numbers in the inference chain below represent the steps in the key.

Half degree grid inference chain:
Clade 3-1: $1 \rightarrow 19 \rightarrow \mathrm{NO}$ : Allopatric fragmentation
Clade 3-2: $1 \rightarrow 2 \rightarrow 11 \rightarrow 17 \rightarrow 4 \rightarrow \mathrm{NO}$ : Restricted gene flow with isolation by distance


Fig. 4.6. Haplotype network with nested clades based on the trnT-L spacer for South Africa samples of Kniphofia (Green= Group 1, Blue= Group 2, Red= Group 4 and Pink= Group 5; refer to text for details).

Clade 3-3: $\quad 1 \rightarrow 2 \rightarrow 3 \rightarrow 5 \rightarrow 6 \rightarrow 13 \rightarrow 14 \rightarrow$ YES: Sampling design inadequate to discriminate between contiguous range expansion, long distance colonisation and past fragmentation

Full-degree inference chain:
Clade 3-1: $1 \rightarrow 19 \rightarrow \mathrm{NO}$ : Allopatric fragmentation
Clade $\quad 3-2: \quad 1 \rightarrow 2 \rightarrow 11 \rightarrow$ YES (Range expansion) $\rightarrow 12 \rightarrow \mathrm{NO}:$ Contiguous range expansion

Clade 3-3: $1 \rightarrow 2 \rightarrow 3 \rightarrow 5 \rightarrow 6 \rightarrow 7 \rightarrow$ YES: Restricted gene flow/ Dispersal with some long distance dispersal

### 4.4. Discussion

It must be emphasised that the results of the TCS and GeoDis analysis should not be over interpreted because of the sampling approaches (see Materials and Methods) and limited sampling done between SA and Ethiopia. Phylogeographical analyses are only meaningful if appropriate sampling is done across the entire distributional range. Small samples sizes or poor sampling may not recover an association between geography and the inferred gene tree (Avise, 2000). More sampling is required from the gaps between SA and Ethiopia to gain better insights. Moreover, not enough is known about how these methods are effected by phenomena such as the hybridisation and incomplete lineage sorting. Thus the interpretation of the results should be regarded as preliminary. Despite these limitations some interesting patterns were found.

Neighbor joining, maximum parsimony and bayesian inference recovered more or less similar groups (Chapter 3). The third level nested clades do not strictly reflect the same lineages recovered by the phylogenetic analyses (Chapter 3). The differences in the results may be due to the different methodological approaches. Phylogeographic approaches take into account the
phenomena experienced at the population level and are not considered hierarchical. Evolutionary processes at the population level such as recombination and hybridisation of lineages generate reticulate relationships (Posada and Crandall, 2001). Traditional phylogenetic methods are bifurcating, and make no allowance for such reticulations. In a broad sense relationships are considered to be hierarchical (Posada and Crandall, 2001). However, the nesting process in TCS (Clements et al., 2000) appears to be hierarchical.

Parsimony is a character based method using only synapomorphic characters to find the tree or set of trees that have a minimum number of steps. Bayesian inference is a model based method that takes into account priors and is also character based. Neighbor joining is a distance based method. It takes into account gaps and missing data and is therefore sensitive to indels and missing data. In the TCS analysis employed, gaps were treated as missing data. It is interesting to note that the only nested clade which matches a phylogenetically delimited group characterised by an indel is Clade 2-8 which corresponds to Group 5. Group 4 which is also characterised by a six bp insertion in the phylogenetic approaches was not recovered as a single unit in the haplotype network irrespective of the level of nesting.

Setting the TCS software parameters to treat the gaps as $5^{\text {th }}$ state characters did not recover a network in both analyses. It is possible that treating gaps as $5^{\text {th }}$ state characters results in numerous sub-networks that do not reach the parsimony limit and are not able to connect and recover a usable network. The other possibility is that $5^{\text {th }}$ state characters resulted in too many haplotypes that were beyond the computational capacity of the program.

### 4.4.1. Distribution of nested clades from haplotype network (Analysis I)

In the anaylsis of the full data set all the third level clades are linked i.e. none are placed at terminal positions in the network. This suggests that the clades may have once formed a single
cohesive unit that has fragmented. This becomes more apparent when the samples from the third level nested clades are mapped (Fig. 4.7.-4.9.).


Fig. 4.7. Vegetation map of Africa (after White, 1983) showing distribution of third level nested clades (based on $\operatorname{trn} T-L$ spacer). Clade $3-1=$ red, Clade 3-2= blue and Clade 3-3= green.

Clade 3-1 is distributed from the Cape Region to the northern limits of southern Africa and the genetic signature is maintained at Afromontane disjuctions in Tropical and East Africa that were sampled in this study. The signature was also recovered for material from Madagascar (Fig. 4.7.). This suggested that the Cape Region and parts of Africa once formed a continuum in the past and this has been broken, with the haplotypes maintaining the genetic signature. All samples that were not southern African are from Afromontane regions. South African samples from Clade 3-1 are from a wide variety of habitats and altitudes (Fig. 4.8. and Fig. 4.9.A.). It is interesting to note that in the phylogenetic analyses (Chapter 3) a sample from Malawi also fell into the Cape clade (Group 2). This sample (K. grantii) was re-extracted and sequenced to rule
out the possibility of mistaken origin. Another sample from Malawi (K. splendida, Chapman 9061) fell into the Tropical Africa clade (Group 3). K. grantii (Group 2) is placed within Clade 2-4, while K. splendida (Chapman 9061) is placed within Clade 2-5. Both Clade 2-4 and Clade 2-5 are nested in Clade 3-1 (Fig. 4.1.). The Cape-Malawi link also suggested that this haplotype extends more northwards than this study has detected.


Fig. 4.8. A detailed map showing distribution of third level clades (based on trnT-L spacer) within South Africa. Clade 3-1= red, Clade 3-2= blue and Clade 3-3= green.

Numerous workers have noted the relationships between the Cape Region and the Afromontane Region (Levyns, 1964; Hedberg, 1965; Cowling, 1983a; Hilliard and Burtt, 1987; Linder, 1990; Linder, 2003, Galley and Linder, 2006). These links mostly concern Cape floral elements which are frequently found in most Afroalpine centres. Galley and Linder (2006) have suggested that Afromontane taxa with southern connections are derived in part from Cape clades either from one or several migrations northwards from the Cape Region. However, Kniphofia is not regarded as a Cape element and this study does not conclusively show that the Tropical and East African clade is derived from Cape clade (Chapter 3).


Fig. 4.9. Map showing distribution of second level clades that form units of third level nested clades (based on trnT-L spacer) for South African samples. A. Clade 3-1: Clade 2-1= pink, Clade $2-4=$ red, Clade $2-7=$ green and Clade $2-8=$ blue. B. Clade 3-2: Clade $2-2=$ blue, Clade $2-6=$ red and Clade 2-10= green. C. Clade 3-3: Clade 2-3= blue and Clade 2-9= red.

As mentioned above limited sampling was done in the Afromontane regions between SA and Ethiopia. Additional sampling is required for the gaps between SA and Ethiopia. It will be interesting to see the placement of additional material from regions not sampled, especially Tropical and East Africa.

In Clade 3-2, K. foliosa (SR 383) from Ethiopia grouped with material from SA (Fig. 4.7., 4.8., 4.9.B.). This also suggests that some SA material is more closely related to East African material rather than other SA samples. This genetic link between southern and East Africa may be more common but was not detected because of poor sampling. It also suggests that more than one genetic signature occurs between southern and East Africa. But more sampling is required to confirm this.

All samples in Clade 3-2 (except $K$. foliosa SR 383) and Clade 3-3 (Fig. 4.7., 4.8., 4.9.C.) are from the Afromontane Region (viz. Drakensberg), the adjacent Drakensberg-MaputolandPondoland transition and the Maputoland-Pondoland Region (within southern Africa). Samples occur from high altitudes in the Drakensberg to the coastal regions in habitats with a grassland affinity. It is generally assumed in phylogeographic studies that the most common haplotype is the also the most ancestral. Haplotype SR 300 ( $\mathrm{n}=27$; Clade 3-2) is the most common haplotype but does not appear to be the most ancestral as it is not centrally placed within the network (Fig. 4.1.). Samples of this haplotype are also not basally placed in the phylogenies, which would support this haplotype being ancestral. It seems plausible to hypothesise that this haplotype is recently derived and has managed to spread in a relatively short period of time. Most of the samples of haplotype SR 300 are from KwaZulu-Natal ( $\mathrm{n}=25$ ) covering a wide range of altitudes (coastal to high montane habitats). In southern Africa there ia a compensation of latitude for altitude. This compensation of latitude for altitude and the results above seem to indicate a range expansion for Kniphofia (see below).

It is also worth noting that $K$. typhoides, $K$. umbrina and $K$. brachystachya that were placed in the segregate genus Notosceptrum did not form a separate cluster in the haplotype network.

### 4.4.2. NCA of South African samples

The discussion below focuses only on the third level nested clades analysed at the full degree grid scale as this gave more meaningful results. This may be due to inadequacies in the half degree grid sampling approach, small samples sizes or poor sampling. At the full degree scale cpDNA haplotypes divided into threee major nested clades that showed some geographical patterns. The distribution of the three nested clades are mapped in Fig. 4.10. Biogeographic scenarios recovered by the GeoDis analysis at the full degree scale become more apparent when samples within the nested clades are mapped out Fig. 4.11.


Fig. 4.10. Map showing distribution of third level nested clades based on the trnT-L spacer for southern Africa representatives of Kniphofia (Orange= Clade 3-1; Blue= Clade 3-2 and Green= Clade 3-3).


Fig. 4.11. Map showing distribution of second level clades that form components of third level nested clades based on the trnT-L spacer for southern Africa representatives of Kniphofia. A. Clade $3-1=2-1$ (blue) $+2-7$ (red); B. Clade $3-2=2-2$ (blue) $+2-4$ (red); C. Clade $3-3=2-3$ (green) $+2-5$ (blue) $+2-6($ red $)$.

Clade 3-1 showed allopatric fragmentation. This suggested that the Cape Region and parts of KZN and Mpumalanga formed a continuum at some stage in the past and these have been broken with the haplotypes maintaining the genetic signature. This does not rule out the possibility that a continuum does exist, and the sampling of this study did not recover it. Most of the samples from the KwaZulu-Natal and Mpumalanga are from the Afromontane Region with the exception of a single sample (K. leucocephala from Richards Bay).

Clade 3-2, showed a range expansion. Samples from this clade are from the Afromontane (Drakensberg) Region, the adjacent Drakensberg-Maputoland-Pondoland transition and the Maputoland-Pondoland Region. Kniphofia has been shown to be a strong Afromontane grassland affinity north of southern Africa. However, in southern Africa, Kniphofia occurs from high altitudes to the coastal regions. The compensation of latitude for altitude may explain a range expansion. Kniphofia may have expanded ranges to lower altitudes especially in the eastern parts of SA in the recent past possibly the last glacial cycle. This range expansion may have been accompanied by a radiation which may account for the high diversity in the eastern part of southern Africa viz. the Afromontane (Drakensberg) Region, the Drakensberg-MaputolandPondoland transition and the Maputoland-Pondoland Region (Chapter 2; also discussed in detail later).

Clade 3-3, showed restricted gene flow and some long distance dispersal. The distribution of this clade extends along the Drakensberg Range with limited samples in the low-lying Drakensberg-Maputoland-Pondoland transition. This pattern is not clear and is unlikely to support the extensive hybridisation suggested previously (Chapter 1 and 3).

### 4.5. Conclusion

The findings presented here should be regarded as preliminary because of the limited sampling. Also the current programs routinely used in phylogeographical studies cater for a single species
or a small number of closely related species. A phylogeographic approach as advocated in this study may not be appropriate due to poor sampling, large spatial gaps between samples, the heterogeneity of morphological entities and the non-monophyly displayed by several species. Coalescent approaches may help in interpreting the data further. However, these were not attempted in this study because of the limiting nature of the data and the problems associated with finding suitable calibration points.

Despite these limitations some interesting patterns were detected. The comparitive study of the nested clades (recovered in the haplotype network of the entire trnT-L matrix) and the phylogenetic reconstruction approaches revealed that the nested clades did not strictly reflect the phylogenetically recovered lineages. Additionally Clade 3-1 appears to show fragmentation between Cape Region, northern parts of SA and the rest of Africa including Madagsacar. Clade 3-2 also indicates a SA-Ethiopia link. The other interesting pattern recovered was in Clades 3-2 and 3-3, which suggests a range expansion.

In the NCA of SA samples one of the nested clades, Clade 3-1, showed allopatric fragmentation between Cape Region and parts of KZN and Mpumalanga. A pattern that point to fragmentation was also detected in the comparitive analysis of the nest clades of the haplotype network and the phylogenetic lineages (above). The other interesting pattern recovered was in Clade 3-2, which points to a range expansion in the Afromontane (Drakensberg) Region, the adjacent Drakensberg-Maputoland-Pondoland transition and the Maputoland-Pondoland Region. Kniphofia may have expanded its range in the recent past possibly the last glacial cycle. This range expansion may have been accompanied by a radiation which may account for the high diversity in the eastern part of SA. The above findings provide valuable insights towards a better understanding of the biogeography and evolutionary history of Kniphofia.

## Chapter 5: Anatomy

### 5.1. Introduction

Anatomical evidence can play an important role in the elucidation of phylogenetic relationships (Ellis, 1983a, 1989; Linder, 2000). Leaf anatomy has been used widely in taxonomically difficult groups to solve problems of relationship and classification (Davis and Heywood, 1973; Ellis, 1974, 1982, 1983b, 1985a, 1985b, 1986a, 1986b, 1987, 1988). Light and scanning electron microscopy have proven to be useful tools for the study of leaf anatomy in Kniphofia (Baijnath, 1980) and other asphodelaceous genera such as Bulbine (Baijnath, 1977; Baijnath, 1992b; Baijnath and Cutler, 1993; Ramdhani, 2002), Aloe (Brandham and Cutler, 1978; Carter et al., 1984; Smith and van Wyk, 1992), Haworthia (Cutler, 1978; Smith et al., 1996), Poellnitzia (Smith and van Wyk, 1992) and Chortolirion (Smith and van Wyk, 1992; Smith et al., 1996).

The above studies have shown that leaf anatomy can be used in conjunction with other sources of evidence to understand relationships. Baijnath (1980) examined the leaf anatomy of 18 species of Kniphofia and two natural hybrids (K. citrina X K. uvaria and K. evansii X K. porphyrantha). It was found that hybrids inherit some leaf surface characters from both parents (Baijnath, 1980). Vascular bundles and crystals were of particular interest. Anatomical data did not support the segregate genus Notosceptrum (Baijnath, 1980). Baijnath's (1980) study also provided additional support for the creation of a separate species status for the V-shaped leaf form of K. northiae, which was later described as $K$. albomontana (Baijnath, 1987). However, juveniles of $K$. northiae have a V-shaped leaf (personal observation).

In the development and execution of this study the focus was to determine phylogenetic relationships using DNA sequence data. At the initial stages of this project it was suggested that leaf anatomical studies may also be useful in understanding relationships. Consequently, anatomical studies were initiated to find characters from both the leaf surface and transverse
sectional anatomy that will be informative in determining phylogenetic relationships. Initial attempts to analyse leaf surface characters phylogenetically was of limited use as there was intraspecific variation and specimens did not group into morphologically based species, a result also obtained for the cpDNA study (Ramdhani et al., 2006). It was decided to attempt a phenetic approach to establish if anatomical characters can define species-specific clusters. Anatomical studies were thus done to determine variation between populations of the same species as well as between species. Anatomical findings were then contextualised in terms of the phylogenetic and phylogeographic frameworks based on DNA sequence data and builds upon on the initial work by Baijnath (1980). A detailed descriptive anatomical account is not provided due to the limitations imposed by time and the confusing results.

### 5.2. Materials and Methods

### 5.2.1. Sampling

Most of the leaf samples used for anatomical studies were collected from the field. A list of samples used is provided in Table 5.1. Leaf portions were selected at a standard level, midway between the base and apex of mature leaves [following Baijnath (1980)]. Fresh material was fixed in FAA (Formalin-Acetic-Alcohol; 85 parts 70\% alcohol: 10 parts $40 \%$ formaldehyde: 5 parts acetic acid) for at least 24 hours. Leaves were also fixed in $50 \%$ ethanol depending on availability of fixative. In some instances herbarium material was used (see below).

Table 5.1. List of specimens used for anatomical studies. Locality and data pertaining to groups based on DNA sequence data from the trnT-L spacer are included. Additional details for collectors, localities, herbaria and additional abbreviations are given in Table 3.3. (Chapter 3).

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer <br> group | Leaf Surface: <br> SEM | Leaf TS: <br> LM |
| :--- | :--- | :--- | :--- | :---: | :---: |
| K. acraea | TD 4626 | Mountain Zebra National Park | 5 | X | X |
| K. albescens | SR \& JB 314 | Dirkiesdorp | 4 | X | X |
| K. angustifolia | SR 542 | Cathedral Peak Nature Reserve | 4 | X | - |
| K. angustifolia | SR 453 | Cathedral Peak Nature Reserve | 4 | X | X |
| K. ankaratrensis | PBP 5676 | Madagascar | 3 | X | - |
| K. baurii | SR 174 | Humansdorp | 4 | X | X |
| K. baurii | SR 275 | Elands Heights | 4 | X | - |
| K. baurii | SR 285 | Naudes Nek | 4 | X | X |
| K. baurii | SR 360 | Port Elizabeth | 4 | - | X |
| K. baurii | NPB 1923 | Alicedale | 2 | - | X |
| K. brachystachya | SR sn | Estcourt | X | X |  |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer group | Leaf Surface: SEM | Leaf TS: <br> LM |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. breviflora | SR 452 | Oliviershoek Pass | 4 | X | X |
| K. breviflora | SR $s n$ | Greytown | - | X | - |
| K. bruceae | SR \& NPB 171 | Komga | 4 | X | X |
| K. buchananii | SR \& BT 305 | Greytown | 4 | - | X |
| K. buchananii | SR \& BT 307 | Greytown | 4 | X | X |
| K. buchananii | SR 458 | Howick | 4 | - | X |
| K. caulescens | SR 270 | Elands Heights | 5 | X | X |
| K. caulescens | SR 278 | Naudes Nek | 5 | - | X |
| K. citrina | SR 176 | Humansdorp | 2 | X | X |
| K. coddiana | SR $s n$ | Umtamvuna Nature Reserve | 4 | X | X |
| K. coddiana | RAL 4820 | Mkambati | - | - | X |
| K. coralligemma | SR 549 | Iron Crown (Wolkberg) | 1 | X | X |
| K. drepanophylla | RJM 1100 | Mkambati | 4 | X | X |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer group | Leaf Surface: SEM | $\begin{array}{\|l} \hline \text { Leaf TS: } \\ \text { LM } \end{array}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. ensifolia subsp. ensifolia | JB $s n$ | Witbank | 4 | X | X |
| K. ensifolia subsp. autumnalis | SR 448 | Harrismith | 4 | X | X |
| K. fibrosa | SR \& AR 297 | Pervensey | 4 | - | X |
| K. fibrosa | PBP 5579 | Dohne Hill | 5 | X | X |
| K. fluviatilis | SR $s n$ | Verloren Vallei | - | X | X |
| K. foliosa | JMG 034 | Sebese Washi, Ethiopia | 3 | X | - |
| K. galpinii | SR 312 | Long Toms Pass, Lydenberg | 4 | X | X |
| K. gracilis | SR \& HB 321 | Durban | 4 | X | X |
| K. gracilis | SR 308 | Arhens | - | X | X |
| K. grantii | CP 4154 | Nyika Plateau, Malawi | 2 | X | - |
| K. hirsuta | SR 282 | Naudes Nek | 4 | X | X |
| K. ichopensis var. ichopensis | SR 242 | Nottingham Road | 4 | X | X |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L <br> spacer group | Leaf Surface: SEM | $\begin{aligned} & \text { Leaf TS: } \\ & \text { LM } \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. ichopensis var. ichopensis | SR 241 | Rosetta | - | - | X |
| K. ichopensis var. ichopensis | SR 409 | Michaelhouse | 4 | - | X |
| K. insignis | SR sn | Tatek, Ethiopia | 3 | X | - |
| K. insignis | Teklehymanot 30 (= TT30) (ETH) | Lege Shekole, Ethiopia | - | X | - |
| K. isoetifolia | JMG 033 | Bale Mountains, Ethiopia | - | X | - |
| K. latifolia | RSS sn | Greytown | 4 | X | X |
| K. laxiflora form B | SR 295 | Kamberg Nature Reserve | 4 | X | X |
| K. laxiflora form B | SR 253 | Himeville | - | X | X |
| K. laxiflora form B | SR 441 | Nottingham Road | 4 | - | X |
| K. laxiflora form B | SR 442 | Michaelhouse | 4 | - | X |
| K. laxiflora form B | SR 468 | Weza | 4 | - | X |
| K. laxiflora form C | SR $s n$ | Wakkerstroom | 4 | - | X |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer group | Leaf Surface: SEM | Leaf TS: <br> LM |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. sp. cf. laxiflora | SR 283 | Naudes Nek | 4 | - | X |
| K. leucocephala | NNBG | Richards Bay | 4 | X | X |
| K. linearifolia | SR 182 | Knysna | - | X | X |
| K. linearifolia | SR 151 | Michaelhouse | - | X | X |
| K. linearifolia | SR 170 | Komga | - | X | X |
| K. linearifolia | SR 269 | Hogsback (Seymour) | 5 | X | X |
| K. linearifolia | SR 287 | Loskop | 4 | X | X |
| K. linearifolia | SR 290 | Rosetta | 4 | X | X |
| K. linearifolia | SR 291 | Kamberg Nature Reserve | 4 | X | X |
| K. linearifolia | SR \& JB 311 | Lydenberg | 4 | X | X |
| K. linearifolia | SR 328 | Mt. Currie Nature Reserve | 4 | X | X |
| K. linearifolia | SR 343 | Hogsback (Seymour) | 5 | X | X |
| K. linearifolia | SR 400 | Mooi River | 4 | X | X |
| K. linearifolia | J Pote $s n$ | Stutterheim | 4 | X | X |
| K. littoralis | SR \& HB 200 | Silverglen Nature Reserve | 4 | X | X |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer group | Leaf Surface: SEM | Leaf TS: <br> LM |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. multiflora | SR \& JB 310 | Lydenberg | 4 | X | X |
| K. northiae | SR 263 | Hogsback (Seymour) | 5 | X | X |
| K. northiae | SR 274 | Naudes Nek | 5 | - | X |
| K. parviflora | SR 268 | Hogsback (Seymour) | 5 | X | X |
| K. parviflora | SR 330 | Mt. Currie Nature Reserve | 4 | - | X |
| K. pauciflora | HB $s n$ | Durban | 4 | X | X |
| K. porphyantha | SR $s n$ | Verloren Vallei | 4 | X | X |
| K. praecox | SR 529 | Jefferys Bay | 2 | X | X |
| K. praecox | TD 4461 | Katberg | - | X | X |
| K. pumila | Friss et al. 1079 (ETH) | Kebre Mengist, Ethiopia | - | X | - |
| K. rigidifolia | SR $s n$ | Lydenberg | 4 | X | X |
| K. ritualis | SR 300 | Pervensey | 4 | X | X |
| K. rooperi | SR 237 | East London | 5 | X | X |
| K. rooperi | SR sn | Cape Recife | - | X | - |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer group | Leaf Surface: SEM | Leaf TS: <br> LM |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. sarmentosa | SR 207 | Hex River Pass | 2 | X | X |
| K. schimperi | JMG 036 | Sebsebe Washe, Ethiopia | 3 | X | - |
| K. splendida | SR 548 | Haenertsberg | 1 | X | X |
| K. stricta | SR 279 | Rhodes | 4 | X | X |
| K. thodei | SR 407 | Kamberg Nature Reserve | 4 | X | X |
| K. thomsonii | JMG 031 | Senatti Plateau, Ethiopia | 3 | X | - |
| K. thomsonii | AAM 2647 | Mt. Elgon, Kenya | 3 | X | - |
| K. thomsonii | CK 4821 | Mt. Kilimanjaro, Tanzania | 3 | X | - |
| K. triangularis subsp. triangularis | SR 264 | Hogsback (Seymour) | 5 | X | X |
| K. triangularis subsp. triangularis | SR 266 | Hogsback (Seymour) | 5 | X | X |
| K. triangularis subsp. triangularis | SR 299 | Pervensey | 4 | X | X |
| K. triangularis subsp. triangularis | SR 267 | Hogsback (Seymour) | - | X | X |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer group | Leaf Surface: SEM | Leaf TS: <br> LM |
| :---: | :---: | :---: | :---: | :---: | :---: |
| K. triangularis subsp. triangularis | SR 304 | Hogsback (Seymour) | - | X | X |
| K. triangularis subsp. obtusiloba | SR $s n$ | Kemps Heights | 4 | X | X |
| K. typhoides | JB 8084 | Witbank | - | X | X |
| K. tysonii subsp. tysonii | SR 302 | Creighton | 4 | - | X |
| K. tysonii subsp. tysonii | SR 199 | Leisure Bay | - | X | X |
| K. tysonii subsp. tysonii | SR 303 | Highflats | 4 | X | X |
| K. tysonii subsp. tysonii | SR 460 | Balito | 4 | - | X |
| K. umbrina | R Gama $s n$ | Forbes Reef, Swaziland | 4 | X | X |
| K. uvaria | SR 165 | Glen Rosa | - | X |  |
| K. uvaria | SR 166 | Port Elizabeth | 2 | - | X |

Table 5.1. continued

| Taxon | Voucher (abbreviation) | Locality | trnT-L spacer <br> group | Leaf Surface: <br> SEM | Leaf TS: <br> LM |
| :--- | :--- | :--- | :--- | :--- | :--- |
| K. uvaria | SR \& NPB 172 | Post Wellington | 5 | X | X |
| K. uvaria | SR 186 | Kurlandsdorp | 2 | X | X |
| K. uvaria | SR 201 | Cape St. Francis | 2 | X | X |
| K. uvaria | SR 203 | Elim | 2 | X | X |
| K. uvaria | SR 211 | Clarkson | 2 | X | X |
| K. uvaria | SR 337 | Hogsback (Seymour) | 5 | X | X |
| K. uvaria | SR 342 | Hogsback (Seymour) | 5 | X | X |
| K. uvaria | SR 344 | Grahamstown | 4 | X | X |
| K. uvaria | SR 471 | Dimbaza | 5 | X | X |
| K. uvaria | SR 477 | Grahamstown | 2 | X | X |
| K. uvaria | TD 4477 | Port Elizabeth | 2 | X | X |

The anatomical studies concentrated on southern African material as this area is the most speciose and most of the field work was done in this area. Every attempt was made to use the same samples as those of the DNA studies for both leaf transverse sectional (TS) and scanning electron microscopy (SEM) studies to build complementary data sets. This approach allows for the detection of genetic and anatomical similarity. Due to time constraints it was not possible to process all of the DNA samples anatomically. Consequently three well sampled species ( $K$. linearifolia, $K$. uvaria and $K$. triangularis) which displayed non-monophyly based on the trnT-L spacer (Chapter 3) were chosen for extensive anatomical sampling.

Some herbarium samples from regions other than southern Africa were also included for scanning electron microscopy. Transverse sectional studies were not possible for these herbarium samples, as these did not re-hydrate satisfactorily.

### 5.2.2. Preparation of transverse sections for light microscopy (LM)

Samples were placed in $50 \%$ alcohol for 12 hours irrespective of prior fixation. Samples were then dehydrated through a series of tertiary butanol: $10 \%, 25 \%, 35 \%, 55 \%$ and $75 \%$ (12 hours each), and $100 \%$ (twice for 12 hours each), after which they were treated with a liquid parafin:tertiary butanol (1:1) mix and $100 \%$ liquid parafin (12 hours each). Samples were kept on a hot plate at c. $40^{\circ} \mathrm{C}$ from the $55 \%$ tertiary butanol to the $100 \%$ liquid parafin stage. Final infiltration was achieved with a liquid parafin:paraplast wax (1:1) mix (12 hours) and pure paraplast wax (thrice for 12 hours each). The liquid parafin:paraplast wax (1:1) mix and pure paraplast wax treatments were done in an oven at $60^{\circ} \mathrm{C}$.

Wax blocks were made with the samples embedded within. These blocks were trimmed and mounted for sectioning. Transverse sections were cut at $15 \mu \mathrm{~m}$ using a steel blade on a Minot rotary microtome (Leitz Wetzlar). Sections were collected and carefully placed into bath of
warm water and mounted onto a glass slide with Haupt's Adhesive and left in an oven to dry overnight. Sections were then conventionally stained with Safranin and Fast Green. Mounted sections were treated with xylol (twice for five minutes each) and a mixture of xylol:absolute ethanol (1:1) for three minutes. Thereafter slides were placed in a decreasing series of ethanol: $100 \%, 95 \%$ and $70 \%$ (three minutes each). Slides were left in Safranin for 12 hours to stain. Slides were placed in $70 \%$ and $90 \%$ ethanol (one minute each), dipped for five seconds in ammoniacal alcohol and placed in $100 \%$ ethanol (twice for two minutes each). Slides were stained with Fast Green for $10-15$ seconds, rinsed in clove oil for 30 seconds and dipped in mixture of clove oil, absolute ethanol and xylol for five seconds. Slides were then treated with xylol (thrice, two minutes each). Permanent slides were made using Canada Balsam mountant. Slides were labeled and dried in an oven at $50^{\circ} \mathrm{C}$ for five to seven days. Measurements for light microscopy (LM) were done with a graticule.

### 5.2.3. Preparation of leaf surface samples for SEM

Leaf portions were selected at standard levels (see above) and cut into manageable pieces. Samples were then dehydrated through a gradually increasing series of ethanol. Samples were finally dehydrated in dry alcohol (twice for 15 minutes each), and then critical point dried.

Leaves samples were mounted on brass stubs with conductive tape and sputter coated with gold. Gold coated samples were examined using a JOEL JSM840 Scanning Electron Microscope and photographed. To aid in interpretation, photographs were taken with the longitudinal axis of the leaf parallel to that of the screen. In an effort to maintain consistency and comparability leaves were photographed in the same region i.e. the central regions between the margin and keel in keeled samples. In triangular samples with no distinct keel, photographs were taken in the central region between one of the adaxial margins and the lower abaxial margin. In samples that were U shape in outline, photographs were taken in the central region between the margin and the medial axis of the leaf. Care was taken to photograph regions between veins for standardised
comparisons. Magnifications of SEM micrographs were recorded as a scale bar by the instrument.

Baijnath (1977), Baijnath and Cutler (1993) and Ramdhani (2002) found that the leaf surfaces were usually similar on both surfaces in Bulbine. However, Baijnath (1980) found that in Kniphofia this was the exception rather than the norm. It was therefore deemed necessary to examine both surfaces for variation. Differences in adaxial and abaxial surfaces increases the range of possible characters. Smith and van Wyk (1992) reported different infra-specific patterning on adaxial and abaxial surfaces especially in the Chortolirion and Aloe bowiea. Based of the above findings it is recommended that both leaf surfaces should be examined in Asphodelaceae.

### 5.2.4. Phenetic analysis

Anatomical characters (both TS and SEM) were selected and coded for phenetic analysis. The systematic anatomical work of Baijnath (1980) for Kniphofia was very detailed despite the small number of taxa examined, and his anatomical terminology well defined. There was no need to redefine the terminology used by Baijnath (1980), which is followed in this study to assist in interpretation and for standardised comparisons. A few additional comments are discussed below.

Characters pertaining to vascular bundles (VBs), especially medial VBs, were selected and coded from mature well developed bundles for standardised comparisons. Two characters that were apparent in the SEM micrographs were not included in the SEM data set: the position of the stomata on both the adaxial and abaxial surfaces, which were better interpreted from TS slides.

Sixty-five characters from leaf TS studies were selected and coded. The characters and coding scheme is given in Table 5.2., while the data matrix is given in Appendix 13.

Table 5.2. Transverse sectional (TS) leaf anatomical characters and characters states for Kniphofia.

|  | CHARACTER: character state |
| :---: | :---: |
| 1 | Leaf outline: $0=\mathrm{U}$-shaped, $1=\mathrm{V}$-shaped, $2=$ triangular |
| 2 | V-shaped leaf angle: $0=<90^{\circ}, 1=90^{\circ}, 2=>90^{\circ}$ |
| 3 | V-shaped leaf flange: $0=$ absent, $1=$ present |
| 4 | Mid-axial groove: $0=$ absent, $1=$ present |
| 5 | Adaxial stomata position: $0=$ superficially sunken, $1=$ sunken |
| 6 | Central mesophyll strip pronounced: $0=$ no, $1=$ yes |
| 7 | Vascular bundles distributed equi-distance from leaf surface: $0=$ no, $1=$ yes |
| 8 | Vascular bundles distributed between chlorenchyma \& central mesophyll: $0=$ no, $1=$ yes |
| 9 | Vascular bundles distributed in alternate pattern: $0=$ no, $1=$ yes |
| 10 | Keel vascular bundle pronounced: $0=$ no, $1=$ yes |
| 11 | Marginal vascular bundle pronounced: $0=$ no, $1=$ yes |
| 12 | Transverse bridging vascular bundles: $0=$ absent, $1=$ present |
| 13 | Mid-adaxial vascular bundle: $0=$ absent, $1=$ present |
| 14 | Keel vascular bundle xylem more or less with T-shaped outline: $0=$ no, $1=$ yes |
| 15 | Keel vascular bundle xylem with stem of T pointing inwards: $0=$ no, $1=$ yes |
| 16 | Marginal vascular bundle xylem more or less with T-shaped outline: $0=$ no, $1=$ yes |
| 17 | Marginal vascular bundle xylem with stem of T pointing inwards: $0=$ no, $1=$ yes |
| 18 | Medial vascular bundle xylem more or less with T-shaped outline: $0=$ no, $1=$ yes |
| 19 | Medial vascular bundle xylem with stem of T pointing inwards: $0=$ no, $1=$ yes |
| 20 | Keel vascular bundle phloem outline: $0=$ T-shaped, $1=$ triangular, $2=$ rectangular |
| 21 | Keel vascular bundle phloem with stem of T away from xylem: $0=$ no, $1=$ yes |
| 22 | Keel vascular bundle phloem with stem of T reduced: $0=$ no, $1=$ yes |
| 23 | Marginal vascular bundle phloem outline: $0=\mathrm{T}$-shaped, $1=$ triangular, $2=$ rectangular |
| 24 | Marginal vascular bundle phloem with stem of T away from xylem: $0=$ no, $1=$ yes |
| 25 | Marginal vascular bundle phloem with stem of T reduced: $0=$ no, $1=$ yes |
| 26 | Medial vascular bundle phloem outline: $0=\mathrm{T}$-shaped, $1=$ triangular, $2=$ rectangular |
| 27 | Medial vascular bundle phloem with stem of T away from xylem: $0=$ no, $1=$ yes |
| 28 | Medial vascular bundle phloem with stem of T reduced: $0=\mathrm{no}, 1=$ yes |
| 29 | Keel vascular bundle outer bundle sheath: $0=$ absent, $1=$ present |
| 30 | Keel vascular bundle outer bundle sheath composed of one layer of parenchyma cells: $0=$ no, $1=$ yes |
| 31 | Marginal vascular bundle outer bundle sheath: $0=$ absent, $1=$ present |
| 32 | Marginal vascular bundle outer bundle sheath composed of one layer of parenchyma cells: $0=$ no, $1=$ yes |
| 33 | Medial vascular bundle outer bundle sheath: $0=$ absent, $1=$ present |
| 34 | Medial vascular bundle outer bundle sheath composed of one layer of parenchyma cells: $0=\mathrm{no}, 1=$ yes |
| 35 | Keel vascular bundle inner bundle sheath: $0=$ absent, $1=$ present |
| 36 | Keel vascular bundle inner bundle sheath complete: $0=$ no, $1=$ yes |
| 37 | Keel vascular bundle inner bundle sheath with inner and outer (2) sclerenchyma caps: $0=$ no, $1=y$ yes |
| 38 | Keel vascular bundle inner bundle sheath with inner sclerenchyma caps having more cell layers than outer cap: $0=$ no, $1=$ yes |

Table 5.2. continued

|  | CHARACTER: character state |
| :--- | :--- |
| 39 | Marginal vascular bundle inner bundle sheath: $0=$ absent, $1=$ present |
| 40 | Marginal vascular bundle inner bundle sheath complete: $0=$ no, $1=$ yes |
| 41 | Marginal vascular bundle inner bundle sheath with inner and outer $(2)$ sclerenchyma caps: $0=$ no, $1=$ <br> yes |
| 42 | Marginal vascular bundle inner bundle sheath with inner sclerenchyma caps having more cell layers <br> than outer cap: $0=$ no, $1=$ yes |
| 43 | Medial vascular bundle inner bundle sheath: $0=$ absent, $1=$ present |
| 44 | Medial vascular bundle inner bundle sheath complete: $0=$ no, $1=$ yes |
| 45 | Medial vascular bundle inner bundle sheath with inner and outer $(2$ ) sclerenchyma caps: $0=$ no, $1=$ yes |
| 46 | Medial vascular bundle inner bundle sheath with inner sclerenchyma caps with more cell layers than <br> outer cap: $0=$ no, $1=$ yes |
| 47 | Keel vascular bundle with parenchyma cells at xylem pole thickened: $0=$ no, $1=$ yes |
| 48 | Marginal vascular bundle with parenchyma cells at xylem pole thickened: $0=$ no, $1=$ yes |
| 49 | Medial vascular bundle with parenchyma cells at xylem pole thickened: $0=$ no, $1=$ yes |
| 50 | Raphide crystals present in chlorenchyma: $0=$ absent, $1=$ present |
| 51 | Keel vascular bundle with tannin cells present at xylem poles: $0=$ no, $1=$ yes |
| 52 | Keel vascular bundle with tannin cells present at phloem poles: $0=$ no, $1=$ yes |
| 53 | Marginal vascular bundle with tannin cells present at xylem poles: $0=$ no, $1=$ yes |
| 54 | Marginal vascular bundle with tannin cells present at phloem poles: $0=$ no, $1=$ yes |
| 55 | Medial vascular bundle with tannin cells present at xylem poles: $0=$ no, $1=$ yes |
| 56 | Medial vascular bundle with tannin cells present at phloem poles: $0=$ no, $1=$ yes |
| 57 | Adaxial stomata position: $0=$ superficially sunken, $1=$ sunken |
| 58 | Epidermal cells of keel apex thickened: $0=$ no, $1=$ yes |
| 59 | Epidermal cells of margin apex thickened: $0=$ no, $1=$ yes |
| 60 | Keel vascular bundle with tannin cells pronounced: $0=$ no, $1=$ yes |
| 61 | Margin vascular bundle with tannin cells pronounced: $0=$ no, $1=$ yes |
| 62 | Epidermal cells above mid-adaxial vascular bundle thickened: $0=$ no, $1=$ yes |
| 63 | Leaf folded upwards in marginal region: $0=$ no, $1=$ yes |
| 64 | Two marginal bundles present: $0=$ no, 1 y yas |
| 65 | Abaxial medial vascular bundles usually larger than adaxial medial vascular bundles: $: 0=$ no, $1=$ yes |

Seventeen characters were selected and coded from leaf SEM micrographs for phenetic analysis.
The characters and coding scheme is given in Table 5.3., while the data matrix is given in Appendix 14. The individual data sets were combined which resulted in a total of 82 characters.

The TS and SEM data sets were analysed individually using NT-SYS version 2.0. (Rohlf, 1998). A similarity matrix was generated using the Simple Matching (SM) co-efficient and clustering was performed using the UPGMA (Unweighted Pair Group Method, Arithmetic Average)
clustering method. Third character states were analysed with the default setting of NT-SYS version 2.0. The SM co-efficient and UPGMA clustering was used as this is a basic approach to determine if further analysis is required and to gain insights into the data. A principle component analysis was done on data sets using NT-SYS. These did not show conflict with the tree plots (phenograms). Thus data were not analysed further by using variable co-efficients and different clustering methods due to the limiting nature of the data. Matching samples for both leaf TS and SEM data sets were used to construct a combined data set, which was analysed as above. The topology of phenograms (separate and combined analyses) were reconstructed using MacClade version 4.06 (Maddison and Maddison, 2000) to trace the distribution of characters.

Table 5.3. Scanning electron microscopy (SEM) leaf anatomical characters and characters states for Kniphofia.

|  | CHARACTER: character states |
| :--- | :--- |
| 1 | Leaf surface dimorphism: $0=$ no, $1=$ yes |
| 2 | Adaxial epidermal cell shape: $0=$ rectangular, $1=$ polygonal |
| 3 | Abaxial epidermal cell shape: $0=$ rectangular, $1=$ polygonal |
| 4 | Adaxial longitudinal striae: $0=$ absent, $1=$ p present |
| 5 | Adaxial transverse striae: $0=$ absent, $1=$ present |
| 6 | Abaxial longitudinal striae: $0=$ absent, $1=$ present |
| 7 | Abaxial transverse striae: $0=$ absent, $1=$ present |
| 8 | Adaxial papillae: $0=$ absent, $1=$ present |
| 9 | Abaxial papillae: $0=$ absent, $1=$ present |
| 10 | Adaxial stomatal rim: $0=$ absent, $1=$ present |
| 11 | Abaxial stomatal rim: $0=$ absent, $1=$ present |
| 12 | Adaxial trichomes: $0=$ absent, $1=$ present |
| 13 | Abaxial trichomes: $0=$ absent, $1=$ present |
| 14 | Adaxial wax: $0=$ absent, $1=$ sparse, $2=$ pronounced |
| 15 | Abaxial wax: $0=$ absent, $1=$ sparse, $2=$ pronounced |
| 16 | Adaxial papillae fused: $0=$ no, $1=$ yes |
| 17 | Abaxial papillae fused: $0=$ no, $1=$ yes |

### 5.3. Results

### 5.3.1. Sampling

A total of 94 leaf samples representing 43 species for Kniphofia from southern Africa were sectioned. Five South African (SA) species were not sectioned due to lack of material. These were K. albomontana, K. evansii, K. tabularis, K. flammula and K. crassifolia. The sample of $K$. albomontana (SR 149) used in the DNA studies was collected prior to the commencement of this study so anatomical material was not collected for this species at that time. It was also decided not to use herbarium material of K. evansii, K. tabularis, K. flammula and K. crassifolia for the SEM studies as they were not included in the genetic studies.

A total of 91 leaf samples were processed for SEM studies representing a total of 51 species of Kniphofia (43 species for Kniphofia from southern Africa and eight from other parts of Africa and Madagascar). Herbarium material was used for: K. ankaratrensis (PBP 5676), K. foliosa, (JMG034), K. grantii (CP 4154), K. insignis (TT 30, SR sn), K. isoetifolia (JMG 033), K. pumila (Friss et al. 1079), K. schimperi (JMG 036) and K. thomsonii (JMG 031, CK 4821, AMM 2647). Most of these specimens had DNA sequence data available except for $K$. insignis (TT 30), $K$. isoetifolia (JMG 033) and K. pumila (Friss et al. 1079).

It was possible to obtain both TS and SEM data for a total of 75 complementary samples representing 43 species from southern Africa. Sixty-four of these samples also had DNA sequence data for the trn $T-L$ spacer. Forty-one species (from SA) had complementary samples for the two anatomical and the cpDNA data sets. These samples represented all four of the trnT$L$ spacer lineages from southern Africa.

### 5.3.2. Characters

Several leaf anatomical characters (from both TS and SEM) warrant additional comments when compared with the study of Baijnath (1980). These may be an additional source of leaf anatomical characters.

### 5.3.2.1. Leaf TS anatomy

Leaf margin prickles were not considered in this study. Only a small number of sections were made per sample in this study. This meant that even when present the likelihood of detection was minimised. These were also not always apparent in the SEM study as the larger leaves had to be trimmed to fit mounting stubs. However, leaf surface macro-hairs were included.

Raphide crystals (Fig. 5.1.A.) were not given detailed attention here. Baijnath (1980) found that both fine and large raphides were present in the genus Kniphofia in contrast to other asphodelaceous genera and considered them to be of particular interest. These crystals are probably composed of calcium oxalate. Only large raphides were present in the related genus Bulbinella (Baijnath, 1980) while only fine raphides occur in the genus Bulbine (Baijnath, 1977; Baijnath and Cutler, 1993). Raphides occur as idioblasts in the chlorenchyma of Chortolirion, but in Poellnitzia and Aloe they are scattered in the chlorenchyma (Smith and van Wyk, 1992). The occurrence of different types of raphides may be more significant for delimitations at the generic level. In the present study crystals were fairly common in chlorenchyma as prismatic/rectangular and needle forms and were recorded as present or absent. The fine needle like crystals are probably raphides while the larger prismatic/rectangular forms are styloids (Prychid and Rudall, 1999). This requires further investigation as they may be useful systematic markers in Kniphofia and/or other related genera. No crystals were observed in the outer bundle sheath as reported by Baijnath (1980) and consequently these were not considered in this study.

Furthermore only a small number of sections were made per sample, this meant that even when present the likelihood of detecting these crystals were minimised. However, raphides require further investigation (Baijnath, 1980).

The order of VBs were not examined in this study. However, for comparative purposes, characters of medial bundles were selected and coded from large, mature and well developed
bundles (Fig. 5.1.D. \& E.). Botha et al. (1982) noted that in grasses so-called bundle orders and sizes intergrade with intermediate forms existing. Little is known about the development and functioning of VBs of different sizes and this aspect that requires further investigation in Kniphofia. Transverse bridging vascular bundles are not always apparent. Only a small number of sections were made per sample, this meant that even when present the likelihood of detecting these were minimised. The outline of phloem poles in mature VBs were not observed as a strictly inverted 'T-shape' (Baijnath, 1980), but either as a triangular or rectangular in outline (Fig. 5.1.E.).


Fig. 5.1. Leaf transverse section of $K$. laxiflora (SR 295). A. raphide crystal (C). B. keel vascular bundle. C. marginal vascular bundle. D. medial vascular bundles. E. mature medial vascular bundle in detail (is= parts of inner sclerenchyma bundle sheath, os= outer parenchyma bundle sheath, $\mathrm{pc}=$ parenchyma cells, $\mathrm{ph}=$ phloem, $\mathrm{xy}=\mathrm{xylem}$ ) (all scale bars $=100 \mu \mathrm{~m}$ ).

### 5.3.2.2. Leaf SEM anatomy

Light microscopy studies on leaf surfaces were not done due to time limits. This was unfortunate as this method does have some merits as it allows for a more detailed examination of leaf surface structures and features less precisely inferred by SEM such as epidermal cell shape and outline. Also the sample size is much reduced as SEM images only cover a small area of the leaf surface depending on the magnification at which the image was captured. All images were taken at the lowest magnification of 300 X with only a limited number of cells in full view in some cases. Longitudinal and transverse anticlinal walls cannot be examined in detail from SEM micrographs and the stomata type and position are difficult to determine accurately from SEM imagery. The above mentioned details are more suitable to interpret if SEM is used in conjunction with LM (Fig. 5.2.A \& B).

Despite the limitations of SEM noted above it is an important and useful tool for inferring characters not visualised by LM. Some features may be too small to be detected by light microscopy or do not show up well in transmitted light. On the other hand there are other features more suited to examination and measurement by SEM (Arora et al., 1982) e.g. wax distribution and striations in Kniphofia. In Kniphofia wax crystals occur as distinct or irregular particles or flakes (Baijnath, 1980). Leaf surface wax (using SEM) was not examined in great detail in this study. Wax was coded as absent, sparse (Fig. 5.2.C.) or pronounced (Fig. 5.2.D.). This is a problematic character as not all samples were fixed in the same manner. Samples were either fixed in FAA or $50 \%$ ethanol depending on availability of fixative. Ideally for such comparisons material should be preserved and processed in exactly the same manner to make standardised comparisons. Additionally differences in wax distribution and amount could be due to developmental difference (e.g. leaf age) or it could be affected by environmental factors. Wax features may be of diagnostic significance but this aspect will require further investigation.


Fig. 5.2. A. leaf transverse section of $K$. linearifolia (SR 287) showing adaxial sunken stomata. B. leaf transverse section of $K$. laxiflora (SR 295) showing abaxial superficially sunken stomata. C. SEM micrograph of adaxial leaf surface of K. linearifolia (SR 269) showing wax crystals. D. SEM micrograph of adaxial leaf surface of $K$. galpinii (SR 312) showing lack of wax crystals (all scale bars $=100 \mu \mathrm{~m})$.

The leaf surface permutations regarding striae and papillae mentioned by Baijnath (1980) were not considered. These permutations were not coded and analysed as they would result in numerous combinations that are difficult to code and interpret. It would also entail the replication of individual characters that would require re-coding depending on the combined nature of the permutation and may be a source of conflict. Instead individual characters that contribute to the permutations were coded separately.

### 5.3.3. Leaf TS phenetic analysis

The phenogram derived for the leaf TS data is shown in Fig. 5.3. The analysis and discussion below will concentrate on species with multiple samples, as these would be expected to cluster together in groups.

Sixteen species had multiple samples for the TS study. These where $K$. linearifolia ( $\mathrm{n}=12$ ), $K$. uvaria $(\mathrm{n}=12)$, K. triangularis $(\mathrm{n}=6)$, K. tysonii subsp. tysonii $(\mathrm{n}=3)$, K. laxiflora $(\mathrm{n}=7), K$. praecox $(\mathrm{n}=2)$, K. fibrosa $(\mathrm{n}=2)$, K. gracilis $(\mathrm{n}=2)$, K. ichopensis var. ichopensis $(\mathrm{n}=3)$, K. northiae ( $\mathrm{n}=2$ ), K. parviflora ( $\mathrm{n}=2$ ), K. buchananii $(\mathrm{n}=3)$, K. caulescens $(\mathrm{n}=2)$, K. baurii $(\mathrm{n}=$ 2), K. ensifolia $(\mathrm{n}=2)$ and $K$. coddiana $(\mathrm{n}=2)$. Details of these samples are given in Table 5.1.


Fig. 5.3. UPGMA phenogram based on leaf TS characters. Clusters of significance are shown numerical on the phenogram (discussed in text). Symbols indicate morphological species of Kniphofia.

The phenogram shows structure but anatomical characters failed to recover species-specific groups. Specimens of only two species, K. northiae [Cluster 1A (SR 263 and SR 274)] and $K$. caulescens [Cluster 2 (SR 270 and SR 278)] clustered (Fig. 5.3.). There was also no correspondence to the four lineages recovered by the $\operatorname{trn} T$ - $L$ spacer from southern Africa. Additionally the clusters did not show any geographic structure, except for $K$. northiae and $K$. caulescens (discussed later). Similarly, none of the nested clades (Chapter 4) correspond to the leaf TS based clusters irrespective of the level of nesting.

### 5.3.4. Leaf SEM phenetic analysis

The phenogram for the leaf SEM analysis is shown in Fig. 5.4. Thirteen species had multiple samples for this study. These where K. linearifolia ( $\mathrm{n}=12$ ), K. uvaria ( $\mathrm{n}=12$ ), K. triangularis $(\mathrm{n}=6)$, K. gracilis $(\mathrm{n}=2)$, K. tysonii subsp. tysonii $(\mathrm{n}=2)$, K. baurii $(\mathrm{n}=3)$, K. angustifolia $(\mathrm{n}=2)$, K. breviflora $(\mathrm{n}=2)$, K. laxiflora $(\mathrm{n}=2)$, K. ensifolia $(\mathrm{n}=2)$, K. rooperi $(\mathrm{n}=2)$, K. praecox $(\mathrm{n}=2)$ and K. thomsonii $(\mathrm{n}=3)$.

Structure was observed in the phenogram but no species-specific clusters were recovered. The major clusters based on the phenetic analysis of SEM characters do not correspond to the five lineages recovered by the $\operatorname{trn} T$ - $L$ spacer and did not show any geographic structure. Furthermore, none of the nested clades (Chapter 4) correspond to the leaf SEM based clusters irrespective of the level of nesting.

### 5.3.5. Combined leaf TS and SEM phenetic analysis

The phenogram for the combined analysis is shown in Fig. 5.5. Nine species had multiple samples for the combined analysis. These were K. linearifolia $(\mathrm{n}=12)$, K. uvaria $(\mathrm{n}=11)$, K. triangularis $(\mathrm{n}=6)$, K. tysonii subsp. tysonii $(\mathrm{n}=2)$, K. gracilis $(\mathrm{n}=2)$, K. praecox $(\mathrm{n}=2), K$. ensifolia $(\mathrm{n}=2)$, K. laxiflora $(\mathrm{n}=2)$ and $K$. baurii $(\mathrm{n}=2)$.


Fig. 5.4. UPGMA phenogram based on leaf SEM characters. Symbols indicate morphological species of Kniphofia.


Fig. 5.5. UPGMA phenogram based on combined leaf TS and SEM characters. Clusters are shown numerical on the phenogram (discussed in text). Symbols indicate morphological species of Kniphofia.

Once again there was much structure in the phenogram but no species-specific clusters were recovered. K. laxiflora was the only species where multiple samples clustered into a group (Fig. 5.5., Cluster 4A). It is also worth noting that five of the nine samples in Cluster 5 (Fig. 5.5.) are K. linearifolia.

The major clusters of the combined analysis did not correspond to the four southern African lineages recovered by the $\operatorname{trn} T$ - $L$ spacer and no geographic structure was apparent. Also none of the nested clades (Chapter 4) correspond to the leaf TS and SEM based clusters irrespective of the level of nesting.

### 5.3.6. Characters that define clusters

Despite the overall inability of the leaf anatomical characters used in this study to cluster morphological species, it is worth mentioning the characters that define major clusters and/or morphological species.

### 5.3.6.1. Leaf TS anatomy

No leaf TS anatomical characters exclusively defined the main clusters except the clusters designated as Cluster 1 and Cluster 2 (Fig. 5.3.). Cluster 2 [K. caulescens (SR 270 and SR 278)] was exclusively defined by a single character viz. the absence of the marginal vascular bundle inner bundle sheath (Fig. 5.6.A.).

Cluster 1 [K. stricta (SR 279) and K. northiae (SR 263 and SR 274)] was exclusively defined by one character, a U-shaped transectional outline (Fig. 5.6.B.). None of the leaf TS anatomical characters exclusively defined the K. northiae cluster (Fig. 5.3., Cluster 1A).


Fig. 5.6.A. leaf transverse section of $K$. caulescens (SR 270) showing the absence of the marginal vascular bundle inner bundle sheath. B. leaf transverse section of K. northiae (SR 263) in central region with no keel i.e. a U-shaped transectional outline. C. leaf transverse section of marginal vascular bundle of K. coralligemma (SR 549) with tannin cells present at the phloem pole. D. leaf transverse section of marginal vascular bundle of $K$. coralligemma (SR 549) with tannin cells present at the phloem and xylem pole (all scale bars $=100 \mu \mathrm{~m}$, except 5.5.B. scale bar $=1 \mathrm{~mm}$ ).

### 5.3.6.2. Leaf SEM anatomy

No leaf surface anatomical characters defined any of the main clusters.
5.3.6.3. Combined TS and SEM anatomy

No characters exclusively defined the main clusters designated as Cluster 5, Cluster 4 and Cluster 3 in the combined analysis (Fig. 5.5.). Cluster 2 was composed only of the single sample of K. coralligemma (SR 549) i.e. is not a strict cluster. It was exclusively defined by three characters: tannin cells present at the phloem pole of keel, margin and medial vascular bundles (Fig 5.6.C.). The importance of tannin cells at the phloem pole is uncertain. Baijnath (1980) found difficulty in interpreting the bundle sheaths in Kniphofia. An exact designation for larger parenchyma cells at the xylem poles is not clear. In Bulbine these cells are not filled with tannin as displayed by some species of Kniphofia (Baijnath, 1977; Baijnath and Cutler, 1993). Baijnath (1980) recorded that in Kniphofia tannins are found mostly in the parenchyma of vascular bundles towards the xylem poles and occasionally at the phloem poles (Fig 5.6.D.). He suggested that these tannin containing cells at the xylem pole might be a third bundle sheath or represent the inwards development of xylem parenchyma. He favored the latter explanation because phloem parenchyma cells between the tracheids and the conductive phloem also contain tannins and secondly the parenchyma cap cells towards the xylem poles in the bundles are heavily thickened and continuous with the inner sclerenchyma bundle sheath (Baijnath, 1980).

Cluster 1 (Fig. 5.5.) was composed of K. stricta (SR 279) and K. northiae (SR 263). It was exclusively defined by one character, a U-shaped transectional outline. The only taxa with more than one sample that clustered as a single species based on combined TS and SEM anatomy was K. laxiflora (Cluster 4A, $\mathrm{n}=2$ ), but no characters exclusively defined this grouping.

The results above indicate extensive anatomical variation within Kniphofia species. To further illustrate this, K. uvaria, which was well sampled is used below. Leaf surface SEM micrographs are shown as these are the easiest graphical means of depicting extensive variation (Fig. 5.7. \& Fig. 5.8.). Only the abaxial surfaces are shown, as Baijnath (1980) found that the abaxial surface in Kniphofia is much more useful than the adaxial surface (discussed below).


Fig. 5.7. SEM micrographs showing abaxial leaf surface variation in K. uvaria. A. SR 165. B. SR 172. C. SR 186. D. SR 201. E. SR 203. F. SR 211.


Fig. 5.8. SEM micrographs showing abaxial leaf surface variation in K. uvaria. A. SR 337. B. SR 342. C. SR 344. D. SR 471. E. SR 477. F. TD 4477.

The SEM micrographs show that the abaxial surfaces of K. uvaria represented by SR 337, SR 342 and SR 471 (Fig. 5.8.A., B. and D. respectively) compare favorably. SR 471 is from Dimbiza while SR 337 and SR 342 are from Hogsback (separate populations). Dimbiza and

Hogsback are in close geographic proximity and this may be reflected anatomically. Also, the abaxial surfaces of K. uvaria represented by SR 344 and TD 4477 (Fig. 5.8.C. and F. respectively) compare favorably. SR 344 is from Grahamstown while TD 4477 is from Port Elizabeth. Grahamstown and Port Elizabeth are in reasonably close geographical proximity and this may be reflected anatomically. The similarities discussed above are not reflected in clusters of the phenetic analysis (Fig. 5.4.). This is because adaxial characters, which are not considered above, were included in the phenetic analysis.

### 5.3.7. Comparison with Baijnath's (1980) results

Baijnath (1980) found that the abaxial surface in Kniphofia exhibits much more richness and complexity in ornamentation than the adaxial surface and emphasised the need for a routine examination of both surfaces. In this study it was found that in seven species (specimen sample size $=7$ i.e. one sample per species) the adaxial surface was more complex in ornamentation than the abaxial surface. In 26 species (specimen sample size=37) the adaxial surface and abaxial surface were more or less similar. In 25 species (specimen sample size $=47$ ) the abaxial surface exhibited more complexity. These results are difficult to consolidate as there is much variation in the leaf surface within a morphological species and several species are found in more than one of the three delimited categories above. However, when the results are compared on a sample basis, free of taxonomic structure, the abaxial surface is generally more complex.

Baijnath (1980) in most cases did not use multiple samples for most Kniphofia species examined. However, he did examine three accessions of K. linearifolia and K. uvaria, and reported intraspecific leaf surface patterning variation. Baijnath (1980) did not provide detailed descriptive anatomical treatments for the species he examined, but his results were presented as a generalised anatomical account. He did provide several SEM plates of leaf surfaces, mostly of abaxial surfaces that make comparisons possible.

Some of the samples (five) examined by Baijnath (1980) were from herbarium material. In reconstructing herbarium material, wall dehydration and shrinkage and subsequent hydration may introduce artifacts making comparisons difficult. Furthermore tilt angles differ which gives a different topological impressions of the leaf surface sculpturing. However, comparisons with Baijnath's (1980) results and the result for this study were possible. K. tysonii subsp. lebomboensis, K. albomontana, K. flammula and K. evansii could not be compared because of lack of material. Baijnath (1980) did not supply SEM micrographs of K. ensifolia subsp. ensifolia and K. parviflora, and the images of K. hirsuta are of low magnification and unsuitable for comparisons.

The following taxa below examined by Baijnath (1980) do not compare favorably with the results of this study for abaxial leaf surface anatomy (SEM). Comparisons and comments on locality data, when possible to trace, are also included.

1. K. northiae. Codd $s n$ [examined by Baijnath (1980)] has papillae absent, with no superficially sunken stomata. SR 263 (this study) has papillae present with superficially sunken stomata. K. northiae is a wide ranging species and the locality data for Codd sn could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy.
2. K. praecox. Codd 9965 [examined by Baijnath (1980)] has papillae present, longitudinal striae are absent and the stomata have a distinct rim. TD 4461 (this study) has papillae present, numerous longitudinal striae present and no stomatal rim. SR 529 (this study) has papillae present, longitudinal striae are present and no stomatal rim. K. praecox is a wide ranging species with a complex and confusing taxonomy (Chapter 1). Codd 9965 was collected in Pretoria and is presumed to be a garden escape as it collected out of its natural range i.e. the locality data is dubious. Thus it is not possible to comment on geographic proximity in relation to anatomy. However, TD 4461 and SR 529 (this study) do not compare favorably. TD 4461 originates from Port Elizabeth and SR 529 from Jefferys Bay.
3. K. sarmentosa. Oliver 4420 [examined by Baijnath (1980)] has papillae present and longitudinal striae are present. SR 207 (this study) has papillae present and longitudinal striae are absent. K. sarmentosa has a fairly restricted distribution in the Cape Region, however, the locality data for Oliver 4420 could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy.
4. K splendida. Admiraal 2227 [examined by Baijnath (1980)] has papillae present, longitudinal striae are present and the stomata have no distinct rim. SR 548 (this study) has papillae present, longitudinal striae are present and the stomata have a distinct rim. $K$ splendida is a wide ranging species and the locality data for Admiraal 2227 could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy.
5. K. tysonii subsp. tysonii. Codd 9364 [examined by Baijnath (1980)] has papillae present, longitudinal striae are present and the stomata are not superficially sunken. SR 303 (this study) has papillae absent, longitudinal striae are present and the stomata are not superficially sunken. SR 199 (this study) has papillae present, longitudinal striae are present and the stomata appear superficially sunken. K. tysonii subsp. tysonii is a wide ranging species. Codd 9364 was collected from Port Shepstone, SR 303 from Highflats and SR 199 from Leisure Bay. These regions are fairly close proximity, however, this is not reflected anatomically.
6. K. baurii. Codd 6797 [examined by Baijnath (1980)] has papillae present and longitudinal striae are present. SR 174 (this study) has indistinct papillae and longitudinal striae are absent. SR 275 and SR 285 (this study) have papillae present and longitudinal striae are present. SR 275 and SR 285 (this study) compare favorably but the micrograph for Codd 6797 [examined by Baijnath (1980)] covers a small area and is difficult to compare with SR 275 and SR 285 in detail. K. baurii is a wide ranging species. Codd 6797 was collected from Nqutu, SR 174 from Jefferys Bay, while SR 275 and SR 285 originate from the Naudes Nek region. SR 275 and SR 285 appear to reflect geographic proximity anatomically.
7. K. gracilis. Baijnath $s n$ [examined by Baijnath (1980)] has papillae present, no longitudinal striae observed and stomata with a distinct rim. SR 308 and SR 321 (this study) has papillae present, longitudinal striae are present and no distinct stomatal rim, but in SR 308 the papillae are more dense. K. gracilis is a fairly restricted species but the locality data for Baijnath $s n$ could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy. However, SR 308 and SR 321 are from Arhens and Durban respectively which are fairly close geographically but this is not reflected anatomically.
8. K. brachystachya. Killick and Vahrmeijer 3630 [examined by Baijnath (1980)] has papillae present, no longitudinal striae were observed and stomata have a rim-like structure (indistinct rim). K. brachystachya SR $s n$ (this study) has papillae present, no longitudinal striae and no distinct stomatal rim. K. brachystachya is a fairly restricted species. Killick and Vahrmeijer 3630 originates from Highmoor while $K$. brachystachya SR sn was collected near Escourt. These areas are fairly close geographically but this is not reflected anatomically.
9. K. citrina. Strey $s n$ [examined by Baijnath (1980)] has papillae present, longitudinal striae are absent and the stomata appear superficially sunken. SR 176 (this study) has papillae present which appear to be fused, longitudinal striae are absent and the stomata appear superficially sunken. K. citrina is a fairly restricted species but the locality data for Strey $s n$ could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy.

The following taxa below examined by Baijnath (1980) compare favorably with the leaf surface anatomy results of this study.

1. K. pauciflora. Samples were similar abaxially in both studies. The plants of both studies were obtained from the same population at the Clairwood Race Course (Durban), which is the only known living wild population.
2. K. linearifolia. The abaxial surface of Marias 1021 [examined by Baijnath (1980)] does not match any of the samples of this study. Codd $s n$ [examined by Baijnath (1980)] resembles SR 290 (this study) for the adaxial surface but not other $K$. linearifolia samples of this study. Furthermore, the abaxial surface of Codd sn [examined by Baijnath (1980)] does not resemble K. linearifolia samples used in this study. Locality data for Codd $s n$ could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy. Bruce 553 [examined by Baijnath (1980)]) has a similar adaxial surface to the adaxial surface of SR 269 and SR 287 (this study). Additionally the abaxial surface of Bruce 553 [examined by Baijnath (1980)] is similar to SR 269, SR 287 and SR 343 (all K. linearifolia, this study). Bruce 553 is from Keiskammahoek, SR 269 and SR 343 are from Hogsback while SR 287 is from Loskop (KwaZulu-Natal). Keiskammahoek and Hogsback are in close proximity which Bruce 553, SR 269 and SR 343 appears to reflect anatomically.
3. K. uvaria. SEM micrographs were not provided by Baijnath (1980) for Admiraal 1001 and Hanekom 2175. Bruce 232 [examined by Baijnath (1980)] is abaxially similar to SR 201 (this study). Bruce 232 originated from Atherstone (Albany District) while SR 201 was from Cape St Francis. These regions are in relatively close proximity which appears to be reflected anatomically.
4. K. porphyrantha. Baijnath $s n$ [examined by Baijnath (1980)] is abaxially similar to $K$. porphyrantha (SR sn, this study). K. porphyrantha is a wide ranging species and the locality data for Baijnath $s n$ could not be traced. Thus it is not possible to comment on geographic proximity in relation to anatomy.
5. K. ensifolia subsp. autumnalis. Van der Haasse sn [examined by Baijnath (1980)] compares well with SR 448 (this study) except that SR 448 has numerous wax crystals. K. ensifolia subsp. autumnalis is a restricted but locality data for Van der Haasse $s n$ could not be traced. It appears that the close proximity of these samples are reflected anatomically except for wax crystals.

### 5.4. Discussion

The comparative exercise with Baijnath's (1980) results and results of this study indicate that leaf surface anatomical variation within morphological defined species of Kniphofia is much greater than has been detected by either study.

Studies on several monocot groups in which multiple samples have been used, show that leaf anatomy is conservative at the species level. Botha et al. (1982) found that internal anatomy in Themeda triandra (Poaceae) from three different climatic zones were similar. Ellis (1983a) found that in Lintonia nutans (Poaceae) leaf anatomy was conservative. Baijnath (1988) reported that the leaf anatomy of Gloriosa superba, Sandersonia auranica and Littonia modesta (Colchicaceae) did not show intra-population differences. Newton (1972) examined the relief markings on the abaxial surface of some Aloe spp. from West Africa and found that clones of $A$. buettneri and A. macrocarpa var. major were similar in their patterns. In A. schweinfurthii a basic pattern could be discerned but there was some variation in leaf surface ornamentation. Whilst intra-specific variation is evident, general patterns were recognised with multiple sampling (Newton, 1972). Baijnath and Cutler (1993) found that in Bulbine, leaf surface characters were diagnostic mainly at the species level. Ramdhani (2002) demonstrated that in most taxa of the closely related broad-leaved Bulbine complex (Bulbine alooides, B. brunsvigiaefolia, B. latifolia and B. natalensis) little or no intra-specific anatomical variation was apparent using multiple samples.

However, some workers have found intra-specific leaf surface variation in asphodelaceous genera. Cutler (1978) showed that there are slight differences in leaf surface sculpturing in Haworthia reinwardtii var. chalumnensis. Triploids and tetraploid plants were distinguishable by means of leaf surface characters. These findings suggested that epidermal surface features were under genetic control. Cutler (1978) also found that glass-house grown plants of Haworthia
reinwardtii var. chalumnensis retained their epidermal features when compared to field collected material. Leaf epidermal patterns have been found to be under genetic control in the some members of the Alooideae and environmental factors have little influence (Cutler and Brandham, 1977; Cutler, 1978; Brandham and Cutler, 1978). Carter et al. (1984) used a multidisciplinary approach to revise the Aloe somaliensis complex [A. hemmingi, A. jucunda, A. peckii and A. somaliensis with two varieties (somaliensis and marmorata)]. A. jucunda displayed leaf surface variation. Anatomical and biochemical evidence showed no difference between A. somaliensis var. somaliensis and marmorata, and the latter was not upheld. The anatomical and biochemical differences between $A$. hemmingi and $A$. somaliensis were considered to be minor, resulting in $A$. hemmingi being reduced to a synonym of A. somaliensis. Smith and van Wyk (1992) reported intra-specific variation in the abaxial leaf surface of $A$. bowiea from different localities.

In this study it was found that in Kniphofia morphologically delimited species exhibited substantial anatomical variation. The phenetic study of anatomical characters for individual and combined data sets showed that anatomical characters do not cluster specimens based on the morphological species classification. Furthermore, the results for the most part do not fit any geographic pattern nor do they reflect the cpDNA groups recovered by the trnT-L spacer (Chapter 3) or the nested clades (Chapter 4).

Two possible factors may account for these results: environmental conditions and hybridisation.

### 5.4.1. Environmental conditions

Anatomical variation and plasticity may be due to environmental conditions. Newton (1972) and Carter et al. (1984) did not discuss the possibility of environmental influences on their results (above). Smith and van Wyk (1992) reported intra-specific variation in the abaxial leaf surface of A. bowiea from different localities, but noted that stomatal elevation (sunken vs superficial) is not a reliable indication of xeromorphy, habitat or climate. This species is restricted to the Coega
area (Eastern Cape) and the habitat and environmental factors for these populations are presumed to be very similar due to the restricted distribution range (Tony Dold, pers. comm.). Cutler (1978) showed that leaf surface sculpturing in Haworthia reinwardtii var. chalumnensis was under genetic control and environmental factors have little influence. This was also found to be the situation in the some members of the Alooideae (Cutler and Brandham, 1977; Cutler, 1978; Brandham and Cutler, 1978).

Baijnath (1980) did not discuss the reasons for intra-specific variation in K. linearifolia and $K$. uvaria. Many species of Kniphofia occur in marshy habitats. Baijnath (1980) found that $K$. gracilis and K. citrina from dry grasslands areas have deeply sunken stomata, while the other species he examined has superficially or slightly depressed stomata. According to Baijnath (1980) the depth of stomata may be a plastic character and could be influenced by habitat.

If environmental factors have a major influence on variation in the leaf anatomy of Kniphofia, it would be expected that samples (and species) in similar habitats and/or close proximity would experience similar environmental conditions and would cluster to some extent. It is reasonable to presume that such clustering would also show geographic or environmental patterns and structure. However, this was not observed in this study, apart from the few exceptions noted above when the results of this study was compared with that of Baijnath's (1980). Additionally a few more exceptions are noted below.

In the leaf TS study (Fig. 5.3.) only two clusters showed geographic and environmental structure: Cluster 3 [K. caulescens (SR 270 and SR 278)] and Cluster 4 [K. stricta (SR 279) and K. northiae (SR 263 and SR 274)]. Both the specimens of K. caulescens (SR 270 and SR 278) are from Naudes Nek and are exposed to similar environmental conditions in Afromontane grasslands. In Cluster 4, K. stricta (SR 279) and K. northiae (SR 274) were also from Naudes Nek, while K. northiae (SR 263) originated from Hogsback. These samples are exposed to similar environmental conditions of Afromontane grasslands.

In the leaf SEM study there was no geographic or environmental structure apparent. While in the combined analysis (Fig. 5.5.) K. stricta (SR 279, Naudes Nek) and K. northiae (SR 263, Hogsback) clustered together which may indicate the effect of environmental adaptation.

Despite the few possible exceptions, leaf anatomical variation does not seem to be influenced by geographical or environmental factors. Variation observed here may thus be under genetic control as reported for other members of the Asphodelaceae (e.g. Cutler and Brandham, 1977; Cutler, 1978; Brandham and Cutler, 1978).

### 5.4.2. Hybridisation

Hybridisation may result in intermediate morphology (Arora et al., 1982; Coetzee et al., 1994) although this is not always the case (Rieseberg, 1995). As already discussed (Chapter 3) in Kniphofia, hybridisation may explain the numerous cases of intermediate and intergrading morphology, and species complexes with extensive variation reported by Codd (1968, 2005) making certain species difficult to delimit morphologically. Incomplete or weak reproductive barriers in taxa of these complexes may promote hybridisation. Sympatry, shared pollinators and/or flowering phenology provides circumstantial evidence supporting hybridisation. Backcrossing could further complicate the issue.

In some studies, intermediate anatomy of leaf surfaces of both parents have been reported in hybrid progeny. These intermediate forms resulting from hybridisation are a potential source of variation. Hybrid material of Heracleum amntegazzianum (Apiaceae) and H. sphondylium had a leaf surface anatomy intermediate between both parents (Arora et al., 1982). Coetzee et al. (1994) also used anatomical data along with other evidence to identify a natural hybrid between Pelargonium tomentosum (Geraniaceae) and P. patulum var. patulum which was intermediate between the two putative parents.

Cutler and Brandham (1977) showed that leaf surface characters were under genetic control in bigeneric hybrids combinations of Aloe, Gasteria and Haworthia (Alooideae). Normally the hybrid plants showed intermediate leaf surface sculpturing between the parents for a number of features. Sometimes new character states were apparent. Genetic control of papillae was also reported (Cutler and Brandham, 1977). Cutler (1978) suggested that in Haworthia reinwardtii var. chalumnensis epidermal surface features were under genetic control (discussed above). Brandham and Cutler (1978) found that hybrids between Aloe rauhii and A. dawei contained either numerical or structural chromosome aberrations. A. rauhii is diploid $(2 n=14)$ while $A$. dawei is tetraploid $(2 n=28)$. Majority of the crosses were triploids but the chromosomes of some hybrids plants differed with either numerical or structural chromosome mutations. Some of these mutations were shown to have a direct effect on leaf surface characteristics, while others had different effects or none. The results suggested that leaf surface features were under genetic control.

Baijnath (1980) examined two natural hybrids viz. K. citrina X K. uvaria and K. evansii X K. porphyrantha. He found that natural hybrids inherited some leaf anatomical characters from both parents. It must be stressed that these were not controlled crosses. Comparisons made by Baijnath (1980) were not made with the exact populations of putative parental species but rather with different accessions of putative parental species. Furthermore, as these were not controlled crosses, Baijnath (1980) was uncertain if the putative hybrids were plants of the $\mathrm{F}_{1}$ generation or products of back-crossing. More detailed studies are required with controlled crossings. Thus, based on the variation found in this study, inferring anatomical patterns of inheritance is not recommended.

As noted above leaf epidermal patterns have been found to be under genetic control in some members of the closely related subfamily Alooideae and that environmental factors have little influence (Cutler and Brandham, 1977; Cutler, 1978; Brandham and Cutler, 1978). Similar
studies which include a cyto-nuclear component are required to show this in Kniphofia (Baijnath, 1980). It will also be interesting to examine population/s of representative taxa to assess interand intra-population variation.

The DNA sequence data results may reflect the same phenomena influencing anatomical variation, viz. hybridisation. Hybridisation has been invoked by taxonomists that have worked on the genus (Codd, 1968, 2005; Marais, 1973). Several species that are non-monophyletic in the DNA study have all been implicated in hybridisation events and hybridisation has been used to explain the extensive non-monophyly in Kniphofia (discussed in Chapter 3). Additionally several of the same species do not cluster in phenetic studies based on leaf anatomy.

Only two species with multiple samples (K. northiae and K. caulescens) clustered based on leaf TS anatomy. Of the remaining taxa (with multiple samples) in this study, K. linearifolia, $K$. uvaria, K. triangularis, K. tysonii, K. laxiflora, K. praecox, K. fibrosa, K. gracilis, K. ichopensis var. ichopensis, K. buchananii, K. baurii and K. ensifolia have all been implicated in hybridisation events (discussed in Chapter 1). It is also worth noting that two species with multiple samples, K. parviflora and $K$. coddiana, that did not cluster have no major taxonomic problems or history of hybridisation.

In the leaf SEM phenetic analysis K. linearifolia, K. uvaria. K. triangularis, K. gracilis, K. tysonii, K. baurii, K. angustifolia, K. ensifolia, K. breviflora, K. laxiflora, K. rooperi, K. praecox and K. thomsonii did not cluster based on morphological delimitations. All of the above species, with the exception of $K$. breviflora have been implicated in hybridisation events (discussed in Chapter 1). K. breviflora does have some taxonomic problems. It shares a close relationship with K. buchananii and K. albescens, and at times specimens of $K$. breviflora are difficult to separate from K. albescens (discussed in detail in Chapter 1). Both K. buchananii and K. albescens were included in this study, but these three taxa did not cluster together (Fig.5.4.) and thus there are no indications of close affinities based on leaf surface characters.

In the combined TS and SEM phenetic study K. linearifolia, K. uvaria, K. triangularis, K. tysonii subsp. tysonii, K. gracilis, K. praecox, K. ensifolia and K. baurii did not group into morphological units. K. laxiflora was the only species that clustered based on leaf TS and SEM anatomy. All of the above species have been implicated in hybridisation events (discussed in Chapter 1).

### 5.5. Conclusion

Studies which have used multiple complementary exemplars of species for both DNA and anatomical data could not be found and so it is difficult to draw parallels. This study has shown that in southern African Kniphofia species leaf anatomy is highly variable for morphologically delimited species and leaf anatomy does not recover species specific-groups. Linder (1986) noted that anatomical evidence from a small number of samples should be treated with caution. Smith and van Wyk (1992) cautioned that taxonomic changes on the basis of leaf anatomy alone should be based on a representative range of samples. The findings of this study supports the cautionary statements of Linder (1986) and Smith and van Wyk (1992).

The anatomical variation does not seem to be influenced by environmental or habitat factors. Hybridisation seems to be a more likely explanation for the substantial anatomical variation in southern African Kniphofia species. Baijnath (1980) noted the need for detailed investigations of the species complexes within Kniphofia based on the anatomical differences in species such as K. linearifolia and the morphological variation reported by Codd (1968, 2005). Such studies will require extensive sampling over the entire distribution range to assess variation. Also because of hybridisation it may not be possible to explain all the variation by examining isolated complexes but will require a much broader approach.

## Chapter 6: Discussion and Conclusion

### 6.1. Summary of Findings

The taxonomic literature on Kniphofia revealed that it has a complicated alpha taxonomy. There are numerous cases of intermediate and intergrading morphology, and species complexes with extensive variation.

Biogeographical and chorological analyses indicate that Kniphofia has six centres of diversity, five of these are regarded as centres of endemism. The South Africa Centre is the most species rich and also the largest centre of endemism. Kniphofia shows a strong Afromontane Grassland affinity in Tropical and East Africa but is occasionally found beyond the boundaries of the Afromontane vegetation. In South Africa it is found from high altitudes to coastal habitats but the most speciose regions for Kniphofia are Afromontane grasslands. It is thus not considered to be an Afromontane element, but rather an Afromontane associate.

Five major lineages were identified using cpDNA, four of which are southern African. The fifth lineage is represented by material from Madagascar, East and Tropical Africa. The nuclear marker failed to provide resolution as many sequences were identical. An intriguing result is that all of the species with multiple samples were resolved as non-monophyletic. This could be due to low sequence divergence, hybridisation and/or incomplete lineage sorting. The five lineages showed some congruence with geographic origin rather than the systematic arrangement based on morphology.

The phylogeographic study did not recover the same lineages as the phylogenetic analyses and should be regarded as preliminary. However, some interesting patterns were detected. In the NCA of SA samples one of the nested clades, Clade 3-1, showed allopatric fragmentation between Cape Region and parts of KZN and Mpumalanga. A pattern that points to fragmentation
was also detected in the comparative analysis of the nest clades of the haplotype network (SA, East and Tropical African material) and the phylogenetic lineages. The other interesting pattern recovered was in Clade 3-2, which points to a range expansion in the Afromontane (Drakensberg) Region, the adjacent Drakensberg-Maputoland-Pondoland transition and the Maputoland-Pondoland Region. Kniphofia may have expanded its range in the recent past possibly the last glacial cycle. This range expansion may have been accompanied by a radiation.

Morphologically delimited species of Kniphofia also exhibited substantial leaf anatomical variation. Phenetic analyses showed that anatomical characters do not cluster species when represented by multiple samples. Furthermore, the anatomical results do not fit any geographic pattern nor do they reflect the cpDNA groups recovered by the $t r n T-L$ spacer or the nested clades of the phylogeographic study. With notably few exceptions, it appears that leaf anatomical variation is not influenced by geographical or environmental factors. Hybridisation may, however, play a role.

The findings above are unusual when compared to most systematic studies which generally find good correspondence with alpha taxonomy and other lines of evidence. In the case of Kniphofia, neither DNA sequence data nor leaf anatomy reflect the alpha taxonomy. Thus, the explanation for the results requires detailed consideration. How can these result be interpreted? It is suggested that the key to understanding and explaining these results lies in the past. Tectonic and climate changes can be invoked as the cause of the biogeographic patterns observed in the molecular data.

### 6.2. Tectonic Events

Generation of a biogeographical hypothesis requires an understanding of the nature, amplitude and timing of palaeo-environmental changes (Cowling, 1983a). The five groups recovered by the trnT-L spacer phylogeny shows some geographic structure. This seems to indicates that
vicariance events fragmented Kniphofia into the five present lineages. The branch lengths and topology of the trnT-L spacer (cpDNA) phylogeny appear to indicate that the five groups have been isolated for a substantial amount of time and the events that resulted in these nodes are old. However, branch lengths, percentages of divergence and dating of such divergence are not available for the trnT-L spacer in the literature. Dating the phylogenies for Kniphofia is not possible as there is no fossil evidence for Kniphofia. Additionally, geological events cannot confidently be used to calibrate the phylogenies as the ages of the different Afromontane islands vary (White, 1983) and the phylogenies are not robust with some poorly supported nodes.

The interplay between landscape development and changing climates since the Cretaceous has had a major influence on the present soil and vegetation distribution in southern Africa. Planation of the African surface was complete by the end of the Cretaceous. After the planation, landscape development in southern Africa was characterised by fragmentation of habitats with successive pulses of uplift and dissection followed by successive cycles of erosion (Partridge, 1997). South Africa has experienced two major uplifts (Partridge and Maud, 1987). The first was at the end of the early Moicene (c. 18 MYA). The magnitude of the uplift was moderate (between 150-300 m) and resulted in a slight westward tilting of the African surface with limited monoclinal warping. The second uplift c. 2.5 MYA was major (up to 900 m ) in the eastern marginal areas. This asymmetrical uplift of the subcontinent resulted in major westward tilting of the interior land surfaces. There was also monoclinal warping along the southern and eastern coast margins (Partridge and Maud, 1987). Over the same period climate changed dramatically culminating in major aridification c. 2.8 MYA. This was followed by the recurrent glacialinterglacial cycles of the Pleistocene. It is postulated that this latter period has had the most effect on Kniphofia.

According to Axelrod and Raven (1978) there were two episodes of rapid speciation in South Africa. The first commenced in the Miocene with the uplift of Africa. The inter-montane valley basins favoured open savanna grasslands at the expense of forest. The open systems expanded
with aridity and many taxa originated in these areas (Axelrod and Raven, 1978). The massive post-Miocene diversification within a large number of lineages has produced a flock of closely related and ecologically uniform species and infra-specific taxa, which is unrivaled in the world (Cowling and Hilton-Taylor, 1997; Linder, 2005). The second burst of speciation resulted from Pliocene-Pleistocene deformation with accompanying fluctuation of climate. As the rim of southern Africa was elevated and the basins enveloped in the interior, the low areas became drier while the mountains wetter. Climate alternated between wetter and drier phases which shifted populations continuously (Axelrod and Raven, 1978). The tectonic events and/or the consequences of them may have fragmented an ancestral Kniphofia into the four southern Africa lineages and the Tropical and East Africa lineage, followed by a burst of speciation as suggested by Axelrod and Raven (1978).

The five lineages show evidence for fairly recent differentiation as the branch length within lineages are small and there is evidence for the non-monophyly of several species. The ITS results for Kniphofia show low sequence divergence which also supports a recent radiation (discussed in detail later) possibly due to more recent glacial-interglacial cycles.

### 6.3. Quaternary Climate Change

Apart from the Miocene and Pleistocene uplift events, Quaternary climate change had a major effect on the African vegetation and flora (Hedberg, 1970; Brenan, 1978; Goldblatt, 1978). Glacial cycles and climate changes have had a great influence on vegetation resulting in shifts in biome composition and boundaries. These shifts were due to fluctuations in temperature, precipitation and seasonal distribution of moisture (Scott et al., 1997).

The glacial-interglacial cycles are largely due to changes in solar oscillations i.e. Milankovitch cycles. These include eccentricity (variations of the Earth's orbit around the Sun), obliquity (variations in the Earth's tilt) and the precession of the equinoxes (Olago et al., 2000). These glacial-interglacial cycles have resulted in 100000 year ice age cycles which define Quaternary
climate change (Wunsch, 2004). These variations effect the Earth's surface temperature by altering latitudinal radiation reception with strong cyclic signals at periods of c. 95800 years (eccentricity), 41000 years (obliquity), and 23000 and 19000 years (precession). Solar radiation reception at low latitudes is mainly affected by variations in eccentricity and precession of the equinoxes, whereas higher latitudes are mainly affected by variations in obliquity (Olago et al., 2000). These orbital parameters are not sufficient to account for the strength of the observed climatic signal. Intrinsic feedback mechanisms amplify extrinsic forcing. These include factors such as the extent of polar sheets, $\mathrm{CO}_{2}$ concentration, changes in atmospheric circulation, displacement of wind belts and changes of moisture flux to continents (Olago et al., 2000). Wunsch (2004) argued that stochastic behavior should also not be discounted in driving glacialinterglacial cycles. Despite these factors, Milankovitch cycle driven climate change has attracted much attention and support in explaining evolutionary history and distribution of species (Dynesius and Jansson, 2000; Jansson and Dynesius, 2002). It is important to note that glacial periods are generally long with shorter interglacials i.e. cold conditions persists for a longer time (Fig. 6.1.).

Also evident are millennial-scale high amplitude flickers during the late Pleistocene termed Dangaard-Oeschger (D-O) cycle oscillations (Roy et al., 1996). These are sudden rapid warming events (by $6-10^{\circ} \mathrm{C}$ ) lasting a few decades followed by millennia of slow cooling periods to form a saw-tooth shaped time series. There is still no explanation for this occurrence (Rial, 2004). These cycles must have repeatedly de-stablised species interactions in communities. However, there is surprisingly little evidence for accelerated extinctions or speciation associated with extreme climate changes (for examples see Roy et al., 1996). The D-O cycles may have profound impacts on plants. Depending on the magnitude and nature of the climatic shifts, species that have not lived together previously may come into contact, conversely co-occurring species may become separated. These individual adjustments of species distributions are common in the Pleistocene record (Roy et al., 1996).


Fig. 6.1. An example of climate change across different time scales and proxy records. Note the extended cool periods and the more brief warm periods [taken from Rial (2004)].

Herbaceous plants will react more readily and have the potential migration speeds to track D-O scale events, but trees exhibit a lag to geographical response. Another paradox is that speciation and extinction rates fail to increase in the face of climatic instability. One interpretation is that species evolutionary stability is a consequence of environmental and ecological instability. As species track the shifting environment, populations break up and regroup thereby circumventing
long-term isolation and genetic differentiation (Roy et al., 1996). In a phylogeographic scenario this will be detected as a fragmentation followed by a coalescent genetic signal if the marker is sensitive enough.

### 6.3.1. Refugia

African biodiversity hotspots have arisen from the persistence of refugia throughout the Quaternary glacial cycles (McClean et al., 2005). The climatic fluctuations during the Quaternary were pronounced but not catastrophic in southern Africa. The persistence of refugia may have contributed to South Africa's species richness and endemism.

Areas of topodiversity are likely to be important climate change refugia (McClean et al., 2005). Mountain systems are frequently viewed as areas of refugia. Linder (1983) suggested that for Disinae, southern African (Cape and Drakensberg) habitats were not lost in the Pleistocene. Carbutt and Edwards (2001) regard the Drakensberg as a historic high-altitude refugium for Cape elements. According to Linder (1983), in East Africa most habitats were lost with the exception of the high Rift Valley mountains. When the climate ameliorated c. 10000 BP these refugia acted as source areas from where Disinae spread.

Other African mountains are also hypothesised to act as refugia (Linder, 1998). Lovett and Friis (1996) considered the Eastern Arc Mountains of Tanzania to be a refuge and have proposed that regions rich in restricted taxa have been climatically and geologically stable. Their explanation requires Pleistocene climatic change fluctuations to explain endemism. The East Usambara Mountains harbor many species which are separated from their closest relatives by wide intervals, suggesting that they have served as refugia for a formerly widespread flora which has become extinct over much of its former area (White, 1983).

The Afromontane vegetation that is now restricted to the isolated, temperate, moist, high altitude refugia ('sky islands') may have been much more widespread over the continent during cooler moist periods. Burgoyne et al. (2005) have suggested that the Afromontane phytochorion may be the largest assemblage of ancient persistent floristic elements in Africa with the grassland as a possible relictual type. Meadows and Linder (1993) have also suggested that Afromontane grasslands may represent 'relict' communities rather than forests in the southern Afromontane Region. Floristically, Afromontane forests and grasslands have nothing in common and do not appear to be interdependent. In some areas Afromontane Forest (with the same species component as in grasslands) occur successfully in a matrix that is floristically very different e.g. Fynbos (Burgoyne et al., 2005). Burgoyne et al. (2005) have suggested that the forests and grasslands are very distinct and unrelated phytochoria that coincidentally happen to occupy the same climate refuge.

It is proposed that the evolutionary and biogeographic history of Kniphofia is strongly linked to climatic cycles and vegetation changes. It seems reasonable to hypothesise that the ancestral area for Kniphofia was much more geographically widespread when high altitude Afromontane grasslands were more extensive during cooler and drier, glacial episodes. During cooler and drier conditions grasslands expanded in Tropical and East Africa and shifted to lower altitudes (Scott, 2002). Kniphofia on the high mountains of Tropical and East Africa would have tracked the Afromontane grasslands and expanded their ranges. This would suggest that Kniphofia had a widespread distribution that covered most of the current disjunctions for the genus.

During wetter and warmer interglacials periods it is proposed that Kniphofia retreated into refugia on the mountains of Tropical and East Africa. In South Africa where latitude compensates for altitude, Kniphofia may have maintained a distribution that extended into the lowlands even during interglacials.

### 6.3.2. Climate and vegetation changes on the African Mountains

The climatic and vegetation diversity of the African mountains renders the interpretation of the palaeo-record extremely difficult (White, 1981). Additionally there is no fossil record for Kniphofia. Since it is not possible to accurately trace cyclic climate change beyond 30000 years (van Zinderen Bakker, 1983), climate change during the Last Glacial Cycle (LGC) and its influence on the Afromontane vegetation is examined in order to gain insights that may assist in explaining the results obtained for Kniphofia. It must be noted that the present glacial-interglacial cycle is one of many that have preceded it, through which Kniphofia has survived. The current in situ diversity and distribution, and molecular data for Kniphofia are used here to demonstrate how a single cycle of climate change could influence the evolutionary and biogeograpical history of Kniphofia. This can be used to gain insights to how Kniphofia would have responded, survived and evolved through preceding cycles depending on the intensity and timing of climate change.

The climatic and vegetation changes for the three most speciose centres of diversity [South Africa, South-central Africa and Rift Valley (including the Ethiopia Subcentre)] are examined below. Each centre covers large spatial areas and it is expected that the climate and vegetation signals would at times be conflicting within a given centre. It is also difficult to untangle the effects of moisture, temperature and $\mathrm{CO}_{2}$ in certain areas. More palaeo-botanical research is required to refine the late Pleistocene and Holocene vegetation history of southern Africa (Scott et al., 1997) and other African regions. Consequently, only broad patterns are taken into account.

The discussion below concentrates on Afromontane vegetation of the Forest Belt because Kniphofia displays a strong Afromontane grassland affinity. The Afromontane Forest Belt is a dynamic mosaic of forest and grassland (Meadows and Linder, 1993). It is not possible to directly infer past distributions of Kniphofia because of the lack of fossil material. Thus in this
study the distribution of the Forest Belt during past prevailing conditions is used as a proxy to gain insights on the past distribution of Kniphofia.

### 6.3.3. The Rift Valley Centre

This region corresponds to the following regional mountain systems as defined by White (1978): the Ethiopian, the Imatongs-Usambara and part of the Kivu-Ruwenzori regional mountain systems. The Rift Valley Centre broadly corresponds mostly to Linder's (1983) East Africa Centre for Disinae. In this study the Ethiopia Subcentre of diversity is treated as a subunit of the Rift Valley Centre. It is also a subcentre of endemism (Chapter 2). Thus the Ethiopia Subcentre was examined in greater detail to determine if there are any features that may explain endemism. Fig. 6.2. summarises the discussion below.

At Lake Sacred (Kenya), Street-Perrot et al. (1997) have recorded dry montane forest (Podocarpus) between 24000 BP (years before present) and 34000 BP. Bonnefille and Chaliẻ (2000) have evidence for a well developed forest belt in the central East African Mountains for this time period. Before 30000 BP the cooling was consistent with the occurrence of montane conifer forest in the Burundi Highlands ( $>2200 \mathrm{~m}$ ) which is now occupied by tropical rainforest (Bonnefille et al., 1990). Bonnefille et al. (1990) have suggested a $4 \pm 2^{\circ} \mathrm{C}$ temperature decrease during 30 000-13 000 BP in several Rift Valley sites. The lowest temperature was recorded between 25 000-15 000 BP (Bonnefille et al., 1990).

According to Street-Perrot et al. (1997) the Last Glacial phase extended from 24 000-13 000 BP, with a decrease in temperature by $5-9^{\circ} \mathrm{C}$. The Last Glacial Maximum (LGM) is recorded at c. 18 200 BP (Street-Perrot et al., 1997). Bonnefille et al. (1990) have suggested that the optimal time for glacial advance in East Africa was c. 21500 BP when the climate was cold but moist.


Rift Valley Centre
Ethiopia Subcentre
Fig. 6.2. Summary of climatic and vegetation changes in the Rift Valley Centre and Ethiopia Subcentre of diversity for Kniphofia during the past 30000 years (details in text). Numbers in parentheses are reference sources which are listed ( $\mathbf{B P}$ on the time axis= 1000 years before present).

However, van Zinderen Bakker (1983) recorded the LGM at c. 18000 BP and noted that the climate in East Africa was dry with a $6^{\circ} \mathrm{C}$ lower temperature then present (van Zinderen Bakker, 1983). There was a decrease in evaporation (25-30\%), aridity increased and conditions were dry (van Zinderen Bakker, 1983). Jolly and Haxeltine (1997) found that there was a $6^{\circ} \mathrm{C}$ decrease in temperature, with $30 \%$ less precipitation (Kashivu, Burundi). Some areas received high rainfall. The area around Lake Kivu received 1364 mm of rainfall per year during the LGM suggesting that it may have served as a refuge for montane forests. During the glacial period 30 000-15 000 BP there was a general $32 \%$ decrease in rainfall relative to present values. In African equatorial regions 'precipitation decrease' is a more appropriate term than 'glacial aridity' (Bonnefille and Chaliẻ, 2000).

In Tropical Africa, displacement of vegetation belts, although difficult to evaluate, depends not only on cooling but also involves precipitation (Bonnefille et al., 1990). According to van Zinderen Bakker (1983), during the LGM the present vegetation belts shifted $1000-1100 \mathrm{~m}$ downwards. Jolly and Haxeltine (1997) noted that the tree line dropped by 1000 m during the LGM. Street-Perrot et al. (1997) found that the vegetation descended by $1000-1100 \mathrm{~m}$ between 24 000-13 000 BP.

The lowland forests being the most sensitive probably survived in limited areas. The three vegetation belts above the moist montane forest viz. the dry montane forests, the Ericaceous Belt and the Afroalpine Belt persisted at a much lower altitude during the hypothermal times. However, according to van Zinderen Bakker (1983) the Afroalpine Belt and the Afromontane Forest Belt could not make contact with the lowlands.

There was a general decrease in forest with open vegetation becoming more prevalent during the Last Glacial. This could have served as a migration route for plant and animals adapted to dry conditions (van Zinderen Bakker, 1983). Cool grassland and xerophytic shrubs increased at the expense of Podocarpus forest (Jolly and Haxeltine, 1997). During the Last Glacial (24 000-13

000 BP ) grassy heathlands composed of $\mathrm{C}_{4}$ grasses and ericaceous shrubs with limited trees have been recorded by Street-Perrot et al. (1997).

Bonnefille and Chalie (2000) found that during the Last Glacial between 16 000-18 000 BP there was a decrease in montane forest trees which were replaced by open-type vegetation in which there was a spread of Ericaceous Belt elements. The grassland was dominated by $\mathrm{C}_{4}$ grasses. Low $\mathrm{CO}_{2}$ levels promoted grassland development and retarded $\mathrm{C}_{3}$ tree cover (Bonnefille and Chaliẻ, 2000).

The glaciations on the East African mountains ended c. 15000 BP. Between 14 500-12 000 BP open arid savanna was recorded at sites presently occupied by lowland forests (van Zinderen Bakker, 1983). The gradual warming had a marked effect c. 12600 BP. Forest elements spread from refuges along the western Rift Valley in an eastern direction probably mostly by long distance dispersal (van Zinderen Bakker, 1983). Bonnefille and Chaliẻ (2000) found an increase in arboreal pollen at c. 13500 BP , and by c. 12000 BP the highland vegetation became more wooded.

At about 10500 BP the temperature on the mountains reached its present level (van Zinderen Bakker, 1983). Street-Perrot et al. (1997) noted that c. 10300 BP there was a dominance of $\mathrm{C}_{3}$ plants (moist montane forest) during the interglacial. Between $9000-8000 \mathrm{BP}$ maximum forest cover was attained, reaching an altitude of 1 820-2 240 m (Bonnefille and Chaliẻ, 2000).

During $6000-7000$ BP there was a decrease in tropical mountain forests with replacement by deciduous or semi-deciduous plants. In the last 6000 years there has been a progressive decrease in arboreal pollen with notable declines at c. 5800 BP and 3000 BP (Bonnefille and Chalié, 2000). At c. 4 000-4 600 BP the temperature on the mountains was higher than at present and forest expansion reached its maximum (van Zinderen Bakker, 1983). Around 3200 BP, Street-

Perrot et al. (1997) noted a dry forest vegetation (Podocapus and Olea) increase at Sacred Lake (Kenya).

During the LGM the abundance of $\mathrm{C}_{4}$ grasslands increased in areas that are currently wooded in equatorial Africa. As $\mathrm{CO}_{2}$ concentration decreased, it gave $\mathrm{C}_{4}$ vegetation the advantage to expand to ranges above the tree line (Scott, 2002). During the LGM, tropical areas that are currently clothed by montane forests, probably resembled subtropical regions (where grasses flourish today) but without the extreme seasonality of the subtropics. Although $\mathrm{C}_{4}$ expansion occurred in the tropics during the LGM, its spread was curbed in the southern direction by subtropical latitudes, regions of high altitude and especially regions with pronounced winter seasonality (Scott, 2002).
6.3.3.1. The Ethiopia Subcentre (summary presented in Fig. 6.2.)

This region corresponds to the Ethiopian regional mountain systems as defined by White (1978). Many studies done in Ethiopia for the LGC (Last Glacial Cycle) have concentrated on Rift Valley lake levels and lake sedimentation to reconstruct climatic changes because of the important hominoid sites in the area. Lake levels and evolution is complex. In additional to climatic factors, lake levels and evolution involves episodic eruptions, faulting, reactivation of faults and pyroclastic accumulation (Williams et al., 1981). These factors may mask climatic variables, so palaeo-data from lakes in the geologically unstable African Rift Valley should be treated with caution.

Barboni et al. (1999) examined phytoliths as palaeo-environmental indicators in the Middle Awash Valley. The Pleistocene was dominated high amounts of Poaceae morphotypes (75\%) and $13 \%$ of dicotyledon morphotypes. The results indicate open grassland with more trees and shrubs which developed under humid conditions or conditions more humid than present. Hurni (1981) found that in the Simien Mountains the temperature was $7^{\circ} \mathrm{C}$ lower than present during
the Last Glacial period which extended between $20000-12000 \mathrm{BP}$. This was a time of poor run off and rainfall. Hurni (1981) postulated that montane forests were at altitudes between 2 900-2 200 m . Egziabher (1986) noted that between $10000-14500$ BP there was an increase in moisture following a long arid phase.

Gillespie et al. (1983) examined lake level sequence in the Ziway-Shala basin during the past 14 000 years. Deglaciation of the mountains was completed before 11500 BP . The lake rose c. 12 000 BP but a major recession was recorded c. 10400 BP. Williams et al. (1981) recorded two major lake level transgressions at Lake Besaka viz. c. $11000-12000$ BP and c. 10000 BP. Gasse and Street (1978) found at least two distinct lacustral phases during the late Pleistocene in the lakes of the northern Rift Valley and Afar. This was followed by a very arid period during which the lakes examined recessed to their present levels or less (Gasse and Street, 1978).

The Holocene is characterised by low content in woody dicotyledons ( $<2 \%$ ) but high Poaceae phytoliths (93\%) (Barboni et al., 1999). Chloridoideae grasslands ( $\mathrm{C}_{4}$ affinity) resembling modern subdesertic shrub steppe and $\mathrm{C}_{3}$ Pooideae grasses would have covered the highlands (Barboni et al., 1999). The climatic shifts during the past 10000 years were considered by Egziabher (1986) to be small with minimal impact to the overall picture with regard to forests. Lamb (2001) found evidence for a savanna dominated vegetation in the Rift Valley (at Lake Tilo) throughout the Holocene despite evidence for strong variations in the moisture regime. The Holocene climate of this area has always been characterised by a wet and dry season (Lamb, 2001).

Gasse and Street (1978) recorded high lake levels c. 10000 BP and a regression between 6 000-4 000 BP. However, no discussion on how this affected vegetation was provided. At Ziway-Shala c. 9000 BP precipitation is estimated to have been at least $25 \%$ greater than today. Around 8500 BP the lake levels began to fall. The lowest level was recorded c. 7 800-7 200 BP (Gillespie et
al., 1983). Lamb (2001) recorded a higher number of trees and shrubs in the savanna c. 7000 BP at Lake Tilo. After 7000 BP there was a decrease in moisture (Lamb, 2001).

Around 5000 BP there was a major recession at Ziway-Shala. Since then dry conditions have prevailed (Gillespie et al., 1983). Grasses increased and the diversity of woody species declined c. 5500 BP. Lake Tilo began to desiccate c. 4500 BP (Lamb, 2001). However, the pollen record shows no marked vegetation response to this climate change. Lamb (2001) recorded distinct vegetation changes c. 2400 BP. Podocarpus, Juniperus and Hagenia increased on the uplands on either side of the Rift Valley. This was interpreted to be a response to a drier climate. Grasses also become more abundant and may reflect the climate changes at Lake Tilo (Lamb, 2001).

Mohammed and Bonnefille (1991) examined the vegetation and climate around Lake Langeno from c. $2500-800 \mathrm{BP}$. During $2500-2100 \mathrm{BP}$ there were humid conditions and high precipitation with maximum development of Podocarpus forests. The humid phase ended c. 2 $100 \pm 220$ BP with drier climatic conditions prevailing. Shoreline vegetation included Cyperaceae, halophytes (Chenopodiaceae and Amaranthaceae) as well as dry evergreen bushland (Olea-Euclea-Dodonaea viscosa). This phase ended c. $1060 \pm 200$ BP. Between 1 060-800 BP conditions became wetter which is indicated by an increase in Podocarpus and Juniperus vegetation. Afromontane forests have been at Wenchi for the last 800 years (Bonnefille and Buchet, 1986).

The palaeo-vegetation data for the Ethiopia Subcentre is rather fragmentary and incomplete. Many of the studies above do not include data on vegetation changes. Thus it is more useful to discuss the implications of past climate and vegetation changes in the broader context of the Rift Valley Centre (of which the Ethiopia Subcentre is a part) until more detailed studies are forthcoming for Ethiopia.

The review of climatic and vegetation changes for the Rift Valley Centre aids in interpreting the situation for Kniphofia during this time frame. Kniphofia on the high mountains of Tropical and East Africa would have tracked Afromontane grasslands and expanded their ranges during cooler and drier conditions. During the LGM in equatorial Africa $\mathrm{C}_{4}$ grasslands increased in abundance and distribution (Bonnefille and Chaliẻ, 2000; Scott, 2002) and shifted to lower altitudes due to the displacement of vegetation belts. This would suggest that Kniphofia had a widespread distribution that covered most of the present disjunctions. The warmer and wet conditions of the interglacial probably did not favor Afromontane grasslands (Meadows and Linder, 1993) and it is most likely that during interglacial times in Tropical and East African forests would have been favored at the expense of grasslands (van Zinderen Bakker, 1983). Afromontane grasslands (including Kniphofia) would have retracted to high altitudes as now seen on the highlands of Tropical and East Africa and would have become limited to high altitude refugia in Tropical and East Africa. This explains the current restricted distribution of Kniphofia to the Afromontane vegetation in these areas as isolated and disjunct populations.

### 6.3.4. The South-central Centre (summary presented in Fig. 6.3.)

This region corresponds to the Uluguru-Mulanje and part of the Kivu-Ruwenzori regional mountain systems as defined by White (1978) and broadly corresponds to Linder's (1983) Southcentral Centre for Disinae.

DeBusk (1998) examined the pollen record for Lake Malawi which spanned over the past 37000 years. The period 37 500-35 900 BP is represented by dry conditions with low lake levels and open vegetation (Hyphaene woodlands, lakeshore marshes and dry wooded grasslands). Between 35 900-34 000 BP montane forests were widespread with woodland reduction but still present indicating a cold moist climate. From 34 000-26 400 BP dry conditions that are slightly cooler than present are evident and forests decreased (DeBusk, 1998).


Fig. 6.3. Summary of climatic and vegetation changes in the South Africa and South-central Centre of diversity for Kniphofia during the past 30000 years (details in text). Numbers in parentheses are reference sources which are listed (BP on the time axis= 1000 years before present).

An increase in Poaceae is recorded with a fairly high percentage of woodland. Montane forests continued to exist in the catchment areas of the lake possibly at high altitudes. The prevailing conditions appear to be intermediate between modern and glacial periods (DeBusk, 1998). Livingstone (1971) examined the pollen record for Lake Young (Ishiba Nganda) in northern Zambia. Neither montane nor moist forest of low or intermediate altitudes appear to have extensive distributions in this part of Zambia during the last 22000 years. Grasses dominate the entire pollen spectra.

The end of the Pleistocene spanning the LGM is characterised by a high percentage of Afromontane elements accompanied by low percentages of woodland and grass taxa, and relatively high percentage of Cyperaceae and evergreen forests at Lake Malawi (DeBusk, 1998). There was an expansion of Afromontane Forest at the expense of woodlands.

Woodlands managed to maintain large coverage in the catchment of the lake. The expansion of the montane forest is accounted for by the depression of altitude belts with the cooling climate. The lowering of the montane forest's altitude by 800-1 000 m implies that most of the catchment of Lake Malawi was within reach of Afromontane Forest (DeBusk, 1998). However, DeBusk (1998) noted that this lowering was not enough to account for the very high percentage of montane forest taxa. It is likely that there were extensive areas of closed montane forests in the catchment of Lake Malawi.

The Holocene reflects a similar climate and vegetation to current conditions. There is evidence for Miombo woodland increase c. 9000 BP (Livingstone, 1971). At c. 3000 BP there was a further decline in the limited evergreen forests (Livingstone, 1971). There are indications for slightly drier conditions between 8 000-6 150 BP at Lake Malawi. Conditions are recorded as slightly wetter between 6 150-3 000 BP. Throughout the Holocene there is evidence for low percentages of forest elements but more extensive woodlands and grasslands (DeBusk, 1998).

Meadows (1984) examined the environmental changes on the Nyika Plateau during the last 12000 years. This period is characterised by a dominance of grasslands over forests. During
some periods the forests did expand but not more than $10 \%$ (present cover of forest is estimated at 5\%). Palaeontological evidence for the South-central centre fails to recover large scale vegetation fluctuations during the LGC and the vegetation on the Nyika Plateau has not altered much during the past 12000 years (Meadows, 1984). Much of the area currently occupied by grasslands on the Nyika Plateau could be above the tree line i.e. in areas that may be too cold and dry for extensive tree growth at present. This situation was probably intensified during the Last Glacial when conditions in most parts of Tropical Africa were cooler and drier than at present. Fire would also have played a role in the maintenance of grasslands (Meadows, 1984).

It is also interesting that the vegetation of the Inyanga Highlands (Zimbabwe Centre) is characterized by grassland from the early Holocene with Brachystegia woodland developing c. 4600 BP (Tomlinson, 1974).

Kniphofia in the South-central Centre is also associated with Afromontane grasslands. A similar situation as hypothesised for the Rift Valley may be applicable to the South-central Centre (discussed above). However, there is a surprising lack of grassland expansion and a notable forest expansion at Lake Malawi during the LGM. According to DeBusk (1998) the Late Pleistocene expansion of the forest patches would corroborate with recent fragmentation of the montane forests by grasslands without conflicting with evidence for the existence of montane grasslands throughout the Holocene (e.g. Meadows, 1984; Meadows and Linder, 1993).

There is a general lack of endemism in plants on the highlands of Malawi. The similarity among areas of montane vegetation in Malawi suggests that there was continuity between the now separate patches of montane vegetation in the recent past (DeBusk, 1998). This centre shows a chorological relationship with the South Africa and Zimbabwe Centres, but has a stronger relationship with the Rift Valley Centre for Kniphofia (Chapter 2). This centre may be associated with an overlap function to the north (Rift Valley Centre), south (Zimbabwe and South Africa Centres) and west (Cameroon Centre) (discussed later).

### 6.3.5. The South Africa Centre (summary presented in Fig. 6.3.)

The Drakensberg regional mountain system as defined by White (1978) and Linder's (1983) Cape and Natal-Transvaal Centres for Disinae fall within the South Africa Centre. In this study the South Africa Centre for Kniphofia was divided into three subcentres of diversity, of which only the Extended Drakensberg Subcentre is a subcentre of endemism. The Extended Drakensberg Subcentre or other areas of diversity recovered in Chapter 2 have not been treated independently in the discussion below as was done for the Ethiopia Subcentre. There is considerable intermingling in the southern Africa flora which partly diminishes the value of treating subcentres independently. The treatment of subcentres may have merits when examining southern Africa in isolation (Chapter 2). However, in the broader African context the South Africa Centre is best treated as a single unit for Kniphofia.

In southern Africa marked cycles of vegetation changes resulted in shifts in biome composition and boundaries in response to glacial cycles. These shifts were caused by fluctuations in temperature, precipitation and seasonal distribution of moisture (Scott et al., 1997). The effects of orbital changes prevailed for a much longer period causing numerous and regular cycles of change in vegetation through the Pleistocene and Tertiary. Both recurring glacials and interglacials must have influenced evolutionary processes in plant communities and contributed to defining the modern vegetation. Vegetation patterns during the LGC which are a reflection of these complex changes can therefore be expected to include a relatively wide diversity of community types (Scott et al., 1997). Climatic oscillations have also caused large scale migrations of vegetation types (as suggested by pollen data). It seems likely that at certain times when new conditions arose, new combinations of species were present (Scott, 1983).

Modern biomes were well established by the Quaternary (Scott et al., 1997). African grasslands have become adapted to Late Tertiary glacial cycles (Scott, 2002). Grasslands in South Africa were well established by the Miocene and have been present consistently throughout the last c. 300000 years in the interior of South Africa. Southern African grasslands are a transition between tropical and temperate (Afroalpine and Afromontane
affinity) grasslands and their compositions must have fluctuated markedly in the past (Scott, 2002). Only broad trends can be identified for the Late Pleistocene. Pollen data from c. 40 000-75 000 BP indicate that forests were more widespread under moist conditions (Scott et al., 1997). Highveld grass types (of tropical affinity) expanded regularly during the Quaternary at the expense of woody vegetation. Pleistocene pollen records for woodlands outside the present Grassland Biome suggests that grassy vegetation occupied a much greater area to the north during past cooler episodes (Scott et al., 1997).

In southern Africa between 30 000-26 000 BP the climate was most likely cool-temperate and the humidity did not vary much from present semi-arid to sub-humid conditions (Scott, 1982). Wonderkrater and Zoutpan show cool moist Podocarpus forest prior to 25000 BP. Before the onset of the LGM, forests near Wonderkrater were replaced by drier, slightly warmer savanna (Scott, 1990). At c. 25000 BP a drastic change occurred which resulted in the Karoo being replaced by grassland (affinity not specified). Higher precipitation and a decrease in temperature were responsible for the ecological change during this period (van Zinderen Bakker, 1978).

During the Last Glacial phase ( $25000-11000 \mathrm{BP}$ ) there was an estimated $5-6^{\circ} \mathrm{C}$ lowering of temperature. This coincides with relatively humid climates, except for the coldest time when it was dry. At Clarens, more Fynbos and swamps are recorded in the wet conditions c. 23 000 BP (Scott, 1990). This was followed by slightly drier times ( $22600-20000 \mathrm{BP}$ ), and a considerably drier and cooler climate c. 20 000-18 000 BP. From the end of the Pleistocene to 10000 BP precipitation fluctuated considerably reaching low levels c. 18000 BP during the LGM. Evaporation rates declined with cooler conditions (Scott et al., 1997). During 21 00018000 BP the southern coast of South Africa was significantly exposed with the shoreline extending c. 100 km further south of the present coast line (Burgoyne et al., 2005). Burgoyne et al. (2005) have suggested that grasslands (tropical affinity) along the south-eastern coast were more extensive. The remnants of these grasslands are now found in the sublittoral grasslands of the Maputoland-Pondoland Region, which are edaphically controlled and probably more extensive today because of frequent fire (Burgoyne et al., 2005).

During the LGM vegetation belts were lowered by c. 1000 m in altitude responding to $\mathrm{c} .5^{\circ} \mathrm{C}$ drop in temperature (Scott et al., 1997; Scott, 1983; Vogel, 1983; van Zinderen Bakker, 1983). In some regions this drop in temperature was as much as $10^{\circ} \mathrm{C}$ (van Zinderen Bakker, 1983; Lewis and Illgner, 2001). Cooler and moist conditions prevailed during this time, similar to the lower parts of the present Afroalpine Belt in Lesotho (van Zinderen Bakker, 1983). Upland grasslands (probably of temperate affinity) and shrublands were present in areas presently occupied by tropical savanna during the glacial phase. This phase was generally humid with a dry spell c. 19000 BP (Scott, 1990). Grasses (presumably of tropical affinity) become more prominent after 18000 BP when wetter conditions gradually returned (Scott, 1990). Climatic changes have generally influenced the long-term history of grasslands. Lower temperatures and less marked seasonal rainfall patterns allowed the downward spread of Afromontane Fynbos and grasses of $\mathrm{C}_{3}$ temperate affinity (Scott et al., 1997).

Mountain Fynbos was restricted to the upper reaches of the higher mountains during cooler and drier glacial conditions in the South-eastern Cape. The lowlands of the South-eastern Cape, which currently supports grassy-fynbos, would have been a mixture of Fynbos, karooid and $\mathrm{C}_{3}$ temperate grassland elements (Cowling, 1983a). Cooler and drier conditions would have promoted fire, favoring Afromontane grasslands rather than Afromontane forests (Meadows and Linder, 1993). Highveld grasslands of tropical affinity were replaced by high altitude grasslands of temperate affinity (Scott, 1982). There is general consensus that temperate grasslands were more extensive during the LGM (Scott et al., 1997) and have dominated most of the summer rainfall montane areas throughout the Holocene (Meadows and Linder, 1993).

During the glacial periods temperate grasslands of the interior were replaced by Alpine grasslands. The coastal plains of the Cape Region were devoid of forest and covered with grassland (not specified but presumably of temperate affinity) (van Zinderen-Bakker, 1978). Alpine vegetation was unable to survive at the highest altitudes. This vegetation only reached current altitudes c. 8000 BP during the Holocene warm up (van Zinderen-Bakker, 1978).

Temperature has ameliorated since c. 13 000-14 000 BP while precipitation increased (van Zinderen Bakker, 1983). Vegetation alternated markedly ranging from woodland savanna during the warm interglacial phases to cool open grasslands including Fynbos elements during the glacial maxima and to mesic woodland with Podocarpus forests during intermediate phases (Scott et al., 1997). Grasslands occupied roughly the same area during the Holocene as it does today with shifts in boundaries. During the climate optimum (i.e. the altithermal) in the middle Holocene c. 7000 BP, on the northern boundary of the main highveld region, bushveld spread southwards over the edge of the plateau (Scott and Vogel, 1983).

In the former Transvaal, tropical savanna elements increased since 11000 BP suggesting a gradual warming until c. 6500 BP . This phase started with moist climates (c. 11000 BP ), indicated by Olea and Podocarpus pollen which was replaced by relatively dry Kalahari thornveld types by c. 8000 BP . Broad leaved savanna elements including Combretaceae became prominent c. 6500 BP indicating wetter conditions (Scott, 1990). Montane forest elements appeared c. 8000 BP when the xeric elements declined and forests have been existing since this time in small patches, similar to the mosaic that exists in the region today (Ellery and Mentis, 1992). Rainfall declined markedly at the start of the Holocene, but by c. 7 000 BP the biomes began to reflect modern conditions although smaller fluctuations continued until recently (Scott et al., 1997).

Meadows and Meadows (1988) have shown that in the Winterberg there are notable signs of drier and cooler conditions with a more xeric element, which indicates a cool grassy-fynbos transitional to Fynbos during the late Pleistocene (c. 12500 BP to the early Holocene). Montane forests first appeared c. 8000 BP during the warmer early Holocene when conditions were probably warmer and moister than present. Forests were not extensive and did not extend beyond localised patches that are reflected in the region today (Meadows and Meadows, 1988). In the South-eastern Cape during the altithermal, Fynbos would have expanded into the lowlands. Subtropical $\mathrm{C}_{4}$ grasses and Afromontane elements would have penetrated into lowland communities on more fertile soils resulting in the present day grassyfynbos (Cowling, 1983a).

Eeley et al. (1999) suggested a significant forest expansion in KwaZulu-Natal during the last altithermal. Some studies in the Maputoland area show extensive Podocarpus forest after the LGM. At c. 5000 BP forests reached maximum extent and retreated from Maputoland in a northerly direction. The forests occupying the upper Mkuze River retreated towards the Drakensberg and the Natal mist belt (Mazus, 2000). Indications are that by the middle Holocene, Podocarpus spread in the interior and has never reached the same proportions as during the phases of the Late and Middle Pleistocene or even the LGM (Scott et al., 1997).

Sites from the former northern Transvaal and the Kalahari show dense woodland and relatively warm conditions by 6500 BP while more open slightly cooler vegetation developed sometime afterwards c. 4 000-2 000 BP. Deposits from Deelpan (Free State) indicate grassland with karooid elements after 4000 BP (Scott, 1990).

In general the past 6500 years started with relatively dense vegetation under warmer, moist conditions in most parts of South Africa. Later in the Holocene there was more open vegetation related to slight cooling and good moisture availability but possible minor dry spells (Scott, 1990). During the late Holocene c. 4 000-1 000 BP the grasslands were very well-developed in the transitional areas suggesting that the grassland boundaries must have been further west (Scott et al., 1997).

Prior to the LGC Kniphofia was probably confined to high altitude Afromontane grassland. During the LGM these grasslands (with Kniphofia) descended and expanded to the lowlands in southern Africa. This may partly explain the present distribution patterns observed for Kniphofia in southern Africa (discussed in Chapter 2). Consequently, the lowering of altitude thresholds has resulted in the wider distribution of Kniphofia in vegetation that is not strictly Afromontane, especially in the Drakensberg-Maputoland-Pondoland transition. As Kniphofia expanded its range into the lowlands of southern Africa, populations may have been trapped in refugial pockets of grassland which possibly promoted morphological divergence. There are 13 Kniphofia species that have a highly restricted grassland distribution. This may have
resulted in an array of recent morpho-species that are too young to have undergone complete lineage sorting and which can potentially still hybridise.

In the South-eastern Cape during the altithermal, Fynbos would have expanded to the lowlands with subtropical $\mathrm{C}_{4}$ grasses and Afromontane elements also penetrating into lowland communities (Cowling, 1983a). Kniphofia could have used these opportunities to spread into the Cape and Karoo regions. It seems likely that Kniphofia tracked the subtropical $\mathrm{C}_{4}$ grasses and Afromontane elements as they penetrated into the south-eastern Cape lowlands and tracked the $\mathrm{C}_{4}$ grasses as they moved further westwards deeper into the Fynbos and Karoo. However, the details of the range expansion without a major morphological divergence into the Cape Region is difficult to interpret but it is interesting to note that tropical $\mathrm{C}_{4}$ elements (Panicoideae and Chloridoideae) can to some extent successfully penetrate the Cape Region but also fail to speciate (Linder, 1989).

Radio isotope examination of 20 Kniphofia samples (representing 19 species) from all five cpDNA lineages by the Stable Light Isotope Unit (Archaeology Department, University of Cape Town) revealed that all samples had a $\mathrm{C}_{3}$ isotope signature. Southern Africa samples represented material collected from coastal to high altitude montane habitats. The samples from Madagascar, Tropical and East Africa were from high altitude montane habitats.

Plants with a $\mathrm{C}_{3}$ metabolism are advantaged in cool conditions, while plants with a $\mathrm{C}_{4}$ metabolism are advantaged in warm conditions (e.g. Cowling, 1983b). Thus, it seems reasonable to postulate that during glacial periods (temperate situations) the $\mathrm{C}_{3}$ condition is advantageous while the $\mathrm{C}_{4}$ condition is advantageous during interglacials (tropical conditions). However, in southern African the situation is complex as grasslands are a transition between tropical and temperate grasslands and their compositions must have fluctuated markedly in the past (Scott, 2002). Currently the most extensive vegetation of the Afromontane Forest Belt in southern Africa is Themeda triandra [a $\mathrm{C}_{4}$ species (Cowling, 1983b)] grassland (White, 1978). In South Africa patterns are obscured because 'temperate' grasses descend much lower and the most abundant tropical grass (Tremeda triandra) ascends relatively high (White, 1983) i.e. Afromontane grasslands in southern Africa are
composed of both temperate and tropical elements with $\mathrm{C}_{4}$ and $\mathrm{C}_{3}$ conditions. More research is required on the (present and past) composition, abundance, affinities and distribution of the temperate and tropical elements of Afromontane grasslands in southern Africa.

Despite these obscurities it appears that in southern Africa Kniphofia was able to compete with $\mathrm{C}_{4}$ grassland elements as they penetrated the Fynbos and Karoo during the Holocene. Kniphofia is also postulated to be able to compete with $\mathrm{C}_{4}$ grassland elements of tropical affinity in the lowlands of eastern South Africa under warm conditions as it expanded its range and radiated.

It is important to note that Afromontane grasslands as a unit may not have existed during the glacials in its present configuration. The LGM was not only colder but also drier with environments different from the present situation. More research is required on the rainfall, temperature and soil requirements for Kniphofia to understand how it survived glacial periods and how this may have effected its evolutionary history.

### 6.4. How can tectonics, climate cycles, genetic and geographic patterns explain the evolutionary history of Kniphofia?

Within the five lineages there is evidence for recent differentiation as the branch length are small and there is evidence for the non-monophyly of several species. Short branch lengths, non-monophyly, and numerous identical haplotypes (both cpDNA and ITS) collectively appear to indicate that a rapid radiation has taken place in southern Africa.

Climate change can create vacant and new niches for evolution of remaining species (Goldblatt, 1978). Knox and Palmer (1995) have reported a radiation for Dendrosenecio from the East African Mountains within the past few million years. D. meruensis is hypothesised to be derived within the past 200000 years (Knox and Palmer, 1995). No dating was provided for giant lobelias radiation/s by Knox and Palmer (1998) but the split of L. sancta/L. ritabeaniana from a L. lukwangulensis-like ancestor is speculated to be 7 MYA (million years ago). These dates are far older than the present study is suggesting for divergence and
morphological speciation in Kniphofia but could easily be the age of the ancestor of the genus as a whole.

The low sequence divergence of ITS has been reported for several taxa in cases where a recent rapid radiation has been implicated (Baldwin and Sanderson, 1998; Harris et al., 2000; Richardson et al., 2001; Malcomber, 2002; Klak et al., 2003b; Howarth and Baum, 2005). The ITS results for Kniphofia show low sequence divergence which supports a hypothesis of recent radiation. While it is not possible to date the Kniphofia radiation, it seems unlikely that all the results obtained for Kniphofia are the consequence of a single glacial cycle (i.e. the LGC), as this will represent an unprecedented fast rate of molecular and morphological evolution. However, the possibility of rapid concerted evolution of ITS sequences cannot be ruled out.

According to Jansson and Dynesius (2002) high orbital forced dynamics (ORD) caused by Milankovitch oscillations are manifested by the following traits: low $\beta$ clades (at least 100 000 years old) with generally high $\alpha$ clades (products of recent cladogenesis nested within $\beta$ clades), low level of spatial genetic divergence in $\beta$ clades, little geographic subdivision and large ranges, high vagility, low specialization, polyploidy and little $\beta$-anagenesis. Kniphofia appears to display most of these traits except that there is geographic structure in the main clades and there is no reported polyploidy in Kniphofia (Webber, 1932; de Wet, 1960; Nayak and Sen, 1992).

The phylogenetic studies of cpDNA showed evidence of non-monophyly for many species suggestive of a history of hybridisation and/or incomplete lineage sorting. If there has been gene flow and/or incomplete lineage sorting then conspecific individuals may be scattered in different lineages, but geographically localised genotypes should be shared between the different species (Whittemore and Schaal, 1991). This appears to be the situation in Kniphofia. Composition of the four southern African groups (and the Tropical and East African group) show more congruence with geographic origin rather than the taxonomic arrangement based on morphology. A recent radiation also suggests that not enough time has passed for currently delimited morphological species to differentiate fully and develop
reproductive barriers, thus promoting hybridisation and mixing of haplotypes. Hybridisation and incomplete lineage sorting may not be mutually exclusive and both these factors may explain the non-monophyly in geographically based cpDNA lineages. Hybridisation may have also promoted concerted evolution of ITS sequences. This may also account for the low sequence divergence of ITS. Thus, the results of this study may reflect the combinational effects of a recent radiation, hybridisation, concerted evolution and incomplete lineage sorting.

If cyclic climatic changes have influenced the evolutionary history of Kniphofia it implies that populations would have (at different phases of the climatic cycle) fragmented and possibly coalesced. Fragmentation and isolation can explain the high number of restricted grassland species in southern Africa.

### 6.4.1. Altitudinal Shifts

Climate changes in mountainous regions can account for altitudinal contractions and expansions resulting in species ascending and descending mountain systems (Knox and Palmer, 1995; Larena et al., 2002). Knox and Palmer (1995) examined diversification of Dendrosenecio in East Africa, which involved repeated altitudinal radiations with Pleistocene climate fluctuations playing a role in altitudinal vegetation distribution. Larena et al. (2002) demonstrated that Pleistocene glacial induced altitudinal migrations in Armeria may have resulted in gene flow and hybrid taxa. Knox and Palmer (1998) also found that in the genus Lobelia (viz. the Giant Lobelias) there is a combination of geographic and repeated altitudinal speciation with hybridisation potentially playing an important role. Knox and Palmer (1998) made no inferences on the influence of past climatic changes.

Altitudinal contractions and expansions result in isolation and secondary contact but also allows for intermediate transient situations. Alternation between long isolations and intermittent establishment in transient habitat would add new species to the same mountain slopes (Fjeldså and Lovett, 1997). A reticulate history may be associated with altitudinal distributional changes in southern Africa for Kniphofia. Repeated expansion and contractions
along altitudinal belts could result in the accumulation of a mixture of genetic signatures (haplotypes) as isolated populations in Kniphofia could have made secondary contact promoting gene flow and possible hybridisation. Altitudinal shifting of the vegetation belts associated with climatic changes in the Pleistocene may account for introgression events that have resulted in the non-monophyly of Kniphofia species.

Altitudinal shifting may have caused instability and disturbance as vegetation transgressed across altitudes. Grimshaw (1998) considered the climatic consequences of global glaciations as a disturbance in the Afromontane Region. The Afromontane vegetation (especially trees) are tolerant of the effects of altitude and regimes of repeated disturbance (Grimshaw, 1998). This implies that many elements of the Afromontane flora are well adapted to overcoming the effects of disturbance and may in fact use it to their advantage. Once these elements become established they may facilitate the introduction and colonisation of other less flexible ecological Afromontane elements.

Steep and complex landscapes are often used to explain gradient, parapatric speciation or microgeographic speciation (Fjeldså and Lovett, 1997). For example new species could be polyploids resulting from hybridisation between different species inhabiting different vegetation/biomes at ecotones or the result of past hybridisations with parents absent from present day floras (Fjeldså and Lovett, 1997). This is proposed to be the case for some African montane taxa. The species flock of Impatiens from the Uluguru Mountains indicate microgeographic as well as reticulate speciation. A similar species flock exists in Saintpaulia where ten of the 19 species inhabit the Usambara Mountains (Fjeldså and Lovett, 1997). This above scenario may also apply to Kniphofia in the Drakensberg.

### 6.4.2. Forest Encroachment

Grassland pockets surrounded by a matrix of forest in some regions may have been a minor contributing factor to fragmentation of grasslands and possibly populations of Kniphofia during the altithermal when forests were more widespread. Forests are a more recent vegetation than grasslands in eastern KwaZulu-Natal (West et al., 2000). Forests have been
existing in small patches during the past 8000 years, similar to the mosaic that exists in the region today (Ellery and Mentis, 1992; Meadows and Meadows, 1988). There is general consensus that forests did not reach the levels suggested by Eeley et al. (1999) and forests have been expanding and are more abundant now than they have been in the recent past (William Bond, pers. comm.). This negates to possibility of forests becoming extensive to a degree that resulted in isolation of grassland pockets. Thus forest encroachment resulting in long-term grassland isolation is unlikely to explain the patterns recovered for Kniphofia in southern Africa.

### 6.4.3. Wetlands Patches

Many Kniphofia species have a strong affinity to moist areas (marshy areas, swamps, vleis and wetlands). Meadows (1988) noted the importance of wetland sediments as tools for reconstruction of palaeo-environments in southern Africa. Rogers (1997) reviewed freshwater wetlands for southern Africa. Unfortunately, no data on palaeo-distributions in relation to climatic cycles were reported in either study. There is a paucity of knowledge of how wetland distribution changed in response to past climate change, but work in this field is currently being initiated (William Ellery, pers. comm.). It is an unfortunate paradox that wetland sediments have contributed much to our understanding of past vegetation in southern Africa but not on wetlands themselves.

Wetlands expand and shrink with variation in water supply, and many may disappear and reappear depending upon the hydrogeomorphic setting. Some wetlands will be more vulnerable to this pattern than others. Wetlands in headwater settings are likely to be vulnerable. These may become geographically isolated during drying, since their integration into the drainage networks are broken. Wetlands that are integrated into the middle and lower reaches of drainage networks are likely to expand and shrink, but unlikely to become insularised (William Ellery, pers. comm.).

According to Grimshaw (1998) swamps could have served as stepping stones for Afromontane vegetation and may explain the crossing of the lowlands gaps between East and

West Africa. During pluvial periods swamps may have been more extensive facilitating dispersal by reducing the distances involved (Grimshaw, 1998). Further work on wetland palaeo-distributions will determine the extent to which wetlands have contracted and expanded under prevailing climatic cycles. This will shed light on their role in isolating plant assemblages or constituent taxa and whether they would have allowed subsequent contacts.

### 6.5. Disjunctions and Migrations

The events responsible for the establishment of the Afromontane Region (and related disjunctions) are complex with a long history, spanning beyond the past 25000 years, and a single model is unlikely to account for the entire Afromontane diversity (White, 1981). Present distribution patterns do not provide all the data to untangle distributions for the Afromontane Region (Grimshaw, 1998). However, it does provide valuable clues and a foundation to build upon.

Disjunctions in the Afromontane flora have been referred to as vicariance events, indicating changes in the distribution patterns of the floras (Friis, 1983; Linder, 1998). Others have alluded to a dispersal explanation. The Drakensberg has been treated as a migration route for both southern (Cape) and tropical elements (van der Schijff and Schoonraad, 1971; Carbutt and Edwards, 2001). Dispersal may be facilitated by stepping stones during cooler periods when habitats on lower peaks were suitable for Afromontane species (Clayton, 1983).

The lowering of vegetation zones during the LGM could have allowed open vegetation to extend from South Africa to Zambia in the present Miombo woodland at 1400 m (Scott, 1983). Hedberg (1969) expressed reservations on the extent of direct migrations between mountains during the Pleistocene and favored independent long distance dispersal events to explain the disjunct Afroalpine floras. He agreed that a shift of vegetation belts must have occurred with direct migrations possible only for some of the forest species between some mountains and not for Afroalpine species. Grimshaw (1998) found that the distances for inter-mountain dispersal were not great considering that the widest gap separating individual islands in the Afromontane archipelago between the Imatong Mountains (Sudan) and

Mulanje (Malawi) is only 140 km . Grimshaw (1998) noted the importance of smaller peaks acting as stepping stones and facilitating dispersal. These would have been clothed with Afromontane vegetation viz. forest in wet periods in the recent past.

It is hypothesised that Kniphofia was more widespread when grasslands were more extensive (glacial times). During wetter and warmer periods (interglacials) Kniphofia retreated into refugia on the mountains of Tropical and East Africa. Vicariance and dispersal seem to accompany the contraction (interglacials) and expansion (glacial) of ranges that are more or less in synchrony with the cyclic climate changes. Under the 'pulse-turnover' hypothesis evolutionary change is expected to be synchronised with climatic change (Lahr and Foley, 2003). When climates change so does habitat distributions. Accordingly populations will either contract or disperse (expand distributions). This provides conditions for selection to act upon and may bring about evolutionary change or extinction (Lahr and Foley, 2003). This has been suggested as a phenomenon experienced by the entire flora of the Afromontane Region (Burgoyne et al., 2005). However, different elements of the flora would have had different responses depending on life history traits, climatic tolerances, vegetation preferences and associations.

According to Axelrod and Raven (1978) southern African genera that have extended their ranges northward along the mountains of East Africa include Kniphofia. It is not possible to judge the centre of origin and the direction of migrations for Kniphofia from this study. The directions moved by Afromontane genera with subsequent radiations and divergence is a matter to be considered for each genus independently.

The disjunction of many Afromontane elements in West Africa warrants additional attention as it also applies to Kniphofia. A single species (K. reflexa) is found in the Cameroon Centre. K. dubia and K. benguellensis from the highlands of Angola are placed in the South-central Centre (Chapter 2). White $(1978,1981)$ postulated that a southern migratory track of montane forests along the highlands of the divide between the Zaire and Zambezi basins by which the Afromontane flora of East Africa reached the isolated West African mountains. Establishment of aridity in the north of the basin precludes a northern exchange route
(DeBusk, 1998). There appears to be a gradient of increasing moisture availability between Equatorial Africa and Lake Malawi during the LGM (DeBusk, 1998). Moist conditions during the LGM in South-central Africa supports the presence of a southern migratory track of montane forest along the highlands of the divide between the Zaire and Zambezi basins supporting the suggestion by White (1978, 1981). Distances separating modern satellite populations are as little as 300 km . During past cooler climatic situations these now disjunct populations could have been continuous. This continuous belt would have allowed migration between Malawi and Angola (DeBusk, 1998) and possibly further north into West Africa. This allowed the Afromontane flora of East Africa to reach the isolated West African mountains.

However, Livingstone (1971) found that in northern Zambia during the last 22000 years, grasslands have dominated. Dupont et al. (1996) examined Podocarpus distribution in West Africa during the Pleistocene, and showed widespread distributions of Podocarpus forest in the highlands of Guinea, Nigeria, Cameroon, Congo (Democratic Republic of Congo) and Angola. These workers found that the direction of spread was from south to north (Angola to Gabon). At c. 24000 BP forests had limited distribution and were centred around Cameroon and Congo probably due to aridity as the LGM approached. After the LGM Podocarpus forests spread northwards to Nigeria and southwards into Angola during the first half of the Holocene. Lowland forest occupied most of the mountains of Cameroon, Gabon and Congo during this time. The dry conditions may account for the decline in Podocarpus forest distribution except in these equatorial highlands. In this area Podocarpus forests may have benefited from the cold climate of the LGM. Expansion of the lowland rain forest would have also pushed mountain forest to higher altitudes (Dupont et al., 1996).

According to DeBusk (1998) the Afromontane expansion occurred under cooler but not drier conditions than present. The interchange would also not have occurred as a continuous belt of forest but rather as stepping stones formed by montane populations. However, data to test this hypothesis would require vegetation and climatic reconstruction of areas such as western Zambia and Angola (Dupont et al., 1996). The paucity of data for this region is unfortunate and additional data is needed to confirm the existence of the southern migratory route.

Many of the disjunctions of Afromontane elements in the Zaire and Zambezi basins are in habitats that are nutrient deficient, either on oligotrophic substrates or in swamps (Grimshaw, 1998). According to Grimshaw (1998) this supports the hypothesis that Afromontane trees are adapted to nutrient poor conditions. Swamps and mountains could have served as stepping stones and may explain the crossing of the lowlands gaps between East and West Africa. During pluvial periods swamps may have promoted dispersal by reducing the distances involved (Grimshaw, 1998). This is particularly relevant to Kniphofia as it has an affinity for wet conditions.

The distance and altitude of major gaps regions were examined for the centers of diversity for Kniphofia. Within the Rift Valley centre the gap between the Ethiopia and Ruwenzori subcentres is c. 200 km . The lowest altitudes are between 200-500 m while the highest peak in the gap is Mt Kanta ( 2518 m). Between the Rift Valley and South-central Africa centres the gap at the shortest distance is c. 200 km . The lowest altitudes are between $500-1000 \mathrm{~m}$, while the highest peak in the gap is Mt Manzanza (2575 m). Within the South-central Africa centre the gap between the Angolan Highlands is c. 800 km . The altitude ranges between 1 $000-2000 \mathrm{~m}$ for this entire region, while the highest region in the gap is South East of Balombo ( 2620 m ). Angola is poorly explored botanically and Kniphofia may occur in this gap region. Between the South-central Africa and Zimbabwe centres the gap is c. 300 km . The lowest altitude is c .200 m , while the highest peak in the gap is Mt Gorongosa ( 1862 m ). The Zimbabwe and South Africa centre are separated by c. 300 km . The lowest altitude is c. 200 m , while the highest region is the Matobo Hills ( 1543 m ). If vegetation belts descended by 1000 m during the last or previous glacials then most of the gaps would have easily bridged by Afromontane grasslands, with the high points between gaps as acting as stepping stones.

The exception is the Cameroon Centre on continental Africa. The nearest centre to the Cameroon Centre is the Rift Valley Centre. The Rift Valley and Cameroon Centre gap is c. 2 000 km . The lowest altitudes are between $500-1000 \mathrm{~m}$, with several peaks that range from 1 000-1 400 m in between [highest peak in the gap is Mt Nqaoui ( 1400 m ) on the Cameroon
border with the Central African Republic). The Cameroon Centre is also c. 2200 km from the nearest highlands in Angola with Kniphofia. The distances from the Rift Valley Centre and Angolan highlands (South-central Africa Centre) are great (2 000 km and 2200 km respectively).

Establishment of aridity in the north most likely precludes a northern exchange route (DeBusk, 1998) for Kniphofia i.e. a Rift Valley-Cameroon linking route. A continuous belt of Afromontane vegetation (Afromontane Grassland) during glacials would have allowed Kniphofia to bridge the present disjunctions with the high points between gaps as acting as stepping stones in southern and East Africa. The Cameroon Centre was most probably reached from the south via the Angolan highlands as a component of the Afromontane vegetation (DeBusk, 1998). It is possible that Kniphofia migrated between Malawi, Zambia and Angola and possibly further north into Cameroon using peaks and swampy regions in between as stepping stones when Afromontane vegetation was more continuous (i.e. glacial periods) or during pluvial periods. No Kniphofia samples were obtained from Angola, Zambia and Cameroon. It will be interesting to see where these samples would be placed in the phylogeny.

Two other disjunctions, Madagascar and Yemen, also merit additional consideration as they are presently linked but not confined to continental Africa.

### 6.5.1. Madagascar

The Africa-Madagascar separation began c. 165 MYA (Jurassic) and ended c. 121 MYA (Rabinowitz et al., 1983). Most African-Madagascan disjunct plant genera are likely to have achieved these distributions by recent long distance dispersal (Goldblatt, 1978; Axelrod and Raven, 1978). The Malagasy disjunction appears to be a result of dispersal from Tropical Africa. Attachment of seeds in mud to the feet of birds was considered to be a mode of long distance dispersal, particularly in plants growing in moist habitats (marshes, mud-flats and streambanks) with small seeds (Carlquist, 1967). This may also apply to Kniphofia as it is frequently found in marshy areas and have relatively small seeds.

### 6.5.2. Yemen

The Afar Plume links Ethiopia, Eritrea, Djibouti and Yemen. This area was covered by Tertiary flood basalts, which were once contiguous from Ethiopia to Yemen (Mohr et al., 1983; Bosworth et al., 2005). Data suggests that rifting was established in the central and eastern parts of the Gulf of Aden c. 30 MYA. Girdler and Styles (1978) have proposed a two stage seafloor spreading for the Gulf of Aden. The first stage was between 30-15 MYA. The second stage started c. 4.9 MYA to the present time. The separation of Arabia from Africa to form the Red Sea and the Gulf of Aden is estimated at 15 MYA (White, 1983). It is possible that the distribution of K. sumarae (Yemen) is not a result of dispersal but rather vicariance that separated Ethiopia and Yemen. This would mean that Kniphofia is old and northern taxa may be relictual. However, samples from Ethiopia were not resolved as basal and are not regarded as old. $K$. sumarae was not included in this study. It will be interesting to see where this sample will be placed in the phylogeny.

### 6.6. Reconciling anatomical data with biogeography

In Kniphofia morphologically delimited species exhibited substantial anatomical variation. The results for the most part do not fit any geographic pattern nor do they reflect the cpDNA groups recovered by the trnT-L spacer. A reticulate history caused by repeated altitudinal shifting of the vegetation belts associated with climatic changes in the Pleistocene may also account for the substantial anatomical variation in southern African Kniphofia species.

Cutler et al. (1980) examined a group of shrubby Aloe species in the East African (termed the East Africa shrubby species). Leaf surface anatomy alone was not sufficient to understand this group. In summary a form very close to $A$. morejensis was postulated to have undergone chromosome doubling somewhere in the southern Rift Valley of Kenya to produce a tetraploid which is now evolving in several directions into the other species of this group. Habitat details were not given, but several taxa appear to be from the lower reaches of the

Afromontane Region. Cutler et al. (1980) also did not discuss the influence of climate cycles on the evolutionary history of this group.

The anatomical work of Ellis (1980a, 1980b, 1981a, 1981b) on the grass genus Merxmuellera (M. disticha, M. stricta, M. drakensbergensis, M. stereophylla, M. macowanii, M. davyi, M. aureocephala) in the Drakensberg of southern Africa is very interesting as it shares some common themes with the proposed evolutionary history of Kniphofia. The details of these studies are not given here but the broad conclusions are of significance to this study. He has shown evidence which supports an adaptive radiation associated with alternating environmental conditions particularly altitudinal effects in the Drakensberg Range for Merxmuellera (Ellis, 1980a). The adaptive radiation is still actively continuing (Ellis, 1980b). He was also able to provide evidence for species of hybrid origin (Ellis, 1981b).

However, there are some notable differences between Mermuellera and Kniphofia. The monophyly of Kniphofia as a whole has not been disputed. Barker et al. $(1999,2003)$ showed that Merxmuellera is polyphyletic. The number of species of Kniphofia are much greater than Merxmuellera, and thus morphological diversity is greater. Ellis' (1980b) work indicates that an adaptive radiation is occurring at high altitudes i.e. an 'upward' radiation for Merxmuellera. The opposite situation seems to apply to Kniphofia where the radiation has been at lower altitudes i.e. a 'downward' radiation.

Additionally Merxmuellera species seem to have a more conservative morphology while displaying much variation in anatomy. Kniphofia on the other hand displays both variable morphology and anatomy. The Merxmuellera adaptive radiation in response to similar environment conditions has resulted in similar phenotypic expression by the ecotypic forms of each species (Ellis, 1981b). The higher altitude Alpine anatomical 'forms' of Merxmuellera species may be experiencing morphological convergent evolution in response to similar environmental conditions (Ellis, 1980b) but retaining distinctive (or ancestral) anatomical types. This may explain the lack of morphological diversity in the adaptive radiation proposed for some Merxmuellera species.

Anatomical data for other Afromontane genera are needed to assess, compare and correlate patterns. Such patterns may reveal how different Afromontane elements have had different responses depending on life history traits, climatic tolerances, vegetation preferences and associations.

### 6.7. Species concepts

What are species, how do they come to be, and how do we discover them? These remain the most elusive and intractable challenges to natural historians (Brower et al., 1996). In groups with reticulate ancestry species concepts with an underlying monophyletic basis are of little use. In a genus where gene flow occurs, the biological species concept is inadequate. The Hennigian species concept applies to reproductively isolated natural populations. Gene flow and possible hybridisation eliminates this application to Kniphofia. The phylogenetic species concept is based on monophyly. This is also not applicable in the case of Kniphofia as species display non-monophyly.

Kniphofia may be regarded as a mixture of more or less discrete morphological units that are inter-fertile. Many taxa appear to be either incipient species or populations that are in the process of diverging to the point of speciation but retaining the potential to interbreed. Early stages of reproductive isolation and genetic differentiation may be taking place among various populations and species complexes. Over time, geographical and phenological discontinuities may arise that reinforce genetic differentiation, thus producing evolutionary entities that can be considered species (Nickrent et al., 2004). In Kniphofia incipient speciation may be occurring and adopting a strict species definition is thus elusive.

### 6.8. Classification

Both the nuclear and chloroplast trees are inconsistent with the sectional classification proposed by Berger (1908) and Codd (1968) and both phylogenies also do not support the segregate genus Notosceptrum.

Reducing the entire genus to a single taxonomic species or five clades/lineages is not considered appropriate, as there is evidence of morphological structure reflected by the alpha taxonomy of many, although not all, taxa. Some species are clearly morphologically definable e.g. K. parviflora but are non-monophyletic according to DNA sequence data. Reduction to a single species may reflect a more accurate evolutionary history but will create a morphologically heterogeneous assemblage, which is considered impractical at this point in time. Recognising five clades/lineages as possible species or subspecies units is also unpragmatic, as all the clades/lineages recovered in this study will be composed of morphologically heterogeneous assemblages. Identification, description and communication of these clades will be problematic. If either of the above steps are advocated it will result in instability in classification. This is of particular importance as not all currently recognised morphological species were sampled so their final placement is uncertain and future research may find different and/or additional lineages. Furthermore, classification based on only one marker especially a cpDNA marker that is maternally inherited in a group that apparently readily hybridises is undesirable and not recommended.

The existing classification may not reflect the evolutionary history and phylogeny of the genus accurately, but does provides a framework for identification, communication and interpretation of additional evidence. Thus, a conservative approach is taken and the existing morphological based classification is upheld and no changes are proposed.

### 6.9. Conclusion

The cpDNA and nuclear phylogenies for Kniphofia do not provide complete resolution and are not robust. Despite these limitations, a phylogenetic hypothesis for Kniphofia using molecular plastid and nuclear DNA sequence data was obtained. The DNA sequence data (especially the cpDNA data) when interpreted in a broader context that encapsulates tectonic, climate change, distributional, phylogenetic, phylogeographic and anatomical patterns does provide evidence to give insights on the evolution, biogeography and complicated alpha taxonomy of Kniphofia.

The present study on the phylogenetic relationships, evolutionary history and biogeographic patterns of Kniphofia has made a significant and much needed contribution to the better understanding of this very popular but little-studied genus of petaloid monocotyledons. This study has provided insights into the potential factors and processes driving evolution in this genus. It has also detected previously unknown phylogenetic and biogeographic patterns and is the first to invoke and incorporate past climatic change across the entire African continent as a mechanism driving diversification and speciation in an angiosperm group. However, many issues need further investigation and attention, and this study provides a framework for additional systematic research. The patterns detected and the explanation of these patterns may need reassessment as information from additional markers and/or samples become available. Thus there is much scope for the improvement, refinement and further development of the hypotheses presented here.

The distribution patterns of Kniphofia in southern Africa and the Afromontane Region is very similar to several other genera (e.g. Alepidia, Freesia, Dierama, Dietes and Knowltonia). It is presumed that they are under the same evolutionary and environmental (both present and past) forces and may have been influenced in a similar manner as Kniphofia. Many of the factors that have influenced the evolutionary history and biogeography of Kniphofia are probably also applicable to these genera. However, no studies with a phylogenetic or phylogeographic framework are currently available for these genera. Such studies may either confirm and strengthen the findings of this study, find different patterns or may provide insights to alternate hypotheses to explain the results obtained for Kniphofia.

According to White (1981), events responsible for the establishment of the Afromontane Region (and related disjunctions) are complex with a long history, spanning beyond the past 25000 years, and a single model is unlikely to account for the entire diversity of Afromontane chorology. Further research may contribute to phytogeographic hypotheses explaining migration routes due to climate change and expansions/contractions. Carbutt and Edwards (2001) noted the importance of species level cladograms in determining and understanding the Afromontane flora and its historic origins. Phylogeographic studies on selected species or groups of closely related species that have an Afromontane distribution
may also be invaluable in understanding the history of the Afromontane Region, directions of migrations and refugial areas for specific taxa.

In the past, work on the Afromontane Region has focused on studies that documented the diversity, vegetation and relationships with other vegetation. Factors driving speciation and evolution in the Afromontane flora have received very little attention. A major challenge is to shift focus from documenting patterns to explaining processes driving speciation and evolution.

Data from tectonics, past climate changes, present distribution patterns, genetic studies, vegetation history, ecology, anatomy and systematics have to be integrated effectively to gain an understanding of origins, evolution and speciation in plant groups of the Afromontane Region. Additionally data from such multidisciplinary research will ultimately contribute to a better understanding of factors that have in the past and are presently shaping the flora of Africa as a whole.

This will also assist zoologists who are studying Afromontane fauna that are restricted to specific Afromontane vegetation types. Research on the fauna of the African mountains has already been initiated. Bowie et al. (2004) showed that how forest expansion driven by climate change in the mid-Pleistocene (1.1-0.7 MYA) have influenced the evolution and biogeography of the olive sunbird (Nectarina olivacea/obscura). Bowie et al. (2006) demonstrated using the starred robin (Pogonocichla stella) how aridification in response to glaciation during the Pleistocene had a major influence on speciation in the mountains of East and Central Africa. Quérouil et al. (2003) examined the phylogeography of two shrew species (Sylvisorex johnstoni and S. ollula) in western central Africa. Both species are tropical forest dwellers. This study suggests that both species originated in the PlioPleistocene and their haplotype distribution reflect forest fragmentation and expansion associated with climatic change in the Pleistocene (Quérouil et al., 2003). These studies do not have a direct bearing on this study (i.e. Kniphofia), but they demonstrate how specific palaeo-climate and vegetation changes can trigger profound effects on evolutionary and
biogeographic history depending on the organism examined. Furthermore, they contribute to a better understanding of how the African biota has survived in space and time.

### 6.10. Future work

In concluding the present study several avenues of investigation are recommended in order to further unravel the threads in the Kniphofia puzzle. Some of the research avenues mentioned below might also contribute to a better understanding of the Afromontane Region and other Afromontane groups that have a similar distribution pattern as Kniphofia.

### 6.10.1. Species complexes

Baijnath (1980) noted the need for detailed investigations of species complexes based on the anatomical differences in species such as $K$. linearifolia and the morphological variation reported by Codd (1968, 2005). Such studies will require extensive sampling over the entire distribution range to assess variation. Also because of the possible prevalence of hybridisation it may not be feasible to explain all the variation by examining isolated complexes but will require a much broader approach.

Morphometric studies of species complexes are needed to identify morphological characters important for identification of difficult species. A phenetic analysis should also be done on floral characters which not only accounts for morphological variation and influences hybridization. Widespread species should be examined for clinal variation in morphology and factors associated with this variation.

### 6.10.2. Ecological studies

More research is required on the ecology of Kniphofia. This should include rainfall, temperature, soil and substrate requirements for Kniphofia species. Pollination studies are also needed (see below). This will help to better understand how Kniphofia survived
changing environmental conditions of glacial periods and how this may have effected its evolutionary and biogeographic history.

### 6.10.3. Reproductive biology and breeding studies

Cronn and Wendel (2003) noted the important contribution that reproductive biology may have in understanding hybridisation. Artificial hybridisation attests to the potential for natural hybridisation between species (Cronn et al., 2003). No studies could be found that have studied the pollination and/or floral biology of Kniphofia. Kniphofias are known to be visited by sunbirds in southern Africa. These may be the likely pollinators. There is ample evidence from horticulture that Kniphofia species hybridise easily. Baijnath (1992a) managed to cross K. leucocephala and K. pauciflora as flowering times overlapped. Both species were predominately self-incompatible. However, hybrid plants were not studied in detail. In the present study no artificial hybridisation experiments where done. More detailed studies are required with controlled crossings. Crossing experiments and comparison (morphological, anatomical, cytological and molecular) of progeny and parents should be done to test hybrid hypotheses and to determine the nature and extent of hybridisation. These studies should also include aspects of floral and pollination biology.

### 6.10.4. Cytology and Polyploidy

Hybridisation and polyploidy are frequent modes of evolution in plants (Baumel, et al. 2002; Soltis et al. 2003; Doyle et al. 2003; Linder and Rieseberg, 2004). There is a well established link between hybridisation and polyploidy in the literature (McDade, 1990; Sang et al., 1995; Church and Taylor, 2005; Ainouche et al., 2003; Guo et al., 2004).

Very few lineages of angiosperms have been unaffected by polyploid events (Valcárcel et al., 2003). Polyploidy is common in plants with estimates ranging from $30-80 \%$ in angiosperms (Hegarty and Hiscock, 2005). Polyploidisation can have major effects on genome structure and function (Soltis et al., 2003; Adams and Wendel, 2005). It can also have major effects on evolution and ecology of taxa involved (Soltis et al., 2003). Hence results from nuclear
markers may be more complex than those of chloroplast markers in cases of polyploidy as nuclear markers can reflect multiple origins in a polyploid taxon (Mason-Gramer, 2004).

Polyploids are also known to hybridise more than their diploid counterparts (Church and Taylor, 2005). Most polyploid plants species that have been examined with molecular markers are polyphyletic, having arisen multiple times from the same diploid species (Soltis and Soltis, 2000b). The success of polyploids is attributed to their genetic variability (Doyle et al., 1999).

Chromosome counts for Kniphofia are few. Webber (1932) examined the karyology of K. aloides (i.e. K. uvaria) in detail. He found that there were six pairs of chromosomes ( $2 n=12$ ). De Wet (1960) noted the somatic chromosome number of $2 n=12$ in 17 species of Kniphofia (viz. South African representatives, including K. typhoides which was previously placed in Notosceptrum). Chromosome morphologies showed little difference and there was no polyploidy. Nayak and Sen (1992) examined the karyology of K. nelsonii (i.e. K. triangularis) and $K$. uvaria clones. Most of the cells examined were diploid $(2 n=12)$, however, these workers found that some cells were aneuploid or tetraploid.

Diploid hybrid speciation has been shown in a number of plant groups (e.g. Wolfe et al., 1998) and cannot be excluded as a possibility for Kniphofia. Future cytotaxonomical investigations are highly recommended to establish if polyploidy exists in this genus and infer primitive versus derived chromosome numbers and morphologies.

Leaf epidermal patterns have been found to be under genetic control as in some members of the Alooideae (Cutler and Brandham, 1977; Cutler, 1978; Brandham and Cutler, 1978). Similar studies which include a cyto-nuclear component are required to show this in Kniphofia (Baijnath, 1980).

### 6.10.5. Multiple sampling

The results of this study emphasise the importance of using multiple exemplars in molecular studies of problematic groups. Sample acquisition is a major problem especially when working on groups with very wide distributions. However, attempts should be made to include as many samples as possible. Sampling only one sample per species may lead to erroneous conclusions and shallow insights of patterns at the species level and below (Soltis et al., 1992). Single exemplar studies may not recover underlying patterns. Insights into reticulation and lineage sorting can only emerge from multiple exemplar sampling approaches. More comprehensive coverage of species and distributional ranges of Kniphofia are needed especially for Tropical and East Africa.

### 6.10.6. Population studies

A component of increased sampling entails studies at the population level. In order to understand evolutionary processes and relationships, examination of population within species or closely related species are needed (Wolfe et al., 1998; Schaal and Olsen, 2000; Ferguson and Jansen, 2002). Increased sampling at the population level is needed to gain a more comprehensive understanding of morphological, anatomical and genetic variation and patterns. Population level studies are needed to confirm putative hybrids and their parental taxa or populations.

### 6.10.7. Application of additional markers and methodologies

Use of more sensitive molecular markers such as amplified fragment length polymorphisms (AFLPs), microsatellites and inter simple sequence repeats (ISSRs) may prove to be useful (Wolfe et al., 1998; Gravendeel et al., 2004; Vriesendorp and Bakker, 2005). Single or low copy nuclear genes appear to be better markers for the parental polymorphisms in hybrids than ITS because of the effects of concerted evolution (Sang and Zhang, 1999). Additional studies with alternative markers may provide scope for determining age of nodes. This data will assist in assessing the role of lineage sorting in Kniphofia. Alternative markers may also contribute to a better understanding of the evolutionary and the biogeographic history of Kniphofia.

Modern molecular cytogenetic techniques such as genomic in situ hybridisation (GISH) (e.g. Chase et al., 2003) and fluorescent in situ hybridisation (FISH) (e.g. Taketa et al., 2005) has opened new avenues to study hybridisation. Linkage disequilibrium studies may also be useful. This involves the search of genetically linked markers. Tightly linked markers in hybrids are more likely to come from the same parent and therefore display linkage disequilibrium (Linder and Rieseberg, 2004). Coalescent approaches may help in interpreting the data further. However, these were not attempted in this study because of the limiting nature of the data and the problems associated with finding suitable calibration points.

The Afromontane Region is under extreme anthropogenic pressure (Burgoyne et al., 2005). Data obtained from multidisciplinary research as outlined above will serve to highlight the importance of conserving the Afromontane Region and to curb the loss of this unique vegetation, its flora and fauna.

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## Appendices

## Appendix 1: Data matrix with Kniphofia species as characters and QDGs as terminal units

| 3424BB | 000010000010000000000000000000000000000000000001 |
| :---: | :---: |
| 3424BA | 000000000010000000000000000000000000000000000000 |
| 3424AA | 000000000000000000000000000000000000000000000001 |
| 3423AB | 000000000000000000000000000100000000000000000000 |
| 3423AA | 000000000000000000000000000000000010000000000001 |
| 3421BC | 000000000000000000000000000000000000000000000001 |
| 3421BA | 000000000000000000000000000000000000000000000001 |
| 3421AD | 000000000000000000000000000000000000000000000001 |
| 3421AC | 000000000000000000000000000000000000000000000001 |
| 3420AD | 000000000000000000000000000000000000000000000001 |
| 3420AB | 000000000000000000000000000000000000000000000001 |
| 3419BD | 000000000000000000000000000000000000000000000001 |
| 3419BC | 000000000000000000000000000000000000000000000001 |
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| 3419AB | 000000000000000000000000000000000000000000000001 |
| 3419AA | 000000000000000000000000000000000000000001000001 |
| 3418BD | 000000000000000000000000000000000000000000000001 |
| 3418BB | 000000000000000000000000000000000000000001000000 |
| 3418AD | 000000000000000000000000000000000000000000000001 |
| 3418AB | 000000000000000000000000000000000000000000000001 |
| 3327BB | 000000000000000000000000000000000000010000000000 |
| 3326DA | 000010000010000000000000000000000000000000000000 |
| 3326CB | 000000000010000000000000000000000000000000000000 |
| 3326BC | 000010000010000000000000000000000000000000000000 |
| 3326AD | 000000000010000000000000000000000000000000000001 |
| 3326AC | 000000000010000000000000000000000000000000000000 |
| 3325DC | 000010000010000000000000000000000000000000000001 |
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2730AA 010000000000000000000000000100000000000000000000 000010000000000000000000000000000000000000000000 000000000000000000000000000000000100000000000000 000000000100000000000000000000000000000000000000 000000000000000000000000000100000000000000000000 000000000000000000000000000000000100000000000000 010000000000000000000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000100000000000000000000000000000000000000 000000000000000000000000000000000000000000000100 000000000000000000000000000000000000000100000000 000000000000000000000000000000000100000000000100 000000000000000000000000000100000000000000000000 000000000000000000000000000100000100000100000010 010000000000000000010000000100000100000000000000 010000000000000000001000000100000000000000000000 000000000000000000000000000000000100000000000000 000000000000000000000000000100000100000100000000 000000000000000000001000000000000100000000000000 000000000000000000000000000000000100000000000000 000000000000000000000000000001000100000000000000 010000000000000000000000000000000000000000000000 010000000000000000000000000000000100000000000000 010000000000000000000000000000000000000000001000 010000000000000000000000000000000100000000000000 000000000000000000000000000000000000000000001000 000000000000000000000000000000000000000000001000 000000000000000000000000000000000000000000001000 000000000000000000000000000000000100000000000000 000000000000000000000000000000000000000000001000 000000000000000000000000000000000100000000000000 000000000000000000000000000000000100000000001000 000000000000000100000000000000000000000000000000 000000000000000000000000000000000000000000001000 000000000000000000000000000000000000000000001000 000000000000000000000000000000000000000000001000 000000000000000100000000000000000000000000001000 000000000000000100000000000000000000000000001000 000000000000000100000000000000000000000000000000 000000000000000000000000000000000100000000000000 000000000000000000000000000000000100000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000000000000000000000000000000000000000100 000000000000000000001000000000000000000000000000 000000000000000000001000000100000100000000000000 000000000000000000001000000001000000000000000000 000000000000000000010000000000000000000000000000 000000000000000000000000000101000000000000000000 000000000000000000001000000101000000000100010000 000000000000000000000000000100000000000000000000 000000000000000000001000000100000000000000000000 000000000000000000001000000100000000000000010000 000000000000000000000000000000000101000000000000 000000000000000000001000000101000001000000011000 000000000000000000010000000000000101000000000000 000000000000000000001000000101000000000000000000 000000000000000000001000000100000000000000010000 000000000000000000000000000101000100000100000000 000000000000000000011000000100000000000100010000 000000000000000000000000000100000001000000000000 000000000000000000010000000100000101000000010000 000000000000000000001000000100000001000000010000 000000000000000100000000000000000001000000000000 000000000000000100000000000000000100000000000000 000000000000000100000000000000000100000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000000000000000000000100000000000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000000000000000000 000000000000000100000000000000000100000000000000 000000000000000100000000000000000100000000000000 000000000000000100000000000000000100000000000000 000000000000000100000000000000000100000000000000 000000000000000100000000000000000100000000000000

| 2527CD | 000000000000000100000000000000000000000000000000 |
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| 2527CB | 000000000000000100000000000000000000000000000000 |
| 2527CA | 000000000000000100000000000000000000000000000000 |
| 2526DD | 000000000000000100000000000000000000000000000000 |
| 2526DA | 000000000000000100000000000000000000000000001000 |
| 2526CA | 000000000000000100000000000000000000000000000000 |
| 2526AD | 000000000000000100000000000000000000000000000000 |
| 2525BD | 000000000000000100000000000000000000000000000000 |
| 2431CC | 000000000000000000000000000100000000000000000000 |
| 2431CA | 000000000000000000000000000001000000000000000000 |
| 2430DD | 000000000000000000001000000101000001000000000000 |
| 2430DC | 000000000000000000001000000100000000000000001000 |
| 2430DB | 000000000000000000001000000100000100000000010000 |
| 2430CD | 000000000000000000000000000000000000000000001000 |
| 2430CA | 000000000000000100000000000000000000000000000000 |
| 2430AB | 000000000000100000000000000000000000000000000000 |
| 2430AA | 000000000000100000000000000000000000000100000000 |
| 2429BB | 000000000000100000000000000000000000000000000000 |
| 2429AA | 000000000000000100000000000000000000000000000000 |
| 2428CD | 000000000000000100000000000000000000000000000000 |
| 2428CB | 000000000000000100000000000000000000000000000000 |
| 2428BC | 000000000000000100000000000000000000000000000000 |
| 2428AC | 000000000000000100000000000000000000000000000000 |
| 2427BC | 000000000000100100000000000000000000000000000000 |
| 2330CC | 000000000000100000000000000001000000000100000000 |
| 2330CA | 000000000000000000000000000001000000000100000000 |
| 2329DD | 000000000000110000000000000001000000000100000000 |
| 2329BB | 000000000000100000000000000000000000000100000000 |
| 2329AA | 000000000000100000000000000000000000000000000000 |
| 2328CB | 000000000000000100000000000000000000000000000000 |
| 2328BB | 000000000000100000000000000000000000000000000000 |
| 2230CD | 000000000000100000000000000001000000000100000000 |
| 2230CC | 000000000000100000000000000000000000000000000000 |
| 2229DD | 000000000000100000000000000001000000000000000000 |

## Appendix 2: Data matrix with Kniphofia species as characters and HDGs as terminal units (with singleton HDGs removed)

3424BX 000010000010000000000000000000000000000000000001 3423AX 000000000000000000000000000100000010000000000001 3421BX 000000000000000000000000000000000000000000000001 3419AX 000000000000000000000000000000000000000001000001 3418BX 000000000000000000000000000000000000000001000001 3326DX 000010000010000000000000000000000000000000000000 3326BX 000010000010000000000000000000000000000000000000 3326AX 00000000001000000000000000000000000000000000000 3325DX 000010000010000000000000000000000000000000000001 3325CX 000000000010000000000000000000000000000000000001 3324CX 000010000000000000000000000000000000000000000001 3323DX 000010000000000000000000000000000010000000000001 3323CX 000010000000000000000000000000000000000000000001 3322DX 000000000000000000000000000000000010000000000001 3319CX 000000000000000000000000000100000000000000000001 3319BX 000000000000000000000000000100000000001000000000 3318DX 00000000000000000000000000000000000000000100000 3228CX 000000000000000000000000000000010000010000000000 3228AX 000010000000000000000000000100000000000000000000 3227DC 000000010010000000000000000100000000010000000001 3227CX 000010000010000001000000000100010000010000010001 3227AX 000000000000000000000000000100010000000000010001 3226DX 00000000000000000000000000010011000000000001000 3226CX 000000000000000000000000000100000000000000010001 3226BX 000010000000000000000000000100100000000000010001 3226AX 000000000100000000000000000100000000000000010001 3225DX 000000000000000000000000000100000000000000010000 3225AX 10000000000000000000000000000000000000000000000 3224BX 000000000000000000000000000100000000000000000001 3222AX 000000000000000000000000000000000010000000000001 3130AX 000000000001000000000000010000010000010000000000 3129DX 000000000000000000000000010100000000000000000000 3129CX 000010000000000000000000000100000000000000000000 3129BX 000000000001001000000100010100010000010000000000 3128DX 000010000000000000000100000100100000000000000100 3128CX 00001000000000000000000000010001000000000000000 3128AX 000010000000000000000000000100110000000000010001 3127DX 000010000000000000000000000000010000000000000000 3127BX 000010000000000000000000000100010000000010010001 3126DX 000000000100000000000000000100100000000010010001 3126BX 000000000000000000000000000100000000000010000000 3030DX 000000000000000000000100000000000000010000000000 3030CX 000010000001000000000100000100000000010000000100 3030BX 000000000000000000000100010010000000010000010100 3030AX 000000000000000000000101010110000000000000000100 3029DX 010011000000001000000100010100010000000000000100 3029CX 000010000000000000000000010100110100000000000000 3029BX 000001000000000000000000010100010000000000000100 3029AX 000010000000000000000000010100010000000000010000 3028CX 000010000000000000000010000100110000100010010000 3028BX 000010000100000000010000000100000000000010010000 3027DX 001000000100000000000010000000100000000010010000 3027CX 000000000100000000000000000000000000000010010000 2931CX 000000000000000000000100010010001000000000000000 2931AX 000000000000000000000000000010000000010000000000 2930DX 000000001000000000000100010110001000000000000100 2930CX 000010001000000000000001010100000000000000010100 2930BX 001000001000000000000101100000000000000000000100 2930AX 000000001000000000010001110100000000000000000100 2929DX 000100000000000001010000010100010000000000010000 2929CX 001001100100000001010001010100100100100000110000 2929BX 001101100100000000010001010100100100100000100000 2929AX 001101000100000011000001010100110100100000110000 2928AX 000000000100000000000010000000000100100000110000 2927BX 000000000100000000000010000000100000100000110000 2832CX 000000000000000000000000001010000000000000000000 2832AX 000010000000000000000100010010000000000000000100 2831DX 000000000000000000000000010010000000000000000000 2831CX 000000001000000000000100100110000000000000000100 2830DX 000000001000000000000100010100000000000000010110 2830BX 000010000000000000000000000000000000000000000100 2830AX 000010000000000000101000000000000000000000000000 2829DX 000001000000000000000100000000000000000000000000

2829CX 001100100100000010000001010101010100100000010000 2829BX 010000000000000000000000000100000000000000010000 2829AX 001000100100000100010000000101000100000000011000 2828DX 001000100100000000000000000100100100100000110000 828CX 000000000100000000000000000000000000100000110000 2828BX 000000000100000000000000000000000000000000010000 2828AX 000000000000000000000000000000000000100000010000
2731CX 000000000000000001000000010100000000000000010100
2730DX 000000000000000000001000000101000100000000001000 2730CX 010000000000000000000000010100000100000000001000 2730BX 010000000000000000000000010000000100000000000000 2730AX 010010000000000000010000010101000100000000000000 2729DX 000010000100000000000000000000000100000000000000 2729BX 010000000000000000000000000000000100000000000000 2728DX 000000000100000100000000000000000000000000000000 2631BX 000000000000000000000000000000000100000000000100
2631AX 000000000000000000000000000100000100000100000010
2630DX 010000000000000000010000000100000100000000000000 2630CX 010000000000000000001000000100000000000000000000 2630BX 000000000000000000001000000100000100000100000000 2630AX 000000000000000000000000000001000100000000000000
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## Appendix 3: PCR reagents

Table 1. Table of PCR reagents and volumes ( $\mu \mathrm{l}$ ) used for PCR reactions

| Mg <br> concentration | $\mathrm{H}_{2} \mathrm{O}$ | 10X <br> Buffer | dNTPs | Primer <br> 1 | Primer <br> 2 | BioTaq | DNA | $\mathbf{M g C l}_{2}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 1$]$ | 35.5 | 5 | 2 | 2 | 2 | 0.1 | 2.5 | 1 |
| 2$]$ | 34.5 | 5 | 2 | 2 | 2 | 0.1 | 2.5 | 2 |
| 3$]$ | 33.5 | 5 | 2 | 2 | 2 | 0.1 | 2.5 | 3 |
| 4$]$ | 32.5 | 5 | 2 | 2 | 2 | 0.1 | 2.5 | 4 |
| 5$]$ | 31.5 | 5 | 2 | 2 | 2 | 0.1 | 2.5 | 5 |

Mg concentration= Magnesium concentration
$\mathrm{H}_{2} \mathrm{O}=\mathrm{PCR}$ quality water
10X Buffer= 10X Bioline $\mathrm{NH}_{4}$ dilution buffer ( $\mathrm{MgCl}_{2}$ free)
BioTaq= Bioline Taq polymerase enzyme
$D N A=$ DNA template
$\mathrm{MgCl}_{2}=50 \mathrm{mM}$ solution of $\mathrm{MgCl}_{2}$ provided with enzyme and enzyme and 10X Buffer

Appendix 4: Final sequence alignments of the $\operatorname{trnT}$ - $L$ spacer



| AATTTCAAAA ATATATTTAT | AAATAGAAAT |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| CAAAA ATATATtTAT | AAATAGAAAT | TTTGATTAAT |  | T |
| A AtAtatttat | AAATAGAAAT | TTTGATTAAT |  |  |
| a Atatatttat | AAATAGA | TTTGAT |  |  |
| an atatatttat | AAATAGAAA | tTTGAT |  |  |
| AA Atat | AAATAGAAA | TTTGATTAAT |  |  |
| Atata | AAATAGA | TTTGATT |  |  |
| AAAA ATATATtTA | AAATAGAAA | TTTGATTAAT | GA |  |
| AtAt | AAAT | TTTGAT |  |  |
| AATTTCAAAA ATAT | AAA | TT |  |  |
| Atat | TAAT | ttagattant | GATAAT---- |  |
| AATtTCAAAA ATATATTTAT | TAATAGAAAT | tTAGATTAAT | GATAAT---- | T |
| AATTTCAAAA ATATATTTAT | AAATAGAAAT | TITGATTAAT |  |  |
| A Atatatttat | TAATAGAAAT | ttagattant | GATAAT---- | T |
| AAA ATATATTTAT | AAATAGAAAT | TTTGATTAAT | G |  |
| AATTTCAAAA ATATA | AAATAGAAA | TTTGATTAAT |  |  |
| AT | TAA | TT |  |  |
| A | taAt | tTAGATTAAT |  |  |
| AATTTCAAAA ATATATTTAT | TAATAGAAAT | tTAGATTAAT | GA | T |
| AAA ATA | AAATAGAAAT | TTTGATTAAT |  |  |
| Atata | AAATAGA | TTTGATTAAT |  | T |
| AATTTCAAAA ATATATTTAT | AAATAGAAAT | TITGATTAAT | GA | T |
| AATTTCAAAA ATA | AAATAGAAAT | TTTGATTAAT | GATAAT---- | T |
| AATTTCAAAA ATA | AAAT | TTTGATTAAT | GATAAT---- |  |
| AAA Atata | AAATAGAAAT | tITGATTAAT |  | T |
| AATTTCAAAA AT | AAA | TTGGATTAAT | GATAAT---- | T |
| A A | AAA | TT | GATA | - |
| AATTTCAAAA ATA | AAAT | TTI |  |  |
| AATTTCAAAA AT | AAATAGA | TT |  |  |
| AATtTCAAAA ATATATTTAT | AAATAGAAAT | TTTGATTAAT | GAT | T |
| AATTTCAAAA ATATA | AAATAGAAA | TTTGATTAAT |  |  |
| AATTTCAAAA ATATA | AAATAGAAA | tTGGATTAAT | GA | T |
| AATTTCAAAA ATA | AAATAGAAAT | TTTGATTAAT | GAT | T |
| AATTTCAAAA ATATATTTAT | AAATAGAAAT | TTGGATTAAT | GA |  |
| AATTTCAAAA ATA | AAAT | TTTGATTAAT | GATAAT---- |  |
| AATTTCAAAA AT | AAATAGAA | TTTGATTAAT | GATAAT---- | T |
| AATTTCAAAA ATATATTTAT | AAATAGAAAT | tITGATTAAT | GATAA | T |
| AATTTCAAAA ATATATTTAT | AAATAGAAAT | TTTGATTAAT | GAtAA | - |
| AATTTCAAAA ATATATtTAT | AAATAGAAAT | TTTGATTAAT | GA |  |
| an atatatttat | AAATAGAAA | TTTGA | GATAAT---- | TTAAGT |
| 70 | 80 | 90 | 100 | 110 |

AATCAGATTT TTGATAATAT CAAATT-TGA TATT---ATG ATTAAAAAAA AAAAAATGGA AATCCTATTT TTGATAATAT CAAATT-TGA TATTATTATG ATTC-----AA AAAAAATGGA


 AATCAAATTT --GATATTAT GA--TTATGA TT--------------------TA AAAAAATAGA AATCAAATTT --GATATTAT GA--TT---- ------------------------TA AAAAAACGGA










 AATCAAATTT --GATATTAT GA--TTATGA TT-------------------TA AAAAAAGA AATCAAATTT --GATATTAT GA--TTATGA TT--------------------TA AAAAAATAGA AATCAAATTT --GATATTAT GA--TTATGA TT-------- -----------TA AAAAAATAGA AATCAAATTT --GATATTAT GA--TT---- ---------------------- AA AAAAATGGA
 AATCAAATTT --GATATTAT GA--TT---- ----------------------AATCAAATTT --GATATTAT GA--TT---- ---------------------AATCCAATTT --GATATTAT GA--TTATGA TT--------- ----------TA AAAAAAGA
 AATCCAATTT --GATATTAT GA--TTATGA TT---------------------TA AAAAAATAGA


 AATCAAATTT --GATATTAT GA--TTATGA TT---------------------TA AAAAAATAGA AATCAAATTT --GATATTAT GA--TT---- -----------------------TT AAAAAATGGA AATCAAATTT --GATATTAT GA--TT---- ------------------------
 AATCAAATTT --GATATTAT GA--TT---- ------------------------ TA AAAAATGGA AATCAAATTT --GATATTAT GA--TT---- ------------------------ TA AAAAAATGGA
 AATCAAATTT --GATATTAT GA--TT---- -------------------------- TA AAAAAATGGA
 AATCAAATTT --GATATTAT GA--TTATGA TT--------- ----------TA AAAAAATAGA


 AATCCAATTT --GATATTAT GA--TTATGA TT------------------- TA AAAAAATAGA



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| K. ichopensis SR289 | AATCAAATTT | --GAtattat | GA--TTATGA |  |  | AAAAAATAGA | [92] |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| K. ichopensis SR409 | AATCAAATTT | --GAtattat | GA--TTATGA |  | TA | AAAAAATAGA | [92] |
| K. insignis SRsn | AATCAAATTT | --GAtattat | GA--TT |  | --------TA | AAAAAATGGA | [86] |
| K. isoetifolia SR386 | AATCAAATTT | --gatattat | GA--TT |  | --------TA | AAAAAATGGA | [86] |
| K. isoetifolia SR388 | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. isoetifolia SR393 | AATCAAATTT | --GAtattat | GA--TT- |  | --------TA | AAAAAATGGA | [86] |
| K. latifolia RSSsn | AATCAAATTT | --GAtattat | GA--TTATGA | TI | --------TA | AAAAAATAGA | [92] |
| K. laxiflora SR283 | AATCAAATTT | --GAtattat | GA--TTATGA | TT | --------TA | AAAAAATAGA | [92] |
| K. laxiflora SR295 | AATCCAATTT | --GAtattat | GA--TTATGA | TT | --------TA | AAAAAATAGA | [92] |
| K. laxiflora SR441 | AATCAAATTT | --gatattat | GA--TTATGA | TT |  | AAAAAATAGA | [88] |
| K. laxiflora SR442 | AATCAAATTT | --GAtATtAT | GA--TTATGA | TI |  | AAAAAATAGA | [92] |
| K. laxiflora SR467 | AATCAAATTT | --GAtattat | GA--TTATGA | TT | A | AAAAAATAGA | [92] |
| K. laxiflora SR468 | AATCAAATTT | --GAtAttat | GA--TTATGA | TT | TA | AAAAAATAGA | [92] |
| K. laxiflorac SRsn | AATCAAATTT | --GAtattat | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. laxiflora NPB1810 | AATCAAATTT | --GAtattat | GA--TTATGA | TT | -TA | AAAAAATAGA | [88] |
| K. leucocephala NNBG | AATCAAATTT | --GAtattat | GA--TT- |  | -TA | AAAAAATGGA | [86] |
| K. linearifolia SR269 | AATCAAATTT | --GAtattat | GA--TT |  | -TT | TAAAAATGGA | [86] |
| K. linearifolia SR287 | AATCAAATTT | --GAtattat | GA--TTATGA | TT-------- | T | AAAAAATAGA | [92] |
| K. linearifolia SR290 | AATCAAATTT | --GAtattat | GA--TTATGA |  | -TA | AAAAAATAG | [92] |
| K. linearifolia SR291 | AATCAAATTT | --GAtattat | GA--TTATGA |  | --------TA | AAAAAATAGA | [92] |
| K. linearifolia SR311 | AATCAAATTT | --GAtattat | GA--TT- |  | TA | AAAAAATGGA | [86] |
| K. linearifolia SR328 | AATCAAATTT | --GAtattat | GA--TTATGA |  | TA | AAAAAATAGA | [92] |
| K. linearifolia SR343 | AATCAAATTT | --GAtattat | GA--TT- |  | T | AAAAAATGGA | [86] |
| K. linearifolia SR400 | AATCAAATTT | --GAtattat | GA--TTATGA |  | --------TA | AAAAAATAGA | [92] |
| K. linearifolia SR558 | AATCAAATTT | --GAtattat | GA--TTATGA |  | --------TA | AAAAAATAGA | [92] |
| K. linearifolia JPsn | AATCAAATTT | --gatattat | GA--TTATGA |  |  | AAAAAATAGA | [92] |
| K. linearifolia TD4638 | AATCCAATTT | --GAtattat | GA--TTATGA |  |  | AAAAAATAGA | [92] |
| K. littoralis SR200 | AATCAAATTT | --GAtattat | GA--TTATGA |  | --------TA | AAAAAATAGA | [92] |
| K. multiflora SR310 | AATCAAATTT | --GAtattat | GA--TT-- |  | --------TA | AAAAAATGGA | [86] |
| K. northiae SR263 | AATCAAATTT | --GAtattat | GA--TT |  | -TT | AAAAAATGGA | [86] |
| K. northiae SR274 | AATCAAATTT | --GAtattat | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. northiae SR446 | AATCAAATTT | --GATATTAT | GA--TT |  | T | AAAAAATGGA | [86] |
| K. pauciflora HBsn | AATCAAATTT | --GAtattat | GA--TTATGA |  | -TA | AAAAAATAGA | [92] |
| K. parviflora SR268 | AATCAAATTT | --GAtattat | GA--TT |  | -TT | AAAAAATGGA | [86] |
| K. parviflora SR330 | AATCAAATTT | --GAtattat | GA--TTATGA |  | T | AAAAAATAGA | [92] |
| K. porphyantha SRsn | AATCAAATTT | --GAtattat | GA--TT---- |  | TA | AAAAAATGGA | [86] |
| K. praecox SR529 | AATCAAATTT | --GAtattat | GA--TT- |  | TA | AAAAAATGGA | [82] |
| K. praecox SR530 | AATCAAATTT | --GAtattat | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. praecox SR532 | AATCAAATTT | --GATATtAT | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. rigidifolia SRsn | AATCAAATTT | --GAtattat | GA--TT- |  | TA | AAAAAATGGA | [86] |
| K. ritualis SR300 | AATCAAATTT | --GAtattat | GA--TTATGA | TT- | TA | AAAAAATAGA | [92] |
| K. rooperi SR237 | AATCAAATTT | --GAtattat | GA--TT |  | --------TT | AAAAAATGGA | [86] |
| K. rooperi SR485 | AATCAAATTT | --GAtattat | GA--TT |  | --------TA | AAAAAATGGA | [86] |
| K. rooperi SR528 | AATCAAATTT | --GAtattat | GA--TT- |  | --------TA | AAAAAATGGA | [86] |
| K. rooperi RAL4227 | AATCCAATTT | --GAtattat | GA--TTATGA |  | --------TA | AAAAAATAGA | [92] |
| K. rooperi TD4559 | AATCCAATTT | --GAtattat | GA--TTATGA | TT-------- | --------TA | AAAAAATAGA | [92] |
| K. sarmentosa SR207 | AATCAAATTT | --GAtattat | GA--TT |  | --------TA | AAAAAATGGA | [86] |
| K. schemperi SR391 | AATCAAATTT | --GAtattat | GA--TT---- |  | --------TA | AAAAAATGGA | [86] |
| K. schimperi JMG036 | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. splendida SR548 | AATCAAATTT | --GAtattat | GA--TT |  | -AA | AAAAAATGGA | [86] |
| K. Splendida Chapman 9061 | AATCAAATTT | --GAtattat | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. stricta SR279 | AATCAAATTT | --GAtattat | GA--TTATGA |  | -TA | AAAAAATAGA | [92] |
| K. thodei SR407 | AATCAAATTT | --GAtattat | GA--TTATGA |  | -TA | AAAAAATAGA | [92] |
| K. thomsonii JMG031 | AATCAAATTT | --GATATTAT | GA--TT |  | -TA | AAAAAATGGA | [86] |
| K. thomsonii AMM2647 | AATCAAATTT | --GATATtAT | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. thomsonii CK4821 | AATCAAATTT | --GAtattat | GA--TT |  | TA | AAAAAATGGA | [86] |
| K. triangularis SR264 | AATCAAATTT | --GAtattat | GA--TT |  | -TT | TAAAAATGGA | [86] |
| K. triangularis SR266 | AATCAAATTT | --GAtattat | GA--TT- |  | T | AAAAAATGGA | [86] |
| K. triangularis SR299 | AATCAAATTT | --GAtattat | GA--TTATGA | TT- | T | AAAAAATAGA | [92] |
| K. triangularia obtusiloba SRsn | AATCAAATTT | --GAtattat | GA--TT |  | - | AAAAAATGGA | [86] |
| K. typhoides NNBG | AATCAAATTT | --GAtattat | GA--TT- |  | -TA | AAAAAATGGA | [86] |
| K. tysonii SR302 | AATCAAATTT | --GAtattat | GA--TTATGA | TT | -TA | AAAAAATAGA | [92] |
| K. tysonii SR303 | AATCAAATTT | --GAtattat | GA--TTATGA | TT | -TA | AAAAAATAGA | [92] |
| K tysonii SR460 | AATCAAATTT | --GAtattat | GA--TTATGA | T | T | AAAAAATAGA | [92] |
| K. umbrina RGsn | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. uvaria SR166 | AATCAAATTT | --GAtATtAT | GA--TT |  |  | AAAAAATGGA | [86] |
| K. uvaria SR186 | AATCAAATTT | --GAtattat | GA--TT---- |  |  | AAAAAATGGA | [86] |
| K. uvaria SR172 | AATCAAATTT | --GAtattat | GA--TT---- |  |  | AAAAAATGGA | [86] |
| K. uvaria SR201 | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. uvaria SR203 | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. uvaria SR211 | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. uvaria SR337 | AATCAAATTT | --GAtattat | GA--TT |  | -TT | AAAAAATGGA | [86] |
| K. uvaria SR342 | AATCAAATTT | --GAtattat | GA--TT |  | -TT | TAAAAATGGA | [86] |
| K. uvaria SR344 | AATCAAATTT | --GAtattat | GA--TT |  | - | AAAAAATGGA | [86] |
| K. uvaria SR471 | AATCAAATTT | --GATATTAT | GA--TT |  | - | AAAAAATGGA | [86] |
| K. uvaria SR477 | AATCAAATTT | --GAtattat | GA--TT |  |  | AAAAAATGGA | [86] |
| K. uvaria TD4477 | AATCAAATTT | --GAtATtAT | GA--TT---- |  |  | AAAAAATGGA | [86] |
| [ |  | 130 | 140 | 150 | 160 | 170 | $180]$ |
| [ |  |  |  |  |  | . |  |
| Bulbine latifolia SR61 | AtGAtttcte | AgAATAT--- | --ttatanai | AAttttgtta | A-----tTAT | Attatagg g | [163] |
| Bulbinella cauda-felis SR204 | Attatticti | AGAAAAGAAA | agttatanca | AAttitgcta | A-----TTAT | Attatagg g | [162] |
| K. acreae TD4626 | Attatticti | AGAATAG--- | --TTATAACA | AAttttgcta | A-----TTAT | Attatagg g | [136] |
| K. albescens SR314 | Attattictc | AGAATAG--- | --ttatanca | AAttttgcta | A-----tTAT | Attatagg g | [136] |
| K. albomontana SR149 | Attatticti | AGAATAG--- | --ttatanca | AAttttgcta | A-----TTAT | Attatagg | [142] |
| K. angustifolia SR453 | Attattictc | AGAATAG--- | --ttatanca | AATtttgcta | A-----tTAT | Attatagg g | [136] |
| K. angustifolia SR542 | Attatticti | AGAATAG--- | --tTATAACA | AAttttgcta | A-----TTAT | Attatagg g | [142] |
| K. ankaratrensis PBP5676 | Attattictc | AGAATAG--- | --ttatanca | AAttttgcta | A-----TTAT | Attatagg g | [136] |
| K. baurii SR174 | Attatticti | AGAATAG--- | --ttatanca | AAttttgcta | A-----TTA | Attatagg g | [142] |
| K. baurii SR202 | Attatticti | AGAATAG--- | --ttatanca | AATtTtGCTA | A-----tTAT | Attatagg g | [136] |
| K. baurii SR275 | Attattictc | AGAATAG--- | --ttatanca | AATtTtGCTA | A-----TTAT | Attatagg | [142] |
| K. baurii SR285 | ATtATtTCTC | AGAATAG--- | --ttatanca | AAttttgcta | A-----TTAT | Attatagg | [142] |
| K. baurii SR360 | ATTATTTCTC | AGAATAG- | --ttatanca | AATTTTGCT |  | ATtATAGGGG | [136] |


|  | baurii SR382 <br> baurii SR398 |
| :---: | :---: |
|  | . baurii NPB1923 |
|  | . baurii RJM1026 |
| K. | . bracystachya SRsn |
| K. | . breviflora SR452 |
| K. | . bruceae SR171 |
| K. | . buchananii SR305 |
| K. | . buchananii SR307 |
| K. | . buchananii SR458 |
| K. | . caulescens SR270 |
| K. | . caulescens SR278 |
| K. | . caulescens NPB1821 |
|  | . caulescens RJM974 |
|  | . citrina SR176 |
|  | . coddiana SRsn |
|  | . corraligemma SR549 |
|  | . drepanophylla RAL4816 |
|  | . drepanophylla RJM1100 |
|  | . ensifolia autumnalis SR448 |
|  | . ensifolia ensifolia JBsn |
|  | . fibrosa SR297 |
|  | . fibrosa PBP5579 |
|  | . foliosa SR383 |
|  | . foliosa SR387 |
|  | . foloisa SR389 |
|  | . foliosa SR390 |
|  | . foliosa JMG034 |
|  | . foliosa JMG038 |
|  | . galpinii SR312 |
|  | . gracilis SR321 |
|  | . gracilis SR561 |
|  | . gracilis NNBG |
|  | . grantii CP4154 |
|  | . hirsuta SR282 |
|  | . ichopensis SR242 |
|  | . ichopensis SR286 |
|  | . ichopensis SR289 |
|  | . ichopensis SR409 |
|  | . insignis SRsn |
|  | . isoetifolia SR386 |
|  | . isoetifolia SR388 |
|  | . isoetifolia SR393 |
|  | . latifolia RSSsn |
|  | . laxiflora SR283 |
|  | . laxiflora SR295 |
|  | . laxiflora SR441 |
|  | . laxiflora SR442 |
|  | . laxiflora SR467 |
|  | . laxiflora SR468 |
|  | . laxifloraC SRsn |
|  | . laxiflora NPB1810 |
|  | . leucocephala NNBG |
|  | . linearifolia SR269 |
|  | . linearifolia SR287 |
|  | . linearifolia SR290 |
|  | . linearifolia SR291 |
|  | . linearifolia SR311 |
|  | . linearifolia SR328 |
|  | . linearifolia SR343 |
|  | . linearifolia SR400 |
|  | . linearifolia SR558 |
|  | . linearifolia JPsn |
|  | . linearifolia TD4638 |
|  | . littoralis SR200 |
|  | . multiflora SR310 |
|  | . northiae SR263 |
|  | . northiae SR274 |
|  | . northiae SR446 |
|  | . pauciflora HBsn |
|  | . parviflora SR268 |
|  | . parviflora SR330 |
|  | . porphyantha SRsn |
|  | . praecox SR529 |
|  | . praecox SR530 |
|  | . praecox SR532 |
|  | . rigidifolia SRsn |
|  | . ritualis SR300 |
|  | . rooperi SR237 |
|  | . rooperi SR485 |
|  | . rooperi SR528 |
|  | . rooperi RAL4227 |
|  | . rooperi TD4559 |
|  | . sarmentosa SR207 |
|  | . schemperi SR391 |
|  | . schimperi JMG036 |
|  | . splendida SR548 |
|  | . splendida Chapman 9061 |
|  | . stricta SR279 |
|  | . thodei SR407 |
|  | . thomsonii JMG031 |
|  | . thomsonii AMM2647 |
|  | . thomsonii CK4821 |
|  | . triangularis SR264 |

    baurii SR382
    baurii
SR398
baurii NPB1923
baurii RJM1026
bracystachya SRsn
breviflora SR4
buchananii SR30
buchananii SR307
buchananii SR458
caulescens SR270
caulescens NPB1821
caulescens RJM974
coddiana SRsn
corraligemma SR549
repanophylla RAL4816
nsifolia autumnalis SR448
nsifolia ensifolia JBsn
ibrosa SR29
fibrosa PBP5579
oliosa SR383
foloisa SR389
foliosa SR390
oliosa JMG034
galpinii SR312
gracilis SR321
gracilis SR561
gracilis NNBG
hrantil CP415
ichopensis SR242
ichopensis SR289
ichopensis SR409
insignis SRsn
isoetifolia SR386
isoetifolia SR388
isoetifolia SR393
latifolia RSSsn
axiflora SR283
laxiflora SR441
axiflora SR442
laxiflora SR467
axiflora SR468
axiflora NPB1810
eucocephala NNBG
nearifolia SR269
inearifolia SR290
inearifolia SR291
inearifolia SR311
linearifolia SR328
inearlfolia SR343
inearifolia SR558
inearifolia JPsn
inearifolia TD463
ittoralis SR200
multiflora SR31
northiae SR26
orthiae SR44
pauciflora HBsn
arviflora SR268
arviflora SR330
praecox SR529
praecox SR530
rigidifolia SRsn
ritualis SR300
rooperi SR237
rooperi SR485
rooperi RAL4227
ooperi TD4559
sarmentosa SR207
schemperi SR391
schimperi JMG036
splendida SR548
plendida Chapman 9061
stricta SR27
homsonii JMG031
homsoni1 AMM261
riangularis SR264

ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG TTATTTCTC AGAATAG-_- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG TTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG

 TTTATTTCTC AGAATAG--- --TTATAACA AATTITGCIA A------1TAT ATIATAGGG ATTATIICIC AGAATAG--- --1TATAACA AATITIGCIA A-----1TAI ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --ITAIAACA AATITIGCTA A-----1TAT ATTATAGGG TTATTTCTC AGAATAG--- --11ATAACA AATHTIGCIA ATIATATIAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTAITTCTC AGAATAG--- --TIATAACA AATITIGCTA A------1TAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG-_- _-TTATAACA AATTTTGCTA A-_-_-TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATATTTTGCTA A-------TTAT ATATATATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------TTAT ATTATAGGGG ATTATTTCTC AGATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG-_- _-TTATAACA AATTTTGCTA A ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG GAMC ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG - TTATAACA AATTTTGCTA A ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATATTTCTC AGATAG--- --TTATAACA AATTTTGCTA A -----TTAT ATTATAGGGG

 MATC ATATC mata ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATITCTC AGAATAG--- --TTATAACA AATYITGCTA A-----11AT ATHATAGGGG ATTATICTC AGAATAG---11ATAACA AAITIGCIA A-- ITAI ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTITGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------1TAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------TTAT ATTATAGGGG ATTATTTCTC AGAATAG-_- _-TTATAACA AATTTTGCTA A--_--TTAT ATTATAGGGG ATTATTTCTC AGAATAG-_- _-TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTCTC AGAATAG--- _-TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A -----TTAT ATTATAGGGG ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG ATATTCTC AGAATAG--- --TTATAACA AATTTGCTA A-----TTAT ATTATAGGG ATATTTCTC AGAATAG--_ --TTATAACA AATTTGCTA A-----TTAT ATTATAGGGG ATMTTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A------TTAT ATTATAGGG ATAFC ATATTTCTC AGAATAG--- - TTATAACA ATTTTGCTA A ---- TTAT ATTATAGGG
 ATTATTTCTC AGAATAG--- --TTATAACA AATTTTGCTA A-----TTAT ATTATAGGGG

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| K. triangularis SR266 | Attattictc agatag | tatanca | AAttttgcta | ttat | AttatagGg | $36]$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| K. triangularis SR299 | ATtATtTCTC AGAATAG | --Ttatanca | AATTTTGCTA | A-----TTAT | ATtATAGGGG | [142] |
| K. triangularia obtusiloba SRsn | ATTATTTCTC AGAATAG | TTATAACA | AATTTTGCTA | A-----TTAT | AttatagGg | [136] |
| K. typhoides NNBG | Attattictc agattag | ttatanca | AATtTtGCTA | A-----TTAT | Attataggeg | [136] |
| K. tysonii SR302 | ATtATtTCTC AgAATAG | --ttatanca | AAttttgcta | A-----TTAT | Attatagg | [142] |
| K. tysonii SR303 | ATTATTTCTC AGAATAG | --ttatanca | AAttttgcta | A-----TTAT | TATAGGGG | [142] |
| K. tysonii SR460 | ATtATtTCTC AgAATAG | ttatamca | AAttttgcta | A-----TTAT | Attatagg | [142] |
| K. umbrina RGsn | ATTATTTCTC AGAATAG | ttatamca | AAttttgcta | A-----TTAT | tatagGg | [136] |
| K. uvaria SR166 | ATtATtTCTC AgAATAG | ttatanca | AATtTTGCT | A-----TTAT | AGGGg | [136] |
| K. uvaria SR186 | Attattictc agantag | --ttatanca | AAttttgc | A-----TT | ATTATAGGGG | [136] |
| K. uvaria SR172 | ATTATTTCTC AGAATAG | --ttatanca | AAttttgcta | A-----TT | Attatagg g | [136] |
| K. uvaria SR201 | Attattictc agaitag | --ttatanca | AATtTtGCTA | A-----TT | AttatagGg | [136] |
| K. uvaria SR203 | ATTATTTCTC AGAATAG | --ttatanca | AAttttgcta | A-----TTA | Attatagg | [136] |
| K. uvaria SR211 | Attatttctc agantag | --ttatanca | AAttttgcta | A-----TTAT | Attatagg | [136] |
| K. uvaria SR337 | ATtATtTCTC AGAATAG | --ttatanca | AAttitgcta | A-----11 | Attatagg | [136] |
| K. uvaria SR342 | Attatticte agatag | --ttatanca | AAttttgcta | A-----TTAT | Attatagg | [136] |
| K. uvaria SR344 | Attattictc agatag | --ttatanca | AATtTtGCTA | ttat | AttatagGg | [136] |
| K. uvaria SR471 | ATTATTTCTC AGAATAG | --ttatanca | AATtTtGCTA | -ttat | AttatagGg | [136] |
| K. uvaria SR477 | Attattictc agattag | --ttatanca | AAttttgcta | -tTAT | Attatagg | [136] |
| K. uvaria TD4477 | ATtATtTCTC AGAATAG- | --ttatanca | AAttttgcta | A-----TTAT | AttatagGg | [136] |
| [ | 190 | 200 | 210 | 220 | 230 | $240]$ |
| [ |  |  |  |  | ] |  |
| Bulbine latifolia SR61 | ATCGGCCCta AgAAAAGTAT | AAGATAAGGA | -TAAAGAT | AATCAAAATT | ctaatgcgac | [222] |
| Bulbinella cauda-felis SR204 | ATAGAGCCTA CGAAAAGTAT | AAGAtAAGGA | ttanagatac | AATCAAAATT | ctantgcaac | [222] |
| K. acreae TD4626 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. albescens SR314 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatgcgac | [195] |
| K. albomontana SR149 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAATGCGAC | [201] |
| K. angustifolia SR453 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. angustifolia SR542 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. ankaratrensis PBP5676 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | Ctatgcgac | [195] |
| K. baurii SR174 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. baurii SR202 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. baurii SR275 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATTC | AATCAAAATT | Ctatatgcal | [201] |
| K. baurii SR285 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. baurii SR360 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CtaAtgccal | [195] |
| K. baurii SR382 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAgatac | AATCAAAATT | ctantgcgac | [195] |
| K. baurii SR398 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | ctantgcgac | [201] |
| K. baurii NPB1923 | AtCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAgatac | AATCAAAATT | ctantgcgac | [195] |
| K. baurii RJM1026 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CtAATGCGAC | [195] |
| K. bracystachya SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatatcgac | [195] |
| K. breviflora SR452 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatgcgac | [195] |
| K. bruceae SR171 | ATCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -TAAAGAT | AATCAAAA | ctantgcgac | [201] |
| K. buchananii SR305 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATA | AATCAAAAT | Ctatatgcal | [201] |
| K. buchananii SR307 | AtCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -taAAGATA | AATCAAAATT | Ctatatgcal | [201] |
| K. buchananii SR458 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGAT | AATCAAAATT | Ctatatgchac | [201] |
| K. caulescens SR270 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | ctatatgcgac | [195] |
| K. caulescens SR278 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. caulescens NPB1821 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatgcgac | [201] |
| K. caulescens RJM974 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | Ctatgcgac | [200] |
| K. citrina SR176 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAAtGCGAC | [195] |
| K. coddiana SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAAtGCGAC | [201] |
| K. corraligemma SR549 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CtaAtgccal | [195] |
| K. drepanophylla RAL4816 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CtaAtgccat | [201] |
| K. drepanophylla RJM1100 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAATGCGAC | [201] |
| K. ensifolia autumnalis SR448 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CtAATGCGAC | [195] |
| K. ensifolia ensifolia JBsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatgcgac | [195] |
| K. fibrosa SR297 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatgcgac | [201] |
| K. fibrosa PBP5579 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | Ctatgcgac | [195] |
| K. foliosa SR383 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | Ctatgcgac | [195] |
| K. foliosa SR387 | AtCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -taAAgatac | AATTAAAATT | ctantgcgac | [195] |
| K. foloisa SR389 | AtCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -taAAgatac | AATGAAAATT | Ctantgcgac | [195] |
| K. foliosa SR390 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATGAAAATT | CTAATGCGAC | [195] |
| K. foliosa JMG034 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATTAAAATT | Ctatatgchac | [195] |
| K. foliosa JMG038 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATTAAAATT | CTAATGCGAC | [195] |
| K. galpinii SR312 | GTCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. gracilis SR321 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAATGCGAC | [201] |
| K. gracilis SR561 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. gracilis NNBG | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. grantii CP4154 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. hirsuta SR282 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. ichopensis SR242 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. ichopensis SR286 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. ichopensis SR289 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. ichopensis SR409 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. insignis SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. isoetifolia SR386 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATTAAAATT | CTAATGCGAC | [195] |
| K. isoetifolia SR388 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATGAAAATT | CTAATGCGAC | [195] |
| K. isoetifolia SR393 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATTAAAATT | CTAATGCGAC | [195] |
| K. latifolia RSSsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAATGCGAC | [201] |
| K. laxiflora SR283 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. laxiflora SR295 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAATGCGAC | [201] |
| K. laxiflora SR441 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [197] |
| K. laxiflora SR442 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CtAATGCGAC | [201] |
| K. laxiflora SR467 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. laxiflora SR468 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAgatac | AATCAAAATT | Ctantgcgac | [201] |
| K. laxifloraC SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. laxiflora NPB1810 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [197] |
| K. leucocephala NNBG | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. linearifolia SR269 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. linearifolia SR287 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. linearifolia SR290 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. linearifolia SR291 | ATCGGTCCTA AgAAAAGT | AGG | -taAAgatac | AATCAAAATT | CtAATGCG | [201] |


| K. linearifolia SR311 | ATCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | 95] |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| K. linearifolia SR328 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. linearifolia SR343 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. linearifolia SR400 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. linearifolia SR558 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. linearifolia JPsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. linearifolia TD4638 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. littoralis SR200 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. multiflora SR310 | GTCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. northiae SR263 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. northiae SR274 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. northiae SR446 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | taAtgcgac | [195] |
| K. pauciflora HBsn | ATCGGTCCTA AgAAAAGTAT | AAGATAAGGA | -taAdGAta | AATCAAAATT | AAtGCGAC | [201] |
| K. parviflora SR268 | ATCGGTCCTA AGAAAAGTA | AAGATAAGGA | -taAAGATAC | AATCAAAATT | Aatgcgac | [195] |
| K. parviflora SR330 | AtcGgtccta aganangtat | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. porphyantha SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. praecox SR529 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [191] |
| K. praecox SR530 | ATCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. praecox SR532 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. rigidifolia SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. ritualis SR300 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. rooperi SR237 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. rooperi SR485 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. rooperi SR528 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -T-AAGATAC | AATCAAAATT | CTAATGCGAC | [194] |
| K. rooperi RAL4227 | AtCGGtccta aganangiat | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. rooperi TD4559 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAT | [201] |
| K. sarmentosa SR207 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAAT | CTAATGCGAC | [195] |
| K. schemperi SR391 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATGAAAATT | CTAATGCGAC | [195] |
| K. schimperi JMG036 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATTAAAATT | CTAATGCGAC | [195] |
| K. splendida SR548 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. splendida Chapman 9061 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. stricta SR279 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. thodei SR407 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. thomsonii JMG031 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATGAAAATT | CTAATGCGAC | [195] |
| K. thomsonii AMM2647 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. thomsonii CK4821 | AtcGgtccta aganangiat | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. triangularis SR264 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. triangularis SR266 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. triangularis SR299 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. triangularia obtusiloba SRsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. typhoides NNBG | ATCGGTCCTA AGAAAAGTAT | AAGAtAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. tysonii SR302 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [201] |
| K. tysonii SR303 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAAT | CTAATGCGAC | [201] |
| K. tysonii SR460 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAAT | taAtgcgac | [201] |
| K. umbrina RGsn | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGA | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR166 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGA | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR186 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAG | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR172 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAA | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR201 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR203 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR211 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -TAAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR337 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR342 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCCAAATT | CTAATGCGAC | [195] |
| K. uvaria SR344 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR471 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria SR477 | AtcGgtccta aganangtat | AAGATAAGGA | -taAAGATAC | AATCAAAATT | CTAATGCGAC | [195] |
| K. uvaria TD4477 | ATCGGTCCTA AGAAAAGTAT | AAGATAAGGA | -T-AAGATAC | AATCAAAATT | CTAATGCGAC | [194] |
| 1 | 250 | 260 | 270 | 280 | 290 | $300]$ |
| [ |  |  |  |  | ] |  |
| Bulbine latifolia SR61 | ATTCCTCTGA TTTCCTTGGA | AAAAGAAGAA | AATAGAGTG- | ---AAAAAAA | AAGAAGAAAG | [278] |
| Bulbinella cauda-felis SR204 | ATtCCttiga titccttgga | AAAAGAAGGA | AATGGGGC-- | -AAAAAAAAA | AAGAAGAAAT | [279] |
| K. acreae TD4626 | ATtCCTCTGA titccttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [252] |
| K. albescens SR314 | Attcctctia titccttaga | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AAtAAGAAAG | [251] |
| K. albomontana SR149 | Attcctctia titcctiaga | AAAAGAAGGA | AATAGGGCG- | - AAAAAAAAA | AAtAAGAAAG | [259] |
| K. angustifolia SR453 | Attcctctia titccttaga | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AAtAAGAAAG | [251] |
| K. angustifolia SR542 | Attcctctia titcctiaga | AAAAGAAGGA | AATAGGGCG- | - AAAAAAAAA | AAtAAGAAAG | [259] |
| K. ankaratrensis PBP5676 | ATTCCTCTGA TTTCCTTAGA | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [253] |
| K. baurii SR174 | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | AAAAAAAAAA | AATAAGAAAG | [260] |
| K. baurii SR202 | ATtCCTCTGA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [252] |
| K. baurii SR275 | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [259] |
| K. baurii SR285 | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [259] |
| K. baurii SR360 | Attcctctia titccttaga | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AAtAAGAAAG | [251] |
| K. baurii SR382 | ATTCCTCTTA TTTCCTTAGA | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AATAAGAAAG | [251] |
| K. baurii SR398 | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [259] |
| K. baurii NPB1923 | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AATAAGAAAG | [251] |
| K. baurii RJM1026 | ATTCCTCTGA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [252] |
| K. bracystachya SRsn | ATtCCTCTGA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [253] |
| K. breviflora SR452 | Attcctctia titcctiaga | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AAtAAGAAAG | [251] |
| K. bruceae SR171 | ATtCCTCTTA titccttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [258] |
| K. buchananii SR305 | Attcctctia titcctiaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AAtAAGAAAG | [259] |
| K. buchananii SR307 | ATtCCTCTTA tttccttaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [259] |
| K. buchananii SR458 | Attcctctia titcctiaga | AAAAGAAGGA | AATAGGGCG- | - AAAAAAAAA | AAtAAGAAAG | [259] |
| K. caulescens SR270 | Attcctcta titccttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [252] |
| K. caulescens SR278 | ATtCCTCTGA TTTCCTTAGA | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AAtAAGAAAG | [252] |
| K. caulescens NPB1821 | Attcctcta titccttaga | AAAAGAAGGA | AATAGGGCG- | ---AAAAAAA | AATAAGAAAG | [257] |
| K. caulescens RJM974 | Attcctcta titccttaga | AAAAGAAGGA | AATAGGGCG- | - AAAAAAAAA | AATAAGAAAG | [258] |
| K. citrina SR176 | Attcctcta titccttaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [253] |
| K. coddiana SRsn | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [258] |
| K. corraligemma SR549 | ATtCCTCTGA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [252] |
| K. drepanophylla RAL4816 | ATtCCTCTTA tTtCCttaga | AAAAGAAGGA | AATAGGGCG- | --AAAAAAAA | AATAAGAAAG | [258] |
| K. drepanophylla RJM1100 | Attcctctita titcctiaga | AAAAGAAGGA | AATAGGGCG- | -AAAAAAAAA | AATAAGAAAG | [259] |
| K. ensifolia autumnalis SR448 | ATtcctctea titccttaga | AAAAGAAGGA | AAtAGGGCG- | --AAAAAAA | AATAAGAAAG | [251] |


|  | ensifolia ensifolia JBsn <br> fibrosa SR297 |
| :---: | :---: |
|  | . fibrosa PBP5579 |
|  | . foliosa SR383 |
|  | . foliosa SR387 |
|  | . foloisa SR389 |
|  | . foliosa SR390 |
|  | . foliosa JMG034 |
|  | . foliosa JMG038 |
|  | . galpinii SR312 |
| K. | . gracilis SR321 |
|  | . gracilis SR561 |
|  | . gracilis NNBG |
|  | . grantii CP4154 |
|  | . hirsuta SR282 |
|  | . ichopensis SR242 |
|  | . ichopensis SR286 |
|  | . ichopensis SR289 |
|  | . ichopensis SR409 |
|  | . insignis SRsn |
|  | . isoetifolia SR386 |
|  | . isoetifolia SR388 |
|  | . isoetifolia SR393 |
|  | . latifolia RSSsn |
|  | . laxiflora SR283 |
|  | . laxiflora SR295 |
|  | . laxiflora SR441 |
|  | . laxiflora SR442 |
|  | . laxiflora SR467 |
|  | . laxiflora SR468 |
|  | . laxifloraC SRsn |
|  | . laxiflora NPB1810 |
|  | . leucocephala NNBG |
|  | . linearifolia SR269 |
|  | . linearifolia SR287 |
|  | . linearifolia SR290 |
|  | . linearifolia SR291 |
|  | . linearifolia SR311 |
|  | . linearifolia SR328 |
|  | . linearifolia SR343 |
|  | . linearifolia SR400 |
|  | . linearifolia SR558 |
|  | . linearifolia JPsn |
|  | . linearifolia TD4638 |
|  | . littoralis SR200 |
|  | . multiflora SR310 |
|  | . northiae SR263 |
|  | . northiae SR274 |
|  | . northiae SR446 |
|  | . pauciflora HBsn |
|  | . parviflora SR268 |
|  | . parviflora SR330 |
|  | . porphyantha SRsn |
|  | . praecox SR529 |
|  | . praecox SR530 |
|  | . praecox SR532 |
|  | . rigidifolia SRsn |
|  | . ritualis SR300 |
|  | . rooperi SR237 |
|  | . rooperi SR485 |
|  | . rooperi SR528 |
|  | . rooperi RAL4227 |
|  | . rooperi TD4559 |
|  | . sarmentosa SR207 |
|  | . schemperi SR391 |
|  | . schimperi JMG036 |
|  | . splendida SR548 |
|  | . splendida Chapman 9061 |
|  | . stricta SR279 |
|  | . thodei SR407 |
|  | . thomsonii JMG031 |
|  | . thomsonii AMM2647 |
|  | . thomsonii CK4821 |
|  | . triangularis SR264 |
|  | . triangularis SR266 |
|  | . triangularis SR299 |
|  | . triangularia obtusiloba SRsn |
|  | . typhoides NNBG |
|  | . tysonii SR302 |
|  | . tysonii SR303 |
|  | tysonii SR460 |
|  | . umbrina RGsn |
|  | . uvaria SR166 |
|  | . uvaria SR186 |
|  | . uvaria SR172 |
|  | . uvaria SR201 |
|  | . uvaria SR203 |
|  | . uvaria SR211 |
|  | . uvaria SR337 |
|  | . uvaria SR342 |
|  | . uvaria SR344 |
|  | . uvaria SR471 |
|  | . uvaria SR477 |
|  | . uvaria TD4477 |

ensifolia ensi
fibrosa SR297
ibrosa PBP5579
foliosa SR383
foliosa SR387
foloisa SR389
oliosa SR390
foliosa JMG038
galpinii SR312
gracilis SR32
gracilis SR56
grantii CP415
hirsuta SR282
ichopensis SR242
ichopensis SR286
ichopensis SR289
insignis SRsn
isoetifolia SR386
isoetifolia SR388
atifolia SRSn
laxiflora SR283
laxiflora SR295
laxiflora SR441
laxiflora SR467
laxiflora SR468
laxiflora NPB1810
leucocephala NNBG
inearifolia SR287
Iinearifolia SR290
linearifolia SR291
linearifolia SR311
linearifolia SR328
linearifolia SR343
inearifolia SR558
linearifolia JPsn
inearifolia TD4638
ittoralis SR200
northiae SR263
northiae SR27
northiae SR44
pauciflora HBsn
parviflora SR330
porphyantha SRsn
praecox SR529
praecox SR532
rigidifolia SRsn
ritualis SR300
rooperi SR237
rooperi SR528
rooperi RAL4227
sarmentosa SR207
schemperi SR391
splendida SR548
splendida Chapman 9061
stricta SR27
thomsonii JMGO31
thomsonii CK4821
triangularis SR264
triangularis SR266
triangularia obtusiloba SRsn
typhoides NNBG
tysonii SR302
tysonii SR303
ysonii SR460
uvaria SR166
uvaria SR186
uvaria SR172
uvaria SR201
varia SR203
varia SR211
varia SR337
uvaria SR342
varia SR47
varia SR477
uvaria TD4477

ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG AIICICIGA信 AHCCM AHCCI ATCCT MCCO TICCICIA ITYCCIMAGA AAAAGAAGGA AATAGGGG - AAAAAA AATAAGAAAG TICCTCTTA TTICCTIAGA AAAAGAAGGA AAIAGGGCG- -AAAAAAAAA AAIAAGAAAG ATCCCTCTTA ITICCTIAGA AAAAGAAGGA AAIAGGGCG- --AAAAAAAA AATAAGAAA AITCCICITA ITICCIIAGA AAAAGAAGGA AAIAGGGCG- AAAAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAA ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCGA AAAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAA ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAA ATTCCTCTTA 11TCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAA ATICCICTIA $11 T C C I T A G A$ AAAAGAAGGA AATAGGGCG- AAAAAAAAAA AATAAGAAAG ATTCCTCTGA TITCCTIAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATICCICTGA TITCCITAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- AAAAAAAAAAA AATAAGAAA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG Attcctctta tttccttaga Aanagangga antagggccg- -AAAAAAAAA AAtanganag ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAA ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- AAAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG Attcctctta tutccttaga Aanagangga Aatagggcg- AAAAAAAAAA AAtAAgAAAg
 ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAA AATAAGAAAG TCCTCTA TTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- AAAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAA AATAAGAAAG ATCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- - AAAAAAAA AATAAGAAAG A ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG -AAAAAAAA AATAAGAAAG ATCCTCTGA TTTCCTTAGA AAAGAGGA AABGGCCG- AAAAAAAAA AATAGAA ATTCCTCTGA TTCCTTAGA AAAAGAAGGA AATAGGGCG---AAAAAAAA AATAAGAAA MAGCA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG AHA ATCCMA GCCCM ATICCTCTGA TTTCCTIAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ITCCTCTGA TITCCITAGA AAAAGAAGGA AAIAGGGCG- --AAAAAAAA AAIAAGAAAG ATICCTCTGA TTICCITAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AAIAAGAAAG ATTCCTCTTA TTTCCITAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAAA AATAAGAAA ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATTCCTCTTA TITCCTIAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAA ATICCTCTGA $11 T c C T I A G A$ AAAAGAAGGA AATAGGGG- AAAAAAAAAA AATAAGAAAG Attcctctga tttccttaga anangaigga antagggcg- -AAAAAAAAA AAtanganag ATICCTCTGA T1TCCITAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAAA AATAAGAAA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATtCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAAG Attcctctta tttccttaga anaigangga antagggcga ananananan antanganag ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- AAAAAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- AAAAAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ----AAAAAAA AATAAGAAAG Attcctctga tttccttaga Aanagangga antagggcg- ---AAAAAAA AAtangaiag ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- ---AAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG Attcctctta tutccttaga Aanagangga Aatagggccg- --AAAAAAAA AAtAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -AAAAAAAAA AATAAGAAAG ATTCCTCTTA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- - - AAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG TTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG
 ATCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- -- AAAAAAAA AATAAGAAA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG AMA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG ATCCTCTIA TTCCTTAGA AAAAGAAGGA AATAGGGCG - AAAAAAA AATAAGAAA ATTCCTCTGA TTTCCTTAGA AAAAGAAGGA AATAGGGCG- --AAAAAAAA AATAAGAAAG
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|  |  | 310 | 320 | 330 | 340 | 350 | $360]$ |
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| [ |  |  |  |  |  | ] |  |
| Bulbine latifolia SR61 | AATATCGCCC | ttttcagtat | TCCAAATCGC | GAtGtaAAAA | CGAAAAAAAA | AAGGGGGGGG | [338] |
| Bulbinella cauda-felis SR204 | AATATCGACC | CTTTCAGTAT | TGCAAATTGC | GATGTAAAA- | --AAAAA | GAGGGGG | [330] |
| K. acreae TD4626 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | GATGTGAAAC | CGAAAAA | -GGGGGGGG | [307] |
| K. albescens SR314 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | gatgtanal | CGAA | GGGGGGGG | [304] |
| K. albomontana SR149 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | gatgtanaic | CG | GGGGGGG | [312] |
| K. angustifolia SR453 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | GGGGGGGG | [304] |
| K. angustifolia SR542 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | GGGGGGG | [312] |
| K. ankaratrensis PBP5676 | AATATTGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAAA | -GGGGGGGGG | [309] |
| K. baurii SR174 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAA | GG | [313] |
| K. baurii SR202 | Agtattgatc | Ctttcagtat | tCCAAATCGC | GATGTAAAAC | CGAAAAAA | GGGGGGGg | [308] |
| K. baurii SR275 | AAtATCGATC | CTITCAGTAT | TCCAAATCGC | AATGTAAAAC | CG | GGGGGGg | [311] |
| K. baurii SR285 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | AATGTAAAAC | CG | GG | [311] |
| K. baurii SR360 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGg | [303] |
| K. baurii SR382 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | gatgtanaic | CGAAA | GGGGGGG | [303] |
| K. baurii SR398 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAA | GGGGGGG | [312] |
| K. baurii NPB1923 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | gatgtanal | CGAAA | GGGGGGG | [303] |
| K. baurii RJM1026 | AGTAttgatc | CTITCAGTAT | TCCAAATCGC | gatgtanal | CGAAAAAA | -GGGGGGGg | [308] |
| K. bracystachya SRsn | AGTATtGATC | CTtTCAGTAT | TCCAAATCGC | gatgtanal | CGAAAAAAA- | -GGGGGGGg | [310] |
| K. breviflora SR452 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | gatgtanaic | CG | GGGGGGGG | [304] |
| K. bruceae SR171 | AATATCGATC | Ctttcagtat | tccaiatcge | AATGTAAAAC | CGAA | GGGGGGG | [310] |
| K. buchananii SR305 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | GGGGGGG | [312] |
| K. buchananii SR307 | AATATCGATC | Ctttcagtat | tCCAAATCGC | GATGTAAAAC | CGAA | GG | [312] |
| K. buchananii SR458 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAA | GGGGGGG | [312] |
| K. caulescens SR270 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAA | GGGGGGG | [306] |
| K. caulescens SR278 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAA | GGGGGGGG | [306] |
| K. caulescens NPB1821 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGA | GGGGGGG | [310] |
| K. caulescens RJM974 | AATATCGATC | CTTTCAGTAT | TCCAAATCGC | GATGTGAAAC | CGAA | GGGGGGGG | [312] |
| K. citrina SR176 | AGTATtGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA | -GGGGGGGGG | [310] |
| K. coddiana SRsn | AATATCGATC | CTITCAGTAT | TCCAAATCGC | AATGTAAAAC | CGAAA | GGGGGGG | [310] |
| K. corraligemma SR549 | AATATtGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAAAA | -GGGGGGGG | [308] |
| K. drepanophylla RAL4816 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | AATGTAAAAC | CGAAA | GGGGGGG | [310] |
| K. drepanophylla RJM1100 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | GGGGGGG | [312] |
| K. ensifolia autumnalis SR448 | AATATCGATC | CTtTCAGTAT | tCCAAATCGC | GATGTAAAAC | CGAA | GGGGGGGG | [304] |
| K. ensifolia ensifolia JBsn | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGA | GGGGGGGG | [304] |
| K. fibrosa SR297 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGA | -GGGGGGGGG | [313] |
| K. fibrosa PBP5579 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAAAA--- | GG | [307] |
| K. foliosa SR383 | AATATtGAtC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | GG | [308] |
| K. foliosa SR387 | AAtAttgatc | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA | GGGGGGg | [308] |
| K. foloisa SR389 | AATATtGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA- | -GGGGGGGG | [310] |
| K. foliosa SR390 | AAtattgatc | CTtTCAGTAT | tCCAAATCGC | gatgtanaic | CGAAAAAA | -GGGGGGGg | [310] |
| K. foliosa JMG034 | AATATtGATC | CTTTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAAAA- | --GGGGGGGG | [309] |
| K. foliosa JMG038 | AATATTGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA-- | --GGGGGGGG | [309] |
| K. galpinii SR312 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGG | [303] |
| K. gracilis SR321 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAA | GGGGGGG | [312] |
| K. gracilis SR561 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAA | -GGGGGGGGG | [312] |
| K. gracilis NNBG | AATATCGATC | Ctttcagtat | TCCAAATCGC | gatgtanal | CGAAAA | -GGGGGGG | [313] |
| K. grantii CP4154 | AGTATtGATC | CTtTCAGTAT | tCCAAATCGC | gatgtanaic | CGAAAAAAA- | GGGGGGG | [308] |
| K. hirsuta SR282 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CG | GGG | [314] |
| K. ichopensis SR242 | AATATCGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAAC | CGA | GGGGGGG | [312] |
| K. ichopensis SR286 | AATATCGACC | Ctttcagtat | tCCAAATCGC | GATGTAAAAC | CGAA | -GGGGGGGGg | [312] |
| K. ichopensis SR289 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGA | GGGGGG | [312] |
| K. ichopensis SR409 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | GGGGGGG | [313] |
| K. insignis SRsn | AATATtGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAAAA | -GGGGGGGg | [308] |
| K. isoetifolia SR386 | AATATtGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA- | --GGGGGGGG | [308] |
| K. isoetifolia SR388 | AATATtGATC | CTTTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAAAA- | --GGGGGGGG | [310] |
| K. isoetifolia SR393 | AATATtGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAAAA | -GGGGGGGG | [308] |
| K. latifolia RSSsn | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGG | [312] |
| K. laxiflora SR283 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGG | [311] |
| K. laxiflora SR295 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | -GGGGGGG | [311] |
| K. laxiflora SR441 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGG | [308] |
| K. laxiflora SR442 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGG | [312] |
| K. laxiflora SR467 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | GGGGGGGGGG | [315] |
| K. laxiflora SR468 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | GGGGGGGGGG | [315] |
| K. laxifloraC SRsn | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA- | --GGGGGGGG | [304] |
| K. laxiflora NPB1810 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGGG | [310] |
| K. leucocephala NNBG | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGg | [305] |
| K. linearifolia SR269 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAAAA | --GGGGGGGg | [307] |
| K. linearifolia SR287 | AATATCGATC | CTTTCAGTAT | TCCAAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGG | [311] |
| K. linearifolia SR290 | AATATCGATC | CTTTCAGTAT | TCCAAAATCGC | GATGTAAAAC | CGAAAA | --GGGGGG | [311] |
| K. linearifolia SR291 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | ---GGGGGGG | [313] |
| K. linearifolia SR311 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAA | --GGGGGGGG | [304] |
| K. linearifolia SR328 | AATATCGATC | Ctttcagtat | TCCAAATCGC | AATGTAAAAC | CGAAA | ---GGGGGGG | [310] |
| K. linearifolia SR343 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAAAA | --GGGGGGGG | [307] |
| K. linearifolia SR400 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | -GGGGGGG | [310] |
| K. linearifolia SR558 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGGG | [312] |
| K. linearifolia JPsn | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | --GGGGGGg | [313] |
| K. linearifolia TD4638 | AATATCGATC | Ctttcagtat | TCCAAATCGC | AATGTAAAAC | CGAAA | ---GGGGGGG | [309] |
| K. littoralis SR200 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA | --GGGGGGg | [311] |
| K. multiflora SR310 | AATATCGATC | CTtTCAGTAT | TCCAAATCGC | GATGTAAAAC | CGAAA | --GGGGGGGG | [304] |
| K. northiae SR263 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAAAA | -GGGGGGGGG | [309] |
| K. northiae SR274 | AATATCGATC | CTTTCAGTAT | TCCAAATCGC | GATGTGAAAC | CGAAAA | --GGGGGGGG | [306] |
| K. northiae SR446 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAAA | -GGGGGGg | [305] |
| K. pauciflora HBsn | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAA- | ---GGGGGGG | [312] |
| K. parviflora SR268 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTGAAAC | CGAAAAA --- | --GGGGGGGG | [307] |
| K. parviflora SR330 | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA | -GGGGGGGGG | [312] |
| K. porphyantha SRsn | AATATCGATC | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAA----- | --GGGGGGGG | [304] |
| K. praecox SR529 | AGTAttgatc | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA-- | --GGGGGGGG | [304] |
| K. praecox SR530 | AGTAttgatc | Ctttcagtat | TCCAAATCGC | GATGTAAAAC | CGAAAAAA-- | --GGGGGGGG | [308] |
| K. praecox SR532 | Agtattgatc | Ctttcagtat | tCCAAATCGC | GATGTAAAAC | CGAAAAAA-- | --GGGGGGGG | [308] |
| K. rigidifolia SRsn | AATATCGATC | CTITCAGTAT | TCCAAATCGC | GATGTAAAA | CGA | --GGGGGGGG | [304] |



AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA----- -GGGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- -- - GGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA--------- AGTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAA-- -GGGGGGGGG
 AGTATTGATC CTHCAT TCCAAATCGC GATGTAAAAC CGAAAAAAA- - GGGGGGG GFATGATC CICAGT TCAAATCGC GAGGAAAC CGAAAAAA AAMTGAC CTTCAGT AAIAITGATC CTITCAGIAT ICCAAATCGC GATGIAAAAC CGAAAAAA--GGGGGG AATATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAA-- --GGGGGGG AATATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAA---- GGGGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA-------- - GGGGGGGG AATAICGAIC CTITCAGIAT TCCAAAICGC GAIGIAAAAC CGAAAA-----GGGGGGG AATATTGATC CITICAGIAT ICCAAAICGC GATGIAAAAC CGAAAAAA-- --GGGGGGG AATATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAAA-- -GGGGGGGGG AATATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAA---- GGGGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- --GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- --GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA----- -GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGIAAAAC CGAAA----- --GGGGGGG AGTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAAA- -GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAA---- ---GGGGGG AATATCGATC CTITCAGTAT TCCAAATCGC GATGTAAAAC CGAAAA---- ---GGGGGG AATATCGATC CTITCAGTAT TCCAAATCGC GATGTAAAAC CGAAAA---- ---GGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA----- --GGGGGGG AGTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAA-- --GGGGGGG AGTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAAA-- --GGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- --GGGGGGGG AgTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAA-- --GGGGGGGG AGTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAAAA -GGGGGGGGG AgTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAA-- --GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- --GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- --GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA--------- AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTGAAAC CGAAAAA--- --GGGGGGGG AATATCGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAA----- -- AGTATTGATC CTTTCAGTAT TCCAAATCGC GATGTAAAAC CGAAAAAA-- -GGGGGGGGG 370 380

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GTAAACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCGCA TATATAT--- -GTAGGATAT ATATATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATATA TGTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA __-_ACCACA _--ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATGAATTGC GGATACATCA -_-_ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA _-_ACCACA ----CCACA --CCACA TATAIALAT- -GIGGGATAT AICIATCTAT AITGAATMC GGATACATCA ---ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ---ACCACA TATATAT--- -GIGGGATAT ATCTAICTAT ATIGAATMC GGATACATCA ---ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATHGAATGGC GGATACATCA ----CCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATtGAATTGC GGATACATCA ----CCACA TATATATAI- -GIGGGATAI ATCTATCTAI ATIGAATIGC GGATACATCA ---ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATIGAATTGC GGATACATCA ---ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ---ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATATA TGTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----CCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATATA TGTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -----CCACA TATATATATA TGTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ----ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -_--ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -_--ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -_--ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA __-_ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -_--ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA -_-_CCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGATTGC GGATACATCA -_--ACCACA TATATAT--- GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA ___-_ACCACA TATATAT-_- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA _-_ACCACA ---ACCACA TATATAT--- -GTGGGATAT ATCTATCTAT ATGAATGC GGATACATCA --ACCACA HAMAT--- -GHGGGATAT ATCTATCTAT AHGAATGC GGAMACACA ----ACCACA TATATATAT- -GTGGGATAT ATCTATCTAT ATTGAATTGC GGATACATCA

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| K. isoetifolia SR386 | ACCACA TATATATAT- | -GTGGGAtAT | atctatctat | ATtGAATTGC | GGATACAT | $62]$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| K. isoetifolia SR388 | -ACCACA tatatatat- | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | $364]$ |
| K. isoetifolia SR393 | ACCACA TATATATAT- | -GTGGGATAT | Atctatctat | ATtGAATTGC | GGAtACATCA | [362] |
| K. latifolia RSSsn | ACCACA tatatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [364] |
| K. laxiflora SR283 | tCCACA tatatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [363] |
| K. laxiflora SR295 | ACCACA tatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [363] |
| K. laxiflora SR441 | accaca tata | -GTGGGAtAT | atctatctat | ATtGAATtGC | Ggatacatca | [360] |
| K. laxiflora SR442 | ACCACA tatat | -GTGGGAtAT | atctatctat | ATtGAATtGC | GGATACATCA | [364] |
| K. laxiflora SR467 | ccaca tatatat | -GTGGGATAT | atctatctat | ATtGAATtGC | Ggatacatca | [366] |
| K. laxiflora SR468 | CCACA TATATAT | -GTGGGATAT | ATCTATCTAT | ATTGAATTGC | GGATACATCA | [366] |
| K. laxifloraC SRsn | ACCACA TATATAT | -GTGGGATAT | ATCTATCTAT | ATTGAATTGC | GGATACATCA | [356] |
| K. laxiflora NPB1810 | CCACA tatatatat- | -GTGGGATAT | ATCTATCTAT | ATTGAATTGC | GGATACATCA | [363] |
| K. leucocephala NNBG | Accaca ta | -GTGGGATAT | Atctatctat | ATtGAATTGC | GGATACATCA | [357] |
| K. linearifolia SR269 | ccaca tatatatata | TGTGGGATAT | atctatctat | ATTGAATTGC | Ggatacatca | [362] |
| K. linearifolia SR287 | ACCACA tatatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [363] |
| K. linearifolia SR290 | ACCACA tatatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [363] |
| K. linearifolia SR291 | accaca tatatat | -GTGGGAT | ATCtatcta | ATtGAATTGC | gatacatca | [365] |
| K. linearifolia SR311 | accaca tatatat | -GTGGGAT | atctatct | ATtGAATTGC | gatacat | [356] |
| K. linearifolia SR328 | ACCACA TAT | -GTGGGATAT | atctatctat | ATTGAATTGC | Ggatacatca | [362] |
| K. linearifolia SR343 | ccaca tatatatata | tgigGgatat | atctatctat | ATtGAATTGC | GGATACATCA | [362] |
| K. linearifolia SR400 | - ${ }^{\text {cheaca }}$ TATATAT--- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [362] |
| K. linearifolia SR558 | -ACCACA tatatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [364] |
| K. linearifolia JPsn | - accaca tatatat | -GTGGGATAT | Atctatctat | ATTGAATTGC | GGATACATCA | [365] |
| K. linearifolia TD4638 | accaca tatatat | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [361] |
| K. littoralis SR200 | accaca tatatat | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [363] |
| K. multiflora SR310 | - ACCACA tatatat- | -GTGGGAtAT | atctatctat | ATtGAATTGC | Ggatacatca | [356] |
| K. northiae SR263 | ccaca tatatatata | tgiggcatat | atctatctat | ATtGAATTGC | Ggatacatca | [364] |
| K. northiae SR274 | ccaca tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | Ggatacatca | [359] |
| K. northiae SR446 | ccaca tatatatata | tgTgGgatat | atctatctat | ATtGAATTGC | Ggatacatca | [360] |
| K. pauciflora HBsn | - ACCACA TATA | -GTGGGATAT | atctatctat | Attganttgc | GGATACATCA | [364] |
| K. parviflora SR268 | CCACA tatatatata | tgTGGgatat | atctatctat | ATtGAATTGC | GgAtACATCA | [362] |
| K. parviflora SR330 | -ccaca tatatat-- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [363] |
| K. porphyantha SRsn | ACCACA tatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [356] |
| K. praecox SR529 | CCACA tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [357] |
| K. praecox SR530 | CCACA tatatatat- | -GTGGGATAT | Atctatctat | ATTGAATTGC | GGATACATCA | [361] |
| K. praecox SR532 | ccaca tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [361] |
| K. rigidifolia SRsn | - ${ }^{\text {ccaca }}$ TATATAT | -GTGGGATAT | atctatctat | ATTGAATTGC | GGAtACATCA | [356] |
| K. ritualis SR300 | -ACCACA tatatat--- | -GTGGGATAT | atctatctat | ATtGAATTGC | Ggatacatca | [364] |
| K. rooperi SR237 | CCACA tatatatata | tgigGgatat | atctatctat | ATtGAATTGC | GGAtACATCA | [362] |
| K. rooperi SR485 | - ${ }^{\text {cheaca }}$ TATATAT--- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [355] |
| K. rooperi SR528 | CCACA tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [361] |
| K. rooperi RAL4227 | accaca tatata | -GTGGGATAT | atctatctat | ATtGAATTGC | GGATACATCA | [362] |
| K. rooperi TD4559 | ccaca tat | -GTGGGAtat | atctatctat | Attganttgc | GGATACATCA | [362] |
| K. sarmentosa SR207 | accaca tatatatat- | -GTGGGATAT | atctatctat | Attganttgc | Ggatacatca | [365] |
| K. schemperi SR391 | accaca tatatatat- | -GTGGGAtat | atctatctat | Attganttgc | GGATACATCA | [363] |
| K. schimperi JMG036 | accaca tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [363] |
| K. splendida SR548 | ccaca tatatatata | tGTGGGATAT | Atctatc | ATtGAATTGC | GGAtACATCA | [363] |
| K. splendida Chapman 9061 | accaca tatatatat- | -GTCGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [363] |
| K. stricta SR279 | -ACCACA tatatat | -GTGGGATAT | atctatctat | ATTGAATTGC | GgAtacatca | [366] |
| K. thodei SR407 | - ${ }^{\text {checaca tatatat- }}$ | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [365] |
| K. thomsonii JMG031 | - ${ }^{\text {chCaca }}$ TATATATAT- | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [364] |
| K. thomsonii AMM2647 | - ${ }^{\text {checaca tatatatat- }}$ | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [361] |
| K. thomsonii CK4821 | accaca tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [361] |
| K. triangularis SR264 | CCACA tatatatata | TGTGGGATAT | Atctatctat | ATTGAATTGC | GGATACATCA | [362] |
| K. triangularis SR266 | ccaca tatatatata | TGTGGGATAT | Atctatctat | ATTGAATTGC | GGATACATCA | [362] |
| K. triangularis SR299 | -CCACA tatatat | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [364] |
| K. triangularia obtusiloba SRsn | accaca tatat | -GTGGGATAT | atctatctat | ATTGAATTGC | Ggatacatca | [356] |
| K. typhoides NNBG | accaca tatatatat- | -GTGGGATAT | Atctatctat | ATTGAATTGC | GGATACATCA | [364] |
| K. tysonii SR302 | accaca tatat | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [363] |
| K. tysonii SR303 | accaca tatata | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [363] |
| K. tysonii SR460 | accaca tata | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [364] |
| K. umbrina RGsn | accaca tatatat | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [356] |
| K. uvaria SR166 | CCACA tatatatat- | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [361] |
| K. uvaria SR186 | CCACA tatatatat- | -GTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [361] |
| K. uvaria SR172 | CCACA tatatatata | TGTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [362] |
| K. uvaria SR201 | CCACA tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [361] |
| K. uvaria SR203 | accaca tatatatat- | -GTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [365] |
| K. uvaria SR211 | CCACA tatatatat- | -GtgGgatat | atctatctat | ATtGAATtGC | Ggatacatca | [361] |
| K. uvaria SR337 | CCACA tatatatata | TGTGGGATAT | atctatctat | ATtGAATTGC | GGAtACATCA | [362] |
| K. uvaria SR342 | -ccaca tatatatata | tGTGGGATAI | atctatctat | ATtGAATTGC | GGAtACATCA | [362] |
| K. uvaria SR344 | -ACCACA tatatat--- | -GTGGGATAT | Atctatctat | ATTGAATTGC | GGATACATCA | [355] |
| K. uvaria SR471 | --ccaca tatatatata | TGTGGGATAT | atctatctat | ATTGAATTGC | GGATACATCA | [362] |
| K. uvaria SR477 | - ${ }^{\text {CCCACA }}$ TATATAT--- | -GTGGGATAT | ATCTATCTAT | ATTGAATTGC | GGATACATCA | [355] |
| K. uvaria TD4477 | -ccaca tatatatat- | -GTGGGATAI | atctatctat | ATtGAATTGC | GGAtACATCA | [361] |
| [ | 430 | 440 | 450 | 460 | 0 | 4801 |
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| Bulbine latifolia SR61 | AtGAtA-GAA TCATtTttGA | ttganacana | tatg ittcag | ACAATAGAGA | tgagatanas | [453] |
| Bulbinella cauda-felis SR204 | ATGATA-GAA TCATtTtTGA | TtGAAACAAA | tatg ittat | ACAATAGAGA | TGAAAT---- | [436] |
| K. acreae TD4626 | AtGAtA-GAA TCATtTtTGA | ttganacaia | TAGGGttcat | ACAATAGAGA | TAAAAT---- | [417] |
| K. albescens SR314 | AtGAtA-GAA TCATtTtTGA | TtGAAACAAA | TGGGGttcat | ACAATAGAGA | TGAAAT---- | [411] |
| K. albomontana SR149 | AtGAtA-GAA TCATtTtTGA | ttganacana | tGGGGttcat | ACAATAGAGA | TGAAAT---- | [419] |
| K. angustifolia SR453 | ATGATA-GAA TCATTTTTGA | TTGAAACAAA | TGGGGTtcat | ACAATAGAGA | TGAAAT---- | [411] |
| K. angustifolia SR542 | ATGATA-GAA TCATTTTTGA | TTGAAACAAA | TGGGGTtcat | ACAATAGAGA | TGAAAT---- | [419] |
| K. ankaratrensis PBP5676 | AtGAtA-GAA TCATtTtTGA | TtGAAACAAA | tagggttcat | ACAATAGATA | tGAAAT---- | [418] |
| K. baurii SR174 | ATGATA-GAA TCATtTtTGA | TtGAAACAAA | TGGGGttcat | ACAATAGAGA | TGAAAT | [420] |
| K. baurii SR202 | AtGAtA-GAA TCATtTtTGA | ttganacana | tagGgttcat | ACAATAGAGA | TGAAAT | [416] |
| K. baurii SR275 | AtGAtA-GAA TCATtTtTGA | ttganacana | tGGGGttcat | ACAATAGAGA | TGAAAT- | [418] |
| K. baurii SR285 | Atgata-gan tcatttttga | TtGAAACAAA | tgGggttcat | ACAATAGAGA | TGAAAT | [418] |
| K. baurii SR360 | Atgata-gan tcatttttga | TtGAAACAAA | tgGggttcat | ACAATAGAGA | TGAAAT- | [410] |
| K. baurii SR382 | Atgata-gan tcatttttga | TtGAAACAAA | tgGggttcat | ACAATAGAGA | TGAAAT- | [410] |
| K. baurii SR398 | ATGATA-GAA TCATtTttGA | TTGAAACAAA | tgGggttcat | ACAATAGAGA | TGAAAT | [419] |
| K. baurii NPB1923 | ATGATA-GAA TCATTTTTGA | tGAAACA | TGGGGTTCA | ACAATAGAG | GAA | [410] |


|  | baurii RJM1026 |
| :---: | :---: |
|  | bracystachya SRsn |
|  | breviflora SR452 |
|  | bruceae SR171 |
|  | buchananii SR305 |
|  | buchananii SR307 |
|  | buchananii SR458 |
|  | caulescens SR270 |
|  | caulescens SR278 |
|  | caulescens NPB1821 |
|  | caulescens RJM974 |
|  | citrina SR176 |
|  | coddiana SRsn |
|  | corraligemma SR549 |
|  | drepanophylla RAL4816 |
|  | drepanophylla RJM1100 |
|  | ensifolia autumnalis SR448 |
|  | ensifolia ensifolia JBsn |
|  | fibrosa SR297 |
|  | fibrosa PBP5579 |
|  | foliosa SR383 |
|  | foliosa SR387 |
|  | foloisa SR389 |
|  | foliosa SR390 |
|  | foliosa JMG034 |
|  | foliosa JMG038 |
|  | galpinii SR312 |
|  | gracilis SR321 |
|  | gracilis SR561 |
|  | gracilis NNBG |
|  | grantii CP4154 |
|  | hirsuta SR282 |
|  | ichopensis SR242 |
|  | ichopensis SR286 |
|  | ichopensis SR289 |
|  | ichopensis SR409 |
|  | insignis SRsn |
|  | isoetifolia SR386 |
|  | isoetifolia SR388 |
|  | isoetifolia SR393 |
|  | latifolia RSSsn |
|  | laxiflora SR283 |
|  | laxiflora SR295 |
|  | laxiflora SR441 |
|  | laxiflora SR442 |
|  | laxiflora SR467 |
|  | laxiflora SR468 |
|  | laxiflorac SRsn |
|  | laxiflora NPB1810 |
|  | leucocephala NNBG |
|  | linearifolia SR269 |
|  | linearifolia SR287 |
|  | linearifolia SR290 |
|  | linearifolia SR291 |
|  | linearifolia SR311 |
|  | linearifolia SR328 |
|  | linearifolia SR343 |
|  | linearifolia SR400 |
|  | linearifolia SR558 |
|  | linearifolia JPsn |
|  | linearifolia TD4638 |
|  | littoralis SR200 |
|  | multiflora SR310 |
|  | northiae SR263 |
|  | northiae SR274 |
|  | northiae SR446 |
|  | pauciflora HBsn |
|  | parviflora SR268 |
|  | parviflora SR330 |
|  | porphyantha SRsn |
|  | praecox SR529 |
|  | praecox SR530 |
|  | praecox SR532 |
|  | rigidifolia SRsn |
|  | ritualis SR300 |
|  | rooperi SR237 |
|  | rooperi SR485 |
|  | rooperi SR528 |
|  | rooperi RAL4227 |
|  | rooperi TD4559 |
|  | sarmentosa SR207 |
|  | schemperi SR391 |
|  | schimperi JMG036 |
|  | splendida SR548 |
|  | splendida Chapman 9061 |
|  | stricta SR279 |
|  | thodei SR407 |
|  | thomsonii JMG031 |
|  | thomsonii AMM2647 |
|  | thomsonii CK4821 |
|  | triangularis SR264 |
|  | triangularis SR266 |
|  | triangularis SR299 |
|  | triangularia obtusiloba SR |

ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----AIGATA-GAA TCAIIIIIGA TIGAAACAAA TGGGGIICAI ACAATAGAGA TGAAAT----AIGATA-GAA TCAIITITGA ITGAAACAAA TGGGGIICAI ACAATAGAGA TGAAAT----AIGAIA-GAA TCATITITGA ITGAAACAAA TAGGGIICAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTICAT ACAATAGAGA TAAAAT----ATGATATGA- TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACTATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTICAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT CCAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----Atgata-gan tcattittga ttganacana tggggitcat acantagaga tganat----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT--ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----Atgata-gan tcatttitga ttganacana tggggticat acantagaga tganat----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT CCAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT CCAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----AtGAtA-GAA tCAtttttga ttganacana tggggttcat acantagaga tganat----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTITGA TCATTTTGA TTGAAACAAA TGGGGTTCAT CCAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGITCAT ACAATAGAGA TAAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT--ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT--ATGATA-GAA TCATTTTTGA ITGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT--ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT--ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----AtGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT CCAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----Atgata-gai tcatttttga ttgaiachan tggggttcat acaitagaga tgaiat----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA T-AAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----
 ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTICAI ACAATAGAGA TGAAAT-----ATGATA-GAA TCATTTTTGA tTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA T-AAAT---TGATA-GAA ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----
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|  | . typhoides NNBG |
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|  | . tysonii SR460 |
|  | . umbrina RGsn |
|  | . uvaria SR166 |
|  | . uvaria SR186 |
|  | . uvaria SR172 |
|  | . uvaria SR201 |
|  | . uvaria SR203 |
|  | . uvaria SR211 |
|  | . uvaria SR337 |
|  | . uvaria SR342 |
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|  | . uvaria SR471 |
|  | . uvaria SR477 |
|  | . uvaria TD4477 |
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|  | ulbine latifolia SR61 |
|  | Bulbinella cauda-felis SR204 |
|  | . acreae TD4626 |
|  | . albescens SR314 |
|  | . albomontana SR149 |
|  | . angustifolia SR453 |
|  | . angustifolia SR542 |
|  | . ankaratrensis PBP5676 |
|  | . baurii SR174 |
|  | . baurii SR202 |
|  | . baurii SR275 |
|  | . baurii SR285 |
|  | . baurii SR360 |
|  | . baurii SR382 |
|  | . baurii SR398 |
|  | . baurii NPB1923 |
|  | . baurii RJM1026 |
|  | . bracystachya SRsn |
|  | . breviflora SR452 |
|  | . bruceae SR171 |
|  | . buchananii SR305 |
|  | . buchananii SR307 |
|  | . buchananii SR458 |
|  | - caulescens SR270 |
|  | . caulescens SR278 |
|  | . caulescens NPB1821 |
|  | . caulescens RJM974 |
|  | . citrina SR176 |
|  | . coddiana SRsn |
|  | . corraligemma SR549 |
|  | . drepanophylla RAL4816 |
|  | . drepanophylla RJM1100 |
|  | . ensifolia autumnalis SR448 |
|  | . ensifolia ensifolia JBsn |
|  | . fibrosa SR297 |
|  | . fibrosa PBP5579 |
|  | . foliosa SR383 |
|  | . foliosa SR387 |
|  | . foloisa SR389 |
|  | . foliosa SR390 |
|  | . foliosa JMG034 |
|  | . foliosa JMG038 |
|  | . galpinii SR312 |
|  | . gracilis SR321 |
|  | . gracilis SR561 |
|  | . gracilis NNBG |
|  | . grantii CP4154 |
|  | . hirsuta SR282 |
|  | . ichopensis SR242 |
|  | . ichopensis SR286 |
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|  | . insignis SRsn |
|  | . isoetifolia SR386 |
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|  | . latifolia RSSsn |
|  | . laxiflora SR283 |
|  | . laxiflora SR295 |
|  | . laxiflora SR441 |
|  | . laxiflora SR442 |
|  | . laxiflora SR467 |
|  | . laxiflora SR468 |
|  | . laxifloraC SRsn |
|  | . laxiflora NPB1810 |
|  | . leucocephala NNBG |
|  | . linearifolia SR269 |
|  | . linearifolia SR287 |
|  | . linearifolia SR290 |
|  | . linearifolia SR291 |
|  | . linearifolia SR311 |
|  | . linearifolia SR328 |
|  | . linearifolia SR343 |

ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----Atgata-gai tcatttttga ttganacana tggggttcat acantagaga tganat----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TGGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA 11GAAACAAA TAGGGIICAT ACAATAGAGA TGAAAT--ATGATA-GAA TCATM ATGATA-GAA ATGATA-GAA TCATTTITGA TTGAAACAAA TAGGGIICAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA TCATTTITGA TTGAAACAAA TAGGGITCAT ACAATAGAGA TAAAAT----ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT----ATGATA-GAA ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TAAAAT---ATGATA-GAA TCATTTTTGA TTGAAACAAA TAGGGTTCAT ACAATAGAGA TGAAAT---$490 \quad 500$

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TGAGAGAGGA TATCCGAAAA AGAAAATAGG AA-CATCCAC TTTTTCAATA TAGGAATCCT GAGAGAGGA TATCCGAAAA AGAAAATAGG AA-CATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAgAGAAGA tatccganaa aganait-gg angcatacac tttttcanta tgggantcat -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA tATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT GAGAGAAGA GAGAGAAGA TATCCGAAAA AGAAT-GG AGCATACAC TTTTCAATA TGGGAATCAT GAGAGAAGA TAICCGAAAA AGAAAAT-GG AAGCAIACAC TIT1TCAATA TGGGAATCAI -GAGAGAAGA TAICCGAAAA AGAAAAI-AG AAGCATACAC ITITICAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCA -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT GAgAgAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAgAgAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAgAgAAGA tatccganai aganait-ag angcatacac tutticanta tgganatcat -GAgAgAAGA tatccganaa aganant-ag angcatacac tttttcanta tgggantcat -GAgAgAAGA tatccganaa agaiant-ag angcatacac tttttcanta tgggantcat -GAgAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-GG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTCAATA TGGGAATCA GAGAGAGA TATCCGAAAA GAAAAT-AG AAGCATACAC TTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TTTTTCAATA TGGGAATCAT -GAGAGAAGA TATCCGAAAA AGAAAAT-AG AAGCATACAC TH1TMAMA TGGGATCAT GAGAGAAGA TATCGAAAA AGAAAM-AG AGCATACAC TITMATA GGGATCA -GAGAGAAGA TATCCGAAAA AGAAAAT-AA AAGCATACAC TTTTTCAATA TGGGAATCAT

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foliosa TMG038 galpinii SR312 gracilis SR321 gracilis NNBG
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insignis SRsn
isoetifolia SR386
soetifolia SR388
atila SR393
latifolia RSSsn
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axiflora NPB1810
leucocephala NNBG
linearifolia SR269
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Inearifolia SR343
linearifolia SR400
inearifolia SR558
inearifolia TD4638
ittoralis SR200
multiflora SR310
northiae SR263
horthiae SR446
pauciflora HBsn
parviflora SR268
porphyantha SRsn
praecox SR529
praecox SR532
rigidifolia SRsn
ritualis SR300
rooperi SR485
cooperi SR528
rooperi TD4559
sarmentosa SR207
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uvaria SR211
varia SR34
uvaria SR344
varia SR471
uvaria SR477
uvaria TD4477

|  | --ATAAA-AA ATT-CAACAG | TTCCAAGATA AATGAAA-GA | GGTGGGTAAA | [523] |
| :---: | :---: | :---: | :---: | :---: |
|  | AA Att-cascag | TTCCAAGATA AATGAAA-GA | GGtGGGTAAA | ] |
|  | ATT-CAACAG | tTCCAAGATA | GG |  |
|  | AT | ttccangata Aatgana-Ga |  |  |
|  | AT | TTC | GGT |  |
|  | TAAA-AA ATT-CAACAG | TTCCAAGATA AATGAAA-G | GGTGGGTAAA | 524] |
|  | tana-AA Att-CAACAG | ttccangata antgana | GGTGGGTAAA | 516] |
|  | Att-CAACAG | TTCCAAGATA AATGAA | gTGGgtana | 525] |
|  | --AtAAA-AA ATT-CAACAG | ttccangata Aatgana-ga | Ggtggatana | ] |
|  |  | ttccangata Aa | GGTGGG-AAA |  |
|  | ATAAA-AA ATT-CAACA | TTCCAAGATA AATGAA | GGTA |  |
|  | --AtAAA-AA ATT-CAAC | tTCCAAGATA AATGAA | GGTGGG- |  |
|  | AT | TTC | GGTGGGTAAA |  |
|  | ATt-CAACAG | TTCCAAGATA AATGAAA | ?????????? | ] |
|  | A | ttccangata Aatgana-ga | GGTGGGTAAA | ] |
|  | AT | tTCCAAGATA AATGAAA-G | GGtGgataia |  |
|  | AT | TTCCAAGATA AATGAAA | GGTGGGTAAA |  |
|  | ATAAA-AA ATT-CAACAG | tccaigata antgana | AA |  |
|  | taAA-AA ATT-CAA | ttccangata antgana | GTAAA | 5] |
|  | ATT-CAA | TCCAAGA | TAA? | 3] |
|  | A-AA ATT-CAA | TCCAAGA | GGTGGGTAAA | ] |
|  | AT | ttccangata antgana | GgtGgatafa | 4] |
|  | AT | ttccaigata AAtgana | GGTGGgtaAA |  |
|  | AT | TTCCAAGATA AATGAAA | GGTGGGTAAA |  |
|  | AA-AA ATT | AA | GgtGgataia |  |
|  | TT | AAGAtA AATGAAA | gGtggatala | $7]$ |
|  | AT | ITCCAAGATA AATGAAA-GA | GGTGGGtaAA | 7] |
|  | AAA-AA ATT-CAAC | CCAA | A | $7]$ |
|  | AT | ttccangata antgana | Ggtggataia |  |
|  |  | ttccaigata AAtGA |  |  |
| TA | ATA | TTCCAAGATA AATGAAA-GA | GGTGGGTAAA |  |
|  | --ATAAA-AA ATT | ttccangata Aatgana-Ga | GGtGgGtaAA | ] |
|  | AT | TTCCAAGATA AATGAAA-GA | Ggtggctana | $4]$ |
|  | AT | TTCCAAGATA AATGAAA-GA | GGTGGGTAAA | 6] |
|  | AT | ttccangata an |  |  |
|  | AT | tTCCAAGATA AA |  |  |
| tatantcatt | AT | ttccangata AATGAAA-G | GGTGGG? ? ? |  |
|  | --ATAAA-AA ATT | ttccangata Aatgata-G | GGTGGGTAAA |  |
|  | Att-CAACAG | ttccaigata matanamana | GGtGgGtaAA |  |
| ? | ?????????? ?????????? | ?????????? ?? | ??? |  |
|  | -AA ATT-CAACAG | AAGA | - |  |
|  | AT | ttccaigata matgan | GGtGgGtaAA |  |
|  | --AtAAA-AA ATT-CAACAG | ttccangata Aatgan |  |  |
| TATAATCATT | A | tTCCAAGATA AATGAAA-GA | GgtGgetaia |  |
|  | Atatana-AA Att | ttccangata Aatgana-Ga | GGtGGgtafa |  |
|  | ATATAAA-AA ATT | TTCCAAGATA AATGAAA-GA | GGTGGGTAAA |  |
|  | AT | TTCCAAGATA AATGAAA-G | GGTGGGTAAA |  |
| tatantcatt | atatana-an at | TTCCAAGATA AATGAAA-G | GGTGGGtAAA | ] |
|  | --ATAAA-AA ATT-CAACAG | ttccangata AAtGAAA-G | Ggtggatala |  |
|  | --AtAAA-AA ATT-CAACAG | ttccangata antgana | GGtGgGtaAA |  |
|  | --AtAAA-AA ATT-CAACAG | ttccangata Aatganatga | GGtGgataia |  |
|  | Att-CAACAG | TTCCAAGATA AAT | TAA |  |
|  | ATT-CAACAG | TTCCAAGATA AATGAAATGA | GGTGGGTAAA | 523] |
|  | Att-CAACAG | ttccangata AATGAAA-G | GGtGgataia | [517] |
|  | AT | ttccangata Aatgana-Ga | GGTGGGTAAA |  |
|  | Atatana-AA A | ttccaigata Aatgana | GgtGgataia |  |
|  | -- | ttccangata antgana | Ggtggatafa |  |
|  | ATAAA-AA ATT-CAA | ttccangata Aatgan | Ggtggataia |  |
|  | TAAA-AA ATT-CAACAG | TCCAAGATA AATGAA | Ggigggiala | 523] |
| ?????????? | ?????????? ?????????? | ?????????? ?????????? | ????? | [536] |
|  | A ATT-CAACAG | ttccangata Aatganatga | TAAA | [527] |
|  | AT | ttccangata antgana | GGtGgGtafa | [524] |
|  | AT | ttccaigata Aatgana |  |  |
|  | -AA ATT-CA | AAA | GGtGgataia | [524] |
|  | Att-CAA | AAGATA AATGAAA | GTAA | 524] |
|  | ATt-CAA | TCCAAGATA AATGAA | GTAAA | [527] |
|  | ATAAA-AA ATT-CAACAG | TCCAAGATA | gtaAA | [526] |
|  | -Atana-AA Att | tTCCAAGATA AATGAA | GGtGgGtaA | [525] |
|  | AtaAA-AA ATT-CA | tTCCAAGATA AATGAAAT | ? | 522] |
|  | --AtAAA-AA --tgCAACAG | TTCCAAGATA AATGAAA | +A | 1] |
|  | ATATAAA-AA ATT-CAACAG | tTCCAAGATA AATGAAA | GGTGGGIAA | ] |
| TA | ATATAAAAAA ATT-CAACA | TTCCAAGATA AATGAAA-G | GTAA | 533] |
|  | AA ATT-CAAC | TTCCAAGATA AATGAAA | TTAAA | [525] |
|  | --AtAAA-AA ATT-CAA | TTCCAAG | GgtGgataia | [517] |
|  | -Atana-AA Att-can |  |  | [526] |
|  | -Atana-AA Att-CAA | ttccaigata AAT |  | 4] |
|  | ATAAA-AA ATT-CAA | TTCCAAGATA AATGAAA | GGIGGGTAA | 4] |
|  | ATAAA-AA ATT-CAACA | tTCCAAGATA AAT-AAA-- | GGTGGGTAA | [523] |
|  | ATAAA-AA ATT-CAACAG | TTCCAAGATA AATGAAA-GA | GGTGGGTAAA | [517] |
|  | -AtAAA-AA ATT-CAACAG | tTCC-AGATA AATGAAATGA | GGTGGGTAAA | [522] |
|  | --AtAAA-AA ATT-CAACAG | tTCCAAGATA AATGAAATGA | GGtGgataia | [523] |
| TT | Atatana-AA Att-CAACAG | tTCCAAGATA AA |  | [532] |
|  | -ATAAA-AA ATT-CAACAG | TTCCAAGATA AATGAAA | GGTGGGTAR | [523] |
|  | -ATAAA-AA ATT-CAACAG | TTCCAAGATA AATGAAA-G | GgtGggtana | 6] |
|  | --AtAAA-AA ATt-CAACAG | TTCCAAGATA AATGAAATGA | GGTGGGTAAA | [523] |
| tatantcatt | ATATAAA-AA ATT-CAACAG | TTCCAAGATA AA | GGTGGGTAAA | [525] |
| ?????????? | ?????????? ?????????? | ?????????? ?????????? | ?????????? | [534] |
|  | --AtAAA-AA ATT-CAACAG | tTCCAAGATA AATGAAA-GA | GGTGGGTAAA | [516] |
| tatantcatt | ATATAAA-AA ATT-CAACAG | TTCCAAGATA AATGAAA- | GG | [532] |
|  | -ATAAA-AA ATT-CAAC | ttccangata Aatgai | GGTGGGIAAA | [516] |
|  | --ATAAA-AA ATT-CCACAG |  |  |  |


| Bulbine latifolia SR61 | ATTACAACT | [569] |
| :---: | :---: | :---: |
| Bulbinella cauda-felis SR204 | ATTACAACT | [551] |
| K. acreae TD4626 | ATTACAACT | [541] |
| K. albescens SR314 | ATTACAACT | [526] |
| K. albomontana SR149 | ATTACAACT | [533] |
| K. angustifolia SR453 | ????????? | [527] |
| K. angustifolia SR542 | ATTACAACT | [534] |
| K. ankaratrensis PBP5676 | At TACAACT | [533] |
| K. baurii SR174 | ATTACAACT | [535] |
| K. baurii SR202 | ATTACAACT | [532] |
| K. baurii SR275 | Attacaict | [533] |
| K. baurii SR285 | Attacaict | [533] |
| K. baurii SR360 | ATTACAACT | [525] |
| K. baurii SR382 | ATTACAACT | [525] |
| K. baurii SR398 | ATTACAACT | [533] |
| K. baurii NPB1923 | ATTACAACT | [525] |
| K. baurii RJM1026 | ATTACAACT | [532] |
| K. bracystachya SRsn | ATTACA??? | [534] |
| K. breviflora SR452 | ATTACAACT | [526] |
| K. bruceae SR171 | ATTACAACT | [532] |
| K. buchananii SR305 | ATTACAACT | [534] |
| K. buchananii SR307 | ATTACAACT | [534] |
| K. buchananii SR458 | ATTACAACT | [534] |
| K. caulescens SR270 | ATTACAACT | [538] |
| K. caulescens SR278 | ATTACAACT | [538] |
| K. caulescens NPB1821 | ATTACAACT | [544] |
| K. caulescens RJM974 | ATTACAACT | [544] |
| K. Citrina SR176 | ATTACAACT | [534] |
| K. coddiana SRsn | ATTACAACT | [532] |
| K. corraligemma SR549 | ATTACAACT | [533] |
| K. drepanophylla RAL4816 | ATTACAACT | [532] |
| K. drepanophylla RJM1100 | ATTACAACT | [534] |
| K. ensifolia autumnalis SR448 | ATTACAACT | [526] |
| K. ensifolia ensifolia JBsn | ATTACAACT | [526] |
| K. fibrosa SR297 | ATTACAACT | [534] |
| K. fibrosa PBP5579 | Attacaict | [541] |
| K. foliosa SR383 | ATTACAACT | [532] |
| K. foliosa SR387 | ATTAC???? | [532] |
| K. foloisa SR389 | Attacaict | [534] |
| K. foliosa SR390 | ATTACAACT | [534] |
| K. foliosa JMG034 | ATTACAACT | [533] |
| K. foliosa JMG038 | ATTACAACT | [533] |
| K. galpinii SR312 | ATTACAACT | [525] |
| K. gracilis SR321 | ATTACAACT | [534] |
| K. gracilis SR561 | ATTACAACT | [534] |
| K. gracilis NNBG | ATTACAACT | [534] |
| K. grantii CP4154 | ATTACAACT | [532] |
| K. hirsuta SR282 | ATTACAACT | [535] |
| K. ichopensis SR242 | ATTACAACT | [534] |
| K. ichopensis SR286 | ????????? | [534] |
| K. ichopensis SR289 | ATTACAACT | [534] |
| K. ichopensis SR409 | ATTACAACT | [535] |
| K. insignis SRsn | ATTACAACT | [532] |
| K. isoetifolia SR386 | ATTACAACT | [532] |
| K. isoetifolia SR388 | ATTACAACT | [534] |
| K. isoetifolia SR393 | ????????? | [532] |
| K. latifolia RSSsn | ATTACAACT | [534] |
| K. laxiflora SR283 | ATTACAACT | [533] |
| K. laxiflora SR295 | ATTACAACT | [533] |
| K. laxiflora SR441 | ATTACAACT | [530] |
| K. laxiflora SR442 | Attacaict | [534] |
| K. laxiflora SR467 | ATTACAACT | [536] |
| K. laxiflora SR468 | ATTACAACT | [536] |
| K. laxiflorac SRsn | Attacaict | [526] |
| K. laxiflora NPB1810 | Attacaict | [533] |
| K. leucocephala NNBG | ATTACAACT | [527] |
| K. linearifolia SR269 | ATTACAACT | [541] |
| K. linearifolia SR287 | ATTACAACT | [533] |
| K. linearifolia SR290 | ATTACAACT | [533] |
| K. linearifolia SR291 | Attacaict | [535] |
| K. linearifolia SR311 | ATTACAACT | [526] |
| K. linearifolia SR328 | ATTACAACT | [532] |
| K. linearifolia SR343 | ????????? | [541] |
| K. linearifolia SR400 | ATTACAACT | [532] |
| K. linearifolia SR558 | ATTACAACT | [535] |
| K. linearifolia JPsn | ????????? | [548] |
| K. linearifolia TD4638 | Attacaict | [531] |
| K. littoralis SR200 | Attacaict | [533] |
| K. multiflora SR310 | ATTACAACT | [526] |
| K. northiae SR263 | ATTACAACT | [543] |
| K. northiae SR274 | ATTACAACT | [538] |
| K. northiae SR446 | ATTACAACT | [539] |
| K. pauciflora HBsn | ATTACAACT | [534] |
| K. parviflora SR268 | ATTACAACT | [541] |
| K. parviflora SR330 | Attacaict | [533] |
| K. porphyantha SRsn | Attacaict | [526] |
| K. praecox SR529 | ATTACAACT | [528] |
| K. praecox SR530 | ATTACAACT | [532 |
| K. praecox SR532 | Attacaict | [532] |
| K. rigidifolia SRsn | ATTACAACT | [526] |
| K. ritualis SR300 | ATTACAACT | [534] |
| K. rooperi SR237 | ATTACAACT | [540] |
| K. rooperi SR485 | ATTACAACT | [525] |


| K. rooperi SR528 | Attacanct | [532] |
| :---: | :---: | :---: |
| K. rooperi RAL4227 | ATTACAACT | [532] |
| K. rooperi TD4559 | ????????? | [545] |
| K. sarmentosa SR207 | ATtACAACT | [536] |
| K. schemperi SR391 | ATTACAACT | [533] |
| K. schimperi JMG036 | ATTACAACT | [533] |
| K. splendida SR548 | AT??????? | [533] |
| K. splendida Chapman 9061 | ATTACAACT | [533] |
| K. stricta SR279 | ATTACAACT | [536] |
| K. thodei SR407 | ATTACAACT | [535] |
| K. thomsonii JMG031 | ATTACAACT | [534] |
| K. thomsonii AMM2647 | ????????? | [531] |
| K. thomsonii CK4821 | ATTACAACT | [530] |
| K. triangularis SR264 | ATTACAACT | [540] |
| K. triangularis SR266 | ATtACAACT | [542] |
| K. triangularis SR299 | ATTACAACT | [534] |
| K. triangularia obtusiloba SRsn | ATtACAACT | [526] |
| K. typhoides NNBG | ATTACAACT | [535] |
| K. tysonii SR302 | ATTACAACT | [533] |
| K. tysonii SR303 | ATTACAACT | [533] |
| K. tysonii SR460 | ATTACAACT | [532] |
| K. umbrina RGsn | ATTACAACT | [526] |
| K. uvaria SR166 | ATTACAACT | [531] |
| K. uvaria SR186 | ATTACAACT | [532] |
| K. uvaria SR172 | Attacanct | [541] |
| K. uvaria SR201 | ATTA????? | [532] |
| K. uvaria SR203 | ????????? | [535] |
| K. uvaria SR211 | ATtACAACT | [532] |
| K. uvaria SR337 | ATTACAACT | [534] |
| K. uvaria SR342 | ????????? | [543] |
| K. uvaria SR344 | ATtACAACT | [525] |
| K. uvaria SR471 | ATTACAACT | [541] |
| K. uvaria SR477 | ATTACAACT | [525] |
| K. uvaria TD4477 | ATTACAACT | [532] |

Appendix 5: Final sequence alignments of the $\operatorname{trn} L$ intron with additional out-groups from Genbank

| [ |  | 10 | 20 | 30 | 40 | 50 | 60] |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| [ |  |  |  |  |  | ] |  |
| Bulbine latifolia SR61 | GAttGgattg | AgCcttatta | TGGAAACCTG | CTAAGTGGTA | TTCCAAAT | AGAGAA | 60] |
| Bulbine semibarbata AJ290259 | ????GGATTG | AgAAttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| Bulbine succulenta AJ290260 | GAttGGAtTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| Bulbine weisei AJ290261 | ????GGATTG | AGCCTTAGTA | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| Bulbinella cuada-felis SR204 | GAtTGGATTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| Bulbinella cauda-felis AJ290262 | GAT?GGATTG | AGC-ttagta | TGGAA-CCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [58] |
| K. acreae TD4626 | GAtTGGATTG | -GCCttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [59] |
| K. albescens SR314 | GATTGGAGTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAA-T | TCAGAGAAAC | [59] |
| K. baurii SR285 | GAttGgattg | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. breviflora SR452 | GAttGGAttg | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. bruceae SR171 | GGTTGGATTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. caulescens SR278 | GAtTGGATTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. corraligemma SR549 | GAtTGGATTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. foliosa JMG034 | AAtTGGATTG | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. insignis SRsn | AATTGGATTG | AGCCttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. isoetifolia SR388 | AAtTGGAttg | agccttagta | TGGAAACCTG | CTAAGTGGTA | ACtTCCAAAT | TCAGAGAAAC | [60] |
| K. laxiflora SR467 | GAttGgattg | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. laxiflora SR468 | GAttGgattg | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACttccanat | tCAGAGAAAC | [60] |
| K. linearifolia SR311 | GATTGGATTG | agccttatta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. multiflora SR310 | GAtTGGATTG | Agccttagta | TGGAAACCTG | CTCAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. northiae SR446 | GAttGgattg | agccttagta | tGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | tCAGAGAAAC | [60] |
| K. parviflora SR268 | GATtGGATTG | AGCCttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. parviflora SR330 | GAttGGAttG | AgCCttagta | TGGAAACCTG | CTAAGTGGTA | ACtTCCAAAT | TCAGAGAAAC | [60] |
| K. ritualis SR300 | GAttGgattg | AGCCTtAGTA | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. schimperi JMG036 | AAttgGattg | agccttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. stricta SR279 | GATtGGATTG | AGCCttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. thomsonii JMG031 | AAttgGattg | AgCcttagta | TGGAAACCTG | CTAAGTGGTA | ACtTCCAAAT | TCAGAGAAAC | [60] |
| K. triangularis SR299 | GATTGGATTG | AgCcttanta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAA-T | TCAGAGAAAC | [59] |
| K. typhoides NNBG | GAttgtattg | Agccttagta | tGGAAACCTG | CtAAGTAGTA | ACttccanat | TCAGAGAAAC | [60] |
| K. tysonii SR302 | GATTGGATTG | G gGctitatta | TGGAA-CCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [59] |
| K. umbrina RGsn | GAttGGAttG | agccttagta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. uvaria SR186 | GATTGGATTG | AGCCtTAGTA | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| K. uvaria SR211 | GATTGGATTG | AgCcttatta | TGGAAACCTG | CTAAGTGGTA | ACTTCCAAAT | TCAGAGAAAC | [60] |
| [ |  | 70 | 80 | 90 | 100 | 110 | 120] |
| [ |  |  |  |  |  | .] |  |
| Bulbine latifolia SR61 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTtTtT | tTttt--AAG | AAAAAA-TGA | [117] |
| Bulbine semibarbata AJ290259 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTT---AG | AAAAAA-TGA | [116] |
| Bulbine succulenta AJ290260 | CCTGGAACTA | AAAATGGGCA | Atcctgagcc | AAATCTITTT | titttit-Ag | AAAAAA-TGA | [118] |
| Bulbine weisei AJ290261 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTT----AG | AAAAAA-TAA | [115] |
| Bulbinella cuada-felis SR204 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTT-----AG | AAAAAA-TGA | [114] |
| Bulbinella cauda-felis AJ290262 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTT-----AG | AAAAAA-TGA | [112] |
| K. acreae TD4626 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTT----G | AAAAAA-TGA | [114] |
| K. albescens SR314 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTITTT | тttt | AAAAAA-TGA | [113] |
| K. baurii SR285 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTtTT---G | AAAAAAATGA | [117] |
| K. breviflora SR452 | CCTGGAACTA | AAAATGGGCA | Atcctgagcc | AAATCTITTT | tTtT | AAAAAA-TGA | [114] |
| K. bruceae SR171 | CCTGGAACTA | AAAATGGGCA | Atcctgagcc | AAATCTTTTT | tittitt--G | AAAAAAATGA | [118] |
| K. caulescens SR278 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | tTttt | AAAAAA-TGA | [116] |
| K. corraligemma SR549 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTTT---G | AAAAAA-TGA | [116] |
| K. foliosa JMG034 | CCTGGAACTA | AAAATGGGCA | Atcctgagcc | AAATCTITTT | tTtTT | AAAAAA-TGA | [115] |
| K. insignis SRsn | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTT | AAAAAA-TGA | [115] |
| K. isoetifolia SR388 | CCTGGAACTA | AAAATGGGCA | AtCCTGAGCC | AAATCTTTTT | tttttt | AAAAAA-TGA | [116] |
| K. laxiflora SR467 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | tttttit- | AAAAAAATGA | [118] |
| K. laxiflora SR468 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | tittitt- | AAAAAAATGA | [118] |
| K. linearifolia SR311 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTT | AAAAAA-TGA | [114] |
| K. multiflora SR310 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | tTttt | AAAAAA-TGA | [115] |
| K. northiae SR446 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTT | AAAAAA-TGA | [115] |
| K. parviflora SR268 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | тTtтT-- | AAAAAA-TGA | [115] |
| K. parviflora SR330 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | tittitt--C | AAAAAAATGA | [118] |
| K. ritualis SR300 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | ttiti- | AAAAAA-TGA | [115] |
| K. schimperi JMG036 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | tttti | AAAAAA-TGA | [115] |
| K. stricta SR279 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTTT---G | AAAAAA-TGA | [116] |
| K. thomsonii JMG031 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTTT---G | AAAAAA-TGA | [116] |
| K. triangularis SR299 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | Ttttitt--C | AAAAAAATGA | [117] |
| K. typhoides NNBG | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTTTT-- | AAAAAA-TGA | [117] |
| K. tysonii SR302 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTIT | TTTT-----G | AAAAAA-TGA | [113] |
| K. umbrina RGsn | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTT-----G | AAAAAA-TGA | [114] |
| K. uvaria SR186 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTTTTT | TTTTTT---G | AAAAAA-TGA | [116] |
| K. uvaria SR211 | CCTGGAACTA | AAAATGGGCA | ATCCTGAGCC | AAATCTITTT | TTTTTTT--G | AAAAAA-TGA | [117] |
| [ |  | 130 | 140 | 150 | 160 | 170 | 180] |
| [ |  | . |  |  |  | .] |  |
| Bulbine latifolia SR61 | TTAATGGGAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTTCTAACGA | [177] |
| Bulbine semibarbata AJ290259 | TTAATGGGAC | AATAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTtCtAACGA | [176] |
| Bulbine succulenta AJ290260 | TTAATGGGAC | AAGAATAAAA | AAGGAtAGg | GCAGAGACTC | AATGGAAGCT | GTtCtAACGA | [178] |
| Bulbine weisei AJ290261 | TTAATG---- | -AGAATAAAA | AAGGATAGGT | GCAGAGACTC | AACGGAAGCT | GTTCTAACGA | [170] |
| Bulbinella cuada-felis SR204 | TTAATCGGAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTTCTAACGA | [174] |
| Bulbinella cauda-felis AJ290262 | TTAATCGGAC | AAGAATAAAA | AAGGAtAGg | GCAGAGACTC | AATGGAAGCT | GTtCtAACGA | [172] |
| K. acreae TD4626 | TTAATCGGAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTTCTAACGA | [174] |
| K. albescens SR314 | TTAATCGGAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTTCTAACGA | [173] |
| K. baurii SR285 | TTAATCGAAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTTCTAACGA | [177] |
| K. breviflora SR452 | TTAATCGGAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTTCTAACGA | [174] |
| K. bruceae SR171 | TTAATCGAAC | AAGAATAAAA | AAGGATAGGT | GCAGAGACTC | AATGGAAGCT | GTtCtancGa | [178] |

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caulescens SR278
foliosa JMG034
insignis SRsn
isoetifolia SR388
laxiflora SR467
laxiflora SR468
linearifolia SR311
multiflora SR31
northiae SR446
parviflora SR268
parviflora SR330
ritualis SR300
schimperi JMG036
stricta SR279
thomsonii JMG031
triangularis SR299
typhoides NNBG
tysonii SR302
umbrina RGsn
. uvaria SR18
k. uvaria SR211
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Bulbine latifolia SR61 Bulbine semibarbata AJ290259 Bulbine succulenta AJ290260 Bulbine weisei AJ290261 Bulbinella cuada-felis SR204 Bulbinella cauda-felis AJ290262
. acreae TD4626
. albescens SR314
. baurii SR285
, breviflora SR452
. bruceae SR171
k. caulescens SR278
k. corraligemma SR549
. foliosa JMG034
insignis SRsn
isoetifolia SR388
laxiflora SR467
laxiflora SR468
inearifor SR311
northiae SR446
norviflora SR2
parviflora SR268
, parvirlora SR330
ritualis SR300
schimperi JMG0
stricta SR279
thomsonii JMG031
triangularis SR299
typhoides NNBG
tysonii SR302
umbrina RGsi
. uvaria SR186

- uvaria SR211
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Bulbine latifolia SR61 Bulbine semibarbata AJ290259 Bulbine succulenta AJ290260 Bulbine weisei AJ290261
Bulbinella cuada-felis SR204 Bulbinella cauda-felis AJ290262 K. acreae TD4626
albescens SR314
baurii SR285
breviflora SR452
bruceae SR171
caulescens SR278
corraligemma SR549
foliosa JMG034
insignis SRsn
isoetifolia SR388
laxiflora SR467
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. linearifolia SR311
multiflora SR310
northiae SR446
northiae SR446
parviflora SR268
ritualis SR300
tuimper JMG036
chimperi JMG0
tricta SR279
thomsonii JMG031
triangularis SR299
typhoides NNB
tysonil SR302

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k. uvaria SR186
K. uvaria SR211
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Bulbine latifolia SR61
Bulbine semibarbata AJ290259
Bulbine succulenta AJ290260
Bulbine weisei AJ290261
Bulbinella cuada-felis SR204 Bulbinella cauda-felis AJ290262
K. acreae TD4626
K. albescens SR314
K. baurii SR285
K. breviflora SR452
k. bruceae SR171
K. caulescens SR278
K. corraligemma SR549
K. foliosa JMG034
K. insignis SRsn
K. isoetifolia SR388
K. laxiflora SR467
K. laxiflora SR468
k. linearifolia SR311
K. multiflora SR310
K. northiae SR446
K. parviflora SR268
. parviflora SR330
K. ritualis SR300
K. schimperi JMG036
K. stricta SR279
K. thomsonii JMG031
k. triangularis SR299
K. typhoides NNBG
K. tysonii SR302
K. umbrina RGsn
k. uvaria SR186
. uvaria SR211

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Bulbine latifolia SR61
Bulbine semibarbata AJ290259 Bulbine succulenta AJ290260 Bulbine weisei AJ290261
Bulbinella cuada-felis SR204 Bulbinella cauda-felis AJ290262 K. acreae TD4626
. albescens SR31
K. baurii SR285
K. breviflora SR452
k. bruceae SR171
K. caulescens SR278
K. Corraligemma SR549
k. foliosa JMG034
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K. multiflora SR310
K. northiae SR446
K. parviflora SR268
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ritualis SR300
schimperi JMG036
stricta SR279
thomsonii JMG031
triangularis SR299
k. typhoides NNBG
. tysonii SR302
. umbrina RGsn
. uvaria SR18
k. uvaria SR211

ATGACCCATA TATCTAATAC ATACGTATAC ATACTGACAT AGCAAACGAT TAATCACGAA ATGACCCATA TATCTAATAC ATACGTATAC ATACTGACAT AGCAAACGAT TAATCACGAA


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CCGAATACAT TATATATATA TATAT----- -----GTATA TGCAAAATTC AGAATTATTG CCGAATACAT TATATATATA TATATA---- -------TATA TGCAAAATTC AAAGTTATTG CCGAATACAT TATATATATA TATATAT--- -----GTATA TGCAAAATTC AGAATTATTG CCGAATACAT TATATATATA TATATATATA T----GTATA TGCAAAATTC AAAGTTATTG CCGAATACAT TATATATATA TAT------------CCGAATACAT TATATATATA TATATATA-- -----GTATA TGCAAAATTC AAAGTTATTG CGGAATACAT TATATATATA TATATATATA TATATGTATA TGCAAAATTC AAAGTTATTG CCGAATACAT TATATATATA TATATATATA TATATGTATA TGCAAAATTC AAAGTTATTG CCGAATACAT TATATATATA TATATAT--_ -_---GTATA TGCAAAATTC AGAATTATTG

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Bulbine latifolia SR61
Bulbine semibarbata AJ290259 Bulbine semibarbata AJ290259 Bulbine weisei AJ290261
Bulbinella cuada-felis SR204 Bulbinella cuada-felis SR204
Bulbinella cauda-felis AJ290262
K. acreae TD4626
K. albescens SR314
. baurii SR285
. breviflora SR452
k. bruceae SR171

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k. corraligemma SR549
k. foliosa JMG034
. insignis SRsn
K. isoetifolia SR388
K. laxiflora SR467
K. linearifolia SR311
K. linearifolia SR31
K. multiflora SR3
K. northiae SR446
K. parviflora SR268
K. ritualis SR300
k. schimperi JMG036
k. stricta SR279
K. thomsonii JMG031
K. triangularis SR299
K. typhoides NNBG
. tysonii SR302
K. umbrina RGsn
k. uvaria SR186
K. uvaria SR211
${ }^{[ }$

Bulbine latifolia SR61 Bulbine semibarbata AJ290259 Bulbine succulenta AJ290260
Bulbine weisei AJ290261
Bulbinella cuada-felis SR204 Bulbinella cauda-felis AJ290262
K. acreae TD4626
K. albescens SR314
K. baurii SR285
K. breviflora SR452
. bruceae SR171
. caulescens SR2
K. caulescens SR278
K. foliosa JMG034
. insignis SRsn
. isoetifolia SR388
. laxiflora SR467
. laxiflora SR468
k. linearifolia SR311
. multiflora SR310
northiae SR446
northiae SR446
parviflora SR268
parviflora SR330
ritualis SR300
schimperi JMG036
stricta SR279
k. thomsonii JMGO31
k. triangularis SR299
. typhoides NNBG
. tysonii SR302
. umbrina RGsn
K. uvaria SR186
. uvaria SR211
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Bulbine latifolia SR61
Bulbine semibarbata AJ290259 Bulbine succulenta AJ290260
Bulbine weisei AJ290261
Bulbinella cuada-felis SR204
Bulbinella cauda-felis AJ290262
K. acreae TD4626
. albescens SR314
baurii SR285
breviflora SR452
bruceae SR171
K. caulescens SR278
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. laxiflora SR468
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parviflora SR3
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schimperi JMGO
stricta SR279
thomsonii JMG031
triangularis SR29
typhoides NNB
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510
520
530

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GGAAAATCCG TCCACTTT-A AAAATCGGGA G [553]
K. uvaria SR186
K. uvaria SR211

GGAAAATCCG TCGACTTT-A AAAATCGTGA G [543]
GGAAAATCCG TCGACTTT-A GAAATCGTGA G [540]

## Appendix 6: Final sequence alignments of the ITS region

| [ |  | 10 | 20 | 30 | 40 | 50 | $60]$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| [ |  |  |  |  |  | . |  |
| Bulbine latifolia SR61 | G | A | A | TC | AGGTGAACCT | GCGGAAGGAT | ] |
| Bulbinella cauda-felis SR204 | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | 60] |
| K. acreae TD4626 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. albescens SR314 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. albomontana SR149 | tatcatttag | AGGAAGGAGA | AgTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. angustifolia SR453 | Atcatttag | AGGAAGGAGA | AGTCGT | AGGTTTCCGT | AGGTGAACC | GGAAGGAT | [60] |
| K. angustifolia SR542 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGA | [60] |
| K. ankaratrensis PBP5676 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. baurii SR275 | tatcatttag | AGGAAGGAGA | AgTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. baurii SR285 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. baurii SR360 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACC | GCGGAAGGAT | [60] |
| K. baurii SR382 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGG | [60] |
| K. baurii NPB1923 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. brachystachya SRsn | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AgGtgaic | GCGGAAGG | [60] |
| K. brevifolia SR452 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. bruceae SR171 | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | ?????????? | [60] |
| K. buchananii SR307 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. caulescens SR270 | tatcatttag | AGGAAGGAGA | AgTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. caulescens SR278 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. caulescens NPB1821 | tatcatttag | AGGAAGGAGA | AgTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. citrina SR176 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. coddiana SRsn | TATCATTTAG | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. corraligemma SR549 | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. drepanophylla RAL4816 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. ensifolia ensifolia JBsn | tatcatttag | AGGAAGGAGA | AgTCGtaACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. ensifolia autumnalis SR448 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. fibrosa SR297 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. fluvitalis SRsn | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. foliosa SR383 | tatcatttag | AGGAAGGAGA | AgTCGtaACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. foliosa SR387 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. foliosa SR389 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. foliosa SR390 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. foliosa JMG034 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. foliosa JMG038 | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. galpinii SR312 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. gracilis SR321 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. gracilis NNBG | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. grantii CP4154 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. hirsuta SR282 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. ichopensis SR242 | tatcatttag | AGGAAGGAGA | AgTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
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| K. latifolia RSSsn | tatcatttag | AGGAAGGAGA | AgTCGtaACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. laxiflora SR283 | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
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| K. laxiflora SR467 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. laxiflora SR468 | tatcatttag | AGGAAGGAGA | AgTCGtaACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. laxifloraC SRsn | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
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| K. leucocephala NNBG | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. linearifolia SR170 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
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| K. stricta SR279 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTTTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. thodei SR407 | tatcatttag | AGGAAGGAGA | AgTCGtaACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
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| K. thomsonii AMM | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. thomsonii CK4821 | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. triangularis SR264 | tatcatttag | AGGAAGGAGA | Agtcgtanca | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGAT | [60] |
| K. triangularis SR266 | tatcatttag | AGGAAGGAGA | AGTCGTAACA | AGGTtTCCGT | AGGTGAACCT | GCGGAAGGA | [60] |


triangularis obtusiloba SRsn typhoides JB8084
typhoides NNBG
tysonii SR302
tysonil SR303
uvaria SR18
uvaria SR201
uvaria SR20
uvaria SR342
uvaria SR34
uvaria SR47

AATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGA
 ATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT IAMAHAG AGGAAGGAGA AGICGIAACA AGGHICCGI AGGYGAACCT GCGGAAGGAI ATCA ATAITAG AGGAGGAGA AGICGIAACA AGGITCGI AGGYGACCI GCGGAAGGA AMAG AGAG AICAIHAG AGGAAGGAGA AGICGIAACA AGGITCCGI AGGIGAACCI GCGGAAGGA ATCATITAG AGGAAGGAGA AGICGIAACA AGGIIICCGI AGGIGAACCI GCGGAAGGA ATCAITIAG AGGAAGGAGA AGICGIAACA AGGIIICCGI AGGIGAACCI GCGGAAGGA ATCAITIAG AGGAAGGAGA AGICGIAACA AGGIHCCGI AGGIGAACCI GCGGAAGGA TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT

100
110

CATTGTCGAG ACCCGAAA-G GACGACCGCG AACCGTTGAT CTCTTTCTAA CGGGCGCCGG ?????????? ?????????? ?????????? ??????????? ?????????? CGGGGGCACATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCTCIC-AA TACTGGCGCC AATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATtGICGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGCCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATtGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACTAGTGAG CTCTCTC-AA AACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ??????????? ?????????? GACGA-CGCG AACCAGTGAG СТСТСТС-АA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA CACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA CACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGTCGTA ACATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTA-AA AAATGGCGCC AMGCG CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTA-AA AAATGGCGCC AMCG CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTA-AA AAATGGCGCC ACG CAIIGICGIA ACIAtAAACG GACGA-CGCG AACCAGIGAG CICICIA-AA AAAIGGCGCC CATM CATIGICGIA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CAIIGICGIA ACTATAAACG GACGA-CGCG AACCAGIGAG CICICIC-AA TACIGGCGCC AIt AATTGICGIA ACTATAAACG GACGA-CGCG AACCAGGAG CICTCTC-AA TACTGGCGC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCTCTC-AA TACTGGCGCC ATIGICGIA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCTCTC-AA TACIGGCGCC AATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTA-AA AAATGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA AAATGGCGCC ATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTA-AA AAATGGCGCC ATIGICGIA ACTATAAACG GACGA-CGCG AACCAGIGAG CICICIA-AA AAATGGCGCc ATtGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGICGIA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCICIC-AA TACrGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGICGIA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCICTC-AA TACIGGCGCC AATTGTCGIA ACTATAAACG GACGA-CGCG AACCAGIGAG CTCICTC-AA TACIGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGCCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATtGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATGTCGTA ACTATAAACG GACGA-CGCG AACCGGTGAG CTCTCTC-AA TACTGGCGCC AATGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CAtTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC AATGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC ATGGTCGA CATTGTCGTA ACTATAAACG GACGA-CGCG AACCAGTGAG CTCTCTC-AA TACTGGCGCC

| sarmentosa SR207 | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTG | CTCTC-AA AACTGGCGC | 8] |
| :---: | :---: | :---: | :---: | :---: |
| K. schemperi SR391 | CATTGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | CTCTCTA-AA AAATGGCGCC | [118] |
| K. schemperi JMG036 | CAttGtcgia actatanacg | GACGA-CGCG AACCAGTGA | СТСтCta-AA AAATGGCGCC | 18] |
| K. splendida SR548 | CATTGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СТСтСtС-AA TACTGGCGCC | [118] |
| K. stricta SR279 | CATTGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СТСТСTC-AA CACTGGCGCC | [118] |
| K. thodei SR407 | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СТСТСTC-AA TACTGGCGCC | [118] |
| K. thomsonii JMG031 | CAttGtcgia actatanacg | GACGA-CGCG AACCAGTGAG | СТСТСta-AA AAATGGCGCC | [118] |
| K. thomsonii AMM | CAttGtcgia actatanacg | GACGA-CGCG AACAAGTGAG | СтСтСtC-AA AACtGGCGCC | [118] |
| K. thomsonii CK4821 | CAttGtcgia actatanacg | GACGA-CGCG AACAAGTGAG | СТСтСTC-AA AACtGGCGCC | [118] |
| K. triangularis SR264 | CAttgicgia actatanacg | GACGA-CGCG AACCAGTGAG | СТСтСTC-AA TACTGGCGCC | [118] |
| K. triangularis SR266 | CATTGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СTCTCTC-AA TACTGGCGCC | [118] |
| K. triangularis SR299 | CATTGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СTCTCTC-AA TACTGGCGCC | [118] |
| K. triangularis obtusiloba SRsn | CATTGTCGTA ACTATTAACG | GACGA-CGCG AACCAGTGAG | СТСТСTC-AA TACTGGCGCC | [118] |
| K. typhoides JB8084 | CATTGTCGTA ACTATAAACG | GACGA-CGCG AACTAGTGAG | СTСTCTC-AA AACTGGCGCC | [118] |
| K. typhoides NNBG | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACcAGTGAg | СтСtСtC-AA TACTGGTGCC | [118] |
| K. tysonii SR302 | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СТСТСTC-AA TACTGGCGCC | [118] |
| K. tysonii SR303 | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СТСТСTC-AA TACTGGCGCC | [118] |
| K. umbrina RGsn | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СТСТСTC-AA TACTGGCGCC | [118] |
| K. uvaria SR186 | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СтСтСtC-AA TACtGGCGCC | [118] |
| K. uvaria SR201 | CAttgtcgia actatanacg | GACGA-CGCG AACCAGTGAG | СтСтСtC-AA TACtGGcgcc | [118] |
| K. uvaria SR203 | CAttgtcgia actatanacg | GACGA-CGCG AACCAGTGAG | СтСтСtC-AA TACtGGcgcc | [118] |
| K. uvaria SR211 | CAttgtcgia actatanacg | GACGA-CGCG AACCAGTGAG | СтСтСtC-AA tactgacgec | [118] |
| K. uvaria SR342 | CATtGTCGTA ACTATAAACG | GACGA-CGCG AACCAGTGAG | СTCTCTC-AA TACTGGCGCC | [118] |
| K. uvaria SR344 | CAttGtcgia actatanacg | GACGA-CGCG AACCAGTGAG | СтСтCTC-AA TACtGGCGCC | [118] |
| K. uvaria SR477 | CAttGtcgia actatanacg | GACGA-CGCG AACCAGTGAG | СТСтСTC-AA TACtGGCGCC | [118] |
| [ | 130 | $140 \quad 150$ | 60 | $0]$ |
| [ |  | . | ] |  |
| Bulbine latifolia SR61 | tGCCtagcte cgacgetche | C | CCTCC-GCG- ACGGGC--GT | 5] |
| Bulbinella cauda-felis SR204 | T-----GCTC CGGCGTTGAC | GCCCCGCCCC GCCGCTCCGT | CCTC--ACGG ATGGAC--GT | [169] |
| K. acreae TD4626 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. albescens SR314 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. albomontana SR149 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. angustifolia SR453 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCT | CCT--TGCGG ATGG-CATGT | [169] |
| K. angustifolia SR542 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. ankaratrensis PBP5676 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. baurii SR275 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. baurii SR285 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. baurii SR360 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. baurii SR382 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. baurii NPB1923 | T-CCGGGCGC CGACGCCGAG | Accccigig GccG | CCT--TGCGG AtGG-CATGT | [169] |
| K. brachystachya SRsn | T-CCGGGCGC CTACGCTGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. brevifolia SR452 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. bruceae SR171 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. buchananii SR307 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. caulescens SR270 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GTCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. caulescens SR278 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. caulescens NPB1821 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GTCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. citrina SR176 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. coddiana SRsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. corraligemma SR549 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG | CCT--TGCGG AtGG-CATGT | [169] |
| K. drepanophylla RAL4816 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. ensifolia ensifolia JBsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG | CCT--TGCGG AtGG-CATGT | [169] |
| K. ensifolia autumnalis SR448 | T-CCGGGcGc CGACGCCGAG | ACCCCGGTGT GCCG | CCT--TGCGG ATGG-CATGT | [169] |
| K. fibrosa SR297 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. fluvitalis SRsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. foliosa SR383 | -CcGggcge cgacgccgag | AACCCGGTGT GCCG | CCT--TGCGG AtGG-CATGT | [169] |
| K. foliosa SR387 | -ccggecge cgacgccgag | AACCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. foliosa SR389 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. foliosa SR390 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. foliosa JMG034 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. foliosa JMG038 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. galpinii SR312 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. gracilis SR321 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG | CCT--TGCGG AtGG-CATGT | [169] |
| K. gracilis NNBG | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. grantii CP4154 | T-CCGGGCGC CGACGCTGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. hirsuta SR282 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. ichopensis SR242 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. ichopensis SR286 | T-ACGGGcGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | ССт--TGCGG ATGG-CATGT | [169] |
| K. ichopensis SR289 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCT-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. insignis SRsn | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. isoetofolia SR386 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. isoetifolia SR388 | T-CCGGGCGC CGACGTCGAG | AACCCGGTGT GCCG-----T | ССт--TGCGG ATGG-CATGT | [169] |
| K. isoetifolia SR393 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT GCCG-----T | ССт--TGCGG ATGG-CATGT | [169] |
| K. latifolia RSSsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. laxiflora SR283 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. laxifolia SR295 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. laxiflora SR467 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. laxiflora SR468 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. laxiflorac SRsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. laxiflora NPB1810 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. leucocephala NNBG | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | ССт--TGCGG ATGG-CATGT | [169] |
| K. linearifolia SR170 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. linearifolia SR269 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. linearifolia SR290 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. linearifolia SR291 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. linearifolia SR311 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. linearifolia SR313 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. linearifolia SR328 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. linearifolia SR343 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG AtGG-CATGT | [169] |
| K. littoralis SR200 | T-CCGGGcGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. multiflora SR310 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT GCCG-----T | CCT--TGCGG ATGG-CATGT | [169] |
| K. multiflora SR315 | T-CCGGGCGC CGACGCCGA | ACCCCGGTGT GCCG | CCT--TGCGG ATGG-CATGT | [169] |


| northiae SR263 | T-CCGGGCGC CGACGCCGAG | CCC | GCCG-----T | T--TGCGG | GG |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| K. northiae SR446 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG- | CCT--TGCGG | ATGG-CATGT | [169] |
| K. parviflora SR268 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | ССт--TGCG | ATGG-CATG | [169] |
| K. paviflora SR330 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | CCT--TGCGG | AtgG-CATGT | [169] |
| K. pauciflora HBsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | CCT--TGCGG | ATGG-CATGT | [169] |
| K. porphyantha SRsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | CCT--TGCGG | ATGG-CATG | [169] |
| K. praecox SR529 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | CCT--TGCGG | ATGG-CATG | [169] |
| K. rigidifolia SRsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | CCT--TGCGG | AtGG-CATG | [169] |
| K. ritualis SR300 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. rooperi SR237 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtgG-CATGT | [169] |
| K. rooperi SR485 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| K. sarmentosa SR207 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCC-----T | CCT--TGCGG | ATGT-CATGT | [169] |
| K. schemperi SR391 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| K. schemperi JMG036 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| K. splendida SR548 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. stricta SR279 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GTCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. thodei SR407 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. thomsonii JMG031 | T-CCGGGCGC CGACGCCGAG | AACCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. thomsonii AMM | T-CCAGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCC--TGCGG | AtgG-CATGT | [169] |
| K. thomsonii CK4821 | T-CCAGGCGC CGACGCCGAG | Accccagta | GCCG-----T | CCT--TGCGG | AtgG-CATGT | [169] |
| K. triangularis SR264 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtgG-CAtgt | [169] |
| K. triangularis SR266 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtgG-CATGT | [169] |
| K. triangularis SR299 | T-CCGTGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. triangularis obtusiloba SRsn | T-CCGGGCGC CGACGCCGAG | ACCCtGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. typhoides JB8084 | T-CCGGGCGC CTACGCtGAg | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. typhoides NNBG | T-CCGGGCGC CTACGCTGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. tysonii SR302 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| K. tysonii SR303 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CAtGt | [169] |
| K. umbrina RGsn | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| K. uvaria SR186 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GC | CCT--TGCGG | ATGG-CATG | [169] |
| K. uvaria SR201 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| K. uvaria SR203 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtgG-CAtgt | [169] |
| K. uvaria SR211 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG | CCT--TGCGG | AtGG-CATGT | [169] |
| K. uvaria SR342 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | AtGG-CATGT | [169] |
| K. uvaria SR344 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG- | CCT--TGCGG | AtGG-CATGT | [169] |
| K. uvaria SR477 | T-CCGGGCGC CGACGCCGAG | ACCCCGGTGT | GCCG-----T | CCT--TGCGG | ATGG-CATGT | [169] |
| [ | 190 | 200 | 210 | 220 | 230 | 2401 |
| [ |  |  |  |  | .] |  |
| Bulbine latifolia SR61 | CTGGG-CGAG -CGGCGAAAC | Atgacceccc | -GGCGCGATT | GGGCGCCAAG | gancacat-C | [231] |
| Bulbinella cauda-felis SR204 | C-GGGACGAG ACGGCGCAAC | AAGACCCCCC | CGGCGCGAT- | GGGCGCCAAG | GAACACAT-C | [226] |
| K. acreae TD4626 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGA | GGGCGCCA | GAACACTtGC | [225] |
| K. albescens SR314 | CCGGGATCTC T--GCGGAAC | AAgACCCCCC | -GGCGCGAT- | GGGCGCCA | GAACACTtGC | [225] |
| K. albomontana SR149 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GgGCGCCAAG | GAACACTtGC | [225] |
| K. angustifolia SR453 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACttgc | [225] |
| K. angustifolia SR542 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACttgc | [225] |
| K. ankaratrensis PBP5676 | CTTGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCC | GAACACTtGC | [225] |
| K. baurii SR275 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. baurii SR285 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. baurii SR360 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. baurii SR382 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. baurii NPB1923 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. brachystachya SRsn | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTTGC | [225] |
| K. brevifolia SR452 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. bruceae SR171 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. buchananii SR307 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTTGC | [225] |
| K. caulescens SR270 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT | GGGCGCCAAG | GAACACTIGC | [225] |
| K. caulescens SR278 | CGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT | GGGCGCCAAG | GAACACTtGC | [225] |
| K. caulescens NPB1821 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT | GGGCGCCAAG | GAACACTIGC | [225] |
| K. citrina SR176 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT | GGGCGCCAAG | GAACACTIGC | [225] |
| K. coddiana SRsn | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. corraligemma SR549 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. drepanophylla RAL4816 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT | GGGCGCCAAG | GAACACttic | [225] |
| K. ensifolia ensifolia JBsn | TCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. ensifolia autumnalis SR448 | CCGGGATCTC T--GCGGAAC | AAgACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. fibrosa SR297 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GgGCGCCAAG | GAACACTtGC | [225] |
| K. fluvitalis SRsn | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. foliosa SR383 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACttgc | [225] |
| K. foliosa SR387 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. foliosa SR389 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. foliosa SR390 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. foliosa JMG034 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. foliosa JMG038 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. galpinii SR312 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. gracilis SR321 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. gracilis NNBG | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. grantii CP4154 | CCGGGATCTC T--GCGGAAC | AATACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. hirsuta SR282 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. ichopensis SR242 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. ichopensis SR286 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. ichopensis SR289 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. insignis SRsn | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. isoetofolia SR386 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. isoetifolia SR388 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACttic | [225] |
| K. isoetifolia SR393 | CCGGGATCTC C--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTIGC | [225] |
| K. latifolia RSSsn | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACttgic | [225] |
| K. laxiflora SR283 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. laxifolia SR295 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | gGgcgccang | GAACACTtGC | [225] |
| K. laxiflora SR467 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTtGC | [225] |
| K. laxiflora SR468 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTTGC | [225] |
| K. laxifloraC SRsn | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTTGC | [225] |
| K. laxiflora NPB1810 | CCGGGATCTC T--GCGGAAC | AAGACCCCCC | -GGCGCGAT- | GGGCGCCAAG | GAACACTTGC | [225] |
| K. leucocephala NNBG | CCGGGATCTC T--GCGGAA | AAGACCCCC | -GGCGCGA | GGGCGCCA | GAACACTTGC | [225] |




|  | . foliosa JMG034 |
| :---: | :---: |
|  | . foliosa JMG038 |
|  | . galpinii SR312 |
|  | . gracilis SR321 |
|  | . gracilis NNBG |
|  | . grantii CP4154 |
|  | . hirsuta SR282 |
|  | . ichopensis SR242 |
|  | . ichopensis SR286 |
|  | . ichopensis SR289 |
|  | . insignis SRsn |
|  | . isoetofolia SR386 |
|  | . isoetifolia SR388 |
|  | . isoetifolia SR393 |
|  | . latifolia RSSsn |
|  | . laxiflora SR283 |
|  | . laxifolia SR295 |
|  | . laxiflora SR467 |
|  | . laxiflora SR468 |
|  | . laxifloraC SRsn |
|  | . laxiflora NPB1810 |
|  | . leucocephala NNBG |
|  | . linearifolia SR170 |
|  | . linearifolia SR269 |
|  | . linearifolia SR290 |
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|  | . linearifolia SR311 |
|  | . linearifolia SR313 |
|  | . linearifolia SR328 |
|  | . linearifolia SR343 |
|  | . littoralis SR200 |
|  | . multiflora SR310 |
|  | . multiflora SR315 |
|  | . northiae SR263 |
|  | . northiae SR446 |
|  | . parviflora SR268 |
|  | . paviflora SR330 |
|  | . pauciflora HBsn |
|  | . porphyantha SRsn |
|  | . praecox SR529 |
|  | . rigidifolia SRsn |
|  | . ritualis SR300 |
|  | . rooperi SR237 |
|  | . rooperi SR485 |
|  | . sarmentosa SR207 |
|  | . schemperi SR391 |
|  | . schemperi JMG036 |
|  | . splendida SR548 |
|  | . stricta SR279 |
|  | . thodei SR407 |
|  | . thomsonii JMG031 |
|  | . thomsonii AMM |
|  | . thomsonii CK4821 |
|  | . triangularis SR264 |
|  | . triangularis SR266 |
|  | . triangularis SR299 |
|  | . triangularis obtusiloba |
|  | . typhoides JB8084 |
|  | . typhoides NNBG |
|  | . tysonii SR302 |
|  | . tysonii SR303 |
|  | . umbrina RGsn |
|  | . uvaria SR186 |
|  | . uvaria SR201 |
|  | . uvaria SR203 |
|  | . uvaria SR211 |
|  | . uvaria SR342 |
|  | . uvaria SR344 |
|  | . uvaria SR477 |
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|  | ulbine latifolia SR61 |
|  | ulbinella cauda-felis SR204 |
|  | acreae TD4626 |
|  | albescens SR314 |
|  | . albomontana SR149 |
|  | angustifolia SR453 |
|  | . angustifolia SR542 |
|  | . ankaratrensis PBP5676 |
|  | baurii SR275 |
|  | baurii SR285 |
|  | baurii SR360 |
|  | baurii SR382 |
|  | baurii NPB1923 |
|  | brachystachya SRsn |
|  | brevifolia SR452 |
|  | bruceae SR171 |
|  | buchananii SR307 |
|  | caulescens SR270 |
|  | caulescens SR278 |
|  | caulescens NPB1821 |
|  | citrina SR176 |

CTCCGGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CTTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CGTCCGCAAC CGAMCACTAC ATAACTCTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CGTCCGCAAC CGAMCACTAC ATAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CICCGCAAC CGAACACIAC AIAAACIGIA TGACICICGG CAACGGATAT CTCGCCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCICT CCTCCGCAAC CGAACACTAC ATAAACTGIA CCGGCAAC CGAACACIAC ATAAACTGIA TGACICICGG CAACGGATAI CICGGCICIC CCCGCAAC CGAACACTAC AIAAACIGIA TGACICICGG CAACGGAIAT CTCGGCTCIC CICGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CICCGCAAC CGAACACIAC ATAAACTGIA TGACICICGG CAACGGATAT CICGGCICIC CGGCAAC CGAACACTAC ATAAACTGIA TGACICICGG CAACGGATAI CTCGGCICIC CGCGAAC CGAACACIAC ATAAACIGIA TGACICGG CAACGAMAT CTCGGCICI CHCGCAAC CGAACACIAC ATAAACTGIA TGACICICGG CAACGGATAT CTCGGCIC1 CGCAC CGAACACIAC ATAAACIGIA TGACICICGG CAACGGATAT CTCGGCICI CICCGCAAC CGAACACIAC ATAAACIGIA TGACICICGG CAACGGATA1 CTCGGCTC CCICGCAAC CGAACACTAC ATAAACTGTA TGACICICGG CAACGGATAT CTCGGCICT CTCCGGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ACAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCI CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC AIAAACTGA GBCTCTCG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCGCAAC CGAACACTAC ATAAACGI CGCTCGG CAACGATAT CTCGGCTC CICCGCAAC CGAACACTAC AIAAACIGIA TGACICICGG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGCCTC CTTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACIAC ATAAACTGTA TGACTCICGG CAACGGATAT CICGGCICTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAI CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACIAC ATAAACTGIA TGACICICGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGIA TGACTCTCGG CAACGGATAT CTCGGCTCT CTCCGCAAC CGAACACIAC ATAAACIGIA TGACICICGG CAACGGATAT CTCGGCTCIC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCT CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC CCTCCGCAAC CGAACACTAC ATAAACTGTA TGACTCTCGG CAACGGATAT CTCGGCTCTC

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GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC TAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAT GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC AAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATCAGA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC CAACAMA MACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC
K. coddiana SRsn
K. corraligemma SR549
K. drepanophylla RAL4816
K. ensifolia ensifolia JBsn
k. ensifolia autumnalis SR448
fibrosa SR297
fluvitalis SRsn
foliosa SR383
foliosa SR387
foliosa SR389
. foliosa SR390
. foliosa JMG034
. foliosa JMG038

- galpinii SR312
. gracilis SR32
. gracilis NNBG
. grantii CP415
. hirsuta SR282
K. ichopensis SR242
K. ichopensis SR286
. ichopensis SR289
. insignis SRsn
isoetofolia SR386
isoetifolia SR388
isoetifolia SR393
latifolia RSSsn
laxiflora SR283
laxifolia SR295
laxiflora SR467
laxiflora SR468
laxifloraC SRsn
laxiflora NPB1810
leucocephala NNBG
linearifolia SR170
linearifolia SR269
linearifolia SR290
linearifolia SR291
linearifolia SR311
linearifolia SR313
linearifolia SR343
inearifolia SR343
luttoralis SR200
multiflora SR315
northiae SR263
northiae SR263
porthiae SR446
parviflora SR268
paviflora SR330
pauciflora HBsn
porphyantha SRsn
praecox SR529
rigidifolia SRsn
ritualis SR300
rooperi SR237
rooperi SR485
sarmentosa SR207
schemperi SR391
schemperi JMG036
splendida SR548
stricta SR279
thodei SR407
thomsonii JMG031
thomsonii AMM
thomsonii CK4821
triangularis SR264
triangularis SR266
triangularis SR299
triangularis obtusiloba SRsn
typhoides JB8084
typhoides NNBG
tysonii SR302
tysonii SR303
umbrina RGsn
uvaria SR186
uvaria SR201
uvaria SR203
uvaria SR21
uvaria SR34
uvaria SR344
uvaria SR477


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Bulbine latifolia SR61
Bulbinella cauda－felis SR204 K．acreae TD4626
K．acreae TD4626
．albescens SR314
albomontana SR149
angustifolia SR453
ankaratrensis PBP567
ankaratrensis PBP5676
baurii SR275

GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGAGA AGAACGTAGC GAAAIGCGAT ACIGGI AATIGCAGAA TCCCGTGAAC CAICGATA GAACGIAGC GAAAMGCGAT ATTGGGG AATGCAGA TCCCGTGAAC CAIGATA AGACGIAGC GAAATGCGAT ACTGG CAICGAIGA AGAACGIAGC GAAAGCGAT ACTGGIG AATCAGA TCCGTGAC
 CAMCATG AGAACGIAGC GAAAGCGAT ACG GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGIGIG AATTGCAGAA TCCCGTGAAC GCAICGAIGA AGAACGIAGC GAAATGCGAT ACTGGTG AAIGCAGAA TCcGGGAAC CCATCGATGA AGAACGIAGC GAAATGCGAT ACITGGIGTG AATIGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTIGGIGIG AATIGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGCGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGIGIG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGIAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGIAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATtGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC CCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC CATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC CCATCGATG AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGA TCCCGTGA CCATCGATGA AGAACGTAGC GAAAGCGAT AGGGG AAGGCAGA TCCCGTGAAC CCATCGATGA AGAACGTAGC GAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAC CCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGGG AAMCAGA TCCGGGAC CAAGAGA AGAACGIAGC GAAATGCGAT ACITGGGG AAITGCAGAA TCCGTGAA CAAGATGA AGACGIAGC GAAAGCGAT ACTMGG AATGCAGA TCGGGAC CCATGA GCAICGAIGA AGAACGIAGC GAAAIGCGAI ACTIGGIGIG AATTGCAGAA TCCCGTGAAC位信 Alt位信信 Aht AATCGATG AGAACGIAGC GAAAIGCGAT ACIIGGIGIG AAIIGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC AAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC AAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC GCATCGATGA AGAACGTAGC GAAATGCGAT ACTTGGTGTG AATTGCAGAA TCCCGTGAAC 430 440 450

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CATCGAGTCT TTGAACGCAA GTTGCGCCCG AGGCCACCCG GCCGAGGGCA CGCCTGCCTG CATCGAGTTT TTGAACGCAA GTTGCGCCCG AGGCCACCCG GCCGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCIGCCC CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGACGCAA GTTGCGCCCG AACCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAMCGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG

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．baurii SR360
．baurii NPB1923
brachystachya SRsn
bruceae SR171
buchananii SR307
caulescens SR278
caulescens NPB1821
citrina SR176
corraligemma SR549
ensifolia ensifolia JBsn
ensifolia autumnalis SR448
fibrosa SR297
foliosa SR389
foliosa SR390
foliosa JMG03
galpinii SR312
gracilis NNBG
grantii CP4154
hirsuta SR282
ichopensis SR286
ichopensis SR2
isoetofolia SR386
isoetifolia SR388
latifolia RSSsn
laxiflora SR283
laxiflora SR467
laxiflora SR468
laxifloraC SRsn
laxiflora NPB1810 linearifolia SR170 inearifolia SR269 inearifolia SR291
linearifolia SR311
linearifolia SR328
inearifolia SR343
littoralis SR200
multiflora SR315
northiae SR263
parviflora SR268
paviflora SR330
porphyantha SRsn
praecox SR529
rigidifolia SRsn
tualls SR30
rooperi SR485
armentosa SR207
schemperi JMG036
splendida SR548
stricta SR279
thomsonii JMG031
thomsonii AMM
thomsonii CK4821
triangularis SR264
triangularis SR299
triangularis obtusiloba SRsn
typhoides JB8084
typhoides NNBG
tysonii SR302
tysonii SR303
umbrina RGs
uvaria SR186
uvaria SR20
varia SR211
uvaria SR34
uvaria SR477

CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT ATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCci ARGAGI THAACGA GIIGCGCCCG AAGCCACIG GCHGAGGCA CGCCIGCCCTG ARGAGIC THAACGA GIIGCGCCCG AAGCCACIG GCHGAGGCA CGCCIGCCCTG CATCGAGTCT HTAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT ARCGAGICT THAACGCAA GITGCGCCCG AAGCCACTG GCGAGGCCA CGCCTGCC ARTGGAGICI 11GAACGCAA GITGCGCCCG AAGCCACICG GCIGAGGGCA CGCcigccic AATCGAGIC1 TTGAACGCAA GIIGCGCCCG AAGCCACICG GCIGAGGGCA CGCCIGCCI CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCIGCCI CAICGAGICI 11GAACGCAA GIIGCGCCCG AAGCCACICG GCTGAGGGCA CGCCIGCCI CATCAGIC ARCGAGIC
信 CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCIGCCT CATCGAGTCT ITGAACGCAA GITGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GITGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCIGCCI CATCGAGTCT ITGAACGCAA GITGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCIGCCI CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCI CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT ATCGAGTCT HTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT FTGAACGCAA GTGGGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT AMCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG AACGACT TGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG ARGAGCT THAACGAA GIIGCGCCCG AAGCCACICG GCHGAGGCA CGCCIGCCCTG ATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGACGGCA CGCCTGCCT CATCGGC AMGAGCT THGAMCAA GIGCGCCCG AGCCCACTCG GCTGAGGCA CGCCTGCCT
 CATCGAGTCT TTGAACGCAA GITGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCIGCCI CAICGAGIC1 1TGAACGCAA GIIGCGCCCG AAGCCACICG GCHGAGGGCA CGCCIGCCI AICGAGIC1 11GAACGCAA GIIGCGCCCG AAGCACR GC1GAGGGCA CGCCrGCC1 g Alct AACGAGIC1 11GAACGCAA GIIGCGCCCG AAGCCACICG GC1GAGGGCA CGccigcct
 Alch信信信 CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GITGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG ATCGAGTCT TTGAACGCAA GITGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT AATCGAGICT TIGAACGCAA GIIGCGCCCG AAGCCACICG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG AATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG ATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCC CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT AACGAG AATCGAGTCT TTGACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCT CATCGAGTCT TTGAACGCAA GTTGCGCCCG AAGCCACTCG GCTGAGGGCA CGCCTGCCTG

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Bulbine latifolia SR61
Bulbinella cauda-felis SR204 K. acreae TD4626
K. albescens SR314
K. albomontana SR149
K. angustifolia SR453
K. ankaratrensis PBP5676
K. baurii SR275
K. baurii SR285
K. baurii SR360
K. baurii SR382
K. baurii NPB1923
K. brachystachya SRsn
k. brevifolia SR452
K. bruceae SR171
k. buchananii SR307
K. caulescens SR270
K. caulescens SR278
K. caulescens NPB1821
. citrina SR176
coddiana SRsn
corraligemma SR549
drepanophylla RAL4816
ensifolia ensifolia JBsn
ensifolia autumnalis SR448
fibrosa SR297
fluvitalis SRsn
foliosa SR383
foliosa SR387
foliosa SR389
foliosa SR390
foliosa JMG034
foliosa JMG038
foliosa JMG038
gracilis SR321
gracilis SR321
gracilis NNBG
hirsuta SR282
ichopensis SR242
ichopensis SR286
insignis SRsn
isoetofolia SR386
isoetifolia SR388
isoetifolia SR393
latifolia RSSsn
laxifolia SR295
laxiflora SR467
laxiflora SR468
laxifloraC SRsn
laxiflora NPB1810
leucocephala NNBG
linearifolia SR170
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linearifolia SR311
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linearifolia SR343
littoralis SR200
multiflora SR310
multiflora SR315
northiae SR263
northiae SR446
parviflora SR268
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schemperi SR391
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thomsonii JMG031
thomsonii AMM
thomsonii CK4821
triangularis SR264
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triangularis SR29
triangularis obtusiloba SRsn

GGCGTCACGC ATCGCGTCGC TCCGCCAA-C CCTAACCCGG GCACAACGTG CTCCGCGGAGGCGTCACGC CTCGCGTCGC TCCGCTCACC CCT--CCCTT T-AGCAC-TA CGTGCTGGAG GGCGTCACGC CTCACGICGC TCCGCGCAGC CCGCCACT-G GCACAAIGIG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACI-G GCACAA G GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACI-G GCACAAIGIG CTIGICGGCG GGCGTCACGC CTCACGICGC TCCGCGCAGC CCGCCACI-G GCACAATGIG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACI-G GCACAAIGIG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCGCGTCGC TCCGCGCAGC CCGCCACA-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCACC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCACC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGGICACGC CTCACGICGC TCCGCGCAGC CCGCCACT-G GCACAAIGTG CTIGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG
 GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GCGTCACGC CTCACGTCGC tCCGCGCAGC CCGCCACT- GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACACTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CGGCACT-G GCACAATGTG CTIGICGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACTG GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGGGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTGTCGGC GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTGTCGGCG GGGIM C Clicce GGGGCACGC © GGGICACGC CICACGICGC ICCGCGCAGC CCGCCACI-G GCACAATGIG CIIGICGGCG ClCACGICGC TCCGCGCAGC CCGCCACI-G GCACAAIGIG CIIGICGGCG ClCACGICGC TCCGCGCAGC CCGCCACI-G GCACAA1GIG CIIGICGGCG ClCACGICGC TCCGCGCAGC CCGCCACI-G GCACAAIGIG CIIGICGGC GGGCACGC CTCACGICGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG Geck GGCGTCACGC CTCACGICGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGICGGCG GCGICACGC CTCACGICGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGcGraiccc CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGICGGCG Ccce GGGICACGC CICACGICGC ICCGCGCAGC CCGCCACI-G GCACAAIGIG CIIGICGGCG GGGICACGC CICACGICGC ICCGCGCAGC CCGCCACI-G GCACAAIGIG CITGICGGCG GGCGICACGC CTCACGTCGC TCCGCGCAGC CCGCCACI-G GCACAA1GIG CIrgicggc GGGICACGC CICACGICGC ICCGCGCAGC CCGCCAC1-G GCACAATGIG CIrgicgacg GGGICACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGICGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG gGcGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCGCGTCGC TCCGCGCAGC CCGCCGCT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACAGTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCACGCACC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-A GCACACTGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG GGCGTCACGC CTCACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGC GGCGTCACGC CTAACGTCGC TCCGCGCAGC CCGCCACT-G GCACAATGTG CTTGTCGGCG

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| K. schemperi JMG036 | tCCCGCGTCG | GG | AGAT | TGGCCCTCCG | G | TGCGGTGGGT | 3] |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| K. splendida SR548 | tcccecgicg | GATGCGG | At | tgGccctccg | tgcctcgcga | TGCGGTGGGT | 573] |
| K. stricta SR279 | tCCCGCGTCG | GAtGCGG | ------AGAT | tgGccctccg | tgcctcgcga | tGCGGTGGGT | [573] |
| K. thodei SR407 | TCCCGCGT | GATGC | -----AGAT | TGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. thomsonii JMG031 | TCCCGCGTCG | GATGCG | GAT | tGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. thomsonii AMM | TCTCGCGTCG | GATGCGG | AGAT | tGGccctccg | tgcctcgcha | TGCGGTGGGT | [573] |
| K. thomsonii CK4821 | TCTCGCGTCG | GATGCGG | AGAT | tGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. triangularis SR264 | TCCCGCGTCG | GATGCGG | AGAT | tGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. triangularis SR266 | TCCCGCGTCG | GATGCGG | T | tGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. triangularis SR299 | TCCCGCGTCG | GATGCGG | AT | tgGccetccg | tGcCtcGcGg | TGCGGTGGGT | [573] |
| K. triangularis obtusiloba SRsn | TCCCGCGTCG | GATGCGG | AGAT | TGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. typhoides JB8084 | TCCCGCGTCG | GATGCGG | AGAT | TGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. typhoides NNBG | TCCCGCGTCG | GATGCGG | AGAT | TGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. tysonii SR302 | TCCCGCGTCG | GATGCGG | GAT | TGGCCCTCCG | tGCCTCGCGG | tGCGGTGGGT | [573] |
| K. tysonii SR303 | CCGCGTCG | GATGCGG | ------AGAT | tgGccetccg | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. umbrina RGsn | TCCCGCGTCG | GATGCGG | AT | tGGCCCTCCG | TGCCtCGCGG | TGCGGTGGGT | [573] |
| K. uvaria SR186 | TCCCGCGTCG | GATGCGG | GAT | tgGccctccg | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. uvaria SR201 | TCCCGCGTCG | GATGCGG | GAT | tGGccetccg | TGCCTCGCGG | TGCGGTGGGT | [573] |
| K. uvaria SR203 | TCCCGCGT | GATGCGG | ------AGAT | tgGccetccg | tgcctcgcha | tGCGGTGGGT | [573] |
| K. uvaria SR211 | tCCCGCGTCG | GATGCGG | ------AGAT | tgGccetccg | tgcctcgcha | tGCGGTGGGT | [573] |
| K. uvaria SR342 | TCCCGCGT | GATGCGG | AG | tgGccetccg | tgcctcgcha | tGCGGTGGGT | [573] |
| K. uvaria SR344 | TCCCGCGT | GATGCGG | AGAT | tggccetccg | tgcctcgcha | tGCGGTGGGT | [573] |
| K. uvaria SR477 | tCCCGCGTCG | GAtGCGG | AGAT | TGGCCCTCCG | TGCCTCGCGG | TGCGGTGGGT | [573] |
| [ |  | 610 | 620 | 630 | 640 | 50 | $60]$ |
| [ |  |  |  |  |  | ] |  |
| Bulbine latifolia SR61 | CGAAGTGTCG | GTCGTCGGTC | GAGCTtGGCA | CGGCGAGTGG | TGGACGGACA | TGAtCCTGAG |  |
| Bulbinella cauda-felis SR204 | CAAAGTGCCG | GTCGCCGGCC | GGGCttGGCA | CGGCGAgtGg | TGGACGGACA | tGctcctgag | [636] |
| K. acreae TD4626 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. albescens SR314 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. albomontana SR149 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. angustifolia SR453 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. angustifolia SR542 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. ankaratrensis PBP5676 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. baurii SR275 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. baurii SR285 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. baurii SR360 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. baurii SR382 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. baurii NPB1923 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGATGGACA | CGCtcctalt | [633] |
| K. brachystachya SRsn | tGAAGTTTCT | CACGTCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. brevifolia SR452 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtg | TGGACGGACA | cgctcctgat | [633] |
| K. bruceae SR171 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. buchananii SR307 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. caulescens SR270 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. caulescens SR278 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtg | TGGACGGACA | cgctcctgat | [633] |
| K. caulescens NPB1821 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cGCtcctgat | [633] |
| K. citrina SR176 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. coddiana SRsn | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. corraligemma SR549 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctectgat | [633] |
| K. drepanophylla RAL4816 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cGctcctgat | [633] |
| K. ensifolia ensifolia JBsn | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtcctgat | [633] |
| K. ensifolia autumnalis SR448 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. fibrosa SR297 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtcctag | [633] |
| K. fluvitalis SRsn | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. foliosa SR383 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtG | TGGACGGACA | cGctcctgat | [633] |
| K. foliosa SR387 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. foliosa SR389 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. foliosa SR390 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctectgat | [633] |
| K. foliosa JMG034 | TGAAGTTTCT | CACACCGGTC | GGGCCAGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. foliosa JMG038 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. galpinii SR312 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctgat | [633] |
| K. gracilis SR321 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. gracilis NNBG | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. grantii CP4154 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | cgctcctant | [634] |
| K. hirsuta SR282 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtg | TGGACGGACA | cgctcctgat | [633] |
| K. ichopensis SR242 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. ichopensis SR286 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. ichopensis SR289 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAt | [633] |
| K. insignis SRsn | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. isoetofolia SR386 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. isoetifolia SR388 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. isoetifolia SR393 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | cgctcctgat | [633] |
| K. latifolia RSSsn | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtcctag | [633] |
| K. laxiflora SR283 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. laxifolia SR295 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. laxiflora SR467 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. laxiflora SR468 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. laxifloraC SRsn | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAT | [633] |
| K. laxiflora NPB1810 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. leucocephala NNBG | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. linearifolia SR170 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. linearifolia SR269 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. linearifolia SR290 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAT | [633] |
| K. linearifolia SR291 | tGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtg | TGGACGGACA | CGCTCCtGAT | [633] |
| K. linearifolia SR311 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtGg | TGGACGGACA | CGCTCCtGAT | [633] |
| K. linearifolia SR313 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtg | TGGACGGACA | CGCTCCtGAt | [633] |
| K. linearifolia SR328 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAgtg | TGGACGGACA | CGCTCCtGAt | [633] |
| K. linearifolia SR343 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCtGAT | [633] |
| K. littoralis SR200 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. multiflora SR310 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. multiflora SR315 | TGAAGTTTCT | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCtCCtGAT | [633] |
| K. northiae SR263 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGCA | CGGCGAGTGG | TGGACGGACA | CGCTCCTGAT | [633] |
| K. northiae SR446 | TGAAGTTTCI | CACGCCGGTC | GGGCCGGGC | CGGCGAGTGG | TGGACGGAC | CGCTCCTGAT | [633] |


| K. parviflora SR268 <br> K. paviflora SR330 |  |
| :---: | :---: |
|  |  |
| K. pauciflora HBsn |  |
|  | porphyantha SRsn |
|  | praecox SR529 |
|  | rigidifolia SRsn |
|  | ritualis SR300 |
|  | rooperi SR237 |
|  | rooperi SR485 |
|  | sarmentosa SR207 |
|  | schemperi SR391 |
|  | schemperi JMG036 |
|  | splendida SR548 |
|  | stricta SR279 |
|  | thodei SR407 |
|  | thomsonii JMG031 |
|  | thomsonii AMM |
|  | thomsonii CK4821 |
|  | triangularis SR264 |
|  | triangularis SR266 |
|  | triangularis SR299 |
|  | triangularis obtusiloba |
|  | typhoides JB8084 |
|  | typhoides NNBG |
|  | tysonii SR302 |
|  | tysonii SR303 |
|  | umbrina RGsn |
|  | uvaria SR186 |
|  | uvaria SR201 |
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| K | uvaria SR477 |
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| Bulbine latifolia SR61 |  |
|  | binella cauda-felis SR204 |
|  | acreae TD4626 |
|  | albescens SR314 |
|  | albomontana SR149 |
|  | angustifolia SR453 |
|  | angustifolia SR542 |
|  | ankaratrensis PBP5676 |
|  | baurii SR275 |
|  | baurii SR285 |
|  | baurii SR360 |
|  | baurii SR382 |
|  | baurii NPB1923 |
|  | brachystachya SRsn |
|  | brevifolia SR452 |
|  | bruceae SR171 |
|  | buchananii SR307 |
|  | caulescens SR270 |
|  | caulescens SR278 |
|  | caulescens NPB1821 |
|  | . citrina SR176 |
|  | coddiana SRsn |
|  | corraligemma SR549 |
|  | drepanophylla RAL4816 |
|  | ensifolia ensifolia JBsn |
|  | ensifolia autumnalis SR44 |
|  | fibrosa SR297 |
|  | fluvitalis SRsn |
|  | foliosa SR383 |
|  | foliosa SR387 |
|  | foliosa SR389 |
|  | foliosa SR390 |
|  | foliosa JMG034 |
|  | foliosa JMG038 |
|  | galpinii SR312 |
|  | gracilis SR321 |
|  | gracilis NNBG |
|  | grantii CP4154 |
|  | hirsuta SR282 |
|  | ichopensis SR242 |
|  | ichopensis SR286 |
|  | ichopensis SR289 |
|  | insignis SRsn |
|  | isoetofolia SR386 |
|  | isoetifolia SR388 |
|  | isoetifolia SR393 |
|  | latifolia RSSsn |
|  | laxiflora SR283 |
|  | laxifolia SR295 |
|  | laxiflora SR467 |
|  | laxiflora SR468 |
|  | laxifloraC SRsn |
|  | laxiflora NPB1810 |
|  | leucocephala NNBG |
|  | linearifolia SR170 |
|  | linearifolia SR269 |

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K. linearifolia SR290 K. linearifolia SR291 . linearifolia SR311
K. linearifolia SR313 K. linearifolia SR343 K. littoralis SR200 . multiflora SR310 K. multiflora SR310
. northiae SR263
k. northiae SR263
. northiae SR446
. parviflora SR26
. pauciflora HBsn
. porphyantha SRsn
. praecox SR529
. rigidifolia SRsn
ritualis SR300
rooperi SR237
rooperi SR485
sarmentosa SR207
schemperi SR391 schemperi JMG036
splendida SR548
stricta SR279
thodei SR407
thomsonii JMG031
thomsonii AMM
thomsonii CK4821
triangularis SR264
triangularis SR266
triangularis SR299
triangularis obtusiloba SRsn
typhoides JB8084
typhoides NNBG
tysonii SR302
tysonii SR303
umbrina RGsn
uvaria SR186
uvaria SR201
uvaria SR203
uvaria SR211
uvaria SR342
uvaria SR34
uvaria SR477

- uvaria SR477


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Bulbine latifolia SR61
Bulbinella cauda-felis SR204 k. acreae TD4626
. albescens SR314
k. albomontana SR149
. angustifolia SR453
. angustifolia SR542
K. ankaratrensis PBP5676
K. baurii SR275
. baurii SR285
. baurii SR360
. baurii SR382
. baurii NPB1923
brachystachya SRsn
brevifolia SR452
bruceae SR171
buchananii SR307
caulescens SR270
caulescens SR278
caulescens NPB1821
citrina SR176
coddiana SRsn
corraligemma SR549
drepanophylla RAL4816
ensifolia ensifolia JBsn
ensifolia autumnalis SR448
fibrosa SR297
fluvitalis SRsn
foliosa SR383
foliosa SR387
foliosa SR389
foliosa SR390
foliosa JMG034
foliosa JMG038
galpinii SR312
gracilis SR321
gracilis NNBG
grantii CP4154
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Hopensis SR242
ichopens1s SR286
ichopensis SR28
insignis SRsn
isoetofolia SR386
. isoetifolia SR388

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K. laxifloraC SRsn
K. laxiflora NPB1810
K. leucocephala NNBG
K. linearifolia SR170
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K. linearifolia SR313
K. linearifolia SR328
K. linearifolia SR343
K. littoralis SR200
K. multiflora SR310
K. multiflora SR315
K. northiae SR263
K. northiae SR446
K. parviflora SR268
K. paviflora SR330
K. pauciflora HBsn
K. porphyantha SRsn
K. praecox SR529
K. rigidifolia SRsn
K. ritualis SR300
K. rooperi SR237
K. rooperi SR485
K. sarmentosa SR207
K. schemperi SR391
K. schemperi JMG036
K. splendida SR548
K. stricta SR279
K. thodei SR407
K. thomsonii JMG031
K. thomsonii AMM
K. thomsonii CK4821
K. triangularis SR264
K. triangularis SR266
K. triangularis SR299
K. triangularis obtusiloba SRsn
K. typhoides JB8084
K. typhoides NNBBG
K. tysonii SR302
K. tysonii SR303
K. umbrina RGsn
K. uvaria SR186
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Bulbine latifolia SR61 Bulbinella cauda-felis SR20 K. acreae TD4626
k. albescens SR314
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. angustifolia SR453
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K. citrina SR176
. coddiana SRsn
k. corraligemma SR549
drepanophylla RAL4816
ensifolia ensifolia JBsn
ensifolia autumnalis SR448
fibrosa SR297
fluvitalis SRs
foliosa SR383
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K. foliosa JMG038

CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG aTCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATGGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCAA CCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGCC GGGACCACCC CCMAACCGA CAAAMC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCAAACCGA AGGGCGCAAC GCGCCATCGG ArCGcGACC CAGGrCAGGC GgGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCAICGG AICGCGACCC CAGGICAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGATCACCC CCAAACCGA AGGGCGCAAC GCGCCAICGG ATCGCGACCC CAGGTCAGGC GGGATCACCC CCCAAACCGA AGgGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCAAACCGA AGGGCGCAAC GCGCCAICGG AICGCGACCC CAGGICAGGC GGGATCACCC CCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGATCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGATCACCC CCCAA CCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGCC GGGACCACCC CCAAACCA AGGGCGCAAC GCGCCAICGG ANGCGACC CAGGICAGGC GGGACCACCC CCAAACCGA AGGGCGCAAC GCGCCAICGG ArCGCGACCC CAGGICAGGC GGGACCACCC CCCAA CCGA MGGCGCAAC GCGCCAMCG ATCGCGACCC CAGGCAGCC GGGACCACCC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC CCCAA CCGA AGGGCGCAAC GCGCCAICG ATCGCGACCC CAGGTCAGGC GGGACCACC CCCAAACCGA AGGGCGCAAC GCGCCATCGG ATCGCGACCC CAGGTCAGGC GGGACCACCC

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GCTGAGCTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATI GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATC GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATI GCTGAGTTTA AGCAIAICAA TAAGGGGAGG AGAAGAAACT AACAAGGATY GCTGAGTTTA AGCATAICAA TAAGCGGAGG AGAAGAAACT AACAAGGATY GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTITA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT gCtgagttta agcatatcan tangcggagg aganganact ancanggatt GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCtGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTA AGCATATCAA TAGCGGAGG AGAAGAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGGGGAGG AGAAGAAACT AACAAGGATT GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAMGAACT AACAAGGETI GCTGAGTTTA AGCATATCAA TAAGCGGAGG AGAAGAAACT AACAAGGATT
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K．gracilis SR321
K．gracilis NNBG
K．grantii CP4154
K．hirsuta SR282
K．ichopensis SR242
K．ichopensis SR286
K．ichopensis SR289
K．insignis SRsn
K．isoetofolia SR386
K．isoetifolia SR388
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K．latifolia RSSsn
K．laxiflora SR283
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K．leucocephala NNBG
K．linearifolia SR170
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K．linearifolia SR343
K．littoralis SR200
K．multiflora SR310
K．multiflora SR315
K．northiae SR263
K．northiae SR446
K．parviflora SR268
K．paviflora SR330
K．pauciflora HBsn
K．porphyantha SRsn
K．praecox SR529
K．rigidifolia SRsn
K．ritualis SR300
K．rooperi SR237
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K．sarmentosa SR207
K．schemperi SR391
K．schemperi JMG036
K．splendida SR548
K．stricta SR279
K．thodei SR407
K．thomsonii JMG031
K．thomsonii AMM
K．thomsonii CK4821
K．triangularis SR264
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K．triangularis SR299
K．triangularis obtusiloba SRsn
K．typhoides JB8084
K．typhoides NNBG
K．tysonii SR302
K．tysonii SR303
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K．uvaria SR186
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. galpinii SR312
K. gracilis SR32
. grantii CP415
K. hirsuta SR282
K. ichopensis SR242
K. ichopensis SR289
. insignis SRsn
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k. latifolia RSSsn
K. laxiflora SR283
. laxifolia SR295
k. laxiflora SR468
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. laxiflora NPB1810
. leucocephala NNBG
. linearifolia SR269
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k. linearifolia SR311
linearifolia SRJ
. linearifolia SR328
K. littoralis SR200
k. multiflora SR315
k. northiae SR263
. northiae SR446
. paviflora SR330
K. pauciflora HBsn
K. porphyantha SR
K. praecox SR529
K. ritualis SR300
K. rooperi SR237
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splendida SR548
stricta SR279
thodei SR407
thomsonii AMM
thomsonii CK4821
K. triangularis SR264
k. triangularis SR299
k. triangularis obtusiloba SRsn
K. typhoides JB808
k typhoides NNB
k. tysonil SR302
K. tysonil SR303
K. umbrina RGsn
K. uvaria SR186
K. uvaria SR20
K. uvaria SR211
k. uvaria SR34
K. uvaria SR477

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## Appendix 7: List of haplotype generated by the TCS analysis for full data set

A total of 56 haplotype were recognised by TCS of which 15 were internal node haplotypes. The remaining 41 are characterised below:

1. Haplotype SR549 ( $\mathrm{n}=1$ ): SR 549

Haplotype SR548 ( $\mathrm{n}=1$ ): SR 548
Haplotype SR468 (n=1): SR 468
Haplotype SR467 (n=3): SR 467, SR 330, SR398
Haplotype SR283 ( $\mathrm{n}=1$ ): SR 283
Haplotype SR311 ( $\mathrm{n}=1$ ): SR 311
Haplotype laxiCSRsn (n=1): K. laxiflora C SR $s n$
Haplotype SR211 (n=4): SR 211, SR 201, RJM 1026, SR530
Haplotype TD4477 (n=1): TD 4477
Haplotype KtypNNBG (n=11): K. typhoides NNBG, SR186, K. brachystachya SR sn, SR 176, SR 529, SR 207, SR 203, SR 202, SR 532, SR 528, SR 166
. Haplotype CP4154 (n=1): CP 4154
Haplotype PBP5676 (n=1): PBP 5676
Haplotype SR275 ( $\mathrm{n}=1$ ): SR 275
. Haplotype SR314 (n=8): SR 314, SR 452, SR 360, SR 382, SR 448, SR 485, SR 344, SR 477
Haplotype NPB 1923 ( $\mathrm{n}=1$ ): NPB 1923
Haplotype NPB 1821 ( $\mathrm{n}=1$ ): NPB 1821
. Haplotype RAL4816 ( $\mathrm{n}=1$ ): RAL 4816
Haplotype SR285 (n=5): SR 285, SR 171, K. coddiana SR sn, TD 4638, RAL 4227
Haplotype SR328 ( $\mathrm{n}=1$ ): SR 328
Haplotype SR282 ( $\mathrm{n}=2$ ): SR 282, SR 295
Haplotype SR300 (n=27): SR 300, SR 279, SR 299, SR 302, SR 149, SR 542, SR 297, SR 321, K. gracilis NNBG, SR 242, SR 289, K. latifolia RSS sn, NPB 1810, SR 291, SR 290, SR 200, K. pauciflora HB sn, SR 407, SR 305, SR 485, RJM 1100, SR 561, SR 409, SR 441, SR 442, SR 558, SR 460

Haplotype SR286 (n=1): SR 286
Haplotype SR400 ( $\mathrm{n}=1$ ): SR 400
Haplotype SR287 (n=1): SR 287
Haplotype SR269 (n=2): SR 269; SR 264
Haplotype TD4626 (n=11): TD 4626, SR 446, SR 268, SR 342, SR 263, SR 237, SR 266, PBP 5579,SR 172, SR 337, SR 471
Haplotype SR391 (n=1): SR 391
Haplotype SR388 (n=4): SR 388, JMG 031, SR 389, SR 390
Haplotype SR174 (n=1): SR 174
Haplotype SR307 (n=1): SR 307
Haplotype Chap9061 ( $\mathrm{n}=1$ ): Chapman \& Chapman 9061
Haplotype insiSRsn (n=3): SR sn K. insignis, AMM 2647, CK 4821
Haplotype JMG034 (n=6): JMG 034, JMG 036, SR 387, JMG 038, SR 386, SR 393
Haplotype SR310 (n=1): SR 310
Haplotype SR312 (n=1): SR 312
Haplotype SR383 (n=1): SR 310
Haplotype KporphSRsn (n=1): K. porphyantha SR sn
38. Haplotype RGsn (n=5): RG sn, K. ensifolia JB sn, K. leucocephala NNBG, K. rigidifolia SR sn, K. triangularis subsp. obtusifolia SR sn
39. Haplotype SR303 ( $\mathrm{n}=1$ ): SR 303
40. Haplotype SR453 ( $\mathrm{n}=1$ ): SR 453
41. Haplotype SR278 ( $\mathrm{n}=4$ ): SR 278, SR 270, RJM 974, SR 274

## Appendix 8: List of haplotype generated by the TCS analysis for South African samples

A total of 42 haplotype were recognised by TCS of which nine were internal node haplotypes. The remaining 33 are characterised below:

1. Haplotype SR549 (n=1): SR 549
2. Haplotype SR549 (n=1): SR 549
3. Haplotype SR468 ( $\mathrm{n}=1$ ): SR 468
4. Haplotype SR467 (n=3): SR 467, SR 330, SR398
5. Haplotype SR283 ( $\mathrm{n}=1$ ): SR 283
6. Haplotype SR311 ( $\mathrm{n}=1$ ): SR 311
7. Haplotype laxiCSRsn $(\mathrm{n}=1)$ : K. laxiflora $C$ SR $s n$ 8. Haplotype SR211 (n=4): SR 211, SR 201, RJM 1026, SR530
8. Haplotype KtypNNBG (n=11): K. typhoides NNBG, SR186, K. brachystachya SR sn, SR 176, SR 529, SR 207, SR 203, SR

202, SR 532, SR 528, SR 166
10. Haplotype TD4477 ( $\mathrm{n}=1$ ): TD 4477
11. Haplotype SR275 ( $\mathrm{n}=1$ ): SR 275
12. Haplotype NPB1923 ( $\mathrm{n}=1$ ): NPB 1923
13. Haplotype SR314 (n=8): SR 314, SR 452, SR 360, SR 382, SR 448, SR 485, SR 344, SR 477
14. Haplotype NPB1821 ( $\mathrm{n}=1$ ): NPB 1821
15. Haplotype RAL4816 ( $\mathrm{n}=1$ ): RAL 4816
16. Haplotype SR285 (n=5): SR 285, SR 171, K. coddiana SR sn, TD 4638, RAL 4227
17. Haplotype SR328 ( $\mathrm{n}=1$ ): SR 328
18. Haplotype SR282 ( $\mathrm{n}=2$ ): SR 282, SR 295
19. Haplotype SR286 ( $\mathrm{n}=1$ ): SR 286
20. Haplotype SR300 ( $\mathrm{n}=27$ ): SR 300, SR 279, SR 299, SR 302, SR 149, SR 542, SR 297, SR 321, K. gracilis NNBG, SR 242,

SR 289, K. latifolia RSS sn, NPB 1810, SR 291, SR 290, SR 200, K. pauciflora HB sn, SR 407, SR 305, SR 485, RJM 1100, SR
561, SR 409, SR 441, SR 442, SR 558, SR 460
21. Haplotype SR287 ( $\mathrm{n}=1$ ): SR 287
22. Haplotype SR400 ( $\mathrm{n}=1$ ): SR 400
23. Haplotype SR269 ( $\mathrm{n}=2$ ): SR 269; SR 264
24. Haplotype TD4626 (n=11): TD 4626, SR 446, SR 268, SR 342, SR 263, SR 237, SR 266, PBP 5579,SR 172, SR 337, SR 471
25. Haplotype SR174 ( $\mathrm{n}=1$ ): SR 174
26. Haplotype SR307 (n=1): SR 307
27. Haplotype SR310 (n=1): SR 310
28. Haplotype SR312 (n=1): SR 312
29. Haplotype SR303 (n=1): SR 303
30. Haplotype SR453 (n=1): SR 453
31. Haplotype RGsn (n=5): RG sn, K. ensifolia JB sn, K. leucocephala NNBG, K. rigidifolia SR sn, K. triangularis subsp. obtusifolia SR sn
32. Haplotype KporphSRsn (n=1): K. porphyantha SR sn
33. Haplotype SR278 ( $\mathrm{n}=4$ ): SR 278, SR 270, RJM 974, SR 274

## Appendix 9: GeoDis input file at half degree grid scale (NCA: South African samples)



```
2333000 S 230000 E
33 Joubertina
1333000 S 23 3000 E
34 Kareedouw
3333000 S 23 3000 E
35 Port Eliza
4333000 S 25 3000 E
36 Grahamstown
5330000 S 26 3000 E
3 7 \text { East london}
1330000 S 27 3000 E
38 Fish River Mouth
1333000 S 2700 00 E
39 Humansdorp
3340000 S 24 3000 E
21
Clade 1-2
3
456
01
4
20191418
1000
1110
0001
Clade 1-3
2
7
10
2
36
10
0
Clade 1-4
3
91011
101
9
3432337939303135
211000000
110112212
000000 0 0 1
Clade 1-6
2
1314
01
5
36683538
10000
31211
Clade 1-8
4
16171819
0100
7
24182823291911
1000000
111111100
0}00000001
0 1 0 0 0 0 1
Clade 1-9
4
20212223
1011
13
111317211581691426122422
100000000000000
3 3 111131241511111
0
```

```
0}00000010000000
Clade 1-10
2
2425
10
6
262537272836
20 0 0 0 0
6
Clade 1-11
2
2627
10
2
3915
10
01
Clade 1-12
2
2829
0
2
10
0}
Clade 1-13
2
3031
0
2
82
10
0}
Clade 1-14
2
3233
0
5
352104
10000
11111
Clade 2-1
2
1-1 1-4
11
1 0
13432337939303135
2000000000
0321112212
Clade 2-2
2
1-2 1-9
1 0
16
201914181113172115816926122422
2}1111110000000000000000000
006 04 3 11 1 3 34111111
Clade 2-3
2
1-3 1-12
0 0
3
364
110
101
Clade 2-4
2
1-5 1-8
10
```

```
7
18242823291911
0 1 0 0 0 0 0
2 21111111
Clade 2-5
2
1-6 1-13
0}
6
3668353821
412110
001001
Clade 2-6
3
1-7 1-15 1-10
0 0 1
9
1 2 1 7 1 8 2 6 2 5 3 7 2 7 2 8 3 6
100000000
0120000000
0 0 0 8 1 1 1 1 1
Clade 2-7
2
1-11 1-14
0}
7
3915352104
1100000
0 0 21111
Clade 3-1
2
2-1 2-7
1 0
1 6
1343233793930313515352104
2321112212000000
00 0 0 001100 0 0 1 211111
Clade 3-2
2
2-2 2-4
1 0
1 9
201914181113172115816926122422282329
2 1 7 1 5 3 1 1 3 3 4111111100 0
0100311000000000003 0 1 1 1
Clade 3-3
3
2-3 2-5 2-6
1 0 1
16
3643683538211217182625372728
211000 0 0 0 0 0 0 0 0 0 0
01043112000000000
00010000113 81111
Total Cladogram
3
3-1 3-2 3-3
1 1 1
3 9
13432337939303135153521042019141811131721816
2612242228232963638253727
2321113212121111000000 0 0 0 0 0 0
0}00000000000000
000001000030000022746311341
141111000000
0000000001102000010030012 308
10010025 2 1 1 1
END
```


# Appendix 10: GeoDis input file at full degree grid scale (NCA: South African samples) 



```
01
2
34
10
01
Clade 1-4
3
91011
110
8
20161545142517
11200000
211111212
00000001
Clade 1-6
2
1314
0 1
5
1845172
1 0000
31211
Clade 1-8
4
16171819
0 0 0 1
7
11813102496
1000000
1111100
0000010
0100001
Calde 1-9
4
20212223
1110
8
68975121122
10000000
711123111
00010000
00001000
Clade 1-10
2
2425
0 1
5
1223191318
2 0 0 0 0
61121
Clade1-11
2
2 6 2 7
0 1
2
207
10
0 1
Clade 1-13
2
3031
0}
2
59
10
0}
Clade 1-14
2
3233
```

```
0
3
3221
10
311
Clade 2-1
2
1-1 1-4
1 1
9
120161545142517
200000000
032311213
Clade 2-2
2
1-2 1-9
10
8
97865121122
31100000
113184111
Clade 2-3
2
1-3 1-12
0}
2
34
11
2
Clade 2-4
2
1-5 1-8
1 0
7
81113102496
1000000
2211111
Clade 2-5
2
1-6 1-13
0 0
6
18451729
412110
001001
Clade 2-6
3
1-7 1-15 1-10
0 0 1
7
681223191318
1000000
0400000
0081121
Clade 2-7
2
1-11 1-14
0}
5
2073221
11000
00411
Clade 3-1
2
2-1 2-7
1 0
1 3
12016154514251773221
2323112130000
```

0100000001411
Clade 3-2
2
2-2 2-4
01
11
97865121122131024
414284111000
10310020111
Clade 3-3
3
2-3 2-5 2-6
101
13
3418517296812231913
3100000000000
0143111000000
0010000148112
Total Cladogram
3
3-1 3-2 3-3
$\begin{array}{lll}1 & 1\end{array}$
25
12016154514251773221986121123131024182319
2423112131411000000000000
00000414000000559131111000
0000230010310141800200511
END

## Appendix 11: GeoDis output file at half degree grid scale (NCA: South African samples)

```
Differentiating population structure from history - Geodis 2.4
(c) Copyright, 1999-2005 David Posada and Alan Templeton
Contact: David Posada, University of Vigo, Spain (dposada@uvigo.es)
```

```
nput file:
```

nput file:
Applications/phylosoft/GeoDis2.4/SYD/Half_deg/geodis_half.txt
Applications/phylosoft/GeoDis2.4/SYD/Half_deg/geodis_half.txt
Kniphofia cpDNA
Kniphofia cpDNA
Tue Jan 31 12:11:33 SAST 2006
Tue Jan 31 12:11:33 SAST 2006
PERMUTATION ANALYSIS OF Clade 1-2
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 6.6667
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.8820
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 1-2 -30 14'24" 29 16'48"
4 -30 30'00" 29 30'00"
5 -30 05'38" 29 20'38"
CLADE }4\mathrm{ (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 5 (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 6 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
llll
PART III. TEST OF INTERIOR VS. TIP CLADES:
TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE -78.4667 0.3370 0.7610
PERMUTATION ANALYSIS OF Clade 1-3
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 2.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 1-3 -26 19'60" 30 00'00"

```

```

PART III. TEST OF INTERIOR VS. TIP CLADES:

| TYPE OF DISTANCE | I-T DISTANCE | PROB. $<=$ | PROB.>= |
| :---: | :---: | :---: | :---: | :---: |
| WITHIN CLADE | 0.0000 | 1.0000 | 1.0000 |
| NESTED CLADE | -74.0597 | 0.5270 | 1.0000 |

PERMUTATION ANALYSIS OF Clade 1-4
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 14.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.7430
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 1-4 -32 24'19" }2416'44
9 -33 30'00" 23 23'05"
10-31 55'27" 24 34'33"
11-33 30'00" 25 30'00'
CLADE }9\mathrm{ (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE 16.4443 0.0050 0.9980
NESTED CLADE 148.2542
CLADE }10\mathrm{ (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE }414.2011 0.9550 0.0500
NESTED CLADE
CLADE }11\mathrm{ (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
PART III. TEST OF INTERIOR VS. TIP CLADES:
$\begin{array}{ccccc}\text { TYPE OF DISTANCE } & \text { I-T DISTANCE } & \text { PROB. }<= & \text { PROB. }>= \\ \text { WITHIN CLADE } & 401.0457 & 0.9970 & 0.0040\end{array}$ $\begin{array}{lllll}\text { WITHIN CLADE } & 401.0457 & 0.9970 & 0.0040\end{array}$ $\begin{array}{llll}\text { NESTED CLADE } & 242.8228 & 0.9910 & 0.0100\end{array}$
PERMUTATION ANALYSIS OF Clade 1-6
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC $=1.4063$
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN

```
```

OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

```

PART II. GEOGRAPHIC DISTANCE ANALYSIS:


PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{ccccc} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & -297.8284 & 0.5580 & 0.8840 \\
NESTED CLADE & -123.6842 & 0.5580 & 0.8840
\end{tabular}
```

PERMUTATION ANALYSIS OF Clade 1-8

```
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC \(=18.4500\)
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.7940

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{2}{|l|}{\multirow[t]{2}{*}{\begin{tabular}{cc} 
GEOGRAPHICAL CENTERS \\
Clade 1-8 & \(-3110^{\prime} 13^{\prime \prime}\) \\
\hline
\end{tabular}}} & ITUDE & \multirow[t]{2}{*}{LONGITUDE} \\
\hline & & 2'24" & \\
\hline 16 -31 00' & -31 00'00" 2930 & & \\
\hline 17 -31 33' & -31 33'50" 28 05' & & \\
\hline 18 -30 00' & -30 00'00" 28 59' & & \\
\hline \(19-2937\) & -29 37'30" 2852 & & \\
\hline CLADE 16 (Interior) & rior) & & \\
\hline TYPE OF DISTANCE & TANCE DISTAN & EE PROB & BB.<= PROB. \(>=\) \\
\hline WITHIN CLADE & ADE 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & LADE 108.8491 & 0.6800 & 0.5260 \\
\hline CLADE 17 (Tip) & & & \\
\hline TYPE OF DISTANCE & TANCE DISTAN & CE PROB & PB.<= PROB. \(>=\) \\
\hline WITHIN CLADE & ADE 91.1247 & 0.1770 & 0.8490 \\
\hline NESTED CLADE & LADE 104.4307 & 0.2000 & 0.8260 \\
\hline CLADE 18 (Interior) & rior) & & \\
\hline TYPE OF DISTANCE & TANCE DISTAN & CE PROB & B. \(<=\) PROB. \(>=\) \\
\hline WITHIN CLADE & ADE 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & LADE 143.1576 & 0.7440 & 0.3620 \\
\hline CLADE 19 (Interior) & rior) & & \\
\hline TYPE OF DISTANCE & TANCE DISTAN & CE PROB & BB.<= PROB. \(>=\) \\
\hline WITHIN CLADE & ADE 107.2991 & 0.9020 & 0.1500 \\
\hline NESTED CLADE & LADE 188.5663 & 0.9790 & 0.0730 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
```

TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=
WITHIN CLADE
lurn

```
```

PERMUTATION ANALYSIS OF Clade 1-9
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 20.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.8990
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 1-9 -29 29'57" 29 41'30"
20 -28 59'60" 29 30'00'
21-29 32'22" 29 42'13"
-28 59'60" }30\mathrm{ 00'00
23-28 30'00" 28 59'60"
CLADE 20 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 21 (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 22 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 23 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
PART III. TEST OF INTERIOR VS. TIP CLADES:
TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=
lull
PERMUTATION ANALYSIS OF Clade 1-10
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 1.4773
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 1-10 -32 31'17" }26\mathrm{ 36'23"
24-32 30'00" }26 30'00"
25 -32 31'21" }26 36'44
CLADE 24 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE 10.2425 0.3640 1.0000

```
```

CLADE 25 (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE 80.3518 0.8640 0.5000
NESTED CLADE 80.4357 0.8640 0.5000

```

PART III. TEST OF INTERIOR VS. TIP CLADES:
```

TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
PERMUTATION ANALYSIS OF Clade 1-11
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 2.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000
PART II. GEOGRAPHIC DISTANCE ANALYSIS:

```

```

PART III. TEST OF INTERIOR VS. TIP CLADES:

```
```

TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=

```
TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=
        WITHIN CLADE 
        WITHIN CLADE 
        NESTED CLADE 118.8253 1.0000 0.5270
PERMUTATION ANALYSIS OF Clade 1-12
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC = 2.0000
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000
```

PART II. GEOGRAPHIC DISTANCE ANALYSIS:

| GEOGRAPHICAL CENTERS | LATITUDE | LONGITUDE |  |
| :---: | :---: | :---: | :---: |
| Clade 1-12 | $-2500^{\prime} 00 "$ | 30 | $24^{\prime} 00 "$ |



```
PERMUTATION ANALYSIS OF Clade 2-1
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC = 1.0000
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0810
PART II. GEOGRAPHIC DISTANCE ANALYSIS
```

```
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
```

GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 2-1 -31 24'14" }24\mathrm{ 50'46
Clade 2-1 -31 24'14" }24\mathrm{ 50'46
-1 -24 00'00" 29 30'00'
-1 -24 00'00" 29 30'00'
1-4 -32 22'10" 24 14'21'
1-4 -32 22'10" 24 14'21'
CLADE 1-1 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 1-4 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE }325.7980 0.0180 0.989
NESTED CLADE
NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

```
    PERMUTATION ANALYSIS OF Clade 2-2
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC \(=28.0000\)
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0180
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{2}{|l|}{GEOGRAPHICAL CENTERS L} & ITUDE & \multirow[t]{2}{*}{LONGITUDE} \\
\hline Clade 2-2 -29 & -29 37'49' 29 & 37'07" & \\
\hline 1-2 -30 14 & -30 14'24" 2916 & & \\
\hline \(1-9 \quad-2929\) & -29 29'57" 2941 & & \\
\hline CLADE 1-2 (Tip) & & & \\
\hline TYPE OF DISTANCE & TANCE DISTA & & . \(<=\) PROB. \(>=\) \\
\hline WITHIN CLADE & ADE 53.0592 & 0.1470 & 0.8530 \\
\hline NESTED CLADE & LADE 95.7345 & 0.5580 & 0.4420 \\
\hline CLADE 1-9 (Interior) & erior) & & \\
\hline TYPE OF DISTANCE & TANCE DISTAN & CE PR & B. \(<=\) PROB.>= \\
\hline WITHIN CLADE & ADE 93.2685 & 0.2080 & 0.7920 \\
\hline NESTED CLADE & LADE 97.1396 & 0.4450 & 0.5550 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>= \(\begin{array}{llll}\text { WITHIN CLADE } & 40.2093 & 0.7770 & 0.2230\end{array}\) \(\begin{array}{llll}\text { NESTED CLADE } & 1.4050 & 0.4420 & 0.5580\end{array}\)
```

PERMUTATION ANALYSIS OF Clade 2-3
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 2.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 2-3 -25 29'60" 30 15'00"
1-3 -26 19'60" 30 00'00"
1-12 -25 00'00" 30 24'00

| CLADE 1-3 (Interior) |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
| TYPE OF DISTANCE | DISTAN |  | B.<= | PR |
| WITHIN CLADE | 98.7463 | 0.8480 | 0.48 |  |
| NESTED CLADE | 132.6438 | 1.0000 |  |  |
| CLADE 1-12 (Interior) |  |  |  |  |
| TYPE OF DISTANCE | DISTANCE PROB.<= PROB.>= |  |  |  |
|  | 16.1090 | 0.4840 | 0.848 |  |
|  | 60.9604 | 0.484 |  |  |

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP
PERMUTATION ANALYSIS OF Clade 2-4
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 2.5926
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000
PART II. GEOGRAPHIC DISTANCE ANALYSIS:

```
```

GEOGRAPHICAL CENTERS LATITUDE LONGITUDE

```
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
    Clade 2-4 -31 09'28" 28 27'25"
    Clade 2-4 -31 09'28" 28 27'25"
            1-5 -31 00'00" 29 30'00'
            1-5 -31 00'00" 29 30'00'
            1-8 -31 10'13" 28 22'24"
            1-8 -31 10'13" 28 22'24"
CLADE 1-5 (Tip)
    TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
        WITHIN CLADE 
        NESTED CLADE 
CLADE 1-8 (Interior)
    TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
        WITHIN CLADE 
        NESTED CLADE 
PART III. TEST OF INTERIOR VS. TIP CLADES:
TYPE OF DISTANCE 
    WITHIN CLADE }1116.3956 0.6140 0.7070 
```

```
PERMUTATION ANALYSIS OF Clade 2-5
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC = 6.5185
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3740
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
```



```
NO INTERIOR/TIP CLADES EXIST IN THIS GROUP
PERMUTATION ANALYSIS OF Clade 2-6
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC = 1.0000
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0190
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
```



PART III. TEST OF INTERIOR VS. TIP CLADES:

| TYPE OF DISTANCE | I-T DISTANCE | PROB.<= | PROB.>= |
| :---: | :---: | :---: | :---: | :---: |
| WITHIN CLADE | -60.0898 | 0.5430 | 0.4570 |
| NESTED CLADE | 117.0343 | 0.9690 | 0.0310 |

```
PERMUTATION ANALYSIS OF Clade 2-7
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 1.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5790
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
```



```
NO INTERIOR/TIP CLADES EXIST IN THIS GROUP
PERMUTATION ANALYSIS OF Clade 3-1
    BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
    OBSERVED CHI-SQUARE STATISTIC = 21.9363
    THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
    OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0410
PART II. GEOGRAPHIC DISTANCE ANALYSIS:
```

```
GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
```

GEOGRAPHICAL CENTERS LATITUDE LONGITUDE
Clade 3-1 -29 41'27" 26 48'33'
Clade 3-1 -29 41'27" 26 48'33'
2-1 -31 24'14" }2450'4\mp@subsup{0}{}{\prime\prime
2-1 -31 24'14" }2450'4\mp@subsup{0}{}{\prime\prime
2-7 -26 46'14" }3009'21
2-7 -26 46'14" }3009'21
CLADE 2-1 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
CLADE 2-7 (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE
PART III. TEST OF INTERIOR VS. TIP CLADES:
TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE

```
```

PERMUTATION ANALYSIS OF Clade 3-2
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 31.1949
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0020
PART II. GEOGRAPHIC DISTANCE ANALYSIS:

| GEOGRAPHICAL CENTERS |  | TUDE | LONGITUDE |
| :---: | :---: | :---: | :---: |
| Clade 3-2 -30 0 | -30 05'23" 29 15'07" |  |  |
| 2-2 -29 37'05 | -29 37'05" 2936 |  |  |
| 2-4 -31 08'14 | -31 08'14" 2826 |  |  |
| CLADE 2-2 (Tip) |  |  |  |
| TYPE OF DISTANCE | DISTAN | CE PROB | PB.<= PROB.>= |
| WITHIN CLADE | ADE 96.2076 | 0.0000 | 1.0000 |
| NESTED CLADE | LADE 117.4931 | 0.0190 | 0.9810 |
| CLADE 2-4 (Interior) | erior) |  |  |
| TYPE OF DISTANCE | TANCE DISTAN |  | B.<= PROB.>= |
| WITHIN CLADE | ADE 114.9561 | 0.3850 | 0.6150 |

PART III. TEST OF INTERIOR VS. TIP CLADES:
TYPE OF DISTANCE
NESTED CLADE 49.7281 0.9580 0.0420
PERMUTATION ANALYSIS OF Clade 3-3
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 58.5556
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000
PART II. GEOGRAPHIC DISTANCE ANALYSIS:

| GEOGRAPHICAL CENTERS | LATITUDE | LONGITUDE |  |
| :---: | :---: | :---: | :---: |
| Clade 3-3 | $-3038^{\prime} 08^{\prime \prime}$ | $2754^{\prime} 04 "$ |  |
| $2-3$ | $-25300^{\prime \prime} 00 "$ | $30155^{\prime \prime} 00^{\prime \prime}$ |  |
| $2-5$ | $-3109^{\prime} 51^{\prime \prime}$ | $2809^{\prime} 06^{\prime \prime}$ |  |
| $2-6$ | $-3158^{\prime} 07 "$ | $2658^{\prime} 19^{\prime \prime}$ |  |

CLADE 2-3 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE 615.9515 0.9980 0.0020
CLADE 2-5 (Interior)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE 304.9098 0.5300 0.4700
CLADE 2-6 (Tip)
TYPE OF DISTANCE DISTANCE PROB.<= PROB.>=
WITHIN CLADE
NESTED CLADE 216.0606 0.0000 1.0000
PART III. TEST OF INTERIOR VS. TIP CLADES:

```

TYPE OF DISTANCE I-T DISTANCE PROB.<= PROB.>= \(\begin{array}{llll}\text { WITHIN CLADE } & 166.9847 & 0.9580 & 0.0420\end{array}\)
\(\begin{array}{llll}\text { NESTED CLADE } & 16.1418 & 0.6110 & 0.3890\end{array}\)
** ANALYSIS FINISHED **
It took 1.0600 seconds.

\section*{Appendix 12: GeoDis output file at full degree grid scale (NCA: South African samples)}

\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 1-3 & -26 23'60" & 30 00'00" & \\
\hline 7 & -25 00'00" & 30 00'00" & \\
\hline 8 & -27 00'00" & 30 00'00" & \\
\hline CLADE 7 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 155.5254 & 1.0000 & 0.5270 \\
\hline CLADE 8 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 66.6537 & 0.5270 & 1.0000 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
NESTED CLADE & 88.8717 & 1.0000 & 0.5270
\end{tabular}

PERMUTATION ANALYSIS OF Clade \(1-4\)
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC \(=10.3636\)

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.9380

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 1-4 & -32 41'47" & 23 12'37" & \\
\hline 9 & -33 10'35" & 23 31'46" & \\
\hline 10 & -32 31'10" & 22 59'60" & \\
\hline 11 & -33 00'00" & 25 00'00" & \\
\hline CLADE 9 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 59.7725 & 0.0300 & 0.9740 \\
\hline NESTED CLADE & 75.6220 & 0.0100 & 0.9940 \\
\hline CLADE 10 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 265.7729 & 0.8940 & 0.1140 \\
\hline NESTED CLADE & 261.8151 & 0.8900 & 0.1180 \\
\hline CLADE 11 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 170.4019 & 0.7680 & 0.4390 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & -210.8395 & 0.5940 & 0.4070 \\
NESTED CLADE & -41.7617 & 0.7140 & 0.2870
\end{tabular}

PERMUTATION ANALYSIS OF Clade 1-6 BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{PART II. GEOGRAPHIC DISTANCE ANALYSIS:} \\
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITU & \\
\hline Clade 1-6 & -28 44'08" & 27 49'2 & \\
\hline 13 & -33 00'00" & 260010 & \\
\hline 14 & -28 22'52" & \(2758^{\prime} 2\) & \\
\hline \multicolumn{4}{|l|}{CLADE 13 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 504.5870 & 0.8990 & 0.5430 \\
\hline \multicolumn{4}{|l|}{CLADE 14 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.> \(=\) \\
\hline WITHIN CLADE & 403.9716 & 0.6590 & 0.7830 \\
\hline NESTED CLADE & 414.5933 & 0.5430 & 0.8990 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB. \(<=\) & PROB. \(>=\) \\
WITHIN CLADE & -403.9716 & 0.7830 & 0.6590 \\
NESTED CLADE & 89.9936 & 0.8990 & 0.5430
\end{tabular}

PERMUTATION ANALYSIS OF Clade \(1-8\) BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC \(=18.4500\)

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.7940

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 1-8 & -31 18'32" & 27 53'07" & \\
\hline 16 & -31 00'00" & 28 59'60" & \\
\hline 17 & -31 33'36" & 27 38'24" & \\
\hline 18 & -30 00'00" & 28 59'60" & \\
\hline 19 & -29 31'35' & 28 28'25" & \\
\hline CLADE 16 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 111.3849 & 0.4440 & 0.7620 \\
\hline CLADE 17 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.> \(=\) \\
\hline WITHIN CLADE & 97.5123 & 0.4630 & 0.5630 \\
\hline NESTED CLADE & 104.4916 & 0.2490 & 0.7770 \\
\hline CLADE 18 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 180.2473 & 0.7440 & 0.3620 \\
\hline CLADE 19 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 73.4280 & 0.7640 & 0.2880 \\
\hline NESTED CLADE & 208.4031 & 0.9590 & 0.0930 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB. \(<=\) & PROB. \(>=\) \\
WITHIN CLADE & -3.7763 & 0.2810 & 0.7220 \\
NESTED CLADE & -92.1045 & 0.0710 & 0.9320
\end{tabular}

PERMUTATION ANALYSIS OF Calde \(1-9\)
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=10.7265\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.5830

PART II. GEOGRAPHIC DISTANCE ANALYSIS:


PERMUTATION ANALYSIS OF Clade 1-10
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=1.4773\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{cccr} 
GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
Clade 1-10 & \(-3225^{\prime} 54^{\prime \prime}\) & \(2603^{\prime} 09 "\) & \\
& 24 & \(-3200^{\prime} 00 "\) & \(2600^{\prime \prime} 00^{\prime \prime}\) \\
& 25 & \(-327^{\prime} 38^{\prime \prime}\) & \(2603^{\prime} 22^{\prime \prime}\) \\
CLADE 24 (Interior) & & & \\
TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & 0.0000 & 0.4010 & 1.0000 \\
NESTED CLADE & 48.1984 & 0.3640 & 1.0000
\end{tabular}
\begin{tabular}{rrrr} 
CLADE 25 (Tip) & & & \\
TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & 94.0316 & 1.0000 & 0.3640 \\
NESTED CLADE & 93.8446 & 0.8920 & 0.4720 \\
& & & \\
PART III. TEST OF INTERIOR VS. TIP CLADES: & \\
& & & \\
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & -94.0316 & 0.3640 & 1.0000 \\
NESTED CLADE & -45.6462 & 0.3640 & 1.0000
\end{tabular}
PERMUTATION ANALYSIS OF Clade1-11
    BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC = 2.0000

THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:


PERMUTATION ANALYSIS OF Clade \(1-13\)
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC = 2.0000
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{cccc} 
GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
Clade \(1-13\) & \(-2911^{\prime} 60 "\) & \(2859^{\prime} 60 "\) & \\
30 & \(-2800^{\prime} 00 "\) & \(2859^{\prime} 60^{\prime \prime}\) & \\
31 & \(-3000^{\prime} 00 "\) & \(2859^{\prime} 60^{\prime \prime}\) & \\
CLADE 30 (Interior) & & & \\
TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
NESTED CLADE & 133.3075 & 1.0000 & 0.5270
\end{tabular}
\begin{tabular}{rrrr} 
CLADE 31 (Interior) & & & \\
TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
NESTED CLADE & 88.8717 & 0.5270 & 1.0000
\end{tabular}

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{PERMUTATION ANALYSIS OF Clade 1-14 BASED ON 1000 RESAMPLES} \\
\hline \multicolumn{4}{|l|}{PART I. PERMUTATIONAL CONTINGENCY TEST:} \\
\hline OBSERVED CHI-SQUAR & STATISTIC = & 0.6000 & \\
\hline \multicolumn{4}{|l|}{THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN
OR EOUAL TO THE OBSERVED CHI-SOUARE \(=1.0000\) OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000} \\
\hline \multicolumn{4}{|l|}{PART II. GEOGRAPHIC DISTANCE ANALYSIS:} \\
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITU & \\
\hline Clade 1-14 & -26 09'60" & \(3023 ' 2\) & \\
\hline 32 & -25 00'00" & 30 00'0 & \\
\hline 33 & -26 14'07" & 3024.4 & \\
\hline \multicolumn{4}{|l|}{CLADE 32 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 135.3351 & 0.6790 & 1.0000 \\
\hline \multicolumn{4}{|l|}{CLADE 33 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 209.8508 & 1.0000 & 0.6790 \\
\hline NESTED CLADE & 208.6634 & 0.8380 & 0.8410 \\
\hline
\end{tabular}

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF Clade \(2-1\)
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=18.0000\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0450

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 2-1 & -31 26'38" & \(2402{ }^{\prime \prime}\) & \\
\hline 1-1 & -24 00'00" & 28 59'60" & \\
\hline 1-4 & -32 41'47" & 23 12'37" & \\
\hline CLADE 1-1 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 0.0750 & 1.0000 \\
\hline NESTED CLADE & 959.5455 & 1.0000 & 0.0050 \\
\hline CLADE 1-4 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 213.5975 & 0.0050 & 1.0000 \\
\hline NESTED CLADE & 305.2983 & 0.0050 & 1.0000 \\
\hline
\end{tabular}

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF Clade 2-2
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=17.2083\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0230

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline \multirow[t]{4}{*}{GEOGRAPHICAL \(\begin{aligned} & \text { CENTERS } \\ & \text { Clade } 2-2 \\ & 1-2 \\ & 1-9\end{aligned}\)} & LATITUDE & LONGITUDE & \\
\hline & -29 32'09" & 29 18'49" & \\
\hline & -29 54'06" & 28 56'04" & \\
\hline & -29 28'15" & 29 22'51" & \\
\hline \multicolumn{4}{|l|}{CLADE 1-2 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 38.2989 & 0.0100 & 0.9900 \\
\hline NESTED CLADE & 75.2995 & 0.1340 & 0.8660 \\
\hline \multicolumn{4}{|l|}{CLADE 1-9 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 106.4647 & 0.7900 & 0.2100 \\
\hline NESTED CLADE & 108.2847 & 0.8700 & 0.1300 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & 68.1658 & 0.9840 & 0.0160 \\
NESTED CLADE & 32.9852 & 0.8970 & 0.1030
\end{tabular}

PERMUTATION ANALYSIS OF Clade \(2-3\) BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=1.3333\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{GEOGRAPHICAL CENTERS LATITUDE LONGITUDE} \\
\hline Clade 2-3 & -25 52'30" & 30 00'00" & \\
\hline 1-3 & -26 23'60" & 30 00'00" & \\
\hline 1-12 & -25 00'00" & 30 00'00" & \\
\hline \multicolumn{4}{|l|}{CLADE 1-3 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 93.3152 & 1.0000 & 0.5100 \\
\hline NESTED CLADE & 116.6440 & 1.0000 & 0.5100 \\
\hline \multicolumn{4}{|l|}{CLADE 1-12 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 0.5100 & 1.0000 \\
\hline NESTED CLADE & 97.2034 & 0.5100 & 1.0000 \\
\hline
\end{tabular}

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{OBSERVED CHI-SQUARE STATISTIC \(=2.5926\)} \\
\hline \multicolumn{4}{|l|}{THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 1.0000} \\
\hline \multicolumn{4}{|l|}{PART II. GEOGRAPHIC DISTANCE ANALYSIS:} \\
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITU & \\
\hline Clade 2-4 & -31 16'07" & \(2753 ' 2\) & \\
\hline 1-5 & -30 00'00" & 280010 & \\
\hline 1-8 & -31 18'32" & \(2753^{\prime \prime}\) & \\
\hline \multicolumn{4}{|l|}{CLADE 1-5 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 141.3188 & 0.7070 & 0.5990 \\
\hline \multicolumn{4}{|l|}{CLADE 1-8 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.> \(=\) \\
\hline WITHIN CLADE & 115.0567 & 0.5990 & 0.7070 \\
\hline NESTED CLADE & 115.4667 & 0.5990 & 0.7070 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB. \(<=\) & PROB. \(>=\) \\
WITHIN CLADE & 115.0567 & 0.5990 & 0.7070 \\
NESTED CLADE & -25.8520 & 0.5990 & 0.7070
\end{tabular}

PERMUTATION ANALYSIS OF Clade 2-5
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=\quad 6.5185\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.3740

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 2-5 & -28 46'49" & 27 56'11" & \\
\hline 1-6 & -28 44'08" & 27 49'23" & \\
\hline 1-13 & -29 11'60" & 28 59'60" & \\
\hline CLADE 1-6 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 421.5012 & 0.9200 & 0.1310 \\
\hline NESTED CLADE & 420.9898 & 0.9200 & 0.1310 \\
\hline CLADE 1-13 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 106.6460 & 0.4210 & 0.6300 \\
\hline NESTED CLADE & 156.2353 & 0.1090 & 0.9420 \\
\hline
\end{tabular}

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF Clade 2-6
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{OBSERVED CHI-SQUARE STATISTIC \(=36.0000\)} \\
\hline \multicolumn{4}{|l|}{THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000} \\
\hline \multicolumn{4}{|l|}{PART II. GEOGRAPHIC DISTANCE ANALYSIS:} \\
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITU & \\
\hline Clade 2-6 & -32 02'52" & 26 21'5 & \\
\hline 1-7 & -28 59'60" & \(2859 ' 6\) & \\
\hline 1-15 & -30 00'00" & 28 00'0 & \\
\hline 1-10 & -32 25'54" & 260310 & \\
\hline \multicolumn{4}{|l|}{CLADE 1-7 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 1.0000 & 1.0000 \\
\hline NESTED CLADE & 422.1125 & 1.0000 & 0.0620 \\
\hline \multicolumn{4}{|l|}{CLADE 1-15 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 0.0000 & 0.0230 & 1.0000 \\
\hline NESTED CLADE & 275.6257 & 0.9990 & 0.0010 \\
\hline \multicolumn{4}{|l|}{CLADE 1-10 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 90.9617 & 0.0030 & 0.9970 \\
\hline NESTED CLADE & 101.1576 & 0.0010 & 0.9990 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES
\begin{tabular}{lrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & -90.9617 & 0.1480 & 0.8520 \\
NESTED CLADE & 203.7655 & 1.0000 & 0.0000
\end{tabular}
```

PERMUTATION ANALYSIS OF Clade 2-7
BASED ON 1000 RESAMPLES
PART I. PERMUTATIONAL CONTINGENCY TEST:
OBSERVED CHI-SQUARE STATISTIC $=1.0000$
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.2030

```

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 2-7 & -26 54'37" & 29 49'37" & \\
\hline 1-11 & -32 56'51" & 25 15'47" & \\
\hline 1-14 & -26 09'60" & 30 23'20" & \\
\hline CLADE 1-11 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 263.0417 & 0.7710 & 0.2560 \\
\hline NESTED CLADE & 810.6780 & 1.0000 & 0.0270 \\
\hline CLADE 1-14 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 204.5896 & 0.1050 & 0.9220 \\
\hline NESTED CLADE & 231.5518 & 0.0550 & 0.9720 \\
\hline
\end{tabular}

NO INTERIOR/TIP CLADES EXIST IN THIS GROUP

PERMUTATION ANALYSIS OF Clade 3-1
BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{OBSERVED CHI-SQUARE STATISTIC \(=22.4792\)} \\
\hline \multicolumn{4}{|l|}{THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0040} \\
\hline \multicolumn{4}{|l|}{PART II. GEOGRAPHIC DISTANCE ANALYSIS:} \\
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITU & \\
\hline Clade 3-1 & -30 06'44" & 254413 & \\
\hline 2-1 & -31 26'38" & 24 02'3 & \\
\hline 2-7 & -26 54'37" & 29 49'3 & \\
\hline \multicolumn{4}{|l|}{CLADE 2-1 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 399.5099 & 0.0190 & 0.9810 \\
\hline NESTED CLADE & 508.8250 & 0.0490 & 0.9510 \\
\hline \multicolumn{4}{|l|}{CLADE 2-7 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB. \(>=\) \\
\hline WITHIN CLADE & 295.0503 & 0.0840 & 0.9160 \\
\hline NESTED CLADE & 640.8546 & 0.9700 & 0.0300 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB. \(>=\) \\
WITHIN CLADE & -104.4596 & 0.2540 & 0.7460 \\
NESTED CLADE & 132.0296 & 0.9720 & 0.0280
\end{tabular}

PERMUTATION ANALYSIS OF Clade 3-2 BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:

OBSERVED CHI-SQUARE STATISTIC \(=24.4286\)
THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0020

PART II. GEOGRAPHIC DISTANCE ANALYSIS:
\begin{tabular}{|c|c|c|c|}
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITUDE & \\
\hline Clade 3-2 & -30 18'21" & \(2840 ' 49 \prime\) & \\
\hline 2-2 & -29 32'09" & 29 18'49" & \\
\hline 2-4 & -31 16'07" & 27 53'20" & \\
\hline CLADE 2-2 (Interior) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 103.3165 & 0.0010 & 0.9990 \\
\hline NESTED CLADE & 144.6634 & 0.1360 & 0.8640 \\
\hline CLADE 2-4 (Tip) & & & \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 116.2646 & 0.3350 & 0.6650 \\
\hline NESTED CLADE & 164.5546 & 0.6510 & 0.3490 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB.>= \\
WITHIN CLADE & -12.9481 & 0.2510 & 0.7490 \\
NESTED CLADE & -19.8912 & 0.3010 & 0.6990
\end{tabular}
```

PERMUTATION ANALYSIS OF Clade 3-3

```
    BASED ON 1000 RESAMPLES

PART I. PERMUTATIONAL CONTINGENCY TEST:
\begin{tabular}{|c|c|c|c|}
\hline \multicolumn{4}{|l|}{OBSERVED CHI-SQUARE STATISTIC \(=56.5083\)} \\
\hline \multicolumn{4}{|l|}{THE PROBABILITY OF A RANDOM CHI-SQUARE BEING GREATER THAN OR EQUAL TO THE OBSERVED CHI-SQUARE = 0.0000} \\
\hline \multicolumn{4}{|l|}{PART II. GEOGRAPHIC DISTANCE ANALYSIS:} \\
\hline GEOGRAPHICAL CENTERS & LATITUDE & LONGITU & \\
\hline Clade 3-3 & -30 07'40" & 27 21'2 & \\
\hline 2-3 & -25 52'30" & 300010 & \\
\hline 2-5 & -28 46'49" & 27 56'1 & \\
\hline 2-6 & -32 02'52" & 2621.5 & \\
\hline \multicolumn{4}{|l|}{CLADE 2-3 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.>= \\
\hline WITHIN CLADE & 109.3538 & 0.1200 & 0.8800 \\
\hline NESTED CLADE & 541.5202 & 0.9930 & 0.0070 \\
\hline \multicolumn{4}{|l|}{CLADE 2-5 (Interior)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.> \(=\) \\
\hline WITHIN CLADE & 395.4836 & 0.8380 & 0.1620 \\
\hline NESTED CLADE & 426.7021 & 0.9110 & 0.0890 \\
\hline \multicolumn{4}{|l|}{CLADE 2-6 (Tip)} \\
\hline TYPE OF DISTANCE & DISTANCE & PROB.<= & PROB.> \(=\) \\
\hline WITHIN CLADE & 130.7005 & 0.0010 & 0.9990 \\
\hline NESTED CLADE & 267.6463 & 0.0000 & 1.0000 \\
\hline
\end{tabular}

PART III. TEST OF INTERIOR VS. TIP CLADES:
\begin{tabular}{lrrrr} 
TYPE OF DISTANCE & I-T DISTANCE & PROB.<= & PROB. \(>=\) \\
WITHIN CLADE & 268.6643 & 1.0000 & 0.0000 \\
NESTED CLADE & 109.2606 & 0.9020 & 0.0980
\end{tabular}

\footnotetext{
** ANALYSIS FINISHED **
It took 0.9330 seconds.
}

Appendix 13: Data matrix used for the leaf transectional phenetic analysis
K. acraea TD4626
k. albesens SR314
K. angustfolia SR453
K. baurii SR174
k. baurii SR285
. baurii SR360
K. baurii NPB1923
K. brachystachya SRs
K. breviflora SR452
. breviflora SR
k. buchananii SR305
. buchananii SR307
k. buchananii SR458
K. caulescens SR270
K. caulescens SR27
k. citrina SR176
K. coddiana SRsn
K. coddiana RAL4820
K. coralligemma SR549
K. coralligemma SR549
K. drepanophylla RJM1100
. ensifolia autumnalis SR448
. ensifolia ensifolia JBsn
fibrosa SR297
fibrosa PBP 5579
fluviatilis SRsn
. gracilis SR308
. gracilis SR321
galpinii SR312
hirsuta SR282
ichopenesis SR241
ichopenesis SR242
ichopensis SR409
latifolia RSSsn
laxiflora SR283
laxiflora SR295
laxiflora SR441
. laxiflora SR442
K. laxiflora SR468
K. laxifloraC SRsn
K. leucocephala NNBG
k. linearifolia SR151
k. linearifolia SR170
k. linearifolia SR182
k. linearifolia SR269
linearifolia SR287
linearifolia SR290
linearifolia SR291
, linearifolia SR311
. linearifolia SR328
. linearifolia SR343
linearifolia SR400
linearifolia JPsn
littoralis SR200
multiflora SR310
northiae SR263
northiae SR274
parviflora SR268
parviflora SR330
pauciflora HBsn
porphyantha SRsn
praecox SR529
praecox TD4461
rigidifolia SRsn
ritualis SR300
rooperi SR237
sarmentosa SR207
splendida SR548
stricta SR279
thodei SR407
triangularis SR264
triangularis SR266
triangularis SR267
triangularis SR299
triangularis SR304
triangularis obtusiloba SRsn
typhoides JB8084
tysonii SR199
tysonii SR302
. tysonii SR303
K. tysonii SR460
K. umbrina RGsn
K. uvaria SR166
k. uvaria SR186
K. uvaria SR201
K. uvaria SR203
K. uvaria SR211
k. uvaria TD4477
. uvaria SR337
\(10 ? 00001111011111120020010010101010001011100011110000000110\) 121001001110111111120010010011111010111011101011110000000110 100000011110111111120010020011111110111011101111110000000110 121101011110111111110010010011111110111011101011110000100110 101001011110111111110010010011101110111011101011110000000110 101110011110111111120010010011111110111011101011110000001110 11000011110111111120010010011111110111011101011110000000110 2100001111011111112002002001111111011101110111111000000011 \(10 ? 100011110111111120020010011111110111011101011110000000110\) 10101101111011111100100100111111011101110101110000001110 100000011110111111120020010010101010111011101011110000000110 100101011110111111120010020011111110111011101111110000000110 120001011110111111200200100101010101101101011110000000110 1200000111101111111001001001111100000000000011111010100110 100010111 10111111 \(001001001110100000000001111111101 ?\)
 1200010 ?11 101111112 001001001010111011101100011110000000110

 1000010?11 1011111110010010011111101101101011110000000110 100001110111111200200100111111011011101011110000000110 10110001111011111110020010011111110111011101011110000000110 22000001111001111120020020011111110111011101011110000000110 22010001111011111110010010011111110111011101111110000000110 1001010111100111112001001001111111011101110111110000000110 \(10 ? 10001111011111110010010011111110111011101011110000000110\) 121100011111111111110010010011111110111011101011110000000110 \(10 ? ? 0001111011111120010010011111110111011101011110000000110\) 1100010111101111111001001001111110111011101011110000000110 121100011110111111110010010011111110111011101011110000000110 121100011110111111110020010011111110111011101011110000000110 111000011110111111110010010011111110111011101011110000000110 101101011110111111110010010011111110111011101011110000000110 121000011110111111110010010011111110111011101011110000000110 101100011110111111110010010011111110111011101111110000000110 \(1010000111101111111100200100111111101110111010111100000001 ? 0\) 10100101111111111120020010010101010111011101011110000000110 101101011111111111120010010010101010111011101011110000000110 11000011111111111110020010011111111111011101011110000000110 101000011110111111120010010011111110111011101011110000000110 220000011110111111110010010011111110111011000011110000000000 101101011110011111120020010011111110111011101011110000000110 11111011110111111120010010011111110111011101011110000000110 21110011110111111120020010011111110111011100011110000001110 01011011110111111110010010011111110111011100011111010101111 11010011110111111110010010011111110111011101011110000000110 101001011110111111120010010011111110111011100011111010100110 101001011110111001110010010011111110111011100011110010100110 101000011110111111110010010011111110111011100011110010100110 101001011110111111120010010011111110111011101011110000100110 01101011110111111110010010011111110111000101011110000000110 101000011110111111120020010011111110111011101011110000000110 101001011110111111120010010011111110111011100011111010100100 10100001111011111112000001001111111011101110001111000000110 101010011111111111110010010011111110111011101011110000000110 00000101101000111100010010000101100001011101001110000000010 00010110 010010111 1000000110 2200000111 10111111 0010001111111110000001110 020000000110 0 \(01.0001111011111200100100101 ? 1 ? 1011101110111110000000110\) 101000011110111111001001001111110110111010111000000000 101001011111111112002001001010110111011101011110000000110 1010010111011111110010010011111101101100011100000100110
 120001111011111100000100111111011011010101110000000110 \(2000011110111111200200100101 ? 1 ? 1011101100001111010100 ? ?\) 200010111011111200100100101101011101101011110000000110
 100001110111111200100100111111011101110111110000000110 0100001110100001010010010011111101101110111110000000110 0100001110111111200100200111111011011101111110000000110 0100011101111110110000000111110 101000011110100111120020020011111110111011101111110000000110 12110101111011111120010010011111110111011101111110000000110 11000011110111111110010010011111110111011101111110000000110 121000011110111111110010010011111110111011101111110000000100 121101011110111111110000010011111110111011101011110000000110 101001011110111111110010010011111110111011101011111010100110 10100101111111111110010010011111110111011100011111010100111 10100101111111111110010010011101110111011101011110010100110 01101011110111111110010010011111110111011101111110000000010 10111101111011111120010010011111110111011101011110000001110 \(01 ? 00011110111111120020010011111110111011101011110000000110\) \(20101011110111111122 ? 2001001111110111011101011110000000110\) 20001011010111111120010010011111110111011100011111010100111 1200010110 101101111011111120020020011111110111011101111110000001110 101011011110111111110020010011111110111011101011110000001110
K. uvaria SR342
K. uvaria SR471
K. uvaria SR172
. uvaria SR47
. uvaria SR344
\begin{tabular}{|c|c|}
\hline K. acraea TD4626 & 0?000 \\
\hline K. albesens SR314 & 0?000 \\
\hline K. angustfolia SR453 & 00000 \\
\hline K. baurii SR174 & 01000 \\
\hline K. baurii SR285 & 00000 \\
\hline K. baurii SR360 & 01000 \\
\hline K. baurii NPB1923 & 01000 \\
\hline K. brachystachya SRsn & 0?000 \\
\hline K. breviflora SR452 & 01000 \\
\hline K. bruceae SR171 & 01010 \\
\hline K. buchananii SR305 & 0?000 \\
\hline K. buchananii SR307 & 0?000 \\
\hline K. buchananii SR458 & 0?000 \\
\hline K. caulescens SR270 & 0?000 \\
\hline K. caulescens SR278 & 13000 \\
\hline K. citrina SR176 & 01000 \\
\hline K. coddiana SRsn & 0?000 \\
\hline K. coddiana RAL4820 & 01000 \\
\hline K. coralligemma SR549 & \(1 ? 000\) \\
\hline K. drepanophylla RJM1100 & 0?000 \\
\hline K. ensifolia autumnalis SR448 & 0?000 \\
\hline K. ensifolia ensifolia JBsn & 01000 \\
\hline K. fibrosa SR297 & 00000 \\
\hline K. fibrosa PBP 5579 & 00000 \\
\hline K. fluviatilis SRsn & 0?000 \\
\hline K. gracilis SR308 & 0?000 \\
\hline K. gracilis SR321 & 01000 \\
\hline K. galpinii SR312 & 0?000 \\
\hline K. hirsuta SR282 & 0?000 \\
\hline K. ichopenesis SR241 & 0?000 \\
\hline K. ichopenesis SR242 & 01000 \\
\hline K. ichopensis SR409 & 0?000 \\
\hline K. latifolia RSSsn & 01000 \\
\hline K. laxiflora SR253 & 01000 \\
\hline K. laxiflora SR283 & 00000 \\
\hline K. laxiflora SR295 & 01000 \\
\hline K. laxiflora SR441 & 01000 \\
\hline K. laxiflora SR442 & 01000 \\
\hline K. laxiflora SR468 & 0?000 \\
\hline K. laxifloraC SRsn & 0?000 \\
\hline K. leucocephala NNBG & 00000 \\
\hline K. linearifolia SR151 & 01100 \\
\hline K. linearifolia SR170 & 01000 \\
\hline K. linearifolia SR182 & 01000 \\
\hline K. linearifolia SR269 & 11000 \\
\hline K. linearifolia SR287 & 01000 \\
\hline K. linearifolia SR290 & 11000 \\
\hline K. linearifolia SR291 & 11000 \\
\hline K. linearifolia SR311 & 11000 \\
\hline K. linearifolia SR328 & 01000 \\
\hline K. linearifolia SR343 & 00000 \\
\hline K. linearifolia SR400 & 01000 \\
\hline K. linearifolia JPsn & 11000 \\
\hline K. littoralis SR200 & 01000 \\
\hline K. multiflora SR310 & 01000 \\
\hline K. northiae SR263 & 00100 \\
\hline K. northiae SR274 & 00000 \\
\hline K. parviflora SR268 & 0?000 \\
\hline K. parviflora SR330 & 00000 \\
\hline K. pauciflora HBsn & 00000 \\
\hline K. porphyantha SRsn & \(0 ? 000\) \\
\hline K. praecox SR529 & 00000 \\
\hline K. praecox TD4461 & 01000 \\
\hline K. rigidifolia SRsn & 0?000 \\
\hline K. ritualis SR300 & 01000 \\
\hline K. rooperi SR237 & 00000 \\
\hline K. sarmentosa SR207 & 13000 \\
\hline K. splendida SR548 & 01000 \\
\hline K. stricta SR279 & 00001 \\
\hline K. thodei SR407 & 0?000 \\
\hline K. triangularis SR264 & 01000 \\
\hline K. triangularis SR266 & 01000 \\
\hline K. triangularis SR267 & \(0 ? 000\) \\
\hline K. triangularis SR299 & 01000 \\
\hline K. triangularis SR304 & 00000 \\
\hline K. triangularis obtusiloba SRsn & 01000 \\
\hline K. typhoides JB8084 & 00000 \\
\hline K. tysonii SR199 & 01000 \\
\hline K. tysonii SR302 & \(1 ? 000\) \\
\hline K. tysonii SR303 & 1 1?000 \\
\hline K. tysonii SR460 & 11000 \\
\hline K. umbrina RGsn & 01000 \\
\hline K. uvaria SR166 & 00000 \\
\hline K. uvaria SR186 & 0?000 \\
\hline K. uvaria SR201 & 00000 \\
\hline K. uvaria SR203 & 10000 \\
\hline K. uvaria SR211 & 0?000 \\
\hline
\end{tabular}
\begin{tabular}{ll} 
K. uvaria TD4477 & \(0 ? 000\) \\
K. uvaria SR337 & 01000 \\
K. uvaria SR342 & 01000 \\
K. uvaria SR471 & 01000 \\
K. uvaria SR172 & 01000 \\
K. uvaria SR477 & \(010 ? 0\) \\
K. uvaria SR344 & 01000
\end{tabular}

Appendix 14: Data matrix used for the SEM leaf surface phenetic analysis
\begin{tabular}{|c|c|c|}
\hline K. acraea TD4626 & 1001110110 & 0002200 \\
\hline K. albescens SR314 & 0001010110 & 0002200 \\
\hline K. angustifolia SR453 & 0000000110 & 0002200 \\
\hline K. angustifolia SR542 & 0001010110 & 0002200 \\
\hline K. ankaratrensis PBP5676 & 1000010010 & 1002200 \\
\hline K. baurii SR174 & 0110000110 & 0001100 \\
\hline K. baurii SR275 & 0001111111 & 1002200 \\
\hline K. baurii SR285 & 1001111110 & 0002211 \\
\hline K. brachystachya SRsn & 0001010110 & 0001100 \\
\hline K. breviflora SR452 & 1000010110 & 0002200 \\
\hline K. breviflora SRsn & 0000000110 & 0002200 \\
\hline K. bruceae SR171 & 1001000111 & 1001100 \\
\hline K. buchananii SR307 & 1000010110 & 0002200 \\
\hline K. caulescens SR270 & 0001010110 & 0002200 \\
\hline K. citrina SR176 & 0000000111 & 1001100 \\
\hline K. coddiana SRsn & 1000010110 & 0001100 \\
\hline K. coralligemma SR549 & 1001111110 & 0002201 \\
\hline K. drepanophylla RJM1100 & 1000010111 & 1002100 \\
\hline K. ensifolia autumnalis SR448 & 1001010110 & 0002200 \\
\hline K. ensifolia ensifolia JBsn & 1001010110 & 0002200 \\
\hline K. fibrosa PBP5579 & 0000000000 & 0001100 \\
\hline K. fluvialitis SRsn & 0110000110 & 0002200 \\
\hline K. foliosa JMG034 & 0??1010110 & 0002200 \\
\hline K. galpinii SR312 & 1001000101 & 1002100 \\
\hline K. gracilis SR308 & 1000010111 & 0001100 \\
\hline K. gracilis SR321 & 1001010011 & 0002200 \\
\hline K. grantii CIP4154 & 0001010110 & 0001100 \\
\hline K. hirsuta SR282 & 1001111111 & 0111200 \\
\hline K. ichopensis SR242 & 0001010110 & 0002200 \\
\hline K. insignis SRsn & 1001000000 & 0002200 \\
\hline K. insignis TT30 & 1001010000 & 0001100 \\
\hline K. isoetifolia JMG033 & 0001010000 & 0002100 \\
\hline K. latifolia RSSsn & 1000010010 & 0001100 \\
\hline K. laxiflora SR253 & 0001010110 & 0002200 \\
\hline K. laxiflora SR295 & 0001010110 & 0002200 \\
\hline K. leucocephala NNBG & 0000000110 & 0001100 \\
\hline K. linearifolia SR151 & 0000000000 & 0002200 \\
\hline K. linearifolia SR170 & 1001010111 & 1001100 \\
\hline K. linearifolia SR182 & 0000000111 & 1001100 \\
\hline K. linearifolia SR269 & 1000010110 & 0001100 \\
\hline K. linearifolia SR287 & 0001010110 & 0001100 \\
\hline K. linearifolia SR290 & 1000010001 & 1001100 \\
\hline K. linearifolia SR291 & 1000011000 & 0001100 \\
\hline K. linearifolia SR311 & 1000000010 & 0001100 \\
\hline K. linearifolia SR328 & 1001011111 & 1002100 \\
\hline K. linearifolia SR343 & 1001011011 & 0001100 \\
\hline K. linearifolia SR400 & 0000000001 & 1002100 \\
\hline K. linearifolia JPSsn & 1110010000 & 0001100 \\
\hline K. littoralis SR200 & 1000010010 & 0002200 \\
\hline K. multiflora SR310 & 1000011011 & 0001200 \\
\hline K. northiae SR263 & 1000100111 & 0002200 \\
\hline K. parviflora SR268 & 0? ?0000000 & 0002200 \\
\hline K. pauciflora HBsn & 0001010110 & 0001200 \\
\hline K. porphyantha SRsn & 1001011110 & 0001100 \\
\hline K. praecox SR529 & 0001010111 & 1001100 \\
\hline K. praecox TD4461 & 1000011111 & 0001200 \\
\hline K. pumila Friss1079 & 1001010100 & 0001100 \\
\hline K. rigidifolia SRsn & 0000000000 & 0001100 \\
\hline K. ritualis SR300 & 1??0010010 & 0001100 \\
\hline K. rooperii SR237 & 1001100111 & 1002200 \\
\hline K. rooperii SRsn & 10?1011110 & 0002200 \\
\hline K. sarmentosa SR207 & 0000000110 & 0002200 \\
\hline K. schemperi JMG036 & 1000010110 & 0001100 \\
\hline K. splendida SR548 & 1001010111 & 0001200 \\
\hline K. stricta SR279 & 0000000111 & 1002100 \\
\hline K. thodei SR407 & 0000000110 & 0002200 \\
\hline K. thomsonii JMG031 & 0001010110 & 0001100 \\
\hline K. thomsonii AMM2647 & 1001010110 & 0002200 \\
\hline K. thomsonii CK4821 & 0001010110 & 0001100 \\
\hline K. triangularis SR264 & 1000010000 & 0002200 \\
\hline K. triangulari sSR266 & 1000010000 & 0001100 \\
\hline K. triangularis SR267 & 1001010000 & 0001200 \\
\hline K. triangularis SR299 & 1000010111 & 0001100 \\
\hline K. triangularis SR304 & 0000000110 & 0002200 \\
\hline K. triangularis obtusifolia SRsn & 1000010010 & 0002200 \\
\hline K. typhoides JB8084 & 0000000110 & 0002200 \\
\hline K. tysonii SR199 & 1001010111 & 1002200 \\
\hline K. tysonii SR303 & 1001010101 & 0001100 \\
\hline K. umbrina RGsn & 1000010110 & 0002200 \\
\hline K. uvaria SR165 & 0000000110 & 0002200 \\
\hline K. uvaria SR172 & 1000011111 & 0001200 \\
\hline K. uvaria SR186 & 1000010111 & 1001100 \\
\hline K. uvaria SR201 & 1000010110 & 1001100 \\
\hline K. uvaria SR203 & 1000000011 & 1001100 \\
\hline K. uvaria SR211 & 1000000110 & 0002201 \\
\hline K. uvaria SR337 & 1001010110 & 0002211 \\
\hline K. uvaria SR342 & 1?01111111 & 1002201 \\
\hline K. uvaria SR344 & 0000000110 & 0002200 \\
\hline K. uvaria SR471 & 1?00011111 & 0001200 \\
\hline
\end{tabular}
K. uvaria SR477
K. uvaria TD4477

00000001100002200
00000001100001100```

